
# Meta Imaging Series®
# MetaMorph
# Drop-in Commands

***Version 7.0 for***
***Microsoft Windows XP®***

## User's Guide

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# List of Available Drop-ins

The following table lists all MetaMorph Drop-ins, the associated commands, the menus from which they are accessed, the assigned drop-in category, and a brief description.

| Drop-in | Menu | Command Name | Category | Description |
|---|---|---|---|---|
| ACQUIRE | Acquire | Acquire | Common | Acquires images from digital cameras. |
| ACQUIRECOLOR | Acquire | Acquire Color | Common | Configures acquisition parameters for single-chip color cameras such as the CoolSNAP camera, and acquires color or grayscale images. |
| ACQUIREULTRA | Acquire | Acquire from ImageXpress Ultra | Special | Acuires images from an ImageXpress Ultra. |
| DCAMVID | Acquire | Digital Camera Video Control | Common | Dialog which controls standard video output of digital cameras. |
| DIGITAL4 | Acquire | Acquire from Digital Camera | Common | Acquires from digital camera. Can be used with any digital camera. |
| FLASHBUS | Acquire | Acquire from Flashbus | Common | Acquires images from the video camera using the Flashbus board. |
| SPOTCAM | Acquire | Acquire from Spot Camera | Common | Acquires 24-bit color images or stacks of 12-bit single-channel monochrome images from a SPOT camera. |
| STREAM | Acquire | Stream Acquisition | Common | Configures and controls high-speed stream acquisition data to RAM or realtime hard disk. |
| TLAPSE | Acquire | Acquire Timelapse | Common | Acquires a series of frames at a specified interval and duration. |
| VIDEVICE | Acquire | Set Acquisition Board-Camera | Common | Selects the video device and switches |

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| | | | | between cameras. |
| CELLVIEW | Acquire | Cellview Acquisition | Legacy | Controls devices and acquisition from Scanalytics EPR system. Use Multi Dimensional Acquisition (ndacquir) instead. this is a legacy drop-in. |
| CFGCCD | Acquire | Configure Digital Camera | Legacy | Configures a digital CCD camera. This is a legacy drop-in. These settings are now made in the Meta Imaging Series Administrator. |
| ZOOMPAN | Acquire | Zoom, Pan and Scroll Video | Legacy | Zooms, pans, and scrolls the video monitor display (requires board). |
| MULTIWAC | Acquire | Acquire Multiple Wavelengths | Occasional | Configures and performs acquisition of images using up to six different sets of settings for wavelength, intensity, and exposure. |
| C240060 | Acquire | Hamamatsu C2400-60 | Special | Allows control of Hamamatsu C2400-60 camera controller. |
| DIGADJ | Acquire | Digital Camera Adjustments | Special | Adjusts black level for Hamamatsu ORCA-Series cameras. |
| DWS | Acquire | Acquire DWS Images | Special | Dual wavelength strobe custom application. |
| FRAME | Acquire | Acquire Image with Frame Transfer Camera | Special | Acquires images with a Frame Transfer Camera. |
| SPECTRAL | Acquire | Acquire Spectral Scan | Special | Acquires a series of images in a range of wavelengths from monochromators. |
| SUM16 | Acquire | Sum 16-bit Image | Special | Sums video into a 16-bit image. (requires Flashbus) |
| TWAINCFG | Acquire | Configure TWAIN | Special | Provides a TWAIN driver configuration dialog to select a Twain-compliant device for image |

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| | | | | acquisition and specify whether to use the device's user interface. |
| SCANSLIDE | Apps | Scan Slide | Special | Automatically scans a user-defined section of a slide and displays the result as a single image. |
| BTIME | Apps | Graph Intensities | Common | Graphs multiple regions from stacks or live video. |
| NDACQUIR | Apps | Multi Dimensional Acquisition | Common | Acquires images or image stacks in multiple dimensions with mulltiple parameters and saves the images in multi dimensional data sets. |
| NDPLAYER | Apps | Review Multi Dimensional Data, Multi Dimensional Set Utilities | Common | Reviews, performs file operations, and runs journals on multi dimensional data sets. |
| FINDSPOTS | Apps | Find Spots | Occasional | Finds Spots On An Image And Highlight Them With Regions |
| TRACKOBJ | Apps | Track Objects | Common | Performs motion analysis by automatically tracking one or more objects through an image stack, or a sequential series of single images. |
| TRACKPTS | Apps | Track Points | Common | Performs motion analysis by manually tracking points through planes in a stack. |
| ASTM | Apps | ASTM Grain Size | Legacy | Performs limited metallurgical ASTM grain size measurements. |
| OPEN4D | Apps | Open 4D Series into Stack | Legacy | Creates a single stack of images selected from a grid that references a series of images on disk. This is a legacy drop-in. Suggest using Review Multi-Dimensional Data |

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|---|---|---|---|---|
| | | | | (ndplayer dropin) instead. |
| CELLCNT | Apps | Count 2 Type of Cells, Count Cells | Occasional | Counts cells, or two groups of cells distinquished by staining. |
| COLOCAL | Apps | Meaure Colocalization | Occasional | Measure colocalization two fluorescent probes in B&W images. |
| CORRPLOT | Apps | Correlation Plot | Occasional | Creates a correlation plot between the intensities of corresponding pixels in two images. |
| MEASGRID | Apps | Measure Grid | Occasional | Performs measurements or runs journals over a user definable grid pattern. |
| MEASXYZD | Apps | Measure XYZ Distance | Occasional | Measures manually the distance between points in different Z-axis planes. |
| MVOLUME | Apps | Measure Volume | Occasional | Measures the volume of a thresholded object through a stack. |
| ODSCALE | Apps | Optical Density (Scaled) | Occasional | Creates scaled optical density images and performs scaled optical density measurements. |
| AQI3DVIEW | Apps | AQI 3D Visualizer | Occasional | AQI 3D Visualizer enables you to more fully visualize 3D stacks. |
| TMACQUIRE | Apps | Tissue MicroArray Acquisition | Special | Locates , identifies, and acquires images of tissue microarray spots, on a semi automated basis. |
| FRET | Apps | FRET Analysis | Occasional | Performs background and bleed through correction to Fluorescence Resonance Energy Transfer (FRET) image sets. |

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| AUTOFO_S | Devices | Autofocus via Software > Adjust Focus, Find Focus | Common | Finds an optimum focal position for the microscope using an intensity measurement algorithm. Used for both Adjust Focus and Find Focus. |
| SHUTTER | Devices | Shutter | Common | Opens/closes/toggles active shutter. |
| CENTROID | Devices | Move Motors, Configure Motors, Refocus Motors, Acquire Centroid Z Series | Legacy | Controls the Centroid motion controller. This is a legacy drop-in for a device that is no longer supported by Molecular Devices. |
| CUSTOMIO | Devices | Custom I/O Control | Occasional | Controls parallel and serial Input/Output devices. This is a custom drop-in. |
| AUTOFCUS | Devices | Autofocus via Hardware | Special | Triggers Ludl or Prior autofocus controller cards to run. |
| KODAK | Devices | Kodak MotionCorder | Special | Acquires and plays back images with the Kodak MotionCorder digital video device. |
| LINKAM | Devices | Linkam MDS600/TMS93 Stage | Special | Linkam MDS600/TMS93 Stage temperature, flow rate, and XY control. |
| MCU | Devices | MCU Driver Parameters | Special | Sets the Zeiss MCU 26/27 XYZ device settings. |
| PIBNC | Devices | Set BNC Output Trigger | Special | Set the BNC trigger on Princeton Instruments cameras. |
| SETICCD | Devices | Intensifier Gain | Special | Configures and controls an intensified CCD and provides ICCD gain control. |
| LEICADM | Devices | Leica DMR Microscope | Special | Leica DMR automated microscope control. |
| NIKON | Devices | Nikon Microscope | Special | Nikon automated microscope control. |
| OLYMPUS | Devices | Olympus Microscope | Special | Olympus automated microscope control. |

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| ZEISSMTB | Devices | Zeiss MTB Microscope | Special | Zeiss automated microscope control. You must have the Zeiss MicroToolbox (MTB) software installed. Version 2.13g or later of the MTB is required. |
| LINKAM | Devices | Linkam Heated Stage | Special | Linkam MDS600/TMS93 Stage temperature, flow rate, and XY control. |
| MMKEYPAD | Devices | MMKeypad | Special | Provides an interface for a Remote Control Keypad |
| ARROW | Display | Graphics > Arrow | Common | Stamps arrows onto image. |
| CALIGN | Display | Color Align | Common | Shifts the red, green, and blue planes of an image independently to bring them into alignment Can be used to align fluorescent images acquired with separate cubes. |
| OVERFLUO | Display | Overlay Images | Common | Combines a grayscale image and up to six fluorescence images into a single image and assigns a different color to each image. |
| SETZOOM | Display | Set Image Zoom | Common | Specifies image zoom, or zooms a region to the size of the image and applies a selected magnification level (1 - 800%). |
| TEXT | Display | Graphics | Common | Draws text on an image. |
| WEDGE | Display | Graphics > Gray Wedge | Common | Draws a gray wedge on the image. |
| COMB_BWC | Display | Combine Into B&W + Color | Legacy | Creates a simple overlay of black and white and color images This is a legacy dropin. Recommend using Overlay Images (overfluo) instead. |

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| FULLSCR | Display | Show/Hide Image at Full Screen | Occasional | Displays the selected image centered against a black background. Useful for presentations. |
| GRID | Display | Graphics > Grid | Occasional | Draws a grid on an image or live video. |
| GRIDBIN | Display | Graphics> Boxes on Binary Image | Occasional | Draws grid/boxes on binary images. |
| ILACE | Display | Interlace Images | Occasional | Interlaces two images into a single image (opposite of de-interlace). |
| STRETCH | Display | Stretch and Mirror | Occasional | Stretches and/or mirrors an image. |
| SUBRGN | Display | Use Region For Background | Occasional | Uses the Region For Background by subtracting the grayscale value in a selected region from each pixel in an image. |
| SUBSHIFT | Display | Sub-Pixel Shift | Occasional | Shifts an image in sub-pixel increments in horizontal and/or vertical directions. |
| MOSAIC | Display | Color Mosaic | Special | Creates color images from black and white images acquired using the Sony color mosaic CCD. |
| SPLITVIEW | Display | Split View | Occasional | Splits apart multiple images from a beam splitter or twin cameras. |
| 24BITCPY | Edit | Duplicate > As Displayed | Common | Duplicates image as a 24 bit color image with overlays. |
| PROPMGR | Edit | Image Properties | Occasional | Defines and or removes image annotation properties to an image or a stack of images. |
| ZEISSINF | Edit | Show Zeiss Image Info | Special | Displays Zeiss confocal image information. |
| EMAIL | File | Send Image as Email Attachment | Occasional | Send image as email attachment. |
| STK2TIFF | File | Convert Stack to TIFF's | Occasional | Saves all stacks in a directory as |

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| | | | | sequential TIFF files. |
| RUNUSER | File | Run User Program | Special | Runs a user-written Visual Basic program from within MetaMorph. |
| MMVAR | Journal | Variables | Common | Creates and applies user-defined variables for advanced journaling. |
| JOURNAL | Journal | Common | Journal menu commands. | Contains most of the Journal commands |
| SAVELOOP | Journal | Loop > Save Original and Result Loop | Legacy | Applies one or two journals to each image in a selected directory, saving both original and result images in a separate directory. |
| JNLYESNO | Journal | Journal Control > Branch On User Input | Occasional | Chooses one of two journals to run based on user input . |
| LPALLDIR | Journal | Loop > Loop for all Images in Directory | Occasional | Loop for all images in a directory. |
| MBRANCH | Journal | Journal Control > Branch on Object Measurement | Occasional | Runs one journal if an object measurement meets a set of criteria, and can run a different journal if it does not. |
| PICKPT | Journal | Recording Tools > Pick Point | Occasional | Special journal function to click on an image and record the coordinates to variables. |
| SELRGN | Journal | Recording Tools > Select Region | Occasional | Specialized function used by journals to select a region by its label. |
| SOUND | Journal | Journal Tools > Play Sound File | Occasional | Plays a sound file. |
| STARTUP | Journal | Journal Control > StartUp Journal | Occasional | Configures a journal to run automatically whenever you start MetaMorph. |
| TLAPSE2 | Journal | Journal Control > Multi-Journal Timelapse | Occasional | Plays multiple journals at varying intervals. |
| EDGELIST | Log | Display EdgeList | Occasional | Creates centroid image and binary |

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| | | Log as Image | | stack by reading an edgelist log file. |
| EXTHRESH | Log | Log Color Threshold | Occasional | Logs the color threshold setting as text. |
| LOGAN | Log | Log Image Annotation | Occasional | Logs image annotation information. |
| LOGHISTO | Log | Log Image Histogram | Occasional | Logs image histogram data. |
| LOGPIX | Log | Log Pixels in Region | Occasional | Logs pixel numeric data. |
| CLRTHRES | Measure | Set Color Threshold | Common | Sets the color threshold range for 24-bit color images using RGB, HSI, or HSL color models. |
| IMA | Measure | Integrated Mophometry and Analysis (IMA) | Common | Counts, measures, and logs measurement data of thresholded objects. |
| ANDIST | Measure | Measure Distance with Annotation | Legacy | Measures distance with annotation to data log. |
| AUTOMEAS | Measure | Morphometry | Legacy | Performs morphometric measurement, analysis, and logging of measurement data from image objects This is a legacy dropin for performing automated measurements. Recommend using the IMA drop-in instead. |
| MSTACK | Measure | Morphometry > Create Classifier Stack | Legacy | Creates a measured stack from the result of Measure Objects<br><br>**Note:** This drop-in requires the AUTOMEAS drop-in to be installed. The Create Classifier Stack command cannot be used in a journal. |
| OBJDIST | Measure | Measure Object Distance | Legacy | Measures the distance of a line region across a thresholded object. |

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| ANMEAS | Measure | Annotate Measured Objects | Occasional | Stamps IMA measurements on image. |
| CALIPERS | Measure | Calipers | Occasional | Measures the straight line distance between a pair of movable "caliper" lines. |
| CUTJOIN | Measure | Cut Objects, Join Objects | Occasional | Cuts or join objects. Use this command before using the Integrated Morphometry Analysis command. |
| THRESHOB | Measure | Internally Threshold Objects | Occasional | Separates objects measured in IMA according to each object's intensity information. |
| TOCKMAN | Measure | Optical Density Application | Special | Customized optical density application. |
| BACKCORR | Process | Produce Background Correction Image | Occasional | Produces a Background Correction Image using a variable-median filter. |
| FFT | Process | FFT | Occasional | Performs Fast Fourier Transform (FFT) filtering of images. |
| KERNEL | Process | Convolve with Image Kernel | Occasional | Creates and convolves large image kernels. |
| 2DDECON | Process | 3D Deconvolution-No Neighbors, 2D Deconvolution-Nearest Neighbors | Common | Functions for performing 2d subtractive deconvolution |
| MEASPSF | Process | Measured Point Spread Function | Occasional | Performs Three-Dimensional iterative deconvolution. |
| 3DDECON | Process | AutoQuant 3D Deconvolution | Occasional | Performs Three-Dimensional iterative deconvolution. |
| MORPHOLOGY | Process | Morphology Filters | Common | Filters and smooths binary and grayscale images. |
| RATIO | Process | Ratio Images | Occasional | Ratio images from two sources (like MetaFluor). |
| TRACEOBJ | Regions | Create Region Around Objects | Common | Creates regions around objects in the currently active |

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| | | | | image. |
| LINERGNS | Regions | Convert Regions to Lines | Occasional | Converts polygon regions to line regions. |
| RGNSEQ | Regions | Resequence Region Labels | Occasional | Renumbers regions in an image, eliminating "gaps" in the sequence left after removing one or more regions.. |
| SEGMENTS | Regions | Create Segement Regions | Occasional | Creates polygon segments along a line region. |
| PLATEACQUIRE | Screening | Plate Acquisition,<br>Plate Acquisition and Control,<br>Plate Acquisition Setup | Special | Acquires images from multi well plates. For MetaXpress systems only. |
| HTPLAYER | Screening | Review Screen Data, Screen Utilities | Special | Reviews, performs file operations, and runs journals on multi well plate data. |
| HTDB_PLAYER | Screening | Review Plate Data, Plate Utilities for Database | Special | Accesses and retrieves images from the database. Reviews, performs file operations, and runs journals on multi well plate data. Runs assays on stored images. |
| 3D | Stack | 3D Reconstruction, View Orthoganol Planes, Topographic Surface. Process > Remove Haze. | Common | Acquires, performs 3D reconstruction of, and analyzes Z-series stacks. |
| KEEPPLN | Stack | Keep Planes | Common | Selects the subset of planes from a stack; chooses which planes in a stack to keep/discard |
| PAIR | Stack | Montage Stacks | Common | Combines 2-4 stacks into a montage stack of 2-4 panels. |
| EQUALIZE | Stack | Equalize Light | Occasional | Equalizes the light levels of a stack of images. |
| INTLEAVE | Stack | Interleave Stacks | Occasional | Interleaves planes from two stacks into a single stack. |
| KYMO2 | Stack | Kymograph | Occasional | Creates a cross- |

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| | | | | sectional view of the intensity values of a line region through the planes in a stack. |
| MAKEAVI | Stack | Make AVI | Occasional | Creates an AVI video file from an image stack. |
| STITCH | Stack | Stitch Stack | Occasional | Takes a stack of calibrated images and stitches them into one image. |
| STEREO | Stack | Stereographic Views | Special | Creates stereographic image pairs from a Z-series stack. |

# Open 4D Series into Stack (File Menu)

**Creates a single stack of images from a collection of images on disk. Allows you to preview images while making your selection.**

**Drop-in: OPEN4D**

Use this command when you want to create a new stack of images that you have selected from a series of image files. This command allows you to create a selection grid that references individual image planes from an image stack, images from files with sequentially numbered names or extensions, and a variety of individual image files. Images can then be selected from this 4D Selection Grid, and selected images will be placed in a new stack. All image file formats supported by MetaMorph can be accessed by the 4D Selection Grid.

Open 4D Series into Stack is best used in situations where a four-dimensional series of images has been acquired. For example, you may have acquired a Z-series (depth) image stack at each of several time points, or may have images that have been acquired at a number of different wavelengths at each of a number of Z-positions.

**Note:** Be sure to choose *Grid Use* to view a display of the keyboard shortcuts and mouse actions for selecting images from the grid.

**Open 4D Series into Stack - Overview**

**Configuring the 4D Grid Layout - Single Series Mode**

**Configuring the 4D Grid Layout - User Defined Mode**

**Selecting Images from the 4D Grid**

## Open 4D Series into Stack - Overview

To create a single stack of images from a collection of images on disk, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the File menu, choose Open 4D Series into Stack. The Open 4D Series Into Single Stack dialog box and 4D Selection Grid will appear. |

| | |
|---|---|
| **2** | Choose *Base File* and use the Select Base File dialog box to select the first file to be displayed in the 4D Selection Grid. The base file should be the file for the image that you wish to be the first one being referenced in the grid. If necessary use the *Look In* list or Up One Level icon button to select a different folder. The selected base file will be displayed in the status line next to the *Base Line* button. |
| **3** | If (1) the base file is a stack or is the first in a continuous series of files with sequentially numbered names or extensions, and (2) this file will be the exclusive source of images for the 4D Selection Grid, select *4D Images Stored as Single Sequential Series,* so that an "X" appears in the check box. The dialog box will change to reflect the Single Series mode.<br><br>**OR**<br><br>If you plan to add image files that have a different base name from the selected *Base File,* leave the *4D Images Stored as Single Sequential Series* check box cleared. The format of the dialog box will stay in User Defined mode. |
| **4** | Decide on a layout for storage of images in the 4D Selection Grid. This will be determined by the number of images in each series or image stack. The configuration process and steps involved in adding images to the 4D Selection Grid will be different depending on whether you are in **Single Series mode** or **User Defined mode** (see Step 3). |
| **5** | When the 4D Selection Grid has been configured and images have been added to it, you may begin **selecting images from the grid** to be used in creating a new image stack. |
| **6** | When you have finished selecting images, choose *OK.* Your new stack of selected images will appear. Images will appear in the stack in the order in which they were selected. |
| **7** | Choose *Close* to close the Open 4D Series into Single Stack dialog box. |

## Configuring the 4D Grid Layout - Single Series Mode

To configure and add images to the 4D Selection Grid in "Single Series" mode, use the following procedure:

| Step | Action |
|---|---|
| **1** | In the *X Max* spin box, enter the number of images available in each row. This will affect the maximum number of columns being |

displayed in the 4D Selection Grid.

**AND**

In the *Y Max* spin box, enter the maximum number of rows to be displayed in the 4D Selection Grid.

**2** If you wish, you may change the X-axis title from the default "Time" by typing a new name in the *X Axis* group's *Title* text box. Similarly, you may change the Y-axis title from the default "Z" by typing a new name in the *Y Axis* group's *Title* text box.

**3** Select the method for indexing the files *(Name, Directory, Extension,* or *Plane)* from the *X Axis* group's *Source* drop-down menu. The files to be added will start at the base file and will be indexed incrementally by the selected file attribute.

**4** If image planes increment from row to row and then by column, select *Incr X by Y Max* from the *X-Y Interaction* group. This selection will make the *Incr. By* and *Y Max* options interactive: changing one will change the other.

**OR**

If image planes increment from column to column and then by row, select *Incr Y by X Max.* This selection will enforce a value of *1* in the *X Axis* group's *Incr. By* spin box.

## Configuring the 4D Grid Layout - User Defined Mode

To configure and add images to the 4D Selection Grid in User Defined mode, use the following procedure:

| Step | Action |
|---|---|
| **1** | If you wish, you may change the X-axis title from the default "Time" by typing a new name in the *X Axis* group's *Title* text box. Similarly, you may change the Y-axis title from the default "Z" by typing a new name in the *Y Axis* group's *Title* text box. |
| **2** | Select the method for indexing the files *(Name, Directory, Extension,* or *Plane)* across rows and down columns from the *X Axis* and *Y Axis* groups' *Source* pull-down menus, respectively. For example, you may wish to index the Y-axis by incrementing the *Extension* numbers and index the X-axis by file *Name.* |
| **3** | If you are only adding images from sequentially indexed files, skip to Step 7. |
| | **OR** |
| | If you are adding images from a user-defined list (i.e. non-sequential), select the *List* check box for the appropriate axis. The *Set List* |

button will now be enabled. You will be able to add files from a user-defined list to only one axis. The *List* check box and *Set List* button for the other axis will be unavailable and will appear dimmed.

**4** Choose *Set List.* The Select File List by Hand dialog box will appear.

**5** Choose *Add File* and select a file to be added to the *Files* list.

**AND**

Choose *OK,* and the file will be added to the list.

**6** Repeat Step 5 for all image files you wish to add, and choose *OK* from the Select File List by Hand dialog box.

**7** Use the *X Axis* and *Y Axis* groups' *Incr. By* spin boxes to specify how to increment the indexing of images (as specified in Step 2) across rows and down columns, respectively. These values typically will both be *1*.

**8** Choose *Auto Range* to set the range to be displayed in the 4D Selection Grid, based on the available files.

## Selecting Images from the 4D Grid

To select images from the 4D Selection Grid for placement in a new image stack, use the following procedure:

| Step | Action |
|---|---|
| **1** | If you have a relatively small number of images being referenced in the 4D Selection Grid, you will probably want to verify that the *Grid Range* in the Open 4D Series into Single Stack dialog box is showing all rows and columns. If you see a box-in-box image in the *Grid Range* region, drag the borders of the inner box until they match the borders of the outer box.<br><br>**OR**<br><br>If you have a large number of images being referenced, you will want to verify that the box-in-box image in the *Grid Range* region is highlighting the area which references images you wish to select next. If necessary, move the box-in-box outline within the *Grid Range* boundaries so that the smaller box encloses the desired images. This smaller region of the entire grid will be represented in the 4D Selection Grid window. |
| **2** | If you wish, you can use the *Grid Size* slider in the Open 4D Series into Single Stack dialog box to change the size of the 4D Selection Grid window. |
| **3** | If you wish to turn off the grid display in the |

4D Selection Grid window, clear the *Grid* check box in the Open 4D Series into Single Stack dialog box. Selecting it again will reenable the grid display.

**4** If you want to see a preview of selected images in a separate window as each image is selected, select the *Show Image* check box. Clearing the check box will disable the preview.

**5** Use your mouse or keyboard to select images from the 4D Selection Grid. If you are using keyboard shortcuts, you will need to make the 4D Selection Grid the active window ( [CTRL] + [TAB] ). **Be sure to choose *Grid Use* for a display of all Selection Grid commands.**

**6** Images will be added to the new image stack in the order in which they were selected. If you wish to automatically reorder the numbering of selected images, choose *Renumber.* Images will be renumbered, with "top-to-bottom" renumbering having priority over "left-to-right" renumbering.

**7** When satisfied with your image selection, choose *OK.* A new image stack will appear, containing your selected images. The image stack window will have a title that is based on the specified *Base File* name, with "4D" and the 4D Selection Grid's starting and ending X-Y coordinates appended to the name.

**8** Choose *Close.*

## Open 4D Series into Stack - Dialog Box Options

### Base File

Brings up a file selector for choosing the first file. This file will form the basis from which subsequent files are selected.

### 4D Images Stored as Single Sequential Series

Select this check box if all images of the 4D series are stored in a single stack or are stored in one continuous set of files with sequentially incremented file names or extensions. When you enable this option, the Open 4D Series dialog will be reconfigured for Single Series mode. The *List, Set List, Source,* and *Incr. By* options in the *Y Axis* group will be disabled, and a new control, *X-Y Interaction,* will appear.

### X Max

Sets the maximum number of available columns.

### Title (X Axis)

This editable text field sets the title for the X-axis of the 4D Selection Grid.

### List (X Axis)

This check box determines if the images to be added to each column will be from a user-defined list. Only one axis may be defined from such a list. When this check box enabled in the *X Axis* group, the corresponding *List* check box and *Set List* button will be disabled in the *Y Axis* group.

### Set List (X Axis)

This button brings up a dialog for adding image files to a user-defined list. The image files will be added to

the 4D Selection Grid, one to a column. The *Source* option for the *X Axis* group will automatically be configured to *Name.* Accordingly, the *Source* option for the *X Axis* group will be unavailable.

### Source (X Axis)

This pull-down menu selects the method for sorting image files across the X-axis of the 4D Selection Grid. Sorting can be performed according to the file's *Name,* the *Directory,* the file's *Extension,* and the *Plane* of an image stack. Sorting will be incremented by the number of steps specified in the *Incr. By* option.

### Incr. By (X Axis)

Specifies the step size for incrementing the file indexing (as selected from the *Source* option) across the rows of the 4D Selection Grid. If the dialog is configured for Single Series mode, changing the number in this *Incr. By* field will set the *X-Y Interaction* option to *Incr X by Y Max* and bring about a corresponding numeric change in *Y Max.*

### Y Max

Sets the maximum number of available rows.

### Title (Y Axis)

This editable text field sets the title for the Y-axis of the 4D Selection Grid.

### List (Y Axis)

This check box determines if the images to be added to each row will be from a user-defined list. Only one axis may be defined from such a list. When this check box enabled in the *Y Axis* group, the corresponding *List* check box and *Set List* button will be unavailable in the *X Axis* group.

### Set List (Y Axis)

This button brings up a dialog for adding image files to a user-defined list. The image files will be added to the 4D Selection Grid, one to a row. The *Source* option for the *Y Axis* group will automatically be configured to *Name.* Accordingly, the *Source* option for the *Y Axis* group will be unavailable.

### Source (Y Axis)

This pull-down menu selects the method for sorting files down the Y-axis of the 4D Selection Grid. Sorting can be performed according to the file's *Name,* the *Directory,* the file's *Extension,* and the *Plane* of an image stack. Sorting will be incremented by the number of steps specified in the *Incr. By* option.

### Incr. By (Y Axis)

Specifies the step size for incrementing the file indexing (as selected from *Source)* down the columns of the 4D Selection Grid.

### Grid Range

This box-in-box control is used to select which portions of the range of images will be displayed in the 4D Selection Grid.

### X-Y Interaction

This option, which only appears when the dialog is in Single Series mode, will determine how images are added to the 4D Selection Grid. When *Incr X by Y Max* is selected, each column will consist of as many rows as is specified in *Y Max,* and each image series (planes of a stack, or sequence of files with the same base file name) will be added to the grid down a column. When *Incr Y by X Max* is selected, each row will consist of as many columns as is specified in *X Max,* and each image series will be added to the grid across a row.

### Status line

Displays the last action taken.

### Auto Range

Automatically sets the displayed Grid Range based on the available files. The files are determined by *Set List* and by the settings in the *X Axis* and *Y Axis* groups. If the appropriate files do not exist, an error message will appear. If neither *List* check box has been selected, the range will be determined by the values specified in *X Max* and *Y Max.*

### Renumber

Reorders the numbering of images that have been selected from the 4D Selection Grids. "Top-to-bottom" renumbering has priority over "left-to-right" renumbering.

### Grid Use

This button displays the keyboard shortcuts and mouse actions for selecting images from the 4D Selection Grid.

### Clear All

Deselects all images from the 4D Selection Grid.

### Grid

Determines if indicator lines separating each element will be displayed in the 4D Selection Grid. If this check box is cleared, only the external outline of the grid will be displayed.

### Show Image

When this check box is selected, a preview of each image will be displayed as it is selected from the 4D Selection Grid.

### Grid Size

This slider selects the size of the grid display. This will not affect the range that is displayed.

### Undo Click

Undoes the effects of the previous mouse click in the grid.

### OK

Creates a stack of the images that have been selected from the 4D Selection Grid. Images will be placed in the stack in the order in which they were selected (unless rearranged by choosing the *Renumber* command). If any selected file can not be found, an error message will be displayed. You may then cancel the operation, fill the stack with images acquired up to the error point, or continue, skipping the missing range altogether.

### Close

Closes the dialog.

### 4D Selection Grid

This separate window displays the grid from which images are selected. The appearance of the grid will be determined by the options that have been selected (*X Max* and *Y Max* settings, *Title* entries for the *X Axis* and *Y Axis* groups, etc.).

# 4D Selection Grid Command Shortcuts

| If you want to… | Then use this shortcut: | Or use this mouse action: |
|---|---|---|
| Select/deselect an image | [SPACEBAR] | Left-click on the image. |
| Move the cursor | Corresponding cursor key | Click the desired image. |
| Deselect all images | [BACKSPACE] | Right-click anywhere. |
| Undo the previous action | [U] | Choose *Undo Click.* |
| Select a row of images | [R] | Click just to the left of the row. |
| Select a column of images | [C] | Click just above the column. |

# Run User Program (File Menu)

**Runs a user-defined Visual Basic routine from within MetaMorph.**

### Drop-in: RUNUSER

Use this command to run a previously created set of Visual Basic functions while in MetaMorph. This command is useful for processing and analyzing your images with MetaMorph's extended set of Visual Basic functions, and provides a powerful and flexible addition to the MetaMorph armament of imaging tools.

The full complement of Microsoft Visual Basic functions are available to you with the Run User Program command, including the ability to run "If…Then…Else" routines, create nested subroutines and loops, and to pass and return values with the imaging system. For a complete description of the use of this command and of creating Visual Basic routines with the MetaMorph programming functions, please refer to the Visual Basic Reference Guide, a written manual which is available to you from Molecular Devices upon request.

> **Note:** You can also use MetaMorph built-in variables in the Run User Program command line. You must enclosing the variables in percent signs when entering them into the command line — for example, %Acquire.FPS% is the correct way to enter the Acquire.FPS variable. For more information about MetaMorph's built-in variables, refer to the Introduction to the Use of Variables help page.

## Running a User-Defined Visual Basic Program

To run a Visual Basic program from MetaMorph, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the File menu, choose Run User Program. The Run User Program dialog box will appear. |
| **2** | From the *Program Name* drop-down list, select the user program you want to run.<br><br>**OR**<br>If the name of the program you want to run does not appear in the *Program Name* list, choose the *Browse* button. The Select Start File dialog box will appear. Select the icon for the program you want to run. If necessary, use the *Look In* drop-down list or the Up One Level icon button to find the appropriate drive and folder. Then choose *Open* to return to the Run User Program dialog box.<br><br>**Note:** If you need to remove the currently highlighted program from the *Program Name* list and unregister it with the system, choose *Remove.* |
| **3** | In the *Command Line* text box, type any parameter you want to pass to the user program. For example, you may wish to specify the name of an image. (See the Visual Basic Reference Guide for details.) |
| **4** | If you want the user program to stay in memory after running, select the *Keep Program in Memory After Execution* check box.<br><br>**OR**<br>If you want the user program to be unloaded after running, clear the *Keep Program in* |

*Memory After Execution* check box.

5 When you are ready to run the user program, choose *OK.* The program will run, and the Run User Program dialog box will close automatically.

## Run User Program - Dialog Box Options

### Program Name

Contains a list of all of your user programs that are currently registered with the system. The entries in the list will be descriptions that were entered in Visual Basic when you created your program. If there is no description, the project name entered in Visual Basic will appear instead.

### Command Line

This is a text field which will be passed to your user program as the parameter for the **Startup** and **DoCommand** functions. You might use this field, for example, to specify the name of an image that your program will then load, convolve with an image filter, threshold, measure, log measurement data from, and close.

### Keep Program in Memory After Execution

This check box determines whether your user program will stay in memory after running, or if it will be unloaded when the routine is completed. If you select the check box, the program will stay in memory, and will therefore run more quickly on subsequent runs. If you clear this check box, the program will be unloaded after running. This may be useful when you are debugging your program, as you can leave both MetaMorph and Visual Basic running at the same time, alternating between running your program and editing it. Visual Basic would not be able to recompile your program if the check box were still selected.

When the *Keep Program in Memory* check box is selected, the **Startup** function will be called the first time your program is run after being loaded. Subsequent runs will call the **DoCommand** function. When this check box is cleared, the **Shutdown** function will be called after the program finishes. If this check box is cleared before you run the program for the first time, the **Startup** and **Shutdown** functions will be called each time you run the program.

### Browse

If the program you want to run does not appear in the *Program Name* list, this command button will allow you to search your system for it. This button opens the Select Start File dialog box, which is a standard file-selection dialog box that has a *Look In* drop-down list, Up One Level icon button, File Name text box, and a table that displays the files in the current folder.

When you create a user program, Visual Basic registers it with the system when it is compiled, and it should then be available in the *Program Name* list. However, if you obtain a program that was compiled elsewhere, it will not have been registered on your system. The *Browse* command button will register the program and insert it in the *Program Name* list.

### Remove

Choosing this button will remove the currently highlighted user program from the *Program Name* list and unregisters it with the system.

### OK

Loads the program selected in the *Program Name* list, passes the parameter you specify in the *Command Line* text box, runs the user program, and closes the Run User Program dialog box.

### Cancel

Cancels any changes made in the Run User Program dialog box and closes it.

# Send Image as Email Attachment (File Menu)

**Generates an email message with the active image attached, then sends the message to the address you specify.**

**Drop-in: EMAIL**

Use this command when you want to attach the active image to an email message. This opens a new message and attaches the image automatically without requiring you to open a separate email application.

**Note:**

You must configure your email settings using before sending mail.

## Sending Images as Email Attachments

To send an image as an email attachment, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the File menu, choose *Send Image as Email Attachment*. The Send Image as Email Attachment dialog box will appear. |
| **2** | Type the email address you would like to send the image to in the *TO:* text box or select a previously used address from the *History* list. An address chosen from this list will automatically appear in the *TO:* text box. |
| **3** | In the *Subject* box, type a brief description of your message, such as the name of the image file you are sending. (optional) |
| **4** | The *Image* list box displays the names of all open images; click on the name of the image you want to send. If the active image is a stack an additional list box will appear. In this box you can choose whether to send the entire stack or just the current plane. |
| **5** | Under *Send As*, select a file type for the image you are sending. |
| **6** | If you want to include a description or other message, enter it in the *Message* box. (optional) |
| **7** | Click the *Send* button to send the email message with the image attached. |
| **8** | Click *Cancel* if you do not wish to send the message. |

## Send Image as Email Attachment - Dialog Box Options

### To

Specifies the address of the message recipient.

### History

Lists recently used addresses that the user can select to be entered in the To: field. Select "Use email address entered in To: field" if you want to send the message to the address listed in the To: field.

### Subject

This space allows the user to type in a brief description of the message and its attachment.

**Image**

This lists all open images and allows the user to select the name of the image to be sent.

**Send As**

Click one of these buttons to determine the file format for the attached image.

**Message**

This text box provides a space for the user to enter a message to be sent with the image.

**Send**

Click this button to send the email message and attached image.

**Cancel**

Click this button to cancel the operation.

# Configure Email Settings (File Menu)

**Configures settings to enable the user to send images as email attachments.**

**Drop-in: EMAIL**

Use the Configure Email Settings command before using the Send Image as Email Attachment command. This function allows you to determine how the messages will be sent.

## Configuring Email Settings

To configure your email settings and enable sending images as email attachments, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the File menu, choose *Configure Email Settings*. The Configure Email Settings dialog box will appear. |
| **2** | Type the name of your local mail server into the *SMTP Server* text box. If you are not sure of the name of your mail server please see your system administrator. |
| **3** | In the *Port* box, type the number of BLANK |
| **4** | Enter the sender's email address in the *Sender's E-mail Address* box. For example *user@domain.com* |
| **5** | From the *Dial-Up Entry to Use* list select the dial-up networking profile you want to use. **Note:** If you are not using a modem you can leave this field blank. |
| **6** | If you are using a dial-up entry and want to provide a password, enter it into the *Optional Password for Dial-Up Entry* box. |
| **7** | If you want to log information about messages (such as error messages) type the path name of the file you want to log the information to. |

**8** To save your settings and close the Configure Email Settings dialog box, click the *Okay* button. To cancel your settings, click the *Cancel* button.

## Configure Email Settings - Dialog Box Options

### SMTP Server

The name of your outgoing mail server, for example mail.yourcompany.com.

### Port

The number of the TCP/IP port used to direct mail to and from your site. If you do not know the number of the port you are using, contact your system administrator.

### Sender's Email Address

Enter your email address (or the address of the user). This will appear as the reply to address for your message.

### Dial-Up Entry to Use

If you have a modem and are using dial-up networking connections select the name of the connection to use when sending mail.

### Optional Password for Dial-Up Entry

You can choose to store a password for the dial-up entry in this field.

### Path to Log File

If you want to keep a record of all information (such as error messages) you receive about your mail, type the path (location) of the file you want to use to store this information. For example C:\log\filename.

### Okay

Click *okay* to save your settings and close the dialog box.

### Cancel

Click *cancel* to exit the dialog box without saving your settings.

# Image Properties (Edit Menu)

**Creates and attaches user-defined properties to an image or a stack of images.**

**Drop-in: PROPMGR**

Use this command to create custom-defined "labels" and their values, and to attach them to a single-plane image or an entire stack of images. Examples of such labels might be the name of the person performing the experiment or the grant associated with the experiment. In these examples, the *Name* for the properties could be "Experimenter" and "Grant," and the *Value* might be "Dr. N. E. Boddy" and "NSF 29758-03," respectively. Defined properties are particularly useful when used by the Find Image Files command (File menu).

Image properties can be text-based or numeric. The Define Image Property secondary dialog box which appears when you choose the *Define* button will retain the last ten definitions that you have created, allowing you quickly to update the *Value* associated with the property *(Name)* for the current image. After you attach a user-defined property to an image or stack, you can remove it easily by highlighting its entry in the Image Properties dialog box and choosing *Remove.* The image properties can be viewed either by choosing the Image Properties command, which allows you to edit the properties, or by choosing the Get Info

command (which does not) from the File menu.

**QUICK TIP:** The pop-up context menu that appears when you right-click in the Define Image Property secondary dialog box contains commands that allow you to cut, copy, paste, or delete text, as well as to undo any changes you make.

**For More Information on Managing Images:**

Find Image Files

Get Info

Annotate Image

## Creating and Attaching Image Properties

To create, edit, remove, or simply view user-defined image properties for an image or stack, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Edit menu, choose Image Properties. The Image Properties dialog box will appear. |
| **2** | If you will be editing or reusing a previously created image property, choose *More >>* to expand the dialog box. |
| **3** | If necessary, use the *Image* selector to select the image whose image properties you want to view, edit, or create. |
| **4** | If the image you select already has user-defined properties attached to it, these will appear in the *Image Properties* table. If you want to remove an existing property, highlight its entry and choose *Remove.* |
| **5** | If you want to create a new property or edit an existing one, choose *Define.* The Define Image Property dialog box will appear.<br>**OR**<br>If you have finished viewing or removing the image properties, skip to Step 11. |
| **6** | In the *Name* text box, type the name for the property label (e.g., "Experimenter"), or select an existing entry from the *Most Recently Used Properties* table. |
| **7** | Depending on whether the user-defined property is text-based or numeric, select either *String* or *Number,* respectively, from the *Value Type* option button group. |
| **8** | In the *Value* text box, type the value for the user-defined label. |
| **9** | Choose *Define* to attach the property to the current image.<br>**OR**<br>Double-click the property's entry in the *Most Recently Used Properties* table to attach the |

property to the image.

**10** Repeat Steps 5 - 9, as needed, for any additional properties you want to attach to the current image.

**11** When you have finished, choose *Close* to close the Define Image Property dialog box. Then choose *Close* to close the Image Properties dialog box.

## Image Properties - Dialog Box Options

**Image**

Selects the image whose image properties you want to view, edit, create, or remove.

**Image Properties**

Lists the user-defined properties currently attached to the selected image.

**Define**

Opens the Define Image Property dialog box, from which you can create a new property or edit an existing one.

**Remove**

Detaches the property currently highlighted in the Image Properties table from the selected image.

**Close**

Closes the dialog box.

## Define Image Property - Dialog Box Options

**Name**

The label for the custom image property. For example, this might be "Experimenter." If you select a property from the *Most Recently Used Properties,* the *Name* text box will update to display the associated label for the property.

**Value**

The "value" for the currently selected property. To use the preceding "Experimenter" example, this might be "Dr. John Doe." If you select a property from the *Most Recently Used Properties,* the *Value* text box will update to display the associated value for the property. If you select *Number* from the *Value Type* radio button group, your *Value* entry must be numeric.

**Value Type**

Selects a format for the *Value* text box: *String* (text-based) or *Number.*

**Most Recently Used Properties**

Displays previously created image properties, up to a maximum of ten entries. When you select a previously defined property by clicking it in this table, the *Name* and *Value* text boxes will update to display the label and value for the property, thereby allowing you to edit them.

**Define**

Attaches the current property *(Name* and *Value)* to the selected image.

**More >>**

Expands the dialog box.

**Less <<**

Condenses the dialog box.

**Close**

Closes the dialog box.

# Convert Regions to Lines (Regions Menu)

**Automatically develops line regions from binary skeletonized images in order to use the line regions for line scans or kymographs.**

**Drop-in: LINERGNS**

This function allows you to take a region that isn't a line and convert it to a line region. Convert Regions to Lines is useful if you have odd regions such as pointing branches in your image. As regions, the lines may be analyzed with a linescan or kymograph.

Creating line regions from a skeletonized image is a two-part process. First you need to create regions in your image. There are three basic ways to do this:

**Create Regions around Objects**

**Skeletonize your Image**

**Trace Regions**

Once you have created regions in your image, you can convert them into lines using Convert Regions to Lines.

## Converting Regions to Lines

To convert regions to lines, use the following procedure:

| Step | Action |
|---|---|
| **1** | Once you have created regions in your image that you want to convert into lines, select *Convert Regions to Lines* from the Regions menu. |
| **2** | Next to *Image*, select the name of the image you would like to use. |
| **3** | In the *Line Regions to Keep* group, select which lines in the image you want to keep when converting them to line regions. |
| **4** | If you have selected *Lines Longer than Minimum*, type a value to use for the minimum length a line must be to be included in the conversion. |
| **5** | In the *Convert* group, select whether you want to apply the conversion to an active region or to all regions. |
| **6** | Click the *Convert* button to convert the regions to lines. |
| **7** | Click the *Close* button to close the dialog box and cancel the conversion process. |

## Convert Regions to Lines - Dialog Box Options

**Image**

Select the image you want to apply the conversion to.

### Line Regions to Keep

From this group, select whether you would like to include all lines, the longest line only, or lines longer than minimum.

### Minimum Length

Select the minimum length in pixels of the lines to include in the conversion process.

### Convert

Select whether to apply the conversion to all of the regions in the image or only to the active region.

### Convert

Click this button to convert the regions to lines.

### Close

Click this button to close the dialog box and cancel the conversion process.

# Resequence Region Labels (Regions Menu)

**Renumbers the regions of interest in an image, starting from "1".**

**Drop-in: RGNSEQ**

Use this command when you have removed one or more of the regions that have been defined on an image, and want to eliminate the "gaps" in the numbering sequence. Regions will be reassigned numbers in the order in which they were created.

**For More Information about Regions:**

**Open Regions**

**Save Regions**

**Clear Regions**

**Transfer Regions**

**Region Tools**

## Resequencing Region Labels

To renumber your regions of interest, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Regions menu, choose Sequence Region Labels. The Sequence Region Labels dialog box will appear. |
| **2** | From the *Image* selector, select the image containing the regions that need to be renumbered. |
| **3** | Choose *OK.* The region numbers will be resequenced. |
| **4** | When you have finished, choose *Close.* |

## Resequence Region Labels - Dialog Box Options

### Image

Selects the image with the regions that you want renumbered.

**OK**

Renumbers the regions in the order in which they were created, starting with "1."

**Close**

Closes the dialog box.

# Create Segment Regions (Regions Menu)

**Creates rectangular regions of a selected length and width along a line region.**

**Drop-in: SEGMENTS**

Use this command to create rectangular regions of a specified length and width along the entire length of a line region. The line region can be drawn with any of the line region tools: Single Line Tool, Multi-Line Tool, or Traced Line Tool. You can configure the command to draw the regions above, below, or along the midline of the line region. If your line region is perfectly vertical, selecting *Top* will draw the regions on the right side of the line region, and selecting *Bottom* draws them on the left side.

**For More Information about Region Tools:**

**Single Line Tool**

**Multi-Line Tool**

**Traced Line Tool**

**Moving a Line Region**

**Resizing or Reshaping a Line Region**

## Creating Segment Regions from a Line

To create segmental regions of interest along a line region, use the following procedure.

**Note:** If you want to label the segment regions, be sure to select the *Draw Labels Next to Regions* check box in the Region Labels tab page of the Preferences dialog box (Edit menu).

| Step | Action |
|---|---|
| **1** | From the Regions menu, choose Create Segment Regions. The Create Segment Regions dialog box opens. |
| **2** | If necessary, select the target image with the *Image* selector. |
| **3** | Use one of the line region tools (Single Line Tool, Multi-Line Tool, or Traced Line Tool) to draw your line region in the target image. |
| **4** | If you are using region labels, type a label prefix in the *Segment Label Prefix* text box. The default prefix is "Segment." The prefix will be given an incrementing region number. |
| **5** | Use the *Segment Length* and *Segment Width* spin boxes to select a length and width, respectively, for each of the segment regions that will be created. |
| **6** | Select the geometric configuration of the segment regions from the *Set Regions Along* radio button group: |

***Top*** will draw the segments above the line region. If the line region is perfectly vertical, the segments will be drawn to the right of the line region.

***Bottom*** will draw the segments below the line region. If the line region is perfectly vertical, the segments will be drawn to the left.

***Top and Bottom*** draws pairs of segments both above and below the line region.

***Center*** draws the segments with their midlines centered along the line region.

**7** If you are creating segment regions successively from more than one line region and want to continue the numbering of the segments from one line region group to the next, select the *Incr. Labels* check box.

**OR**
If you want to start each line region's set of segments with number 1, clear the *Incr. Labels* check box.

**8** If you want to remove the original line region during the creation of the segment regions, select the *Delete Line* check box.

**9** When you are ready to create the segments, choose *Create Regions.* The segments will be drawn for the currently active line region.

**Note:** If you need to clear the segments for the currently active line region, choose *Clear Polygons.*

**10** When you have finished, choose *Close.*

## Create Segment Regions - Dialog Box Options

### Image

Selects the image in which the segment regions are to be created.

### Segment Label Prefix

Specifies the prefix for the segment region labels. The prefix will be given an incrementing region number. The default prefix is "Segment."

### Segment Length

Specifies the length (parallel to the line region) of each segment region.

### Segment Width

Specifies the width (perpendicular to the line region) of each segment region.

### Set Regions Along

Selects the geometric configuration of the segment regions:

***Top*** will draw the segments above the line region. If the line region is perfectly vertical, the segments will be drawn to the right of the line region.

***Bottom*** will draw the segments below the line region. If the line region is perfectly vertical, the segments will be drawn to the left.

***Top and Bottom*** draws pairs of segments both above and below the line region.

***Center*** draws the segments with their midlines centered along the line region.

### Incr. Labels

Continues the numbering of the segments from one line region group to the next when you are creating segment regions successively from more than one line region. If you want to restart each line region's set of segments with number 1, leave this check box cleared.

### Delete Line

Removes the original line region during the creation of the segment regions.

### Clear Polygons

Clears the segments for the currently active line region.

### Create Regions

Draws the segment regions along the line region.

### Close

Closes the dialog box.

# Select Region (Regions Menu)

**Programmatically selects a region from defined regions in open images during the running of a journal or lets a user specify a region selection during a programmed pause while running the journal.**

### Drop-in: SELRGN

Use this command to select a region programmatically in a journal. Place this command in your journal to select a predefined region or use it to enable the person running the journal to select a region within an image during a programmed pause. You can specify the name of the region in the Regions Label field or you can pass the name of the region you want to select to the Regions Label field as a variable.

## Selecting Regions

The following procedure assumes that you are recording a journal or editing a journal.

To select a region in a journal, use the following procedure:

| Step | Action |
|---|---|
| **1** | If you are recording a journal, ensure one or more images are open in MetaMorph and that you have defined one or more regions. If you are editing a journal using the Journal Editor, go to step 2. |
| **2** | From the Regions menu, choose Select Region. The Select Region dialog box opens.<br><br>**OR**<br><br>From the Journal Editor, drag or add the Select Region function to your journal dialog. When the Add Function dialog box opens, choose *Yes* or *No* for Interactive Mode. The Select Region dialog box opens. |
| **3** | Click the Image box to identify the image that you want the journal to select. The image drop-down list opens. |

4 Click the image you want to have the journal select, or choose the appropriate method of image selection for the journal to use.

- Choose *Last Result* to use the last resulting image created by this journal.
- Choose *Current at Start* to use the image open and selected at the start of running the journal. (This option is active only in journal edit mode.)
- Choose *Specified* to enable you to change the image selection in the journal editor.
- Choose *Select on Playback* if you want to enable the user running the journal to be able to select or change the image selection while playing the journal.

**Note:** See Image Selectors and Image Selector Structure for Journals for more information about image selectors.

5 In the Region Label box, type the name of the region that you want to select or enter the variable name for the region. Be sure to enclosed the variable name in percent symbols (%*variable_name*%).

6 Click Select to complete adding this function to your journal.

7 Click Cancel to discontinue adding this function to your journal.

## Select Regions - Dialog Box Options

### Image

Identifies the image containing the region that you want to select and to be used by the journal you are running. You can apply a region selection while recording your journal, apply or change an region selection using the Image Editor, or enable the user to apply an region selection when running the journal. Click the image you want to have the journal select, or choose the appropriate method of image selection for the journal to use.

*Last Result* uses the last resulting image created by this journal.

*Current at Start* uses the image open and selected at the start of running the journal. (This option is active only in journal edit mode.)

*Specified* enables you to change the image selection in the journal editor.

*Select on Playback* enables the user running the journal to be able to select or change the image selection while playing the journal.

**Note:** See Image Selectors and Image Selector Structure for Journals for more information about image selectors.

### Region Label

Specifies the name of the region that you want to select. As an alternative, you can enter a variable in this field and programmatically replace the variable with the name of the region you want to select.

### Select

Applies the Select Region function and the associated settings to your journal.

**Cancel**

Discontinues applying the Select Region function to your journal.

# Acquire Timelapse (Acquire Menu)

**Acquires a series of frames at a specified interval and duration. The acquired frames can be placed in a stack or stored on disk. If you are acquiring image(s) using a journal, you are not required to save the acquired images.**

**Drop-in: TLAPSE**

Use Acquire Timelapse to acquire timelapsed images of an experiment. If you are using a RS-170 video camera, this command uses the acquisition settings from the Acquire Image command. If you are using a digital camera, Acquire Timelapse uses the acquisition settings from the Acquire from Digital Camera command.

**Note:** Acquire Z Series, Acquire Timelapse and Acquire Spectral Scan can be running at the same time. However, you will not be able to carry out a command within itself (such as running a timelapse within a timelapse).

## Acquiring Timelapsed Images

**Configuring a Timelapse Acquisition**

**Acquiring Timelapsed Images**

## Configuring a Timelapse Acquisition

To configure a timelapse acquisition, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Acquire Timelapse. The Acquire Timelapse dialog box opens. |
| **2** | Select the desired amount of time between frames using *Time Interval.* Select the desired unit of time from the drop-down list.<br><br>**Note:** Use *Time Interval* = 0 to acquire images as rapidly as possible. In this case, Acquire Timelapse cannot be guaranteed to acquire at precisely regular intervals. |
| **3** | Type the number of frames you want to acquire in the *Number of Planes to Acquire* text box. When you make a change to this setting, the *Duration* settings will be updated automatically.<br><br>**OR**<br>Select the desired duration for the entire timelapse acquisition using *Duration.* Select the desired unit of time from the drop-down list.<br><br>**Note:** Use *Duration* = 0 to acquire images for an unlimited duration (subject to running out of memory or disk space). |

| | |
|---|---|
| **4** | Select the desired type of storage from the *Image Storage* group. Use *None* to perform a timelapse operation without storing images. |
| **5** | If you selected *Stack,* select the destination stack using the *Destination* image selector.<br><br>**OR**<br><br>If you selected *Disk,* choose the *Save File Name* command button to select a file name. Type the file name in the *File Name* text box and select the desired file type using *Files of Type.* Choose *Save* when you have finished.<br><br>**Note:** If Acquire Z Series on Each Interval is set, the file type must be a multiple image format, such as .stk or .pic. |
| **6** | If you want to run a journal between acquisitions, choose the command button next to *Journal to Run.* Select the desired journal name from the *File Name* list. Choose *OK.*<br><br>**Note:** To clear a journal from the selection, choose this command button again and choose *Cancel.* The command button will display *Press to Select* rather than the journal name. |
| **7** | Select any of the desired check box options if necessary. (See Acquire Timelapse Dialog Box Options for more information.) |
| **8** | If you are using a shutter, select the illumination setting associated with your illumination hardware from the *Illumination* list.<br><br>**OR**<br>Otherwise, select "*[None]*." |
| **9** | Choose *OK*. The Timelapse Acquisition dialog box will appear.<br><br>**Note:** If you are recording this command in a journal, choosing *OK* will display a dialog box that asks whether or not you want to record the journal without actually acquiring the images. Choose *Yes* if you want to merely save the journal without acquiring. Choose *No* if you want to both save the journal and acquire the images. |

## Acquiring Timelapsed Images

To collect images using the Timelapse Acquisition dialog box, use the following procedure:

| Step | Action |
|---|---|
| **1** | Configure the acquisition using the procedure presented in Configuring a Timelapse Acquisition**.** |
| **2** | The Timelapse Acquisition dialog box will appear, and the acquisition will start |

immediately.

**3** If you want to change the *Duration* and *Time Interval* settings during acquisition, choose *Interval.* When you have adjusted the duration and time Interval as necessary in the Change Timelapse Interval dialog box, choose *OK* to begin acquisition again.

**4** To control the acquisition process, you can use the *Pause/Resume, Acquire,* and *Stop* command buttons. Use *Acquire* when you want to acquire some of the specified frames manually, rather than at the specified interval.

**5** MetaMorph will close the Timelapse Acquisition dialog box automatically when the acquisition is completed.

## Acquire Timelapse - Dialog Box Options

### Time Interval

Specifies the amount of time between acquired frames. The drop-down list next to the text box specifies the unit of time. Use a *Time Interval* setting of *0* if you want to acquire images as rapidly as possible. However, if you select *0,* Acquire Timelapse cannot be guaranteed to acquire at precisely regular intervals. For best results in acquiring images as rapidly as possible, disable frame-averaging, *Update Image Window,* and *Show Live Between Acquisitions.*

### Duration

Specifies the duration for the entire timelapse acquisition. The drop-down list next to the text box specifies the unit of time. Use a *Duration* setting of *0* if you want to acquire images for an unlimited duration of time (subject to running out of memory or disk space).

### Number of Planes to Acquire

Specifies the number of frames to acquire. The maximum number of frames that can be acquired is 32767. When you make a change to this setting, the *Duration* settings will be updated automatically. If you selected *Acquire Z-Series on Each Interval,* this option title will change to *"Number of Stacks to Acquire."*

### Image Storage

Specifies the type of image storage for the timelapsed images. Select

***None*** if you want to perform a timelapse acquisition without storing images.

***Stack*** to create a stack.

***Disk*** to store images in a file on disk.

### Save File Name

Specifies the file name for image storage when *Disk* is selected as the *Image Storage* type. If a file name has not been selected, the words *"Press to Select"* will appear on the command button. Otherwise the file name will appear on the command button.

### Destination

Specifies the destination stack if *Stack* is selected as the *Image Storage* type.

### Journal to Run for Each Time Interval

Specifies the journal to run between timelapse intervals If a journal file name has not been selected, the words *"Press to Select"* will appear on the command button. Otherwise, the journal name will appear on the command button. **Note:** If you are recording this command in a journal, choosing *OK* will display a dialog box that asks whether or not you want to record the journal without actually acquiring the images. Choose *Yes* if you want to merely save the journal without acquiring. Choose *No* if you want to both save the journal

and acquire the images.

### Update Image Window

Updates the image window after each acquisition when images are stored to a stack. If this option is disabled, the image window will not update until after the timelapse is completed.

### Warn If Interval Time Exceeded

Displays a warning if the selected time interval is exceeded and prompts you to change the interval and duration.

### Acquire Z Series on Each Interval

Expands the dialog box to include the appropriate options from the **Acquire Z Series** dialog box. Acquires a Z-series of images at specified time intervals. This option will not be available if the "3D" drop-in has not been installed. The options that will appear include

***Number of Planes*** - Specifies the number of planes that will be acquired using the spacing selected with the *Step(s)* option. The default is *1.*

***Step(s)*** - Specifies the spacing between planes. This option is based on the Z-axis device driver presently configured. The units of measurements are calibrated using the Focus command.

***Start At*** - specifies the starting location of the stage for the Z-series acquisition. *Current Position* is the stage's present location: *Origin, Top, Bottom,* and *Home* are positions you set with the Focus command.

***Move To*** - Specifies the ending location of the stage for the Z-series acquisition. *Planes * Spacing* specifies the ending location as the *Number of Planes* x *Step(s).* The *Origin, Top, Bottom,* and *Home* options are positions you set with the Focus command.

***After*** - Specifies the location to which the stage will be moved after an acquisition is successfully completed. The *Origin, Top, Bottom,* and *Home* options are positions you set with the Focus command.

***Journal to Run for Each Plane in Z-Series Acquisition*** - Specifies the journal to run between acquisitions. If a journal file name is not selected, the words *"Press to Select"* will appear on the command button. Otherwise, the journal name will appear on the command button.

### Illumination

Selects the illumination setting as defined in the Configure Illumination dialog box.

### OK

Opens the Timelapse Acquisition dialog box and starts the timelapse acquisition. This dialog box displays the progress of the timelapse acquisition. You can change the interval during acquisition from the Timelapse Acquisition dialog box. An *Acquire* button is provided so that you can manually acquire some of the images at any interval, rather than at the specified interval. You can also *Pause, Resume,* and *Stop* the acquisition.

### Close

Closes the dialog box.

# Acquire Spectral Scan  (Acquire Menu)

**Acquires a series of images in a range of excitation wavelengths. The acquired images can be placed in a stack or stored on disk. If you are acquiring image(s) using a journal, you are not required to save the acquired images.**

**Drop-in: SPECTRAL**

Use this command to acquire images while changing excitation wavelengths at a specified increment. For example, you could use this command to acquire images from the wavelengths of 310 to 420 nm using increments of 10 nm to evaluate a system's ability to illuminate a fura-2 sample. (This task could be performed manually by changing the wavelength in the Illumination dialog box and then acquiring the image.)

If you are using a RS-170 video camera, this command will use the acquisition settings from the Acquire Image command. If you are using a digital camera, Acquire Spectral Scan will use the acquisition settings from the Acquire from Digital Camera command.

This command requires a hardware device that uses continuous wavelength control, such as a monochromator. You must first install and configure the hardware using the Meta Series System Administrator.

**Note:** Acquire Z Series, Acquire Timelapse, and Acquire Spectral Scan can be running at the same time. However, you will not be able to perform a command within itself (such as running a timelapse within a timelapse).

## Acquiring a Spectral Scan

To configure a spectral scan acquisition, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Acquire Spectral Scan. The Acquire Spectral Scan dialog box opens. |
| **2** | Select the starting wavelength for the acquisition using *Starting Wavelength.* |
| **3** | Select the ending wavelength for the acquisition using *Ending Wavelength.* |
| **4** | Select the wavelength increment between acquisition using *Wavelength Increment.* |
| **5** | If you are using a shutter, select the illumination setting from the *Illumination* list.<br>**OR**<br>Otherwise, select "*[None]*." |
| **6** | Select the desired type of storage from the *Image Storage* group. Use *None* to perform an acquisition without storing images. |
| **7** | If you selected *Disk,* choose *Save File Name* to select a file name. Type the file name in the *File Name* text box and select the desired file type using *Files of Type.* Choose *Save* when you have finished. |
| **8** | If you want to run a journal between acquisitions, choose the command button next to *Journal to Execute.* Select the desired journal name from the *File Name* list. Then choose *OK.*<br>**Note:** To deselect a journal, choose this command button again and choose *Cancel.* The command button will display *Press to Select,* rather than the journal name. |
| **9** | Select *Update Image Window* if you want the image window updated after each acquisition (for a stack). |
| **10** | Select *Show Live Between Acquisitions* if you want to display the live video on the video monitor between acquisitions. |
| **11** | Choose *OK.* The Spectral Scan Acquisition |

dialog box will appear.

**Note:** If you are recording this command in a journal, choosing *OK* will display a dialog box that asks whether or not you want to record the journal without actually acquiring the images. Choose *Yes* if you want to merely save the journal without acquiring. Choose *No* if you want to both save the journal and acquire the images.

To collect images using the Spectral Scan Acquisition dialog box, use the following procedure:

| Step | Action |
|---|---|
| **1** | Configure the acquisition using the procedure presented in the preceding table. |
| **2** | The Spectral Scan Acquisition dialog box will appear. |
| **3** | Choose *Begin* to start the acquisition. |
| **3** | If you want to change the *Ending Wavelength* or the *Wavelength Increment* during acquisition, choose *Change.* When you have adjusted the options as needed in the Change Scan dialog box, choose *OK* to begin acquisition again. |
| **4** | To control the acquisition process, you can use the *Pause/Resume* and *Stop* command buttons. |
| **5** | MetaMorph will close the Spectral Scan dialog box when the acquisition is completed. |

## Acquire Spectral Scan - Dialog Box Options

### Starting Wavelength

Specifies the starting wavelength (nanometers) for the spectral scan acquisition.

### Ending Wavelength

Specifies the ending wavelength (nanometers) for the spectral scan acquisition.

### Wavelength Increment

Specifies the number of wavelengths to move the wavelength changer between each acquisition.

### Status

Displays the number of images that will be acquired during the spectral scan acquisition.

### Image Storage

Specifies the type of image storage for the spectral scan images. Select

*None* if you want to perform a spectral scan acquisition without storing images,

*Stack* to create a stack,

*Disk* to store images in a file on disk.

### Save File Name

Specifies the file name for image storage when *Disk* is selected as the *Image Storage* type. If a file name is not selected, the words *"Press to Select"* will appear on the command button. Otherwise the file name will appear on the command button.

### Illumination

Selects an illumination setting as defined in the Configure Illumination command.

### Journal to Run

Specifies the journal to run between acquisitions If a journal file name has not been selected, the words *"Press to Select"* will appear on the command button. Otherwise the journal name will appear on the command button.

> **Note:** If you are recording this command in a journal, choosing *OK* will display a dialog box that asks whether or not you want to record the journal without actually acquiring the images. Choose *Yes* if you want to merely save the journal without acquiring. Choose *No* if you want to both save the journal and acquire the images.

### Update Image Window

Updates the image window after each acquisition when images are stored to a stack. If this option is disabled, the image window will not update until after the spectral scan is completed.

### Show Live Between Acquisitions

Displays live video between image acquisitions. If this option has not been selected, the last acquired image will remain frozen on the video monitor until the next acquisition.

### OK

Starts the spectral scan acquisition and opens the Spectral Scan Acquisition dialog box. This dialog box displays the progress of the acquisition. You can change the ending wavelength and the wavelength increment during acquisition from the Spectral Scan Acquisition dialog box. You can also *Pause, Resume,* and *Stop* the acquisition.

### Close

Closes the dialog box.

# Multi-Journal Timelapse (Journal Menu)

**Runs up to five journals simultaneously. Similar to Define Stopwatch Sequences (Journal menu), but uses a much simpler interface with fewer features.**

**Drop-in: TLAPSE2**

Use this command when you want to run a number of journals at the same time. This command is used both to create a timelapse sequence and to run it.

**Define Stopwatch Sequences**

## Creating a Multi-Journal Timelapse

To create and use a multi-journal timelapse, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Journal Control>Multi-Journal Timelapse. The Multi-Journal Timelapse dialog box will appear. If the command has been run previously, the last group of settings will still be available for immediate execution. |
| **2** | Choose *Add Jnl.* The Select a Journal to Run During Timelapse dialog box will open. |
| **3** | Select the desired journal name from the *File Name* list. |
| | **AND** |

Choose *OK.* The name of the journal will be displayed in the *Journal* list and will appear highlighted.

**4** Use the *Interval* spin box and drop-down list box to define the time between executions of the journal highlighted in the *Journal* list. The time interval will appear in the *Interval (sec.)* list, across from its associated journal name in the *Journal* list.

**5** If necessary, repeat Steps 2 - 4 for all journals you want to run simultaneously. If you want to remove a journal from the list, click on its name in the *Journal* list and choose *Remove.*

**6** Use the *Expt. Duration* spin box and drop-down list box to define the overall length of time that you want the journals to run.

**7** To run the journals, choose *Start.* The Multi-Journal Timelapse dialog box will close, the Timelapse Control dialog box will open, and the journals will run. The Timelapse Control dialog box will allow you to *Pause* (and *Resume)* or *Stop* journal timelapse execution.

When the timelapse is completed or stopped, the Timelapse Control dialog box will close automatically.

## Multi-Journal Timelapse - Dialog Box Options

### Journal

Lists the names of journals that have been added to the Multi-Journal Timelapse sequence.

### Interval (sec.)

Lists the time between executions of its corresponding journal listed in the *Journal* list.

### Interval

Defines the time unit *(Seconds, Minutes,* or *Hours)* for the interval (time between executions) of the journal highlighted in the *Journal* list. Use the spin box to set the number of time units (maximum of 999).

### Expt. Duration

Defines the time unit *(Seconds, Minutes,* or *Hours)* for the duration (total time of execution) of the entire Multi-Journal Timelapse sequence. Use the spin box to set the number of time units (maximum of 999).

### Start

Opens the Timelapse Control dialog box and starts execution of the Multi-Journal Timelapse sequence.

### Cancel

Cancels all current changes made to the Multi-Journal Timelapse dialog box and closes the dialog box.

### Add Jnl

Opens the Select a Journal to Run During Timelapse dialog box. Allows you to add a previously created journal to the timelapse sequence.

### Remove

Removes the journal highlighted in the *Journal* list from the timelapse sequence.

# Equalize Light (Stack Menu)

**Changes the average, minimum, or maximum light intensity in each plane in an image stack to equal the average, minimum, or maximum intensity, respectively, of the current plane or of a user-defined active region of interest in the current plane.**

**Drop-in: EQUALIZE**

Use this command when the background levels vary greatly between the various planes in an image stack. *Equalize Light* will change the intensities in an image stack so that the average, minimum, or maximum intensity in each plane in the stack will be equal to the average, minimum, or maximum intensity, respectively, in the current stack or in a user-defined region of the current stack. You can specify how this command is carried out by choosing between an algorithm that adds a constant value to the entire image and one that multiplies each plane by a constant.

**WARNING:**
This command should not be used if you are performing a quantitative analysis of intensities or optical densities, because it will alter the grayscale data in your image.

## Equalizing Light Levels in a Stack

To equalize the light levels throughout all planes in a stack, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Stack menu, choose Equalize Light. The Equalize Light dialog box will appear. |
| **2** | Select a source image stack using the *Source* image selector. |
| **3** | Select the destination image using the *Dest* image selector. You can overwrite or add to the existing image or you can place the results in a new image window. |
| **4** | If you want to use a region of interest within the current image plane to determine the intensity characteristics of the entire stack, draw the region using a Region Tool**.**<br>**OR**<br>If you want to use the entire current plane to determine the light levels of the stack, move on to Step 5. |
| **5** | Select an equalization function from the *Equalize* group: *Average, Minimum,* or *Maximum.* |
| **6** | If you wish, you can select the algorithm to be used to perform the light level equalization, either by *Addition* of a constant to each plane or by *Multiplication* of each plane by a constant. |
| **7** | Choose *Apply* to carry out the equalization operation. |
| **8** | Choose *Close* to close the dialog box. |

## Equalize Light - Dialog Box Options

### Source

Selects the source image stack.

### Dest

Selects the destination for the equalized stack. You can overwrite the existing stack or place the results in a new image window.

### Equalize

Specifies the intensity characteristic that you wish to make equal between all of the planes in the selected image stack.

> ***Average****:* Sets the average intensity of the entire image stack to equal the average intensity in the current plane or region of interest. Typically used for transmitted light images.
>
> ***Minimum****:* Sets the minimum intensity in the stack to equal the minimum intensity in the current plane or region of interest. Typically used for fluorescent images.
>
> ***Maximum****:* Sets the maximum intensity in the stack to equal the maximum intensity in the current plane or region of interest.

### Equalize By

Selects the algorithm by which the equalization is performed. You can equalize either through *Addition* (arithmetic addition or subtraction of a constant to or from the grayscale value of each pixel) or through *Multiplication* (arithmetic multiplication of the grayscale value of each value by a constant).

### Apply

Carries out the equalization operation. The dialog box will stay open.

### Close

Closes the dialog box.

# Keep Planes (Stack Menu)

**Selects planes in an image stack that are to be kept and discards the rest.**

**Drop-in: KEEPPLN**

Use the *Keep Planes* command when you want to remove unwanted planes from a stack of images. Using this command will allow you to use a journal to remove planes within a specified range within the stack. The advantage to using this command rather than the Remove Plane command is that you do not need to know the total number of planes in a stack before removing them in a journal.

## Using Keep Planes

To use the Keep Planes command, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Stack menu, choose Keep Planes. The Keep Planes dialog box will appear. |
| **2** | Select a source image stack using the *Image Stack* image selector. |
| **3** | Select the image planes to be retained by clicking on the desired images' plane numbers in the Plane Selection Table. A check mark will appear next to each selected plane number.<br>**OR**<br>Select a range of image planes to be retained by specifying the number of the |

lowest plane to be kept in the *Low Range* spin box and number of the highest plane in the range in the *High Range* spin box and choose *Select Planes in Range.* You can choose every *nth* plane by selecting the number of planes to jump, from the *Step Size* spin box. This method of selecting planes can be used in journals.

**4** Choose *Apply.* The dialog box will close and the non-selected planes will be removed from your image stack.

**Note:** If you have configured the Keep Planes dialog box to remove all planes (that is, no planes have been selected), a message box will appear, stating that nothing will be done to the image stack. Similarly, if you configure the Keep Planes dialog box to retain all planes, a message will appear, stating that the image stack will not be altered and asking if you wish to continue.

## Keep Planes - Dialog Box Options

### Image Stack

Selects the source image stack for processing by the Keep Planes command.

### Low Range

Specifies the lowest numbered plane in a range of planes to be kept.

### Step Size

Selects every *nth* plane in the range that is specified by the values in *Low Range* and *High Range.*

### High Range

Specifies the highest numbered plane in a range of planes to be kept.

### Select Planes in Range

Selects the planes to be discarded. The range will start with the plane specified by *Low Range* and end with the plane specified by *High Range.* Every *nth* plane will be marked, as specified by the *Step Size* setting. A check mark will also appear in the Plane Selection Table next to the numbers of the planes in the selected range.

### Clear All

Deselects all planes and unchecks all plane numbers in the Plane Selection Table.

### Plane Selection Table

Displays the numbers of the planes in the image stack. Individual planes can be selected and deselected directly from this list with a single mouse click. The numbers of the planes to be kept will have a check mark next to them.

### Apply

Removes and discards all but the selected planes in the image stack, and closes the dialog box.

### Close

Closes the dialog box.

# Stitch Stack (Stack Menu)

**Attaches and combines individual images of a calibrated stack into one image.**

**Drop-in: STITCH**

Use this dialog box to combine related images in a calibrated stack into one complete image. This dialog takes into account the orientation of the camera. By selecting the setting that indicates the relative angle of the camera to the stage, you can correct for different camera orientations. You can also compensate for reversed left-to-right orientation that has resulted from a mirror image effect.

In addition, you can apply a Flatten background algorithm to compensate for an uneven background in individual images. As a result, the background of the combined images will be continuously smooth and even.

**Note:** The calibrated stack image must use the same calibration units as your stage. The stack image can be calbrated using the Calibrate Distances command. The stage is calibrated using the Meta Imaging Series Administrator program.

## Stitching Stacks

To combine separate related images of a stack in to a single image, complete the following steps:

| Step | Action |
|---|---|
| **1** | Ensure that at least one appropriate, calibrated image stack is open. |
| **2** | From the Stack menu, choose *Stitch Stack*. The *Stitch Stack* dialog box opens. |
| **3** | If more than one image stack is open, click *Source stack* to select the appropriate calibrated image stack. |
| **4** | Click the image selector for *Dest image* to start a new stack or add images to an existing stack. |
| **5** | In the *Camera Orientation* area, click *Images are mirror* to specify that you want to change the left-to-right image orientation. |
| **6** | In the *Camera Orientation* area, click the appropriate relative camera angle. |
| **7** | Click *Flatten background* if the background intensity is uneven and you want to have an even background intensity in the destination image. |
| **8** | In the *Flatten Background* area, click *Fluorescence* if the image stack was acquired with a fluorescence light source; click *Transmitted* if the image stack was acquired with a transmitted light source. |
| **9** | In the *Object size* box, type or select the smallest size object in microns that you want to consider as image data. |
| **10** | Click *Quick Paint* to create the completed stitched image without applying smoothing. |
| **11** | In the *Background pixel fill value area*, click the appropriate setting to fill any non-image |

areas with a value that closely matches either the darkest (for fluorescence), brightest (for brightfield), or average intensity found among all the images to be stitched.

**12** After all settings have been made, click *Stitch* to generate the new stitched image as the destination image.

**13** Click *Close* to close the Stitch Stack dialog box.

## Stitch Stack - Dialog Box Options

### Source stack

Selects the image stack that you want to stitch from the available open stacks.

### Dest image

Selects whether you want to start a new stack or add new images to an existing stack.

### [Warning Text]

Alerts you that the loaded stack is not a calibrated stack.

### Camera Orientation

Provides options to adjust for the camera's relative orientation to the stage.

***Images are mirror images*** – Reverses images that are mirrored or flipped left-to right from the desired orientation.

***Relative angle of camera to stage*** – Selects the appropriate camera angle setting of either 0, 90, 180, or 270 degrees, depending on the camera's orientation to the stage. By selecting the correct angle, the stitching algorithm can correctly determine which edges of the images are to be compared.

### Flatten background

When checked, applies the *Flatten Background* algorithm.

#### Illumination

Specifies the type of illumination used to acquire the image, either Fluorescence or Transmitted. This selection determines the appropriate *Flatten Background* algorithm to be applied to the image stack.

***Fluorescence*** – Applies the Fluorescence *Flatten Background* algorithm.

***Transmitted*** – Applies the Transmitted *Flatten Background* algorithm.

#### Object size

Specifies the object size of the smallest object in the image for the *Flatten Background* algorithm.

### Quick Paint

Creates the completed stitched image without applying any smoothing. This option enables you to create the stitched image faster than with smoothing applied.

### Background pixel fill value

Fills non-image areas with an appropriately selected maximum dark or light value. When images are stitched, areas can occur where there is no original image information. These areas are usually close the edges of the new stitched image or in small spaces created where three or four overlapping images intersect. Choose one of the following settings appropriate for your images:

***Min*** – Fills non-image areas of Fluorescence stitched images with the lowest intensity value of all the images.

***Max*** – Fills non-image areas of Brightfield stitched images with the highest intensity value of all the

images.

***Average*** – Fills non-image areas of stitched images with the average intensity value of all the images.

### Stitch

Applies the stitching algorithm and creates a stitched *Dest image*.

### Close

Closes the *Stitch Stack* dialog box.

# Make Movie (Stack Menu)

**Creates multimedia .avi video or QuickTime movie files from a stack of image planes.**

**Drop-in: MAKEAVI**

Use this command to create full motion Video for Windows (*.avi) files or QuickTime movie (*.MOV) files of the images in a stack. These video files can be played back similarly to a MetaMorph Movie, but do not require the use of MetaMorph for playback. AVI (audio video interleaved) files can be replayed automatically with the Media Player (Mplayer.exe) that comes bundled with Microsoft Windows. These multimedia files can also be embedded in a word processor document or spreadsheet, or placed on a Web page.

Digital video information can consume large amounts of memory, particularly if the video frames are in 24-bit color. Because of this, you may want to use one of the compression formats, or codecs (compression-decompression engines), if you save your movie as an .avi file. The .avi format has several standard compression codecs to choose from after creating an .avi movie. The number and type of codecs available will vary depending on your operating system version, video card, and other software installed on your system. The following codecs are installed with all versions of Windows:

**Radius Inc. Cinepak**: A 32-bit video codec that works best for compressing 24-bit color video images. This format provides greater compression, higher resolution, and faster playback than the Microsoft Video codec. You can specify your desired tradeoff between image quality and compression.

**Intel Indeo Video**: Another 32-bit video codec that works best for compressing 24-bit color video images. As with the Cinepak codec, you can specify your desired tradeoff between image quality and compression.

**Microsoft Video 1**: A 32-bit lossy compressor that works best with 8-bit and 16-bit images. You can specify separate settings for your desired tradeoffs between (1) compression and image quality, and (2) compression and temporal resolution.

**Microsoft RLE**: A 16-bit compressor that uses run-length encoding. Best for binary or 8-bit high-contrast images.

There is also an option to save an uncompressed, full frames version of the .avi file.

**Note:** If you are saving the movie as an .avi file or a QuickTime file and want to play it back on a Macintosh or use the Apple QuickTime (TM) player, you must use the included Radius, Inc. Cinepak codec so that the appropriate look-up table values are saved along with the .avi or QuickTime information.

## Making a Movie Video File

To create an .avi or QuickTime movie file from a stack of images, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Stack menu, choose Make Movie. |

The Make Movie dialog box opens.

**2** Use the *Source Stack* image selector to select the stack containing the images you want to include in the .avi or QuickTime file.

**3** In the *Play Each Frame for… 1/30th of a Second* text box, type the frame time, measured in 1/30ths of a second.

**Note:** The default value is *1* ("video rate"). This option allows you to play back the frames at a slower rate.

**4** In the *Save* radio button group, select *Selected* to include the planes you select in Step 5 in the .avi file.

**OR**
Select *Unselected* to exclude the planes you select from the .avi file.

**Note:** To use the Make Movie command in a Journal, you must ensure that no planes are selected in the Plane Selection Table and that *Unselected* is selected in the Save field. This ensures the journal will create complete movies from stacks containing different numbers of planes.

**5** To select the planes that you want to include in the .avi video file, you can click the number of the plane in the Plane Selection Table.

**OR**
Alternatively, you can use the *Low Range* and *High Range* edit boxes to specify the lowest and highest plane number, respectively, in the range of planes to be included. You can choose every *nth* plane by selecting the number of planes to jump from the *Step Size* edit box. Then choose *Select Planes in Range.*

**Note:** Choose the *Clear All* button to deselect all planes.

**6** In the Movie Formats box, click *AVI* to make an .avi format movie,

**OR**

Click *QuickTime* to make a QuickTime movie.

**7** Choose *Save.* The Make Movie dialog box will close, and the Save AVI or Save QuickTime movie dialog box will open. Type a name for the new .avi or .mov file in the *File Name* text box. If necessary, use the *Save In* drop-down list or Up One Level icon button to select an appropriate drive and folder for storing the file. Then choose *Save.*

**8** If you are creating a QuickTime movie, the make movie command will build the .mov file using the Radius, Inc. Cinepak codec and

save it to the selected folder.

**OR**

If you are creating an .avi movie, the Save AVI dialog box will close, and the Video Compression dialog box will appear.

Select the desired compression format from the *Compressor* drop-down list. Where available, use the *Compression Quality* slider to select a setting for the tradeoff between compression and image quality. A setting of *100* will provide the highest image quality at the expense of compression. You can also use the *Configure* option on some codecs to open a dialog box specific to that codec.

**9** Choose *OK.* The selected compression codec will automatically build the .avi file and save it in the location you specified in Step 7.

## Make Movie - Dialog Boxes

**Make Movie**

**Video Compression**

## Make Movie - Dialog Box Options

### Source Stack

Selects the stack of images to be used for creating the .avi full motion video file or QuickTime video file.

### Play Each Frame for… 1/30th of a Second

Specifies a display time, in one-thirtieths of a second, for each frame in the .avi or QuickTime file. The default value is *1* ("video rate").

### Low Range

Selects the lowest plane number for a range of planes to be selected.

### Step Size

Selects every *nth* plane in the range specified by *Low Range* and *High Range.*

### High Range

Selects the highest plane number for the range of planes to be selected.

### Select Planes in Range

Selects the planes to be marked. The range will start with the plane specified by *Low Range* and end with the plane specified by *High Range.* Every *nth* plane will be marked, as specified by the *Step Size* setting. A check mark will also appear in the Planes List Box next to the numbers of the planes in the selected range. If the *Save* radio button group has been set to *Selected,* the marked planes will be included in the .avi file. On the other hand, if the *Save* radio button group has been set to *Unselected,* the marked planes will be excluded from the .avi file.

### Clear All

Deselects all planes and unchecks all plane numbers in the Plane Selection Table.

### Plane Selection Table

Displays the numbers of the planes in the image stack. Individual planes can be marked and unmarked directly from this list with a single mouse click. The numbers of the selected planes will have a check mark next to them.

**Note:** To use the Make Movie command in a Journal, you must ensure that no planes are selected in the Plane Selection Table and that *Unselected* is selected in the Save field. This ensures the journal will create complete movies from stacks containing different numbers of planes.

### Movie Formats

Specifies the video format that you want to create when you export an image stack using this command. Click *AVI* to create an AVI format movie, click *QuickTime* to create a QuickTime movie.

### Save (radio button group)

Specifies whether marked planes are to be excluded *(Unselected)* or included *(Selected)* in the .avi or QuickTime file.

**Note:** To use the Make Movie command in a Journal, you must ensure that no planes are selected in the Plane Selection Table and that *Unselected* is selected in the Save field. This ensures the journal will create complete movies from stacks containing different numbers of planes.

### Save (command button)

Opens the Save AVI of Save QuickTime dialog box, from which you can select a name and storage location for the file. After you choose *Save* from the Save AVI dialog box, the Save AVI dialog box will close, and the Video Compression dialog box will appear.

**Note:** If you are creating a QuickTime movie, the *Save* command will build the .mov file using the Radius, Inc. Cinepak codec and save it to the selected folder without opening the Video Compression dialog box.

### Close

Closes the Make Movie dialog box without creating the movie.

## Video Compression - Dialog Box Options

### Compressor

Selects a codec (compression/decompression engine) for creation and playback of the .avi file. The number and type of codecs available will vary depending on your operating system version, video card, and other software installed on your system. There will always be the option to choose an uncompressed, full frames version of the .avi file.

### Compression Quality

Selects a setting that determines the tradeoff between compression and image quality. Moving the slider "thumb" to the right selects a higher quality image at the expense of storage space.

### Configure

Opens a proprietary configuration dialog box for codecs that support them.

### About

Displays a proprietary message box for codecs that support them.

### OK

Closes the Video Compression dialog box and creates the configured .avi file, saving it in the location specified in the Save AVI dialog box.

### Cancel

Cancels the command.

# Kymograph (Stack Menu)

**Creates a cross-sectional view of the intensity values of a user-defined line region through the planes in a stack. This can be used to create either a Z-axis view of objects in a through-focus sequence of images or a view of object movement in a time-based series of images.**

**Drop-in: KYMO2**

Use this command to create a cross-sectional view through the planes in an image stack. Such images typically represent either a Z-axis through-focus series of images or a sequence of images taken over time. The grayscale intensity values along a line region, or "transept," which has been drawn in the source stack image window will be displayed in the result image. The values from the first plane will be displayed in the first row of the result image, the values from the second plane in the second row, and so on. The effect of this command is somewhat similar to that of the View Orthogonal Planes command, but additionally allows you to configure the line region to have any length, angle, or location that you wish.

The Kymograph dialog box contains an option to increase the width of the line region from its default setting of one pixel. Accordingly, you can select between the average or the maximum grayscale value within that line width.

**Note:** If you are simultaneously using the Linescan command (Measure menu) and the line region you have drawn on the image is being shared by both the Kymograph and Linescan commands, the *Line Width* setting in the Kymograph dialog box will follow the width set in the Linescan dialog box. Conversely, if you make a change to the width of the line in the Kymograph dialog box, the *Scan Width* setting in the Linescan dialog box will be updated.

## Using Kymograph

To create a cross-sectional display of the intensity values of a line region as it passes through a stack, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Stack menu, choose Kymograph. The Kymograph dialog box will appear. |
| **2** | Use the *Kymograph Image* selector to specify the destination image. You can overwrite or add to the existing source stack, or you can place the results in a new image window. |
| **3** | Select the source image stack with the *Source Stack* image selector. |
| **4** | With the Single Line Tool (or any other open-ended line region tool), draw a line region across the area of interest in the image window.<br><br>**Note:** Because the Kymograph command draws the result image starting with the line region values in the topmost plane in the stack, you should verify that you are looking at the top plane in your stack; otherwise, your result image may appear to be inverted. |
| **5** | If you want to display the grayscale intensities from all planes in the source stack, select the *All Planes* check box. |

**OR**
If you want to use a restricted range of planes in the source stack, clear the *All Planes* check box. Then use the *Plane… to…* spin boxes to select the starting and ending plane.

**6** By default, line regions drawn with a line region tool will have a width of one pixel. If you want to use a wider line region, select the width with the *Line Width* spin box.

**AND**
From the *Value Across Width* option button group, select which grayscale value across the width of the line that you want to display: *Average* or *Maximum.*

**7** Choose *Create Kymograph.* The cross-sectional image will be displayed.

**8** When you have finished, click the Close button in the upper right corner of the Kymograph dialog box.

## Kymograph - Dialog Box Options

### Kymograph Image

Selects the destination for the cross-sectional image. You can overwrite the existing image or place the results in a new image window. Or you can add the result image as a plane to the existing stack.

### Source Stack

Selects the source image.

### All Planes

Specifies whether all planes in the source stack are to be included in the cross-sectional image. When selected, all planes will be used, and the *Plane… to…* spin boxes will be unavailable. When this check box is cleared, the *Plane… to…* spin boxes will become available.

### Plane… to…

Selects a starting and ending plane for inclusion in the result image. When the *All Planes* check box is selected, these spin boxes will be unavailable.

### Line Width

Selects a width for the line region to be used as the cross-sectional transept. When a width greater than *1* is specified, the *Value Across Width* option button group will become available.

### Value Across Width

Selects which grayscale value across the width of the line region is to be displayed: *Average or Maximum.* If a *Line Width* of *1* has been specified, this option button group will be unavailable.

### A line has not been selected in the source image stack.

Displayed if no line region is present and selected on the stack.

### Create Kymograph

Creates the cross-sectional image, using a width corresponding to the length of the line region and a height corresponding to the number of selected planes in the source stack.

### Cancel

Cancels the command.

# Montage Stacks (Stack Menu)

**Uses two to four stacks to create a two- or four-pane montaged stack that consists of one image from each original stack in each plane of the montaged stack.**

**Drop-in: PAIR**

Use this command when you want to compare two stacks, plane-by-plane, in a montage format. This command is useful for presentations because you can create a movie of a montaged stack. You can use the Select Plane command to see each plane in the montaged stack.

Montage

## Montaging Stacks

To create a montage from two stacks, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Stack menu, choose Montage Stacks. The Montage Stacks dialog box will appear. |
| **2** | If the desired *Resultant Montage* is not displayed, select it using the image selector. You can overwrite or add to an existing image or you can place the results in a new image window. |
| **3** | Select two to four source stacks for the montage using the *Source Stacks* image selectors. If you want arrange a side-by-side montage, use the *Top Left* and *Top Right* selectors (or the *Bottom Left* and *Bottom Right),* and select *None* from the other image selectors. If you want to create a vertically arranged montage (one above the other) use the *Top Left* and *Bottom Left* selectors (or the *Top Right* and *Bottom Right).* |
| **4** | Choose *Apply.* |
| **5** | When you have finished, choose *Close.* |

## Montage Stacks - Dialog Box Options

### Resultant Montage

Specifies the destination montage. You can add to or overwrite an existing image or stack. You can also specify a new stack.

### Source Stacks

Use these image selectors to specify the source stacks for the montage: *Top Left, Top Right, Bottom Left,* and/or *Bottom Right.* You can select from two to four stacks.

### Apply

Creates the montage from the specified source stacks.

### Close

Closes the dialog box.

# Convert Stacks to TIFFs (File Menu)

**Converts each stack image file in a source directory to TIFF files with sequentially numbered file names or extensions.**

**Drop-in: STK2TIFF**

Use this command to save each plane in your stack image (*.stk) files as separate .tif files. You can save the single-plane image files using either a sequential file name format (e.g., file001.tif, file002.tif, etc.) or a sequential file extension format (e.g., filename.001, filename.002, etc.). This command will be particularly useful when you need to convert your stack files for processing by a third-party software program, such as a deconvolution program, that does not recognize the Molecular Devices Corporation stack file format.

## Converting Stacks to Sequential TIFF Files

To save the individual planes in all of the stacks in a directory as separate sequential .tif files, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the File menu, choose Convert Stacks to TIFFs. The first page of the Convert Stacks to TIFFs wizard opens. |
| **2** | Choose *Source Directory.* The Browse for Folder dialog box opens. |
| **3** | In the *Select Source Directory* tree window, find the folder containing the stack files you want to convert and click it so that the closed folder icon is displayed as an open folder icon.<br><br>**AND**<br>Choose *OK.* The Browse for Folder dialog box will close and the Convert Stacks to TIFFs wizard reopens. |
| **4** | If you want to save the stack planes as sequentially named .tif files (e.g., file001.tif, file002.tif, etc.), select *Save with Sequential Names* from the *Destination TIFF Images* option button group.<br><br>**OR**<br>If you want to save the stack planes as sequentially numbered .tif files (e.g., filename.001, filename.002, etc.), select *Save with Sequential Extensions.* |
| **5** | Choose *Destination Directory.* The Browse for Folder dialog box opens. |
| **6** | In the *Select Destination Directory* tree window, find the folder in which you want to save the .tif files and click it so that the closed folder icon is displayed as an open folder icon. If necessary, you can create a new subfolder under an "open" folder by choosing *New.*<br><br>**AND**<br>Choose *OK.* The Browse for Folder dialog box will close and the Convert Stacks to |

TIFFs wizard reopens.

**7** Choose *Next >>.* The second page of the Convert Stacks to TIFFs wizard opens. This page contains information about the number of stack files in the source directory, the amount of disk space to be used by the new .tif files, and the amount of free space that will remain on your hard disk.

If necessary, choose << *Back* to return to the preceding page of the wizard.

**8** When you are ready to start the conversion process, choose *OK.* The conversion will proceed automatically. A third page of the wizard will appear, providing you with a real-time progress report as the files are processed.

**9** When you have finished, choose *OK* to close the Convert Stacks to TIFFs dialog box.

## Convert Stacks to TIFFs - Dialog Box Options

### Source Directory

Opens the Browse for Folder dialog box, from which you select the folder containing the stack files to be converted to single-plane .tif files.

### Destination TIFF Images

Selects a name format for the .tif files. *Save with Sequential Names* saves the files using sequential file names (e.g., file001.tif, file002.tif, etc.). *Save with Sequential Extensions* saves the files using sequential file extensions (e.g., filename.001, filename.002, etc.).

### Destination Directory

Opens the Browse for Folder dialog box, from which you select the folder in which you want to save the sequential .tif files. You can create a new subfolder by selecting an existing folder and choosing *New.*

### Information

This status text box, which appears in the second page of the Re-Save Stacks as TIFFs wizard, provides information regarding the number of stack files in the source directory, the amount of disk space to be used by the new .tif files, and the amount of free space that will remain on your hard disk.

### Status

Provides real-time feedback regarding the progress of the file conversion process. A progress meter will indicate the status of the conversion of each stack file, and a status line will display the stack file and plane currently being converted.

### Next >>

Proceeds to the next page in the wizard.

### << Back

Returns to the preceding page in the wizard.

### Cancel

Cancels the Re-Save Stacks as TIFFs command and closes the dialog box.

# Interleave Stacks (Stack Menu)

**Creates a new stack of interleaved images from two source stacks, with all odd-numbered planes coming from one source stack and all even-numbered planes coming from the**

**other source stack.**

### Drop-in: INTLEAVE

Use this command to combine two stacks of images into a single stack. The interleaved planes can be placed in a new destination stack, or can be added to an image stack that is currently open on the desktop.

The number of interleavings is limited by the number of planes in the smaller source stack. Thus, for example, if you combine a stack of four images with a stack of 50 images, the resulting stack will consist of eight planes, four from each source stack.

If you combine two stacks with different sizes, the new stack planes will have dimensions corresponding to the larger width and the larger height. For example, if you combine a stack of 200 x 300 images with a stack of 300 x 200 images, the resulting stack will consist of 300 x 300 planes. The additional space around the image data will be filled with black pixels.

If you combine two stacks with different bit-depths, the new stack will consist entirely of images of the greater bit-depth. For example, if you combine a binary stack with a 24-bit color stack, the planes in the resulting stack that came from the binary source stack will consist of pixels with "color values" of either 0,0,0 or 255,255,255.

## Interleaving Stacks

To interleave two stacks of images into a single stack, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Stack menu, choose Interleave Stacks. The Interleave Stacks dialog box will appear. |
| **2** | With the *Source Stack 1 (Odd Planes)* image selector, select the source stack whose images will give rise to Planes 1, 3, 5, etc., in the new set of stack planes. |
| **3** | With the *Source Stack 2 (Even Planes)* image selector, select the source stack whose images will give rise to Planes 2, 4, 6, etc., in the new set of stack planes. |
| **4** | With the *Destination Stack* image selector, specify a target destination for the interleaved planes. You can specify a *New* stack or *Add To* an existing one. |
| **5** | Choose *OK* to start the interleaving. |
| **6** | When you have finished, choose *Close.* |

## Interleave Stacks - Dialog Box Options

### Source Stack 1 (Odd Planes)

Selects the source stack whose images will give rise to Planes 1, 3, 5, etc., in the new set of stack planes.

### Source Stack 2 (Even Planes)

Selects the source stack whose images will give rise to Planes 2, 4, 6, etc., in the new set of stack planes.

### Destination Stack

Specifies the destination for the new, interleaved stack of images. You can specify a *New* stack or *Add To* one that is currently open in the MetaMorph application workspace.

**OK**

Carries out the interleaving process.

**Close**

Closes the dialog box.

# Stereographic Views (Stack Menu)

**Creates paired stereographic images from an image stack.**

**Drop-in: STEREO**

Use this command to create stereo pair images for viewing (1) with stereographic goggles, (2) with red/green or red/blue glasses, or (3) as side-by-side stereo pairs. The Stereographic Views command works best when the source stack that it uses is one that was created with the 3D Reconstruction command. You can configure the arrangement of the stereo pair images for optimal viewing with either Stereographics Viewer synchronizing goggles (contact your Molecular Devices Corporation representative for information), red/green viewing glasses, or red/blue glasses. The output of this command is particularly suited for viewing as side-by-side stereo pairs or for use in creating a movie .

The # *of Planes Between Image Pairs* option determines the way in which images are paired up. For example, if you select *1*, the starting image pair will consist of planes 1 and 2 of the original source stack. If you select *2*, the starting image pair will consist of planes 1 and 3. If you select *3*, the starting pair will comprise planes 1 and 4, and so on.

The *Create Pair for Every nth Image* option determines which source stack planes will be used as the first image of each resulting pair. Thus, if you select *1*, the first of the paired images in the result stack will be planes 1, 2, 3, etc., from the original source stack. If you select *2*, the first member of the pairs will be planes 1, 3, 5, etc., from the source stack, and so on.

Result images can be saved as an individual stack or as separate TIFF files.

## Creating Stereographic Views

To create a set of stereographic image pairs from a stack of images, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Stack menu, choose Stereographic Views. The Stereographic Views dialog box will appear. |
| **2** | Select the source image stack with the *Image Stack from Which to Create Stereo Pairs* image selector. |
| **3** | If you are using an external monitor, select the *Transfer Image to Video While Previewing* check box so that a check mark appears in it. You should use this option if you wish to export the images to an optical memory disk recorder. |
| **4** | Use the # *of Planes Between Image Pairs* spin box to specify the way in which images are paired up.<br><br>EXAMPLES:<br>If you select *1*, the first image pair that is created will come from planes 1 and 2 of the original source stack. If you select *2*, the first image pair will be made up of planes 1 and 3. If you select *3*, the first pair will be planes 1 and 4, and so on. |

**AND**

Use the *Create Pair for Every nth Image* spin box to specify which source stack planes will be used as the first image of each resulting pair.

**EXAMPLES**:
If you select *1,* the first image in the resulting pairs will be come from planes 1, 2, 3, etc., of the original source stack. If you select *2*, the first image in the resulting pairs will come from planes 1, 3, 5, etc., from the source stack, and so on.

**5** Select the number of resulting image pairs you want to create using the *# of Stereo Pairs to Create* spin box.

**6** If there are not enough planes in the source stack to create a complete set of image pairs from a single pass using the configuration you specified in the preceding two steps, select the *Wrap Around Until All Planes Are Exhausted* check box so that a check mark appears in it.

This will prompt MetaMorph to begin using source planes at the beginning of the stack when there would otherwise not be enough planes in the stack to complete the stereo pairs as configured.

**7** By default, stereo pairs will be arranged vertically in a stack, and the destination image will be 512 x 480. If you want to reconfigure the size, placement, color rendering or spacing of the stereo pairs, or specify a different format for saving the images, choose *More >>.* The dialog box will expand, revealing more options.

**OR**

If you wish to use these default configuration settings, skip to Step 13.

**8** Depending on your method of viewing, specify an arrangement and color rendering of the stereo pairs by selecting from the *Type of Stereo Pairs to Create* list. The selection you make will determine the spacing options that appear just below this box. Select

*Stereographics Stacked* to create pairs that are arranged vertically (one above the other)--select this if you are using a pair of Stereographics Viewer goggles;

*Side by Side* to create pairs that are arranged horizontally--select this if you will be using another method to view the stereo pairs such as "cross-eyed" viewing;

*Red/Green Anaglyph* to create a pair of red and green "superimposed" stereo images--select this if you are using a pair of red/green

viewer glasses; or

*Red/Blue Anaglyph* to create a pair of red and blue "superimposed" stereo images--select this if you are using a pair of red/blue viewer glasses.

**9** If you selected *Stereographics Stacked* in Step 8, use the *Vertical Spacing Between Images* spin box to specify the space, in pixels, between the upper and lower image. Then use the *Horizontal Offset of Bottom Image* spin box to specify the shift of the lower image relative to the upper image, in pixels.

**AND**

Use the *X* and *Y* spin boxes in the *Dest. Image Size* option group to specify the horizontal and vertical dimensions, in pixels, of the destination image. You can choose *Preview* to see the arrangement of the images and make adjustments as necessary while wearing your viewer goggles. Then skip to Step 12.

**10** If you selected *Side by Side* in Step 8, a resizable region of interest will be defined on the source image. If you wish, choose *Center, Set Region to Half Image Width.* This will automatically center the region and resize it horizontally so that it is half the width of the original image (the height will stay the same). When the stereo pairs are created, only this region of the source image planes will be used in the result images.

**AND**

Use the *Spacing Between Images* spin box to specify the space, in pixels, between the left and right image. If you wish, you can switch the left and right images by selecting the *Flip Views* check box. You can choose *Preview* to see the arrangement of the images while wearing your viewer goggles and make adjustments as necessary. Now skip to Step 12.

**11** If you selected *Red/Green Anaglyph* or *Red/Blue Anaglyph* in Step 8, select the offset between the red image and the green or blue image with the *Horizontal Offset of Bottom Image* spin box. You can choose *Preview* to see the arrangement of the images while wearing your viewer goggles and make adjustments as necessary.

**12** From the *Save* radio button group, select the format for saving the stereographic image(s). Select

*Stack* if you want the pairs saved in a single stack (*.stk) file (you will perform the actual Save operation when you close the image).

*Disk* if you want to save the images as separate TIFF files. Then choose the *Select* command button and type a base name for the series of images in the *File Name* text box of the Select File for Timelapse Storage dialog box that appears and choose *Save.*

**13** When you are satisfied with your configuration, choose *OK.* The stereographic pair images will be generated and the Stereographic Views dialog box will close automatically.

**Note:** If you want to hide the Image Window Tools, right-click in the stereographic image window and choose Hide Image Window Toolbar from the pop-up context menu that appears.

## Stereographic Views - Dialog Box Options

### Image Stack from Which to Create Stereo Pairs

Selects the image stack from which planes will be used to generate the stereographic pair image(s).

### Wrap Around Until All Planes Are Exhausted

If there are not enough planes in the source stack to create a complete set of image pairs from a single pass using the current configuration, this option prompts MetaMorph to begin using planes at the beginning of the stack to complete the stereo pairs as configured. This option is only valid if the original 3D stack was created for the full 360 degrees, such that the last image differs from the first image by the same number of degrees as the first image differs from the second.

### # of Planes Between Image Pairs

Specifies the number of planes by which the images in a stereo pair differ. For example, if you start with plane 1 in the source stack, setting this option to *1* will result in the first pair being taken from planes 1 and 2 of the source stack. If you set this option to *2,* the first pair will consist of planes 1 and 3 from the source stack.

### Create Pair for Every *n*th Image

Specifies which planes from the source stack will be used as the first image of each resulting pair. For example, if you set this option to *1,* the first image in the resulting pairs will come from planes 1, 2, 3, etc., in the source stack. If you set this option to *3,* the first image in the resulting pairs will come from planes 1, 4, 7, etc.

### # of Stereo Pairs to Create

Specifies the number of stereographic image pairs that will be created.

### Transfer Image to Video While Previewing

If you are using an external video monitor, selecting this check box will send the Preview image to the monitor, rather than to an image window on the computer monitor.

### Preview

Places a sample view of the first stereo pair in an image window on the computer monitor (or on an external monitor if you selected the *Transfer Image to Video While Previewing* check box). This will allow you to obtain quick visual feedback on the arrangement that will result from your current set of configuration settings.

### More >>

Expands the dialog box, revealing additional options for image pair configuration, arrangement, and saving.

### Less <<

Condenses the dialog box.

### Type of Stereo Pairs to Create

Selects the format for the stereographic pair images.

> ***Stereographics Stacked*** arranges the images in a column, one above the other. This format is best when you are using a pair of Stereographics Viewer goggles. **(Note:** Because of their synchronization rate, these goggles work best when you use an external video monitor.)
>
> ***Side by Side*** arranges the images in a horizontal row. This format is used if you will be using another method to view the stereo pairs such as "cross-eyed" viewing.
>
> ***Red/Green Anaglyph*** arranges the image pair in a "superimposed" configuration, with one image rendered in red and the other in green, with a user-specified offset between the two. This format is ideal when you are using a pair of red/green viewing glasses.
>
> ***Red/Blue Anaglyph*** is similar to the *Red/Green Anaglyph,* but renders the second image in blue, rather than green. This format is used when you have a pair of red/blue viewing glasses.

### Vertical Spacing Between Images

Selects the space between the upper and lower image, in pixels. This spin box appears only when *Stereographics Stacked* is selected as the *Type of Stereo Pairs to Create.* This option is used to set the alignment of the images when they are superimposed by the stereographics hardware.

### Horizontal Offset of Bottom Image

Selects the shift of the lower image, in pixels, relative to the upper image. This spin box appears only when *Stereographics Stacked* is selected as the *Type of Stereo Pairs to Create.* This option is used to set the alignment of the images when they are superimposed by the stereographics hardware.

### Dest. Image Size

Selects the horizontal *(X)* and vertical *(Y)* size of the result image, in pixels. This set of spin boxes appears only when *Stereographics Stacked* is selected as the *Type of Stereo Pairs to Create.*

### Save

Selects a format for saving the resulting sets of image pairs.

> ***Stack*** saves the pairs as planes in a single stack (*.stk) file. The actual saving operation will be carried out when you close the stack.
>
> ***Disk*** saves the image pairs as separate TIFF files on disk.

### Spacing Between Images

Selects the space between the left and right image, in pixels. This spin box appears only when *Side by Side* is selected as the *Type of Stereo Pairs to Create.* This option is useful for creating a border between the images.

### Region

Indicates the starting (upper left) X and Y coordinate (in the first set of parentheses) and the ending (lower right) X and Y coordinate (in the second set of parentheses) for the currently defined region of interest on the source image. This region will appear automatically, and this status line will only appear, when *Side by Side* is selected as the *Type of Stereo Pairs to Create.*

### Flip Views

Switches the placement of the left and right images. When this option is disabled, images will be "read in" to the image pair from left to right. When this option is selected , images will be "read in" from right to left. This check box appears only when *Side by Side* is selected as the *Type of Stereo Pairs to Create.* This allows you to determine whether the pairs are to be viewed "cross-eyed" or "wall-eyed."

### Center, Set Region to Half Image Width

Centers the region of interest within the source image and resizes it horizontally so that it is half the width of the original image (the height will stay the same). When the stereo pairs are created, only this region of the source image planes will be used in the result images. This command button appears only when *Side by*

*Side* is selected as the *Type of Stereo Pairs to Create.*

### File Name (*Select* command button)

Displays the Select File for Timelapse Storage dialog box, from which you can specify the base name for images being saved as TIFF files on disk. This command button appears only when *Disk* is selected as the *Save* format.

### OK

Creates the stereo image pairs as configured. The Stereographic Views dialog box will close automatically.

### Close

Closes the dialog box.

# Acquire (Acquire Menu)

**Configures image acquisition for and acquires images from a variety of digital cameras.**

### Drop-in: ACQUIRE

Use this command for acquiring images from several different types of digital cameras. This dialog box combines multiple functions and options into a single, multi-tabbed dialog box. It enables you to specify settings that control image acquisition, image correction, image annotation, image display, and special settings including digitizing speed, gain, and camera shutter selection. Also use this command to acquire 24-bit color images. Acquisition of 24-bit color images is enabled when the *Bit Depth* on the Special tab is set to 24-Bit, and the correct video channel is selected in the *Set Video Channel* dialog box.

The Acquire command now features a Live Replay tab for capturing real time events such as in FRAP studies and other laser-based events, time lapse experiments, live cell imaging and digital video microscopy. When Live Replay is enabled, MetaMorph buffers the live stream to memory. This enables you to start recording a stream when something of interest occurs. You can configure Live Replay to include frames in the stack that occurred before the capture point.

Live Replay also enables you to select an optional journal to run at the capture point. One example of using Live Replay with a journal is to record recovery data during a FRAP experiment. You can configure a journal to photobleach at the capture point. The resulting stack can be used to measure recovery because it contains frames from before and after the photobleaching.

> **Note**: The Live Replay feature is only available for supported cameras. Refer to the MetaMorph Support site for a list of supported cameras.

The Acquire dialog box has the following two formats:

- A minimized format that contains only the controls for acquisition region selection, exposure, binning, live activation (Show Live), plus Acquire, and Save Image.
- A standard format that contains all the controls in the minimized dialog box, plus tabbed areas containing controls for Acquisition (Acquire), Display, Image Correction (Correct), Image annotation (Annotate), and special settings for Digitizer speed (Digitizer), Gain, Bit Depth, and Camera Shutter.

Using the default image selector settings, the acquire command acquires images to an image window called "Acquired." If additional images are acquired, and the last image acquired is not saved and closed, the name of newly acquired image becomes Acquired-2. Subsequently acquired images are incremented with Acquired-3, Acquired-4, and so on. The image selector enables you to specify the name of the acquired image. The assigned name is also automatically incremented when additional images are acquired. If you set the Image Selector to Overwrite, acquired images are called "Acquired" or a name that you designate in the image selector, and will overwrite the currently displayed image. Optionally, using the image selector, you can specify that subsequently acquired images are accumulated into an image stack (.stk) file.

**Note:** You can cancel an ongoing acquisition at any time by pressing the [Esc] key.

**Procedures**

**Dialog Box Options**

## Acquiring Images

**Acquiring Images - Main Dialog Box**

**Acquiring Images - Display Tab**

**Acquiring Images - Acquire tab**

**Acquiring Images - Correct Tab**

**Acquiring Images - Annotate Tab**

**Acquiring Images - Special Tab**

**Acquiring Images - Live Replay Tab**

**Acquiring Images - Color Tab (Brightfield)**

**Acquiring Images - Color Tab (Fluorescence)**

## Acquiring Images – Main Dialog Box

The configuration steps for many types of simple image acquisitions can be completed on the Acquire main, minimized (Less<<) dialog box. Additional acquisition requirements can be specified on one or more of the five Acquire dialog box tabs. This Acquire dialog box enables you to configure and acquire images rapidly because some of the buttons simultaneously set a specific acquisition requirement and acquire an image.

To Configure acquisition and acquire one or more images, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, click *Acquire*, the Acquire dialog box opens. |
| **2** | If the dialog box is not maximized, click the *More>>* button. |
| **3** | If you are loading a previously save group of settings in a setting file, click *Load…* The *Acquire: Load Setting* dialog box opens. If you are making a new group of Acquire settings, Skip to step 5. |
| **4** | From the *Acquire: Load Setting* dialog box, check the settings that you want to load from your saved settings file. Click once to check a setting, click again to uncheck. Then click *Load.* |
| **5** | Determine if you will be acquiring a single image, an image stack, or a sequence of images. If you are acquiring single images, an image stack, or are overwriting your acquired images, configure the image selector accordingly. If you are acquiring a sequence of images, click *Save w/* |

*Sequence*. If you are acquiring only a single image, Skip to step 8.

**6** If you clicked *Save w/ Sequence*, Click *Set Save…*to specify the sequence base name and specify Set Saving options.

**7** In the *Acquire: Set Saving* dialog box, type the base file name in the Base Name box. In the *If image already exists* box, choose an option. Click *Directory…* to specify the directory where you want to save the images. Click *Show Saved Image* to have the saved image displayed in an Image window. Click *OK* to close the *Acquire: Set Saving* dialog box.

**8** Click the **Acquire tab** to make settings for auto exposure and to select an external shutter.

**9** On the Acquire Main dialog, type or select an exposure time and unit of measure.

**10** If you are auto exposing, click *AutoExpose*. The acquire command calculates the exposure time and acquires an image.

**11** If you need to shorten the exposure time, type or enter a value in the Binning box.

**12** In Camera Area, specify the acquisition region. Remember, each of these buttons simultaneously sets the acquisition region and acquires an image.

Click *Full Chip* to set the acquisition region to the entire area of the chip, and acquire an image.

Click *Center Quad* to set the acquisition region to the center quadrant of the chip, and acquire an image.

Click *Use Active Region* to assign the acquisition region to the current active region, and acquire an image.

**13** Click *Show Live* to continuously acquire images to enable you to set the exposure time and to focus the microscope.

**14** If *Show Live* is active, type or select a value in the *Live Bin* box. Increasing the Live Bin increases the frame rate within the live window.

**15** To acquire the image after configuring the settings as needed (see the help file for each Acquire tab) click *Acquire* to begin acquiring the image.

> **Note:** You can cancel an ongoing acquisition at any time by pressing the [Esc] key.

**16** To save settings made in the Acquire dialog box, click *Save As…* when saving to a new

setting file. Click *Save* to save settings to the setting file shown in the *Setting (Modified):* box. To choose a different setting file, click the arrow button in the Setting box, and click the mane of the setting file.

## Acquiring Images - Display Tab

Use the settings on this tab to configure the images shown on your display.

To configure the Acquire Display dialog box, complete the following procedure:

| Step | Action |
|---|---|
| **1** | Click the Display tab. The display options and settings move to the front. |
| **2** | If autoscale is on, type or select values for the Low percent and the High percent.<br><br>**OR**<br><br>If Autoscale is off, in the Image Scaling area, type or select values for the Low and High limits. |
| **3** | If you want scaling to be performed in the active region on acquire, click *Scale within the active region.* |
| **4** | To apply gamma correction to your viewed image, move the Image Gamma slider left or right to decrease or increase the image gamma below or above 1.00. To reset the gamma to 1.00, click **=1**. When you decrease or increase the gamma, the line chart indicates the change. |
| **5** | To select a different chart or turn off the chart, click the appropriate button on the right side of the chart. |
| **6** | To change the chart configuration, click the down arrow button under the lower left corner of the chart. A list box opens containing a list of options that you can use to customize the chart, print the chart, copy the chart, or save it as a bitmap file. |
| **7** | From the list box, click *Configure Plot*. The configure plot dialog box opens. Use this dialog box to change the visual and physical attributes of the chart including the background and border colors. |
| **8** | From the list box, you can also select *Y axis*, *X axis*, *Title*, *X Title*, and *Y Title*. Use these options to modify and customize settings and colors for each of these individual parts of the graph. |
| **9** | Click *Reset Display* to reset all values in the display to their previous default values. |

## Acquiring Images - Color Tab (Brightfield)

To set the color balance for acquiring 24-bit brightfield color images, complete the following procedure:

| Step | Action |
|---|---|
| **1** | Click the *Color* tab, the Color options move to the front. |
| **2** | Select *Brightfield* if you are acquiring brightfield images illuminated by a transmitted light source (dark objects against a white background). |
| **3** | Click *Show Live* to begin acquiring images from which to preview your settings. |
| **4** | Without a specimen in place or with an active region created that defines an area to be interpreted as white, Click *Measure White Balance* to establish an initial white balance level. |
| **5** | With a specimen in place, adjust the *Brightness* control to achieve the best overall level of brightness. |
| **6** | Adjust the controls for each individual color *(Red, Green, Blue)* to obtain the best color balance. |
| **7** | Click *Stop Live*, and acquire a sample image to verify the accuracy of your settings. |

## Acquiring Images - Color Tab (Fluorescence)

To set the color balance for acquiring 24-bit fluorescence color images, complete the following procedure:

| Step | Action |
|---|---|
| **1** | Click the *Color* tab, the Color options move to the front. |
| **2** | Select *Fluorescence* if you are acquiring fluorescent images illuminated by a reflected light source (light objects against a dark background). |
| **3** | Click *Show Live* to begin acquiring images from which to preview your settings. |
| **4** | With a specimen in place, create an active region that defines an area that should be interpreted as white, Click *Measure White Balance* to establish an initial white balance level. |
| **5** | For each color *(Red, Green, Blue)*, adjust the *Min* and *Max* values to obtain the best color balance and image quality. |
| **6** | Click *Stop Live*, and acquire a sample image to verify the accuracy of your settings. |

## Acquiring Images - Acquire tab

To configure for auto exposure settings and select an external shutter, complete the following procedure:

| Step | Action |
|---|---|
| **1** | Click the *Acquire* tab. The acquire options and settings move to the front. |
| **2** | In the *Target Intensity* box, type or select a target intensity that is the desired percentage of the maximum intensity capability of the camera. (Typically, 75 percent of maximum is chosen.)<br><br>OR,<br><br>Optionally, set a value in the *% of Max* box to specify what percentage of the maximum intensity the target intensity should be. |
| **3** | In the *Maximum Exposure* box, type or select an exposure time that you do not want to exceed. |
| **4** | In the *Increment Exposure by* box, type or select a value by which you want the exposure time setting on the main dialog box to increment. |
| **5** | In the *Shutter* box, type of select the name of the external shutter you are using (If applicable). |
| **6** | Click *Zoom live image if binning is different* to keep the image windows for Show Live and Acquire the same size when Live and Acquire have different binning values. |
| **7** | Click *Use setting name as image name* to assign the name of the Save Setting file as the name of the image file. |

## Acquiring Images - Correct Tab

To apply corrections to your image(s) during acquisition, complete the following procedure:

| Step | Action |
|---|---|
| **1** | Click the *Correct* tab. The Correction options and settings move to the front. |
| **2** | If you want to apply background subtraction, click the background subtraction option that you want to apply. |
| **3** | In the *Offset Value* box, type or select the amount of offset you want to apply to the image to set the level considered to be "black" above the background noise level. |
| **4** | Click *Keep Shutter Closed* if you need to have complete darkness for acquiring a background image. |
| **5** | Click *Acquire Background* to acquire the background image that you want subtracted from your image. |
| **6** | Click *Display Background* Image to see the background image that was acquired. |

| | |
|---|---|
| **7** | Click *Do correction when live is running* to apply Background Subtraction and Shading Correction to the live image while it is actively acquiring images. |

## Acquiring Images - Annotate Tab

To apply annotations to your image(s) during acquisition, complete the following procedure:

| Step | Action |
|---|---|
| **1** | Click the Annotate tab. The Annotate options and settings move to the front. |
| **2** | Under *Automatic Image Annotation* click to add or delete any annotations that you want included with your image(s). |
| **3** | Under *User Annotation* type any additional annotations that you want included with your image(s). Be sure to enclose any variables within percent symbols. |

## Acquiring Images - Special Tab

To set special controls on your camera, complete the following procedure:

| Step | Action |
|---|---|
| **1** | Click the Special tab. The Special options and settings move to the front. |
| **2** | Set the appropriate controls for your specific camera. (The following steps guide you through the example.) |
| **3** | In the *Digitizer* box, select the appropriate digitizer speed (Slow or Fast). |
| **4** | In the *Gain* box, select the appropriate gain level (Low, High, or Super High). |
| **5** | In the *Bit Depth* box, select the appropriate bit depth (10, 12, 14, or 16-bit). |
| **6** | In the *Camera Shutter* box, select the appropriate Camera Shutter option (Open for Expose, Always Closed, or Always Open). |
| **7** | In the *Digital4 Visibility* box, click *Hide Digital4 menu* to exclude the Digital4 commands from the Acquire menu.<br>**OR**<br>Click *Show Digital4* and set as acquisition handler to include the Digital4 commands on the Acquire menu. |

## Acquiring Images - Live Replay tab

To configure and start capturing live images, complete the following procedure:

| Step | Action |
|---|---|
| **1** | Click the *Live Replay* tab to bring it to the |

front.

**2** Select *Enable Live Replay*.

**Note**: This option should be unchecked when you finish using Live Replay to free up system memory.

**3** To specify a name for the resulting stack, click *Untitled* in the *Image Stack* field, then select *Specified* and enter or select a name for the stack.

**4** To select a journal to run when *Capture Live Images* is activated, click *Browse* and navigate to the journal.

**5** In the *Before the capture point* field, enter the number of frames to add to the stack that were acquired before activating *Capture Live Images* mode.

**Note**: The number of frames possible in the stack is dependent on the amount of memory on your computer. See the *Memory Acquisition Information* fields for the amount of memory available.

**6** In the *After the capture point* field, enter the number of frames to add to the stack that were acquired after activating *Capture Live Images* mode.

**7** Click *Show Live* to open a live image window. The Timing Acquisition Information will update.

**8** Click *Capture Live Images* (or press F11) to start recording based on the current settings. The stack image window opens when the capture is complete.

**9** Click *F2: Stop Live* to close the live image window.

**10** After you are done using the Live Replay tab, uncheck *Enable Live Replay* to free up system memory.

## Acquire - Dialog Box Options

**Acquire - Main Dialog Box Options**

**Acquire Dialog Box Options - Display Tab**

**Acquire Dialog Box Options - Acquire Tab**

**Acquire Options Dialog Box -- Annotate Tab**

**Acquire Dialog Box- Correct Tab**

**Acquire Dialog Box Options - Special Tab**

**Acquire Dialog Box Options - Live Replay Tab**

**Acquire Dialog Box Options - Color Tab**

## Acquire – Main Dialog Box Options

### Acquire

Acquires an image using the current settings. The image is always acquired into an image window called "Acquired." Newly acquired images automatically overwrite previously acquired images in this window. The "Acquired" window stores information about its last position, size, zoom, gamma, LUT, and other image display attributes. Journals that include the Acquire button do not record any information about the settings. On playback, the acquisition occurs using the dialogs latest configuration.

> **Note:** You can cancel an ongoing acquisition at any time by pressing the [Esc] key.

### Save Image

Saves the image currently in the "Acquired" window. If there is no "Acquired" window, this button is inactive. If Save w/Sequence is not checked, this opens a "Save as" dialog box to save the image. The image defaults to TIFF format using the *Configure Default Paths* last saved path for *Save Images*. If *Save w/Sequence* is checked, the image is saved to the file location indicated on the "Save to" line in the expanded dialog box. Click *More* to fully expand the Acquire dialog box. Click Set Save to open the Acquire: Set Saving dialog box and specify an image sequence name.

> **Note:** If the Show Saved Image option is checked, when an image is saved, it is displayed in a new window, and the Acquire window remains open.

### Save w/Sequence

Enables the Save with Sequence option. This option allows you to save sequentially acquired images as a sequentially numbered group of images in a single directory. Click *Save w/ Sequence*, then acquire an image. In the upper right corner of the maximized (*More*>>) Acquire dialog box, click *Set Save…*

### Set Save

Opens the Acquire: Set Saving dialog box. Use this dialog box to name a sequential sequence of images. Type the Base Name (for example, Image001) in the Base Name field. Set the option that specifies what to do if the image name already exists. Click *Show Image when Overwrite Saved* if you want overwritten saved images to open in a new window.

### Image

Specifies the name and destination for the acquired image and selects whether the image should be saved as a new image, overwrite an existing image with the same name, or add the image to an existing image in order to make a stack of two or more images. This is a standard Meta Imaging Series Image Selector. Though this image selector is not visible in Less<< mode, settings made to the image selector are still active in Less<< mode.

### Exposure Time

Selects and indicates the exposure time for the current acquisition. Possible units are, ms, sec, and min. This will be available depending on what range the camera driver supports. The exposure time will be the basis for the exposure of the live window. If live binning is different than the acquire binning, the time used for live exposure will be a value calculated from the displayed exposure.

> **Note:** If you set an exposure time greater than five seconds, a status bar will display on the bottom of the MetaMorph desktop when you click *Acquire* to indicate the progress of the exposure.

### AutoExpose

Calculates and sets the autoexposure time for individually acquired images and for the Set Live image window. The AutoExpose button works differently depending on whether the Live window is open. If the Live window is open, AutoExpose seeks an exposure time that achieves the auto-expose parameters specified on the Acquire tab and uses the resulting exposure time. If the Live window is not open, the same AutoExpose calculations occur and an image is acquired just as if the Acquire button was pressed. The initial exposure attempted by AutoExpose is the value specified in the Exposure field. If AutoExpose can not

achieve the target intensity, the status line shows an error message accompanied by a red error "light". The following are the possible warnings that can be displayed.

***Low Signal*** – If no exposure reached or exceeded the target intensity this message appears. Several circumstances can produce this message, including: The camera or controller is not turned on; the light source is not turned on; the shutter is not turned on; or the wrong shutter is specified on the Acquire tab, and the maximum exposure duration is too small for the actual sample present.

***Target Not Achieved*** – If an exposure exceeded the target intensity but no exposure was within range of the target this message appears. Possible causes including a changing image due to changes in illumination or sample while the exposure was being calculated.

***No Hardware Image*** – If AutoExpose can not obtain an image from the camera this message will appear. The most likely cause of this message is that the device is not properly configured in the Meta Imaging Series Administrator.

### Binning

Sets the binning used by the Acquire command. Horizontal and vertical binning are always set the same. The settings are limited to binning choices supported by the camera driver.

### Camera Area

Defines the area that will be the acquisition region and acquires an image from the defined region. Use one of the following buttons to define the acquisition region and acquire an image:

***Full Chip*** – Defines the entire chip area as the acquisition region and acquires an image.

***Center Quad***. – Defines the center quadrant of the chip as the acquisition region and acquires an image.

***Use Active Region*** – Defines the designated active region as the acquisition region and acquires an image.

When one of the buttons is pressed, the defined camera area is used and a new image is acquired, resizing the image to fit the appropriate acquire image.

**Note:** For the Active Region button to perform any action, there must be an active region on the active window on the desktop.

### Show Live (and F2: Stop Live)

Rapidly acquires new image data in to a new window; in effect, showing a live image. Pressing F2 or this button again stops updating the image.

**Note:** If you change the position of the stage (X, Y, or Z axes) using any of the commands within MetaMorph the live window will pause until the stage reaches its destination.

### Live Bin

Sets the binning value for live acquisition. If live binning is different than acquire binning, the time used for live exposure will be a value calculated from the acquire exposure. For example, if the live binning is 2 and the acquire binning is 1, then the exposure used when acquiring the live image will be one-fourth the displayed exposure. This compensates for the intensity increase that results from combined pixels and enables the live image to update at a faster rate. This feature is not available if the *Do correction when live is running* checkbox is checked in the *Correct* tab.

### Status Line

Displays the current camera status, such as, "Exposing…" or "Transferring…". If the camera is not in a process, it displays the camera CCD chip temperature. It also displays an error message if AutoExpose could not reach its target.

### Status Lights

Indicates whether background subtraction or shading correction are in use. If a setting is not active, no light or text will be displayed. A green light is displayed if the setting is active and valid. A yellow light indicates the setting was modified (and serves as a reminder that to keep the modifications, the setting should be

resaved). A red light indicates the active setting cannot be used due to a configuration error. Explanations that accompany warnings and errors can be seen on the "Correct" tab.

### Setting

Displays the names of the most recently saved or loaded settings. Up to eight settings can be displayed in the order of most recent usage. Selecting a setting load the values for the setting into the dialog for the next acquisition, immediately updating the controls and the display of the current "Acquired" image. If any value in the dialog is changed after a setting has been loaded, the title of the setting control will change from "Setting:" to "Setting [Modified]:" to indicate that the dialog differs from the currently listed settings.

When loading a setting the load dialog may appear so that the portion of the setting to be loaded can be configured. If the load dialog does not appear, the portion used will be the same as the portion used the last time a setting was loaded. To get the Load dialog to appear when selecting a setting from the popup, hit the "Load…" button on the more section of the dialog and set the "When using the pop list to load" radio buttons.

### Load

Opens the Acquire Load Setting dialog box.

### Save

Saves the current settings state into the existing state file for subsequent re-use. The previously saved settings are overwritten.

### Save as

Opens the Acquire State save dialog box. Use this option and dialog box to assign a name to a new setting file or to create a duplicate setting file that you can modify.

### Close

Closes the Acquire dialog box.

### More>>/Less<<

Expands (maximizes) or reduces (minimizes) the Acquire dialog box.

## Acquire: Load Setting - Dialog Box Options

### Exposure

Check this box to use the *Exposure Time* value from the setting you are loading.

### Binning

Check this box to use the *Binning* value from the setting you are loading.

### Camera Area

Check this box to use the Camera Area selection from the setting you are loading.

### Illum

Check this box to use the *Illumination* setting defined in the *Acquire* tab from the setting you are loading.

### Use Setting for name

Check this box to set the image name of acquired images to the name of the state file when the state file is loaded. The state file name appears in the Setting drop-down box.

### Display

Check this box to use the values defined in the *Display* tab from the setting you are loading.

### Image Saving

Check this box to use the Save values from the setting you are loading. The Save values affected are set in the following commands: *Save w/Sequence*, *Set Save*, and *Image*.

### Correction Settings

Check this box to use the values defined in the Correct tab from the setting you are loading.

### Correction Images (if applicable)

Check this box to use the correction images acquired in the Correct tab from the setting you are loading.

### Annotation

Check this box to use the values defined in the *Annotation* tab from the setting you are loading.

### Special Parameters

Check this box to use the values defined in the *Special* tab from the setting you are loading.

### Scaling for Color Camera

Check this box to use previously saved color camera scaling settings.

### When using the popup list to load

#### Show this dialog

Check this box to view this dialog when selecting a setting from the *Setting* list.

#### Use latest selections. Skip this dialog.

Check this box to immediately switch to the selected settings without opening this dialog box. The default (all) settings will be loaded.

### Load

Opens the Acquire: Load Setting dialog box used to select a saved Acquire state (.AST) file.

### Cancel

Cancels the command.

## Acquire Dialog Box Options – Display Tab

### Image Scaling

Sets 16-bit image scaling and specifies the range within which to either automatically or manually scale the image bit density.

> **Note:** As you make settings changes, the *Acquired* window (if open) reflects the changes. Any subsequent images acquired will use these settings.

### Low

Specifies the scaling value for the lower end of the range. If Autoscale is selected, the value is specified as a percentage of the range to be excluded from the lower end of the scale. If Autoscale is not selected, the value is specified as the exact low cutoff point on the image scale.

### High

Specifies the scaling value for the upper end of the range. If Autoscale is selected, the value is specified as a percentage of the range to be excluded from the upper end of the scale. If Autoscale is not selected, the value is specified as the exact high cutoff point on the image scale.

### Autoscale

Activates or deactivates image autoscaling. If Autoscale is on, the low and high percentage values specify the percentages of the lower and upper areas of the image histogram to ignore; if Autoscale is off, the low and high values specify the direct gray range within which to scale.

### Scale within the active region

Scales the acquired image based on its active region. The scaling is applied immediately upon checking the box or adding an active region to a live or acquired image.

### Image Gamma

Displays and sets the Gamma for the "Acquired" image. Use the slider control to set the gamma value.

**=1**

Resets the gamma value to 1.

### Graph

Shows the histogram of the current "Acquired" image or the Gamma curve used in displaying the image. The histogram will be scaled to the available bit depth of the camera in use. The histogram enables you to identify which portion of the dynamic range of the camera is in use. This is useful for setting the exposure while in live mode.

### Reset Display

Resets the display of the "Acquired" image to defaults. This includes the scaling and gamma values set on this tab as well as the other display values such as LUT, zoom and contrast settings.

## Acquire Dialog Box Options - Color Tab

### Image Type

Selects either ***Brightfield*** or ***Fluorescence*** as the image type based on the illumination source the you intend to use. The default for this setting is ***Brightfield***, which enables you to make settings for Brightness and/or the color intensity of individual primary colors. If you select ***Fluorescence*** as the image type, the settings options on the Color tab change to a different group of settings.

#### *Settings for Fluorescence*

##### Red

Provides controls to set the minimum and maximum scaling values to control the red sensitivity. Move the pointed sliders along the red scale or type or select minimum and/or maximum scaling values in the associated boxes.

##### Green

Provides controls to set the minimum and maximum scaling values to control the green sensitivity. Move the pointed sliders along the green scale or type or select minimum and/or maximum scaling values in the associated boxes.

##### Blue

Provides controls to set the minimum and maximum scaling values to control the blue sensitivity. Move the pointed sliders along the blue scale or type or select minimum and/or maximum scaling values in the associated boxes.

##### Min

Sets the lower limit of the scaling range for the associated color. This specifies the minimum gray scale sensitivity for this color. Type or select a value in this box, or move the associated pointer along the associated color scale slider.

##### Max

Sets the upper limit of the scaling range for the associated color. This specifies the maximum gray scale sensitivity for this color. Type or select a value in this box, or move the associated pointer along the associated color scale slider.

#### *Settings for Brightfield*

##### Brightness

Adjusts the overall intensity levels of all three color channels simultaneously. Moving this slider is equivalent to moving the Red, Green, and Blue sliders by equal amounts.

##### Red

Adjusts the intensity levels of the red values in the image.

#### Green

Adjusts the intensity levels of the green values in the image.

#### Blue

Adjusts the intensity levels of the blue values in the image.

#### Reset

Resets all sliders to the default midpoint position.

### Measure Black Reference

Measures a black reference region in the image and records the measurement in the Black Reference window.

### Measure White Balance

Measures a white reference image, which is used to correct for the differences between red, green, and blue values of the image. For the region that you specify as white, MetaMorph scales the intensity value indicated for each channel to equal the maximum intensity for that color.

## Acquire Dialog Box Options – Acquire tab

### Auto-Expose Settings

Sets the target and limits for auto-expose calculations.

### Target Intensity

Sets the maximum intensity value for the acquired image. The target intensity value default is 75 percent of the maximum gray level that the camera driver reports as possible to obtain (For example, 75 percent of 4096 is 3072).

### % of Max

Specifies the percentage of the maximum gray level needed to achieve the target intensity. The default value is 75 percent.

### Maximum Exposure

Sets the maximum exposure time that you want to allow. Time can be in milliseconds (ms), seconds (sec), or minutes (min).

### External Shutter Linked to Camera

Specifies the name of the external shutter that is in use.

### Illumination

Lists the available shutters for you to use to acquire images. When acquiring an image, the shutter is opened, the image is acquired, and then the shutter is closed. When in Live mode, the shutter is locked open.

### Preferences

Provides settings for available preferences.

### Amount to adjust exposure when using the arrows in the edit box

Sets a value by which the exposure time can be incremented for each up or down click of the *Exposure Time* settings buttons. The default value for this is 25.

### Zoom live image if binning is different

Enables you to maintain the Live image window at the same size as the Acquire image window. If the Live binning value is greater or less than the Acquire binning value the image window size will be increased or

decreased by a proportionate percentage.

### Use setting name as image name

Sets the image name in the image selector to the name of the state file when the state file is loaded. The state file name appears in the Setting drop-down box.

## Acquire Dialog Box Options – Correct tab

### Background Subtraction

This can be off or use a constant, region, or image as a background.

***None*** – Applies no background subtraction.

***Constant*** -- Applies a constant value for background subtraction. Select or type a value in the Constant Value box.

***Region*** -- Applies a background subtraction value that is the average value of the defined region. You must first define a region on the Acquire image. The average value of the region is subtracted from the image. If you specify a value in the Offset Value box, this value can be added to the image. This is useful for transmitted light images in which the background level should be high and not 0.

If a region has never been established or is not valid for the image, the Define Region button places a region on the "Acquired" image, acquiring the image if necessary. Once the background region is on the image it can be moved or resized (but not deleted) as necessary. If the binning changes and a new image is acquired the region will resize to position itself so that it covers the same portion of the image. The region will maintain its proper position if the camera area changes. If the camera region area changes so that the background is no longer on the image the region will become invalid. Only the region created through the Define Region button can be used as the subtraction region, other user defined regions will not be used.

***Image*** – Acquires an image for the background using the current exposure and binning. The background image acquired will always be the full chip area of the camera. When an acquisition is done using some portion of the image, the correct portion of the background image is subtracted. In this way, you can select new camera regions without having to acquire new background images.

***Acquire Background*** – Acquires a background image.

***Keep Shutter Closed*** – Prevents the shutter from opening during acquisition. Use this option to acquire a background image to be used for background subtraction.

***Display Background Image*** – Shows the portion of the background image that corresponds to the current acquisition area. Altering, deleting, or saving the displayed image will have no effect on the actual background image. If a new background image is acquired the window displaying the previous background will not close, update or change. To see the newly-acquired background image, click *Display Background Image*.

***Load Background Image*** – Loads the acquired background image to be used for background subtraction. If you acquire a new background image, you must click *Load Background Image* again to use the most recently acquired background image.

***(Error and Warning Text)*** – Provides error and warning information about the image background. Under some circumstance a background setting may not be functional or appropriate. In these cases a light matching that on the main tab will appear with some explanatory text. The following messages may appear for Background subtraction:

[Red] Background image not acquired or loaded

[Red] Background binning differs from acquire

[Red] Background image size differs from camera

[Red] Background region not valid for acquisition

[Yellow] Background exposure differs from acquire

Errors, [Red], indicate that no subtraction will occur when the image is acquired. Warnings [Yellow] indicate that subtraction will be performed but may not be appropriate under all circumstance.

**Note:** Background and shading images are saved when exiting MetaMorph and reloaded when MetaMorph restarts. In addition these images are saved with Setting files and can be loaded with the Setting files.

### Shading Correction

***None*** – Applies no shading correction.

***Image*** – Enables the shading correction option and applies shading correction to your image.

***Acquire Shading Reference*** -- Acquires an image for shading correction using the current exposure and binning. The shading image acquired will always be the full chip area of the camera. When an acquisition is done using some portion of the image, the correct portion of the shading image is used for correction. In this way you can select new camera regions without having to acquire new shading images.

***Display Shading Image*** -- Shows the portion of the shading image that corresponds to the current acquisition area. Altering, deleting, or saving the displayed image will have no effect on the actual shading image. If a new shading image is acquired the window displaying the previous shading image will not close, update or change. To see the newly-acquired shading image, click *Display Shading Image*.

***Load Shading Image*** – Loads the most recently acquired shading image for use by the Shading Correction option. If you acquire a new shading image, you must click *Load Shading Image* again to use the most recently acquired shading image.

***Error and Warning Text*** -- Provides error and warning information about the image shading. Under some circumstance a shading setting may not be functional or appropriate. In these cases a light matching that on the main tab will appear will some explanatory text. The following messages may appear for shading correction:

[Red] Shading image not acquired or loaded

[Red] Shading binning differs from acquire

[Red] Shading image size differs from camera

Errors, [Red], indicate that no shading correction will occur when the image is acquired.

### Do correction when live is running

Applies *Background Subtraction* and *Shading Correction* to the live image when "live" is actively acquiring images. Activating this disables the *Live Bin* field.

## Acquire Dialog Box Options – Annotate tab

### Automatic Image Annotation

Enables you to select and include information that can automatically be placed into the acquired image's annotation. Click each checkbox for the information that you want to include.

### User Annotation

Enables you to append text and/or variables you want to the acquired image's annotation. All variables must be enclosed between percent symbols (%*variable*%). Type the text and/or variable into the User Annotation box.

## Acquire Dialog Box Options – Special tab

### Various Controls

Any control unique to the camera driver can appear on this tab. The options available will vary depending on the camera installed.

### Sensor Mode

Defines the operational mode of the camera.

### Digitizer

Sets the range of speed with in which the digitizer will acquire images.

### Gain

Sets the range of sensitivity and signal strength for the camera. The gain setting works both for image acquisition and Show Live. In Show Live mode, the value displayed is the live gain. In non-live mode, the value displayed is the gain value to be used for image acquisition.

### Intensifier Gain

Controls the output gain on specific cameras or controls the gain of image intensifiers on certain cameras.

### Bit Depth

Sets the image bit depth. Enables you to expand the camera image bit depth range to fit within the bit depth range of the acquired image. Set this value to 24-Bit to enable color image acquisition from qualified color video cameras.

### Sharpness

Controls camera hardware to increase or decrease camera sharpness.

### Camera Shutter

Sets the state of the camera shutter to one of the following states: *Open for Exposure, Always Closed,* and *Always Open.*

### Clear Mode

Defines when to clear the camera chip.

### Clear Count

Specifies the number of frames to clear when clearing the camera chip.

### Frames to Average Field

Specifies the number of frames to combine for frame averaging cameras.

### Offset

Adjusts the black level reference above the zero level to reduce or eliminate background noise.

### Sensitivity

Turns on/off camera sensitivity capability and specifies a sensitivity value to control the camera's internal image intensifier.

### Light Mode

Specifies the relative brightness of the light source and raises or lowers the camera's sensitivity accordingly.

### Cooler On

Activates internal camera cooling.

### Use Contrast Knobs

Enables the camera-mounted contrast controls.

### External Trigger/Trigger Mode

Enables the camera's external trigger capability.

### Flat Field Correction

Activates the camera's internal shading correction capability.

### Get Flatfield

Acquires an image for flatfield (shading) correction.

### Noise Filter

Activates the camera's internal noise correction capability.

### Compute exposure and gain on live startup

Computes exposure and gain whenever the *Live* button is pressed.

### Quality/Speed

Enables you to achieve an ideal balance between *Quality* and *Speed* when continuously updating "live" images. The better the quality, the slower the speed; conversely, the faster the speed, the lesser the quality.

### Image Type

Selects the image's type of illumination: *Brightfield* or *Darkfield.*

### Show Focus Indicator

Displays a value on the live image that reflects focus accuracy, where the highest value equals the most accurate focus.

### Reset

Resets certain camera settings to default values.

### Digital4 Visibility

Enables a special option for those users who have Digital4 loaded in addition to the Acquire drop-in. Users may want to switch to the new Acquire dialog, but have journals they want run that were recorded with Acquire from Digital Camera. In this case the "Hide Digital4 menu" may be appropriate.

### Hide Digital4 menu

Hides digital4 from the menu, but journals using Digital4 will still run. The acquire icons on the toolbar and other functions such as Acquire Timelapse will run through the settings of the Acquire dialog.

If the user selects the option to show the digital4 menu then the acquire icons on the toolbar and other functions such as Acquire Timelapse will run through the settings in the Acquire from Digital Camera dialog.

### Show Digital4 and set as acquisition handler

Makes the Digital4 commands *Acquire From Digital Camera* and *Basic Digital Acquire* visible on the Acquire menu, and sets them to function as the valid acquisition handler.

## Acquire Dialog Box Options - Live Replay Tab

### Enable Live Replay

Enables the Live Replay command when in Live mode. This checkbox must be selected to use the Live Replay feature.

### Image Stack

Specifies the name and destination for the Live Replay image stack and selects whether the stack should be saved as a new image, overwrite an existing image with the same name, or add the image to an existing image in order to make a stack of two or more images. This is a standard Meta Imaging Series Image Selector.

### Capture point journal

Displays the name of the journal to be run at the capture point. This is an optional step.

### Browse

Enables you to select a journal to run at the capture point.

### Number of frames to capture

#### Before the capture point

Sets the number of images to add to the stack that were acquired before activating *Capture Live Images* mode.

**After the capture point**

Sets the number of images to add to the stack that were acquired after activating *Capture Live Images* mode.

**Timing Acquisition Information**

**Note**: The Timing Acquisition Information is determined by the values set in the *Number of frames to capture* fields. The time is also effected by acquisition factors such as binning, camera area, and digitizer speed.

**Amount of time before capture point**

Displays the amount of time (in seconds) from before the capture point that will be in the resulting stack. This information is only displayed while in Live mode.

**Amount of time after capture point**

Displays the amount of time (in seconds) from after the capture point that will be in the resulting stack. This information is only displayed while in Live mode.

**Memory Acquisition Information**

Displays memory usage statistics based on the current settings.

**Capture Live Images**

Begins capturing the live images into a stack based on the current settings. The *Number of frames to capture* fields must be set before starting this command. You can also use the F11 key to activate this command.

# Digital Camera Adjustments

**Eliminates background noise and redefines the black level value by repositioning the zero reference level above the background noise.**

**Drop-in: DIGADJ**

Use this command to eliminate background noise in images acquired with MV-1500 ORCA cameras. Moving the slider or typing a new value in the settings box enables you to reposition the image's zero pixel level reference point. When repositioned at a value above zero, all chip pixel information below the new level setting is ignored, and the new level value is considered to be zero.

## Adjusting Digital Camera Offset

To use the Digital Camera Adjustments, perform the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Digital Camera Adjustments. The Digital Camera Adjustments dialog box opens. |
| **2** | Acquire continuous images using Show Live in the Acquire from Digital Camera dialog box, the Basic Digital Acquire dialog box, or the Acquire dialog box. |
| **3** | Close the shutter, cover the lens, or place a lens cap on the lens to eliminate any outside light source. |

4 While observing the image view, adjust the offset control (slider) to obtain the best possible black image.

5 Click Close when you are finished setting the offset value. The offset dialog box closes.

## Digital Camera Adjustments - Dialog Box Options

### Offset

Adjusts the black level reference above the zero level to reduce or eliminate background noise.

### Close

Closes the dialog box.

# Hamamatsu C2400-60 (Acquire Menu)

**Allows you to control the Hamamatsu C2400-60 camera controller from either the controller or the computer.**

### Drop-in: C240060

You can use this command to specify *Remote* (computer) control or *Local* (controller box) control of some of the camera controller functions, such as contrast enhancement. (There are several functions of the controller that cannot be controlled from the computer. These include the SHADING control knobs and the DETAIL control knob.)

**Note:** You must complete the following steps before using this command:

- Close MetaMorph and open the Meta Imaging Series Administrator program.
- Load the video camera driver using the Configure Acquisition command.
- Close the Meta Imaging Series Administrator program and open MetaMorph.
- Select the camera with the Select Camera/Board command (Acquire menu).

*Local* mode disables all of the dialog box options except the *Local/Remote* toggle command button. All of these functions can then be controlled using the controller. *Remote* mode enables the dialog box options. Except for *Gain* and *Offset,* all functions that can be controlled by the computer will be disabled on the controller. The *Contrast* list allows you select whether the Gain and Offset functions are adjusted using the dialog box, the controller knobs, or by *Auto Enhance.*

Functions Controlled by the Hamamatsu C2400-60 Command:

| Controller Name | Dialog Box Option |
|---|---|
| REMOTE (LED) | *Remote/Local* toggle command button |
| GAIN | *Gain* |
| OFFSET | *Offset* |
| AUTO ENHANCE | *Auto Enhance* selection in *Contrast* list |
| BOOST - HIGH | *High (0.45)* selection in *Gamma* list |
| BOOST - LOW | *Low (0.75)* selection in *Gamma* list |
| BOOST - OFF | *Off (1.00)* selection in *Gamma* list |
| NEGA | *Negate* |
| AGC | *Auto-Gain* |
| GRAY SCALE | *Gray Scale* |
| SHADING MODE - DIAG | *Diagonal* selection in *Shading* list |
| SHADING MODE - | *Normal* selection in *Shading* list |

| | |
|---|---|
| NORM<br>SHADING MODE - OFF | *Off* selection in *Shading* list |

## Using the Hamamatsu C2400-60 Camera Controller

To control the Hamamatsu C2400-60 camera controller from MetaMorph, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Hamamatsu C2400-60. |
| **2** | If this is the first time that this command has been used, the Serial Communications dialog box will appear.<br><br>Select the communication port being used by the camera controller from the *Comm. Port* list. Select *9600* baud from the *Baud Rate* list. Choose *OK* when you have finished.<br><br>This allows MetaMorph to set up the appropriate Data Stream that it will create exclusively for this command. This step will be skipped during subsequent uses of the Hamamatsu C2400-60 command. |
| **3** | Using the *Remote/Local* toggle command button, select whether the controller *(Local)* or computer *(Remote)* controls the Hamamatsu C2400-60 functions. The dialog box options will be available when the button is labeled as *Remote*. The dialog box options will be unavailable when the button is labeled as *Local.* |
| **4** | If you selected *Local* in Step 3, you can operate the controls using the controller box. Otherwise, continue to Step 5. |
| **5** | To set the *Gain* and *Offset* manually, choose either *Set via Dialog Controls* or *Set via Controller Knobs* from the *Contrast* list.<br><br>**OR**<br><br>Select *Auto Enhance* from the *Contrast* list if you want the controller to determine the best *Gain* and *Offset* settings. Then skip to Step 7. |
| **6** | Use the *Gain* and *Offset* options to set the gain and offset levels. If you selected *Set via Controller Knobs* in Step 5, use the knobs on the controller box instead. |
| **7** | Select the desired *Shading Mode (Normal, Diagonal,* or *Off)* from the *Shading* list. |
| **8** | Select the desired *Boost* setting *(High, Low,* or *Off)* from the *Gamma* list. |
| **9** | You can select the *Auto-Gain, Negate,* and/or *Gray Scale* options to turn on their respective functions *(AGC, NEGA,* and |

*GRAY SCALE)* on the controller box.

To turn off one of these functions, deselect the appropriate option so that its check box is cleared.

**10** Choose *Close* when you have finished.

## Hamamatsu C2400-60 - Dialog Box Options

### Remote/Local

Specifies whether the applicable camera controller functions are set from the controller box *(Local)* or from this dialog box *(Remote).* When *Remote* is displayed as the label on the button, the functions will be controlled by the computer. When *Local* is displayed as the label, the dialog box options (except this one) will be disabled.

### Contrast

Specifies how the *Gain* and *Offset* will be set: with the control knobs on the controller box, with the dialog box options, or to be determined by the controller using *Auto Enhance* to select the best settings. If you select *Auto Enhance, Gain* and *Offset* will be unavailable.

### Gain

Specifies the gain setting. You can select a value from *0* to *10.*

### Offset

Specifies the offset setting. You can select a value from *0* to *10.*

### Shading

Specifies the shading mode. You can select *Normal, Diagonal,* or *Off.*

### Gamma

Specifies the boost mode. You can select *High, Low,* or *Off.*

### Auto Enhance

Enables and disables the AUTO ENHANCE function.

### Negate

Enables and disables the NEGA function.

### Gray Scale

Enables and disables the GRAY SCALE function.

### Close

Closes the dialog box.

# Configure Digital Camera (Acquire Menu)

**Configures basic settings for use of a digital camera.**

### Drop-in: CFGCCD

Use this command to set a digital camera's temperature, shutter speed, and sensor mode. This command is used after installation of a charge-coupled device (CCD), or "digital camera." After the camera's use has been configured, you probably will not need to use this command again for that camera.

**Note:** These configuration settings are currently only supported by the Photometrics PVCam driver and by Princeton Instruments cameras (EXAMPLES: MicroMAX, PentaMAX).

**Note:** You must complete the following steps before using this command:

- Close MetaMorph and open the Meta Imaging Series Administrator program.
- Load the video camera driver using the Configure Acquisition command.
- Close the Meta Imaging Series Administrator program and open MetaMorph.
- Select the camera with the Select Camera/Board command (Acquire menu).

## Configuring a Digital Camera

To configure the use of your digital camera, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Configure Digital Camera. The Configure Digital Camera dialog box will appear. |
| **2** | The temperature setting of your camera is shown in the *Current Camera Temperature Is* text line. To change the temperature setting for your camera, enter the new temperature with the *Set Camera Temperature To* spin box. This will be determined by the particular camera driver being used. Typical settings for the Photometrics PVCam camera are in the range of -5 to -25 degrees Centigrade. |
| **3** | Select a sensor mode from the *Sensor Mode* drop-down list box. Again, the particular camera driver you are using will determine your choice. Possible choices are: *Normal, FT* (frame transfer), *MPP* (a camera option available on certain PVCam cameras), and *FT MPP* (a frame transfer version of an MPP camera). |
| **4** | If you are using a camera that has a shutter, set the shutter's open and close delays in the *Shutter Open Delay* and *Shutter Close Delay* text boxes. Typical delays for the PVCam are 15 ms to open and 30 ms to close. |
| **5** | Choose *OK.* |

## Configure Digital Camera - Dialog Box Options

### Current Camera Temperature Is

Indicates the current temperature setting for your digital camera.

### Set Camera Temperature To

Changes the temperature setting of your digital camera to your specified new temperature.

### Sensor Mode

Sets the sensor mode of your camera. The particular camera driver you are using will determine your choice. The choices are: *Normal, FT* (frame transfer), *MPP* (a camera option available on certain PVCam cameras), and *FT MPP* (a frame transfer version of an MPP camera).

### Shutter Open Delay

Sets the delay time for the opening of your camera's shutter. The typical delay for a Photometrics PVCam camera is 15 ms.

### Shutter Close Delay

Sets the delay time for the closing of your camera's shutter. The typical delay for a Photometrics PVCam camera is 30 ms.

### OK

Closes the dialog box and implements the option changes that were selected.

### Cancel

Closes the dialog box and cancels the option changes that were selected.

# Acquire from Digital Camera (Acquire Menu)

**Acquires and transfers images from a digital camera. Allows focusing with a digital camera.**

### Drop-in: DIGITAL4

Use this command when you want to acquire images from a digital camera.

*Expose* acquires the current image, placing it in memory (replacing the image previously stored in memory).

*Transfer* transfers the most recently acquired image stored in the video board's memory to an image window in MetaMorph.

*Expose & Transfer* performs both of these tasks.

**WARNING:**
You must run all mechanical shutters at a cycle time greater than 25 ms. Uniblitz, Lambda 10, Metaltek, Ludl, and cooled CCD shutters are driven by a high voltage which takes time to dissipate. Running these shutters at a cycle length shorter than 25 ms will cause a build-up of heat, leading to eventual jamming. Neither Molecular Devices nor any manufacturers of these shutters will honor warranties on equipment that has been damaged by improper use. Operation of these shutters at a cycle length shorter than 25 ms is considered improper use.

**Note:** You must complete the following steps before using this command:

- Close MetaMorph and open the Meta Imaging Series Administrator program.
- Load the video camera driver using the Configure Acquisition command.
- Close the Meta Imaging Series Administrator program and open MetaMorph.
- Select the camera with the Select Camera/Board command (Acquire menu).

Acquire from Digital Camera allows you to create and define multiple sets of acquisition settings that you can select quickly as needed during an acquisition work session. Each set of acquisition settings can include specific settings for exposure time, binning, gain, speed, bit-depth, region size, shutter, background subtraction, and shading correction. After you define them using the Define Acquisition Settings dialog box, three separate sets of acquisition settings can be selected using the *Acquisition Settings* lists. You can then switch between these three sets as needed by selecting the check box next to the desired set. You can save and load settings.

You can also use this command to focus the image while acquiring from the digital camera. The *Focus* command quickly updates and displays the image in an image window. This allows you to see an image while focusing on your specimen. The focusing image will be displayed in the Focus window at 100% zoom, regardless of the zoom you select for the acquisition image window. The *Focus* command derives its acquisition settings from those that are selected in the *Focus Acquisition Settings* list. Acquisition configurations such as binning settings will be disabled while you are focusing.

If you include the *Focus* command as part of a journal, you will have the option of configuring a brief message that will appear during playback. This message will then be displayed in a dialog box that provides you with two buttons: *Cancel Journal* and *Stop Focusing.*

The Acquire from Digital Camera dialog box expands to include three image selectors. The first one, *Destination Image,* is used to select the destination image for *Expose & Transfer* and *Transfer.* The *Background Reference* image selector is used to specify the background reference image to be used if you selected *Subtract Background* from the Define Acquisition Settings dialog box. Likewise, the *Shading Reference* image selector is used to specify the shading reference image if you selected *Correct Shading.* (These image selectors will be unavailable if you did not select the pertinent option in the Define Acquisitions Settings dialog box. Background and shading reference images are acquired as for any other image, using the *Expose & Transfer* command. You may wish to save them for future work sessions using the Save command in the File menu.)

**Acquiring Digital Images**

**Opening the Acquire from Digital Camera Dialog Box**

**Defining Acquisition Settings**

**Configuring Region Settings**

**Focusing a Digital Camera**

**Acquiring Images**

**Acquiring Reference Images**

## Opening the Acquire from Digital Camera Dialog Box

To transfer an image or a stack to video, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Acquire from Digital Camera. The Acquire from Digital Camera dialog box opens. |
| **2** | If you want to set the preferences for the digital camera, select *Prefs.* The Digital Camera Preferences dialog box will appear.<br>**AND**<br>Select *Warn If Exposure Time Is Long* if you want to be warned when the camera's exposure is longer than the *Exposure Time Warning Threshold* value. Then select the desired number of milliseconds for the *Exposure Time Warning Threshold.* |
| **3** | Choose *OK*. |

## Defining Acquisition Settings

To define the acquisition settings for Acquire from Digital Camera, use the following procedure:

| Step | Action |
|---|---|
| **1** | **Open the Acquire from Digital Camera dialog box.** |
| **2** | Choose *Define Acquisition Settings.* The Define Acquisition Settings dialog box will appear. |
| **3** | To create a new set of acquisition settings, choose *New Setting.* "Untitled 1" will appear in the *Setting Name* text box. You can replace this name with a name of your |

choice.

**OR**

Select an existing set of settings from the *Stored Setting* list. Its name will appear in the *Setting Name* text box.

**4** Select the desired exposure time and the unit of time using *Exposure Time*.

**5** Use the **Configure Region** command to configure the desired region size.

**AND**

Select the desired region from the *Region* list.

**6** If your camera supports **binning,** you can select the desired type of binning from the *Binning* list. Select *Sep H and V* for separate horizontal and vertical binning, *Same H and V* for the same horizontal and vertical binning, or *None* for no binning. (The options available in this drop-down list box will vary depending on the type of binning supported by your camera.)

**AND**

Select the desired *Horz.* and *Vert.* values.

**7** You can select settings for *Gain, Speed,* and *Bit Depth* if your camera supports any of these options. The settings will vary depending on the type of camera. In some cases, the settings available in one of these options will depend on the setting(s) in the other option(s).

Bit-depth specifies the gray level scale resolution of the camera. Although setting the bit-depth to a lower value may result in faster acquisition, the image quality will suffer if you do not use the highest bit value.

**8** If you are using an external shutter, select a shutter state from the *Shutter* list: *Open for Expose, Always Closed,* or *Always Open.*

**AND**
Select the shutter associated from the *External Shutter* list.

**9** If much of your image data resides in a narrow band at the lower or upper end of the grayscale range, you may need to rescale the image to be able to discriminate intensity differences. You can select *Auto Scale 16-Bit Image* if you want MetaMorph to choose the range of gray levels used for scaling.

**OR**

Deselect *Auto Scale 16-Bit Image* to choose the range manually. Then select the darkest gray level for the image using the *Low* text box and select the brightest gray level using

the *High* text box.

**10** Select *Subtract Background* if you want to enable background subtraction. The background image will be subtracted whenever an image is acquired. This option will use the reference image selected from the *Background Reference* image selector in the Acquire from Digital Camera dialog box.

**AND**

If the image resulting from background subtraction is too dark (less than gray level 0), select a *Subtraction Offset* value to be added back to the image's values.

(See also: **Acquiring Reference Images.)**

**11** If your hardware supports camera chip clearing, use the *Clear Chip Count* spin box to specify the number of times the chip is to be cleared before exposure.

**12** Select *Correct Shading* if you want to enable shading correction. This option will use the reference image selected from the *Shading Reference* image selector in the Acquire from Digital Camera dialog box.

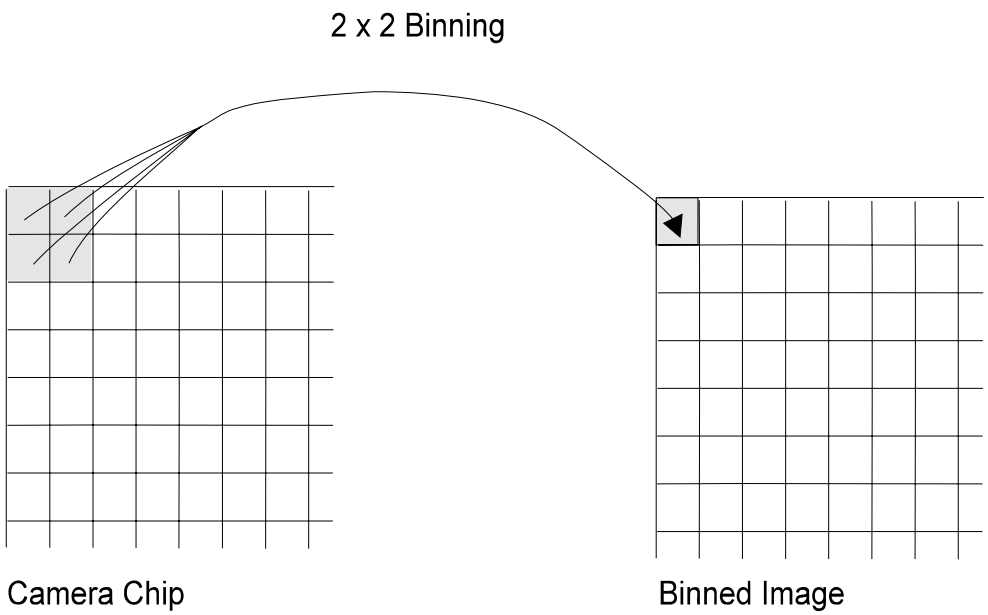
**13** Repeat Steps 3 - 12 for as many sets of acquisition settings as you need for acquisition and focusing. You can use the *Delete Setting* command to delete any settings if necessary.

**14** Choose *Close* when you have finished.

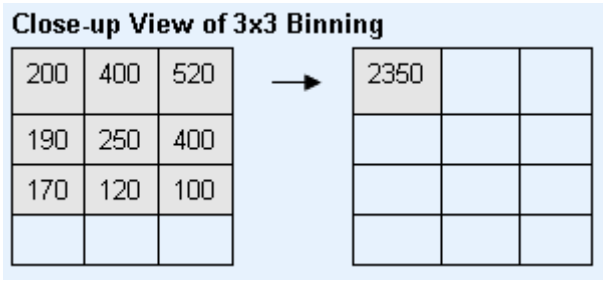
# Understanding Binning

Binning is the process of combining data from groups of image pixels into a single pixel during acquisition. Binning results in a higher signal to camera noise ratio in the resulting image, with a corresponding increase in intensity, or brightness, for the resulting image. For example, 2 x 2 binning causes the signal to camera noise ratio to increase 4x in the resulting image, with a 4x decrease in the image size. 3 x 3 binning results in a 9x increase in signal to camera noise ratio and a 9x decrease in image size. Because the image is smaller, the time required to transfer the image and the image file size are significantly reduced.

In the example below, two horizontal pixels and two vertical pixels are combined in 2 x 2 binning. Usually the number of horizontal and vertical pixels that are used are the same.




When you select the *Horz*. and *Vert*. binning values, it is important that you consider the final value of the resulting pixel. If your camera is a 12-bit camera, you would calculate the maximum brightness as: $2 * 2^{12} - 1 = 4095$. Thus, the sum of the binned pixels cannot exceed that value for a 12-bit camera. Binned pixels will lose some spatial resolution but no intensity data loss unless the resulting pixel values exceed the maximum brightness value.




## Configuring Region Settings

To configure the region settings for Acquire from Digital Camera, use the following procedure:

| Step | Action |
|---|---|
| 1 | **Open the Acquire from Digital Camera dialog box.** |
| 2 | Choose *Define Acquisition Settings.* The Define Acquisition Settings dialog box will appear. |
| 3 | Choose *Configure Regions.* The Configure Regions dialog box will appear. |
| 4 | To create a new region, choose *New Region.* "Default" will appear in the *Region Name* text box. You can replace this name with a name |

of your choice.

**OR**

Select an existing region from the *Stored Region* list. Its name will appear in the *Region Name* text box.

5 If you want to use the active rectangular region from an image on the desktop, rather than defining the region with the dialog box options, select the desired image using the *Image* selector.

**AND**

Choose *Use Action Region Defined on Image.*

6 You can use *Left, Top, Width,* and *Height* to specify the size and location of the region on the chip. Choose *Entire Chip* to create a region that is the size of the chip, or choose *Center Quadrant* to create a region centered on the chip that is the size of one quadrant. Choose *Ctr* to center the region. You can use the << or >> options to shrink or enlarge a region by a factor of 2.

**OR**

Specify the size and location of region using the box-in-box option on the left side of the dialog box. The smaller box can be sized and moved just as for a region of interest.

7 Repeat Steps 4 - 6 for each region you want to create.

8 Choose *Close* when you have finished.

## Focusing the Microscope with a Digital Camera

The *Focus* command instructs MetaMorph to acquire an image continuously into an image window while you are focusing the microscope, so that you can verify that your specimen is visible and in focus. It is important to use the *Focus* command because what can be seen through the microscope's eyepiece and what the camera acquires are not always the same. A few digital cameras have gain and offset controls. These too can be adjusted while using the *Focus* command.

MetaMorph will use the acquisition settings defined in the selected *Focus Acquisition Setting* to acquire images continuously until you choose *Stop Focusing* (or press the [F2] key). Acquisition configurations such as binning settings will be disabled while you are focusing.

| Step | Action |
|---|---|
| **1** | **Open the Acquire from Digital Camera dialog box.** |
| **2** | Choose *Define Acquisition Settings.* The Define Acquisition Settings dialog box will appear. |
| **3** | Define a set of acquisition settings suitable for focusing using the procedure outlined in **Defining Acquisition Settings.** When you select a **region size** for focusing, the smaller |

the region, the faster the Focus image window can update.

After you have finished defining the settings for focusing, choose *Close* to close the Define Acquisition Settings dialog box.

**4** Select the desired acquisition settings from the *Focus Acquisitions Settings* list.

**5** Choose *Focus.* The Focus image window will appear.

**6** Focus your microscope.

**7** Choose *Stop Focusing* or press the [F2] key to stop the focusing acquisition. MetaMorph will stop acquiring images and will close the Focus image window.

## Acquiring Images from a Digital Camera

To acquire images from Acquire from Digital Camera, use the following procedure:

| Step | Action |
|---|---|
| **1** | **Open the Acquire from Digital Camera dialog box.** |
| **2** | Define the desired set(s) of acquisition settings using the procedure outlined in **Defining Acquisition Settings.** |
| **3** | Choose *More >>* to select the desired images for your acquisition.<br>**AND**<br>Select the desired destination image using the *Destination* image selector. If you selected *Subtract Background* as one of your acquisition settings, you can select a reference image using the *Background Reference* image selector. If you selected *Shading Correction* as one of your acquisition settings, you can select a reference image using the *Shading Reference* image selector. |
| **4** | Select the desired sets of acquisition settings that you want to display in the three *Acquisition Settings* lists during acquisition.<br>**AND**<br>Select the check box next to the set that you want to use first during the acquisition. You can quickly change to a different set at any time by selecting the appropriate check box. |
| **5** | Choose *Expose & Transfer* to acquire an image and transfer the image to an image window.<br>**OR**<br>Choose *Expose* if your camera supports the video monitor display of the image in the video board's memory. Then choose |

*Transfer* when you want to place the displayed image in an image window.

If you plan to switch between *Acquisition Settings* sets that have different region sizes, you should use *Clear* command before each *Expose* command to clear the contents of video board's memory. (Otherwise, your monitor will display part of the previous image.)

**6** Choose *Close* when you have finished.

## Acquire from Digital Camera - Dialog Box Options

### Acquisition Settings

The Acquire from Digital Camera command allows you to create and define multiple sets of acquisition settings that you can select quickly as needed during an acquisition work session. Each set of acquisition settings can include specific settings for exposure time, binning, gain, speed, bit-depth, region size, shutter, background subtraction, and shading correction. After you have configured them using the Define Acquisition Settings dialog box, you can select from three separate sets of acquisition settings using the *Acquisition Settings* lists. You can switch between these three sets as needed by selecting the check box next to the desired set.

### Focus Acquisition Settings

Specifies the set of acquisition settings MetaMorph will use for focusing.

### Define Acquisition Settings

Opens the Define Acquisition Settings dialog box.

### Expose & Transfer

Acquires the current image from the digital camera, placing it in the video board's memory (replacing the previous image stored in memory) and then transfers it to an image window in MetaMorph.

### Expose

Acquires the current image from the digital camera, placing it in the video board's memory (replacing the image previously stored in memory). If the digital camera supports display of the video board's memory on a video monitor, the image will be displayed on the monitor.

### Transfer

Transfers the last acquired image stored in the video board's memory to an image window in MetaMorph.

### Clear

Clears the contents of the video board's memory. If you plan to switch between *Acquisition Settings* sets that have different region sizes, you should use *Clear* command before each *Expose* command to clear the monitor. Otherwise, your monitor will display part of the previous image.

### More >>

Expands the dialog box.

### Less <<

Condenses the dialog box.

### Focus

The *Focus* command instructs MetaMorph to acquire an image continuously into an image window while you are focusing the microscope so that you can verify that your specimen is visible and in focus. It is important to use the *Focus* command because what can be seen through the microscope's eyepiece and what the camera acquires are not always the same. A few digital cameras have gain and offset controls. These too can be adjusted while using the *Focus* command. MetaMorph will use the acquisition settings defined in the selected *Focus Acquisition Setting* to acquire images continuously until you choose *Stop Focusing* (or press

the [F2] key). The journal function for this option, "ADC: Focus," acts as a toggle--the first time it is activated, images will be acquired into a Focus window. A subsequent call to the function or a press of the [F2] key will terminate acquisition.

### Stop Focusing

Stops the digital camera acquisitions used for focusing. Pressing the [F2] key also stops the acquisitions. Choosing *Expose & Transfer, Expose, Transfer,* or selecting an *Acquisition Setting* check box will also stop the focus acquisitions.

### Prefs

Opens the Digital Camera Preferences dialog box.

### Destination

Specifies the destination image for the acquisition. You can add to or overwrite an existing image or stack. You can also specify a new image.

### Background Reference

Specifies the background reference image for the acquisition. You can add to or overwrite an existing image or stack. You can also select *None.* This image selector can be used only if you selected *Subtract Background* from the Define Acquisition Settings dialog box. You will not be able to select the same image for both background reference and shading reference.

### Shading Reference

Specifies the shading reference image for the acquisition. You can add to or overwrite an existing image or stack. You can also select *None.* This image selector can be used only if you selected *Correct Shading* from the Define Acquisition Settings dialog box. You will not be able to select the same image for both background reference and shading reference.

### Load

Loads a set of acquisition settings previously saved with the *Save* command. This command opens the Load Acquisition Settings dialog box.

### Save

Saves the current acquisition settings on disk. You can open the settings at a later date using the *Load* command. This command opens the Save Acquisition Settings dialog box.

### Close

Closes the dialog box.

## Digital Camera Preferences - Dialog Box Options

### Warn If Exposure Time Is Long

Instructs MetaMorph to warn you before an acquisition starts if the camera's exposure setting is longer than the value specified in the *Exposure Time Warning Threshold.*

### Exposure Time Warning Threshold (ms)

Specifies the minimum exposure time that is to be considered a long exposure. You will be warned if the exposure time equals or exceeds this limit.

### OK

Sets the digital camera preferences.

### Cancel

Cancels the command.

## Define Acquisition Settings - Dialog Box Options

### Stored Setting

Specifies the stored setting currently displayed in the Define Acquisition Settings dialog box.

### Setting Name

Lists the name of the new or existing setting that you are editing. You can edit the name of the current setting using this option's text box. The name for each setting must be unique.

### Delete Setting

Deletes an acquisition setting set from the *Stored Setting* list. Will not allow you to delete the last remaining setting.

### New Setting

Creates a new setting based on the last setting displayed in the dialog box.

### Exposure Time

Specifies the length of the exposure and unit of time for each acquisition.

### Region

Specifies the region for the acquisition.

### Configure Regions

Opens the Configure Regions dialog box which configures regions for use with various sets of acquisition settings.

### Binning

Specifies the type of binning used if your camera supports binning.

### Horz

Specifies the horizontal value for binning if your camera supports binning.

### Vert

Specifies the vertical value for binning if your camera supports binning.

### Gain

Specifies the gain used if your camera supports this option. Select a higher gain value if you want a brighter image. The settings will vary depending on the type of camera. In some cases, the settings available will depend on other setting(s) selected.

### Speed

Specifies the speed used if your camera supports this option. The settings will vary depending on the type of camera. In some cases, the settings available will depend on other setting(s) selected.

### Bit Depth

Specifies the grayscale resolution of the camera if your camera supports this option. Although setting the bit-depth to a lower value results in faster acquisition, the image quality will be better if you use the highest bit value. The settings will vary depending on the type of camera. In some cases, the settings available will depend on other setting(s) selected.

### Shutter

Selects a shutter state for an external shutter, if one is available. This option is available only if your camera has its own shutter and it can be controlled from MetaMorph.

***Open for Expose*** will open the shutter only during active acquisition of an image.

***Always Closed*** leaves the shutter closed. This can be used for acquiring a dark reference image.

***Always Open*** leaves the shutter open continuously.

### Auto Scale 16-Bit Image

Instructs MetaMorph to choose the range of gray levels used for scaling a 16-bit image.

### **Low** and **High**

Sets the range for scaling a 16-bit image manually. Select the darkest gray level for the image using the *Low* text box and select the brightest gray level using the *High* text box option.

### Subtraction Offset

If the image resulting from background subtraction is too dark ("less" than gray level 0), this option specifies the value that will be added back to the image's values.

### Clear Chip Count

This option erases the camera chip to gray level 0 the specified number of times before each acquisition. If your camera supports this option, consult your camera's documentation to determine the correct value for this option.

### Subtract Background

Enables background subtraction.

### Shading Correction

Enables shading correction.

### Use Contrast Knobs

This option is only available if your camera's control box has contrast knobs. When this option is selected, the camera will use the settings from the contrast knobs. Otherwise, these settings will be ignored.

### External Shutter

Selects the shutter. If you are not using a shutter, select *"[None]."*

### Transfer as 8-Bit Image

Scales the image to 8-bit depth and transfers it to the destination image window. This option uses the *Auto Scale 16-Bit Image* settings.

### Close

Closes the dialog box.

## Acquire from Digital Camera: Configure Regions - Dialog Box Options

### Stored Region

Specifies the stored region currently displayed in the Define Region dialog box.

### Region Name

Lists the name of the new or existing region that you are editing. You can edit the name of the current region using this option's text box. The name for each region must be unique.

### Delete Region

Deletes a region from the *Stored Region* list.

### New Region

Creates a new region based on the last region displayed in the dialog box.

### Box-in-Box Interactive Display of Region

Allows you to click on the smaller box with the left mouse button and then drag the pointer to resize and move the chip region box, as you would for a region of interest.

### Image

Specifies the image to use for the *Use Active Region Defined on Image* command.

### Use Active Region Defined on Image

Defines a region for the chip based on the active region of interest in the image selected with the *Image*

selector. The box-in-box display is updated as well as the region's *Left, Top, Width,* and *Height* values.

**Left**
Specifies the region's leftmost point.

**Top**
Specifies the region's topmost point.

**Width**
Specifies the region's width.

**Height**
Specifies the region's height.

**Entire Chip**
Creates a region that is the size of the entire chip.

**Center Quadrant**
Creates and centers a region that is the size of one quadrant of the chip.

**<<** and **>>**
Shrinks or enlarges the region by a factor of two.

**Ctr**
Centers the region on the chip.

**Close**
Closes the dialog box.

# Stop Focusing (Acquire Menu)

**Stops acquiring images used during focusing with a digital camera.**

**Drop-in: AUTOFCUS, ACQSCCC**

Use this command after you have finished using the *Focus* command in the Acquire from Digital Camera or Acquire Color dialog boxes. This command is the same as the *Stop Focusing* command button in that dialog box.

**Note:** This command has no relationship with the Device menu's Auto-Focus or Focus commands or with the Stack menu's Acquire Z Series command.

**Note:** You must load the appropriate digital camera driver in the Meta Imaging Series Administrator and select it with the Select Camera/Board command (Acquire menu) for this command to be available.

**Shortcut: [F2]**

## Stop Focusing a Digital Camera

To stop focusing a digital camera, use the following procedure:

| Step | Action |
|---|---|
| 1 | From the Acquire menu, choose Stop Focusing. |
| 2 | MetaMorph will stop image acquisition and close the Focus image window. |

# Basic Digital Acquire (Acquire Menu)

**Acquires an image from a digital camera. Can be configured to calibrate the exposure time automatically.**

**Drop-in: DIGITAL4**

This command allows you to acquire an image from a digital camera. You can also use the command to calculate the exposure time for you automatically.

**WARNING:**
You must run all mechanical shutters at a cycle time greater than 25 ms. Uniblitz, Lambda 10, Metaltek, Ludl, and cooled CCD shutters are driven by a high voltage which takes time to dissipate. Running these shutters at a cycle length shorter than 25 ms will cause a build-up of heat, leading to eventual jamming. Neither Molecular Devices nor any manufacturers of these shutters will honor warranties on equipment that has been damaged by improper use. Operation of these shutters at a cycle length shorter than 25 ms is considered improper use.

**Note:** You must complete the following steps before using this command:

- Close MetaMorph and open the Meta Imaging Series Administrator program.
- Load the video camera driver using the Configure Acquisition command.
- Close the Meta Imaging Series Administrator program and open MetaMorph.
- Select the camera with the Select Camera/Board command (Acquire menu).

This command has a simpler interface than the powerful Acquire from Digital Camera and, as such, is particularly useful for newer users. It is also useful for streamlining the acquisition process because it allows you to calculate the exposure for the session automatically. Once you have performed this at the beginning of a session, you can condense the dialog box and acquire images by using the options in the upper half of the dialog box. This command is ideal for use in journals.

This command uses the settings that were set in the Acquire from Digital Camera command. Basic Digital Acquire provides access to Acquire from Digital Camera's Define Acquisition Settings dialog box so that you can define settings as needed. The Configure Regions dialog box is also accessible from the Basic Digital Acquire dialog box so that you can specify the size of the region used for acquisition.

When the exposure is calculated, multiple acquisitions are performed until MetaMorph produces an exposure value that satisfies the image intensity criteria that you have defined in the dialog box. Typically, the targeted intensity value will be obtained by the third or fourth acquisition in the series.

The first exposure uses the *Initial Exposure Duration* value set in the dialog box. Based on the brightest pixel in this image and the desired *Target Intensity* value, MetaMorph will adjust the exposure time, extrapolating from the values of the first exposure, to arrive at a calculated exposure value that meets your criteria. It then acquires the second exposure. An image will be deemed acceptable if the maximum pixel intensity in it is within 10% (+/-) of the target intensity value.

A safety feature has been included. If the calculated exposure is greater than a previous one which exceeded the target intensity or is shorter than one which fell short of the target, the command will resort to a binary search. The newly calculated exposure will be halfway between the longest exposure that didn't reach the target intensity and the short exposure that went beyond the target.

## Using Basic Digital Acquire

**Setting Autoexposure Options**

**Acquiring Images**

## Setting Autoexposure Options

To set up the autoexposure options for Basic Digital Acquire, use the following procedure:

| Step | Action |
|---|---|
| 1 | From the Acquire menu, choose Basic Digital Acquire. The Basic Digital Acquire dialog box opens. Then choose *More >>* to expand the dialog box. |
| 2 | Select your camera's maximum intensity value from the first *Target Intensity* list.<br><br>Then use the second text box to select the percentage of the maximum value that the brightest pixel in the image should equal. This will update the third text box to display the brightest pixel's target intensity value. (If desired, you can select actual value first, and let MetaMorph update the percentage for you.) |
| 3 | Select the length of the first exposure attempt from *Initial Exposure Duration.* |
| 4 | Select the maximum and minimum duration lengths from *Duration of Exposure.* |
| 5 | Select the size of the region used to test the brightest pixel value against its neighbors from *Region Size for Testing Noisy Pixel.* |
| 6 | If you want to change the acquisition settings choose **Acquisition Settings.**<br><br>**Note:** *Background Subtraction* and *Shading Correction* are not available in Basic Digital Acquire. |
| 7 | When you are satisfied with the settings, you are ready to **acquire images using Basic Digital Acquire.** |

## Acquiring Images Using Basic Digital Acquire

To acquire images using Basic Digital Acquire, use the following procedure.

**Note:** If you are using the autoexposure options and want to log the exposure calculations, open a data log file prior to using this procedure.

| Step | Action |
|---|---|
| 1 | From the Acquire menu, choose Basic Digital Acquire. The Basic Digital Acquire dialog box opens. |
| 2 | Select the desired image from the *Destination Image* selector. |
| 3 | Select the desired set of stored acquisition settings that you want to use for the acquisition from the *Acquire Digital Camera Settings* list.<br><br>To change the acquisition settings for a particular set, you can expand the dialog box using *More >>* and choose ***Acquisition Settings*.**<br><br>**Note:** Background subtraction and shading correction are not available in Basic Digital |

Acquire.

4 Choose **Configure Regions** if you want to define the region size used for the acquisition.

5 If you want to acquire images using the Basic Digital Acquire auto exposure feature, set up the **Auto Exposure options,** if you have not already done so.

Select *Calculate Exposure and Update Settings* to enable the autoexposure options. Then skip to Step 7.

**OR**
If you do not want to use the autoexposure options, skip this step.

6 If you are not using the autoexposure options, use the *Next Exposure* text box to set the length of your next exposure.

7 Choose *Acquire.*

If you have enabled *Calculate Exposure and Update Settings,* the command will perform multiple acquisition until your criteria are met. If it cannot meet your criteria, an **error message** will appear, explaining the cause of the error. In most cases, you will be allowed to continue trying or you can accept the last acquisition which will be placed in the destination image window.

If you are not using the autoexposure options, the acquisition will be placed the destination image window.

8 Choose *Close* when you have finished.

# Error Conditions That Generate Error Messages in Basic Digital Acquire

The following conditions will generate error messages when using autoexposure options in Basic Digital Acquire.

- Null image: A camera has not yet been selected using the Meta Imaging Series Administrator.
- Several saturation exposures have occurred.
- Initial value is very low compared to camera's maximum value on attempts of increasing duration.
- Repeat values of the maximum pixel intensity occurred more than once.
- The exposure to be performed has a duration that is outside the duration range set by the user.
- Too many attempts have been performed.

These error messages appear because the command's algorithm tests for the following items:

- No camera selected in the Video Driver Manager: Indicated by presence of null image.

- Saturation: If 1% or more of the image has a value equal to the maximum pixel intensity.
- Initial low values: If the initial exposure result is low then it is not a good choice for choosing a maximum pixel.
- Repeat values of maximum pixel: Indicates that camera is turned off or some other type camera error has occurred.
- Duration outside of range: Next exposure is longer than selected maximum value.
- Duration outside of range: Next exposure is shorter than selected minimum value.
- Number of attempts without success: A limit on the number of times that the command will attempt to reach the target without success has been defined. This prevents the program from oscillating between a several exposures.

**Print this page**

## Basic Digital Acquire - Dialog Box Options

### Destination Image

Specifies the destination for the acquired image. You can add to or overwrite an existing image or stack. You can also specify a new image.

### Acquire Digital Camera Settings

Displays the names of the stored settings in the Acquire from Digital Camera dialog box so that you can select the desired set to be used by the Basic Digital Acquire command. If you want to use whichever set is currently active in the Acquire from Digital Camera dialog box, rather than selecting a specific set, you can choose *Current.* The name of the current set will then be displayed below the *Acquire Digital Camera Settings* list and will be updated if you change it with Acquire from Digital Camera. (The *Current* option is useful for creating a journal that performs an autoexposure using the current settings.)

### Last Exposure/Next Exposure

If you select *Calculate Exposure and Update Settings, "Last Exposure"* will be displayed, along with the maximum pixel value of the last exposure. The duration of the last exposure will be displayed in its text box. If you leave the *Calculate Exposure and Update Settings* check box cleared, or if you change the duration value for the text box (which disables *Calculated Exposure and Update Settings),* the *Last Exposure* label will change to *"Next Exposure."* The duration value will then be used for the next exposure.

### Configure Region

Opens the Configure Regions dialog box which configures regions for use with various sets of acquisition settings.

### Calculate Exposure and Update Settings

This option causes *Acquire* to calculate the length of time necessary for a proper exposure and acquire an image for this exposure. If *Acquire* is successful in reaching the target intensities, the exposure time for the setting is set to the exposure time used in the automatic exposure.

### Acquire

Performs an exposure. If you select *Calculate Exposure and Update Settings,* multiple exposures will be performed until the program produces an exposure that satisfies the criteria set using the Auto Exposure options.

### More >>

Expands the dialog box to include the auto exposure options in addition to the *Configure Log* and *Acquisition Settings* buttons.

### Less <<

Condenses the dialog box.

### Target Intensity % Of (autoexposure option)

Specifies a maximum gray value for the image and two text boxes. The first text box allows you to specify

the percentage of the maximum gray value that you want the brightest pixel in the image to equal. Changing this value will update the second text box, which selects the actual value that you want to use as the brightest pixel. You can change either the first or second text box. The command will adjust the other value for you.

### Initial Exposure Duration (autoexposure option)

Specifies the length of the first exposure attempt when you select *Calculate Exposure and Update Settings.*

### Duration of Exposure: Maximum and Min (autoexposure option)

Specifies the longest and shortest durations to use for calculated automatic exposures before issuing a warning.

### Region Size for Testing Noisy Pixel (autoexposure option)

Because bad pixels in a camera can give falsely high values, MetaMorph tests each pixel against its neighbors to ensure that the values are consistent when determining the brightest pixel in the image. This option specifies the region size around the brightest pixel used to test it against its neighbor's values. If the region around the brightest pixel is not at least 80% of the brightest pixel, it will be determined that the brightest pixel is giving a false value due to some type of noise. In such cases, the program then tests the next brightest pixels in order until it finds one that passes this test. That pixel is used for the calculations.

### Acquisition Settings

Opens the Define Acquisition Settings dialog box.

### Configure Log (for logging exposure calculations)

Opens the Configure Log dialog box so that you can select the parameters to be logged to the log file.

### Close

Closes the dialog box.

# Set BNC Output Trigger (Device Menu)

**Configures the signal state for the BNC output from a Princeton Instruments PentaMAX camera.**

### Drop-in: PIBNC

Use this command to set the BNC output trigger state for the PentaMAX camera. An example of such an output might be that to a Sutter DG4 filter wheel controller. This configuration step will only need to be performed once at the beginning of an acquisition session.

**Note:** You must complete the following steps before using this command:

- Close MetaMorph and open the Meta Imaging Series Administrator program.
- Load the video camera driver using the Configure Acquisition command.
- Close the Meta Imaging Series Administrator program and open MetaMorph.
- Select the camera with the Select Camera/Board command (Acquire menu).

## Setting the BNC Output Trigger State

To set the signal state for the BNC output from a PentaMAX camera, use the following procedure:

| Step | Action |
|---|---|
| 1 | From the Acquire menu, choose Set BNC Output Trigger. The Set BNC Output Trigger dialog box will appear. |
| 2 | Select the trigger state from the *BNC Output Trigger* list. Your selection will depend on your hardware configuration. (See the user's |

manual for your camera for more details.)

**3** Choose *Close.*

## Set BNC Output Trigger - Dialog Box Options

### BNC Output Trigger

Specifies the trigger state used in BNC output of the camera: *Not Scan, Cleaning, Shutter, Not Ready to Accept Sync, Not Frame Transfer Shift, Logic 0, Logic 1,* or *Reserved.* Your selection will depend on your hardware configuration. (For details, consult the user's manual for your camera.)

### Close

Closes the dialog box.

# Acquire with Frame Transfer Camera (Acquire Menu)

**Controls the sequencing and acquisition of images from a frame transfer camera.**

### Drop-in: FRAME

This command controls the sequencing and acquisition from a Frame Transfer camera through the use of scripts. A script is a sequence of acquisition and device control commands. You can define up to four scripts of commands. Once defined, you can then acquire images from the frame transfer camera by running the desired script. You can select the script commands to be added and the order of the script commands for each script. You can add the same script command more than once to a script. Each time you add a command to a script, its parameters will appear at the bottom of the dialog box, where they can be configured.

You can run a script by choosing the appropriate *Acquisition Scripts* button or you can record the script to a journal and then run it from the journal. The journal will only record the button to be used, not the actual script commands.

**Sample Script 1**

**Sample Script 2**

Because this command provides a great deal of flexibility in the order of the script commands, you should check your scripts carefully to make sure that the commands are in a sensible order.

**Note:** You can also use the Acquire from Digital Camera or Basic Digital Acquire commands. These commands automatically perform the exposure, shift, transfer, and copy to image window. However, when acquiring multiple images as rapidly as possible, the Acquire with Frame Transfer Camera command will perform faster.

## Acquiring Images with a Frame Transfer Camera

To acquire images with a frame transfer camera, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Acquire with Frame Transfer Camera. The Acquire with Frame Transfer Camera dialog box will appear. Choose *More* >> to expand the dialog box so that you can access the script definition options. |
| **2** | Select the script that you want to define from the *Script* # list. |
| **3** | Select the first script command you want to add to the script from the *Acquisition* |

*Commands* list. Then choose *Add.*

**OR**
Double-click the script command name in the *Acquisition Commands* list.

The script command will appear in the script list box and its parameter setting options will appear in the bottom of the dialog box. (Refer to **Acquire with Frame Transfer Camera - Dialog Box Options** for more information.)

**4** Select the desired parameter settings for the script command.

**5** Repeat Steps 3 and 4 for each script command you want to add. The command will be added above the currently highlighted item in the script list box. Therefore, if you want to add an command to the end of the script, highlight ***End of Script*** before you choose *Add.*

**6** Type the name of the script in the *Script Name* text box and press the [TAB] key. The name will appear in the *Script #* list and on the *Acquisition Script* button.

**7** Repeat Steps 2 - 6 for each script that you want to define. You can then choose << *Less* to condense the dialog box, if desired.

**8** The image selectors are used to define the image windows used by the *Copy to Image Window* commands in your script. Select the desired image for each. You can overwrite or add to the existing image or you can place the results in a new image window.

**9** To run a particular script, choose its *Acquisition Scripts* button.

## Acquire with Frame Transfer Camera - Dialog Box Options

### Acquisition Scripts

Runs the script assigned to its button. You can define up to four scripts.

### Image #1, Image #2, Image #3, and Image #4

These image selectors are used to hold images from the four frames that can be acquired and copied to image windows. The image windows are assigned when you define the *Copy to Image Window* parameters. You can overwrite the existing image or place the results in a new image window. Or you can add the image as a plane to an existing image or stack.

### More >>

Expands the dialog box.

### Less <<

Condenses the dialog box.

### Close

Closes the dialog box.

### Acquisition Commands

Lists the acquisition commands available for scripts.

### Script #

Specifies the script that is currently being defined in the script list box.

### Script List Box

Lists the script commands that have been defined and added to the script selected from the *Script* # list. The parameters for the currently selected command will appear at the bottom of the dialog box.

### Add

Adds the currently selected command in the *Acquisition Command* list to the script list box.

### Remove

Removes the currently selected command from the Script List Box.

### Clear

Clears all commands from the currently selected script and returns it to a state of *"<Undefined>."*

### Script Name

Specifies the name of the current script and displays that name in the *Script* # list and the corresponding *Acquisition Scripts* button.

### Parameters

This option group is used to configure the individual *Acquisition Commands.* The options that appear here will be determined by the script command that is currently selected and by your hardware set-up.

## Acquisition Commands for Acquire with Frame Transfer Camera

### Set Exposure

Sets the *Exposure Time* of the camera in milliseconds, seconds, or minutes.

### Set Illumination

Selects the illumination setting as defined in the Configure Illumination dialog box.

### Run Journal

Allows you to run any journal.

### Expose

Exposes the unmasked portion of the camera's chip. The video driver determines the number of frames that are available. For example, the Princeton Instruments Video Driver (PI.vin) has storage for four frames. Use the *Frame* slider to select the number of frames you want to acquire.

The camera will be exposed for the exposure time set by the *Set Exposure* command (if in the script) or, if did not select *Set Exposure,* the exposure last set in the Acquire from Digital Camera command will be used. When exposed, the image will be shifted under the camera's mask. If *Expose* is used again or *Transfer* is used, the image that is currently under the mask will be read out of the chip and into the appropriate frame memory in the camera driver. Therefore, you must perform a *Transfer* command after the last *Expose* command. See Example

### Transfer

Transfers the area of the chip under the camera's mask to the host computer. You must use this command after the last *Expose* command. See Example

### Copy to Image Window

Copies image data from the host computer's video driver to one of the image windows. You can specify which frame is to be copied and the window its image is to be placed in from one of the four image selectors. There may be fewer image selectors than there are frames on the video driver.

A *frame transfer camera* has a factory-installed mask over half of its imaging sensor. When an image is acquired, it is quickly transferred to the side of the sensor behind the mask. A new image can then be acquired. This setup makes it possible to perform high speed acquisition of multiple images.

To capture two images, you would write a script that used *Expose 1,* followed by *Expose 2,* followed by *Transfer.* The first *Expose* will capture an image and shift it underneath the camera's mask. That image will be "tagged" to go into frame 1 of the video driver's memory. The second *Expose* will cause the image already under the mask to go into the desired frame buffer (in this case, frame 1) and will capture a second image and shift it underneath the mask. The second image is now "tagged" to go into frame 2. The *Transfer* command will force the second image, which is still under the mask, to be read into its frame buffer.

### Sample Script 1

One typical operation is to acquire two wavelengths. Available hardware for this might consist of the frame transfer camera, a shutter on the light source, and a filter wheel. An acquisition script would look like this:

| | |
|---|---|
| Set Exposure | (100 milliseconds) |
| Set Wavelength | (wavelength #1) |
| Set Shutter | (open) |
| Expose | (frame 1) |
| Set Shutter | (closed) |
| Set Exposure | (500 milliseconds) |
| Set Wavelength | (wavelength #2) |
| Set Shutter | (open) |
| Expose | (frame 2) |
| Set Shutter | (closed) |
| Transfer | |
| Copy to Image Window | (frame 1, image 1) |
| Copy to Image Window | (frame 2, image 2) |

### Sample Script 2

If you wanted to acquire three wavelengths, you could use the following acquisition script:

| | |
|---|---|
| Set Exposure | (100 milliseconds) |
| Set Wavelength | (wavelength #1) |
| Set Shutter | (open) |
| Expose | (frame 1) |
| Set Shutter | (closed) |
| Set Wavelength | (wavelength #2) |
| Set Shutter | (open) |
| Expose | (frame 2) |
| Set Shutter | (closed) |
| Set Exposure | (500 milliseconds) |
| Set Wavelength | (wavelength #3) |
| Set Shutter | (open) |
| Expose | (frame 3) |
| Set Shutter | (closed) |
| Transfer | |
| Copy to Image Window | (frame 1, image 1) |
| Copy to Image Window | (frame 2, image 2) |
| Copy to Image Window | (frame 3, image 3) |

# Twain Configure (Acquire Menu)

**Selects a TWAIN-compliant device for image acquisition and specifies whether to use the device's user interface.**

**Drop-in: TWAINCFG**

Use this command to select a different TWAIN device and user interface mode (interactive or non-interactive) for image acquisition without the need for exiting MetaMorph to use the external Meta Imaging Series Administrator program. The Configure Twain Driver dialog box that this command displays is the same as that used in the Configure Acquisition program.

**Note:** You must complete the following steps before using this command:

- Close MetaMorph and open the Meta Imaging Series Administrator program.
- Load the TWAIN driver using the Configure Acquisition command.

- Close the Meta Imaging Series Administrator program and open MetaMorph.
- Select the TWAIN driver with the Select Camera/Board command (Acquire menu).

The dialog box that this command displays provides a list of the TWAIN-compliant devices that you have installed on your system. This list will reflect the TWAIN device files that reside on your system, regardless of whether the equipment is still actually connected. Examples of TWAIN-compliant devices include certain CCDs, video cameras, and flat-bed scanners.

A typical use of this command is to select the *Show Device User Interface* check box so that the acquisition options for the TWAIN device will appear before each acquisition. Most TWAIN devices can save the acquisition settings from their respective device user interfaces. You can use this feature to fine-tune your acquisition settings, save the settings, and then clear the *Show Device User Interface* check box in this dialog box and proceed to acquire your images in non-interactive mode, typically with the use of a journal.

### Configuring Use of a TWAIN-Compliant Device

To configure use of a TWAIN device for image acquisition, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Twain Configure. The Configure Twain Driver dialog box will appear. |
| **2** | Select the TWAIN device you want to use from the *Installed Devices* table. |
| **3** | Select the *Show Device User Interface* check box so that a check mark appears in it. Then choose *OK.* The Configure Twain Driver dialog box will close. |
| **4** | From the Acquire menu, choose Acquire Image. The Acquire Image dialog box will appear. The image selector and the *Acquire Image* command button will be the only options that are valid for use with the TWAIN device. |
| **5** | Choose *Acquire Image.* The user interface dialog box for your selected TWAIN-compliant device will appear. |
| **6** | Make any adjustments to the acquisition settings that are necessary, and acquire and transfer some images through the use of the device's user interface. |
| **7** | If you want to acquire images using the device's user interface, you are done. Simply proceed to acquire your experimental images using the user interface.<br><br>**OR**<br>If you want to acquire images automatically in non-interactive mode, choose Twain Configure again from the Devices menu. The Configure Twain Driver dialog box will reappear. |
| **8** | Clear the *Show Device User Interface* check box. Then choose *OK.* |
| **9** | If desired, **create a journal** for image acquisition that uses the *Acquire Image* |

journal function.

**10** When you are ready to acquire your images automatically, run the image acquisition journal you created in Step 9, or choose *Acquire Image* from the Acquire Image dialog box.

## Twain Configure - Dialog Box Options

### Installed Devices

Lists the TWAIN-compliant devices (EXAMPLES: FlashPoint video acquisition board, scanners, etc.) that you have installed on your system. Select the device that you want to use from this list before acquiring images with the TWAIN device.

### Show Device User Interface

Selecting this check box will configure your system to display the TWAIN device's interface when you choose the acquisition command. You should acquire an image in this fashion at least once to adjust your image acquisition settings before you subsequently acquire images in non-interactive mode with this check box cleared.

### OK

Accepts your selection of a TWAIN-compliant device from the *Installed Devices* table and closes the Configure Twain Driver dialog box.

### Cancel

Cancels any changes you have made to the settings in the Configure Twain Driver dialog box and closes the dialog box.

# Acquire from Spot Camera (Acquire Menu)

**Acquires 24-bit true color images or stacks of 12-bit single-channel monochrome images with the Spot camera. Configures the exposure balance between the red, green, and blue acquisition channels.**

### Drop-in: SPOTCAM

Use this command to configure acquisition from the Spot camera and to acquire images. You can acquire images (1) as a single-plane, 24-bit color image consisting of image data from one, two, or all three color channels, (2) as a single-plane, single-color 12-bit image, or (3) as a two- or three-plane stack of single-channel, single-color (red, green, and/or blue) 12-bit images.

> **Note:** To use the Spot camera to acquire images with MetaMorph, you must first load the Spot Camera driver Using the Meta Imaging Series Administrator's Configure Acquisition command and select it with the Select Camera/Board command (Acquire menu). You must also install the SPOTCAM drop-in for this command to be available. The DIGITAL4 drop-in commands (Acquire from Digital Camera, Basic Digital Acquire) and other commands that rely on them (for example, Acquire Multiple Wavelengths) will be unavailable unless you select at least one of the three 12-bit color channels from the Acquire from Spot Camera dialog box.

> **Note:** The Spot camera driver automatically bins at 2x2 to increase the frame rate. This means that the live image window is half width and half height. However, the Region of Interest (ROI) used by the live image is the same as the full chip when you have *Full Chip* selected as the *Camera Area*.

Depending on your camera model, you can acquire single-plane images using the *Acquire 24-Bit Image* command or the *Acquire 12-Bit Image* command. You can use the *Acquire 24-Bit Image* command for three-color or single-color images. Single-plane images will always be acquired as 24-bit images, regardless of the number of color channels you activate. For example, if you select just the red channel

for acquisition, the brightest possible pixel will have a color value of 255,0,0.

As an alternative, you can acquire 12-bit images, using the *Acquire 12-Bit Image* command, either as a single plane or as a stack of planes. This mode of acquisition relies on the *Acquire 12-Bit Image/Acquire 12-Bit Stack* command button. When you select a single color channel for acquisition, the command button will read *"Acquire 12-Bit Image."* If you select two or three color channels for acquisition, the button title will change to *"Acquire 12-Bit Stack."* If you are conducting fluorescence imaging, it may not be necessary to acquire a 24-bit color image--such images are limited to 255 intensity levels per color channel. You can select a single channel through which to acquire a 12-bit image, and in this way use the full dynamic range of the camera (4096 intensity levels).

**QUICK TIP:** If your image moves between acquisitions from the various color channels, you can use the Color Align drop-in command (Display menu) to align the component colors in a 24-bit color image. If you are acquiring images in a stack, you can use the Align Stack command (Stack menu).

Configuration of acquisition time is a two-stage process. The first step is to compute the relative ratio between the exposures of the red, green, and blue channels. This is performed by choosing the *Compute White Balance* command button while your *Exposure Settings* are in *Auto-Exposure* mode. Your image should contain at least some white regions for this to be effective. Once the white balance has been computed, you will need to compute the actual exposure times for the three color channels. Exposure time is determined automatically by choosing the *Compute /Gain* command button while your *Exposure Settings* are in *User-Defined* mode. Exposure time selection is an iterative process that uses the ratios which you just obtained from the white balance computation.

You can also use this command to focus the image while acquiring live video from the Spot camera. The *Focus* command is used to display and update the image quickly from the camera and display it on the video monitor. This allows you to see an image while focusing on your specimen. It is important to use the *Focus* command because what can be seen through the microscope's eyepiece and what the camera acquires are not always the same. Acquisition configurations such as binning settings will be disabled while you are focusing.

## Acquiring Images with a Spot Camera

**Acquiring Images with a Spot Camera - Main Dialog**

**Acquiring Images with a Spot Camera - Acq Setup Tab**

**Acquiring Images with a Spot Camera - Live Setup Tab**

**Acquiring Images with a Spot Camera - Display Tab**

**Acquiring Images with a Spot Camera - Process Tab**

**Computing Exposure Times (Spot Camera**)

## Acquiring Images with a Spot Camera - Main Dialog

To acquire images using the Spot Camera, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire Menu, choose Acquire from SPOT. The Acquire from SPOT dialog box opens. |
| **2** | If you have a settings file with a group of previously saved settings that you want to load, click Load Settings. The Load Spot Camera State dialog box opens. Select the settings file that you want to use, and click Open. |

**3** Set the Acquisition region that you want to use to acquire images by clicking on the appropriate area.

- To use the entire chip, Click *Full Chip*.
- To use the center quadrant of the chip, click *Center Quad*.
- To use a custom area, first acquire an image using Full Chip or Center Quad, then use an appropriate region tool to select a custom area. After you define an active region, click *Use Active Region.*

**4** If the Acquire from SPOT Camera Dialog box is not fully expanded, click *More>>* to expand the dialog box.

**5** Click the **Acq Setup** tab to make acquisition settings.

**6** Click the **Live Setup** tab to make live acquisition settings.

**7** Click the **Display** tab to make display settings.

**8** Click the **Process** tab to make process settings.

**9** Click *Show Live* to continuously acquire images that will enable you to set or check the microscope focus.

**10** Click *Acquire* to acquire the type of image for which the Acquire from SPOT dialog box is configured.

**11** To save your Spot Camera settings, click *Save Settings*. The Save Spot Camera State dialog box opens. Assign a name to the state file, and click *Save*.

## Acquiring Images with a Spot Camera - Acq Setup Tab

To configure the Acq Setup tab, complete the following procedure:

| Step | Action |
|---|---|
| **1** | Click the Acq Setup tab. The Acq Setup options move to the front. |
| **2** | In the *Image Bit Depth* box, select the image bit depth that you need. Color 24BPP yields 24-bit color images that consist of three separate exposures that are combined by the SPOT Camera program. Monochrome 12BPP are 12-bit images that can be acquired separately and used as individual monochrome images or can be acquired into a stack through color filters and combined into a single, high quality color image. |
| **3** | In the *Exp Mode* box, select the exposure mode that you want to use. |
| **4** | In the *Gain* box, type or select the camera |

gain that you want to apply to your image.

**5** In the *Binning* box, type or select the level of binning that you want to apply.

**6** If you selected Auto for the exposure mode, in the *Exp Adjust* box, type or select the amount of exposure adjustment that you want to apply.

**7** If you selected *Auto* for the exposure mode, in the *Image Type* box select the image type for the image you are acquiring. For brightfield images, select *Brightfield*, for darkfield, select *Darkfield*, For fluorescence images, select *Fluorescence*.

**8** If you selected *Auto* for the exposure mode and you are acquiring a color image, in the *White Balance* area you need to set the color order and compute the white balance. Click the arrow in the Color Order box, and choose the appropriate color order. One reason for selecting a different color order might be that in your experiment, a particular color fades faster than the others.

**9** To compute the white balance for a color image, ensure that the Red, Green, and Blue check boxes are checked, then click *Compute White Balance*. The Compute White balance Values information box opens and reports the results. If the white balance values are acceptable, click Yes, otherwise click No.

**10** If you selected Manual for the Exp. Mode and you are acquiring a color image, a Compute Exp/Gain button will be in place of the *Compute White Balance* button, and the area will be called *Exposure*.

**11** To compute the exposure, ensure that the Red, Green, and Blue checkboxes are checked, set the color order that you want to use, then click Compute Exp/Gain. A Compute Exposure Time and Gain information box is displayed. If the exposure times are acceptable, click Yes, otherwise click No.

**12** In the Illumination box, click the down arrow, and select the illumination setting that you want to use.

## Acquiring Images with a Spot Camera - Live Setup Tab

**Note:** Settings you make on the Live Setup tab affect only the appearance of the Live (continuously acquired) image. This image is for aligning the position of your specimen, selecting an appropriate objective, or setting the microscope focus. Settings made on this tab do not affect your acquired image result.

To configure the Live Setup tab, complete the following procedure:

| Step | Action |
|---|---|

1. Click the Live Setup tab. The Live Setup options move to the front.
2. Click *Compute exposure and gain on live startup* if you want these values computed each time you click *Show Live*.
3. If *Compute exposure and gain on live startup* is checked, move the Quality/Speed slider to an appropriate setting.
4. Click *Show Live*. If Compute exposure and gain on live startup is not checked, type or select an appropriate exposure value in the Exp. Time box.
5. Click *Show Live*. In the *Gamma* box, type or select an appropriate gamma setting to improve the appearance of the image.
6. Click *Show Live*. In the *Brightness* box, type or select an appropriate brightness setting to improve the appearance of the image.
7. In the *Binning* box set an appropriate binning value if you need to reduce the live mode exposure time.
8. In *Camera area for live mode*, select the appropriate acquisition region.
   - To use the entire chip, Click *Full Chip*.
   - To use the center quadrant of the chip, click *Center Quad*.
   - To use a custom area, first acquire an image using Full Chip or Center Quad, then use an appropriate region tool to select a custom area and click *Use Active Region*. Then, select *Same as Acquire Area* on the Live Setup tab.
9. In the LC Filter Position box, click the Liquid Crystal filter that you want to use for live mode. Choose *Red*, *Green*, or *Blue*. If your camera is the SPOT RT, you can also choose *Clear* or *RGB*.

   **Note:** RGB continuously cycles through the Red, Green, and Blue filters.

## Acquiring Images with a Spot Camera - Display Tab

**Note:** The settings on this tab apply to 12-bit Monochrome images only.

To configure the Display tab, complete the following procedure:

| Step | Action |
|---|---|
| 1 | Click the Display tab. The Display options move to the front. |
| 2 | In the *Monochrome (12 BPP) Image scaling* box, click Autoscale to select or deselect autoscaling, as appropriate.<br><br>**Note:** If autoscaling is not selected, the |

values you enter into the low and high settings boxes are high and low limits in bit density. If autoscaling is selected, the low and high settings are a percentage of the overall scale range.

**3** In the *Lo* box, enter a lower scaling limit for the scaling range.

**4** In the *Hi* box, enter an upper scaling limit for the scaling range.

## Acquiring Images with a Spot Camera - Process Tab

To configure the Process tab, complete the following procedure:

| Step | Action |
|---|---|

**1** Click the Process tab. The Process options move to the front.

**2** Click *Chip Defect Correction* to automatically apply the Chip Defect Correction Table to all images.

**3** Click *Fluorescence Color Handling* to apply fluorescence color handling to darkfield images that are fluorescence images.

**4** Click *Flatfield Correction*, then click *Get Flatfield* to apply Flatfield correction to the acquired images.

Flatfield correction has two purposes, depending on the type of camera used. Use Flatfield correction with a RT or SPOT camera to adjust images with uneven intensity or coloration in illumination, or to correct for artifacts in the optical system.

OR

Use Flatfield correction with an Insight camera to correct uneven color density ("halo" effect) in low contrast images.

> **Note:** Flatfield correction using the SPOT camera refers only to the correction of uneven lighting. It should not be confused with the correction of optical field flatness

**5** In the Gamma Adjustment area, click *Gamma Adjust* and type or select a gamma adjustment value in the Gamma Adjust box.

**6** If you checked *Fluorescence Color Handling*, click *RGB* to apply the gamma correction to the Red, Green, and Blue channels, or click *Luminance* to apply the gamma correction to the Luminance channel.

## Computing Exposure Times (Spot Camera)

To compute the exposure times for image acquisition with the Spot camera, use the following procedure:

| Step | Action |
|---|---|
| **1** | Choose the *Compute Exp/Gain* command button. The Computing Exposure Time and Gain dialog box opens. |
| **2** | The *White Balance* option group will display the current selection of active color channels, as well as the current white balance ratio values. If desired, you can alter these settings before computing the exposure times. |
| **3** | Verify that the *Image Type* drop-down list indicates the appropriate selection of image illumination: *Brightfield* or *Darkfield.* |
| **4** | If desired, you can adjust the overall brightness of the acquired images with the *Exp Adjust* spin box.<br><br>The default value is *1.00.* Increasing the value will yield a brighter image, while decreasing the value will result in a darker image. |
| **5** | When you are ready, choose *Compute.*<br><br>The Spot Camera Status message box will appear, displaying messages that indicate the progress of the procedure. MetaMorph will perform an iterative process to determine the optimum exposure times for each color channel, using the current set of white balance ratios and exposure adjustment values, and taking the image type into account. |
| **7** | When the process is complete, the Spot Camera Status message box will close, and a second message box will appear, asking whether you want to save the computed values. Choose *Yes* to store the exposure times.<br><br>The exposure times will appear in the *Exposure* option group spin boxes of the Acquire from Spot Camera dialog box. |

## Acquire with Spot Camera - Dialog Box Options

**Acquire from Spot Camera - Main Dialog Box Options**

**Acquire from SPOT Camera Dialog Box Options - Acq Setup tab**

**Acquire from SPOT Camera Dialog Box Options - Live Setup tab**

**Acquire from SPOT Camera Dialog Box Options - Display tab**

**Acquire from SPOT Camera Dialog Box Options - Process tab**

**Computing Exposure Times (Spot Camera) - Dialog Box Options**

## Acquire from Spot Camera - Main Dialog Box Options

### Acquire 12-Bit Image / Acquire 24-Bit Image / Acquire 12-Bit Stack

Acquires a single-plane, single-channel 12-bit image, a single-plane 24-bit image, or a two-, three-, or four-plane 12-bit image stack (depending on which color channels are selected), where each plane corresponds to a color channel. Images are acquired using the current settings. By default, the image is acquired into an image window with the name "acquired." Click the Image Name button to rename the file.

### Image Name (Destination)

Specifies the image name and destination to apply to the image. You can add to or overwrite an existing image or stack, or you can specify a new image.

### Status

Indicates the Acquisition status.

### Show Live/F2: Stop Live

Acquires a continuously updating image into an image window. Use this command to interactively set the microscope's focus or color balance. Click F2: Stop Focus to discontinue live image capture.

### Camera Area

Specifies the active region.

*Full Chip* – Specifies that the entire chip be used to acquire the image.

*Center chip* – Specifies that only the center portion of the chip be used to acquire the image. MetaMorph creates and centers a region that is the size of one quadrant of the chip.

*Use Active Region* – Specifies that the active region is used to acquire the image.

### Load Settings

Opens the *Load SPOT Camera State* dialog box to enable you to load a saved settings file.

### Save Settings

Opens the *Save SPOT Camera State* dialog box to enable you to save your current settings to a file.

### Close

Closes the Acquire from SPOT RT or SPOT Camera dialog box.

### More>>/Less<<

Expands the Acquire from SPOT RT Camera dialog box to full size; if expanded, *Less*<< sets the dialog box to its minimized size.

## Acquire from SPOT Camera Dialog Box Options - Acq Setup tab

### Image Bit Depth

Selects either Color (24BPP) or Monochrome (12BPP)

### Exp Mode

Selects an acquisition mode: *Manual* or *Auto*:

*Manual* mode, acquisition will proceed based on the specified exposure times for each color channel. The *Exp Adjust* option is unavailable in this mode.

*Auto* mode, acquisition will be an iterative process that determines the optimum exposure for each image. This mode is used for computing white balance and for acquisition of images in which the speed of acquisition is not a concern.

### Gain

Specifies a conversion factor for transforming acquired 12-bit source images to an 8-bit range. Increasing the *Gain* value will yield a brighter image. You may need to adjust the *Gain* value if the brightness of the specimen changes or if the exposure times that are computed are too long to be practical. This option is available only in *Manual* exposure mode.

### Binning

Selects a number of pixels in the horizontal and vertical direction to be binned during acquisition. This will increase the speed of acquisition and conserve memory.

For example, if you select *2,* a 2 x 2 binning region will be used. This will nearly quadruple acquisition speed while consuming just 25% of the memory space that an unbinned image would use.

> **Note:** As binning is increased, image resolution decreases.

### Exp Adjust

Adjusts the calculated exposure times. For example, if you select *1.25* and MetaMorph has calculated an exposure time of 100 ms, the returned value will be 125 ms. This option will be available in this dialog box only in *Auto* exposure mode.

### Image Type

Selects the image's type of illumination: *Brightfield, Darkfield,* or *Fluorescence.* This option is available only in *Auto* exposure mode.

### Exposure (Monochrome mode only)

#### Use Filter

Enables use of color filters for capturing separate monochrome (gray scale) images that can be recombined later into a color image.

*Red* – Controls the image exposure time through red filtration. If Manual exposure mode is selected, sets the exposure time in the red filter box.

*Green* – Controls the image exposure time through green filtration. If Manual exposure mode is selected, sets the exposure time in the green filter box.

*Blue* – Controls the image exposure time through blue filtration. If Manual exposure mode is selected, sets the exposure time in the blue filter box.

*Clear* – Controls the image exposure time with no filter in place. If Manual exposure mode is selected, sets the exposure time in the clear box (RT only).

#### B/W

Specifies image capture in monochrome (gray scale) only. No color filters are used for this option.

### White Balance (Color mode only)

Provides the controls for setting the white balance for Color (24-bit) images.

> **Note:** To change these values while in Live mode, ensure that *Exp. Mode* is set to *Auto* and *Compute exposure and gain on live startup* is enabled in the *Live Setup* tab.

#### Red

Enables the red color channel. In *Manual* mode, the spin box specifies an exposure time or adjusts the computed exposure time. In *Auto* mode, the spin box specifies a white balance value or adjusts the computed one.

#### Green

Enables the green color channel. In *Manual* mode, the spin box specifies an exposure time or adjusts the computed exposure time. In *Auto-Exposure* mode, the spin box specifies a white balance value or adjusts the computed one.

#### Blue

Enables the blue color channel. In *Manual* mode, the spin box specifies an exposure time or adjusts the computed exposure time. In *Auto* mode, the spin box specifies a white balance value or adjusts the computed one.

#### Color Order

Selects the order in which the camera's color channels will be sampled during image acquisition: *RGB, RBG,* etc.

### Compute White Balance

Calculates the relative exposure ratios between the color channels that are necessary to achieve the same intensity at each channel. This command button appears when the system is in *Auto-Exposure* mode, and becomes the *Compute Exposure Times* button when you switch to *User-Defined* mode. Be sure to move the specimen to a blank area of the slide before you compute the white balance.

### Compute Exp/gain

Opens the Computing Exposure Time and Gain dialog box under the following conditions:

- The *Exp. Mode* is set to *Manual*
- The camera is acquiring 24-bit color images or 12-bit Red, Green, Blue, and/or Clear stacks.

Use the Computing Exposure Times and Gain dialog box to turn on/off colors and adjust the *White Balance*, Image Type, and *Exp Adjust* values to calculate the exposure times and gain values for the active color channels.

If camera is in monochrome mode, The *Compute Exp/Gain* command will directly compute exposure time and gain values for image acquisition without opening the Compute Exposure Time and Gain dialog box.

### Use 'Acquire' as default when channel is 12bit

Enables the Acquire drop-in to be used as well as the Acquire from SPOT drop-in when acquiring images with a SPOT camera. When this is selected the settings from the Acquire dialog are used by default when acquiring images using other commands in MetaMorph.

### Illumination

Selects the desired illumination setting from among those that are available. To use the active setting, select [*Current Shutter*]. If you are not using an illumination device, select *"[None]."*

## Acquire from SPOT Camera Dialog Box Options - Live Setup tab

### Compute exposure and gain on live startup

Automatically calculates the correct exposure and gain values whenever you click *Show Live*. Uncheck this box to manually set exposure and gain settings for Live mode.

### Recalc Exposure Time

Recalculates exposure time and gain in Live mode.

### Quality/Speed

Enables you to achieve an ideal balance between *Quality* and *Speed* when continuously updating "live" images. The better the quality, the slower the speed; conversely, the faster the speed, the lesser the quality. This feature is disabled if *Compute exposure and gain on live startup* is selected.

### Gamma

Sets the image gamma value for live-mode image viewing.

### Binning

Sets the camera pixel binning value for live-mode image viewing.

**Note:** The binning value can be changed while in Live mode.

### Live Gain

Sets the gain for the live-mode viewing. This feature is disabled if *Compute exposure and gain on live startup* is selected.

### Exp Time

Sets the exposure time (in milliseconds) for live images. This feature is disabled if *Compute exposure and gain on live startup* is selected.

### Camera area for live mode

Provides controls to designate the live-mode image viewing area.

#### Full Chip

Assigns the entire chip as the live-mode image viewing area.

#### Center Quad

Assigns the chip's center quadrant as the live-mode image viewing area.

#### Same as Acquire Area

Assigns the selected active region as the live-mode image viewing area.

### LC Filter Position

Selects the filter to have in place for live-mode image viewing.

**Note:** If the Image Bit Depth is set to Color (24BPP) in the Acq Setup tab, you can change the LC Filter Position while in Live mode and it will update.

#### Red

Selects the red filter for live-mode image viewing.

#### Green

Selects the green filter for live-mode image viewing.

#### Blue

Selects the blue filter for live-mode image viewing.

#### Clear

Selects no filters to be in place for live-mode image viewing.

#### RGB

Selects all filters to be in place for live-mode image viewing.

## Acquire from SPOT Camera Dialog Box Options - Display tab

### Monochrome (12BPP) Image Scaling

Provides controls to set image scaling for monochrome images.

### Low

Selects the gray value to be used as the lower limit of the range being autoscaled.

### High

Selects the gray value to be used as the upper limit of the range being autoscaled.

### Autoscale

Enables the autoscale function. When active, autoscaling calculates the correct exposure limited by high and low limits specified as a percentage of the total range. When autoscale is off, *Low* and *High* are fixed scaling values on a linear scale.

## Acquire from SPOT Camera Dialog Box Options - Process tab

### Black Level Subtraction (12 BBP)

Turns off the automatic Black Level Subtraction function. This function can be deactivated only when capturing 12 BBP (12-bit mono) images. For other capture formats, this option is always on. When active, this function ensures that any part of the image that is attributed to the image background (such as noise or minor pixel level differences) will be black (level 0). However, image accuracy might be sacrificed when Black Level Subtraction is on. To ensure greater image accuracy, turn off Black Level Subtraction.

### Chip Defect Correction

When this check box is selected, MetaMorph will correct for chip defects automatically, based on the settings in the ChipInfo.dat file. However, if you have not copied the ChipInfo.dat file correctly to the Spot camera's VInput folder and this check box has been selected, your image acquisition may be considerably slowed.

### Flatfield Correction

Applies Flatfield correction to the acquired image. Click *Get Flatfield*, then acquire an image to apply the Flatfield correction.

Flatfield correction has two purposes, depending on the type of camera used. Use Flatfield correction with a RT or SPOT camera to adjust images with uneven intensity or coloration in illumination, or to correct for artifacts in the optical system.

OR

Use Flatfield correction with an Insight camera to correct uneven color density ("halo" effect) in low contrast images.

### Get Flatfield

Click this button when the Flatfield Correction box is checked, then acquire an image to apply the Flatfield correction.

### Color Enhancement

Applies automatic color enhancement of acquired image.

> **Note:** Color enhancement will not be applied to live images.

### Fluorescence Color Handling

Applies compensations to the settings to optimize image capture for fluorescence color images. The *Color Enhancement* checkbox must be checked for this option to be enabled.

### Gamma Adjustment

Provides controls for enabling and setting a Gamma correction value for monochrome images or RGB or Luminance to color images. Typical gamma values range between 1.3 and 1.7.

### Gamma Adjust

Enables and sets the value for the gamma correction curve to be applied to images.

#### RGB

Check this button to apply the gamma correction curve separately to the red, green, and blue pixel values. Use this option for monochrome images

#### Luminance

Check this button to apply the gamma correction curve to HSL (hue, saturation, and luminance) values. Use this option for all color images except fluorescence images.

## Computing Exposure Times (Spot Camera) - Dialog Box Options

### Red

The check box enables use of the red channel during calculation of exposure time. The spin box indicates the calculated white balance value, which you can adjust as desired.

### Green

The check box enables use of the green channel during calculation of exposure time. The spin box indicates the calculated white balance value, which you can adjust as desired.

### Blue

The check box enables use of the blue channel during calculation of exposure time. The spin box indicates the calculated white balance value, which you can adjust as desired.

### Image Type

Selects the image's type of illumination: *Brightfield* or *Darkfield.*

### Exp Adjust

Adjusts the calculated exposure times. For example, if you select *1.25* and MetaMorph calculates an exposure time of 100 ms, the returned value will be 125 ms.

### Compute

Begins calculating the exposure time for each active channel, based on the current set of white balance ratios and exposure adjustment values, and taking the image type into account. The Spot Camera Status dialog box will appear, and will close automatically when acquisition is complete.

### Abort

Terminates the exposure time calculation process.

# Acquire from Flashbus (Acquire Menu)

**Acquires and displays images from a video camera connected to the Flashbus MV video acquisition board. Configures the acquisition region, frame integration and averaging, and image display.**

### Drop-in: FLASHBUS

Use this command to configure acquisition with the Flashbus MV video acquisition board, and to display "live video" or acquire single-frame images (NTSC = 33 ms/frame exposure duration, PAL = 40 ms/frame) with a video camera. This easy-to-use interface enables integration and averaging of both the "live" display and of acquired frames.

The Flashbus frame grabber board supports the use of RS-170, CCIR, RGB, Composite, and S-Video input signals. Acquisition will be performed using the channel that you select with the Set Video Channel command (Acquire menu). If you select the NTSC (US) format when you configure the Flashbus driver in the Meta Imaging Series Administrator, you can select an RS-170, RGB, Composite, or S-Video input channel from the Set Live Video Channel dialog box. If you select the PAL (EC) format when you configure the driver, you can select CCIR, RGB, Composite, or S-Video.

The Flashbus frame grabber and video driver also support the use of a VCR as the video input device. If you are using a VCR for transmission of images to the Flashbus board, it is recommended that you configure the Flashbus driver for optimum synchronization, using the Configure Acquisition command (see below).

**Note:** You must complete the following steps before using this command:

- Close MetaMorph and open the Meta Imaging Series Administrator program.
- Load the video camera driver using the Configure Acquisition command.
- Close the Meta Imaging Series Administrator program and open MetaMorph.
- Select the camera with the Select Camera/Board command (Acquire menu).

The Acquire from Flashbus command uses the concept of State files to restore previous settings. This enables you to configure acquisition settings and save them to a State file that can be loaded the next time you use the command. There is no limit to the number of State files you can save. This help file contains procedures for both manually configuring the dialog box or loading a saved State file:

Performing Acquisition with the Flashbus Video Board using Manual Settings

Performing Acquisition with the Flashbus Video Board Using a State File

The Acquire from Flashbus dialog box settings enable you to reduce or eliminate background image information that is constant and not part of your experiment image information. This feature incorporates three settings designed to work together. The Acquire Background button acquires and stores an image of the background data. When you check the Subtract Background function, this information is subtracted from the acquired image information to remove any constant background information associated with the microscope, its objectives, or the illumination sources. If the image intensity is excessively reduced from background subtraction, type or select a value in the Offset box to restore image brightness and add a base level of intensity to the image.

## Performing Acquisition with the Flashbus Video Board Using a State File

To configure acquisition and acquire images with a camera connected to the Flashbus video acquisition board using a previously saved State file, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Acquire from Flashbus. The Acquire from Flashbus dialog box opens. |
| **2** | Click *Load State* to open the Load Acquisition State dialog box. |
| **3** | Click the selections for the settings you want to load from the state file. The settings that are not checked will not be loaded. Click *Select All* if you want to load all saved settings. |
| **4** | Click *Load*. The Open dialog box opens. |
| **5** | Navigate to the state file (.fbs) you want to open and click *Open*. The state file you selected will load and the Load Acquisition State dialog box will close. |
| **6** | Click the *Dest* image selector to select a destination image. To acquire images into a stack, configure the selector to acquire images into an image stack. |
| **7** | If you need to make adjustments to the image display, click *Start Live* to display a "live" image. (Adjust the microscope focus, if necessary). Then click *Adjustments.* The Video Adjustments dialog box opens.<br><br>Depending on the type of signal your video input device generates, different sets of adjustment options will be available in the dialog box.<br><br>**OR**<br>If you do not need to adjust the image, skip to Step 11. |
| **8** | For all signal types, adjust the *Contrast* and *Brightness* sliders for an optimal image.<br><br>If you are using the 8-bit RS-170 signal, you can select *Use Saturation LUT* to display the image with undersaturated pixels (grayscale value 0 or "below") displayed in blue and saturated pixels (grayscale value 255 or |

"above") displayed in red.

**9** For Composite or S-Video signals, use the *Hue, Saturation,* and *Gain* sliders to adjust the image display. If you are using a Composite signal, you can also use the *Sharpness* slider.

**10** When you have finished adjusting the display, choose *Close* to return to the Acquire from Flashbus dialog box.

**Note:** To return all video adjustment settings to their defaults, choose *Reset* before closing the Video Adjustments dialog box.

**11** To acquire the Active Region, define the active region for the image area that you want on an image window using the Rectangular Region tool, then Click *Set using Active Region*.

**OR**

To acquire the entire image area after you have already specified the active region as the acquisition, click *Reset to Full Frame*.

**12** To display a "live" video image at any time, such as when you want to adjust the focus or change the visual field, choose *Start Live.*

**OR**
To acquire an image, choose *Acquire.* An image will be captured and saved using the name you specified with the *Dest* image selector.

**13** When you have finished your acquisition session, choose *Close* to close the Acquire from Flashbus dialog box.

## Performing Acquisition with the Flashbus Video Board using Manual Settings

To manually configure acquisition and acquire images with a camera connected to the Flashbus video acquisition board, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Acquire from Flashbus. The Acquire from Flashbus dialog box opens. |
| **2** | Click the *Dest* image selector to select a destination image. To acquire images into a stack, configure the selector to acquire images into an image stack. |
| **3** | To perform frame averaging, in the *Average* box, type or select the number of frames to average for each image.<br><br>**Note:** If you are performing both integration and averaging, MetaMorph will first perform the frame integration and then perform |

averaging on the integrated images.

**4** If you used the Configure Acquisition command to configure the Flashbus driver for integration and want to integrate your acquired images, click *Enable Integration.* Then, in the *Integrate* box, type or select the number of frames to be integrated for each image.

**Note:** Integration is supported only by RS-170, CCIR, and RGB inputs, but not by Composite or S-Video.

**5** Click *Acquire Background* to store a background image to be subtracted from your acquired images.

**Note:** Ensure that no specimen is in place and that all illumination and objective settings are already made before acquiring a background image.

**6** If you need to make adjustments to the image display, click *Start Live* to display a "live" image. (Adjust the microscope focus, if necessary). Then click *Adjustments.* The Video Adjustments dialog box opens.

Depending on the type of signal your video input device generates, different sets of adjustment options will be available in the dialog box.

**OR**
If you do not need to adjust the image, skip to Step 10.

**7** For all signal types, adjust the *Contrast* and *Brightness* sliders for an optimal image.

If you are using the 8-bit RS-170 signal, you can select *Use Saturation LUT* to display the image with undersaturated pixels (grayscale value 0 or "below") displayed in blue and saturated pixels (grayscale value 255 or "above") displayed in red.

**8** For Composite or S-Video signals, use the *Hue, Saturation,* and *Gain* sliders to adjust the image display. If you are using a Composite signal, you can also use the *Sharpness* slider.

**9** When you have finished adjusting the display, choose *Close* to return to the Acquire from Flashbus dialog box.

**Note:** To return all video adjustment settings to their defaults, choose *Reset* before closing the Video Adjustments dialog box.

**10** Click *Subtract Background* to enable subtraction of the stored background image.

**11** In the *Offset* box, type or select a base intensity value to be added to the image intensity value. Use this setting to

compensate for image intensity reduced by the Subtract Background function.

**12** Select *Continue Live after Acquisition* to leave the Live window open after acquiring an image.

**13** Select a live average method from the *Live Average* drop-down list, or select *No Average*.

**14** If you are using an illumination device, select its setting from associated from the *Illum* drop-down list.

**OR**
If you are not using an illumination device, select *"[None]."*

**15** To acquire the Active Region, click *Start Live* to open a live image window. Define the active region for the image area that you want using the Rectangular Region tool, then Click *Set using Active Region*.

**Note:** You can also press the F2 key to start and stop live acquisition.

**OR**

To acquire the entire image area after you have already specified the active region as the acquisition, click *Reset to Full Frame*.

**16** To display a "live" video image at any time, such as when you want to adjust the focus or change the visual field, choose *Start Live.*

**OR**
To acquire an image, choose *Acquire.* An image will be captured and saved using the name you specified with the *Dest* image selector.

**17** To save the current settings, click *Save State* to open the Save Acquisition State dialog box and save the State file.

**18** When you have finished your acquisition session, choose *Close* to close the Acquire from Flashbus dialog box.

## Acquire from Flashbus - Dialog Box Options

**Acquire from Flashbus**

**Video Adjustments**

**Load Acquisition State**

## Acquire from Flashbus - Dialog Box Options

### Dest

Specifies or selects the name of the acquired image and the file location for the new image file. You can

create a new image file or stack, overwrite the existing image file or stack, or add images to an existing image stack.

### Average

Selects the number of frames (integrated or non-integrated) to be averaged together for each image. This can be used to reduce image pixel "noise."

### Acquire

Acquires a single image, using any integration and averaging settings and the acquisition region you have selected, and saves it using the name you specified with the *Dest* image selector.

### Enable Integration

Enables integration of images using the number of frames specified in the *Integrate* box. When you select the *Enable Integration* check box, the *Integrate* spin box will become available. You must configure the Flashbus driver for integration using the Configure Acquisition command in the Meta Imaging Series Administrator for the *Enable Integration* check box to be available.

**Note:** Integration is supported only by RS-170, CCIR, and RGB inputs, but not by Composite or S-Video.

**Note:** If you are configuring the Acquire from Flashbus command from within the Journal Editor, there is a function in the *Builtin Functions* Tab called Acquire from FlashBus - Adjust FTI that accepts fractional numbers and, when run in a journal, sets a value such that each subsequent acquisition from the Flashbus has the integration value incremented by that amount. For example, if you enter 0.50 in the Adjustment factor field of the Adjust FTI function dialog box and run the journal, each subsequent acquisition from the FlashBus has the integration value incremented by 0.50 (whether acquiring from within a journal or in normal mode). Because frame integration requires an integer value, the value used in the acquisition is truncated to an integer. So, if you start with integration = 2 and acquire 5 images, the following parameters are used:

| Image | Integration Value | Actual Number of Frames Integrated |
|---|---|---|
| 1 | 2.0 | 2 |
| 2 | 2.5 | 2 |
| 3 | 3.0 | 3 |
| 4 | 3.5 | 3 |
| 5 | 4.0 | 4 |

### Start Live

Acquires continuous "live" images of the preparation at video rate (NTSC = 30 frames/s, PAL = 25 frames/s) and displays them in the Live Image window.

**Note:** You can also press the F2 key to start and stop live acquisition.

### Integrate

Selects the number of frames to be "added" together for each image. This may be particularly useful if you are acquiring images under low light conditions.

### Adjustments

Opens the Video Adjustments dialog box, from which you can adjust the contrast, brightness, hue,

saturation, sharpness, and gain of your image display. (Depending on the type of signal your video input device generates, different sets of adjustment options will be available in the dialog box.)

### Subtract Background

Enables background subtraction. Use this function to subtract the image background data captured with the Acquire Background function from the entire acquired image area. The result is an image absent of all constant background image information. This option is only enabled if a background has been acquired using the *Acquire Bkgd* command.

### Offset

If the image resulting from background subtraction is too dark ("less" than gray level 0), this option specifies a base image intensity value to be added back to the image's values.

### Acquire Bkgd

Acquires and stores a background image. With no specimen present, click Acquire Background to acquire a background image to be stored in memory. When Subtract Background is selected, this image data is subtracted from the captured specimen image data to remove all information that is not part of the specimen image.

### Continue Live after Acquisition

Leaves the Live image window open after acquiring an image.

### Live Average

Determines the method of averaging frames while in Live mode. Valid choices include:

- **No Average** — no averaging is performed.
- **Jumping Average** — Applies average to groups of frames in sequence, such as 0-2, 3-5, and 6-8.
- **Running Average** — Applies average to groups of frames that overlap, such as 0-2, 1-3, and 2-4.

### Acquire Region

Contains settings to define the limits of the region to be acquired.

#### Reset to Full Frame

Sets the acquisition region to include the entire image frame.

#### Set Using Active Region

Configures image acquisition within the image area defined by an active region of interest. Use the Rectangular Region Tool, in the Region Tools window, to define the acquisition region in the image window.

### Illum

Selects an Illumination Setting to use when acquiring. If you are not using an illumination device, select *"[None]."*

### Load State

Opens the Load Acquisition State dialog box. This option enables you to reuse Flashbus dialog box options that you previously saved to a state file.

### Save State

Saves the current Flashbus dialog box settings to a file. Load these settings from the file to reuse them using the *Load State* option.

### Close

Closes the Acquire from Flashbus dialog box (and the Video Adjustments dialog box, too, if it is open).

## Acquire from Flashbus: Load Acquisition State - Dialog Box Options

These checkboxes enable or disable the loading of specific settings from a state file. The Settings listed in the Load Acquisition State dialog box are configured on the Acquire from Flashbus dialog box. The following

table lists each setting and where they are set in the Acquire from Flashbus dialog box:

| Setting | Fields/Comments |
|---|---|
| **Enable Integration** | *Enable Integration*<br>You must configure the Flashbus driver for integration using the Configure Acquisition command in the Meta Imaging Series Administrator for the *Enable Integration* check box to be available. |
| **Frames to Integrate** | *Integrate*<br>*Enable Integration* must be checked for this option to be valid. |
| **Enable Bkgd Subtraction** | *Subtract Background* |
| **Bkgd Offset** | *Offset*<br>*Enable Bkgd Subtraction* must be checked for this option to be valid. |
| **Illumination Setting** | *Illum* |
| **Acquisition Region** | This loads the region used when the state file was created. |
| **Continue Live after Acquisition** | *Continue Live after Acquisition* |
| **Live Average Mode** | *Live Average* |

### Load

Opens the Load Flashbus state dialog box used to select a saved Flashbus state (.fbs) file.

### Select All

Selects all checkboxes.

### Clear All

Deselects all checkboxes.

### Cancel

Cancels the command and closes the dialog box.

## Video Adjustments (Flashbus) - Dialog Box Options

### Contrast

Adjusts the contrast (degree of change of the grayscale or color intensity levels) of the image.

### Brightness

Adjusts the overall brightness or darkness of the image.

### Hue

Adjusts the color in the image. This option is available only for Composite and S-Video inputs.

### Saturation

Adjusts the level of color saturation (degree of "dilution" or vividness of the color) in the image. This option is available only for Composite and S-Video inputs.

### Sharpness

Adjusts the sharpness of the display of grayscale transitions, or object edges, in the image. This option is

available only for Composite inputs.

### Gain

Adjusts the level of signal amplification. To some extent, this is equivalent to increasing the contrast and brightness of the image. This option is available only for Composite and S-Video inputs.

### Use Saturation LUT

Displays the image with a look-up table in which undersaturated pixels (grayscale value 0 or "below") are displayed in blue and saturated pixels (grayscale value 255 or "above") are displayed in red. This option is applicable only for RS-170 inputs.

### Reset

Reverts all settings to their default values. The actual values will depend on the specific input type (RS-170, CCIR, RGB, Composite, or S-Video).

### Close

Closes the Video Adjustments dialog box.

# Sum 16-Bit Image (Acquire Menu)

**Sums the specified number of images together to create a 16-bit image.**

**Drop-in: SUM16**

Use this command to enhance the contrast and reduce the noise of faint or dim images.

**Note:** This command can be used only if you are using a Matrox Image Series frame-grabber board and a compatible RS-170 or CCIR camera.

The images summed together should have a narrow range of gray levels for this command to work well. For very dim images, you may need to sum together a large number of frames. For this reason, Sum 16-Bit Image may not be ideal for fast-moving specimens. To determine how much time is needed to acquire an image using an RS-170 camera, divide the number of frames by 30 (divide by 25 for a CCIR camera).

When the image is acquired, you can adjust its contrast using the Scale 16-Bit Image command.

## Summing a 16-Bit Image

To sum acquired 8-bit frames into 16-bit images, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Sum 16-Bit Image. The Sum 16-Bit Image dialog box will appear. |
| **2** | Select the desired destination image using the *Destination* image selector. |
| **3** | Select the desired number of frames to sum together using *Number of Frames.* |
| **4** | Choose *Acquire.* |
| **5** | Choose *Close* when you have finished. |

## Sum 16-Bit Image - Dialog Box Options

### Destination

Specifies the destination image for the 16-bit image. You can add to or overwrite an existing 16-bit image or stack. You can also specify a new 16-bit image.

### Number of Frames

Specifies the number of frames to sum together to create the 16-bit image.

### Acquire

Acquires the specified number of frames and sums them together to create the 16-bit image.

### Close

Closes the dialog box.

# Select Camera/Board (Acquire Menu)

**Selects a current video device from the list of devices that have been installed using the Meta Imaging Series Administrator.**

### Drop-in: VIDEVICE

Use this command to change the current video device from the default selection without using the Configure Acquisition command in the Meta Imaging Series Administrator. This command's dialog box will display only those video devices that are currently installed. If you want to use a video device that is not currently installed, you must quit MetaMorph and use the Meta Imaging Series Administrator program to install it. This command temporarily changes the current video device for the current work session.

## Selecting a Camera/Board

To select a video device as the current device, use the following procedure:

| Step | Action |
|---|---|
| **1** | Before starting MetaMorph, install the desired video device drivers using the Configure Acquisition command in the Meta Imaging Series Administrator. |
| **2** | From the Acquire menu, choose Select Camera/Board. The Select Camera/Board dialog box opens. |
| **3** | Select the desired device from the *Video Device* list. |
| **4** | Choose *OK.* |

## Select Camera/Board - Dialog Box Options

### Video Device

Selects an active video device from the list of currently installed video device drivers. Alternatively, the dummy driver, "Demonstration Video," can be selected.

### OK

Changes the current video device based on the *Video Device* selection.

### Cancel

Cancels the command.

# Acquire Color Camera (Acquire Menu)

**Configures acquisition parameters for single-chip color cameras and acquires color or grayscale images. Configures the exposure balance between the red, green, and blue acquisition channels, and interactively sets the focus.**

### Drop-in: ACQUIRECOLOR

Use this command to configure single-chip color camera acquisition parameters and acquire color or grayscale images.

**Note:** To use this command, before you start MetaMorph, load the Photometrics Camera device driver using the *Configure Acquisition* dialog box in the Meta Imaging Series Administrator, and click *This camera uses a color mosaic chip.* After you start MetaMorph, from the Acquire menu, click *Select Camera/Board*, then choose the name of the *Video Device* that you are using.

Single-chip Color Cameras typically acquire images at 12 bits per channel or 10 bits per channel. When you acquire color images, input from the three color channels will be converted into 24-bit true color images (8 bits per channel). The Color Balancing tab page of the Acquire Color dialog box contains an option for measuring a "white reference" image. This measurement determines the contributions from the red, green, and blue channels. This will in turn be used to balance the input from each channel, thereby achieving true color balance. Other options allow you to "tweak" the settings by adjusting the brightness of each color channel separately, or by adjusting the brightness of the entire image.

Grayscale images retain their native 12-bit image depth, which MetaMorph handles as 16-bit images. If you configure acquisition of grayscale images, an additional set of options will appear in the Acquire tab page which allow you to configure image intensity scaling either manually or automatically and apply the scaling to all images during focusing or acquisition. In addition, you can scale the image display after acquisition with the Scale 16-Bit Image command.

## Acquiring Color Images



## Overview of Configuration and Acquisition from Single-chip Color Cameras

These procedures guide you in setting up your Single-chip Color Camera configuration, saving and/or loading a configuration, making adjustments to color and focus, and acquiring images. This procedure contains links to subordinate procedures for setting acquisition parameters, setting color balance, and setting focus. Be sure to follow the links to complete these procedures, and then return to the main procedure after you have finished the subordinate procedure.

**Note:** Many of the options and controls in the Acquire Color dialog box will function interactively during the focusing procedure.

To acquire images using Single-chip Color Camera, complete the following procedures:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Acquire Color. The Acquire Color dialog box opens. |
| **2** | On the Acquire tab, click the *Image* selector to select the destination image window. |
| **3** | If you have previously saved a set of Single-chip Color Camera acquisition settings and you want to use these settings, choose *Load* to open the Load Settings dialog box and configure which features of a setting to load, or select a setting from the *Settings* drop-down box. Then, skip to Step 5. |

**OR**
If you are configuring a new set of acquisition settings, continue with Step 4.

**4** Complete the procedure for **setting acquisition parameters.** The settings you use depend on whether you are acquiring color or grayscale images and your type of illumination.

**5** If you are acquiring brightfield color images, you need to **check the brightfield color balance** for your images.

**6** If you are acquiring fluorescence color images, you need to **check fluorescence color balance** for your images.

**7** Before you begin acquiring images, check the focus of the microscope. Follow the procedure for **focusing** the image sample.

**8** Now you are ready to acquire your experimental images. Click *Acquire.* A single image will be acquired.

**9** If you want to save your acquisition settings, click *Save As.* The Save Single-chip Color Camera Settings As dialog box opens.

**AND**
Type a name for the settings (.csn) file in the *File Name* text box and click *Save.*

**10** Click *Close.*

## Setting Acquire Color Acquisition Parameters

To configure acquisition parameters for the Single-chip Color Camera, use the following procedure:

| Step | Action |
|---|---|
| **1** | On the Acquire Color dialog box Acquire tab, select the Image Type and Exposure that you want to use.<br><br>Click *Color Image* if you are acquiring a color image; click *Intensity Image* if you are acquiring a grayscale image. |
| **2** | Type or select an exposure time interval in milliseconds (ms) in the active exposure time box.<br><br>**OR**<br><br>If you want MetaMorph to calculate your exposure time, click Auto Expose. This exposure time is based on the exposure time in milliseconds that you entered limited by the Target maximum pixel value on the Preferences tab. |
| **3** | If you selected Intensity Image, the Intensity Image settings became active. If you want MetaMorph to automatically set the grayscale image intensity range, click |

*Autoscale Image*.

**4** If you selected Autoscale image, you can specify the low and high limits of the intensity range as a percentage. Image data below the low value will be interpreted as no signal and above the high value will be interpreted as maximum signal; thus, both will be excluded. Type or select percentages for either or both the low and high ends of the image intensity scale.

**OR**

If you did not select Autoscale Image, you can specify the low and high limits of the image intensity range. Type or select a value for either or both the low and high ends of the image intensity scale.

**Note:** The low value must be less than the high value.

**5** If you are using an illumination device, select the device from the *Illum* drop-down list.

**OR**

If you are not using an illumination device, select *"[None]."*

**6** Click the Preferences tab.

**7** If you are acquiring color images, select a target maximum pixel intensity value from one of the *Color Image* boxes. You can select the target value either as a percent of the 4095-level maximum for the 12-bit range (left box) or as an actual pixel color intensity value (right box).

**OR**

If you are acquiring grayscale images, select a target maximum pixel intensity value from one of the *Intensity Image* boxes. You can select the target value either as a percent of the 4095-level maximum for the 12-bit range (left box) or as an actual pixel grayscale value (right box).

**8** If you want MetaMorph to display an overexposure warning, select the *Warn If Exposure Time Is Long* check box. Then select the desired *Exposure Time Warning Threshold (ms).*

**AND**

If you want to display a warning graphic on overexposed focusing images, select the *Draw Warning Text Onto Focus Image If Image Is Overexposed* check box.

**9** Click the Region tab.

**10** If you want to use the active rectangular region from an image on the desktop, rather than defining the region with the dialog box

| | |
|---|---|
| | options, select the desired image using the *Image* selector.<br><br>**AND**<br><br>Use the Rectangular Region Tool from the Region Tools window to draw an acquisition region in the image window. Then choose *Use Active Region Defined on Image.* |
| **12** | You can use *Left, Top, Width,* and *Height* to specify the size and location of the region on the chip. Choose *Entire Chip* to create a region that is the size of the chip, or you can choose *Center Quadrant* to create a region centered on the chip that is the size of one quadrant. Choose *Ctr* to center the region. You can use the << or >> options to shrink or enlarge a region by a factor of two.<br><br>**OR**<br><br>Specify the size and location of region using the *Box-in-Box* option on the left side of the dialog box. The smaller box can be sized and moved similarly to a region of interest. |
| **13** | Now you are ready to proceed with configuring the image display scaling, focusing, and acquisition. |

## Acquire Color - Color Balancing for Brightfield Images

Color balancing adjusts the relationship between the red, green, and blue channels. This is necessary only if you are acquiring color images. To adjust color balance effectively, you need to expose the camera to an evenly illuminated, all-white visual field, or have a white region within the visual field. If your field does not contain a clear (white) region, you might only need to move the microscope slide to a clear region.

To configure the color balance for image acquisition with the Single-chip Color Camera, use the following procedure:

| Step | Action |
|---|---|
| **1** | Click *Start Focusing*. A focus image window opens. |
| **2** | Click the Place Active Region for Color Balancing button (next to the Close button). The Color Balancing tab moves to the front, and an active square region is placed in the focus image window. |
| **3** | In the focus image window, move and/or size the region so it is surrounds an all-white area or an area that you want to define as white.<br><br>**OR**<br><br>Provide an evenly illuminated, all-white visual field, or move the specimen slide to a clear region. |
| **4** | Set the Image Type to *Brightfield*. |

**5** Click *Measure White Ref.* MetaMorph assigns the intensity value of the active region as the white value for the image. All other color intensity values should fall below this. All values higher will also be considered as white.

**6** Adjust the *Brightness* slider to obtain the level of brightness you want in your image. To cancel the adjustment you made, click the associated Reset button.

**Note:** Moving the *Brightness* slider is equivalent to moving all three individual color sliders an identical distance.

**7** Adjust the three individual sliders for *Red*, *Green*, and *Blue* to fine tune the color balance that you want in your image. To cancel the adjustment you made, click the associated Reset button.

**8** If it was necessary to move your specimen slide to a clear area to achieve correct white balance, return the specimen to its original position.

**9** Click the Preferences tab.

**10** If an active color balance region is still present, move the region and size it to a black area in your image or an area that you want to define as black.

**11** Click *Measure Black Reference*.

**OR**

Type or select a value in the Black Reference box.

**Note:** You can also use this setting to fine-tune the black reference value after you have clicked the Measure Black Reference button.

**12** Click the Color Balancing tab.

**13** Make any necessary final adjustments to the Brightness or to the individual color settings.

**14** Check *Scale Every Image* if you want MetaMorph to recalculate scaling for each acquired image. MetaMorph will compensate for image intensity variations from one image to the next, while retaining the color balance setting that you made.

**15** When you have finished making your adjustments, click *F2: Stop Focusing,* or press the [F2] function key on your keyboard. The Focus window will close.

## Acquire Color - Color Balancing for Fluorescence Images

If you are acquiring fluorescence images, you need to set the color balance (color channel scaling). The MetaMorph Acquire Color *Color Balancing* tab enables you to select an image type of either Brightfield or

Fluorescence. When you choose Fluorescence, the settings on the Color Balancing tab change to controls that enable you to either automatically or manually set fluorescence color channel scaling. Because fluorescence images are darkfield images, a white balance setting is not applicable.

To set the color balance for fluorescence images, complete the following procedures:

| Step | Action |
|---|---|
| **1** | From the Acquire Color dialog box, click the *Color Balancing* tab. |
| **2** | In the Image Type list, click *Fluorescence*. The fluorescence color balancing controls appear. |
| **3** | On the *Scale Range* list, select the camera scale range to use to scale your images. MetaMorph converts the camera scale of 12-bits to 8-bit (0-255) format. By using 12-bits per channel instead of 10-bits per channel, you can achieve greater control over the range you select to convert to 8-bit format. |
| **4** | In the *Image Scaling* box, click on *Auto* to enable automatic image scaling for all color channels. If you need to manually control image scaling for one or more channels, click *Custom*. |
| **5** | If you selected Auto Image Scaling, skip to step 7.<br>**OR**<br>If you selected Custom Image Scaling, click the *Autoscale* check box for the channels that you want to control manually. |
| **6** | Click *Start Focusing*, then using the focus image as a guide, set the minimum (Low) and maximum (High) levels for each channel for which you turned off Autoscale. You can change the low and high values by typing the value into the box, clicking the scroll buttons on the right side of the box, or moving the sliders under the channel's color strip. |
| **7** | For channels that you allowed MetaMorph to autoscale, you can type or select percentage values that specify the low and high limits into the Low and High boxes. |
| **8** | Click *F2:Stop Focusing* when you have finished setting your color balance. |

## Focusing with Single-chip Color Cameras

The *Start Focusing* command instructs MetaMorph to acquire an image continuously into an image window while you are focusing the microscope to verify that your specimen is visible and in focus. Some digital cameras have gain and offset controls which can be adjusted while using the *Start Focusing* command.

**Note:** Because of a potential difference between the microscope eyepiece view and the camera view, it is recommended that you complete the following procedure to ensure accurate focus and image content.

To focus the microscope, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire Color dialog box, click the Acquire tab. |
| **2** | Focusing is more effectively performed with intensity images, rather than color images. If *Color Image* is currently selected, change the selection to *Intensity Image.* |
| **3** | Click *Start Focusing.*<br><br>MetaMorph begins to acquire a continuous stream of images that are displayed and updated in a Focus Image window.. The *Start Focusing* button becomes the *F2: Stop Focusing* button, and the Stop Focusing command entry in the Devices menu is active. |
| **4** | Focus your microscope while monitoring the appearance of the images in the Focus window.<br><br>**Note:** Exposure, scaling, and color balancing options will remain available. |
| **5** | When you have finished adjusting your microscope's focus, click *F2: Stop Focusing,* or press the [F2] function key on your keyboard. The Focus window closes. |
| **6** | Now you are ready to acquire your experiment images. If you are acquiring color images, select *Color Images* as the Image Type. |

## Acquire Color Dialog Box Options

### Acquire (tab)

Moves the Acquire tab to the front and enables the functions and settings on the Acquire tab.

### Region (tab)

Moves the Region tab to the front and enables the functions and settings on the Region tab.

### Color Balancing (tab)

Moves the Color Balancing tab to the front and enables the functions and settings on the Color Balancing tab.

### Preferences (tab)

Moves the Preferences tab to the front and enables the functions and settings on the Preferences tab.

### Start Focusing/ F2: Stop Focusing

Acquires an image continuously into an image window while you are focusing the microscope to verify that your specimen is visible and in focus. Images will be acquired continuously until you choose *F2: Stop Focusing,* which discontinues acquisition of focusing images. As an alternative to using this button in the

dialog box, you can press the [F2] function key on your keyboard or choose the Acquire Color Stop Focusing command from the Acquire menu.

 **(Adjust Digital Contrast)**

Opens the Adjust Digital Contrast dialog box.

 **(Place Region for Color Balance)**

Places a preset size rectangular region in the center of the continuously updating focusing image to be used as the color balancing region. You can resize or move this region anywhere within the acquisition region. Click this button after you click Start Focusing. This function is active only when the focusing function is active and when acquiring a color image. When you click this button, the Color Balancing tab is automatically displayed.

### Close

Closes the dialog box.

## Acquire Color: Acquire - Dialog Box Options

### Image

Selects the destination image window.

### Image Type and Exposure

Selects the type of images that you plan to acquire and an exposure time for the selected image type. If you select *Color Image,* the Color Balancing options are available. If you select *Intensity Image,* additional intensity scaling options *(Autoscale Image, Low, High)* appear. However, the options on the Color Balancing tab page will not be operative.

### Intensity Image

Contains settings for monochrome images, including Autoscale Image and Low (or Low %) and High (or High%).

### Autoscale Image

Configures Acquire Color to scale the acquired images automatically to a 256-level intensity display. The lowest grayscale value in the image will be assigned grayscale value 0 (black), the highest value will be assigned value 255 (white) and all intensity values between the lowest and highest value will be scaled accordingly. The result will be an increase in image contrast. This may be necessary if much of your image data resides within a relatively narrow range of the camera's 12-bit (4096 intensity levels) overall intensity range. If you select the *Autoscale Image* check box, the *Low* and *High* spin boxes will become *Low%* and *High%* spin boxes, respectively. The *Autoscale Image* check box will be available only when *Intensity Image* has been selected as the image type.

> **Note:** If image intensity settings are set too high, the image will be over-saturated and a warning message will appear in each corner of the image window to indicate that the image will be over exposed if the current settings are used to acquire an image.

### Low / High

If the *Autoscale Image* check box is unchecked, the *Low* and *High* boxes are used for selecting an intensity range manually, to be scaled to a 256-level image intensity display. *Low* selects the image's lowest grayscale value, which will be reassigned to grayscale value 0 (black), and the *High* spin box selects the highest grayscale value, which will be reassigned to grayscale value 255 (white). The result will be an increase in image contrast. This may be necessary if much of your image data resides within a relatively narrow range of the camera's 12-bit (4096 intensity levels) overall intensity range. If you select the *Autoscale Image* check box, the *Low* and *High* spin boxes will become *Low%* and *High%* spin boxes, respectively. The *Low / High* spin boxes will be available only when *Intensity Image* has been selected as the image type and the *Autoscale Image* check box has been left cleared.

### Low% / High%

If you select the *Autoscale Image* check box, the *Low* and *High* boxes become *Low%* and *High%* boxes, respectively. These exclude a selected percentage of pixels in the image from the lower and upper end, respectively, of the range of values being autoscaled. For example, you could exclude the bright nucleus of a cell in a fluorescence image by setting the *High%* spin box to *10.* The *Low% / High%* spin boxes will be available only when *Intensity Image* has been selected as the image type and the *Autoscale Image* check box has been selected.

### Acquire

Acquires an image with the Color camera, using the current acquisition region and exposure settings. The image will be placed in the video board's memory (replacing the previous image stored in memory) and then transferred to an image window, where it will be displayed using the scaling settings you configured.

**Note:** In certain instances, if you press the Esc key, stream acquisition or other processes in progress may be halted.

### Auto-Expose

Initiates an autoexposure and adjusts the exposure time as determined by the target intensity preference.

### Load

Opens the Load Setting dialog box. Use this dialog box to load all or part of a set of Color Camera acquisition settings, previously saved as a .csn file with the *Save* or *Save As* command. You can also configure this dialog to determine if it is opened each time a setting is changed using the *Setting* drop-down box.

### Save

Save changes to a previously loaded .csn file. If you want to save the changes to a new or different .csn file, click *Save As*.

### Save As

Saves all current acquisition settings on disk, including color balance settings. You can open the settings at a later date using the *Load* command. This command opens the Save Acquire Color Settings As dialog box.

### Setting

Displays the names of the most recently saved or loaded settings. Up to eight settings can be displayed in the order of most recent usage. Selecting a setting load the values for the setting into the dialog for the next acquisition, immediately updating the controls and the display of the current "Acquired" image.

When loading a setting, the Load Setting dialog may appear so that the portion of the setting to be loaded can be configured. If the Load Setting dialog does not appear, the portion used will be the same as the portion used the last time a setting was loaded. To get the Load Settings dialog to appear when selecting a setting from the popup, open the Load setting dialog box and select *Show this dialog* from the *When using the pop list to load* fields.

### Illumination

Selects the illumination setting. If you are not using a shutter, select *"[None]."* If a shutter is selected, it will open the shutter before acquiring an image and close the shutter after exposure (before transfer.)

## Acquire Color: Region - Dialog Box Options

### Acquisition

Selects the region settings that are to be applied to the acquisition region.

### Focus

Selects the region settings that are to be applied to the focus region.

### Use Single Region

Indicates that a single region will be used for all acquired images, including images acquired in focusing mode.

**Note:** When this box is checked (default state), the Focus radio button is inactive.

### Chip Size

Indicates the size of the camera chip, in pixels.

### Box-in-Box Interactive Display of Region

Allows you to click on the smaller box with the left mouse button and then drag the pointer to resize and move the chip region box, as you would for a region of interest.

### Image

Specifies the image to use for the *Use Active Region Defined on Image* command.

### Use Active Region Defined on Image

Defines a region for the chip based on the active region of interest in the image selected with the *Image* selector. The box-in-box display is updated as well as the region's *Left, Top, Width,* and *Height* values.

### Left

Specifies the region's leftmost point.

### Top

Specifies the region's topmost point.

### Width

Specifies the region's width.

### Height

Specifies the region's height.

### Entire Chip

Creates a region that is the size of the entire chip.

### Center Quadrant

Creates and centers a region that is the size of one quadrant of the chip.

### **<<** and **>>**

Shrinks or enlarges the region by a factor of two.

### Ctr

Centers the region on the chip.

## Acquire Color: Color Balancing - Dialog Box Options

### Image Type

Selects either *Brightfield* or *Fluorescence* as the image type based on the illumination source the you intend to use. The default for this setting is *Brightfield*, which enables you to make settings for Brightness and/or the color intensity of individual primary colors. If you select *Fluorescence* as the image type, the settings options on the Color Balancing tab change to a different group of settings.

### Brightness

Adjusts the overall intensity levels of all three color channels simultaneously. Moving this slider is equivalent to moving the *Red, Green,* and *Blue* sliders by equal amounts.

### Adjustment

Adjusts color intensity levels of each individual color:

***Red*** – Adjusts the intensity levels of the red values in the image.

***Green*** – Adjusts the intensity levels of the green values in the image.

***Blue*** – Adjusts the intensity levels of the blue values in the image.

### Reset

Resets the associated slider(s) to the original midpoint position setting. If you choose the *Reset* button for the *Brightness* slider, this will also simultaneously reset all three of the color sliders.

### Scale Every Image

Forces MetaMorph to recalculate the minimum and maximum scaling values for all color channels every time you acquire an image. This effectively recalculates the brightness for the view or region to compensate for variations in image brightness, while maintaining the original, overall color balance of the image.

### Measure White Ref

Measures a "white reference" image, which is used to correct for the differences between red, green, and blue values of the image. For the region that you specify as white, MetaMorph scales the intensity value indicated for each channel to equal the maximum intensity for that color.

### Image Scaling

Selects either automatic scaling for the entire image or selected image region or custom scaling that you can set for each color.

### Scale Range

Selects the appropriate custom scaling range to apply to your color image. Select either 10 bits (1024 colors) or 12 bits (4096 colors).

### Autoscale

Enables automatic scaling of a color. When Autoscale is selected for a specific color, type or select a low and high percentage value. When Autoscale is not selected, type or select a both a low and high scaling range or move the pointers on the color scale to select the scaling range that you want to use.

### Scaling Range

Accepts both low and high scaling range values. If Auto scale is selected, type or select a low and high scaling range as a percentage value. If Autoscale is not selected, type or enter appropriate low and high bit range values, or move the sliders to select appropriate low and high values.

## Acquire Color: Preferences - Dialog Box Options

### Warn If Exposure Time Is Long

Instructs MetaMorph to warn you when an acquisition starts if the camera's exposure setting is longer than the value specified in the *Exposure Time Warning Threshold.*

### Exposure Time Warning Threshold (ms)

Specifies the minimum exposure time that is to be considered a long exposure. You will be warned if the exposure time equals or exceeds this limit.

### Draw Warning Text Onto Focus Image If Image Is Overexposed

Draws an overexposure warning in the corners of the Focus image if any portion of the acquired image is saturated.

### Color Image

Selects a maximum pixel intensity for image acquisition. The target intensity will determine the exposure time. You can select the target value either as a percent of the 4095-level maximum for the 12-bit range (left spin box) or as an actual pixel color intensity value (right spin box).

### Intensity Image

Selects a maximum pixel intensity for image acquisition. The target intensity will determine the exposure time. You can select the target value either as a percent of the 4095-level maximum for the 12-bit range (left spin box) or as an actual pixel grayscale value (right spin box).

### Black Reference

Establishes the maximum image intensity value for black in the image.

### Measure Black Reference

Initiates a measurement of a black reference region in the image and records the measurement in the Black Reference window.

# Stream Acquisition (Acquire Menu)

**Enables you to configure MetaMorph for rapid acquisition of images as a continuous data stream, activate image streaming, and transfer the images into a stack.**

### Drop-in: STREAM

Use this command to configure Stream Acquisition settings and to acquire an image stream based on the settings. When configuring Stream Acquisition, you can specify the number of frames to acquire, the camera state, shutter mode, and the clear mode settings. Stream Acquisition captures images as a continuous data stream at the fastest possible rate and stores the image data in RAM, or directly to the hard drive. Once all of the frames have been acquired, MetaMorph transfers the image data into an image stack, performing the image saving and data logging tasks as specified by the settings in the Configure Digital Camera dialog box.

**Note:**
Be sure to configure your digital camera correctly in the Configure Digital Camera dialog box before beginning stream acquisition.

After Configure Stream Acquisition and Configure Digital Camera settings are defined, use the Acquire button on the Acquire tab to begin streaming your image data. When all of the frames have been acquired, MetaMorph will transfer the image data into planes in a stack.

Stream Acquisition also enables you to acquire through-focus Z-series images as a stream. To do so, both your video driver and focus motor driver must be capable of supporting streaming sequences. Currently, only the Princeton Instruments digital camera driver and the Physik focus motor device driver support streaming. When these conditions have been met, an enhanced version of the Configure Stream Acquisition dialog box is displayed. This provides additional options for setting the starting and ending position of the focus motor, based on the position settings that have been configured with the Focus command (Devices menu).

**Note:** To use this command, your camera must support image streaming.

**WARNING:**
If you attempt to use a streaming exposure time that is shorter than the fastest readout time your camera can handle, your actual exposure time will be limited by the readout time. If this occurs, MetaMorph will display a warning message.

**WARNING:**
You must run all mechanical shutters at a cycle time greater than 25 ms. Uniblitz, Lambda 10, Metaltek, Ludl, and cooled CCD shutters are driven by a high voltage which takes time to dissipate. Running these shutters at a cycle length shorter than 25 ms will cause a build-up of heat, leading to eventual jamming. Neither Molecular Devices nor any manufacturers of these shutters will honor warranties on equipment that has been damaged by improper use. Operation of these shutters at a cycle length shorter than 25 ms is considered improper use.

## Stream Acquisition - Procedures

To acquire images as a stream, complete the following procedure:

| Step | Action |
|---|---|
| 1 | From the Acquire menu, choose Stream Acquisition. The Stream Acquisition dialog box opens. |
| 2 | In the Acquisition Mode box on the Acquire |

dialog tab, choose the image stream storage method. If sufficient RAM memory is available, choose *Stream to RAM*, otherwise choose *Stream to Hard Disk*.

**Note:** The default acquisition method, *Stream to RAM,* sends the entire image stream to RAM and transfers the images to disk afterwards. You can also stream the acquired frames to hard disk in real-time by selecting *Stream to Hard Disk.*

**3** In the *Number of Z sets* box, select the number of z sets (frames) you want to acquire.

**Note:** MetaMorph displays the amount of memory the stream will use (based on the size and number of images that you want to acquire). It will also display the amount of memory the resulting stack will use and the amount of memory available. This information will help you to determine how many frames you can acquire in one stream.

**4** Select an illumination setting from the *Initial Illum* list.

**OR**
If you are not using a shutter, leave this selection as *"[None]."*

**5** Select *Use with High-Speed Focus Motor* to enable the *Focus Motor* options. The settings in this option group will be based on the position settings that have been configured with the Focus command (Devices menu).

**6** Select Use with high-speed Wavelength changer, if you have a high-speed wavelength changer installed and configured.

**7** Click the Focus tab, The focus tab dialog is displayed.

**8** From the *Start At* drop-down list, select the starting position for the Z-series image stream: *Current Position, Origin, Top, Bottom,* or *Home.*

**9** From the *Move to* list select the Z-position for the last image in the stream series from one of the following: *Origin, Top, Bottom,* or *Home.*

**10** From the *After* drop-down list, select the position to which the focus motor should return after the Z-series stream has been acquired: *Current Position, Origin, Top, Bottom,* or *Home.*

**11** Click the Wavelength tab. The Wavelength tab dialog is displayed.

**12** From the Wavelength tab dialog, type or choose the number of wavelengths to include in your image stream in the *Number of*

*Wavelengths* box.

**13** In the *Wavelengths Selection* area, set the wavelength value for each wavelength that you want to acquire in your image stream.

**14** Click the Camera Parameters tab. The Camera Parameters tab dialog is displayed.

**15** Select the acquisition mode from the *Acquisition Mode* section. The options available will vary depending on the camera used.

**16** Select the desired state for the camera from the *Camera State* list. The options available will vary depending on the camera used. The suggested setting for the PVCam camera is *HALT, CLEAR*.

**17** Select the desired shutter mode for the camera from the *Shutter Mode* list. The options available will vary depending on the camera used. The suggested setting for a frame transfer camera operating in the FT mode is *OPEN PRE SEQUENCE.* If you do not have a frame transfer camera, you should set this option to *OPEN PRE EXPOSURE.*

**18** Select the desired clear mode for the camera from the *Clear Mode* list. The options available will vary depending on the camera used. The suggested setting for a frame transfer camera operating in the FT mode is *CLEAR PRE EXPOSURE.*

**19** After you complete all settings on all tabs, click the Acquire tab, and then click *Acquire* to capture your image stream based on your settings.

> **Note:** To cancel a Stream Acquisition before it finishes, press the *Esc* key. If your camera driver supports it, you are given the option to save the images already collected to a stack on the MetaMorph desktop.

**20** When you have finished, click Close to close the dialog box.

## Stream Acquisition – Main Dialog Box Options

### Acquire Tab

Select the dialog box tab that contains the principle acquire configuration settings and displays the Acquisition Information.

### Focus Tab

Selects the dialog box tab that contains the focus motor configuration settings.

### Wavelength Tab

Selects the dialog box tab that contains the wavelength configuration settings.

### Camera Parameters Tab

Selects the dialog box tab that contains the camera parameter settings.

### Status

Displays a message regarding the status of the stream acquisition and alerts you to any problems that may occur.

> **Note:** The types of messages that display depend on your camera and driver. For example, the Orca ERG supports the display of Readout time per frame information.

### Record Configuration State

> Applies any new configuration settings.

### Acquire

Begins image streaming acquisition.

> **Note:** To cancel a Stream Acquisition before it finishes, press the *Esc* key. If your camera driver supports it, you are given the option to save the images already collected to a stack on the MetaMorph desktop.

### Close

Closes the Stream Acquisition dialog box and disregards the new configuration settings.



## Stream Acquisition - Acquire Dialog Box Options

### Acquisition Mode

This option enables you to select between (1) the default method of acquiring frames rapidly to RAM and then processing the images afterwards on disk *(Stream to RAM),* and (2) acquiring the image frames directly to disk by high-speed streaming *(Stream to Hard Disk).*

### Number of Z Sets

Specifies the number of image frames to acquire.

### Browse

Opens the Select Stream File dialog box to enable you to select or assign a file name for the acquired stream. By default, the file will be saved as a stack (.stk) file. This option is only available when *Stream to Hard Disk* is selected in the Acquisition Mode field.

### Filename

Displays the path and name of the stack file for the streamed acquisition. You can edit this path directly, or use the Browse command to choose the path. This option is only available when *Stream to Hard Disk* is selected in the Acquisition Mode field. This field must be filled before you can acquire a stream using the *Stream to Hard Disk* option.

### Acquisition Information:

> **Your Current Acquisition Region Is**

Displays the size of the acquisition region. (The acquisition region is defined in the Configure Digital Camera dialog box.)

### Each Pixel Will Use

Displays the amount of memory that each pixel will use. This value depends on the capabilities of the channel used to acquire the stream. Channels that acquire 8-bit images will use 1 byte per pixel.

### Each Frame Will Use

Displays the amount of memory that each frame will use. This value is a result of the size of the acquisition region and the number of bytes used per pixel.

### Total Number of Frames

Indicates the number of frames planned to acquire. Change the number of frames in the Number of z sets window. As you change the Number of z sets value, the value displayed by the *Amount of Memory Stream Will Use* is automatically updated.

### Amount of Memory Stream Will Use

Displays the amount of memory needed to complete the stream acquisition. If the amount of memory used by the stream exceeds the total amount of free memory, acquisition will not be possible. When this situation occurs, a message will be displayed on the *Status* line.

### Amount of Memory Stack Will Use

Displays the amount of memory that will be used by the resulting stack.

### Amount of Memory Available

Displays the amount of memory available for use by the stream during acquisition.

### Readout Time Per Frame

Displays the amount of time it takes to read the image out of the camera and into system memory.

**Note**: This field is only displayed if your camera can provide frame readout times.

### Acquisition Time Per Stream

Displays the total amount of time that the stream acquisition will require.

**Note**: This field is only displayed if your camera can provide frame readout times.

### Initial Illum

Selects the illumination setting to use when acquiring the stream. Illumination settings are defined using the Configure Illumination command. If you are not using a shutter, select *"[None]."*

### Use with High-Speed Focus Motor

Enables the *Focus Motor* options *(Start At, Move To, After),* which are used for selecting the starting, ending, and return position settings of a Z-series of through-focus images. The *Focus Motor* options and *Use with High-Speed Focus Motor* check box will be displayed only if your video and focus motor device drivers support Z-streaming. Currently, only the Princeton Instruments video driver and the Physik focus motor device driver support streaming.

### Use with High-Speed Wavelength Changer

Indicates that a high speed wavelength changer is connected and enabled.

## Stream Acquisition - Focus Dialog Box

### Focus Motor:

Specifies the parameters that determine the focus motor Z plane movements.

### Start At

Selects the starting position for the Z-series image stream: *Current Position, Origin, Top, Bottom,* or *Home.* These settings are based on the position settings that have been configured with the Focus command (Devices menu).

### Move To

Selects the Z-position for the last image in the stream series: *Origin, Top, Bottom,* or *Home.* These settings are based on the position settings that have been configured with the Focus command.

### After

Selects the position to which the focus motor should return after the Z-series stream has been acquired: *Current Position, Origin, Top, Bottom,* or *Home.* These settings are based on the position settings that have been configured with the Focus command.

### Plane Distance

Indicates the between-plane distance of the Z-series images, based on the starting and ending Z-positions and the *Number of Frames to Acquire.*

### Total Distance

Indicates the total distance between the starting and ending position in the Z-series.

## Stream Acquisition - Wavelength Dialog Box

### Number of Wavelengths

Indicates the number of wavelengths that you want to acquire.

### Wavelength Selection:

Displays a control for each wavelength that you want to acquire.

### Wavelength #1…4

Indicates the wavelength value in nanometers for the first wavelength, and provides a slider for setting the wavelength value.

## Stream Acquisition - Camera Dialog Box

### Acquisition Mode

### Acquire Images at frame Rate

Specifies image acquisition to occur at the frame rate determined by the current exposure time.

### Acquire Images from Each External Trigger

Specifies image acquisition to occur in synchronization with a separate external trigger source for each frame of the stream.

### Acquire Images from First External Trigger

Specifies image acquisition to occur in synchronization with a single external trigger source.

### Number of Frames to Skip

Specifies the number of frames to skip before beginning to acquire a continuous stream of frames.

### Camera State

Specifies the camera state used during acquisition. The options available will vary depending on the camera used. The suggested setting for the PVCam camera is *HALT, CLEAR.*

### Shutter Mode

Specifies the shutter mode used during acquisition. The options available will vary depending on the camera used. The suggested setting for a frame transfer camera operating in the FT mode is *OPEN PRE SEQUENCE.* In this mode, the shutter will open before the acquisition and stay open until the acquisition is done. If you do not have a frame transfer camera, you should set this option to *OPEN PRE EXPOSURE.*

This will open the shutter before each frame is acquired and then close it after the acquisition.

### Clear Mode

Specifies the mode used to clear the camera chip. The options available will vary depending on the camera used. The suggested setting for a frame transfer camera operating in the FT mode is *CLEAR PRE SEQUENCE.* This setting clears the chip prior to starting the first exposure. If you do not have a frame transfer camera, you should set this option to *CLEAR PRE EXPOSURE.* This setting clears the chip before each frame is exposed.

# Digital Camera Video Control (Acquire Menu)

**Controls the RS-170 video output of a digital camera.**

**Drop-in: DCAMVID**

Use this command to focus digital cameras that use an external video monitor. The command allows you to set the zoom level, panning, exposure time, and intensity scaling for the video output. The Digital Camera Video Control command also allows you to focus the camera so that you can set up the video output while focusing.

**Note:** This command is supported only by the Princeton Instruments MicroMAX and PentaMAX cameras.

**WARNING:**
You must run all mechanical shutters at a cycle time greater than 25 ms. Uniblitz, Lambda 10, Metaltek, Ludl, and cooled CCD shutters are driven by a high voltage which takes time to dissipate. Running these shutters at a cycle length shorter than 25 ms will cause a build-up of heat, leading to eventual jamming. Neither Molecular Devices nor any manufacturers of these shutters will honor warranties on equipment that has been damaged by improper use. Operation of these shutters at a cycle length shorter than 25 ms is considered improper use.

## Configuring Digital Camera Video Control

To configure digital camera video control, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Digital Camera Video Control. The Digital Camera Video Control dialog box will appear. |
| **2** | If you are using an external shutter, select the Illumination setting associated with your shutter from the Illumination setting list. The shutter will open automatically when focusing begins, and will restore itself to its previous state when focusing ends.<br>**OR**<br>If you are not using a shutter, leave the Illumination setting list selection as "*[None]*." |
| **3** | From the *Zoom* group, select the desired zoom level at which you want to display the video output.<br>The *2x* and *4x* zoom levels come with the additional option of using *Binning,* in which the camera chip pixels are binned in X-Y fashion to produce the video image, or *Decimation,* in which every Nth pixel is used for the final image. |
| **4** | If you selected one of the *2x* or *4x* options from the *Zoom* group, the *Pan* group will |

become available, as you will not be able display the entire zoomed image. You can pan to one of nine separate positions in the image.

**5** In the *Exposure Time (ms)* text box, type the amount of time that you want the chip to be exposed.

**6** From the *Intensity Scaling* list, select the scaling factor that you want to use to scale the camera's 12-bit image to an 8-bit display (if necessary).

**7** Choose *Focus* to focus the image.

When you have finished, choose *Stop Focus.*

**8** Choose *Close.*

## Digital Camera Video Control - Dialog Box Options

### Zoom

Zooms the image displayed on the video monitor. You can select *1x, 2x,* or *4x.* The *2x* and *4x* zoom levels come with the additional option of using *Binning,* in which the camera chip pixels are binned in X-Y fashion to produce the video image, or *Decimation,* in which every Nth pixel is used for the final image. If you select one of the *2x* or *4x* options, the *Pan* radio buttons will become available, allowing you to select which part of the image to display.

### Pan

Allows you to pan to one of nine separate positions in the image when the image is zoomed up to 2x or 4x. This option is available only when the *Zoom* factor is either *2x* or *4x.*

### Exposure Time (ms)

Specifies the amount of time the chip is exposed, in milliseconds. The Princeton Instruments camera optimizes the focus algorithm internally, so that it may not seem as though the value selected here corresponds to the exposure time of the chip.

### Intensity Scaling

Specifies the scaling factor to use to scale the camera's 12-bit image to an 8-bit display. This may be necessary when your images' intensity values are skewed toward one end of the grayscale. If you are using a MicroMAX camera, you will be able to choose the range of gray levels to display. However, the PentaMAX camera uses look-up tables instead to determine the display.

### Illumination

Selects the Illumination setting associated with the external shutter you are using.

### Focus

Starts camera focusing. This button's text will change to *"Stop Focus."*

### Stop Focus

Stops camera focusing.

### Close

Closes the dialog box.

# Acquire Multiple Wavelengths (Acquire Menu)

**Configures and performs acquisition of images using up to six different sets of settings for wavelength, intensity, and exposure duration.**

**Drop-in: MULTIWAC**

Use this command to acquire images automatically, using up to six sets of illumination, exposure, and offset settings. The images can be saved as planes in a stack, or acquired as images of individual wavelengths.

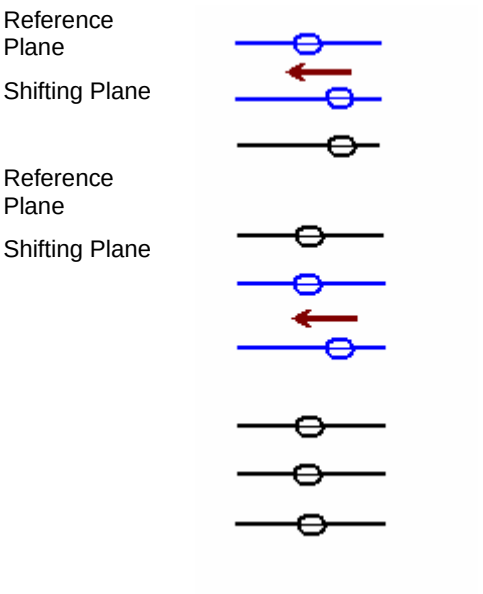
For digital cameras, you can set different exposure times for the acquisitions, or you can specify that autoexposures be performed for each.

**Note:** If you use the *Acquire* command to acquire images, this command uses the *Acquire* acquisition settings. If you use the *Acquire from Spot* or *Acquire from Digital Camera* commands to acquire images, this command uses acquisition settings from those commands. If you are using a Spot Camera, you must also select at least one of the three color channels from the Acquire from Spot Camera dialog box to be able to use Acquire Multiple Wavelengths. Background subtraction and shading correction will not be performed when images are acquired with this command, regardless of the setting used.

The planes in the acquired stack can be shifted relative to one another to align the objects in the images. This is similar to the process used in the Align Stack command. When you perform an alignment, only that the area of the image that is common to all wavelengths will be saved. Thus, the result image may be smaller than the original acquisition area.

## Aligning a Stack

**Aligning a Stack**




1. A horizontal shift of ten pixels to the left is selected and applied to the middle plane, using the top plane as a reference.

2. A horizontal shift of ten pixels to the left is selected and applied to the bottom plane, using the middle plane as a reference.

## Acquiring Multiple Wavelengths - Procedures

**Acquiring Multiple Wavelengths**

**Aligning a Multiple-Wavelength Stack**

## Acquiring Multiple Wavelengths

To configure and perform multiple wavelength acquisitions, use the following procedure. If you are using a digital camera, the acquisition settings not configured here will be taken from the settings in the Acquire from Digital Camera dialog box.

| Step | Action |
|---|---|

**1** From the Acquire menu, choose Acquire Multiple Wavelengths. The Acquire Multiple Wavelengths dialog box opens.

**2** If you have previously saved a state file containing the dialog box settings that you want to use, choose *Load State* and select the desired .amw file from the Load Acquire Multiple Wavelength File dialog box that appears. Then choose *OK* to return to the Acquire Multiple Wavelengths dialog box and skip to Step 9.

**OR**
If you will be using a new set of dialog box settings, continue to Step 3.

**3** With the # *of Waves* spin box, specify the number of different sets of illumination settings that will be used.

**4** Select an illumination setting for each of the wavelengths in the *Illumination* drop-down boxes.

**5** If you are using a digital camera, you can specify an exposure duration in the *Exposure (ms)* spin box for each wavelength.

**OR**

If you want MetaMorph to determine an appropriate exposure duration, select the *Auto* check box for the first wavelength. Then specify a maximum grayscale value for the acquired image in the *Target Intensity* spin box.

**6** If you want to specify an exposure duration for each wavelength, continue to Step 7.

**OR**

Select an exposure duration mode. Choose

***Auto Balance*** if you want all wavelength acquisitions to use the autoexposure feature. A check mark will appear in the *Auto* check boxes for all wavelengths.

***Balance = 1*** if you want all wavelength acquisitions to use the exposure duration set for Wavelength 1. A check mark will appear in the *Balance* check boxes for all wavelengths, and the *Balance* spin boxes will all update to display a "1".

***Use Balance*** if you want Wavelengths 2 and higher to use an exposure duration that is a specific ratio or multiple of the exposure for Wavelength 1 (to be specified in the next step).

**7** Repeat Steps 4 - 8, as appropriate, for each successive wavelength acquisition set.

If you want to configure an exposure duration for each wavelength acquisition that is a

| | |
|---|---|
| | specific ratio or multiple of the exposure for Wavelength 1, verify that a check mark appears in its *Balance* check box and enter a ratio value in its *Balance* spin box.<br><br>**OR**<br><br>If you want to specify an exposure duration for each wavelength acquisition, verify that there are no check marks in either its *Balance* or *Auto* check boxes, and type the duration, in milliseconds, in the *Exposure (ms)* spin box. |
| **8** | If you want to save the current set of dialog box settings, choose *Save State.* Type a file name for the .amw state file in the *File Name* text box of the AMW State dialog box that appears. Then choose *Save.* |
| **9** | To acquire all configured wavelength images as planes in a single stack, select the destination stack with the *Stack* image selector and click the *Acquire* icon in the *All Wavelengths* section (upper-left corner of dialog box).<br><br>**OR**<br><br>If you want to acquire an image using a single wavelength's settings, click the Acquire icon next to the settings for that wavelength.<br><br>If you have enabled autoexposure, the *Exposure* and/or *Balance* settings will be updated following the acquisition(s). |
| **10** | If you want to align the acquired set of images, follow the procedure for **aligning the stack planes.** |
| **11** | Choose *Close* when you have finished. |

## Aligning a Multiple-Wavelength Stack

To align the planes in a multiple-wavelength image stack, use the following procedure.

| Step | Action |
|---|---|
| **1** | From the Acquire Multiple Wavelengths dialog box, choose *Set Alignment.* The AMW: Set Alignment dialog box will appear. |
| **2** | Select the desired image from the *Stack* image selector. |
| **3** | If you are starting with a stack that was not aligned during acquisition, select *Set Initial Alignment* from the *Change to Multiple Wavelengths* option button group.<br><br>**OR**<br>If you are editing a stack that was aligned during acquisition with the main dialog box's *Image Alignment and Cropping* spin boxes *(X* and *Y),* and you want to add a set of offset values to the values already in the main |

dialog box, select *Adjust Current Values.*

**4** Select an alignment display from the *Display* group:

*Subtract* uses subtraction to show the difference between the reference plane and the shifting plane.

*Average* uses averaging to display this difference.

**5** Use the *Horizontal Shift* and *Vertical Shift* text boxes or sliders to adjust the alignment of the plane displayed in the alignment image window. The plane will be moved in one-pixel increments.

If you are using the *Subtract* display, the plane will be aligned when there is a nearly uniform grayscale level throughout the entire image.

If you are using *Average,* the aligned plane should look like the original plane with as little blurring as possible.

If you need to start over, choose *Zero Shift* to reset the sliders to zero and repeat Step 5 as needed.

**6** Choose the *Next* or the *Previous* command button to advance to the next or the preceding plane (depending on whether you are starting with the first or last plane).

**7** Repeat Steps 5 and 6 until you have aligned each plane in the stack.

**8** Choose *Close* when you have finished.

## Acquire Multiple Wavelengths - Dialog Box Options

### Acquire Multiple Wavelengths (main dialog box)

### AMW: Set Alignment

## Acquire Multiple Wavelengths - Dialog Box Options

### All Wavelengths

### # of Waves

Sets the number of separate wavelengths which can be configured.

### Auto Balance

Enables *Auto* for all wavelengths. Not recorded in journals.

### Use Balance

Sets all wavelengths to use their respective *Balance* values. For each wavelength, excluding Wavelength 1, the *Balance* check box will be enabled. This is used to best effect after an autoexposure is performed for all wavelengths.

### Balance = 1

Sets the exposure time to be the same for all wavelengths. For each wavelength, excluding Wavelength 1, the *Balance* check box will be selected, *Auto* will be deselected, the *Balance* value will be set to 1, and the

exposure of the wavelength being configured will be set to the exposure duration of Wavelength 1.

### Stack

Sets the destination for the stack of images generated by *Acquire* in the All Wavelengths section.

### Specific Wavelengths

### Illumination

Selects the illumination setting for the wavelength being configured.

### Auto

Determines whether a new exposure duration is to be calculated for the current wavelength. If you select the check box for this option, MetaMorph will perform multiple exposures to determine an appropriate exposure setting. The algorithm used is the same as that used for Basic Digital Acquire. If you deselect this option and an appropriate exposure is achieved, the new exposure duration will be represented in the *ms* spin box, and the *Balance* value for the wavelength will be set.

### Exposure (ms)

Specifies the exposure duration for the corresponding wavelength. If you select *Auto,* this option will merely display the duration to be used once an acquisition time has been determined.

### Balance (check box)

Determines whether changes to the settings for Wavelength 1 are to affect the current wavelength. If this check box is selected while Wavelength 1 is calculated or simply changed, the exposure for the current wavelength will be calculated as a ratio of the exposure duration of Wavelength 1. If this check box is cleared while *Auto* is simultaneously disabled, the exposure value for the current wavelength will be the time, indicated in milliseconds, and the *Balance* value will simply reflect the calculated ratio.

### Balance (spin box)

Displays and sets the ratio of the exposure time for the current wavelength, relative to Wavelength 1. Changing this value will change the exposure time. If *All to Use Balance* has been chosen, changes to the settings for Wavelength 1 will automatically affect the exposure duration of the current wavelength. If *Auto* is enabled, the *Balance* spin box will merely display the ratio to be used once an acquisition time has been determined.

### Offset

### X

Specifies a horizontal alignment shift for the currently active image plane. Positive numbers will shift the image to the right, negative numbers to the left. When you perform an alignment, only that the area of the image that is common to all wavelengths will be saved. Thus, the result image may be smaller than the original acquisition area.

### Y

Specifies a vertical alignment shift for the currently active image plane. Positive numbers will shift the image downwards, negative numbers will shift upwards.

### Destination

Determines the destination for acquisition of the current wavelength only.

### Acquire

Acquires an image for the current wavelength, based on the active settings.

### Target Intensity

Sets the maximum value of the intensity during autoexposures. This field affects all wavelengths.

### Set Alignment

Opens the AMW: Set Alignment dialog box**,** from which you can configure alignment of the planes in the acquired multiple-wavelength stack.

### Load State

Opens the Load Acquire Multiple Wavelength File dialog box, from which you can select and load a saved state file (*.amw) which stores the Acquire Multiple Wavelengths settings. These settings include the exposure times, the balances, the wavelengths to autoexpose, illumination information for each wavelength, and image alignment information.

### Save State

Saves the current set of dialog box settings in an .amw state file.

### Close

Closes the dialog box.

## AMW: Set Alignment (Acquire Multiple Wavelengths) - Dialog Box Options

### Stack

Selects the stack that will be aligned.

### Change to Multiple Wavelengths

Determines whether the stack being edited was aligned with the *X* and *Y* spin box values in the main dialog box's *Image Alignment and Cropping* option group. If you are starting with a stack that was not created with a set of alignment values, select *Set Initial Alignment.* If the stack was acquired with the *X* and *Y* offset values and you want to add a value set to the existing values, select *Adjust Current Values.*

### Display

Selects the method to be used to display differences between the reference plane and the shifting plane:

*Subtract* uses subtraction to show the difference between the reference plane and the shifting plane. The planes will be aligned when there is a nearly uniform grayscale level throughout the entire image.

The *Average* display uses averaging to display the offset between the two planes. The aligned plane should look like the original plane with as little blurring as possible.

### Horizontal Shift (text box and top slider)

Adjusts the horizontal alignment of the plane in one-pixel increments.

### Vertical Shift (text box and left slider)

Adjusts the vertical alignment of the plane in one-pixel increments.

### Previous

Places the previous plane in the alignment image window.

### Next

Places the next plane in the alignment image window.

### Zero Shift

Resets the *Horizontal Shift* and *Vertical Shift* to zero.

### Cancel

Cancels the command and returns to the main Acquire Multiple Wavelengths dialog box.

### OK

Accepts the new settings and closes the dialog box. Any values you enter in the *Horizontal Shift* and *Vertical Shift* boxes will be added to the *Y* and *X* spin boxes, respectively, in the main dialog box's *Image Alignment and Cropping* option group.

# Configure Intensifier Gain Control (Acquire Menu)

**Configures the control of the intensifier CCD camera settings when using computer-**

**controlled gain.**

### Drop-in: SETICCD

Use this command before using the Set Intensifier Gain command and the Set Camera Level and Gain command with an intensified CCD camera (and the PI Video ICCD Settings command, if you are using a Princeton Instruments video camera). Configure Intensifier Gain Control allows you to specify the camera model, serial port, and baud rate. It also allows you to select whether the camera is controlled by the computer or by the front-panel knobs on the intensifier. The camera must be controlled by the computer to use the intensifier gain commands in MetaMorph.

You must use this command before using the Set Intensifier Gain, the Set Camera Level and Gain, and PI Video ICCD Settings commands. These three commands will be unavailable until you do so.

Prior to using this command, you must install its drop-in, SETICCD, using the Configure Drop-ins/Toolbars Command in the Meta Imaging Series Administrator.

## Configuring the Intensifier Gain Control

To configure the intensifier gain control for use with an intensified CCD camera, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Configure Intensifier Gain Control. The Configure Intensifier Gain Control dialog box will appear. |
| **2** | Select your camera's model name from the *Intensifier Model* drop-down list. |
| **3** | Select the serial port used to connect the camera from the *Serial Port* drop-down list. |
| **4** | Select the appropriate baud rate for the connection from the *Baud* drop-down list. |
| **5** | To control the intensified CCD camera using the other intensified gain control commands in MetaMorph, select *Computer* from the *Camera Control* group.<br><br>**Note:** If your camera requires manual control for some features on the camera, select *Manual* before performing those operations. |
| **6** | Choose *OK.* |

## Configure Intensifier Gain Control - Dialog Box Options

### Intensifier Model

Specifies the intensified CCD camera model name.

### Serial Port

Specifies the serial port used to connect the camera to the computer.

### Baud

Specifies the baud rate used for the connection between the camera and the computer.

### Camera Control

Switches between manual control and computer control of the camera. Use *Manual* when you want to change settings on the camera that can only be accessed on the camera when it is not controlled by the computer. Use *Computer* when you want to control the camera from MetaMorph.

### OK

Configures the intensifier gain control options.

### Cancel

Cancels the command.

# Set Intensifier Gain (Acquire Menu)

**Sets the intensifier gain for use of an intensified CCD camera.**

**Drop-in: SETICCD**

You can use this command to set the intensifier gain interactively during acquisition. The same gain is used for all wavelengths that are acquired. You can change the gain setting by using a slider. Once you have changed the slider's value, the camera will use that value for all wavelengths acquired, starting with the next acquisition.

Prior to using this command, you must install its drop-in, SETICCD, using the Configure Drop-ins/Toolbars command in the Meta Imaging Series Administrator. You must also configure the intensifier using the Configure Intensifier Gain Control command and specify that the camera is controlled by the computer.

## Setting the Intensifier Gain

To set the intensifier gain, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Set Intensifier Gain. The Set Intensifier Gain dialog box will appear. |
| **2** | If you want a delay to occur after the gain has been changed, select the desired length using *Delay After Setting Gain.* |
| **3** | Begin your experiment. |
| **4** | To set the gain interactively, select the desired gain from the *Intensifier Gain* slider. |
| **5** | Choose *Close* when you have finished. |

## Set Intensifier Gain - Dialog Box Options

### Intensifier Gain

Selects an intensifier gain interactively.

### Delay After Setting Gain

Specifies the length of the delay to occur before the next acquisition once the gain is changed. This allows the intensifier system to lock in to the new setting before acquisition proceeds.

### Close

Closes the dialog box.

# Set Camera Level and Gain (Acquire Menu)

**Sets the intensified CCD camera's black level and video gain.**

**Drop-in: SETICCD**

Use this command to change the ICCD camera's black level and video gain. The control of the black level and video gain depends on the particular camera used. For some cameras, the black level and

video gain can be set only by using the computer after control of the camera has been turned over the computer. In some cases, you may be allowed to change the black level and video gain either manually or with this command. However, some cameras do not support this command, and you must change the black level and video gain manually.

Prior to using this command, you must install its drop-in, SETICCD, using the Configure Drop-ins/Toolbars command in the Meta Imaging Series Administrator. MetaMorph Drop-in Manager. You must also configure the intensifier using the Configure Intensifier Gain Control command and specify that the camera is controlled by the computer.

### Setting the Camera Level and Gain

To set the ICCD camera black level and gain, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Acquire menu, choose Set Camera Level and Gain. The Set CCD Level and Gain dialog box will appear. |
| **2** | Select the desired black level value from the *Black Level* slider. |
| **3** | Select the desired video gain value from the *Video Gain* slider. |
| **4** | Choose *Close.* |

### Set Camera Level and Gain - Dialog Box Options

#### Black Level

Specifies the current black level value used by the intensified CCD camera. For some cameras, the black level and video gain can be set only by using the computer after control of the camera has been turned over the computer. In some cases, you may be allowed to change the black level and video gain either manually or with this command. However, some cameras do not support this command and you must change the black level and video gain manually.

#### Video Gain

Specifies the current video used by the intensified CCD camera. For some cameras, the black level and video gain can be set only by using the computer after control of the camera has been turned over the computer. In some cases, you may be allowed to change the black level and video gain either manually or with this command. However, some cameras do not support this command and you must change the black level and video gain manually.

#### Close

Closes the dialog box.

## PI Video ICCD Settings (Acquire Menu)

**Changes the ICCD operating temperature and resets the intensifier if it shuts off due to overload. If you configured the system for direct gain control from the computer, additional setting controls that appear on the controller front panel will also be available.**

#### Drop-in: SETICCD

Use this command to reset the intensifier if it shuts itself down due to overload. You can also use the PI Video ICCD Settings command to specify an operating temperature for the cooled ICCD (the default setting is -10 degrees Centigrade).

If you specified direct control of the ICCD by the computer with the Configure Intensifier Gain Control command, a dozen other setting controls will appear in the PI Video ICCD Settings dialog box. For the most part, these correspond to controls that appear on the ICCD controller box, and include such options as enabling or disabling Auto-Black Level, Automatic Gain Control, responsiveness to external triggers,

Micro-Channel Plate (MCP) protection circuitry, and the like.

Prior to using this command, you must install its drop-in, SETICCD, using the Configure Drop-ins/Toolbars command in the Meta Imaging Series Administrator. You must also configure the intensifier using the Configure Intensifier Gain Control command.

## Specifying the PI Video ICCD Settings

To specify the PI video ICCD settings, use the following procedure:

| Step | Action |
|---|---|
| **1** | Follow the directions for **configuring the intensifier gain,** selecting one of the PI Video ICCD entries from the *Intensifier Model* drop-down list. |
| **2** | From the Acquire menu, select PI Video ICCD Settings. The PI Video ICCD Settings dialog box will appear. |
| **3** | If you want to change the operating temperature of the ICCD, use the *Temperature Set Point* spin box to specify the new temperature. The default setting is -10 degrees C. |
| **4** | If the intensifier has shut down due to overload, choose *Reset Intensifier If It Shut Off Due to Overload.* (Be sure all input to the camera is off!) |
| **5** | If you selected control of the ICCD by the control box *(PI Video ICCD - Control Box)* when you used the Configure Intensifier Gain Control command, you have finished. Now skip to Step 9.<br><br>**OR**<br><br>If you selected direct control of the ICCD by the computer *(PI Video ICCD - Direct)* when you used the Configure Intensifier gain Control command, you will see some additional options in the PI Video ICCD Settings dialog box. Continue to Step 6. |
| **6** | Depending on your experimental conditions, select or clear the *Advanced Settings* check boxes as necessary.<br><br>**Note:** If you are performing quantitative densitometric or ratiometric analysis of your images, you should leave *Enable Automatic Gain Control* deselected. |
| **7** | If you are using an integrating ICCD, you can specify the number of video frames to be integrated by using *Frames to Integrate.* |
| **8** | If you need to reset *Advanced Settings* or *Frames to Integrate* to the default values, choose *Reset to Defaults.* |
| **9** | Choose *Close* to close the dialog box. |

## PI Video ICCD Settings - Dialog Box Options

### Temperature Set Point

Specifies the operating temperature of the cooled ICCD. The default setting is *-10* degrees C.

### Reset Intensifier If It Shut Off Due to Overload

If your ICCD becomes saturated due to an overload of input, it will shut down as a protective measure. When this happens, you can reset it by choosing this command button.

### Advanced Settings

These check boxes can be selected or cleared independently, thereby emulating the controls on the ICCD controller box. These options will only be displayed if you selected *PI Video ICCD - Direct* as the *Intensifier Model* in the Configure Intensifier Gain Control dialog box. These options include the following:

*Enable Micro-Channel Plate Protection Circuitry* (default = enabled)
*Enable Gamma of 0.45* (default = disabled)
*Enable Automatic Gain Control* (default = disabled)
*Enable Continuous (CW) Intensifier Mode* (default = enabled)
*Positive Polarity EXT Trigger* (default = enabled)
*Not EXT Trigger Enabled* (default = enabled)
*Enable Odd Field for Trigger and Integration* (default = enabled)
*Enable Any Field for Trigger and Integration* (default = enabled)
*Turn Off Auto-Black Level* (default = enabled)
*INVERT Valid Polarity* (default = enabled)

### Frames to Integrate

If you are using an integrating ICCD, this option specifies the number of video frames to be integrated. A setting of *0* specifies no integration.

### Reset to Defaults

Resets the *Advanced Settings* check boxes and *Frames to Integrate* spin box to their default settings.

### Close

Closes the dialog box.

# Nikon Microscope

**The Nikon drop-in controls any Nikon automated microscope that uses the Nikon driver.**

### Drop-in: NIKON

The Nikon drop-in enables you to control the following:

- Fluorescence settings
- Transmitted light settings
- Light path settings
- Magnification settings

In addition, the drop-in lets you annotate an image with the Microscope settings, and log current settings.

**Notes:**

- The XY stage and Z focus are not controlled by this drop-in; they are controlled by the normal Stage and Focus settings.

Before you install the Nikon drop-in, remove the SCOPE drop-in using the Configure Drop-ins/Toolbar command in the Meta Imaging Series Administrator. These two drop-ins conflict with each other.

## Using the Nikon Microscope

To use a Nikon microscope, use the following procedure:

> **Note:** Some dialog box options in this procedure may not be enabled; this depends on the hardware components you are using. The Record buttons are enabled only when you are recording a journal.

| Step | Action |
|---|---|
| 1 | On the Devices menu, select Nikon Microscope. The Nikon Microscope dialog box opens.<br><br>**Note:** On the left is a status list of all your current settings. Use the different tabs on the dialog box to change these settings. |
| 2 | To configure the Fluorescence settings, click the *Fluor* tab. |
| 3 | To open/close the fluorescence shutter, check the *Shutter* box. |
| 4 | In the Filter Block group, select the fluorescence filter cube. |
| 5 | To configure the Transmitted settings, click the *Trans* tab. |
| 6 | Check the Lamp Shutter box to turn on/off the Halogen (transmitted) lamp. |
| 7 | In the Condenser group, choose a position for the condenser. |
| 8 | Use the Lamp Voltage slider to select the lamp voltage. |
| 9 | Select the position of the Analyzer from the Analyzer group. |
| 10 | To configure light path settings, click the *Light Path* tab. |
| 11 | Select the position of the optical path filter from the optical path group. |
| 12 | To configure magnification settings, click the *Magnification* tab. |
| 13 | In the Objective group, select the objective to use. |
| 14 | Use the *Z Res* slider to select the zoom level. |
| 15 | If you want to annotate your image with the status information under Current Settings, use the Image Annotation group. Select an image from the list next to *Image.* |
| 16 | Click the *Annotate Image with* |

*Microscope Settings* button to annotate your image.

17 Click the *Configure Log* button to select the parameters you want to send to a data log file.

18 To select a destination for the logged information, click the *Open Log* button.

19 If you make any changes to your microscope or its components, click the *Resync* button to update your settings in MetaMorph.

The *Resync* button will read the current microscope settings and reconfigure the dialog to reflect these settings.

20 Click *Close* to close the dialog box when you are finished.

## Nikon Microscope - Dialog Box Options

### Current Settings

#### Fluorescence

The parameters in this group are related to the fluorescence shutter (shutter), and the filter block.

#### Transmitted

This group contains parameters related to the halogen lamp (shutter), condenser, and analyzer.

#### Light Path

This group contains parameters related to the optical path filter.

#### Magnification

These parameters represent the objective and the zoom level.

### Fluor (Fluorescence) Tab

### Trans (Transmitted) Tab

### Light Path Tab

### Magnification Tab

### Image Annotation

#### Image

Selects an image to annotate with the microscope's current settings.

#### Annotate Image with Microscope Settings

Annotate your image with the status information under *Current Settings*.

### ReSync

Reads the current microscope settings and updates the dialog to reflect these settings.

### Configure Log

Allows the selection of image characteristics and data that are to be enabled or disabled from data logging. Also allows a choice of whether column titles are to be included and if data are to be listed on a single line.

### Open Log

Opens a data log file and/or a DDE link to an open spreadsheet application for logging data. This command will change to F9: Log Data when a log file is open.

### Close

Closes the dialog box.

## Nikon Microscope Dialog Box Options - Fluorescence Tab

> **Note:** The options listed on this tab will vary depending on the configuration of your microscope.

### Shutter

The shutter check box is used to open/close the fluorescence shutter.

### Record Shutter

Records the setting to a journal. This option is only enabled when recording a journal.

### Filter Block

Selects the active magnification setting for the filter.

### Record Filter

Records the setting to a journal. This option is only enabled when recording a journal.

## Nikon Microscope Dialog Box Options - Transmitted Tab

> **Note:** The options listed on this tab will vary depending on the configuration of your microscope.

### Lamp Shutter

Turns the lamp shutter on/off. The lamp functions as a virtual shutter, and is turned on and off as requrired.

### Record Lamp

Records the setting to a journal. This option is only enabled when recording a journal.

### Condenser

Selects the active condenser filter.

### Record Condenser

Records the setting to a journal. This option is only enabled when recording a journal.

### Lamp Voltage

Adjusts the illumination intensity of the transmitted light source. When the shutter is open, changes in intensity are instaneous; when the shutter is closed, intensity changes are applied when the shutter is opened. **Note:** The displayed lamp voltage value provides a reference to which the voltage can be reset either manually or using a journal.

### Record Voltage

Records the setting to a journal. This option is only enabled when recording a journal.

### Analyzer

Selects the position of the Analyzer.

### Record Analyzer

Records the setting to a journal. This option is only enabled when recording a journal.

### Nikon Microscope Dialog Box Options - Light Path Tab

> **Note:** The options listed on this tab will vary depending on the configuration of your microscope.

**Optical Path**
Selects the active optical path filter.

**Record Default Path**
Records the setting to a journal. This option is only enabled when recording a journal.

### Nikon Microscope Dialog Box Options - Magnification Tab

> **Note:** The options listed on this tab will vary depending on the configuration of your microscope.

**Objective**
Selects the active objective.

**Record Objective**
Records the setting to a journal. This option is only enabled when recording a journal.

**Z Res**
Selects the zoom level to the camera.

**Record Z Res**
Records the setting to a journal. This option is only enabled when recording a journal.

# Olympus Microscope

**The Olympus drop-in controls any Olympus automated microscope that uses the Olympus driver.**

**Drop-in: OLYMPUS**

The Olympus drop-in enables you to control the following:

- Fluorescence settings
- Bright Field settings
- Transmitted light settings
- Light path settings
- Magnification settings

In addition, the drop-in lets you annotate an image with the Olympus settings, and log current settings.

**Notes:**

- The XY stage and Z focus are not controlled by this drop-in; they are controlled by the normal Stage and Focus settings.

Before you install the OLYMPUS drop-in, remove the SCOPE drop-in using the Configure Drop-ins/Toolbar command in the Meta Imaging Series Administrator. These two drop-ins conflict with each other.

## Using the Olympus Microscope

To use an Olympus microscope, use the following procedure:

> **Note:** Some dialog box options in this procedure may not be enabled; this depends on the hardware components you are using. The Record buttons are enabled only when you are recording a journal.

| Step | Action |
|---|---|
| 1 | On the Devices menu, select Olympus Microscope. The Olympus Microscope dialog box opens.<br><br>**Note:** On the left is a status list of all your current settings. Use the different tabs on the dialog box to change these settings. |
| 2 | To configure the Fluorescence settings, click the *Fluor* tab. |
| 3 | To open/close the fluorescence shutter, check the *Shutter* box. |
| 4 | In the Filter Cube group, select the fluorescence filter cube. |
| 5 | To configure the Bright Field settings, click the Bright Field tab. |
| 6 | In the Top Lens group, select a filter. |
| 7 | In the Condenser group, select a filter. |
| 8 | Select an aperture location from the Aperture group. |
| 9 | To configure the Transmitted settings, click the *Trans* tab. |
| 10 | Check the *Halogen Lamp* box to turn on/off the halogen (transmitted) lamp. |
| 11 | Use the Lamp Voltage slider to select the lamp voltage. |
| 12 | Check the *DIA Shutter* box to turn on/off the DIA shutter. |
| 13 | Select the position of ND filter wheel 1 from the ND Filter Wheel 1 group. |
| 14 | Select the position of ND filter wheel 2 from the ND Filter Wheel 2 group. |
| 15 | To configure light path settings, click the *Light Path* tab. |
| 16 | Select the camera filter to use from the Camera Port group. |
| 17 | To configure magnification settings, click the *Magnification* tab. |
| 18 | In the Objective Lens group, select the |

objective to use.

19 Use the *Z Res* slider to select the zoom level.

20 If you want to annotate your image with the status information under Current Settings, use the Image Annotation group. Select an image from the list next to *Image.*

21 Click the *Annotate Image with Microscope Settings* button to annotate your image.

22 Click the *Configure Log* button to select the parameters you want to send to a data log file.

23 To select a destination for the logged information, click the *Open Log* button.

24 If you make any changes to your microscope or its components, click the *Resync* button to update your settings in MetaMorph.

The *Resync* button will read the current microscope settings and reconfigure the dialog to reflect these settings.

25 Click *Close* to close the dialog box when you are finished.

## Olympus Dialog Box Options - Main

### Current Settings

#### Fluorescence

The parameters in this group are related to the fluorescence shutter (shutter) and the filter cube.

#### Bright Field

The parameters in this group are related to the top lens, aperture and condenser.

#### Transmitted

This group contains parameters related to the halogen lamp (shutter), two neutral density filter wheels, and the DIA shutter.

#### Light Path

This group contains parameters related to the camera port filter.

#### Magnification

These parameters represent the objective lens.

### Fluor (Fluorescence) Tab

### Bright Field Tab

### Trans (Transmitted) Tab

**Light Path Tab**

**Magnification Tab**

**Image Annotation**

**Image**

Selects an image to annotate with the microscope's current settings.

**Annotate Image with Microscope Settings**

Annotate your image with the status information under *Current Settings*.

**ReSync**

Reads the current microscope settings and updates the dialog to reflect these settings.

**Configure Log**

Allows the selection of image characteristics and data that are to be enabled or disabled from data logging. Also allows a choice of whether column titles are to be included and if data are to be listed on a single line.

**Open Log**

Opens a data log file and/or a DDE link to an open spreadsheet application for logging data. This command will change to F9: Log Data when a log file is open.

**Close**

Closes the dialog box.

## Olympus Microscope Dialog Box Options - Fluorescence Tab

**Note:** The options listed on this tab will vary depending on the configuration of your microscope.

**Shutter**

The shutter check box is used to open/close the fluorescence shutter.

**Record Shutter**

Records the setting to a journal. This option is only enabled when recording a journal.

**Filter Cube**

Selects the active filter cube.

**Record Cube**

Records the setting to a journal. This option is only enabled when recording a journal.

## Olympus Microscope Dialog Box Options - Bright Field Tab

**Note:** The options listed on this tab will vary depending on the configuration of your microscope.

**Top Lens**

Selects the active top lens filter.

**Record Top Lens**

Records the setting to a journal. This option is only enabled when recording a journal.

**Aperture**

Adjusts the opening size of the aperture.

### Record Aperture

Records the aperture setting to a journal. This option is only enabled when recording a journal.

### Condenser

Selects the active condenser filter.

### Record Condenser

Records the setting to a journal. This option is only enabled when recording a journal.

## Olympus Dialog Box Options - Transmitted Tab

> **Note:** The options listed on this tab will vary depending on the configuration of your microscope.

### Halogen Lamp

Turns the halogen (transmitted lamp) on/off.

### Record Lamp

Records the lamp selection setting to a journal. This option is only enabled when recording a journal.

### Lamp Voltage

Adjusts the illumination intensity of the transmitted light source. When the shutter is open, changes in intensity are instaneous; when the shutter is closed, intensity changes are applied when the shutter is opened. **Note:** The displayed lamp voltage value provides a reference to which the voltage can be reset either manually or using a journal.

### Record Voltage

Records the lamp voltage setting to a journal. This option is only enabled when recording a journal.

### DIA Shutter

Opens/Closes the DIA shutter.

### Filter Wheel 1

In this group, choose an ND filter for transmitted light from ND wheel 1.

### Filter Wheel 2

In this group, choose an ND filter for transmitted light from ND wheel 2.

### Record ND1/2

Records the setting to a journal. This option is only enabled when recording a journal.

## Olympus Dialog Box Options - Light Path Tab

> **Note:** The options listed on this tab will vary depending on the configuration of your microscope.

### Camera Port

Selects the active camera port.

### Record Camera Port

Records the setting to a journal. This option is only enabled when recording a journal.

## Olympus Dialog Box Options - Mag Tab

**Note:** The options listed on this tab will vary depending on the configuration of your microscope.

**Objective Lens**

Selects the active objective.

**Record Objective**

Records the setting to a journal. This option is only enabled when recording a journal.

# Zeiss MTB Microscope

**The ZEISSMTB drop-in controls any Zeiss automated microscope that uses the Zeiss MTB driver.**

**Drop-in: ZEISSMTB**

The Zeiss MicroToolBox (MTB) driver and ZeissMTB drop-in enable you to control the following:

- Fluorescence settings
- Transmitted light settings
- Light path settings
- Magnification settings

In addition, the drop-in lets you annotate an image with the Zeiss settings, and log current settings.

**Notes:**

- The XY stage and Z focus are not controlled by this drop-in; they are controlled by the normal Stage and Focus settings.
- The Zeiss driver update enables support for switching between the front and back camera ports on microscopes that are configured with dual motorized ports.
- The Zeiss MTB device driver optionally auto-lowers the stage before any objective change. This feature can be enabled from the device configuration dialog for the driver.
- Before you install the ZEISSMTB drop-in, remove the SCOPE drop-in using the Configure Drop-Ins dialog box in the Meta Imaging Series Administrator. These two drop-ins conflict with each other.

## Using the Zeiss MTB Microscope

To use a Zeiss MTB microscope, use the following procedure:

**Note:** Some dialog box options in this procedure may not be enabled; this depends on the hardware components you are using. The Record buttons are enabled only when you are recording a journal.

| Step | Action |
|---|---|
| **1** | On the Devices menu, select Zeiss MTB Microscope. The Zeiss MTB Microscope dialog box opens.<br><br>**Note:** On the left is a status list of all your current settings. Use the different tabs on the dialog box to change these settings. |
| **2** | To configure the Fluorescence settings, click |

the *Fluor* tab.

3. To open/close the fluorescence shutter, check the *Shutter* box.
4. In the Reflector Turret group, select the fluorescence filter cube.
5. In the Exciter Filter group, select the wavelength from the excitation filter wheel.
6. In the Barrier Filter group, select the emission filter for fluorescence you wish to use from the Barrier Filter wheel.
7. To configure the Transmitted settings, click the *Trans* tab.
8. Check the Halogen Lamp box to turn on/off the Halogen (transmitted) lamp.
9. In the Filter Wheel 1 group, choose an ND filter for transmitted light from ND wheel 1.
10. In the Filter Wheel 2 group, choose an ND filter for transmitted light from ND wheel 2.
11. To configure the light path settings, click the *Light Path* tab.
12. In the Lower Prism group, select the lower prism filter to use.
13. In the Upper Prism group, select the upper prism filter to use.
14. To configure magnification settings, click the *Magnification* tab.
15. In the Objective Nosepiece group, select the objective you want to use.
16. In the Zoom group, select the zoom level.
17. If you want to annotate your image with the status information under Current Settings, use the Image Annotation group. Select an image from the list next to *Image.*
18. Click the *Annotate Image with Microscope Settings* button to annotate your image.
19. Click the *Configure Log* button to select the parameters you want to send to a data log file.
20. To select a destination for the logged information, click the *Open Log* button.
21. If you make any changes to your microscope or its components, click the

*Resync* button to update your settings in MetaMorph.

The *Resync* button will read the current microscope settings and reconfigure the dialog to reflect these settings.

22 Click *Close* to close the dialog box when you are finished.

## Zeiss MTB Microscope Dialog Box Options - Main

### Current Settings

#### Fluorescence

The parameters in this group are related to the fluorescence shutter (shutter), the reflector turret (filter cube), and the excitation and barrier (emission) filter wheels.

#### Transmitted

This group contains parameters related to the halogen lamp (shutter) and two neutral density filter wheels.

#### Light Path

The objects in this group are related to the two prisms (upper and lower) that direct light to either the documentation port (cameras) or to the eyepieces, and when light is going to the documentation port, to the appropriate camera port on that module.

#### Magnification

These parameters represent the objective nosepiece and the zoom.

### Fluor (Fluorescence) Tab

### Trans (Transmitted) Tab

### Light Path Tab

### Magnification Tab

### Image Annotation

#### Image

Selects an image to annotate with the microscope's current settings.

#### Annotate Image with Microscope Settings

Annotate your image with the status information under *Current Settings*.

### ReSync

Reads the current microscope settings and updates the dialog to reflect these settings.

### Configure Log

Allows the selection of image characteristics and data that are to be enabled or disabled from data logging. Also allows a choice of whether column titles are to be included and if data are to be listed on a single line.

### Open Log

Opens a data log file and/or a DDE link to an open spreadsheet application for logging data. This command will change to F9: Log Data when a log file is open.

### Close

Closes the dialog box.

## Zeiss MTB Microscope Dialog Box Options - Fluorescence Tab

> **Note:** The options listed on this tab will vary depending on the configuration of your microscope.

### Shutter

The shutter check box is used to open/close the fluorescence shutter.

### Record State

Records the setting to a journal. This option is only enabled when recording a journal.

### Reflector Turret

Selects a reflector turret.

### Record Refractor

Records the setting to a journal. This option is only enabled when recording a journal.

### Exciter Filter

Selects the wavelength you want to use from the excitation filter wheel.

### Record Exciter

Records the setting to a journal. This option is only enabled when recording a journal.

### Barrier Filter

This group is used for selecting the emission filter for fluorescence you want to use from the Barrier Filter Wheel.

### Record Barrier

Records the setting to a journal. This option is only enabled when recording a journal.

## Zeiss MTB Microscope Dialog Box Options - Transmitted Tab

> **Note:** The options listed on this tab will vary depending on the configuration of your microscope.

### Halogen Lamp

Turns the halogen (transmitted lamp) on/off.

### Record Lamp

Records the setting to a journal. This option is only enabled when recording a journal.

### Filter Wheel 1

In this group, choose an ND filter for transmitted light from ND wheel 1.

### Filter Wheel 2

In this group, choose an ND filter for transmitted light from ND wheel 2.

### Record ND1/2

Records the setting to a journal. This option is only enabled when recording a journal.

## Zeiss MTB Microscope Dialog Box Options - Light Path Tab

**Note:** The options listed on this tab will vary depending on the configuration of your microscope.

**Lower Prism**

In the Lower Prism group (the lower prism is the output prism), select where the light should be directed. For example, 100% doc would send all of the light to the camera, while 100% visual would send it all to the eyepiece.

**Record Lower Prism**

Records the setting to a journal. This option is only enabled when recording a journal.

**Upper Prism**

In the Upper Prism group, select which camera will receive the image.

**Record Upper Prism**

Records the setting to a journal. This option is only enabled when recording a journal.

### Zeiss MTB Microscope Dialog Box Options - Magnification Tab

**Note:** The options listed on this tab will vary depending on the configuration of your microscope.

**Objective Nosepiece**

Selects the active objective.

**Record Objective**

Records the setting to a journal. This option is only enabled when recording a journal.

**Zoom**

Select the zoom to the camera.

**Record Zoom**

Records the setting to a journal. This option is only enabled when recording a journal.

# Leica DMR Microscope

**The LEICADM drop-in controls any Leica automated microscope that uses the Leica DMR driver.**

**Drop-in: LEICADM**

The Leica drop-in enables you to control the following:

- Fluorescence settings
- Transmitted light settings
- Light path settings
- Magnification settings

In addition, the drop-in lets you annotate an image with the Leica settings, and log current settings.

**Notes:**

- The XY stage and Z focus are not controlled by this drop-in; they are controlled by the normal Stage and Focus settings.

- Before you install the LEICADM drop-in, remove the SCOPE drop-in using the Configure Drop-Ins dialog box in the Meta Imaging Series Administrator. These two drop-ins conflict with each other.

## Using the Leica DMR Microscope

To use the Leica DMR, use the following procedure:

**Note:** Some dialog box options in this procedure may not be enabled; this depends on the hardware components you are using. The Record buttons are enabled only when you are recording a journal.

| Step | Action |
|---|---|
| **1** | On the Devices menu, select Leica DMR Microscope. The Leica DMR Microscope dialog box opens.<br><br>**Note:** On the left is a status list of all your current settings. Use the different tabs on the dialog box to change these settings. |
| **2** | To configure the Fluorescence settings, click the *Fluor* tab. |
| **3** | To open/close the fluorescence shutter, check the *Shutter* box. |
| **4** | In the Filter Cube group, select the fluorescence filter cube. |
| **5** | To configure the Transmitted settings, click the *Trans* tab. |
| **6** | Check the *Top Lens* box to turn on/off the top lens. |
| **7** | Use the Lamp Voltage slider to select the lamp voltage. |
| **8** | Use the Field Diaphragm slider to select the field diaphragm position. |
| **9** | Use the Aperture Diaphragm slider to select the aperture diaphragm position. |
| **10** | To configure the Light Path settings, click the *Light Path* tab. |
| **11** | In the Prism #1 group, select a port to receive light from the prism. |
| **12** | In the Prism #2 group, select a port to receive light from the prism. |
| **13** | In the Tube Optic group, select the position of the tube optic. |
| **14** | In the Beam Splitter group, select the position of the beam splitter. |
| **15** | To configure magnification settings, click the *Magnification* tab. |
| **16** | In the Objective Turret group, select the objective you want to use. |

17 In the Zoom group, select the zoom level.

18 If you want to annotate your image with the status information under Current Settings, use the Image Annotation group. Select an image from the list next to *Image.*

19 Click the *Annotate Image with Microscope Settings* button to annotate your image.

20 Click the *Configure Log* button to select the parameters you want to send to a data log file.

21 To select a destination for the logged information, click the *Open Log* button.

22 If you make any changes to your microscope or its components, click the *Resync* button to update your settings in MetaMorph.

The *Resync* button will read the current microscope settings and reconfigure the dialog to reflect these settings.

23 Click *Close* to close the dialog box when you are finished.

## Leica DMR Dialog Box Options - Main

### Current Settings

#### Fluorescence

The parameters in this group are related to the fluorescence shutter (shutter) and the filter cube.

#### Transmitted

This group contains parameters related to the top lens, lamp voltage, field diaphragm and aperture diaphragm.

#### Light Path

This group contains parameters related to the two prisms (upper and lower) that direct light to either the documentation port (cameras) or to the eyepieces, as well as the tube optic and beam splitter.

#### Magnification

These parameters represent the objective nosepiece and the zoom level.

### Fluor (Fluorescence) Tab

### Trans (Transmitted) Tab

### Light Path Tab

### Magnification Tab

### Image Annotation

#### Image

Selects an image to annotate with the microscope's current settings.

#### Annotate Image with Microscope Settings

Annotate your image with the status information under *Current Settings*.

### ReSync

Reads the current microscope settings and updates the dialog to reflect these settings.

### Configure Log

Allows the selection of image characteristics and data that are to be enabled or disabled from data logging. Also allows a choice of whether column titles are to be included and if data are to be listed on a single line.

### Open Log

Opens a data log file and/or a DDE link to an open spreadsheet application for logging data. This command will change to F9: Log Data when a log file is open.

### Close

Closes the dialog box.

## Leica DMR Microscope Dialog Box Options - Fluor Tab

**Note:** The options listed on this tab will vary depending on the configuration of your microscope.

### Shutter

The shutter check box is used to open/close the fluorescence shutter.

### Record State

Records the setting to a journal. This option is only enabled when recording a journal.

### Filter Cube

Selects the active filter cube.

### Record Cube

Records the setting to a journal. This option is only enabled when recording a journal.

## Leica DMR Microscope Dialog Box Options - Trans Tab

**Note:** The options listed on this tab will vary depending on the configuration of your microscope.

### Top Lens

Opens and closes the top lens.

### Record Top Lens

Records the setting to a journal. This option is only enabled when recording a journal.

### Lamp Voltage

In this group, choose an ND filter for transmitted light from ND wheel 1.

### Record Voltage

Records the setting to a journal. This option is only enabled when recording a journal.

### Field Diaphragm

Adjusts the position of the field diaphragm.

### Record Field

Records the setting to a journal. This option is only enabled when recording a journal.

### Aperture Diaphragm

Adjusts the position of the aperture diaphragm.

### Record Aperture

Records the setting to a journal. This option is only enabled when recording a journal.

## Leica DMR Microscope Dialog Box Options - Light Path Tab

> **Note:** The options listed on this tab will vary depending on the configuration of your microscope.

### Prism #1

Selects which port the light should be directed to. For example, select *Visual Port* to send all of the light to the eyepiece.

### Record Prism #1

Records the setting to a journal. This option is only enabled when recording a journal.

### Prism #2

Selects which port the light should be directed to. For example, select *Visual Port* to send all of the light to the eyepiece.

### Record Prism #2

Records the setting to a journal. This option is only enabled when recording a journal.

### Tube Optic

Selects the position of the tube optic.

### Record Tube Optic

Records the setting to a journal. This option is only enabled when recording a journal.

### Beam Splitter

Selects the position of the beam splitter.

### Record Beam Splitter

Records the setting to a journal. This option is only enabled when recording a journal.

## Leica DMR Microscope Dialog Box Options - Magnification Tab

> **Note:** The options listed on this tab will vary depending on the configuration of your microscope.

### Objective Turret

Selects the active objective.

### Record Objective

Records the setting to a journal. This option is only enabled when recording a journal.

### Zoom

Select the zoom to the camera.

### Record Zoom

Records the setting to a journal. This option is only enabled when recording a journal.

**Immersion Mode**

**Immersion Mode**

Enables you to select any of the immersion objectives as defined in the Leica software.

**Dry Mode**

Enables you to select any of the dry objectives as defined in the Leica software.

**Record Immersion Mode**

Records the setting to a journal. This option is only enabled when recording a journal.

# Linkam MDS600/TMS93 Stage

**Controls the settings and configuration to create the initial setup to use the Linkam MDS 600 Motor-Driven, Heated Stage, and the Linkam TMS 93 Temperature Programmer, which provides an interface for programmatic temperature control of the Linkam MDS 600 Motor-Driven Stage. The Heating Sequence dialog box enables you to make settings and schedule journals to control the Linkam TMS 93 Temperature Programmer.**

**Drop-in: LINKAM**

Use this dialog box to make the initial settings for the Linkam MDS 600 Motor-Driven Stage. This dialog box controls the activation and setting of the stage heating unit and the Liquid Nitrogen Pump (LNP) stage cooling unit. These two units combine to provide precise stage temperature control and the ability to quickly and accurately raise or lower the temperature of the stage. The heating unit can be used without the LNP Pump, but cooling time will be longer. The heating unit can be set to hold a specific temperature, or to raise or lower the stage temperature. The LNP Pump enables you to more easily maintain a specific temperature or to raise or lower the temperature at a very precise rate.

## Using the Linkam MDS600/TMS93 Stage

To use the controls on the primary area of the Linkam MDS600/TM93 Stage dialog box, complete the following steps.

| Step | Action |
|---|---|
| **1** | From the Devices menu, click Linkam MDS600/TM93 Stage, the Linkam MDS600/TM93 Stage dialog box opens. |
| **2** | Click the appropriate arrow button to change the stage position, or click *Go To Origin* to move the stage to the Origin position set in either the *Move Stage to Absolute Position* or the *Move Stage to Relative Position* dialog box. |
| **3** | Click the appropriate radio button in the Heater box. Click *On* to turn on the heater, *Off* to turn off the heater, or click *Hold* to hold the currently set temperature. |
| **4** | Observe the current stage temperature in Celsius next to *Temp*: |
| **5** | In the *LNP Pump* box, click the appropriate button to either turn on or turn off the LNP pump. |
| **6** | If the dialog box is minimized, click *More* to |

maximize the dialog box and access the Settings. Click *Less* to minimizes the dialog box.

**7** Click *Close* after all settings are complete or to close the dialog box.

## Setting the Linkam MDS600/TMS93 Stage

To make settings in the Linkam MDS600/TM93 Stage dialog box, complete the following steps.

| Step | Action |
|---|---|
| **1** | From the Devices menu, click Linkam MDS600/TM93 Stage, the Linkam MDS600/TM93 Stage dialog box opens. |
| **2** | In the *Stage increment* box, type or select the stage increment value to specify the distance you want the stage to move for a single click on any arrow button. |
| **3** | In the *Stage Temperature* box, type or select using the small arrow buttons or the slider control, the temperature that you want to achieve or maintain on the stage. |
| **4** | In the *Heating Rate* box, type or select using the small arrow buttons or the slider control, the heating rate in degrees Celsius that you want to achieve or that you want to maintain on the stage. |
| **5** | In the *LNP Pump Speed* box, type or select using the small arrow buttons or the slider control, the pump speed at which you want to operate the LNP Pump. |
| **6** | In the *LNP Pump Mode* box, click *Manual* to operate the LNP Pump manually, or *Automatic* to operate the pump automatically. In manual mode, you must set the *LNP Pump speed* to control how much cooling you are delivering to the stage. In automatic mode, the controller regulates the LNP pump speed to maintain or change the temperature. |

## Linkam MDS0600/TMS93 Stage - Dialog Box Options

### (Arrow Keys)

Moves the stage to the selected position. Use either the *Move Stage to Absolute Position* or *Move Stage to Relative Position* dialog box to specify the limits of movement for this stage.

### Go To Origin

Moves the stage directly to the predefined origin position. Use either the *Move Stage to Absolute Position* or *Move Stage to Relative Position* dialog box to set the location of the Origin position.

### Heater

Controls the heater unit in the stage. Use these controls to turn the heat on or off, or hold the heat at the current temperature.

***On*** – Turns on the stage heating unit.

***Off*** – Turns off the stage heating unit.

***Hold*** – Holds the stage temperature at the current temperature setting.

### Temp

Indicates the current stage temperature in degrees Celsius.

### LNP Pump

Specifies whether the Liquid Nitrogen Pump is off or on.

### Less<</More>>

Minimizes (collapses) or Maximizes (expands) the dialog box.

### Close

Closes the dialog box.

### Settings

#### Stage Increment

Specifies the amount of increment for stage movements when using the arrow buttons in this dialog box. This value is based on values set in either the *Move Stage to Absolute Position* or *Move Stage to Relative Position* dialog box.

#### Stage Temperature

Specifies the target temperature of the stage in degrees Celsius.

#### Heating Rate

Specifies the rate in degrees Celsius at which you want the specimen to reach the target temperature.

#### LNP Pump Speed

Specifies the pump speed to control the liquid nitrogen's rate of circulation.

#### LNP Pump Mode

Specifies the liquid nitrogen pump mode.

***Manual*** – Causes the pump to run continuously when *LNP Pump* is set to *On*. To control the temperature in manual mode, you must adjust the *LNP Pump Speed* manually to obtain the correct amount of cooling to maintain the desired temperature or to raise or lower the temperature.

***Automatic*** – Enables the LNP Pump to be controlled by the program. The controller determines when the LNP Pump needs to run to maintain the current temperature or to raise or lower the temperature.

# Heating Sequence

**Defines one or more heating sequences for the Linkam MDS600 Heated Stage and sends commands to the TMS93 Stage Controller to complete the sequences.**

#### Drop-in: LINKAM

Use this dialog box to specify sequential heating and cooling sequences for the Linkam MDS600 heated stage. Sequences that you specify in this dialog box are sent to the TMS93 Stage Controller for execution. You can create sequences that contain as many as 9,999 steps. Each step can initiate a journal to run either at the beginning of the ramping of the heating sequence, or at the end of the temperature holding interval. Journals can be either run once during a heating sequence step, or run repeatedly during a single step at an interval that you specify as the Journal Rate in seconds for a maximum 32,767 seconds interval.

## Setting up a Heating Sequence

To configure a heating sequence for the Linkam MDS600 Heated Stage and the TMS93 Stage Controller, complete the following procedure.

| Step | Action |
|---|---|
| **1** | From the Devices menu, click *Heating Sequence*. The Heating Sequence dialog box opens. |
| **2** | If you have a prepared heating sequence that you want to use, click *Load Sequence* to open the Select Sequence dialog box, then select the sequence that you want to use and click *Open*. Use the remaining steps to modify your sequence as needed. If you are creating a new sequence, skip this step. |
| **3** | In the *Loops in Sequence* dialog box, type or select the number of loops that you want to occur in your heating sequence. |
| **4** | In the *# of Steps in Sequence* box, type or select the number of steps that you want to include in your sequence. |
| **5** | If you are running one or more journals in conjunction with any step(s), in the *Journal Acquisition Style* box, select the option that initiates your journal at the appropriate time. Click *Run Journal during Ramping* if you want to start your journal at same time that ramping starts; click *Run Journal after Holding* if you want to start your journal after the Holding Time is complete. |
| **6** | For each sequence step that you have enabled, ensure that the activation box is checked.<br><br>**Note:** You can uncheck this box to temporarily exclude this step from the sequence, without loosing the setting for that step. |
| **7** | In the *Heating Rate* box, type or select the appropriate heating rate in degrees Celsius per minute. |
| **8** | In the *Target Temperature* box, type or select the appropriate target temperature that you want to attain for your sample. |
| **9** | In the *Holding Time* box, type or select the time in seconds for which you want to hold the target temperature. |
| **10** | Click *Select Journal* to assign a journal to be run in conjunction with this step. |
| **11** | Click *Run Once* if you will be running your selected journal only one time in conjunction with this step. |
| **12** | In the *Journal Rate* box, type or select the reoccurrence rate in seconds. |

**13** After you have configured your heating sequence, click *Save Sequence* to save it to a Save Sequence file (.hss)

**14** Click *Run Sequence* to run the heating sequence that you configured or loaded.

## Heating Sequence - Dialog Box Options

### # of Loops in Sequence

Specifies the number of loops in the sequence that you want to occur during your experiment.

### Sequence

Enables you to specify the attributes for the heating sequence.

### #Steps in Sequence

Specifies the number of heating steps that you want to occur during each loop in the heating sequence. A maximum of 9,999 steps can be included in the heating sequence.

### Journal Acquisition Style

Specifies whether the journal that you selected to run with this heating sequence step is to be run during the heating ramp-up or down or once the sequence step has completed the specified holding time.

### Run Journal during Ramping

Causes your selected journal to be run in conjunction with ramping from the previous temperature to the target temperature. The journal is started when ramping begins.

### Run Journal after Holding

Causes your selected journal to be run at the end of the specified holding time.

#

Indicates the step number in the sequence.

### (checkbox)

When checked, specifies that the step be included in the sequence. Uncheck this box to exclude the step from the sequence.

### Heating Rate [C/min]

Specifies the rate in degrees Celsius per minute at which the temperature of the stage and sample is raised or lowered.

### Target Temperature [C]

Specifies the temperature in degrees Celsius to which you want to raise or lower the temperature of the sample or stage.

### Holding Time

Specifies the amount of time that the sample and stage will be kept at the target temperature.

### Select Journal

Selects the journal that you want to run in conjunction with the associated step.

### Journal Name

Indicates the name of the journal that you selected to be run in conjunction with this specific heating sequence step.

### Run Once

When checked, specifies that the selected journal is to be run only once in conjunction with the associated step. Uncheck this box to enable you to specify the number of times to run the journal.

### Journal rate [sec]

Specifies the number of seconds between the repeated initiation of the journal that you selected to run in conjunction with a specific step in the heating sequence. The repeat interval can be set for a maximum of 32,767 seconds.

### Save Sequence

Opens the *Save Sequence File* dialog box. Use this option to save detailed heating sequences that you plan to reuse or to save and modify for future use. Type the name that you want to assign to Sequence File in the file name box, and click *Save*.

### Load Sequence

Opens the *Load Sequence File* dialog box. Use this option to open and load a previously saved Sequence File.

### Run Sequence

Runs the currently loaded sequence.

### Cancel

Closes the *Heating Sequence* dialog box.

# Kodak MotionCorder (Devices Menu)

**Acquires and plays back images with the Kodak MotionCorder digital video device.**

### Drop-in: KODAK

Use this command in either of two ways: *Live* or *Playback.* The *Live* mode configures acquisition for the Kodak MotionCorder and acquires images using the specified acquisition rate and frame size. Acquired images can then be played back on the MotionCorder using the *Playback* buttons in the same manner as the buttons on a VCR. However, the transfer of images from the camera controller into MetaMorph for processing and analysis requires that you follow one of two possible additional procedures. In one method, you first must create a journal to step the controller forward by one frame (see the "Creating a Journal for Image Transfer" procedure). You will then need to "loop" the journal by a number of iterations appropriate to the number of frames you want to transfer (see the "Running the Image Transfer Journal" procedure). In the second method, you will need to use the Kodak Readcam program which is supplied with your MotionCorder. This program will download the images from the MotionCorder via your computer's SCSI interface.

# Kodak MotionCorder Procedures

**Acquiring Images**

**Playing Back Images**

**Creating a Journal for Image Transfer**

**Running the Image Transfer Journal**

**Configuring Communications**

## Acquiring Images with the Kodak MotionCorder

To configure acquisition and then acquire images with the Kodak MotionCorder, use the following procedure. Before you start, however, you should first create a Data Stream setting with a serial communications component by using the Install and Configure Devices command (Devices menu).

| Step | Action |
|---|---|

**1** From the Devices menu, choose Kodak MotionCorder. The Kodak MotionCorder dialog box will appear.

**2** If you have not already done so, **configure the serial communications** between the camera controller and your computer. You should only have to perform this routine once.

**3** Select *Live* from the *Playback/Live* radio button group.

**4** Use the *Acquisition Rate* drop-down list to configure the acquisition rate for time-lapse recording. This does not need to be the same as the display rate you intend to use for playback (the latter typically being the 30 frames/s "video" rate).

**5** Use the *Acquisition Size* drop-down list to set the frame size for acquisition.

**Note:** Depending on the acquisition rate you selected in Step 4, the camera may not be able to acquire frames above a certain size.

**6** If you want to adjust the linearity of the relationship between pixel intensity and the video signal amplitude, adjust the gamma value by selecting from the *Gamma Adjustment* drop-down list.

The appropriate value will depend on the characteristics of the specimen, but, for most part, a value at or near 1.0 will be sufficient.

**7** If you are using an illumination setting , select the setting from the *Illumination* drop-down list. Otherwise, select *"[None]."*

**8** If you want to specify an acquisition region, choose *Set Acq Rgn.* The Set Acquisition Region dialog box will appear, and a full-frame image will be acquired and displayed.

**AND**
Use the Rectangular Region Tool to draw a region on the image. Then choose *Use Active Region.* This region will be used for all subsequently acquired image frames.

**9** Click the *Record Ready* button to put the camera controller into the ready state.

The *Record Ready* button will become the *Cancel* button, which will allow you terminate the recording.

**10** When you are ready to record, click *Trigger Record.*

The MotionCorder will acquire frames into the MotionCorder controller until all memory is filled or until you choose *Cancel.*

**11** Choose *Close* to close the dialog box.

## Playing Back Images with the Kodak MotionCorder

To play back the current image data recorded by the MotionCorder, use the following procedure. Note, however, that this will only display the frames. If you want to save the frames in a stack file for subsequent processing and analysis, you will need to follow the procedures for creating and then running an image transfer journal.

| Step | Action |
|---|---|
| **1** | From the Devices menu, choose Kodak MotionCorder. The Kodak MotionCorder dialog box will appear. |
| **2** | Select *Playback* from the *Playback/Live* radio button group. |
| **3** | Select a playback rate from the *Display Rate* drop-down list. Typically, this will be *30fps.* |
| **4** | Use the buttons in the Playback option group to control the playback of the image frames.<br><br>You can play in reverse, stop, play forward, step backward, and step forward by clicking the appropriate button. You can go to a specific frame by selecting the frame number with the *Go to Frame* spin box and clicking the *Go to Frame* command button. |

## Creating a Journal for Image Transfer

To transfer images from the MotionCorder controller to MetaMorph, you will first need to create a journal that steps the camera controller forward by one step. You will then need to run the journal in a loop.

To create the stepping journal, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Devices menu, choose Kodak MotionCorder. The Kodak MotionCorder dialog box will appear. |
| **2** | From the Journal menu, choose Start Recording. The MetaMorph Imaging System title bar will display the message "[Recording]." |
| **3** | From the first part of the *Dest* image selector, choose *Add To.*<br><br>**AND**<br>In the second part, select *Specified.* Then type a name for the image in the Specify Image Name dialog box that appears and choose *OK.* |
| **4** | In the Kodak MotionCorder dialog box, choose *Acquire.* |
| **5** | Choose the Step Forward button (it will be marked with an asterisk). |
| **6** | From the Journal menu, choose Stop Recording. The Save Journal As dialog box will appear. |

| Step | Action |
|---|---|
| **7** | Type the desired file name for the journal in the *File Name* text box, such as "Kodak." MetaMorph will assign the file extension ".jnl" to your file name. If necessary, use the *Save In* list or Up One Level button to locate the appropriate drive and folder.<br><br>**AND**<br><br>Choose *Save.* |
| **8** | When the Record Journal dialog box appears, choose *No* to skip adding the newly created journal to the current taskbar. |

## Running the Image Transfer Journal

To run the stepping journal in a loop, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Kodak MotionCorder dialog box's *Display Rate* drop-down list, select *30fps.* |
| **2** | Use the *Go to Frame* spin box and command button, or the Step Forward button, to go to the specific frame at which you want to start image transfer. |
| **3** | From the Journal menu, choose Loop a Journal. The Loop a Journal dialog box will appear. |
| **4** | Select the desired number of loops using the *Number of Loops* text box or spin control. This should correspond to the number of frames you want to transfer from the MotionCorder. |
| **5** | If you want to confirm each loop during playback, select *Confirm Each Loop.*<br><br>The message "Continue Looping Journal?" will appear before each loop during playback, to which you can choose *Yes* or *No.* |
| **6** | Choose *Press to Select the Journal to Loop* to select the journal that you want looped. The Select Journal dialog box will appear.<br><br>**AND**<br><br>Select the journal file. If necessary, use the *Look In* list or Up One Level button to locate the correct drive and folder. Then choose *Open.*<br><br>The journal's file name will be displayed in the *Loop a Journal* dialog box. |
| **7** | If you want to specify an interval between loops, select *Loop on Interval.*<br><br>**AND**<br><br>Select the length and type of interval using the text box and drop-down list next to *Loop on Interval.* |

| | |
|---|---|
| **8** | If you want to specify a prompt on playback for the number of loops, select *On Journal Playback, Prompt for Number of Loops.*<br><br>The default prompt, "How many times do you want to loop the journal?" is displayed in the text box below this option. You can change the text if you wish. |
| **9** | To loop the stepper journal, choose *Continue.* The **Loop Control Panel** dialog box will appear while the journal is looping. |
| **10** | When you have finished, the MotionCorder image data will be saved as a MetaMorph stack file. |

### Configuring Communications for the Kodak MotionCorder

To configure serial communications between the camera controller and your computer, use the following procedure. Note, however, that this procedure is best left to your MDC representative, and, once performed, these settings should not be altered without explicit instruction from your representative.

| Step | Action |
|---|---|
| **1** | In the Kodak MotionCorder dialog box, choose *More >>* to expand the lower portion of the dialog box. |
| **2** | To select the Data Stream setting for handling serial communications, choose Select. |
| **3** | To configure the serial port parameters, choose *Configure.* The Kodak MotionCorder Configuration Options dialog box will appear. |
| **4** | Make your selections, as required, from the *Comm. Port, Baud Rate, Parity, Data Bits,* and *Stop Bits* configuration options.<br><br>When you have finished, choose *OK* to return to the Kodak MotionCorder dialog box. |
| **5** | If required, use the *Serial Delay (ms)* spin box to configure a delay in the transmission of commands to the camera controller. This may be necessary to adjust for overly fast rates of transmission that might otherwise be missed by the controller. |
| **6** | When you have finished, you can choose *<< Less* to condense the Kodak MotionCorder dialog box. |

# Kodak - Dialog Box Options

**Kodak MotionCorder**

**Set Acquisition Region**

## Kodak MotionCorder - Dialog Box Options

### Display Rate

Sets the display rate for playback of the currently acquired images.

### Acquisition Rate

Sets the acquisition rate for time-lapse recording with the Kodak MotionCorder.

### Acquisition Size

Sets the frame size for acquisition.

### Gamma Adjustment

Adjusts the linearity of the response of the camera (i.e., the relationship between the amplitude of the camera output signal and image pixel intensities).

### Illumination

Selects the Illumination setting associated with your external shutter. If you are not using a shutter, select *"[None]."*

### Playback / Live Selector (radio buttons)

Selects between the playback and acquisition modes. When you select *Playback,* the video output will display the current recorded image data, and the *Playback* option group buttons will be functional. When you select *Live,* the video output will display the current live video image at the current frame size and frame acquisition rate.

### Record Ready

Puts the MotionCorder camera controller into the ready state. When you choose this button, it will become the *Cancel* button.

### Trigger Record

Initiates recording of images to the Kodak MotionCorder controller until all memory is filled, or until you choose *Cancel.*

### Playback Reverse Button 

In Playback mode, this button plays the image frames backwards from the MotionCorder controller. Playback will continue until the first frame is reached or until you press the Stop Button. If nothing has been recorded, only "noise" will be displayed.

### Stop Button 

Stops playback of image frames from the camera controller.

### Playback Forward Button 

In Playback mode, this button plays the image frames forwards from the MotionCorder controller. Playback will continue until the last frame is reached or until you press the Stop Button. If nothing has been recorded, only "noise" will be displayed.

### Step Left Button 


Jumps the display back by one image frame.

### Step Right Button 


Jumps the display forward by one image frame.

### Go to Frame

Jumps the display to a specified frame. The spin box selects the image frame number, and the command button jumps the display to that frame.

### Acquire

Initiates image frame transfer from the video output of the camera controller to MetaMorph. This button is used only during recording of the "stepper" journal, which must be looped to transfer MotionCorder image data to MetaMorph for processing and analysis (see the "Creating a Journal for Image Transfer" procedure).

### Dest

Selects an image window for transfer of the image frames being acquired, played back, or transferred to MetaMorph.

### Set Acq Rgn

Specifies an acquisition subregion. Choosing this button will acquire a full-frame image, on which you can draw the acquisition subregion with the Rectangular Region Tool.

### More >>

Expands the dialog box downwards, revealing the options used for configuring serial communications.

### << Less

Condenses the dialog box.

### Select / Unselect

Selects the available Data Stream setting for use in handling serial communications. After you choose this button, it will become the *Unselect* button, which you can click to "unload" the setting.

### Configure

Opens the Kodak MotionCorder Configuration Options dialog box, from which you can reconfigure your serial communications parameters *(Comm. Port, Baud Rate, Parity, Data Bits,* and *Stop Bits).*

### Serial Delay (ms)

Configures a delay in the transmission of commands to the camera controller. This may be necessary to adjust for overly fast rates of transmission that might otherwise be missed by the controller.

### Close

Closes the dialog box.

## Set Acquisition Region (Kodak MotionCorder) - Dialog Box Options

### Use Active Region

Configures MetaMorph to use the rectangular region you drew in the image window as the acquisition region.

### Cancel

Cancels the command and closes the dialog box.

# Auto-Focus via Hardware (Devices Menu)

**Emulates triggering the AUTO FOCUS button on the front panel of the Ludl or Prior Z-motor controller.**

#### Drop-in: AUTOFCUS

Use this command to emulate an autofocus button on a stage Z-motor controller. This command can be used in a journal. A typical journal might include this command to autofocus while scanning each well on

a multiple-well plate.

You must install and configure a Z-motor device driver before using this command. This command can only be used Ludl and Prior controllers which have the autofocus card installed in them.

**QUICK TIP:** When used in a journal, the autofocusing procedure can be greatly improved by using the Acquire Image command's *Record Settings* journal function (available only as a command button in the dialog box), which you can use to fine-tune frame averaging without actually acquiring images.

**WARNING:**
It is recommended that you set the range of the autofocus Z-motor for the Prior stage with the Configure Auto-Focus command before you use the Auto-Focus command. The Auto-Focus command should be used only if an object is guaranteed to be found within the autofocus region. The ending location of the focus cannot be determined or guaranteed unless an object is inside the autofocus region before autofocusing.

**WARNING:**
The use of a CCD camera, rather than a tube camera, is recommended with this command because tube cameras (Newvicon or SIT) have a substantial lag time that interferes with the autofocus algorithm.

### Auto-Focusing the Microscope

To autofocus the microscope using your imaging system, use the following procedure.

**Note:** If you are using a Prior stage Z-motor, you should first use the Configure Auto-Focus command.

| Step | Action |
|---|---|
| **1** | Install and calibrate a Z-Motor device using the Meta Imaging Series Administrator.<br><br>**Note:** You must exit MetaMorph to start the Meta Imaging Series Administrator. |
| **2** | Start MetaMorph and Select the Z-Motor device in the Device Control dialog box |
| **3** | If you are using a Ludl Auto-Focus card, verify that an object is inside the image region on the Ludl Controller video output (the region's size and position is configured using the front panel of the Ludl autofocus controller). |
| **4** | From the Devices menu, choose Auto-Focus. MetaMorph will autofocus the microscope. |

# Configure Auto-Focus via Hardware (Devices Menu)

**Configures the focus range for the Prior stage Z-motor.**

**Drop-in: AUTOFCUS**

Use this command to configure the range of Z-axis movement for the Prior stage during focusing. This should be done before using the Auto-Focus command (Devices menu). The setting you choose will depend on the magnification of the microscope objective you are using.

## Configuring Auto-Focus via Hardware

To configure the Z-axis focusing range of the Prior stage Z-motor, use the following procedure:

| Step | Action |
|---|---|
| **1** | Install and configure a Z-Motor device in the Meta Series System Administrator. |
| **2** | From the Devices menu, choose Configure Auto-Focus. The Configure Auto-Focus dialog box will appear. |
| **3** | Use the *Autofocus Range* slider to select a range of Z-axis movement. The ranges are in arbitrary units, with higher magnification requiring a lower number and a lower magnification requiring a higher number. |
| **4** | Choose *OK.* |

## Configure Auto-Focus via Hardware - Dialog Box Options

### Autofocus Range

Selects a Z-axis range over which focusing movements can be made by the Prior stage Z-motor. Ranges are given in arbitrary units from *0* to *5*. Higher magnifications (for example, 60x and 100x objectives) will require a lower number, whereas lower magnifications (for example, 5x and 10x objectives) will require a higher number.

### OK

Sets the focusing range and closes the dialog box.

### Cancel

Cancels the command.

# Auto-Focus via Software (Devices Menu)

**Finds the best focal position of the microscope, using a Z-motor and an algorithm to estimate image resolution.**

**Drop-in: AUTOFO_S**

Use this command to focus the microscope when a previously focused image has become blurred. If you are just starting an experiment and need to make large adjustments to the focus, you should adjust your microscope while using the Focus command, or use the Auto-Focus drop-in command.

The Auto-Focus via Software commands rely on the *Move Increment* (step size) settings configured in the Focus dialog box. Before you can perform autofocusing with either Auto-Focus via Software command, you must calibrate and configure your step sizes with the Focus command. This should only need to be performed once after you install your stage Z-motor hardware.

There are two Auto-Focus via Software commands, each with a slightly different purpose:

**Adjust Focus** is useful for making minor adjustments when the stage has drifted slightly or the image has otherwise been put slightly out of focus.

**Find Focus** is intended for finding the best focus position within a broader Z-axis range but with a minimum number of acquisitions. This will be particularly useful for acquiring images from a multi-well or similar application in which there are many stage movements and the focus may change significantly after each move.

To assess the best focal position, the Auto-Focus via Software commands use the Brenner algorithm to measure a "focus value" that is based on the sum of squares of the intensity difference between a pixel and a pixel that is two pixels away from it. This provides a rough estimate of image resolution such that, the higher the value at a given pixel, the sharper the grayscale transitions must be surrounding that pixel. When making a comparison of this value for a pixel across several focal planes, the image with the highest focus value will be the sharpest. When the best focus value has been determined, the focus

motor will be moved to the corresponding focal plane.

For more information on automated measurement of focus values, see **Firestone et al., 1991** or **Price and Gough, 1994.**

Firestone, L., Cook, K., Culp, K., Talsania, N., and Preston, K., Jr. Comparison of autofocus methods for automated microscopy. *Cytometry* 12: 195 - 206, 1991.

Price, J.H. and Gough, D.A. Comparison of phase-contrast and fluorescence digital autofocus for scanning microscopy. *Cytometry* 16: 283 - 297, 1994.

## Configuring Auto-Focus via Software

**Adjusting Focus**

**Finding Focus**

## Configure Auto-Focus via Software - Dialog Box Options

**Adjust Focus**

**Find Focus**

# Adjust Focus (Auto-Focus via Software)

**Makes fine adjustments to the focal position of the microscope, using a Z-motor and an algorithm to estimate the sharpest image resolution.**

**Drop-in: AUTOFO_S**

Use this command to make minor adjustments to the focus of the microscope when a previously focused image has become slightly blurred. If you are just starting an experiment and need to make large adjustments to the focus, you should adjust your microscope while using the Focus command, or use the Auto-Focus drop-in command. If you need to find the best focus position within a broader Z-axis range but with a minimum number of acquisitions, you should instead use the Find Focus command.

The Auto-Focus via Software commands rely on the *Move Increment* (step size) settings configured in the Focus dialog box. Before you can perform autofocusing with either Auto-Focus via Software command, you must calibrate and configure your step sizes with the Focus command. This should only need to be performed once after you install your stage Z-motor hardware.

The *Number of Steps, Increment Step(s),* and *Range, Current +/-* options in the Adjust Focus dialog box will have an interactive effect on one another. The *Number of Steps* will always be an odd number, and will include the current focus position. To use an example, if the *Number of Steps* has been set to *5,* there will be two focus positions above the current plane and two below it. If you set *Increment Step(s)* to *3,* the *Range, Current +/-* option will update to reflect the fact that there will be a total range of six steps above the current focus position and six steps below it. Altering any of these three options will bring about a change in the setting of one of the other two options.

By default, the Auto-Focus via Software commands uses the Brenner algorithm as the standard method to assess the best focal position. The Brenner algorithm measures a "focus value" that is based on the sum of squares of the intensity difference between one pixel and another that is two pixels away from it. This provides a rough estimate of image resolution such that, the higher the value at a given pixel, the sharper the grayscale transitions must be surrounding that pixel. When making a comparison of this value for a pixel across several focal planes, the image with the highest focus value will be the sharpest. The Find Focus command differs from the Adjust Focus command in that it attempts to use the fewest possible acquisitions to obtain the optimal focal position. It does so by taking images at the limits of the selected range and recursively dividing the step size in half.

An alternative to the Standard algorithm is the Directional Average algorithm. If you are not getting good results using the Brenner algorithm, try switching to the Directional Average algorithm using the *Algorithm* drop-down box. This algorithm gives more accurate focus values with some high magnification objectives.

For more information on automated measurement of focus values, see **Firestone et al., 1991** or **Price and Gough, 1994.**

## Adjusting Focus (Auto-Focus via Software)

To make minor adjustments to the focal position for the microscope and set the Z-position to that plane, use the following procedure. **(Note:** You must first calibrate and configure the Z-motor step sizes with the Focus command.)

| Step | Action |
|---|---|
| **1** | In the Devices menu, point to the entry for Auto-Focus via Software. A secondary menu will open, displaying two focusing subcommand entries. |
| **2** | Choose Adjust Focus. The Adjust Focus dialog box will appear. |
| **3** | Select an algorithm to use from the *Algorithm* drop-down list. Valid choices are *Standard* and *Directional Avg*.<br><br>**Note:** The Standard algorithm will produce the best results under most conditions and should be used first. |
| **4** | With the *Number of Steps* spin box, select the overall number of images to acquire to test the focus. This will be an odd number that includes the current focal position. |
| **5** | Using the *Increment Step(s)* spin box, select the number of steps the Z-motor should be moved between each acquisition. The size of these steps will be determined from the *Move Increment* setting in the Focus dialog box. The *Range, Current* +/- spin box will update to reflect the *Number of Steps* and *Increment Step(s)* settings you have selected.<br><br>**OR**<br>Select an overall range, in Z-motor steps, from the *Range, Current* +/- spin box. The size of these steps will be determined from the *Move Increment* setting in the Focus dialog box. The *Increment Step(s)* spin box will update to reflect the *Number of Steps* and *Range, Current* +/- settings you have selected. |
| **6** | If you want to use backlash compensation to minimize focus position drift due to the effect of gravity on the Z-motor gears, select the *Backlash Compensation* check box. If your Z-motor hardware has this compensation already built in, you can leave this check box cleared. |

7 If you want to see each focus test image as it is acquired, select the *Display Images Being Acquired* check box.

8 When you are ready, choose *Auto-Focus.* MetaMorph will acquire the focus test images, measure a focus value for each, determine the best value, and move the Z-motor to the corresponding focal plane.

9 When you have finished, choose *Close.*

## Adjust Focus (Auto-Focus via Software) - Dialog Box Options

### Algorithm

Specifies the algorithm used to find focus. The options are Standard or Directional Avg. The default is Standard and uses the Brenner algorithm. Directional Avg gives more accurate focus values with some high magnification objectives.

### Number of Steps

Specifies the number of focus test images to be acquired to determine the best focal position. This will always be an odd number, and will include the current focus position. Changing this value will change the total focus range in the *Range, Current +/-* spin box based on the Z-axis increment selected in the *Increment Step(s)* spin box.

### Increment Step(s)

Specifies the Z-distance to move between each focus test image. The size of each step will be determined from the *Move Increment* setting in the Focus dialog box. The number of steps to move between acquisitions should be small enough to provide a well-focused image at one of the positions. Changing this value will change the total focus range in the *Range, Current +/-* spin box based on the selected *Number of Steps.*

### Range, Current +/-

Specifies how far above and below the current Z-position that the Z-motor will be moved while acquiring focus test images. This distance is specified in steps, as defined by the Focus command. Changing this value will change the Z-axis increment in the *Increment Step(s)* spin box, based on the total *Number of Steps.*

### Backlash Compensation

Selecting this check box will enable a Z-motor movement protocol whereby the focus motor will be moved to a Z-axis position slightly below the target position, and then moved against gravity to the target position. This may be desirable so that the Z-motor gears will be fully engaged, thereby avoiding drift due to slippage of the gears. Some focus devices will have a built-in backlash compensation, but there will be no harm in leaving this option selected.

### Display Images Being Acquired

Displays the focus test images in an image window during autofocusing. The image window will be closed when autofocusing is complete.

### Auto-Focus

Initiates the autofocusing protocol and moves the Z-motor to the best focus position, as determined by the Auto-Focus algorithm.

### Close

Closes the dialog box.

# Find Focus (Auto-Focus via Software) (Devices Menu)

**Finds the optimal focal position of the microscope within a broad Z-axis range, using the fewest possible acquisitions.**

**Drop-in: AUTOFO_S**

Use this command to find the best focal position of the microscope with the fewest Z-axis steps. This will be particularly useful for acquiring images from a multi-well plate or similar application in which there are many stage movements and the focus may change significantly after each move. If you are just starting an experiment and need to make large adjustments to the focus, you should adjust your microscope while using the Focus command, or use the Auto-Focus drop-in command. If you need to make minor adjustments of the focus position within a narrower Z-axis range, you should instead use the Adjust Focus command.

The Auto-Focus via Software commands rely on the *Move Increment* (step size) settings configured in the Focus dialog box. Before you can perform autofocusing with either Auto-Focus via Software command, you must calibrate and configure your step sizes with the Focus command. This should only need to be performed once after you install your stage Z-motor hardware.

By default, the Auto-Focus via Software command uses the Brenner algorithm as the standard method to assess the best focal position. The Brenner algorithm measures a "focus value" that is based on the sum of squares of the intensity difference between one pixel and another that is two pixels away from it. This provides a rough estimate of image resolution such that, the higher the value at a given pixel, the sharper the grayscale transitions must be surrounding that pixel. When making a comparison of this value for a pixel across several focal planes, the image with the highest focus value will be the sharpest. The Find Focus command differs from the Adjust Focus command in that it attempts to use the fewest possible acquisitions to obtain the optimal focal position. It does so by taking images at the limits of the selected range and recursively dividing the step size in half.

An alternative to the Standard algorithm is the Directional Average algorithm. If you are not getting good results using the Brenner algorithm, try switching to the Directional Average algorithm using the *Algorithm* drop-down box. This algorithm gives more accurate focus values with some high magnification objectives.

For more information on automated measurement of focus values, see **Firestone et al., 1991** or **Price and Gough, 1994.**

## Finding Focus (Auto-Focus via Software)

To find the best focal position for the microscope within a broad Z-axis range using the least number of acquisitions, use the following procedure. **(Note:** You must first calibrate and configure the Z-motor step sizes with the Focus command.)

| Step | Action |
|---|---|
| **1** | In the Devices menu, point to the entry for Auto-Focus via Software. A secondary menu will open, displaying two focusing subcommand entries. |
| **2** | Select Find Focus. The Find Focus dialog box opens.<br><br>Select an algorithm to use from the *Algorithm* drop-down list. Valid choices are *Standard* and *Directional Avg*.<br><br>**Note:** The Standard algorithm will produce the best results |

under most conditions and should be used first.

3. Select an overall range, in Z-motor steps, from the *Range, Current +/-* spin box. The size of these steps will be determined from the *Move Increment* setting in the Focus dialog box.
4. With the *Accuracy: Step(s)* spin box, select the smallest movement size at which the focus is to be tested.
5. If you want to use backlash compensation to minimize focus position drift due to the effect of gravity on the Z-motor gears, select the *Backlash Compensation* check box. If your Z-motor hardware has this compensation already built in, you can leave this check box cleared.
6. If you want to see each focus test image as it is acquired, select the *Display Images Being Acquired* check box.
7. When you are ready, choose *Find Focus.* MetaMorph will acquire the focus test images, measure a focus value for each, determine the best value, and move the Z-motor to the corresponding focal plane.
8. When you have finished, choose *Close.*

## Find Focus (Auto-Focus via Software) - Dialog Box Options

### Algorithm

Specifies the algorithm used to find focus. The options are Standard or Directional Avg. The default is Standard and uses the Brenner algorithm. Directional Avg gives more accurate focus values with some high magnification objectives.

### Range, Current +/-

Specifies how far above and below the current Z-position that the Z-motor will be moved while acquiring focus test images. This distance is specified in steps, as defined by the Focus command.

### Accuracy: um(s)

Specifies the Z-distance to move (in ums). Changing this value will change the *Number of Z Moves.*

### Number of Z Moves

Indicates the total number of acquisitions that will be made, based on the settings selected with the *Range, Current +/-* and *Accuracy: Step(s)* spin boxes.

### Status

Displays the current status of the Find Focus command. When the command is complete the current Z position is shown in um(s) — for example, *Focused at 5.25*.

### Backlash Compensation

Selecting this check box will enable a Z-motor movement protocol whereby the focus motor will be moved to a Z-axis position slightly below the target position, and then moved against gravity to the target position. This may be desirable so that the Z-motor gears will be fully engaged, thereby avoiding drift due to slippage of the gears. Some focus devices will have a built-in backlash compensation, but there will be no harm in leaving this option selected.

### Display Images Being Acquired

Displays the focus test images in an image window during autofocusing. The image window will be closed when autofocusing is complete.

### Find Focus

Initiates the autofocusing protocol and moves the Z-motor to the best focus position, as determined by the Auto-Focus algorithm.

### Close

Closes the dialog box.

# Resync Focus Dialog with Olympus Z-Motor (Devices Menu)

**Updates the Focus command's dialog box to reflect the current Z-distance setting on the Olympus microscope Z-motor. This may be particularly necessary if you make changes manually to the Z-motor settings.**

**Drop-in: OLAXRF**

Use this command to resynchronize the Focus dialog box settings with the current Z setting on the Olympus microscope's Z-motor.

## Resynchronizing the Focus Dialog with the Olympus Z-Motor

To updates the Focus command's dialog box to reflect the current Z-distance setting on the Olympus microscope Z-motor, use the following procedure:

| Step | Action |
|---|---|
| 1 | From the Devices menu, choose Resync Focus Dialog with Olympus Z Motor. The Resync Focus Dialog with Olympus Z-Motor dialog box will appear. |
| 2 | Choose *Resync.* The settings in the Focus dialog box will be updated. |
| 3 | You can leave the Resync Focus Dialog with Olympus Z-Motor dialog box open if you expect to make more changes manually to the Z-motor settings. When you have finished, choose *Close.* |

## Resync Focus Dialog with Olympus Z-Motor - Dialog Box Options

### Resync

Updates the Focus command's dialog box to reflect the current Z-distance setting on the Olympus microscope Z-motor.

### Close

Closes the Resync Focus Dialog with Olympus Z-Motor dialog box.

# Custom I/O Control (Devices Menu)

**Provides control over devices that utilize data streams.**

**Drop-in: CUSTOMIO**

There are four commands in the Custom I/O Control secondary menu: Send Serial Data, Wait for Serial

Data, Set Digital I/O, and Wait for Digital I/O. A serial communications port must be installed and configured using the Meta Imaging System Administrator before using Send Serial Data and Wait for Serial Data.

# Send Serial Data (Devices Menu)

**Sends a sequential stream of data from the computer to another device via a serial port.**

**Drop-in: CUSTOMIO**

Use this command to send recognized command strings for controlling another device to the device from the serial port. For example, some intensified CCD cameras have camera gain-switching abilities which are controlled by two gain (or sensitivity) knobs, and a toggle switch which determines which knob is active. After you set the gain for each knob, you can instruct the camera to switch between the two settings by sending the appropriate command string using the Send Serial Data command.

The documentation for the device receiving the data will include the appropriate commands needed to control the various components of the device. These are typed into a text box in the Send Serial Data dialog box using the syntax rules followed by MetaMorph. You can use hexadecimal, decimal, or ASCII Control Codes in your command strings.

**Syntax Rules**

**ASCII Control Code Chart**

## Sending Serial Data

To send serial data to a peripheral device, use the following procedure:

| Step | Action |
|---|---|
| **1** | Install and configure a serial communications port using the Meta Imaging System Administrator. |
| **2** | From the Devices menu, choose Custom I/O. Then choose Send Serial Data from the secondary menu that appears. The Send Serial Data dialog box will appear. |
| **3** | Choose *Select.* This will open the Select Serial Device dialog box. If there are several serial devices installed, you can select one from the drop-down list. |
| **4** | If the device receiving data sends back each character after the device receives it, select *Wait for Echo After Each Character.*<br>**AND**<br>Select the amount of time (seconds) that MetaMorph should wait for the echo before notifying you about the missing echo using *Timeout.* |
| **5** | Type the command string(s) to be sent to the device in the text box at the top of the dialog box. Your command string(s) must follow the syntax rules used by MetaMorph.<br>**Syntax Rules**<br>**ASCII Control Code Chart** |

**6** Choose *Send.*

**7** Choose *Close* to close the dialog box.

## Send Serial Data - Dialog Box Options

### Device Name

Displays the name of the open serial device. If no serial device is open, *"<None>"* will be displayed instead.

### Command String Text Box

Use this text box to type the command string(s) to be sent to the device. Refer to the device's documentation to determine the necessary commands to control the device. Your command strings must follow the **syntax rules** used by MetaMorph. An **ASCII Control Code Chart** is provided in this online help for your convenience.

### Wait for Echo After Each Character

Instructs MetaMorph to wait for an echo from the other device before sending the next character and to warn you if the character is not received. Use this option if the device receiving data sends back each character after the device receives it.

### Timeout

Specifies how long MetaMorph should wait for an echo from the other device before warning you that the character was not received. You must select *Wait for Echo After Each Character* before using this option.

### Select

Opens the Select Serial Device dialog box. If there are several serial devices installed, you can select one from the drop-down list.

### Send

Sends the command string(s) typed in the Command String Text Box to the device.

### Close

Closes the dialog box.

# Wait for Serial Data  (Devices Menu)

**Waits for a sequential stream of data from another device by way of a serial port.**

**Drop-in: CUSTOMIO**

Use this command to specify command strings that MetaMorph should wait for from another device via the serial port.

The documentation for the device sending the data will include the appropriate commands used by the device. These are typed into a text box in the Wait for Serial Data dialog box using the syntax rules that are followed by MetaMorph. You can use hexadecimal, decimal, or ASCII control codes in your command strings.

**Syntax rules**

**ASCII Control Code Chart**

## Waiting for Serial Data

To configure the system to wait for serial data from a peripheral device, use the following procedure:

| Step | Action |
|---|---|
| **1** | Install and configure a serial communications port using the Meta Imaging System |

Administrator.

**2** From the Devices menu, choose Custom I/O. Then choose Wait for Serial Data from the secondary menu that appears. The Wait for Serial Data dialog box will appear.

**3** Choose *Select.* This will open the Select Serial Device dialog box. If there are several serial devices installed, you can select one from the drop-down list.

**4** If you want to log the data received from the device, select *Write Received Data to Log File* and the dialog box expands to include logging options. (A data log file must be open to log the data.)

**AND**

Specify the logging format for the data using the *Log Format* text box. You can add text as desired; the "$" will be replaced by the data received from the device each time data are logged.

**5** Specify the amount of time MetaMorph should wait for each character before warning you about the missing character using *Timeout.*

**6** Type the command string(s) to be sent by the device in the text box at the top of the dialog box. Your command string(s) must follow the syntax rules used by MetaMorph.

**Syntax Rules**

**ASCII Control Code Chart**

**7** Choose *Wait.*

**8** Choose *Close* to close the dialog box.

## Wait for Serial Data - Dialog Box Options

### Device Name

Displays the name of the open serial device. If no serial device is open, *"<None>"* will be displayed instead.

### Command String Text Box

Use this text box to type the command string(s) to be sent from the device. Refer to the device's documentation to determine the appropriate commands. Your command strings must follow the **syntax rules** used by MetaMorph. An **ASCII Control Code Chart** is provided in this online help for your convenience.

### Write Received Data to Log File

Instructs MetaMorph to log the received data to the open data log file.

### Log Status

Lists the status of the data log file, including the name of the name of log file, if one is open.

### Log Format

Specifies the logging format used to log data received from the device. When you have added the desired text, use the "$" to represent the data that were received from the device. This option will appear when you select the *Write Received Data to Log File* check box.

### Timeout

Specifies how long MetaMorph should wait for each character from the device before warning you that the character was not received.

### Select

Opens the Select Serial Device dialog box. If there are several serial devices installed, you can select one from the drop-down list.

### Wait

Instructs MetaMorph to wait for the string specified in the Command String Text Box.

### Close

Closes the dialog box.

# Set Digital I/O (Devices Menu)

**Controls a peripheral digital I/O device by sending signals to it from the computer.**

**Drop-in: CUSTOMIO**

Use this command to send TTL-level voltage signals from MetaMorph by a parallel port or a digital I/O board. For example, you could use this command to control a valve that opens when the hardware receives a +5 V signal and closes when the hardware receives a 0 V signal.

## Setting Digital I/O

To configure digital I/O communication lines, use the following procedure:

| Step | Action |
|---|---|
| **1** | Install and configure a digital I/O device using the Meta Imaging System Administrator. |
| **2** | From the Devices menu, choose Custom I/O. Then choose Set Digital I/O from the secondary menu that appears. The Set Digital I/O dialog box will appear. |
| **3** | Choose *Configure* to set the configuration options appropriate for the parallel port or the digital I/O board.<br>**AND**<br>Choose *OK* when you have finished. The Set Digital I/O dialog box will update when you choose *OK.* |
| **4** | Select *Continuous Update of Line Assignments* if you want the line states to be set automatically as you change the status of the line states in the dialog box. If this option is deselected, line states will only be changed when you choose *Set Lines.* |
| **5** | The line numbers and their corresponding pin position on the parallel port or digital I/O card will be displayed at the bottom of the dialog box. The *Set State* radio buttons next to each line specify how each line should be set each time you choose *Set Lines.*<br>Select *On* to set a line to high signal (+5 V). |

Select *Off* to set a line to low signal (0 V). Or select *Ignore* to leave a line set to its present setting.

**6** Repeat Step 5 for each line used by the device. You may need to use the scroll bar on the right side of the dialog box to view the line assignments for some lines.

**7** Choose *Set Lines.*

**Note:** If you select *Continuous Update of Line Assignments,* the digital I/O signal will be set each time a line state is changed.

**8** Repeat Steps 5 - 7 as necessary. Choose *Close* when you have finished.

## Set Digital I/O - Dialog Box Options

### Device Name

Lists the name of the open Digital I/O device. If no Digital I/O device is open, *"<None>"* will be displayed instead.

### Number of Output Lines

Lists the number of output lines available for the installed digital I/O device.

### Continuous Update of Line Assignments

Automatically changes the state of a line whenever its status is changed in the dialog box, rather than waiting for the user to select *Set Lines.*

### Line #

Lists the line numbers available for the installed digital I/O device.

### Pin #

Lists the corresponding pin number for each line used by the digital I/O device.

### Set State

Specifies the state for each line. A line can be set to *On* (high signal, +5 V), *Off* (low signal, 0 V), or *Ignore* (remains in present state).

### Slider

Scrolls through the list of available lines.

### Set Line

Sets each line to the state *(On, Off,* or *Ignore)* specified by the *Set State* radio buttons.

### Configure

Specifies the appropriate configuration options for the parallel port or digital I/O board installed. Use this option to specify the parallel port.

### Close

Closes the dialog box.

# Wait for Digital I/O (Devices Menu)

**Sends signals to the computer from a digitally-controlled device.**

**Drop-in: CUSTOMIO**

Use this command to receive TTL-level voltage signals from a digitally controlled device by a parallel

port or a digital I/O board.

**QUICK TIP:** If you have installed the MMVAR drop-in, you can use the Branch on Variable drop-in command (Journal menu) to run one journal when the system receives the digital I/O signal or run a different journal if a timeout occurs. To do so, use the "$Device.DigitalIO.Timeout$" system variable in the Branch on Variable dialog box. In the absence of a timeout, this variable will be set to a value of 0. If a timeout occurs, the variable will be reset to 1.

## Waiting for Digital I/O Signals

To configure the system to wait for signals from a peripheral digital I/O device, use the following procedure:

| Step | Action |
|---|---|
| **1** | Install and configure a digital I/O device using the Meta Imaging System Administrator. |
| **2** | From the Devices menu, choose Custom I/O. Then choose Wait for Digital I/O from the secondary menu that appears. The Wait for Digital I/O dialog box will appear. |
| **3** | Choose *Configure* to set the configuration options appropriate for the parallel port or the digital I/O board.<br><br>**AND**<br><br>Choose *OK* when you have finished. The Wait for Digital I/O dialog box will update itself when you choose *OK.* |
| **4** | Use *Timeout* to specify the amount of time MetaMorph should wait for signals that match the selected line states before warning you. |
| **5** | The line numbers and their corresponding pin position on the parallel port or digital I/O card will be displayed at the bottom of the dialog box. The *Wait State* radio buttons next to each line specify the expected line signal that should be received each time you choose *Wait.*<br><br>Select *On* to set a line to high signal (+5 V). Select *Off* to set a line to low signal (0 V). Or select *Ignore* to leave a line set to its present setting. |
| **6** | Repeat Step 5 for each line used by the device. You may need to use the scroll bar on the right side of the dialog box to view the line assignments for some lines. |
| **7** | Choose *Wait.* The Wait for Digital I/O dialog box will appear, indicating the MetaMorph is waiting for the digital I/O signals that match the line states specified the dialog box.<br><br>If the signals are not received before the time specified by *Timeout* has elapsed, a message dialog box will appear, stating that the input lines are not set. |

**8** Repeat Steps 5 - 7 as necessary. Choose *Close* when you have finished.

## Wait for Digital I/O - Dialog Box Options

### Device Name

Lists the name of the open Digital I/O device. If there is no Digital I/O device open, *"<None>"* will be displayed instead.

### Number of Input Lines

Lists the number of input lines available for the installed digital I/O device.

### Timeout

Specifies the amount of time MetaMorph should wait for digital I/O signals that match the selected line states in the dialog box before warning you.

### Line #

Lists the line numbers available for the installed digital I/O device.

### Pin #

Lists the corresponding pin number for each line used by the digital I/O device.

### Wait State

Specifies the state for which to wait for each line. A line can be set to *On* (high signal, +5 V), *Off* (low signal, 0 V), or *Ignore* (remains in present state).

### Slider

Scrolls through the list of available lines.

### Wait

Instructs MetaMorph to wait for digital signals from the device that match the line states specified by the *Wait State* radio buttons *(On, Off,* or *Ignore).*

### Configure

Specifies the appropriate configuration options for the parallel port or digital I/O board installed. Use this option to specify the parallel port.

### Close

Closes the dialog box.

# Syntax Rules

| Code | Result |
|---|---|
| $ | "Escape" Character, which is ASCII 27 in Decimal. |
| ^A thru ^Z | "Control" Character, which is ASCII 1 for ^A through ASCII 26 for ^Z. |
| \c | Sends the character after the slash. In this example, the character "c" would be sent. Useful for sending ^, \, or $ characters. |
| \ddd | Sends ASCII digits in Decimal. EXAMPLE: \192. |

| | |
|---|---|
| \xdd | Sends ASCII digits in Hexadecimal.<br>EXAMPLE: \x27. |
| (dddd) | Delays for specified number of milliseconds.<br>EXAMPLE: (1000) |

**Note:** If you need to send more than one command at a time, command strings can be appended serially.
EXAMPLE: "\xdd\xdd\x04".

# ASCII Control Code Chart

| Hex | Dec | Key | Name | Description | | Hex | Dec | Key | Name | Description |
|---|---|---|---|---|---|---|---|---|---|---|
| 00 | 0 | ^@ | NUL | Null | | 10 | 16 | ^P | DLE | Data Link Escape |
| 01 | 1 | ^A | SOH | Start of Header | | 11 | 17 | ^Q | DC1 | Device Control 1 |
| 02 | 2 | ^B | STX | Start of Text | | 12 | 18 | ^R | DC2 | Device Control 2 |
| 03 | 3 | ^C | ETX | End of Text | | 13 | 19 | ^S | DC3 | Device Control 3 |
| 04 | 4 | ^D | EOT | End of Transmission | | 14 | 20 | ^T | DC4 | Device Control 4 |
| 05 | 5 | ^E | ENQ | Inquiry | | 15 | 21 | ^U | NAK | Negative Acknowledge |
| 06 | 6 | ^F | ACK | Acknowledge | | 16 | 22 | ^V | SYN | Synchronous Idle |
| 07 | 7 | ^G | BEL | Bell | | 17 | 23 | ^W | ETB | End Transmission Block |
| 08 | 8 | ^H | BS | Backspace | | 18 | 24 | ^X | CAN | Cancel |
| 09 | 9 | ^I | HT | Horizontal Tab | | 19 | 25 | ^Y | EM | End of Medium |
| 0A | 10 | ^J | LF | Line Feed | | 1A | 26 | ^Z | SUB | Substitute |
| 0B | 11 | ^K | VT | Vertical Tab | | 1B | 27 | | ESC | Escape |
| 0C | 12 | ^L | FF | Form Feed | | 1C | 28 | | FS | File Separator |
| 0D | 13 | ^M | CR | Carriage Return | | 1D | 29 | | GS | Group Separator |
| 0E | 14 | ^N | SO | Shift Out | | 1E | 30 | | RS | Record Separator |
| 0F | 15 | ^O | SI | Shift In | | 1F | 31 | | US | Unit Separator |

# MMKeyPad

**Provides a customizable set of program controls on a standard remote keypad device that can be remotely located from the workstation.**

**Drop-in: MMKEYPAD**

Use the MMKeyPad drop-in to enable you to execute commands for controlling your microscope and associated devices including Z-motor, filtration, and shutter, and for executing journals and acquiring images. This drop-in enables you to connect a standard, wired, 17-key keypad to a serial com port, and to assign a maximum of 16 different journals to the keypad keys, as well as use the pre-assigned functions. Pressing the Num Lock key switches the keypad from its pre-assigned functions to user assigned journals. The pre-assigned numeric keys are used to initiate from one to nine user-defined keys on a single taskbar.

## Configuring the MMKeyPad

To configure and use the MMKeyPad, complete the following procedure.

| Step | Action |
|---|---|
| **1** | If the *Active on COM #* check box is on (checked), click the checkbox to deactivate the MM Keypad, which enables you to make configuration settings. |
| **2** | If the MMKeyPad dialog box is minimized, click *More>>.* The complete MMKeyPad dialog box opens. |
| **3** | In the *Serial Port* box, select the number of the Com port to which the keypad is connected.<br><br>**Note:** To use your keypad, you must first install the appropriate keypad driver. |
| **4** | You can assign journals to any keypad key except the Num Lock key. Click the key to which you want to assign the journal. The *Select a Journal to run for each Interval* dialog box opens. |
| **5** | Select the folder containing the journal that you want to associate with the selected key, then select the journal and click *Open*. |
| **6** | Repeat steps 4 and 5 for each key to which you want to assign a journal. |
| **7** | After you have finished assigning journals to keys, click *Active* to activate the keypad. |
| **8** | Press the Num Lock key to change from Journal mode to Standard mode. |
| **9** | Open or create a task bar set up with the dialog boxes that you want to run remotely. |
| **10** | To initiate a task on a task bar, press the remote keypad key associated with the task. Keys 1 through 9 on the key pad will activate tasks 1 through 9 on the task bar (top to bottom). |

## MMKeyPad - Dialog Box Options

### Active

Activates the Keypad on the selected com port. Select the com port that you want to use first, then click this check box. To configure keypad keys to run specific journals, you must ensure that this "Active" checkbox is not checked.

### More>> / <<Less

Switches the dialog box between its "minimized" and "maximized" format. In minimized format, only the Active checkbox, the More>> button, and the Close button are available. After all settings have been made, you can minimize the dialog box to occupy less area on your display.

### Close

Closes the MMKeyPad dialog box.

### Serial Port

Specifies the Com port to which the Keypad is connected.

**NumLk/(Num Lock)**
Switches the keypad between its pre-assigned functions and user assigned journals.

**Pop Images (/)**
Brings the currently open image window(s) to the front.

**Pop Dialogs (*)**
Brings the currently open dialog boxes to the front.

**DecZMotor (-)**
Moves the Z-Motor (Focus) up one step for each key press.

**IncZMotor (+)**
Moves the Z-Motor (Focus) down one step for each key press.

**Tog Shttr (Enter)**
Opens and closes the selected shutter device.

**Dec Filter (Ins)**
Moves the selected filter device (wheel, dichroic, or cube) to the next filter in a negative direction.

**Inc Filter (Del)**
Moves the selected filter device (wheel, dichroic, or cube) to the next filter in a positive direction.

**Taskbar 1-9 (1-9)**
Activates the appropriate taskbar command on the active (open) taskbar.

# Color Align (Display Menu)

**Shifts the red, green, and blue planes of an image independently to bring them into alignment.**

**Drop-in: CALIGN**

Use this command to shift the color planes in a single 24-bit color image, or in individual 8-bit or 16-bit images (red, green, and/or blue), to correct for image registration misalignment, such as are caused by chromatic aberrations or mechanical components such as filter wheels. This feature will be particularly useful for users of large-chip CCDs who acquire RGB images through separate acquisition steps.

Your method of selecting the source image(s) depends on whether you are working with a single 24-bit image or with individual "color component" source images. When you first choose the Color Align command, the Color Align dialog box will be condensed when it appears. If you are using a 24-bit source image, the *Image* selector, located in the upper, left corner of the dialog box, is used to select the source image. If, however, you are using two or three individual "color component" source images, you need to expand the dialog box by choosing *More >>.* On the right half of the expanded dialog box, you will see three *Image* selectors, which you will use to select the red, green, and/or blue source images.

This command offers two ways to alter alignment values. One method relies on horizontal and vertical sliders, which you can drag with your pointer to the desired position. The other method involves a table of values, in which you can enter the number of pixels to move in the horizontal or vertical direction for each of the color components. A negative value indicates a shift upwards or to the left.

To assist you in the alignment process, a Preview image window is provided so that you can see the effect of your alignment changes before you apply them permanently. The Preview window centers on a 256 x 256 region in the source image(s), which you can change with a box-in-box region selection control.

When the alignment operation is performed on 8-bit or 16-bit source images, the results will be saved in a new 24-bit image. However, when the source image is a single 24-bit color image, the results of the alignment operation will overwrite the original. Thus, if you want to retain the original 24-bit image, you

should make a copy of it with the Duplicate Image command (Edit menu), and perform your alignment on the copied image.

If you are working with 16-bit source images, the Color Align command allows you to adjust the contrast of the displayed images. You can have MetaMorph adjust the contrast automatically, or you can perform the adjustments manually. These controls are similar to those found in the Scale 16-Bit Image dialog box.

## Aligning Colors

**Three 8-Bit Images**

**Three 16-Bit Images**

**A Single 24-Bit Image**

## Color-Aligning Three 8-Bit Images

To align three 8-bit color component images, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Display menu, choose Color Align. The Color Align dialog box opens. |
| **2** | If the dialog box is condensed, choose *More >>* to expand it. |
| **3** | In the *Red, Green,* and *Blue* option groups on the right side of the dialog box, use the pertinent *Image* selectors to select the source images representing the red, green, and/or blue component images, respectively. If you are using only two color source images, select *None Selected* for the color plane that will not be involved. |
| **4** | If you wish, use the *Destination* image selector to specify a name for the aligned result image. |
| **5** | With the *Preview Image* box-in-box control, select the region of the source image to be displayed in the Preview image window by dragging the inner box with your pointer. |
| **6** | From the *Alignment* group, select the check box for the color component image (*Red, Green,* or *Blue*) that you want to shift. You can select check boxes for two of the color components if you want to apply the same shift to both images. |
| **7** | To adjust the position of the selected image(s), drag the sliders in the left half of the dialog box to the desired location while watching the movement of the colored objects in the Preview window.<br>**OR**<br>Click the appropriate cell of the Alignment Table and type the desired shift, in pixels, in the cell. Type a negative number in the *H Shift* column to specify a shift to the left. Similarly, type a negative number in the *V Shift* column to specify a shift upwards. |

If you want to reset the shift values to zero at any time, choose *Zero Shift.*

**8** If necessary, repeat Steps 6 and 7 for the other color components. If you need to bring one of the source images "to the front" in your desktop display while doing so, choose the "*S*" button in its option group on the right side of the dialog box.

**9** When you are satisfied with the new alignment, choose *Apply.*

**10** Choose *Close.*

## Color-Aligning Three 16-Bit Images

To align three 16-bit color component images, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Display menu, choose Color Align. The Color Align dialog box opens. If the dialog box is condensed, choose *More >>* to expand it. |
| **2** | In the *Red, Green,* and *Blue* option groups on the right side of the dialog box, use the pertinent *Image* selectors to select the source images representing the red, green, and/or blue component images, respectively. If you are using only two color source images, select *None Selected* for the color plane that will not be involved. |
| **3** | If desired, use the *Destination* image selector to specify a name for the aligned result image. |
| **4** | If you need to, you can adjust the contrast of the source image to assist you with visual discrimination during the alignment. To do so, continue to Step 5.<br>**OR**<br>If you do not need to adjust the contrast of the source images, skip to Step 7. |
| **5** | If necessary, you can specify gray levels for contrast scaling that are outside of the default range of the image's lowest and highest gray levels (*Image Min/Max*). Select *10-Bits (0 - 1023), 12-Bits (0 - 4095), 14-Bits (0 - 16383),* or *16-Bits (0 - 65535)* from the *Scale Range* list. This will change the range to include all of the gray values available in the specified image depth type. |
| **6** | If you want MetaMorph to choose the range of gray levels used for scaling the contrast, select *Auto* for the pertinent source image.<br>**OR**<br>To adjust the contrast manually, select the darkest gray level for the source images using the pertinent *Min* sliders or text boxes, and select the brightest gray level using the |

corresponding *Max* options.

**7** With the *Preview Image* box-in-box control, select the region of the source image to be displayed in the Preview image window by dragging the inner box with your pointer.

**8** From the *Alignment* group, select the check box for the color component image (*Red, Green,* or *Blue*) that you want to shift. You can select check boxes for two of the color components if you want to apply the same shift to both images.

**9** To adjust the position of the selected color component image(s), drag the sliders in the left half of the dialog box to the desired location while watching the movement of the colored objects in the Preview window.

**OR**
Click the appropriate cell of the Alignment Table and type the desired shift, in pixels, in the cell. Type a negative number in the *H Shift* column to specify a shift to the left. Similarly, type a negative number in the *V Shift* column to specify a shift upwards.

If you want to reset the shift values to zero at any time, choose *Zero Shift.*

**10** If necessary, repeat Steps 8 and 9 for the other color components. If you need to bring one of the source images "to the front" in your desktop display while doing so, choose the "*S*" button in its option group on the right side of the dialog box.

**11** When you are satisfied with the new alignment, choose *Apply.*

**12** Choose *Close.*

## Color-Aligning a Single 24-Bit Image

To align the color components in a 24-bit color image, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Display menu, choose Color Align. The Color Align dialog box opens. If the dialog box is expanded, choose *Less* << to condense it. |
| **2** | If necessary, select the source image with the *Source* selector. |
| **3** | With the *Preview Image* box-in-box control, select the region of the source image to be displayed in the Preview image window by dragging the inner box with your pointer. |
| **4** | From the *Alignment* group, select the check box for the color component (*Red, Green,* or *Blue*) that you want to shift. You can select check boxes for two of the color components if you want to apply the same shift to both. |

**5** To adjust the position of the selected color component(s), drag the Alignment Sliders in the left half of the dialog box to the desired location while watching the movement of the colored objects in the Preview window. Repeat as necessary, for the other color components.

**OR**
Click the appropriate cell of the Alignment Table and type the desired shift, in pixels, in the cell. Type a negative number in the *H Shift* column to specify a shift to the left. Similarly, type a negative number in the *V Shift* column to specify a shift upwards.

If you want to reset the shift values to zero at any time, choose *Zero Shift.*

**6** When you are satisfied with the new alignment, choose *Apply.*

**7** Choose *Close.*

## Color Align - Dialog Box Options

### Source

Selects a source image for the alignment procedure. This image selector will appear only when you are using a 24-bit source image. This source image will be overwritten by the alignment operation. Thus, if you want to retain the original 24-bit image, you should make a copy of it with the Duplicate Image command (Edit menu), and perform your alignment on the copied image.

### Destination

Selects a destination for the alignment result image. This image selector will appear when you are using 8-bit or 16-bit source images.

### Horizontal and Vertical Alignment Sliders

The sliders shift the selected color component to the left or right (horizontal slider) and up or down (vertical slider). Changes made with the sliders will be reflected in the Alignment Table. You can apply a shift of up to 10 pixels in any direction with the sliders. Shifts selected with the sliders will be added to any offsets you have already specified in the Alignment Table.

### Alignment (check boxes)

Selects the color component to be shifted. You can select check boxes for two of the color components if you want to apply the same shift to both.

### Alignment Table

Use this table to specify the horizontal (*H Shift*) and vertical (*V Shift*) offsets for the red, green, and blue color components. There is no limit to the size of the shift you can apply (you can even shift a color component completely out of the image). Type a negative number in the *H Shift* column to specify a shift to the left. Similarly, type a negative number in the *V Shift* column to specify a shift upwards.

### Preview Image (box-in-box control)

Allows you to drag the smaller box with the pointer to select a region in the source image to be displayed in the Preview window.

### Apply

Applies the configured alignment shift to the image. If you are using 8-bit or 16-bit color component source images, a new image will be created with the name you selected with the *Destination* image selector. If you are using a 24-bit color source image, this image will be overwritten by the alignment operation.

### Close

Closes the dialog box.

### More >>

Expands the dialog box to the right, revealing image selectors for 8-bit and 16-bit source images and contrast scaling options for 16-bit source images.

### Less <<

Condenses the dialog box and reveals the image selector for 24-bit source images.

### Zero Shift

Resets the horizontal and vertical shifts to zero for all color component(s).

### Image (*Red, Green,* and *Blue* option groups)

Selects the 8-bit or 16-bit source images for the red, green, and/or blue components, respectively.

### Min (*Red, Green,* and *Blue* option groups)

Selects the darkest gray level for the 16-bit color component source image for which you are manually scaling the contrast. These options (text box and scaling wedges) will be unavailable and will appear dimmed if you are using 8-bit or 24-bit source images.

### Max (*Red, Green,* and *Blue* option groups)

Selects the brightest gray level for the 16-bit color component source image for which you are manually scaling the contrast. These options (text box and scaling wedges) will be unavailable and will appear dimmed if you are using 8-bit or 24-bit source images.

### Auto (*Red, Green,* and *Blue* option groups)

Allows MetaMorph to choose the grayscale range to be scaled when you are adjusting the contrast of a 16-bit color component source image. This button will be unavailable and will appear dimmed if you are using 8-bit or 24-bit source images.

### "S" command button (*Red, Green,* and *Blue* option groups)

Brings the corresponding 8-bit or 16-bit color component source image to the front on your desktop.

### Scale Range

Changes the range of gray levels available for scaling to include the maximum possible number of levels for the selected image type *(10-Bits [0-1023], 12-Bits [0-4095], 14-Bits [0-16383],* or *16-Bits [0-65535]),* rather than restricting the range to the values between the image's minimum and maximum gray levels *(Image Min/Max).* In journal record/edit mode, a check box will accompany this list box, which you can select to specify that the recorded 16-bit scaling values are to be used on playback.

# Color Mosaic (Display Menu)

**Converts color mosaic images to 24-bit color images.**

**Drop-in: MOSAIC**

Use this command to convert color mosaic images to true 24-bit color images. The color mosaic Interline CCD chip acquires images in which 2 x 2 arrays of pixels encode the red, green, and blue information in each image. The Color Mosaic command takes the information in these arrays and reencodes it into single pixels containing 24-bit color information.

The Color Mosaic dialog box can be expanded to display options for configuring the intensity scaling of the red, green, and blue components of the result image. If you wish, you can direct MetaMorph to perform the scaling for you automatically. Autoscaling can be applied to any or all of the three color components. When the dialog box is expanded, the Color Mosaic Preview image window will appear and the *Preview Image* box-in-box region selector will become available, which you can use to select a region on the source image for display in the Color Mosaic Preview window. When you are satisfied with

your scaling configuration settings, you can condense the dialog box again to save desktop space.

**Note:** Because of the arrangement of the CCD chip elements and the resulting color mosaic image information, the conversion process will not work correctly if the source image has been binned.

## Converting a Color Mosaic Image to a 24-Bit Color Image

To converting a Sony Color Mosaic image to a 24-bit color image, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Display menu, choose Color Mosaic. The Color Mosaic dialog box opens. |
| **2** | Select the Color Mosaic source image with the *Source* image selector. If the source image is a stack of images, use the secondary selector to select the *Current* plane or *All Planes* in the stack. |
| **3** | Specify a destination for the 24-bit color image with the *Result* image selector. You can *Add To* or *Overwrite* the existing image, or you can specify a *New* image. |
| **4** | If you need to change the mosaic format, select one from the *Mosaic Format* drop-down list.<br><br>**Note:** The normal format for raw mosaic image data is RGGB. However, if the camera driver is set to flip the image either horizontally or vertically, the format will be different. If the preview image does not display as expected, try changing the Mosaic Format value. Valid values are: RGGB, GRBG, BGGR, AND GBRG. |
| **5** | If you want MetaMorph to perform intensity scaling in the result image for you automatically, select *Auto* from the *Image Scaling* option button group. Then skip to Step 10.<br><br>**OR**<br>If you want to specify the intensity scaling ranges in the result image, select *Custom* from the *Image Scaling* option button group. |
| **6** | Choose *Preview* >> to expand the dialog box, revealing the color component scaling options on the right. When you do so, the Color Mosaic Preview image window will appear, and the *Preview Image* box-in-box region selector will become available. |
| **7** | If you have already defined a region of interest on the Color Mosaic source image, that region will be displayed in the Color Mosaic Preview image window. Otherwise, |

drag the inner box in the *Preview Image* box-in-box control to select a region for display in the preview window.

**8** For each color component option group, use the slider or text boxes to specify a lower and upper color intensity value for the scaling range.

**OR**
If you want MetaMorph to perform the scaling for you automatically, based on the minimum and color maximum intensity values in the image, select the *Autoscale* check box.

**9** If you need to specify intensity values that are outside of the default range of the image's lowest and highest intensity values *(Image Min/Max),* select *10-Bits [0-1023], 12-Bits [0-4095], 14-Bits [0-16383],* or *16-Bits [0-65535]* from the *Scale Range* list.

This will change the range to include all of the intensity values available for the pertinent color component image depth. Repeat Step 8 as necessary. When you are satisfied with the result, you can choose *Preview* << to condense the dialog box, if you wish.

**10** To create the 24-bit result image, choose *Apply.* The new 24-bit image will appear.

**11** When you have finished, choose *Close.*

## Color Mosaic - Dialog Box Options

### Source

Selects the Sony Color Mosaic source image.

### Result

Specifies a destination for the 24-bit color result image. You can *Add To* or *Overwrite* the existing image, or you can specify a *New* image.

### Mosaic Format

Selects the mosaic format to use when processing the image. The normal format for raw mosaic image data is RGGB. However, if the camera driver is set to flip the image either horizontally or vertically, the format will be different. If the preview image does not display as expected, try changing the Mosaic Format value. Valid values are: RGGB, GRBG, BGGR, AND GBRG.

### Preview Image (box-in-box control)

Selects a region of interest on the source image for display in the Color Mosaic Preview image window. Drag the inner box to the desired location, as you would a region of interest that has been defined on an image. **Note:** If you have already defined a region of interest on the Color Mosaic source image, that region will be displayed in the Color Mosaic Preview image window.

### Image Scaling

Selects an intensity scaling mode for the result image:

When you select *Auto,* MetaMorph will select the scaling range for each color component automatically, based on the minimum and maximum color intensity values already present in the image. Accordingly, the red, green, and blue component scaling option groups in the expanded right half of the Color Mosaic dialog box will be unavailable.

When you select *Custom,* the color component scaling option groups will become available, which you can

use to select a minimum and maximum intensity value for each of the three scaling ranges. **Note:** You will still have the option of autoscaling each color component intensity range individually by selecting the pertinent *Autoscale* check box.

### Preview >>

Expands the dialog box, revealing the *Red Component Scaling, Green Component Scaling,* and *Blue Component Scaling* option groups. The Color Mosaic Preview image window will also appear, displaying the region of the source image that has been selected by the *Preview Image* box-in-box region selector.

### Preview <<

Condenses the dialog box. The Color Mosaic Preview image window will also close.

### Red Component Scaling

Specifies a minimum and maximum red intensity value for the scaling range in the result image. The minimum value will be displayed in the result image as intensity (0,X,X), and the maximum value will be displayed as intensity value (255,X,X). Drag the lower and upper handles of the slider to select a minimum and maximum value, or type the values in the upper and lower text boxes, respectively. Alternatively, you can direct MetaMorph to use the minimum and maximum values in the image to define the scaling range.

### Green Component Scaling

Specifies a minimum and maximum green intensity value for the scaling range in the result image. The minimum value will be displayed in the result image as intensity (X,0,X), and the maximum value will be displayed as intensity value (X,255,X). Drag the lower and upper handles of the slider to select a minimum and maximum value, or type the values in the upper and lower text boxes, respectively. Alternatively, you can direct MetaMorph to use the minimum and maximum values in the image to define the scaling range.

### Blue Component Scaling

Specifies a minimum and maximum blue intensity value for the scaling range in the result image. The minimum value will be displayed in the result image as intensity (X,X,0), and the maximum value will be displayed as intensity value (X,X,255). Drag the lower and upper handles of the slider to select a minimum and maximum value, or type the values in the upper and lower text boxes, respectively. Alternatively, you can direct MetaMorph to use the minimum and maximum values in the image to define the scaling range.

### Scale Range

Changes the color intensity levels available for scaling to include all of the levels available in the selected image type *(10-Bits [0-1023], 12-Bits [0-4095], 14-Bits [0-16383],* or *16-Bits [0-65535]*), rather than restricting the range to the values between the image's minimum and maximum gray levels *(Image Max/Min).*

### Apply

Creates the 24-bit color image.

### Close

Closes the dialog box.

### Color Mosaic Preview (image window)

This image window displays a preview of the converted 24-bit result image. If a region of interest has been defined on the source image, that are will be displayed in the Color Mosaic Preview image window. Otherwise, the area displayed will be that which has been selected with the *Preview Image* box-in-box region selector.

# Split View (Display Menu)

**Separates multiple wavelength images that have been acquired using an emission splitter device and a single camera. Acquired images can be either two or four separate wavelengths of the same sample projected onto a single camera chip.**

**Dropin: SPLITVIEW**

Use this drop-in to separate and organize multiple wavelength images of a single sample originally

acquired as one image or image stack using a single camera. This dialog can split into two or four separate images or stacks a single image or stack composed of either two or four discrete image areas acquired at either two or four discrete wavelengths. When the images are separated, they can be overlaid and combined into a single image composed of each individual wavelength assigned to a discrete representative color. The images can be organized into a single stack, or they can be organized into two or four separate images or stacks.

The advantage of using an image splitter is to enable you to simultaneously acquire two or four images of the same sample at different wavelengths. Each image is identical to the others and each can be acquired using a different emission filter. The images can then have appropriate overlay colors assigned to make it easier to identify the separate wavelengths.

Two different image splitters are available for use with MetaMorph. The Optical Insights splitter can split an image into either two or four discrete pathways, and direct the individual images onto two or four separate areas of the camera chip. The Hamamatsu splitter can split the image into only two discrete pathways.

**Procedures:**

Splitting Images - Split Tab

Splitting Images - Align Tab

Splitting Images - Configure Tab

Splitting Images - Overlay Tab

**Dialog Box Options:**

Split View Dialog Box Options - Split Tab

Split View - Dialog Box Options - Align Tab

Split View - Dialog Box Options - Configure Tab

Split View - Dialog Box Options - Overlay Tab

## Splitting Images - Split Tab

To begin setting up an image or image stack to be split, complete the following steps, then click the Configure tab.

| Step | Action |
|---|---|
| **1** | Ensure that the image or image stack that you want to split into separate images or stacks is open.<br><br>**OR**<br><br>Open the *Acquire* dialog and begin acquiring a *Live* image. |
| **2** | From the *Display* menu, click *Split View*. The Split View dialog box opens. |
| **3** | If you are using a live image to align your views, open the live image in the *Source* image selector. If you are splitting a stack, you can choose whether to split the entire stack or only a single image in the stack. |

| | |
|---|---|
| **4** | On the *Split* tab, choose whether you want to organize your destination images as a single stack, or a single image or a single stack for each wavelength. |
| **5** | Click the Configure tab. |

## Split View - Dialog Box Options - Split Tab

### Source

Selects and opens the image or image stack that you want to split into separate views. When both the source and destination images are stacks, the wavelengths will be interleaved in the destination stack. Therefore, when a two wavelength image is saved as a single stack, the image order is Image1, Wave1; Image1, Wave2; Image2, Wave1; Image2, Wave2, and so on. When selecting images from a stack, you can select a single image, or the entire stack.

### Destination

Designates where the destination images will be placed. The destination image or stack can be organized into a single stack combining all images and all wavelengths, or they can be organized into two or four discrete images or stacks.

#### *Stack*

Saves the images as a single stack of two or four images or a stack of interleaved wavelength images. Use the associated image selector to designate an image name.

#### *Separate Images*

Saves the separated image or image stack as either two or four separate images or image stacks with each specific wavelength assigned to a specific stack.

### Apply

Applies the settings that you made on the four split view tabs and creates the designated destination images or image stacks.

### Close

Closes the Split View dialog box.

## Splitting Images - Align Tab

To make settings on the *Align* tab in preparation for splitting one or more images, complete the following steps, then click the *Overlay* tab if you are using the Color Overlay method to align your images.

| Step | Action |
|---|---|
| **1** | Click the *Align* tab. The *Align* page is displayed. |
| **2** | In the Image Selector, choose the image that you want to use to complete your image alignment steps.<br><br>**Note:** Be sure to select the entire stack or a single image, as appropriate. |
| **3** | In the Alignment Options box, Click *Subtraction* to use a grayscale subtraction alignment image.<br><br>**OR** |

Click *Color Overlay* to use the color overlay image alignment method.

**4** In the Alignment image box, click *Show alignment image* to visually align two or four images.

**5** If you are splitting four images and using the subtraction alignment method, one wavelength at a time, choose each of the image wavelengths that you need to align with the Wavelength 1 image.

**6** In the *Region size* box, set the size of the region that you want to include in your destination image.

**7** To help you visually align your images, click *Crosshair*. A centered crosshair is placed on the image.

**8** In the image window, you should see either two or four rectangular regions. These regions can be resized and moved to achieve a visual alignment. As you move the regions, the values for *H Shift* and *V Shift* will change. If you resize the regions, the Region size values for Height and Width will change.

**9** To reset the values on the Align tab to their default values, click *Reset*.

**10** If you chose the *Color Overlay* alignment method, click the *Overlay* tab. Otherwise, once you have finished aligning your images, Click the Split tab, then click *Apply*.

## Split View - Dialog Box Options - Align Tab

### Source Image

Selects the source image to be used for image alignment. If this dialog is being used in conjunction with the Acquire dialog box, you can use the *Live* image to ensure that all acquired images are properly aligned.

### Alignment Options

Provides two different alignment options that you can use, depending on the bit depth of the image.

***Subtraction*** – Creates a grayscale overlay image in which the selected image is subtracted from the Wavelength 1 image. You can change the subtraction value from the default of 128 to improve the visual quality of the subtraction alignment image. When an image is correctly aligned using the subtraction alignment method, all image details that are completely identical will cancel each. Therefore, in those areas, only a shade of gray will be visible.

***Color Overlay –*** Creates an overlay image for images that are less than 24 bits in bit depth. Use the color overlay option to achieve a precise image alignment.

### Alignment Image

Provides settings that enable you to select the images to use for Subtraction alignment and enables you to display an interactive alignment image.

***Show Alignment Image –*** Overlays the images that you chose as alignment images.

Wavelength 1 is the reference image; Wavelength 2, 3, or 4 is the subtraction image.

***W1 and W2*** – Aligns Wavelength 2 image with Wavelength 1

***W1 and W3 –*** Aligns Wavelength 3 image with Wavelength 1

***W1 and W4 –*** Aligns Wavelength 4 image with Wavelength 1

**Region Size**

Specifies the region size in height and width. Type or select values in the Height and Width boxes to change the size of the region. This region size is applied equally to all of the image regions (either two or four).

**H Shift**

Specifies the left position of the upper left corner of the image.

**V Shift**

Specifies the top position of the upper left corner of the image.

**Multiplier**

Multiplies the overall image intensity of the wavelength image with which it is associated.

**Subtraction Constant**

Specifies a subtraction constant to use when aligning the image. The default constant is 128 for 8-bit images, and 1000 for 16-bit images.

**Crosshair**

Places a centered crosshair on the image. This is a centered vertical and centered horizontal line. Use this option as a visual reference when adjusting and aligning the image regions.

**Reset**

Resets the region sizes, region positions, multiplier values and subtraction constant to default values.

## Splitting Images - Configure Tab

To make settings on the *Configure* tab in preparation for splitting one or more images, complete the following steps, then click the *Align* tab:

| Step | Action |
|---|---|
| **1** | Click the *Configure* tab. The *Configure* page is displayed. |
| **2** | In the *Splitter optics* box, choose either 2 Wavelenghts or 4 Wavelengths, depending on the type of splitter. |
| **3** | If you chose 2 Wavelengths, choose the correct image orientation (Left/Right) or (Top/Bottom).<br><br>**Note:** If your installation uses a twin camera configuation, check *Twin Camera* mode. |
| **4** | If you want the wavelength values for your wavelengths annotated into the *Image Info* area of each image, type the wavelength values for each emission filter into the *Filters* boxes. |
| **5** | Click the Align tab. |

## Split View - Dialog Box Options - Configure Tab

### Splitter optics

Selects the number of pathways into which the device splits the image. Choose either two or four wavelengths.

***2 Wavelengths –*** Creates two equal size image areas. The orientation of these images areas can be either left-to-right (horizontial) or top-to-bottom (vertical).

***4 Wavelengths –*** Creates four equal size image areas. Use the settings on the align tab to change the size and position of the image areas.

***Left/Right –*** Selects left-to-right (vertical) image orientation.

***Top/Bottom –*** Selects top-to-bottom (horizontial) image orientation.

### Filters

Specifies the wavelengths used to acquire the associated image.

### Twin Camera mode

Provides a different 2-image configuration from a single source image in which there is no margin between the two images. This option is intended to be used with image splitters that use two cameras that combine the two images into a single image. When *Left/Right* is selected, the images are arranged diagonally (lower left/upper right). When Top/Bottom is selected, the images are touching.

## Splitting Images - Overlay Tab

Complete the following steps to specify the hue to assign to each overlay color applied to individual wavelengths. You should make these setting before beginning to use the Color Overlay method to align your images.

**Note:** Settings on the overlay tab apply only to using the color overlay for image alignment, and have no effect on the appearance of the destination image.

| Step | Action |
|---|---|
| **1** | Click the *Overlay* tab. The Overlay page is displayed. |
| **2** | To change the intensity of any individual hue relative to the other hues, type or select an appropriate color in the associated *Balance* box. |
| **3** | To assign a predefined color, click the Hue dropdown list, then select the appropriately named color hue. The *Hue* indicator box for the selected wavelength will change to the newly selected hue. |
| **4** | If you want to assign a named color that is not on the list of predefined colors, click Edit hue list. The Edit hue list dialog box opens. |
| **5** | If you want to assign a color other than those named in the Hue drop-down list, simply move the Hue slider control to the location of the color you want to use. |
| **6** | To let MetaMorph automatically control the intensity of each color, check *Auto Balance*. |
| **7** | If you want to enhance or brighten the areas where fluorescence probes overlap, check |

*Boost Colocalization*.

**8** To change the overall brightness of the image, type or select an appropriate value in the *Brightness* box. The default value is 50.

**9** To use a specific wavelength as the grayscale "background" (transmitted light) image in the alignment image, click *Gray* adjacent to the wavelength that you want to assign as grayscale.

**10** Click the Align tab to return to the image alignment setting, and complete the image alignment.

## Split View - Dialog Box Options - Overlay Tab

### Hue

Indicates the overlay color assigned to the selected wavelength. The region corresponding to that wavelength on the source image will be set active. The Hue slider will also indicate the selected Hue.

### Balance

Sets a scaling factor for each overlay. The default is 50.

### Gray

Sets the wavelength to be used as the grayscale "background" (transmitted-light) image in the alignment image.

### No Gray

Turns of all Gray settings

### Hue

Sets the overlay color for the selected wavelength that you are configuring. When a specific wavelength is selected, move the slider to choose a color to be assigned to the wavelength. If you move the slider to a position corresponding to one of the default colors, the Hue List will automatically update to display the name of the color. Otherwise, the list will display "Unnamed." The color box will be updated with the selected color.

### Edit hue list

Opens the Edit Hue List dialog box. Use this to customize and name a hue that is not currently listed.

### Auto Balance

Sets the intensity balance between the wavelengths automatically. If you select this option, the Balance settings will be deactivated .

### Boost Colocalization

Enhances the intensity of the areas in which two or more fluorescence probes overlap.

### Brightness

Sets the intensity in the alignment image, based on the intensities of the two wavelengths. The default setting is 50.

# Interlace Images (Display Menu)

**Creates a single image from the even-field scan lines from one source image and the odd-field scan lines from a second source image.**

**Drop-in: ILACE**

This command can be used to combine ratio image pairs from Image-1/FL. Images from Image-1/FL are stored so that the first wavelength will be in the even scan lines and the second wavelength will be in the odd scan lines of the saved image. Interlace Images can be applied to stacks, provided that both source stacks have the same number of planes.

**Note:** This command does not support 24-bit color images.

## Interlacing Images

To interlace two images or two stacks, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Display menu, choose Interlace Images. The Interlace Images dialog box opens. |
| **2** | Select the first image using the *Numerator* image selector. If the selected image is a stack, select *All* (planes) or the *Current* plane from the image selector. |
| **3** | Select the second image using the *Denominator* image selector. If the selected image is a stack, select *All* (planes) or the *Current* plane from the image selector. |
| **4** | Select the desired destination and name for the interlaced image using the *Interlaced* image selector. |
| **5** | Choose *OK*. |
| **7** | Choose *Close* when you have finished. |

## Interlace Images - Dialog Box Options

**Numerator**

Specifies the first source image or stack for the interlaced image.

**Denominator**

Specifies the second source image or stack for the interlaced image.

**Interlaced**

Specifies the destination and name used for the resulting interlaced image.

**OK**

Interlaces the selected images or stacks.

**Close**

Closes the dialog box.

# Duplicate as Displayed (Edit Menu)

**Creates a 24-bit image from an existing image that includes all graphical elements.**

**Drop-in: 24BITCPY**

Use this command to create 24-bit true color images from binary, 8-, 16-, 24-, or 48-bit images. All graphical elements will become part of the image: thresholding overlays, object overlays, labels, arrows, text, region outlines, etc.

**Note:** If a region of interest is active in the source image, as indicated by a blinking, dashed outline, just the area delineated by the region's bounding rectangle (determined by a rectangular region's outline or by bounding an elliptical or irregularly shaped region with a rectangular box) will be used to create the new image. If you have drawn regions on the source image and do not want to restrict the size of the destination image, be sure to use the Locator Tool to click outside of the regions so that none of the regions is still active.

**QUICK TIP:** If you want to specify the colors of region outlines, first convert your source image to 24 bits using the Color Combine command, then use the Paint Region command to paint the region outlines, setting the *Paint Mode* to *Region Outline Only* and selecting your preferred color with the *Color* command button. Be sure to delete the regions from your source image when you have finished by right-clicking the region outline and choosing Delete Region from the pop-up menu that appears.

## Duplicating a Displayed Image

To create a 24-bit image from an image of another bit-depth, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Edit menu, choose Duplicate as Displayed. The Duplicate as Displayed dialog box opens. |
| **2** | Select the source image with the *Source Image* selector. |
| **3** | Select the desired destination image using the *Destination Image* selector. You can *Overwrite* or *Add To* the existing image, or you can place the results in a *New* image window. |
| **4** | When you are ready, choose *OK.* The 24-bit image will be created. |
| **5** | When you have finished, choose *Close.* |

## Duplicate as Displayed - Dialog Box Options

### Source Image

Selects the image to be converted to 24 bits.

### Destination Image

Selects the destination for the 24-bit image. You can *Overwrite* the existing image or place the results in a *New* image window. Or you can *Add To* the existing image or stack as a plane.

### OK

Creates the 24-bit presentation image.

### Close

Closes the dialog box.

# Arrow (Display Menu)

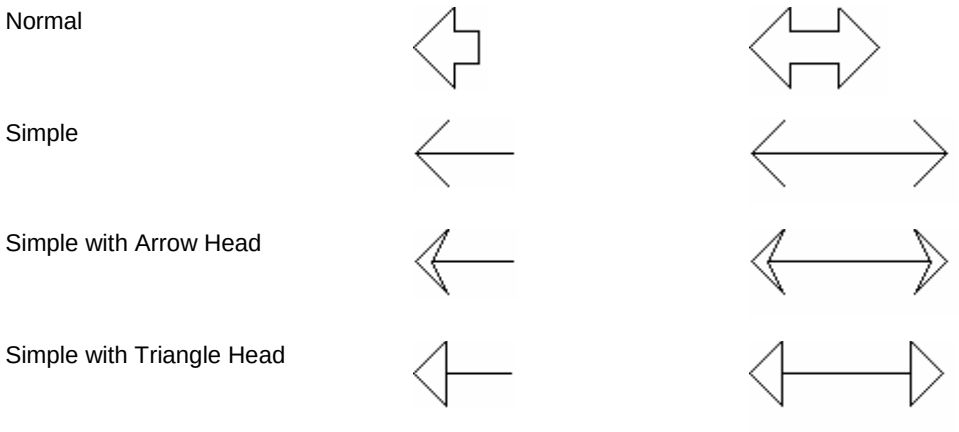
**Draws an arrow symbol onto the selected image.**

**Drop-in: ARROW**

Use this command to add a graphical arrow symbol to your image to prepare it for publication or for presentation. You can specify the arrow's shape, size, position, angle of rotation, grayscale value

(binary, 8-bit, and 16-bit images) or color (24-bit images), and the proportions of the arrow's head and tail. In addition, you can add a text label to each arrow.

You can choose from four different shapes of arrow. Each can be either single- or double-headed:




**WARNING:**
The arrow and its label will become a permanent part of the image. If you need to make morphometric or densitometric measurements of the image, you should do so before drawing an arrow on the image. Alternatively, you might want to consider making a copy of the original image with the Duplicate Image command (Edit menu), and draw the arrow on the duplicate image.

**For More Information about Images:**

**Duplicate Image**

**Stamp Date/Time**

**Stamp Calibration Bar**

## Painting an Arrow onto an Image

To paint an arrow symbol onto an image, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Display > Graphics menu, choose Arrow. The Paint Arrow dialog box opens and an arrow-shaped region in the currently specified configuration will appear on the image. |
| **2** | If necessary, use the *Image* selector to select the image onto which you want to draw the arrow. |
| **3** | Select a shape for the arrow from the *Arrow Type* button group.<br>**AND**<br>If you want a double-headed arrow, select the *Double-Headed* check box. |
| **4** | If you want the arrow to consist of its head only, with no tail, clear the *Include Arrow Tail* check box.<br>**OR**<br>If you want the arrow to have a tail, leave the |

*Include Arrow Tail* check box selected and select a size for the tail (8 - 250) with the *Tail Size* spin box.

**5** To change the overall length of the arrow (head and tail, combined), select a size (1 - 100) from the *Length* spin box or slider.

**AND**
To change the width of the arrow (head and tail), select a size (1 - 100) from the *Width* spin box or slider.

**6** To change the angle of rotation of the arrow, select an angle (0 - 359) from the *Rotation* spin box or slider. An angle of *0* specifies an arrow pointing to the left.

**7** Position the arrow in the image by dragging it with your pointer, or use the *X* and *Y* spin boxes in the *Position* option group to select the X and Y coordinates, respectively.

**8** To specify a grayscale value (binary, 8-bit, and 16-bit images) or a color (24-bit images) for the border and interior of the arrow, choose *Colors.* The Arrow Colors dialog box will appear.

**OR**
If you want to use the default color scheme of a black border and white interior, skip to Step 12.

**9** If you want the image to show through the interior of the arrow, clear the *Paint Arrow Interior* check box.

**OR**
If you want to specify a "fill" for the arrow, select the *Paint Arrow Interior* check box. If you are drawing the arrow on a grayscale image (binary, 8-bit, or 16-bit), use the *Color* spin box to select a grayscale intensity value for the interior of the arrow. If you are drawing the arrow on a 24-bit color image, choose the *Color* command button and select a color from the Color dialog box that appears and choose OK.

**10** If you do not want to draw a border for the arrow, clear the *Paint Arrow Border* check box.

**OR**
If you want to draw a border for the arrow that differs in color from its interior, select the *Paint Arrow Border* check box. Then specify a grayscale value (binary, 8-bit, or 16-bit images) or color (24-bit images) for the border with the *Color* option, as in Step 9.

**11** Choose *OK* to return to the Paint Arrow dialog box.

**12** If you want to label the arrow, select the *Paint Region Label* check box. Then type the

desired label in the *Label Text* box.

**13** To apply the arrow to the selected image, choose *Paint.*

If you change your mind, you can choose *Undo* to remove the arrow. You can also repaint the removed arrow by choosing *Redo.*

**14** Choose *Close.*

## Arrow - Dialog Box Options

### Image

Selects the image on which you want to draw the arrow.

### Arrow Type

Selects a shape for the arrow.

### Double-Headed

Specifies that the arrow be double-headed (back-to-back).

### Include Arrow Tail

Specifies that the arrow is to be drawn with a tail. If you clear this check box, just the head of the arrow will be drawn.

### Tail Size

Specifies a length for the tail of the arrow. The size can range from 8 to 250.

### Position

Specifies the X and Y coordinate of the center of the arrow. If the arrow region is off of the image, you may need to adjust these settings before you can drag the region with your pointer.

### Length

Selects an overall length for the arrow (head and tail, combined). This value can range from 1 to 100. The default size is 20.

### Width

Selects an overall width for the arrow (head and tail). This value can range from 1 to 100. The default size is 20.

### Rotation

Selects an angle of rotation for the arrow. This value can range from 0 to 359. An angle of 0 will specify an arrow that is pointing to the left; an angle of 90 will point the arrow towards the top of the image.

### Paint Region Label

Labels the arrow with the text you type in the *Label Text* box.

### Label Text

Specifies a text label for the arrow. To label the arrow, you will also need to select the *Paint Region Label* check box.

### Paint

Draws the arrow, as configured, onto the image. The arrow will become a permanent part of the image.

### Undo/Redo

Undoes or redraws the arrow.

### Colors

Opens the Arrow Colors dialog box, from which you can specify a color for the arrow's interior and border.

### Close

Closes the Paint Arrow dialog box. If the Arrow Colors dialog box is open, you will need to close it before you can close the Paint Arrow dialog box.

## Arrow Colors - Dialog Box Options

### Paint Arrow Interior

Specifies a "fill" for the interior of the arrow. If you clear this check box, the image will show through the interior of the arrow.

### Color spin box (Paint Arrow Interior)

Selects a grayscale value for the interior of the arrow. If you clear the *Paint Arrow Interior* check box, this option will be unavailable. If you are drawing on a binary (1-bit) image, you can select either 0 (black) or 1 (white). If you are drawing on an 8-bit image, you can select an intensity between 0 and 255. If you are drawing on a 16-bit image, you can select a value between 0 and 65535. If you are drawing on a 24-bit color image, this spin box will be replaced by a *Color* command button, which opens the Color dialog box.

### Color command button (Paint Arrow Interior)

Opens the Color dialog box, from which you can select a color for the interior of the arrow. This option will be available only for 24-bit color images.

### Paint Arrow Border

Specifies that a border be drawn for the arrow. This allows you to select an intensity value or color for the outline of the arrow that differs from that of the interior.

### Color spin box (Paint Arrow Border)

Selects a grayscale value for the border of the arrow. If you clear the *Paint Arrow Border* check box, this option will be unavailable. If you are drawing on a binary (1-bit) image, you can select either 0 (black) or 1 (white). If you are drawing on an 8-bit image, you can select an intensity between 0 and 255. If you are drawing on a 16-bit image, you can select a value between 0 and 65535. If you are drawing on a 24-bit color image, this spin box will be replaced by a *Color* command button, which opens the Color dialog box.

### Color command button (Paint Arrow Border)

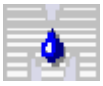
Opens the Color dialog box, from which you can select a color for the border of the arrow. This option will be available only for 24-bit color images.

### OK

Accepts any changes you made to the settings in the Arrow Colors dialog box and closes the dialog box.

### Cancel

Cancels any changes you made to the settings in the Arrow Colors dialog box and closes the dialog box.



# Grid (Display Menu)

**Draws a grid of a specified gray level on a grayscale or color image in an image window or on an external video monitor, using the selected number of vertical and horizontal lines.**

#### Drop-in: GRID

Use this command to draw a grid on an image or stack. This command allows you to specify the number of vertical and horizontal lines, as well as the line color and line width. You can *Undo* a grid drawn on an image or the current plane of a stack.

When a video monitor is selected as the location of the grid, this command will draw the grid on a frozen image displayed on the monitor, not on the live video display. You can use the Live Video dialog box to

restore live video and clear the grid from the monitor. If you want the grid to be part of the acquired images, you can draw a grid on a blank image and then use that image as background subtraction image for live video. (Remember to reverse the colors of the grid lines when you draw the grid--use the line color 255 on a dark image to create a grid with black lines when using background subtraction.) Or you can draw the grid on the live video image window and then copy that image.

## Drawing a Grid

To draw a grid on an image or video monitor, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Display > Graphics menu, choose Grid. The Draw Grid dialog box opens. |
| **2** | If you are drawing the grid on an image in an image window, select the desired image using the *Image* selector. If the selected image is a stack, select *All* (planes) or the *Current* plane from the image selector. |
| **3** | Select whether you want the grid drawn on the *Selected Image* or the *Video Monitor* from the *Draw On* group. |
| **4** | Select the desired number of vertical and horizontal lines using *Horizontal Lines* and *Vertical Lines.* The number of boxes that will be drawn with the current selections will be displayed next to these options. |
| **5** | Select the thickness of the grid lines using *Line Thickness.*<br>**AND**<br>Select the grayscale value of the grid lines using *Line Color.* |
| **6** | Choose *Draw Grid.* |
| **7** | Choose *Close* when you have finished. |

## Grid - Dialog Box Options

### Image

Specifies the image window for the grid if the image is to be displayed on the computer monitor.

### Draw On

Specifies whether the grid will be drawn on the selected image or the external video monitor (if a video board is being used).

### Vertical Lines

Specifies the number of vertical lines for the grid.

### Horizontal Lines

Specifies the number of horizontal lines for the grid.

### Line Thickness

Specifies the thickness of the grid lines, in pixels.

### Line Color

Specifies the grayscale value for the grid lines.

### Draw Grid

Draws the specified grid on the selected image or on the video monitor.

### Undo/Redo

Undoes/redoes the last grid drawn on an image or the current plane of a stack.

### Close

Closes the dialog box.

# Stretch and Mirror (Display Menu)

**Resizes an image by rescaling it in the X and Y axis directions. Creates a mirror image by flipping the image horizontally or vertically.**

#### Drop-in: STRETCH

Use this command when you want to scale an image proportionally to a larger or smaller size, or when you want to stretch or compress an image using different vertical and horizontal proportions. You can also flip the image horizontally and/or vertically while stretching it. If a region of interest is active, the command will resize and/or flip the area in the region of interest.

The *Horizontal Mirror* and *Vertical Mirror* options which flip the image are similar to MetaMorph's Flip: Horizontal and Flip: Vertical commands on the Graphics menu.

**Image-1/AT:** You can convert Image-1/AT images, acquired using rectangular pixels, for use in MetaMorph by stretching the image horizontally to 125%.

**For More Information about Editing Images:**

**Duplicate Image with Zoom**

**Flip**

**Rotate**

## Stretching and Mirroring an Image

To stretch and mirror an image, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Display menu, choose Stretch and Mirror. The Stretch and Mirror dialog box opens. |
| **2** | Select the source image using the *Source Image* selector. If the selected image is a stack, select *All* (planes) or the *Current* plane from the image selector. |
| **3** | Select the destination image using the *Destination Image* selector. You can overwrite or add to the existing image or you can place the results in a new image window. |
| **4** | Select the desired percentages of horizontal and vertical stretch or compression using *Stretch Horizontally* and *Stretch Vertically.*<br><br>To enlarge or reduce the image proportionally, select the same value for both options. |
| **5** | You can select *Interpolate When Stretching* for images that are stretched greater than |

100 percent.

**6** You can select *Horizontal Mirror* and/or *Vertical Mirror* to flip the image in the desired direction.

**7** Choose *OK* when you have finished.

## Stretch and Mirror - Dialog Box Options

### Source Image

Selects the source image that you want to stretch, compress, and/or flip.

### Destination

Selects the destination for the stretched/compressed image. You can overwrite the existing image or place the results in a new image window. Or you can add the stretched/compressed image as a plane to an existing image or stack.

### Stretch Horizontally

Specifies the percentage that MetaMorph should stretch or compress the image horizontally.

### Stretch Vertically

Specifies the percentage that MetaMorph should stretch or compress the image vertically. To reduce or enlarge the image proportionally, select the same value for this option as *Stretch Horizontally.*

### Interpolate When Stretching

Select this option when you are stretching an image larger than 100%. Interpolation computes what the grayscale or color values between the original values should be in the stretched image. Normally, when a stretch of 400% is applied to an image, sixteen times as many pixels are used to represent each pixel's value. This creates a chunky, pixelated image that is not as smooth as the original image. Rather than applying the same value to a larger block of pixels, interpolation computes the intensity values for each new pixel based on the original values surrounding it.

### Horizontal Mirror

Flips the image on its vertical axis, thereby creating a mirror image. You can use *Horizontal Mirror* and *Vertical Mirror* at the same time.

### Vertical Mirror

Flips the image on its horizontal axis, thereby turning the image upside down. You can use *Horizontal Mirror* and *Vertical Mirror* at the same time.

### OK

Stretches and/or mirrors the selected image.

### Cancel

Cancels the command.

# Boxes on Binary Image (Display Menu)

**Places a fixed grid of boxes of user-specified height, width, and line thickness on a selected binary image and indicates the image size, the total number of boxes, and total area in pixels inside each box.**

**Drop-in: GRIDBIN**

Use this command to place a line grid on a binary image. You can set the width, height and line thickness dimensions in pixels for the grid of boxes, or you can use the default values which are 100 X 100 with a line thickness of 1 pixel. The dimension settings for box width and height control the spacing between lines on the grid. The image values displayed in the dialog box indicate the image size in pixels, the area in pixels inside each box, and the number of boxes. The smaller the dimensions for

width and height, the greater the number of boxes in the image. Depending on the image background, you can select either white or black lines for the image grid. The default setting is black lines. If your background is black, select white lines.

You can overlay applications of the grid boxes on a single binary image. This can be useful for creating a graduated grid with the smallest spacing using the thinnest lines. For example, you might first apply a grid of 10 X 10 with a line width of 1 pixel, then apply a grid of 20 X 20 with a line width of 2 pixels. Thus, every second line in the grid is a heavier weight.

**Note:** Boxes on Binary Image can only be applied to a binary image. If your image is not binary (only black and white pixels) you can convert it to a binary image using the Binary Image Operations command.

**Note:** Boxes on Binary Image makes only complete boxes of the specified dimension in your image. No partial boxes are created. Any image area not included in a complete box is discarded. Therefore, your final image with the grid applied might be smaller than the original, and might have areas excluded that were part of the original image.

## Drawing Boxes on a Binary Image

To apply a grid of either black or white boxes to a binary image, complete the following procedure:

| Step | Action |
|---|---|
| **1** | Open a binary image to which you want to apply a box grid. |
| **2** | From the Display > Graphics menu, choose Boxes on Binary Image. The Draw Boxes on Binary Image dialog box opens. |
| **3** | If more than one binary image is open, click the Image box, and choose the binary image to which you want to apply the box grid. |
| **4** | In the Box Width box, type or select the box width in pixels. |
| **5** | In the Box Height box, type or select the box height in pixels. |
| **6** | In the Line Thickness box, type or select the line thickness for the grid lines. |
| **7** | Click Rename Image if you want the command to rename your image to a name composed of the values for the number of boxes, and the width, height, and the area in pixels within each box |
| **8** | In the Color area, click White for white lines on a black background, or click Black for black lines on a white background. |
| **9** | Click Draw Boxes to apply the specified grid to your binary image. |
| **10** | Click Revert to undo the application of the grid to your binary image. |
| **11** | Click Close to close the dialog box after you have completed all steps and are satisfied with the results. |

## Boxes on Binary Image - Dialog Box Options

### Image

Indicates the name of the binary image available for application of the grid boxes. Boxes on Binary Image can only be applied to a binary image. If more that one binary image is open, the Image drop-down list will contain the names of all open binary images.

### Image Size

Indicates the size of the image in pixel width and height.

### Box Width

Specifies the width setting to be applied to the box grid.

### Box Height

Specifies the height setting to be applied to the box grid.

### Line Thickness

Specifies the line thickness to be applied to the box grid.

### Rename Image

Indicates that you want to rename the image to a name that incorporates the values for the number of boxes, the width, height, and the area in pixels within each box.

### Box Area

Indicates the area in pixels within each box.

### # Boxes

Indicates the total number of boxes within the image.

### Color

Specifies whether to apply a box grid of white lines or black lines. Apply white lines to a black background; apply black lines to a white background.

### Draw Boxes

Applies the box grid to the image based on the settings made in the dialog box.

### Revert

Removes the most recently applied box grid from the image.

**Note:** Once you close the dialog box, Revert cannot remove the last grid applied.

### Close

Closes the dialog box and permanently applies all grids to the image.

# Text (Display Menu)

**Draws text of a specified gray value on the selected image.**

**Drop-in: TEXT**

Use this command when you want to place text on an image at a specified location. You can place one line of text at a time on the selected image. You can erase the image area behind the text and specify a background color for that area.

You can place the text anywhere on the image prior to drawing. This command creates an active region to mark the location of the text, so that you can see where the text will be drawn. You can use the options in the dialog box to change the location of the text or you can move and resize the region, as

with a rectangular region (the region defines the space for the text). Once you choose *Draw,* the text will become part of the image, overwriting any image data under it.

This command can be applied to 1-bit, 8-bit, 16-bit, or 24-bit images. You can use the *Undo* option in the dialog box to undo it or you can use the Undo command in the Edit menu.

**QUICK TIP:** Text boxes can be copied and pasted into successive images in precisely the same location as in the original image. When you right-click the text region in the source image, a pop-up context menu will appear, from which you can choose Copy Region. You can then right-click in the destination image and choose Paste Region from the context menu. The region outline will be pasted into the destination image, and you can then apply the Draw Text command by selecting the destination image from the image selector and choosing *Draw.* You can also use the Arithmetic command to merge text onto an image.

**WARNING:**
When you apply the text to an image, the text will become a part of the image itself. If you subsequently attempt to perform a densitometric or morphometric measurement on the entire image, the grayscale values and morphometric characteristics of the text will be measured along with the objects in the image. Be sure to perform your measurements before you apply any graphics to the image. If there is any chance that you will need to reanalyze the original image, you should make a copy of the original and apply the text to the copy.

**WARNING:**
If you are drawing text on a 16-bit image that has already been scaled using the Scale 16-Bit Image command's *Auto Scale* option, you should select grayscale values for the text and background colors that do not differ greatly from the high and low intensity values in the image itself. Because the text becomes part of the image, the image intensities will be rescaled when an excessively bright or dark text label is applied to an autoscaled image. This can have the undesirable effect of severely reducing the apparent contrast in the image and making it too dark or too bright.

**For More Information about Editing Images:**

**Paint Region**

**Transfer Regions**

**Copy**

**Paste**

**Arithmetic**

## Drawing Text on an Image

To draw text on an image or stack, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Display > Graphics menu, choose Text. The Text dialog box opens. |
| **2** | Select the desired image using the *Image* selector. If the selected image is a stack, select *All* (planes) or the *Current* plane from the image selector. |
| **3** | Select the X and Y coordinates of the desired location for the text using the *X* and *Y* options.<br>**OR**<br>Click in the middle of the text region and drag the region outline to the desired location. The values in the *X* and *Y* text boxes will change accordingly. |

4 Select the desired gray value for the text color using *Text Color.*

5 If you want to erase the image area behind the text, select *Erase Image Behind Text.*

**AND**

Select the desired gray value for the background color behind the text using *Back. Color.*

6 Type the desired text in the *Text* box.

7 Choose *Draw.*

**Note:** If you write text that goes beyond the bounds of the image, an error message will be displayed.

8 Choose *Close* when you have finished.

## Text - Dialog Box Options

### Image
Specifies the image or stack for the drawn text.

### X
Specifies the X-coordinate on the image for the location of the text.

### Y
Specifies the Y-coordinate on the image for the location of the text.

### Text Color
Specifies the grayscale value to be used for the text.

### Erase Image Behind Text
Erases the image area behind the text and fills it in with the grayscale value specified by *Back. Color*.

### Back. Color
Specifies the grayscale value used behind the text if you selected *Erase Image Behind Text.*

### Text
Use this text box to type the desired text to be drawn on the image.

### Draw
Draws text on the selected image or stack.

### Close
Closes the dialog box.

### Undo
Undoes the last text drawn on an image.

# Gray Wedge (Display Menu)

**Draws a reference grayscale "wedge" on the specified image, using your choice of starting and ending grayscale or color values.**

**Drop-in: WEDGE**

Use this command when you want to place a reference grayscale or color wedge on an image. This

command places the wedge in the active region in the image, or uses the entire image if there is no active region. If necessary, you can use the Flip or Rotate commands (Display > Graphics menu) to change the orientation of the wedge.

Once you have placed a wedge in an image, you can use it to verify that your video monitor is adjusted correctly by transferring the image to the monitor with the Transfer Image to Video command.

The starting value (gray level or color) will be on the left side of the wedge and the ending value will be on the right side. If the height is greater than the width, the wedge will be drawn vertically, with the starting value at the lower edge.

**Note:** This command does not support binary (1-bit) images.

**For More Information about Editing Images:**

**Flip**

**Rotate**

**Transfer Image to Video**

## Drawing a Gray Wedge on an Image

**Drawing a Gray Wedge on a Grayscale Image**

**Drawing a Color Wedge on a 24-Bit Image**

## Drawing a Gray Wedge on a Grayscale Image

To draw a reference gray wedge on an 8-bit or 16-bit image or stack, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Display > Graphics menu, choose Gray Wedge. The Gray Wedge dialog box opens. |
| **2** | Select the desired image using the *Image* selector. If the selected image is a stack, select *All* (planes) or the *Current* plane from the image selector. |
| **3** | Define an active region on the source image using a Region Tool**.**<br><br>The width of the active region, in pixels, should equal the number of gray levels that you want to represent (for example, the region should be 256 pixels wide for a wedge that represents grayscale values 0 - 255). Otherwise, not all of the grayscale levels in the specified range can be represented. |
| **4** | Select the desired starting gray value for the left side (or lower edge) of the gray wedge using *Starting Gray Value.* |
| **5** | Select the desired ending gray value for the right side (or upper edge) of the gray wedge using *Ending Gray Value.* |
| **6** | Choose *Apply.* The reference gray wedge will be drawn on the image.<br><br>If you change your mind, you can choose *Undo* to remove the gray wedge. You can also repaint the removed wedge by choosing |

*Redo.*

**7** Choose *Close* when you have finished.

## Drawing a Color Wedge on a 24-Bit Image

To draw a reference color wedge on a 24-bit color image or stack, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Display > Graphics menu, choose Gray Wedge. The Gray Wedge dialog box opens. |
| **2** | Select the desired image using the *Image* selector. If the selected image is a stack, select *All* (planes) or the *Current* plane from the image selector. |
| **3** | Define an active region on the source image using a Region Tool**.**<br><br>The width of the active region, in pixels, should equal the number of color levels that you want to represent (for example, the region should be 256 pixels wide for a wedge that represents red values 0 - 255). Otherwise, not all of the levels in the specified range can be represented. |
| **4** | Choose the *Color* command button in the *Starting Color* row. The Color dialog box will appear. |
| **5** | Select the desired color from the *Basic Color* palette displayed at the top of the dialog box, so that the selected color's outline is highlighted.<br><br>**OR**<br>Use the *Red, Green,* and *Blue* text boxes to define the values for a custom color and then choose *Add to Custom Colors.*<br><br>**OR**<br>Click inside the *Color Refiner Box* to select a color and then choose *Add to Custom Colors.* |
| **6** | Choose *OK* to return to the Draw Gray Wedge dialog box. |
| **7** | Repeat Steps 4 - 6, choosing the *Color* command button in the *Ending Color* row. |
| **8** | Choose *Apply.*<br><br>If you need to undo the color wedge you just created, you can choose *Undo.* Choose *Redo* to reapply the color wedge. |
| **9** | When you have finished, choose *Close.* |

# Gray Wedge - Dialog Box Options

### Grayscale Images

### 24-Bit Color Images

## Gray Wedge (Grayscale Images) - Dialog Box Options

### Image
Specifies the image or stack for the gray wedge.

### Starting Gray Level
Specifies the first grayscale level displayed on the left side (or lower edge, depending on its orientation) of the gray wedge. The default initial value for this option will match the limits of the selected image.

### Ending Gray Level
Specifies the last grayscale level displayed on the right side (or upper edge, depending on its orientation) of the gray wedge. The default initial value for this option will match the limits of the selected image or grayscale value 255, whichever is lower. This value can be increased for 16-bit images.

### Apply
Draws the gray wedge on the specified image in the active region, if available, or on the entire image.

### Undo/Redo
Undoes or redraws the gray wedge.

### Close
Closes the dialog box.

### Gray Wedge (Color Images) - Dialog Box Options

### Image
Specifies the image or stack for the color wedge.

### Starting Color
Opens the Color dialog box, from which you can select the starting color displayed on the left side (or lower edge, depending on its orientation) of the color wedge.

### Ending Color
Opens the Color dialog box, from which you can select the ending color displayed on the right side (or upper edge, depending on its orientation) of the color wedge.

### Apply
Draws the color wedge on the specified image in the active region, if available, or on the entire image.

### Undo/Redo
Undoes or redraws the color wedge.

### Close
Closes the dialog box.

# Show/Hide Image at Full Screen (Display Menu)

**Centers the selected single-plane image and displays it against a black background.**

**Drop-in: FULLSCR**

Use this command to display an image by itself, without any other desktop elements. The image will be centered in the screen and displayed against a black or white background. The Show Image at Full Screen command will be particularly useful for preparing images for screen capture or for display during a presentation. The display will revert back to its original state when you press any keyboard key or the left mouse button.

You will not be able to use this command simultaneously with the Movie command (Stack menu).

However, the Movie window has a control to play back at full screen.

**QUICK TIP:** If you use this command in a journal, you can use the Delay command to specify the length of time that each image is to be displayed.

**Note:** This command does not alter the size of the image. If you want to display the image at its maximum possible size, you will first need to use the Zoom Tool.

### Showing an Image at Full Screen

To display an image at full screen, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Display > Graphics menu, choose Show/Hide Image at Full Screen. The Show/Hide Image at Full Screen dialog box opens. |
| **2** | If necessary, select the image to be displayed with the *Image* selector. |
| **3** | From the *Background Color* group, select whether you would like the image to appear on a black or white background. |
| **4** | Choose *OK.* The image will be displayed at full screen.<br><br>**Note:** If the image is already being displayed at full screen, clicking *OK* will hide the image. Conversely, if the image is currently hidden, clicking *OK* will show the image at full screen. |
| **6** | If you are performing a screen capture, move the cursor off-screen and perform your screen-capture routine. |
| **7** | To revert back to the original display state, press a keyboard key or the left mouse button.<br><br>**OR**<br>Select Hide from the *Action* radio button group and choose *OK.* |
| **8** | Choose *Close.*<br><br>**Note:** If you are playing back this command in a journal, the dialog box will have closed automatically when you chose *OK* in Step 4. |

## Show Images at Full Screen - Dialog Box Options

### Image

Specifies the image or stack to be displayed at full screen.

### Background Color

Selects whether the image will be shown on a black or a white background.

### OK

Displays the selected image

### Close

Closes the dialog box.

# Graph Settings

**Use the Graph Settings command to configure graphs in MetaMorph.**

This command contains settings that affect the features and display of the active graph in MetaMorph.

Opening the Graph Settings Dialog Box

Using the Graph Settings Command

Graph Setting - Dialog Box Options

## Opening the Graph Settings Dialog Box

Use one of the following procedures to open the Graph Settings dialog box from any graph in MetaMorph:

| Step | Action |
|---|---|
| **1** | Click the Down Arrow button directly below the left side of the graph and select Graph Settings from the drop-down menu.<br>OR<br>Double-click in any portion of the graph.<br>OR<br>Right-click on any portion of the graph that does not contain data and select Graph Settings from the drop-down menu. |
| **2** | The Graph Settings dialog box opens. |

## Using the Graph Settings Command

Use the following procedures to configure graph settings:

Configuring a Trace Line

Configuring Graph Titles

Configuring the Background

## Configuring a Trace Line

To configure a trace line, use the following procedure:

| Step | Action |
|---|---|
| **1** | Click the Down Arrow button directly below the left side of the graph and select Graph Settings from the drop-down menu. The Graph Settings dialog box opens. |
| **2** | Click the *Traces* tab. |
| **3** | Configure the appearance of the trace line using the *Name*, *Show*, *Color, Line Style, Width, Mark Style,* and *Size* options.<br>The *Name* field can be edited. *Color* displays the color of the trace line. *Line Style* specifies |

the type of line used. *Width* specifies the pixel width of the line. *Mark Style* selects the style of marker to be used. *Size* selects the size of the mark style, if applicable

**4** If you want to change the color of a trace line, click the color box for the trace to open the Color dialog box. Select a color and click *OK.*

OR

To make all trace lines the same color, right-click the color heading and select Change All Colors to select a color.

**5** If you want to change the line style of the trace, click the drop-down arrow in the *Line Style* column and select the desired style.

OR

To make all trace lines have the same line style, right-click the Line Style heading, select Change All Line Styles, and choose a style from the drop-down list.

**6** If you want to change the mark style of points on a trace, click the drop-down arrow in the *Mark Style* column and select the desired style.

OR

To make all trace lines have the same mark style, right-click the Line Style heading, select Change All Mark Styles, and choose a style from the drop-down list.

**7** To save the settings you created so they can be loaded later, click *Save* to open the Save Graph Settings dialog box and save your settings.

**8** Click *Close* when you have finished.

## Configuring Graph Titles

To change a graph title, use the following procedure:

| Step | Action |
|---|---|
| **1** | Click the Down Arrow button directly below the left side of the graph and select Graph Settings from the drop-down menu. The Graph Settings dialog box opens. |
| **2** | Click the *Graph* tab. |
| **3** | Type a new title name in the *Title* text box. |
| **4** | To save the settings you created so they can be loaded later, click *Save* to open the Save Graph Settings dialog box and save your settings. |
| **5** | Choose *Close* when you have finished. |

## Configuring the Background

To configure the background, use the following procedure:

| Step | Action |
|---|---|
| **1** | Click the Down Arrow button directly below the left side of the graph and select Graph Settings from the drop-down menu. The Graph Settings dialog box opens. |
| **2** | Click the *Appearance* tab. |
| **3** | Click *Background Color* to open the Color dialog box. Select a color and click *OK*. |
| **4** | To save the settings you created so they can be loaded later, click *Save* to open the Save Graph Settings dialog box and save your settings. |
| **5** | Choose *Close* when you have finished. |

## Graph Settings – Dialog Box Options

The following tabs are used in the Graph Settings dialog box:

Samples Tab

Graph Tab

Traces Tab

X-Axis Tab

Y-Axis Tab

Legend Tab

Appearance Tab

### Undo Changes

Reverses any changes made in the current session.

### Load

Opens the Load Graph Settings dialog box. Use this to load previously saved graph settings to apply to the active graph.

### Save

Opens the Graph Settings dialog box. Enables you to save the graph settings of the active graph to a file (*.cfg) for subsequent re-use using the Load command.

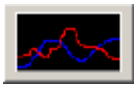
### Close

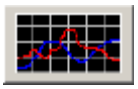
Closes the dialog box.

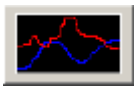
## Graph Settings Dialog Box Options - Samples Tab

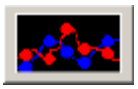
### Click a button to change the way the graph looks

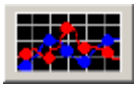
Enables you to change the appearance of graphs. The following choices, from left to right, are available:

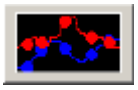

Trace lines

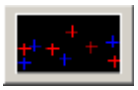

Trace lines and grid lines

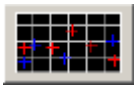

Trace lines and vertical space traces

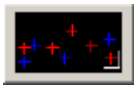

Trace lines and circle point marks

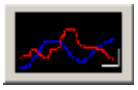

Trace lines, circle point marks, and grid lines

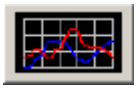

Trace lines, circle point marks, and vertical space traces

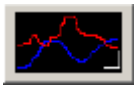

Plus point marks


Plus point marks and grid lines


Plus point marks and bar markers

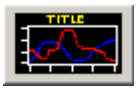

Trace lines and bar markers


Trace lines, grid lines, and autoscale X- and Y-axes

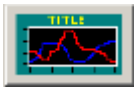

Trace lines, bar markers, and vertical space traces

### Color scheme

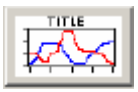
Enables you to change the color scheme of graphs. The following choices, from left to right, are available:



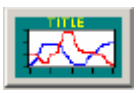
Black background and white foreground.



Black background and colorful outside background. You can edit the background colors in the Appearance tab.



White background and black foreground.



White background and colorful outside background. You can edit the background colors in the Appearance tab.

## Graph Settings Dialog Box Options - Graph Tab

### Title
Default name of graph. This name can be edited.

### Graph Type
Selects a graph type to use. The following choices are available: Linear (default), Log x linear y, Linear x log y, Log x log y, and Bar.

### Show Title
Toggles the graph title on and off.

### Show Border
Toggles the graph border on and off.

### Show Border One Pixel Outside Graph Interior
Ensures the border is outside the graph interior.

### Space Traces Vertically
Divides the visible vertical space by each trace (line on graph) and separates the traces so each trace is clearly visible.

### Margins

#### Top
Specifies the top margin of the graph (in pixels).

#### Bottom
Specifies the bottom margin of the graph (in pixels).

#### Left
Specifies the left margin of the graph (in pixels).

#### Right
Specifies the right margin of the graph (in pixels).

## Graph Settings Dialog Box Options - Traces Tab

### Key
Displays the color and style of each trace.

### Name
This field can be edited.

### Show
Toggles the display of the trace.

### Color
Displays the color of each trace. Click the color box to edit.

### Line Style
Selects the line style of the trace. Solid is the default.

### Width
Selects the width (in pixels) of the trace. Click the box to edit.

### Mark Style

Selects the mark style of the trace. None is the default.

### Size

Selects the size of the mark style if applicable.

## Graph Settings Dialog Box Options - X-Axis Tab

### Title

Displays the title of the X-Axis. This field can be edited.

### Range

Selects the X-Axis range for the graph. The From and To fields are only enabled when *Auto scale range to encompass the data* is not selected.

### Auto scale range to encompass the data

Automatically sets the range of the axis to be equal to the minimum and maximum data point values.

### Invert range values on axis

Inverts the range value on the X-Axis.

### Show

Toggles the following features of the X-Axis:

**Title**

**Major ticks**

**Axis line**

**Minor ticks**

**Bar marker**

**Tick label**

### Grid lines

Selects the vertical grid lines displayed on the graph.

### Tick Marks

#### Use automatic placement of ticks

Automatically places ticks on the X-Axis based on the amount and range of data in the graph.

#### Major ticks

Selects the number of major ticks on the X-Axis. This field is only enabled when Use automatic placement of ticks is unchecked.

#### Minor ticks

Selects the number of minor ticks on the X-Axis.

#### Decimal digits

Selects the number of decimal points to use in the X-Axis values.

#### Rotate tick labels 90 degrees

Rotates the X-Axis tick labels 90 degrees.

#### Location of ticks

Selects whether tick marks are located outside or inside the graph, or both.

## Graph Settings Dialog Box Options - Y-Axis Tab

### Title

Displays the title of the Y-Axis. This field can be edited.

### Range

Selects the Y-Axis range for the graph. The From and to fields are only enabled when *Auto scale range to encompass the data* is not selected.

### Auto scale range to encompass the data

Automatically sets the range of the axis to be equal to the minimum and maximum data point values.

### Invert range values on axis

Inverts the range value on the Y-Axis.

### Show

Toggles the following features of the Y-Axis:

#### Title

#### Major ticks

#### Axis line

#### Minor ticks

#### Bar marker

#### Tick label

### Grid lines

Selects the vertical grid lines displayed on the graph.

### Tick Marks

#### Use automatic placement of ticks

Automatically places ticks on the Y-Axis based on the amount and range of data in the graph.

#### Major ticks

Selects the number of major ticks on the Y-Axis. This field is only enabled when Use automatic placement of ticks is unchecked.

#### Minor ticks

Selects the number of minor ticks on the Y-Axis.

#### Decimal digits

Selects the number of decimal points to use in the Y-Axis values.

#### Rotate tick labels 90 degrees

Rotates the Y-Axis tick labels 90 degrees.

#### Location of ticks

Selects whether tick marks are located outside or inside the graph, or both.

## Graph Settings Dialog Box Options - Legend Tab

### Show legend

Toggles the legend on or off.

### Columns

Selects the number of columns in the legend.

### Show legend border

Toggles the legend border on or off.

### Show legend background

Toggles the legend background on or off.

### Key

#### Trace and marker

Displays the trace in the key as it is represented on the graph—the line width and marker will match the graph.

#### Square

Displays each trace as a colored square in the key.

### Traces

#### Key

Displays the color and style of each trace on the legend.

#### Name

Displays the name of each trace shown on the legend. This field can be edited. You can also drag a name to rearrange the order shown in the legend.

## Graph Settings Dialog Box Options - Appearance Tab

### Simple

Enables you to select the foreground color, background color, and font for the active graph.

### Custom font and color for each graph item

Opens a table that enables you to select a custom font and color for each graph item.

### Foreground Color

Opens the Color dialog box to change the foreground color.

### Background Color

Opens the Color dialog box to change the background color.

### Font

Opens the font dialog box to change the font and font size for the active graph.

# Set Color Threshold (Measure Menu)

**Selects a threshold range for 24-bit color images.**

*Availability: Available for MetaMorph Basic and MetaMorph Premier*

#### Drop-in: CLRTHRES

Use this command to select upper and lower limits for a continuous threshold for a 24-bit color image. You can select the Red-Green-Blue (RGB) color space model, the Hue-Saturation-Intensity (HSI) color space model, or the Hue-Saturation-Luminosity (HSL) color space model, and perform thresholding manipulations through each color component, or "channel," of that model.

The Set Color Threshold command also has an "interactive" *Set by Example* mode, which allows you to select a threshold range based on the values of the pixels in the image that you click on with your mouse

cursor. This is somewhat similar to the use of Color Threshold Mapping Tool in the Region Tools window. The major difference is that, with the Set Color Threshold command, you can select a pixel that represents the lower end of the threshold range, and then select a pixel that represents the upper end. MetaMorph will automatically select all values that are between the values of the two pixels. By contrast, the Color Threshold Mapping Tool specifies just the values of the selected pixels, providing a discontinuous threshold range.

Color threshold range settings that are configured with the Set Color Threshold command can be saved and loaded as color threshold range (*.ctr) state files.

## Setting a Color Threshold

To set a color threshold, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Measure menu, choose Set Color Threshold. The Set Color Threshold dialog box opens. |
| **2** | Select the image to be thresholded using the *Image* selector. |
| **3** | If you want to use a previously saved set of color threshold settings, choose *Load Range.* Otherwise, skip to Step 5. |
| **4** | The Load Color Threshold Range dialog box opens.<br><br>**AND**<br>Select the icon for the desired color threshold range (*.ctr) state file. If necessary, use the *Look In* list or Up One Level icon button to locate the correct drive and folder. Then choose *Open.* The threshold settings will be applied to your image. Now skip to Step 11. |
| **5** | From the *Color Model* list, select the color model you want to use for setting the color threshold: *RGB, HSI,* or *HSL.* Your selection will determine the options you see in the lower half of the dialog box. |
| **6** | If you selected *HSI* or *HSL* as your color model in Step 5, use the *Hue Range* radio button group to select whether the ranges between the upper and lower limits are to be included in the threshold range *(Inclusive)* or if the ranges outside of the upper and lower limits are to be thresholded *(Exclusive).* |
| **7** | Use the sliders or the left and right spin boxes for each of the color channels (Red-Green-Blue, Hue-Saturation-Intensity, or Hue-Saturation-Luminosity) to select the lower and upper threshold range values. As you adjust the settings, the distribution of the red thresholding overlay that covers pixels with the selected values will change. |
| **8** | If you want to use the interactive "point-and-click" method of selecting the threshold range, choose *Set by Example.* The dialog box will expand, revealing two more options. If necessary, reset the threshold range by |

selecting the *Reset Color Threshold Range on Next Click* check box, so that a check mark appears in it.

**AND**

Use the pointer to select pixels in the image that have the values that you want to include in the threshold range. As you click the pixels, they will be covered by the red thresholding overlay, and the color channel sliders and spin boxes will update to display the new values.

If you want to remove the values of the pixels you selected last, choose *Undo Last Click.*

**9** By default, the thresholding *State* is in *Inclusive* mode, which is to say that the range that you have selected is included in the threshold range and will be highlighted by a red thresholding overlay. If you want to reverse the selection so that the pixels in the range you have selected are excluded from thresholding and all other pixels are included instead, select *Exclusive* from the *State* option button group.

**10** If you want to save the threshold settings, choose *Save Range.* The Save Color Threshold Range dialog box will appear. Type a name for the color threshold range (*.ctr) state file in the *File Name* text box. If necessary, use the *Save In* list or Up One Level icon button to locate the correct drive and folder. Then choose *Save.*

**11** When you have finished, choose *Close.*

## Set Color Threshold - Dialog Box Options

**Image**

Selects the image to be thresholded.

**Color Model**

Selects a color space model *(RGB, HSI,* or *HSL)* to use for configuring threshold range settings. The model you choose will determine the "color channels" that are to be used for selecting the threshold range. The *RGB* model will use the *Red, Green,* and *Blue* channels. The *HSI* model will use the *Hue, Saturation,* and *Intensity* channels. The *HSL* model will use the *Hue, Saturation,* and *Luminosity* channels.

**Hue Range**

Selects whether the ranges between the upper and lower limits are to be included in the threshold range *(Inclusive)* or if the ranges outside of the upper and lower limits are to be thresholded *(Exclusive).* This option is only available when the *HSI* or the *HSL* color space model is selected.

**Red**

Sets the lower and upper limits for the threshold range values of the red color channel. You can use either the "arrows" on the slider or the left (lower value) and right (upper value) spin boxes to set these range limits. This option is available only when the *RGB* color space model is selected.

**Green**

Sets the lower and upper limits for the threshold range values of the green color channel. You can use either the "arrows" on the slider or the left (lower value) and right (upper value) spin boxes to set these range

limits. This option is only available when the *RGB* color space model is selected.

**Blue**

Sets the lower and upper limits for the threshold range values of the blue color channel. You can use either the "arrows" on the slider or the left (lower value) and right (upper value) spin boxes to set these range limits. This option is only available when the *RGB* color space model is selected.

**Hue**

Sets the lower and upper limits for the threshold range values of the hue channel. The colors on the slider indicate which hues in the image will be selected. You can use either the "arrows" on the slider or the left (lower value) and right (upper value) spin boxes to set these range limits. The black bar under the slider indicates the color range that will be thresholded. If you selected an *Inclusive* range, the bar will span between the slider's lower and upper arrows. If you selected an *Exclusive* range the bar will be outside of one of the arrows. This option is available when either the *HSI* or the *HSL* color space model is selected.

**Saturation**

Sets the lower and upper limits for the threshold range values of the saturation channel. This determines how "undiluted" the selected colors will be. Colors with lower saturation will be paler, with a pastel appearance. Colors with higher saturation will be purer and more vivid. The "gray wedge" on the slider indicates which saturation values in the image will be selected. You can use either the "arrows" on the slider or the left (lower value) and right (upper value) spin boxes to set these range limits. This option is available when either the *HSI* or the *HSL* color space model is selected.

**Intensity**

Sets the lower and upper limits for the threshold range values of the intensity channel. This determines how bright or dark the selected colors will be. Colors with lower intensity will be darker, while those with higher intensity will be brighter. The "gray wedge" on the slider indicates which intensity values in the image will be selected. You can use either the "arrows" on the slider or the left (lower value) and right (upper value) spin boxes to set these range limits. This option is available when either the *HSI* or the *HSL* color space model is selected.

**Luminosity**

Sets the lower and upper limits for the threshold range values of the luminosity channel. As with the *Intensity* value, the *Luminosity* value determines the brightness of the selected colors, but the associated HSL color space model takes into account the eye's differential sensitivity to light of different wavelengths. The "gray wedge" on the slider indicates which luminosity values in the image will be selected. You can use either the "arrows" on the slider or the left (lower value) and right (upper value) spin boxes to set these range limits. This option is available when either the *HSI* or the *HSL* color space model is selected.

**Load Range**

Opens the Load Color Threshold Range dialog box, from which you can select a color threshold range (*.ctr) state file. When you load the settings file, the threshold settings will be applied automatically to the selected image.

**Save Range**

Opens the Save Color Threshold Range dialog box, which you can use to specify a name for a color threshold range (*.ctr) state file and save your current threshold settings.

**State**

Selects a thresholding state for the image:

*Inclusive* thresholds pixels with color values that have been selected by the three range sliders,

*Exclusive* reverses the thresholding, such that pixels with color values that have been selected by the sliders are excluded, and all other pixels are included in the thresholding, and

*Off* disables thresholding.

**Set by Example >>**

Places the Set Color Threshold command into an interactive mode. The values of image pixels that you click with the pointer will be added to the threshold range. If you select a pixel that represents the lower end of the threshold range, and then select a pixel that represents the upper end, MetaMorph will automatically select

all values that are between the values of the two pixels for the three color channels that make up the color space model. When you choose *Set by Example >>,* the Set Color Threshold dialog box will expand, revealing the *Reset Color Threshold on Next Click* check box and the *Undo Last Click* command button.

**Set by Example <<**

Condenses the Set Color Threshold dialog box and takes the command out of interactive mode.

**Reset Color Threshold on Next Click**

When this check box is selected, the next mouse click in the image window will clear the threshold range.

**Undo Last Click**

Removes the values of the pixel that was last selected from the threshold range.

**Close**

Closes the dialog box.

# Combine into B&W + Color (Display Menu)

**Combines two grayscale images (8-bit or 16-bit) into a single 8-bit image containing both gray values and color values. Converts a single grayscale image into an 8-bit image containing both grayscale and color values. This command can also be applied to a single plane or to all planes in an image stack.**

**Drop-in: COMB_BWC**

Use this command when you want to add color to a specific range of gray values in a grayscale image to make them stand out, or when you want to combine two grayscale source images into a single image which displays the contribution from one source image in color. You can specify that the colored regions be transparent (that is, will show brightness variations) or be opaque (that is, will be at a uniformly maximum intensity level).

You can select a palette for the colored region from an available list. The list includes color palettes of a particular color *(Red, Green,* or *Blue),* a palette that varies from white (highest intensity) to red *(Red-White),* a palette that varies from red to blue *(Blue-Red),* and a "pseudocolor" palette that comprises the entire color spectrum *(RainBow).* Pseudocolor (Display Mode Tool). Alternatively, you can select a user-defined palette that you have saved previously.

The resulting image will have a custom look-up table with 127 palette levels, a portion of which are grayscale and the remainder of which are in color. By default, these are roughly evenly divided between grayscale and color, but the relative proportions can be changed to suit your needs.

**Note:** This command does not support 24-bit color images.

## Using Combine into B&W + Color

To convert one or two grayscale images into a single image containing both grayscale and color values, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Display menu, choose Combine into B&W + Color. The Combine 2 Images into B&W + Color dialog box opens. |
| **2** | Select the desired destination image using the *Destination* image selector. You can overwrite or add to the existing image, or you can place the results in a new image window.<br>**AND**<br>From the *Source for Color* image selector, select a source image or stack to serve as the source for the creation of the color |

portion of the destination image.

**3** If necessary, use # *Color Values* to specify the number of color values into which the color portion of the result image is to be divided. This number, plus the number in # *Gray Values,* will always equal 127. Alterations of one will produce automatic reciprocal changes in the other.

**4** Use *Clear Level* to enter a threshold value below which the result pixels are to be displayed in grayscale.

**AND**

Use *Saturation* to enter a value above which result pixels are to be displayed at the maximum color value. This will also specify the maximum color value.

**5** Select a color palette *(RainBow, Blue-Red, Red-White, Red, Green,* or *Blue)* from the *Look Up Table* list.

**OR**

Select the *Use .LUT File* check box and choose *Select LUT.* Select an icon for a previously stored look-up table file (*.lut) from the Select a LUT File dialog box that appears and choose *Open.*

**6** From the *Source for Gray Scale* image selector, select a source image or stack to serve as the source for the creation of the grayscale portion of the destination image. If you are converting a single source image into an image with grayscale and color values, this will be the same as the image selected in the *Source for Color* image selector.

**7** Use *Black Level* to enter a threshold value below which the result pixels are to be displayed as black.

**AND**

Use *White Level* to enter a value above which the result pixels are to be displayed as white.

**8** If you wish, select the *Auto Scale* check box to have MetaMorph automatically scale the display of gray levels.

**9** Choose *OK.*

**10** Choose *Close* when you have finished.

## Combine into B&W + Color - Dialog Box Options

**Destination**

Selects a destination for the result image. You can add to or replace the existing image or stack of images, or you can place the results in a new image window.

**Source for Color**

Selects the image or stack of images that will serve as the source for the creation of the color portion of the resulting image.

**# Color Values**

Specifies the number of values into which the color portion of the look-up table is divided. The total number of values in the color and grayscale portions will equal 127. Changing the number of values in one portion will automatically cause a reciprocal change in the other.

**Clear Level**

Specifies a threshold value for the color source below which the result pixel will be displayed in grayscale. This value is also used to specify the minimum color value in the color portion of the result image.

**Saturation**

Specifies a value for the color source above which the result pixel will be displayed at the maximum color value. This value is also used to specify the maximum color value in the color portion of the result image.

**Look Up Table**

Selects the palette to be used to create the color range of the result image. You can select from the following:

***RainBow**:* a "pseudocolor" palette that includes the entire color spectrum.

***Blue-Red**:* a palette that varies from red (highest intensity) to blue.

***Red-White**:* a palette that varies from white (highest intensity) to red.

***Red**:* a palette entirely of red values.

***Green**:* a palette entirely of green values.

***Blue**:* a palette entirely of blue values.

**Use .LUT File**

Allows you to load a previously stored look-up table (*.lut) file to apply to the color portion of the new image when you choose *Select LUT.*

**Select LUT**

Opens the Select a LUT File dialog box, from which you can load a previously stored look-up table to apply to the color portion of the new image. This option will be unavailable and will appear dimmed unless *Use .LUT File* is selected.

**Source for Gray Scale**

Selects the image or stack of images that will serve as the source for the creation of the grayscale portion of the resulting image.

**# Gray Values**

Specifies the number of values into which the grayscale portion of the look-up table is divided. The total number of values in the grayscale and color portions will equal 127. Changing the number of values in one portion will automatically cause a reciprocal change in the other.

**Black Level**

Specifies a threshold value for the grayscale source below which the result pixel will be displayed as black.

**White Level**

Specifies a value for the grayscale source above which the result pixel will be displayed as white.

**Auto Scale**

Automatically expands the range of grayscale values in the new image.

**OK**

Executes the command.

**Close**

Closes the dialog box.

# Log Color Threshold (Log Menu)

**Exports the 24-bit color threshold settings as text to a log file or by Dynamic Data Exchange to another application, such as a spreadsheet. The color values of the upper and lower threshold range limits will be stored for each color channel (red, green, and blue).**

**Drop-in: EXTHRESH**

Use this command to save a text-based record of the threshold settings for a 24-bit color image. This command will save the settings for the threshold in "bins," the number of which is determined by the binning sensitivity used by the Threshold Image command.

## Logging a Color Threshold

To save a 24-bit color threshold as text, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Log menu, choose Log Color Threshold. The Log Color Threshold dialog box opens. |
| **2** | Select a source image from the *Image* selector. |
| **3** | Choose *Open Log.* The button title will change to *F9: Log Data,* and the Open Data Log dialog box will open. |
| **4** | Determine whether you want to save the thresholding information to a text file, by DDE link to an open spreadsheet, or both, by selecting the *Dynamic Data Exchange (DDE)* and/or *A Text File* check boxes.<br><br>**Note:** If you are opening a DDE link, you must already have the spreadsheet application running and the desired worksheet open. |
| **5** | If you selected *A Text File* in Step 4, the Open Data Log File dialog box will appear.<br><br>Select the icon for the desired log file or type a new file name in the *File Name* text box. If the desired folder is not listed at the top of the dialog box, use the *Save In* list or Up One Level icon button to change to the correct location. Then select a file name. If you select an existing log file name, the Log File Exists dialog box will appear. You can *Overwrite* the contents of the file, *Append* new data, or *Cancel.*<br><br>**AND**<br><br>Choose *Save.* |
| **6** | If you selected *Dynamic Data Exchange (DDE)* in Step 4, the Export Log Data dialog box will appear. |

Select the desired spreadsheet program from the *Application* list. Choose *Default* to use the default settings for the selected application.

Choose *OK* to open the DDE link. Once the application is open, MetaMorph will reappear.

**7** To configure the data log file for logging, choose *Configure Log* from the Export 24-Bit Threshold dialog box. The Configure Log dialog box will appear.

**AND**

From the *Configuration* list, select the parameters you want to log so that each is marked by a check mark next to its name (you can choose *Enable All* or *Disable All* if you want to include or exclude all of the parameters listed). Then choose *OK* to return to the Export 24-Bit Threshold dialog box.

**8** Choose *F9: Log Data* to save the threshold settings.

**9** Choose *Close.*

## Log Color Threshold - Dialog Box Options

**Image**

Selects the 24-bit color source image whose threshold settings you want to save.

**Open Log**

Opens a data log and/or a DDE link to an open spreadsheet for logging data. This command changes to *F9: Log Data* when a log file is open.

**F9: Log Data**

Sends the color threshold settings to an open data log.

**Configure Log**

Allows the selection of image and threshold bin data that are to be included or omitted from data logging. Also allows a choice of whether column titles are to be included and if data are to be listed on a single line.

**Close**

Closes the dialog box.

# Save Original and Result Loop (Journal Menu)

**Applies a journal to each image in a selected directory, saving each original image and its corresponding result image in a separate directory, using a user-supplied base file name and numeric suffix. A second journal is then applied to perform measurements on the result images and log data automatically.**

### Drop-in: SAVELOOP

Use this command to loop through all images in a directory, applying a journal to each image in turn and saving both the original image and its corresponding result image in another separate directory.

The first original image will be saved in the selected directory as *Basename0001.xxx* and its corresponding result image will be saved in the same directory as *Basename0002.xxx.* The next original image will be saved as *Basename0003.xxx,* and so on. The base name is specified in the Save Original

and Result Loop dialog box in the *Subject ID* text box, which can accommodate up to four characters.

Each original image in the specified directory will be displayed in turn in an image window that receives a window title which you supply with the *Open into Image Named* text box. This is intended to simplify journal writing so that the journal can be configured to use the specified image window name. The image will be processed by the journal you select using the *Select Journal One* command button, and the result image will be displayed in a second image window that receives a window title which you supply with the *Save Image Named* text box.

A second journal is then invoked with the *Select Journal Two* command button. This journal can be applied to either the original image or the result image, depending on how you configure the journal. A typical use might be to perform thresholding and measurement of the result image and to log the measured data to a data log or an object log. The effects of this second journal on the images themselves will not be saved.

## Saving Original and Result Images from a Journal Loop

To apply the Save Original and Result Loop command, use the following procedure.

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Save Original and Result Loop. The Save Original and Result Loop dialog box opens. |
| **2** | To select the directory containing the original images to be processed, choose *Set Open Directory.* The Set Open path dialog box will appear.<br><br>**AND**<br><br>Select an icon for any image that is in the source image directory. If necessary, use the *Save In* list or Up One Level icon button to select the correct drive and folder. Then choose *Save.* |
| **3** | To select the folder where the both the original images and the processed result images are to be saved, choose *Set Save Directory.* The Set Save Path dialog box will appear.<br><br>**AND**<br><br>Use the *Save In* list or Up One Level icon button to select the correct drive and folder. If necessary use the Create New Folder icon button to create a new folder under the currently selected one. Then choose *Save.*<br><br>**WARNING:**<br>Be sure *not* to specify the original source folder for saving the result images. If you do, the journal may continue to operate on the newly saved images, resulting in an infinite loop routine as the source images continue to be loaded from the folder in which result images are being stored. |
| **4** | In the *Subject ID* text box, type a base name that is to be applied to all images (original and result) that will be saved in the result directory. The base name can be any combination of alphanumeric characters. |

**5** In the *Open into Image Named* text box, type a name for the image window that will be used to display the original source images.

**6** Choose *Select Journal One.* The Select Journal One dialog box will appear.

**AND**

Select the icon for the journal that is to be applied to each original source image. This journal should already exist, and should be written so as to operate on the image window specified by the *Open into Image Named* text box. If necessary, use the *Look In* list or Up One Level icon button to select the correct drive and folder for the desired journal. Then choose *Open.*

**7** In the *Save Image Named* text box, type a name for the image window that will be used to display the processed image that results from the actions of the journal selected in Step 6.

**8** A second journal is then applied to either the original or result images, depending on how you write the journal. A typical use might be to open a data log or object log, perform thresholding and measurement on each image, and then log the data that is obtained.

To select the second journal, choose *Select Journal Two.* The Select Journal Two dialog box will appear.

**AND**

Select the icon for the journal that is to be applied to each image. If necessary, use the *Look In* list or Up One Level icon button to select the correct drive and folder for the desired journal. Then choose *Open.*

**9** When you are ready to apply the journal loop, choose *Run.* Each original image will be opened and displayed in an image window, the selected journal will be applied to it, and the result image will be displayed in a second image window. Both the original and result images will then be saved to the new folder. The process will repeat until all images in the source folder are used.

**10** When you have finished, choose *Close.*

## Save Original and Result Loop - Dialog Box Options

**Set Open Directory**

Selects the folder that contains the original source images. Once selected, the path for the folder will be displayed in the text box to the right. If you wish, the path can be typed directly into this text box, and the text can be edited.

**Set Save Directory**

Selects the folder into which both source images and result images will be saved, using the base file name

specified in the *Subject ID* text box. If you wish, the path can be typed directly into this text box, and the text can be edited.

**WARNING:**

Be sure *not* to specify the original source folder for saving the result images. If you do, the journal may continue to operate on the newly saved images, resulting in an infinite loop routine.

**Subject ID**

Sets the base file name that will be used for storing the original and result images in the new folder. This base can be any combination of alphanumeric characters. The first original image will be saved in the selected folder as *Basename0001.xxx* and its corresponding result image will be saved in the same folder as *Basename0002.xxx.* The next original image will be saved as *Basename0003.xxx,* and so on.

**Open into Image Named**

Specifies the name for the image window that will be used to display the original source image. The journal that is to be applied should be configured so as to use this window's name.

**Select Journal One**

Selects the journal to be applied in turn to each source image. The name of the selected journal will be displayed in a status line to the right of this command button.

**Save Image Named**

Specifies the name for the image window that will be used to display the processed result image.

**Select Journal Two**

Selects the second journal, to be applied to either the original or the result images (depending on how you write the journal). If you want to apply the journal to all of the result images, the journal should reference the name of the window as specified by the *Save Image Named* option. A typical use of a second journal might be to threshold and measure each image and save the measurement data in a log file. Any effects of this second journal on the images themselves will not be saved.

**Run**

Starts the journal loop for all images in the selected folder, saving the original and result images in the new folder.

**Close**

Closes the dialog box.

# Set Image Zoom (Display Menu)

**Applies a selected magnification level to an image that is between 1 and 800 percent of the original. Zooms a region of the image so that it fills the image window.**

**Drop-in: SETZOOM**

Use this command to specify a precise zoom level for an image between 1 and 800 percent of the original magnification, or to zoom a region of the image so that it fills the entire image window. This command, which is fully journalizable, allows you to set a zoom level that is not available in the Zoom Tool pop-up menu.

**Note:** If you want to save a copy of the resized image, use the Edit menu's Duplicate Image with Zoom command.

## Setting an Image Zoom Level

To specify and apply a zoom level to an image with the Set Image Zoom command, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Display menu, choose Set Image |

Zoom. The Set Image Zoom dialog box opens.

**2** Use the *Image* selector to select the image for which you want to change magnification.

**3** If you want to change the image magnification to a specific zoom level, use the *Zoom* spin box to select a value for the new zoom level, expressed as a percent of the original magnification. Then choose *Set Zoom.* The image will be displayed at the new zoom level. Now skip to Step 5.

**OR**
If you want to zoom in on a region of the image, continue to Step 4.

**4** To zoom in on a region of the image, use the **Rectangular Region Tool** to draw the region. Then choose *Zoom to Fit.* The region will be zoomed to fill the image window.

**5** Choose *Close* to close the dialog box.

**Note:** If you want to save a copy of the image at the new zoom level, choose Duplicate Image with Zoom from the Edit menu.

## Set Image Zoom - Dialog Box Options

**Image**

Selects the source image for which you want to change the zoom level.

**Zoom**

Specifies a zoom level to be applied to the selected image. The values are expressed as a percent of the original magnification. You can select any integer between 1 and 800.

**Set Zoom**

Applies the specified zoom level to the selected image.

**Zoom to Fit**

Zooms in on a defined rectangular region, filling the image window.

**Close**

Closes the dialog box.

# FFT (Process Menu)

**Performs fast Fourier transform filtering of images in the frequency domain, rather than in the spatial domain.**

**Drop-in: FFT**

Use this command to remove or enhance patterns of periodic "noise" in an image. This command can also be used to apply a *Blur, High Pass,* or *Homomorphic* Fourier filter to images in the frequency domain. When you apply the filter, the source image will first be transposed from the spatial domain into the frequency domain using the Fast Fourier Transform. The filter will then be applied to the image. Finally, the image will be processed using an inverse fast Fourier transform that returns it back to the spatial domain.

Because images processed with this command are filtered in the frequency domain and not the spatial domain (the way the Sharpen and Low Pass filters function), you don't need to correct for problems with

pixels that are within a distance of half the width of the kernel from the edge of the image. This avoids the problem with convolution filters in which a bordering band of pixels remains unprocessed around the periphery of the image.

The *Remove Patterns* filter is particularly useful for removing periodic noise from an image, such as might be caused by the photomultiplier tube in an intensifier. Enhance Patterns accentuates such patterns. Click here for a pair of "before" and "after" images which illustrate the effects of the *Remove Patterns* filter.

The effects of the *Blur* filter are similar to those of the Low Pass filter in that high spatial frequency elements in the image will be removed, leaving a hazy image containing low frequency information only. This operation attenuates image information above a selected spatial frequency. The cutoff frequency is selected with the *Radius* slider. Click here for an example showing the effects of the filter.

The *High Pass* filter attenuates the low frequency information in the source image. The result of this operation is an image that retains much of its edge information, and the resulting images can resemble those that result from application of an edge-detection convolution. As with the *Blur* filter, the *Radius* slider is used to select the degree of filtering. Click here for a figure which illustrates the effects of the filter.

The *Homomorphic* filter simultaneously performs a contrast enhancement and a compression of the dynamic range of intensities. The resulting effect is somewhat similar to that of the Unsharp Mask command. This figure illustrates the effects of the filter.

## Applying a Fast Fourier Transform Filter

To apply an FFT filter to an image, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Process menu, choose FFT. The FFT dialog box will appear. |
| **2** | From the *Source image* selector, select a source image to be filtered. |
| **3** | Select a destination for the filtered image with the *Result image* selector. |
| **4** | From the *Operation* group, select the FFT filter you want to apply: *Blur, High Pass, Homomorphic, Remove Patterns,* or *Enhance Patterns*. |
| **5** | Use the *Radius* slider to specify the cutoff frequency (percent) for the filter. |
| **6** | Choose *Apply.* |
| **7** | When you have finished, choose *Close.* |

## FFT - Dialog Box Options

**Source image**

Selects the source image you want to filter.

**Result image**

Selects the destination for the filtered image. You can place the results in a new image window or in a new plane appended to an existing image or stack, or you can overwrite an existing image.

**Operation**

Selects the filter to be applied to the source image:

***Blur*** selects the image smoothing operations that attenuate high-frequency components.

***High Pass*** attenuates the low-frequency components.

***Homomorphic*** simultaneously performs a contrast enhancement and a compression of the brightness dynamic range (similar to Unsharp Mask).

***Remove Patterns*** removes periodic patterns of "noise" from the image.

***Enhance Patterns*** accentuates periodic patterns in the image.

**Radius**

Specifies the radius, expressed as a percentage, of the filter cutoff frequency.

**Apply**

Applies the selected filter operation to the source image.

**Undo/Redo**

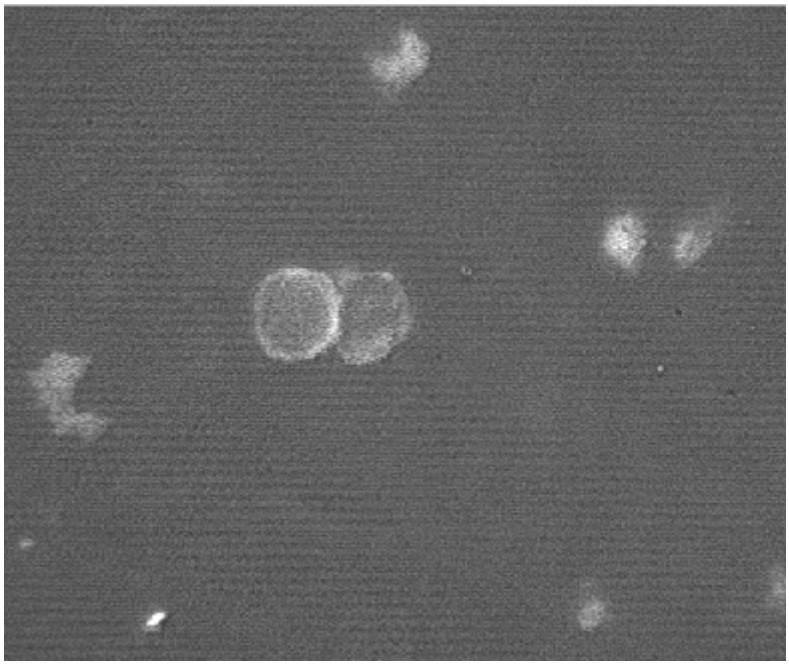
Undoes or redoes the last applicable command that did not create a new result image. (The *Undo/Redo* buttons in MetaMorph apply to any previously applied command, as if you selected it from the Edit menu, not just to the last command in the dialog box where the button was chosen.)

**Close**

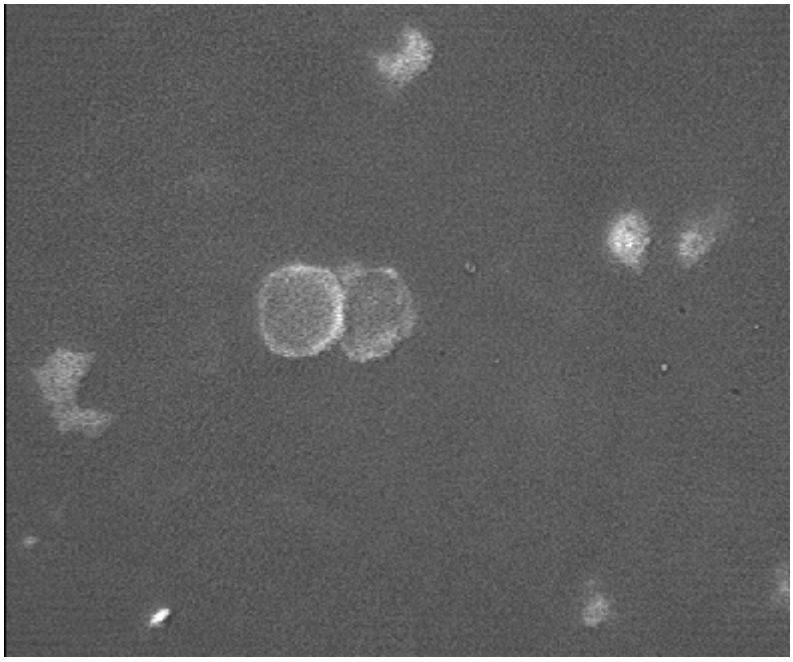
Closes the dialog box.

# The FFT *Pattern Removal* Filter

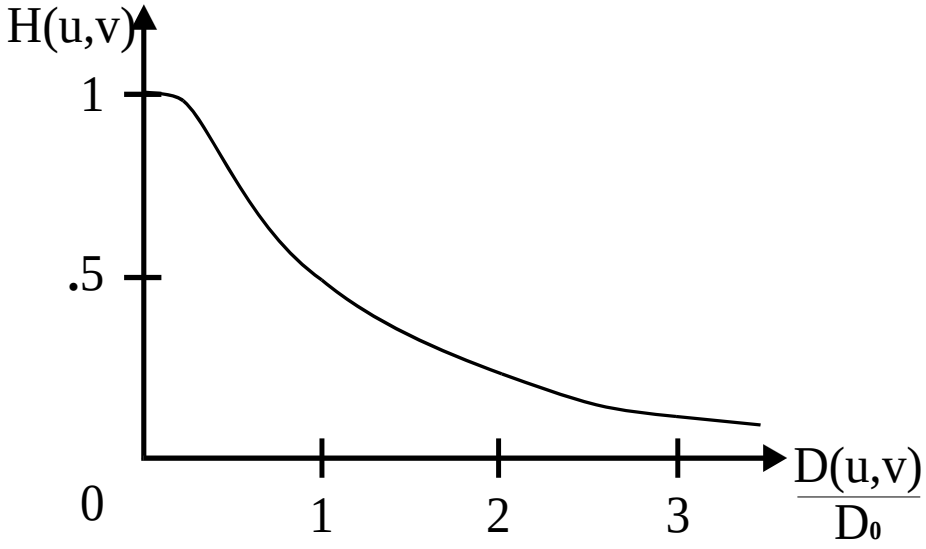
**Before:**



**After:**



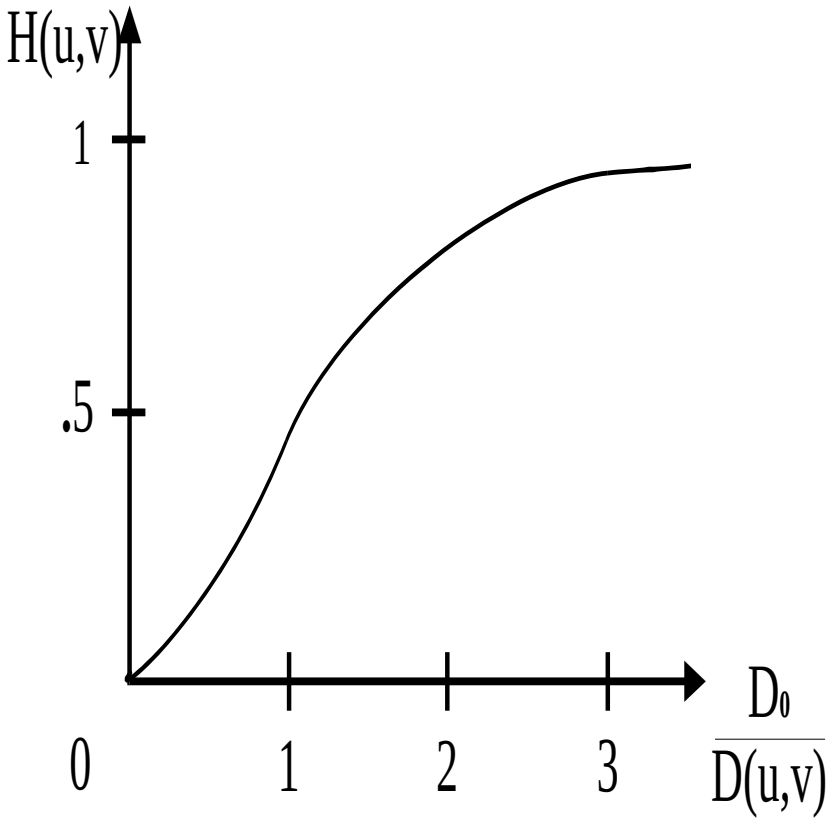
## The *Blur* FFT Filter




The equation for the *Blur* (Butterworth Low Pass) filter is:

$$H(u,v) = \frac{1}{1 + [D(u,v)/D_0]^{2n}}$$

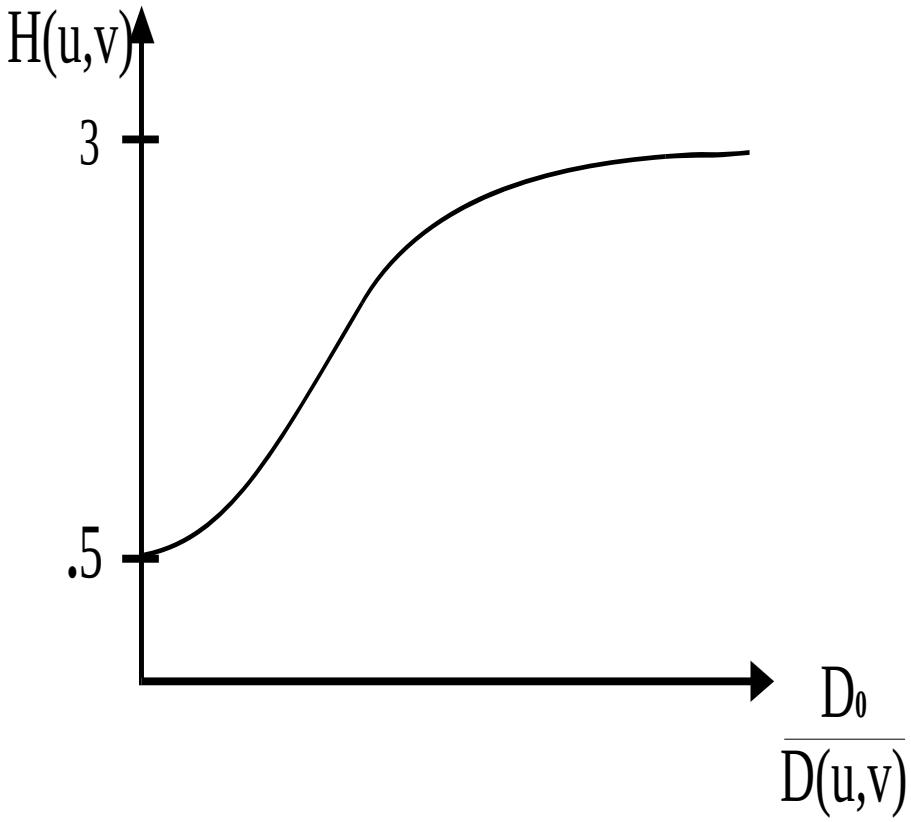
# The *High Pass* FFT Filter



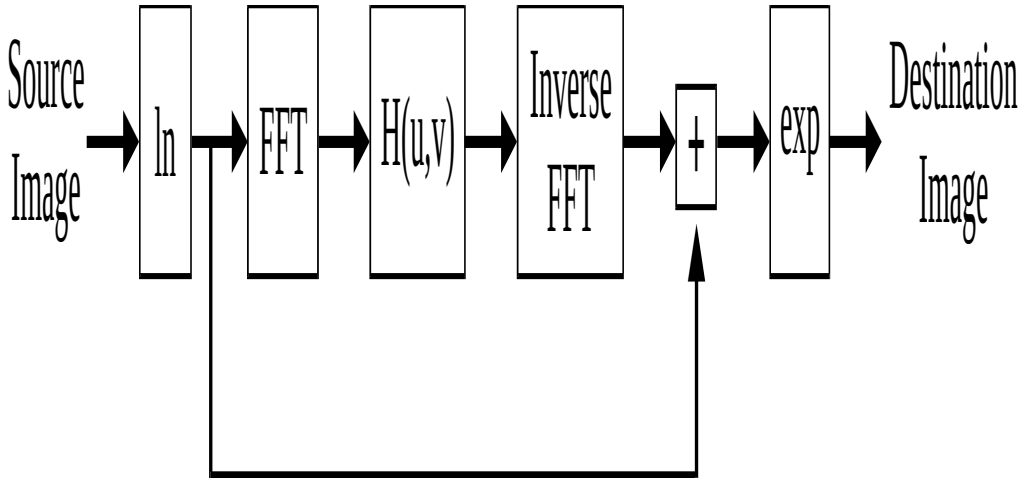

The equation for the *High Pass* (Butterworth High Pass) filter is:

$$H(u,v) = \frac{1}{1+[D_0 / D(u,v)]^{2n}}$$

# The *Homomorphic* FFT Filter




The process applied by the *Homomorphic* filter is:



# Convolve with Image Kernel (Process Menu)

**Allows you to define and/or convolve an image with a custom convolution kernel. You can create a custom image kernel using part of another image as a template for the new image kernel. You can also use this command to load kernels from a file on disk.**

**Drop-in: KERNEL**

Use this command when you want to use or define the values to be used in a custom convolution kernel for processing images. This command allows you to define a region on an image and then copy its values into a table of values for a kernel that is the same size as the region.

When you open this dialog, the drop-in will create its own region upon the image to be used to create the image kernel (the default will be the active image when the dialog was opened). The size of this region is determined by the *Width* and *Height* defined in the dialog box. *Width* and *Height* also define the size of the image kernel, so that kernel size and region size are identical. The custom kernel can range in size from 2x2 to 128x128 elements. Each element can be individually set to a value between -32,768 and 32,767. The Convolve with Image Kernel dialog box includes a table of the element values, which can be edited by right-clicking on the desired element in the table. You can use the scroll bars to examine the parts of kernels that are larger than the table window.

Once you have defined your custom image kernel, you can apply it to an image specified by the *Source* image selector and/or *Save* the new kernel for future use. You can also *Print* a copy of the image kernel.

When a custom convolution kernel is applied to a selected image, the result image is formed by convolving the image with the kernel divided by the *Element* scale. Then the result image is divided by the *Result* scale factor. In some cases, you may want specify an *Offset* value to be added to all pixel gray values after the convolution is completed, so that all pixel values remain within the image's grayscale range (0 - 255 for 8-bit images, or 0 - 32767 for 16-bit images).

*Absolute Value* allows you to determine how negative gray level results will be dealt with. For example, a kernel that would create a value of -50 in an 8-bit image would set the pixel's gray level value to zero if *Absolute Value* were set to *No,* or would set the value to 50 ( |150-200| = 50 ) if *Absolute Value* were set to *Yes.*

**Image-1/AT:** This drop-in can be used to load and apply kernels created with Image-1/AT.

## Convolving with an Image Kernel

To define a custom convolution and apply it to an image, use the following procedure.

**Note:** Once you have defined a custom kernel, you can skip steps 4 - 10 if you want to apply that kernel to an image later during the same session.

| Step | Action |
|---|---|
| **1** | From the Process menu, choose Convolve with Image Kernel. The Convolve with Image Kernel dialog box will appear. |
| **2** | Select the desired source image using the *Source image* selector.<br><br>**AND**<br><br>If you plan to define the kernel by copying values from a region to the kernel editor, select the image you want to use from the *Image with Region to Copy* image selector. |
| **3** | Select the desired destination using the *Result image* selector. |

**4** If the dialog box is condensed, choose *More >>* so that you can see the kernel size options. A region will appear that that is the same size as the kernel size.

**5** To change the size (pixels) of the kernel, type the desired dimensions in the *Width* and *Height* text boxes. You can also change the size of the kernel by resizing the region.

The kernel must be at least 2x2 elements, but cannot be larger than 128x128 elements.

**6** Once you have the region positioned over the part of image you want to use for the kernel, choose *Copy* to copy its values into the kernel table.

To see the kernel table, choose *View.* The dialog box will expand to include a table. You can edit the kernel elements by clicking inside the table cells.

**7** If necessary, use *Element* to change the element scaling from the default value of *1.* The result of the kernel element divided by the *Element* scale should not be much less than 1.0. Otherwise, significant numerical inaccuracies can occur.

**8** If necessary, use *Result* to change the result scaling from the default value of *1.* The result of the convolution will be divided by this value. The value of the *Result* scale must be set so that the sum of elements becomes as close to 1 as possible, otherwise the image values will overflow the frame buffer.

**OR**

Select the *Autoscale Result* check box so that the best *Result* value can be determined for you automatically.

**9** If you want an intensity offset value to be added to the final image pixel values after the convolution, select the desired value using *Offset.*

**10** Select *Yes* in the *Absolute Value* group to use the absolute value of each resulting pixel. Select *No* to clip any negative values to zero.

**11** Choose *Apply* to apply the custom kernel.

## Convolve with Image Kernel - Dialog Box Options

**Source image**

Selects the source image to which you want to apply the convolution kernel.

**Result image**

Selects a destination for the result image. You can overwrite the existing image or place the results in a new image window. Or you can add the resulting image as a plane to an existing image or stack.

**Image with Region to Copy**

Select the image for the region used by the *Copy* command. Whenever the Convolve with Image Kernel dialog box is in its expanded state, a region for the kernel will appear on this image.

**More >>**

Expands the dialog box to include the kernel element options.

**Less <<**

Condenses the dialog box.

**Width**

Specifies the width of the kernel. The width can range from 2 to 128 elements.

**Height**

Specifies the height of the kernel. The height can range from 2 to 128 elements.

**Element**

Specifies the number by which each kernel element should be divided before the convolution is to be performed. The total of the kernel elements divided by the *Element* scale should not deviate much from 1.0. Otherwise, the resulting image will be too dark or too light.

**Result**

The result of the convolution is divided by this value. The value of the *Result* scale must be set so that the sum of elements becomes as close to 1.0 as possible. For example, if the sum of the elements is 8, set *Result* to *8* so that the value of sum of the elements becomes 1.

**Offset**

Specifies the intensity offset value that is to be added to the final pixel values in the image after the convolution is completed so that all gray values will remain within the image's range (for example, 0 - 255 for 8-bit images).

**Absolute Value**

Specifies whether absolute values of each resulting pixel will be used or whether negative values are to be clipped to zero. Select *No* to clip the negative values to zero.

**Autoscale Result**

Instructs MetaMorph to determine the best *Result* value automatically, so that the sum of elements becomes as close to 1 as possible.

**Copy**

Copies the values in the region created by Convolve with Image Kernel to the kernel table. You should use this command after you specify the kernel's size and other elements.

**Load**

Loads a previously saved kernel so that you can edit it or apply it to the selected image.

**Save**

Saves the current kernel to disk so that you can use it during future sessions.

**Print**

Prints a copy of the Kernel Table to the default printer.

**View**

Displays the Kernel Table.

**Kernel Table**

Displays the elements for the current kernel. You can edit these values by clicking inside the desired table cell. Each element can be individually set to any value between -32,768 and 32,767. The size of the kernel is governed by the *Width* and *Height* options.

**Apply**

Applies the current convolution kernel to the specified image.

**Close**
Closes the dialog box.

# 2D Deconvolution (Process Menu)

**Removes image haze from a stack by performing either No Neighbors or Nearest Neighbors deconvolution on a plane in a stack of images.**

**Drop-In: 2DDECON**

Use this command when you want to reduce the effects of out-of-focus haze from a stack. This command can be applied to 8-bit or 16-bit images.

The **No Neighbors** command performs the operation without considering out-of-focus information contained in adjacent planes. Instead, an unsharp mask operator that utilizes a convolution kernel based on the properties of the imaging system is used to blur the image plane. This kernel is an estimation of the 3D PSF. The blurred input is then subtracted from the original input, removing the added blurred component along with the original out of focus information.

The **Nearest Neighbors** command also uses an estimation of the three-dimensional Point Spread Function (PSF) to compute the contributions from out-of-focus planes, and uses this to generate an image in which the effects of out-of-focus information are reduced.

Stacks that lend themselves well to haze-removal will have planes that were acquired with the same analog adjustment settings, and will have a maximum dynamic range. The command is useful for stacks where planes are not spaced closely together. If the planes are spaced closely together (for example, less than one-half micron for a stack imaged with an oil objective) the results obtained using the Nearest Neighbors and No Neighbors commands will be very similar.

MetaMorph enables you to control how much out-of-focus information (Scaling Factor) is removed and how much scaling is performed on the output image (Result Scale). The greater the Scaling Factor, the greater the amount of haze that is removed from the original, and thus the lower the amount of brightness from the original will be retained. If you select Auto Result Scale, MetaMorph selects a Result Scale that matches the selecting Scaling Factor so that the brightness reduction is automatically minimized.

MetaMorph also enables you to enter experimental settings that help specify the PSF (filter kernel) used to approximate the contribution of out-of-focus information from adjacent planes. As a general rule, these settings are determined by the data collection and are not typically altered to change the quality of results.

**Note:** Results obtained from the Nearest Neighbors command are not suitable for use in quantitative gray level analysis.

## The Process of Nearest Neighbors Deconvolution

The process that MetaMorph uses to remove haze from a plane can be broken down into four steps:

**Note:** This table describes the Nearest Neighbors deconvolution process that MetaMorph carries out. It is not a procedure for you to follow and complete.

| Step | Result |
|---|---|
| **1** | Convolving the adjacent planes with the PSF approximates the out-of-focus haze in the desired plane. To speed up computations, MetaMorph averages the adjacent planes before the convolution operation. |
| **2** | Before MetaMorph subtracts the estimate of the haze from the target plane; it first |

decreases the intensity range of haze estimate so that the result of the subtraction operation will be greater than zero (if the result was zero, the image would be uniformly black). Thus, rather than:

**100% - 100% = 0** intensity range, the subtraction operations will look similar to the following if a Scaling Factor of 0.75 is applied:

**100% -75% = 25%** intensity range (approximately).

**3** Next, MetaMorph subtracts the scaled haze estimate from the target plane to remove the out-of-focus information.

**4** After the out-of-focus haze estimate has been subtracted, the contrast of the result plane can be "stretched" by multiplying the output by the Result Scale. This will not restore the number of gray levels to that of the original image, but the range can be approximately restored.

## 2D Deconvolution Procedures

**No Neighbors**

**Nearest Neighbors**

## 2D Deconvolution - No Neighbors

To perform no neighbors deconvolution on an image stack, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Process menu, choose 2D Deconvolution->No Neighbors. The No Neighbors dialog box opens. |
| **2** | If the desired source stack is not displayed in the *Source image* selector, select it using the image selector. |
| **3** | If the desired destination image is not displayed in Result image selector, select it using the image selector. You can place the results in a new image window. |
| **4** | Select Auto Result Scale if you want to attempt to automatically select an appropriate result scale that matches the scaling factor. |
| **5** | Use Filter Size to select the spatial size of the kernel. Larger kernel sizes lead to less sever subtraction of hazy features. |
| **6** | Select a Scaling Factor. This selects how much out-of-focus information will be subtracted from the target image. The larger the Scaling Factor, the more information is |

subtracted from the target image.

**7** Use the Result Scale option to increase the contrast lost during the subtraction of the blur estimate from the original image. Note: Skip this step if you selected Auto Result Scale in Step 5. Changing the Result Scale setting will disable Auto Result Scale.

**8** Choose Apply.

**9** Choose Close when you have finished.

## 2D Deconvolution - Nearest Neighbors

To perform nearest neighbors deconvolution on an image stack, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Process menu, choose 2D Deconvolution->Nearest Neighbors. The Nearest Neighbors dialog box will appear. |
| **2** | If the desired source stack is not displayed in the *Source image* selector, select it using the image selector. Note: If the source stack contains only one plane the Nearest Neighbors operation will still operate, but will produce the same output as the No Neighbors command. |
| **3** | If the desired destination image is not displayed in the Result image selector, select it using the image selector. You can overwrite or add to the existing image or you place the results in a new image window. |
| **4** | Select Auto Result Scale if you want to attempt to automatically select an appropriate result scale that matches the scaling factor. |
| **5** | Use Filter Size to select the spatial size of the low pass filter. Larger kernel sizes lead to less sever subtraction of hazy features. |
| **6** | Select a Scaling Factor. This selects how much out-of-focus information will be subtracted from the target image. The larger the Scaling Factor, the more information is subtracted from the target image. |
| **7** | Use the Result Scale option to increase the contrast lost during the subtraction of the blur estimate from the original image. Note: Skip this step if you selected Auto Result Scale in Step 5. Changing the Result Scale setting will disable Auto Result Scale. |
| **8** | Choose Apply. |
| **9** | Choose Close when you have finished. |

## 2D Deconvolution Dialog Box Options

The following options are used for both the No Neighbors and Nearest Neighbors versions of the 2D Deconvolution command.

### Main tab

Select the primary 2D Deconvolution dialog box options.

### Settings tab

Selects the 2D Deconvolution settings dialog box options.

### Source image

Selects the source stack to deconvolve.

### Result image

Selects the destination for the result image. You can place the results in a new image window or you can add the resulting image as a plane to an existing image or stack.

### Apply

Applies the selected filter to the source image.

### Close

Closes the dialog box.

## 2D Deconvolution Dialog Box Options – Main tab

The following options are used for both the No Neighbors and Nearest Neighbors versions of the 2D Deconvolution command.

> **Note:** Filter Size, Scaling Factor, and Result Scale are preset to recommended default settings appropriate for most images. The recommended values are noted for each option.

### Filter Size

Specifies the size of the filter applied the stack. Larger filter sizes lead to the detection of larger, lower intensity areas and thus less severe intensity subtraction. (Recommended minimum Filter (Kernel) Size is equal to 7)

### Scaling Factor

Specifies the scaling factor applied to the stack after the filter is applied. Smaller numbers allow more of the original image features to be retained in the result image. (Recommended Scaling Factor should be equal to or greater than .9)

### Result Scale

Restores the contrast lost during the subtraction of the haze estimate from the original image. (Recommended Result Scale should be 2 or 3.) **Note:** If you type a fractional value in the *Result Scale* box, it will automatically be rounded to the nearest integer when the entry is processed.

### Auto Result Scale

Enables MetaMorph to select a Result Scale value that matches the selected Scaling Factor.

### Supress Noise

Activates background noise suppression.

### Set To Defaults

Resets all settings to the original values.

## 2D Deconvolution Dialog Box Options – Settings tab

The following options are used for both the No Neighbors and Nearest Neighbors versions of the 2D Deconvolution command.

### Distance

Selects whether the dimensions of the image (XY Spacing and Z Spacing) are taken from MetaMorph's calibrations (Calibration), or set manually (User Specified) in the dialog.

### XY Spacing

Sets, in microns, the size of a pixel laterally. Editable only if Distance is set to User Specified.

### Z Spacing

Sets, in microns, the distance between adjacent planes. Editable only if Distance is set to User Specified.

### Numerical Aperture

Sets the numerical aperture of the objective lens used to collect the image.

### Refractive Index

Sets the refractive index of the medium in which the object lens is dipped. Typically, this is 1.0 (air), 1.3333 (water), or 1.515 (oil). **Note:** You must type or select a refractive index value greater than 1. This option will not accept a refractive index value of less than 1.

### Wavelength

Sets the peak wavelength of the detected intensity spectrum (in nanometers).

# Measured PSF Decon (Process Menu)

**Deconvolves image stacks using a measured point spread function (PSF) obtained from a stack containing fluorescent microspheres.**

*Availability: Available for MetaMorph Basic and MetaMorph Premier*

**Drop-in: MEASPSF**

Use the Measured PSF Decon dialog to deconvolve image stacks distorted by blur due to a Point Spread Function (PSF). This PSF is a characteristic of the microscope's optics. The optical properties are such that individual points of light (fluorescent intensity) originating in the sample cannot be resolved onto the camera chip without some amount of blur being introduced into the image by the microscope optics. The amount of blur is determined, measured, and corrected using the Measured PSF deconvolution algorithm.

The Measured PSF Decon dialog uses a reference stack of either a single bead or multiple beads to determine the PSF required for effective deconvolution of an image. To use this dialog you must open both the stack that you want to deconvolve and the bead stack.

Once both stacks are opened and selected, chose the algorithm that you want apply, the number of iterations that you want to occur, and where applicable, the typical values for Sigma and Frequency that you want to use.

You must chose whether you are using a single bead or multiple beads specified by regions. If your bead stack has multiple beads, regions should be drawn around one or more of the beads and *Extract PSF from one or more regions* should be selected. Whereas, if you have only one bead that has already been processed by the Measured PSF Decon dialog, select *Use PSF stack directly for deconvolution.*

If you want to view the processed bead stack used as the PSF for deconvolution in addition to your deconvolved stack, click *Display PSF stack result*.

**Note:** The *Measured PSF Decon* command might have difficulty processing very large images. If you attempt to process images that are too large, the program displays the message "Maximum Image Limits Exceeded."

## Deconvolving an Image using the PSF

Use the following typical procedure to guide you in using the *Measured PSF Decon* dialog box for deconvolving images:

| Step | Action |
|---|---|
| **1** | Open the source image stack that you want to deconvolve. |
| **2** | Open the bead stack that you want to use for the point spread function. |
| **3** | Click *Source image* and select the source image from the list of open images. |
| **4** | Click *Bead Stack* and select the bead stack from the list of open images. |
| **5** | In the *Algorithm* box, select the *Fast* algorithm. |
| **6** | In the *Iterations* box, type or select an iteration number from two to ten. A typical iteration value is six or seven. |
| **7** | In the *Sigma* box, type or select a value at or close to 0.7. |
| **8** | In the *Frequency* box, type or select an appropriate value for how often you want smoothing to run. A typical value is 4 or 5. This causes the smoothing function to run during the fourth or fifth iteration. |
| **9** | In the *Bead Stack Process* area, click *Use PSF stack directly for deconvolution* if you have a PSF stack previously created by the *Measured PSF Decon* operation.<br>**OR**<br>Click *Extract PSF from one or more regions* if you created one or more regions to identify the bead or beads on which to base the Measured PSF Decon operation. Choose this option even if you have a bead stack containing only a single bead that is not centered. |
| **10** | If you chose *Extract PSF from one or more regions*, you can click *Display PSF stack result* to create and view the stack used as a PSF in the deconvolution performed by the *Measured PSF Decon* function. |
| **11** | Click *Apply* to process your source image using the settings that you made. |
| **12** | Click *Close* to close the *Measured PSF Decon* dialog box. |

## Measured PSF Decon - Dialog Box Options

***The following are the three image selectors:***

### Source image

Displays the name of the image to be deconvolved.

### Bead Stack

Indicates the name of the image that will be used for the Point Spread Function (PSF) bead stack or image stack.

### Result image

Specifies the name of the image that will be created during deconvolution. This is the standard destination image selector. The destination specifies location of the deconvolved image result.

### Algorithm

Specifies the algorithm to be used for the deconvolution. Algorithms are *Fast, Medium,* or *Slow (Most Robust)*. The slow algorithm is the most robust.

***The following three settings are configurations for each algorithm.***

### Iterations

Specifies the number of times that the algorithm is to be repeated during the deconvolution process. Suggested values when using the Fast algorithm are between 5 and 7. Suggested values when using the medium algorithm are between 10 and 20, and for the slow, most robust algorithm are between 20 and 100.

### Sigma

Sets the smoothing factor for the deconvolution process. This is how much smoothing is applied to the image during deconvolution to prevent noise build-up. Sigma is used for only the fast and medium algorithms; and is not visible when Algorithm is set to *Slow (Most Robust).* Typical value is 0.7 for both the fast and medium algorithms.

### Frequency

Specifices how often the *Sigma* smoothing factor will be applied during the deconvolution process. For example a frequency of 2 indicates that smoothing will be applied every other iteration. This option is used for only the fast and medium algorithms. Typical values are either 4 or 5 for both the fast and medium algorithms. This value can be used to control how often and/or how soon you want to apply smoothing during deconvolution. Thus, it might be better to apply smoothing later rather than earlier during deconvolution.

### Bead Stack Processing

Controls how the PSF or bead stack is used.

#### *Use PSF stack directly for deconvolution*

Processes the PSF "as-is" (single bead, no regions in the latest image window.)

#### *Extract PSF from one or more regions*

For regions placed around some or all beads in the bead stack, a PSF is created by averaging intensities from all regions using the location (x, y, and z) of the each region's maximum intesnity as the center averaging coordinate.

#### *Display PSF stack result*

Produces an additional stack along with the deconvolved result. The second stack is the PSF used for the deconvolution operation, and is called, "**PSF Result**."

> **Note:** This control is available only when ***Extract PSF from one or more regions*** is enabled.

### Apply

Applies the selected settings and creates the specified deconvolved image and optional PSF result.

### (Status)

Indicates the current status of the Measured PSF Deconvolution process.

### Close

Closes the *Measured PSF Decon* dialog box.

# Batch Deconvolution (Process Menu)

**Provides Measured Point Spread Function (PSF) 3D deconvolution processing of multiple image stacks both on a single system or on multiple systems in a shared processing environment using network connections.**

**Drop-in: 3DDECON**

Use this dialog box when you want to process more than a single image stack using the Measured Point Spread Function (PSF) deconvolution method. Each image stack can be processed for multiple wavelengths. You can choose the most appropriate algorithm as defined by its processing speed and processing iterations. In addition, you can specify a smoothing factor (Sigma) and how often in the iterations loop it should be applied. When processing multiple stacks, you need to specify the first stack in a folder and the total number of stacks to be processed.

Batch Deconvolution requires PSF image stacks of microspheres (beads). These stacks can be with or without square regions surrounding the microspheres. For each wavelength that you are processing, you need one PSF stack.

If you are processing a large number of stacks and your system is part of a network with other MetaMorph systems with the same software version, you can initiate the distributed processing feature that is part of this command and share the processing capabilities of several processors in your network. Using this feature enables you to deconvolve more image stacks in the same time as you would deconvolve one stack on a single system. When using this feature, you have the option of doing all of your image stack deconvolution processing on server systems only, using the client system only for managing server processing tasks.

This dialog box is divided into the following four areas:

- ***Algorithms Tab*** – Contains the settings for selecting one of three PSF Deconvolution algorithms and the most appropriate settings for the number of iterations to run and the amount of smoothing to apply.
- ***Directories Tab*** – Contains the settings for the location and names of the source stacks, destination for deconvolved stacks, and number of source stacks to be processed.
- ***Wavelengths Tab*** – Enables you to specify a maximum of four wavelengths. The Wavelengths tab is used to specify the relevant microsphere (bead) stack PSF image for the selected wavelength.
- ***Networking Tab*** – Enables you to establish network connections to other systems in your network in order to create a distributed or shared system environment.

## Summary Procedure

1. Select a deconvolution method (Fast, Medium, or Slow).
2. Select the directories containing your stacks for deconvolution.
3. Designate PSF stacks with or without square regions surrounding microspheres. Select one PSF for each wavelength.
4. If you are using distributed batch deconvolution, configure your network settings. This includes ensuring

that a copy of MetaMorph is installed on each server system that will process image stacks, and that you have started ***hmagserv.exe*** on each system.

5. Process your image stacks.

## Configuring Batch Deconvolution

To configure Batch Deconvolution to process your image stacks, complete the following procedure:

| Step | Action |
|---|---|
| **1** | In the Batch Deconvolution dialog box, click the ***Algorithms*** tab. |
| **2** | In the *Algorithm* box, select the speed of the algorithm that you want to use. Choose the *Fast* algorithm for fastest processing. Obtaining the best results from this algorithm requires that your image quality is the best possible. For more robust processing, choose the *Medium* algorithm. For the best quality and most robust processing, choose the *Slow* algorithm. |
| **3** | In the Iterations box, type or select an interaction number from two to ten. A typical iteration value is six or seven. |
| **4** | In the Sigma box, type or select a value at or close to 0.7. |
| **5** | In the Frequency box, type or select an appropriate value for how often you want smoothing to run. A typical value is 4 or 5. For example, a value of 4 causes smoothing to run during the fourth iteration. |
| **6** | Click the ***Directories*** tab, then click *Select First File*. The S*elect Base File* dialog box opens. |
| **7** | In the S*elect Base File* dialog box select the folder containing the image stacks that you want to process, then click the first stack file that you want to process, and click *Open*. |
| **8** | Click *Destination*. The *Browse for Folder* dialog box opens. Choose an existing folder, or create a new folder in which to store your processed images. |
| **9** | In the *Number of Source Stacks* dialog box, type or select the total number of stacks in your selected directory that you want to process. |
| **10** | Click the ***Wavelengths*** tab. |
| **11** | In the *Number of Wavelengths* box, type or select the number of wavelengths in the stacks. All stacks should have the same number of wavelengths and the wavelength frequency values for all stacks should match. |

**12** For each wavelength that you are going to process, click *Select Psf for Wavelength n.* The S*elect Base File* dialog box opens.

**13** Select the directory folder where your PSF stacks that you want to use are stored, then choose the appropriate PSF stack for the wavelength number to which you are assigning it.

**14** If you are using distributed Batch Deconvolution processing, click the ***Networking*** tab, otherwise, skip to Step 17.

**15** Before adding Server IP addresses to the client system, make sure that each system that will be included in the Batch Deconvolution distributed processing network has a copy of the same version of MetaMorph that is on the client sytem installed.

***Note:*** The MetaMorph sofware only needs to be installed, but not running. A memory key is needed only when installing or running MetaMorph. A memory key is not needed on remote servers that are processing image stacks using Batch Deconvolution.

**16** Before adding server IP addresses to the Client system, run ***hmagserv.exe*** on each server system that will be processing image stacks. ***Note:*** Click Start, then choose *Run* and type ***hmagserv.exe*** in the Run box, and click *OK*.

**17** On the ***Networking*** tab, in the *New IP Address* box, type a static IP address for each system that you want to add to the *Server IP Addresses* list, then click *Add Server IP*.

***Note:*** All IP addresses added to the *Server IP Addresses* list must be static IP addresses. Ask you system administrator to be sure that all IP addresses that you want to use are *Static*.

**18** If you do not want to use the Client system to process image stacks, uncheck *Use this computer for processing*.

**19** After all configuration steps have been completed on all tabs, click *Apply* to begin processing your image stacks.

**20** To close the *Batch Deconvolution* dialog box and discontinue processing, click *Close*.

## Batch Deconvolution - Dialog Box Options

**Batch Deconvolution - Dialog Box Options - Algorithms**

**Batch Deconvolution - Dialog Box Options - Directories**

## Batch Deconvolution - Dialog Box Options - Algorithms

### Algorithm

Specifies the algorithm to be used for the deconvolution. Algorithms are Fast, Medium, or Slow (most robust). The slow algorithm is the most robust.

The following three settings are configurations for each algorithm.

### Iterations

Specifies the number of times to repeat the algorithm during the deconvolution process. Suggested values when using the Fast algorithm are between 5 and 7. Suggested values when using the medium algorithm are between 10 and 20, and for the slow, most robust algorithm are between 20 and 100.

### Sigma

Sets the smoothing factor for the deconvolution process. This is how much smoothing is applied to the image during deconvolution to prevent noise build-up. Sigma is used for only the fast and medium algorithms; it is not visible when Algorithm is set to Slow (most robust). Typical value is 0.7 for both the fast and medium algorithms.

### Frequency

Specifies how often the Sigma smoothing factor will be applied during the deconvolution process. For example, a frequency of 2 indicates that smoothing will be applied every other iteration. This option is used for only the fast and medium algorithms. Typical values are either 4 or 5 for both the fast and medium algorithms. This value can be used to control how often and/or how soon you want to apply smoothing during deconvolution. Thus, it might be better to apply smoothing later rather than earlier during deconvolution.

## Batch Deconvolution - Dialog Box Options - Directories

### Select First File

Opens the *Select Base File* dialog box. Use this dialog box to select the first stack from your batch for deconvolution. The number of stacks processed is determined by the value in the *Number of Source Stacks* box. All stack files to be processed must be located in the same folder.

### Destination

Selects the directory where the stacks from batch deconvolution will be saved. A message on this tab indicates: *Output filenames will be the input filenames with a prefix of "decon_"*.

### Number of Source Stacks

Specifies the total number of source stacks to process from the selected folder.

## Batch Deconvolution - Dialog Box Options - Wavelengths

### Select Psf for Wavelength #

Selects the file corresponding to the processed PSF (bead stack) to use in the batch deconvolution. Text below the button indicates either ***<None>*** or the filename of the PSF stack.

### Number of Wavelengths

Sets the number of wavelengths, and thereby the number of PSF's, to be used for batch deconvolution. This setting determines how many ***Select PSF for Wavelength #...*** buttons appear in the dialog. The minimum value is 1 and the maximum value is 4.

## Batch Deconvolution - Dialog Box Options - Networking

> ***Note:*** When running batch processing in a distributed processing configuration, systems in the configuration can process only complete image stacks. Therefore, the best processing performance is achieved when you have a large number of stacks for processing. There is no benefit in processing single stacks in this configuration.

### New IP Address

Accepts a numeric IP address in the format of *uint.uint.uint.uint*, where the 4-numbered string designates the *static* IP address of a machine to be used to batch deconvolution.

> ***Note:*** You must configure each system used for distributed batch deconvolution processing to a ***static IP address***. You can include any system accessable to your system by way of an IP address, as long as the address is static.

### Add Server IP

Adds the current value of **New IP Address** to the **Server IP Addresses** list.

### Remove Server IP

Removes the currently selected IP address from the **Server IP Addresses** list.

### Server IP Addresses

Displays a list of IP address of machines that can be used for batch deconvolution.

### Use this computer for processing

Controls whether this system (the client computer on which MetaMorph is *running*, and which is controlling the selection of server systems), is used for processing.

### Apply

Applies all settings that you have made and initiates Batch Deconvolution processing.

### Close

Closes the Batch Deconvolution dialog box. If Batch Deconvolution is currently processing, clicking close will discontinue processing.

# 3D Deconvolution (Process Menu)

**Provides Several 3D Deconvolution methods using the AutoQuant 3D Deconvolution Algorithms.**

*Availability: Available for MetaMorph Basic and MetaMorph Premier*

**Drop-in: 3DDECON**

Use this drop-in to deconvolve image stacks acquired from Widefield, Confocal, and Two-Photon microscopes. This dialog controls the AutoQuant 3D Deconvolution software and takes into consideration a variety of factors including objective lens parameters such as Numerical Aperture and Refractive Index. It also enables you to limit and control your degree of participation in making optional settings.

This dialog box contains the following four tabbed pages:

***Settings*** – Accepts basic algorithm settings and shows the current microscope settings.

***Optics*** – Specifies settings relative to the optical properties of the microscope.

***PSF*** – Provides options and settings for choosing the PSF processing method.

***Expert*** – Provides options and setting for applying advanced PSF Deconvolution techniques. (Not recommended for normal use.)

## 3D Deconvolution Procedures

The AutoQuant 3D Deconvolution Procedures are divided into five parts. The first part is for the non-tabbed area of the dialog box. The remaining four parts correspond to the dialog box tabs: Settings, Optics, PSF, and Expert. Complete these procedures in sequence. For additional information, refer to the corresponding dialog box options.

To deconvolve an image stack using either an associated PSF bead stack or a calculated PSF, complete the following procedures:

| Step | Action |
|---|---|
| **1** | From the Process menu, choose 3D Deconvolution>AutoQuant Decon. The 3D Deconvolution dialog box opens. |
| **2** | Open the image stack that you want to deconvolve; or if you have more that one image open at the time, click the Source image selector in the 3D Deconvolution dialog box to choose the image that you want to deconvolve. |
| **3** | If you want to designate a unique name for the destination image, click the Result image selctor. Use the standard MetaMorph image selector conventions to rename your destination image. |
| **4** | If you have a saved State File of a previous 3D Deconvolution dialog box state that you want to use or modify, click Load State and choose the appropriate state file. |
| **5** | Go to the procedure for the *Settings Tab*. |

## Setting Parameters - Settings Tab

To configure the Settings Tab, complete the following procedure:

| Step | Action |
|---|---|
| **1** | On the Settings tab, in the Algorithms Settings area, in the Total Iterations box, type or select the number of iterations that you want to run. To determine the appropriate number of iterations, refer to the tables for Total Iterations on the Dialog Box Options page. |
| **2** | If you want to use the default Algorithm settings, click *Set Defaults* and proceed to step 5. |
| **3** | Determine the noise level present in your image stack, and choose the appropriate noise level setting in the Noise Level box. The choices available are *Low*, *Medium*, and *Other*. Low and Medium set predefined |

default values. Other enables you to type a custom value in the *Noise Value* box. The default value for Low is 2; the default value for Medium is 20.

**4** If you chose *Other* as the Noise Level, type an appropriate custom value in the Noise Value box.

**5** If you need to have faster processing, and you are willing to accept some amount of reduced resolution, click the performance check box to place a check for *Faster Processing/Reduced Resolution*.

**6** It is recommended that you initially use the expert setting built into the software. However, if you decide that you want to manually designate the expert-level PSF settings, uncheck *Use Recommended Expert Settings*. Otherwise, leave this checkbox checked.

**7** Go to the procedure for the *Optics Tab*.

## Setting Parameters - Optics Tab

To configure the Optics Tab, complete the following procedure:

| Step | Action |
|---|---|
| **1** | Click the *Optics* tab. The optics tab page is displayed. |
| **2** | In the Modality box, choose the appropriate microscope modality that corresponds to the type of microscope that was used to acquire your image stack. Refer to *Modality* in the 3D Deconvolution Dialog Box Options. |
| **3** | In the Objective lens area, choose whether you want to enable the program to obtain the values for Numerical Aperture (NA) and Refractive Index from the image data or whether you want to set these values manually. |
| **4** | If you chose Manual for the Objective Lens settings, in the Lens NA and Refractive Index boxes select the Numeric Aperture and Refractive Index for the lens that was used to acquire the image stack. |
| **5** | In the Image spacings area, choose whether you want to enable the program to obtain the values for X, Y, and Z image spacing from the image calibration data or whether you want to manually specify these values in microns. |
| **6** | If you choose Manual for Image spacings, type or select the correct image spacing values for X, Y, and Z. For additional information about making these settings refer to Image Spacings on the Dialog Box |

Options topic page.

**7** In the Emissive Wavelength area, choose whether you want to enable the program to obtain the values for each channel from the image data, or whether you want to manually specify these values for Channels 1, 2, and 3.

**8** If you choose Manual for Emissive Wavelength, in the Ch(x) boxes, type or select the appropriate wavelength value in nanometers for each active channel.

**9** Go to the procedure for the PSF Tab.

## Setting Parameters - PSF Tab

To configure the PSF Tab, complete the following procedure:

| Step | Action |
|---|---|
| **1** | Click the *PSF* tab. The PSF tab page is displayed. |
| **2** | For the PSF Starting Point, click *Theoretical PSF* if you do not have a PSF image of a bead plate. If you have a bead plate PSF image, click *Measured PSF*. |
| **3** | If you chose Theoretical PSF, you can correct for spherical aberrations. Click the Spherical Aberrations check box, then choose either *Detect SA* or *Calculate SA*. |
| **4** | Detect SA determines a Spherical Aberration correction value from the image. Click Detect SA to enable the software to determine the SA correction value to apply; OR click Calculate SA to open the Spherical Aberration dialog box. |
| **5** | If you chose Calculate SA, the *Spherical Aberration* dialog box opens. |
| **6** | In the Spherical Aberration dialog box, in the *Sample embedding medium RI box*, type or select the Refraction Index (RI) value for the embedding medium you are using. |
| **7** | In the Spherical Aberration dialog box, in the *Depth from coverslip(um)* box type or select the value in microns for your coverslip thickness, then click OK. |
| **8** | If you chose *Measured PSF* as your *PSF Starting Point*, in the Measured PSF area on the PSF tab click the *Select PSF* button for each active channel and select the appropriate PSF file for channel. |
| **9** | If you chose *Measured PSF* as your *PSF Starting Point*, in the Measured PSF area on the PSF tab you can specify whether you want to use PSF spacings from your image data or whether you want to manually |

specify PSF spacing values.

| | |
|---|---|
| **10** | To use PSF spacing values from the image data, under PSF Spacings(um), click *From Image*; to manually specify your image spacing values, click *Manual*, then type or select your image spacing values in the X, Y, and Z boxes. |
| **11** | If the *Use Recommended Expert Settings* checkbox is not checked, go to the procedure for the *Expert* tab. |

## Setting Parameters - Expert Tab

**Notes:**

- This tab will be available only if you have not checked *Use recommended Expert Settings* on the Settings tab.
- If you make any setting changes on the Expert tab, you must leave the setting *Use Recommended expert Settings* on the Settings tab checked. If you uncheck this setting, all your settings on the Expert tab will be reset to their default values.

**Hint:** Once you have made settings on the Expert tab that you want to recall and reuse, click *Save State* and save a state file with a name that indicates that it contains a unique group of expert settings.

To modify the settings on the Expert tab for their default values, complete the following procedure:

| Step | Action |
|---|---|
| **1** | Click the *Expert* tab. The Expert tab page is displayed. |
| **2** | Uncheck the XY Montage box if your deconvolution results are creating rigid, box-like artifacts (pixelation) in your images. The Default for this setting is *On* (checked). |
| **3** | Check the Z Montage box to divides your dataset into subsections along the optical axis and deconvolve these subsections separately. This option should only be turned *On* for image stacks with a large *Depth* setting, such as a stack containing more than 100 planes. The Default for this setting is *Off* (unchecked). |
| **4** | Uncheck Dynamic subvolumes if you do not want the available system memory to determine the largest subvolume allowable. This option is enabled only when XY Montage is checked. The Default for this setting is *On* (checked). |
| **5** | If you need to define a different value for *Subvolume overlap*, type the new value in the Subvolume overlap box. This value specifies the amount of pixel seam overlap in a montage. Increase this value to reduce artifacts. The default value is 10. |

**6** To change the amount of padding around image borders, type a new value in the XY Guardband box. Larger borders decrease the amount of edge and ringing artifacts. The default value is 10.

**7** To change the number of planes that will be added to the top and bottom of the subvolume, type a new value into the Z Guardband box. This guardband prevents artifacts at the seams of these subvolumes. The default value is 6.

**8** Uncheck the Intensity Correction box to turn off intensity correction. Intensity Correction, when turned on, prevents abrupt intensity changes from one image plane to another. Turn this option off if it is interfering with the accuracy of your image data. The default for this option is *On* (checked).

**9** Uncheck Minimum Intensity Removal to turn this option off. Leaving this box checked removes the erroneous background intensity level from the image data. Uncheck this box if your image stack has no erroneous image data. The default for this option is *On* (checked).

**10** Check *Accelerated SA Detection* if you want to reduce the time it takes to detect and correct for Spherical Aberration (SA). If you check this box, binning is applied to the image during SA detection. Using *Accelerated SA Detection* can influence the accuracy of the correction. The Default for this setting is *Off* (unchecked).

**11** Check *Pre-condition Imported PSF* to precondition the bead stack image whenever the measured PSF algorithm is applied. The default, and recommended setting for this option is *Off* (unchecked).

**12** In the *Object First Guess* box Choose the appropriate setting: *Flat Sheet*, *Linear Filtered Data*, or *Original Data*. See the detailed explanation of these choices on the *Expert Tab – Dialog Box Options* page. This option is used to select the starting point for the iterative estimation process of the object.

**13** In the PSF Processing area, type a new value for the Axial Stretch Factor. This applies a stretch factor to the Z axis when Theoretical PSF is selected. See the detailed explanation of this setting on the *Expert Tab – Dialog Box Options* page.

**14** In the PSF Processing area, type a new value for the PSF Waist, measured in Airy Discs. The PSF waist is the narrowest part of the PSF measurement area. See the detailed explanation of this setting on the *Expert Tab – Dialog Box Options* page.

**15** Click *disable PSF Constraints* to discontinue PSF limitations that are set on the PSF tab.

**16** In the *Result Save Interval* box, type or select a higher or lower value than the default value. This value must be a multiple of the Total Iterations value. See the detailed explanation of this setting on the *Expert Tab – Dialog Box Options* page.

**17** After all settings on all the tabs have been completed, Click *OK*; your selected source image stack will be processed accord to the settings you made in this dialog box.

**18** Click *Cancel* to discontinue using the AutoQuant 3D Deconvolution and close the dialog box.

## 3D Deconvolution - Primary Dialog Box Options

### Source image

Opens the image selector for the Source image. This is the image that you want to deconvolve. Select the name of the image that you want to deconvolve from the list of images that are open.

### Result image

Indicates the name of the Destination image. By default, this name is "Deconvolved." Click on the "Specified" image name(deconvolved) to rename the destination image name or to select and overwrite an existing image name.

### OK

Runs the AutoQuant 3D Deconvolution command using the settings that you made.

### Load State

Opens the Load State dialog box. This option enables you to load a previously saved state file and apply one or more of the saved settings to the option settings in this dialog box. The settings of controls in the main and settings dialogs can be all loaded from a state file.

### Save State

Opens the "Save State file as" dialog box. Use this option to store the current AutoQuant settings for this dialog box.

### Cancel

Closes the 3D Deconvolution Options dialog box.

## 3D Deconvolution - Dialog Box Options - Settings Tab

### Microscope Settings

Summarizes the current microscope-related settings that have been made in the dialog box .

***Modality*** – Indicates the type of microscope that was used to acquire the image(s).

***Lens NA*** – Indicates the Numerical Aperture (NA) of the objective used to acquire the image.

***Refractive Index*** – Indicates the refractive index value for the objective used to acquire the image.

***Image Spacings (um)*** – Indicates the assigned image spacing values microns. These values are based on either the image calibration values if the image is calibrated and *Calibrated* is selected for Image spacings on the Optics tab, or the values specified for X, Y and Z if Manual is selected for

Image spacings on the Optics tab.

***Wavelengths (nm)*** – Indicates the selected wavelength values for each channel in nanometers.

***PSF*** – Indicates that the Point Spread Function will be used to deconvolve the image.

### Algorithm Settings

Provides the primary settings that you can use to optimize the performance of your selected PSF algorithm. You can use the default values as a starting point. By combining different setting values from the available settings, you can determine settings that are most ideal for your images.

#### Set Defaults

Resets all values in the Algorithm Settings box to their original, default values. These values are different when Performance: Faster Processing/Reduced Resolution is checked.

#### Total Iterations

Specifies the number of times that you want the algorithm to run to complete the deconvolution process. A greater number of iterations can improve the resolution of the result image. The default value for this setting changes when *Faster Processing/Reduced* resolution is selected.

Different modalities (microscope configurations) can require different iteration values in order to optimize the deconvolution image processing process. Values set higher than the recommended setting might not provide any additional improvement to the image.

If you choose standard performance, (*Faster Processing/Reduced Resolution* is **not** checked), the following recommended Total Iterations setting values apply:

*For Widefield Data:*

| Total Iterations | Determining Factors |
|---|---|
| **1 – 80** | Minimum range of values; specify a value in this range if speed is the most important consideration. |
| **80 – 100** | Recommended range for an optimal choice between processing speed and processing accuracy and as an initial range of values. |
| **100 – 350** | Maximum recommended range under normal conditions; specify a value in this range if resolution is the most important consideration. |
| **350 – 500** | Typically not recommended; these higher iteration settings might provide better resolution, but require images with very low noise levels. |

*For Confocal Data:*

| Total Iterations | Determining Factors |
|---|---|
| **1 – 40** | Minimum range of values; specify a value in this range if speed is the most important consideration. |
| **40 – 60** | Recommended range for an optimal choice between processing speed and processing accuracy and as an initial range of values. |
| **60 – 100** | Maximum recommended range under normal conditions; specify a value in this range if resolution is the most important consideration. |

If you check *Faster Processing/Reduced Resolution*, the following recommended Total Iterations setting values apply:

*For Widefield Data:*

| Total Iterations | Determining Factors |
|---|---|
| **1 – 40** | Minimum range of values; specify a value in this range if speed is the most important consideration. |

| | |
|---|---|
| **40 – 70** | Recommended range for an optimal choice between processing speed and processing accuracy and as an initial range of values. |
| **70 – 100** | Maximum recommended range under normal conditions; specify a value in this range if resolution is the most important consideration. |

*For Confocal Data:*

| **Total Iterations** | **Determining Factors** |
|---|---|
| **1 – 10** | Minimum range of values; specify a value in this range if speed is the most important consideration. |
| **10 – 20** | Recommended range for an optimal choice between processing speed and processing accuracy and as an initial range of values. |
| **20 – 40** | Maximum recommended range under normal conditions; specify a value in this range if resolution is the most important consideration. |

**Noise Level**

Specifies the amount of noise suppression to apply to your image. Choose the amount of noise suppression that you want to apply based on the amount of noise that you visually detect. Depending on the setting that you choose, the program will apply the specified amount of smoothing during image processing to suppress the noise in your image. Choose from the following settings to specify the amount of noise suppression that you want to apply.

***Low*** – specifies that noise is minimally detected in you image. Choose this setting to apply a noise suppression value of 2. typically use this setting for Widefield images.

***Medium*** – specifies that moderate amounts of noise are visible in your image. Choose this setting to apply a noise suppression value of 20. Typically use this setting for Confocal images.

***Other*** – specifies a noise-suppression value different than the available preset values.

***Guidelines:***

- Widefield images usually contain lower noise levels.
- Confocal images usually contain moderate noise levels.

**Noise Value**

Indicates or specifies a value that represents the amount of noise suppression that will be applied to your image. If you choose a noise suppression level of *Low*, a value of 2 is displayed in this settings box; if you choose a noise suppression level of *Medium*, a value of 20 is displayed in this settings box. To choose a different noise suppression level, choose *Other* as the Noise Level setting, and type an appropriate value in this box.

**Performance: Faster Processing/Reduced Resolution**

Improves the processing time, while slightly reducing the resolution. When it is necessary to use a high number of iterations or when this dialog will be used to process a large number of images, you can decrease the total processing time by checking this box.

**Note:** It is recommended that you leave this box unchecked when you are processing images from thick samples or noisy data sets.

**Use Recommended Expert Settings**

Applies the recommended expert settings and hides the Expert page tab. It is recommended that you leave this checkbox checked. Uncheck this checkbox and click the Expert tab to modify the default expert settings.

**Notes:**

- Before making any modifications to the settings on the Expert tab, you should become familiar with all of the settings on this tab and how each setting affects your results. Generally, you should not change the Expert settings from their default values. Change these settings only for special situations.
- To reset the settings on the Expert tab to their default values, uncheck this checkbox, then check it again. When you re-select the Expert tab, any values or settings that you made on the Expert tab will have been reset to their original default values.

## 3D Deconvolution - Dialog Box Options - Optics Tab

### Microscope

Specifies the type of microscope from which your image was acquired.

### Modality

Specifies the imaging method used to acquire your images based on the microscope configuration.

Modality choices are the following:

- ***Confocal – Point Scan***
- ***Confocal – Spinning Disk***
- ***Two photon***
- ***Widefield – Fluorescence***
- ***Widefield – Transmitted***

### Objective Lens

Accepts optical properties information about the objective in use.

***Image –*** Specifies that the values for Numerical Aperture and Refractive Index will be obtained from the data stored with the image, if available. The Numerical Aperture and Refractive Index boxes are deactivated.

***Manual –*** Specifies that the values typed or selected in the Numerical Aperture and Refractive Index boxes will be used.

***Lens NA –*** Specifies the numerical aperture (NA) of the objective that you used to acquire your image.

***Refractive index –*** Specifies the refractive index of the objective that you used to acquire your image.

### Image spacing

Specifies whether a calibrated or user-defined spacing will be used.

***Calibrated***– Specifies that the program will use the calibration values for X, Y and Z stored with the image.

***Manual (um)***– Enables you to specify the calibration settings for Z that you want to use, but uses the values for X and Y stored with the image.

***X, Y, and Z***

Indicates the X, Y, and Z spacing of the image to which the source image selector points.

#### X Spacing

Defines the X dimension of a single pixel in microns. Calculate X Spacing by dividing the width of the image in microns by its width in pixels. Type or select the X Spacing width in microns. Decimal values can be typed in. The value you enter should be accurate to within 3% of the total width.

#### Y Spacing

Defines the Y dimension of a single pixel in microns. Calculate Y Spacing by dividing the height of the image in microns by its height in pixels. Type or select the Y Spacing height in microns. Decimal values can be typed in. The value you enter should be accurate to within 3% of the total height.

#### Z Spacing

Defines the calibrated vertical, Z-distance between the acquisition points in an image stack. Calculate Z Spacing by dividing the total Z-distance of the image stack by the total number of image planes in the stack. The value you enter should be accurate to within 3% of the total Z-distance.

### Emissive Wavelength

Specifies the emissive wavelength of the fluorescent dye used to stain the sample. This value should be the wavelength at which the image was acquired. Use a value of 520 for brightfield data. Default value is 520.

Specifies whether the image wavelength values used will be the values stored in the image data or manually entered values.

### Image

Specifies that the image processing will use the wavelength values stored in the image.

### Manual (um)

Specifies that the image processing will use the wavelength values that you enter or select in the separate boxes for each channel.

### Ch1

Accepts the wavelength value for Channel 1 in nanometers.

### Ch2

Accepts the wavelength value for Channel 2 in nanometers.

### Ch3

Accepts the wavelength value for Channel 3 in nanometers.

## 3D Deconvolution - Dialog Box Options - PSF Tab

### PSF Starting Point

Chooses either a theoretical PSF or a measured PSF to complete your image deconvolution.

#### Theoretical PSF

Selects the theoretical PSF. Use the Theoretical PSF settings to complete your PSF configuration. This is a *Blind* deconvolution process.

#### Measured PSF

Selects the measured PSF. Use the Measured PSF settings to complete your PSF configuration. This is a *Non-Blind* deconvolution process.

### Theoretical PSF

Specifies settings for theoretic PSF deconvolution processing.

#### Spherical Aberrations

Indicates the amount of spherical aberration present in the objective used to acquire the image. If you know the value, you can type it into the box.

#### Detect SA

Detects the amount of spherical aberration present in the image you are processing.

#### Calculate SA

Calculates the amount of spherical aberration based on the values you enter in the Spherical Aberration dialog box. Click Calculate SA to open the Spherical Aberration dialog box.

### Measured PSF

Specifies settings for measured PSF deconvolution processing.

#### Ch1, Ch2, Ch3 – Select PSF

Specifies and selects the PSF files for each channel that will be used for deconvolution.

#### PSF Spacings

Specifies whether the PSF spacing values will be from the image data or manually typed into the provided boxes for X, Y, and Z.

#### From Image

Specifies that the PSF spacing values will be derived from the image data.

#### Manual

Specifies that the PSF spacing values will be manually typed into the boxes for X, Y, and Z.

#### X, Y, Z

Accepts image pixel spacing values for X, Y, and Z.

## 3D Deconvolution - Dialog Box Options - Expert Tab

### Subvolume

#### XY Montage

Divides the data into subvolumes along the XY dimensions. This option reduces the amount of RAM required by the deconvolution application. The default value for this setting is *On* (checked).

**Note:** The default for this setting is *On*. It should be turned *Off* only if the deconvolution application is producing rigid, box-like artifacts in your data.

#### Z Montage

Divides the dataset into subsections along the optical axis and deconvolves these subsections separately.

**Note:** The default for this option is *Off* (unchecked). It should only be turned *On* for image stacks with a large Depth setting, such as a stack containing more than 100 planes. This option reduces the amount of RAM required by the deconvolution process. It might also be useful in instances where the sample thickness causes the PSF to change along the Z axis. In these cases, Z Montage enables blind deconvolution to find different PSF solutions for different depths.

#### Dynamic subvolumes

Specifies that the size of the subvolume will be determined based on the available memory, if this is checked. This option uses the available system memory to determine the largest subvolume allowable. This will be enabled only if *XYMontage* is on. The default value, if *XY Montage* is on is *True* (checked).

### Subvolume overlap (Pixels)

Specifies the number of pixels by which the adjacent sub volumes will overlap. Larger values result in longer processing but reduce edge and seaming artifacts, should they occur. The default value is 10.

### XY Guardband (Pixels)

Specifies the amount of padding around image borders. The XY Guardband provides a region of pixels around the border of the processed image stack or subvolume, but disregards this region before the results of deconvolution are returned. Larger values increase processing time, but decrease edge and ringing artifacts. The default value is 10.

### Z Guardband (Pixels)

Specifies the number of planes that will be added at the top and bottom of the subvolume. This guardband prevents artifacts at the seams of these subvolumes. The possible values are integers from 0 to N/2, where N is the depth of the XZ or YZ field. The default value is 6.

**Note:** The Z-Guardband should never be larger than the subvolume overlap region. A value of 6 is appropriate for most image stacks.

## Pre-processing

### Intensity Correction

Applies correction to fluctuations in the image intensity values across the depth of an image stack. This option should be checked if the image intensity changes abruptly from one image plane to another in areas where image intensity should be constant from one image plane to the next. Fluctuations can result from minor variations in illumination intensity. The default value is *On* (checked).

### Minimum Intensity Removal

Specifies that the program will automatically calculate and remove the erroneous background intensity level in the data. The most common cause of this erroneous background intensity is the electronic dark current (background electrical signal level) of the photodetector or CCD camera. Other causes are the bias voltage of amplifiers in the camera, back-scattered light that penetrates the emission filter and nonspecific dye that can leak into the embedding medium, among other causes. The default for this option is *On* (checked).

### Accelerated SA Detection

Reduces the time required to detect Spherical Aberrations in your image stack by applying binning to the image data. In this instance, each group of four pixels is treated as one. Applying acceleration might influence the calculated spherical aberration correction value to not represent the true amount of spherical aberration present in the objective used to acquire the image stack. The default for this option is *Off* (unchecked).

### Pre-Condition Imported PSF

Applies a preconditioning algorithm to the PSF image whenever you have selected Measured PSF and are using a bead stack. The default, and recommended setting for this option is *Off* (unchecked).

### Object First Guess

Specifies the most appropriate deconvolution method for the 3D Deconvolution algorithm to use to produce the initial guess to restore the image. This option is used to select the starting point for the iterative estimation process of the object. Choose one of the following options:

***Flat sheet*** – Provides a constant-valued volume; use this setting for extremely noisy data.

***Original data*** – Specifies the default value; use this setting for the majority of your images.

***Linear filtered data*** – Runs the source image through a linear filter to remove some blur; use this setting for images containing a very strong fluorescent signal.

### PSF Processing

Contains the expert level settings used to configure PSF processing.

#### Axial Stretch Factor

Specifies the factor by which the theoretical (calculated) PSF is lengthened along the Z axis to more accurately initiate the first guess, particularly for a confocal data set. The default Axial Stretch Factor is 1 for Widefield data and 3 for Confocal.

#### PSF Waist (Airy Disc's)

Specifies size of the narrowest part of the PSF, usually measured in Airy Disc diameters. The default setting is 1 for both Widefield and Confocal.

#### Disable PSF Constraints

Removes the limitations placed on the Point Spread Function (PSF).

### Result Save Interval

Specifies the number of iterations that will occur between storage of intermediate deconvolution results on disk. For example, if the number of Total Iterations is 20 and the Save Interval is 5, the deconvolution results will be saved at 5, 10, 15 and 20 iterations. The program automatically checks for available disk space before beginning deconvolution. This setting is available only when the Expert tab is active.

# Sub-Pixel Shift (Process Menu)

**Shifts an image by a selected sub-pixel distance in the horizontal and/or vertical direction.**

#### Drop-in: SUBSHIFT

Use this command to shift an image by a non-integer number of pixels. This function may useful in cases in which your regions of interest, which are defined over precise pixels, do not line up exactly in register with objects you want to measure. This command is also helpful in aligning planes in a stack when they are out of register with one another by a fraction of a pixel. Shifts are measured in one-twentieths of a pixel.

## Applying a Sub-Pixel Shift

To apply a shift to an image or stack plane by a non-integer number of pixels, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Process menu, choose Sub-Pixel Shift. The Sub-Pixel Shift dialog box will appear. |
| **2** | With the *Source image* selector, select the image or stack plane you want to shift. |
| **3** | Select a destination for the result image with the *Result image* selector. You can add to or overwrite the existing source image or stack, or you can specify a new image. |
| **4** | Select a whole number for the offset, measured in one-twentieths of a pixel, with the *Horizontal Shift* and/or *Vertical Shift* spin boxes. |
| **5** | Choose *Apply* to apply the shift. |

**6** When you have finished, choose *Close*.

## Sub-Pixel Shift - Dialog Box Options

**Source image**

Selects the image or stack plane to be shifted.

**Result image**

Selects a destination for the shifted result image. You can add to or overwrite the existing source image or stack, or you can specify a new image.

**Horizontal Shift**

Selects a shift in the X-axis direction, measured in one-twentieths of a pixel. The number you enter should be an integer.

**Vertical Shift**

Selects a shift in the Y-axis direction, measured in one-twentieths of a pixel. The number you enter should be an integer.

**Apply**

Applies the shift to the selected image or stack plane.

**Close**

Closes the dialog box.

# Optical Density (Scaled) (Process Menu)

**Performs quantitative densitometry on an entire image.**

**Drop-in: ODSCALE**

Use this command to display the optical densities of a brightfield source image in a scaled 8-bit or 16-bit image. You will need both a "raw" source image and a bright shading-correction reference image. A background reference image is optional. The grayscale value of each pixel in the output image will be determined by the following formula:

**Result = ( -Log10 [ { Source - Background + Offset } / Shading ] ) * (Scaling Factor)**

The *Scaling Factor* option will be useful for adjusting the brightness of the resultant image, so as to allow the optical densities to be displayed at image intensities that are more easily discerned by eye. Adjustment of the *Scaling Factor* will have a reciprocal effect on the *Max OD Result* control--a lower *Max OD Result* will be correlated with a higher *Scaling Factor* and a brighter result image.

The optical density scaling operation can also be performed on 24-bit color source images. The intensity values from color images are weighted equally (Intensity = [R + G + B] / 3).

**Note:** The *Auto Calibrate* option will set the MetaMorph system calibration to use optical density (OD) units. The Calibrate Gray Levels data table will contain two entries, one for an OD of zero and one for an OD of 1, along with their corresponding gray values. This calibration will affect measurements of all images, and involves all densitometric commands, such as Show Region Statistics, Integrated Morphometry Analysis, Morphometry Histogram, Measure Pixel, and Linescan. If you subsequently need to disable the optical density system calibration, you must open the Calibrate Gray Levels dialog box (Measure menu) and clear the *Use Calibration* check box.

**Image-1/AT:** The Optical Density (Scaled) command is similar to the Optical Density operation in the MVPMATH program from Image-1/AT.

## Using Optical Density (Scaled)

To perform quantitative densitometry on an entire image, use the following procedure.

**Note:** You must have both a source image and a shading image. The source image should be a brightfield image.

| Step | Action |
|---|---|
| **1** | From the Process menu, choose Optical Density (Scaled). The Optical Density (Scaled) dialog box will open. |
| **2** | Select a source image from the *Source image* selector. |
| **3** | Select an image for shading using the *Shading image* selector.<br><br>**Note:** Before applying the Shading Image, using the *Background Image* selector, select a background image to be subtracted from the source image. |
| **4** | Use the *Result Image* selector to select a destination image. |
| **5** | If you wish, specify an offset value in the *Offset* spin box. This value will be added to the grayscale value of each pixel before its optical density is calculated. |
| **6** | In the *Scaling Factor* spin box, select a value by which the optical density of each pixel is multiplied before being displayed in the destination image.<br><br>**OR**<br>Use the *Max OD Result* spin box to select what you expect to be the maximum optical density in the measured image, so that an appropriate range of intensities will be used in the displayed image.<br><br>**Note:** Adjustment of the *Scaling Factor* will have a reciprocal effect on the *Max OD Result* control--a lower *Max OD Result* will be correlated with a higher *Scaling Factor* and a brighter result image. |
| **7** | Choose between an 8-bit and a 16-bit output from the *Result Depth* group. |
| **8** | If you want to use the scaled optical density calibration for other images, select the *Auto Calibrate* check box to set the MetaMorph system calibration to use the optical density units. |
| **9** | Choose *Apply.* The scaled optical density result image will be displayed. If you selected the *Auto Calibrate* check box in Step 8, the system will also be set to use the measured optical density units. |
| **10** | When you have finished, choose *Close* to close the dialog box. |

## Optical Density (Scaled) - Dialog Box Options

**Source image**

Selects the source image. This should be a brightfield image.

**Background image**

Selects a background reference image. Use of a background image is optional.

**Shading image**

Selects a shading correction image. This image is required. **Note:** Before applying the shading image, it should have the background subtracted from it.

**Result image**

Selects the destination for the scaled optical density image. You can create a new image or you can overwrite or add to an existing image.

**Offset**

Specifies an offset value for the equation if there are pixels in the source image that have a lower gray value than the pixels in the shading image.

**Scaling Factor**

Specifies a scaling factor to be applied to the optical density value of each pixel in the destination image.

**Max OD Result**

Selects the maximum optical density in the measured image, so that an appropriate range of intensities will be used in the displayed image. **Note:** The *Scaling Factor* and the *Max OD Result* options have a reciprocal effect on one another--a lower *Max OD Result* will be correlated with a higher *Scaling Factor* and a brighter result image.

**Auto Calibrate**

Sets the MetaMorph system calibration to use the measured optical density (OD) units. This will affect measurements of all images, and will involve all densitometric commands. If you need to disable the optical density system calibration, you will need to open the Calibrate Gray Levels dialog box (Measure menu) and clear the *Use Calibration* check box.

**Result Depth**

Specifies the bit-depth of the *Destination Image.*

**Apply**

Executes the quantitative densitometry calculations and displays the scaled optical density image.

**Close**

Closes the dialog box.

# Ratio Images (Process Menu)

**Computes a ratio image from two images (or stacks).**

### Drop-in: RATIO

Use this command when you want to analyze a ratio image in MetaMorph or when you want to build a ratio image but do not have access to MetaFluor. It can also be used simply to correct shading errors in images.

You can specify the minimum and maximum permissible ratio.

This command also allows you to specify use of the IMD (Intensity-Modulated Display) mode palette for the ratio image, as well as which source image to use for the intensity. The IMD mode is an alternative to the Pseudocolor palette. The IMD palette uses a custom look-up table that associates color *hues* with the ratio values, and the *intensities* of each hue with source image brightness.

**Note:** For some images, you may find it desirable to threshold the denominator and numerator images before building the ratio image.

**Note:** This drop-in does not support the active region.

# IMD Display

The *Intensity-Modulated Display* (IMD) display mode, devised by Dr. Roger Tsien, is an alternative to the Pseudocolor display palette. The IMD palette associates color hues with the ratio image, and the intensity of each hue with wavelength intensity.

## Building a Ratio Image

To build a ratio image, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Process menu, choose Ratio Images. The Ratio Images dialog box will appear. |
| **2** | Select the desired image for the numerator using the *Numerator* image selector.<br><br>EXAMPLE:<br>If you have images from 340 and 380 nm wavelengths, you would select the 340 nm wavelength image with the *Numerator* image selector. |
| **3** | Select the desired image for the denominator using the *Denominator* image selector.<br><br>EXAMPLE:<br>If you have images from 340 and 380 nm wavelengths, you would select the 380 nm wavelength image with the *Denominator* image selector. |
| **4** | Select the destination image for the ratio using the *Ratio Image* selector. |
| **5** | Select the desired IMD display using the *IMD Display* list.<br><br>Your choice will depend mostly upon whether the ratio image's values are expected to be evenly distributed throughout the ratio range or clustered around one ratio value. If most of the values are clustered, the *64 Ratios with 4 Intensities* selection will produce the best results.<br><br>**OR**<br><br>If you want to use a pseudocolor display instead of the IMD display, select *None* from the list and skip Step 6. |
| **6** | Select the desired source for the IMD intensity from the *IMD Intensity* list. You should select the intensity from the brighter image. If you are using this command in a journal and do not know which image will be brighter, you can select *Average Num. and Denom*. to use an average from the two images. |
| **7** | Select the minimum and maximum values for the ratio using the *Min Ratio* and *Max Ratio* |

options.

**8** Choose *Apply* to create the ratio image.

**9** Choose *Close* when you have finished.

## Ratio Images - Dialog Box Options

**Numerator**

Selects the numerator image of the pair of images used to build the ratio image.

**Denominator**

Selects the denominator image of the pair of images used to build the ratio image.

**Ratio Image**

Selects the destination for the ratio image. You can create a new image or you can overwrite or add to an existing image.

**IMD Display**

Selects the desired IMD display for the ratio image. The IMD display will use a custom look-up table that consists of hues corresponding to the selected number of ratios, with each hue having its own range of intensities. For example, a ratio image that was built using the *8 Ratios with 32 Intensities* option will have eight distinct hues, each with 32 intensities visible in its contrast/threshold slider (as opposed to the continuous range of values visible for a pseudocolor image).

**IMD Intensity**

Selects the source for the intensity values. Select the brighter image. If you are using a journal and do not know which image will be brighter, select *Average Num. and Denom*. instead.

**Min Ratio**

Selects the minimum ratio value for the ratio image.

**Max Ratio**

Selects the maximum ratio value for the ratio image.

**Apply**

Builds the ratio image.

**Close**

Closes the dialog box.

# Use Region for Background (Legacy)

> **Note**: The Use Region for Background command is now available through the Background and Shading Correction command in the Process menu. The stand alone Use Region for Background command is no longer available from the MetaMorph desktop and can only be accessed through the Journal Editor.

**Takes the maximum, minimum, or average intensity value of a selected region of interest and subtracts it from each pixel in the current image plane or in an entire stack of images.**

**Drop-in: SUBRGN**

Use this command to perform background subtraction based on the intensity levels in a selected region of interest. Once you have delineated a region, you can subtract the maximum, minimum, or average intensity value in that region from each pixel in the image. If you have defined several regions of interest in the image, you can determine the one that gives the best results by selecting from among them and observing the result images. You can add to or replace the existing image or stack of images, or you can place the results in a new image window.

**Note:** If your source image is a stack and you choose *All Planes*, the calculated background value from this current plane will be subtracted from all of the planes of the stack. To calculate a different background value for each plane, record this command into a journal using the current plane in the image selector. Then run the journal using the Loop for All Planes command.

## Using a Region's Intensity Value for Background Subtraction in an Image

To subtract a region's intensity value from each pixel in an image or stack of images, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Process menu, choose Use Region for Background. The Use Region for Background dialog box will appear. |
| **2** | Select the desired image from the *Source* image selector. If the source image is a stack of image planes, select either the *Current* plane or *All Planes.* |
| **3** | Selects a destination for the processed image with the *Result* image selector. You can place the results in a new image window or in a new plane appended to an existing image or stack, or you can overwrite the existing image. |
| **4** | If there is more than one region of interest defined in the image, select the desired region with the *Region to Be Used as Background* spin box. |
| **5** | Select which intensity value in the region that you want to subtract: *Average, Minimum,* or *Maximum.* |
| **6** | Choose *Apply.* |
| **7** | When you have finished, choose *Close.* |

## Use Region for Background - Dialog Box Options

**Source**

Selects the image or stack of images that you wish to process. If the source image is a stack, you can select either the *Current* plane or *All Planes* in the stack.

**Note:** If your source image is a stack and you choose *All Planes*, the calculated background value from this current plane will be subtracted from all of the planes of the stack. To calculate a different background value for each plane, record this command into a journal using the current plane in the image selector. Then run the journal using the Loop for All Planes command.

**Result**

Selects a destination for the result image. You can add to or replace the existing image or stack of images, or you can place the results in a new image window.

**Region to Be Used as Background**

Selects the region of interest to use for determining the intensity value to be subtracted. The region number may not match the region label. See Sequence Region LabelsSequence Region Labels.

**Region Measurement to Use**

Select which intensity value in the selected region that you want to subtract: *Average, Minimum,* or *Maximum.*

**Apply**

Applies the intensity value subtraction process to the *Source* image.

**Undo / Redo**

Undoes or redoes the last applicable command that did not create a new result image. (For this command, it undoes the results of overwriting the source image with the background-subtracted result image.) *Undo* / *Redo* buttons in MetaMorph apply to any previously applied command that did not create a new result image, not just the last use of the command in the dialog box from which the button was chosen.

**Close**

Closes the dialog box.

# Overlay Images (Display Menu)

**Overlays up to seven images, assigning a different color to each. Typically, one of the images is a grayscale transmitted-light image, and the other overlay images are fluorescence images.**

**Drop-in: OVERFLUO**

Use this command to combine a "background" image, such as a brightfield transmitted-light image, with up to six fluorescence "probe" images. The images will be combined in such a way that the information available in the background image can be seen through the fluorescence images. Each fluorescence image can be assigned a different color in the result image, and the relative contribution of each image ("balance") to the final result can be adjusted. The source images can be either 8-bit or 16-bit images, and can be individual, single-plane images or they can be selected planes in an image stack. The result image will be a 24-bit color image. The Overlay Preview image window displays a 256x256 preview of the overlay result.

The resulting overlay image will, to a large extent, have the image characteristics of the source images. For example, if you have significant contribution from background in your fluorescence images, this may show up in the final image as an unwanted, diffuse coloration of the entire image. To avoid such an effect, you should be sure to threshold the image so as to include only the objects of interest in the threshold range. Overlay Images will take thresholding into account, adding only the thresholded areas of interest in the source images to the final result. Similarly, if the contrast is too low in the source images, you should perform any appropriate enhancements to the source images, such as increasing contrast or scaling of the intensity range, before combining them in the final overlay image.

This command does not directly handle binary (1-bit) images. If you want to use a binary source image, you must first convert it to an 8-bit mask image with the Threshold Image command (Process menu).

**Note:** The Overlay Images command is somewhat similar to Combine into B&W + Color in that it creates a combined color and B&W image. However, Overlay Images allows you to create images in which the colored regions that represent the dye probes are transparent, and the Black & White "background" image can be seen "through" the fluorescence images. Combine into B&W + Color, on the other hand, is useful for combining one or two grayscale images into a single color/B&W image with a complex custom palette.

## Overlay Images - Procedures

**Overlaying Images**

**Editing the Hue List**

## Overlaying Images

To create a fluorescence "overlay" image, use the following procedure. Be sure to have all images already open in the application workspace, and perform any thresholding or contrast enhancements before proceeding.

| Step | Action |
|---|---|
| **1** | From the Display menu, choose Overlay Images. The Overlay Images dialog box opens. |
| **2** | With the # *Images* spin box, select the number of images that you will be combining, including the transmitted light image (optional), if any. |
| **3** | If you have a stack of fluorescent images of different wavelength, select the *Images from Stack* check box. Otherwise, leave the check box cleared.<br><br>**Note:** If your stack contains more image planes than you want to include in the overlay, make sure that the images you do intend to include are in the topmost planes of the stack. |
| **4** | If your source images are individual, single-plane images, use the first image selector in the *Source* column to select the grayscale "background" (transmitted light) image. If you are not using a "background" image, select *[None].*<br><br>**OR**<br>If your source images are planes in an image stack, select the source stack with the *Source* image selector. Then select the radio button that indicates which plane will be the "background" image. If you are not using a "background" image, select *No Plane Is Gray Image.* |
| **5** | For each color overlay image, select the image or plane you are about to configure by clicking its *Hue* color box. If the image is a single-plane image, use the corresponding *Source* image selector to select the desktop image.<br><br>**AND**<br>Use the *Hue* slider or the drop-down Hue List in the *Hue Selection* option group to select a color to be assigned to the image.<br><br>If you move the slider to a position corresponding to one of the default colors, the Hue List will automatically update to display the name of the color. Otherwise, the list will display *"Unnamed."* Conversely, if you select a different color from the Hue List, the slider will move to the corresponding location on the *Hue* color bar.<br><br>**Note:** If necessary, you can **edit the default** |

**list of available colors.**

**6** If you want MetaMorph to set the intensity balance between the images automatically, select *Auto Balance.*

**OR**

If you want to adjust the intensity balance between the images manually, clear the *Auto Balance* check box and use the *Bal* spin boxes to adjust the relative contribution from each of the images.

**7** If necessary, use the *Overlay Brightness* spin box to change the overall brightness of the result image.

This may be necessary because the algorithm used to encode the intensity in the result image derives its values from the grayscale "background" image, and any pixel with an intensity value of 0 (black) will be assigned an intensity of 0 in the result image, resulting in loss of overlay image information.

**8** If you want to enhance the areas in which the fluorescent probes overlap, select *Boost Colocalization.*

**9** Select a destination for the result image with the *Dest* image selector.

**10** A 256x256 Overlay Preview image window will display a preview of the overlay result. If your source images are larger than 256x256, use the box-in-box control in the upper right of the Overlay Images dialog box to select a different region to display in the preview window, as desired.

**11** When you are ready, choose *Apply* to create the new overlay image.

**Note:** If you want to hide the Image Window Tools, right-click in the overlay image window and choose Hide Image Window Toolbar from the pop-up context menu that appears.

**12** Choose *Close* to close the dialog box.

## Editing the Overlay Images Hue List

To edit, add to, or remove a color from the hue list, use the following procedure.

| Step | Action |
|---|---|
| **1** | From the Overlay Images dialog box, choose Edit Hue List. The Edit Hue List dialog box will appear. |
| **2** | To add a new color to the Hue List, drag the handle of the *Hue* slider to the desired position. The color box in the upper right corner will update as you do so.<br>**AND**<br>Type the name for the new color in the *Name* |

*of Hue* text box. You may want to use the name of the fluorescent dye to which the assigned image corresponds (for example, "DAPI" or "Rhodamine"). Then choose *Add.*

**3** If you want to remove an entry in the Hue List, highlight the entry, and then choose *Remove.*

**4** If you want to edit the name or hue of an existing entry, double-click the entry's name in the Hue List. The *Hue* slider and *Name of Hue* text box will update.

**AND**
Make your changes to the *Hue* slider setting or the name in the *Name of Hue* text box. Then choose *Add.*

**5** When you have finished, choose *OK* to return to the Overlay Images dialog box.

## Overlay Images - Dialog Box Options

**Overlay Images**

**Edit Hue List**

## Overlay Images - Dialog Box Options

**# Images**

Selects the number of images, including any grayscale "background" (i.e., transmitted-light) image, that you want to include in the overlay image. This number will determine how many image configuration rows *(Hue/Bal/Source)* are displayed in the dialog box.

**Auto Balance**

Sets the intensity balance between the source images automatically. If you select this option, the *Bal* and *Overlay Brightness* spin boxes will be unavailable.

**Images from Stack**

Reconfigures the Overlay Images dialog box for use with a stack source image. When you select this check box, a single *Source* image selector will be displayed, and each image configuration row will be labeled for its corresponding stack plane. The *Plane* N *Is Gray Image* radio button group will also be displayed.

**Boost Colocalization**

Enhances the intensity of the areas in which two or more fluorescence probes overlap.

**Show Preview**

Select this option to view an overlay image preview window. The overlay section displayed can be manipulated using the Preview Image control.

**Overlay Brightness**

Sets the intensity in the result image, based on the intensities of the overlay source. the default setting is *50.* Increasing this value will produce a brighter result image. This may be necessary because the algorithm used to encode the intensity in the result image derives its values from the grayscale "background" image, and any pixel with an intensity value of 0 (black) will be assigned an intensity of 0 in the result image, resulting in loss of overlay image information.

**Hue (colored boxes)**

Displays the hue currently assigned to the image or plane. The image being configured is selected by clicking its corresponding *Hue* box. An arrow at the left indicates which image or plane is the one currently being configured. If the source images are single-plane images, the first (topmost) box will always be gray,

because this configuration row is reserved for the grayscale (transmitted-light) image.

**Bal**

Sets a scaling factor for each overlay, relative to the *Bal* factors of the other overlay images. The default setting is *50.*

### X Align

Changes the X position of the source image or plane.

### Y Align

Changes the Y position of the source image or plane.

**Source**

If the source images are single-plane images, this column of image selectors selects the source images for the final overlay image. By default, the first (topmost) selector selects the grayscale image. If the source images are planes in a stack, just one *Source* image selector will be displayed, which will be used for selecting the source image stack.

**Plane *N* Gray**

Selects a stack plane to be used as the grayscale "background" (transmitted-light) image in the final overlay image. If you are incorporating only fluorescence images into a color overlay image, and do not want a "background" image, select *No Gray.* This column of radio buttons will appear only when you have selected the *Images from Stack* check box.

**Destination**

Specifies the result image.

### Preview Image (Box-in-Box Control)

Selects a subregion of the source images to display in the 256x256 Overlay Preview image window. This option will be disabled if the source images do not have a width or height greater than 256 pixels across.

**Hue (slider)**

Selects a color to be assigned to the image or plane currently being configured. If you move the slider to a position corresponding to one of the default colors, the Hue List will automatically update to display the name of the color. Otherwise, the list will display *"Unnamed."* Conversely, if you select a different color from the Hue List, the slider will move to the corresponding location on the *Hue* color bar.

**Hue List**

Displays the currently selected color. Clicking the "down arrow" will open the drop-down list, from which you can select a different color to be assigned to the image or plane currently being configured. If you select a different color, the *Hue* slider will move to the corresponding location on the color bar. Conversely, if you move the *Hue* slider to a position corresponding to one of the default colors, the Hue List will automatically update to display the name of the color. Otherwise, the list will display *"Unnamed."*

**Edit Hue List**

Opens the Edit Hue List dialog box**,** from which you can change a Hue List entry's name or assigned color, add a new entry, or remove an existing entry.

**Apply**

Creates the overlay image.

**Close**

Closes the dialog box.

## Edit Hue List - Dialog Box Options

**Hue List**

Displays the current set of entries in the Hue List. (Note: This list does *not* have an interaction with the *Hue* slider or entry name text box in the manner seen in the Overlay Images dialog box.)

**Hue (slider)**

Selects a color for a new entry that you want to add to the Hue List.

**Name of Hue**

Specifies a name for a new entry that you are adding to the Hue List. By default, this text box displays *"New Hue."*

**Remove**

Removes the entry currently highlighted in the Hue List. You can remove any entry, with the exception of the *Unnamed* entry.

**Add**

Adds a new entry to the Hue List, using the color selected with the *Hue* slider and using the name specified in the *Name of Hue* text box. If you assign a different color to an entry whose name is already in use, a message box will appear, asking if you wish to overwrite the existing entry.

**OK**

Accepts the currently configured Hue List and closes the Edit Hue List dialog box.

# Produce Background Correction Image (Legacy)

**Note**: The Produce Background Correction Image filter is now available through the Basic Filters command in the Process menu. The stand alone Background Correction Image command is no longer available from the MetaMorph desktop and can only be accessed through the Journal Editor.

**Creates a background correction image for a source image or stack. The background image is generated from the source through a series of user-specified operations. This function helps to remove noise and unwanted fluorescence from large objects (background) in an image.**

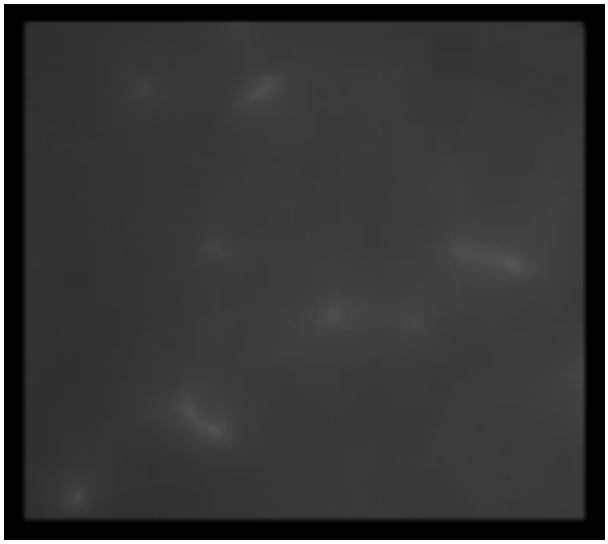
### Dropin: BACKCORR

Background Correction is similar to the Median Filter in that it works by selecting the median pixel value of the pixels in an *NxM* pixel area and replacing the center pixel with this value. This process is repeated for every pixel in the image unless the user specifies a sub-set ratio. The resulting background image is then subtracted from the source image to produce a new image with less background noise and unwanted fluorescence. While the Median Filter uses only the true median pixel at the 50% mark, the background correction process may be set to use an arbitrary percentage by the user.

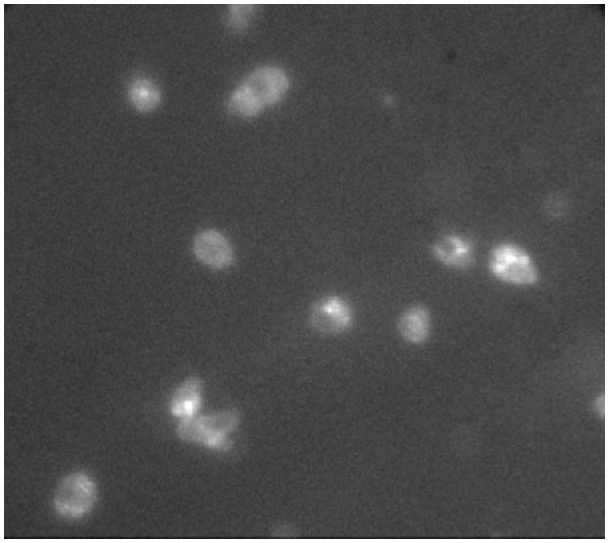
The filter works to 12-bit accuracy. If the original image is a true 16 bits deep, the least significant 4 bits will be ignored. The function lets the user specify that the operation be performed on a lower bit range to accommodate cameras that use only 12 or 14 bits.

**Note:** This function operates exclusively on the full image, ignoring any active region on the source image.

The following images show the effects of producing a background image and subtracting it from a source image, using a kernel size of 3 x 3:



Original Image



Correction Image

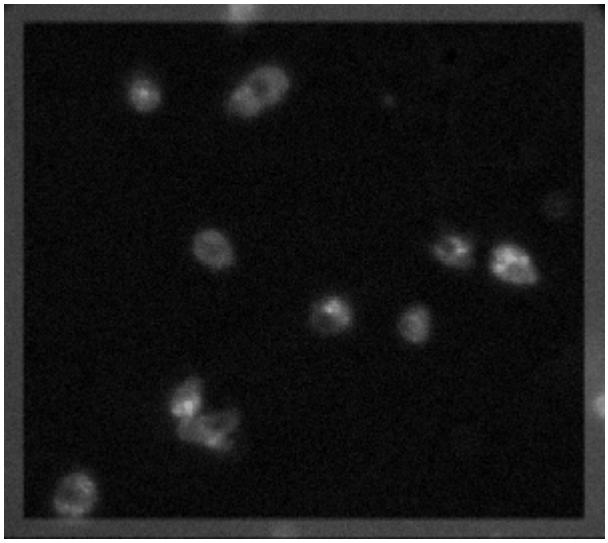


Image after
Subtraction

## Producing a Background Correction Image

To create a background correction image:

| Step | Action |
|---|---|
| **1** | From the Process menu, choose Produce Background Correction Image. The Background Correction dialog box will appear. |
| **2** | Select the desired source image or stack using the *Source* image selector. |
| **3** | Select the desired destination image using the *Dest* image selector. You can overwrite or add to the existing image or you can place the results in a new image window. |
| **4** | Use *Size* to select the size of the convolution kernel. |
| **5** | If you want to speed up processing by using a sub-sample, select the desired value using *Sub-Sample Ratio.* |
| **6** | Select the pixel intensity (p%) to be used in creating the background. A percentage of 100 will produce a background of maximum intensity, while a percentage of 0 will produce a background based on minimum intensity. |
| **7** | Under *True Depth of 16 it Image* select which bits of the image will be used to determine the median (or p%) pixel. |
| **8** | Click apply to create the background image. |
| | **Note:** When performing background correction on a large stack, the progress of the operation is indicated in the MetaMorph status bar. You can cancel the operation by pressing the <Esc> key. |
| **9** | From the Process menu, choose Arithmetic. The arithmetic dialog box will appear. |
| **10** | Select your source image as *Source 1* and the background image as *Source 2,* then select a destination for the new image. |
| **11** | Select the *subtract* operation and click *apply*. |
| **12** | Click *close* to close the dialog box. |

## Produce Background Correction Image - Dialog Box Options

### Source

Selects the image from which the background will be produced.

### Destination

Selects a destination for the resulting background image.

### Size (pixels n x n)

Specifies the size (in pixels) of the regions (kernels) used to determine each median (or p%) pixel.

### Sub-sample Ratio

Speeds up the processing of large kernels by limiting the calculations to a sub-sample, rather than every pixel. For example, if *2* is selected as the sub-sample, the pixels in columns/rows 1, 3, 5, 7, etc. will be used (1/4th the pixels). If *3* is selected, the pixels in columns/rows 1, 4, 7, 10, etc. will be used (1/9th). Likewise, if *8* is selected, the pixels in columns/rows 1, 9, 17, etc. will be used (1/64th). The maximum sub-sample value is *16* (samples 1 in 256 pixels).

### %(50 = median)

Determines which pixel's intensity value will be used in each kernel to create the background. A value of 50 indicates the median pixel will be used. Other values indicate that the pixel at the given percentage in the list will be used.

### True depth of 16-bit image:

Lets you select which bits of the image will be used for determining the median (or p%) pixel. The calculations are only done to 12-bit precision, so it is preferable to perform the calculations on the most significant bits in the image. Since some cameras use only the lowest 12 or 14 bits for the data, which can not be determined by MetaMorph, the user can direct the procedure to operate on the proper set of 12 bits.

### Apply

Performs the operations used to produce the background image.

### Close

Closes the dialog box.

# Log Image Annotation (Log Menu)

**Saves an annotation, along with the data from the current image or stack plane, to a data log.**

### Drop-in: LOGAN

Use this command to save image data with an annotation to a data log for the current plane of a selected image. You must first create the annotation with the Annotate Image command from the File menu. The Log Image Annotation command will allow you to open and configure the data log file. This command is fully journalizable, and can be particularly useful when used in combination with the Loop for All Planes command from the Journal menu.

### For More Information about Logging Images:

**Annotate Image**

**View Current Data Log**

**Close Data Log**

**Loop for All Planes**

## Logging Image Annotations

To save image annotations to a data log, use the following procedure:

| Step | Action |
|---|---|
| **1** | If you have not yet created an annotation for the image plane being measured, create one with the **Annotate Image** command from the File menu. |
| **2** | From the Log menu, choose Log Image Annotation. The Log Image Annotation dialog box will appear. |

**3** Use the *Image* selector to select the image whose data and annotation are to be logged.

**4** If necessary, choose *Configure Log.* The Configure Log dialog box will appear.

**AND**

From the *Configuration* list, select the items that you want to be logged. Then choose *OK.* The Log Image Annotation dialog box will reappear.

**5** Choose *Open Log* and open a text-based data log, a DDE link to an open spreadsheet, or both. The button's title will change to *"F9: Log Data."*

**6** Choose *F9: Log Data* to log your data.

**7** Choose *Close.*

## Log Image Annotation - Dialog Box Options

**Image**

Selects the image whose data and image annotation are to be logged.

**Data Log**

Indicates the file name of the data log to which the annotation and data are to be logged. If no log file is open, the line will read "Data log not open." After logging the data and annotation, the line will read "Logged to *Filename*.xxx."

**Open Log**

Opens a data log file and/or a DDE link to an open worksheet for logging data. This command changes to *F9: Log Data* once a log file is open.

**F9: Log Data**

Sends data measurements to an open data log.

**Configure Log**

Allows the selection of image characteristics and data that are to be enabled or disabled from data logging. Also allows a choice of whether column titles are to be included and if data are to be listed on a single line.

**Close**

Closes the dialog box.

# Log Image Histogram (Log Menu)

**Stores grayscale or color value data from the Histogram Tool's histogram in a data log.**

**Drop-in: LOGHISTO**

Use this command to save grayscale or color value histogram data from an entire image. The number of pixels in the image at each grayscale level or RGB color value will be stored. You have the option of defining the range of grayscale or color value bins on the basis of the minimum and maximum values in the image *(Image Min/Max),* an 8-bit range (0 - 255 for the grayscale values or for each of the color channels), a 10-bit range (0 - 1023), a 12-bit range (0 - 4095), a 14-bit range (0 - 16383), or a 16-bit range (0 - 65535). If you select one of the bit-defined ranges, you can fine-tune the minimum and maximum bin values using the *Start* and *End* spin boxes, respectively.

## Logging Image Histogram Data

To save grayscale or color value data, on a bin-by-bin basis, to a data log, use the following

procedure:

| Step | Action |
|---|---|
| **1** | From the Log menu, choose Log Image Histogram. The Log Image Histogram dialog box will appear. |
| **2** | With the *Image* selector, select the image from which you want to save grayscale or color value bin data. |
| **3** | From the Range drop-down list, select the range of grayscale or color bin values for which you want to log data. Select<br><br>*Image Min/Max* if you want to restrict the range to those values actually present in the image,<br><br>*8 Bit or RGB* if you want to use a range of 0 - 255 (for 8-bit images or for each color channel in a 24-bit image),<br><br>*10 Bit* if you want to use a range of 0 - 1023,<br><br>*12 Bit* if you want to use a range of 0 - 4095,<br><br>*14 Bit* if you want to use a range of 0 - 16383, or<br><br>*16 Bit* if you want to use a range of 0 - 65535. |
| **4** | If you selected one of the bit-defined ranges in Step 3, you can fine-tune the range further by selecting a minimum and maximum bin value with the *Start* and *End* spin boxes, respectively. |
| **5** | If you want to select the parameters to be logged to the log file, choose *Configure Log.* The Configure Log dialog box will appear.<br><br>**AND**<br>From the *Configuration* table, click the entries for the parameters to be logged, so that a check mark appears next to them. Then choose *OK* to return to the Log Image Histogram dialog box. |
| **6** | Open a data log by clicking the ***Open Log*** command button.<br><br>Once the data log is open, the text on the *Open Log* button will change to *F9: Log Data.* |
| **7** | When you are ready to save the grayscale or color value data, choose *F9: Log Data,* or press the [F9] function key on your keyboard. The data will be sent to the open data log.<br><br>If you sent your data to a text-based data log, you can inspect the data immediately by choosing View Current Data Log from the Log menu. |
| **8** | When you have finished, choose *Close.* |

## Log Image Histogram - Dialog Box Options

**Image**

Selects the image from which you want to store grayscale or color value pixel count (binned) data.

**Range**

Select the range of grayscale or color bin values for which you want to log data:

*Image Min/Max* restricts the range to those values actually present in the image.

*8 Bit or RGB* uses a range of 0 - 255 (for 8-bit images or for each color channel in a 24-bit image).

*10 Bit* uses a range of 0 - 1023.

*12 Bit* uses a range of 0 - 4095.

*14 Bit* uses a range of 0 - 16383.

*16 Bit* uses a range of 0 - 65535.

**Start**

Selects the minimum (starting) value for the range of bins to be logged. This option becomes available when you select one of the bit-defined ranges from the *Range* drop-down list.

**End**

Selects the maximum (ending) value for the range of bins to be logged. This option becomes available when you select one of the bit-defined ranges from the *Range* drop-down list.

**Configure Log**

Opens the Configure Log dialog box, from which you can select the parameters to be logged to the data log file.

**Open Log**

Opens a data log and/or a DDE link to an open spreadsheet application for logging data. This command will change to *F9: Log Data* when a log file is open.

**F9: Log Data**

Sends the binned data to the open data log.

**Close**

Closes the dialog box

# Open Object Log (Log Menu)

**Opens an existing or new object log for storing morphometric measurement data.**

### Drop-in: AUTOMEAS or IMA

Use this command to open an object log for logging morphometric measurement data. Measure Objects, Measure Objects with Mask, Recalculate Object Parameters, Measure Single Object, Integrated Morphometry Analysis, and Show Individual Object Data are commands whose measurements are logged to an object log. You can log the data to a text file, by Dynamic Data Exchange (DDE) to an open worksheet in a spreadsheet program**,** or to both.

For MetaMorph to log measurement data, it must know *where* you want the data stored, that is, which text file or open, DDE-linked spreadsheet to use. This information is supplied by the Open Object Log command. *What* is logged will be based on the types of measurement data selected using the Configure Object Measurements command. *Which* object measurements are logged will be based on the filters you defined using the Configure Object Classifiers command, excluding or including objects based on selected parametric criteria. For commands which log individual object data, even if a log file is open and configured, nothing will be logged until you choose **Log** Data from the Log menu. This command allows you to log measurement data selectively *when* you need it. For morphometric measurement of many objects, logging occurs when the measurement is performed.

You can view the logged data by

(1) Opening the current text-based object log using View Current Object Log**,**

(2) Opening a comma-delimited text file in a text editor, or

(3) Switching to an external DDE-linked spreadsheet application.

## Opening an Object Log File

To open an object log, use the following procedure. If you want to log data to an external spreadsheet application, you must first start the spreadsheet program and open the desired worksheet.

| Step | Action |
|---|---|
| **1** | From the Log menu, choose Open Object Log. The Open Object Log dialog box will appear. |
| **2** | Select *Dynamic Data Exchange (DDE)* to log directly to an open spreadsheet program. Select *A Text File* to log the data to a text file.<br><br>**Note:** You can select both options. |
| **3** | If you selected *A Text File* in the previous step, the Open Object Log File dialog box will appear.<br><br>Select an icon for an existing log file or type a new file name in the *File Name* text box. (If necessary, use the *Look In* list or Up One Level icon button to change the current drive and folder to the correct location.) Then choose *Open.* |
| **4** | If you selected an existing log's file name in Step 3, the Log File Exists dialog box will appear. You can *Overwrite* the contents of the file, *Append* new data, or *Cancel.* |
| **5** | If you selected *Dynamic Data Exchange (DDE)* in Step 2, the Export Log Data dialog box will appear.<br><br>Select the desired application from the *Application* list. Choose *Default* to use the default settings for the selected application.<br><br>Choose *OK* to open the DDE link. |

**Additional Information About DDE:**

**Using a New Microsoft Excel Worksheet**

**Using a Microsoft Excel Worksheet Other Than the Default**

**Creating a DDE Link to Lotus 1-2-3, Borland Quattro Pro, or MicroCal Origin**

**Linking to Another Application**

## Open Object Log - Dialog Box Options

Open Object Log File

Export Log Data

## Open Object Log File - Dialog Box Options

**File Name**

Lists the name of selected file.

**Files of Type**

Determines the file format of the files displayed in the *File Name* list. For opening log files, the default is **.LOG.* Select *All Files (*.*)* to display all file names.

**Save In**

Displays the currently selected folder. Click the icon for the desired folder to display its files. Click the Up One Level icon button to go up one level in the directory structure.

**Save**

Opens the log file.

**Cancel**

Cancels the command.

# Close Object Log (Log Menu)

**Closes the current object log (text file) or the dynamic data exchange (DDE) link to a running spreadsheet program.**

**Drop-in: AUTOMEAS or IMA**

Use this command to close the current object log when you have finished logging data or before you open a new object log. All log files will be closed automatically by MetaMorph upon exiting.

**Note:** This command closes the DDE link to the spreadsheet program--it does not close or save worksheet files. You must switch to the spreadsheet program to perform these tasks.

## Closing an Object Log File

To close an object log, use the following procedure:

| Step | Action |
|---|---|
| 1 | Select the Log menu. |
| 2 | Choose Close Object Log. |

# Pause Object Logging (Log Menu)

**Pauses logging of data to the current object log.**

**Drop-in: AUTOMEAS or IMA**

Use this command before you make measurements that you do not want to log. Use the Resume Object Logging command when you want to continue logging data to the current log file.

## Pausing Object Data Logging

To pause object data logging, use the following procedure:

| Step | Action |
|---|---|
| 1 | Select the Log menu. |

| | |
|---|---|
| **2** | Choose Pause Object Logging. |

# Resume Object Logging (Log Menu)

**Resumes logging of data to the current object log.**

**Drop-in: AUTOMEAS or IMA**

Use this command when you want to continue logging data to an object log after using the Pause Object Logging command.

## Resuming Object Data Logging

To resume object data logging, use the following procedure:

| Step | Action |
|---|---|
| **1** | Select the Log menu. |
| **2** | Choose Resume Object Logging. |

# View Current Object Log (Log Menu)

**Displays the contents of the current object log (text file) in table format using the Viewer window.**

**Drop-in: AUTOMEAS or IMA**

Use this command to view the current object log within MetaMorph. If the current object log contains no data, a message dialog box will appear, stating "The file *Filename.log* could not be viewed because it has no columns or rows." Empty log files can occur if you open an existing log file and then close it without actually logging any data or if you log to a new log file with all of the logging parameters disabled.

The Viewer window has adjustable column widths so that you can resize the columns to best fit on your screen. (Drag the vertical cell borders between the column labels.) The maximum width of a column is about 1/4 the width of the screen. As with any other window, you can also adjust the Viewer window's size by dragging its borders.

If you want to print a table in the Viewer, there is a Print Table command in the Viewer's Control Menu (click the icon in the window's upper left corner). This command will print the text that is visible within the each column's width. Thus, you should adjust the columns before printing. The size of the window and the position of the scroll bars will have no effect on what is printed; if the data can fit in the column width, it will be printed.

You can also view a log file by using a spreadsheet program or a text editor, such as the Windows WordPad program.

**Note:** If you are currently logging to an open, DDE-linked spreadsheet application, you will need to switch to that application to view the data. View Current Object Log displays text files only.

## Viewing the Current Object Log

To view the current object log, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Log menu, choose View Current Object Log. The Viewer window will open. |
| **2** | The Viewer window can be resized by dragging its borders, as with other windows.<br><br>If the labels or data are not entirely visible, you can adjust the width of the columns in the table by dragging the vertical cell border |

between the labels until the columns are the desired width. The maximum width of a column is 1/4 of the width of the screen.

**3** To close the Viewer window, click the Close button in its upper right corner.

# Log All Object Data (Log Menu)

**Logs all object data from an image after it has been measured.**

**Drop-in: AUTOMEAS**

Use this command to log object data from a measured image if an object log was not open at the time you measured the image. If, after you have measured the image, you decide that you want to log its object data, you can open the log file and choose Log All Object Data without measuring the image again. This command does not apply to measurements made by single measurement commands.

## Logging All Object Data

To log all object data, use the following procedure:

| Step | Action |
|---|---|
| **1** | Measure the desired image using the Measure Objects, Measure Single Object, or Measure Objects with Mask command. |
| **2** | Open the object log file using the Open Object Log command in the Log menu. |
| **3** | From the Log menu, choose Log All Object Data. |
| **4** | If the data have already been logged, the message, "You have already logged this measurement set. Do you really want to write another set to this log?" will appear.<br><br>Choose *Yes* if you want to log the measurement set again, or choose *No* to cancel the logging. |

# Open Summary Log (Log Menu)

**Opens an existing or new summary log for storing summaries of morphometry statistics.**

**Drop-in: AUTOMEAS or IMA**

Use this command to open a summary log for logging summary data of morphometric measurements collected with the Show Classifier Statistics command. You can log the data to a text file, by Dynamic Data Exchange (DDE) to an open worksheet in a spreadsheet program**,** or to both.

For MetaMorph to log measurement data, it must know *where* you want the data stored, that is, which text file or open, DDE-linked spreadsheet to use. This information is supplied by the Open Summary Log command. *What* is logged will be based on the types of measurement data selected using the Configure Object Measurements command. *Which* object measurements are logged will be based on the filters you defined using the Configure Object Classifiers which exclude or include objects based on selected parametric criteria. Logging occurs when the measurement is performed.

You can view the logged data by

(1) Opening the current text-based summary log using View Current Summary Log**,**

(2) Opening a comma-delimited text file in a text editor, or

(3) Switching to an external DDE-linked spreadsheet application.

## Opening a Summary Log File

To open a summary log, use the following procedure. If you want to log data to an external spreadsheet application, you must first start the spreadsheet program and open the desired worksheet.

| Step | Action |
|---|---|
| **1** | From the Log menu, choose Open Summary Log. The Open Summary Log dialog box will appear. |
| **2** | Select *Dynamic Data Exchange (DDE)* to log directly to an open spreadsheet. Select *A Text File* to log the data into a text file.<br><br>**Note:** You can select both options. |
| **3** | If you selected *A Text File* in the previous step, the Open Summary Log File dialog box will appear.<br><br>Select an icon for an existing log or type a new file name in the *File Name* text box. (If necessary, use the *Look In* list or Up One Level icon button to change the current drive and folder location to the correct location.) Then choose *Open.* |
| **4** | If you selected an existing log file name in Step 3, the Log File Exists dialog box will appear. You can *Overwrite* the contents of the file, *Append* new data, or *Cancel.* |
| **5** | If you selected *Dynamic Data Exchange (DDE)* in Step 2, the Export Log Data dialog box will appear.<br><br>Select the desired application from the *Application* list. Choose *Default* to use the default settings for the selected application.<br><br>Choose *OK* to open the DDE link. |

**Additional Information about DDE:**

**Using a New Microsoft Excel Worksheet**

**Using a Microsoft Excel Worksheet Other Than the Default**

**Creating a DDE Link to Lotus 1-2-3, Borland Quattro Pro, or MicroCal Origin**

**Linking to Another Application**

## Open Summary Log - Dialog Help

Open Summary Log File
Export Log Data

## Open Summary Log File - Dialog Box Options

**File Name**

Lists the name of the currently selected file.

**Files of Type**

Determines the file format of the files displayed. For opening log files, the default is **.LOG.* Select *All Files (*.*)* to display all file names.

**Save In**

Displays the currently selected folder. Click the icon for the desired folder to display its files. Click the Up One Level icon button to go up one level in the directory structure.

**Save**

Opens the log file.

**Cancel**

Cancels the command.

# Close Summary Log (Log Menu)

**Closes the current summary log (text file), or closes the dynamic data exchange (DDE) link to a running spreadsheet program.**

**Drop-in: AUTOMEAS or IMA**

Use this command to close the current summary log when you have finished logging data or before you open a new summary log. All log files will be closed automatically by MetaMorph upon exiting.

**Note:** This command closes the DDE link to the spreadsheet program--it does not close or save worksheet files. You must switch to the spreadsheet program to perform these tasks.

## Closing a Summary Log File

To close a summary log, use the following procedure:

| Step | Action |
|---|---|
| 1 | Select the Log menu. |
| 2 | Choose Close Summary Log. |

# Pause Summary Logging (Log Menu)

**Pauses logging of summary data to the current summary log.**

**Drop-in: AUTOMEAS or IMA**

Use this command before you make measurements that you do not want to log. Use the Resume Summary Logging command when you want to continue logging data to the current log file.

## Pausing Summary Data Logging

To pause summary data logging, use the following procedure:

| Step | Action |
|---|---|
| 1 | Select the Log menu. |
| 2 | Choose Pause Summary Logging. |

# Resume Summary Logging (Log Menu)

**Resumes logging of summary data to the current summary log.**

**Drop-in: AUTOMEAS or IMA**

Use this command when you want to continue logging data to a summary log after using the Pause Summary Logging command.

### Resuming Summary Data Logging

To resume summary data logging, use the following procedure:

| Step | Action |
|---|---|
| 1 | Select the Log menu. |
| 2 | Choose Resume Summary Logging. |

# View Current Summary Log (Log Menu)

**Displays the contents of a current summary log (text file) in table format using the Viewer window.**

**Drop-in: AUTOMEAS or IMA**

Use this command to view the current summary log within MetaMorph. If the current summary log contains no data, a message dialog box will appear, stating "The file *Filename.log* could not be viewed because it has no columns or rows." Empty log files can occur if you open an existing log file and then close it without actually logging any data or if you log to a new log file with all of the logging parameters disabled.

The Viewer window has adjustable column widths so that you can resize the columns to best fit on your screen. (Drag the vertical cell borders between the column labels.) The maximum width of a column is about 1/4 the width of the screen. As with any other window, you can also adjust the Viewer window's size using its borders.

If you want to print a table in the Viewer, there is a Print Table command in the Viewer's Control Menu (click the icon in the window's upper left corner). This command will print the text that is visible within the each column's width. Thus, you should adjust the columns before printing. The size of the window and the position of the scroll bars will have no effect on what is printed; if the data can fit in the column width, it will be printed.

You can also view a log file by using a spreadsheet program or a text editor, such as the WordPad program.

**Note:** If you are currently logging to a DDE-linked spreadsheet application, you will need to switch to that application to view the data. View Current Summary Log displays text files only.

### Viewing the Current Summary Log

To view the current summary log, use the following procedure:

| Step | Action |
|---|---|
| 1 | From the Log menu, choose View Current Summary Log. The Viewer window will open. |
| 2 | The Viewer window can be resized by dragging its borders, as with other windows.<br><br>If the labels or data are not entirely visible, you can adjust the width of the columns in the table by dragging the vertical cell border between the labels until the columns are the desired width. The maximum width of a column is 1/4 of the width of the screen. |
| 3 | To close the Viewer window, click the Close button in its upper right corner. |

# Open EdgeList Log (Log Menu)

**Opens an existing or new edgelist log for storing each object's centroid and vertex X,Y-**

**coordinate data in an image.**

**Drop-in: AUTOMEAS or IMA**

Use this command to open an edgelist log for logging edgelist data. Data will logged to an open edgelist log when any of the following measurement commands are used: Measure Objects, Recalculate Object Parameters, and Log All Object Data. Measure Single Object, and Show Individual Object Data. You can log the data to a text file, by Dynamic Data Exchange (DDE) to an open worksheet in a spreadsheet program**,** or to both.

For MetaMorph to log measurement data, it must know *where* you want the data stored, that is, which text file or open, DDE-linked spreadsheet to use. This information is supplied by the Open EdgeList Log command. *Which* object measurements are logged will be based on the filters you defined using the Configure Object Classifiers which exclude or include objects based on selected parametric criteria. Unlike other log files, the edgelist log is always configured to log the same measurement data (object number, number of vertices, centroid X, centroid Y, and then on following lines, the vertex X and Y coordinates, starting with the pixel at the top, leftmost point and moving clockwise around the object). Data in an edgelist log are always reported in pixel values, even if the image has been calibrated.

There are two options in the Preferences dialog box (Measure Objects tab page) that will help you when you are working with edgelist logs. By selecting both *Draw Object Border* and *Draw Centroid Mark,* you will see a visual representation on the measured image of the centroid and object borders being logged.

You can view the logged data by

(1) Opening the current text edgelist log using View Current EdgeList Log,

(2) Opening a comma-delimited text file in a text editor, or

(3) Switching to an external DDE-linked application.

## Opening an EdgeList Log File

To open an edgelist log, use the following procedure. If you want to log data to an external spreadsheet application, you must first start the spreadsheet program and open the desired worksheet.

| Step | Action |
|---|---|
| **1** | From the Log menu, choose Open EdgeList Log. The Open EdgeList Log dialog box will appear. |
| **2** | Select *Dynamic Data Exchange (DDE)* to log directly to an open spreadsheet. Select *A Text File* to log the data into a text file.<br><br>**Note:** You can select both options. |
| **3** | If you selected *A Text File* in the previous step, the Open EdgeList Log File dialog box will appear.<br><br>Select an icon for an existing log or type a new file name in the *File Name* text box. (If necessary, use the *Look In* list or Up One Level icon button to change the current drive and folder to the correct location.) Then choose *Open.* |
| **4** | If you selected an existing log file name in Step 3, the Log File Exists dialog box will appear. You can *Overwrite* the contents of the file, *Append* new data, or *Cancel.* |
| **5** | If you selected *Dynamic Data Exchange (DDE)* in Step 2, the Export Log Data dialog |

box will appear.

Select the desired application from the *Application* list. Choose *Default* to use the default settings for the selected application.

Choose *OK* to open the DDE link.

**Additional Information about DDE:**

**Using a New Microsoft Excel Worksheet**

**Using a Microsoft Excel Worksheet Other Than the Default**

**Creating a DDE Link to Lotus 1-2-3, Borland Quattro Pro, or MicroCal Origin**

**Linking to Another Application**

## Open EdgeList Log - Dialog Box Options

Open EdgeList Log File
Export Log Data

## Open EdgeList Log File - Dialog Box Options

**File Name**

Lists the name of the selected file.

**Files of Type**

Determines the file format of the files displayed in the *File Name* list. For opening log files, the default is **.LOG.* Select *All Files (*.*)* to display all file names.

**Save In**

Displays the currently selected folder. Click the icon for the desired folder to display its files. Click the Up One Level icon button to go up one level in the directory structure.

**Save**

Opens the log file.

**Cancel**

Cancels the command.

# Close EdgeList Log (Log Menu)

**Closes the current edgelist log text file, or closes the dynamic data exchange (DDE) link to a running spreadsheet program.**

**Drop-in: AUTOMEAS or IMA**

Use this command to close the current edgelist log when you have finished logging data or before you open a new edgelist log. All log files will be closed automatically by MetaMorph upon exiting.

**Note:** This command closes the DDE link to the spreadsheet program--it does not close or save worksheet files. You must switch to the spreadsheet program to perform these tasks.

## Closing an EdgeList Log File

To close an edgelist log, use the following procedure:

| Step | Action |
|---|---|
| **1** | Select the Log menu. |
| **2** | Choose Close EdgeList Log. |

# Pause EdgeList Logging (Log Menu)

**Pauses logging of data to the current edgelist log.**

Use this command before you make measurements that you do not want to log. Use the Resume EdgeList Logging command when you want to continue logging data to the current log file.

## Pausing EdgeList Data Logging

To pause edgelist data logging, use the following procedure:

| Step | Action |
|---|---|
| 1 | Select the Log menu. |
| 2 | Choose Pause EdgeList Logging. |

# Resume EdgeList Logging (Log Menu)

**Resumes logging of edgelist data to the current edgelist log.**

**Drop-in: AUTOMEAS or IMA**

Use this command when you want to continue logging data to an edgelist log after using the Pause EdgeList Logging command.

## Resuming EdgeList Data Logging

To resume edgelist data logging, use the following procedure:

| Step | Action |
|---|---|
| 1 | Select the Log menu. |
| 2 | Choose Resume EdgeList Logging. |

# View Current EdgeList Log (Log Menu)

**Displays the contents of the current edgelist log (text file) in table format with the Viewer window.**

**Drop-in: AUTOMEAS or IMA**

Use this command to view the current edgelist log within MetaMorph. If the current edgelist log contains no data, a message dialog box will appear, stating "The file *Filename.log* could not be viewed because it has no columns or rows." Empty log files can occur if you open an existing log file and then close it without actually logging any data or if you log to a new log file with all of the logging parameters disabled.

The Viewer window has adjustable column widths so that you can resize the columns to best fit on your screen. (Drag the vertical cell borders between the column labels.) The maximum width of a column is about 1/4 the width of the screen. As with any other window, you can also adjust the Viewer window's size using its borders.

If you want to print a table in the Viewer, there is a Print Table command in the Viewer's Control Menu (click the icon in the window's upper left corner). This command will print the text that is visible within the each column's width. Thus, you should adjust the columns before printing. The size of the window and the position of the scroll bars will have no effect on what is printed; if the data can fit in the column width, it will be printed.

You can also view a log file by using a spreadsheet program or a text editor, such as the WordPad program.

**Note:** If you are currently logging to a DDE-linked spreadsheet application, you will need to switch to that application to view the data. View Current EdgeList Log displays text files only.

### Viewing the Current EdgeList Log

To view the current edgelist log, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Log menu, choose View Current EdgeList Log. The Viewer window will open. |
| **2** | The Viewer window can be resized by dragging its borders, as with other windows.<br><br>If the labels or data are not entirely visible, you can adjust the width of the columns in the table by dragging the vertical cell border between the labels until the columns are the desired width. The maximum width of a column is 1/4 of the width of the screen. |
| **3** | To close the Viewer, click the Close button in its upper right corner. |

# Display EdgeList Log as Image (Log Menu)

**Creates a binary stack of the edgelists and an 8-bit pseudocolored image of the centroids stored in the selected edgelist log.**

**Drop-in: EDGELIST**

Use this command to create a binary stack of the edgelist data stored in an edgelist log file so that you can view the data in a graphical representation. This command is useful for timelapses of single objects.

The binary stack has a default size of 512 x 512 pixels (which you can alter), and consists of one edgelist per plane.

The 8-bit centroid image marks the center of mass for each object. Each centroid in this image is assigned a gray value between 1 and 255 to represent its object number. If there are more than 255 centroids, the gray value will loop back to 1 and continue incrementing. If you have trouble viewing this image, you may want to Binarize the image.

You may use this command to load arbitrary data into a MetaMorph image window, provided that the data uses the format of an edgelist log file. This may be of particular use in photon-counting experiments conducted over time. The edgelist log file format is

Object number, number of points, centroid X, centroid Y

*X1, Y1* (X and Y vertices in pixels of the edgelist points)

*....*

*Xn, Yn*

**Note:** The edgelist log **must** be closed before using this command.

If you want to view the edgelists for all of the objects in a single image, you may perform a Stack Arithmetic operation (select *Maximum)* on the stack of edgelists to create one image.

### Displaying an EdgeList Log File as an Image

To display an edgelist file as an image, use the following procedure:

| Step | Action |
|---|---|
| **1** | If the edgelist log is open, close it before using the Display EdgeList Log as Image |

command.

**2** From the Log menu, choose Display EdgeList Log as Image. The Display EdgeList Log as Image dialog box will appear.

**3** Choose *Set Filename* and select the icon for the desired edgelist log file from the Select Log File dialog box that appears. (If necessary, use the *Look In* list or Up One Level icon button to locate the appropriate folder.) Then choose *Open.*

**4** From the *Draw EdgeList Points As* group, select *Vectors* to display the edgelists as connected outlines.

**OR**

If you are working with data that has been converted from a photon-counting experiment, select *Points* to display the edgelists as single pixels.

**5** *Image Width* and *Image Height* each display the default value of 512 pixels as the dimensions used for the destination images. You will only need to change these default values if the data in the edgelist log cannot be displayed within that range.

If the data from the edgelist log cannot be displayed using the specified dimensions, an error message will appear. This error message will suggest the minimum values needed to display the edgelist log data.

**6** Choose *OK.* MetaMorph will create the binary stack of the edgelists and the 8-bit image of the centroids stored in the selected edgelist log.

## Display EdgeList File as Image - Dialog Box Options

**Draw EdgeList Points As**

Selects between *Vectors* (the default), which displays the edgelists as connected dots, and *Points,* which displays the edgelists as single pixels. Generally, there will be no difference in the displays for most edgelist data. However, if you are using a text file of converted "photon-counting" data as your source file, you should select *Points.*

**Image Width**

Specifies the width of the edgelist and centroid image windows. This value must be large enough to accommodate all of the data in the edgelist log. If the data from the edgelist log cannot be displayed using the specified dimensions, an error message will appear. This error message will suggest the minimum values needed to display the edgelist log data. The default value is *512.*

**Image Height**

Specifies the height of the edgelist and centroid image windows. This value must be large enough to accommodate all of the data in the edgelist log. If the data from the edgelist log cannot be displayed using the specified dimensions, an error message will appear. This error message will suggest the minimum values needed to display the edgelist log data. The default value is *512*.

**Filename**

This status line lists the currently loaded edgelist log.

**Set Filename**

Opens the Select Log File dialog box, from which you may select the edgelist log that you want to use.

**OK**

Creates the binary edgelist stack and the 8-bit centroid image.

**Cancel**

Cancels the command.

# Configure Object Classifiers (Measure Menu)

**Configures measurement filters for measuring objects by defining value ranges that either include or exclude certain objects.**

**Drop-in: AUTOMEAS**

Use the Configure Object Classifiers command when you want to measure certain objects in the image while excluding other classes of objects from measurements.

You can measure all of the objects in the image first by using the Measure Objects command and then using the Show Individual Object Data command to determine measurement parameters and values that you want to use to define the filters in the Configure Object Classifiers dialog box. After you define the filters, you can then remeasure the objects. If you know how you want to define the filters, you can configure them in the Configure Object Classifiers dialog box first and then measure the objects. You can save and load classifier sets for future measurements.

**Measurement Term Definitions**

**Statistical Term Definitions**

## Configuring Object Classifiers

**Overview of Configuring Object Classifiers**

**Configuring Object Classifiers**

**Creating New Object Classifier Sets**

**Loading and Saving Object Classifier Sets**

## Overview of Configuring Object Classifiers

The Configure Object Classifiers command has a complicated dialog box. To help you use it in the most efficient manner, a basic overview of the procedure is presented in this table while separate steps are explained in separate procedure tables.

| Step | Action |
|---|---|
| **1** | From the Measure menu, choose Configure Object Classifiers. The Configure Object Classifiers dialog box will appear. |
| **2** | When you first start MetaMorph, the *Default* classifier set current listed in the *Classifiers* list will be the only available set.<br><br>You can create a new classifier set using the *New Classifier* command (refer to **Creating New Object Classifier Sets),** or load a previously saved classifier set using the *Load Set* command (refer to **Loading and Saving** |

**Object Classifier Sets).**

**3** Configure each classifier set as necessary for measurement (refer to **Configuring Object Classifiers).**

**4** Select the *Active* option above the *Classifier* list for each classifier set that you want to use for measurements and logging.

**5** Measure the objects using the desired measurement command from the Measure menu.

**6** If necessary, use the Classifier Statistics dialog box to view the classifier statistics generated from the active classifier set(s) and/or log the classifier statistics generated from the active classifier sets.

**7** If necessary, you can reconfigure existing active classifier sets or select new sets as the active classifier sets.

Choose *Recalc* to update the measurements whenever you want to use a revised or new classifier set. *Recalc* will update the measurement data. (The *Recalc* button replaces the *Measure* button once an image has been measured.)

**8** Choose *Close* when you have finished.

## Configuring Object Classifiers

To configure object classifiers for the image you want to measure, use the following procedure (refer to Overview of Configuring Object Classifiers for an overview of this command).

| Step | Action |
|---|---|

**1** Select the classifier set you want to configure so that it is visible at the top of the *Classifiers* list in the Configure Object Classifiers dialog box.

**2** Select the desired filters for this set from the *Filters* list so that each is marked by a check mark. Deselect any previously selected filters that are unnecessary.

**Note:** You can select *Show Descriptive Text* to display a description of each filter. You can also select *Active* from the *Show Filters* group if you want only the selected filters displayed in the *Filters* list.

**3** Select *Inclusive* from the *Filter Values* group if you want to include only those objects whose measurements fall inside the filter range when recording measurement data.

**OR**

Select *Exclusive* to exclude all objects whose measurements fall inside the filter range when recording measurement data.

**4** Type the desired filter range values in the

*Filter Range* text boxes (the text boxes allow you to set the range by completing the equation shown in the dialog box).

**5** If you want to change the default color set *(Random)* to another color for the selected classifier set, select a new color set from the drop-down color list located next the *Reset* command. This color set will be used to paint the measured objects.

**6** Repeat Steps 1 - 5 for each classifier set that you want to configure.

## Creating New Object Classifier Sets

To create a new classifier set, use the following procedure (refer to Overview of Configuring Object Classifiers for an overview of this command).

**Note:** Before creating a new classifier set using the *Point to Object* option, you must threshold and measure the image with the Measure Objects command. You should also confirm that you have selected a filter from the *Filter* list to use for the initial values and that this filter is also selected in the Preferences dialog box by using the *Preferences* command button in the Configure Object Classifiers dialog box.

| Step | Action |
|---|---|
| **1** | From the Configure Object Classifiers dialog box, choose *New Classifier.* The New Classifier dialog box will appear. |
| **2** | Type a name for the new classifier in the *Classifier Name* text box. |
| **3** | Select the desired option from the *Initial Filter Values* group:<br>*Duplicate Current Classifier* uses the current classifier values.<br>*Use Default Filter Values* uses MetaMorph's stored values.<br>*Point to Object* uses the selected object within the image window. |
| **4** | If you selected *Point to Object,* position the pointer over the desired object and click the left mouse button. *(Point to Object* finds the nearest centroid when you select the object.) The New Classifier dialog box will display the selected object number. |
| **5** | Choose *OK.* MetaMorph will create the new classifier and close the New Classifier dialog box. |

## Loading and Saving Object Classifier Sets

To load a previously saved object classifier set, use the following procedure (refer to Overview of Configuring Object Classifiers for an overview of this command).

| Step | Action |
|---|---|
| **1** | From the Configure Object Classifiers dialog box, select *Load Set File.* The Load |

Classifier Set dialog box will appear.

| | |
|---|---|
| **2** | Select the icon for the desired file. If necessary, select the correct folder or drive using the *Look In* list or Up One Level button. |
| **3** | Choose *Open.* MetaMorph will load the classifier set file, adding its name to the bottom of the *Classifier* list. MetaMorph will also close the Load Classifier Set dialog box. |
| **4** | Open the *Classifier* list to select the newly added classifier for configuring. |

To save a new object classifier set, use the following procedure:

| Step | Action |
|---|---|
| **1** | Select the desired object classifier set, if it is not currently displayed at the top of the *Classifiers* list. |
| **2** | From the Configure Object Classifiers dialog box, select *Save Set File.* The Save Classifier Set As dialog box will appear. |
| **3** | Type the desired file name in the *File Name* text box. (MetaMorph will assign the appropriate file name extension.) If necessary, select the correct folder or drive using the *Save In* list or Up One Level button. |
| **4** | Choose *Save.* MetaMorph will save the classifier set and close the Save Classifier Set As dialog box. |

## Configure Object Classifiers - Dialog Box Options

### Classifier Set File

Indicates the currently loaded classifier filter set file, if any.

### Classifiers

Selects the current classifier set. The text box below the drop-down list can be used to edit the name of the currently selected classifier (except *Default).*

### Active

Enables or disables the selected classifier set for use during measurements. Sets which are noted as "(active)" in the *Classifiers* list will be used for measurements.

### New Classifier

Opens the New Classifier dialog box, which is used to create new classifier sets. You can create a new classifier set by duplicating the current classifier by using default filter values or by using the pointer to "point" to a measured object that has the desired classifier characteristics. The last option is not available until you have thresholded the image and then measured it using the Measure Objects command.

### Remove Classifier

Removes the current classifier from the *Classifiers* list.

### Load Set File

Loads a previously saved classifier set (*.cls) file.

### Save Set File

Saves the currently selected classifier set as a .cls file.

### Reset

Resets the current classifier set to the "factory" settings.

### Color Drop-Down List Box *("Random")*

Selects the color used for painting the items that pass the selected classifier set. The default is *Random.*

### Filters

Selects the filters that make up each classifiers set. The *Filter Values* option is used in conjunction with the *Filter Range* to select the limiting conditions for screening objects through each filter.

### Show Descriptive Text

Displays a description of each filter at the bottom of the dialog box.

### Show Filters

Displays all filters in the *Filters* list if the *All* option is displayed. Otherwise only the active filters are displayed.

### Filter Values

If *Inclusive* is selected, this option includes only those objects whose measurements fall inside the filter range when recording measurement data. If *Exclusive* is selected, this option excludes all objects whose measurements fall inside the filter range when recording measurement data.

### Filter Range

Specifies the filter range of acceptable values used to determine if an object passes the filter.

### Measure/Recalc

Measures or recalculates the objects in the current image which pass the selected object classifiers. *Recalc* is faster than *Measure* because the objects do not have to be traced. The *Recalc* command is the same as Recalculate Object Parameters in the Measure menu.

### Preferences

Opens the Preferences dialog and displays the New Classifier tab page. The New Classifier preferences allow you to select which classifier parameters you want used in the "point to object" mode of the Create New Classifier command. Any parameter that you select (by double-clicking on the parameter name so that a check mark appears next to it) from the *Auto Classifier Definition* list will be used to define a new classifier when using the "point to object" mode.

### Description

Provides a brief description of the selected parameter in the *Filters* table. This status box will be displayed when you select the *Show Descriptive Text* check box.

### Closes

Closes the dialog box.

# Configure Object Measurements (Measure Menu)

**Selects which object measurements will be recorded in log files and displayed in the Show Individual Object Data dialog box and the Show Classifier Statistics dialog box.**

#### Drop-in: AUTOMEAS

Use this command before measuring data that will be logged to object and/or summary logs. This command selects which type of object measurements will be logged and displayed. MetaMorph completes and stores all object measurements in its temporary buffer, whether or not you configure them for your use. This allows you to log only a selected set of measurements, but use other measurements as object classifiers to filter out ranges of objects. You can save and load object measurement sets for future use.

The Configure Object Measurements dialog box includes a list of measurements that you can enable or disable. Descriptions of these measurements can be seen by enabling *Show Descriptive Text.* You can save and load object measurement sets (*.mes). The summary log is configured separately using the *Configure Summary Log* command in the Configure Object Measurements dialog box.

**Measurement Term Definitions**

**Statistical Term Definitions**

## Configuring Object Measurements - Procedures

**Configuring Object Measurements**

**Creating New Measurement Sets**

**Loading and Saving Measurement Sets**

## Configuring Object Measurements

To configure object measurements, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Measure menu, choose Configure Object Measurements. The Configure Object Measurements dialog box will appear. |
| **2** | Select the measurement set you want to configure from the *Measurement Sets* list so that its name appears in the text box below *Measurement Sets.*<br><br>If you do not want to use the *Default* set, you can create a new measurement set using the **New Set** command or load a previously saved set using the **Load Set** command. |
| **3** | Double-click each measurement in the *Measurements to Log* list that you want to include in the current set. Those included will be marked with a check mark. Deselect any measurements marked with a check mark that you do not want to include.<br><br>You can use *All On/All Off* to enable or disable all measurements. You can also use the *Reset* button to reset the measurement values to "factory" settings. |
| **4** | While you are selecting measurements, you can enable *Show Descriptive Text* if you want to read a short description of each measurement. You can also select *Active* from the *Show Log Measurements* group box to limit the measurements listed in the *Measurements to Log* list to those which have been selected for the current set. |
| **5** | Select the numeric format for the measurement data from the drop-down list below *Measurements to Log.* |
| **6** | Repeat Steps 2 - 5 for each measurement set you want to configure. |
| **7** | To configure a summary log for logging, choose *Configure Summary Log.* The |

**Configure Log** dialog box will appear.

**8** Once you have configured the desired measurement set(s) and the summary log, you can select the measurement set you want to use for the next group of measurements so that it is displayed in the *Measurement Sets* list.

**AND**
Choose *Close* to close the dialog box.

## Creating New Measurement Sets

To create a new measurement set, use the following procedure:

| Step | Action |
|---|---|
| **1** | Select *New Set* from the Configure Object Measurements dialog box. The New Measurement Set dialog box will appear. |
| **2** | Type the desired name in the *Set Name* text box. |
| **3** | Select *Duplicate Current Set* from the *Initial Logging Values* group to use the current set's values as the initial values for the new set.<br>**OR**<br>Select *Default Logging Values* to use the MetaMorph's stored values as the initial values. |
| **4** | Choose *OK.* The new set will be created and added to the bottom of the *Measurements Set* list in the Configure Object Measurements dialog box. |

## Loading and Saving Measurement Sets

To load a previously saved measurement set, use the following procedure:

| Step | Action |
|---|---|
| **1** | Select *Load Set File* from the Configure Object Measurements dialog box. The Load Measurement Set dialog box will appear. |
| **2** | Select the icon for the desired file. If necessary, select the appropriate drive or folder with the *Look In* list or Up One Level icon button. |
| **3** | Choose *Open.* The measurement set will be loaded and its name will be added to the bottom of the *Measurement Sets* list. |
| **4** | If you want to configure the new set, open the *Measurement Sets* list and select the set so that its name appears in the text box below *Measurements Sets.* |

To save the measurement sets currently listed in the *Measurement Sets* list together in one file, use the following procedure:

| Step | Action |
|---|---|
| 1 | Select *Save Set File* from the Configure Object Measurements dialog box. The Save Measurement Set As dialog box will appear. |
| 2 | Type the icon for the desired file name. If necessary, select the appropriate drive or folder with the *Save In* list or Up One Level icon button. |
| 3 | Choose *Save* to save the measurement set. |

## Configure Object Measurements - Dialog Box Options

### Measurement Set File

Lists the measurement set file currently loaded and displayed in the *Measurement Sets* list.

### Measurement Sets

Lists of the measurement sets that have created during the current work session or loaded with the current measurement set file.

### Measurement Sets Text Box

Allows you to rename a user-created measurement set. You may not rename the *Default* set.

### New Set

Creates a new measurement set that uses either current set's values or the default logging values as the initial values for the new set.

### Remove Set

Removes the current set from the *Measurement Sets* list.

### Load Set File

Loads a measurement set file (*.mes), consisting of one or more measurement sets, into the *Measurement Sets* list.

### Save Set File

Saves a measurement set file consisting of all of the measurement sets currently listed in the *Measurement Sets* list.

### Reset

Resets the *Measurements to Log* selections to the factory default settings.

### All On/All Off

Enables or disables the selection of all measurements in the *Measurements to Log* list.

### Show Descriptive Text

Displays a description at the bottom the dialog for the currently selected measurement in the *Measurements to Log* list.

### Show Log Measurements

Displays all available measurements in the *Measurements to Log* list when *All* is selected. Displays only those which are marked with a check mark if *Active* is selected.

### Configure Summary Log

Opens the Configure Log File dialog box so that you can select the parameters to be logged to the log file.

### Measurements to Log

Specifies which measurements will be included in the current set and used for logging by marking them with

a check mark.

### Numeric Format

Specifies the numeric format used to log and data measurement data.

### Close

Closes the dialog box.

# Annotate Measured Objects (Measure Menu)

**Adds annotations to measured objects in an image.**

**Drop-in: ANMEAS**

Use this command to label individual measured objects in an image with a selected annotation. You can simply identify objects with a character such as an asterisk, or label them with an ID number, or you can label each object with such measurement parameters as its area, centroid X and Y coordinates, average gray value, shape factor, etc. Alternatively, you can add your own annotation to each object. More than one parameter or comment can be used in the annotation.

**WARNING:**
When you apply the annotation to an image, the text will become a part of the image itself. If you subsequently attempt to perform a densitometric or morphometric measurement on the entire image, the grayscale values and morphometric characteristics of the text will be measured along with the objects in the image. Be sure to perform your measurements before you apply any graphics to the image. If there is any chance that you will need to reanalyze the original image, you should make a copy of the original and apply the annotation to the copy.

**Note:** To annotate objects with their measurement parameters, you must first set the image threshold and measure the objects with the Measure Objects command (Measure menu). This command can be also used to annotate images that have not been measured, but any measurement parameters that are specified in the annotation will be read as "0".

Measurement Term Definitions

## Annotating Measured Objects

To annotate measured objects in an image, use the following procedure. To include measurement parameters in the annotation, you must first set the image threshold and measure the objects with the Measure Objects command (Measure menu).

| Step | Action |
|---|---|
| **1** | From the Measure menu, choose Annotate Measured Objects. The Annotate Measured Objects dialog box opens. |
| **2** | Select the image to be annotated with the *Image to Annotate* image selector. |
| **3** | Choose *Show Codes.* The Annotate Measured Image Codes dialog box will appear, displaying the object parameter codes that you will use to specify what will appear in the object annotations. |
| **4** | In the *Annotation Text* box, enter the code for the measurement parameter you want to appear in each measured object's annotation. For example, if you want to label objects with their area, type "$02". Be sure to include the dollar sign. |

**Note:** You can specify several parameters to be used as object labels, separating the codes that you type into the *Annotation Text* box with a comma, space, or hyphen. The default entry for *Annotation Text* is "$*", which labels objects with an *Object ID* number. If you delete the dollar sign, this command will label each object with the asterisk that remains. You can change the label to any keyboard character, such as a plus sign (+).

**5** You can specify the number of decimal points to be displayed in the annotation. For example, to display Total Area to one decimal point, you would set the *Annotation Text* field to "$00.1". The "$00" will specify the display of the variable "Total Area," while the ".1" restricts the output of this variable to one decimal place. Each variable can have its own precision.

**6** If you want to change the color of the annotation label, enter the value in the *Foreground Color* spin box. If your selected image is one that has been measured, the values will correspond to the Pseudocolor scale associated with the image. Thus, for example, a *Foreground Color* value of 200 will produce a red annotation.

**7** If you want the areas behind the annotations to be opaque, select the *Fill Background* check box, so that a check mark appears in it. If your selected image has been measured, the values will again correspond to the pseudocolor scale associated with the image. Thus, for example, a *Background Color* value of 150 will produce a yellow background.

**8** Choose *Annotate.* The annotations will appear in the image window.

**9** To undo the annotation, click *Undo*.

**10** Choose *Close.*

## Annotate Measured Objects - Dialog Box Options

### Image to Annotate

Selects the image to be annotated.

### Annotation Text

Enter the code here for the measurement parameter you want to appear in the annotation.

### Foreground Color

Selects a color for the annotation label text. If your selected image is one that has been measured, the values will correspond to the pseudocolor scale associated with the image. For example, a value of 200 will produce a red annotation.

### Fill Background

Fills the annotation label background with the color corresponding to the value entered in the *Background Color* spin box.

### Background Color

Selects a color for the annotation label background. If your selected image is one that has been measured, the values will correspond to the pseudocolor scale associated with the image. For example, a value of 150 will produce a yellow background.

### Annotate

Labels the image objects with the selected annotation.

### Show Codes

Displays a list of the object measurement parameters and their codes. The code for your selected measurement parameter is entered in the *Annotation Text* box.

### Undo/Redo

Removes/replaces the annotation.

### Close

Closes the dialog box.

# Measure Objects (Measure Menu)

**Compiles selected measurements about objects in a binary image or a thresholded image using the thresholded or binary values to distinguish objects from the background.**

**Drop-in: AUTOMEAS**

Use this command to measure all objects in a selected image or region and display them in a new 8-bit image window using a pseudocolor display. The selected image must be a binary image or must be thresholded first. This command ignores single pixel objects.

When an image is measured, MetaMorph stores the measurements in a temporary buffer until they are replaced by the results from the next measurement command. There are two ways to use the measurements compiled by Measure Objects: (1) View them immediately using Show Individual Object Data or Show Classifier Statistics before performing another measurement command, or (2) Store them permanently in an object log or summary log.

Prior to using Measure Objects you will need to select the desired types of measurements using Configure Object Measurements. You may also want to configure filters to include or exclude objects from measurements using Configure Object Classifiers.

**WARNING:**
The maximum number of objects that can be measured in an image is 16,300. If there are more objects in an image than this maximum, MetaMorph will display an error message dialog box and abort the Measure Objects command.

**Shortcut: CTRL + M**

**An Introduction to Automatic Object Analysis**

Creates a boundary between the objects and the background (non-object image information) of the image on the basis of the image's gray values. When an image is thresholded, all of its objects can be measured using an automatic object analysis command such as Measure Objects.

## Measuring Objects

To measure objects, use the following procedure:

| Step | Action |
|---|---|

1. If you want to save the measurement data generated by the Measure Objects command, open an object, summary, or edgelist log from the Log menu.
2. If the image is an 8-bit, 16-bit, or 24-bit image, threshold it using the **Threshold Tool** or the **Threshold Image** command so that the threshold values clearly delineate the desired objects from the background.

   **OR**

   If the image is a binary image, continue to Step 3.
3. From the Measure menu, choose Measure Objects.

   **Note:** If you have already applied an automatic measurement command to any image during the current work session, MetaMorph will display a message dialog box to remind you that it will erase the data in the buffer with the new measurements. (The display of this prompt can be turned off using the **Preferences** command.)
4. When MetaMorph has finished measuring objects, it will display the objects it found in a new image window using a pseudocolor display.

   **WARNING:**
   The maximum number of objects that can be measured is 16,300. If there are more objects that this maximum, MetaMorph will display an error message and abort the measurement command.

# Recalculate Object Parameters (Measure Menu)

**Recalculates the objects in the current image which pass the selected object classifier filters defined by Configure Object Classifiers and previously calculated with the Measure Objects command.**

**Drop-in: AUTOMEAS**

An image must be measured with an automatic measurement command such as Measure Objects before Recalculate Object Parameters can be used. Because this command recalculates objects and then redraws the image without tracing the objects, Recalculate Object Parameters should be used when defining classifiers to determine if the classifiers are defined properly. Since Recalculate Object Parameters does not trace the objects, it is faster than using the original measurement command again.

This command is the same as the *Recalc* command button found in the Configure Object Classifiers dialog box.

**Shortcut: CTRL + SHIFT + M**

**An Introduction to Automatic Object Analysis**

## Recalculating Object Parameters

To recalculate object parameters, use the following procedure:

| Step | Action |
|---|---|
| 1 | Reconfigure the object parameters you want to change using the **Configure Object Classifiers** command. |
| 2 | From the Measure menu, choose Recalculate Object Parameters. |
| 3 | The objects that pass the active classifiers will be recalculated and then redrawn in the image window. |

# Measure Objects with Mask (Measure Menu)

**Compiles selected measurements about objects in an image using a matching 1-bit or 8-bit mask image (consisting only of gray values 0 and 255) to distinguish objects (gray value 255) from the background (gray value 0) in the image. After MetaMorph measures the source image's objects, it will display the objects that were defined by the matching mask image in a new 8-bit image window using a pseudocolor display.**

### Drop-in: AUTOMEAS

Use this command to measure objects in a source image (8-bit, 16-bit, or 24-bit) using a matching mask image, and display the measured image using a pseudocolor display. The power of this command comes from its ability to use mask images, such as those created from the source image using the Binary command (Process menu). For example, you could use the Binary command's *Segment* option to apply a "watershed" segmentation of objects in the image prior to measuring them with the Measure Objects with Mask command.

Once an image is measured, MetaMorph stores the measurements in a temporary buffer until they are replaced by the results from the next measurement command. There are two ways to use the measurements compiled by Measure Objects with Mask: (1) View them immediately using Show Individual Object Data or Show Classifier Statistics before performing another measurement command, or (2) Store them permanently in an object log.

Prior to using the Measure Objects with Mask command you will need to select the desired types of measurements using Configure Object Measurements. You may also want to configure object classifier filters to include or exclude objects from measurements using Configure Object Classifiers.

**WARNING:**
The maximum number of objects that can be measured in an image is 16,300. If there are more objects in an image than this maximum, MetaMorph will display an error message dialog box and abort the Measure Objects with Mask command.

### An Introduction to Automatic Object Analysis

## Measuring Objects Using a Mask Image

To measure objects in an image using a matching mask image, use the following procedure:

| Step | Action |
|---|---|
| 1 | Threshold the image you want to measure, to separate the objects from their background. |
| 2 | Create a binary mask image with the Process menu's **Binary** command. |
| 3 | Open an object, summary, or edgelist log from the Log menu if you want to save the measurement data generated by the Measure Objects with Mask command. |
| 4 | From the Measure menu, choose Measure |

Objects with Mask. The Measure Objects with Mask dialog box will appear.

**Note:** If you have already applied an automatic measurement command to any image during the current work session, MetaMorph will display a message dialog box to remind you that it will erase the data in the buffer with the new measurements. (The display of this prompt can be turned off using the **Preferences** command.)

**5** Select the image to be measured (8-bit, 16-bit, or 24-bit) from the *Gray Image* selector.

**6** Select the desired 1-bit or 8-bit binary mask image from the *Mask Image* selector.

**7** If the desired destination image is not displayed in the image selector next to *Destination,* select it using the image selector. You can overwrite or add to an existing 8-bit image or you can place the results in a new 8-bit image window.

**8** Choose *OK.*

**9** When MetaMorph has finished measuring the thresholded objects in the *Gray Image,* based on the mask overlay in the *Mask Image,* it will display the objects it found in the selected destination image window using a pseudocolor display.

**WARNING:**
The maximum number of objects that can be measured is 16,300. If there are more objects that this maximum, MetaMorph will display an error message and abort the measurement command.

## Measure Objects with Mask - Dialog Box Options

### Gray Image

Selects the source image (8-bit, 16-bit, or 24-bit) for measuring objects with a matching mask image. This image should be thresholded before measuring, so that objects can be separated from background.

### Mask Image

Selects the 1-bit or 8-bit binary mask image. This will determine where on the *Gray Image* objects will be measured.

### Destination

Selects the destination image for the pseudocolor measured result image. You can place the results in a new 8-bit image window or overwrite/append to an existing 8-bit image.

### OK

Measures the selected image.

### Cancel

Cancels the command.

# Measure Single Object (Measure Menu)

**Compiles selected measurements about a single object selected with the Locator Tool in a binary image or a thresholded image (8-bit, 16-bit, or 24-bit).**

**Drop-in: AUTOMEAS**

Use this command when you want to measure (and log data from) one or more objects in the image. When you apply Measure Single Object to an image or active region, MetaMorph will find all of the objects in the image using the threshold values. It will then display the objects it found, using a pseudocolor display in a new image window. All of the objects will be marked with the same pseudocolor.

MetaMorph will also open the Measure Single Object dialog box, which includes a list of the current measured object's data. An object can be measured by positioning the pointer over the object and clicking the left mouse button. The object's data will appear in the Measure Single Object dialog box and you can then log the data. After an object is measured, MetaMorph will paint the object a color based on the classifier sets you are currently using.

Once an image is measured, MetaMorph stores the measurements in a temporary buffer until they are replaced by the results from the next measurement command. There are two ways to use the measurements compiled by Measure Single Object: (1) View them immediately using the command's dialog box or using the Show Classifier Statistics command before performing another measurement command, or (2) Store them permanently in an object log or summary log.

Prior to using Measure Objects you will need to select the desired types of measurements using Configure Object Measurements. You may also want to configure object classifier filters to include or exclude objects from measurements using Configure Object Classifiers.

**WARNING:**
The maximum number of objects that can be measured in an image is 16,300. If there are more objects in an image than this maximum, MetaMorph will display an error message dialog box and abort the Measure Single Object command.

**Shortcut: CTRL + SHIFT + M**

**Configuring Drop-ins/Toolbars**

**An Introduction to Automatic Object Analysis**

**Measurement Term Definitions**

**Statistical Term Definitions**

## Measuring a Single Object

To measure a single object, use the following procedure:

| Step | Action |
|---|---|
| **1** | If the image is an 8-bit, 16-bit, or 24-bit image, threshold it using the **Threshold Tool** or **Threshold Image** command so that the threshold values clearly delineate the desired objects from the background.<br><br>**OR**<br><br>If the image is a binary image, continue to Step 2. |
| **2** | From the Measure menu, choose Measure Single Object.<br><br>**Note:** If you have already applied an |

automatic measurement command to any image during the current work session, MetaMorph will display a message dialog box to remind you that it will erase the data in the buffer with the new measurements. (The display of this prompt can be turned off using the **Preferences** command.)

**3** MetaMorph will find all of the objects in the image using the threshold values. When MetaMorph has finished, it will display the objects it found in a new pseudocolor image window. All of the objects will share the pseudocolor. It will also open the Measure Single Object dialog box.

**WARNING:**
The maximum number of objects that can be measured is 16,300. If there are more objects that this maximum, MetaMorph will display an error message and abort the measurement command.

**4** If you want to log the measurement data, open an object log or edgelist log using the corresponding *Open Log* command button in the dialog box or the **Open Object Log** or **Open EdgeList Log** command from the Log menu.

*Open Log* will be replaced by the *F9: Log Data* command button.

**5** Select the object you want to measure from the pseudocolored image window by clicking in the center of the object. MetaMorph will paint it a color based on the classifier filter sets you are currently using and then will number it.

The Measure Single Object dialog box displays the measured object data.

**6** Choose *F9: Log Object* or press the [F9] key to log the displayed object or edgelist data. MetaMorph will log the data and gray out the *F9: Log Object* command button until you select a different object.

**7** To review the data for a previously measured object, use the *Object* spin box to select the object. MetaMorph will display the selected object's data in the dialog box and its object number stamp in the pseudocolor image window.

**8** Choose *Close* when you have finished.

## Measure Single Object - Dialog Box Options

### Open Log

Opens an object log or edgelist log for logging data if desired. Changes to *"F9: Log Data"* when a log file is open.

### F9: Log Data

Logs the currently selected object or edgelist data to the open object log or edgelist log. You can also press the [F9] function key to log these data. After the current data have been logged, the command button will be unavailable and will appear dimmed until you select another object.

### Object

Specifies the object ID number of a previously measured object and displays that object's data in the dialog box. Also marks the object number stamp on the selected object in the pseudocolor image window.

# Morphometry Histogram (Measure Menu)

**Displays a histogram of measurement data from the last automatic measurement in bar graph form.**

**Drop-in: AUTOMEAS**

Use this command to display a histogram of data from a selected measurement parameter. This command allows you to see a visual representation of the data from automatic measurements in the form of a bar graph. This command is similar to Image-1/AT's Data Histogram command or MetaMorph's Integrated Morphometry Analysis command. You can configure autoscaling and the number of bins in the graph.

Prior to using this command, you must threshold and measure an image to provide data for the histogram. The menu item for this command will remain disabled until you have measured an image.

You can log data from this command to an open data log. First use the Open Data Log command to open a data log. You can use the Log Data command or its keyboard shortcut, the [F9] function key, to log the data.

**An Introduction to Automatic Object Analysis**

**Measurement Term Definitions**

## Displaying a Morphometry Histogram

To display a morphometry histogram, use the following procedure:

| Step | Action |
|---|---|
| **1** | If you have not measured the image, threshold it using the **Threshold Tool** or the **Threshold Image** command.<br><br>**AND**<br><br>Measure the image using the desired automatic measurement command. |
| **2** | From the Measure menu, choose Morphometry Histogram. The Morphometry Histogram dialog box will appear. |
| **3** | If you want to log data, open a data log using the ***Open Log*** command.<br><br>Once the data log is opened, the text on the *Open Log* button will change to *F9: Log Data*, and you can then log measurement parameter data whenever you desire. |
| **4** | To configure data logging, choose ***Configure Log*****.** |
| **5** | If you want to autoscale the data displayed in the graph so that plot's X and Y axis is based on the range of the measured object data, select *Auto-Scale Graph.* |

**6** Select the number of bins you want to display in the graph using *Number of Bins.*

**7** To display the data from a measurement parameter, select its name from the *Measurement* list.

When you select a different measurement parameter, MetaMorph will update the graph to display the applicable data.

**8** Choose *Close* when you have finished.

## Morphometry Histogram - Dialog Box Options

### Measurement

Selects the measurement parameter data to be displayed in the graph. You can select from any of the parameters that are currently selected in the Configure Object Measurements dialog box. The data in the graph are automatically updated when you select a new measurement parameter.

### Number of Bins

Specifies the number of bins of data displayed in the graph.

### Auto-Scale Graph

Automatically displays the data in the graph so that the plot's X and Y axis are based on the range of the measured object data.

### Open Log

Opens a data log and/or a DDE link to an open spreadsheet application for logging data. This command changes to *F9: Log Data* once a log file is open.

### F9: Log Data

Logs the currently displayed data from the dialog box to an open data log or to an open spreadsheet application via a DDE link. To assist you in logging the appropriate data when several measurement dialog boxes are open, *"F9"* will be added to the name of this option in the active dialog box to indicate which data will be logged when you press [F9].

### Configure Log

Opens the Configure Log dialog box so that you can select the parameters to be logged to the log file.

### Close

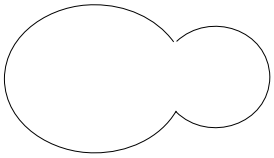
Closes the dialog box.

# Cut Objects (Measure Menu)

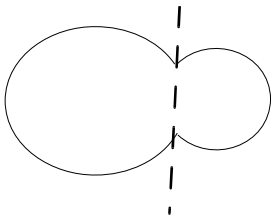
**Cuts an area of an image thresholded as "one object" into two or more objects based on the placement of a region, so that the thresholded "object" will be counted and measured as mul*tip*le objects.**

### Drop-in: CUTJOIN

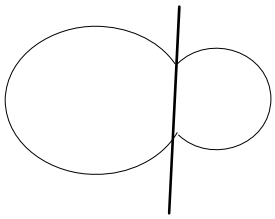
This command can be used in situations where two or more objects are thresholded as one object because they overlap or are otherwise not well-defined. This command cuts by "painting" a "cut" line in the segmentation overlay, which MetaMorph will sense while performing image segmentation.



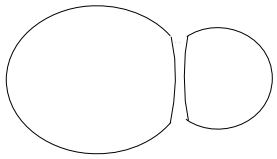
1. Multiple objects thresholded as one "object."



2. A line region is placed to mark the location of the cut mark.



3. The Cut Objects command is applied.



4. The "object" is measured as two objects.

To apply the Cut Objects command more than once to an image, you can write a journal, using the Loop for All Regions command, that allows you to threshold the image and place regions on the all of the desired cut mark locations before the marked objects are cut.

**Shortcut: [F7]**

## Cutting Objects

To separate overlapping objects prior to image segmentation and measurement, use the following procedure:

| Step | Action |
|---|---|
| **1** | Threshold the image for measurement using the **Threshold Image** command (Process menu). |
| **2** | Draw a region through the desired cut mark location using a Line Region Tool. The region must completely bisect the edges of the object. |
| **3** | From the Measure menu, choose Cut Objects. The object marked with the active region will be "cut." |
| **4** | Repeat Steps 2 and 3 for each "cut" you want to create. |

**5** Turn off the active region indicator by choosing the Selector Tool and right-clicking with the mouse.

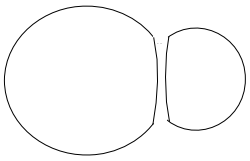
**6** Measure the image as desired.

# Join Objects (Measure Menu)

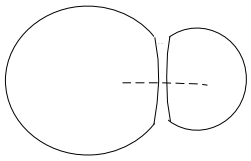
**Joins areas of an image, thresholded as mul*tip*le objects, into a single object, so that the thresholded "objects" will be counted and measured together.**

**Drop-in: CUTJOIN**

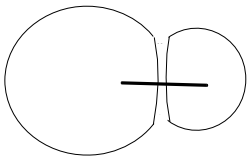
This command can be used in situations where a single object is thresholded as mul***tip***le "objects" because it is not well-defined. This command joins by "painting" a "join" line between the objects in the segmentation overlay, which MetaMorph will sense while performing image segmentation.



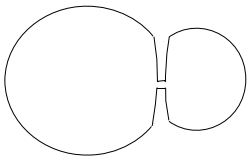
1. One object thresholded as multiple "objects."



2. A line region is placed to mark the location of the join mark.



3. The Join Objects command is applied.



4. The "objects" are measured as one object.

To apply the Join Objects command more than once to an image, you can write a journal, using the Loop for All Regions command, that allows you to threshold the image and place regions on all of the desired join mark locations before the marked objects are joined.

**Shortcut: CTRL + [F7]**

## Joining Objects

To join objects prior to image segmentation and measurement, use the following procedure:

| Step | Action |
|---|---|
| **1** | Threshold the image for measurement using the **Threshold Image** command. |
| **2** | Link the separated parts of an object by using a Line Region Tool to draw a line region that starts inside the first part and ends inside the second part . |
| **3** | From the Measure menu, choose Join Objects. The objects marked with the active region will be "joined." |
| **4** | Repeat Steps 2 and 3 for each "join" you want to create. |
| **5** | Turn off the active region indicator by choosing the Selector Tool and right-clicking with the mouse. |
| **6** | Measure the image as desired. |

# Integrated Morphometry Analysis (Measure Menu)

**Opens the Integrated Morphometry Analysis (IMA) interface for the measurement, display, and logging of morphometric data.**

**Drop-in: IMA**

**Measurement Term Definitions**

**Statistical Term Definitions**

Use this command when you want to perform morphometric measurements of objects in your image. You can select parameters for measurement or define classifier filters which restrict your measurements to just those objects that meet your set criteria.

You can select any of four ways to display morphometric data:

(1) In a table showing the data for each measured object,

(2) In a table showing a statistical summary of the data collected from all of the objects,

(3) In a histogram showing distribution of the data in a bar graph, or

(4) In a scatterplot showing the relationship between any two parameters that you have measured.

The IMA interface has four interactive modes that allow you to "point-and-click" as you work back and forth between the objects in the image window and the data being displayed in the IMA data table, histogram, or scatterplot:

(1) *None* allows you to use region tools or the Cut and Join Drawing Tools, change image magnification, set thresholding, or apply graphics stamps to the image without altering the current set of measurements.

(2) *Single* allows you to add or remove individual objects from the current set of measurements by clicking them in the image window.

(3) *Teach* allows you to use the parameters of objects you click in the image window to configure the classifier filter criteria.

(4) *Find* allows you to find an object's entry in the object measurement data table, its bin in the histogram, or its data point on the scatterplot by clicking the object in the image window.

Conversely, an object can be found in the image window by clicking its entry in the data table, its bin in the histogram, or its data point in the scatterplot.

State files contain your Integrated Morphometry Analysis dialog box configuration settings, such as the threshold levels of your color images, classifier filter settings, interactive mode, and the list of parameters you have selected for measurement and/or classification. These files can be saved to or loaded from disk. Additionally, you can configure log files and perform data logging from within the Integrated Morphometry Analysis dialog box.

## Using Integrated Morphometry Analysis



## Overview of Morphometric Analysis

To perform a morphometric analysis with the Integrated Morphometry Analysis drop-in, use the following procedure.

**Note:** Unless your image is a binary image or a binarized "mask" image (i.e., an 8-bit image that has only two pixel intensity values, such as 0 and 255), it must first be thresholded before conducting your analysis.

| Step | Action |
|---|---|
| **1** | From the Measure menu, choose Integrated Morphometry Analysis. The Integrated Morphometry Analysis dialog box will appear. |
| **2** | Select a source image or stack plane from the *Image* selector. |
| **3** | Select your parameters for measurement from the Parameters List Box. |
| **4** | If desired, you can restrict your analysis to image objects that meet a set of specific quantitative criteria by using classifier filters. This can be performed either by setting the classifier filter ranges **manually** or **interactively.** |
| **5** | Perform your measurements. Measurements can be performed for the entire image **automatically,** or you can select image objects for measurement **interactively.** |
| **6** | You can use the *Display* drop-down list to select between<br>(1) A view of the morphometric data |

| | |
|---|---|
| | associated with each thresholded image object *(Objects),*<br><br>(2) A view of the statistical data for the entire group of objects *(Summary),*<br><br>(3) A view of a histogram of the object measurements *(Histogram),* or<br><br>(4) A scatterplot showing the relationship between any two parameters that you have measured *(Scatterplot).* |
| **7** | If you want to relate the data displayed in the object measurement data table, histogram, or scatterplot more clearly to an individual object in the image window, you can **"find" objects interactively.** |
| **8** | When you are satisfied with your data, you can log the data**.** |
| **9** | If desired, you can save and load the Integrated Morphometry Analysis dialog box settings or **Color Threshold** level settings (if a 24-bit image was being analyzed) by choosing **Save State** and **Load State,** respectively. |
| **10** | When you have finished, choose *Close.* |

## Performing Measurements in IMA

To measure objects automatically throughout your image, use the following procedure. Be sure that your image has been thresholded first.

| Step | Action |
|---|---|
| **1** | If the data region at the right of the IMA dialog box is hidden, choose *Show Data* to expand the dialog box. If you later want to hide the data table, you can choose *Hide Data.* |
| **2** | Select *Measuring* from the *Setup Parameters For* group. |
| **3** | In the Parameters List Box, select which measurement parameters you want to analyze by double-clicking the desired entries. A check mark will appear next to each selected entry. |
| **4** | If desired, you can change the number of significant digits in your data from the *Format* drop-down list box. This can be done for each parameter you have selected from the Parameters List Box. |
| **5** | Choose *Measure.* A green object overlay will appear over the measured objects in the source image, and the morphometric measurements will appear in the object measurement data table, histogram, or scatterplot. |
| **6** | If you need to redo the measurement, choose *Reset Current,* make any necessary |

changes in the Parameters List Box and the *Format* or *Filter Range* boxes, and then choose *Measure* again.

**7** If you perform another measurement, the most recently measured data set will be displayed in the data table and histogram. If you want to view or log all of the data from successive measurements, select *Accumulated* from the *Show/Log Data* group. (The *Reset Accumulated button will replace the Reset Current* button.) Each new data set will be added to the previously displayed set, and will appear in the data table or histogram.

**8** If you need to undo the last measurement, select *Current* from the *Show/Log Data* option button group and choose *Reset Current.*

**OR**
If you want to undo all measurements, select Accumulated from the *Show/Log Data* option button group and choose *Reset Accumulated.*

## Performing Measurements Interactively in IMA

To measure objects interactively with the Integrated Morphometry Analysis interface, use the following procedure. Be sure that your image has been thresholded first.

| Step | Action |
|---|---|
| **1** | In the Parameters List Box, select the parameter that you want to measure by double-clicking its entry. A check mark will appear next to the entry. |
| **2** | Select *Single* from the *Image Interactive Modes* section. |
| **3** | In the image window, click the object you want to measure. A green object overlay will appear over the measured object, and its measurements will appear in the object measurement data table and histogram. |
| **4** | Repeat Step 3 for all objects in the image window that you want to measure. As each object is selected, the data table, histogram, or scatterplot will display the additional measurement for each of the newly selected objects. |

## Setting the Classifier Filter Range

When classifying objects, a range of acceptable values for each classifier filter needs to be defined. Only objects which pass the criteria set by the filter range will be included when classifying objects. Each classifying parameter has its own *Filter Range.* This option will appear only when the *Classifying* radio button is selected in the *Setup Parameters For* group.

A range can be defined to include objects whose measurements fall within a specified range. To do so, select *< = N < =* from the *Filter Range* list. However, if you want to analyze all objects outside a particular range, select *> N or N >* instead. Set the limits of your range by entering desired values in the text boxes on either side of the drop-down list box.

## Setting the Classifier Filter Range Interactively

To add an object's parameters to the Integrated Morphometry Analysis classifier filter range interactively, use the following procedure. Be sure that your image has been thresholded first.

| Step | Action |
|---|---|
| **1** | In the Parameters List Box, select the parameter that you want to use as a classifier filter by double-clicking its entry. A check mark will appear next to the entry. |
| **2** | Select *Teach* from the *Image Interactive Modes* section. |
| **3** | In the image window, click the object whose measurements you want to use to define the classifier filter criteria. A yellow object overlay will appear over each selected object, and its measurements for the currently highlighted parameter will appear in the *Filter Range* text boxes. Simultaneously, all of the selected classifier filters will be updated. Other image objects with measurements that "pass" the updated classifier filters will appear with a green object overlay in the image window.<br><br>**Note:** If you have selected *Draw Failed Classifier Objects* in the Measure Objects page of the Preferences dialog box (Edit menu), objects that fail the classifier filter will be displayed with a blue object overlay. If this option has not been selected, failed objects will simply show the red thresholding overlay. |
| **4** | Repeat Step 3 for all objects in the image window that you want to use to update the classifier filter criteria. As each object is selected, the *Filter Range* text boxes will reflect the measurements of the selected objects for the currently highlighted parameter. |

## Relating Image Objects to Their Data

The *Find* interactive mode allows you highlight an object's entry in the data table, its bin in the histogram, or its data point on the scatterplot by selecting the object in the image with your pointer. Conversely, you can click a data entry, histogram bin, or scatterplot data point, and the associated object(s) will become marked in the image window with a yellow object overlay.

To "find" the object associated with a data entry in the IMA data table, or to "find" the data associated with a particular object in the image window, use the following procedure. Be sure that your image has first been thresholded and measured.

| Step | Action |
|---|---|
| **1** | Select *Find* from the *Image Interactive Modes* section. |
| **2** | To find the data entry for an object in the image window, use the pointer to select the object. A yellow object overlay will appear over the selected object, and the data table will "scroll" to the object's data entry, which will be highlighted. |

| | |
|---|---|
| **3** | To find an object associated with an entry in the data table or point on the scatterplot, or all objects associated with a particular bin in the histogram, use your pointer to select the data table entry, histogram bin, or data point. A yellow object overlay will appear in the image window over the object(s) associated with the selected table entry, histogram bin, or data point.<br><br>**Note:** You can select a range of adjacent table entries or histogram bins by clicking the first entry or bin, holding down the [SHIFT] key, and selecting the ending table entry or bin. Similarly, you can select an assortment of table entries, histogram bins, scatterplot points, or image objects by holding down the [CTRL] key while you make your selections. |

## Logging IMA Data

To log data from the IMA interface, use the following procedure. IMA will automatically select the data to be logged from the list of parameters you have selected from the Parameters List Box. Accordingly, Steps 1 - 4 in the following table are optional.

| Step | Action |
|---|---|
| **1** | Choose *Configure Log.* The Configure Log dialog box will appear. |
| **2** | From the list of parameters, select the parameters you want to log by double-clicking their entries so that each is marked by a check mark. |
| **3** | You can choose *Enable All* or *Disable All* if you want to select or deselect all of the parameters listed. |
| **4** | Choose *OK* to return to the IMA dialog box. |
| **5** | Choose *Open Log.* The button's title will change to *"F9: Log Data."* Each of the displays available in the *Display* group has a different type of data associated with it. Therefore, depending on the *Display* option currently selected, you will be specifying a log file for logging either **object, summary,** or data measurement data. Switch between the displays and specify the log file names for the other two data types after you have selected the first one. |
| **6** | Choose *F9: Log Data* to log your data. If you have selected different file names for the different data types, you will need to switch between the displays and log the data separately for each. |

## Saving a State File

To save the settings you used in your Integrated Morphometry Analysis dialog box, use the following procedure:

| Step | Action |
|---|---|

| Step | Action |
|---|---|
| **1** | Select *Save State.* The Integrated Morphometry: Save State dialog box will appear. |
| **2** | If you want to save your Integrated Morphometry Analysis dialog box settings (for example, the *Setup Parameters For* and *Display* modes, interactive mode, parameter selections, classifier filters, etc.), select the check box next to *Integrated Morphometry State.* A check mark will appear in the box. These settings will be saved in an .ima file. |
| **3** | If you are working with a 24-bit color image, the *24 Bit Image Threshold* check box will be available for selection. Otherwise the check box and title will be unavailable and will appear dimmed. To save color image threshold level settings and their associated image overlays, select the *24 Bit Image Threshold* check box. These settings will also be saved in the .ima file. |
| **4** | If you want to save the criterion settings for a classifying filter range separately from the .ima file, select the *Classifier Filters in a *.cls File* check box.<br><br>(Although these settings can also be saved in the .ima file along with the dialog box and color threshold settings, saving the classifier filter settings to a .cls file allows you to use them independently of the parameter measurement settings.) |
| **5** | Finally, if you want to save the settings for selection of measurement parameters separately from the .ima file, select the *Parameter Measurements in a *.mes File* check box.<br><br>(These settings, too, can also be saved in an .ima file, but saving them to an .mes file allows you to use them independently of the classifier filter settings.) |
| **6** | When you have finished, choose *OK.* |

## Loading a State File

To load a previously saved Integrated Morphometry Analysis (IMA) State File (*.ima, *.mes, *.cls), use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Integrated Morphometry Analysis dialog box, select *Load State.* The Load State File dialog box will appear. |
| **2** | Select the icon for the desired state file. If the desired folder is not currently displayed, use the *Look In* list or Up One Level icon button to change the current folder.<br><br>**Note:** The dialog box defaults to a display of files with the .ima file name extension. If you |

want to select an .mes or .cls file, you will need to select *All Files (*.*)* from the *Files of Type* list.

**3** Choose *Open.*

## Integrated Morphometry Analysis - Dialog Box Options

### Image

Lists the images available to be selected for analysis by the Integrated Morphometry Analysis interface.

### Setup Parameters For

Selecting *Measuring* from this option allows you to pick parameters from the Parameters List Box that are to be measured. Selecting *Classifying* allows you to pick parameters that are to be used for applying a classification filter to the displayed data. Superficially, the *Measuring* and *Classifying* parameter lists that appear in the Parameters List Box as a result of this selection will look identical. However, they serve very different functions. When you select *Measuring,* items selected in the Parameters List Box will be measured, and the *Format* list will appear. When you select *Classifying,* items selected in the Parameters List Box will be used to define object classifier filters, using the values entered in the *Filter Range* boxes that appear.

### Parameters List Box

Lists the morphometric parameters available for either measurement or for defining an object classifier filter (depending on the selection made in the *Setup Parameters For* option button group). (Click here for Measurement Term Definitions.)

### Format

Specifies the number of decimal places to be displayed in the morphometry data. You can specify that figures be carried out to anywhere between zero and eight decimal places. This option is only visible when you select *Measuring* from the *Setup Parameters For* group.

### Filter Range

Sets quantitative criteria for objects, filtering out those objects that do not have morphometric parameters that fall within the specified range from those that do. This option is available only when *Classifying* is selected from the *Setup Parameters For* group.

> **Note:** If the range set in the Filter Range field filters out all objects, or there are no legitimate objects to measure, summary variables are set to 0 when logged.

### Show/Log Data

Determines whether the data to be displayed or logged are from the current measurement set (*Current*) or from the accumulation of measurement sets *(Accumulated).*

### Reset Current/Reset Accumulated

Removes measurements from the active image and clears the data display. When *Show/Log Data* is set to *Current,* the button will be labeled *Reset Current,* and only the most recent measurement will be removed. When *Show/Log Data* is set to *Accumulated,* the labeling will switch to *Reset Accumulated,* and all measured data will be cleared when you click the button. This option is journalizable.

### Image Interactive Modes

This option allows you to pick one of the following four modes for manipulation of morphometric data:

***None*** allows you to use Region Tools such as the Cut and Join Drawing Tools, to change zoom magnification, or to apply graphics stamps to the image without affecting measurements.

***Single*** allows you to add or remove individual objects from the current measurement set by clicking them in the image window.

***Teach*** allows you to add the parameters of objects you click in the image window to the classifier filter criteria.

***Find*** allows you to find objects in the object measurement data table, or find their histogram bin, by

clicking an object in the image window, and vice versa.

### Measure

Measures all objects in the active image window, applying any specified classifier filters, and displays the specified measurement parameters in the data display at the right of the dialog box. This option is journalizable.

### Open Log

Initiates the process of logging data to a text file, via Dynamic Data Exchange (DDE) to an open spreadsheet, or to both. When you choose *Open Log,* its title will change to *"F9: Log Data."* The data that will be logged is determined by the *Display* mode:

When you select ***Objects*** or ***Scatterplot****,* an object log file will be opened, and the data associated with each object will be logged to this file.

When you select ***Summary****,* a summary log file will be opened, and the statistical data for the entire group of objects will be displayed.

When you select ***Histogram****,* a data log file will be opened, and the histogram will be displayed.

### F9: Log Data

Logs the data for all selected parameters. (See preceding entry for *Open Log.)* This option is journalizable.

### Configure Log

Selects the image characteristics and data to be logged.

### Show Data

Expands the dialog box to the right to reveal the IMA data.

### Hide Data

Condenses the dialog box to hide the IMA data.

### Reset Filters

Unchecks all selected items in the Classifying parameters list box and returns the *Filter Range* values to default.

### Save State

Saves the Integrated Morphometry dialog box settings as state files. When you choose this command, the Integrated Morphometry: Save State dialog box will appear.

Selecting one of the upper two check boxes (*Integrated Morphometry State* or *24 Bit Image Threshold)* will specify whether you want to save the dialog box settings, the 24-bit color image threshold level settings, or both, to an .ima file. (The associated image must be a 24-bit color image for you to be able to use the *24 Bit Image Threshold* option.)

The lower two check boxes *(Classifier Filters in a *.cls File* and *Measurement Parameters in a *.mes File)* can be selected if you want to save your measurement parameter choices to a measurement set (*.mes) file or if you want to save classifier filter settings to a classifier set (*.cls) file, respectively.

### Load State

Loads previously saved state files that specify Integrated Morphometry Analysis dialog box settings (*.ima), color image threshold level settings (*.ima), measurement parameter choices (*.mes), and/or classifier filter settings (*.cls) for a particular image. This option is journalizable.

### Preferences

Displays the MetaMorph Preferences dialog box with the Measure Objects page selected. This is the same page as is displayed when you choose Preferences from the Edit menu.

### Display

Selects between the following choices:

***Objects*** – a view of a table of morphometric data for each thresholded image object

***Summary*** – a table of the statistics regarding the entire group of objects

***Histogram*** – a histogram of the object data. Contains the following fields:

**X**

This drop-down box will appear when *Histogram* or *Scatterplot* has been selected from the *Display* list. When more than one object parameter has been measured, this drop-down box allows the selection of the measurement parameter to be graphed on the X-axis. Only measurement parameters activated in the Parameters List Box will be displayed here.

**# of Bins**

Changes the number of bins (and the width of the bins) that are displayed in a histogram. This spin box will appear when *Histogram* has been selected from the *Display* list.

**Set Filter Range from Histogram Calipers**

This command button will appear when *Histogram* has been selected from the *Display* list. Use this command after moving the histogram calipers to the desired location in the histogram display to interactively reset the classifier *Filter Range* for a measurement parameter. Objects that fall outside of the filter range will revert to the red thresholding overlay (or will change to a blue object overlay if you have selected *Draw Failed Classifier Objects* in the Measure Objects page of the Preferences dialog box); objects that stay within the filter range will retain the green object overlay.

***Scatterplot*** – a scatterplot showing the relationship between any two parameters that you have measured. Contains the following fields:

**X**

This drop-down box will appear when *Histogram* or *Scatterplot* has been selected from the *Display* list. When more than one object parameter has been measured, this drop-down box allows the selection of the measurement parameter to be graphed on the X-axis. Only measurement parameters activated in the Parameters List Box will be displayed here.

**Y**

This drop-down box will appear when *Scatterplot* has been selected from the *Display* list. When more than one object parameter has been measured, this drop-down box allows the selection of the measurement parameter to be graphed on the Y-axis. Only measurement parameters activated in the Parameters List Box will be displayed here.

**Use X Calipers**

This command button will appear when *Scatterplot* has been selected from the *Display* list. Use this command after moving the scatterplot calipers to the desired location on the X-axis of the scatterplot display to interactively reset the classifier *Filter Range* for the measurement parameter represented on the X-axis. Objects that fall outside of the filter range will revert to the red thresholding overlay (or will change to a blue object overlay if you have selected *Draw Failed Classifier Objects* in the Measure Objects page of the Preferences dialog box); objects that stay within the filter range will retain the green object overlay.

**Use Y Calipers**

This command button will appear when *Scatterplot* has been selected from the *Display* list. Use this command after moving the scatterplot calipers to the desired location on the Y-axis of the scatterplot display to interactively reset the classifier *Filter Range* for the measurement parameter represented on the Y-axis. Objects that fall outside of the filter range will revert to the red thresholding overlay (or will change to a blue object overlay if you have selected *Draw Failed Classifier Objects* in the Measure Objects page of the Preferences dialog box); objects that stay within the filter range will retain the green object overlay.

**Close**

Closes the dialog box.

# Internally Threshold Objects (Measure Menu)

**Thresholds objects so that pixels below a selected percentage of the maximum intensity in each object are removed, and then measures the processed objects.**

**Drop-in: THRESHOB**

Use this command during morphometric analysis to separate fluorescent objects that overlap. This command uses the internal intensity maximum of each object as initially thresholded, and then sets a new threshold for each object based on a percentage of its maximum. Pixels that fall below the selected percentage are omitted from the new threshold range. The objects are then measured as delineated by the new threshold.

**CAUTION:** This method for splitting objects will shrink object sizes and alter many morphological measurements. Measurements made after internal thresholding will be made on the basis of the resulting objects after processing, not on the original objects as depicted in the original source image.

All single-pixel objects are ignored by MetaMorph during measurement. If the original thresholding of the source image produces single-pixel objects, these objects will be ignored. As an added feature, the dialog box for this command contains an option that allows you to perform a dilation convolution prior to measurement, which will "grow" single-pixel objects and thereby prevent their loss. You should be aware, however, that this convolution will expand the boundaries of other objects, as well, and your data may become skewed as a result.

## Internally Thresholding Objects

To threshold and measure objects on the basis of their individual intensity maxima, use the following procedure:

| Step | Action |
|---|---|
| **1** | Threshold the image by applying the Threshold Image command from the Measure menu or by using the **Threshold Tool.** |
| **2** | Select the desired source image using the *Source* image selector. |
| **3** | Select the desired destination image using the *Dest* image selector. You can overwrite or add to the existing image, or you can place the results in a new image window. |
| **4** | With the *Percent of Objects' Internal Max* spin box, select a percentage of the maximum intensity in each object below which pixels will be removed from the final thresholding range. |
| **5** | If you want to carry out a dilation convolution prior to measurement, select *Dilate Objects to Save Those Reduced to Single Pixel.* |
| **6** | When you are ready, choose *Measure.*<br><br>The internal thresholding will be carried out and a new set of object measurements will be made. When MetaMorph has finished remeasuring the objects, it will display the objects in a new image window using a pseudocolor display. |
| **7** | When you have finished, choose *Close.* |

## Internally Threshold Objects - Dialog Box Options

### Percent of Objects' Internal Max

Selects a percentage of the maximum intensity in each object below which pixels will be removed from the final thresholding range. Pixels that fall below the selected percentage will be omitted from the new threshold range.

### Dilate Objects to Save Those Reduced to Single Pixel

Performs a dilation convolution prior to measurement. This will "grow" single-pixel objects and thereby prevent their loss during subsequent measurement.

### Source

Selects the image to be processed and measured.

### Dest

Selects the destination for the pseudocolor measurement image. You can overwrite the existing image or place the results in a new image window. Or you can add the measurement image as a plane to an existing image or stack.

### Measure

Carries out the internal thresholding procedure, measures the objects as delineated by their respective new threshold ranges, and displays the measured objects in a new pseudocolor image.

### Close

Closes the dialog box.

# Create Regions Around Objects (Regions Menu)

**Draws regions around objects in the currently active image or plane in a stack. You must first threshold the image or apply the Measure Objects or Integrated Morphometry Analysis commands.**

**Drop-in: TRACEOBJ**

Use this command to trace the outlines of objects automatically in the current image or the current plane of an image stack. MetaMorph will determine the edges of objects based on the distribution of the thresholding overlay and will draw a region of interest around each object. This feature can be useful for embellishing an image in preparation for presentation.

**Note:** You must first threshold the image or apply the Measure Objects or Integrated Morphometry Analysis commands before you can apply the Create Regions Around Objects command. Consequently, object tracing with this command requires that either the AUTOMEAS or IMA drop-in also be installed.

One particularly powerful application of this command is in the analysis of subregions within regions, such as in the study of subnuclear components within a cell. This can be accomplished with a combined approach involving use of the Configure Object Classifiers and Integrated Morphometry Analysis commands.

**Note:** The regions that are drawn are "true" regions that can be moved and resized as with any other region. You can move and modify these regions using the Region Toolbar .

## Automatically Tracing Regions Around Objects

To create regions automatically around all objects in an image, use the following procedure:

| Step | Action |
|---|---|
| 1 | Threshold the image by applying the **Threshold Image** command from the Measure menu or by using the **Threshold** |

**Tool.**

**2** If you want to number the regions that will be drawn, select the *Draw Labels Next to Regions* check box from the Region Label tab page of the Preferences dialog box (Edit menu).

**Note:** This step can also be performed after the Create Regions Around Objects command has been applied.

**3** From the Regions menu, choose Create Regions Around Objects. MetaMorph will determine the locations of the edges of each object and will draw a region automatically around each object. The region numbers will correlate with the object numbering system that MetaMorph uses, starting in the upper left corner.

# Show Classifier Statistics (Measure Menu)

**Displays measurement statistics based on the objects in a measured image that pass the selected classifier filter.**

**Drop-in: AUTOMEAS**

Use this command when you want to display or log measurement data from objects that pass a particular classifier filter. The image used for this command can be one that was created when measurements were made with the Measure Objects or Measure Objects with Mask command.

The Classifier Statistics dialog displays the following statistics for each type of measurement: Count, Average, Standard Deviation, Minimum, Maximum, and Total.

The Show Classifier Statistics dialog box can display statistics from the current measured image or an accumulated total from all measured images. The accumulated total will include statistics compiled from all measured images created since the beginning of the worksession or after the last time the *Reset* command button was chosen.

The statistics are compiled from the selected classifier or from all classifiers combined together if the *All Classifiers* option is selected. *All Classifiers* should be used only when the active classifier sets do not have overlapping filters that would cause an object to be counted more than once. Data for *Std. Dev*. (standard deviation) will not be available when *All Classifiers* is selected.

**An Introduction to Automatic Object Analysis**

**Statistical Term Definitions**

## Showing Classifier Statistics

To show classifier statistics, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Measure menu, choose Show Classifier Statistics. The Classifier Statistics dialog box will appear. |
| **2** | If you plan to log data, choose *Open Log* to open a log file or DDE link. When you have opened a log file. the *Open Log* command will be change to "*F9: Log Data*." |
| **3** | Select the desired classifier set from the *Classifier* list. You can select *All Classifiers* if |

you want to view statistics compiled from all of the classifier sets.

MetaMorph displays the statistics for the selected classifier set(s) at the top of the Classifier Statistics dialog box. Only objects that pass the classifier filters in the classifier sets will be included the statistics.

**4** If you only want to display statistics from the current measured image, select *Current* from the *Show* group.

**OR**

If you want to display an accumulated total from all measured images, select *Accumulated* from the *Show* group. The accumulated total will include statistics compiled from all measured images created since the beginning of the work session or after the last time the *Reset* command button was chosen.

**5** Choose *F9: Log Data* or press the [F9] function key if you want to log the data displayed in the dialog box. If the selected classifier set has already been logged, the command button will be unavailable until you select another classifier set.

**6** Repeat Steps 3 - 5 for each classifier set you want to view.

The image window and the data in the *Classifier Statistics* dialog box will be automatically updated whenever you select a new set from the *Classifier* list or modify the classifier filters using the Configure Object Classifiers command.

**7** Choose *Close* when you have finished.

## Show Classifier Statistics - Dialog Box Options

### Classifier Statistics Table

Displays the following statistics for each type of measurement: Count, Average, Standard Deviation, Minimum, Maximum, and Total. The width of each column can be adjusted by dragging the column dividers to the desired width.

### Open Log

Opens a summary log and/or a DDE link to an open spreadsheet application for logging data. This command will change to *F9: Log Data* when a log file is open.

### F9: Log Data

Logs the currently displayed data from the dialog box to an open summary log or open spreadsheet application by way of a DDE link. To assist you in logging the appropriate data when several measurement dialog boxes are open, *"F9"* will be added to the name of this option in the active dialog box to indicate which data will be logged when you press [F9].

### Classifier

Selects the classifier set for display in the Classifier Statistics Table. The statistics are compiled from the selected classifier, or from all classifiers combined together if you select *All Classifiers.* You should use *All Classifiers* only when the active classifier sets do not have overlapping filters that would cause an object to

be counted more than once.

**Note:** Data for *Std. Dev.* (standard deviation) will not be available when you select *All Classifiers.*

### Show

Displays statistics from the current measured image or an accumulated total from all measured images. The accumulated total will include statistics compiled from all measured images created since the beginning of the work session or after the last time the *Reset* command button was chosen.

### Reset

If you selected *Accumulated* from the *Show* group, this button resets the accumulated total for the statistics from all measured images.

### Close

Closes the dialog box.

# Show Individual Object Data (Measure Menu)

**Displays measurement data for an individual object in the last measured image created by the Measure Objects or Measure Objects with Mask commands.**

**Drop-in: AUTOMEAS**

Use this command when you want to display or log data for one or more objects in a measured image. An object's data can be viewed by positioning the pointer over the object and pressing the left mouse button, or you can select the object ID number from the *Object Number* spin box. The data will appear in the Individual Object Data dialog box and an object ID number stamp will label the object in the image. When an object's data are displayed, the measurements can be logged to an object log or an edgelist log.

**Note:** The Show Individual Object Data dialog box will close if you reset the object measurements with the Reset Object Measurements command, since there will be no object data to display in the Individual Object Data dialog box.

**An Introduction to Automatic Object Analysis**

**Measurement Term Definitions**

**Statistical Term Definitions**

## Showing Individual Object Data

To show individual object data, use the following procedure:

| Step | Action |
|---|---|
| **1** | Measure the desired image using the Measure Objects or Measure Objects with Mask command. |
| **2** | From the Measure menu, choose Show Individual Object Data. The Show Individual Object Data dialog box will appear.<br><br>An object ID number stamp will appear on the image. (The first object is determined by finding the topmost object, left to right.) |
| **3** | If you plan to log data, choose *Open Log* to open an object log or DDE link to an open spreadsheet application.<br><br>Once you have opened a log file, the *Open* |

*Log* command will be replaced by the *F9: Log Data* command.

**4** To display an object's data in the Show Individual Object Data dialog box, select its object number using *Object* and press the [TAB] key.

If you do not know the object's number, you can position the pointer over the desired object in the measured image and click the left mouse button. MetaMorph will display the object ID number and data for the closest object (based on the position of its centroid).

The object ID number for the displayed object will be stamped on the measured image window for identification purposes.

**5** Choose *F9: Log Data* to log the data displayed in the Show Individual Object Data dialog box. If the data for the selected object have just been logged, the command button will be unavailable until you select another object.

**6** Choose *Close* to close the Show Individual Object Data dialog box.

## Show Individual Object Data - Dialog Box Options

### Individual Object Data Table

Displays the selected object's measurement values. The width of each column can be adjusted by dragging the column dividers to the desired width.

### Open Log

Opens an object log and/or a DDE link to an open spreadsheet application for logging data. This command will change to *F9: Log Data* when a log file is open.

### F9: Log Data

Logs the currently displayed data from the dialog box to an open object log or DDE-linked spreadsheet. To assist you in logging the appropriate data when several measurement dialog boxes are open, *"F9"* will be added to the name of this option in the active dialog box to indicate which data will be logged when you press [F9].

### Object

Selects and lists the object whose data is displayed in the Individual Object Data Table at the top of the dialog box. Objects can also be selected for display by positioning the pointer over the object in the measured image and pressing the left mouse button.

# Reset Object Measurements (Measure Menu)

**Clears the classifier statistics summaries derived from the last measured image.**

**Drop-in: AUTOMEAS**

Use this command when you want to clear the temporary buffer that MetaMorph uses to store classifier statistics summaries from the last measured image.

**Note:** This command is independent of the Integrated Morphometry Analysis command functions. Choosing this command will not reset the IMA measurements, and choosing the IMA *Reset* button will not reset measurements made with the Measure Objects command.

## Resetting Object Measurements

To reset object measurements, use the following procedure:

| Step | Action |
|---|---|
| 1 | From the Measure menu, choose Reset Object Measurements. |
| 2 | If there are measurements stored in MetaMorph's buffer the Object Measurements dialog box will appear.<br><br>**Note:** This dialog box will not appear unless you select the *Warn User When Measurement Data Will Be Erased* check box in the Preferences dialog box's Measure Objects tab page. |
| 3 | Choose *Yes* if you want to reset the object measurements.<br><br>**OR**<br><br>Choose *No* to cancel the command. |

# Create Classifier Stack (Measure Menu)

**Creates a stack from the results of the Measure Objects command, displaying objects that match each classifier filter in separate planes.**

**Drop-in: MSTACK**

Use this command after you have applied the Measure Objects command to an image and want to display the objects that pass each filter in separate planes of an image stack.

Before using this command, you must configure the classifier filters to include or exclude objects from measurements using the Configure Object Classifiers command, and then measure the objects using the Measure Objects command.

## Creating a Classifier Stack

To create a classifier stack, use the following procedure:

| Step | Action |
|---|---|
| 1 | Configure the desired classifier filters using the **Configure Object Classifiers** command. |
| 2 | Measure the objects using the **Measure Objects** command. |
| 3 | Select the measured image so that it is the active image. |
| 4 | From the Measure menu, choose Create Classifier Stack. A stack will be created, consisting of the objects that match each filter displayed in a separate plane. |

# Measure Distance with Annotation (Measure Menu)

**Displays the distance of a line drawn with a Line Region Tool and logs the current text annotation whenever the distance measurement data are logged.**

**Drop-in: ANDIST**

Use this command to determine the distance of an object or area of interest within an image, and to log an annotation each time a distance measurement is logged with the Log Data command.

The distance measurement can be displayed in units which have been calibrated with the Calibrate Distances command. If the units have not been calibrated, MetaMorph will display the distance in pixels.

You can log each measurement to an open data log if desired. First, use the Open Data Log command to open a data log. You can use Log Data or its keyboard shortcut, the [F9] function key, to log the data.

## Measuring Distance with Annotation

To measure the distance of a line and make an annotation, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Measure menu, choose Measure Distance with Annotation. The Measure Distance with Annotation dialog box will appear. |
| **2** | Select the desired image using the *Image* selector. |
| **3** | Choose *Open Log* to open a data log. |
| **4** | To configure the data log for logging, choose *Configure Log.* The Configure Log dialog box will appear.<br>**AND**<br>From the *Configuration* list, select the parameters you want to enable for logging, so that each is marked by a check mark next to its name (you can choose *Enable All* or *Disable All* if you want to select or deselect all of the parameters listed).<br>Choose *OK* to return to the Measure Distance with Annotation dialog box. |
| **5** | Draw a line of the desired distance using the Single Line Tool, Multi-Line Tool, or Traced Line Tool. |
| **6** | Select the desired line so that it is the active region. MetaMorph will measure the distance of the line and display it in the dialog box. |
| **7** | Type the desired annotation in the *Log Annotation* text box. You can delimit entries that are to be sent to separate columns in the data log by separating them with commas when you enter them into the annotation text box. |

**8** When you want to log the measured distance and annotation, choose *F9: Log Data.*

**9** To measure another distance, create and select another line using the process described in Steps 5 - 8.

**Note:** You can edit the distance of the line by double-clicking the line using the left mouse button and dragging the round handles that appear.

**10** Choose *Close* when you have finished measuring the distances.

## Measure Distance with Annotation - Dialog Box Options

### Image

Selects the image for measuring distances and annotating.

### Distance

Displays the data from the current distance measurement.

### Log Annotation

Specifies the annotation to be logged when you choose *F9: Log Data.* You can delimit entries that are to be sent to separate columns in the data log by separating them with commas when you enter them into the annotation text box.

### Open Log

Opens a data log and/or a DDE link to an open spreadsheet program for logging data. This command changes to *F9: Log Data* when a log file is open.

### F9: Log Data

Logs the currently displayed data from the dialog box to an open data log or to an open spreadsheet by way of a DDE link. To assist you in logging the proper data when several measurement dialog boxes are open, *"F9"* will be added to the name of this option in the active dialog box to indicate which data will be logged when you press [F9].

### Configure Log

Opens the Configure Log dialog box so that you can select the parameters to be logged to the data log. Parameters marked with a check mark will be logged for subsequent measurements.

If you select ***Log Column Titles****,* a line listing the measurement titles will be logged (1) the first time you use the configured measurement, (2) whenever you enable/disable measurement parameters, or (3) whenever the logged measurement is different from the previous measurement in the log file.

If you select ***Place Log Data on Current Line****,* subsequently logged data will be appended to the current line in the log file, rather than on a new line. *Log Column Titles* will be unavailable when you select this option.

### Close

Closes the dialog box.

# Graph Intensities (Apps Menu)

**Measures and logs intensity data from selected regions in an image stack or in a series of live video images.**

*Availability: Available for MetaVue; included in MetaMorph Premier and MetaMorph Basic*

### Drop-in: BTIME

Use this command to measure intensity values

(1) Over time from a series of live video images,

(2) Over time, plane number, Z-axis distance, or wavelength from a stack of images.

Intensity data that can be measured, graphed, and logged include such parameters as are displayed in the Show Region Statistics dialog box. These include the average intensity, standard deviation of the intensity, integrated intensity (summed over all pixels in the region), maximum and minimum grayscale levels, thresholded area (expressed as either numbers of pixels or as a percent of total), and the like. The HSI intensity value is used for logging color images.

For a stack, intensity measurements over time are determined by the acquisition time of each plane in the stack. Alternatively you can measure the intensity values over plane number. Intensity measurements can be made over wavelength for stacks acquired using the Acquire Spectral Scan command.

If you are using a digital camera, Measure Brightness uses the acquisition settings from the Acquire from Digital Camera command.

## Graphing Intensities

To measure brightness over time, plane, or frame, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, choose Graph Intensities. The Configure Graph Intensities dialog box opens. |
| **2** | From the *Measure From* group, select *Live Video, Disk,* or *Stack.* |
| **3** | From the *Measurement* drop-down list, select the intensity statistic you want to measure and graph. |
| **4** | If you want to measure only those pixels that fall within the threshold settings, select *Use Threshold for Intensity Measurements.* |
| **5** | If you selected *Live Video* in Step 2, choose *Acquisition* to open the Configure Acquisition dialog box and chose acquisition options.<br>**Additional Acquisition Options** |
| **6** | If you selected *Stack* in Step 2, select the desired stack from the *Image* selector.<br>AND<br>From the *Measure Regions Over* group, select *Time, Plane Number, Z Distance,* or *Wavelength.* |
| **7** | If you selected *Disk* in Step 2, choose *Select Files* and select the image files you want to measure. |
| **8** | Select *X Axis* to configure the X-axis. Select the desired *Range, Number of Planes,* or *Number of Frames* (as applicable). Select the number of *Tick Marks* for the axis. The number should be divisible by the range or number of tick marks. |

If you are measuring regions over time, select the unit of time using *Time Scale.* Then choose *OK.*

**9** Choose *Y Axis* to configure the Y-axis. Select the *Minimum Gray Level* and *Maximum Gray Level* values. Then select the desired number of *Tick Marks.*

If you want to use calibrated values, select *Use Calibrated Gray Values.* Then choose *OK.*

**10** To configure an open data log for logging, choose ***Configure Log*.**

**11** Choose *OK* to continue to the Measure Regions dialog box.

After you have completed the preceding steps, use the following procedure to measure region intensity values.

**Note:** If you are working with a stack, you need to have the Select Plane dialog box opened and positioned in an accessible location during brightness measurements.

| Step | Action |
|---|---|
| **1** | If you are working with live video, choose *Refresh Video.* This will update the image presented in the Live Video image window with the most recently acquired frame. It is recommended that you do this before creating new regions.<br><br>**Note:** You **must** choose *Refresh Video* to update the video window, or else have open the live video window for the acquisition command appropriate for your video device (Live Video, Acquire from Digital Camera, Acquire from Flashbus, etc.). |
| **2** | Create region(s) in the live video or stack image window using a Region Tool. You can modify or add regions at any time during the measurements.<br><br>**Note:** Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaMorph. |
| **3** | To log data to an open data log, choose *F9: Log Data.* |
| **4** | Choose *Begin* to start measuring brightness and graphing the intensity values for the regions in the Measure Regions graph.<br><br>If you are using a stack, the Select Plane dialog box will appear. *Begin* will change to *Play Stack* after it has been selected. You can use the options in the Select Plane dialog box to start and stop the stack. Choosing *Play Stack* resets the stack to the first plane and plays it.<br><br>**OR** |

If you are using live video, the *Begin* button will change to a *Pause/Resume* button.

**5** You can attach event notes at any time by typing a short text description in the text box at the bottom of the dialog box and choose *New Event.*

A line marker will be placed inside the graph at the appropriate spot along the X-axis.

**6** If you want to change the X-axis or Y-axis configuration settings during acquisition, choose *Change X-Axis* or *Change Y-Axis.* When you have adjusted the options as desired in the appropriate dialog box, choose *OK* to begin acquisition again.

**7** When you have graphed the data, you can click and hold the pointer over any point within the graph to view a summary of that point's data. The scroll bar will change to a data display. The bullets preceding the data and the trace line in the graph are color-coded to match the region outline.

For more information about configuring graph, refer to Working with Graphs .

You can choose *Clear* if you want to clear the data from the graph.

**8** Choose *Close* to close the dialog box and graph when you have finished.

## Selecting Files from Disk for Measuring Regions

### Selection Type

Selects the method by which you specify which files to measure:

***Quick Select*** will allow you to pick the first file in a series that is numbered by file name or by extension, and will automatically select all other files in that series.

***Numbered Names* and *Numbered Extensions*** allow you to select the first and last numbered files in their respective series.

### Select File

This button will appear when *Quick Select* has been selected as the *Selection Type.* Choose this to select the first file in a series of files that have numbered file names or extensions. MetaMorph will automatically select all subsequent files in that series.

### Select First

This button will appear when either *Numbered Names* or *Numbered Extensions* is selected as the *Selection Type.* Choose this to select the first file in a series of files that have numbered file names or extensions.

### Select Last

This button will appear when either *Numbered Names* or *Numbered Extensions* is selected as the *Selection Type.* Choose this to select the last file in a series of files that have numbered file names or extensions.

## Graph Intensities - Options

### Configure Graph Intensities

### Configure Acquisition

**Graph Intensities**

## Configure Graph Intensities - Dialog Box Options

### Measure From

Specifies whether the brightness measurements will be made from a stack, from image files residing on disk, or from live video.

### Measure Regions Over

Specifies whether intensity values will be measured over *Time, Plane Number, Z Distance, Wavelength* or *Frame. Time* is the only option available for live video.

### Measurement

Selects an intensity statistic to be measured, graphed, and logged over image frames. The selections include: *Thresholded Area, Average Intensity* (which, in 24-bit color images, takes the arithmetic mean for all three channels), *Standard Deviation, Signal to Noise, Integrated, Minimum, Maximum, % Thresholded Area, Average Red, Average Green, Average Blue,* and *Average (Red, Green, Blue)* (which gives the mean for each of the three channels simultaneously).

### Use Threshold for Region Measurements

Specifies that only pixels whose gray values fall within the threshold settings will be included in the intensity measurements. For example, if you want view the average gray value of all non-zero pixels in an 8-bit image, set the image's threshold range from 1 - 255 and select this option.

### Image

Specifies the stack to measure if *Stack* is selected in the *Measure From* group.

### Select Files

Opens the Select Files dialog box which is used to select which files on disk are to be measured.

### Acquisition

Opens the Configure Acquisition dialog which is used to select the desired acquisition interval and additional acquisition options.

### X-Axis

Configures the X-axis. If you are measuring brightness over time, the unit of time is selected with the *Time Scale* option. For all measurements, the desired range or number of planes and the number of tick marks for the axis are selected. You can select a tick mark value between the range of 2 to 1000.

### Y-Axis

Configures the Y-axis. The minimum and maximum gray values and the tick marks for the axis are selected. The use of calibrated gray values is also enabled using this dialog box. You can select a tick mark value between 2 and 1000.

### Configure Log

Opens the Configure Log File dialog box so that you can select the parameters to be logged to the log file.

> **Note:**
>
> The New Event button in the Measure Regions dialog box will only be enabled if you turn on "Event" in the list of parameters to measure in the Configure Log File dialog box.

### OK

Sets the configuration options for measuring brightness and opens the Measure Regions dialog box.

### Cancel

Cancels the command.

## Configure Log - dialog box options

### Configuration

Specifies the parameters to be logged. Parameters marked with a check mark will be logged in subsequent measurements. Double-click the desired parameter to mark it with a check mark.

### Enable All

Enables all parameters in the *Configuration* list.

### Disable All

Disables all parameters in the *Configuration* list.

### Log Column Titles

If *Log Column Titles* is selected, a line listing the measurement titles will be logged (1) the first time you use the configured measurement, (2) whenever you are enable/disable measurement parameters, or (3) whenever the logged measurement is different from the previous measurement in the log file.

### Place Log Data on Current Line

When you select this option, subsequently logged data will be appended to the current line in the log file, rather than to a new line. The *Log Column Titles* option will become unavailable when you select this option.

### OK

Reconfigures the log file.

### Cancel

Cancels the Configure Log command.

## Configure Acquisition (Graph Intensities) - Dialog Box Options

### Measure Every

Selects the number and unit *(Frames, Milliseconds, Seconds, Minutes,* or *Hours)* for the time interval between measurements from Live Video.

### Illum

Selects the illumination setting associated with the shutter. If you are not using a shutter, leave this selection as *"[None]."*

### Force Image Acquisition

Acquires an image into the frame buffer and then measures the brightness from the acquired image, rather than directly from the Live Video image. This is useful when using a digital camera or when acquiring over a very long time interval. Measure Regions uses the settings from Acquire Image, rather than from Live Video, if you are not using a digital camera.

### Force Image Update

Redraws the image window for each measurement taken on the graph. Checking this box can slow down the Graph Intensities command.

### Use Journal for Acquisition

Runs the selected journal instead of the Acquire Image command. The journal must include an image acquisition command.

### OK

Accepts the current acquisition configuration .

### Cancel

Cancels the acquisition configuration and closes the Configure Acquisition dialog box.

## Graph Intensities - Dialog Box Options

### Log Data

Enables/disables logging of data to the open data log.

### Begin

Starts measuring brightness and graphing intensity values for the selected region to the Measure Regions graph. If you are measuring from a stack, the Select Plane dialog box will appear and *Begin* will change to *Play Stack.* If you are measuring from live video, *Begin* will change to *Pause/Resume.*

### Pause/Resume

If you are measuring from live video, the *Begin* button changes to a *Pause/Resume* button. When live video is paused using *Pause,* the *Refresh Video* command will be carried out for you automatically.

### Change X Axis

Changes the X-axis configuration options. If you are measuring brightness from a stack, you will need to choose *Stop* in the Select Plane dialog box to pause the playing of the stack before choosing this command.

### Change Y Axis

Changes the Y-axis configuration options. If you are measuring brightness from a stack, you will need to choose *Stop* in the Select Plane dialog box to pause the playing of the stack before choosing this command.

### Clear Graph

Clears the data from the Measure Regions graph.

### Refresh Video

Updates the image in the live video image window with the most recently acquired frame.

> **Note:**
>
> You must choose *Refresh Video* to update the video window, or else have open the live video window for the acquisition command appropriate for your video device (Live Video, Acquire from Flashbus, etc.).

### New Event

Records the event text in the text box next to the command in the Measure Regions graph and in the log file, if applicable. A marker is placed at the appropriate spot along the graph's X-axis.

> **Note:**
>
> The New Event button in the Measure Regions dialog box will only be enabled if you turn on "Event" in the list of parameters to measure in the Configure Log File dialog box.

### Close

Closes the Measure Brightness dialog box and the graph.

# Calipers (Measure Menu)

**Measures the distance between a pair of movable "caliper" lines.**

> **Drop-in: CALIPERS**

Use this command to measure the distance between a pair of parallel lines that can be dragged displayed on an image. This may be particularly useful for making quick determinations of distances in live images during focusing or acquisition. The length of the two caliper lines can be resized, and the location, distance between, and angle of the calipers can be altered by dragging various points on the H-shaped caliper display with your pointer. Both the caliper lines and their measured distance values can

be stamped directly onto the image. Distance measurements can be logged to a data log, which you can open and configure from within the Calipers dialog box.

**Note:** To express the distance measurement in "real" units, be sure to apply the Calibrate Distances command before stamping the image or logging the measurement.

## Performing Distance Measurements with Calipers

To use the calipers for distance measurements, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Measure menu, choose Calipers. The Calipers dialog box will appear, and an H-shaped set of caliper lines will appear in the active image window. |
| **2** | If necessary, use the *Image* selector to select the image to be measured. |
| **3** | If desired, you can select the color in which the calipers and the measured distance value are displayed by using the *Color* drop-down list. This will affect only the color of the caliper display, not the stamp (see Step 7). |
| **4** | The calipers can be moved by single-clicking the cross-bar so that it is displayed as a blinking line, indicating that it is active, and then dragging the cross-bar to the desired location with your pointer. |
| **5** | You can adjust the distance between the two caliper edge lines in one of three ways. The distance between the calipers will be displayed on the image, and the value in the *Distance* spin box will be updated:<br><br>(1) Single-click one of the caliper edge lines so that it is displayed as a blinking line. Then drag the line to the desired distance. The other caliper line will remain anchored.<br><br>(2) Double-click the caliper cross-bar so that "nodes" appear at each end. With your pointer, drag one of nodes away from the other to the desired distance.<br><br>(3) Use the *Distance* spin box to specify the distance between the caliper lines. |
| **6** | Similarly, you can change the angle of the calipers in one of three ways. The value in the *Angle* spin box will be updated:<br><br>(1) Double-click one of the caliper edge lines so that nodes appear at each end. With your cursor, drag one of nodes up or down until the desired angle is achieved. The other node will act as a pivoting anchor. **Note:** You can extend the length of the end lines simultaneously while you adjust the angle of the calipers by dragging the edge line node.<br><br>(2) Double-click the caliper cross-bar so |

that nodes appear at each end. With your pointer, drag one of nodes up or down until the desired angle is achieved. The other node will act as a pivoting anchor. **Note:** You can lengthen the cross-bar simultaneously while you adjust the angle of the calipers by dragging the cross-bar node.

(3) Use the *Angle* spin box to specify the angle of the calipers. Angle values can range from -180 to 180 degrees, rotating counterclockwise from the horizontal.

**7** If you wish to stamp the caliper lines and the measured distance value directly on the image, you can specify a color for the stamp that differs from the color of the caliper display. This will be particularly useful if you plan to make several measurements.

To specify a color for the stamp, select the desired color from the *Overlay Color* drop-down list.

**8** If you do not wish to log the measurement values, you can stamp the values and the caliper lines directly on the image by choosing *Stamp.* If necessary, you can remove the stamps by choosing *Clear Overlay.* (Alternatively, you can choose Clear Measurement Stamps from the Graphics menu, or use its keyboard shortcut, [ALT] + [C].) Repeat Steps 6 - 8 as desired for additional measurements and stamps. Then skip to Step 11.

**OR**
If you plan both to log the measurement data and to stamp the image, you have the option of stamping the image automatically when you log the data. To configure the Calipers command to do so, select the *Stamp as Data Is Logged* check box.

**9** Open a data log using the **Open Log** command button. When the data log is open, the text on the *Open Log* button will change to *F9: Log Data.*

**AND**
To configure the data log for logging, choose ***Configure Log*****.** Select the parameters you want to log by double-clicking their entries in the *Configuration* list and choose *OK* to return to the Calipers dialog box.

**10** When you are ready, choose *F9: Log Data,* or press the [F9] function key.

**11** When you have finished, choose *Close.*

## Calipers - Dialog Box Options

### Image

Selects the image to be measured.

### Distance

Specifies a distance between the two caliper edge lines. This spin box will update when the arrangement of the caliper lines is changed in the image window.

### Angle

Specifies an angle for the caliper lines. Angle values can range from -180 to 180 degrees, rotating counterclockwise from the horizontal. This spin box will update when the arrangement of the caliper lines is changed in the image window.

### Color

Selects the color in which the calipers and the measured distance value are to be displayed. This will not affect the color of the overlay stamp.

### Open Log

Opens a data log and/or a DDE link to an open spreadsheet application for logging data. This command will change to *F9: Log Data* when a log file is open.

### F9: Log Data

Logs the currently displayed data from the dialog box to an open data log or to an open spreadsheet application via a DDE link. To assist you in logging the appropriate data when several measurement dialog boxes are open, *"F9"* will be added to the name of this option in the active dialog box to indicate which data will be logged when you press [F9].

### Configure Log

Opens the Configure Log dialog box so that you can select the parameters to be logged to the log file.

### Overlay Color

Specifies a color for the stamp. This will be particularly useful if you plan to make several measurements.

### Stamp as Data Is Logged

Configures the Calipers command to stamp the image automatically when you log distance measurement data.

### Clear Overlay

Removes all stamps from the image.

### Stamp

Stamps the image with the current arrangement of caliper lines and the measurement value. This stamp will become a permanent part of the image overlay unless you click the *Clear Overlay* button or choose Clear Measurement Stamps from the Graphics menu.

### Close

Closes the dialog box.

# Count Cells (Apps Menu)

**Counts single cells automatically in a clear 8-bit, 16-bit, or 24-bit image.**

*Availability: Available for MetaVue and MetaMorph Basic; included in MetaMorph Premier*

**Drop-in: CELLCNT**

Use this command for simple cell-counting based on

(1) Threshold-based detection of discrete cell boundaries,

(2) Use of an interactively selected Standard Area, or

(3) Use of an interactively selected integrated pixel intensity value.

If you need to perform more complicated morphometric measurements, it may be better to do so by using standard morphometric methods, such as the Integrated Morphometry Analysis command (Measure menu). To separate two types of cells from each other based on their staining, such as when measuring live vs. dead cells, you should use the Count 2 Types of Cells command, also located in the Measure menu.

> **Note:** Because of its reliance on threshold ranges, Count Cells is not appropriate for use with binary images.

Boundary-based detection uses the fact that objects in an image that are of importance tend to have pixel intensities that are significantly different from background. When you define a threshold range, the Count Cells command can measure the number of cells in your image automatically, based on the boundaries of what MetaMorph perceives as discrete, single objects.

Counting objects is often complicated by the fact that objects overlap or clump together when thresholded, resulting in counts that are lower than the actual number of objects present. Using the second cell-counting method allows you to define a Standard Area. This is a value that will be used to represent the size of a typical cell, based on the assumption that the cells you want to count are of fairly uniform size (area). You can select a Standard Area interactively with the Count Cells command by clicking objects in the image window. MetaMorph will calculate an average size for all of the cells you click. Objects will then be counted by dividing the total thresholded area of each clump by the Standard Area. Clumps that are less than half the Standard Area will be omitted from the final cell count.

The third method is in some ways similar to the second in that a standard is selected interactively by clicking objects in the image. This method, however, is based on the integrated pixel intensity, that is, the arithmetic sum of the intensity values of all pixels in the thresholded area. This value will be used as a standard for the subsequent measurement and counting. This may be useful under conditions in which moderately fluorescent cells are clumped together, resulting in a region that is smaller than in size than the number of cells present, but the fluorescence of the clumped cells is noticeably additive. As with the use of a Standard Area, thresholded objects that have an integrated intensity less than half the standard value will be omitted from the final cell count.

Integral to each of these methods is the selection of a threshold range. Regardless of the method you choose, you will need to define a threshold range to separate the objects of importance from their background. Count Cells keeps its own threshold, ignoring any other current thresholding in the image. To set or adjust the thresholding used by Count Cells, use the *Set Threshold* button in the Count Cells dialog box for 24-bit images. Use the Image Window Threshold Slider to adjust the thresholding used in 8- or 16-bit images. The *Measure* button in the Count Cells dialog box will then apply the thresholding automatically during the measurement process. If you need to change the thresholding, you can use the dialog box's *Set Threshold* command button. When you have finished, the measurement will proceed automatically.

## Counting Cells



## Overview of Single-Cell Counting

To perform simple single-cell counting, use the following general procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, choose Count Cells. The Count Cells dialog box opens. |
| **2** | If necessary, select the image containing the cells you want to count with the *Source Image* selector. |
| **3** | If you want to view an image that highlights the counted cells and displays the number of cells in each cluster next to it, select the *Display Result Image* check box. This may be useful for comparison with the original source image to determine how good your configuration and thresholding was. |
| **4** | Configure your measurement by choosing the *Configure* button. Your configuration procedure will depend on whether you want to base your measurement on<br>**Detection of cell boundaries,**<br>**Use of a Standard Area,** or<br>**Use of a standard integrated intensity.** |
| **5** | If the image is 24-bit and you need to adjust the thresholding, click *Set Threshold* to open the Set Color Threshold dialog box. |
| **6** | Click *Use Images's Threshold* to use the image's own threshold and automatically run the measure command. The number of cells that are detected will appear in the *Count* status line of the Count Cells dialog box. |
| **7** | When you are ready to carry out the measurement, choose *Measure.* The number of cells that are detected will appear in the *Count* status line of the Count Cells dialog box. |
| **8** | If you want to save the measurement data, you can **open a data log.** The *Open Log* button will become a *Log Data* button. When you are ready to log the count data, choose *Log Data.* |
| **9** | When you have finished counting cells, choose *Close.* |

## Configuring Cell-Count Measurement Based on Object Boundaries

To configure cell-counting based on the boundaries of thresholded objects, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Count Cells dialog box, choose *Configure.* The Set Counting Method dialog box will appear. |
| **2** | From the *Counting Method* radio button group, select *Count Each Discrete Object* |

*as 1.*

## Configuring Cell-Count Measurement Based on Standard Area

To configure cell-counting based on a Standard Area, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Count Cells dialog box, choose *Configure.* The Set Counting Method dialog box will appear. |
| **2** | From the *Counting Method* radio button group, select *Use Standard Area to Estimate Objects in a Cluster.* |
| **3** | Choose *Next >.* The Set Counting Method dialog box will close, and the Set Standard dialog box will appear. Simultaneously, a blue overlay will appear over any thresholded regions of the image. |
| **5** | As necessary, use the *Source Image* selector to select the image containing objects that you want to use to define the standard. This does not need to be the same image as the one containing the cells you want to count. For example, you could use an image containing objects of a known size, such as microspheres. |
| **6** | If you need to adjust the thresholding for the image, click *Set Threshold*. The Set Color Threshold dialog box opens if the image is in color; if not, a red overlay is placed over the image and you can adjust the threshold using the Image Windows threshold slider. After you finish selecting the threshold range and have closed the Set Threshold dialog box, you will return to the Set Standard dialog box.. |
| **7** | In the image window, click several objects that are of a "typical" size. As you select the objects, a yellow overlay will appear over them in the image window, and the *Total Area, Count,* and *Standard Area* spin boxes will update, displaying the total area selected, the number of selected cells, and the average computed area, respectively. You can modify these values directly, if desired. |
| **8** | When you have finished, choose *Next >.* The Set Standard dialog box will close, and the Count Cells dialog box will reappear. |

## Configuring Cell-Count Measurement Based on Integrated Intensity

To configure cell-counting based on a standard integrated intensity value, use the following procedure:

| Step | Action |
|---|---|
| 1 | From the Count Cells dialog box, choose *Configure.* The Set Counting Method dialog box will appear. |
| 2 | From the *Counting Method* radio button group, select *Use Integrated Intensity to Estimate Objects in a Cluster.* |
| 3 | Choose *Next >.* The Set Counting Method dialog box will close, and the Set Standard dialog box will appear. Simultaneously, a blue overlay will appear over any thresholded regions of the image. |
| 4 | As necessary, use the *Source Image* selector to select the image containing objects that you want to use to define the standard. This does not need to be the same image as the one containing the cells you want to count. For example, you could use an image containing objects of a known brightness, such as fluorescent beads of a specific diameter. |
| 5 | If you need to adjust the thresholding for the image, click *Set Threshold*. The Set Color Threshold dialog box opens if the image is in color; if not, a red overlay is placed over the image and you can adjust the threshold using the Image Windows threshold slider. After you finish selecting the threshold range and have closed the Set Threshold dialog box, you will return to the Set Standard dialog box. |
| 6 | In the image window, click several objects that are of a "typical" brightness and size. As you select the objects, a yellow overlay will appear over them in the image window, and the *Total Intensity, Count,* and *Unit Intensity* spin boxes will update, displaying the total integrated intensity, the number of selected cells, and the average integrated intensity, respectively. You can modify these values directly, if desired. |
| 7 | When you have finished, choose *Next >.* The Set Standard dialog box will close, and the Count Cells dialog box will reappear. |

## Configuring Data Logging for Cell-Count Measurement

To configure logging of your single-cell count data, use the following procedure. You can follow this procedure either before or after you perform the actual measurement.

| Step | Action |
|---|---|
| 1 | From the Count Cells dialog box, choose *Open Log.* The Open Data Log dialog box will appear. |
| 2 | Select *Dynamic Data Exchange (DDE)* to log |

directly to an open spreadsheet program. Select *A Text File* to log the data to a text file.

**Note:** You can select both options.

**3** If you selected *A Text File* in the previous step, the Open Data Log File dialog box will appear.

Select an existing log file or type a new file name in the *File Name* text box. (If necessary, use the *Look In* list or Up One Level button to change the current drive and folder to the correct location.)

**AND**

Choose *Open.*

**4** If you selected an existing log's file name in Step 3, the Log File Exists dialog box will appear. You can *Overwrite* the contents of the file, *Append* new data, or *Cancel.*

**5** If you selected *Dynamic Data Exchange (DDE)* in Step 2, the Export Log Data dialog box will appear.

Select the desired application from the *Application* list. Choose *Default* to use the default settings for the selected application.

Choose *OK* to launch the external application. After the application opens, click the MetaMorph Imaging System button in the Windows Taskbar to return to MetaMorph.

**6** If necessary, choose *Configure Log* from the Count Cells dialog box to select which data to log. The Configure Log dialog box will appear.

**AND**
From the *Configuration* table, select the data types that you want to log. Then choose *OK.*

**7** The *Open Log* button will have changed to a *Log Data* button. To log your cell count data, choose this button after you have performed your measurement.

**8** If necessary, click the *Back* button at the top of this Help window to return to the procedure you were just reading.

## Count Cells - Dialog Box Options

### Configure

Opens the Set Counting Method dialog box, from which you can select the counting method (boundary-based, Standard Area-based, or integrated intensity-based).

### Set Threshold (24-bit Images Only)

Opens the Set Color Threshold dialog box, from which you can change the thresholding range. This should only be necessary for images in which the thresholding has never been set, or in cases where you want to change the thresholding.

### Source Image

Selects the image containing the cells you want to count.

### Use Image's Threshold

Applies the image's own threshold and automatically run the measure command. The number of cells that are detected will appear in the *Count* status line of the Count Cells dialog box.

### Open Log

Opens a text-based data log file or an OLE connection to an open spreadsheet program for logging count data if desired. Changes to *"Log Data"* when a log file is open.

### Log Data

Sends your cell-count measurement data to the open data log or spreadsheet.

### Configure Log

Opens the Configure Log dialog box, from which you can select the types of data to be stored in the data log.

### Display Result Image

When selected, displays a measurement image in a separate window, showing only the objects that have been counted, along with a number next to each cluster which indicates the number of cells in the cluster.

### Count

This status line indicates the number of cells that have been counted.

### Measure

Carries out the measurement, based on the configuration of the counting method and the current threshold state of the image. (Note: If you change the threshold range with the *Set Threshold* command button, measurement will proceed automatically when you have finished.)

### Close

Closes the Count Cells dialog box.

## Set Counting Method (Count Cells) - Dialog Box Options

### Counting Method

Selects a cell-counting method:

*Count Each Discrete Object as 1* - Bases the cell-count measurement on what MetaMorph perceives to be the object boundaries, as defined by thresholding. When you select this option, choosing *Next >* will return you to the Count Cells dialog box, from which you should then select a thresholding range. After you select the threshold range, measurement will proceed automatically.

*Use Standard Area to Estimate Objects in a Cluster* - Bases the cell-count measurement on a selected Standard Area. When you select this option, choosing *Next >* will open the Set Standard dialog box, which you will use to select the Standard Area.

*Use Integrated Intensity to estimate Objects in a Cluster* - Bases the cell-count measurement on a standard integrated intensity value. When you select this option, choosing *Next >* will open the Set Standard dialog box, which you will use to select the standard integrated intensity.

### Cancel

Cancels your selection of cell-counting method and closes the Set Counting Method dialog box, returning you to the Count Cells dialog box.

### Next >

Accepts your selection of cell-counting method and closes the Set Counting Method dialog box. If you selected *Count Each Discrete Object as 1,* you will return to the Count Cells dialog box. If you selected either of the other two methods, the Set Standard dialog box will appear.

## Set Standard (Count Cells) - Dialog Box Options

### Source Image

Selects the image containing the cells you want to use to define the standard area or integrated intensity. This does not need to be the same image as the one containing the cells you want to count. For example, you could use an image containing objects of a known size or brightness, such as microspheres or fluorescent beads of a specific diameter.

### Set Threshold

**24-bit Images:**

Opens the the Set Color Threshold dialog box, from which you can change the thresholding range for the image you are using to define your standard (which could be the same as the image containing the cells you want to count).

**8- or 16-bit Images:**

Places a red overlay over the image. You can adjust the thresholding using the Image Windows threshold slider.

### Clear Counts

Clears the *Total Area* or *Total Intensity* settings and the *Count* setting.

### Total Area

Indicates the summed area, in pixels, of all objects that have been selected. This option is seen when you select the Standard Area-based measurement method.

### Total Intensity

Indicates the summed intensity values of all pixels in the objects that have been selected. This option is seen when you select the integrated intensity-based measurement method.

### Count

Indicates the number of objects that have been selected for defining the standard value.

### Standard Area

Indicates the size of the Standard Area, based on the *Total Area* and the *Count.* This option is seen when you select the Standard Area-based measurement method.

### Unit Intensity

Indicates the unit intensity value, based on the *Total Intensity* and the *Count.* This option is seen when you select the integrated intensity-based measurement method.

### < Back

Cancels the standard selection process and returns you to the Set Counting Method dialog box.

### Next >

Accepts the standard selection and returns you to the Count Cells dialog box.

### Cancel

Cancels the standard selection process and returns you to the Count Cells dialog box.

# Count 2 Types of Cells (Apps Menu)

**Counts two types of cells automatically in separate 8-bit or 16-bit images or in a single 24-bit image.**

**Drop-in: CELLCNT**

Use this command to count cells that have been labeled with two different fluorescent dyes. In particular, this command has been optimized to distinguish between live and dead cells based on their appearance

after staining with a Live/Dead or bacterial staining kit. Measurement of the two types of cells will then proceed based on

(1) Threshold-based detection of discrete cell boundaries,

(2) Use of an interactively selected Standard Area, or

(3) Use of an interactively selected integrated pixel intensity value.

If you need to perform complicated morphometric measurements, it may be better to do so by using standard morphometric methods, such as the Integrated Morphometry Analysis command (Measure menu). To perform simple single-cell counting, you should use the Count Cells command, also located in the Measure menu.

**Note:** Because of its reliance on threshold ranges, Count 2 Types of Cells is not appropriate for use with binary images.

Boundary-based detection uses the fact that objects in an image that are of importance tend to have pixel intensities that are significantly different from background. When you define a threshold range for one of your cell types, the Count 2 Types of Cells command can measure the number of those cells in your image automatically, based on the boundaries of what MetaMorph perceives as discrete, single objects.

Counting objects is often complicated by the fact that objects overlap or clump together when thresholded, resulting in counts that are lower than the actual number of objects present. Using the second cell-counting method allows you to define a Standard Area. This is a value that will be used to represent the size of a typical cell, based on the assumption that the cells you want to count are of fairly uniform size (area). You can select a Standard Area interactively with the Count 2 Types of Cells command by clicking objects in the image window. MetaMorph will calculate an average size for all of the cells you click. Objects will then be counted by dividing the total thresholded area of each clump by the Standard Area. Clumps that are less than half the Standard Area will be omitted from the final cell count.

The third method is in some ways similar to the second in that a standard is selected interactively by clicking objects in the image. This method, however, is based on the integrated pixel intensity, that is, the arithmetic sum of the intensity values of all pixels in the thresholded area. This value will be used as a standard for the subsequent measurement and counting. This may be useful under conditions in which moderately fluorescent cells are clumped together, resulting in a region that is smaller than in size than the number of cells present, but the fluorescence of the clumped cells is noticeably additive. As with the use of a Standard Area, thresholded objects that have an integrated intensity less than half the standard value will be omitted from the final cell count.

## Counting 2 Types of Cells

### Overview of Two Cell Type Counting

### Changing the Threshold Range:

#### 8-Bit or 16-Bit Images

#### 24-Bit Images

### Configuring Data Logging

## Changing the Threshold Range

### 8-Bit or 16-Bit Images

### 24-Bit Images

## Overview of Two Cell Type Counting

To perform counting of two types of cell, use the following general procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, choose Count 2 Types of Cells. The Count 2 Types of Cells dialog box opens. |
| **2** | If you are using a single, 24-bit source image, select the image containing the cells you want to count with the *Source Image* selector, if necessary.<br><br>**OR**<br>If you are using separate 8-bit or 16-bit source images, select the appropriate images using the respective image selectors in the *LIVE Cells* and *DEAD Cells* option groups. (You will be able to change these default names in Step 6, if desired.) If you only see a single image selector, the command is still in a single-image configuration. The appropriate image selectors will appear when you finish the configuration process (Steps 3 - 10). |
| **3** | If you want to view an image that highlights the counted cells and displays the number of cells in each cluster next to it, select the *Display Result Image* check box. This may be useful for comparison with the original source image to determine how good your configuration and thresholding was. |
| **4** | Configure your measurement by choosing the *Configure* button. The Configure page of the Count 2 Types wizard will appear. This page will display a message informing you of the purpose of the Count 2 Types of Cells command. Choose *Next >.* The Set Kit page of the wizard will appear. |
| **5** | From the *Kit* drop-down list, select the staining kit that was used for staining the cells in your image. Then choose *Next >.* The Set Names page of the wizard will appear. |
| **6** | Depending on the kit you selected in Step 5, default names will be assigned to the two cells types. If you want to change these names, type the desired names in the *Type 1 Name* and *Type 2 Name* text boxes. Then choose *Next >.* The Set Sources page of the wizard will appear. |
| **7** | If you are using a single, 24-bit source image, select *One 24 Bit Image for Both Types of Cells* from the *Source Images* option button group.<br><br>**OR**<br>If you are using separate 8-bit or 16-bit source images, select *Separate Images for Each Type.*<br><br>When you have finished, choose *Next >.* |
| **8** | If you selected *[Other]* when you picked a |

staining kit in Step 5, the Staining page of the wizard will appear. This page is used for informing MetaMorph whether one of the two stains used is binding non-specifically to all cells or if two independent stains are binding to mutually exclusive sets of cells.

From the *Staining* option button group, select *Type1/Type2, All Cells/Type1,* or *All Cells/Type2.* Then choose *Next >.*

**9** The Set Counting Method page will appear. Select a cell-counting method from the Counting Method group:

***Count Each Discrete Object as 1*** configures cell-counting based on the boundaries of thresholded objects,

***Use Standard Area to Estimate Objects in a Cluster*** configures cell-counting based on a Standard Area, and

***Use Integrated Intensity to Estimate Objects in a Cluster*** configures cell-counting based on a standard integrated intensity value.

**10** Your configuration procedure will proceed depending on the counting method you selected in Step 9. If you selected

*Count Each Discrete Object as 1,* you will return to the Count 2 Types of Cells dialog box. Proceed to Step 11.

*Use Standard Area to Estimate Objects in a Cluster,* the Set Standard page of the wizard for Standard Areas will appear. You will next need to **configure use of a Standard Area** for each type of cell.

*Use Integrated Intensity to Estimate Objects in a Cluster,* the Set Standard page of the wizard for Standard Areas will appear. You will next need to **configure use of a standard integrated intensity** for each type of cell.

**11** When you are ready to carry out the measurement, choose *Measure.* The number of cells that are detected will appear in the *Count* status line of the Count 2 Types of Cells dialog box.

**12** If you are using separate 8-bit or 16-bit source images, select *Use Image Threshold* for each image to use the image's threshold to determine the cell count. You can then adjust the thresholding using the image window toolbar or the Threshold Image command and select *Use Image Threshold* again to recount the cells, if necessary.

OR

If you are using a single, 24-bit source image, select Set Threshold to open the Set

| | |
|---|---|
| | Color Threshold dialog box and set the threshold for the image. |
| **13** | If you want to save the measurement data, you can **open a data log.** The *Open Log* button will become a *Log Data* button. When you are ready to log the count data, choose *Log Data.* |
| **14** | When you have finished counting cells, choose *Close.* |

## Configuring Cell-Count Measurement Based on Standard Area

To configure cell-counting based on a Standard Area, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Set Standard page of the Count 2 Types of Cells wizard, use the *Source Image* selector to select the image containing objects that you want to use to define the standard. This does not need to be the same image as the one containing the cells you want to count. For example, you could use an image containing objects of a known size, such as microspheres. |
| **2** | If you need to adjust the thresholding for the image you are using to select a standard, choose *Set Threshold* and **select an appropriate threshold range** from the Set Threshold dialog box which appears. You may find that adjusting the threshold is easier if you select the *Use Transparent Thresholds* check box from the Windows tab page of the Preferences dialog box (Edit menu). After you finish selecting the threshold range and have closed the Set Threshold dialog box, you will return to the Set Standard dialog box. |
| **3** | In the image window, click several objects that are of a "typical" size. As you select the objects, a yellow overlay will appear over them in the image window, and the *Total Area, Count,* and *Standard Area* spin boxes will update, displaying the total area selected, the number of selected cells, and the average computed area, respectively. You can modify these values directly, if desired. |
| **4** | When you have finished, choose *Next >.* The Set Standard dialog box will close, and the Count 2 Types of Cells dialog box will reappear. |

## Configuring Cell-Count Measurement Based on Integrated Intensity

To configure cell-counting based on a standard integrated intensity value, use the following procedure:

| Step | Action |
|---|---|
| 1 | From the Set Standard page of the Count 2 Types of Cells wizard use the *Source Image* selector to select the image containing objects that you want to use to define the integrated intensity standard. This does not need to be the same image as the one containing the cells you want to count. For example, you could use an image containing objects of a known brightness, such as fluorescent beads of a specific diameter. |
| 2 | If you need to adjust the thresholding for the image you are using to select a standard, choose *Set Threshold* and **select an appropriate threshold range** from the Set Threshold dialog box which appears. You may find that adjusting the threshold is easier if you select the *Use Transparent Thresholds* check box from the Windows tab page of the Preferences dialog box (Edit menu). After you finish selecting the threshold range and have closed the Set Threshold dialog box, you will return to the Set Standard dialog box. |
| 3 | In the image window, click several objects that are of a "typical" brightness and size. As you select the objects, a yellow overlay will appear over them in the image window, and the *Total Intensity, Count,* and *Standard Intensity* spin boxes will update, displaying the total integrated intensity, the number of selected cells, and the average integrated intensity, respectively. You can modify these values directly, if desired. Clicking a selected object will undo the selection. |
| 4 | When you have finished, choose *Next >.* The Set Standard dialog box will close, and the Count 2 Types of Cells dialog box will reappear. |

## Changing the Threshold Range for 8-Bit or 16-Bit Images (Count 2 Types of Cells)

To adjust a threshold range for 8-bit or 16-bit source images, use the following procedure. If your image has never been thresholded, a red overlay will initially be displayed over the entire image.

| Step | Action |
|---|---|
| 1 | In the Count 2 Types of Cells dialog box, choose the *Use Image's Threshold* button for the first cell type. |
| 2 | Adjust the image's threshold using the image window toolbar or the Threshold Image command in the Measure menu. |
| 3 | Repeat Steps 1 - 2 for the second cell type. |
| 4 | If necessary, click the *Back* button at the top |

of this Help window to return to the procedure you were just reading.

## Changing the Threshold Range for 24-Bit Images (Count Cells)

To adjust a threshold range for a single 24-bit color source image, use the following procedure:

| Step | Action |
|---|---|
| **1** | In the Count 2 Types of Cells dialog box, choose the *Set Threshold* button. The Set Color Threshold dialog box opens. |
| **2** | If you want to use a previously saved set of color threshold settings, choose Load Range. Otherwise, skip to Step 4. |
| **3** | The Load Color Threshold Range dialog box opens.<br><br>AND<br><br>Select the icon for the desired color threshold range (*.ctr) state file. If necessary, use the Look In list or Up One Level icon button to locate the correct drive and folder. Then choose Open. The threshold settings will be applied to your image. Now skip to Step 10. |
| **4** | From the Color Model list, select the color model you want to use for setting the color threshold: RGB, HSI, or HSL. Your selection will determine the options you see in the lower half of the dialog box. |
| **5** | If you selected HSI or HSL as your color model in Step 4, use the Hue Range radio button group to select whether the ranges between the upper and lower limits are to be included in the threshold range (Inclusive) or if the ranges outside of the upper and lower limits are to be thresholded (Exclusive). |
| **6** | Use the sliders or the left and right spin boxes for each of the color channels (Red-Green-Blue, Hue-Saturation-Intensity, or Hue-Saturation-Luminosity) to select the lower and upper threshold range values. As you adjust the settings, the distribution of the red thresholding overlay that covers pixels with the selected values will change. |
| **7** | If you want to use the interactive "point-and-click" method of selecting the threshold range, choose Set by Example. The dialog box will expand, revealing two more options. If necessary, reset the threshold range by selecting the Reset Color Threshold Range on Next Click check box, so that a check mark appears in it.<br><br>AND<br><br>Use the pointer to select pixels in the image that have the values that you want to include |

| | |
|---|---|
| | in the threshold range. As you click the pixels, they will be covered by the red thresholding overlay, and the color channel sliders and spin boxes will update to display the new values.If you want to remove the values of the pixels you selected last, choose Undo Last Click. |
| **8** | By default, the thresholding State is in Inclusive mode, which is to say that the range that you have selected is included in the threshold range and will be highlighted by a red thresholding overlay. If you want to reverse the selection so that the pixels in the range you have selected are excluded from thresholding and all other pixels are included instead, select Exclusive from the State option button group. |
| **9** | If you want to save the threshold settings, choose Save Range. The Save Color Threshold Range dialog box will appear. Type a name for the color threshold range (*.ctr) state file in the File Name text box. If necessary, use the Save In list or Up One Level icon button to locate the correct drive and folder. Then choose Save. |
| **10** | When you have finished, choose Close. |
| **11** | If necessary, click the *Back* button at the top of this Help window to return to the procedure you were just reading. |

## Configuring Data Logging for Cell-Count Measurement

To configure logging of your single-cell count data, use the following procedure. You can follow this procedure either before or after you perform the actual measurement.

| Step | Action |
|---|---|
| **1** | From the Count 2 Types of Cells dialog box, choose *Open Log.* The Open Data Log dialog box will appear. |
| **2** | Select *Dynamic Data Exchange (DDE)* to log directly to an open spreadsheet program. Select *A Text File* to log the data to a text file.<br><br>**Note:** You can select both options. |
| **3** | If you selected *A Text File* in the previous step, the Open Data Log File dialog box will appear.<br><br>Select an existing log file or type a new file name in the *File Name* text box. (If necessary, use the *Look In* list or Up One Level button to change the current drive and folder to the correct location.)<br><br>**AND**<br><br>Choose *Open.* |

**4** If you selected an existing log's file name in Step 3, the Log File Exists dialog box will appear. You can *Overwrite* the contents of the file, *Append* new data, or *Cancel.*

**5** If you selected *Dynamic Data Exchange (DDE)* in Step 2, the Export Log Data dialog box will appear.

Select the desired application from the *Application* list. Choose *Default* to use the default settings for the selected application.

Choose *OK* to launch the external application. After the application opens, click the MetaMorph Imaging System button in the Windows Taskbar to return to MetaMorph.

**6** If necessary, choose *Configure Log* from the Count Cells dialog box to select which data to log. The Configure Log dialog box will appear.

**AND**
From the *Configuration* table, select the data types that you want to log. Then choose *OK.*

**7** The *Open Log* button will have changed to a *Log Data* button. To log your cell count data, choose this button after you have performed your measurement.

**8** If necessary, click the *Back* button at the top of this Help window to return to the procedure you were just reading.

## Count 2 Types of Cells - Dialog Box Options

## Count 2 Types of Cells - Dialog Box Options

### Configure

Opens the Set Counting Method dialog box, from which you can select the counting method (boundary-based, Standard Area-based, or integrated intensity-based).

### Source

Selects the single 24-bit source image that contains the cells you want to count. If you have configured the cell count to use two separate 8-bit or 16-bit images, two unlabeled image selectors will appear in the Count 2 Types of Cells dialog box, one in each cell type's option group (image selector, *Use Image's Threshold* button, and *Count* status line).

### Use Image's Threshold

Uses the image's current threshold settings when counting cells. You can adjust thresholding for the image using the Threshold button on the image's tool bar or by using the Threshold Image command in the Measure menu.

### Count

These status lines indicate the numbers of each type of cell that have been counted.

### Set Threshold

Opens the Set Color Threshold dialog box. Use this command to change the threshold on a 24-bit image.

### Open Log

Opens a text-based data log file or an OLE connection to an open spreadsheet program for logging count data and threshold values if desired. Changes to *"Log Data"* when a log file is open.

### Log Data

Sends your cell-count measurement data to the open data log or spreadsheet.

### Configure Log

Opens the Configure Log dialog box, from which you can select the types of data to be stored in the data log.

### Measure

Carries out the measurement, based on the configuration of the counting method and the current threshold state of the image.

### Close

Closes the Count 2 Types of Cells dialog box.

## Set Kit (Count 2 Types of Cells) - Dialog Box Options

### Kit

Selects the staining kit that was used to stain the cells in the source image. This will affect the way Count 2 Types of Cells expects cells to be stained.

### < Back

Cancels the kit selection process and returns you to the Configure message page of the wizard.

### Next >

Accepts the kit selection and forwards you to the Set Names page of the wizard.

### Cancel

Cancels the kit selection process and returns you to the Count 2 Types of Cells dialog box.

## Set Names (Count 2 Types of Cells) - Dialog Box Options

### Type 1 Name

Specifies a name for the type 1 cells. This name will be used for both the counting result image and for the pertinent labels in the Count 2 Types of Cells dialog box and wizard pages, as well as data logging.

### Type 2 Name

Specifies a name for the type 2 cells. This name will be used for both the counting result image and for the pertinent labels in the Count 2 Types of Cells dialog box and wizard pages, as well as data logging.

### < Back

Returns you to the Set Kit page of the wizard.

### Next >

Accepts the source image format selection and forwards you to the Set Sources page of the wizard.

### Cancel

Cancels the source image format selection process and returns you to the Count 2 Types of Cells dialog box.

## Set Sources (Count 2 Types of Cells) - Dialog Box Options

### Source Images

Selects the source image format. Select

***One 24 Bit Image for Both Types of Cells*** if you are using a single 24-bit source image, or

***Separate Images for Each Type*** if you are using separate 8-bit or 16-bit source images.

**< Back**

Returns you to the Set Names page of the wizard.

**Next >**

Accepts the source image format selection. If you select *[Other]* as the staining kit, you will be forwarded to the Staining page of the wizard. If you selected one of the staining kits, you will be forwarded to the Set Counting Method page of the wizard.

**Cancel**

Cancels the source image format selection process and returns you to the Count 2 Types of Cells dialog box.

## Staining (Count 2 Types of Cells) - Dialog Box Options

> **Note:** This page of the configuration wizard will appear only if you have selected *"[Other]"* as the staining kit.

**Staining**

This option informs MetaMorph whether one of the two stains is binding non-specifically to all cells or if two independent stains are binding to mutually exclusive sets of cells. Select *Type1/Type2, All Cells/Type1,* or *All Cells/Type2.*

**< Back**

Returns you to the Set Sources message page of the wizard.

**Next >**

Accepts the staining format selection and forwards you to the Set Counting Method page of the wizard.

**Cancel**

Cancels the staining format selection process and returns you to the Count 2 Types of Cells dialog box.

## Set Counting Method (Count 2 Types of Cells) - Dialog Box Options

**Counting Method**

Selects a cell-counting method:

***Count Each Discrete Object as 1 -*** Bases the cell-count measurement on what MetaMorph perceives to be the object boundaries, as defined by thresholding. When you select this option, choosing *Next* > will return you to the Count 2 Types of Cells dialog box.

***Use Standard Area to Estimate Objects in a Cluster*** - Bases the cell-count measurement on a selected Standard Area. When you select this option, choosing *Next* > will open the Set Standard page of the configuration wizard, from which you can select the Standard Area.

***Use Integrated Intensity to estimate Objects in a Cluster*** - Bases the cell-count measurement on a standard integrated intensity value. When you select this option, choosing *Next* > will open the Set Standard page of the configuration wizard, from which you can select the standard integrated intensity.

**Cancel**

Cancels your selection of cell-counting method and closes the Set Counting Method dialog box, returning you to the Count 2 Types of Cells dialog box.

**Next >**

Accepts your selection of cell-counting method and closes the Set Counting Method dialog box. If you

selected *Count Each Discrete Object as 1,* you will return to the Count 2 Types of Cells dialog box. If you selected either of the other two methods, the Set Standard page of the configuration wizard will appear.

## Set Standard (Count 2 Types of Cells) - Dialog Box Options

### Source Image

Selects the image containing the cells you want to use to define the standard area or integrated intensity. This does not need to be the same image as the one containing the cells you want to count. For example, you could use an image containing objects of a known size or brightness, such as microspheres or fluorescent beads of a specific diameter.

### Set Threshold

Opens the Set Threshold dialog box, from which you can change the thresholding range for the image you are using to define your standard (which could be the same as the image containing the cells you want to count).

### Clear Counts

Clears the *Total Area* or *Total Intensity* settings and the *Count* setting.

### Total Area

Indicates the summed area, in pixels, of all objects that have been selected. This option is seen when you select the Standard Area-based measurement method.

### Total Intensity

Indicates the summed intensity values of all pixels in the objects that have been selected. This option is seen when you select the integrated intensity-based measurement method.

### Count

Indicates the number of objects that have been selected for defining the standard value.

### Standard Area

Indicates the size of the Standard Area, based on the *Total Area* and the *Count.* This option is seen when you select the Standard Area-based measurement method.

### Integrated Intensity

Indicates the average integrated intensity value, based on the *Total Intensity* and the *Count.* This option is seen when you select the integrated intensity-based measurement method.

### < Back

Cancels the standard selection process and returns you to the Set Counting Method dialog box.

### Next >

When determining the standard for the first type of cell, this button leaves up the Set Standard dialog box so that you can proceed to select the standard for the second type of cell. When you have selected the second standard, *Next >* accepts the standard selection and returns you to the Count 2 Types of Cells dialog box.

### Cancel

Cancels the standard selection process and returns you to the Count 2 Types of Cells dialog box.

# Measure Colocalization (Apps Menu)

**Provides quantitative data regarding regions of overlap of two fluorescent probes in an image. The area, average intensity, and integrated intensity in the region of overlap can be measured and saved to a log file.**

*Availability: Available for MetaVue and MetaMorph Basic; included in MetaMorph Premier*

**Drop-in: COLOCAL**

Use this command to display and save measurements of the area of overlap between two fluorescent probes, or the average or integrated intensities in the region of overlap. The intensity of pixels in 24-bit color images is calculated as the mean of the red, green, and blue intensities. Typically, two source images are used. The same view of the preparation must be used for both images, but they should be acquired at different excitation or transmission wavelengths, as appropriate for the respective probes being used. If desired, you can use a Region Tool to define and select a specific region of interest for measurement. This should be defined in the *Source A* image. Both source images must be thresholded prior to performing the measurement.

**Note:** Areas of overlap will be expressed in pixels. To express the area in "real" units (square microns, millimeters, etc.), use Calibrate Distances before using this command.

## Measuring Colocalization

To measure the region of overlap between two fluorescent probes, use the following procedure. The two source images must first be thresholded.

| Step | Action |
|---|---|
| **1** | From the Apps menu, choose Measure Colocalization. The Measure Colocalization dialog box opens. |
| **2** | If desired, use a Region Tool to define a region of interest for the measurement. This must be defined in the source image that will be selected with the *Source A* image selector.<br><br>**Note:** Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaMorph. |
| **3** | Use the *Source A* and *Source B* image selectors to select the two views of the preparation. These will correspond to the wavelength image for each of the two respective fluorescent probes. |
| **4** | In the *Method* group, select the parameter you want to measure. Select<br><br>*Area* if you want to measure the area of overlap,<br><br>*Average* if you want to measure the average pixel intensity, or<br><br>*Integrated* if you want to measure the integrated pixel intensity. |
| **5** | If you want to log the measurement data, open a **data log file.** Then, if desired, choose *Configure Log* from the Measure Colocalization dialog box and **configure** your data logging. Otherwise continue to Step 6. |
| **6** | Choose *Measure.* Once measured, the data will be displayed in the dialog box. If you have opened a data log, the data will also be logged automatically. |
| **7** | Choose *Close* to close the dialog box. |

## Measure Colocalization - Dialog Box Options

### Source A

Selects the image for the first probe. If you want to define regions of interest for the measurement, they must be drawn on this image. This image must be thresholded prior to measurement.

### Source B

Selects the image for the second probe. This image must be of the same sample as in the *Source A* image, differing only in the wavelength used for imaging the second dye. Any regions of interest drawn on this image will be ignored. As with the *Source A* image, the image for *Source B* must be thresholded prior to measurement.

### Value for All A

Displays the value of the parameter selected in the *Method* group *(Area, Average* pixel intensity, or *Integrated* pixel intensity) for the entire thresholded region in the *Source A* image. The intensity of pixels in 24-bit color images is calculated as the mean of the red, green, and blue intensities.

### A Overlapping B

Displays the value of the selected parameter for that part of the thresholded *Source A* image that is also thresholded in the *Source B* image. This may not necessarily be the same as the *B Overlapping A* measurement for average or integrated pixel intensity because the *B Overlapping A* measurement is derived from the *Source B* image, not the *Source A* image, and may therefore have different intensity values in the regions of interest.

### A Not Overlapping B

Displays the value of the selected parameter for that part of the thresholded *Source A* image that is outside of the thresholded region in the *Source B* image.

### Value for All B

Displays the value of the parameter selected in the *Method* group *(Area, Average* pixel intensity, or *Integrated* pixel intensity) for the entire thresholded region in the *Source B* image.

### B Overlapping A

Displays the value of the selected parameter for that part of the thresholded *Source B* image that is also thresholded in the *Source A* image. This may not necessarily be the same as the *A Overlapping B* measurement for average or integrated pixel intensity because the *A Overlapping B* measurement is derived from the *Source A* image, not the *Source B* image, and may therefore have different intensity values in the regions of interest.

### B Not Overlapping A

Displays the value of the selected parameter for that part of the thresholded *Source B* image that is outside of the thresholded region in the *Source A* image.

### Method

Selects the parameters to be measured. The intensity of pixels in 24-bit color images is calculated as the mean of the red, green, and blue intensities.

> ***Area*** measures the area of overlap between the two probes. This will be expressed in pixels unless you apply the Calibrate Distances command (Measure menu) prior to using the Measure Colocalization command.
>
> ***Average*** measures the average pixel intensity in the regions of overlap.
>
> ***Integrated*** measures the integrated pixel intensity in the regions of overlap (equivalent to the sum of all grayscale values for every pixel in the region).

### Configure Log

Displays the Configure Log dialog box, which allows you to select the image parameters to be saved in an open data log. You must use the Open Data Log command (Log menu) to log Measure Colocalization data.

### Measure

This command performs and displays the measurement of the colocalization data that you specified in *Method.* The data will be displayed in the Measure Colocalization dialog box. If a data log is open, the data will be logged automatically when you choose the *Measure* command.

### Close

Closes the dialog box.

# Morphology Filters

**Provides a set of tools that can be used to transform binary and grayscale images through morphology filtering and analysis.**

**Drop-in: MORPHOLOGY**

Use this drop-in to smooth noisy images or to simplify overly complex images. Both of these processes can make the data that you need to extract from your images more accessible.

Morphology analysis also provides the ability for you to extract specific features from images.

Morphology operations rely on processes based on shape.The filters are interrelated, with most constructed as combinations of the simplest pieces: Dilate and Erode. Dilate acts by growing bright pixels, while erode does the opposite by growing dark pixels, functioning as the morphological complement of dilate.

Several of the image filters use dilate or erode as their foundation, but add more generalized functionalities to achieve more powerful filtering abilities.

**Application Note PDF** — *Using the MetaMorph Morphology Filters*

**Note:** Morphology Filters can be used individually. However, complex tasks, such as segmentation, are often best performed through a combination of several steps. For example, you can combine filters to remove noise, estimate and remove background, and extract features. You can benefit most by creating journals containing several Morphology filters.

**Note:** Morphology Filters can be used on either an entire image, or an active region of interest.

**Definition:**

**Neighborhood** – A region surrounding each pixel in the source image defined by the filter shape and diameter, width, or area, and centered on the pixel on which the filter is acting.

## Applying Morphology Filters

To process an image using one or more morphology filters, complete the following procedure.

| Step | Action |
|---|---|
| **1** | From the Process Menu, click Morphology Filters. The Morphology Filters dialog box opens. |
| **2** | Open one or more images that you want to process. |
| **3** | If more that a single image is open, click |

*Source Image* to select the image that you want to process.

**4** Using the *Result Image* selector, choose whether you want to Overwrite, Add to, or create a New destination image. The destination image name by default is the name of the filter used to process the image.

**5** Click the image name button, then click specified to open the Specify Image Name dialog box. Type an image name, then click OK.

**6** Using the Morphology Filters Use Comparison Chart, determine the filter or filters that are best suited for the image(s) that you need to process.

**7** Click the filter that you want to use.

**8** If there are parameter settings associated with the filter, set these parameters according to the guidelines in the ***Morphology Image Filters Use Comparison Chart*** and in the Dialog Box Options. Where applicable, choose the most appropriate filter shape. The circle is well suited for most biological samples.

**9** Set the most appropriate filter size. Refer to the guidelines in the ***Morphology Image Filters Use Comparison Chart***.

**10** If applicable to your selected filter and your image(s), choose *Use sequential filtering* and/or *Use reconstruction.*

**11** Click Apply to process your image(s) with the Image Filter and settings that you have selected.

**12** Click Undo to undo the last filter that was applied to the image.

**13** Click Close to close the Morphology Filters dialog box.

## Morphology Filters - Dialog Box Options

### Source Image

Opens the Image Selector for the source image. Morphology Filters can process images of any grayscale bit depth, including binary, but not color images. When processing image stacks, each image in the stack is processed before proceeding to the next image, until all images in the stack have been processed.

### Result Image

Opens the Image Selector for the destination image.

### Operation

Defines the action of the parameters and the filter.

### Image Filters

Lists the available morphology filter types for smoothing and simplifying images. Click to select the filter that

you want to use.

### Dilate

Performs morphological dilation (neighborhood max). This is the complementary operation of erode. This filter grows bright blobs and shrinks dark blobs. ***Applicable Parameters:*** *Filter shape and size.*

### Erode

Performs morphological erosion (neighborhood min). Complementary operation of dilate. This filter grows dark blobs and shrinks light blobs. ***Applicable Parameters:*** *Filter shape and size.*

### Close

Performs morphological close. Filters out dark blobs by first applying dilate then erode. Complementary operation of open. ***Applicable Parameters:*** *Filter shape and size, reconstruction on/off if circle or square filter shape.*

### Open

Performs morphological open. Filters out bright blobs by first applying erode then dilate. Complementary operation of close. ***Applicable Parameters:*** *Filter shape and size, reconstruction on/off if circle or square filter shape.*

### Close-open

Performs morphological close-open. Filters out dark then light blobs by first applying close then open. Complementary operation of open-close. ***Applicable Parameters:*** *Filter shape and size, sequential filtering on/off, reconstruction on/off if circle or square filter shape.*

### Open-close

Performs morphological close-open. Filters out light then dark blobs by first applying open then close. Complementary operation of close-open. ***Applicable Parameters:*** *Filter shape and size, sequential filtering on/off, reconstruction on/off if circle or square structuring element.*

### Center filter

Performs morphological center filter, a pointwise median of 3 images: the original, its close-open-close, and its open-close-open. This filter combination simultaneously smoothes both dark and bright blobs. ***Applicable Parameters:*** *Filter shape and size, sequential filtering on/off, reconstruction on/off if circle or square structuring element.*

### Lomo filter

Performs morphological lomo filter, a pointwise mean of 2 images: the close and the open of the original. This filter automatically repeats itself until smoothness is achieved. This filter combination simultaneously smoothes both dark and bright blobs. ***Applicable Parameters:*** *Filter shape and size, sequential filtering on/off, reconstruction on/off if circle or square filter shape.*

### Reconstruct from below

Performs a grayscale reconstruction of original from the Marker image. The Marker image expands intensities from below the original intensity signal, retaining only the bright blobs into which they grow, filling the remaining area with darkness. Complementary operation of reconstruct from marker above. ***Applicable Parameters:*** *Marker Image.*

### Reconstruct from above

Performs a grayscale reconstruction of original from Marker image. The Marker image expands from above the original intensity signal, retaining only the dark blobs into which they grow, filling the remaining area with brightness.. Complementary operation of reconstruct from marker below. ***Applicable Parameters:*** *Marker Image.*

## Extract Features

Lists the available image analysis tools for enhancing and extracting visually important features. Click to select the analysis tool that you want to use. For guidelines on using these features, refer to the ***Morphology Extract Features Use Comparison Chart***.

### Gradient

Performs morphological gradient for edge detection: dilate minus erode. ***Applicable Parameters:*** *Filter shape (not area) and size.*

### Top hat

Detects bright spots that are removed by open filtering by subtracting the open of original from the original. Complementary operation of bottom hat. ***Applicable Parameters:*** *Filter shape and size, reconstruction on/off if circle or square filter shape*

### Bottom hat

Detects dark spots that are removed by close filtering by subtracting the original from the close of original. Complementary operation of top hat. ***Applicable Parameters:*** *Filter shape and size, reconstruction on/off if circle or square filter shape.*

### Regional max

Detects local maxima points and plateaus and returns them white on black background. ***Applicable Parameters:*** *none.*

### Regional min

Detects local minima points and plateaus and returns them white on black background. ***Applicable Parameters:*** *none.*

### H-dome

Detects bright spots using a gray level threshold relative to each pixels local surroundings. The absolute difference between the original and its reconstruction by the marker image created by subtracting a constant intensity from the original. ***Applicable Parameters:*** *threshold.H is for Height threshold*.

### H-basin

Detects dark spots using a gray level threshold relative to each pixels local surroundings, but returns them as bright on zero background. The absolute difference between the original and its reconstruction by the marker image created by adding a constant intensity to the original. ***Applicable Parameters:*** *threshold. H is for Height threshold*.

### Watershed lines

Performs a watershed transform and returns the thin watershed boundaries as white and the region interiors as black. Useful for segmentation problems. The grayscale input should represent the boundaries between objects to be segmented, so edge detectors (including morphological gradient on this same dialog) are commonly used for this purpose. **Note:** Only very smooth or simple input images give a small number of basins without the use of a marker image. With a marker image, each non-black marker blob becomes a region in the final result. ***Applicable Parameters:*** *Marker Image if checkbox selected.*

### Watershed basins

Performs watershed transform, resulting in each pixel given an integer label (may not be unique if more region labels than can be represented at your destination image's bit-depth) **Note:** Only very smooth or simple input images give a small number of basins without the use of a marker image. With a marker image, each non-black marker blob becomes a region in the final result. . ***Applicable Parameters:*** *Marker Image if checkbox selected.*

## Parameters

Defines the available options.

**Filter Shape** – Indicates the available filter shapes. Click to select the filter shape that you want to use.

Filter Shape defines the spatial neighborhood around each pixel within which calculations are performed to locally smooth and simplify the image. Each filter has an associated settable parameter that defines its relative size. For example, for the Circle filter, you can set the diameter of the circle in pixels.

***Circle*** – Applies a filter in the shape of a circle. The dimension of the circle is defined by the Diameter parameter.

***Square*** – Applies a filter in the shape of a square. The dimension of the square is defined by the Width parameter.

***Diamond*** – Applies a filter in the shape of a diamond. The dimension of the diamond is defined by the Width parameter.

***Area*** – Specifies a threshold value in adjacent pixels (squared) that make up an area of contiguous pixels, commonly referred to as a blob. Depending on the filter using this value, blobs of fewer pixels than specified are removed from the image.

***Diameter*** – Specifies the diameter of the circle filter shape.

***Width*** – Specifies the width of a square or diamond filter shape.

**Use sequential filtering** – Applies a sequence of progressively larger filter shapes, until the user-specified size is reached. Produces visually better smoothing results than single application of the user-specified filter shape, however, more processing time is required. This option can be used with close-open, open-close, center, and lomo filters only.

**Use reconstruction** – Removes blobs without distorting and rounding off the edges of those blobs that remain. After applying a fixed-shape filter (circle, diamond, or square) to remove blobs, retains or "reconstructs" the original shape of remaining blobs. This option can be used with open, close, top hat, bottom hat, close-open, open-close, center, and lomo filters only. This option is inactive if the "area" filter shape, which already includes reconstruction.

**Threshold –** Edit box for graylevel height offset threshold. Hidden except for use with H-dome/H-basin.

## Morphology Image Filters Use Comparison Chart

| Filter Name | Best for… | Use for… | Best Filter Shape | Filter Size | Cautions |
|---|---|---|---|---|---|
| ***Dilate*** | *Growing* bright objects<br>*Shrinking* dark objects | *Enhancing* bright features or shrinking dark features<br>*Joining* nearby bright blobs or separating dark blobs<br>*Expanding* a binary mask to include a larger masked region | Usually you should use circles since biological applications tend not to have right angles or any particular orientation.<br>Diamond or square of width 3 gives some precise control over very small neighborhoods | Spatial extent to which bright pixels expand. Similar to selecting a paintbrush size.<br>Repeated application of a small structuring element is done more easily with a single large one. | Dilate grows bright pixe of noise as well as the bright objects your interested in, so use other filters (median, lo pass, other morpholog filters such as close-open, etc.) to remove noise and small unwanted bright spots prior to dilating.<br>Dilate moves the spati location of object edge so if edge localization i important, use one of t edge-preserving morphological filters (open, close, lomo, center…). |
| ***Erode*** | *Growing* dark objects<br>*Shrinking* bright objects | *Enhancing* dark features or shrinking bright features<br>*Joining* nearby dark blobs or separating bright blobs<br>*Shrinking* a binary mask to include a smaller masked region | Usually you should use circles since biological applications tend not to have right angles or any particular orientation.<br>Diamond or square of width 3 gives some precise control over very small | Spatial extent to which dark pixels expand. Similar to selecting a paintbrush size.<br>Repeated application of a small structuring element is done more easily with | Erode grows dark pixe of noise as well as the dark objects your interested in, so use other filters (median, lo pass, other morpholog filters such as close-open, etc.) to remove noise and small unwanted dark spots |

| | | | | | |
|---|---|---|---|---|---|
| | | | neighborhoods | a single large one. | prior to eroding.<br>Erode moves the spati location of object edge so if edge localization i important, use one of t edge-preserving morphological filters (open, close, lomo, center…). |
| ***Open*** | Functions like an enhanced erode<br>Bettter edge localization than erode.<br>Does not expand noise as much as erode. | *Splitting* connected bright blobs (or connecting nearby dark blobs)<br>*Removing* unwanted bright blobs and noise. | Need to determine which blobs that you want to mark for deletion<br>The shape is required to "fit" entirely within a bright blob to prevent its removal. | Determine filter size according to the demension required to "fit" the filter element entirely within a bright blob to prevent its removal.<br>Use reconstruction for retaining detail of the remaining blobs. | Sensitivity to noise—even a small number o dark specs can cause loss of a bright blob.<br>You may need one of t more advanced filters (open-close, close-ope center, lomo, etc.) if yo haven't already remove the dark noise pixels. |
| ***Close*** | Functions like an enhanced dilate.<br>Better edge localization than dilate.<br>Does not expand noise as much as dilate. | *Growing* bright objects<br>*Shrinking* dark objects<br>*Moving* edges (object boundaries) and Expanding bright noise and small features. | Need to determine which blobs that you want to mark for deletion.<br>The shape is required to "fit" entirely within a dark blob to prevent its removal. | Determine filter size according to the demension required to "fit" the filter element entirely within a dark blob to prevent its removal.<br>Use reconstruction for retaining detail of the remaining dark blobs. | Sensitivity to noise—even a small number o bright specs can cause the loss of a dark blob.<br>You may need one of t more advanced filters (open-close, close-ope center, lomo, etc.) if yo haven't already remove the bright noise pixels. |
| ***Close-Open*** | Combines Close and Open | *Smoothing* dark, then light objects using the selected filter shape. | Determine which blobs, (both bright and dark) to mark for deletion. | Use sequential filtering for best visual results.<br>Use reconstruction to retain more details of remaining blobs. | Open-Close has a slightly different intensi bias than Open-Close due to the filtering orde |
| ***Open-Close*** | Combines Open and Close | *Smoothing* light, then dark objects using the selected filter shape. | Determine which blobs, (both bright and dark) to mark for deletion. | Use sequential filtering for best visual results.<br>Use reconstruction to retain more details of remaining blobs. | Open-Close has a slightly different intensi bias than Close-Open due to the filtering orde |
| ***Center Filter*** | *Smoothing* light and dark | Uses (at each pixel location) the middle | Determine which blobs, (both bright and dark) | Use sequential filtering for best | Similar to the median filter, this filter might no |

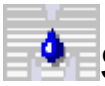
| | | | | | |
|---|---|---|---|---|---|
| | objects simultaneously. | intensity value of three choices: open-close, close-open, and the original image. | to mark for deletion. | visual results.<br>Use reconstruction to retain more details of remaining blobs. | smooth certain texture or patterns. Use the Lomo Filter for better smoothness. |
| ***Lomo Filter*** | *Smoothing* light and dark objects simultaneously. | Uses (at each pixel location) the mean intensity value between open and close filters, and reapplies itself until smoothness is reached. | Determine which blobs, (both bright and dark) to mark for deletion. | Use sequential filtering for best visual results.<br>Use reconstruction to retain more details of remaining blobs. | Slightly slower than Close-Open, Open-Close, and Center filte |
| ***Reconstruct from Above*** | *Expanding* marker image intensities from above the source image, marking blobs in one image from blobs in another. | *Reconstructing* cells fluorescently stained in one wavelength by their nuclei stained in another wavelength, removing those cells or other blobs without a nuclear stain. | Use Reconstruct from Above if you are marking dark blobs to retain, allowing unmarked ones to become bright.<br>After a filtering process, use this filter to regenerate the details of the remaining blobs/objects. | N/A | The software automatically cuts off your marker image intensities to be entirel above or below (as you selected) in case you input a marker that is i some places above an other places below the source image.<br>The actual marker ima is not altered by this intermediate calculatio |
| ***Reconstruct from Below*** | *Expanding* marker image intensities from below the source image, marking blobs in one image from blobs in another. | *Reconstructing* cells fluorescently stained in one wavelength by their nuclei stained in another wavelength, removing those cells or other blobs without a nuclear stain. | Use Reconstruct from Below if you are marking bright blobs to retain, allowing unmarked ones to become dark.<br>After a filtering process, use this filter to regenerate the details of the remaining blobs/objects. | N/A | The software automatically cuts off your marker image intensities to be entirel above or below (as you selected) in case you input a marker that is i some places above an other places below the source image.<br>The actual marker ima is not altered by this intermediate calculatio |

## Morphology Extract Features Use Comparison Chart

| Extract Feature | Best for… | Use for… | Best filter shape | Filter size | Cautions |
|---|---|---|---|---|---|
| ***Gradient*** | Showing local contrast:<br>Reports intensity of the amount of local contrast computed by dilate minus erode | Detecting edges between bright and dark regions of an image.<br>Segmentation: Creating a "topographic surface" image for use in watershed segmentation | Usually you should use circles, since biological applications tend not to have right angles or any particular orientation.<br>A diamond or square with a width of three gives some precise control over very small neighborhoods | The typical width of a bright to dark or dark to bright transition between objects (may not be a perfectly sharp transition due to focus, and so on.) | Sensitive to noise, so you might want to use another morphology fil to smooth your image before using gradient. |

| | | | | | |
|---|---|---|---|---|---|
| ***Top Hat*** | Enhancing small bright objects by computing the original image minus the result of the open filter | Detecting bright features of a known spatial size or shape. | Determine the shape of the *Open* filter that removes your objects of interest. The top hat result show what the open filter removed. | Determine the size of the Open filter that removes your objects of interest. The top hat result show what the *Open* filter removed. | Sensitive to noise, so you may want to use another morphology fil to smooth your image prior to using top hat. |
| ***Bottom Hat*** | Reporting brightness where small dark objects were detected by computing the result of the close filter minus the original image | Detecting dark features of a known spatial size or shape. | Determine the shape of the *Close* filter that removes your objects of interest. The bottom hat result shows what the close filter removed. | Determine the close filter's size that removes your objects of interest. The bottom hat result shows what the close filter removed. | Sensitive to noise, so you may want to use another morphology fil to smooth your image prior to using bottom h |
| ***Regional max*** | Reporting a bright spot for all local "peaks" in intensity (pixels of a constant intensity value completely surrounded by lower intensity pixels) | Detecting bright spots.<br>***Tip***: Use *Euclidean distance i*n the Binary dialog box to find the furthest points away from the white pixels—maybe the furthest points inside your cells as "markers" for watershed | ***n/a*** | ***n/a*** | Sensitive to slight intensity fluctuations: Returns a bright spot even for the slightest local "peak" in intensity To set a threshold amount of how high a local peak of intensity must be, use H-dome. |
| ***Regional min*** | Reporting a bright spot for all local "valleys" of intensity (pixels of a constant intensity value completely surrounded by higher intensity pixels) | Detecting dark spots. | ***n/a*** | ***n/a*** | Sensitive to slight intensity fluctuations: Returns a bright spot even for the slightest local "valley" in intensi To set a threshold amount of how deep a local valley of intensity must be, use H-basin. |
| ***H-dome*** | Reporting bright spots where significant intensity "peaks" occur. Similar to regional max, but allows control over how "significant" the peaks. | Detecting local bright spots whose intensity "peaks" are at least the threshold amount brighter than their surrounding background.<br>Finding bright spots on a non-uniform background. | None: Useful for when you don't have knowledge of spatial shape, but do know the intensity above local background of the bright spots you're looking for. | None: Useful for when you don't have knowledge of spatial size, but do know the intensity above local background of the bright spots you're looking for. | Requires intensity knowledge of how brig above background you spots are—if instead y have spatial knowledg consider top hat as we |
| ***H-basin*** | Reporting bright spots where significant intensity "valleys" occur. | Detecting local dark spots whose intensity "valleys" are at least the threshold amount | None: Useful for when you don't have knowledge of spatial shape, but do know the intensity below | None: Useful for when you don't have knowledge of spatial size, but do know the | Requires intensity knowledge of how dark below background you spots are—if instead y have spatial knowledg |

| | | | | | |
|---|---|---|---|---|---|
| | Similar to regional min, but allows control over how "significant" the valleys. | darker than their surrounding background.<br>Finding dark spots on a non-uniform bright background. | local background of the dark spots you're looking for. | intensity below local background of the dark spots you're looking for. | consider bottom hat as well. |
| ***Watershed Lines*** | Partitioning an "intensity surface" image where the brighter intensity "ridges" (watershed lines) separate the watershed valleys or basins. | Segmentation of an image that can be visualized as a topographical surface upon which rain flows into separate watersheds.<br>***Tip***: Use the Binary dialog's Euclidean distance to make surfaces from binary images. | ***n/a*** | ***n/a*** | Use the marker option avoid over-segmentati (many little regions). Each marker blob give single watershed region—no more, no less. |
| ***Watershed Basins*** | Partitioning an "intensity surface" image where the brighter intensity "ridges" (watershed lines) separate the watershed valleys or basins. Each basin is given a different color for visualization. | Segmentation of an image that can be visualized as a topographical surface upon which rain flows into separate watersheds.<br>***Tip***: Use *Euclidean distance* in the Binary dialog box to make surfaces from binary images. | **n/a** | ***n/a*** | Use the marker option avoid over-segmentati (many little regions). Each marker blob give single watershed region—no more, no less.<br>Select individual basin of interest by using the Binarize function in the Binary dialog box, sett both Low and High values to the basin number that you see a the bottom of the scree from placing your mou pointer over the basin the watershed image. |



# Segment Image (Process Menu)

**Provides access to the Segment Image Modifications dialog box, and saves, stores, and retrieves Image segmentation settings.**

**Drop-in: SEGIMAGE**

Use this dialog box to access the *Segment Image Modifications* dialog box to define settings for image segmentation. You can apply your image segmentation results to individual images directly from the *Segment Image Modifications* dialog box, or you can use this Segment Image dialog box to store satisfactory image segmentation settings for future use. Also use this dialog box to retrieve and load previously stored image segmentation settings. The stored and loaded settings can be applied to images directly from this dialog box. Use the output selections to specify whether you want to create a binary mask from the processed image data or create a clipped version of the original image.

## Segmenting Images

To segment an image, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps Menu, click Segment Image. The Segment Image dialog box opens. |
| **2** | From the File menu, open the image that you want to segment. The image name is displayed on the *Source image* button. If you open more than one image, use the *Source image* button to select the image you want to segment. |
| **3** | If you have a prepared Setting file that you want to use, click the *Load* button. The *Segment Image: Load Setting* dialog box opens. |
| **4** | In the *Segment Image: Load Setting dialog box*, click the name of the setting (*.AST) file that you want to load. Repeat this step to load additional setting files into Segment Image. You can then select the file that you want to use in the Active Setting box. |
| **5** | Use the *Result image* selector to assign the image name and characteristics to the segmentation output image file. You can specify *New* to create a new image file each time you save, *Overwrite*, to overwrite an image file with the same name, or *Add To* in order to create an image stack from sequentially saved images. |
| **6** | In the Active Setting list box, select the active setting that you want to apply to your image. The description associated with the setting will appear in the *Setting Description* box. |
| **7** | In the *Setting Description* box, view the details of the setting assigned to the selected active setting. Based on the setting information, determine if you can apply the setting in its present state, or if you need to modify the setting. |
| **8** | If you do not have a Setting file or you need to modify your loaded setting file, click *Modify.* The *Segment image modifications* dialog box opens. |
| **9** | After you have made modifications in the *Segment image modifications* dialog box and accepted the modifications, the *Segment Image* dialog box reopens. Click *Save* to save your modifications to either a new or existing Setting (*.AST) file. |
| **10** | Determine the appearance that you want in the final segmented image output : If you want to output the image as a Binary Mask (Binarize), click *Create Binary Mask*.<br><br>OR, |

If you want the segmented image data to retain its gray-level value, while setting the value of all non-segmented image data to 0, click *Clip to gray level*.

**11** Click *Apply* to Apply the setting values as described in the Setting Description box.

OR,

Click *Close* to close this dialog box and disregard any settings.

## Segment Image - Dialog Box Options

### Source image

Specifies the name of the source image file.

### Result image

Specifies the name of the destination image file and enables you to select creating a new image file, overwrite an existing image file, or create an image stack from saved images.

### Setting

Defines the name and description assigned to the currently active setting.

### Active Setting

Defines the name of the currently active setting.

### Setting Description

Provides a summary of the segmentation settings in the currently active set.

### Load

Loads an existing segmentation settings file.

### Save

Saves your current segmentation settings to a segmentation settings file.

### Modify

Opens the *Segment Image Modifications* dialog box to enable you to modify the currently active settings and preview results of the new settings.

### Output

Specifies that one of the following treatments is applied to your image output.

***Create binary mask*** – Outputs your image segmentation result as a binary mask. With this setting, details detected as image features are set to maximum level (white), and details not detected as image features and also the background information are set to a zero level (black), similar to the binarize operation.

***Clip to gray level*** – Outputs your image as a clipped version of the original image. With this setting, details detected as image features remain at their original gray levels, while details not detected as image features are set to a zero level (black).

### Apply

Applies the segmentation settings stored in the currently active segmentation settings file to the currently open image.

### Close

Closes the *Segment Image* dialog box.

# Segment Image Modifications

**Provides a set of options for making image segmentation settings based on a standard set of questions designed to determine the types of objects present in your image and their general characteristics.**

**Drop-in: SEGIMAGE**

Use this dialog box to evaluate the general and specific characteristics of an image and to determine the image segmentation settings that it must apply to achieve successful image segmentation. Compare the conditions described on each tab of this dialog box with the actual conditions present in your image. Make radio button selections according to the conditions that exist in the image. After selections have been made on the Image Features and More Settings tab, you can test and modify your settings on the Adjustments tab before making your segmentation settings permanent.

To effectively segment an image type for which you have not already created a setting file, complete the appropriate settings on the Image Features tab and the More Settings tab. Settings made on the Adjustments tab and the Diagnostics tab are optional. The Adjustments tab enables you to interactively view and modify the resulting segmented image using two controls. The Sensitivity Control enables you determine how much of or how many of the objects in your original image will appear in your segmented output image. The Segment Length control enables you to change the value for the maximum length of a detectable object. By reducing the length of isolated segments, you can enable the program to better distinguish and separate objects that are touching or overlapping. The Diagnostics tab enables you to make additional modifications to the results of the settings you achieved on the other tabs.

## Setting Segment Image Modifications

Use the following procedure to set options in the Segment image modifications dialog box to specify your segmentation criteria. As you make settings, click Preview to update the output image to enable you to see the results of your selections.

| Step | Action |
|---|---|
| **1** | From the Segment Image dialog box, click *Modify*. The *Segment image modifications* dialog box opens. |
| **2** | Click the *Source* button to select a different source image from the list (optional). |
| **3** | Click the appropriate image selector button for the output image file destination to make any changes. You can designate a New output image file name or Overwrite or Add To an existing image. (optional). |
| **4** | On the *Image Features* tab, click the appropriate Illumination setting. Choose *Fluorescent* for images created using a fluorescence light source or for light objects on a dark background. Choose *Transmitted* for images created using a transmitted light source or for dark objects on a light background. |
| **5** | On the *Image Features* tab, click the best method for *Distinguishing objects from background and other objects*. Refer to the individual descriptions on the Dialog Box |

Options page.

**6** On the *More Settings* tab, in the *Halos* box, click *Correct halos around objects* to reduce or remove the halo effect that can be created by light passing though transparent mediums. If no halos are present or if it is not necessary to remove them, click *Objects do not exhibit halos.*

**7** On the *More Settings* tab, in the *Shading Correction* box, click *Uneven illumination requires correction*, if there is a varying level of illumination across the image. If the illumination in the image appears even, click *No illumination shading artifacts.*

**8** On the *More Settings* tab, in the *Segmentation Goals* box, click one of the following buttons: *Detect discrete object* , *Detect linear objects and structures*, or *Segment into several homogeneous layers or areas*. Refer to the individual descriptions on the Dialog Box Options page.

**9** On the *More Settings* tab, in the *Separate Objects* box, click Try to break apart joined objects to separate objects that are touching or overlapping. Click Do not adjust objects if no objects are touching, or if it is not necessary to separate touching objects.

**10** Click *Preview* to review the your segmentation results so far. A Preview image window opens. The image in this window will have the current segmentation settings applied. Compare the results in the output window with the image in the source image window. Make the necessary changes to create the segmented image that you want. If the results are satisfactory, click *Accept* to return to the *Segment Image* dialog box where you can save your settings ans create your final result.

**11** If further segmentation settings are needed, click the *Adjustments tab*. The Adjustments tab moves to the front.

**12** Click *Display preview as an overlay on the image* to enable you to view the preliminary output image superimposed on a copy of the original image.

**13** Click *Overlay On/Off* to toggle the image overlay. This enables you to compare the original image with the segmented image.

**14** In the *Feature Adjustments* box, adjust the *Sensitivity* control according to the results that you want. Move the slider, click the buttons on the spin box, or type a value in the spin box. The greater the sensitivity (100%) the more features are detected.

**15** In the *Feature Adjustments* box, adjust the

*Length* control according to the results that you want. Move the slider, click the buttons on the spin box, or type a value in the spin box. The lesser the length the greater the separation will be between objects that are touching.

**16** In the *Feature Adjustments* box, click *Try a range of settings*. The *Try a range of settings* dialog box opens along with an image window showing a grid of nine different thumbnail images that show nine different segmentation possibilities.

**17** In the *Try a range of settings* dialog box, click the button that corresponds to the best possible image. The selected image is shown by itself in the image window. Click *Accept* to accept this image,

**OR**,

Click *Cancel* to close the dialog box and the image window without choosing an image.

**18** Click the *Diagnostics* tab to try a different approach for obtaining the best segmented image.

**19** On the *Diagnostics* tab, click one or more symptom descriptions that describe the visual symptom or problem that you are observing in your image. Highlight the symptom description to view an explanation of the expected improvement to the image when the selected symptom is checked.

**20** Click *Preview* to view the changes that have resulted from applying fixes for the selected symptoms.

**21** When the Preview image is satisfactory, click *Accept*. The *Segment image modifications* dialog box closes, and the *Segment Image* dialog box opens.

## Segment Image Modifications - Dialog Box Options

### Image Features Tab

Selects and shows the Image Features tab. Use this tab to make settings based on the type of image illumination and object characteristics that can be used to more effectively separate the objects from the background.

### More Settings Tab

Selects and shows the More Settings tab. Use this tab to apply algorithms to the image that can correct for the appearance of halos, uneven illumination, detect objects that are completely separate from other objects, and attempt to separate touching objects.

### Adjustments Tab

Selects and shows the Adjustments tab. Use this tab to place an overlay of the proposed new image on top of the original image. The Feature adjustments on this tab enables you adjust the grayscale threshold and to set a value for the maximum acceptable line segment length.

### Diagnostics Tab

Selects and shows the Diagnostics tab. Use this tab to choose from a list of symptoms that best describe the symptoms that you are observing in your preview image. View the corrections interactively, then click *Accept* to accept the recommend changes based on the symptoms you selected.

### Source

Specifies the name of the source image file.

### Dest

Specifies the name of the destination image file and enables you to select creating a new image file, overwrite an existing image file, or create an image stack from saved images.

### Preview

Opens an image preview window to view the results of the changes you selected for your image before the changes are permanently applied to the image.

### Cancel

Cancels applying your selected changes and closes the dialog box.

### Accept

Accepts and applies your selected changes and closes the dialog box.

## Image Features Tab - Dialog Box Options

### Illumination

Defines the type of light source used to create the image, or defines the general appearance of the lighting in the image. This selection is mutually exclusive. Click one choice.

> ***Fluorescent*** – Indicates that the image was produced using a fluorescence light source or has the appearance of a light colored object on a dark background.
>
> ***Transmitted*** – Indicates that the image was produced using a transmitted light source or has the appearance of a dark colored object on a light background.

### Distinguishing objects from background and other objects

Defines the algorithm that will be applied to improve the visual separation of objects from the background and other objects. This selection is mutually exclusive. Click one choice.

> ***Detect objects by intensity*** – Isolates objects that have a difference in intensity from the background. The difference in object texture is not considered.
>
> ***Detect objects by texture*** – Isolates objects that have a different texture than the background. The difference in object intensity is not considered.
>
> ***Detect objects by intensity and texture*** – Uses the difference in values in both intensity and texture to isolate objects from the background.
>
> ***Detect objects by color*** – Isolates objects from the background that have a different color than the background.

## More Settings Tab - Dialog Box Options

### Halos

Removes or reduces the appearance of halos around objects in the image that have resulted from excessive light diffusion through glass or other light transmissive media.

> ***Correct halos around objects*** – Applies remove halo to Image features. Click when halos are observed.

***Objects do not exhibit halos*** – Deactivates halo removal. Click when no halos are observed.

### Shading Correction

Enables or disables automatic background correction. If the image background is uneven, this option attempts to adjust the gray level of the background to the same level of intensity across the entire image.

***Uneven illumination requires correction*** – Enables automatic background correction. Click if the image background is uneven.

***No illumination shading artifacts*** – Deactivates automatic background correction. Click if the image background appears to be even.

### Segmentation goals

Selects one of three criteria for segmenting objects in an image.

***Detect discrete objects*** *(blobs)* – Detects large, unstructured binary objects that are completely discrete (not touching or attached to other objects).

***Detect linear objects and structures*** – Detects lines and coarse structures.

***Segment into several homogeneous layers or areas*** – Separates individual objects or structures into separate layers or areas.

*Overlay colors on output* – Assigns a different color overlay for each segment layer or area.

*Output mul**tip**le layers as stack planes* – Organizes mul***tip***le layers of segmentation as separate planes in a stack.

### Separate objects

Enables or disables the option for separating objects that are touching or connected.

***Try to break apart joined objects*** – Enables the option for separating joined or touching objects into separate objects.

***Do not adjust objects*** – Ignores objects that are joined or touching.

## Adjustments Tab - Dialog Box Options

### Display preview as an overlay on the image data

Overlays the Preview image on a copy of the original image. Use this option to help you determine how well your segmentation results match their data.

### Overlay On/Off

Activates or deactivates the Preview image overlay feature. Use this button to toggle the overlaid preview image on and off to get a comparison of the two images.. This button is inactive if *Display preview as an overlay on the image data* is unchecked.

### Feature Adjustments

Use these options to interactively view your segmentation results. You can either accept your results or adjust the segmentation settings on this tab to produce updated segmentation results.

***Sensitivity (%)*** – Controls the level of grayscale threshold (either local or global) applied to the image.

***Length (units)*** – Specifies the estimated length of the largest object to be segmented in the image.

***Try a range of settings on the image*** – Applies a range of settings for sensitivity and length to the image and displays a panel of images from which you can select the best result.

## Diagnostics Tab - Dialog Box Options

### Select symptoms that describe the segmentation result

Enables you to select and apply a number of symptoms from this list that best describe your anticipated segmentation result.

### Objects missing from the result

Defines the objects that were excluded from your segmentation result by your segmentation settings selection.

# Correlation Plot (Apps Menu)

**Measures and displays the correlation between the intensities of corresponding pixels in two images. Provides a correlation coefficient (*r*) of the pixel intensity data.**

*Availability: Included in MetaMorph Basic and MetaMorph Premier*

**Drop-in: CORRPLOT**

Use this command when you want to display a graphical representation of the correlation between corresponding pixel intensities in two images and log the measured correlation coefficient. The correlation plot that is created represents a large amount of data in a resizable scatterplot. For every image pixel being analyzed, MetaMorph examines the intensity of the corresponding pixels in the two images, and uses the two intensity values as the X and Y coordinates in the scatterplot.

The Correlation Plot dialog box displays the correlation coefficient (*r*) of the data. This is defined as

$$r = \frac{\sum xy}{NS_x S_y}$$

where

*r* = correlation coefficient,
*xy* = product of deviation scores,
*N* = sample size,
Sx = standard deviation of X (intensities in first image), and
Sy = standard deviation of Y (intensities in second image).

The range of values of the correlation coefficient is -1.0 to +1.0. A value of 1.0 shows that the data are perfectly correlated with one another. This will only happen if the two images are identical. A correlation coefficient of -1.0 is observed when there is an inverse relationship between intensities in the two images.

Before you make your measurements, you may wish to use a Region Tool to define and select a specific region of interest for measurement. MetaMorph allows you to move, resize, or switch between regions while you follow the resulting measurements.

You also have the option of using thresholding in either or both of the images. Only pixels that have intensities that are outside of the threshold range of *both* images will be excluded from the measurement. If thresholding is turned off in *either* image, *all* pixels in the active region will be measured. Thresholding will affect measurement of the correlation coefficient.

The correlation scatterplot that is displayed can be adjusted in two ways. First, the size of the plot window can be adjusted up or down by using the *Plot Size* slider. Second, the range of gray values that are displayed in the plot can be adjusted. The range of values selected in the first image will affect the X-axis, and the range selected from the second image will affect the Y-axis. You can select the range

manually by using the *Min* and *Max* spin boxes, or you can have MetaMorph select the ranges for you automatically by selecting the *Auto Scale* check box. The range that you select will affect only the display of the plot; it will not affect the measurement of the correlation coefficient.

**QUICK *TIP*:** To hide the Image Window Tools on the Plot window, right-click in the window and choose Hide Image Window Toolbar from the pop-up context menu that appears.

## Plotting Intensity Correlations

To display or print a correlation scatterplot of corresponding pixel intensities in two images, use the following procedure:

| Step | Action |
|---|---|
| **1** | If desired, define at least one region for measurement. If regions have been defined, skip to Step 2. |
| **2** | From the Apps menu, choose Correlation Plot. The Correlation Plot dialog box and a Plot window opens.<br><br>The currently selected region and the correlation coefficient will be displayed in the Correlation Plot dialog box, and the Plot window will display a scatterplot of the intensity correlation data.<br><br>**QUICK *TIP*:** To hide the Image Window Tools on the Plot window, right-click in the window and choose Hide Image Window Toolbar from the pop-up context menu that appears. |
| **3** | If necessary, use the *X* and *Y* image selectors to select the appropriate images. |
| **4** | If you have applied thresholding and want to restrict the data analysis to pixels that have not been excluded from both images by thresholding, select the *Use Thresholds* check box. (Pixels that are included in the threshold range in either image will be measured.) The scatterplot and displayed correlation coefficient will be updated automatically. |
| **5** | To change the size of the Plot window, use the *Plot Size* slider to specify a size. A smaller size will be specified by sliding it to the left. |
| **6** | If you want the X and Y axis of the Plot to be scaled automatically, based on the intensities in the two images, select *Auto Scale*.<br><br>**OR**<br>Use the *Min* and *Max* spin boxes in the *X* and *Y* option groups to select the lower and upper grayscale intensities to be plotted from each image. |
| **7** | If you want to store the correlation measurement in a log file and you do not have a data log open, choose *Open Log* to select a text-based log file or to open a DDE |

link to an open spreadsheet..

**8** To configure data logging, choose *Configure Log.* The Configure Log dialog box will appear.

**AND**

From the *Configuration* table, select the parameters you want to log, so that each is marked by a check mark next to its entry (you can choose *Enable All* or *Disable All* if you want to select or deselect all of the parameters listed). Then Choose *OK* to return to the Log Pixels in Region dialog box.

**9** When you are ready to log the correlation measurement, choose *F9: Log Data.*

**10** You can select another region to be plotted by clicking it directly in the image window, or you can resize the currently active region by dragging its outline. The Correlation Plot dialog box and the Plot window will update automatically to reflect the new measurement. Choose *F9: Log Data* whenever you want to save the measurements.

**11** When you have finished, choose *Close* from the Correlation Plot dialog box. Both the dialog box and the Plot window will close.

## Correlation Plot - Dialog Box Options

### X (image selector)

Selects the first image, which will have its pixel intensities plotted as the X-coordinates of the points in the correlation plot.

### Y (image selector)

Selects the second image, which will have its pixel intensities plotted as the Y-coordinates of the points in the correlation plot.

### Min

Manually selects a minimum pixel intensity for the corresponding image (X or Y) to be plotted in the correlation plot.

### Max

Manually selects a maximum pixel intensity for the corresponding image (X or Y) to be plotted in the correlation plot.

### Image Whose Region Should Be Used

This option appears only in Journal Edit mode. Selects which image's region, if any, to use in the correlation plot *(Image X, Image Y,* or *None).*

### Auto Scale

Scales the X and Y axes of the correlation plot to include the entire range of pixel intensities available in the two images. If there is an active region and/or thresholding has been applied, only pixels in the region and/or with intensities within the threshold range will be plotted.

### Use Thresholds

Restricts the data analysis to pixels with intensities in the threshold range of either of the images. Only pixels

with grayscale values outside of both threshold ranges will be excluded from measurement.

### Plot Size

Changes the size of the Plot window.

### Correlation Coefficient

Displays the correlation coefficient value *(r)* for the current correlation measurement data set.

### Open Log

Opens a data log and/or a DDE link to an open spreadsheet application for logging data. This command changes to *F9: Log Data* when a log file is open.

### F9: Log Data

Logs the pixel data from the active region to an open data log or to an open spreadsheet application via a DDE link. To assist you in logging the proper data when several measurement dialog boxes are open, *"F9"* is added to the name of this option in the active dialog box to indicate which data will be logged when you press [F9].

### Configure Log

Opens the Configure Log dialog box so that you can select the parameters to be logged to the data log. Parameters marked with a check mark will be logged for subsequent measurements.

> If you select ***Log Column Titles***, a line listing the measurement titles will be logged (1) the first time you use the configured measurement, (2) whenever you enable/disable measurement parameters, or (3) whenever the logged measurement is different from the previous measurement in the log file.
>
> If you select ***Place Log Data on Current Line***, subsequently logged data will be appended to the current line in the log file, rather than to a new line. *Log Column Titles* will be unavailable when you select this option.

### Close

Closes the Correlation Plot dialog box and the Plot window.

# Log Pixels in Region (Log Menu)

**Logs pixel grayscale data to a data log from the active region of interest of an image or current plane of a stack. Copies pixel grayscale data from the active region to the Kernel Editor if the region is smaller than 15 x 15 pixels.**

> **Drop-in: LOGPIX**
>
> Use this command to log pixel grayscale data to a data log. You can also use this command to copy the pixel grayscale data into the Kernel Editor. If you want to copy the data into the Kernel Editor, the region must be smaller than 16 x 16 pixels. Before applying the kernel, you will need to change the *Result* option in the Edit Kernel dialog box to the number of pixels in the kernel.
>
> The Log Pixels in Region dialog box provides options for specifying the location and size of the region. This dialog box also provides some region control options that, when enabled, provide full control over the specified region characteristic, but will restrict it when disabled.
>
> **Note:** Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaMorph.
>
> Use the Open Data Log command to open a data log before using this command. You can use the Log Data command or its keyboard shortcut, the [F9] function key, to log the data.

## Logging Pixel Gray Scale Values from a Region

To log pixel intensity values from a region, use the following procedure:

| Step | Action |
|---|---|
| 1 | From the Log menu, choose Log Pixels in Region. The Log Pixels in Region dialog box opens. |
| 2 | Select the desired image for logging pixels using the *Image* selector. |
| 3 | Choose *Open Log* to open a data log, if one is not already open. |
| 4 | To configure data logging, choose *Configure Log.* The Configure Log dialog box will appear.<br>**AND**<br>From the *Configuration* list, select the parameters you want to log, so that each is marked by a check mark next to its entry (you can choose *Enable All* or *Disable All* if you want to select or deselect all of the parameters listed).<br>Choose *OK* to return to the Log Pixels in Region dialog box. |
| 5 | Draw a region of interest on the active image using a Region Tool. Select it so that it is the active region of interest.<br>**Note:** Regions drawn with a two-dimensional Region Tool (Rectangular, Ellipse, Trace, or Auto-Trace) must be at least 2x2 in size to be valid in MetaMorph. |
| 6 | *Left, Top, Width,* and *Height* display the location and size of the region. You can use these options to reposition or resize the region if desired.<br>The ***Region Control*** options provide greater control over the region. You can enable or disable these as desired. |
| 7 | If you want to copy the pixel data from the active region to the Kernel Editor, choose *Copy to Kernel Editor.* |
| 8 | Choose *F9: Log Data* to log the pixel data from the region. |
| 9 | To measure another region, create and select another region using the process described in Steps 5 - 8. |
| 10 | Choose *Close* when you have finished . |

## Log Pixels in Region - Dialog Box Options

### Image

Specifies the image from which regional pixel gray values will be logged.

### Left

Specifies the X-coordinate for the upper left corner of the region to be used for logging the pixels.

### Top

Specifies the Y-coordinate for the upper left corner of the region to be used for logging the pixels.

### Width

Specifies the width of the region to be used for logging the pixels.

### Height

Specifies the height of the region to be used for logging the pixels.

### Horiz. Movement

Allows horizontal movement of the region when enabled.

### Vert. Movement

Allows vertical movement of the region when enabled.

### Keep in Image

Forces the entire region to stay inside image when enabled.

### Horiz. Resizing

Allows horizontal resizing of the region when enabled.

### Vert. Resizing

Allows vertical resizing of the region when enabled.

### Deletable

Region can be deleted when enabled.

### Copy to Kernel Editor

Copies the pixel data into the Kernel Editor. If you want to copy the data into the Kernel Editor, the region must be smaller than 16 x 16 pixels. Before applying the kernel, you will need to change the *Result* option manually in the Edit Kernel dialog to the number of pixels in the kernel.

#### Open Log

Opens a data log and/or a DDE link to an open spreadsheet application for logging data. This command changes to *F9: Log Data* when a log file is open.

#### F9: Log Data

Logs the pixel data from the active region to an open data log or to an open spreadsheet application via a DDE link. To assist you in logging the proper data when several measurement dialog boxes are open, *"F9"* is added to the name of this option in the active dialog box to indicate which data will be logged when you press [F9].

#### Configure Log

Opens the Configure Log dialog box so that you can select the parameters to be logged to the data log. Parameters marked with a check mark will be logged for subsequent measurements.

> If you select ***Log Column Titles***, a line listing the measurement titles will be logged (1) the first time you use the configured measurement, (2) whenever you enable/disable measurement parameters, or (3) whenever the logged measurement is different from the previous measurement in the log file.
>
> If you select ***Place Log Data on Current Line***, subsequently logged data will be appended to the current line in the log file, rather than to a new line. *Log Column Titles* will be unavailable when you select this option.

#### Close

Closes the dialog box.

# Measure Grid (Apps Menu)

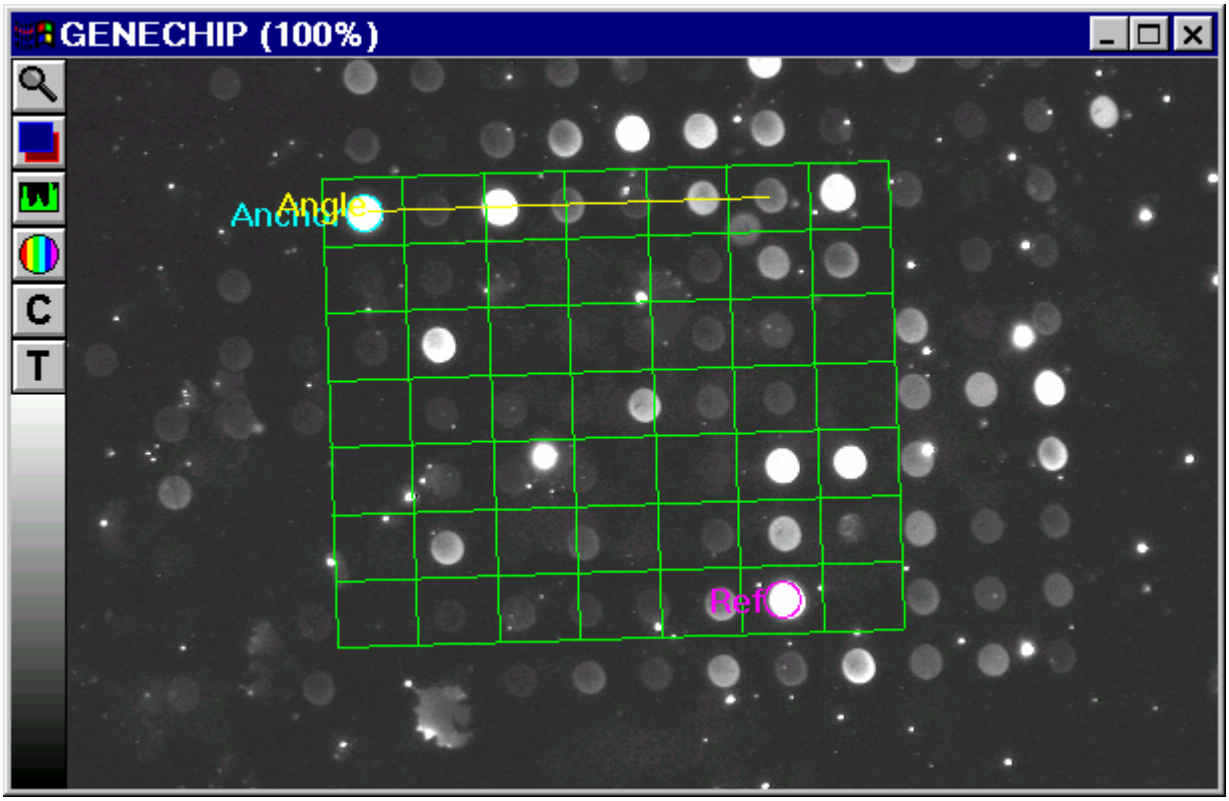
**Creates a user-configurable measurement grid on an image and performs measurements, or runs a journal to process and/or measure the image regions, within each element in the grid.**

*Availability: Included in MetaMorph Basic and MetaMorph Premier*

### Drop-in: MEASGRID

Use this command to divide an image into evenly spaced regions and run a journal to measure each region. This function is particularly useful for images with regularly spaced areas of interest, such as those from a gene chip. Measurement parameters include the location (coordinates of the upper left corner), width, height, area, perimeter, thresholded area, average, minimum, maximum, and integrated intensity value, standard deviation and signal-to-noise level of the intensity, and the percent thresholded area.

When you first open the Measure Grid dialog box, the measurement grid and three regions will appear in an overlay on the selected image: an Anchor region, an Angle line region, and a Reference region. The following sample figure illustrates the placement of the Measure Grid regions and measurement grid:




In their default configuration, the Anchor and Reference regions appear in the upper left and lower right corners of the measurement grid, respectively. Their placement defines the initial horizontal and vertical extent of the measurement grid. These two regions can be resized and moved in the same manner as any other region of interest. Alternatively, you can use the *Horizontal Spacing* and *Vertical Spacing* spin boxes in the Measure Grid dialog box to define the size of each element in the grid, and then set the number of rows and columns with the *Columns* and *Rows* spin boxes in the *Grid* option group. To apply a change you have made from the dialog box, click another option in the dialog box, or press the [TAB]

key.

The Angle line region is used to change the angle of the grid to correspond to the angle of the data regions. This may be necessary for images in which such regions are not arranged in a perfectly vertical and horizontal orientation. After you make the Angle line region the active region by clicking it, you can move the region by dragging it. You can change its angle or length by double-clicking one end of the line and dragging the rounded handle that appears. Alternatively, you can use the *Grid Angle* spin box to select an angle with respect to the X-axis. Again, to apply the configured change, click another option in the dialog box, or press the [TAB] key.

Many of the settings in the *Grid, Anchor Region,* and *Reference Region* option groups have an interactive effect on one another and the on the size and placement of the measurement grid. For example, if you increase the *Column* setting in the *Reference Region* group, the setting in the *Columns* spin box in the *Grid* option group will also increase. Simultaneously, the number of columns in the measurement grid will increase, and the *Horizontal Spacing* setting will decrease, reflecting the diminished width of each element in the grid. If you then decrease the *Column* setting in the *Reference Region* group, the number of columns in the measurement grid will stay at its higher setting, and the Reference region will stay in its current location on the image, but the size and width of the measurement grid will increase. You will need to experiment to find your optimum settings for the various options in the dialog box.

After you have configured the measurement grid to your specifications, you will need to configure the size and shape of the measurement regions within each grid element using the *Measure Each* option button group and the *Region Width* and *Region Height* spin boxes.

Regions by their nature must lie on discrete pixels. In practice, however, samples in the image rarely align with discrete pixels. For this reason, the grid spacing can be set to non-integer values. When regions are placed on the image, the vertices will fall on discrete pixels. Thus, because the size of the regions will not change, the *Unit Square* sizes may differ slightly from the displayed grid.

A data log can be opened and configured directly from the Measure Grid dialog box. Choosing *F9: Log Data* will measure the region within each grid element. You can use the *Configure Log* button to select which measurement values to record. If you need measurements other than those provided in the Configure Log dialog box, or if you wish to perform some additional processing, you can use the *Run Journal for Each Region* option. (But you will not be able to run Measure Grid in a journal from within itself: doing so will generate an error message.) When you use *Run Journal for Each Region,* a region will be placed over the first grid element and made active. The journal will then be run for that region. The region is then moved to the next element, and the process will be repeated for each grid element in turn. Nothing will be logged, however, unless the journal being run explicitly performs the logging function.

**Measuring Grids**

**Overview of Grid Measurement**

**Configuring the Grid**

## Overview of Grid Measurement

To use the Measure Grid command to measure an image, use the following procedure as a general guideline. If you intend to run a journal that makes measurements, be sure to threshold the image before proceeding.

| Step | Action |
|---|---|
| **1** | From the Apps menu, choose Measure Grid. The Measure Grid dialog box opens, and a measurement grid (green), an Anchor region (cyan), an Angle line region (yellow), and a Reference region (magenta) appears in an overlay on the active image. |

**2** If necessary, use the *Image* selector to select the image you want to measure.

**3** Follow the directions for **configuring the measurement grid.**

**4** If you want to measure the regions and simultaneously log your measurements, choose *Open Log* and select a data log. The *Open Log* button label will change to "*F9: Log Data.*" Continue to Step 5.

**OR**
If you want to run a journal to process or measure each measurement region, skip to Step 6.

**5** To configure the data log for logging, choose *Configure Log.* The Configure Log dialog box will appear.

**AND**

From the *Configuration* list, select the parameters you want to enable for logging, so that each is marked by a check mark next to its name (you can choose *Enable All* or *Disable All* if you want to select or deselect all of the parameters listed). Then choose *OK* to return to the Measure Grid dialog box. Now skip to Step 7.

**6** To run a journal to process or measure each measurement region, choose *Select a Journal.* The Select Journal dialog box will appear.

**AND**
Select the icon for the desired journal. (You will not be able to run Measure Grid in a journal from within itself: doing so will generate an error message.) If necessary, use the *Look In* list or Up One Level icon button to select the pertinent drive and folder. Choose *Open.* Then *c*ontinue to Step 7.

**7** If you are running a journal, choose *Run Journal for Each Region.* The selected journal will be run for each region in the grid.

**OR**
If you are logging data, choose *F9: Log Data,* or press the [F9] function key. The selected measurements will be made and logged to the data log.

**8** When you have finished, choose *Close.*

## Configuring the Grid

To configure the measurement grid, use the following procedure:

| Step | Action |
|---|---|
| **1** | Click the Anchor region (a cyan-colored elliptical region in the upper left corner of the grid) to make it the active region. Then use |

the pointer to drag the Anchor region to the uppermost, leftmost sample that you want to measure in the image.

Alternatively, you can use the *X Position* and *Y Position* spin boxes in the *Anchor Region* option group to specify the placement of the Anchor region. Be sure the *Column* and *Row* spin boxes in the *Anchor Region* option group correspond to the column and row in the measurement grid that you want to use to define the Anchor position (typically Column 1, Row 1).

**2** Click the Angle region (a horizontal yellow line region across the first row in the grid) to make it the active region. Drag the Angle region up, down, to the left, or to the right to move it to the desired position.

To change the line's length and angle, double-click one end of the line and drag the round handle that appears. It is best to place the left handle over the center of the leftmost sample and the right handle over the rightmost sample.

Alternatively, you can change the Angle region's angle setting by selecting a value with the *Grid Angle* spin box in the Measure Grid dialog box's *Grid* option group.

**3** Click the Reference region (a magenta-colored elliptical region in the lower right corner of the grid) to make it the active region. Then use the pointer to drag the Anchor region to another clearly identifiable sample. This typically will be the lowermost, rightmost sample that you want to measure in the image (but does not need to be, as long as it is not in the same row or column as the Anchor region).

**4** Adjust the *Reference Region* option group's *Column* and *Row* settings to reflect the configuration of the image samples. If necessary, readjust the *Grid* option group's *Column* and *Row* settings. These must have a width and height of at least two pixels.

**5** For added visual feedback, select the *Show Complete Grid* check box to see the grid lines for all of the measurement grid's columns and rows. Clear the check box if you want to see just the bounding rectangle of the grid and the Anchor and Reference regions' rows and columns.

**6** From the *Measure Each* option button group, select the type of region that you want to measure for each grid element. Select

*Unit Square* if you want to measure the entire rectangular region that is defined by the column and row grid lines. If you select this option, the *Region Width* and *Region Height*

spin boxes will become unavailable, and will appear dimmed.

**OR**
*Circle Region* if you want to measure an elliptical region that corresponds in size to the magenta-colored Reference region. You can adjust the dimensions of the Reference region by dragging its outline. Alternatively, you can use the *Region Width* and *Region Height* spin boxes to specify the respective horizontal and vertical sizes, in pixels, of the measurement regions. These must have a width and height of at least two pixels.

**7** If your image has been thresholded and you want to measure just the thresholded regions, select the *Use Threshold for Measurement* check box.

**OR**
If you want to measure all the entire area in each grid element, leave the *Use Threshold for Measurement* check box cleared.

**8** If necessary, adjust your settings for optimum size and placement of the measurement grid and its elements. Then continue with Step 4 of the procedure described in the **Overview of Grid Measurement.**

## Measure Grid - Dialog Box Options

### Image
Selects the image to be measured.

### GRID
This option group specifies the size, angle, and number of rows and columns of the measurement grid.

### Grid Angle
Determines the angle of the measurement grid, in degrees, with respect to the X-axis (horizontal). The grid will be rotated around the Anchor region, which serves as the pivot point. Changing the grid angle will change the position of the Reference region.

### Columns
Selects the number of columns of samples in the horizontal (X-axis) direction that will be measured.

### Rows
Selects the number of rows of samples in the vertical (Y-axis) direction that will be measured.

### Vertical Spacing
Specifies the vertical size, in pixels, of each element in the measurement grid. This measurement is based on the distance between the centers of two successive elements in a column. Changing this distance will change the position of the Reference region, and vice-versa. The spacing must be at least two pixels high.

### Horizontal Spacing
Specifies the horizontal size, in pixels, of each element in the measurement grid. This measurement is based on the distance between the centers of two successive elements in a row. Changing this distance will change the position of the Reference region, and vice-versa. The spacing must be at least two pixels wide.

### Show Complete Grid

Determines the display of the measurement grid. If you select this check box, all of the elements of the measurement grid will be displayed. This is useful for checking the configuration of the grid before you perform the measurement. If you clear this check box, only the outermost rows and columns of the grid will be displayed, along with the lines marking the row and column of the Anchor and Reference regions.

### ANCHOR REGION

This option group specifies the location of the Anchor region on the image and its position within the measurement grid.

### Column (Anchor Region)

Selects the horizontal position of the Anchor region within the measurement grid. Increasing this setting will move the grid and the Reference region to the left, one column at a time. Decreasing the setting will move the grid and the Reference region to the right.

### Row (Anchor Region)

Selects the vertical position of the Anchor region within the measurement grid. Increasing this setting will move the grid and the Reference region upwards one row at a time. Decreasing the setting will move the grid and the Reference region downwards.

### X Position

Specifies the vertical location of the center of the Anchor region, in pixels, from the top border of the image.

### Y Position

Specifies the horizontal location of the center of the Anchor region, in pixels, from the left border of the image.

### REFERENCE REGION

This option group specifies the location of the Reference region on the image and its position within the measurement grid.

### Column (Reference Region)

Selects the horizontal position of the Reference region within the measurement grid. Increasing this setting will increase the number of columns in the grid and decrease the width of the columns without moving the Anchor or Reference regions on the image. Decreasing this setting without altering the *Grid* option group's *Columns* setting will increase the width of the columns, and the Reference region will be positioned one column to the left in the measurement grid, although the Reference region will not itself be moved from its location on the image.

### Row (Reference Region)

Selects the vertical position of the Reference region within the measurement grid. Increasing this setting will increase the number of rows in the grid and decrease the height of the rows without moving the Anchor or Reference regions on the image. Decreasing this setting without altering the *Grid* option group's *Rows* setting will increase the height of the rows, and the Reference region will be positioned one row higher in the measurement grid, although the Reference region will not itself be moved from its location on the image.

### Measure Each

Determines the type of region that will be measured at each element in the measurement grid. *Unit Square* will measure the entire rectangular region that is defined by the column and row grid lines. If you select this option, the *Region Width* and *Region Height* spin boxes will become unavailable, and will appear dimmed. *Circle Region* will measure an elliptical region, centered within each grid element, that corresponds in size to the dimensions of the Reference region. Use the *Region Width* and *Region Height* spin boxes to specify the respective horizontal and vertical sizes, in pixels, of the measurement regions.

### Use Threshold for Measurement

When selected, this check box specifies that only the thresholded regions in each grid element are to be measured. When this check box is cleared, the entire area within each cell will be measured.

### Region Width

Specifies the horizontal size of each elliptical measurement region. This option is available only when you select *Circle Region* from the *Measure Each* group. The width must be at least two pixels across.

### Region Height

Specifies the vertical size of each elliptical measurement region. This option is available only when you select *Circle Region* from the *Measure Each* group. The height must be at least two pixels across.

### Open Log

Opens a data log and/or a DDE link to an open spreadsheet program for logging data. This command changes to *F9: Log Data* when a log file is open.

### F9: Log Data

Measures the samples and logs the measured data to an open data log or to an open spreadsheet by way of a DDE link. The values that can be measured are similar to those for the Show Region Statistics command. Measurements themselves will not be displayed. To assist you in logging the pertinent data when several measurement dialog boxes are open, *"F9"* is added to the name of this option in the active dialog box to indicate which data will be logged when you press the [F9] function key.

### Configure Log

Displays the Configure Log dialog box, from which you can select the parameters to be logged to the data log. Parameters marked with a check mark will be logged for subsequent measurements.

If you select *Log Column Titles,* a line listing the measurement titles will be logged (1) the first time you use the configured measurement, (2) whenever you enable/disable measurement parameters, or (3) whenever the logged measurement is different from the previous measurement in the log file.

If you select *Place Log Data on Current Line,* subsequently logged data will be appended to the current line in the log file, rather than on a new line. *Log Column Titles* will be unavailable when you select this option.

### Select a Journal

Displays the Select Journal dialog box, from which you can select the processing and/or measurement journal that you want to run for each measurement region in the grid.

### Run Journal for Each Region

Starts the grid measurement process, placing an active measurement region over each element in the measurement grid in turn, and running the selected journal at each position. This function will not log any data unless the journal being run does so explicitly.

### Close

Clears the grid and region overlays from the image and closes the dialog box.

# Measure XYZ Distance (Apps Menu)

**Measures the spatial distance between pairs of points in different image planes in a stack. Plays back three-dimensional wireframe rotations of the track lines. Distance measurements are displayed in the dialog box, and can be saved in a data log.**

### Drop-in: MEASXYZD

Use this command to measure the straight-line distance between pairs of points or between endpoints of a multi-plane track. The points can be in the same image or image plane, or they can be in different planes in an image stack.

Distances can be expressed either in pixels or in distance units that have been calibrated with the Calibrate Distances command (Measure menu). Similarly, the Z-distance between successive planes can be set to use either calibrated units or a user-specified distance.

Measurement lines and their distance values are drawn in a measurement overlay. You can show or hide the overlay as desired. You can also select whether to show all lines and values, or just those for the last distance measured, and you can enable or disable display of the measurement values on the image. All measurements and their associated overlay elements will be retained even when their display

is disabled.

An additional feature of the Measure XYZ Distance command is its ability to play back the three-dimensional tracks between points in a wireframe rotation. The wireframe can be rotated along either the horizontal or vertical axis. This feature is similar to those seen with the 3D Reconstruction drop-in. This command can also used simultaneously with the View Orthogonal Planes drop-in command, which is also a component of the 3D Module. The tracking line can be observed as an overlay on both the original image stack and on the XZ and YZ view orthogonal plane stacks.

> **Note:** The measurement overlay will be retained with the image stack if you choose to save it. If you do not want the overlay to be saved with the stack, be sure to choose *No* from the Image Has Been Modified dialog box when you close the stack.

**Measuring XYZ Distance**

**Measuring XYZ Distance - Overview**

**Configuring Display**

**Performing a Wireframe Rotation**

## Measuring XYZ Distance - Overview

To measure multi-plane distances between pairs of points, use the following procedure.

> **Note:** If you want to use calibrated distance values, you will need to apply the Calibrate Distances command (Measure menu) before using this procedure.

| Step | Action |
|---|---|
| **1** | From the Apps menu, choose Measure XYZ Distance. The Measure XYZ Distance dialog box opens. |
| **2** | Use the *Stack* image selector to select the image you want to measure. **(Note:** The image does not need to be an image stack; this command works equally well on single-plane images.) |
| **3** | From the *Display* option group, **configure the display** for the points and the tracks between them. |
| **4** | Use the *Use Z Distance* radio button group to select the method for calculating distance. (The X and Y values will always be the calibrated values.) Select<br><br>*Calibrated* if you want to use the currently active distance calibration (this will be measured in pixels if you have not applied the Calibrate Distances command, or in calibrated units if you have), or<br><br>*User Specified* if you want to specify the between-plane distance. Then specify the Z-distance with the *Z Step* spin control that appears in the dialog box. |
| **5** | If you want to log the distance measurements, choose *Open Log* and select the icon for the desired data log you want to overwrite or append, or type a name for a |

new data log in the *File Name* text box. Then choose *Save*. The label on the *Open Log* command button will change to *"Log Data."*

**AND**

If necessary, choose *Configure Log* and select the data parameters that you want to log from the *Configuration* list. Then choose *OK.*

**6** Open the Change Plane dialog box for the image to be measured by choosing its Change Plane Tool.

**7** Select the plane for the starting point of the distance you want to measure and click the first point with the pointer using the *left* mouse button.

**8** Select the plane for the next point in the track whose distance you are measuring and click the point. If this is the last point in the track, use the *left* mouse button. If you make a mistake or otherwise want to remove a point you have just added, choose *Undo Click.*

**OR**
If this is *not* the last point in the track, use the *right* mouse button to click the point. Then when you finally come to the last point in the track, use the *left* mouse button to click the point.

**9** Depending on the display selections you made, measurement lines and values will appear in the image overlay, and the distance will be displayed in the Measure XYZ Distance dialog box's *Distance* status line.

**10** Repeat Steps 7 - 9 for all distance measurements you want to make.

**11** If you opened a data log, choose *Log Data* to store all your measurement data.

All the points displayed will be logged. If you select *Accumulated*, all distances measured for the image since the dialog box was opened, or since you last chose the *Clear Measurements* command button, will be logged. If you leave *Accumulated* cleared, only the last distance measured will be stored.

**12** If you want to perform a three-dimensional wireframe rotation of the images and the measurement track overlays, follow the procedure for **performing a wireframe rotation.**

**13** When you have finished, choose *Close.*

## Configuring Display for Measure XYZ Distance

To configure the point and measurement track display for XYZ distance measurements, use the

following procedure:

| Step | Action |
|---|---|
| **1** | If you have not already done so, follow the first two steps in the **Measuring XYZ Distance Overview.** |
| **2** | To enable display of the measurement overlay, select the *Show Overlay* check box so that a check mark appears in it. |
| **3** | If you want to display measurement lines (and values) for all pairs of points, select the *Accumulated* check box.<br><br>**OR**<br><br>If you only want to display the measurement lines (and values) for the most recently measured pair of points, leave the *Accumulated* check box cleared. |
| **4** | If you want to display the individual points along a multi-point track, select the *Show Nodes* check box. |
| **5** | If you want to display measurement values directly in the image overlay, select the *Stamp Distances* check box.<br><br>**OR**<br><br>If you want to display only the measurement lines in the image overlay, but not the distance values, leave the *Stamp Distances* check box cleared. (The distance measurement will still be displayed in the *Line Length* status line in the Measure XYZ Distance dialog box.) |
| **6** | Make your color selections for the measurement overlay. Select a color for the distance line from the *Line Color* drop-down list box. Then select a color for the starting point of the distance being measured from the *Pt 1 Color* list. |

## Performing a Wireframe Rotation with Measure XYZ Distance

To perform a three-dimensional wireframe rotation of the images and the measurement track overlays, use the following procedure:

| Step | Action |
|---|---|
| **1** | If you have not already done so, follow the first 11 steps in the **Measuring XYZ Distance Overview.** |
| **2** | To adjust the angle settings for the wireframe view, use the *Angle of First View, Angle of Last View,* and *Angle Between Views* options to select the desired settings. |
| **3** | From the *Rotation* option button group, select a wireframe rotation direction: *Horizontal* or |

*Vertical.*

**4** If you want to see a preview of the wireframe before building the actual three-dimensional display, select the *Show Preview* check box. A preview of the wireframe will be displayed in a Preview window.

**AND**
Then select an angle of view from the *Preview Angle* spin box. This angle will also be affected by the direction of rotation you selected in Step 3.

**5** When you are ready to build the wireframe display, choose *Build Wireframe.* The three-dimensional wireframe representation will be created and displayed in a Wireframe window.

**6** You can "play" the wireframe display back and forth by choosing the Change Planes Tool from among its Image Window Tools and manipulating the slider in the Change Plane dialog box that appears.

## Measure XYZ Distance - Dialog Box Options

### Stack

Selects the image stack to be used for measuring distance.

### Show Overlay

Enables or disables display of the measurement overlay.

### Accumulated

Determines whether all distance measurements will be displayed in the image overlay (checked) or only the last measurement (unchecked). Clearing this check box may aid in viewing image details without interference from the overlay.

> **Note:** All measurements and their graphical elements will be retained even if the *Accumulated* check box is left cleared. If you then select the *Accumulated* check box, all measurements and their graphical elements will be displayed.

### Show Nodes

Determines whether or not the individual points in a measurement track are to be indicated by a dot in the measurement track overlay.

### Stamp Distances

Determines whether or not measurement values will be displayed in the image overlay. Note: The distance will be still be displayed in the Measure XYZ Distance dialog box's *Line Length* status line.

### Line Color

Selects a color for the distance measurement lines and their values displayed in the image overlay.

### Pt 1 Color

Selects a color for the starting point of the distance measurement lines. This color will also be used to display the current point while you are selecting a series of points.

### Use Z Distance

Determines how the Z-distance is to be calculated. *Calibrated* will use the currently active distance

calibration (measured in pixels if you have not applied the Calibrate Distances command, or in calibrated units if you have). *User Specified* allows you to specify the between-plane distance with the *Z Step* spin box.

### X:Y:Z Units

Indicates the ratio between X, Y, and Z distances, as well as the units used. If the Calibrate Distances command has not been applied, distances will be expressed in pixels.

### Z Step

Specifies a step size for the between-plane Z-distance. This option will only appear if you select *User Specified* from the *Use Z Distance* group.

### Line Length

Indicates the distance between the last measured pair of endpoints.

### Preview Angle

Selects an angle of view for the wireframe Preview window. This view will also be affected by your selection from the *Rotation* option button group.

### Show Preview

Displays a wireframe preview of the measurement track in a separate Preview window.

### Angle of First View

Sets the angle by which the first reconstructed view will be offset from zero degrees.

### Angle of Last View

Sets the angle by which the last reconstructed view will be offset from zero degrees.

### Angle Between Views

Sets the interval between adjacent views in the stack.

### Rotation

Selects a direction *(Horizontal* or *Vertical)* for the wireframe rotation.

### Build Wireframe

Creates a three-dimensional wireframe representation of the measurement track and displays it in a separate Wireframe window.

### Undo Click

Removes the last point added. You can use this command button repeatedly to remove several points you have just added.

### Clear Data

Clears all measurements and removes the image overlay.

### Log Total Line Only

When this option is selected, only the data for the entire measurement track will be logged. If you leave this check box cleared, the distance data for each pair of points in the track will also be logged.

### Open Log/Log Data

Opens a data log and/or a DDE link to an open spreadsheet application for logging data. This command will change to *F9: Log Data* when a log file is open. Subsequently choosing the button or pressing the [F9] key will save the distance measurement data for all currently displayed distance lines (distances, starting and ending XYZ coordinates, elapsed time, etc.) in the data log. If you selected the *Accumulated* check box, all distances measured for the image since the dialog box was opened, or since the you last chose *Clear Measurements,* will be logged. If did not select *Accumulated,* only the last distance measured will be stored.

### Configure Log

Opens the Configure Log dialog box so that you can select the parameters to be logged to the data log.

Parameters marked with a check mark in the *Configuration* list will be logged during subsequent measurements.

If you select *Log Column Titles*, a line listing the measurement titles will be logged (1) the first time you use the configured measurement, (2) whenever you enable/disable measurement parameters, or (3) whenever the logged measurement is different from the previous measurement in the log file.

If you select *Place Log Data on Current Line*, subsequently logged data will be appended to the current line in the log file, rather than to a new line. *Log Column Titles* will be unavailable when you select this option.

### Close

Closes the dialog box.

# Measure Volume (Apps Menu)

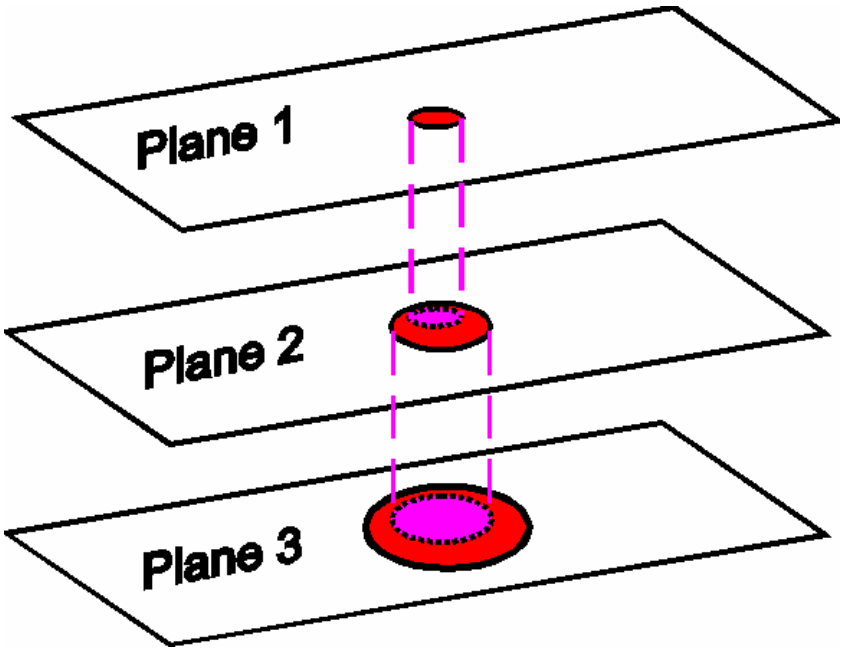
**Measures the volume of a thresholded object through all of the planes in a stack.**

*Availability: Available for MetaMorph Basic; included in MetaMorph Premier*

**Drop-in: MVOLUME**

Use this command to measure the volume of an object in a Z-series stack of through-focus images. This measurement requires that the images first be thresholded to separate the object to be measured from its background. You must also draw a region of interest around the object, to delineate the extent of the thresholded region.

The thresholded area in each image plane will be used to determine the volume of the object between that plane and the next. MetaMorph does not interpolate the outline of the object as it passes between planes, but, rather, simply extends the outline of the object from one plane to the next, as illustrated in the following figure. Thus, the measured volume is only an approximation of the actual volume of the object.



**Note:** The algorithm used for measuring object volume instructs MetaMorph to assume that small, poorly defined objects may not show up in every image plane. Thus, if an object in one plane is "missing" in the very next plane but reappears in the subsequent plane, MetaMorph will interpolate the volume of the object from its positions in the first and third planes.

If you have not used the Calibrate Distances command, volumes will be expressed in terms of pixel volumes (voxels). If you want to express volume in another unit, such as cubic microns, you must first apply Calibrate Distances.

If necessary, you should also specify a between-planes distance in the Change Plane dialog box's *Z Distance* text box. Alternatively, you can specify a user-defined relationship between the Z-distance unit on the one hand, and the X and Y distance unit on the other.

## Measuring Volume

To measure the volume of an object through a stack, use the following procedure.

**Note:** If you want the volumes to be expressed in calibrated distance units, rather than pixels, you must first use the Calibrate Distances command.)

| Step | Action |
|---|---|
| **1** | Threshold the image stack using either the Threshold Tool and Slider or the Threshold Image command (Process menu). |
| **2** | Define a region of interest around the object using the Rectangular Region**, Ellipse Region,** or Trace Region Tool. |
| **3** | If necessary, specify a between-plane distance using the Set Plane Z Distance command. |
| **4** | From the Apps menu, choose Measure Volume. The Measure Volume dialog box opens. The *Volume* status line will inform you if any steps have been overlooked, and will automatically indicate the measured volume. |
| **5** | Use the *Image* selector to select the image stack that contains the object to be measured. |
| **6** | To specify how the Z-distance units relate to the X and Y units, select *Calibration* from the *Relationship of Z to X-Y* group if you want the relationship to be defined by the Calibrate Distances command and the *Z Distance* setting in the Change Planes Tool's dialog box.<br><br>**OR**<br>Select *Custom* if you want to use a user-defined Z-distance. Then specify the arithmetic relationship of the Z-distance unit to the X and Y distance unit in the *1 XY Unit* = text box.<br><br>The *X:Y:Z* status line will indicate the selected relationship between distances in the three dimensions. |
| **7** | If you want to save the volume measurement in a log file, choose Open Log and open |

either a text-based data log file or a Dynamic Data Exchange (DDE) link to an open spreadsheet. The *Open Log* button text will change to *"F9: Log Data."*

**OR**
If you do not want to log the measurement data, skip to Step 10.

**8** If desired, choose *Configure Log* to select the measurement parameters to be logged. The Configure Log dialog box will appear. Double-click the entries in the *Configuration* list to select the parameters you want to log. Then choose *OK* to return to the Measure Volume dialog box.

**9** When you are ready to log the measurement data, choose *F9: Log Data,* or press the [F9] function key to use the keyboard shortcut.

**10** When you have finished, choose *Close.*

## Measure Volume - Dialog Box Options

### Image

Selects the image stack that contains the object you want to measure.

### Volume

Displays the measured volume. If the image has not been thresholded, or if a region of interest has not been defined, this status line will indicate this to you.

### Relationship of Z to X-Y

Specifies the method by which the Z-distance is to be related to the X and Y distances. Select *Calibration* if the Z-distance is to be derived from the calibration applied with the Calibrate Distances command and the Change Planes Tool's *Z Distance* option. Select *Custom* if you want to specify a relationship in the *1 XY Unit* = text box.

### 1 XY Unit =

If you selected *Custom* from the *Relationship of Z to X-Y* radio button group, this text box specifies the arithmetic relationship between the Z-distance units and the X and Y distance units. If you type *1,* the Z-distance units will be equal to the X and Y distance units.

### X:Y:Z

Indicates the relationship between the X, Y, and Z distance units.

### Open Log/Log Data

Opens a data log and/or a DDE link to an open spreadsheet application for logging volume measurement data. This command will change to *F9: Log Data* when a log file is open. Subsequently choosing the button or pressing the [F9] key will save the volume measurement data in the data log.

### Configure Log

Opens the Configure Log dialog box so that you can select the parameters to be logged to the data log. Parameters marked with a check mark in the *Configuration* list will be logged when you choose *F9: Log Data.*

### Close

Closes the dialog box.

# Measure Object Distance (Measure Menu)

**Displays the distance of that part of a drawn line which is within the boundaries of a binary or thresholded object.**

**Drop-in: OBJDIST**

Use this command in conjunction with a region drawn with a Line Region Tool to determine the distance of a thresholded or binary object. The line region should pass through the object completely. Assuming the line is straight, this command reports both the distance of the entire line and the distance of the line over the object.

The distance measurement is displayed in calibrated units, which can be selected using the Calibrate Distances command. If the units have not been calibrated, MetaMorph will display the distance in pixels.

You can log each measurement to an open data log if desired. First, use the Open Data Log command to open a data log. You can use the Log Data command or its keyboard shortcut, the [F9] function key, to log the data.

Measure Object Distance allows you to measure the length of a thresholded or binary object for all planes in a stack, all images in a directory, or from live video at near video rate (for example, a contracting cell whose edges are thresholded). To measure objects in a stack, use this command with Loop for All Planes (Journal menu). To measure objects in all images in a directory, use this command with Loop for All Images in Directory (Journal menu). To measure objects in Live Video, use the Loop a Journal command (Journal menu) with an extended line region drawn over the thresholded Live Video image window.

## Measuring Object Distance

To measure the distance of an object, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Measure menu, choose Measure Object Distance. The Measure Object Distance dialog box will appear. |
| **2** | Select the desired image using the *Image* selector.<br>**AND**<br>Threshold the image using the Threshold Tool. |
| **3** | If you want to log measurement data, open a data log using the **Open Data Log** command. |
| **4** | To configure the data log for logging, choose *Configure Log.* The Configure Log dialog box will appear.<br>**AND**<br>From the *Configuration* list, select the parameters you want to enable for logging, so that each is marked by a check mark next to its entry (you can choose *Enable All* or *Disable All* if you want to select or deselect all of the parameters listed).<br>Choose *OK* to return to the Measure Object Distance dialog box. |

5 Draw a line across the object using the Single Line Tool, Multi-Line Tool, or Traced Line Tool. The line must cross the object completely.

6 Select the desired line so that it is the active region. MetaMorph will measure the distance of the line and display it in the dialog box.

7 If you want to log the measured distance, choose *F9: Log Data.*

8 To measure another distance, create and select another line using the process described in Steps 5 - 7.

**Note:** You can edit the distance of the line by double-clicking the mouse pointer on the line with the left mouse button and moving the vertices.

9 Choose *Close* when you have finished.

## Measure Object Distance - Dialog Box Options

### Image

Selects the image for measuring object distances.

### Distance

Displays the data from the distance measurement for the entire line region.

### Object Distance

Displays the data from the current object distance measurement, based on the thresholding. This assumes that the original line is straight.

### Open Log

Opens a data log and/or a DDE link to an open spreadsheet application for logging data. This command changes to *F9: Log Data* when a log file is open.

### F9: Log Data

Logs the currently displayed data from the dialog box to an open data log or to an open spreadsheet application by way of a DDE link. To assist you in logging the proper data when several measurement dialog boxes are open, *"F9"* is added to the name of this option in the active dialog box to indicate which data will be logged when you press [F9].

### Configure Log

Opens the Configure Log dialog box so that you can select the parameters to be logged to the data log. Parameters marked with a check mark will be logged for subsequent measurements.

If you select ***Log Column Titles***, a line listing the measurement titles will be logged (1) the first time the you use the configured measurement, (2) whenever you enable/disable measurement parameters, or (3) whenever the logged measurement is different from the previous measurement in the log file.

If you select *Place* ***Log Data on Current Line***, subsequently logged data will be appended to the current line in the log file, rather than to a new line. *Log Column Titles* will be unavailable when you select this option.

### Close

Closes the dialog box.

# Track Objects (Apps Menu)

**Tracks one or more selected objects through each image in an image stack or a sequential series of single images. You can derive measurements of the paths, positions, and velocities of the points.**

*Availability: Available for MetaMorph Basic; included in MetaMorph Premier*

### Drop-in: TRACKOBJ

Use this command when you want to measure the movement of one or more objects between successive images in a stack or a sequential series of images. You can track the movements over time of individual tagged particles, such as fluorescently labeled cell surface molecules, microtubules, nucleic acids, or lipids. Typically, this procedure will be performed to determine whether or not the molecules being tracked are stationary, move in a straight line, or move in a "random walk."

This procedure works particularly well when used with differential interference contrast (DIC) images, as the tracking region can be made to encompass both the white and black "spots" produced by this method. This gives you twice as many pixels to track, leading to a proportionate increase in the tracking precision.

After you have configured the tracking parameters and defined the objects to be tracked, MetaMorph will determine the intensity centroids of the defined target regions, and track their displacements automatically through the planes in the source image stack. Each particle is imaged as an Airy disk covering many pixels. The Airy disk is imaged with high contrast, and its position is determined with sub-pixel accuracy. The image of the particle is then tracked using a cross-correlation centroid-finding algorithm to determine the best match of the particle position in successive images. A search based on image thresholding is also available.

**WARNING:**
You will not be able to switch to another stack or add a plane to an image stack after you have already measured it. Doing so will generate an error message informing you the track data are no longer valid, and the data will be cleared and all data displays will be closed.

As you select your options to configure the object tracking, you will also have the opportunity to select a journal to run during the tracking. In this way, you can perform processing on the image prior to tracking each point. Because you will need to configure your processing journal to overwrite the original images (so as to retain the original images' file name), we suggest that you make duplicates of the original images and track the objects in *them* if you do not want the original images to be altered by the journal.

For dealing with displacement data, you will need to define an "origin point" to which object positions can be referred. One option *(Corner of Image)* is to select the upper left corner of the image. In this mode, all positions for each object will be expressed as absolute positions in the image. A second option *(First Point in Track)* uses the location of the object at its first position in the track. In this mode, the position of each object will be expressed in terms of its starting point. The third option *(Corresponding Point of First Object)* expresses the positions of all objects in terms of the location of the first defined object within the same image plane. This method can be particularly useful for accounting for drift when the first "object" is a fixed point. Alternatively, you can use this method to measure such phenomena as elongation of microtubules or transport of labeled proteins.

> **QUICK *TIP*:** You can erase all tracks with the Clear Measurement Stamps command (Graphics menu) or use the keyboard shortcut, ALT + C.

When the tracking procedure is complete, you will have the opportunity to edit the data. You can change the coordinates of a point or delete it altogether. You can then display and print the motion measurements that have been derived, such as particle X and Y coordinates, velocity, mean displacement, and mean vector length. Other measures include the mean angle (the angle of the mean vector of the object) and the angular deviation (analogous to the standard deviation in linear statistics). The selected variables can be displayed in a configurable and printable scatterplot graph, which, like the data tables, can be sent to a printer or copied to the Clipboard for use in a graphics or word processing program.

**Note:** This command does not support 24-bit color or binary (1-bit) images.

## Tracking Objects - Procedures

**Overview of Object Tracking**

### Configuring Object Tracking:

**Configuring Data Logging**

**Configuring the Search**

**Configuring the Time Intervals**

**Configuring the Track Overlays**

**Selecting and Tracking Objects**

### Viewing and Editing Object Tracking Data:

**Viewing Track Data**

**Viewing Point-by-Point Data**

**Viewing Data Graphs**

**Editing Track Data**

## Overview of Object Tracking

To track objects and measure their movement through a series of images, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, choose Track Objects. The Track Objects dialog box opens. |
| **2** | From the *Source for Images* option button group, select the type of images that you will use for object tracking: *Stack,* or *Sequential Files.* |
| **3** | If you selected *Stack* in Step 2, use the *Stack* image selector to select the stack that you want to use. Then use the *Plane… to…* spin boxes to select which planes in the stack that you want to use.<br>**OR**<br>If you selected *Sequential Files* in Step 2, choose the *Select Files* button, and select the first and last image in the series from the Select First Image and Select Last Image dialog boxes that appear. |
| **4** | If you want to run a journal to process each source image before the tracking protocol is applied, choose *Select Journal,* and choose the journal from the Select a Journal to Run dialog box that appears. Then choose *Open.* |
| **5** | If you wish, **open and configure a data log** to store the object tracking data.<br>**Note:** You can also perform this step after you have already carried out the tracking |

procedure.

**6** Choose *Search Options,* and **configure the search algorithm** and its behavior from the Search Options dialog box that appears.

**7** If you want to **configure the time units** in the data tables and displays, choose *Set Interval* and make your selections from the Track Objects Interval Options dialog box that appears.

If you want to configure the origin, click Set Origin to open the Set Origin dialog box.

**8** If necessary, use the *Set Overlay* command to **configure the track overlays** that will be displayed during object tracking.

**9** To define an "origin point" to which object positions can be referred, choose *Set Origin.* Then make a selection from the Origin Options dialog box that appears: *Corner of Image, First Point in Track,* or *Corresponding Point of First Object.*

When you have finished, choose *OK* to return to the Track Object main dialog box.

**10** When you are ready, choose *Track,* and **select the objects to be tracked. Object tracking will then proceed automatically.**

**11** If you are saving the tracking data in a data log (see Step 5), choose *Log Data* when object tracking has completed.

**12** You can choose any of three ways to display the object position and path data.

Choose ***Display Statistics*** to view a table that shows data for each object's entire path.

Choose ***Display Data*** to view a table that shows the frame-by-frame data for each object.

Choose ***Graph Data*** to view configurable scatterplots that show point or path data for each of the objects.

**13** If you need to edit the data points after tracking is complete, you will have the opportunity to do so. Choose *Edit Data,* and **make your changes** from the Track Objects: Edit Data dialog box.

**14** If, for clarity's sake, you want to view an otherwise blank image that displays the track overlays, click the *Duplicate Overlay* button that has appeared in the dialog box.

**15** When you have finished, choose *Close.*

## Configuring Data Logging for Track Objects

To configure logging of object tracking data, use the following procedure:

| Step | Action |
|---|---|
| 1 | If you have not already done so, follow the procedures in the first five steps of the **Overview of Object Tracking.** |
| 2 | If you want to organize the tracking data in the data log by frame, select the *Log by Frame* check box.<br><br>**OR**<br>If you want to organize the data by object number, leave the *Log by Frame* check box cleared. |
| 3 | If you are processing a large number of images (several hundreds) and need to conserve program memory, select the *Log Only: No Data Display* check box. This will prevent the display of the tracking data, and will accordingly reduce the risk of memory resource depletion.<br><br>**OR**<br>If memory usage is not a consideration, leave the *Log Only: No Data Display* check box cleared. |
| 4 | Choose *Open Log* and **select a log file** for data storage. When you have finished, the title on the *Open Log* button will change to *"Log Data."* |
| 5 | Choose ***Configure Log*** to select which data are to be enabled for logging. |
| 6 | After you finish configuring the other tracking options and carry out the tracking, you can choose *Log Data* to save the tracking data in the log file. |
| 7 | If you want to save the statistical summary data for each object's entire path, you will be able to open and configure a summary log and log the data from the **Track Objects Statistics dialog box.** |

## Configuring the Search Options for Track Objects

To configure the Track Objects search algorithm and its behavior, use the following procedure:

| Step | Action |
|---|---|
| 1 | If you have not already done so, follow the procedures in the first four steps of the **Overview of Object Tracking.** |
| 2 | Choose *Search Options.* The Search Options dialog box will appear. |
| 3 | From the *Algorithm* drop-down list, select the method by which MetaMorph decides whether an object it finds is the same as the object it found in the preceding frame:<br><br>***Template Match*** filters each new frame using a convolution mask that is based |

on the object's intensity values in the preceding frame. The centroid of the object will be determined from the convolved image's intensity peak, using a percentage of the original peak intensity (specified by *Minimum % for Match)* as the lowest acceptable value. (For more on image convolution and masks, be sure to read the chapter on *Using Image Filters* in your *MetaMorph Task Guide.)*

***Threshold Result*** simply detects the center of the intensity peaks within the thresholded target areas and determines which object in the preceding frame is closest.

**4** To configure the search behavior for cases in which MetaMorph is unable to find an object in a given frame, make a selection from the *If Object Not Found* option button group. Select

***Click on Position*** if you want to use your pointer to click on what you perceive to be the object,

***Quit Object*** if you want MetaMorph to stop searching for the object in all subsequent frames, or

***Skip Frame*** if you want MetaMorph to omit this frame as a data point for the object and to continue the search for the object in the next frame.

**5** If you want MetaMorph to extrapolate where the center of an object should be based on the "velocity" of its movement calculated from the previous two frames, select *Use Velocity for Center of Next Search.*

**6** If you want to slow down the tracking process, for example to be able to catch errors as they occur, use the *Delay* spin box to specify the time, in seconds, that each frame will be displayed before proceeding to the next.

**7** If you selected *Template Match* in Step 3, several more options will be displayed. Continue to Step 8.

**OR**
If you selected *Threshold Result* in Step 3, skip to Step 10.

**8** Ordinarily, the Template Match algorithm will use the same convolution mask for every frame. If you are tracking objects that change in shape and intensity, you will need to have the template recalculate the convolution mask for each frame. If so, select the *Update Template Each Frame* check box.

**9** In performing the Template Match convolution, MetaMorph looks for intensity maxima in the convolved image. If an

object's shape or intensities change considerably from their original state, the intensity levels in the convolved image may be quite low. Accordingly, you will need to inform MetaMorph of a cutoff intensity level below which it should discard the object "match."

Use the *Minimum % for Match* spin box to specify what the cutoff should be, expressed as a percentage of the original peak object intensity. Then skip to Step 11.

**10** If you selected *Threshold Result* as the search algorithm in Step 3, the *Object Size Match Requirement (as %)* spin box will appear. Because an object may appear to change in size from frame to frame, you should use this option to select a range of object sizes within which an object detected in a subsequent frame will be considered as a "positive match."

**11** When you have finished, choose *OK* to return to the Track Objects dialog box.

## Configuring the Time Interval Options for Track Objects

To configure the time units used for the object tracking data, use the following procedure:

| Step | Action |
|---|---|
| **1** | If you have not already done so, follow the procedures in the first six steps of the **Overview of Object Tracking.** |
| **2** | Choose *Set Interval.* The Track Objects Interval Options dialog box will appear. |
| **3** | From the *Table Time Units* drop-down list, select the time units to be used: *Milliseconds, Seconds, Minutes,* or *Hours.* |
| **4** | From the Time Interval Options button group, select the method by which the image time is to be determined. Select<br><br>***Time Image was Created*** if you want to take the image time from the image's timestamp, which stores the time of creation or last modification, or<br><br>Check ***User Defined*** if you want to specify a time interval between frames. If you select this option, the *Time Interval* spin box and drop-down list will become available. |
| **5** | If you selected *User Defined* in Step 4, select a time interval between frames from the *Time Interval* spin box and drop-down list. Otherwise, continue to Step 6. |
| **6** | When you have finished, choose *OK* to return to the Track Objects dialog box.<br><br>If you have already performed object |

tracking, the values in the displayed table and graphs will be updated based on the new time intervals, and subsequent logging of data will use the updated values. Data already logged will not be changed.

## Configuring the Overlay Options for Track Objects

To configure the track overlays that will be displayed during object tracking, use the following procedure:

| Step | Action |
|---|---|
| **1** | If you have not already done so, follow the procedures in the first seven steps of the **Overview of Object Tracking.** |
| **2** | Choose *Set Overlay.* The Track Overlay Options dialog box will appear. |
| **3** | To change the color of the Track Objects marker and path, select the desired color from the *Track Points Color* list. To change the shape of the Track Points marker, select the desired shape from the *Point Marker Type* list.<br><br>If you choose a *Circle* marker and want to fill in the circle, select the *Fill Circle Markers* check box. You can change the size of the Track Points marker with the *Point Marker Size* spin box.<br><br>**Note:** The size of the *Dot* marker can not be changed. For a larger dot marker, select a *Circle* marker shape and select the *Fill Circle Markers* check box. |
| **4** | Use the *Point Marker Display Mode* radio button group to choose between an overlay display that shows all points in a track (*Display All Points*) and one that shows only the point in the current frame or image plane (*Display Point on Current Plane*). |
| **5** | The track path and number can be displayed or hidden by selecting or clearing the *Display Track Path* and *Display Track Numbers* check boxes, respectively. |
| **6** | A track "pattern" can be added to the overlay, showing lines that connect the points in an individual plane. The patterns from each plane will be displayed simultaneously. This option can be enabled and disabled from the *Display Track Pattern* check box.<br><br>**AND**<br>The color of the pattern in the plane being viewed can be changed in the *Track Pattern Color* list. The patterns from other planes will continue to be displayed in red. This option also controls the color of the track numbers displayed in the image window overlay. |
| **7** | When you are satisfied with all of your |

selected graphics options, choose *OK.* Your options will then take effect.

## Selecting and Tracking Objects

To select the image objects to be tracked and to perform the tracking, use the following procedure:

| Step | Action |
|---|---|
| **1** | If you have not already done so, follow the procedures in the first eight steps of the **Overview of Object Tracking.** |
| **2** | When you are ready to select the objects to be tracked, choose *Track.* The Select Objects dialog box will appear. |
| **3** | Select the objects in the first image frame by holding down the [CTRL] button and clicking the objects using the left mouse button.<br><br>A rectangular object region will appear around each object as you click it, and a larger search region will appear around the object region. |
| **4** | If you want to use the same size for all object and search regions, select the *Lock Region Sizes* check box.<br><br>**OR**<br>If you want to use different sized object and search regions for the various objects, leave the *Lock Region Sizes* check box cleared. |
| **5** | You should configure the search region so that it is large enough that no part of the object will extend beyond the edge of the search region in the subsequent frame. You can modify the size of the object and search regions by dragging the outlines with your pointer. Alternatively, you can use the four spin boxes in the Select Object dialog box to specify the object and search region heights and widths, expressed in pixels.<br><br>**AND**<br>You can move object and search regions by clicking inside the region and dragging it to the desired location. |
| **6** | When you are satisfied with the size and placement of the object and search regions, choose *OK.*<br><br>Object tracking will proceed automatically. Colored object tracks will be drawn in the images, updating as successive frames are processed.<br><br>**WARNING:**<br>You will not be able to switch to another stack or add a plane to an image stack after you have already measured it. Doing so will generate an error message informing you the track data are no longer valid, and the data |

will be cleared and all data displays will be closed.

**7** If an object is "lost" during the tracking, MetaMorph will proceed according to your selection in the *If Object Not Found* options group in the Search Options dialog box.

You can also stop object tracking by pressing the [ESC] key. Doing so will display the Tracking Halted dialog box, from which you can make a selection for what MetaMorph should do next: *End Tracking, Quit Object, Skip This Point, Step Back, Update Position and Continue,* or *Update Template and Continue.* If you need to resize the tracking regions, make sure that the *Lock Region Sizes* check box is cleared and use the Locator Tool to drag the edges of the regions. You also have the option of switching the tracking overlay on and off with the *Overlay* option buttons.

**Note:** The *Update Template and Continue* option will be available only when *Template Match* has been selected as the *Algorithm* in the Search Options dialog box.

## Viewing Track Data

To display the track statistics for individual points, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Track Objects dialog box, choose *Display Statistics.* The Track Objects Statistics dialog box opens. |
| **2** | You can use the horizontal and vertical sliders, at the lower edge and at the right of the data table, respectively, to scroll from side to side or up and down through the table to see the data for each object.<br><br>If necessary, you can increase the size of the Track Objects Statistics dialog box by dragging its borders. You can also change the width of the data columns by placing the pointer between the columns at the top of the table and dragging the column border. |
| **3** | If you want to log the track data, choose *Open Log*. When you have finished selecting a summary log file, the text on the *Open Log* button will change to *"Log Data."*<br><br>**AND**<br>If you want to select which track parameters to store in the summary log, choose *Config Log* and make your selection from the *Configuration* table of the Configure Log dialog box that appears. Then choose *OK* to return to the Track Objects Statistics dialog box. |
| **4** | To save the track data in the summary log |

you opened in Step 3, choose *Log Data.*

**5** If you want to print the data table, choose *Print Table.* A message box will appear, asking you to confirm the print request. Choose *Yes* to proceed with the printing.

**6** When you have finished, choose *Close.*

## Viewing Point-by-Point Data

To display the point-by-point measurement data for individual objects, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Track Objects dialog box, choose *Display Data.* The Track Objects Data dialog box will appear. |
| **2** | You can use the horizontal and vertical sliders, at the lower edge and at the right of the data table, respectively, to scroll from side to side or up and down through the table to see the data for each object at each position. Clicking on a table entry will switch the image display to the corresponding frame.<br><br>If necessary, you can increase the size of the Track Objects Data dialog box by dragging its borders. You can also change the width of the data columns by placing the pointer between the columns at the top of the table and dragging the column border. |
| **3** | To switch between **data views,** make a selection from the *Data Type* option button group. |
| **4** | If you want to print the data table, choose *Print Table.* A message box will appear, asking you to confirm the print request. Choose *Yes* to proceed with the printing. |
| **5** | When you have finished, choose *Close.* |

## Viewing Data Graphs

To display scatterplot graphs showing object data over time or plane number, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Track Objects dialog box, choose *Graph Data.* The Track Objects Graph dialog box will appear. |
| **2** | From the *Data Type* option button group, select the data that you want to display.<br><br>***Position****:* The X and Y coordinate at each position.<br><br>***Distance****:* The absolute distance between each position and the one immediately preceding. |

***DeltaXY**:* The change in X and Y values at each position, relative to the immediately preceding position.

***Velocity**:* The distance moved per time unit or from one position to the next.

***Angle**:* The angle of the path taken by the object from its previous position. Angles are measured from the "nine o'clock" position and will range from 0 to 180 degrees. Downward angles are expressed as positive numbers, and upward movements are expressed as negative numbers.

***Dist. to Origin**:* The straight-line distance between the object's current position and the Origin.

**3** If you want to display the data points for just a single object, select the *Graph Single Object* check box. Then use the associated spin box to select the number of the desired point.

**OR**
If you want to display the data points for all objects, leave the *Graph Single Object* check box cleared.

**4** To display the data in terms of time, select *Time* from the *X-Axis* radio button group.

**OR**

To display the data in terms of plane number, select *Plane* from the *X-Axis* radio button group.

**5** To change the colors or labeling in the graph, click the Down Arrow button and choose the appropriate command from the configuration pop-up menu that appears.

**6** If you want to print the graph, click the Down Arrow button and choose Print from the configuration pop-up menu.

**7** You can also copy the graph to the Clipboard, so that you can paste it into another program, such as a graphics or word processing program. To do so, click the Down Arrow button and choose Copy to Clipboard from the configuration pop-up menu. Then use the appropriate Paste command to import the graph to the other program (most programs support the CTRL + V keyboard shortcut).

**8** When you have finished, choose *Close.*

## Editing Object Tracking Data

To edit the object tracking data, use the following procedure:

| Step | Action |
|---|---|

1. From the Track Objects main dialog box, choose *Edit Data.* The Track Objects: Edit Data dialog box will appear.

2. Use the *Frame* # spin box to select the frame containing the "bad" or missing point, and use the *Object* # spin box to select the number of the object being tracked.

   Clicking on the data in the graph or either data table will also update the source stack and the Edit Data dialog box to the appropriate plane. Changing the plane in the source stack will also update the setting in the *Frame* # spin box.

3. To change the location of a "bad" point, use the *Position on Image* spin boxes to select the position, relative to the Origin. Then choose *Done,* or go to the next frame.

   **OR**
   Alternatively, you can hold down the [CTRL] key and click on the location in the image where a "bad" or missing point ought to be.

4. If you need to undo your change and you have not yet moved to a new frame or object, choose *Reset Point.*

5. If you need to remove the active point (that is, the currently selected point), choose *Delete Point.*

   **CAUTION:** Be sure to verify that you have selected the point to be removed. The *Object* # spin box should be set to the desired object before you remove the current point.

6. When you have finished, choose *Done.*

## Track Objects - Dialog Box Options

### Track Objects Main Dialog Box

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## Track Objects: Overview of Dialog Box Options

### Source for Images

Selects the type of images that will be used for object tracking: *Stack* or *Sequential Files. I*f you select *Stack,* the *Stack* image selector will appear. If you select *Sequential Files,* the *Select Files* command button will appear.

### Plane… to…

Selects the range of planes in the source stack that you want to use in the object tracking. This set of spin boxes becomes available when you select *Stack* from the *Source for Images* option button group.

### Stack

Selects the image stack to be used for object tracking. This option appears only if you select *Stack* from the *Source for Images* option button group.

### Select Files

Opens the Select First Image dialog box, from which you can select the first image in the sequential series to be used for object tracking. After you choose *OK,* the Select Last Image dialog box will appear, from which you can select the last image to be included in the tracking procedure. The first and last image that you select must be from the same sequential image series. The *Select Files* button appears only if you select *Sequential Files* from the *Source for Images* option button group.

### Select Journal

Opens the Select a Journal to Run dialog box, from which you can select a journal to process each source image before the tracking protocol is applied to it. Because you will need to configure your processing journal to overwrite the original images (so as to retain the original images' file name), we suggest that you make duplicates of the original images and track the objects in *them* if you do not want the original images to be altered by the journal.

### Log by Frame

When you select this check box, data being sent to a data log will be organized by position (image frame) number. If you leave this check box cleared, the data will be organized by object number.

### Log Empty Lines

When selected, the log will show a line for every frame even when no object is found. This ensures that every frame will be logged.

### Log Only: No Data Display

When selected, this check box prevents the display of the tracking data, thereby conserving system memory resources. Data will be logged automatically, but the data tables and graphs will be unavailable, as will their corresponding *Display Statistics, Display Data,* and *Graph Data* command buttons. When you use *Log Only,* be sure that the interval is set correctly before tracking, because changing an incorrect interval after tracking will not correct any data that are already logged.

### Open Log/Log Data

Opens a data log for storing the frame-by-frame object data. After you open the data log, the text on this button will change to *"Log Data."* Choosing this button will then save the object tracking data set to the data log.

### Config Log

Allows the selection of tracking data that are to be included or excluded from data logging. Also allows a choice of whether column titles are to be included and if data are to be listed on a single line.

### Search Options

Opens the Search Options dialog box, from which you can configure the search algorithm and its behavior.

### Set Interval

Opens the Track Objects Interval Options dialog box, from which you can configure the time units used for the object tracking data.

### Set Overlay

Opens the Track Overlay Options dialog box, from which you can configure the track overlays that will be displayed during object tracking.

### Set Origin

Opens the Origin Options dialog box, from which you can configure the origin that the object will be measured against.

### Display Statistics

Opens the Track Objects Statistics dialog box, which displays the track statistics for each object's entire path.

### Display Data

Opens the Track Objects Data dialog box, which displays the frame-by-frame data for each object.

### Graph Data

Opens the Track Objects Graph dialog box, which displays configurable scatterplot graphs showing point or path data for each of the objects.

### Edit Data

Opens the Track Objects: Edit Data dialog box, which you can use after tracking to change the position of a point or to remove the point altogether.

### Duplicate Overlay

Creates a blank image with a copy of the track overlays, as currently displayed on the tracking image.

### Track

Opens the Select Objects dialog box**,** which you use to define regions that contain objects that you want to track. After you create and configure the object and search regions and choose *OK,* the tracking procedure and data analysis will proceed automatically.

### Close

Closes the Track Objects dialog box.

## Track Objects: Search - Dialog Box Options

### Algorithm

Selects the method by which MetaMorph decides whether an object it finds is the same as the object it found in the preceding image frame. Two methods are available:

> ***Template Match*** filters each frame using a convolution mask that is based on the object's grayscale intensity values in the preceding frame. The centroid of the object will be determined from the convolved image's intensity peak, using a percentage of the original peak intensity (specified by *Minimum % for Match)* as the lowest acceptable value. When you select *Template Match,* the *Update Template Each Frame* check box and *Minimum % for Match* spin box will appear. (For more on image convolution and masks, be sure to read the chapter on *Using Image Filters* in your *MetaMorph Task Guide.)*
>
> ***Threshold Result*** detects the center of the intensity peaks in the current image frame and determines which object in the preceding frame is closest.

### If Object Not Found

Configures the search behavior for cases in which MetaMorph is unable to find an object in a given frame.

> ***Click on Position*** allows you to use your pointer to click on what you perceive to be the object. If

MetaMorph fails to find the object, a message box will appear, instructing you to click on the position of the "missing" object. If you can not find the object, you can choose *End Tracking* to terminate the entire object tracking procedure. (If you selected *Template Match* as the search algorithm, this message box will also have an *Update Template* check box, which you can select to recalculate the template.) Otherwise, you can simply choose *OK,* and a second message box will appear, instructing you to click the *Cancel* button in the search image window. The search will then continue for the other objects if they can still be found.

***Quit Object*** instructs MetaMorph to stop searching for the object in all subsequent frames.

***Skip Frame*** directs MetaMorph to interpolate the object track between the last found location and the next location it can detect.

### Use Velocity for Center of Next Search

Directs MetaMorph to extrapolate where the center of an object should be based on the "velocity" of its movement calculated from the previous two frames. This extrapolated position will be used as the center of the search region at the next position.

### Delay

Slows down the tracking process (for example, to monitor the tracking and halt it when an error occurs) by specifying a time, in seconds, that each frame will be displayed before proceeding to the next.

### Update Template Each Frame

Ordinarily, the Template Match algorithm will use the same convolution mask for every frame. The *Update Template Each Frame* check box configures the template to recalculate the convolution mask for each frame. This may be necessary if you are tracking objects that are changing shape or intensities. This option appears only when you select *Template Match* from the *Algorithm* drop-down list.

### Minimum % for Match

In performing the Template Match convolution, MetaMorph looks for intensity maxima in the convolved image. If an object's shape or intensities change considerably from the previous position, the intensity levels in the convolved image may be quite low. The *Minimum % for Match* spin box selects a cutoff intensity level below which MetaMorph is to discard the object "match." This option appears only when you select *Template Match* from the *Algorithm* drop-down list.

### Use Derivative of Image

Uses the derivative of the equation that describes transitions of grayscale values within the image to assist in determining locations of object centroids. This option may be more useful in images that have *not* been acquired with such contrast enhancement methods as differential interference contrast microscopy, and will appear only when you select *Template Match* from the *Algorithm* drop-down list.

### Object Size Match Requirement (as %)

Selects a range of object sizes within which an object detected in a subsequent frame will be considered to be a "positive match" with the object in the preceding frame. This size-matching procedure is often necessary because an object may appear to change in size from frame to frame. This option appears only when you select *Threshold Result* from the *Algorithm* drop-down list.

### OK

Closes the Search Options dialog box.

## Track Objects: Interval - Dialog Box Options

### Table Time Units

Selects the time units to be used for the object tracking data: *Milliseconds, Seconds, Minutes,* or *Hours.*

### Time Interval Options

Selects the method by which the image time is to be determined:

***Time of Image Creation*** takes the image time from the image's timestamp, which stores the time of creation or last modification.

***User Defined*** specifies a user-defined time interval between frames. If you select this option, the *Time Interval* spin box and drop-down list will become available.

### Time Interval

Selects a user-defined time interval between frames. The drop-down list selects the time units, and the spin box selects the number of time units between frames.

### OK

Accepts the currently configured time interval and closes the Track Objects Interval Options dialog box.

### Cancel

Rejects any changes to the time interval configuration and closes the Track Objects Interval Options dialog box.

## Track Overlay Options - Dialog Box Options

### Track Point Color

Selects the color of the Track Objects marker. The default setting is *Red.*

### Point Marker Type

Selects the shape of the Track Objects marker. The default setting is *Cross.*

### Point Marker Size

Selects the size, in pixels, of the Track Objects marker. The default setting is *7.*

### Point Marker Display Mode

Selects between an overlay display that shows all points in a track *(Display All Points)* and one that shows only the point in the current image plane or frame *(Display Point on Current Plane).*

### Fill Circle Markers

If the Track Points marker is changed to a circle, this option will fill the circle, so that it looks like a large dot.

### Display Track Path

Enables or disables the display of track paths.

### Display Track Numbers

Enables or disables the display of track numbers in the image window.

### Display Track Pattern

Enables or disables a display of track patterns, which show a line connecting the points in a plane.

### Track Pattern Color

Selects the color of the track pattern and number as displayed in the image window. The default setting is *Red*. Select *Alternating* if you want MetaMorph to select a color for each track pattern by cycling through the eight colors that are available.

### OK

Closes the dialog box and implements the option changes that were selected.

### Cancel

Closes the dialog box and cancels the option changes that were selected.

## Origin Options (Track Objects) - Dialog Box Options

### Origin Options

Defines an "origin point" to which object positions can be referred:

***Corner of Image*** - Selects the upper left corner of the image. In this mode, all positions for each object will be expressed as absolute positions in the image.

***First Point in Track*** - Uses the location of the object at its first position in the track. In this mode, the position of each object at the second and all subsequent positions will be expressed in terms of its starting point.

***Corresponding Point of First Object*** - Expresses the positions in terms of the location of the first defined object within the same image plane.

### OK

Accepts the *Origin Options* setting and closes the dialog box.

## Track Objects: Select Objects - Dialog Box Options

### Object Region Width

Specifies a width, in pixels, of the region around the object. Alternatively, you can modify the size of the object regions by dragging their outlines with your pointer. The region size spin boxes will become unavailable when you select the *Lock Region Sizes* check box.

### Object Region Height

Specifies a height, in pixels, of the region around the object. Alternatively, you can modify the size of the object regions by dragging their outlines with your pointer. The region size spin boxes will become unavailable when you select the *Lock Region Sizes* check box.

### Search Region Width

Specifies a width, in pixels, of the search region that is defined around the object region. Alternatively, you can modify the size of the object regions by dragging their outlines with your pointer. The region size spin boxes will become unavailable when you select the *Lock Region Sizes* check box. You should configure the search region so that no part of the object will extend beyond the edges of the search region at subsequent positions.

### Search Region Height

Specifies a height, in pixels, of the search region that is defined around the object region. Alternatively, you can modify the size of the object regions by dragging their outlines with your pointer. The region size spin boxes will become unavailable when you select the *Lock Region Sizes* check box. You should configure the search region so that no part of the object will extend beyond the edges of the search region at subsequent positions.

### Lock Region Sizes

Specifies that the same size be used for all object and search regions.

### OK

Accepts the selection of objects and the current configuration of object and search regions, closes the Select Objects dialog box, and starts the object tracking procedure.

### Cancel

Rejects all object and region selections and returns you to the Track Objects dialog box.

## Track Objects: Tracking Halted - Dialog Box Options

### Overlay

Selects a display mode for the tracking overlays:

***On*** - Displays the overlay for all objects.

***Off -*** Removes all tracking overlays.

***This Object*** - Displays the overlay only for the currently selected object.

### Lock Region Sizes

Specifies that the same size be used for all object and search regions. If you locked region sizes prior to selecting objects to track, and now need to change the tracking regions, clear this check box and use the Locator Tool to drag the edges of the regions.

### End Tracking

Stops all object tracking.

### Continue One Step and Pause

Check this box to pause between frames. You can then choose from the following options for each frame:

### Quit Object

Stops the tracking for just the "missing" objects, and continues tracking all others.

### Skip This Point

Skips the current image frame for this object and continues tracking in the subsequent frames.

### Step Back

Moves to the previous object, reversing by one frame if necessary. Stepping back will clear the position data of the points being viewed. This option will be unavailable if you selected the *Log Only: No Data Display* check box in the Track Objects main dialog box.

### Update Position and Continue

After you move the object or search regions, this option will update the position of the object and continue tracking.

### Accept Position and Continue

Accepts the current position of the object and continues tracking.

### Update Template and Continue

After you move the object or search regions, this option will update the template used by the tracking algorithm, and then continue tracking. This option will be available only when *Template Match* has been selected as the *Algorithm* in the Search Options dialog box.

# Track Objects: Stat Table - Dialog Box Options

### Track Objects Statistics Table

Displays the statistical data for each object's track. The following parameters are displayed:

***Mean X ()*** - the mean value of the X-coordinates of all positions of the object (measured at its centroid).

***Mean Y ()*** - the mean value of the Y-coordinates of all positions of the object (measured at its centroid).

***Mean Distance ()*** - the mean of the frame-by-frame distances moved by the object.

***STD Distance ()*** - the standard deviation of the mean distance.

***Mean Angle*** - the mean of the frame-by-frame path angles moved by the object (relative to the location in the preceding frame). Angles are measured from the "nine o'clock" position and will range from 0 to 180 degrees. Downward angles are expressed as positive numbers, and upward movements are expressed as negative numbers.

***Mean Angular Vector*** - the mean angle of the motion vector (relative to the starting point).

***Angular Deviation*** - the standard deviation of the mean angle.

***Mean Velocity (/sec)*** - the distance moved per time unit or plane number.

### Open Log/Log Data

Opens a summary log for storing the track data for all objects . After you open the summary log, the text on this button will change to *"Log Data."* Choosing this button will then save the track statistical data in the summary log.

### Config Log

Allows the selection of track data to be included or excluded from data logging. Also allows a choice of whether column titles are to be included and if data are to be listed on a single line.

### Print Table

Prints the Track Objects Statistics data table.

### Close

Closes the Track Objects Statistics dialog box.

## Track Objects: Data Table - Dialog Box Options

### Track Objects Data Table

Displays the point-by-point measurement data for individual objects. Data for an object will be represented down a column. The frame-by-frame data that is displayed is determined by the selection you make from the *Data Type* option button group. Clicking an entry in the table will update the displayed image with the selected frame.

### Data Type

Selects the type of data to be displayed in the data table. Because the measurements that are displayed by selecting *Distance, Time Interval, Velocity,* or *Angle* are calculated by using information from the immediately preceding plane, the first entry in the Track Objects Data table will display "N/A". If the object was not tracked at a particular frame, the data table will display "- - -".

***Position*** - Displays the X and Y coordinates of each object at each position.

***Distance*** - Displays the distances between successive positions of each object from frame to frame.

***Interval*** - Depending on the options you selected in the Track Objects Interval Options dialog box, this displays either the image timestamps or the between-frame time interval you specified to determine the elapsed time between successive image frames.

***Velocity*** - Displays the movement velocity of the object, which is determined from the displacement of the object, relative to its previous position, and the image timestamps.

***Angle*** - Displays the angle of the path taken by the object from frame to frame. Angles are measured from the "nine o'clock" position and will range from 0 to 180 degrees. Downward angles are expressed as positive numbers, and upward movements are expressed as negative numbers.

***Dist. to Origin*** - Displays the straight-line distance between the object's current position and the Origin.

### Print Table

Prints the Track Objects Data table.

### Close

Closes the Track Objects Data dialog box.

### Track Objects Data Display

**The *Track Objects Data* dialog box allows you to change between views of data in the data table. Five views are available.**

**Position:** This is the default view. In it, the X and Y coordinates of each object will be displayed.

**Distance:** This view displays the distances between successive positions of each point from frame to frame.

**Interval:** Depending on the options you selected in the Track Objects Interval Options dialog box, Track Objects uses either the image timestamps or the between-frame time interval you specify to determine

the elapsed time between successive image frames. This calculated time interval will be displayed in the Track Objects Data table.

**Velocity:** When this view is selected, MetaMorph uses the displacement of the point and the image timestamps (or the time interval defined by the user with the *Set Interval* command) to determine the movement velocity of the point.

**Angle:** This view displays the angle of the path taken by the object from frame to frame. Angles are measured from the "nine o'clock" position and will range from 0 to 180 degrees. Downward angles are expressed as positive numbers, and upward movements are expressed as negative numbers.

**Dist. to Origin:** This view displays the straight-line distance between the object's current position and the Origin.

Because the measurements that are displayed by selecting *Distance, Time Interval, Velocity,* or *Angle* are calculated by using information from the immediately preceding plane, the first entry in the Track Objects Data table will display "N/A". Data may also be unavailable because no match was found, or because of cancellation by the user. For these points, the data table will display "- - -".

## Track Objects Graph - Dialog Box Options

### Track Objects Scatterplot Graph

Displays object data over time or plane number in graphical format. For displays other than position, clicking on the graph will update the displayed image with the frame corresponding to the nearest data point.

### Data Type

Selects the data to be displayed:

***Position*** - The X and Y coordinate at each position.

***Distance*** - The absolute distance between each position and the one immediately preceding.

***DeltaXY*** - The change in X and Y values at each position, relative to the immediately preceding position.

***Velocity*** - The distance moved per time unit or plane number.

***Angle*** - The angle of the path taken by the object from its previous position. Angles are measured from the "nine o'clock" position and will range from 0 to 180 degrees. Downward angles are expressed as positive numbers, and upward movements are expressed as negative numbers.

***Dist. to Origin*** - The straight-line distance between the object's current position and the Origin.

### Graph Single Object

When you select this check box, the data points for just a single object will be displayed. When you clear this check box, data points for all objects will be displayed.

### X-Axis

Selects between a display of the data in terms of *Time* or *Plane* number.

### Down Arrow Configuration Menu

The Down Arrow button opens a pop-up configuration menu, which you can use to configure the graph colors, titles, or axis ranges. The menu also has commands for printing the graph and copying it to the Clipboard for use in another Windows-based program, such as a graphics or word processing program.

### Close

Closes the Track Objects Graph dialog box.

## Track Objects: Edit Data - Dialog Box Options

### Frame #

Selects the frame containing the "bad" or missing point.

### Object #

Selects the number of the object corresponding to the "bad" or missing point.

### Position on Image

Selects a new location for the active point. The position is expressed relative to the selected Origin. When you change the position of a point with the *Position on Image* option group's *X* and *Y* spin boxes, the *Reset Point* button will become available, allowing you to undo the change.

### Calibrated Position from Origin

This status text gives the current location of the active point, relative to the Origin. The position is expressed in terms of pixels in uncalibrated images, or in terms of an actual distance in images that have been calibrated with the Calibrate Distances command.

### Reset Point

Reverts the currently active point to the original position before it was edited.

### Delete Point

Removes the currently active point.

### Done

Accepts the newly edited data and closes the dialog box.

# Track Points (Apps Menu)

**The Track Points command allows you to track one or more selected points through each frame in a series of images and to derive measurements of the paths, positions, and velocities of the points.**

*Availability: Available for MetaVue and MetaMorph Basic; included in MetaMorph Premier*

#### Drop-in: TRACKPTS

Use this command when you want to measure the movement of one or more objects between frames in a series of images. You can also track the position of an object with respect to a user-defined point in the image. Track Points can be used to track movement between planes in an image stack or frames from a continuously acquired live image. Data regarding the X and Y coordinates, displacement, and velocity of the objects will be displayed in a table, and can be logged to disk or sent to a printer.

**Note:** If the time interval = 0 for a measurement, velocity will be logged as -1 to indicate that it is invalid. This applies to the first plane in a stack as well, as the velocity can not be reported for the first plane, having no previous frame of reference. If you are performing statistics on logged data with a spreadsheet application, you should be able to eliminate all non-positive velocity values.

**WARNING:** You will not be able to switch to another stack or add a plane to an image stack after you have already measured it. Doing so will generate an error message informing you the track data are no longer valid, and the data will be cleared and all data displays will be closed.

**Note:** This command does not support 24-bit color images.

## Tracking Points - Procedures

### Overview of Track Points

### Deleting a Track

**Setting the Track Points Origin**

**Setting Track Points Graphics Options**

**Setting Track Points Interval Options**

## Overview of Track Points

To track and measure the movement of an object through a stack of images, use the following procedure. If you want to see a table of the keyboard shortcuts at any time after you start a track, choose *Keyboard Commands*.

**WARNING:**
Do **not** switch to another stack or add/remove planes after tracks have been added, or all track information will be lost!

| Step | Action |
|---|---|
| **1** | From the Apps menu, choose Track Points. The Track Points dialog box opens. |
| **2** | Select the image source from the *Source* radio button group. |
| **3** | If you want to track the position of an object with respect to a particular location within the image, choose **Set Origin** and define the origin point. |
| **4** | When you are ready to add a track, choose *Add Track.* |
| **5** | If you selected *Stack* in Step 2, this will display the first image in your stack's image window. If you want the track to be considered complete when a point is selected in the final image plane, select the *Entering Point on Last Frame = Done* check box.<br>**OR**<br>If you selected *Updating Image* in Step 2, the Track Points: Start Tracking dialog box will appear. From the *Tracks* spin box, select the number of tracks you want to measure and then choose *OK.* |
| **6** | Using your pointer, click the point in the first image that you want to track. The next plane will be displayed automatically. |
| **7** | Add the next point in this plane, and repeat for all planes in the stack. Your points will be indicated by an image window overlay, and the data associated with the points will be displayed horizontally in the Track Points table. Additional tracks can be defined, as needed. |
| **8** | From the *Overlay* group, select<br>***All Tracks*** if you want to see the track overlays for all of the tracks,<br>***Current*** if you only want to see the current track, or |

***None*** if you want to turn off all track overlays.

**Note:** You can toggle between these three selections by pressing the [7] key on your keyboard.

**9** If you want to display a specific plane to add a point, use *Plane* to select the desired plane.

**10** To change between views of the different sets of data, select a data set from the Data Type group box. If you want to clear the table, choose *Clear All.* The data table and the track overlay(s) will be removed.

**11** To save the Track Points data, open a data log by choosing **Open Data Log** from the Log menu. You can now log your data by choosing the *Log Data* command in the Track Points dialog box. To view your data, choose **View Current Data Log** from the Log menu.

**12** When you have finished, choose *Close***.**

## Deleting a Track

To delete a Track Points track and all of its associated data, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Track Points dialog box, choose *Delete Track.* The Track Points Select dialog box opens. |
| **2** | In your stack image window, use your mouse pointer to click the track you want to delete. You will be prompted for confirmation of the deletion. |
| **3** | Click *OK* to delete or *No* to cancel. If you chose *OK,* the selected track will disappear from the image window, its data will disappear from the Track Points table, and all track numbers will be renumbered accordingly. |

## Setting the Track Points Origin

You can measure the position of an object with reference to a particular point in your image by defining an "origin" point. It is not necessary to perform this step before defining your tracks. To set a Track Points origin, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Track Points dialog box, choose *Set Origin.* |
| **2** | In your stack image window, click the location of the point to which you want to refer all coordinates. The Track Points table, viewed in *Position* mode, will now display coordinates relative to the origin point. |

| Step | Action |
|---|---|
| **3** | If you want to remove an origin point after setting it, choose *Set Origin* again from the Track Points dialog box. In the image window, choose *Cancel.* You will be prompted for confirmation of the removal of the origin point. |

## Setting Track Points Graphics Options

If you want to change the default settings of the Track Points marker size, color, or shape, or aspects relating to the display of track paths, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Track Points dialog box, choose *Set Graphics.* The Track Points Graphics Options dialog box will appear. |
| **2** | To change the color of the Track Points marker and path, select the desired color from the *Track Points Color* list. To change the shape of the Track Points marker, select the desired shape from the *Point Marker Type* list.<br><br>If you choose a *Circle* marker and want to fill in the circle, select the *Fill Circle Markers* check box. You can change the size of the Track Points marker with the *Point Marker Size* spin box.<br><br>**Note:** The size of the *Dot* marker can not be changed. For a larger dot marker, select a *Circle* marker shape and select the *Fill Circle Markers* check box. |
| **3** | Use the *Display Mode* radio button group to choose between an overlay display that shows all points in a track (*Display All Track Points*) and one that shows only the last point in the track (*Display Last Track Point Only*). |
| **4** | The track path and number can be displayed or hidden by selecting or clearing the *Display Track Path* and *Display Track Number* check boxes, respectively. |
| **5** | A track "pattern" can be added to the overlay, showing lines that connect the points in an individual plane. The patterns from each plane will be displayed simultaneously. This option can be enabled and disabled from the *Display Track Pattern* check box. The color of the pattern in the plane being viewed can be changed in the *Track Pattern Color* list. The patterns from other planes will continue to be displayed in red. This option also controls the color of the track numbers displayed in the image window overlay. |
| **6** | The default coordinate system considers the origin of the Y-axis to be at the top of the image window. If you want to switch to a coordinate system that places the Y-axis |

origin at the bottom of the window, select the *Y-Coordinate Increases up the Screen* check box.

**7** When you are satisfied with all of your selected graphics options, choose *OK.* Your options will then take effect.

### Setting Track Points Interval Options

To configure the settings associated with the time intervals between planes in your Track Points stack, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Track Points dialog box, choose *Set Interval.* The Track Points Interval Options dialog box will appear. |
| **2** | To change the time units in the Track Points table from the default *Seconds,* select the desired time unit from the *Table Time Units* list. |
| **3** | By default, MetaMorph obtains the time interval between planes in a stack by reading the image timestamps. However, you can specify your own between-plane time interval in the *Time Interval* spin box and drop-down list box.<br><br>To choose between these two types of time interval, select *Time of Image Creation* or *User Defined* from the *Time Interval Options* group. |
| **4** | When you are satisfied with all of your selected interval options, choose *OK.* |

### Track Points - Dialog Box Options

**Track Points Summary**

**Track Points Graphics Options**

**Track Points Interval Options**

# Track Points Data Type Display

**The *Data Type* group box allows you to change between views of data in the Track Points table. The following views are available.**

**Position:** This is the default view. In it, the X and Y coordinates of the selected object(s) will be displayed. If you defined your own origin point, the coordinates that are displayed when *Position* is selected will be given relative to the origin point.

**Distance:** Displays the displacement of a point from the previous point to current point.

**Interval:** By default, Track Points uses the image timestamps to determine the actual time which elapsed between successive stack planes. This calculated time interval will be displayed in the Track Points table.

**Delta XY** Displays the displacement of a point from the previous point as a coordinate pair (displacement in X, displacement in Y).

**Velocity:** Displays the movement velocity of a point (derived from the distance and time interval between planes).

**Angle** Displays the orientation from the previous point to the current point.

**Dist To Orig.** Displays the distance from the relative origin (0, 0) to the point.

Since the measurements brought up by selecting *Distance, Time Interval,* or *Velocity* are calculated by using information from the last selected plane, the first column in the Track Points table will display "N/A."

## Track Points - Dialog Box Options

### Source (radio button group)

Selects the source of the images to be used for tracking: *Stack, Updating Image.*

### Source (image selector)

Selects between image acquisition windows or open stacks for analysis by Track Points.

### # of Periods

Displays the number of planes in the stack.

### # of Tracks

Displays the number of track points per plane that have been defined.

### Plane

Specifies a stack plane to be displayed. Use this option to select the plane you want to mark next.

### Overlay

Specifies which track overlay(s) are displayed on the image. Select *All Tracks* to display the overlays for all tracks that have been created, select *Current* to display only the overlay for the track currently being created, or select *None (7 = Toggle)* to display no tracks. When the image is the active item on the desktop, you can press the [ 7 ] key to toggle the track display on or off. (The option selected in the *Overlay* group box will be updated when you change the display with the [ 7 ] key.) The appearance of the track overlay can be set using *Set Graphics* prior to starting the track.

### Entering Point on Last Frame = Done

Specifies that a track is to be considered complete when a point is added in the last plane of the stack. When this check box is selected, the track will be completed and added to the table when a point is selected on the last plane in the stack. This is the default setting for this option. Clearing this check box allows you to continue adding points until you choose *Done.*

### Done

Stops tracking and adds the track to the table.

### Cancel

Stops tracking without adding the track to the table.

### Track Points Table

Displays the Track Points data, with data for each track listed horizontally and data for each plane listed vertically. The data set that is displayed *(Position, Distance, Time Interval,* or *Velocity)* is determined by the *Data Type* option.

### Data Type

Selects between the data to be viewed in the Track Points table.

***Position*** displays the X and Y coordinates of each point.

***Distance*** displays the displacement of a point from the previous point to current point.

***Interval*** displays the time interval between planes.

***Delta XY*** displays the displacement of a point from the previous point as a coordinate pair (displacement in X, displacement in Y).

***Velocity*** displays the movement velocity of a point (derived from the distance and time interval between planes).

***Angle*** displays the orientation from the previous point to the current point.

***Dist To Orig.*** displays the distance from the relative origin (0, 0) to the point.

### Add Track

Used for defining the track of a point from plane to plane in a stack.

### Delete Track

Opens the Track Points Select dialog box and enables you to select a track to delete.

### Clear All

Deletes all of the data in the Track Points table and removes all points and tracks from the image window.

### Print Table

Prints the data in the Track Points table.

### Config Log

Selects image characteristics and data that you want to be included or excluded from data logging. Also allows you to choose whether column titles are to be included, and if data are to be listed on a single line.

### Open Log

Sends data measurements to an open data log file.

### Set Interval

Configures the settings associated with the time intervals between planes in a stack.

### Set Overlay

Opens the ***Track Points Overlay Options*** dialog box. Use this dialog box to change the default settings of the Track Points marker and path displays.

### Set Origin

The default origin of the coordinate system used in image windows is in upper left corner of the window (or lower left, if you chose *Y-Coordinate Increases up the Screen* in the Set Graphics dialog box). *Set Origin* allows you to obtain point coordinates that are defined in terms of a particular location in the image that you select as a different point of origin.

### Duplicate Overlay

Creates a blank image with a copy of the track overlay, as currently displayed on the tracking image.

### Graph Data

Opens the ***Track Objects Graph*** dialog box.

### Close

Closes the dialog box.

## Track Points Overlay Options - Dialog Box Options

### Track Point Color

Selects the color of the Track Points marker. The default setting is *Red.*

### Point Marker Type

Selects the shape of the Track Points marker. The default setting is *Cross.*

### Point Marker Size

Selects the size, in pixels, of the Track Points marker. The default setting is *7.*

### Display Mode

Selects between an overlay display that shows all points in a track *(Display All Track Points)* and one that shows only the last point in the track *(Display Last Track Point Only).*

### Fill Circle Markers

If the Track Points marker is changed to a circle, this option will fill the circle, so that it looks like a large dot.

### Display Track Path

Enables or disables the display of track paths.

### Display Track Pattern

Enables or disables a display of track patterns, which show a line connecting the points in a plane.

### Display Track Numbers

Enables or disables the display of track numbers in the image window.

### Track Pattern Color

Selects the color of the track pattern and number as displayed in the image window. The default setting is *Red*. Select *Alternating* if you want MetaMorph to select a color for each track pattern by cycling through the eight colors that are available.

### Y-Coordinate Increases up the Screen

The default location of the Y-axis origin is at the top of the image window. This option places the Y-axis origin at the bottom of the image window.

### OK

Closes the dialog box and implements the option changes that were selected.

### Cancel

Closes the dialog box and cancels the option changes that were selected.

## Track Points Interval Options - Dialog Box Options

### Table Time Units

Changes the time units in the Track Points table. The default setting is *Seconds.*

### Time Interval Options

Selects a time interval. To use an interval that is based on the images' timestamps, select *Time of Image Creation.* If you want instead to use an interval that you have specified in *Time Interval,* select *User Defined.*

### Time Interval

Specifies a time interval between image planes. Use the drop-down list box to select the time unit *(Milliseconds, Seconds, Minutes,* or *Hours).* Use the spin box to specify the number of time units in the interval. The *Time Interval* option will be unavailable and will appear dimmed unless you select *User Defined* in *Time Interval Options.*

### OK

Closes the dialog box and implements the option changes that were selected.

### Cancel

Closes the dialog box and cancels the option changes that were selected.

# Show Zeiss Image Info (Edit Menu)

**Displays information about the microscope settings used during the acquisition of a TIFF image (*.tif) with a Zeiss confocal microscope.**

**Drop-in: ZEISSINF**

Use this command to display microscope and filter settings that were used during the acquisition of a TIFF image, or when you want to save such information to a data log. This feature requires the use of the Zeiss LSM 410 software package during image acquisition with a Zeiss confocal Laser Scan Microscope. For further information, consult your Zeiss microscope user's manual.

**Note:** The *X Scale* or *Y Scale* information can be used for calibrating distances in your image by defining a region of interest with a width of a known number of pixels and then mul*tip*lying the *X Scale* or *Y Scale* setting by that number. The result will give the length or width of the region of interest, in microns.

## Showing Zeiss Image Information

To display or log the settings used by your Zeiss confocal Laser Scan Microscope during acquisition of a TIFF image, use the following procedure.

**Note:** You must use the Zeiss LSM 410 software package to store the microscope setting information at the time you acquire the TIFF image.

| Step | Action |
|---|---|
| **1** | From the Edit menu, choose Show Image Zeiss Info. The Show Zeiss Image Info dialog box opens. The microscope settings are displayed automatically. |
| **2** | If you want to display the information from a different .tif file which has had its settings stored with the Zeiss LSM 410 software package, select the name of the image from the *Image* selector. Its settings will be displayed automatically. |
| **3** | If you want to store the microscope settings in a data log, choose ***Configure Log*** to select which data are to be enabled for logging. Then choose ***Open Log*** and log your microscope settings. |
| **4** | Choose *Close* to close the Show Zeiss Image Info dialog box. |

## Show Zeiss Image Info - Dialog Box Options

### Image

Selects the TIFF image for which you want to display microscope settings.

### Source

Displays the Zeiss LSM microscope mode that was used during image acquisition: Conventional, Laser Scan, Reflected, Transmitted, Confocal, Fluorescence, TV, or OBIC.

### Contrast

Displays the contrast setting of the microscope.

### Brightness

Displays the brightness setting of the microscope.

### Pinhole
Displays the Pinhole setting of the microscope.

### Emission Filter
Describes the emission filter that was used during image acquisition: long-pass vs. bandpass, and peak transmission wavelength (nm).

### Zoom
Displays the Zoom setting of the microscope.

### Lens
Displays the type of lens that was used by the microscope during image acquisition.

### Magnification
Displays the total lateral magnification of the image.

### Aperture
Displays the Aperture setting of the microscope.

### X Scale
Displays the horizontal size of each pixel, in microns.

### Y Scale
Displays the vertical size of each pixel, in microns.

### Attenuation Filters
Displays the neutral density filters that were used in the microscope.

### Laser
Displays the wavelength (nm) of the laser used by the microscope in Laser Scan mode.

### Scanning Time
Displays the scanning time specified during image acquisition.

### Real Scan Time (sec)
Displays the actual scan time taken during image acquisition.

### Frames Averaged
Displays the number of frames that were averaged during image acquisition. A "1" indicates that no averaging was performed.

### Bandwidth
Displays the bandwidth setting of the microscope.

### Beam Splitter
Describes the beam splitter used during image acquisition: CBS (chromatic beam splitter), DM (dichroic mirror), TK *(Teiler Kante,* or "edge splitter"), FT *(Farb Teiler,* or "color splitter"), or RKP *(Reflexion kurz Pass,* or "reflection short-pass"), and peak reflection and transmission wavelengths (nm).

### X Motor (steps)
Displays the number of steps from the origin that were taken by the motorized stage translator in the X (horizontal) axis.

### Y Motor (steps)
Displays the number of steps from the origin that were taken by the motorized stage translator in the Y (vertical) axis.

### Z Motor (steps)

Displays the number of steps from the zero plane that were taken by the motorized stepper in the Z (depth) axis.

### Date and Time

Displays the date and time of image acquisition.

### Open Log

Opens a data log and/or a DDE link to an open spreadsheet application for logging data. This command will change to *F9: Log Data* when a log file is open.

### F9: Log Data

Sends data measurements to an open data log.

### Configure Log

Opens the Configure Log dialog box. Enables you to select image characteristics and data categories that you want to include in the log file. Also enables you to choose whether column titles are to be included and data are to be listed on a single line or multiple lines.

### Close

Closes the dialog box.

# Start Recording (Journal Menu)

**Creates a journal by recording commands as you use them. After you choose Stop Recording, the sequence of commands and the dialog box settings will be saved in the journal file of your choice.**

**Drop-in: JOURNAL**

Use this command to record commonly used commands and settings so that they can be easily run at any time from the Journal menu or from a taskbar.

The MetaMorph Imaging System title bar will display the message "[Recording]" when this command is in use. Once you start recording, execute the commands in the order they should be played back. As each command is carried out, select the proper options, destination image, and source image before choosing the command's *OK, Apply,* or *Record* command to record the selected command. In complex dialog boxes, there may be more than one command button that can be recorded. In this situation, use the command as you normally would and the current operation will be recorded.

While you are creating a journal, keep in mind that you are recording commands and option settings, not mouse clicks. Shorter journals will be easier to manage and troubleshoot (if necessary) than long journals.

Use the Pause Recording command to pause journal recording. When you are done recording the desired command(s), use the Stop Recording command to end the recording and to save the journal.

When the journal is saved, MetaMorph assigns the file extension ".jnl" to the journal file. After the journal is saved, you can assign it to the current taskbar or you can assign it to a different taskbar later.

You can use the Edit Journal command to edit the newly created journal.

## Starting a Journal Recording

To start recording a journal, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Start Recording. The MetaMorph Imaging System title bar will display the message |

"[Recording]."

**2** Execute the journal's commands in the order they should be played back. As you carry out each command, you should verify that the correct options, source image, and destination image have been selected.

**Note:** Remember to choose each dialog box's *OK, Apply,* or *Record* command (as applicable); otherwise, the command will not be recorded. This also applies to commands that issue dialog boxes from the main dialog box.

**3** If you want to pause the recording of the journal, use the **Pause Recording** command.

**4** To stop recording the journal, use the **Stop Recording** command.

# Stop Recording (Journal Menu)

**Terminates the recording of journal commands and saves the recorded commands and settings in the journal file of your choice.**

**Drop-in: JOURNAL**

Use this command when you want to stop recording commands and save them in a journal file. When you select a file name for the journal file in the Save Journal As dialog box, MetaMorph will assign the extension ".jnl" to your file name. You will be given the option of adding the newly created journal to the current taskbar. Otherwise you can run the journal using the Run Journal command or you can assign the journal to any taskbar later.

## Stopping a Journal Recording

To stop recording a journal, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Stop Recording. The Save Journal As dialog box will appear. |
| **2** | Type the desired file name for the journal in the *File Name* text box. MetaMorph will assign the file extension ".jnl" to your file name. If necessary, use the *Save In* list or Up One Level icon button to locate the appropriate drive and folder.<br>**AND**<br>Choose *Save.* |
| **3** | When the Record Journal dialog box appears, choose *Yes* to add the newly created journal to the current taskbar.<br>**OR**<br>Choose *No* if you want to run the journal from the Run Journal command or add it to a taskbar later. |
| **4** | If you chose *Yes* in the previous step, the |

Taskbar Editor dialog box will appear. Follow the procedure in **Editing a Taskbar** to add the new journal to a taskbar.

# Pause Recording (Journal Menu)

**Temporarily stops the recording of a journal so that other commands can be executed without being recorded as part of the journal.**

**Drop-in: JOURNAL**

Use this command to halt the recording of a journal when you need to execute one or more commands in preparation for commands that will be recorded. While a journal is paused, the MetaMorph Imaging System title bar will display "[Recording - Paused]." When you choose Resume Recording, you can continue recording the remainder of the desired commands in the journal.

## Pausing Journal Recording

To stop recording to a journal temporarily, use the following procedure:

| Step | Action |
|---|---|
| 1 | From the Journal menu, choose Pause Recording. The message "[Recording Paused]" will appear in the MetaMorph Imaging System title bar. |
| 2 | Complete the commands that you need to carry out but do not want to record in the journal. |
| 3 | Use the **Resume Recording** command to resume recording of the journal. |

# Resume Recording (Journal Menu)

**Continues recording a journal after the recording has been paused with the Pause Recording command.**

**Drop-in: JOURNAL**

Use this command to continue recording a journal after pausing to execute commands that you did not want to be recorded in the journal.

## Resuming Recording a Journal After Pausing

To resume recording to a journal, use the following procedure:

| Step | Action |
|---|---|
| 1 | From the Journal menu, choose Resume Recording. |
| 2 | The message "[Recording]" will reappear in the MetaMorph Imaging System title bar. |

# Run Journal (Journal Menu)

**Plays back a selected previously saved journal.**

**Drop-in: JOURNAL**

Use this command to run journals that you do not use frequently. For journals that you use frequently, it is best to assign them to a taskbar with other similar journals and run them directly from the taskbar as

needed, rather than using the Run Journal command.

**Note:** You can record the Run Journal command as part of a larger journal. This allows you to create small journals and reuse them as modules for multiple journals.

**WARNING:**
When you use journals during a procedure that involves an illumination device, you should close the Illumination dialog box before starting the journal. Attempts to run a journal while the Illumination dialog box is still open may cause your system to appear to "freeze." This is due to the "modal" nature of dialog boxes that are opened in journal playback mode, combined with the Illumination dialog box's need to reconfigure itself by closing itself and reopening when a new illumination device is selected.

If you want to cancel a journal after you have chosen the Run Journal command, press the [CTRL] + [BREAK] keys or press the [ESC] key while the progress meter is displayed. To dismiss a dialog box that is displayed during interactive journal playback, press the [ESC] key twice.

## Running a Journal

To run a journal, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Run Journal. The Run Journal dialog box will appear. |
| **2** | Select the file for the desired journal. If necessary, use the *Look In* list or Up One Level button to locate the correct drive and folder. Then choose *Open.* |
| **3** | MetaMorph will run the journal. |

## Run Journal - Dialog Box Options

**File Name**
Lists the name of the selected journal.

**Files of Type**
Determines the file format of the files displayed in the *File Name* list. For opening journal files, the default is ".jnl".

**Look In**
Displays the currently selected folder. Click the icon for the desired folder to display its files. Click the Up One Level button to go up one level in the directory structure.

**Open**
Opens the journal file and runs the journal.

**Cancel**
Cancels the command.

# Edit Journal (Journal Menu)

**Edits a previously recorded and saved journal using the Journal Editor dialog box.**

**Drop-in: JOURNAL**

Use this command to modify an existing journal or to create a new journal. Use this command to add journal entries or to edit the parameters for a journal entry. Also use this command to add programming actions that cannot be created in a journal by recording the journal.

The Journal Editor dialog box contains two main panes. The pane on the left side of the dialog box contains the following three tabs:

- Builtin Functions — contains all of the available functions that initiate specific commands
- Recorded Journals — contains a tree view of your file system open to the Journal folder
- Actions — contains a list of programming commands that you can include in your journal

The *Journal* pane on the right contains a Functions tab that shows each step of the currently selected journal, in the order that they will occur. It also contains a Description tab that contains the same information as the Functions tab, but also includes any assigned variables and parameter settings.

**Edit Journal Procedures**

**Application Note** — ***How to use image file variables to save images in a journal without user interaction***

**Index of Journal Functions**

**Notes:**

- You cannot use this command while in Record mode.
- The *Edit Journal* command enables you to select any journal for editing. However, if you want to edit a journal that is part of the current taskbar, simply press and hold the [SHIFT] key while you click the associated button on the taskbar. This opens the Journal Editor with the selected journal ready for editing.
- The Command ***"***End of Journal***"*** indicates the end of the journal. This is the last command in every journal and is a required command. You cannot delete this command.
- Journals created with MetaMorph software earlier than version 6.0 can be edited with this version. When you edit a pre-6.0 journal with a file extension of .jnl, a copy of this journal is automatically saved with the extension .prv. Using Windows Explorer, you can change the .prv extension back to .jnl to make it available for pre-6.0 versions.

## Edit Journal - Procedures

**Editing a Journal**

**Adding a Journal Entry**

**Cutting, Copying, and Pasting Journal Entries**

**Editing Entries in a Journal**

## Editing a Journal

To edit one or more journals, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Edit Journal. The *Select a Journal to Edit* dialog box opens. |
| **2** | Select the journal that you want to edit. If necessary, use the *Look In* list or Up One Level button to locate the correct drive and folder. Then choose *Open.* The Journal Editor dialog box will appear. |
| **3** | If you want to edit more than one previously saved journal at once, choose *File>Open.* The Open a Journal to Edit dialog box will open. Select the file for the desired journal and choose *Open.* |

(You can open previously saved journals at any time while editing a journal.)

**4** The last journal open will become the current journal, as listed in the drop-down list in the upper right corner of the dialog box.

You can edit another open journal by selecting it from the drop-down list or, if the taskbar to which it has been assigned is currently displayed, you can "shift-click" its assigned button (hold down the [SHIFT] key and click the taskbar button using your left mouse button). The status text at the top of the dialog box will note how many journals are currently open.

**5** From the *View* list, select the view you want to use; either *Alphabetical* or *Menu.*

**6** You can add entries to your journal by dragging functions to either the Functions or Descriptions box on the right or by using another appropriate editing method, such as Copy and Paste.

**7** *Playback Interactively* will change an entry's interactive status. If there is an "I" displayed in front of the entry, the journal will stop during playback to display a dialog box, so that you can modify the function's parameters (if there are any parameters that can be edited). This command is the same as the Toggle Interactive command in the Journal menu.

**8** To save a journal after editing, click *Save* or choose *File>Save.* This will overwrite the existing version of the journal.

**OR**

Choose *File>Save As* to save the edited journal under a new name so that the original file remains intact. Type the file name in the *File Name* text box in the Save Journal As dialog box and choose *Save.*

**9** To print your completed journal, choose *File>Print*. The entire journal is sent to the currently selected Windows printer. Icons are not shown in the printed version, only text.

**10** To run the current journal open in the editor, click Run Journal. You must save the joural before running it..

**11** You can use the *File>Close* command to close any journals you do not want to use. (Remember to save first!)

**12** When you have finished editing and saving your journals, click *Exit* or choose *File>Exit* to close the Journal Editor dialog box.

## Adding a Journal Entry

To add a journal entry to the journal you are editing, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the *View* list, select the view you want to use to display the Function List: either *Alphabetical* or *Menu.* |
| **2** | From the Built-in Functions list on the left, select the function that you want to add to your journal.<br><br>**AND**<br><br>Choose *Edit>Copy* or drag the function from the Built-in Functions List to the list of journal entries on the right. |
| **3** | If the command has parameters or settings that you can edit (such as an enabled/disabled state), its dialog box will appear so that you can change its options. Choose *OK* when you have finished.<br><br>If there are no parameters or options to set, a dialog box with the message "This journal entry is not editable" will appear. |
| **4** | The function will now appear in the right column before the previously selected entry.<br><br>If you selected *Playback Interactively, a*n "I" will appear before the function name in both the *Functions* tab and the *Descriptions* tab. |

## Cutting, Copying, and Pasting Journal Entries

If you want to remove the currently highlighted entry from your journal list, choose *Edit>Cut,* OR right-click and choose *Cut*, OR Ctrl+X, OR Press the Delete key.

If you want to copy the currently highlighted entry from your journal list, choose *Edit>Copy,* OR right-click and choose *Copy*, OR Ctrl+C.

If you want to paste the last entry that was cut or copied, highlight the entry and choose *Edit>Paste*, OR right-click and choose *Paste*, OR Ctrl+V*.* The pasted entry will appear above the currently highlighted entry.

You can cut and copy multiple entries from the journal. To select a set of adjacent entries, select the first entry and then hold down the [SHIFT] key while selecting the last entry in the list. All entries in between the two selected entries will also be selected. To select multiple entries scattered throughout the list, select the first entry, and then hold down the [CTRL] key while selecting the other desired entries. Only these entries will be highlighted. You can cut and copy as described above.

## Editing Entries in a Journal

As you edit a journal, you can decide to change the parameters or settings that you selected for a particular function. To change the parameters or settings, highlight the desired entry in the list on the right and click *Edit Function Setting.* You will then be able to change the function's editable parameters in its dialog box. Choose *OK, Apply,* or *Record* (as applicable) to record the changes in the function's parameters.

If you want to change a particular journal entry's interactive mode status, highlight the desired entry in the right-hand list and choose *Play Interactively.* This will toggle the entry's status to on ("I") or off (no "I")

depending on its current state.

**Note:** When you change the setting in a command's dialog box while editing a journal, you **must** choose its *OK, Apply or Record,* or other applicable command button for your changes to be recorded.

## Edit Journal - Dialog Box Options

### File

Provides a menu that contains a set of commands to enable you to originate new journals and to edit, save and print existing journals.

#### New

Creates a new journal file.

#### Open

Opens the selected journal file.

#### Close

Closes the active journal.

#### Save

Saves the current journal, overwriting the contents of the journal file if it has been previously saved.

#### Save As

Saves the current journal using a different file name of your choice.

#### Revert to Saved

Restores the currently displayed journal to the condition it was in when it was last saved.

#### Print

Opens the Windows Print dialog box and enables you to print a copy of the journal to the selected Windows device. Icons are not shown in the printed version, only text.

#### Exit

Discontinues any running journal and closes the Journal Editor dialog box.

### Edit

Provides a menu that contains commands for cutting, copying, pasting, and controlling journal functions.

#### Cut

Deletes the selected function from the current journal.

#### Copy

Copies the selected function from the current journal.

#### Paste

Pastes the most recently cut or copied function to the current journal, placing it above the currently selected function in the list of functions.

#### Delete

Permanently removes the selected journal function.

#### Disable

Temporarily deactivates the selected journal function.

#### Interactive

Turns on or off (toggles) the interactive journal mode. A check next to this setting indicates that it is active (on).

### Override Settings

Enables you to temporarily override the current settings and replace them with new settings.

## Built-in Functions

Lists functions that can be added to a journal. Functions can be added by double-clicking a function name in the table, or by dragging a function from the table to the list of functions in the current journal.

### View

Selects a view for the display of the journal functions in the Function Table: Alphabetical or by Menu.

## Recorded Journals

Shows your currently accessed folder and associated path. Use this tab to move your folder selection from one folder to another. From the appropriate journal folder, double-click the name of the journal that you want to open for viewing or editing, or to run. You can also double-click or drag journals from these folders into the currently open journal to run journals from within a journal or to loop a journal.

## Actions

Shows a list of programming commands that you can include in your journal. Double-click the name of the command or drag the command into the appropriate location in your journal

## Built-in Functions

Lists functions that can be added to a journal. Functions can be added by double-clicking a function name in the table, or by dragging a function from the table to the list of functions in the current journal.

## Journal

Lists the active journals that are open for editing. The journal name displayed is the current journal. The status text next this option lists how many journals are open for editing.

## Functions

Lists all of the functions in the current journal. Click an entry once to select it for cutting, copying, pasting, editing, or toggling the interactive mode. Double-click an entry to edit the entry. Select the entry and press Delete to remove the entry, or right click and select Delete. Only the function names are shown in this window. Choose the Description tab to see any variables or parameters assigned to the function or action. This table is located on the right side of the dialog box.

## Descriptions

Shows the same information as the Functions tab, but also includes any assigned variables and parameter settings.

For both the ***Functions*** tab and the ***Descriptions*** tab, the following selections are available for most functions. Selected programming actions will show entry boxes for all applicable parameters, variables and settings for the programming action.

### Playback Interactively

Enables interactive journal editing. With interactive journal editing, you can modify function settings during journal playback. For any functions that have modifiable settings, the journal will pause and open a dialog box for each function for which you have checked *Playback Interactively*.

### Disable

Deactivates the selected function without removing it from the journal or changing any of the function settings.

### Edit Function Settings

Opens the associated settings dialog box for the selected function.

### Select Settings to Override

For specific function settings in specific function dialog boxes, enables you to temporarily select and override certain settings with new values.

### Undo

Resets all settings for the function to the previously set values.

### Save

Saves the current journal, overwriting the contents of the journal file if it has been previously saved.

### Run Journal

Runs the current open journal. The journal must be saved before it will run.

### Exit

Closes any running journal and closes the Journal Editor dialog box.

# Loop a Journal (Journal Menu)

**Repeats a journal a specified number of times at the specified interval.**

**Drop-in: JOURNAL**

Use this command when you want to repeat a journal a specified number of times. You can specify the interval between the loops (each repetition of the journal) and confirmation of each loop. The maximum number of journal loops is 30,000. If necessary, you can add a prompt that appears during playback which requests the number of times the journal should loop.

You can use the Loop a Journal command to loop a previously created journal a specified number of times, or you can record the Loop a Journal command as part of a journal. You can also loop a journal that contains a loop, up to a nesting level of ten loops. If you want to add a Loop a Journal command to a previously saved journal, it can be added to a journal from the *Function* list in the Journal Editor.

After you have configured the loop using the Loop a Journal dialog, MetaMorph will display the Loop Control Panel dialog box while recording the loop. This dialog box lists the journal's name, interval between loops, current loop count, and status of the loop. You can change the journal's interval and loop count using this dialog box. You can also pause (and resume) or stop the journal.

If you want to cancel a journal loop after you have chosen this command, press the [CTRL] + [BREAK] keys or press the [ESC] key while the progress meter is displayed.

## Looping a Journal

To loop a journal, use the following procedure.

**Note:** If you are recording a loop as part of a journal, the Loop a Journal Confirmation dialog box will appear after you have added the loop to the journal. The purpose of this dialog box is to allow you either to execute the journal while recording the loop or only to record the loop. You should select the former option if the next command to be journalized requires the results of the looped journal.

| Step | Action |
|---|---|
| **1** | If you are recording the loop as part of a journal, start the recording of the journal and record all commands that occur prior to the journal that will be looped. |
| **2** | From the Journal menu, choose Loop a Journal. The Loop a Journal dialog box will appear. |

**3** Select the desired number of loops using the *Number of Loops* text box or spin control.

**4** If you want to confirm each loop during playback, select *Confirm Each Loop.*

The message "Continue Looping Journal?" will appear before each loop during playback, to which you can choose *Yes* or *No.*

**5** Choose *Press to Select the Journal to Loop* to select the journal that you want looped. The Select Journal dialog box will appear.

**AND**

Select the icon for the journal. If necessary, use the *Look In* list or Up One Level icon button to locate the correct drive and folder. Then choose *Open.*

The journal's file name will be displayed in the *Loop a Journal* dialog box.

**6** If you want to specify an interval between loops, select *Loop on Interval.*

**AND**

Select the length and type of interval using the text box and drop-down list next to *Loop on Interval.*

**7** If you want to specify a prompt on playback for the number of loops, select *On Journal Playback, Prompt for Number of Loops.*

The default prompt, "How many times do you want to loop the journal?" is displayed in the text box below this option. You can change the text if you wish.

**8** Choose *Continue* to loop the specified journal. The **Loop Control Panel** dialog box will appear while the journal is looping.

## Loop a Journal - Dialog Box Options

### Number of Loops

Specifies the number of times to loop (repeat) the specified journal. The maximum number journal loops is 30,000.

### Confirm Each Loop

Displays a dialog box before each loop that allows you to either continue looping the journal or stop looping it.

### Press to Select the Journal to Loop

Specifies the journal to loop with the Select Journal dialog box, and displays the selected journal in the Loop a Journal dialog box.

### Loop on an Interval

Enables the use of the selected interval between loops. Use the text box and the drop-down list to specify the length and type of interval.

### On Journal Playback, Prompt for Number of Loops

Prompts on playback for the number of times to loop the journal.

### Continue

Starts the journal loop. Displays the Loop Control Panel while the journal is looping.

### Cancel

Cancels the command.

The Loop Control Panel dialog box displays the journal's name, interval between loops, current loop count, and status of the loop.

### Pausing/Resuming a Journal

Choose *Pause* to stop a journal temporarily. *Pause* will be replaced by *Resume.*

### Enabling and/or Changing the Journal's Interval

Choose *Interval.* The Change Interval dialog box will appear. Select *Loop on Interval.* Select the length and type of interval using the text box and drop-down list. Then choose *OK.*

### Changing the Journal's Loop Count

Choose *Count*. The Change Count dialog box will appear. Select the desired number of loops using *Number of Loops.* If you want to confirm each loop during playback, select *Confirm Each Loop*. Then choose *OK*.

### Stopping the Journal

Choose *Stop* to stop the journal.

# Loop for All Planes (Journal Menu)

**Repeats a journal for each plane of the selected stack.**

**Drop-in: JOURNAL**

Use this command when you want to repeat a journal for each plane of the selected stack. You can use the Loop for All Planes command to loop a previously made journal for a selected stack or you can record the Loop for All Planes command as part of a journal. If you want to add the Loop for All Planes command to a previously saved journal, it can be added to a journal from the *Function* list in the Journal Editor.

If you want to cancel a journal loop once you have chosen this command, press the [CTRL] + [BREAK] keys or press the [ESC] key while the progress meter is displayed.

## Looping a Journal for All Planes in a Stack

To loop a journal for all planes of the current image stack, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Loop for All Planes. The Loop for All Planes dialog box will appear. |
| **2** | Select the desired stack using the *Image* selector. |
| **3** | Choose *Select Journal.* The Select Journal dialog box will appear.<br>**AND**<br>Select the icon for the desired journal. If necessary, use the *Look In* list or Up One Level icon button to locate the correct drive |

and folder. Then choose *Open.*

The journal's file name will be displayed in the Loop for All Planes dialog box.

**4** Choose *OK.* The journal will loop through all planes of the current image stack.

## Loop for All Planes - Dialog Box Options

### Image

Selects the image stack for the Loop for All Planes command.

### Journal

Lists the journal to be looped for all planes of the active image.

### Select Journal

Selects the journal to be looped for all planes of the active image using the Select Journal dialog box.

### OK

Loops the selected journal for all planes.

### Cancel

Cancels the command.

# Loop for All Regions (Journal Menu)

**Repeats a journal for each region on the selected image.**

**Drop-in: JOURNAL**

Use this command when you want to repeat a journal for each region of interest that is defined in the selected image. You can use the Loop for All Regions command to loop a previously created journal for all regions in the image, or you can record the Loop for All Regions command as part of a journal. If you want to add the Loop for All Regions command to a previously saved journal, it can be added to a journal from the *Function* list in the Journal Editor.

If you want to cancel a journal loop once you have chosen this command, press the [CTRL] + [BREAK] keys or press the [ESC] key while the progress meter is displayed.

## Looping a Journal for All Regions in an Image

To loop a journal for all regions in the current image, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Loop for All Regions. The Loop for All Regions dialog box will appear. |
| **2** | Select the desired image using the *Image* selector. |
| **3** | Choose *Select Journal.* The Select Journal dialog box will appear.<br>**AND**<br>Select the icon for the desired journal. If necessary, use the *Look In* list or Up One Level icon button to locate the correct drive and folder. Then choose *Open.* |

The journal's file name will be displayed in the Loop for All Regions dialog box.

**4** Choose *OK.* The journal will loop for all regions in the current image.

## Loop for All Regions - Dialog Box Options

### Journal

Lists the journal to be looped for all regions in the active image.

### Image

Selects the image or stack for the Loop for All Regions command. This command only works on the current plane of a stack.

### Select Journal

Selects the journal to be looped for all regions in the active image using the Select Journal dialog box.

### OK

Loops the selected journal for all regions.

### Cancel

Cancels the command.

# Loop for All Images in Directory (Journal Menu)

**Repeats a journal for each image in a selected directory.**

**Drop-in: LPALLDIR**

Use this command when you want to repeat a journal for each image in a directory. You should first be sure that all images In the directory are appropriate for the journal that is to be applied.

**WARNING:**
Be careful to avoid creating the "infinite loop" in which you save images to the end of the directory from which you are loading images.

## Looping a Journal for All Images in a Directory

To loop a journal for all images in a directory, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Loop for All Images in Directory. The Loop for All Images in Directory dialog box will appear. |
| **2** | Choose *Select Directory.* The Set Open Path dialog box will appear.<br>**AND**<br>Select the directory of the images to which you want to apply a journal. Then choose *Save.* The name of the directory will appear in the *Dir:* text box of the Loop for All Images in Directory dialog box. |
| **3** | Choose *Select Journal.* The Select Journal dialog box will appear.<br>**AND** |

Select the file for the desired journal. If necessary, use the *Look In* list or Up One Level button to locate the correct drive and folder. Then choose *Open.*

**4** From the *Close Each Loop* group, select

*Nothing* if you want to keep all images in the directory open after they are processed,

*Opened Image* if you want to close only the image that is opened at the beginning of the loop, or

*All Images* if you want all images to be closed after they are processed.

**5** From the *Close Options* group, select

*Save Nothing* if you do not want to save any images when they are closed,

*Query Modified* if you want to be queried as to whether to save each newly created or modified image before closing, or

*Auto Save Modified* if you want to save each newly created or modified image automatically before closing it.

**6** If you want to produce a query before the journal proceeds to the next image, select the *Confirm Each Loop* check box.

**7** Choose *Run.* The selected journal will be run for each image in the selected directory.

**8** Choose *Close.*

## Loop for All Images in Directory - Dialog Box Options

### Dir

Displays the selected image directory.

### Select Directory

Selects a directory of images to which the selected journal will be applied.

### Journal

Displays the journal that has been selected.

### Select Journal

Selects a journal to apply to the selected directory of images.

### Close Each Loop

Determines which images will be closed before continuing on to the next image in the loop. The *Close Each Loop* setting is journalizable.

***Nothing*** indicates that no images will be closed, although the journal that is run can perform a closing operation of its own. If you select *Nothing,* the *Close Options* group will become unavailable.

***Opened Image*** indicates that the image opened at the beginning of the loop will be closed at the end of the loop. If the image no longer exists, no error message will be displayed.

***All Images*** will close all displayed images at the end of each loop.

### Close Options

Determines how images that are being closed will be saved. The *Close Options* group will be disabled if *Nothing* has been selected from *Close Each Loop.* The *Close Options* setting is journalizable.

***Save Nothing*** specifies that any image that is closed should not be saved, regardless of whether it has been newly created or modified.

***Query Modified*** specifies that the program should ask whether or not to save each newly created or modified image that is being closed.

***Auto Save Modified*** will automatically save each image being closed. However, if the image is newly created, you will still be asked to provide a file name.

### Confirm Each Loop

Selecting this check box will produce a query to confirm the actions of the journal before proceeding to the next image. This option is journalizable.

### Run

Carries out the command, opening each image and running the specified journal for each in turn. This button is journalizable.

### Close

Closes the dialog box.

# Toggle Interactive (Journal Menu)

**Enables the display of the next command's dialog box during playback, allowing you to make changes to the dialog box settings.**

**Drop-in: JOURNAL**

Use this command during recording when you want the next command's dialog box to appear during playback. This will allow you to change its settings. If a command does not have a dialog box, the Toggle Interactive command will be ignored.

The *Toggle Interactive* command in the Journal Editor can also enable and disable the interactive display of a command's dialog box during playback. A journal command which was recorded with Toggle Interactive will be marked with an "X" in the Journal Editor dialog box. Choosing the *Toggle Interactive* command will turn off the display of that dialog box.

**Note:** Image-1/AT users may recognize this command as equivalent to its *Query During Playback* command.

## Toggling Interactive Mode

To record the "Interactive" state for a journal function, use the following procedure:

| Step | Action |
|---|---|
| **1** | Start the recording of the journal and record all commands that occur prior to the command that will have an interactive display of its dialog box. |
| **2** | From the Journal menu, choose Toggle Interactive. A check mark will be placed next to this menu item. |
| **3** | Record the command for which you want the dialog box displayed when the journal is played back. |
| **4** | Continue recording the journal. |

# Show Message and Wait (Journal Menu)

**Records a message in a journal that is displayed during the journal playback; the message display pauses the journal playback and allows user input.**

**Drop-in: JOURNAL**

Use this command when you want to pause a journal and display a message or instructions for the user during playback of a journal. Typical uses include displaying instructions about upcoming commands, pausing the journal and prompting the user for input, and helping to test the journal. The Image Window Tools and Region Tools are accessible while this command's message is displayed.

This command also enables you to add a timeout value to the message so that the journal automatically continues after a specified amount of time, even if the user has not responded to the message. After you type the message text and title, you can preview your message using the *Preview* button in the Show Message and Wait dialog box.

This command is the same as the *Show Message and Wait* command available through the Journal Editor.

## Using Show Message and Wait

To record Show Message and Wait in a journal, use the following procedure:

| Step | Action |
|---|---|
| **1** | Begin recording a journal and record all commands that occur before the display of the message you want to record. |
| **2** | From the Journal menu, choose Show Message and Wait. The Show Message and Wait dialog box opens. |
| **3** | From the *Display Buttons* group, click *Continue, Cancel* or *Both* to select which button(s) you want to be displayed in the message dialog box. |
| **4** | You may want to add a timeout feature to the message dialog box so that the journal is automatically continued even if a response to the message is not given. To do so, select *Use Timeout.*<br>**AND**<br>Select the number of seconds using the *Timeout* text box. |
| **5** | Type the desired message in the *Message* text box.<br>**Note:** Press the [CTRL] and [ENTER] keys simultaneously to add a paragraph return in the *Message* text box. |
| **6** | Choose *Preview* to display a sample Message dialog box based on the options you have selected. |
| **7** | Choose *OK.* |
| **8** | Continue recording the journal. |

## Show Message and Wait - Dialog Box Options

### Display Buttons

Specifies the command button(s) displayed in the message box when the journal is played back. You can display a *Continue* button, a *Cancel* button, or both.

### Use Timeout

Enables the timeout specified with the *Timeout* option.

### Timeout

Specifies the length of the timeout (in seconds) that occurs before the journal is automatically continued if a response to the message is not made.

### Title

Specifies the title of the message dialog box.

### Message

Specifies the message that will be displayed during playback of the journal.

### Preview

Displays a preview of the message dialog box based on the options selected in the Show Message and Wait dialog box.

### OK

Records the Show Message and Wait command.

### Cancel

Cancels the command.

# Record Image State (Journal Menu)

**Records the present state of the active image as it exists when the Record Image State journal function is run.**

**Drop-in: JOURNAL**

Use this command when you want to record the state of the active image, such as information about its display, current plane, contrast, thresholding, or display state of the Image Window Toolbar.

The window size, window location, horizontal/vertical scroll bar settings, window or icon status, and zoom setting can be recorded. The active plane will also be recorded. The display information that is recorded are the image's contrast, brightness, look-up table (LUT) model and settings (contour, quantization, invert, and subtract), and palette entries. The threshold information recorded include the image's Threshold Tool mode, low threshold setting, and high threshold setting.

**Note:** LUT settings and palette entries apply only to 8-bit and 16-bit images.

**Note:** This command should be added only when recording a journal. Although it can be deleted from a journal using the Journal Editor, the recorded state settings can not be modified using the Journal Editor. Thus, you should set up the active image carefully prior to recording the image state.

## Recording an Image's State During Journal Playback

To configure recording of the active image's state during playback of a journal, use the following procedure:

| Step | Action |
|---|---|

| | |
|---|---|
| **1** | Start the recording of the journal and record all commands that occur prior to the image state you want to record. |
| **2** | Set up the current image window in the exact state you want played back.<br><br>**Note:** The recorded state settings can not be modified using the Journal Editor. Thus, you should carefully set up the image prior to recording its state. |
| **3** | From the Journal menu, choose Record Image State. |
| **4** | Continue recording the journal. |

# Select Image (Journal Menu)

**Programmatically selects an image from the open images during the running of a journal or lets the user specify an image selection during a programmed pause while running the journal.**

**Drop-in: JOURNAL**

Use this command to select an image programmatically in a journal. Place this command in your journal to select a predefined image or use it to enable the person running the journal to select an image during a programmed pause. If you activate interactive mode before recording your journal, you can enter a text message in the Text box to be displayed when the journal is run. The text message should prompt the user to complete a step or action before continuing the execution of the journal.

**Note:** Select Image sets the image selected as the Last Result. This means that the next action done to an image in the journal will be the selected image by default.

## Selecting an Image

The following procedure assumes that you are recording a journal or editing a journal.

To select an image in a journal, use the following procedure:

| Step | Action |
|---|---|
| **1** | If you are recording a journal, ensure one or more images are open in MetaMorph. If you are editing a journal using the Journal Editor, go to step 2. |
| **2** | From the Journal > Recording Tools menu, choose Select Image. The Select Image dialog box opens.<br><br>**OR**<br><br>From the Journal Editor, drag or add the Select Image function to your journal dialog. When the Add Function dialog box opens, choose *Yes* or *No* for Interactive Mode. The Select Image dialog box opens. |
| **3** | Click the Image box to identify the image that you want the journal to select. The |

image drop-down list opens.

4 Click the image you want to have the journal select, or choose the appropriate method of image selection for the journal to use.

- Choose *Last Result* to use the last resulting image created by this journal.
- Choose *Current at Start* to use the image open and selected at the start of running the journal. (This option is active only in journal edit mode.)
- Choose *Specified* to enable you to change the image selection in the journal editor.
- Choose *Select on Playback* if you want to enable the user running the journal to be able to select or change the image selection while playing the journal.

**Note:** See Image Selectors and Image Selector Structure for Journals for more information about image selectors.

5 If Interactive Mode is enabled, type an instruction or message for the user into the Text box.

6 Click Select to complete adding this function to your journal.

7 Click Cancel to discontinue adding this function to your journal.

## Select Image - Dialog box Options

### Image

Selects the image to be used by the journal you are running. You can apply an image selection while recoding your journal, apply or change an image selection using the Image Editor, or enable the user to apply an image selection when running the journal. Click the image you want to have the journal select, or choose the appropriate method of image selection for the journal to use.

***Last Result*** uses the last resulting image created by this journal.

***Current at Start*** uses the image open and selected at the start of running the journal. (This option is active only in journal edit mode.)

***Specified*** enables you to change the image selection in the journal editor.

***Select on Playback*** enables the user running the journal to be able to select or change the image selection while playing the journal.

**Note:** See Image Selectors and Image Selector Structure for Journals for more information about image selectors.

### Text

Enables you to display a text message in conjunction with the Select Image function when the associated journal is run.

### Select

Applies the Select Image function and the associated settings to your journal.

### Cancel

Discontinues applying the Select Image function to your journal.

# Change Plane (Journal Menu)

**Records a relative change in plane position of a specified number of planes for the specified image.**

**Drop-in: JOURNAL**

Use this command to record a relative change in plane positions in a journal. You can specify a forward or backward move in plane position (until the first or last plane is reached). If you want to specify a particular plane number, you should record the Select Plane command in the Stack menu instead.

## Recording a Change of Planes in a Journal

To record a change in planes to a journal, use the following procedure:

| Step | Action |
|---|---|
| **1** | Start the recording of the journal and record all commands that occur prior the change in plane position. |
| **2** | From the Journal menu, choose Change Plane. The Change Plane dialog box will appear. |
| **3** | Select the desired image using the *Image* selector. |
| **4** | Type the desired number of planes to move forward *(x)* or backward *( -x)* in the *Change Plane By* text box. |
| **5** | Choose *OK.* |
| **6** | Continue recording the journal. |

## Change Plane - Dialog Box Options

**Image**

Selects the stack image for changing plane position.

**Change Plane By**

Specifies the relative change in plane position to perform when the journal is played back. Use a positive number to move forward in the stack from the current position or use negative number to move backward in the stack.

**OK**

Records the change in plane position.

**Cancel**

Cancels the command.

# Beep (Journal Menu)

**Adds the computer's system "beep" sound to the journal being recorded.**

**Drop-in: JOURNAL**

Use this command when you want to record an audible tone before or after another command as an audio "alert." This command is the same as the *Beep* function in the Journal Editor.

**Note:** This command relies on the correct configuration of your computer to emit an audible sound, and uses the computer's built-in system tone, rather than any sound card.

## Adding the Computer's "Beep" Sound to a Journal

To add the computer's "beep" sound to a journal, use the following procedure:

| Step | Action |
|---|---|
| **1** | Start the recording of the journal and record all commands that occur prior to the "beep." |
| **2** | From the Journal menu, choose Beep. The computer will emit its "beep" sound. |
| **3** | Continue recording the journal. |

# Delay (Journal Menu)

**Adds a delay of the specified length to the journal being recorded.**

**Drop-in: JOURNAL**

Use this command when you want to record a delay after another command. This command is the same as the *Delay* function in the **Journal Editor.**

This command has an accuracy on the order of milliseconds, but performance is computer-dependent. You can time this (and other) commands with the help of the Stopwatches command (Journal menu).

## Adding a Delay to a Journal

To add a delay to a journal, use the following procedure:

| Step | Action |
|---|---|
| **1** | Start the recording of the journal and record all commands that occur prior to the delay. |
| **2** | From the Journal menu, choose Delay. The Delay dialog box will appear. |
| **3** | Select the length of the delay using *Delay Time.* |
| **4** | Choose *OK.* |
| **5** | Continue recording the journal. |

## Delay - Dialog Box Options

**Delay Time**

Specifies length of the delay. The delay can be in milliseconds, seconds, minutes, or hours.

**OK**

Records the delay.

**Cancel**

Cancels the command.

# Pick Point (Journal Menu)

**Displays a dialog box prompting the user to click on an image. The X and Y coordinates at that position are then measured. The coordinates' values are returned and the dialog box closed automatically.**

### Drop-in: PICKPT

This command is enabled only during the recording of a journal. Use it to create a dialog box that prompts the user to click on a position in an image and then returns the values of the coordinates to the variables $PickPoint.X$ and $PickPoint.Y$. A third value, returned to the variable $PickPoint.Success$, will be 1 if the user successfully clicks on the image and 0 if the user clicks on cancel.

Pick Point is similar to the Measure Pixel function in that it measures X and Y coordinates. However, once the user clicks on the image, the Pick Point dialog box closes automatically.

## Using Pick Point

To add a pick point dialog box to a journal, use the following procedure:

| Step | Action |
|---|---|
| **1** | Start the recording of the journal and record all commands that occur before the point needs to be picked. |
| **2** | From the Journal menu, choose Pick Point. The Pick Point set-up dialog box will appear. |
| **3** | In the *Title* text box, type the text that you want to appear in the title bar of the Pick Point message box |
| **4** | Type the message (prompt) that you want the user to see in the message box in the *Prompt* text box. |
| **5** | Click the *Pick* button to open the message box you've created so you or the user can pick a point to be measured. |
| **4** | Choose *Cancel* to cancel the operation. |
| **5** | Continue recording the journal. |

### Pick Point - Dialog Box Options

**Title**

Specifies the text that will appear in the title bar of the Pick Point message box.

**Prompt**

Specify the text that will be used to prompt the user to pick a point on the active image.

**Pick**

Click this to display the Pick Point message box so you can click on a position in the image that you want to measure the X,Y values of.

**Cancel**

Cancels the operation and closes the Pick Point set-up dialog box.

# Branch on User Input (Journal Menu)

**Creates a user-configured dialog box that runs one of two specified journals, depending on whether you click a *Yes* or *No* command button in the dialog box. This command will appear in the Journal menu only while you are in "journal recording" mode.**

**Drop-in: JNLYESNO**

Use this command to configure a simple dialog box that runs one journal if you click the *Yes* command button during playback or a different journal if you click the *No* button. You can also configure the dialog box to contain a *Cancel* button, which, when clicked, will terminate playback of the entire journal. You can specify the *Caption Text,* which will appear on the dialog box's title bar, and the *Message Text,* which will appear in the body of the dialog box.

EXAMPLE: You could create a journal that successively displays the individual planes in a stack. By using the Branch on User Input function, you could create a dialog box, entitled "Copy to Save Folder," which displays the message, "Do You Want to Copy This Plane?" You could configure the dialog box so that clicking the *Yes* button would run the *Save As* function to copy the current plane to a specified folder, whereas clicking the *No* button will run the *Change Plane* function to jump to the next plane in the stack.

## Running Journals Based on User Input

To configure a dialog box that uses a branching condition to select a journal to run on the basis of your input, use the following procedure.

**Note:** You must be in "journal recording" mode for this command to appear in the Journal menu. You should also already have created the journals that you intend to run from the new dialog box.

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Branch on User Input. The Branch on User Input dialog box will appear. |
| **2** | In the *Caption Text* box, type a title for the dialog box that you want to appear during playback. |
| **3** | In the *Message Text* box, type a message that you want to appear in the body of the dialog box. This typically will be in the form of a question. |
| **4** | To see a sample preview of what the dialog box will look like, choose *Preview.* The dialog box will appear in the application window. Click any of the buttons to close it.<br><br>If necessary, repeat Steps 2 or 3 to reconfigure the title or message in the dialog box. |
| **5** | Select the *Yes/No* radio button to configure the dialog box to display a *Yes* and a *No* command button.<br><br>**OR**<br>Select the *Yes/No/Cancel* radio button if you want to configure the dialog box to have a *Cancel* button, which, when clicked will |

cancel playback of the entire journal.

**6** Choose the *Press to Select* button next to the *Run YES Journal* label to select a journal that will run when you click the *Yes* button during playback. This will open the Select a Journal dialog box. Select the desired journal file and choose *Open.*

**AND**
Choose the *Press to Select* button next to the *Run NO Journal* label to select a journal that will run when you click the *No* button.

**Note:** Although it is not necessary to assign a journal to both buttons, you must assign a journal to at least one of them.

**7** When you have finished, choose *Close.*

## Branch on User Input - Dialog Box Options

**Caption Text**

Specifies a title for the new dialog box. This text will appear in the title bar at the upper edge of the dialog box.

**Message Text**

Specifies a message that will appear in the body of the dialog box. Typically, this will be in the form of a question ("Do you want to do such-and-such?").

**Yes/No**

Select this radio button to configure the dialog box to have a *Yes* and a *No* command button.

**Yes/No/Cancel**

Select this radio button to configure the dialog box to have a *Yes* button, a *No* button, and a *Cancel* button. When clicked, the *Cancel* button will terminate playback of the entire journal.

**Press to Select (Run YES Journal)**

Opens the Select a Journal dialog box, from which you can select the file for a journal that will run when you click the *Yes* button during playback.

**Press to Select (Run NO Journal)**

Opens the Select a Journal dialog box, from which you can select the file for a journal that will run when you click the *No* button during playback. **Note:** Although it is not necessary to assign journals to both the *Yes* and the *No* button, you must assign a journal to at least one of them.

**Preview**

Displays a sample preview of the new dialog box, as currently configured. Click any of the buttons in the dialog box to close it.

**OK**

Accepts the current configuration of your new dialog box, and closes the Branch on User Input dialog box.

**Close**

Rejects the current configuration, and closes the Branch on User Input dialog box.

# Branch on Object Measurement (Journal Menu)

**Runs a specified journal if (1) a selected object's morphometric data meets a particular quantitative criterion, (2) a selected object passes at one classifier filter, or (3) a summary data parameter for all of the objects in the image meets a particular quantitative criterion. Runs a different specified journal if one of these three selected conditions is not met.**

### Drop-in: MBRANCH

Use this command to run one or the other of two selected journals, depending on whether or not a specified measurement criterion has been met. This procedure is carried out in the manner of an "IF…THEN…ELSE" condition, with one journal being run if the "IF" condition is met and the other journal being run if the condition is not met.

One of three types of branch conditions can be configured:

(1) The first type of condition is based on whether or not the specified morphometric parameter for an object (e.g., area, perimeter, shape factor, average gray value, etc.) meets a particular quantitative criterion, that is, whether it is equal to, not equal to, greater than, or less than a specified value, or is between two specified values.

(2) A second method is simply based on whether or not the specified morphometric parameter for a selected object passes any classifier filter, as configured by the Configure Object Classifiers command (Measure menu).

(3) The third possible condition is based on whether or not the specified summary data parameter for the objects in the image (e.g., the average, minimum, maximum, or standard deviation of the object areas, shape factors, gray values, etc.) meets a particular quantitative criterion.

If you use one of the first two types of branch conditions, basing the outcome on the characteristics of a selected object, you can select the object based on either its object ID number or on its location within the image.

**Note:** Before you can carry out the Branch on Object Measurement command, three criteria must be met: (1) an image must be open and active in the application workspace, (2) the image must already have been measured with either the Measure Objects or the Integrated Morphometry Analysis command, and (3) the journals you select must already be created and saved to disk.

### Measurement Term Definitions

## Running Journals Based on the Outcome of Object Measurements

To configure a "TRUE/FALSE" branching condition that selects a journal to run on the basis of object measurements, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Branch on Object Measurement. The Branch on Object Measurement dialog box will appear. |
| **2** | From the *Source* option button group, select the type of branching condition to apply. Select<br><br>***Object Data*** to base the journal selection on whether or not the specified morphometric parameter for an object meets a particular quantitative criterion,<br><br>***Object Classifier*** to base the journal selection on whether or not the specified morphometric parameter for a selected object passes any classifier filter (as configured by the Configure Object Classifiers command), or<br><br>***Classifier Summary*** to base the journal selection on whether or not the specified summary data parameter for the objects in the image meets a particular quantitative criterion. |

**3** If you selected either *Object Data* or *Object Classifier* as the condition type in Step 2, the *Object Selected By* option group will be available. Please continue to Step 4.

**OR**
If you selected *Classifier Summary* as the condition type in Step 2, the *Object Selected By* option group will become unavailable. You should now skip to Step 5.

**4** From the *Object Selected By* group, select *Object #* to select the object on the basis of its object ID number. Then select its number from the *Object #* spin box that appears.

**OR**
Select *Object Location* to select the object on the basis of its location in the image. Then specify the desired X and Y coordinates, respectively, using the *X Pos* and *Y Pos* spin boxes that appear.

**5** If you selected either *Object Data* or *Classifier Summary* as the condition type in Step 2, the *IF* section of the *Branch* configuration group will be available. Please continue to Step 6.

**OR**
If you selected *Object Classifier* as the condition type in Step 2, the *IF* section of the *Branch* configuration group will display "If the selected object passes at least one classifier…." The outcome of the branch condition will be determined by whether the selected object passes the first active classifier set listed in the *Classifiers* list of the **Configure Object Classifiers** dialog box. You should now skip to Step 8.

**6** Use the *Branch* configuration group to configure the branching condition. From the *IF* drop-down list, select the object or summary data parameter that will be used to determine if the condition is met.

If you selected *Object Data* as the condition type in Step 2, you will select a single-object parameter, such as the object's total area, average gray value, perimeter, etc.

**OR**
If you selected *Classifier Summary* as the condition type in Step 2, you will select a grouped object statistical parameter, such as the average area, the standard deviation of the shape factor, the minimum fiber length, etc.

**7** Use the options on the next line to configure the filtering that will be used for the selected parameter.

From the drop-down list, select *Equal To, Not Equal, Less Than, Greater Than,* or

*Between.*

**AND**
Use the spin box to pick a value for the selected parameter. If you selected *Between,* two spin boxes will appear, which you can use to select the minimum and maximum value for the criterion range.

**8** To select the journal that will be run if the object measurement passes the configured condition, choose the *Press to Select* button next to *Run TRUE Journal.* The Select a Journal dialog box will appear.

**AND**
Select the file for the desired journal. If necessary, use the *Look In* list or Up One Level button to select the appropriate drive and folder. Then choose *Open* to return to the Branch on Object Measurement dialog box.

**9** To select the journal that will be run if the object measurement fails the configured condition, choose the *Press to Select* button next to *Run FALSE Journal.* The Select a Journal dialog box will again appear.

**AND**
Select the file for the desired journal. If necessary, use the *Look In* list or Up One Level button as before to select the appropriate drive and folder. Then choose *Open* to return to the Branch on Object Measurement dialog box.

**10** When you are ready to run the conditional journals, choose *OK.*

**11** When you have finished, choose *Close.*

## Branch on Object Measurement - Dialog Box Options

**Source**

Selects the type of branching condition to apply:

***Object Data*** bases the journal selection on whether or not the specified morphometric parameter for an object meets a particular quantitative criterion,

***Object Classifier*** bases the journal selection on whether or not the specified morphometric parameter for a selected object passes any classifier filter (as configured by the Configure Object Classifiers command), and

***Classifier Summary*** bases the journal selection on whether or not the specified summary data parameter for the objects in the image meets a particular quantitative criterion.

**Object Selected By**

Selects a measured object on the basis of its object ID number *(Object #)* or its position in the image *(Object Location).* This option button group will appear only if you select *Object Data* or *Object Classifier* from the *Source* option button group. If you select *Object #,* the *Object #* spin box will become available. If you select *Object Location,* the *X Pos* and *Y Pos* spin boxes will become available.

**Object #**

This spin box specifies the object ID number (up to a maximum of 32767) of the object whose measured morphometric value will be used to determine the journal that will be run. This option will appear only if you

select *Object #* from the *Object Selected By* option button group.

**X Pos**

Specifies the X-coordinate of the object whose measured morphometric value will be used to determine the journal that will be run. This option will appear only if you select *Object Location* from the *Object Selected By* option button group.

**Y Pos**

Specifies the Y-coordinate of the object whose measured morphometric value will be used to determine the journal that will be run. This option will appear only if you select *Object Location* from the *Object Selected By* option button group.

**IF**

This group of drop-down lists and spin box(es) configures the values for the morphometric filter that will be used to determine the branching of the TRUE/FALSE condition. This option group will appear only if you selected *Object Data* or *Classifier Summary* as the *Source.*

The first drop-down list selects the morphometric parameter that will be used by the branching filter. If you selected *Object Data* as the *Source,* this list will select a single-object parameter, such as the object's total area, average gray value, perimeter, etc. If you selected *Classifier Summary* as the *Source,* this list will select a grouped object statistical parameter, such as the average area, the standard deviation of the shape factor, the minimum fiber length, etc.

The second drop-down list selects the method of comparison that will be used by the branching filter: *Equal To, Not Equal, Less Than, Greater Than,* or *Between.*

If you selected *Equal To, Not Equal, Less Than,* or *Greater Than* from the second drop-down list, a single spin box will be available, which you can use to pick a value for the selected parameter. If you selected *Between,* two spin boxes will appear, which you can use to select the minimum and maximum value for the criterion range.

**Run TRUE Journal**

Opens the Select a Journal dialog box, from which you can select the journal to run if the morphometric measurement passes the configured condition.

**Run FALSE Journal**

Opens the Select a Journal dialog box, from which you can select the journal to run if the morphometric measurement fails the configured condition.

**Current Value**

Displays the value of the currently selected parameter. If you selected *Object Classifier* as the *Source,* this status line will read either TRUE or FALSE.

**Range**

Displays the range of values for the selected parameter.

**OK**

Applies the IF…THEN…ELSE branching condition to the active image, running the TRUE journal if the conditions are met and running the FALSE journal if they are not.

**Close**

Closes the dialog box.

# Introduction to the Use of Variables

## What Are Variables?

One of the characteristics that sets MetaMorph above other imaging programs is its ability to measure and make use of a vast number of measurement parameters. MetaMorph provides you with the means of creating complex expressions that use these parameters. These expressions can be used to define a variable, which you can use in an almost limitless way to perform advanced measurements or to evaluate some aspect of an image and use the result of the evaluation to perform some action, typically

by running a journal. Variables can be built using both numeric expressions, which are based on selected measurement parameters, and character-based expressions, such as an image's annotation. In some cases, you may wish to create a variable that uses other variables you have already defined.

### Creating Variables and Assigning Values

The MMVAR drop-in module provides you with the means of creating variables and using them to calculate and log measurements. In the past, such tasks may have required you to switch to another application, such as a spreadsheet or database program. The Assign Variable command, which is the first to appear in the Journal menu's block of variables commands, lets create your own variable by performing arithmetic, relational comparisons, logical operations, and conversions (e.g., string to number, number to string, absolute values, etc.) on any collection of MetaMorph measurement parameters. To see an example of using image file variables, refer to the following application note:

**Application Note** — ***How to use image file variables to save images in a journal without user interaction***

### Using Variables in MetaMorph: An Example

A simple use of variables might be to create your own variable by writing an equation that uses one or more existing MetaMorph variables, and to log the calculated value during a runtime measurement of an image. For example, you might want to log the "average intensity" value of a single 24-bit image pixel. To do so, you would open the image and apply the Measure Pixel command (Measure menu). Next you would use the Assign Variable command to create the equation:

> "(Measurement("Measure Pixel (24-bit)", "Red Value")+Measurement("Measure Pixel (24-bit)", "Green Value")+Measurement("Measure Pixel (24-bit)", "Blue Value")) / 3"

To create this equation, you would open the Assign Variable dialog box and type a name for the variable in the *Variable Name* text box. The name can by up to 30 characters long and must not contain spaces. From the Measurement tab page, you would then scroll down until you found the entry for Measure Pixel (24-Bit), and expand the "tree" view to display the variable entries. You would then successively use the *Insert* button to add the measurement variables for the Red Value, Green Value, and Blue Value, typing a "+" between each expression. After placing opening and closing parenthesis marks around the entire expression, you would type "/ 3" at the end, to divide the summed color intensities by the number of color channels. Finally, you would enter the expression into the Variables tab page table by choosing *Assign.* After you have created the variable in this way, you could then log the value as needed with the Log Variable command after any time you select a pixel in a 24-bit color image using the Measure Pixel command (Measure menu).

### Using Variables to Run Journals in Conditional Branches and Loops

A particularly powerful use of variables is to make a comparison between a variable's value, as derived from a measurement that you make on an image, and a standard value that you set ahead of time. This is performed by using the Branch on Variable command. You could then use comparisons between the standard value (or range) and subsequent measurements to execute a set of conditional runtime journals through the use of an "If/Then/Else" (TRUE/FALSE) branching decision. When the condition is met, one journal will run, and if the condition is not met, a different journal will run. This is somewhat similar to the conditional branching used by the Branch on Object Measurement command (Journal menu). The difference, of course, lies in the ability to use variables to create your own custom equation to set the conditions.

A similar command, Loop Variable, uses "For/Next," "While/Wend," "Do/Until," and "Do/While" logical constructs to run a selected journal when specific conditions have been met and to stop when a particular criterion has been reached (e.g., number of steps).

### Advanced Uses of the Variables Commands

Text-based values of variables can be used in some of MetaMorph's other commands. For example, you could use a variable named "Dist" that has been assigned a measurement of the distance between objects. With the Draw Text drop-in command (Graphics menu), you could type "%Dist%" in the *Text* box

(without the quotation marks, but remember to use the percentage signs). This will stamp the active image with the actual measured distance. Variables can also be used in this manner with the Show Message and Wait command (Journals menu).

### The Variables Commands:

**Assign Variable**

**Delete Variable**

**Log Variable**

**Branch on Variable**

**Loop Variable**

# Using Variables - Procedures

**Assigning Variables**

**Deleting Variables**

**Logging Variables**

**Branching on Variables**

**Looping Variables**

## Introduction to Using Variables - Dialog Box Options

**Assign Variable**

**Delete Variable**

**Log Variable**

**Branch on Variable**

**Loop Variable**

# Assign Variable (Journal Menu)

**Creates custom variables, assigns values to variables, and performs arithmetic, string, number, and logical operations. These variables can then be used when creating and executing journals.**

**Drop-in: MMVAR**

Use this command to define your own variables, or change the values of MetaMorph variables. Values using variables are assigned to equations that may include selected MetaMorph defined parameters and/or operators. These parameters include, but are not limited to, hardware settings, image parameters, and measurement results.

Variables can be used following measurements that you make on an image to make calculations and the calculated value can be saved in a log file by using the Log Variable command. Variable values can also be compared to a standard value or range, or a set of conditions, and the results of the comparison used for branching or looping of a journal.

For more information on branching and looping, refer to the Branch on Variable and Loop Variable help files. To see an example of using image file variables, refer to the following application note:

**Application Note** — ***How to use image file variables to save images in a journal without user interaction***

**Note:** Functions for branching and looping of journals based on variables, as well as assignment of variables, can also be incorporated directly into a journal using the *Actions* Tab of the of journal editor.

There are two major categories of variables within MetaMorph – Custom and Built-in. Custom variables are defined by the user mostly by using the Assign Variable command. You can use existing parameters from MetaMorph's built-in variables and/or define new variables using built-in operators. Custom variables can be deleted and are automatically deleted when you exit MetaMorph. They will not be available the next time MetaMorph is opened unless you created them again.

**Note:** Custom variables must start with a letter. Numbers are valid characters in custom variable names as long as the name begins with a letter. Spaces and non-alphanumeric characters are not valid characters.

Built-in variables are associated with images, regions, installed hardware, and measurements. The following types of built-in variables are available in MetaMorph:

- **Image** variables refer to parameters of the active plane of the active image. The Assign Variable dialog box displays the current values of these variables.
- **Measurements** variables contain the last logged measurement. The software must log measurement data before you can access it in the Assign Variable dialog box. All measurement variables are read-only.

  **Note:** Within a journal, a log can be not open (or open and paused) and variables will still be updated.
- **Program** variables contain acquisition and installed hardware settings. You can assign new values to these variables to change, among other things, the magnification, stage position, illumination, and exposure settings during an image acquisition journal.
- **Region** variables refer to parameters of the active region of interest. The dialog box displays the current values of these variables.

All Image, Measurements, and Region variables have the following attributes, or permissions:

- **Read-only (R)** variables cannot be assigned new values. All measurement variables are read-only.
- **Readable/Writable (RW)** variables can be read and changed.
- **Readable/Writable/Executable (RWX)** variables can be changed and the result is immediate. For example, if you change the Region.Width variable for an active region on an image and click OK, the region changes width to reflect the change.

These permissions are listed in the *Variable* table for each variable type.

**Note:** As of version 6.0 and above, the naming convention for built-in variables in MetaMorph has changed. Built-in variables names are no longer be enclosed with "$" characters (for example, $Region.Width$ is now displayed as Region.Width).

 For more information about variables, be sure to read Introduction to the Use of Variables.

## Assign Variables: Creating Custom Variables

Complete the following procedure to create a custom variable:

**Note:** Custom variables that you create are only available for the current MetaMorph session and will not be available the next time MetaMorph is started.

| Step | Action |
|---|---|
| 1 | From the Journal menu, choose Assign Variable. The Assign Variable dialog box opens with the Variables tab selected. |
| 2 | Select *Custom* from the Variable Type Drop Down Box. The Custom Variable Table opens. |
| 3 | Type a name for the new variable in the *Variable* box on the right side of the dialog box.<br><br>**Note:** Custom variables must start with a letter. Numbers are valid characters in custom variable names as long as the name begins with a letter. |
| 4 | Use the *Variable Expression* Field to create an expression for your variable. Your expression can contain any defined variable from the Variables tab, as well as any operators from the Operator tab. You can also type directly into this field or use the **>** and bottom **>>** button to add or replace existing variables or operators. |
| 5 | To add an existing variable to your expression, select the variable type from the Variable Type Drop Down Box, then select the variable from the Variable Table. |
| 6 | Click **>** to add the selected variable to your expression or click the bottom **>>** button to replace your expression with the selected variable.<br><br>**Note:** You can also double-click the variable to insert it, or right-click on the variable and select *Insert into Expression*. |
| 7 | To add an operator to your expression, either type it into the Variable Expression field or Click the Operator tab and select the operator from the tree view.<br><br>**Note:** Selecting an operators opens a description and an example below the tree view. |
| 8 | Click **>,** or double-click, to add the selected operator to your expression or click the bottom **>>** button to replace your expression with the selected operator. |
| 9 | When you are satisfied with the equation and the *Result* status line indicates that it is valid, click *OK* to add the variable to the Custom Variables table. |
| 10 | Click *Close* to close the Assign Variable dialog box. |

## Assign Variables: Assigning New Values to MetaMorph Variables

Complete the following procedure to assign a new value to a MetaMorph variable:

**Note:** Measurement variables cannot be changed, they are read-only. Image and region variables are only valid for the active image/region.

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Assign Variable. The Assign Variable dialog box opens with the Variables tab selected. |
| **2** | Select the variable type from the *Variable Type* Drop Down Box, then select the variable to edit from the Variable Table. |
| **3** | Click the top **>>** button to add the selected variable to the *Variable* field.<br><br>**Note:** You can also right-click the variable and select *Set as Variable Name* to insert it in the *Variable* field. |
| **4** | Assign a new value in the Expression field to create a valid result. The expression can include existing variables, operators, text, or numbers depending on the variable type. |
| **5** | When you are satisfied with the equation and the *Result* status line indicates that it is valid, click *OK* to update the variable. |
| **6** | Click *Close* to close the Assign Variable dialog box. |

## Assign Variable - Dialog Box Options

### Variable Tab

### Operators Tab

#### >>

Replaces the variable listed in the *Variable* field with the active one from the *Variables* tab.

#### >

Adds the active value or operator from the *Variables* or *Operators* Tab to the Variable Expression field.

#### >>

Replaces the entire expression with the active selection in the Variables or Operators Tab.

**Note:** You can also right-click a selected variable and select either *Insert into Expression* or *Set as Variable Name* to perform these actions.

### Variables and Expression

#### Variable

Specifies a name for a new variable, or provides a list of previously defined variables which you can select for assignment.

**Note:** Custom variables must start with a letter. Numbers are valid characters in custom variable names as long as the name begins with a letter.

### Variable Expression Field

The expression to be assigned to the variable listed in the *Variable* drop-down box above. Expressions are built by inserting variables from the Image, Measurements, Programs, Regions, or Customs Variable groups, and/or by typing or inserting operators from the Operators tab in the appropriate place in the equation being built in the *Variable Expression* box.

**Note:** For character-based variables, you must enclose the string in quotation marks.

### Current Value

Indicates the value for the variable specified by *Variable Name.* If the variable has not yet been assigned, this status line will read "<Variable is undefined>".

### Result

Displays the currently calculated value for the equation entered in the *Variable Expression* box. If an equation has not been entered, this status line will read "Null expression." If the equation is invalid or missing an expression or other equation element, the *Result* status line will indicate this.

### OK

Enters a properly defined variable to the Variables Table.

### Close

Closes the dialog box.

## Assign Variable: Variables Tab - Dialog Box Options

### Variable Type Drop Down Box

Select the type of variable to assign. The following choices are available:

- **Custom** variables are those defined by the user.
- **Image** variables refer to defined variables of the active image. The dialog box displays the current values of these variables.
- **Measurements** variables contain the last logged measurement data for an image. You must open an appropriate log and log measurement data before you can access it in the Assign Variable dialog box. All measurement variables are read-only.
- **Program** variables contain settings for installed hardware. You can edit these variables to change, among other things, the magnification, illumination, and exposure settings during an image acquisition journal.
- **Region** variables refer to defined variables of the active region. The dialog box displays the current values of these variables.

**Note:** With the region variables only the rectangle, circle (oval), line, and polyline region shapes are writable. Therefore, these are the only shapes that can be resized by entering a different value for the variables: Region.Width, Region.Height, Region.Top, and Region.Left.

### Variable Table

Contains the following information about the variable type selected in the Variable Type drop-down box:

- **Name** lists the names of the selected variable type. Note that MetaMorph no longer uses $ symbols to distinguish built-in variables. For example, the variable for a region's height is now Region.Height, and not $Region.Height$.
- **Current Value** contains the current value of the selected variable. Note that values can be text (string) or numeric. Text values are always defined in quotation marks.
- **Attrib** contains the attributes, or permissions, for the selected variable. Possible attributes are: R (read-only), RW (read-write), and RWX (read, write, and execute). Read-only variables cannot be

changed. Changing the value of a RWX variable will automatically update the parameter.

## Assign Variable: Operators Tab - Dialog Box Options

### Operators Tree View

Contains a tree list of operators and functions that you can use while building custom variable equations. Use the + and – boxes to expand or collapse the view for each type of operator or function. Select an item and a description, an explanation of its return value, and an example is displayed at the bottom of the tab. Use the **>** button to insert the Operator into the variable definition box or the **>>** button to replace the current value with the selected operator. Double-clicking on an item in the tree list will also insert the item into the variable definition box.

**Description**

Describes the currently selected operator or function.

**Returns**

Indicates what value the selected operator or function will pass to the expression it is in.

**Example**

Provides an example of how you might use the selected operator or function.

The following operators and functions are available:

### Arithmetic Operators:

* – Multiplication

/ – Division

+ – Addition

- – Subtraction

### Relational Operators:

= – Equal to

<> – Not equal to

> – Greater than

>= – Not less than

< – Less than

<= – Not greater than

### Logical Operators:

NOT – Logical not

AND – Logical and

OR – Logical or

( and ) – Opening and closing parenthesis

IF – Evaluate condition and return T or F expression

ImageExists – Returns 1 if the image exists, returns 0 if the image cannot be found.

VariableExists – Returns 1 if the variable exists, returns 0 if the variable cannot be found.

CheckIfKeyPressed – Returns 1 if the KeyName was pressed, 0 otherwise

> **Note:** The characters 0-9 and A-Z (upper and lower case) are the only valid characters for the CheckIfKeyPressed operator. The Shift, Control, and Alt keys, as well as all functions keys, are not valid.

### String Functions:

VAL – Converts a character-based representation of a number to its actual numeric value.

VALHEX – Converts a hexadecimal string to a number.

ASC – Converts the first character of a string to an ASCII number.

LEN – Gives the number of characters in a string.

LEFT – Returns the leftmost ‘count’ characters in a string.

RIGHT – Returns the rightmost ‘count’ characters in a string.

MID – Returns a string, starting at the specified position and ending a specified number of characters to the right.

INSTR – Returns the position number of a substring within a specified string.

### Number Functions:

STR – Converts a number to its equivalent character string.

STRHEX – Converts a number to its equivalent hexadecimal number.

CHR – Converts a number to its equivalent ASCII string.

CEILING – Converts a number "x" to the smallest whole value greater than or equal to number "x."

FLOOR – Converts a number "x" to the largest whole value less than or equal to number "x."

ABS – Converts number "num" to its absolute value.

LOG – Calculates the natural logarithm of the number "x."

LOG10 – Calculates the base 10 logarithm of the number "x."

SQRT – Calculates the positive square root of the number "x."

EXP – Calculates the exponential "e" to the "x."

POW – Calculates the number "x" to the power "y."

MOD – Calculates the remainder of "x" divided by "y."

RAND – Generates a random number between 0 and 1.

### Trigonometry Functions:

ACOS – Calculates the arc cosine of the number "x."

ASIN – Calculates the arc sine of the number "x."

ATAN – Calculates the arc tangent of the number "x."

COS – Calculates the cosine of degree "x."

SIN – Calculates the sine of degree "x."

TAN – Calculates the tangent of degree "x."

# Enter Variable (Journal Menu)

**Allows you to provide, during playback of a journal, a dialog box for assigning values to variables.**

**Drop-in: MMVAR**

Use this command when you want to display a message prompting the user to enter or select a value for a variable during playback of a journal. This command is similar to the *Show Message and Wait* command.

The Enter Variable command allows you to choose how to format the message box that will be displayed during journal playback. You may choose to use a Yes/No message box, a message box that lets the

user select one or more of several options, or a message box that asks the user for a string or number.

After you have typed in the desired prompt text and title and specified the format for your message box, you can preview your message using the *Preview* option provided in the Enter Variable dialog box.

## Entering Variables

To create a message box prompting a user to enter a new variable, use the following procedure:

| Step | Action |
|---|---|
| **1** | In the space next to *Variable Name,* type a name for the variable you are assigning a value to. |
| **2** | In the *Dialog Type* list box, select a format for your message box. |
| **3** | Choose a format from the links below for more help creating your message box:<br>• Yes/No Message Box<br>• Yes/No/Cancel Message Box<br>• Message box with a string field<br>• Message box with a number field<br>• Message box with radio buttons<br>• Message box with a text list |

## Creating a Yes/No Message Box

To create a message box prompting a user to select yes or no, use the following procedure:

| Step | Action |
|---|---|
| **1** | Type a title for your message box in the space next to *Title Text*. Your title will appear on the message box. |
| **2** | In the *Prompt Text* box, type the message you want to use to prompt the user to select yes or no. |
| **3** | To preview your message box, click the *Preview* button. |
| **4** | To save your settings and close the Enter Variables dialog box, click *Okay*. |
| **5** | To cancel your settings and close the Enter Variables dialog box, click *Cancel*. |

## Creating a Yes/No/Cancel Message Box

To create a message box prompting a user to select yes, no, or cancel, use the following procedure:

| Step | Action |
|---|---|
| **1** | Type a title for your message box in the |

space next to *Title Text*. Your title will appear on the message box.

**2** In the *Prompt Text* box, type the message you want to use to prompt the user to select yes or no.

**3** To preview your message box, click the *Preview* button.

**4** To save your settings and close the Enter Variables dialog box, click *Okay*.

**5** To cancel your settings and close the Enter Variables dialog box, click *Cancel*.

## Creating a Message Box with a String Field

To create a message box prompting a user to enter a string of characters, use the following procedure:

| Step | Action |
|---|---|
| **1** | Type a title for your message box in the space next to *Title Text*. Your title will appear on the message box. |
| **2** | In the *Prompt Text* box, type the message you want to use to prompt the user to select yes or no. |
| **3** | To preview your message box, click the *Preview* button. |
| **4** | To save your settings and close the Enter Variables dialog box, click *Okay*. |
| **5** | To cancel your settings and close the Enter Variables dialog box, click *Cancel*. |

## Creating a Message Box with a Number Field

To create a message box prompting a user to enter a numeric value, use the following procedure:

| Step | Action |
|---|---|
| **1** | Type a title for your message box in the space next to *Title Text*. Your title will appear on the message box. |
| **2** | In the *Prompt Text* box, type the message you want to use to prompt the user to select yes or no. |
| **3** | Check the *Force number to be between min and max* button if you want the user to enter a value that is within a range you specify. |
| **4** | If you checked the *Force number to be between min and max* button enter a minimum and maximum value you want |

the user to enter.

5 To preview your message box, click the *Preview* button.

6 To save your settings and close the Enter Variables dialog box, click *Okay*.

7 To cancel your settings and close the Enter Variables dialog box, click *Cancel*.

## Creating a Message Box with Radio Buttons

To create a message box that lets the user choose one of several options, use the following procedure:

| Step | Action |
|---|---|
| 1 | Type a title for your message box in the space next to *Title Text*. Your title will appear on the message box. |
| 2 | In the *Prompt Text* box, type the message you want to use to prompt the user to select yes or no. |
| 3 | In the Item List box, enter a list of options that you want the user to choose from.<br><br>**Note:** You must press <CTRL> + Enter to insert a carriage return in the list box. |
| 4 | To have the variable return the selected item as the variable value, click *Return text from item list*, otherwise the variable will return a letter (A, B, or C and so on) as the variable value. |
| 5 | To preview your message box, click the *Preview* button. |
| 6 | To save your settings and close the Enter Variables dialog box, click *Okay*. |
| 7 | To cancel your settings and close the Enter Variables dialog box, click *Cancel*. |

## Creating a Message Box with Text Lists

To create a message box that lets the user choose one *or more* of several options, use the following procedure:

| Step | Action |
|---|---|
| 1 | Type a title for your message box in the space next to *Title Text*. Your title will appear on the message box. |
| 2 | In the *Prompt Text* box, type the message you want to use to prompt the user to select |

yes or no.

3 In the *Item List* box, enter a list of options that you want the user to choose from.

**Note:** You must press <Ctrl> + Enter to insert a carriage return in the list box.

4 To have the variable return the selected items as the variable value, click *Return text from item list*, otherwise the variable will return one or more letters (A, B, C and so on) as the variable value.

5 To preview your message box, click the *Preview* button.

6 To save your settings and close the Enter Variables dialog box, click *Okay*.

7 To cancel your settings and close the Enter Variables dialog box, click *Cancel*.

## Enter Variable - Dialog Box Options

### Variable Name

In this space, enter the name of the variable to which you are assigning a value.

### Dialog Type

Select the type of dialog box you want to use when prompting the user to enter a variable.

### Title Text

Specifies the title of the message dialog box.

### Prompt Text

Specifies the text that will be used to prompt the user to make a selection or enter text. For example, the text "Enter the width of the region here:" would be used to prompt the user to enter a value into a field on the dialog box.

### Force Number to be between Min and Max

Forces the Message Box Number field to accept only values between the selected Min and Max values. In the Message Box, you can type or select the appropriate value. Selected values are between the Min and Max values. If you type a value greater than the Max value or less than the Min value, values less than Min are set to the Min value; values greater than the Max are set to the Max value.

### Min

Specifies the minimum value that can be entered when the message box appears.

### Max

Specifies the minimum value that can be entered when the message box appears.

### Item List

Provides a space for the user to create a list of items that can be chosen by either clicking on radio buttons or selecting one or more items in a list. Note that when you are entering your items into this list you must hit <CTRL> + Enter to insert a carriage return and move down to the next line.

**Return text from item list**

Check this box to have the variable pass the name of the item that the user checks on the Item List as the variable variable value. If this box is not checked, the variable passes a letter (A, B, C, and so on) as the variable value. If the items are part of a Text List, and more than one box is checked, all selected items are passed, separated by commas. If the items are on a Radio Button list, only the value or letter for the selected radio button is passed.

### Preview

Displays a preview of the message dialog box based on the options selected in the Enter Variable dialog box.

### Okay

Records your settings and closes the dialog box.

### Close

Cancels your settings and closes the dialog box.

# Delete Variable

**Removes a user-defined variable from use by MetaMorph.**

**Drop-in: MMVAR**

Use this command to remove a variable that you have created with the Assign Variable command. You can either select an individual variable for removal or remove all variables simultaneously.

 For more information about variables, be sure to read Introduction to the Use of Variables.

## Deleting a Variable

To remove user-defined variables from use by MetaMorph, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Delete Variable. The Delete Variable dialog box will appear. |
| **2** | From the *Variable Name* drop-down list, select the name of the variable you want to remove.<br>**OR**<br>Select the *Delete All Variables* check box to remove all currently active variables. |
| **3** | When you have finished, choose *OK.* |

## Delete Variable - Dialog Box Options

**Variable Name**

Selects the name of an individual variable to be removed.

**Delete All Variables**

When selected, this check box prompts MetaMorph to remove all currently assigned variables.

**OK**

Removes the selected variable from use by MetaMorph.

**Cancel**

Cancels the command.

# Log Variable

**Opens an existing or new data log for storing calculated data from a user-defined variable.**

**Drop-in: MMVAR**

Use this command to store measurement data derived from a variable that you have defined with the Assign Variable command. The Log Variable dialog box provides the means of both opening a log file or DDE link to an open spreadsheet and of sending the data to the file.

For more information about variables, be sure to read Introduction to the Use of Variables**.**

## Logging Variables

To log the data from a user-defined variable, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Log Variable. The Log Variable dialog box will appear. |
| **2** | From the *Variable Name* drop-down list, select the name of the variable for which you want to log data. |
| **3** | Open a data log by clicking the ***Open Log*** command button.<br><br>Once the data log is open, the text on the *Open Log* button will change to *F9: Log Data.* |
| **4** | When you are ready to save the variable data, choose *F9: Log Data,* or press the [F9] function key on your keyboard. The data will be sent to the open data log.<br><br>If you sent your data to a text-based data log, you can inspect the data immediately by choosing View Current Data Log from the Log menu. |
| **5** | When you have finished, choose *Close.* |

## Log Variable - Dialog Box Options

**Log File**

Selects the type of log to log the variable to.

**Variable Name**

Selects the name of a variable for which you want to log data.

**Log variable name as header**

When selected, the variable name is logged as header along with the variable value.

**Log value on one line**

When you select this option, subsequently logged data will be appended to the current line in the log file,

rather than to a new line. The *Log variable name as header* option is unavailable if you select this option.

### Open Log

Opens a data log and/or a DDE link to an open spreadsheet application for logging data. This command will change to *F9: Log Data* when a log file is open.

#### F9: Log Data

Sends the data for the selected variable to the open data log.

#### Close

Closes the dialog box.

# Branch on Variable

**Runs a specified journal if a selected variable's value meets a specific criterion. Runs a different specified journal if the condition is not met.**

**Drop-in: MMVAR**

Use this command to run one or the other of two selected journals, depending on whether or not a selected variable meets a specified criterion. This procedure is carried out in the manner of an "IF…THEN…ELSE" condition, with one journal being run if the "IF" condition is met and the other journal being run if the condition is not met. This situation is similar to that for the Branch on Object Measurement command (Journal menu). If you do not select a journal for the FALSE condition, the conditional testing will behave in the same manner as the *While/Wend* paradigm in the Loop Variable command, and will do nothing unless the TRUE condition has been met.

For quantitative variables, condition testing will determine if the variable's value is equal to, not equal to, greater than, not greater than, less than, or not less than a specified value (or the value of a variable, whose name you specify in the text box), or if the value lies between two specified values. For character-based variables, condition testing will determine if the variable's value is equal to, or not equal to, a specified character string. Character string values must be enclosed in quotation marks.

 For more information about variables, be sure to read Introduction to the Use of Variables.

## Branching on a Variable

To configure a "TRUE/FALSE" branching condition that selects a journal to run on the basis of a selected variable's value, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Branch on Variable. The Branch on Variable dialog box will appear. |
| **2** | From the *IF* drop-down list, select the variable you want to use for the condition testing. |
| **3** | From the drop-down list just below the *IF* list, select the appropriate operator for the condition testing.<br><br>For quantitative variables, select from *Equal to, Not Equal, Greater Than, Not Less, Less Than, Not Greater,* or *Between… and….* |

For character-based variables, select between *Equal (String)* and *Not Equal (String).*

**4** If you selected *Between… and…* in Step 3, two spin boxes will appear next to the drop-down list of operators. Use these spin boxes to select the minimum and maximum values for the inclusive range.

**OR**
If you selected one of the other quantitative operators in Step 3, a single text box will appear. Use this to select the value against which the variable's value is to be tested. Alternatively, you can select the name of another variable.

**OR**
If you selected an operator for a character-based variable, a text box will appear. Type a character string in this box. Be sure to enclose the character string in quotation marks.

**5** To select a journal for the TRUE condition, choose the button next to the *Run TRUE Journal* label. If journals have not yet been assigned, the button will read *"Press to Select."*

**6** The Select a Journal dialog box will appear. Select the journal you want to use. If necessary, use the Up One Level button or *Look In* list to locate the appropriate folder. When you have selected the journal, choose *Open* to return to the Branch on Variable dialog box. The *Press to Select* button label will now display the name of the selected journal.

**Note:** To remove a journal assignment, choose the journal button and then choose *Cancel* from the Select a Journal dialog box.

**7** If you want to assign a journal to the FALSE condition, repeat Steps 5 and 6 for the *Run FALSE Journal* entry.

**8** When you are ready to run the condition testing paradigm, choose *OK.* The variable value will be evaluated and the TRUE/FALSE branching will be carried out, running the appropriate journal.

**9** When you have finished, choose *Close.*

## Branch on Variable - Dialog Box Options

**IF**

This option group sets the condition to be tested for the selected variable. The drop-down list in the first row select the variable to be tested. The drop-down list in the second row selects an operator. For quantitative variables, you can select from *Equal to, Not Equal, Greater Than, Not Less, Less Than, Not Greater,* and *Between… and….* For character-based variables, you can select between *Equal (String)* and *Not Equal (String).* The operator you select will determine the format of the third option. If you selected *Between…*

*and…,* two spin boxes will appear, which you will use to select the minimum and maximum values for the inclusive range. If you selected one of the other quantitative operators, a single text box will appear, in which you can enter a value against which the variable's value is to be tested. Alternatively, you can type the name of another variable, whose value will be used for the comparison. If you selected an operator for a character-based variable, a text box will appear, in which you will type a character string. Be sure to enclose the character string in quotation marks.

**Press to Select (Run TRUE Journal)**

Opens the Select a Journal dialog box, from which you can select the journal to run if the TRUE condition is met.

**Press to Select (Run FALSE Journal)**

Opens the Select a Journal dialog box, from which you can select a journal to run if the TRUE condition is *not* met. If you do not select a journal, the conditional testing will behave in the same manner as the *While/Wend* paradigm in the Loop Variable command.

**Current Value**

This status line indicates the value of the selected variable. If no variable has been selected, this will read "<Undefined>."

**OK**

Runs the condition-testing paradigm, evaluating the current value of the variable and running the TRUE journal if the condition is met or running the FALSE journal (if one has been selected) if the TRUE condition is *not* met.

**Close**

Closes the dialog box.

# Loop Variable

**Repeats a journal a specified number of times or for as long as the value of a selected variable fulfills a specified condition.**

**Drop-in: MMVAR**

Use this command to loop a selected journal using one of four methods of condition-testing:

***For/Next*** - Acts in the fashion of an iterative counter, running the journal a number of times that is determined by setting the counter to run from *X* to *Y,* where you select the starting number, *X,* and the ending number, *Y.* If desired, you can specify that the counter jump by an amount greater than 1. For example, you might set the *STEP* spin box to *2* and run the journal while incrementing from 1 to 10 in steps of 2. Although not currently supported, future enhancements will allow you to use this, for example, to apply the actions of a journal to every other plane of a ten-plane stack. When you configure a For/Next paradigm, you can enter either a name for a counter or the name of an appropriate variable. **QUICK TIP:** You can use the name of another variable to specify a value for the starting or ending step number.

*While/Wend* - Runs the selected journal for as long as the variable fulfills the configured condition *(Equal to, Greater Than, Between… and…,* etc.). This is somewhat similar to the Branch on Variable command when a journal has been selected only for the TRUE condition.

***Do/Until*** - Runs the selected journal until the variable meets the configured condition. The journal will be run at least one time.

***Do/While*** - Runs the selected journal for as long as the variable fulfills the configured condition. The journal will be run at least one time. This is similar to the While/Wend paradigm, with the difference that the condition is evaluated after each replication of the journal under the Do/While paradigm, whereas the condition is tested first before running the journal under the While/Wend paradigm. Thus, the journal will run at least once under the Do/While paradigm, even if the condition is not met, whereas the journal may not run at all under the While/Wend paradigm.

**Note:** You can use negative numeric values as conditions when setting up your loop.

**Note:** This command can be nested, using a loop within another loop.

After you have configured the loop using the Loop a Journal dialog, MetaMorph will display a Loop Variable control panel while recording the loop. This message box lists the journal's name, interval between loops, current loop count, and status of the loop. If you want to cancel a journal loop after you have chosen this command, press the [CTRL] + [BREAK] keys or press the [ESC] key while the progress meter is displayed.

 For more information about variables, be sure to read Introduction to the Use of Variables.

## Looping Variables

To loop a journal based on the value of a variable, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Loop Variable. The Loop Variable dialog box will appear. |
| **2** | From the Loop Construct option button group, select a loop condition-testing paradigm:<br><br>***For/Next*** acts in the fashion of an iterative counter, running the journal a selected number of times. If you select this option, continue to Step 3.<br><br>***While/Wend*** runs the selected journal for as long as the variable fulfills the configured condition (Equal to, Greater Than, Between… and…, etc.). If you select this option, skip to Step 4.<br><br>***Do/Until*** runs the selected journal until the variable meets the configured condition. If you select this option, skip to Step 5.<br><br>***Do/While*** runs the selected journal for as long as the variable fulfills the configured condition. (This is similar to the *While/Wend* paradigm, with the difference that the condition is evaluated after each replication of the journal under the *Do/While* paradigm, whereas the condition is tested first before running the journal under the *While/Wend* paradigm.) If you select this option, skip to Step 5. |
| **3** | If you selected *For/Next* in Step 3, type a name for either an existing quantitative variable or a counter variable in the *FOR* text box.<br><br>**AND**<br>Select a starting and ending count for the variable in the text boxes in the following row of the dialog box. If desired, select an |

incrementing step size with the *STEP* spin box. Now skip to Step 5.

**4** If you selected *While/Wend* in Step 3, select a variable from the *WHILE* drop-down list.

**AND**
From the drop-down list in the following row of the dialog box, select the appropriate operator for the loop condition-testing. For quantitative variables, select from *Equal to, Not Equal, Greater Than, Not Less, Less Than, Not Greater,* or *Between… and….* For character-based variables, select between *Equal (String)* and *Not Equal (String).* Then type a test value for the variable in the accompanying text box. If the variable is character-based, be sure to enclose the character string value in quotation marks.

**5** Choose the *Run Journal* command button. The Select a Journal dialog box will appear.

**AND**
Select the journal you want to loop. If necessary, use the Up One Level button or *Look In* list to locate the appropriate folder. When you have selected the journal, choose *Open* to return to the Branch on Variable dialog box. The *Press to Select* button label will now display the name of the selected journal.

If you chose either *For/Next* or *While/Wend* in Step 3, skip to Step 8. If you chose *Do/Until,* continue to Step 6. If you chose *Do/While,* skip to Step 7.

**Note:** To remove a journal assignment, choose the journal button and then choose *Cancel* from the Select a Journal dialog box.

**6** If you selected *Do/Until* in Step 3, select a variable from the *UNTIL* drop-down list.

**AND**
From the drop-down list in the following row of the dialog box, select the appropriate operator for the loop condition-testing. For quantitative variables, select from *Equal to, Not Equal, Greater Than, Not Less, Less Than, Not Greater,* or *Between… and….* For character-based variables, select between *Equal (String)* and *Not Equal (String).* Then type a test value for the variable in the accompanying text box. If the variable is character-based, be sure to enclose the character string value in quotation marks.

**7** If you selected *Do/While* in Step 3, select a variable from the *WHILE* drop-down list.

**AND**
From the drop-down list in the following row of the dialog box, select the appropriate operator for the loop condition-testing. For

quantitative variables, select from *Equal to, Not Equal, Greater Than, Not Less, Less Than, Not Greater,* or *Between… and….* For character-based variables, select between *Equal (String)* and *Not Equal (String).* Then type a test value for the variable in the accompanying text box. If the variable is character-based, be sure to enclose the character string value in quotation marks.

**8** When you are ready to run the journal loop, choose *OK.*

**9** When you have finished, choose *Close.*

## Loop Variable - Dialog Box Options

**Loop Construct**

Selects the type of condition-testing under which the journal is to be looped:

***For/Next*** acts in the fashion of an iterative counter, running the journal a selected number of times. If desired, you can specify that the counter jump by an amount greater than 1 (e.g., 1 to 15 in steps of 3).

***While/Wend*** runs the selected journal for as long as the variable fulfills the configured condition *(Equal to, Greater Than, Between… and…,* etc.).

***Do/Until*** runs the selected journal until the variable meets the configured condition.

***Do/While*** runs the selected journal for as long as the variable fulfills the configured condition. This is similar to the While/Wend paradigm, with the difference that the condition is evaluated after each replication of the journal under the *Do/While* paradigm, whereas the condition is tested first before running the journal under the *While/Wend* paradigm.

**FOR/NEXT**

This option group, which appears when you select *For/Next* from the *Loop Construct* option button group, consists of (1) a text box in the first row, in which you specify the name of an existing quantitative variable or type a name for a counter variable, (2) a pair of text boxes in the second row, which you use to specify a starting and ending value for the increment counter, and (3) a *STEP* spin box to select the size of the step increments. The final element in this option group is the *Run Journal* command button which opens the Select a Journal dialog box, from which you will select the journal to be looped.
EXAMPLE: You could set a variable called COUNTER to increment from 3 to 18 in steps of 5.

**WHILE/WEND**

This option group, which appears when you select *While/Wend* from the *Loop Construct* option button group, consists of (1) the WHILE drop-down list in the first row, from which you will select the name of a variable, (2) an operator selector drop-down list in the second row, from which you select an operator (*Equal to, Not Equal, Greater Than, Not Less, Less Than, Not Greater,* and *Between… and…),* and (3) a text box, which you use to enter the test value of the variable. If you are using a string (character-based) variable, be sure to enclose the entry for the string in quotation marks. The final element in this option group is the *Run Journal* command button which opens the Select a Journal dialog box, from which you will select the journal to be looped.

**DO/UNTIL**

This option group, which appears when you select *Do/Until* from the *Loop Construct* option button group, comprises three rows of options. The first row consists of the *Run Journal* command button which opens the Select a Journal dialog box, from which you will select the journal to be looped. The second row consists of the UNTIL drop-down list, from which you will select the name of a variable. The final row contains an operator selector drop-down list, from which you select an operator (*Equal to, Not Equal, Greater Than, Not Less, Less Than, Not Greater,* and *Between… and…),* and a text box, which you use to enter the test value of the variable.

**DO/WHILE**

This option group, which appears when you select *Do/While* from the *Loop Construct* option button group, comprises three rows of options. The first row consists of the *Run Journal* command button which opens the

Select a Journal dialog box, from which you will select the journal to be looped. The second row consists of the WHILE drop-down list, from which you will select the name of a variable. The final row contains an operator selector drop-down list, from which you select an operator (*Equal to, Not Equal, Greater Than, Not Less, Less Than, Not Greater,* and *Between… and…),* and a text box, which you use to enter the test value of the variable.

**Current Value**

Displays the current value of the selected variable being incremented.

**OK**

Runs the loop condition-testing paradigm, evaluating the current value of the variable and running the journal as configured by the loop paradigm.

**Close**

Closes the dialog box.

# StartUp Journal (Journal Menu)

**Runs a journal automatically whenever MetaMorph is opened.**

**Drop-in: STARTUP**

Use this command to configure a journal of your choice to run automatically every time you start MetaMorph. This can be useful for performing a sequence of configurations that set up MetaMorph to your specific needs, such as opening a particular combination of windows or dialog boxes, loading a state file, moving a stage to a specific position, or selecting a video device and channel.

## Configuring a StartUp Journal

To configure a journal to run automatically whenever MetaMorph is started, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose StartUp Journal. The StartUp Journal dialog box will appear. |
| **2** | Choose *Select Journal.* The Select a Journal to Run dialog box will appear. |
| **3** | Select the file for the journal you want to run at startup. If necessary, use the *Look In* list or Up One Level button to select the appropriate drive and folder.<br>**AND**<br>Choose *Open.* The Select a Journal to Run dialog box will close, the StartUp Journal dialog box will reappear, and the name of the selected journal will appear in the status line below the *Select Journal* button.. |
| **4** | If you have a startup journal loaded already and no longer want to run it at startup, choose *Clear File.* |
| **5** | When you have finished, choose *OK.* |

## StartUp Journal - Dialog Box Options

**Select Journal**

Opens the Select a Journal to Run dialog box, from which you can select the journal you want to run automatically whenever you start MetaMorph.

**Clear File**

Unloads the currently selected journal.

**OK**

Accepts the current configuration and closes the dialog box.

**Cancel**

Rejects any changes and closes the dialog box.

# Import Journal Suite

**Imports a complete set or "suite" of journals contained in a specially prepared Zip file created by the *Export Journal Suite* dialog box in MetaMorph and reestablishes the correct file links and hierarchy necessary to enable the journals to function correctly.**

**Drop-in: Journal**

Use this dialog box to import MetaMorph Journals and their related files from a special version Zip file created by MetaMorph. Journal suites stored in ***.jzp*** Zip files can contain Journal files and all associated files including related image files.

The journal suite can be permanently stored in the Zip file and imported or re-imported any time it is needed. The Journal Suite feature is primarily intended to be used for "transporting" complete sets of related journals and their associated files and images to systems other than the originating system, ensuring that the intended functionality and capabilities of the journals in the suite are preserved. This ensures that journals prepared on one system will perform identically on other similar systems.

***Note:*** Files imported to a new location from a Journal Suite Zip file retain the file linking and hierarchal information from the original location which ensures that this structure is correctly reestablished when the journal suite is imported.

## Importing Journals

To import Journals and their related files and images from a Journal Suite Zip file, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal Menu, click *Import Journal Suite*. The Import Journal Suite dialog box opens. |
| **2** | Click *Select Journal Suite*. The Select Import Suite File Name dialog box opens. |
| **3** | In the *File name* box, type or select the name of the Journal Suite Zip file that you want to import, then click *Open*. |
| **4** | In the *files to be imported* dialog box, verify the names of the journals that you want to import. |
| **5** | Under *Location to import to*, verify that the Import Journal Suite dialog box is set to import to the correct location. |
| **6** | To specify a different location, click *Select Import Location*. The *Browse for folder* dialog box opens. |

7 Click the name of the folder to which you want to export the files located in the selected Journal Suite Zip file or click *New* to create a new folder, then click *OK*.

8 Click *Import* to import the Journals and files into the selected folder.

9 Click *Close* to close the Import Journal Suite dialog box.

## Import Journal Suite - Dialog Box Options

### Select journal suite to import

Indicates the file name and path of the currently selected Journal Suite (Zip) file. Click *Select Journal Suite* to choose a different file.

### Select Journal Suite

Opens the Import Suite File Name dialog box. Type or select the name of a listed file, then click *Open*. This dialog box contains a *Description* field that shows the description that was entered when the Zip file was created.

### Files to be imported

Lists all journals, files, and images stored in the currently selected journal suite.

### Location to import to

Indicates the location to which the journals in the currently selected Journal Suite (Zip) file will be placed.

### Select Import Location

Opens the Browse for Folder dialog box. Use this dialog box to select the location to which you want to import the journal files and related files from the Journal Suite (Zip) files. To designate a new folder location, click *New*.

### Import

Imports all files in the currently selected journal suite into the specified location. If the folder into which you are importing journals already contains journals and files, the new journals and files will be added to those files.

### Close

Closes the Import Journal Suite dialog box.

# Export Journal Suite (Journal Menu)

**Creates a transportable Journal Suite by exporting selected Journals and their related files from MetaMorph into a single Zip file while retaining internal file linking information.**

**Drop-in: Journal**

Use this dialog box to export journal files located in a single folder into a Zip file. The Zip file that is created is given the file extension ***.jzp*** to indicate that it was created by MetaMorph. This zip file is intended to be used exclusively with MetaMorph. MetaMorph provides two special dialog boxes for both storing files into a special type of Zip file and extracting files from this zip file.

Use the *Files to be exported* list to choose the files that you want to include in the Zip file. After creating the Zip file, you can update the file to include additional journals or exclude journals by revising the *file to be exported* list and exporting the journals again. Each time you export files to an existing .jzp file, all journals that were in that file are deleted and the new selection of journals is added.

***Note:*** Files exported to a Journal Suite Zip file retain the information needed to ensure that identical file linking and hierarchal structure will be correctly reestablished when the journal suite is imported to a new location.

## Exporting a Journal Suite

To export a selected group of journals and related files, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal Menu, click *Export Journal Suite*. The Export Journal Suite dialog box opens. |
| **2** | Click *Select Export File*. The Select Export Suite File Name dialog box opens. |
| **3** | In the *Save in* box, choose the folder into which you want to store your new journal or choose the folder that contains a Zip file that you want to modify.<br><br>**Note:** When you export your Journals to an existing Zip file, all files previously stored in that Zip file are erased. |
| **4** | In the *File name* box type or select the name of the Zip file into which you want to store your Zip file, then click *Save.* |
| **5** | Under *Select export options*, check *Include image files* to include all image files associated with your selected journals. |
| **6** | Click Select Directory. The Browse for Folder dialog box opens. |
| **7** | In the Browse for Folder dialog box, click the folder containing the journals that you want to export, then click *OK*. |
| **8** | In the Files to be exported dialog box uncheck any journals or files that you do not want to include in the Zip file, and check any that you want to include, but are not currently included. |
| **9** | Verify that no warnings exist in the Warning box. Correct any warning conditions that are listed. |
| **10** | Click *Export* to export your selected journal suite. |
| **11** | Click *Close* to close the Export Journal Suite dialog box. |

## Export Journal Suite - Dialog Box Options

### Name of export file to create

Specifies the name of the Zip file to be created. Type or select the name of the file. If you are creating a new file, you must type the file name; if you are using an existing file you can select the name of the file from the file list.

**Note:** If you use an existing file, any journals stored in the file will be overwritten by the new selection of journal files. The files stored in the Zip file are not limited to Journal files. Any files required to enable the journals to function can be included.

### Description

Accepts text to enable you to type a description of the Zip file, its intended use, and its contents. This information is displayed when the Zip file is imported.

### Select export options

Provides options for journal and image file selection.

### Include image file

Finds and includes image files associated with the selected journals in the currently selected directory path.

### Select directory tree to export

Displays the currently selected directory path.

### Select Directory

Opens the Browse for Folder dialog box. Use this dialog box to choose the folder from which you want to export journals and related files.

### Files to be exported

Selects and specifies the journals to be included in the Zip file. The default setting is all boxes checked. Uncheck checkboxes for any files that you do not want to include in the Zip file.

**Note:** If you accidentally included or exclude any journal or file in the exported Zip file, simply re-export the Zip file again with the correct settings. The previous contents of the Zip file will be discarded.

### Warnings

Displays warnings to indicate any selected journals or files that might not export correctly.

### Export

Activates the export process and creates the Zip file.

### Close

Closes the Export Journal Suite dialog box.

# Create Taskbar (Journal Menu)

**Creates a taskbar that consists of buttons which allow you to run journals or commands, or summon other taskbars, by choosing the appropriate button on the taskbar.**

**Drop-in: JOURNAL**

Use this command to create taskbars for accessing frequently used groups of related journals, commands, or other taskbars. Each taskbar can consist of up to 48 buttons in a configuration of rows and columns of your choosing. You can mix and match journals, commands, or taskbars within the same taskbar. You can create and save as many taskbars as you wish, but only one can be used at any one time.

As with journals, commands and taskbars can be assigned directly to the taskbar buttons. This allows you to load a new taskbar directly from another taskbar. When you choose a command from a taskbar, it behaves just as it would if you had chosen it from its menu. Your access to the menu or other windows is not restricted.

When you use this command, the Taskbar Editor dialog box and a Taskbar window will appear. The

Taskbar window is an interactive window which allows you to change the width and height of the taskbar by dragging its outer border. Likewise, you can resize the width of the buttons on the taskbar by dragging the active button's sides. The active button will have a thicker border like that of the Taskbar window.

Commands, journals, and taskbars can added to a new taskbar by selecting the button on the taskbar and then double-clicking the entry for the function, journal, or taskbar in the Taskbar Editor's list table. The item will be added to the taskbar automatically.

The button's name will be the same as the function's, journal's, or other taskbar's name in the Taskbar Editor's list box. However, you can change the name by clicking the button so that it is the active button. As such, the button becomes a text box in which you can type a new label. The taskbar shortcuts can be displayed on the buttons if you wish. These are the same as those assigned by the Taskbar Shortcuts command.

> **Note:** After you have customized the text for a button, it will not change, even if you replace the existing item with a new item. You must edit the text again manually.

We recommend that you use one "main" taskbar which has buttons that are assigned to load other taskbars. Each of these "secondary" taskbars can be dedicated to a single application (EXAMPLES: "Fluorescence Acquisition," "Stage Movements," "Morphometry") or to a single user.

You can use the Edit Taskbar command to assign items to the taskbar buttons later.

## Creating a Taskbar

To create a taskbar, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Create Taskbar. The Taskbar Editor dialog box and Taskbar window will appear. Position them so that you can see both the dialog box and the window at the same time. |
| **2** | Select the number of rows and columns for the taskbar by dragging the thick border of the Taskbar window until the desired number of rows and columns appear in the window.<br><br>Select the width of the buttons in the taskbar by dragging the thick border of the active button until the buttons are the desired width. |
| **3** | Select the desired category for the first item you want to add the taskbar from the *Category* group.<br><br>If you selected *Journal* or *Taskbar* as the *Category,* the directory names will be displayed in square brackets in the list box below *Category.* Double-click a directory name to display the appropriate files in that directory or double-click the double period ("..") to go up one level in the directory structure. |
| **4** | When you have located the item you want to add to the taskbar, double-click its entry in the list box to add it to active button in the taskbar. |
| **5** | Repeat Steps 3 and 4 for each item you want to add to the taskbar.<br><br>You can use the *Undo* command to undo the |

last item you added or you can use *Clear Button* to clear an item from the active button if needed.

**6** If you want to rename the taskbar, choose *Rename Taskbar.* The Rename Taskbar dialog box will appear.

**AND**
Type the desired name in the text box. Then choose *OK.*

**7** Once you have finished, choose *Save* to save the taskbar.

Type the desired file name in the *File Name* text box. You can use the *Save In* list or Up One Level button to select the appropriate drive and folder, if necessary. Then choose *Save.*

**8** To use the new taskbar (or a different taskbar) immediately, choose *Load.* Select the desired taskbar file. You can use the *Look In* list or Up One Level button to select the appropriate drive and folder, if necessary. Then choose *Open.* The taskbar will be loaded when you close the Taskbar Editor dialog box.

**9** Choose *Close.*

## Create Taskbar - Dialog Box Options

**Taskbar File**

Displays the path and file name for the current taskbar. If the taskbar is new, this status text area will be empty.

**Category**

Selects the category of items displayed in the list box that can be added to the taskbar. You can add commands *(Function),* journals *(Journal),* or other taskbars *(Taskbar)* to the taskbar.

**Item List Box**

Displays the items that can be added to the taskbar. If you are adding commands, the text above the list box will indicate if the command is part of MetaMorph or part of a particular drop-in. If you are adding journals or taskbars, the text will indicate the current path. If you selected *Journal* or *Taskbar* as the *Category,* the directory names will be displayed in square brackets in the list box below *Category.* Double-click a directory name to display the appropriate files in that directory or double-click the double period ("..") to go up one level in the directory structure.

**Load**

Loads the selected taskbar. This is the same as the Load Taskbar command in the Journal menu. This button allows you to load a newly created or edited taskbar without using the Load Taskbar command.

**Save**

Saves the taskbar. If the taskbar is new, it will open the dialog box for the Save As command so you can select a file name for the new taskbar. Otherwise, it will save the changes to the existing file name.

**Save As**

Saves the taskbar to a different file name of your choice.

**Rename**

Allows you to change the default name that appears in the title of the taskbar.

**Undo**

Undoes the last change. This change could be an addition of a new item, the resizing of the taskbar or its buttons, or the renaming of the toolbar. You can choose *Undo* multiple times to undo all of the changes you have made.

**Discard Changes**

Discards all changes made in this session and reloads the last saved version of the taskbar. A warning dialog box will appear, asking you to confirm the discard changes command.

**Function Run by Selected Button**

Displays the name of the command assigned to the active taskbar button.

**Journal Run by Selected Button**

Displays the name of the journal assigned to the active taskbar button.

**Taskbar Run by Selected Button**

Displays the name of the taskbar assigned to the active taskbar button.

**Show Shortcuts**

Displays the shortcuts assigned to each button on the button. These are the same the shortcuts assigned by the Taskbar Shortcuts command in the Journal menu. Thus, to use the shortcut, you would press the [CTRL] key and the number listed.

**Clear Button**

Clears the assigned item from the active taskbar button.

**Close**

Closes the dialog box.

# Edit Taskbar (Journal Menu)

**Assigns journals to empty buttons, replaces existing journals assigned to buttons with new journals, and clears journals from the selected taskbar. Adds new rows or columns of buttons to the selected taskbar.**

### Drop-in: JOURNAL

Use this command when you want to add or change the journals assigned to a taskbar's buttons or you want to change the size of the taskbar. This command allows you to edit existing taskbars which access frequently used groups of related journals, commands, or other taskbars. This command loads the current taskbar into the Taskbar Editor so that you can edit it. However, you can load a different taskbar for editing once the Taskbar Editor is open.

When you use this command, a Taskbar Editor dialog box and a Taskbar window will appear. The Taskbar window is an interactive window which allows you to add more rows and columns for new items by dragging its outer border. Likewise, you can resize the width of the taskbar's buttons by dragging the active button’s sides. The active button will have a thicker border like that of the Taskbar window.

Commands, journals, and taskbars can added to a new taskbar by selecting the button on the taskbar and then double-clicking the entry for the function, journal, or taskbar in the Taskbar Editor's list table. The item will be added to the taskbar automatically.

The button's name will be the same as the item's name in the Taskbar Editor's list box. However, you can change the name by clicking the button so that it is the active button. As such, the button becomes a text box so you can type in a new label.

**Note:** Once the text for a button has been customized, it will not change, even if you replace the existing item with a new item. You must manually edit the text again.

The taskbar button shortcuts be displayed on the buttons if needed. These are same as those assigned by the Taskbar Shortcuts command.

## Editing a Taskbar

To edit a taskbar, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Edit Taskbar. The Taskbar Editor dialog box and Taskbar window will appear. Position them so that you can see both the dialog box and the window at the same time. |
| **2** | If you do not want to edit the current taskbar, choose *Load.* Select the desired taskbar file. You can use the *Look In* list or Up One Level button to select the appropriate drive and folder, if necessary. Then choose *Open.* |
| **3** | You can resize the taskbar by dragging the thick border of the Taskbar window until the desired number of rows and columns appear in the window.<br><br>You can resize the width of the buttons in the taskbar by dragging the thick border of the active button until the buttons are the desired width. |
| **4** | Select the desired category for the first item you want to add the taskbar from the *Category* group.<br><br>If you selected *Journal* or *Taskbar* as the *Category,* the directory names will be displayed in square brackets in the list box below *Category.* Double-click a directory name to display the appropriate files in that directory or double-click the double period ("..") to go up one level in the directory structure. |
| **5** | Once you have located the item you want to add to the taskbar, double-click its entry in the list box to add it to active button in the taskbar. |
| **6** | Repeat Steps 3 and 4 for each item you want to add or replace in the taskbar.<br><br>You can use the *Undo* command to undo the last item you added or you can use the *Clear* button to clear an item from the active button if desired. |
| **7** | If you want to rename the taskbar, choose *Rename Taskbar.* The Rename Taskbar dialog box will appear.<br><br>**AND**<br>Type the desired name in the text box. Then choose *OK.* |
| **8** | When you have finished, choose *Save* to save the taskbar.<br><br>Type the desired file name in the *File Name* text box. You can use the *Save In* list or Up One Level button to select the appropriate |

drive and folder, if necessary. Then choose *Save.*

**9** To use the taskbar (or a different taskbar) immediately, choose *Load.* Select the desired taskbar file. You can use the *Look In* list or Up One Level button to select the appropriate drive and folder, if necessary. Then choose *Open.* The taskbar will be loaded into MetaMorph when you close the Taskbar Editor dialog box.

**10** Choose *Close.*

## Edit Taskbar - Dialog Box Options

**Taskbar File**

Displays the path and file name for the current taskbar. If the taskbar is new, this status text area will be empty.

**Category**

Selects the category of items displayed in the list box that can be added to the taskbar. You can add commands, journals, or other taskbars to the taskbar.

**Item List Box**

Displays the items that can be added to the taskbar. If you are adding commands, the text above the list box will indicate if the command is part of MetaMorph or part of a particular drop-in. If you are adding journals or taskbars, the text will indicate the current path. If you selected *Journal* or *Taskbar* as the *Category,* the directory names will be displayed in square brackets in the list box below *Category.* Double-click a directory name to display the appropriate files in that directory or double-click the double period ("..") to go up one level in the directory structure.

**Load**

Loads the selected taskbar. This is the same as the Load Taskbar command in the Journal menu. This allows you to load a newly created or edited taskbar without using the Load Taskbar command.

**Save**

Saves the taskbar. If the taskbar is new, it will open the dialog box for the Save As command so you can select a file name for the new taskbar. Otherwise, it will save the changes to the existing file name.

**Save As**

Saves the taskbar to a different file name of your choice.

**Rename Taskbar**

Allows you to change the default name that appears in the title of the taskbar.

**Undo**

Undoes the last change. This change could be an addition of a new item, the resizing of the taskbar or its buttons, or the renaming of the taskbar. You can choose *Undo* multiple times to reverse all of the changes you have made.

**Discard Changes**

Discards all changes made in this session and reloads the last saved version of the taskbar. A warning dialog box will appear, asking you to confirm the *Discard Changes* command.

**Function Run by Selected Button**

Displays the name of the command assigned to the active taskbar button.

**Journal Run by Selected Button**

Displays the name of the journal or command assigned to the active taskbar button.

**Taskbar Run by Selected Button**

Displays the name of the taskbar assigned to the active taskbar button.

**Show Shortcuts**

Displays the shortcuts assigned to each button on the button. These are the same the shortcuts assigned by the Taskbar Shortcuts command in the Journal menu. To use the shortcut, press the [CTRL] key and the number listed.

**Clear Button**

Clears the assigned item from the active taskbar button.

**Close**

Closes the dialog box.

# Load Taskbar (Journal Menu)

**Loads the selected taskbar as the active taskbar.**

**Drop-in: JOURNAL**

Use this command when you want to load a new taskbar as the active taskbar. Once a taskbar is loaded, you can run any of the journals or commands assigned to its buttons. You can assign other taskbars to a taskbar's buttons using the Edit Taskbar command (Journal menu). For example, a button on the "Main" taskbar can be assigned to open a "Morphometry" taskbar (and vice versa).

The active taskbar is saved when you quit MetaMorph so that, when you start MetaMorph the next time, it will be displayed again. This means that you should only need to use the Load Taskbar command to switch between taskbars.

## Loading a Taskbar

To load a taskbar, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Load Taskbar. The Select a Taskbar dialog box will appear. |
| **2** | Select the desired taskbar file. You can use the *Look In* list or Up One Level button to select the appropriate drive and folder, if necessary. Then choose *Open.* |
| **3** | The selected taskbar will appear. |

## Load Taskbar - Dialog Box Options

**File Name**

Lists the name of the currently selected file.

**Files of Type**

Determines the file format of the files displayed in the *File Name* list. The default is **.JTB.* Select *All Files (*.*)* to display all file names.

**Look In**

Displays the currently selected folder. Click the icon for the desired folder to display its files. Click the Up One Level button to go up one level in the directory structure.

**OK**

Opens the taskbar file.

**Cancel**

Cancels the command.

# Taskbar Shortcuts (Journal Menu)

**Displays a secondary menu that lists keyboard shortcuts that can be used to run the journals or commands on the current taskbar.**

**Drop-in: JOURNAL**

Use this command to view the keyboard shortcuts for each journal or command on the taskbar. While you can run them by selecting a menu item from the secondary menu, this command's primary purpose is to provide you with a list of the keyboard shortcuts assigned to the current taskbar.

The shortcuts are CTRL + 1 through CTRL + 9 and CTRL + 0 for the first ten taskbar journals. Assigned shortcuts will display the journal name as it appears on the taskbar. Shortcuts which are not assigned to a taskbar button will display the message "Not Available" in text that appears dimmed.

Once you know the shortcuts associated with each journal or command, you won't need to use the Taskbar Shortcuts command. The keyboard shortcuts work the same way as other keyboard shortcuts listed in the menus do: press and hold the first key and then press the second key listed.

EXAMPLE:
The first taskbar button on an active taskbar could be named "Acquire." Its shortcut will be CTRL + 1. Press and hold the [CTRL] key and then press the number [1] on your keyboard.

## Using the Taskbar Shortcuts

To see a list of keyboard shortcuts for the active taskbar, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Journal menu, choose Taskbar Shortcuts. A secondary menu will appear.<br><br>The menu lists the taskbar's commands or journals and the keyboard shortcut assigned to each position on the taskbar. Shortcuts which are not assigned to a taskbar button display the message "Not Available" in dimmed text. |
| **2** | To run a particular journal or command, choose it from the secondary menu.<br><br>**OR**<br><br>Use the keyboard shortcut listed next to the name; press and hold the [CTRL] and then press the assigned numeric key. |

# Show Taskbar (Journal Menu)

**Displays the current taskbar.**

**Drop-in: JOURNAL**

Use this command when you want to display a taskbar that has been hidden using the Hide Taskbar command. This command will be unavailable and will appear dimmed if there is no active taskbar. Use the Load Taskbar command to load a taskbar.

## Shortcut: [F4]

## Showing the Active Taskbar

To show the active taskbar after hiding it, use the following procedure:

| Step | Action |
|---|---|
| 1 | Select the Journal menu. |
| 2 | Choose Show Taskbar. The active taskbar will appear. The Show Taskbar command in the Journal menu will be replaced by the Hide Taskbar command. |

# Hide Taskbar (Journal Menu)

**Hides the current taskbar from view.**

**Drop-in: JOURNAL**

Use this command when you want to hide the current taskbar to gain additional desktop space or because it is not currently needed. This command may be preferable to closing the taskbar because you only need to choose the Show Taskbar command to display it again, rather than going through all of the steps involved in reloading it.

## Hiding the Active Taskbar

To hide the active taskbar, use the following procedure:

| Step | Action |
|---|---|
| 1 | From the Journal menu, choose Hide Taskbar. |
| 2 | The active taskbar will disappear. The Hide Taskbar command in the Journal menu will be replaced by the Show Taskbar command. |

# Graph Variable Value (Journal Menu)

**Creates a graph representing the changing value(s) one or more variable(s).**

**Drop-in: JOURNAL**

Use this command to graph the values of one or more variables in a journal. Up to two variables can be displayed per graph and multiple graphs can be displayed and updated at the same time. This command can display the value of multiple variables in the following ways:

- Using one graph with separate traces for each variable
- Opening separate graphs for each variable

The most effective way to use the Graph Variable Value command is as part of a journal. For example, if you have a journal that measures the intensities of a region in a stack, you can add a step to the journal using the Graph Variable Value command to graph the changes in intensities. Any variable that returns a numeric value can be graphed, and you can graph one value on the Y-axis and another on the X-axis of the same graph. Once the graphs are created, they can be configured using the Graph Settings commands available to all graphs in MetaMorph. To open the Graph Settings command, double-click inside an active graph or click the Show Graph Menu arrow on the bottom left corner of the graph and select Graph Settings.

**Note:** Variables used in the Graph Variable Value command must be valid MetaMorph or custom variables that have a numeric value. For example, the variable *ShowRegionStatistics.Average is* valid because it is a built-in MetaMorph variable that returns a numeric value—the average intensity of the active region. The variable

Image.FileName is not valid because the value it returns is not numeric—it returns the file name of the active image. For more information on variables, refer to the Introduction to the Use of Variables help section.

## Graph Variable Value - Dialog Box Options

### Graph Options

#### Line

Creates a standard graph with tracers and an X- and Y-Axis.

#### Scatter Plot

Creates a graph that plots data as individual points

#### Bar

Creates a graph that displays the value of a measurement by the length of a rectangular bar.

**Note:** Changes made to the graph using the Graph Settings dialog box will override any settings made in the *Graph Options* fields.

### Graph

Selects the graph to display. You can select an existing graph and add points to it or enter a new name to create a new graph. You can create up to 10 graphs.

**Note:** You can only select existing graphs that were created using the Graph Variable command.

### Trace

Selects the trace to display. You can select an existing trace and add points to it or enter a new name to create a new trace. You can create up to 10 traces.

### Trace Color

Opens the Color dialog box to select a custom color for the trace line(s). The trace color will be applied to the trace selected in the *Trace* field.

### Increment X axis by 1

Automatically adds an increment of 1 for each point on the X-axis. This option is used when only one variable is being graphed.

### Use Variable for X axis

Enables the *X Axis* drop-down list.

### X Axis

Enter or select the variable to use for the X-axis. The variable must be a valid, MetaMorph or custom defined variable. For more information on variables in MetaMorph, refer to the Introduction to the Use of Variables help section.

### Y Axis

Enter or select the variable to use for the Y-axis. The variable must be a valid, MetaMorph or custom defined variable with a numeric value. For more information on variables in MetaMorph, refer to the Introduction to the Use of Variables help section.

### Apply

Creates the graph based on the current settings. Clicking *Apply* again, without changing the image or variables and when *Increment X axis by 1* is selected, will add another point to the graph with the same Y value and an X value incremented by 1.

**Note:** If you select a new graph from the *Graph* field and open it using *Apply*, the new graph will have the same dimensions as the last graph opened — so if you resize a graph, the next graph opened will have the same dimensions as the resized graph.

### Load Graph Settings

Opens the Load Graph Settings dialog box. Use this dialog box to load and apply previously saved graph settings to the active or selected graph. You can choose to load all or select settings.

### Close Graph

Closes the graph currently selected in the Graph field.

### Close

Closes the dialog box.

## Graphing Variable Values

To graph one or more variable values, use the following procedure:

| Step | Action |
|---|---|
| **1** | Ensure that the image or stack that contains the data to graph is open on the MetaMorph desktop. |
| **2** | From the Journal menu, select Variable>Graph Variable Value. The Graph Variable Value dialog box opens. |
| **3** | Select a graph format from the *Graph Options* field. Valid choices are *Line*, *Scatter Plot*, or *Bar*. |
| **4** | Select a Graph name from the *Graph* drop-down list or type in a new name. |
| **5** | Select a Trace name from the *Trace* drop-down list or type in a new name. |
| **6** | To select a color for the trace, click *Trace Color* and choose a color from the Color dialog box. |
| **7** | If you are only tracking one variable, select *Increment X axis by 1*. The variable will be tracked on the Y axis. |
| **8** | If you are tracking two variables, select *Use Variable for X axis*. This will enable the X Axis field. |
| **9** | If you are graphing a variable on the X axis, enter its name in the *X Axis* field or select a name from the drop-down list. |
| **10** | Enter a valid variable name in the *Y Axis* field or select a name from the drop-down list. |
| **11** | Click *Apply* to create the graph. Clicking *Apply* again, without changing the image or variables and when *Increment X axis by 1* is selected, will add another point to the graph with the same Y value and an X value incremented by 1. |
| **12** | To load previously saved graph settings to the active or selected graph, click *Load* |

| | |
|---|---|
| | *Graph Settings*, select which settings to load, and browse for the saved graph settings file (*.GCF). to open. |
| **13** | To configure the graph settings, double-click inside the graph or click the Show Graph Menu arrow on the bottom left corner of the graph and select Graph Settings. |
| **14** | Click *Close Graph* to close the active graph. |
| **15** | Click *Close* to close the dialog box. |

# Find Spots

**Locates discrete spots in an image and arranges them in a coordinate system.**

*Availability: Included in MetaMorph Premier*

**Drop-in: FINDSPOTS**

Use the Find Spots command to locate and isolate discrete spots in an image and arrange the identified spots within a specified coordinate structure. Experiments that could benefit the greatest from this command are those involved with tissue micro array or gene chip acquisition and analysis.

## Finding Spots

Use the following procedure to locate and isolate discrete spots or texture in a sample:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click *Find Spots*. The *Find Spots* dialog box opens. |
| **2** | Open an appropriate image in which you want to find either bright spots, dark spots, or texture. |
| **3** | Click *Show Sample Region* to place a single sample region in the image. |
| **4** | Click and drag the region over one of the spots in the image. |
| **5** | Click and drag the region boundary to change the region size, OR type or select an appropriate region size value in the *Region Size dialog box.* |
| **6** | In the *Spot cutoff* box, type or select an appropriate cutoff value. This value controls the find spots sensitivity. Lower threshold sensitivity values results in more spots being found; higher threshold sensitivity values results in fewer spots being found. |
| **7** | In the *Find spots by* box, choose *Bright Spots* to find bright spots on a dark background, choose *Dark Spots* to find dark spots on a bright background, and choose *Texture* to locate textured areas. |
| **8** | To apply a coordinate system to the found spots, click *Apply coordinate system*. |

**9** In the *Coordinate system* box, click *Create regions for missing spots* to place regions in the area of the grid where no spots are located.

**10** In the *Coordinate system* box, click *Specify rows and columns* to indicate the number of rows and columns that you want to include in your selected sample area.

**11** In the *Number of Rows* box, type or select the number of rows that you want to include in your coordinate system grid selection.

**12** In the *Number of Columns* box, type or select the number of Columns that you want to include in your coordinate system grid selection.

**13** Click *Find Spots* to run the find spots command.

**14** To clear the regions from the image, click *Clear Spot regions*.

**15** Click *Close* to close the *Find Spots* dialog box.

## Find Spots - Dialog Box Options

### Image

Selects the source image on which to perform Find Spots. Images can be of 8, 16 or 24-bit depths. The image selector can select only a single plane.

### Show Sample Region

Displays a resizable region on the image. Circular regions are used to identify the spots.

### Region Size

Specifies the size of the region to be used. You can resize the region by selecting or typing a different region size in the Region Size box, or by clicking and dragging the region boundary in the image window. When you click and drag to change the size of the region boundary, the region size field updates automatically.

### Spot cutoff

Controls the spot-finding threshold sensitivity. Lower threshold sensitivity values results in more spots being found; higher threshold sensitivity values results in fewer spots being found.

### Find Spots by

Searches for spots that are predominately ***bright***, ***dark***, or defined by ***texture***. The default setting is texture.

### Apply Coordinate System

Assigns an optional grid and coordinate system to the spots that have been found. This option uses region labels to clearly identify the region's coordinates.

### Coordinate system

#### Create Region for missing Spots

Fills in any missing regions in the coordinate system.

#### Specify Rows and Columns

Enables the option for specifying the number of rows and columns of spots in the image to be included for finding spots.

**Numbers of rows**

Specifies the numbers of rows that you want to find.

**Numbers of columns**

Specifies the numbers of columns that you want to find.

### Clear Spot Regions

Clears all ellipse regions from the entire image area.

### Find Spots

Begins the spot finding process using the current settings.

### Close

Closes the Find Spots dialog box.

# Tissue MicroArray Acquisition

**Locates , identifies, and acquires images of tissue microarray spots, on a semi automated basis. Images can be acquired using transmitted or fluorescence light sources, as required.**

*Availability: Available for MetaMorph Premier; included in MetaXpress*

### Drop-in: TMACQUIR

Use this dialog box to create the images you need when running Tissue MicroArray experiments. This Drop-in enables you to locate individual tissue spots and identify each spot by surrounding it with a region.

The Tissue MicroArray Acquisition dialog box provides a set of tools to complete the steps for scanning and locating tissue spots on a slide, identifying them using regions at a low-power magnification, acquiring multiple images at multiple wavelengths of small areas within the regions at higher magnifications, then stitching the acquired images together to form a complete image. Final images are accumulated into images files for later viewing, processing, and analyzing.

This dialog box is divided into five tabbed areas that separate specific acquisition tasks into a logical, easy-to-use organization.

Use the *Main* tab to record a general description of your experiment and to define the naming convention to be used for your experiment's file names.

Use the *Acquisition* tab to define the number of wavelengths and the specific wavelength value assigned to each one.

Use the *Positions* tab to define the spot positions that you want to include in your experiment data.

Use the *Scan* tab to define the criteria and settings for scanning a slide to determine the range of spots that you want to scan.

Use the *Correction* tab to make background and shading correction settings.

The following is a general list of procedures that you need to complete when acquiring TMA images:

- Define and describe your experiment.

- Define the wavelengths at which you will acquire your images.
- Define the scan area.
- Define the scan wavelength.
- Define the scan magnification.
- Define the spot size.
- Scan the TMA slide to locate the spots.
- Apply background subtraction and/or shading correction to the scanned image.
- Verify that the regions have been correctly placed on the spots.
- Set the spot size, distance, number of columns and number of rows.

## Describing and Naming your Experiment - Main Tab

To begin defining your TMA experiment acquisition settings, complete the following steps:

| Step | Action |
|---|---|
| **1** | From the Apps Menu, click *Tissue MicroArray Acquisition.* The *Tissue MicroArray Acquisition* dialog box opens. |
| **2** | On the *Main* tab, in the *Description* box, type a general description of you experiment and any other information that you think is important to notate about your experiment. |
| **3** | Click *Select Directory*. The *Browse for Folder* dialog box opens. |
| **4** | In the *Browse for Folder* dialog box, select or create a folder into which to store your multidimensional images. |
| **5** | In the *Base Name* box, type the base name that you want to use for all images acquired for your experiment. |
| **6** | Check *Increment if file exists* to create a series of files with incrementing file names. For example, you could use TMA_001 to TMA_999. |
| **7** | Click the Wavelengths tab to define the wavelengths, exposure, alignment, and binning settings for your experiment. |

## Tissue MicroArray Acquisition - Dialog Box Options

### Snap

Captures a single full-frame image from the camera using the current wavelength settings. Captured images are displayed in the Tissue Micro Array Snap window, which is overwritten with each snap. Snapping an image halts focusing. The image size depends on the wavelength selected. If the scan wavelength is selected, the image will be a region of 80 percent of the

camera dimensions with an offset of ten percent.

**Live**

Continuously acquires images from the camera at the current stage position. The dimensions of the image will match those of the TMA Snap image.

**Wavelength**

Selects and indicates the wavelength of the image currently being acquired when you are acquiring multiple wavelengths. The selected wavelength also determines the exposure settings for the next exposure.

**Bin**

Determines the size of the bin that the camera will use.

**Gain**

Sets the camera gain on cameras that support this feature.

**Save State**

Saves the current settings for this dialog box into a state file. The saved state file can be loaded later to enable you to recall and reuse the settings or so that you can modify the settings by using the saved settings as the basis for the new settings.

**Load State**

Opens the Load State File dialog box. Use this dialog box to load an existing state file. Existing state files can be loaded to enable you to recall and reuse settings or so that you can modify saved settings and use them as the basis for new settings. When loading the state file, the Load State File dialog box enables you to select various groups of settings for loading into the present dialog box.

**Scan**

Finds spots and allows you to inspect preliminary Images and change settings accordingly before acquiring final versions of each image. This is a low-level scan. After the scan is finished, MetaMorph will stitch the collected images together and run the sample finding algorithm to locate the samples.

**Preview**

Acquires an image of the currently selected wavelength. If the current wavelength is set to one of the acquisition wavelengths then all acquisition wavelengths will be acquired. This enables you to specify where you want your image windows to be placed. After you make this setting, you can click acquire to start the acquisition or click cancel. If you click cancel, all open image windows will be closed.

**Acquire**

Starts the acquisition.

**Scan + Acquire**

Successively finds spots and acquires images in one action. Use this option when you want to acquire final images at the same time that the program is finding spots.

**Close**

Closes the Tissue MicroArray Dialog box.

## TMA - Dialog Box Options - Main Tab

**Description**

Enables you to enter and save text that describes the acquisition.

**Select Directory**

Selects the drive location where the files will be saved.

**Base name**

Assigns a unique name that you designate to a file that you intend to save.

**Increment if File exists**

When checked, automatically increments the file name for that file you want to save if the file name already exists. The program will notify you if the file name does not exist.

## Defining Wavelength Settings - Acquisition Tab

To define your TMA wavelength settings, complete the following steps:

| Step | Action |
|---|---|
| **1** | In the *Magnification* box, choose the magnification setting that you want to use to acquire images of each TMA spot. |
| **2** | In the *Images per sample box*, choose the number of images that you want to acquire for each sample. Choose 1, 4, 9, or Entire Sample. |
| **3** | In the *Number of wavelengths* box, type or select the number of wavelengths that you are including in your experiment. |
| **4** | In the *Current Wavelength* box choose the wavelength that you want to use as the current wavelength. |
| **5** | In the *Illumination* box for the current wavelength, select the illumination setting that you are using. Your illumination setting defines filtration for both excitation wavelengths and emission wavelengths, as well as a shutter, neutral density, and dichroic selections. |
| **6** | Check *Auto focus* if you are using the TMA/MetaMorph autofocus feature/algorithm. |
| **7** | Click *Auto Focus…* The TMA: Auto Focus dialog box opens. Refer to the procedure for making the TMA: Auto Focus settings. |
| **8** | In the Exposure area, type or select the relevant values for *Exposure time* and *Target intensity*,<br><br>**OR**<br><br>Click *Auto Expose* to allow MetaMorph to set the exposure value automatically. |
| **9** | In the Alignment area, type or select the appropriate values for X and Y alignment in the *Alignment cropping X* and the Alignment cropping Y boxes, or click Set Alignment to |

open the TMA: Set Alignment dialog box.

**10** In the Camera binning box, type or select a value from 1 to 4 for a camera binning value depending on how quickly you want to acquire your image and the image quality that you need.

**11** In the Gain box, choose *Low*, *High*, or *Super High* depending on the image quality that you need.

**12** Click the *Sampling* tab to display the Sampling settings part of this dialog box. The Sampling tab is displayed.

## TMA - Dialog Box Options - Acquisition Tab

**Magnification**

Specifies the level of magnification to use during the acquisition.

**Images per sample**

Specifies the number of images that will be acquired for each sample. Options are 1, 4, 9, or the entire sample.

**Number of Wavelengths**

Specifies how many wavelengths will be acquired.

**Current Wavelength**

Changes the wavelength setting from the current wavelength to a different wavelength setting.

**Illumination**

Selects the illumination setting that you want to use.

**Auto Focus**

Determines whether an *Auto focus* operation will be done.

**Auto Focus…**

Opens the ***Auto Focus*** dialog to enable you to configure auto focus.

**Exposure time**

Sets the exposure time for the wavelength.

**Target Intensity**

Specifies the intensity level that the auto expose uses to calculate the appropriate exposure time.

**Auto Expose**

Determines the appropriate exposure time.

**Alignment Cropping**

Compensates for image shifting that occurs at different wavelengths. Type or select values for X and/or Y to realign an image at one wavelength with a similar image at another wavelength. Due to refractive color shifting, images of the same sample at different wavelengths might not precisely converge. Use these settings to correct for this. To apply this correction interactively, click Set Alignment.

***X*** – Crops the specified amount in microns from either the left side or the right side of the image, depending on whether the value is positive or negative. Negative crops the left side; positive crops the right side.

***Y*** – Crops the specified amount in microns from either the top or the bottom of the image, depending on whether the value is positive or negative. Negative crops the bottom; positive crops the top.

**Set Alignment**

Opens the ***TMA: Set Alignment*** dialog box. Use this dialog box to interactively apply color shift correction (Alignment Cropping) to one or more images of the same sample acquired at different wavelengths. Set alignment creates a stack from the acquired images and applies horizontal and vertical shift values to each selected image.

## TMA: Auto Focus - Dialog Box Options

**Range, Current +/-**

Specifies how far above and below the current Z position that the Z motor will be moved while acquiring focus test images. This distance is specified in steps, as defined by the Focus command.

**Accuracy: Step(s)**

Specifies the Z distance to move. Changing this value changes the Number of Z Moves.

**Number of Z Moves**

Indicates the total number of acquisitions that will be made, based on the settings selected with the Range, Current +/- and Accuracy: Step(s) spin boxes.

**Current Position**

Indicates the current Z position of the objective.

**Backlash Compensation**

Enables the Backlash Compensation Z motor movement protocol which directs the focus motor to move to a Z axis position slightly below the target position and then move against gravity to the target position. This ensures that the Z motor gears are fully engaged, avoiding drift due to slippage. This option has no effect on focus devices with built-in backlash compensation.

**Update Z position with Result**

Resets the Z position to the best focus point.

**Test**

Activates auto focus at the current position.

**OK**

Saves settings and closes the dialog box.

# TMA: Set Alignment

**Aligns images from two or more wavelengths to compensate for the image shift that results from the variation in light transmission at different wavelengths.**

**Drop-in: TMACQUIR**

Use this dialog box to interactively apply color shift correction (Alignment Cropping) to one or more images of the same sample acquired at different wavelengths. Set alignment creates a stack from the

acquired images and applies horizontal and/or vertical shift values to each selected image.

**TMA: Setting Alignment**

**TMA: Set Alignment - Dialog Box Options**

## TMA: Setting Alignment

To align the images of two or more wavelengths, complete the following procedure.

| Step | Action |
|---|---|
| **1** | From the Screen Acquisitions Acquisition tab, click *Set Alignment*. The *Screen: Set Alignment* dialog box opens. · |
| **2** | Click *Acquire Alignment Stack*. · |
| **3** | In the Display group, choose *Subtract* or *Average*. |
| **4** | Move the horizontal and vertical sliders to set the Horizontal Shift and Vertical Shift values. |
| **5** | Click *Zero Shift* to reset the horizontal and vertical values to Zero. |
| **6** | Click *Previous* or *Next* to change images. |
| **7** | Click *OK* when you are finished setting the alignment of your wavelengths. |

## TMA: Set Alignment - Dialog Box Options

**Acquire Alignment Stack**

Acquires an image at each wavelength and builds a stack that can be used in adjusting alignment.

**Display**

Selects the method to be used to display differences between the reference plane and the shifting plane:

***Subtract*** uses subtraction to show the difference between the reference plane and the shifting plane. The planes will be aligned when there is nearly a uniform grayscale level throughout the entire image.

***Average*** uses averaging to display the offset between the two planes. The aligned plane should look like the original plane with as little blurring as possible.

**Horizontal Shift (text box and slider)**

Adjusts the horizontal alignment of the plane in one-pixel increments

**Vertical Shift (text box and slider)**

Adjusts the vertical alignment of the plane in one-pixel increments.

**Zero Shift**

Resets the horizontal and vertical shift to zero.

**Previous**

Places the previous plane in the alignment image window.

**Next**

Places the next plane in the alignment image window.

**Cancel**

Cancels your adjustments and closes the dialog box.

**OK**

Applies the shift to all of the wavelengths.

## Setting Sample Positions - Positions Tab

To specify the spot positions for which you want to acquire detailed images, complete the following steps:

| Step | Action |
|---|---|
| **1** | Click *Positions*; the *Positions* tab is displayed. |
| **2** | Click *Show Region Labels* to display the region label information for each region. |
| **3** | Right-click on individual positions to turn off acquisition for one or more specific locations. |
| **4** | Click on one or more column or row buttons to turn off acquisition for one or more rows or columns. |
| **5** | To adjust the position of a specific region relative to a spot, type or select new values In the Position X and Y boxes, or drag the region to a different location. The values shown in the Position X and Y boxes indicate the X. Y location of the currently selected spot region. |
| **6** | To change Row and Column locations of specific regions, or to delete specific regions, click *Adjust Row/Column*. The *Adjust Rows and Columns* dialog box opens. |

## TMA - Dialog Box Options - Positions Tab

**Show Region Labels**

Overlays spots with the stored region label information to identify the individual spots and to clarify the row and column designations.

**[Grid Area]**

Identifies the locations of valid spots. Use this grid to select the spots that you want to acquire in detail. An X indicates a position containing a spot that will be acquired. Right-click on the X to turn-off the spot and excludes the spot from the acquisition. Right click again to turn it on. Click column or row buttons on the top and left side to turn off or on an entire row or column.

**Edit Position**

Moves the position of the region associated with a specific spot. Click the region that you want to move. You can either drag the region to a new location, or type or select the new X and Y

coordinates in the X and Y boxes.

**Adjust Row/Column**

Opens the ***Adjust Rows and Columns*** dialog box. Use this dialog box to arrange the respective positions of the images to be acquired. By arranging and moving selections, you can consolidate images that are distributed over a larger area into a smaller grouping. This arrangement is reflected in the organization of the acquired images when viewing them using the *Review Screen Data* dialog box.

## Adjusting Rows and Columns

To adjust rows and columns on the Adjust Rows and Columns dialog box, complete the following steps:

| Step | Action |
|---|---|
| **1** | From the Positions tab, click *Adjust Row/Column*. The Adjust Rows and Columns dialog box opens. |
| **2** | If the region labels are currently not visible on your image, click *Show Region Labels*. |
| **3** | Click appropriate column or row buttons to turn off any columns or rows that contain image data that you do not want to include in your experiment for viewing, then click *Delete*. |
| **4** | Click any appropriate individual spot locations that contain image data that you do not want to include for viewing, then click *Delete*. |
| **5** | Select one or more rows or columns that you need to move to create a better arrangement of your images, then use the arrow buttons to move your selected regions. |
| **6** | Select any individual regions that you need to move to create a better arrangement of your images, then use the arrow buttons to move your selected regions. |
| **7** | Click *Apply* to keep the changes that you made in this dialog box, or click *Close* to discard the changes and restore your previous settings. |

## Adjust Rows and Columns - Dialog Box Options

**Show Region Labels**

Overlays spots with the stored region label information to identify the individual spots and to clarify the row and column designations.

**[Grid Area]**

Enables you to identify and select the spots that you want to include.

**[Arrow Buttons]**

Moves the selected spot to a different location on the grid.

**Delete**

Deletes selected positions from the grid. Click to highlight one or more positions, then click Delete. To complete the deletion process, click *Apply* to permanently remove your selections. If you decide not to remove your selections, click *Close*, then re-open this dialog box.

> **Note:** You can also delete regions from directly within the scanned image. Click to select the region, then press the Delete key.

**Apply**

Applies all settings made in the *Adjust Rows and Columns* dialog box.

**Close**

Closes the Adjust Rows and Columns dialog box. If you made changes, and you want them to take effect, you must first click *Apply*, otherwise the changes will be lost.

## Configuring TMA Sampling

Use the Scan tab to make acquisition settings that apply specifically to images acquired to create the sampling image for each TMA.

To configure the Sampling settings, complete the following steps:

| Step | Action |
|---|---|
| **1** | In the *Scan Wavelength* box, choose the wavelength that you want to use for sampling. |
| **2** | In the *Magnification* box, choose the objective magnification that you want to use. |
| **3** | In the *Illumination* area, click *Transmitted* or *Fluorescence* to select the type of light source. |
| **4** | In the *Exposure* area, type or select the relevant values for *Exposure time* and *Target intensity*,<br>**OR**<br>Click *Auto Expose* to allow MetaMorph to set the exposure values automatically. |
| **5** | Use the settings in the *Scan area* box to set the Scan Area boundaries. You can type or select the X and Y coordinates for both the *Top Left* corner and *Bottom Right* corner of the scan area, or you can position your cursor at the location where you want the Scan Area to begin; then click *Set to Current* for the Top Left corner. Repeat this procedure for the Bottom Right corner. |
| **6** | Use the settings in the Spot information area to define the average size and distance information for each spot on your TMA slide. Also, specify the number of rows and columns of spots.<br>• In the Spot size box, type or select the spot size in microns. |

- In the Spot distance boxes, type or select the distance in microns between adjacent spots for both the X and Y axis.
- In the Number of columns and the Number of rows boxes, type or select the number of columns and rows that you want to include in your image.
- In the Signal to noise box, type or select a value to specify the level of sensitivity to be used when locating spots.

**7** Click *Recalculate* to reapply spot information criteria after you have changed any Spot information values.

**8** Click *Load Sample File*. The *Load Sample File* dialog box opens. Choose an appropriate sample file

**9** Click *Clear Sample File* to clear all currently loaded Sample File Data.

## TMA - Dialog Box Options - Scan Tab

**Scan Wavelength**

Selects the wavelengths to use for the scan.

**Magnification**

Chooses the magnification to use for the scan.

**Exposure**

**Exposure Time**

Selects and indicates the exposure time for the scan acquisition.

**Target Intensity**

Sets the maximum intensity value for the acquired scan image. The target intensity value default is 75 percent of the maximum gray level that the camera driver reports as possible to obtain (For example, 75 percent of 4096 is 3072).

**Auto Expose**

Automatically determines the appropriate exposure time for the scan image and snaps an image to the desktop.

**Illumination**

**Transmitted**

Select if using a transmitted light source.

**Fluorescence**

Select if using a fluorescent light source.

**Scan Area**

Defines the physical boundaries of the Scan area.

***Top Left (X and Y)***

Determines the top left corner of the slide.

***Bottom Right (X and Y)***

Determines the bottom right corner of the slide.

***Set to Current***

Sets the current position of either the top left or bottom right corner of the slide.

***Go To***

Automatically moves to the stage positions set with the *Set to Current* command

**Spot Information**

Specifies the parameters used to locate and identify TMA spots.

**Spot Size**

Specifies the spot size that you want to use. The value that you specify should create a rectangular region into which each spot that you included in your sample image fits.

**Spot Distance**

Specifies how much distance should be between each spot.

**Columns**

Specifies the number of columns to be included in the image.

**Rows**

Specifies the number of rows to be included in the image.

**Spot Cutoff**

Determines the level of sensitivity to use when separating spots from background during a scan. The higher the value, the more filtering is done and less objects will be counted as spots. The filtering is based on the intensity levels of each object in the image. The valid range of values for this field is 0-66535.

**Recalculate**

Recalculates the grid spacing on the image.

**Load Sample File**

Loads TARP3 format files as Comma-Separated-Value (.csv) files exported from Excel, and stores selected data as region labels and annotation data.

**Clear Sample File**

Clears all currently loaded Sample File Data.

## Making Correction Settings

To make background and shading correction settings, complete the following steps:

| Step | Action |
|---|---|
| **1** | Click *Correction*. The Correction tab is displayed. |
| **2** | In the Background subtraction area, click scan to apply background subtraction to only the scanned images, or click acquisition to apply background subtraction to only the Acquired images, or click both Scan and |

Acquisition to apply Background subtraction to both.

3 In the Shading correction area, click scan to apply shading correction to only the scanned images, or click acquisition to apply shading correction to only the Acquired images, or click both Scan and Acquisition to apply shading correction to both.

4 In the *Acquire shading images at* box, click Left of scan area to begin applying shading correction on the left side of the image, or, click user specified position, and type or select the position in the Position box.

5 If you selected *User specified position*, type or select the appropriate X and Y coordinates, or place the cursor in the image window at the position in the image window where you want shading correction to begin, then click *Set to current*.

## TMA - Dialog Box Options - Correction Tab

### Background subtraction

Applies background subtraction to scanned images, acquired images, or both.

***Scan*** – Applies background subtraction to scanned images.

***Acquisition*** – Applies background subtraction to acquired images.

### Shading Correction

Applies shading correction to scanned images, acquired images, or both.

***Scan*** – Applies shading correction to scanned images.

***Acquisition*** – Applies shading correction to acquired images.

### Acquire shading image at

Specifies the location in the image where MetaMorph will begin to apply shading correction.

***Left of scan area*** – Specifies that the shading correction will begin on the left (default) side of the scan area.

***User specified position*** – Enables you to manually specify the location where you want the shading correction to begin.

### Position

For the *Acquire shading image at* option, when *User specified position* is chosen, Use these settings to specify the exact position.

***X*** – Specifies the X axis position

***Y*** – Specifies the Y axis position

***Set to Current*** – Specifies that shading correction position will begin at the current cursor location.

***Go To*** – Automatically moves to the shading image acquisition area stage defined with the *Set to Current* command.

# Multi Dimensional Acquisition (Apps Menu)

**Opens the Multi Dimensional Acquisition interface for the acquisition of images from a series of times, stage positions, wavelengths, and/or Z-positions. This function is used to acquire large and complex data sets without requiring the use of journals.**

*Availability: Available for MetaMorph Basic; included in MetaMorph Premier*

**Drop-in: NDAQUIR**

Use this command when you want to acquire images using multiple parameters. Choose the dimensions that you want to acquire and configure the parameters for acquisition by selecting the appropriate setup tabs in the dialog box. Each tab represents a different dimension. The dialog box shows setup tabs only for the dimensions you select on the Main tab. The dimensions available for selection on the Main tab will vary depending on which devices you have installed on your computer.

Depending on installed devices, you can choose any or all of the following options:

- Do Timelapse
- Multiple Stage Positions
- Multiple Wavelengths
- Do Z Series
- Stream
- Run Journals
- Minimize images during acquisition

Setup tabs are shown for each dimension checked on the main tab. After you configure the appropriate values for each dimension, use the Snap button to display a snapshot of your image using the current parameters. Click the Live button to display an image continuously. Click the Preview button to display an image for each wavelength you have configured (8 maximum). The Preview button is more advanced than the Snap and Live buttons in that it memorizes the image position and display preferences (for example, scaling). This enables you to close and re-open the dialog box without losing your settings.

After you adjust the parameters as necessary to make monitoring the experiment convenient, including adjusting the images' zoom, position, and scaling, you can save your settings in a state (.MDS) file. This enables you to repeat your experiment without re-configuring your settings.

The program begins acquiring data when you click the Acquire button. For each full set of acquisitions, a base file is created to store information about the experiment. This file has the extension .nd and tells MetaMorph where the acquired images are stored. This file is then used by the Review Multi Dimensional Data command to display the information collected during Multi Dimensional Acquisition.

## Multi Dimensional Acquisition - Dialog Box Options

**Do Timelapse, Multiple Stage Positions, Multiple Wavelengths, Do Z Series, Stream, Run Journals, and Minimize images during acquisition.**

Indicates the active dimensions based on the installed devices. Also indicates available functions and options, such as *Stream* and *Run Journals*. Click the boxes of the dimensions you want to include in your acquisition. The dimensions and options in this list will vary depending on which devices are installed on your computer. For example, if you do not have a Z motor installed, the *Do Z Series* option and tab will not be present. To activate the associated settings tab for the dimension or option, click the checkbox next to the dimension or option name.

***Minimize images during acquisition*** minimizes each open image window and places

their title bars at the bottom of the MetaMorph window.

**Description**

Attaches an annotation to your experiment data set. Type a description of your experiment in this box (optional).

**Select Directory**

Opens the Browse for Folder dialog box. Select the directory in which you want to store your data set.

**Increment base file name if file exists**

Detects an existing *.nd file and assigns the detected name as a base file name for new experiments. If checked, MetaMorph looks for an existing *.nd file, and increments the numerical value sequentially. If a *.nd file without a numerical value is detected, a one (1) is appended to the file name. Check this box to use an existing *.nd file name in the current directory as your experiment name. MetaMorph will use and automatically increment this name until you specify a different base file name in the Base Name box. If a *.nd file is not detected, type a name in the Base Name box.

**Base Name**

Indicates a default base file name (experiment1.nd) and enables you to change the base file name. Type the name of the file you want to assign as your base file name to your experiment.

**Note:** If your base file name includes reserved characters such as "/" or "%," the reserved character is replaced by a hyphen (-) when the file is saved; however, the reserved characters are maintained in the .nd file so they can be used by the Review Screen Data and the Screen Data Utilities commands.

**Snap**

Opens an image display box and acquires and displays a single new image. Click Snap to acquire a single image using the current device positions and settings. You can use your acquired image as a guide for adjusting your settings.

**Bin**

Adjusts binning for acquisition. Specifies the pixel value for X and Y. The value specifies the number of pixels for each dimension to be binned. The total number of pixels binned is the square of the value in the bin box. Therefore, a bin value of 1 = 1 pixel, a bin value of 2 = 4 pixels, a bin value of 3 = 9 pixels, and so on. See Binning.

**Live**

Opens an image display box and continuously acquires new images. Click Live to continuously acquire images using the current settings. After you press this button, its caption changes to F2:Stop. Click F2:Stop or press the F2 key to stop the live acquisition.

**Bin [Live]**

Adjusts the binning value for *Live*. Enables you to have separate binning values for *Live* and image acquisition. The *Live* binning value is also used as the binning value during autofocus. ***Note:*** If the live binning value is different than the acquisition binning value, the time used for live exposure will be based on the acquisition exposure time, using the binning difference as a factor.

**Full Chip**

Selects the largest image the camera can produce as the acquisition region.

**Center Quad.**

Selects only the center quadrant of the camera's chip as the acquisition region.

**Active Region**

Selects only the active region of the snap or live image as the acquisition region.

**Summary**

Opens the Multi Dimensional Acquisition Summary dialog box. This dialog box indicates your current MDA Acquisition configuration including the number time points, the time interval, the acquisition duration, the number of Z steps, step spacing, step range, the total number of images, and the amount of memory required. Also, for each wavelength, the wavelength illumination name, the frequency of collection, whether it is a Z series, the exposure time, and whether autofocus is on or off.

**Save State**

Opens the MDA State (save file) dialog box. Use Save State to save your data set acquisition configuration settings to a *.MDS state file. All configurable information in the dialog is saved to the state file, including image display parameters. States saved from an experiment can be applied to new experiments using Load State.

**Load State**

Opens the Load MDA State dialog box. Use Load State to load data set conditions saved in a *.MDS state file during a previous experiment. Click the appropriate state check box(es) to include or exclude specific states from loading into your new experiment.

**Preview**

Acquires and displays an initial image for each wavelength. Preview enables you to make adjustments to the image settings and position before you start to acquire your series of images. From the Preview dialog box, you can click Start to begin acquiring images, or click Cancel to return to the Multi Dimensional Acquisition dialog box. The Preview dialog box (labeled Multi Dimensional Acquisition) also enables you to revise the acquisition interval value and the the number of wavelengths that you plan to acquire.

> **Note:** During a Z-series Acquisition, the Pause and Cancel buttons on the Preview dialog box are inactive. To discontinue image acquisition during a Z-series, press the **Esc** key on your keyboard.

**Acquire**

Starts image data acquisition.

**Close**

Closes the Multi Dimensional Acquisition dialog box.

## Multi Dimensional Acquisition - Procedures

To acquire a multi dimensional image sequence, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, choose Multi Dimensional Acquisition. The Multi Dimensional Acquisition Dialog Box opens. |
| **2** | On the Main tab, click the dimensions that you want to measure and the functions that you want to run.<br><br>**Note:** The list of dimensions will vary depending on which devices are installed on your computer. For example, if you do not have a Z motor installed, the Do Z Series option will not be visible. |

**3** Click Load State to load a state file created during a previous experiment. The Load MDA State dialog box opens.

Click the check boxes for the conditions from your previous experiment that you want to apply to your new experiment, then click Load. The Load Multi-D Acquisition State dialog box opens.

Click the MDS file that contains the conditions you want to apply to your new experiment, then click Open. The MDS conditions are loaded to the Multi Dimensional Acquisition dialog box.

**4** In the Description box, type a description of your experiment (optional). This serves as an annotation for your image.

**5** Click *Select Directory*. The browse for Folder dialog box opens. Select a directory or create a new directory for storing your images.

**6** Click *Increment base name if file exists* if you want your experiment to be saved with a new name without having to specify it manually.

**7** Configure the parameters for acquisition for each dimension by clicking the appropriate setup tabs. See the following separate procedures for each dimension's setup tab.

**8** Click Snap to acquire a single image using the current position and settings. Use the acquired image as a guide for adjusting your settings.

**9** Click Live to continually acquire images using the current state. When this button is pressed, its caption changes to "F2: Stop". Click this button or press the F2 key to stop the live acquisition.

**10** In the separate *Bin* boxes for both Snap and Live, type or select the binning value for the number of both X and Y pixels that you want to bin. The number of actual pixels binned is the square of the value in the bin box. For example, 1 = 1 pixel, 2 = 4 pixels 3 = 9 pixels, and so on.

**11** Set the region for acquisition by clicking either Full Chip, Center Quad, or Active Region.

**12** Click the arrow in the Wavelength box to choose the wavelength that you want to preview.

**13** Click Save State to save your configuration settings in a state file. The MDA State save file dialog box opens. Select or enter the file name for the file to which you want to save the MDA state.

**14** Click Preview to acquire and display an

image for each wavelength. The Status dialog box opens and shows an image preview. Use the controls in this box to adjust image positioning before the data set is acquired.

**15** Click *Summary* to open the MDA Summary dialog box, which shows a summary of the MDA settings that currently exist, including acquisition time points, z-steps, and wavelength settings.

**16** In the Status dialog box, click Start to begin acquiring data or click Cancel to return to the Multi Dimensional Acquisition dialog box.

**17** Click Acquire to begin acquiring your experiment data.

**18** If you are not running a timelapse or multiple stage position experiment, click Save Images to save the acquired images.

**19** Click Close to close the Multi Dimensional Acquisition dialog box.

## Timelapse - Dialog Box Options

### Number of Time Points

Specifies the total number of times you want an image to be acquired. Enter 0 if you want the experiment to run until halted.

### Duration

Specifies the time it will take to acquire the number of times points based on the interval. Changing this field updates the *Number of time points field* by calculating the number of time points from the time interval and the duration. Set the time units in the adjacent box. The available units are msec, seconds, minutes, and hours. This setting is not visable if either the *Number of time points* or the *Time Interval* is set to 0.

### Time Interval

Specifies the amount of time to wait between acquisitions. In the box next to the Time Interval box, select the unit of time you want to use.

> **Note:** If the acquisition cannot be accomplished in a time period shorter than the interval, acquisition will move to the next time point as soon as the current time point is completed.

### Estimate minimum interval

Indicates the estimated minimum time interval caluculated from the sum of the exposure time for the wavelength multipled by the number of images that need to be acquired for each loop.

### [Warning Message]

If the specified interval is not sufficient, an appropriate warning message is displayed, such as *Interval specified is below the calculated minimum*.

## Timelapse - Procedures

To configure the parameters for acquiring images at specific intervals of time, use the following procedure:

| Step | Action |
|---|---|
| 1 | On the Main tab, click Do Timelapse. |
| 2 | On the Timelapse tab, select the total number of times you want an image to be acquired in the Number of Time Points box. |
| 3 | In the Time Interval box, specify the amount of time to wait between acquisitions.<br><br>**Note**: If the acquisition cannot be accomplished in a time period shorter than the interval, acquisition will move to the next time point as soon as the current time point is completed. |
| 4 | In the box next to the Time Interval box, select the units of time you want to use.<br><br>**Note**: If time streaming is on, the time interval will be determined by exposure and this text box will not be available on the Timelapse tab. |

## Stage - Dialog Box Options

### X, Y, and Z

Indicates the coordinates of the current stage position. You can change the stage position by entering new values into these boxes or by using a joystick.

### Position Label

Designates a unique identifier for the currently displayed position. Use the default position label or enter your own label name.

### Add

Adds the current position to the Positions box. The Positions Box lists the Position Label name and the X, Y, and Z coordinates of the position.

### Remove

Removes the selected position from the Positions box.

### Positions

Contains the currently defined positions. Double-clicking a position will move the stage to the selected position.

### Move to Position

Moves the stage to a specific position without adding the new position to the positions box or moves the stage to the position selected in the Positions box.

## Stage - Procedures

To configure the parameters for acquiring images at multiple stage positions, use the following procedure:

| Step | Action |
|---|---|
| 1 | On the Main tab, click Stage Position. |

**2** On the Stage tab, the X, Y, and Z boxes show the current stage position. You can change the stage's position by entering new values in these boxes or by using a joystick.

**3** Set the coordinates for the first position of acquisition in the X, Y and Z boxes.

**4** Type a position label name for the position in the Position Label box, or use the pre-filled, default label name, Position(n).

**5** Click *Add* to add the position to the Positions box on the right.

**6** Click *Remove* to remove positions from the Positions box.

**7** To move the stage to a specific position without adding the new position to the Positions box, click Move to Position.

**8** To move the stage to one of the positions in the Positions box, select the position, and click Move to Position.

## Wavelengths - Dialog Box Options

### # of Waves

Specifies the number of different sets of wavelenth settings to be used. You can specify a maximum of 8 wavelengths.

### Current Wavelength

Selects and indicates the Current Wavelength. Select the wavelength you want to configure first. The default names for the wavelengths are 1:Illum Setting #1, 1:Illum Setting #2, and so on. The remaining settings on the Wavelengths tab can be configured separately for each wavelength.

### Illumination

Selects the illumination setting to be used for the current wavelength. Illumination settings are defined using the Configure Illumination command located in the Devices menu.

### Exposure

Specifies the exposure time in milliseconds. If you are using a video camera, the units will be frames. If you are auto focusing, this value will be the initial exposure attempted for the first auto expose.

### Auto Expose

Activates auto exposure and specifies the auto exposure interval. Choose from the follwing selection of settings:

***Every Acquisition*** – Auto exposes for all images acquired for the current wavelength.

***First time point*** – Auto exposes for only the first time point for each stage position.

***Never*** – Turns off Auto Expose.

***Every Nth Acquisition*** – Auto exposes images at the time point interval that you specified in the adjacent box.

### Target Intensity

Specifies the intensity value of the gray level you are trying to achieve. This setting is active only when Auto Expose is enabled.

**Note:** Auto Expose is inactive in Live mode.

**Acquire**

Specifies the acquisition interval for the currently selected wavelength. Choose from the following selection of acquisition intervals:

***Every Time Point*** – Acquires an image for all time points for the current wavelength as specified by the settings on the Timelapse tab.

***First Time Point*** – Acquires an image for the first time point for the current wavelength.

***First and Last Time Point*** – Acquires images for the first and last timepoints of the current wavelength.

***Every Nth Time Point*** – Acquires images at the time point interval that you specified in the adjacent box.

**Auto Focus**

Activates auto focus for the currently selected wavelength and specifies the Auto Focus interval.

***Every Acquisition*** – Auto focuses for all images acquired for the current wavelength.

***First time point*** – Auto focuses for only the first time point for each stage position.

***Never*** – Turns off Auto Focus.

***Every Nth Acquisition*** – Auto focuses images at the time point interval that you specified in the adjacent box.

**Configure**

Opens the ***MDA: Auto Focus*** dialog box.

**Do Z Series**

Specifies that the images for the current wavelength are captured and included in the data set as a Z series.

**Note:** You must also select Do Z Series on the Main tab to enable this function.

**Alignment Cropping**

Adjusts the image alignment for the current wavelength, set the X and Y values to which you want your image aligned in the Alignment Cropping group. Or click the *Set Alignment* button to interactively shift pixels in order to align wavelengths.

**Set Alignment**

Opens the ***MDA:Set Alignment*** dialog box. Click this button to interactively set the precise alignment of images between two or more different wavelengths.

## Multiple Wavelengths

To configure the parameters for acquiring images at multiple wavelengths, use the following procedure:

| Step | Action |
|---|---|
| **1** | On the Main tab, click Multiple Wavelengths. |
| **2** | On the Wavelength tab, set the number of different illumination settings that will be used in the # of Waves box. You can specify a maximum of 8 wavelengths. |

**3** In the Current Wavelength box, click the wavelength you want to configure first. When you select a new wavelength from this list, the configuration settings in the dialog box change to reflect the current setting for the selected wavelength.

**4** In the Illumination field, select an illumination setting to use with the current wavelength.

**5** Click Do Z Series if you are doing a Z series and you want the current wavelength to be part of the Z series.

**Note:** For this check boxes to be present, you must check Do Z Series on the Main tab.

**6** Click Stream if you are streaming your image sequence and you want the current wavelength to be part of the stream.

**Note:** For this check boxes to be present, you must check Stream on the Main tab.

**7** Check the Auto Focus check box to use Auto Focus while acquiring images.

**OR**

If you do not want to Auto Focus, go to Step 9.

**8** Click Auto Focus, the click Configure Auto Focus to configure the range and accuracy of auto focus.

**9** To auto expose, choose Every Loop to auto exposes at every time point;

**OR**

Choose First Loop to auto exposes only on the first time point at each stage position;

**OR**

Choose Never If you do not want to Auto Expose, and then go to Step 12.

**10** In the Target Intensity box, set the intensity value of the gray level you are trying to achieve.

**11** In the Exposure box, select how long to expose the image (in ms). If you are using a video camera, the units will be frames.

**12** To adjust the image alignment for the current wavelength, set the X and Y to the values to which you want your image aligned in the Alignment Cropping boxes.

**OR**

Click Set Alignment. The MDA Set Alignment dialog box opens.

- Click Acquire Alignment Stack.
- In the Display group, choose Subtract or

Average.

- Move the horizontal and vertical sliders to set the Horizontal Shift and Vertical Shift values.
- Click Zero Shift to reset the horizontal and vertical values to Zero.
- Click Previous or Next to change images.
- Click OK when you are finished setting alignment.

## Z Series - Dialog Box Options

### Interactive Settings

#### Current Position

Sets and indicates the current Z position coordinate. Select or type a new value in this box to change the position. The units of calibration are shown to the right of this box.

#### Increment

Sets and indicates an increment value for the Current Position box. The value in the Increment box controls the increment size when using the arrow buttons to set the current position in the Current Position box.

**Note:** The range value always equals the number of steps minus one multiplied by the step size.

### Settings for Acquisition Series

#### Loop Order

Determines the integrated order of priority of wavelength acquisitions and Z-series acquisition in a series containing both.

***Acquire wavelength set at each Z*** – For a specific Z position, all wavelengths to be acquired for that location are acquired before moving to the next Z position. This is the default setting.

***Acquire Z series for one wavelength at a time*** – All Z positions for a specific wavelength are acquired before moving to the next wavelength.

***Keep shutter open between steps*** – Keeps the shutter open during a continuous Z series acquisition for a single wavelength. The option, *Acquire Z series for one wavelength at a time* must be selected to enable this option.

#### Range

Sets and indicates the total range in units between the top and bottom Z positions. Use this setting to automatically set the Top and Bottom positions based on the current position. The top and bottom positions are based on the current position being the center position. To set a value for Range, Range Around Current must be checked.

#### Range Around Current

Enables you to automatically specify the top and bottom Z positions based on the value you enter in the Range box. The current position is applied as the center position of the range. When you change the current position, the values for top and bottom are automatically updated. Set the range value in the Range box.

**Note:** When Range Around Current is selected, the Top and Bottom settings boxes, and the Set Top To Current, Set Bottom To Current, and Center Around Current buttons are deactivated

**Top**

Sets and indicates the position for the top of the Z series. To set the top of the series value to the current position value, click Set Top to Current.

**Set Top to Current**

Sets the top position value to the current position.

**Bottom**

Sets and indicates the position for bottom of the Z series. To set the bottom of the series value to the current position value, click Set Bottom to Current.

**Set Bottom to Current**

Sets the bottom position value to the current position.

**Step Size**

Sets and indicates the distance to move the Z motor between steps.

**Note:** As you change the value for either step size or number of steps the other value changes proportionally.

**Center Around Current**

Sets the current Z position as the center of the Z series. The values set for Top and Bottom will change based on the new value applied as the center. The range established between the top and the bottom remains constant. This function enables you to reposition the center of the Z series without affecting the range, step size, or number of steps.

**Number of Steps**

Sets and indicates the number of steps in the Z series.

## Z Series - Procedures

To configure the parameters for acquiring a Z series, use the following procedure:

| Step | Action |
|---|---|
| **1** | On the Main tab, click Do Z Series. |
| **2** | Select the Z Series tab. The value in the Current Position box indicate the current Z position. Select or type a new value into the Current Position box to change the Z position. |
| **3** | Select or type a value in the Increment box to set an increment value for the Current Position box.<br><br>***For example***, if you set Increment to 5, the Current Position value will then change in increments of five units when you click either the up or down arrow buttons for Current Position. |
| **4** | To automatically set the top and bottom of |

the range values for your experiment, click Range Around Current, and select or type a range value in the Range box.

**5** Select or type a value for the top of the Z series range in the Top box.

**OR**

Click Set Top To Current to set the current position as the top of the Z series range.

**6** Select or type a value for the bottom of the Z series range in the Bottom box.

**OR**

Click Set Bottom To Current to set the current position as the bottom of the Z series range.

**Note:** You must change the current position to bottom position before clicking Set Bottom To Current.

**7** Select or type the distance to move between steps in the Step Size box.

**Note:** When you change the step size, the number of steps changes proportionally.

**8** Select or type the number of steps to take in the Number of Steps box.

**Note:** When you change the number of steps, the step size changes proportionally.

**9** Click Center Around Current to use the current Z position as the center of the Z series.

## Stream - Dialog Box Options

### Stream Time

Designates that MetaMorph collect your experiment image sequence as single continuous data stream to RAM. When streaming time, the entire experiment is stored in RAM before being saved. In this case, all other dimensions used in the experiment must be able to stream as well. If you chose not to stream time, you can still stream other dimensions. However, in this instance, the other dimensions are streamed and then saved to disk before moving to the next time point. This enables you to capture Z and/or wave "snapshots." Using this method, you do not need to consider the amount of available RAM when determining the number of time points to acquire. As a benefit of this method, you can specify a time delay between snapshots.

### Stream Z

Designates that MetaMorph collect your Z series-based experiment image sequence as a continuous data stream to RAM. You must have a Z motor that supports streaming for this option to be available.

### Stream Multiple Wavelengths

Designates streaming of multiple wavelengths. When streaming multiple wave lengths, you must set the Stream Exposure Time in milliseconds.

### Stream Exposure Time (ms)

Sets the streaming exposure time (in milliseconds).

**Status**

Displays a status message about any conflicts resulting from your current Multi Dimensional Acquisition dialog box configuration for streaming. When a conflict exists, a yellow or red square is displayed on the Stream tab. Yellow indicates that acquisition can occur, but that there are settings that are not ideally configured. Red indicates a major setting conflict; acquisition cannot be completed. If neither a yellow or red square are present on the Stream tab, the Status should indicate OK, and normal Stream Acquisition can begin.

**Stream To**

Specifies the location to which to stream your image data. For example, choose RAM or choose real-time hard disk.

## Stream - Procedures

To configure the parameters for acquiring images in a stream, use the following procedure:

| Step | Action |
|---|---|
| **1** | On the Main tab, click Stream. |
| **2** | On the Stream tab, click Stream Time if you want to stream a time-based sequence of experiment images to RAM, and all your devices support streaming.<br>**OR**<br>Click Stream Z-Series if you want to stream a Z Series-based sequence of experiment images to RAM, and your Z-motor supports streaming.<br>**OR**<br>Click Stream Multiple Wavelengths to stream images from more than one wavelength to RAM, and your wavelength devices supports streaming.<br>**Note:** If you check the Stream Time box, the Time Interval option is removed from the Timelapse tab. |
| **3** | Set the stream exposure time in the Stream Exposure Time box. |
| **4** | Select the location to which to stream in the Stream To box. For example, most users will be streaming to RAM. |

## Journal - Dialog Box Options

**Journal Step**

Enables you to assign journals to run at specific time points during MDA image acquisition. Only one journal can be assigned to each time point. Click the check box next to the time point that you want to use, then click *Select* to assign the journal to the time point. After you have assigned a journal to a time point, you can temporarily deactivate the running of the journal by deselecting (unchecking) the check box for the time point. To reactive a pre-assigned journal, simply click the check box.

***Before each Image*** – Runs the selected journal only during acquisition, after illumination changes for wavelength and focusing are complete, but before the image is acquired.

***After each image*** – Runs the selected journal only during acquisition, after the shutter is closed and before images are saved.

***Start of Z Series*** – Runs the selected journal just before a Z series acquisition begins for a specific wavelength.

***End of Z Series*** – Runs the selected journal just after a Z series acquisition for a specific wavelength has completed.

***Start of stage position*** – Runs the selected journal during acquisition each time the stage moves to a new position.

***End of stage position*** – Runs the selected journal when acquisition has completed for the current stage position before the stage moves to the nextr position.

***Start of time point*** – Runs the selected journal only during the acquisition loop, at the beginning of each time point, before any images are acquired for a time point.

***End of time point*** – Runs the selected journal only during the acquisition loop, at the end of each time point, after all images have been acquired for a time point.

***Start of Stream*** – Runs the selected journal just before streaming begins.

***End of Stream*** – Runs the selected journal immediately after streaming stops.

***Start of Acquisition*** – Runs the selected journal just before acquisition begins.

***End of Acquisition*** – Runs the selected journal immediately after acquisition stops.

**Journal**

Lists the names of the journals that you have assigned to each time point.

**Select**

Opens the Select MDA Journal dialog box. Use this dialog box to select and assign a journal to a time point. Also use this dialog box to deselect or unassign a journal to a time point. To assign a journal, click the checkbox for the acquisition step, click *Select*.

## MDA: Set Alignment - Dialog Box Options

**Acquire Alignment Stack**

Acquires an image at each wavelength and builds a stack that can be used in adjusting alignment.

**Display**

Selects the method to be used to display differences between the reference plane and the shifting plane:

***Subtract*** uses subtraction to show the difference between the reference plane and the shifting plane. The planes will be aligned when there is nearly a uniform grayscale level throughout the entire image.

***Average*** uses averaging to display the offset between the two planes. The aligned plane should look like the original plane with as little blurring as possible.

**Horizontal Shift (text box and slider)**

Adjusts the horizontal alignment of the plane in one-pixel increments

**Vertical Shift (text box and slider)**

Adjusts the vertical alignment of the plane in one-pixel increments.

**Zero Shift**

Resets the horizontal and vertical shift to zero.

**Previous**

Places the previous plane in the alignment image window.

**Next**

Places the next plane in the alignment image window.

**Cancel**

Cancels your adjustments and closes the dialog box.

**OK**

Applies the shift to all of the wavelengths.

# Binning

The process of combining data from multiple pixels (for example, 2 x 2 regions) into a single pixel during acquisition. Directing the camera to use binning causes the resulting acquired image to be brighter and smaller, but the resolution will be lower as a result. Because the image is smaller, the time required to transfer the image is significantly reduced.

## MDA: Auto Focus

**Algorithm**

Specifies the algorithm that you want to use for autofocus. Use ***Standard*** for acquiring images of normal brightness using lower magnification objectives; use ***Directional Avg*** for acquiring images of reduced brightness that can result from using objectives with magnification of 20X and greater.

**Range, Current +/-**

Specifies how far above and below the current Z position that the Z motor will be moved while acquiring focus test images. This distance is specified in steps, as defined by the Focus command.

**Accuracy: Step(s)**

Specifies the Z distance to move. Changing this value changes the Number of Z Moves.

**Number of Z Moves**

Indicates the total number of acquisitions that will be made, based on the settings selected with the Range, Current +/- and Accuracy: Step(s) spin boxes.

**Backlash Compensation**

Enables the Backlash Compensation Z motor movement protocol which directs the focus motor to move to a Z axis position slightly below the target position and then move against gravity to the target position. This ensures that the Z motor gears are fully engaged, avoiding drift due to slippage. This option has no effect on focus devices with built-in backlash compensation.

**Update Z position with Result**

Resets the Z position to the best focus point.

**Test**
Activates auto focus at the current position.

**OK**
Saves settings and closes the dialog box.

# Acquiring Multiple Dimensions

Main

Timelapse

Stage Position

Multiple Wavelengths

Z Series

Stream

# Multi Dimensional Acquisitions - Options

Main Options

Timelapse Options

Stage Position Options

Multiple Wavelengths Tab Options

Z Series Tab Options

Stream Tab Options

# Review Multi Dimensional Data (Apps Menu)

**Enables you to view, filter, organize, and analyze MetaMorph images in a Multi Dimensional Data Set (*.nd) and generate new images based on your viewing criteria.**

*Availability: Available for MetaMorph Basic; included in MetaMorph Premier*

**Drop-in: NDPLAYER**

Use the Review Multi Dimensional Data command to view multi dimensional data sets (*.nd) and/or generate new images based on your viewing criteria. Typically, these are data sets created using the Multi Dimensional Acquisition dialog box. They can also be created using the Build .nd Set dialog box, which is used to assemble and organize individual sequential images. In addition, you can combine images from two or more data sets into a single data set for viewing using Multi Dimensional Data Set Utilities.

A Multi Dimensional Data Set is a group of sequential images containing one, several, or all of the dimensional properties of your captured experiment. These properties include wavelengths, time points,

X/Y stage positions, and Z-positions.

As you browse your data with the viewer, an image is opened for each wavelength that you choose. Operations can be performed on these images similar to other images in MetaMorph. The viewer enables you to run process, measurement, and analysis functions on data from a multi dimensional sequence without having to search through and individually open your image files. For each data set, the number of time points, stage positions wavelengths, and Z-positions are already recorded in the data set base file (*.nd file), enabling you to view, filter, organize, and analyze the images.

You can also create a montage image of thumbnails for all or some of the timepoints in a wavelength. When the montage image is displayed, you can click a thumbnail in the montage image to open the full image.

The Review Multi Dimensional Data dialog box contains elements that represent the organizational structure of the data set dimensional information. The largest area in this dialog box is the image grid which organizes the images according to time point and Z-position in the image sequence. The remaining dimensions X and Y, and image groupings according to wavelength are identified in the Stage Position box and the Wavelength box. These boxes enable you to narrow your viewing preferences.

If you used or plan to use the Build .nd Set dialog box to create a multi dimensional data set from existing sequential experiment images, you can use Build .nd Data and the Multi Dimensional Data Set Utilities dialog boxes in conjunction with Review Multi Dimensional Data to follow an interactive path that enables you to make changes to the data set in Build .nd Set, and immediately view these changes with Review Multi Dimensional Data. If you determine that it is necessary to refine the structure of the data set, you can modify and update the data set using the Build .nd Set dialog box. Also, if the original .nd file is lost, you can recreate this file using the Build .nd Set dialog box.

**Note:** To view an image in the data set, you must select at least one wavelength in the Wavelengths box. It is not necessary to select a stage position. The stage position default is Position1.

# Review Multi Dimensional Data - Options



## Review Multi Dimensional Data - Dialog Box Options

**Select Base File**

Opens the Multi Dimensional Data Set Utilities dialog box. Use this dialog to select the file that you want to use as the base file for your multi dimensional data. Chose the file from the list of the data sets in the Data Sets (*.nd) box or click Select Directory to select a data set from a different directory.

**Note:** After you select or change your base file selection, you must click View to implement your selection and return to the Review Multi Dimensional Data dialog box.

**Wavelengths**

Displays the wavelengths that are included in your data set. If two or more wavelengths are included in the structure of your data set, your images will be organized according to wavelengths. Select a wavelength to display the images associated with that wavelength. You can select more than one wavelength at a time. An image window will open for each wavelength you select.

**Z**

Player controls for playing and viewing images in a single Z projection.

**Stage Position**

Selects the stage position that you want to view. Click on the drop-down box to see all stage positions included in the data set, then click on the position that you want to view.

**Note:** You can view only one stage position at a time.

**Time**

Player controls for playing and viewing all images associated with a specific time point in the Multi Dimensional Data.

**Enable Montage**

Activates and deactivates the Montage controls and creates a montage image of thumbnails for each timepoint in a wavelength. If multiple wavelengths are selected, a montage image is created for each wavelength. After the montage image is displayed, click a thumbnail in the montage image to open a full image. You must have at least one wavelength selected to use this feature. If you click Enable Montage before selecting any wavelengths, a montage for a specific wavelength will be generated when you select the wavelength.

**# of Thumbnails**

Specifies the dimensional configuration of the montage images. For example, specifying 5X4 selects 5 rows horizontally by 4 rows vertically, and creates a thumbnail view of 20 thumbnail images. To specify the number of thumbnails in the montage image, type or select the horizontal and vertical dimensions of the montage area, then click *Apply* to update the settings and generate the montage. This montage area can be superimposed anywhere within the image grid; its position is controlled by the four arrow buttons to the right of the # of Thumbnails selection boxes.

**Montage Controls**

Controls which timepoints are shown in the montage image. Use these controls to move the gray (selected) timepoints in the image viewing grid. The current gray area represents what is displayed in the montage(s). The four controls can move the montage area up, down, left, and right, as indicated by the direction of the arrow on each button.

**Apply**

Updates the settings specified in the # of Thumbnails selection boxes and generates a new montage image. If a montage image is currently displayed, the image is replaced with the new image. If you change the settings for # *of Thumbnails*, it is not necessary to click Apply; the settings will automatically be applied and the montage will be updated after about five seconds. If one or more wavelengths are selected before you click *Enable Montage*, you must click *Apply* to generate the montage(s).

**Selections (X's) (Tab)**

Selects and displays controls for the Selections (X's) functions.

**Display (Tab)**

Selects and displays the controls for the Display functions.

**Z Projection (Tab)**

Displays the controls for the Z Projection functions.

## Review Multi Dimensional Data - Selection (X's) Tab Options

**The following dialog box options are on the Selection (X's) Tab.**

**Load Image(s)**
Loads selected images into MetaMorph as new images and displays the images. The image can be saved to a file using the Set Up Sequential File Name dialog box.

**Select Best Focus**
Selects the best focused image from the Z-planes at each time point.

**Clear**
Clears all the image selections from the image grid.

## Review Multi Dimensional Data - Display Tab Options

**The following dialog box options are on the Display Tab.**

**Full Image**
Displays an entire image without regard to active regions.

**Active Region**
Displays only the active region of an image.

**Color Composite**
Combine the defined and selected image sources into a single image, assigning the selected primary color to each of the respective images.

**Source R:**
Assigns the primary color Red to the wavelength that you select as source. After you assign a wavelength to this source, you can turn this image selection off and on in the Image Window.

**Source G:**
Assigns the primary color Green to the wavelength that you select as source. After you assign a wavelength to this source, you can turn this image selection off and on in the Image Window.

**Source B:**
Assigns the primary color Blue to the wavelength that you select as source. . After you assign a wavelength to this source, you can turn this image selection off and on in the Image Window.

**Note:**

For a wavelength to appear in the color image, it must be selected in the wavelength list as well as selected as a source color.

## Review Multi Dimensional Data - Z Projection Tab Options

**The following dialog box options are on the Z Projection Tab.**

**Z Projection**
Activates the Z-projection function, and applies the parameters entered or selected on the Z Projection dialog tab.

**Create Rotation**

Opens the ***Create Rotational Sequence*** dialog box, which enables you to create a stack of 3D Reconstruction images using one angle for each time point. Use this option to view your 3D reconstruction as either a slow rotation stack, or a stack that rotates back and forth.

**Type**

Specifies the reconstruction type of either Minimum or Maximum. Use Minimum for brightfield planes (dark objects on a bright background). Use Maximum for darkfield or fluorescence planes (bright objects on a dark background).

**Orientation**

Specifies either a horizontal or vertical axis orientation for a reconstructed 3D image view.

**Border Color**

Changes the value assigned by MetaMorph for unfilled areas of the 3D reconstruction image if the image data from the source stack does not fill all parts of the image.

**Angle**

Specifies a specific view angle for the image extracted from the 3D stack.

**Interpolate**

Fills in the gaps between planes so that the resulting 3D reconstruction will be smoother. Be sure that the computer has sufficient memory resources before using this option, especially for larger stacks. Because of the additional calculations to interpolate between planes, 3D reconstruction takes longer with interpolate enabled.

**Z Distance**

Sets the Z-axis distance value to the calibrated Z-axis distance value (if the stack was calibrated with the Calibrate Distances command prior to acquisition), or to a user-specified distance, in pixels.

**Z Dist**

Specifies a user value for Z Distance.

**Planes**

Specifies the range of planes to be included in the Z projection image.

**All Planes**

Specifies that all planes are included in the Z projection image.

**Reset Image Displays**

Resets all the values for the controls in the Image Display window.

**Run Journal Loop**

Opens the Run Journal for Multi Dimensional Data dialog box. Use this dialog box to select a journal to assist in organizing and processing your image data.

**Close**

Closes the Review Multi Dimensional Data dialog box.

## Review Multi Dimensional Data - Procedures

### Viewing a Data Set

## Viewing a Data Set

To view a data set, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, Choose Review Multi Dimensional Data. The Review Multi Dimensional Data dialog box opens. |
| **2** | Click Select Base File. The Multi Dimensional Data Set Utilities dialog box opens. The Data Sets (*.nd) box should list the name of the data set you want to open. |
| **3** | If the name of the data set that you want to open is not listed in the Data Sets (*.nd) box, click Select Directory. If the data set is listed, go to step 5. |
| **4** | Click the directory containing the data set you want to open, then click OK. |
| **5** | In the Data Sets box, double-click the name of the data set that you want to open. |
| **6** | Click View to return to the Review Multi Dimensional Data dialog box. |
| **7** | Click one or more wavelengths to select the primary image group(s) that you want to view. An image view opens for each wavelength you select. |
| **8** | Click the rectangle in the image viewing grid that represents the image you want to view. The image displayed in each open view will change to the selected image. |

**Note:** When an image window for a selected wavelength opens, it retains the zoom factor from the last multi dimensional review session. When you use Review Multi Dimensional Data for the first time on an image, the zoom factor is 100 percent. The zoom settings for images viewed using Review Multi Dimensional Data will not follow the zoom settings that appear on the Windows tab in Preferences.

## Modifying Viewing Criteria

After you have opened your data set in the Review Multi Dimensional Data dialog box, you can change or limit the viewing criteria to provide you with images that contain the information you want to view.

In the Review Multi Dimensional Data dialog box, perform the following steps for each criteria or limit that you want to apply to your images:

| Step | Action |
|---|---|

| Step | Action |
|---|---|
| 1 | To specify wavelengths, click on the wavelengths that you want to select or deselect. You can select one or more wavelengths. |
| 2 | To select a different stage position, click the Stage Position arrow to open the stage position box, then click the new position number that you want to use. |

## Creating a Montage Image

After you have opened your data set in the Review Multi Dimensional Data dialog box, you can create a montage image of thumbnails for each of the timepoints in a wavelength. When the montage is displayed, you can click a thumbnail to open the full image.

In the Review Multi Dimensional Data dialog box, perform the following steps to create a montage image:

| Step | Action |
|---|---|
| 1 | Select the wavelength(s) to include in the montage(s) from the Wavelengths field. You must have at least one wavelength selected to create a montage. |
| 2 | Select Enable Montage. The *# of Thumbnails* and Montage controls become enabled. |
| 3 | Use the *# of Thumbnails* fields to configure the layout of the montage images. For example, specifying 5X4 selects 5 rows horizontally by 4 rows vertically, and creates a thumbnail view of 20 thumbnail images. |
| 4 | Click *Apply* to view the montage image(s). |
| 5 | Click a thumbnail on the montage to open the full image. |
| 6 | To change which timepoints are displayed on the montage, use the montage control arrow buttons. |

## Viewing Images as a Stack

This procedure assumes that you have opened the Review Multi Dimensional Data dialog box and selected the base file for the data set you want to view.

To view and save selected images as a stack, use the following procedure:

| Step | Action |
|---|---|
| 1 | In the Wavelengths box, select the wavelengths from which you want to select your images. |
| 2 | In the Stage Positions box, select the stage position from which you want to select your images. |
| 3 | In the Z-plane/Time point image grid, right - click on the images you want to include in |

| | |
|---|---|
| | your new image stack. An X is displayed on the image position. To include an entire row or column, right-click the numbered button for the row or column. To deselect an image, right-click again on the image; the X is turned off. |
| **4** | Click the Selections (X's) tab. |
| **5** | If you want to select the best focus for each time point, click Select Best Focus. |
| **6** | Click Load Images. A new image opens for each currently displayed wavelength. If more than one Z-position is selected for any time point, a separate stack will open for each time point that has images selected. |
| **7** | Click the appropriate player buttons to play the new image stack. |
| **8** | From the File menu, choose Save to save your new stack file. |
| **9** | Click Clear to clear all of the image selections from the image grid. |

## Creating a Color Composite

This procedure assumes that you have opened the Review Multi Dimensional Data dialog box and selected the base file for the data set you want to view.

To create a color composite from two or more wavelengths, use the following procedure:

| Step | Action |
|---|---|
| **1** | In the Wavelengths box, select a maximum of three wavelengths that you want include in your composite image. |
| **2** | In the Stage Positions box, select the stage position for which you want to create the composite image. |
| **3** | Click the Display tab, and click Color Composite. |
| **4** | Select the wavelengths that you want to assign to each source color (Red (R), Green (G), and Blue (B)). |

## Creating a Z Projection

This procedure assumes that you have opened the Review Multi Dimensional Data dialog box and selected the base file for the data set for which you want to create a Z projection.

To create a 3D Z projection from your multi dimensional data set, use the following procedure:

| Step | Action |
|---|---|
| **1** | In the Wavelengths box, select the wavelengths from which you want to create a Z projection. |

**2** In the Stage Positions box, select the stage position for which you want to create a Z projection.

**3** Click the Z Projection tab.

**Note:** You can either click Z Projection first, and then set the values on the Z Projection tab, or you can set the values, and then click Z Projection to apply them. The Z Projection check box can be checked or unchecked at any time to apply or turn off all the settings simultaneously.

**4** Click the Z Projection box. This enables the Z projection functions.

**5** Click *Create Rotation* to create a rotation stack of 3D reconstruction images. The *Create Rotational Sequence* dialog box opens. (optional)

**6** In the Type box, Click Maximum or Minimum. Use Maximum for darkfield or fluorescent planes (bright objects on a dark background). Use Minimum for brightfield planes (dark objects on a bright background).

**7** In the Angle box, type or select the 3D view angle to be applied to the Z projection image.

**8** Click Interpolate if you want the 3D reconstruction to have a smoother appearance. Interpolate fills the gaps between planes by interpolating the image data between each image plane. This process requires additional memory resources.

**9** In the Planes settings boxes, indicate the range of planes to be included in the 3D image. Click All Planes if all planes are to be included.

**10** In the Orientation area, click Horizontal or Vertical to specify either a horizontal or vertical axis orientation for your reconstructed 3D image view.

**11** In the Border Color box, type or select a value between 0 and 255 if you need to assign a color value to the unfilled areas of the 3D reconstruction.

**12** In the Z Distance area, click Calibrated to set the Z-axis value to the calibrated Z-axis distance. Click User Specified to set the Z-distance to a value that you specify.

**13** In the Z Dist: box, type or select your Z distance value if you selected User Specified for Z Distance.

# Create Rotational Sequence (Apps Menu)

**Builds a rotational sequence of images from a Z projection created by the Review Multi Dimensional Data command.**

*Availability: Available for MetaMorph Basic; included in MetaMorph Premier*

**Drop-in: NDPLAYER**

Use this dialog box to create a rotational sequence of images from a Review Multi Dimensional Data Z Projection. The sequence can contain images for all time points in the data set or only images for user-selected time points. You can specify the start angle (offset in degrees from zero), the final angle (also offset in degrees from zero), and the increment amount for each angle. You can also specify the type of rotation you want to use when displaying the stack: either a single slow rotation from beginning to end, or a back and forth rotation from the Start Angle to the Final Angle.

## Creating a Rotational Sequence

To create a rotational sequence, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Z-Projection tab in the Review Multi Dimensional Data dialog box, click *Create Rotation*, the Create Rotational Sequence dialog box opens. |
| **2** | Type or select the *Start Angle*. This is the angle by which the first reconstructed view will be offset from zero degrees. |
| **3** | Type or select the *Angle Increment*. This angle is the interval between adjacent views in the stack. |
| **4** | Type or select the *Final Angle*. This is the angle by which the last reconstructed view will be offset from zero degrees. |
| **5** | In the *Time Points* box, click *User Selected* to use only the images for the time points that you selected on the Selections [X's] grid, OR, click *All Time Points* to use all time points in the data set. |
| **6** | In the *Rotational Style* box, click *Rotate from Start to Final Angle* to create a slow rotation stack that moves only in the forward direction from the Start Angle to the Final Angle, OR, click *Rock between Start and Final Angle* to create a stack that rotates back and forth between the Start Angle and the Final Angle. |
| **7** | Click *Create* to create and display the rotational sequence, OR click *Cancel* to disregard settings and close the Create Rotational Sequence dialog box. |

## Create Rotational Sequence - Dialog Box Options

**Start Angle**
Sets the angle by which the first reconstructed view will be offset from zero degrees.

**Angle Increment**
Sets the interval between adjacent views in the stack.

**Final Angle**
Sets the angle by which the last reconstructed view will be offset from zero degrees.

**Time Points**
Specifies whether the rotational stack will use only the *User Selected* time points or *All Time Points*:

> ***User Selected*** – Uses only the images for the time points that you selected on the Selections [X's] grid.
>
> ***All Time Points*** – Uses all time points in the data set.

**Rotational Style**
Specifies the method that will be used to rotate the stack:

> *Rotate from Start to Final Angle* – Creates a slow rotation stack that moves only in the forward direction from the Start Angle to the Final Angle.
>
> *Rock between Start and Final Angle* – Creates a stack that rotates back and forth between the Start Angle and the Final Angle.

**Create**
Creates and displays the rotational sequence according to the settings in the dialog box.

**Cancel**
Disregards settings and closes the Create Rotational Sequence dialog box.

# Multi Dimensional Data Set Utilities (Apps Menu)

**Enables you to select data sets for viewing, run a journal in conjunction with selected data sets, perform file system functions on your data set including appending two or more data sets into a single data set, delete data sets, copy data sets, move data sets to different directories, and build Multi Dimensional Data Sets.**

*Availability: Available for MetaMorph Basic; included in MetaMorph Premier*

**Drop-in: NDPLAYER**

Use the Multi Dimensional Data Set Utilities command to initiate multi dimensional data file system functions, which includes the data set appending function, or to access the Build .nd Set command. This dialog box is closely connected with both the Review Multi Dimensional Data dialog box and the Build .nd Set dialog box.

From the Multi Dimensional Data Set Utilities dialog box, you can access either the Review Multi Dimensional Data dialog box or the Build .nd set dialog box, depending on what you want to do. If you are creating a new data set, begin in this dialog box.

If you are performing file system manipulations for a set of data or multiple sets of data, select the source directory of the data, then select the set(s) to be manipulated and run the appropriate function.

If you are preparing to view an existing data set, you can begin in this dialog box or in the Review Multi Dimensional Data dialog box. If you begin here, click Select Directory to select the directory where your data set is located, select your data set in the Data Sets (*.nd) window, then click View to open the Review Multi Dimensional Data dialog box.

If you are building a new data set, click Build Set to open the Build .nd Set dialog box. Complete the steps to build your data set in the Build .nd Set dialog box. Then close the Build .nd Set dialog box to return to the Multi Dimensional Data Set Utilities dialog box. From there you can append your new set to an existing data set, copy or move the set to another directory, or run a journal on the selected data set.

> **Note:** The Multi Dimensional Data Set Utilities dialog box shows the defined data set parameters for selected, existing .nd sets. These include Time Points, Stage Positions, Wavelengths, and Z Steps.

## Multi Dimensional Data Set Utilities - Dialog Box Options

**Select Directory**

Opens the Browse for Folder dialog box to select an existing directory or create a new directory in which to locate new Multi Dimensional (*.nd) data sets.

**Build Set**

Opens the Build .nd Set dialog box. Use this dialog box to define the properties of and create a new .nd data set.

**Data Sets (*.nd)**

Lists the .nd data sets in the directory you selected using Select Directory.

**Description:**

Displays the description associated with the highlighted data set in the Data Sets (*.nd) window.

**Time:**

Indicates the number of time point increments specified in the highlighted data set. This field is only available if applicable for the selected data set.

**Stage Positions:**

Indicates the stage positions specified in the highlighted data set. This field is only available if applicable for the selected data set.

**Wavelengths:**

Indicates the wavelengths specified in the highlighted data set. This field is only available if applicable for the selected data set.

**ZSteps:**

Indicates the number of Z steps specified in the highlighted data set. This field is only available if applicable for the selected data set.

**Config Log**

Selects the parameters of the data set to be logged.

**Open Log/F9: Log Data**

Initiates the process of logging data to a text file, via Dynamic Data Exchange (DDE) to an open spreadsheet, or to both. When you choose Open Log, its title will change to "F9: Log Data." This option can be used with journals.

**Run Journal**

Opens the Run Journal for Multi Dimensional Data dialog box. Use this dialog box to select, define the operating parameters for, and run a predefined journal to provide further processing on the selected multi dimensional data set.

**View**

Opens the Review Multi Dimensional Data dialog box. Use this dialog box to view the images within and the structure of the .nd data set.

**Append Sets**

Opens the Append Multi Dimensional Sets dialog box to combine two or more selected data sets together. You must first double-click to select each data set that you want to append. Data sets should have similar properties in order to be appended. All images are either combined into the first data set in the list that you selected or copied to a new file name. If you chose to combined into the first data set, the data sets from where the images were extracted are deleted. If you chose to copy the selected data sets to a new file, the original data sets are not deleted.

**Delete Set(s)**

Removes the selected dataset file(s) (*.nd) from the directory.

**Copy Set(s)**

Copies the selected data set file(s) to the location specified in the Browse for Folder dialog box.

**Move Set(s)**

Moves the selected dataset file(s) to the location specified in the Browse for Folder dialog box.

**Build Thumbnails**

Creates thumbnail images of all images in the selected data set and saves them to the directory specified in the Select Directory path.

**Close**

Closes the Multi Dimensional Data Set Utilities dialog box.

## Multi Dimensional Data Set Utilities - Procedures

Appending Data Sets

Deleting, Copying, and Moving Data Sets

## Appending Data Sets

To append two or more data sets, use the following procedure.

| Step | Action |
|---|---|
| **1** | From the Apps menu, choose Multi Dimensional Data Set Utilities. The Multi Dimensional Data Set Utilities dialog box opens. |
| **2** | Click Select Directory. The Browse for Folder dialog box opens. |
| **3** | Click the folder that contains the data sets that you want to append. |
| **4** | Click OK. The Browse for Folder dialog box closes and the list of data sets contained in |

the folder appears in the Data Sets (*.nd) box.

**5** In the Data Sets (*.nd) box, double-click the names of the data sets that you want to append. A check mark appears next to each data set *.nd file on the list that you checked.

**Note:** The uppermost data set in the list will be the data set to which the other data sets are appended.

**6** Click Append Sets. The Append Multi Dimensional Sets dialog box opens. Click *Merge files into first file* to combine the selected data sets into the uppermost data set and delete the original data sets.

OR

Click *Copy files to a new name* to merge the selected data sets to a new file and save the original files.

**7** Edit the *Result Base Name* text field, if necessary.

**8** Click OK to append the data sets and close the Append Multi Dimensional Sets dialog box.

## Deleting, Copying, or Moving Data Sets

To delete, copy, or move data sets, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, choose Multi Dimensional Data Set Utilities. The Multi Dimensional Data Set Utilities dialog box opens. |
| **2** | Click Select Directory. The Browse for Folder dialog box opens. |
| **3** | Click the folder that contains the data sets that you want to delete, copy, or move. |
| **4** | Click OK. The Browse for Folder dialog box closes and the list of data sets contained in the folder appears in the Data Sets (*.nd) box. |
| **5** | In the Data Sets (*.nd) box, double-click the names of the data set(s) that you want to delete, copy, or move. A check mark appears next to each data set *.nd file on the list that you checked. |
| **6** | If you are deleting one or more data sets, click Delete Set(s). The data set(s) are deleted.<br>**OR**<br>If you are copying or moving one or more data sets to a different folder, click Copy |

Set(s) or Move Set(s). The Browse for Folder dialog box opens.

**7** Click the folder to which you want to copy or move your selected data sets or click New to create a new folder.

**8** Click OK. The selected data sets are moved to the selected folder.

# Build .nd Set (Apps Menu)

**Enables you to create a multi dimensional data set from a group of sequential images of an experiment or to recreate a .nd data set file for a multi dimensional data set that is missing its .nd file.**

*Availability: Available for MetaMorph Basic; included in MetaMorph Premier*

**Drop-in: NDPLAYER**

Use the Build .nd Set command to create new multi dimensional data sets from sequential experiment images. These can be images captured during a single session or can be combinations of multiple sessions. They can be individual image files, MetaMorph .STK files, or MetaFluor files. When creating a new data set, you need to know the sequence of the image files that you intend to place into the data set. Use this information to determine the settings to make in the Build .nd Set dialog box. You can also use the Build .nd Set command to replace a missing *.nd file. In this instance, the Build .nd Set command determines the setting values.

Before you configure and build your data set, you need to know the file name structure and whether the file name or the file name extension increments in the series. File name incrementing can be accomplished using either numbers or letters. Your experiment can consist of individual image files, such as .TIF or .IMG, or stack (*.STK) files. The file name extension can be used to specify incrementing file names (For example, Tblood.001 through Tblood.999). Also, numerical incrementing can be included in the file name (For example, Image001.tif through Image045.tif. Typically, incrementing will occur in the body of the file name so that the extension indicates the file type.

If a single sequence indicator is used to keep track of more than one dimension, you need to know the numerical counts for each of these dimensional components of your experiment. For example, if image001.tif through image045.tif represents a set of Z-series, each comprised of five planes, you must know that each Z-series contains five images. You also need to know which dimensional attributes are associated with the images that you want to place in the data set. These are the time points, stage positions, Z-positions and wavelength components of the images.

## Build .nd Set - Example

**Consider the following example:**

The sequence of images from this sample experiment contains 20 time points. Within each of those time points are nine stage positions, for a each stage position, there are five Z-positions, and finally, for each Z-position there are two wavelengths.

Therefore, the number of images in the experiment is:

20 (time points) x 9 (stage positions) x 5 (Z-positions) x 2 (wavelengths) = 1800 images.

Use this information to determine the increment values you need to enter into the Build .nd Set dialog box as follows:

- For each time point, there are 9 (stage positions) x 5 (Z-positions) x 2 (wavelengths) = 90 images

Therefore, Time Changes Every 90 (images).

- For each stage position, there are 5 (Z-positions) x 2 (wavelengths) = 10 images

Therefore, Stage Position Changes Every 10 (images).

- For each Z-position, there are 2 wavelengths = 2 images

Therefore, Z Changes Every 2 (images).

- For each wavelength, there is 1 image

Therefore, Wavelength Changes Every 1 (image)

These are the values you would enter for this experiment in the respective Changes Every boxes.

## Build .nd Set - Dialog Box Options

**Image Source**

Chooses the appropriate image source:

***ND Data Set missing .nd file***

Builds a new .nd dataset from files that were formerly an ND dataset. Choose this option if you have an existing ND dataset that does not have a .nd file associated with it. A new .nd file will be created.

***Sequential Images***

Builds an .nd dataset from two or more images that comprise a sequence. Choose this option to import for a file or group of files that you want to include in a new .nd dataset.

***MetaFluor File***

Builds an .nd data set from two or more MetaFluor files. Choose this option to import MetaFluor image files into an .nd dataset for viewing, processing, and analyzing.

***Nikon C1 Confocal image file***

Builds an .nd dataset from one or more Nikon C1 Confocal format files. Choose this option to make a new .nd dataset from Nikon C1 Confocal files.

**Time**

Specifies the total number of images for each time point grouping.

**Stage Position**

Specifies the total number of images for each stage position grouping.

**Z**

Specifies the total number of images for each Z step grouping.

**Wavelength**

Specifies the total number of images for each wavelength grouping.

**Increment By**

Select the identifier to use to determine the incremental order of the images. Name uses the file

name (all characters before the dot) Extension uses the file extension (the three characters after the dot). Plane is used only when copying stack images into the data set.

**Change Every**

Specifies the number of images belonging to the image grouping for a particular dimension.

**Select First File**

Opens the Select Base File dialog box. Select or enter the first file in a series of images from which you want to create an ND data set.

**Destination**

Opens the Browse for folder dialog box. Select or enter the folder name in which you will store the data set.

**Name**

Specifies the name of the data set file that you want to create.

**Preview**

Opens the Build .nd Preview dialog box. This dialog box shows the file names for each of the files assigned to each dimension element. It also shows the arrangement of the images by file name as they will appear in the Multi Dimensional Data View dialog box.

**Create .nd file and Copy Images**

Creates the .nd data set and copies the selected files into it.

**Close**

Closes the Build .nd Set dialog box.

## Building or Replacing a Data Set

To build a new data set or to replace a missing *.nd file, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, Choose Multi Dimensional Set Utilities. The Multi Dimensional Data Set Utilities dialog box opens. |
| **2** | Click Build Set. The Build .nd Set dialog box opens. |
| **3** | Click Sequential images to create a new Multi Dimensional Data Set, Click MetaFluor File to create an .nd data set from a MetaFluor file, or click ND Data Set missing .nd file to replace the *.nd file for an existing data set. If you are replacing an *.nd file for an existing data set, go to step 7. |
| **4** | Click the appropriate check boxes for each type of dimension contained in the data set. |
| **5** | Select the Increment By method for each selected dimension. |
| **6** | Type or select the appropriate increment value for each selected dimension in the corresponding Changes Every box. |

7. Click Select First File. The Select Base File dialog box opens.
8. Click the first file in the image sequence for your experiment, then click Open. The Select Base File dialog box closes.
9. Click Destination. The Browse from Folder dialog opens.
10. Select the folder in which you want to place your data set or click New to create a new folder. The Browse from Folder dialog closes.
11. In the name box, type the name for your new data set.
12. Click Preview to see the names of the files that will be included in the data set and the dimension group to which it is assigned.
13. Click Create .nd file and Copy Images to create your multi dimensional data set.
14. Click Close to close the Build .nd Set dialog box.

# Run Journal for Multi Dimensional Data

**Enables you to associate a specific journal to run at one or more designated instances while viewing your multi dimensional data set or while generating new images based on your viewing criteria.**

*Availability: Available for MetaMorph Basic; included in MetaMorph Premier*

**Drop-in: NDPLAYER**

Use the Run Journal for Multi Dimensional Data command to run a specific, user designated journal at intervals within your data set associated with either time points or Z-positions. This function steps through the data set as specified, enabling you to run a journal for images at each point in the entire data set or for selected portions of the data set.

Your selected journal can be synchronized to run at each time point or time points within a specified range or at each Z-position or at Z-positions within a specified range. In addition, you can specify that the journal run for only a single stage position, or for all stage positions. You can also specify that your journal run for one wavelength, a number of selected wavelengths, or for all wavelengths.

## Run Journal for Multi Dimensional Data - Dialog Box Options

### Select File

Selects a data set to associate with a journal or changes the data set selection.

### Wavelengths

Lists the available wavelengths. Select one or more wavelengths for which you want to run the journal. An image will be opened for each wavelength when the journal is run. The name of each image will be the same as the name of the wavelength in the list.

**Run a journal for each (Time or Z)**

Specifies the image sequence for triggering your selected journal. If time is chosen, the function will move through the range of Z-positions before moving to the next time point. If Z is chosen, the function will move through the range of time points before moving to the next Z-position.

**Load as Stack**

Determines if a journal will be run for each Time or Z position or if a stack will be loaded before running a journal and advancing to the next stack. If Load as Stack is selected while Run a journal for each Time is selected, a Z-stack will be opened, and then the journal will be run for each time point in the range. If Load as Stack is selected while Run a journal for each Z is selected, a time stack will be loaded, and a journal will be run for each Z position.

**Z Range**

Specifies the range of Z-positions within which to allow the selected journal to run. Select All Planes to enable the journal to be run for all Z-positions.

**All Planes**

Enables the journal to be run for all Z-positions.

**Time Points**

Specifies the range time points within which to allow the selected journal to run. Select All to enable the journal to be run for all time points.

**All**

Enables the journal to be run for all time points.

**Stage Pos**

Specifies the stage position for which to enable the journal to run. Select All to run for all positions.

**Note**: A journal can be run for either one stage position or all stage positions only.

**All Pos**

Enables the journal to be run for all stage positions.

**Select Journal**

Opens the Select Journal Dialog box. Select or enter the name of the journal that you want to run.

**View**

Opens the Review Multi Dimensional Data dialog box. Use this dialog box to view the .nd data set for which you want to attach a journal.

**Run**

Runs the journal attached to the dataset.

**Close**

Closes the Run Journal for Multi Dimensional Data dialog box.

## Applying a Journal to a Data Set

To apply a journal to a data set, use the following procedure.

| Step | Action |
|---|---|

**1** From the Apps menu, choose View Multi Dimensional Data. The View Multi Dimensional Data dialog box opens.

**OR**

From the Apps menu, choose Multi Dimensional Data Set Utilities. The Multi Dimensional Data Set Utilities dialog box opens.

**2** In the View Multi Dimensional Data dialog box, click Run Journal Loop. The Run Journal for Multi Dimensional Data dialog box opens.

**OR**

In the Multi Dimensional Data Set Utilities dialog box, click Run Journal. The Run Journal for Multi Dimensional Data opens.

**3** If you have not selected your ND file to which to apply the Journal, click Select File. The Multi Dimensional Data Set Utilities dialog box opens.

**4** If your data set file is not listed in the Data Set (*.nd) box, click Select Directory and select the directory containing your data set; otherwise, in the Data Set (*.nd) box, double-click the name of the data set to which you want to apply the journal.

**5** Click Run Journal, the Run Journal for Multi Dimensional Data dialog box re-opens.

**6** In the Wavelengths box, double-click the wavelength(s) to which to apply the journal.

**7** In the Run a journal for each box, click Time to trigger your selected journal to run at each Z-plane in sequence from the first to the last time point in the range;

**OR**

Click Z to trigger your selected journal run for each time point in the Z-plane range before advancing to the next Z-plane.

**8** Click Load As Stack to set the journal trigger points to only occur for each complete Z-stack if Run journal for each Time is selected or to only occur once for each stack at the end of the time range if Run a journal for each Z is selected.

**9** In the Z Range boxes, set the upper and lower limits of the range, or click All Planes to enable the entire range of Z-planes.

**10** In the Time Points boxes, set the beginning and ending limits of the time point range, or click All to enable the entire range of time points.

**11** In the Stage Pos box, select the stage position to which the journal will be applied,

or click All Pos to apply the journal to all stage positions.

**12** Click Select Journal. The Select Journal dialog box opens.

**13** Click the name of the journal file that you want to run, then click open.

**14** Click View to open the Review Multi Dimensional Data dialog box at any time.

**15** Click Run to run the journal using the settings in the Run Journal for Multi Dimensional Data dialog box.

**16** Click Close to close in the Run Journal for Multi Dimensional Data dialog box.

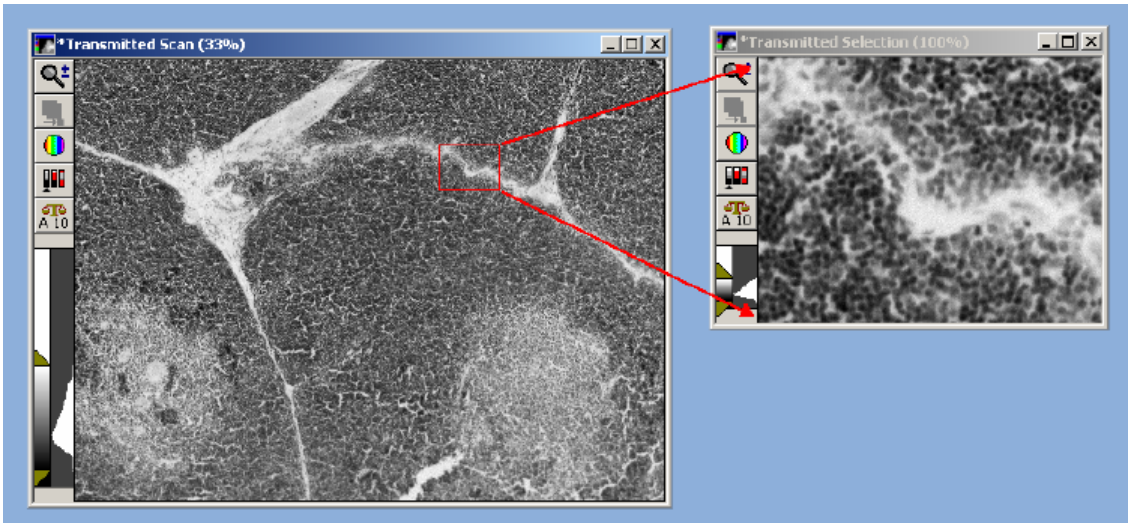
# Scan Slide

**Automatically scans a user-defined section of a slide and displays the result as a single image.**

*Availability: Available for MetaMorph Basic, MetaMorph Premier and MetaXpress.*

### Drop-in: SCANSLIDE

Use the Scan Slide command to acquire high resolution images from a defined section of a slide. A single overview image shows the entire scanned area. You can then use the Scan Slide data review tools to open a full resolution image of any selected region of the scan, as shown below:




The Scan Slide Data Review tab also enables you to automatically move the stage to an area of interest on the scan for further acquisition.

The Scan Slide command has the following features:

- Acquires multiple wavelengths

- Supports laser and image-based autofocusing
- Saves scan images as a single .nd file that can be viewed from within the Scan Slide dialog box or the Review Multi Dimensional Data dialog box
- Supports shading correction
- Scan is not limited by RAM memory since the high resolution images are kept on disk and only loaded to view sections of the data
- Supports advance journaling options:
    - Journals can be run at steps along the acquisition
    - Review journals include the ability to creating a stage list from the regions on the scan image for further acquisition
    - The scan area can be set via program variables
- One time calibration wizard ensures easy setup, acquisition accuracy and helps speed acquisition

**Notes**:

- When configuring the Scan Slide dialog box, you will encounter settings highlighted either in yellow or red. A yellow highlight can mean that an optional field is not filled in or could indicate another minor error. A red highlight means that a required field is either not filled in or contains invalid data that should be changed. These visual reminders help when configuring an experiment.
- This command can be used with either a traditional microscope stage or with the ImageXpress line of cellular imaging products. If you are using the Scan Stage command with an ImageXpress system, you must use the slide holder that came with your system to load the slide.
- The calibration procedure must be completed any time after the camera angle changes. This includes after removing and replacing the camera, or any time after the camera is jarred.

Scan Slide Procedures

Scan Slide Dialog Box Options

## Scan Slide - Procedures

Setting Up a Scan

Selecting Journals

Calibrating the Stage without an Existing Calibration Setting

Calibrating the Stage with an Existing Calibration Setting

Setting the Scan Area

Reviewing the Data

## Scan Slide Procedures - Setting Up a Scan

Complete the following procedure to set up a scan:

**Note**: This procedure assumes that you have a slide loaded on the stage.

| Step | Action |
|---|---|
| **1** | From the Apps menu, click Scan Slide. The Scan Slide dialog box opens. |
| **2** | Select the *Main* tab. |
| **3** | Select the objective to use from the *Scan magnification* drop-down list.<br><br>**Note**: You need to calibrate the stage, using the commands on the *Calibration* tab, for each objective used. |
| **4** | Type a description of your experiment settings into the *Descriptions* field. |
| **5** | Click *Select Directory*. The browse for Folder dialog box opens. Select a directory or create a new directory for storing your .nd image file. The default location is the Images folder in your root MetaMorph directory. |
| **6** | Edit the *Base name* field as needed. |
| **7** | Click the *Acquisition* tab to highlight it. |
| **8** | Select the amount of binning from the *Camera Binning* field. |
| **9** | Select the total number of wavelengths to use during acquisition in the *Number of wavelengths* field. Up to eight wavelengths are supported. |
| **10** | Select *Perform shading correction* to enable shading correction. Then select the type to be performed:<br><br>**Based on magnification setting**<br><br>Select this if the shading correction file is based only on the magnification setting. The file must be named in the following format:<br><br>**shading_<magnification setting>.tif**. For example: **shading_10x.tif**.<br><br>**OR**<br><br>**Based in magnification and illumination settings**<br><br>Select this if the shading correction file is based on both the magnification and illumination setting. The file must be named in the following format:<br><br>**shading_<magnification setting>_<wavelength>_.tif**. For example: **shading_10x_DAPI.tif**.<br><br>For most microscopes shading per magnification setting is sufficient However, on some systems, such as an ImageXpress Micro, shading can vary between wavelengths enough to warrant individual shading images for each illumination setting.<br><br>Correction images for each magnification and/or illumination setting must be stored in the location specified in the *Select Directory* command. |

**11** To enable Laser autofocusing for each stage position in the first wavelength, select *Each stage position* from the *Laser auto focus* drop-down list.

**12** Click the *W1* tab to highlight it.

**13** In the *Illumination* box, select the illumination setting for this wavelength. The illumination settings are defined in the Configure Illumination dialog box.

**14** If the *Intensifier gain* field is enabled, select a new value if needed.

If supported by your camera, this controls the output gain on specific cameras, or controls the gain of image intensifiers on other cameras. Consult your camera documentation for more information.

**15** In the *Exposure* field, type or select an exposure time in milliseconds or, if you have an appropriate sample in view, you can click *Auto Expose* to set this value automatically. You will need to set the target intensity (next step) first.

**16** Enter a value for the Target Intensity in the *Target Intensity* field or use the default value. This value sets the intensity that auto exposure should attempt to attain for the brightest pixel in the image. When *Auto Expose* is selected, the target intensity value default is 75 percent of the maximum gray level that the camera driver reports as possible to obtain.

**17** To perform image-based autofocusing for this wavelength, select *Image auto focus* and configure the values as needed. Refer to the Scan Slide Dialog Box Options - Wavelength Tab help file for more information about these fields.

**18** If you are using a shading correction image for this wavelength, confirm that the image is in the correct location from the *Shading Correction* field.

**19** If you want to assign journals to run at different stages of the Scan Slide operation, click the *Run Journal* tab to highlight it and continue to the next procedure — Selecting Journals.

**OR**

If you are not running journals during the Scan Slide operation, click the *Calibration* tab to highlight it and continue to the next procedure — Calibrating the Stage without an Existing Calibration Setting.

## Scan Slide Procedures - Selecting Journals

To configure the *Run Journal* tab, complete the following procedure:

**Note:** If you do not need to run any journals during the acquisition, skip this procedure.

| Step | Action |
|---|---|
| **1** | Click the checkbox next to the Acquisition step where you want to run a journal. |
| **2** | Click the folder icon next to the selected acquisition step. The Select Journal dialog box opens with the contents of the Journals folder displayed by default. |

| | |
|---|---|
| **3** | Choose the journal that you want to run at the selected acquisition step, and click *Open*. If the journal is not located in the Journals folder, browse to the folder it is in, select it and click *Open*. |
| **4** | Repeat steps 1-3 for as needed to assign journals to run at additional acquisition points. |
| **5** | Click the *Calibration* tab to highlight it and continue to the next procedure — Calibrating the Stage without an Existing Calibration Setting. |

## Scan Slide Procedures - Calibrating the Stage without an Existing Calibration Setting

Complete the following procedure to calibrate the stage if you do not have an existing calibration settings applied for the selected magnification setting, or if the calibration has changed because the camera or its angle was modified:

**Notes**:

- Once this procedure has been completed for a magnification setting, you do not need to run it again unless the camera is moved or it angle changed. If the camera angle changes even slightly, for example when if the camera were to be removed and replaced, or nudged, you must run the procedure again for each magnification setting.
- Make sure the correct Magnification Setting is selected for the system and on the *Acquisition* tab before starting this calibration process.
- Before running the calibration procedure, ensure that the x-axis of the camera is aligned within 15° of the x-axis of the stage. If needed, rotate the camera.
- If you have an existing calibration settings applied for the selected magnification setting, complete the Calibrating the Stage a with an Existing Calibration Setting procedure instead of this one.
- You must complete the procedure Setting Up a Scan before starting this procedure.
- MDC recommends setting that the *Binning* field on the *Acquisition* tab to 1 for the calibration. However, this is not required and results may be acceptable at higher binning, especially if the scan is performed at the same binning as the calibration.

| Step | Action |
|---|---|
| **1** | Ensure that the *Calibration* tab is selected. |
| **2** | Click *Calibrate*. The Scan Slide Calibration dialog box opens. |
| **3** | To use live mode to calibrate the stage, select *Use live mode to position objects for calibration* and click *Next*. A live window opens with a region in the center.<br><br>**Note**: If the live window is not exposed properly, click *Cancel* and adjust your settings in the first three tabs as needed. |
| **4** | Use a joystick, or the Move Stage to Absolute Position command, to move the stage so that an object of interest is |

centered in the region. Click *Next*. The Live window updates with a new region. A Reference Object window also opens.

**5** In the Live window, move the stage left or right so that the object you centered in step 4 is again centered in the region. Click *Next*. The Live window closes and a Scan Slide Snap window opens with a new region.

**6** Use your mouse to move the region so that the object is centered again. Click *Next*. The calibration begins and the Scan Slide Snap window updates.

> **Note**: If an error message opens, ensure that the x-axis of the camera is aligned within 15° of the x-axis of the stage. If needed, rotate the camera and restart this procedure.

**7** Move the region up or down to again center the object. Click *Next*.

**8** The Scan Slide Calibration dialog box closes and the *X*, *Y*, and *Angle* fields of the *Calibration* tab are filled in.

**9** Continue to the next procedure — Setting a Scan Area.

## Scan Slide Procedures - Calibrating the Stage with an Existing Calibration Setting

Complete the following procedure to calibrate the stage and set the scan area if you have an existing calibration settings applied for the selected magnification setting or if the calibration has changed because the camera or its angle was modified:

**Notes**:

- Once this procedure has been completed for a magnification setting, you do not need to run it again unless the camera is moved or it angle changed. If the camera angle changes even slightly, for example when if the camera were to be removed and replaced, or nudged, you must run the procedure again for each magnification setting.
- Make sure the correct Magnification Setting is selected for the system and on the *Acquisition* tab before starting this calibration process.
- Before running the calibration procedure, ensure that the x-axis of the camera is aligned within 15° of the x-axis of the stage. If needed, rotate the camera.
- You must have a calibration setting for the selected objective defined and applied in the Calibrate Distances dialog box to complete this procedure. If you do not have a calibration setting defined, complete the Calibrating the Stage without an Existing Calibration Setting procedure instead of this one.
- Ensure that a clearly defined object is centered in the stage before starting this procedure.
- MDC recommends setting that the *Binning* field on the *Acquisition* tab to 1 for the calibration. However, this is not required and results may be acceptable at higher binning, especially if the scan is performed at the same binning as the calibration.
- You must complete the procedure Setting Up a Scan before starting this procedure.

| Step | Action |
|---|---|
| **1** | Ensure that the *Calibration* tab is selected. |

**2** Click *Calibrate*. The Scan Slide Calibration dialog box opens.

**3** Select *Start with existing magnification calibration and object in center of stage* and click *Next*. A scan slide snap window opens with a region on the image.

> **Note**: If the image is not focused, click *Cancel* and adjust your settings in the first three tabs as needed.

**4** Use your mouse to move the region over the object in the center of the image window. again. Click *Next*. The Live window updates with a new region. A Reference Object window also opens.

**5** Move the region over the same object again. The object should now be near the edge of the image. Click *Next*.

**6** Move the region up or down to again center the object. Click *Next*. The calibration begins and the Scan Slide Snap window updates.

**Note**: If an error message opens, ensure that the x-axis of the camera is aligned within 15° of the x-axis of the stage. If needed, rotate the camera and restart this procedure.

**7** The Scan Slide Calibration dialog box closes and the *X*, *Y*, and *Angle* fields of the *Calibration* tab are filled in.

**8** Continue to the next procedure — Setting the Scan Area.

## Scan Slide Procedures - Setting the Scan Area

Complete the following procedure to set the area of the slide to be scanned:

| Step | Action |
|---|---|
| **1** | Ensure that the *Scan Area* tab is selected. |
| **2** | Click *Live* to open a live window. |
| **3** | Move the stage to the upper left corner of the area that you want to scan, then click *Set to current* in the *Upper left* filed. The current X and Y stage values are entered in the Upper left field. |
| **4** | Next, move the stage to the lower right corner of the area that you want to scan, then click *Set to current* in the *Lower right* filed. The current X and Y stage values are entered in the Lower right field.<br><br>The total number of images to be acquired, as well as the amount of disk space required, is shown beneath the X and Y fields. |
| **5** | Click *Scan* to begin the scan. Images are acquired and combined into a montage image on the desktop. When the scan is complete the *Data Review* tab is selected. |
| **6** | Continue to the next procedure — Reviewing the Data. |

## Scan Slide Procedures - Reviewing the Data

Complete the following procedure to review the data:

**Note**: You must complete the previous procedures before starting this procedure.

| Step | Action |
|---|---|
| **1** | Ensure that the *Data Review* tab is selected. |
| **2** | To toggle the display of individual wavelengths, click the corresponding checkboxes in the *Display wavelengths* list. |
| **3** | To view a high resolution image of a defined area of interest, draw a region on the scan image and click *Show Image*. A high resolution image window opens. If no region is active on the image the high resolution image will be of the entire scan.<br><br>**Note**: If the resulting scan image is too large to be displayed, A warning message opens. Decrease the value in the *Size %* field and click *Show Image*. |
| **4** | To move the stage to a defined area of the high resolution region image, use the *Move stage* controls. These controls can be used to help configure a new scan based on the region. |
| **5** | To save your current settings, click Save Settings, enter a name in the File Name field and click Save. |
| **6** | Click Close to exit the dialog box. |

## Scan Slide - Dialog Box Options

Main

Acquisition

WI..W8 (Wavelengths)

Run Journal

Calibration

Slide Area

Data Review

**Note**: When configuring the Scan Slide dialog box, you will encounter settings highlighted either in yellow or red. A yellow highlight can mean that an optional field is not filled in or could indicate another minor error. A red highlight means that a required field is either not filled in or contains invalid data that should be changed. These visual reminders help when configuring an experiment.

## Scan Slide Dialog Box Options - Main Tab

**Load Settings**

Opens the Load Settings dialog box.

**Scan magnification**

Selects the objective to use during the scan. Objectives are configured in the Configure Magnification dialog box.

> **Note**: You need to calibrate the stage, using the commands on the *Calibration* tab, for each objective used.

**Description**

Enables you to enter and save text that describes the current Scan Slide settings.

**Save Directory**

> Opens the Browse for Folder dialog box, which enables you to specify the location of saved Scan Slide images.

**Base Name**

Assigns a unique name to the Scan Slide image. This field can be edited.

**Increment base name if file exists**

Automatically increments the file name if the file name already exists.

**Live**

Continuously acquires images from the camera at the current stage position.

**Snap**

Captures a single full-frame image from the camera using the current settings.

**Save Settings**

Opens the Save dialog box. This enables you to save the current settings to a file that can be loaded using the *Load Settings* command.

**Scan**

Starts the scan using the current settings.

**Close**

Closes the dialog box.

## Scan Slide Dialog Box Options - Acquisition Tab

**Camera binning**

Sets the binning used by the camera during the scan.

**Number of wavelengths**

Specifies how many wavelengths will be acquired. Eight is the maximum number allowed.

**Shading correction**

Enables shading correction for the acquisition. Correction images for each magnification and/or illumination setting must be stored in the location specified in the *Select Directory* command. The naming convention for the shading correction file must match one of the selections below:

> **Based on magnification setting**
>
> Select this if the shading correction file is based only on the magnification setting. The file must be named in the following format:
>
> **shading_<magnification setting>.tif**. For example —**shading_10x.tif**.

**Based in magnification and illumination settings**

Select this if the shading correction file is based on both the magnification and illumination setting. The file must be named in the following format:

**shading_<magnification setting>_<wavelength>_.tif**. For example — **shading_10x_DAPI.tif**.

**Select directory**

Opens the Browse for Folder dialog box, which enables you to specify the location of shading correction images.

**Acquire Shading Image**

Acquires a shading image using the current settings. For a transmitted light image, ensure the stage is located on an empty area of the slide before acquiring the shading image. For a fluorescent image, ensure that you have an even fluorescent target, such as a fluorescent plastic slide. You will be prompted to save the file to the folder specified by the *Select directory* command. The file will be automatically named in the correct format based on the current magnification setting.

**Laser auto focus**

Enables laser autofocusing for each stage position for the first wavelength. If you select this, ensure that a laser autofocus offset is set in the *W1* tab. Configuration of the laser auto focus is done using the Focus dialog box.

**Note**: The laser auto focus option is only available if the system has a recognized laser auto focus (LAF) component present. Some microscopes have optional LAF support. The following MDC hardware supports LAF:

- ImageXpress 5000a
- ImageXpress MICRO
- Discovery-1

## Scan Slide Dialog Box Options - Wavelength Tab

**Illumination**

Selects an illumination setting to be used with the active wavelength. Illumination settings are defined in the Configure Illumination dialog box.

**Intensifier gain**

If supported by your camera, this controls the output gain on specific cameras, or controls the gain of image intensifiers on other cameras. Consult your camera documentation for more information.

**Exposure**

Specifies the exposure time in milliseconds to be associated with the active wavelength. Type a value in this box or click *Auto Expose* to automatically determine an exposure time.

**Auto Expose**

Automatically determines the exposure time for the currently loaded sample, and applies it as the exposure value.

**Target intensity**

Sets the intensity that auto exposure should attempt to attain for the brightest pixel in the image. The default target intensity value is 75 percent of the maximum gray level that the camera driver reports as possible to obtain.

**Image auto focus**

Enables image-base autofocusing for the wavelength. The following options are available to configure:

**Range**

Specifies how far above and below the current Z position that the Z motor will move while acquiring focus test images. This distance is specified in steps, as defined in the Focus command.

**Accuracy**

Specifies the Z distance to move. Changing this value changes the Number of Z Moves.

**Camera binning**

Sets the binning used by the camera during the Auto Focus command.

**Backlash compensation**

Enables the backlash compensation Z motor movement protocol. This protocol directs the focus motor to move to a Z axis position slightly below the target position and then move against gravity to the target position. This ensures that the Z motor gears are fully engaged, avoiding drift due to slippage. This option has no effect on focus devices with built-in backlash compensation.

**Algorithm**

Enables you to select the algorithm to use when focusing. Valid choices include the following:

- Standard – Algorithm based on a standard group of settings including a normal camera signal level. (Default)
- Directional average – This algorithm gives more accurate focus values with some high magnification objectives.

**Shading Correction**

Displays the current status of shading correction. This text is only visible if shading correction is enabled on the *Acquisition* tab.

## Scan Slide Dialog Box Options - Run Journal Tab

**Acquisition step**

Specifies that a journal should be run for the selected step. Only one journal can be assigned to each step. Click the check box next to the step that you want to use, then click File Open to assign the journal to the step. After you have assigned a journal to a step, you can temporarily deactivate the running of the journal by unchecking the check box for the step.

**File Open** 

Opens the Select Journal dialog box. Use this dialog box to select and assign a journal to a step. To assign a journal, click the checkbox for the acquisition step and click the *File Open* button.

**Journal to run**

Lists the names of the journals assigned to each step.

## Scan Slide Dialog Box Options - Calibration Tab

**Image to stage calibration and orientation**

**Calibrate**

Opens the Scan Slide Calibration wizard. For more information about the Scan Calibration wizard, refer to the Scan Slide procedures.

**X (um/pixel)**

Displays the X image to stage calibration. This value is calculated using the Scan Slide Calibration wizard.

**Y (um/pixel)**

Displays the Y image to stage calibration. This value is calculated using the Scan Slide Calibration wizard.

**Angle**

Displays the angle of the camera in relationship to the stage. This value is calculated using the Scan Slide Calibration wizard.

## Scan Slide Dialog Box Options - Slide Area Tab

**Slide Area**

**Upper left**

**X and Y**

Displays the X and Y stage coordinates for the top left corner of the area to be scanned.

**Set to Current**

Sets the upper left corner of the area to be scanned to the X and Y stage coordinates. Use this command after you have moved the stage to the desired location while in *Live* mode.

**Move to**

Moves the stage to the coordinates displayed in the *Upper Left X* and *Y* fields.

**Lower Right**

**X and Y**

Displays the X and Y stage coordinates for the bottom right corner of the area to be scanned.

**Set to Current**

Sets the lower right corner of the area to be scanned to the X and Y stage coordinates. Use this command after you have moved the stage to the desired location while in Live mode.

**Move to**

Moves the stage to the coordinates displayed in the *Lower Right X* and *Y* fields.

**Number of images in scan**

Displays the total number of images to be acquired during the scan based on the current *Slide Area* settings. The disk space required for the scan is also listed.

**Scan**

Starts the scan based on the current settings.

## Scan Slide Dialog Box Options - Data Review Tab

**Data source**

**Current scan**

Displays the scan that was just acquired.

**File**

Enables the *Open* button.

**Open**

Opens the Load Scan dialog box. Use this to locate and open saved scans (scans are saved in the .nd file format).

**Displayed wavelengths**

Lists the wavelengths acquired in the scan. Use the checkboxes to toggle the display of the image on and off.

**Show selected area at high resolution**

**Show image**

Opens a high resolution version of the scan. To view a section of the scan, use the region tools to create a region on the overview image, then click *Show Image*.

**Size %**

Sets the zoom percentage of the high resolution image.

**Move stage (only for current scan**

**Move to Region Upper Left**

Moves the stage to the upper left corner of the selected region.

**Move to Region Center**

Moves the stage to the center of the selected region.

**Move to Region Lower Right**

Moves the stage to the lower right corner of the selected region.

**Note**: The Move stage commands are only valid if *Current scan* is selected in the *Data Source* field.

# AQI 3D Visualizer

**Enables you to view 3D images of Stack Images in MetaMorph.**

*Availability: Available for MetaMorph Basic and MetaMorph Premier*

**Drop-in: AQI3DVIEW**

Use the AQI 3D Visualizer to display a three-dimensional representation of any 8-, 16-, or 24-bit stack image in MetaMorph. Features available in the 3D Visualizer Window include the following:

- View, create, and save movies of the stack image with multiple rotations.
- Apply different color maps types to the image, including types based on predefined look-up tables, popular fluorescent dyes, and user-defined wavelengths.
- Create and move oblique and orthogonal slices through the image.

## AQI 3D Visualizer - Dialog Box Options

**Source**

Chooses the stack image from the desktop to open in the AQI 3D Visualizer. Use this to select from any stack images on the desktop.

> **Note:** You must have a stack image open on the MetaMorph desktop to view it in the AQI 3D Visualizer.

**Destination**

Specifies or selects the name of the processed image and the file location for the new image file. You can create a new image file or stack, overwrite the existing image file or stack, or add images to an existing image stack.

**Settings**

**Use default**

Opens the stack with no rotation.

**Use last saved**

Opens a stack that was previously processed and saved in the AQI 3D Visualizer with the settings used when the stack was saved.

**Preserve Acquired Aspect Ratio**

Opens the selected stack image in its original aspect ratio. If this is checked, the x, y and z values are all set to 1 (regardless of their true values) and the visualizer does not adjust the stack's aspect ratio. If this is not checked, the visualizer adjusts the height, width, and/or number of planes in the image to maintain the x to y and y to z ratio at 1.

**Apply**

Opens the selected stack in the AQI 3D Visualizer.

**Close**

Closes the dialog box.

## Using the AQI 3D Visualizer

Viewing a Stack Image

Navigating the 3D Visualizer Window

Using Menu Commands

Using the AQI 3D Visualizer Control Panel

## Viewing a Stack Image with the AQI 3D Visualizer

To view an open stack image using the AQI 3D Visualizer, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, Click AQI 3D Visualizer. The AQI 3D Visualizer dialog box opens. |
| **2** | If there is more than one stack image open, click *Source* and select the stack you want to view.<br><br>**Note:** You must have a stack image open on the MetaMorph desktop to view it in the AQI 3D Visualizer. |
| **3** | Select *Preserve Acquired Aspect Ratio* to maintain the image's current aspect ratio when opened in the AQI 3D Visualizer. |
| **4** | Click *Apply*. The 3D Visualizer Window opens with the stack you selected. |

## Navigating the 3D Visualizer Window

The 3D Visualizer window opens with the stack you selected from the AQI 3D Visualizer dialog box, as shown below:

{bmct aqi3d.shg}

**Click the items in the 3D Visualizer window for a brief description.**

Rotating an Image

Zooming an Image

Keyboard/Mouse Shortcuts

## Rotating an Image

To rotate an image in the 3D Visualizer window, use the following procedure:

| Step | Action |
|---|---|
| **1** | Click and drag using the left mouse button from anywhere in the window. The image and X-, Y-, and Z- axes will rotate in the same direction your mouse moves. |
| **2** | To make the image continue to rotate automatically, release the left mouse button before you stop moving the mouse.<br><br>**Note:** The rate the image rotates is in direct proportion the speed you move the mouse. |
| **3** | To stop the image from auto-rotating, click anywhere in the 3D Visualizer window. |

## Zooming an Image

To zoom an image in the 3D Visualizer window, use the following procedure:

| Step | Action |
|---|---|
| 1 | To zoom into an image, click the right mouse button and drag the cursor from the bottom to the top of the 3D Visualizer window. Repeat as needed. |
| 2 | To zoom out of an image, click the right mouse button and drag the cursor from the top to the bottom of the 3D Visualizer window. Repeat as needed. |

## Menu Commands

**The following menu commands can be used within the 3D Visualizer window:**

**View**

**Projection**

**Axis**

**Oblique**

**Movie**

**Color Map**

**Save**

**Options**

## Menu Commands - View

**Volume Projection – Software**

Opens a single 2D projection of the 3D volume as seen from your perspective at the object's selected orientation. This option uses the 3D Visualizer software to process the image.

**Volume Projection – Hardware**

Opens a single 2D projection of the 3D volume as seen from your perspective at the object's selected orientation. This option uses the hardware on your video card to process the image.

**Orthogonal Slices**

Displays three single orthogonal planes, oriented to the object's XY, XZ, and ZY perspectives. Adjust these planes by holding down the [**Ctrl**] key, selecting a plane, and dragging.

**Cube Surface**

Displays a cube with a painted projection of the image on each surface. The view of each projection is the image as it would be seen from directly above that surface. The projection shown is based on the active selection from the Projection menu.

**IsoSurface**

Computes and draws a surface within a volumetric data field, on a 3D surface corresponding to

points with a single scalar value.

**Height Map**

Opens a 3D height map of the image

## Menu Commands - Projection

**The projection commands enable you to view the image with different projection settings.**

> **Note:** Projection commands are only enabled when Volume Projection or Cube Surface is active in the View menu.

**Maximum**

Highlights edges and prominent bright features. The Maximum command takes parallel rays, perpendicular to the viewing surface, and casts them through the image. The maximum voxel value encountered along each ray is taken for the projection pixel value, and the resulting image is made up of each maximum voxel value.

**Sum**

Creates a projection of all the summed values. The Sum command takes all the voxel values along each parallel ray, perpendicular to the viewing surface, and adds their intensity values. Sum projections provide volumetric representations of the data in which more information from the data is considered than with the Maximum projection. Background noise will also be included in this type of projection.

**Minimum**

Produces the opposite effect of the Maximum command. The Minimum command takes parallel rays, perpendicular to the viewing surface, and casts them through the image. The minimum voxel value encountered along each ray is taken for the projection pixel value and the resulting image is made up of each minimum voxel value.

**Voxel Gradient**

Finds the first voxel that is above the intensity threshold for each parallel ray, perpendicular to the viewing surface, then computes the dot product of the gradient at this point. These dot product values make up the pixel intensity values in the projection. The Voxel Gradient command is ideal for examining the surfaces of objects.

**Alpha Blending**

Simulates the effects of transparency, translucency, and opacity in the image.

**Best Focus**

Creates an image out of the regions within a volume that best stands out against their neighbors. This is useful for observing more subtle features in an image, which may be not as pronounced in other projections.

**Surface Slice**

Enables you to view the first slice from each side of your image. For example, with darkfield data that was scanned well above and well below the sample, its surface slice projection will have a dark slice projected for the top view and bottom view of the Surface Slice projection.

## Menu Commands - Axis

**Free**

Produces unconstrained rotations of the object. An unconstrained rotation is produced when the image is rotated about multiple axes simultaneously; your image will rotate in any direction your

mouse moves.

**Object**

Makes the object rotate around one of its three axes. To specify which axis, select Object, then select X axis, Y axis, or Z axis from the Axis menu. The object axes are in relation to the image (object) itself. The object X, Y, and Z axes always run along the width, height, and optical sections of the image, respectively, regardless of how the image is currently oriented.

**Screen**

Constrains available rotations to the screen axis selected. To specify which axis, select Screen, then select X axis, Y axis, or Z axis from the Axis menu. The screen axes are in relation to your display. The screen X- and Y- axes always run along the width and height of the screen, respectively, and the screen Z-axis always runs along your line of sight, regardless of how the data set is currently oriented.

**Best Axis**

Determines the single axis that is closest to the mouse's direction of movement when a rotation is being performed by dragging the mouse across the object. This allows you to rotate around one axis at a time without having to continually specify the explicit axis of rotation.

**X, Y, and Z Axis**

Confines the rotation to the enabled axis. All mouse movements made to rotate the object will only rotate the object along the selected axis.

**Go to View**

Change the image to the XY, XZ, or ZY view.

**Rotate 90**

Rotate the view by 90 degrees around the X, Y, or Z Axis.

## Menu Commands – Oblique

**These features enable you to view and manipulate an oblique plane (slice) through the image.**

**Display Slice**

Toggles the display of an oblique slice through the image.

> **Note:** The *Display Slice* command must be active to enable the rest of the Oblique commands.

**Go to Origin**

Sets the oblique origin to the last selected orthogonal slice intersection point.

**Fix Plane**

Fixes the position and orientation of the oblique plane relative to the screen when the object rotates. This means that while the object rotates, the oblique plane is continually updated with the current view of the data on that fixed cutting plane.

**Flip Plane**

Reverses the oblique plane is set to remove one part of the object and it will retain everything below or behind the slice. Flip plane reverses the part that is removed. The XY, XZ, and ZY buttons allow you to automatically orient the object to the standard perspectives of XY, XZ, or ZY. Additionally, the "Flip" button allows you to view the current orientation from the rear of the sample, effectively rotating the object 180 degrees about the screen's Y-axis.

**XY, XZ, ZY**

Positions the oblique plane through the object's XY, ZY, or XZ plane.

**Parallel**

Positions the Oblique Slice (the cutting plane) so it is parallel to the 3D Visualizer screen.

## Menu Commands - Movie

**Play Movie**

Starts rotating the image from its default position. By default, a movie in the 3D Visualizer window is complete when the image has rotated 360 degrees.

> **Note:** The Movie commands used in the 3D Visualizer window are unrelated to those used with stack images on the MetaMorph desktop.

**Stop Movie**

Stops rotating the image.

**Quick Movie**

Generate a movie quickly by rotating the image on the X or Y-axis using predetermined angles.

> **Rotate Y Axis**
>
> Choose a degree range for the object to rotate on the Y-axis. You can choose from the following ranges: +/- 30, +/-45, +/-60, +/-90, or +/-180 degrees.
>
> **Rotate X Axis**
>
> Choose a degree range for the object to rotate on the X-axis. You can choose from the following ranges: +/- 30, +/-45, +/-60, +/-90, or +/-180 degrees.
>
> **Original View**
>
> Resets the position of the image to its original orientation.

**Set Start Point**

Sets the current position of the image as the start point of the movie. Position the image, select Set Start Point, then select Play Movie.

**Set Mid Point**

Sets the image in the position you want it to be when the movie is 50 percent complete. Position the image, select Set Mid Point, then select Play Movie.

**Mid Point Active**

Indicates that the rotating image will pass through the defined midpoint. Set by default if the Set Mid Point command is used. If Mid Point Active is not set the movie will take the shortest path from the start to the end point.

**Set End Point**

Sets the image in the position you want it to be when the movie ends. Position the image, select Set End Point, then select Play Movie.

**Set Step Angle**

Choose the size of the rotation angle between successive frames of the movie. You can choose from the following angles: 5, 10, or 15 degrees. A smaller step size will cause more movie frames to be generated between points.

**Go to Point**

Go to the Start, Mid, or End point of the movie.

**Loop Mode**

This feature plays the movie from start to end and continues repeating.

**Rock Mode**

Plays the movie from start to end, then end to start, and continues repeating.

**Opposite Path**

Sets the rotational path of the image to the longer of two possible paths. When there are two possible rotational paths between any two points (Start, Mid, or End) the movie generator takes the shortest path by default. When this box is checked, the movie generator will take the opposite or longer rotational path.

**Create Movie**

Creates a stack image of the movie created with the Movie commands. This stack can be saved and processed into a movie using the Make Movie command in MetaMorph.

## Menu Commands - Color Map

**Color Selection**

The Color Map commands enable you to select a color map for the image. The following color maps are available:

- Gray
- Cool
- Jet
- Red
- Blue
- Yellow
- Hot
- Copper
- Spectrum
- Green
- Cyan
- Magenta

**Wavelength**

Selects a single wavelength to use as a color map for your image. The following wavelengths are available:

- 400nm
- 500nm
- 600nm
- 700nm
- 450nm
- 550nm
- 650nm
- 750nm

**Probe**

Selects a color map based on popular fluorescent dyes. Use this feature to view an image as it appeared after staining. The following list displays available dye colors and their associated wavelengths:

- Dapi (456nm)
- Cy2 (506nm)
- Fitc (520nm)
- GFP (540nm)
- Rhodamine (576nm)
- Propidium Iodide (500nm)
- Fluorescein (519nm)
- Lucifer Yellow (528nm)
- Cy3 (570nm)
- DsRed (583nm)

- Cy3.5 (596nm)
- Texas Red (620nm)
- Cy5 (670nm)
- Cy5.5 (694nm)
- Cy7 (767nm)

**Reverse**

Reverses the order of the color map intensity scale of the selected color map. Color map points that normally indicate high intensity areas will indicate low-intensity areas, and vice versa.

## Menu Commands - Save

**Current View**

Creates an image file on the MetaMorph desktop of the current display in the 3D Visualizer window.

**Rotated View**

Creates a new stack image on the MetaMorph desktop of the rotated view that is displayed in the 3D Visualizer window.

**To Clipboard**

Creates an image file of the current display in the 3D Visualizer window and sends it to the Windows clipboard. Use this feature to paste the image into other applications.

## Menu Commands - Options

**Control Panel**

Opens the Control Panel.

**Whole Volume**

Restores the image to its full dimensions after it has been cropped from a subvolume.

**Full Res View**

Generates a full resolution view of the image. This option is only enabled when Volume Projection view is active. If you rotate the image, the rendering returns to the lower resolution version.

**Correct Aspect**

Adjusts the visible dimensions of the image based on pixel spacings. When enabled, the displayed voxels will be cubic in dimension.

**Auto Rotate**

Continually rotates the image once it has been set in motion.

**Display Floor**

Displays the black and white checkered floor pattern. The floor is not visible when the Perspective View is off or the Volume Projection view is on.

**Perspective View**

Displays the view of the image in a depth perspective, so that closer objects appear larger than distant objects.

**Stereo Mode**

Offers the following choices:

**Off**

Default setting.

**Anaglyph**

Shows contrasting colors that appear 3-dimensional when superimposed. Only gray-scale images can be viewed in anaglyph stereo mode.

**LCD Glasses**

This feature is for systems with Open GL enabled Stereo Viewing capability (e.g. StereoGraphics CrystalEyes LCD glasses). This capability splits the image into two alternating components to produce a 3D effect that is visible through special stereo goggles; since this method does not require special coloring, color images as well as grayscale images may be viewed in this mode.

**Note:** This option will only be available if you have the hardware necessary to support it.

**Anaglyph Colors**

Splits the image into two differently colored components to produce a 3-dimensional effect. The following color pairs are available: Red/Cyan, Red/Blue, Red/Green, Cyan/Red, Blue/Red, Green/Red, Left Only, and Right Only. Since the image is specially colored, only grayscale images can be viewed in anaglyph stereo mode.

## Menu Commands - Help

**Keys**

Displays a list of keyboard shortcuts to use in the 3D Visualizer .

## AQI 3D Visualizer Control Panel

Use the Control Panel to configure the AQI 3D Visualizer. The control panel contains settings that affect the display and playback of images in the 3D Visualizer , including all the commands available on each menu.

The following tabs are used in the AQI 3D Visualizer Control Panel:

**Display**

**Subvolume**

**Rotation**

**Oblique**

**Movies**

**Color Maps**

**Settings/Stereo**

**Help**

## Control Panel Dialog Box Options - Display Tab

## Rendering View

### Rendering

Select how your data is displayed. Valid choices include:

**Volume Projection - Software** – Opens a single 2D projection of the 3D volume as seen from your perspective at the object's selected orientation. This option uses the 3D Visualizer software to process the image.

**Volume Projection - Hardware** – Opens a single 2D projection of the 3D volume as seen from your perspective at the object's selected orientation. This option uses the hardware on your video card to process the image.

**Orthogonal Slices** – Opens three single orthogonal planes, oriented to the object's XY, XZ, and ZY perspectives. Adjust these planes by holding down the [Ctrl] key, clicking a plane, and dragging.

**Cube Surface** – Opens a cube, which has on each surface a painted projection of the image as would be seen from directly above that surface or view. The projection shown depends on the projection selected.

### IsoSurface

Computes and draws a surface within a volumetric data field, on a 3D surface corresponding to points with a single scalar value.

### Height Map

Opens a 3D height map of the image

### Projection

Select 2D projection. Valid choices include:

**Maximum Projection** – Highlights edges and prominent bright features. The Maximum command takes parallel rays, perpendicular to the viewing surface, and casts them through the image. The maximum voxel value encountered along each ray is taken for the projection pixel value, and the resulting image is made up of each maximum voxel value.

**Minimum Projection** – Produces the opposite effect of the Maximum command. The Minimum command takes parallel rays, perpendicular to the viewing surface, and casts them through the image. The minimum voxel value encountered along each ray is taken for the projection pixel value and the resulting image is made up of each minimum voxel value.

**Sum Projection** – Creates a projection of all the summed values. The Sum command takes all the voxel values along each parallel ray, perpendicular to the viewing surface, and adds their intensity values. Sum Projections provide volumetric representations of the data in which more information from the data is considered than with the Maximum projection. Background noise will also be included in this type of projection.

**Voxel Gradient Shading** – Finds the first voxel that is above the intensity threshold for each parallel ray, perpendicular to the viewing surface, then computes the dot product of the gradient at this point. These dot product values make up the pixel intensity values in the projection. The Voxel Gradient command is ideal for examining the surfaces of objects.

**Alpha Blending** – Simulates the effects of transparency, translucency and opacity in the image.

**Best Focus** – Creates an image out of the regions within a volume that best stands out against their neighbors. This is useful for observing more subtle features in an image, which may not be as pronounced in other projections.

**Surface Slice** – Enables you to view the first slice from each side of your image. For example, with darkfield data that was scanned well above and well below the sample, its surface slice projection will have a dark slice projected for the top view and bottom view of the Surface Slice projection.

**Full Res –** Generates a full resolution view of the image. This option is only enabled when Volume Projection view is active. If you rotate the data set, the rendering returns to the lower resolution version.

**Save Current**

N/A

**Image Appearance**

**Render Range (0-100%)**

The following settings specify the maximum and minimum image rendering thresholds, as well as the alpha value of the image:

**Maximum –** Sets the maximum rendering thresholds for the image. Intensities above this setting will not be displayed.

**Minimum –** Sets the minimum rendering thresholds for the image. Intensities below this setting will not be displayed.

**Alpha** – Sets the Alpha value to be applied to Alpha projections displayed in the 3D Visualizer .

**Display Range (0-100%)**

The following settings adjust image brightness levels to improve visibility of features of interest:

**Brightness** – Sets a threshold percentage above which all intensities are increased to maximum.

**Darkness** – Sets a threshold percentage below which all intensities are decreased to minimum.

**Gamma –** Sets a gamma correction factor to the image.

**Zoom** – Sets a zoom factor for the image. Higher values enlarge the image and lower values reduce the image.

**Apply**

Applies changes made in the Render and Display fields.

**Default**

Resets the Image Appearance values to their defaults.

**Properties**

**Correct Aspect**

Rescales the image along Z so as to more accurately reflect the actual proportions of the object, as defined through its X, Y, and Z spacings. On by default.

**Plane Outline**

Outline the boundaries of the image around orthogonal planes, and around subvolume boundaries. This aids in judging the orientation of the dotted line, and in judging the relative positions of the planes and the subvolumes. On by default.

**Transparency**

This feature allows you to toggle between whether the background areas of the object

are transparent or not.

**Display Floor**

Displays checkered floor pattern in the 3D Visualizer . On by default. Not available when Volume Projection is set.

**Perspective View**

Displays the view of the image in a depth perspective, so that closer objects appear larger than distant objects. On by default. Not available when Volume Projection is set.

**Auto Rotate**

Continually rotates the image once it has been set in motion. On by default. Not available when Volume Projection is set.

**Stereo View**

Enables 3D stereo-split view. Off by default.

## Control Panel Dialog Box Options – Subvolume Tab

**Subvolume**

Specifies the first and last slices included in the subvolume's region along each of the X-, Y-, and Z- axes.

**Note:** When in surface cube and projection modes, subvolumes can also be selected in the 3D Visualizer window by holding **[Ctrl]** while clicking and dragging any one of the volume surfaces.

**X –** Sets the first and last slices included in the subvolume's region along the X-axis.

**Y –** Sets the first and last slices included in the subvolume's region along the Y-axis.

**Z –** Sets the first and last slices included in the subvolume's region along the Z-axis.

**Apply**

Applies changes made in X, Y, and Z fields.

**Whole Volume**

Restores the full volume to the image.

**Orthogonal Slices**

Toggles the display of the X, Y, and Z planes while in Orthogonal Slices view. Also enables you to select which slice along each of the X-, Y-, and Z- axes is displayed. Valid values range from 1 to the number of slices along the corresponding axis.

**Note:** These settings are only enabled when the Orthogonal Slices view is active.

**X Slice –** Selects which slice along the X-axis is displayed. Use the checkbox to toggle the display of the X plane.

**Y Slice –** Selects which slice along the Y-axis is displayed. Use the checkbox to toggle the display of the Y plane.

**Z Slice** – Selects which slice along the Z-axis is displayed. Use the checkbox to toggle the display of the Z plane.

**Apply**

Applies changes made in X, Y, and Z Slice fields.

The drop-down box include the following image rotational center settings:

**Manual Input**

Sets the image rotational center manually using the X, Y, and Z Slice boxes.

**Whole Volume**

Sets the image rotational center to the geometric center of the data set's full volume.

**Subvolume**

Sets the image rotational center to the geometric center of the currently selected subvolume.

**Center of Mass**

Sets the image rotational center to the data set's center of mass.

**Object Center**

Sets the image rotational center to image's center of mass.

**Note:** When in orthogonal slice mode, the orthogonal planes can also be selected and moved in the 3D Visualizer window holding down the [**Ctrl**] key, clicking a plane, and dragging.

**Object Center**

Specifies the rotation center of the image. Use the X,Y, and Z fields to set the center of rotation for each plane. Valid values range from 1 to the number of slices along the corresponding axis.

**X –** Sets the rotation center of the image along the X-axis.

**Y –** Sets the rotation center of the image along the Y-axis.

**Z –** Sets the rotation center of the image along the Z-axis.

**Apply**

Applies changes made in X, Y, and Z fields.

The drop-down box include the following image rotational center settings:

**Manual Input**

Sets the image rotational center manually using the X, Y, and Z Slice boxes.

**Whole Volume**

Sets the image rotational center to the geometric center of the data set's full volume.

**Subvolume**

Sets the image rotational center to the geometric center of the currently selected subvolume.

**Center of Mass**

Sets the image rotational center to the data set's center of mass.

**Ortho Slices**

Sets the image rotational center to the coordinates specified in the Orthogonal Slices frame.

## Control Panel Dialog Box Options - Rotation Tab

**Rotate**

Determines whether the remaining controls on this tab adjust the positioning of the image or the Oblique Slice of the image. The Oblique Slice option is only enabled when Display Slice is

checked in the Oblique menu.

**Current Rotation**

**Anchor**

Selects the axis about which the object rotates when the slider bar is used to rotate the image. Use the slider bar to spin the object about the selected axis.

**Angle**

Specifies an exact angle of rotation about the X-, Y-, and Z- axes. The order in which these rotations are executed depends on the anchor axis chosen.

**Apply**

Applies changes made in the Angle fields.

**Reset**

Resets the image to its original orientation.

**Round**

Rounds the image rotation angles to the nearest whole degree interval, as specified in the accompanying drop-down menu. The following choices are available in the drop-down menu: 1, 5, 10, 15, 30, 45, or 90.

**Present Views**

**XY**

Automatically orients the image to the XY perspective.

**ZY**

Automatically orients the image to the ZY perspective.

**XZ**

Automatically orients the image to the XZ perspective.

**Flip**

Flips the current orientation – rotates the image 180 degrees about the screen's Y-axis.

**Rotate Object**

The feature allows you to control the incremental rotations about a particular axis.

**Axis**

Selects the rotation axis for the image.

**Angle**

Selects the angle by which to rotate the object about the specified axis.

**Apply**

Rotates the image the selected number of degrees about the selected object axis.

**Constrain Rotation**

Selects whether the image rotates freely when manipulated (i.e., rotate about multiple axes simultaneously), or if the rotation of the object is constrained to one axis at a time. This can be useful to prevent unwanted rotations from occurring due to slightly imprecise mouse movements.

**Around**

Selects the anchor of rotation your image is constrained to. The following options are

available:

**Free** – Enables you to view unconstrained rotations of the image. An unconstrained rotation is produced when the image is rotated about multiple axes simultaneously; your image will rotate in any direction your mouse moves.

**Object** – Makes the object rotate around one of its three axes. To specify which axis, select *Object*, then select *X*, *Y*, or *Z* from the *Axis* field. The object axes are in relation to the image (object) itself. The object X, Y, and Z axes always run along the width, height, and optical sections of the image, respectively, regardless of how the image is currently oriented.

**Screen** – Constrains available rotations to the screen axis selected. To specify which axis, select *Screen*, then select *X*, *Y*, or *Z* from the Axis field. The screen axes are in relation to your display. The screen X and Y axes always run along the width and height of the screen, respectively, and the screen Z axis always runs along your line of sight, regardless of how the image is currently oriented.

**Axis**

Selects which axis you would like the object rotated about. It only is active if Object or Screen is selected for the Around type.

## Control Panel Dialog Box Options – Oblique Tab

### Oblique Slice

**Display Oblique Slice**

Click to display an oblique slice through the image.

**Fix to Screen**

Fixes the position and orientation of the oblique plane relative to the screen when the object rotates. This means that while the object rotates, the oblique plane is continually updated with the current view of the data on that fixed cutting plane.

### Preset Views

**XY**

Positions the oblique plane through the image's XY plane.

**ZY**

Positions the oblique plane through the image's ZY plane.

**Origin**

Repositions the Oblique Slice to pass through the image's origin.

**XZ**

Positions the oblique plane through the image's XZ plane.

**Flip**

Reverses the direction of the cutting plane; the visible portion of the image becomes the cut portion.

**Parallel**

Positions the Oblique Slice (the cutting plane) so it is parallel to the 3D Visualizer screen.

### Oblique Origin

The controls in this group determine the image coordinates that serve as the oblique origin. The oblique plane and parallel slice are calculated with respect to this origin.

**X Slice**
Sets the X coordinate of the oblique origin.

**Y Slice**
Sets the Y coordinate of the oblique origin.

**Z Slice**
Sets the Z coordinate of the oblique origin.

**Calculate origin from**
Selects the method for determining the oblique origin. The following options are available:

**Manual Input**
Set the image's rotational center manually.

**Subvolume**
Set the image's rotational center to be the geometric center of the currently selected subvolume.

**Center of Mass**
Repositions the oblique slice to pass through the origin of the data set.

**Ortho Slices**
Resets the oblique origin to the last selected orthogonal slice intersection point.

**Object Center**
Resets the oblique origin to the image's center of rotation.

**Apply**
Sets the oblique origin to the coordinates set in the X, Y, and Z slice edit boxes.

**Offset from Origin**
Moves the oblique slice from its original location. *Display Oblique Slice* must be active to enable this option.

**Equation (Ax + By + Cz + D > 0)**
Use the edit boxes to select values to use in the planar equation. Used to specify an exact oblique plane by specifying its planar equation.

**Apply**
Sets the oblique plane per the planar equation.

## Control Panel Dialog Box Options – Movie Tab

**Quick Movie**
Use the Quick Movie options to create a simple rotational movie with the following parameters:

**Rotate current view about**
Sets the rotation of the movie around the screen or the object.

**Axis**
Choose the axis the image rotates on using predetermined angles.

**From**
Choose the starting angle of the image for the movie.

**To**

Choose the ending angle of the image for the movie.

**Apply**

Starts the movie.

**Control Movie Points**

Use the Control Movie Points options to create a movie that rotates about more than one angle.

**Start**

Check this box to use the Set and Go to commands for the movie starting point.

**Set**

Sets the current position of the image as the Start, Mid, or End point of the movie. Position the image in the 3D Visualizer and click *Set*.

**Go to**

Go to the set Start, Mid, or End point of the movie.

**Mid**

Check this box to use the Set and Go to commands for the middle of the movie.

**End**

Check this box to use the Set and Go to commands for the end of the movie.

**Opposite path**

Sets the rotational path of the image to the longer of two possible paths. When there are two possible rotational paths between any two points (Start, Mid, or End) the movie generator takes the shortest path by default. When this box is checked, the movie generator will take the opposite or longer rotational path.

**Low res/faster**

N/A

**Play Movie**

**Stop**

Stops the movie.

**Play**

Starts the movie.

**Step Angle**

Choose the size of the rotation angle between successive frames of the movie. Angle values can range from 1 to 45 degrees. A smaller step size will cause more movie frames to be generated between points.

**Rock Mode**

Plays the movie from start to end, then end to start, and continues repeating.

**Frames/sec**

Sets the frame rate of the movie.

**Create Movie**

N/A

## Control Panel Dialog Box Options - Color Maps Tab

### Current Color Maps

#### Display

Selects which channel(s) of a multichannel image is displayed. Use the checkbox next to each channel label to toggle the display of that channel.

#### Select

Indicates that a new color map can be selected for that channel. This feature is enabled when only one channel is active.

#### Color Map

Displays the current color map for the associated channel. The choices are: *Gray, Red, Green, Blue, Cyan, Yellow, Magenta, Orange*, and *Custom*.

#### Oblique

Enabled when *Display Slice* is selected in the Oblique menu. Select *Oblique* to choose a color map for the oblique slice.

#### Display Color Bar

Toggles the color map indicator bar along the right side of the 3D Visualizer display.

#### Monochrome

Toggles grayscale display of multi-channel images. Activating this option enables a color image to be rendered in a stereo view using split-color anaglyphs.

### Select Color Map

Selects a color map for the image when one channel is selected in the Display fields.

#### Color

Select a color map based on a single color or define a custom color. The drop-down list offers the following choices: Gray, Red, Green, Blue, Cyan, Yellow, Magenta, Orange, and Custom.

#### Look Up Table

Select a color map based on multiple colors. The drop-down list offers several maps that vary in color as intensity changes. The preset colors or color spectrums are as follows: *Gray, Copper, Cool, Hot, Jet,* and *Spectrum*.

#### Fluorescent Probe

Select a color map based on colors of popular fluorescent dyes. Use this feature to view a sample in the color in which it appears after staining.

#### Wavelength

Generates a color map based on a wavelength. You can use this feature to view an image in the color of its emissive wavelength.

#### Reverse Color Map

Reverses the intensity scale of the selected color map. The color map points that normally indicate high intensity areas indicate low-intensity areas, and vice versa.

#### Apply

Applies changes made in the Select Color Map fields to the channel or oblique slice selected in the Current Color Maps fields.

## Control Panel Dialog Box Options - Settings/Stereo Tab

### Pixel Spacing

**X**

Specifies the X pixel scale size (in micrometers).

**Y**

Specifies the Y pixel scale size (in micrometers).

**Z**

Specifies the Z pixel scale size (in micrometers).

### Aspect Ratio

Specifies the image's ratio between the Z and X dimensions.

### Apply

Applies the selected settings.

### Auto Calculate Ratio

Automatically calculates and displays the image in the resulting ratio of the Z-spacing to the X-spacing. Enabled by default. Uncheck to edit the *Aspect Ratio* field.

### Correct Aspect Ratio

Adjusts the visible dimensions of the image to take into account the pixel spacings in addition to the number of pixels in the X, Y, and Z dimensions.

### Stereo View

### Mode

Select between the following stereo view modes:

#### Anaglyph

Shows contrasting colors that appear 3-dimensional when superimposed. Only gray-scale images can be viewed in anaglyph stereo mode.

#### LCD Glasses

This feature is for systems with Open GL enabled Stereo Viewing capability (e.g. StereoGraphics CrystalEyes LCD glasses). This capability splits the image into two alternating components to produce a 3D effect that is visible through special stereo goggles; since this method does not require special coloring, color images as well as grayscale images may be viewed in this mode.

**Note:** This option will only be available if you have the hardware necessary to support it.

### Filter

Splits the image into two differently colored components to produce a 3-dimensional effect. The following color pairs are available: *Red/Cyan, Red/Blue, Red/Green, Cyan/Red, Blue/Red, Green/Red, Left Only*, and *Right Only*. Since the image is specially colored, only grayscale images can be viewed in anaglyph stereo mode.

### Angle (+/-)

Sets the angular offset between the two images of the stereo pair. This number can be adjusted to optimize the 3-dimensional effect for a particular user (the optimum value for this is affected by your distance from the monitor and the distance between your eyes).

### Stereo On

Activates the stereo view in the 3D Visualizer window.

**Apply**
Applies the adjustments made in the Stereo View fields.

**Display**

**Perspective Angle**
Sets the angular size of the 3D Visualizer 's field of view. Reducing the angular size decreases the size of the field of view of the 3D Visualizer . It also reduces the 3D Visualizer 's visible area around the image and the perspective effect on what is visible. Conversely, increasing the angle increases the field size and the perspective effect.

**Apply**
Applies changes made in the *Perspective Angle* field.

## Control Panel Dialog Box Options - Help

This tab contains Keyboard/Mouse Shortcuts used to manipulate images in the 3D Visualizer :

To rotate the object:

*Left click on the image and drag in the desired direction.*

To crop the volume or move an Orthogonal/Oblique Slice:

*Hold down the Ctrl key, left click on the plane/slice and drag.*

To rotate the Oblique Slice:

*Hold down the Shift key, left click on the Oblique Slice and drag.*

To zoom the displayed image:

*Right click on the image and drag from bottom right to top left (or vice-versa).*

The X-, Y-, and Z- axes of the stack are represented by
the red, green, and blue lines, respectively.
This is the orthogonal slices
view of a stack. To change views, click the View menu
and select from the list.

The Color bar displays the current color map for the image.

The floor is used as a 3-D point of reference.
To remove the floor, click Options and uncheck Display Floor.

# FRET Analysis (Apps Menu)

**Performs background and bleed through correction to Fluorescence Resonance Energy Transfer (FRET) image sets.**

*Availability: Included in MetaMorph Basic and MetaMorph Premier*

**Drop-in: FRET**

The FRET drop-in enables correction of FRET image sets. The FRET technique involves observing the energy transfer between an excited donor fluorophore and a nearby acceptor fluorophore. This energy transfer is dependent on the overlap of excitation spectrum of the acceptor with the emission spectrum of the donor, as well as the distance between the fluorophores. Energy transfers can only occur when a donor and acceptor are very close together (within nanometers) and their spectra sufficiently overlap. Due to the overlap in spectra of the donor and acceptor, bleed through between observed wavelength channels (filter sets) influence FRET observations.

Use this command to correct FRET image sets. The FRET command will correct for background and

bleed through between fluorophore filter sets using either of the following methods:

- Fully Sensitized Emission — use this method, with or without background subtraction, if you measured the bleed through of the acceptor signal and donor signal through the FRET filter set when you acquired the FRET images.
- Specified Bleed Through — use this method, with or without background subtraction, if you calibrated your system for fully specified bleed through correction; that is, you measured all possible contaminators into the FRET channel.

The following is an overview of the steps used to create a corrected FRET image in MetaMorph:

1. Acquire images of the donor, FRET, and acceptor using an appropriate filter set for each image. This data can be three separate images, or combined into a stack.

   **Note:** You can use the Multi Dimensional Acquisition or Acquire Multiple Wavelengths commands to efficiently acquire images using multiple filters.
2. Designate the source stack or images in the FRET Correction dialog box.
3. Determine the FRET correction method to use — Fully Sensitized Emission or Specified Bleed Through.
4. Enter the correction coefficients and select the background subtraction method to be used when creating the corrected FRET image. You can create a background region, specify a constant value, or select a separate background image for the donor, acceptor, and raw FRET images.
5. Create the corrected image(s).

**Determining FRET Coefficients**

## Determining FRET Coefficients

Both the Sensitized Emission and Fully Specified Bleed through methods for correcting FRET require calibration procedures. These calibrations provide the coefficients for correcting the acquired FRET images. Perform these calibrations for each wavelength filter set used for FRET experiments. After the coefficients values are determined for each filter set, they can be used in subsequent FRET experiments.

Calibrations for both methods require controls which contain only donor fluorophore (a Donor-only control) and only acceptor fluorophore (an Acceptor-only control). These samples are used to determine bleed through between filter configurations.

**Determining FRET Coefficients for Sensitized Emission**

**Determining FRET Coefficients for Fully Specified Bleed Through Correction**

## Determining FRET Coefficients - Sensitized Emission

To use the Sensitized Emission method to correct FRET image sets, you must determine values for coefficients A and B.

Coefficient A represents the degree to which the Acceptor signal is contaminating observations made in the FRET channel. This value is determined by dividing the average thresholded intensity of the image obtained using the FRET filter configuration by the average thresholded intensity of the image obtained using the Acceptor filter configuration:

$$\text{Coefficient A} = \frac{\text{Average thresholded intensity of Acceptor image from FRET filter set}}{\text{Average thresholded intensity of Acceptor image from Acceptor filter set}}$$

Coefficient B represents the degree to which the Donor signal is contaminating observations made in the FRET channel. This value is determined by dividing the average thresholded intensity of the image obtained using the FRET filter configuration by the average thresholded intensity of the image obtained using the Donor filter configuration:

$$\text{Coefficient B} = \frac{\text{Average thresholded intensity of Donor image from FRET filter set}}{\text{Average thresholded intensity of Donor image from Donor filter set}}$$

To determine the coefficient A for Sensitized Emission, complete the following procedure:

**Note:** This procedure assumes you have collected sample donor, FRET, and acceptor images using the appropriate filter set for each image.

| Step | Action |
|---|---|
| 1 | Use the Threshold Tool and Slider to threshold the Acceptor images acquired from both the FRET and Acceptor filter sets.<br><br>**Note:** You must threshold the images to include as much signal as possible in the threshold. The only areas of the images that should not be thresholded are areas that are clearly background. Failure to threshold all the signal will result in an incorrect coefficient. |
| 2 | Use the Show Region Statistics command to determine the average thresholded intensity of both Acceptor images. |
| 3 | Divide the average thresholded intensity of the image obtained using the FRET filter set by the average thresholded intensity of the image obtained using the Acceptor filter set. The result of this division is coefficient A. |

To determine the coefficient B for Sensitized Emission, complete the following procedure:

| Step | Action |
|---|---|
| 1 | Use the Threshold Tool and Slider to threshold the Donor images acquired from both the FRET and Donor filter sets.<br><br>**Note:** You must threshold the images to include as much signal as possible in the threshold. The only areas of the images that should not be thresholded are areas that are clearly background. Failure to |

threshold all the signal will result in an incorrect coefficient.

2 Use the Show Region Statistics command to determine the average thresholded intensity of both Donor images.

3 Divide the average thresholded intensity of the image obtained using the FRET filter set by the average thresholded intensity of the image obtained using the Donor filter set. The result of this division is coefficient B.

## Determining FRET Coefficients - Fully Specified Bleed Though

To use the Fully Specified Bleed Through method to correct FRET image sets, you must determine values for some or all of the following coefficients:

**Donor in Acceptor**

**Acceptor in Donor (if applicable)**

**Donor in FRET**

**Acceptor in FRET (if applicable)**

## Determining FRET Coefficients - Donor in Acceptor

The Donor in Acceptor coefficient represents the degree to which signal from the Donor filter set is bleeding into observations made using the Acceptor filter set. This value is determined by dividing the average thresholded intensity of the Acceptor-only image obtained using the Donor filter set by the average thresholded intensity of the Acceptor-only image obtained using the Acceptor filter set:

$$\text{Donor in Acceptor} = \frac{\text{Average thresholded intensity of Acceptor image from Donor filter set}}{\text{Average thresholded intensity of Acceptor image from Acceptor filter set}}$$

To determine the Donor in Acceptor coefficient for Specified Bleed Through, complete the following procedure:

**Note:** This procedure assumes you have collected sample donor, FRET, and acceptor images using the appropriate filter set for each image.

| Step | Action |
|---|---|
| 1 | Use the Threshold Tool and Slider to threshold the Acceptor images acquired from both the Donor and Acceptor filter sets. |

**Note:** You must threshold the images to include as much signal as possible in the threshold. The only areas of the images that should not be thresholded are areas that are clearly background. Failure to threshold all the signal will result in an incorrect coefficient.

2 Use the Show Region Statistics command to

determine the average thresholded intensity of both Acceptor images.

**3** Divide the average thresholded intensity of the image obtained using the Donor filter set by the average thresholded intensity of the image obtained using the Acceptor filter set. The result of this division is the Donor in Acceptor coefficient.

## Determining FRET Coefficients - Acceptor in Donor

The Acceptor in Donor coefficient represents the degree to which signal from the Acceptor filter set is bleeding into observations made using the Donor filter set. This value is determined by dividing the average thresholded intensity of the Donor-only image obtained using the Acceptor filter set by the average thresholded intensity of the Donor -only image obtained using the Donor filter set:

$$\text{Acceptor in Donor} = \frac{\text{Average thresholded intensity of Donor image from Acceptor filter set}}{\text{Average thresholded intensity of Donor image from Donor filter set}}$$

To determine the Acceptor in Donor coefficient for Specified Bleed Through, complete the following procedure:

**Note:** This procedure assumes you have collected sample donor, FRET, and acceptor images using the appropriate filter set for each image.

| Step | Action |
|---|---|
| **1** | Use the Threshold Tool and Slider to threshold the Donor images acquired from both the Acceptor and Donor filter sets.<br><br>**Note:** You must threshold the images to include as much signal as possible in the threshold. The only areas of the images that should not be thresholded are areas that are clearly background. Failure to threshold all the signal will result in an incorrect coefficient. |
| **2** | Use the Show Region Statistics command to determine the average thresholded intensity of both Donor images. |
| **3** | Divide the average thresholded intensity of the image obtained using the Acceptor filter set by the average thresholded intensity of the image obtained using the Donor filter set. The result of this division is the Acceptor in Donor coefficient. |

## Determining FRET Coefficients - Donor in FRET

The Donor in FRET coefficient represents the degree to which signal from the Donor filter set is bleeding into observations made using the FRET filter set. This value is determined by dividing the average thresholded intensity of the Donor-only image obtained using the FRET filter set by the average

thresholded intensity of the Donor -only image obtained using the Donor filter set:

$$\text{Donor in FRET} = \frac{\text{Average thresholded intensity of Donor image from FRET filter set}}{\text{Average thresholded intensity of Donor image from Donor filter set}}$$

To determine the Donor in FRET coefficient for Specified Bleed Through, complete the following procedure:

**Note:** This procedure assumes you have collected sample donor, FRET, and acceptor images using the appropriate filter set for each image.

| Step | Action |
|---|---|
| 1 | Use the Threshold Tool and Slider to threshold the Donor images acquired from both the FRET and Donor filter sets.<br><br>**Note:** You must threshold the images to include as much signal as possible in the threshold. The only areas of the images that should not be thresholded are areas that are clearly background. Failure to threshold all the signal will result in an incorrect coefficient. |
| 2 | Use the Show Region Statistics command to determine the average thresholded intensity of both Donor images. |
| 3 | Divide the average thresholded intensity of the image obtained using the FRET filter set by the average thresholded intensity of the image obtained using the Donor filter set. The result of this division is the Donor in FRET coefficient. |

## Determining FRET Coefficients - Acceptor in FRET

The Acceptor in FRET coefficient represents the degree to which signal from the Acceptor filter set is bleeding into observations made using the FRET filter set. This value is determined by dividing the average thresholded intensity of the Acceptor-only image obtained using the FRET filter set by the average thresholded intensity of the Acceptor -only image obtained using the Acceptor filter set:

$$\text{Acceptor in FRET} = \frac{\text{Average thresholded intensity of Acceptor image from FRET filter set}}{\text{Average thresholded intensity of Acceptor image from Acceptor filter set}}$$

To determine the Acceptor in FRET coefficient for Specified Bleed Through, complete the following procedure:

**Note:** This procedure assumes you have collected sample donor, FRET, and acceptor images using the

appropriate filter set for each image.

| Step | Action |
|---|---|
| 1 | Use the Threshold Tool and Slider to threshold the Acceptor images acquired from both the FRET and Acceptor filter sets.<br><br>**Note:** You must threshold the images to include as much signal as possible in the threshold. The only areas of the images that should not be thresholded are areas that are clearly background. Failure to threshold all the signal will result in an incorrect coefficient. |
| 2 | Use the Show Region Statistics command to determine the average thresholded intensity of both Acceptor images. |
| 3 | Divide the average thresholded intensity of the image obtained using the FRET filter set by the average thresholded intensity of the image obtained using the Acceptor filter set. The result of this division is the Acceptor in FRET coefficient. |

## Using FRET to Correct Images - Sensitized Emission

To correct a FRET image using the Sensitized Emission method, complete the following procedure:

| Step | Action |
|---|---|
| 1 | From the Apps menu, click FRET. The FRET dialog box opens to the *Setup* tab. |
| 2 | Select *Sensitized Emission* from the FRET Method field. |
| 3 | If you are using separate images for the donor, acceptor, and raw FRET images select *Component* from the *Source* field.<br><br>OR<br><br>If you are using a stack with planes that contain the donor, acceptor, and raw FRET images, select *Stack* from the *Source* field. |
| 4 | If you selected *Component* in Step 3, Select the desired donor, acceptor, and raw FRET images using the *Donor, Acceptor, and Raw FRET* image selectors and skip to Step 6.<br><br>OR<br><br>If you selected *Stack* in Step 3, select the stack using the Source Stack image selector. |
| 5 | If you selected *Stack* in Step 3, select the plane offset to use for the Donor, Acceptor, and Raw FRET frames. |

**Note:** For more information on determining the plane offset, see FRET Dialog Box Options - Setup Tab.

**6** Click the Image Correction tab.

**7** From the *Background Subtraction* group, select the background subtraction you want to apply from the following: *None*, *Constants*, *Regions*, or *Images*.

**8** If you selected *Constants* in Step 7, Select the level of background subtraction applied to the Donor, Acceptor, and Raw FRET images using the spin-boxes for each value.

OR

If you selected *Regions* in Step 7, move or resize the region if needed. This will move or resize the region for each plane in all source stacks.

OR

If you selected Images in step 7, use the Donor, Acceptor, and Raw FRET image selectors to select an image to be used for background subtraction.

**9** From the *Sensitized Emission Constants* group, enter values for *Constant A* and *Constant B*.

**10** Click *Apply* to create a correct FRET image using the settings you entered.

**11** Click *Close* to exit the FRET dialog box.

## Using FRET to Correct Images - Specified Bleed Through

To correct a FRET image using the Specified Bleed Through method, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, select FRET. The FRET dialog box opens to the *Setup* tab. |
| **2** | Select *Specified Bleed Through* from the FRET Method field. |
| **3** | If you have a donor image but no acceptor image, select *Donor Only* from the FRET Method field.<br><br>OR<br><br>If you have both a donor and an acceptor image, select *Donor + Acceptor* from the FRET Method field. |
| **4** | If you are using separate images for the |

donor, acceptor (if applicable), and raw FRET images select *Component* from the *Source* field.

OR

If you are using a stack with planes that contain the donor, acceptor (if applicable), and raw FRET images, select *Stack* from the *Source* field.

**5** If you selected *Component* in Step 4, Select the desired donor, acceptor (if applicable), and raw FRET images using the *Donor, Acceptor, and Raw FRET* image selectors and skip to Step 7.

OR

If you selected *Stack* in Step 4, select the stack using the Source Stack image selector.

**6** If you selected *Stack* in Step 4, select the plane offset to use for the Donor, Acceptor, and Raw FRET frames.

**Note:** For more information on determining the plane offset, see FRET Dialog Box Options - Setup Tab.

**7** Click the Image Correction tab.

**8** From the *Background Subtraction* group, select the background subtraction you want to apply from the following: *None*, *Constants*, *Regions*, or *Images*.

**9** If you selected *Constants* in Step 8, Select the level of background subtraction applied to the Donor, Acceptor (if applicable), and Raw FRET images using the spin-boxes for each value.

OR

If you selected *Regions* in Step 8, move or resize the region if needed. This will move or resize the region for each plane in all source stacks.

OR

If you selected Images in step 8, use the Donor, Acceptor (if applicable), and Raw FRET image selectors to select an image to be used for background subtraction.

**10** From the *Spectral Bleed Though Coefficients* group, check *Bleed Through Correction* to perform additional correction on the image.

**11** From the *Spectral Bleed Though Coefficients* group, enter coefficient values for the following fields:

- *Donor in Acceptor*

- *Acceptor in Donor* (if applicable)
- *Donor in FRET*
- *Acceptor in FRET* (if applicable)

**12** Click *Apply* to create a correct FRET image using the settings you entered.

**13** Click Close to exit the FRET dialog box.

## FRET - Dialog Box Options

**Setup Tab**

**Image Correction Tab**

**Apply**

Creates the corrected FRET image using the settings you entered.

**Close**

Closes the FRET dialog box.

## FRET Dialog Box Options - Setup Tab

**Source**

**Component**

Enables you to use separate images for the Donor, Acceptor, and Raw FRET selections.

**Stack**

Enables you to select a source stack for the FRET analysis. If the source image is a stack, two image selectors are enabled – one for the Source Stack and the other for the FRET destination. The remaining image selectors are disabled.

If the source image is a stack, there will be edit boxes displayed for the user to specify the plane numbers which form the donor, acceptor and raw FRET images.

**FRET Method**

**Sensitized Emission**

Uses a simplified formation of the fully specified bleed through correction method. It corrects for bleed through from the donor and acceptor into the FRET channel.

**Specified Bleed Through**

Use this method if you calibrated your system for fully specified bleed through correction; that is, you measured all possible contaminators into the FRET channel.

**Donor Only**

Enables you to select the Donor and Raw FRET source images or stacks. This option is only available if *Specified Bleed Through* is selected in the *Source* field.

**Donor + Acceptor**

Enables you to select the Donor, Acceptor, and Raw FRET source images or stacks. This option is only available if *Specified Bleed Through* is selected in the

*Source* field.

**Source Stack**

Selects the source stack for the FRET analysis. This option is only available if Stack is selected in the Source field.

**Donor**

Selects the Donor image. This option is only enabled when *Component* is selected as the Source.

**Acceptor**

Selects the Acceptor image. This option is only enabled when *Component* is selected as the Source.

**Raw FRET**

Selects the raw FRET image. This option is only enabled when *Component* is selected as the Source.

**FRET Dest**

Selects the name of the corrected FRET image.

**Plane Offset**

Determines which frames of the stack are used as the Donator, Acceptor, and Raw FRET images. If you have only three planes in the stack, the correct Plane offset for the donor would be 1, the plane offset for the acceptor would be 2, and the plane offset for the raw FRET would be 3.

However, if your stack contains more than three planes, you must determine the correct offset for the planes. For example, if you have a stack of 12 planes and the first four planes are donor images, the second four planes acceptor images, and the last four planes FRET images, then your plane offsets would be:

Donor: 1

Acceptor: 5

Raw FRET: 9

## FRET Dialog Box Options - Image Correction Tab

**Background Subtraction**

**None**

No background subtraction performed on the image.

**Constants**

Enables you to select the level of background subtraction applied to the Donor, Acceptor, and Raw FRET images.

**Regions**

Creates a region on each of the source images. The region is used for background subtraction. If your image is a stack, moving or resizing the region will move or resize for each plane in the stack.

**Images**

Enables you to select images to use for background selections. Use this option when you have selected Components as your Source images.

**Sensitized Emission**

**Note:** These options are only available if *Sensitized Emission* is selected in the *FRET Method* section of the Setup tab.

**Constant A**

Enable you to select the value of Constant A.

**Constant B**

Enable you to select the value of Constant B.

**Spectral Bleed Through Coefficients**

**Note:** These options are only available if *Specified Bleed Through* is selected in the *FRET Method* section of the Setup tab.

**Bleed through correction**

Check this box to apply bleed through correction.

**Donor in Acceptor**

Selects the bleed through coefficient of the donor wavelength through the acceptor filter set.

**Acceptor in Donor**

Selects the bleed through coefficient of the acceptor wavelength through the donor filter set. This option is only enabled if *Donor + Acceptor* is selected in the *FRET Method* section of the Setup tab.

**Donor in FRET**

Selects the bleed through coefficient of the donor signal in the raw FRET image.

**Acceptor in FRET**

Selects the bleed through coefficient of the acceptor signal in the raw FRET image. This option is only enabled if *Donor + Acceptor* is selected in the *FRET Method* section of the Setup tab.

Sensitized Emission

FRET = RawFRET - (A * Acceptor) - (B * Donor)

Where A and B = Coefficient A and Coefficient B

Sensitized Emission if background subtraction is used

$$\frac{\begin{array}{l}(\text{RawFRET} - \text{Correction Factor}) \\ - \ A * (\text{Acceptor} - \text{Correction Factor}) \\ - \ B * (\text{Donor} - \text{Correction Factor}\end{array}}{= \quad \text{FRET}}$$

Specified Bleed Through
with Donor and Acceptor

$$\frac{\begin{array}{l}\text{RawFRET} \\ - \ [\text{Acceptor} - (\text{Donor in Acceptor} * \text{Donor})] * [\text{Acceptor in FRET}] \\ - \ [\text{Donor} - (\text{Acceptor in Donor} * \text{Acceptor})] * [\text{Donor in FRET}]\end{array}}{= \quad \text{FRET}}$$

Specified Bleed Through

if background subtraction is used

$$\frac{\begin{array}{l}[\text{RawFRET} - \text{Correction Factor}] \\ -[(\text{Acceptor} - \text{Correction Factor}) - (\text{Donor in Acceptor} * (\text{Donor} - \text{Correction Factor}))] * [\text{Acceptor in FRET}] \\ -[(\text{Donor} - \text{Correction Factor}) - (\text{Acceptor in Donor} * (\text{Acceptor} - \text{Correction Factor}))] * [\text{Donor in FRET}]\end{array}}{= \quad \text{FRET}}$$

# Plate Acquisition Setup

**Guides the setup of acquiring images from a multi-well plate using MetaXpress software. After setup is complete, the settings file can be saved and you can begin acquisition.**

### Drop-in: PLATEACQUIRE

*Availability: Exclusive to MetaXpress*

Use this dialog box to step through the process of setting up the system for screening. These settings are then saved and used to acquire images using the Plate Acquisition command. The *Previous* and *Next* buttons in the Plate Acquisition Setup dialog box guide you step-by-step through the process of configuring the system for plate acquisition. You can also use the vertical tabs on the left side of the dialog box for quick access to each tab and edit settings files created earlier.

**Note:** MDC recommends that new users step through the interface in order and use the online help (available by clicking the question mark icon on the bottom of each tab).

There are several possible workflows available for your acquisition. One typical workflow for multi-well plate acquisition is as follows:

- Configure and save your settings file using the Plate Acquisition Setup dialog box.
- Use the Plate Acquisition and Control dialog box or toolbar to do the following:
    - Load your settings file and review the settings using the Summary button.
    - Confirm your settings if needed using the available tools.
    - Enter an experiment base name.
    - Start the acquisition. During acquisition, the acquired images are saved into the database.
- Perform any post-acquisition analysis using the Review Plate Data or Plate Data Utilities dialog boxes. This can be configured to start automatically from the Plate Acquisition Setup dialog box if desired.

**Plate Acquisition Setup Step-by-step Tutorials**

The links below provide several Plate Acquisition Setup tutorials, including a step-by-step tutorial for each tab in the Plate Acquisition Setup dialog box. You can access the tutorial for each tab by using the Previous and Next links at the bottom of each Help page or by clicking the question mark icon  on the bottom of each tab.

**Tutorial Links**

Complete step-by-step tutorial for creating new settings

Creating a new plate layout

Using a Saved Setting on a New Plate

Plate Acquisition Setup Keyboard Shortcuts

**PDF Application Notes**

Application Note — Defining Plate Types in MetaXpress: ImageXpress$^{MICRO}$ hardware platform

Application Note — Defining Plate Types in MetaXpress: ImageXpress 5000A hardware platform

Application Note — Defining Plate Types in MetaXpress: Discovery-1 hardware platform

**Other Useful Links**

Plate Acquisition Setup - dialog box options

Plate Acquisition and Control main help file

Plate Acquisition main help file

Plate Acquisition Toolbar help file

**Notes:**

- You can use the Plate Control and Acquisition dialog box or the Plate Acquisition Toolbar to view a test image at any time while using the Plate Acquisition Setup dialog box. This is useful to immediately check the results of any changes you made to image acquisition settings.
- When configuring the Plate Acquisition Setup dialog box, you will encounter settings highlighted either in yellow or red. A yellow highlight can mean that an optional field is not filled in or could indicate another minor error. A red highlight means that a required field is either not filled in or contains invalid data that should be changed. These visual reminders help when configuring an experiment.

## Plate Acquisition Setup - Keyboard Shortcuts

**<Alt+l> = Load Settings**

**<Alt+s> = Save Settings**

**<Alt+u> = Summary**

**<Alt+p> = Previous**

**<Alt+c> = Close**

**<Alt+n> = Next**

## Plate Acquisition Setup Tutorial Links

The following links go to the tutorial page for each tab of the Plate Acquisition Setup dialog box. You can navigate between the pages using the Previous and Next links at the bottom of each page or by clicking the question mark icon  on the bottom of each tab in the Plate Acquisition Setup dialog box:

Experiment

Names and Descriptions

Objective Camera

Plate

A1 Center

Wells to Visit

Sites to Visit

Time Lapse

Fluidics

Acquisition Loop

Autofocus

W1. . . W8

Journals

## Plate Acquisition Setup Tutorial - Experiment Tab

**Plate Acquisition Setup Home  Next**

When you open the Plate Acquisition Setup dialog box, it opens to the Experiment tab. The Experiment tab enables you to create a new setting or load a previous setting for revision. If you are creating new settings, click *Next* to move to the next tab. To load an existing settings file, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Screening menu, select Plate Acquisition Setup. The Plate Acquisition Setup dialog box opens with the Experiment tab displayed. |
| **2** | Select *Load existing setting*. The Load Settings button appears. |
| **3** | Click *Load Settings*. The Load Plate Acquisition Settings dialog box opens. |
| **4** | If you are loading a settings file that was saved to the database, select the settings file to load from the Settings File drop-down list. Then select the individual settings to load from the file using the check boxes next to each settings group.<br><br>**OR**<br><br>If you are loading a settings file that was saved outside the database, select *From File* in the drop-down list and then select the individual settings to load from the file using the check boxes next to each settings group.<br><br>**Note:** The settings listed here are configured on various tabs of the Plate Acquisition Setup dialog box. |
| **5** | If you are using a settings file from the database, click *Load*. The file will load from the database and the Load Plate Acquisition State dialog box will close.<br><br>**OR**<br><br>If you are loading a settings file saved outside the database, click *Load*. The Load Screen state dialog box opens. Navigate to the state file (.HTS) you want to open and click *Open*. The state file you selected will load and the Load Screen Acquisition dialog |

box will close.

**6** Click *Load*. The selected settings will load and the Load Screen Acquisition dialog box will close.

**7** Click *Summary* to view the *Summary* tab and confirm you settings.

**OR**

Click *Next* to move to the next tab.

**Plate Acquisition Setup Home Next**

## Plate Acquisition Setup Tutorial - Names and Description Tab

**Previous Plate Acquisition Setup Home Next**

The Names and Description tab enables you assign an author name, base name, and description to your settings file. This information is stored with the settings file in the database. To add an author name, base name, and description, complete the following procedure:

| Step | Action |
|---|---|
| **1** | Type an experiment base name into the *Experiment base name* field. |
| **2** | Select a location where screening images are saved from the *Storage Location* Drop-down list.<br>**Note:** Image locations are configured in the using the Meta Imaging Series Administrator command Database Utilities command. |
| **3** | Type a description into the *Description* field. |
| **4** | Click *Next* to move to the next tab. |

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## Plate Acquisition Setup Tutorial - Objective/Camera Tab

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Use the Objective/Camera tab to select magnification, binning, and gain settings for your acquisition. To change these settings, complete the following procedure:

Notes:

- The settings available in the *Magnification* drop-down list were created in the

Configure Magnification dialog box in the Devices menu. Magnification settings have a calibration and assign X and Y offset values and a Z escape distance to a specific objective.

- The Camera Gain and binning option are not available for the ImageXpress.

| Step | Action |
|---|---|
| **1** | Select the Magnification setting to use from the *Magnification* drop-down list at the top of the dialog box. |
| **2** | Select the amount of binning from the *Camera Binning* spin box.<br><br>**Note:** Binning is the process of combining data from multiple pixels (for example, 4 pixels — 2 x 2) into a single pixel during acquisition. Directing the camera to use binning causes the resulting acquired image to be brighter and smaller, but the resolution will be lower as a result. Because the image is smaller, both the time required to transfer the image and the storage requirement are significantly reduced. See the Understanding Binning page for more information. |
| **3** | Select the amount of gain from the *Camera gain* drop-down box (if applicable). The gain value specifies the amplification to be applied to the camera output.<br><br>**Note:** For most experiments, use the highest gain possible for increased sensitivity. |
| **4** | Click *Next* to move to the next tab. |

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## Plate Acquisition Setup Tutorial - Plate Tab

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Use the Plate tab to configure and save plate settings for your acquisition. In order for laser auto focusing to work within MetaXpress, you must have valid plate values in the *Plate* tab of the Plate Acquisition Setup dialog box. These values are also needed by the optional Live Cell component for the fluidics robot to operate correctly. For a basic overview on creating and saving plate settings, complete the following procedure. For more detailed instructions on configuring plate types refer to the linked Application note PDF for your hardware platform:

**PDF Application Notes**

Application Note — Defining Plate Types in MetaXpress: ImageXpress$^{MICRO}$ hardware platform

Application Note — Defining Plate Types in MetaXpress: ImageXpress 5000A hardware platform

Application Note — Defining Plate Types in MetaXpress: Discovery-1 hardware platform

**Notes:**

- The MetaXpress CD comes with a variety of common plate type already configured. Check the Plates directory of the MetaXpress CD for included plate files. In order to use these plate files in MetaXpress, you must copy them from the Plates folder on the CD into the plates directory of your MetaXpress installation (C:\MX\plates by default). These plate types will then be available from the plate name drop-down list.
- You must know the optical thickness and bottom variation for your plate. The optical thickness is a value in microns that is the average thickness for the well bottom — see the graphic next to the *Optical Thickness* field. The bottom variation is a value in microns that is the maximum variation in Z direction between adjacent well bottoms. For instructions on determining the optical thickness and bottom variation for your plate, refer to the appropriate Application Note PDF for your hardware type listed above.

| Step | Action |
|---|---|
| **1** | Select a plate that corresponds with the type you are using in the *Plate name* drop-down list.<br><br>The options available to configure depend on the plate type selected and the hardware platform you are using.<br><br>If you select *Custom*, all options will be available for configuration. |
| **2** | Select your square or circular well shape from the *Well shape* drop-down list. The graphics of the plate are updated to reflect your selection. |
| **3** | Enter values in the *Optical Thickness* and *Bottom Variation* fields (see note above). |
| **4** | If needed, change the values in the *Well depth* and *Plate height* fields (see note above). |
| **5** | If you selected *Custom*, enter other values as needed. |
| **6** | After you have configured the plate settings, click *Save Configuration* to enter a name for your setting and save the plate configuration. Configurations that have been saved are then available to select in the *Plate Name* drop-down list. |
| **7** | Click *Next* to move to the next tab. |

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## Plate Acquisition Setup Tutorial - A1 Center Tab

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Use the A1 Center tab to configure the A1 center position for your stage. This is necessary because the AI center location may vary depending on the type of plate you are using. To set A1 center, complete the following procedure:

**Notes:**

- The A1 Center Tab is only available for the Gen2 hardware platform. If you are using an ImageXpress, skip this page.
- Setting A1 center only has to be done the first time you create settings with each plate type. Be sure to create and save a settings file for each plate that can be reloaded as needed.
- Note: the following method involves going into Live mode and moving the stage using the joystick. Live mode opens a window that shows the current view of the camera. Use the Plate Control and Acquisition dialog box or the Plate Acquisition toolbar *Show Live* option to enter Live mode.

**Warning**: Do not look into the microscope objective or look into the well over the objective as damage to eyes can occur. This lamp produces concentrated, high-intensity Ultraviolet (UV) light, which can permanently damage eyes. Use appropriate safety precautions in the presence of UV light.

| Step | Action |
|---|---|
| **1** | Mark the center of the A1 well on a test plate and load the plate. |
| **2** | With the microscope in "Live" mode, use the joystick to move the mark to the center position. |
| **3** | Click *Set A1 Center to Current Position*. |
| **4** | Click *Next* to move to the next tab. |

**Warning:** Do not look down through the well into the objective at any time. The potential exists for bright light to pass through if the shutter is opened accidentally.

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## Plate Acquisition Setup Tutorial - Wells to Visit Tab

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The Wells to Visit tab enables you to configure which wells to acquire on your plate. You can also enable acquisition from multiple sites per well from this tab. To configure which wells to visit, complete the following procedure:

**Note:** If you select Visit multiple sites per well, the Sites to Visit tab will become available and opens when you click *Next*.

| Step | Action |
|---|---|
| **1** | In the Wells To Visit box, click to select the wells that you want to visit:<br>▪ Click individual wells to select or deselect each well.<br>▪ Click lettered buttons to select or deselect an entire row.<br>▪ Click numbered buttons to select or deselect an entire column.<br>▪ Click the *All* button in the upper-left corner to select or deselect all wells on the plate.<br>▪ Right-click a well to move the stage to the well. |
| **2** | To enable visiting multiple sites per well. Click *Visit multiple sites per well*. The *Sites to Visit* tab becomes available. |
| **3** | Click *Next* to move to the next tab. |

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## Plate Acquisition Setup Tutorial - Sites to Visit Tab

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The Sites to Visit tab enables you to configure acquiring images from multiple sites in a well. You can also configure the vertical and horizontal spacing between sites in an image. Both positive and negative values can be entered. This enables you to either add a barrier between images (using positive values) or overlap images using negative values. To configure the Sites to visit tab, complete the following procedure:

**Notes**

- The Sites to Visit tab is only available if you selected *Visit multiple sites per well* on the Wells to visit tab.
- The focus for individual sites is set on the *Autofocus* tab.

| Step | Action |
|---|---|
| **1** | Enter a value in the *Site arrangement in well (NxN)* field:<br>▪ Select 2 to acquire up to four sites<br>▪ Select 3 to acquire up to nine sites |

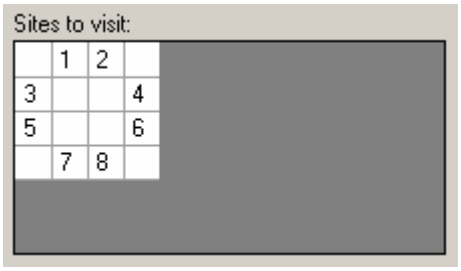
- Select 4 to acquire up to 16 sites, etc.

**Note**: The box in the *Sites to Visit* field will grow or shrink according to the value entered in the *Site arrangement in well (NxN)* field.

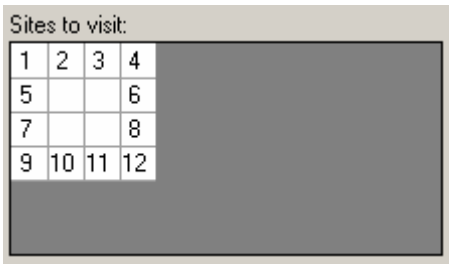
In the *Sites to Visit* field click individual sites to turn off any sites that you do not want to acquire or to turn on any sites that are turned off. Right-click a site to move the stage to that site.

**Example:**

If you want to acquire images at the edges of a **round** well configured with 16 sites, the configure the *Sites to visit* field as shown below:




To acquire images at the edges of a **square** well configured with 16 sites, the configure the *Sites to visit* field as shown below:




**3** If you want to include a distance between adjacent sites, enter the X and Y values of the spacing in the *Spacing between images (um) field*.

**Note:** Entering a negative value in these fields will result in overlapping data between adjacent images.

You can view the current image size, well size, and image spread values on the lower half of the tab. These value update to reflect changes in configuration.

**4** Click *Next* to move to the next tab.

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## Plate Acquisition Setup Tutorial - Time Lapse Tab

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The Time Lapse tab enables you to set the loop order to use when acquiring images at multiple time points. It also enables you to set the number of time points to acquire and the total time needed for all time points. To configure the Timelapse tab, complete the following procedure:

**Notes:**

- If you are not using multiple time points in your acquisition, set the *Number of Timepoints* value to *1* and click *Next* to move to the next tab.
- The values in the *Number of Timepoints*, *Interval*, and *Duration*, fields have the following relationship:

  **Number of Timepoints * Interval = Duration**

  Changing any of values will automatically update the others as needed.

| Step | Action |
|---|---|
| **1** | Select the number of time points to acquire in the *Number of Timepoints* field. Note that changing this value updates the *Duration* field as needed (see note above). |
| **2** | Select a loop order to use when acquiring images at multiple time points from the *Perform time series for* field. The following options are available:<br>▪ ***One site, then next*** – Acquires all the images at the site and then collects the next set of wavelength images after the interval has elapsed. Once the series is collected, the next site is acquired. No refocusing is done after the first timepoint. This option is useful to collect large amounts of data from a well when image alignment is vital — for example, when performing cell mobility analysis.<br>▪ ***One well, then next*** – A set of wavelength images is acquired at each site in the well at each time point. No refocusing is done. This option is most common for rapid acquisition from a well if multiple sites are selected. All sites are acquired per timepoint.<br>▪ ***One row, then next*** – All the images in one row's worth of wells are collect at each time point. Once the series is collected the next row is acquired. No refocusing done. This option requires a longer time interval because all the wells in a row are acquired.<br>▪ ***One column, then next*** – All the images in one column's worth of wells are collected at each time point. Once the series is collected, the next column is |

acquired. No refocusing done after the first timepoint.

- ***All selected wells*** – Every well selected for acquisition is acquired at each time points The well selection is determined at the start of the first acquisition. No refocusing is done after the first timepoint.

**3** Set the amount of time between the start of acquisition at one time point to the start of acquisition at the next time point in the *Interval* fields. Use the drop-down box to select a time unit. Units can be ms, sec, min, or hr.

**Notes:**

- If the time interval is shorter than the length of time required to actually acquire the images, the next acquisition will occur as soon as possible once the first acquisition is complete. No warning notice will be given if the acquisition time is longer than the specified interval. Under these circumstances, the actual duration will be longer than the duration time shown in the *Duration* field
- The *Interval* field can be set to 0 to acquire images as fast as possible. If the interval is set to 0, the duration will be set according to the approximate minimum time, and the *Duration* field will be inactive.

**4** If needed, change the amount of time for the entire time series in the *Duration* field. Note that the *Duration* field value is the result of multiplying the number of time points by the interval.

**Note:** The Number of timepoints field will automatically update as the Duration field is changed.

**5** To activate or deactivate the Fluidics tab, click *Perform fluidics experiment* . To run a fluidics experiment, follow the procedure in the tutorial for the ***Fluidics*** tab.

**6** Click *Next* to move to the next tab.

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## Plate Acquisition Setup Tutorial - Fluidics Tab

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**Note**: Fluidics control is currently only available on the ImageXpress 5000A hardware platform.

The following procedures explain how to define initial fluidics settings and then make experiment-specific settings. Fluidics events are synchronized with MetaXpress plate acquisition time points. To enable the Fluidics tab, select the *Timelapse* tab on the Plate Acquisition Setup dialog box, then click *Perform fluidics experiment.* Click the *Fluidics* tab to highlight it.

To configure a fluidics experiment, complete the following procedure:

| Step | Action |
|---|---|
| **1** | To begin the process to configure a fluidics experiment, from the screening menu, click *Plate Acquisition Setup*. The Plate Acquisition Setup dialog box opens. |
| **2** | Follow the tutorial guidelines for the other Plate Acquisition Setup dialog box tabs to set up an experiment. |
| **3** | After all other aspects of your experiment have been configured, click the *Timelapse* tab. |
| **4** | On the Timelapse tab, click *Perform fluidics experiment*. The Fluidics tab will appear. |
| **5** | Click the *Fluidics* tab to begin to make the necessary settings to run a Fluidics experiment. |
| **6** | On the *Plate Acquisition Setup - Fluidics* tab, click *Configure Stations*. The *Configure Fluidic Station* dialog box opens. |
| **7** | Follow the procedure to configure a fluidics station. Before configuring settings on the ***Configure Fluidic Station*** dialog box, Click *Define Tips* if no tips are currently defined. The Define Tips dialog box opens. |
| **8** | Follow the procedure for the ***Define Tips*** dialog box to define as many different types of disposable pipette tips as needed, and assign each one a unique, descriptive name. |
| **9** | After completing your settings, click *Save*, the *Plate Acquisition-Save Configuration* dialog box opens. Use the default name (20, 50, 100, 200µl), or type a unique, descriptive name. |
| **10** | In the ***Configure Fluidic Station*** dialog box, click *System Properties*. The *Fluidics System Properties* dialog box opens. |
| **11** | Follow the procedure to configure the fluidic station settings in the ***Fluidics System Properties*** dialog box. Typically, you can use the default settings for this dialog box. However, your experiment might have unique requirements. |
| **12** | After you complete the settings in the ***Fluidics System Properties*** dialog box, Click *OK* or *Cancel* to return to the *Configure Fluidic Stations* dialog box. |
| **13** | Configure the settings in the ***Configure Fluidic Stations*** dialog box. |
| **14** | Click *Close* when you have completed your settings in the *Configure Fluidic Stations* dialog box. |
| **15** | On the *Plate Acquisition Setup - Fluidics* tab, click *Add New Event*. The Fluidic Event |

dialog box opens.

**16** Follow the procedure to *Add New Event* using the *Fluidic Event* dialog box.

**17** To edit an existing event, in the Scheduled Events area, click the event that you want to edit, then click Edit Event. The ***Fluidic Event*** dialog box opens. This is the same dialog box that you used to schedule an event, however, this version of it is connected to the event you selected for editing.

**18** To delete an event, click the event that you want to delete, then click *Delete Event*. The event is immediately deleted. No confirmation message is displayed.

## Plate Acquisition Setup Tutorial - Acquisition Loop Tab

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The Acquisition Loop tab enables you to configure options for what happens during a single acquisition loop. Here you configure the number of wavelengths to acquire, autofocus options, and whether to use shading correction during acquisition. To configure the Acquisition Loop tab, complete the following procedure:

**Note:** The selections made in this tab determine what options are available in the *Autofocus* and *Wavelength* tabs as well as the number of *Wavelengths* tabs.

| Step | Action |
|---|---|
| **1** | Select the total number of wavelengths to use during acquisition in the *Number of wavelengths* field.<br><br>**Note:** You must enable at least one wavelength in this field. |
| **2** | In the *Autofocus options* field, select the autofocus options to enable in the Autofocus tab — laser-based, image-based, or both.<br><br>**Notes:**<br><br>• **Laser-based**: Finds the bottom of the well and moves a specified distance up from the bottom. This method is normally fastest and will not cause photo damage to your specimen. This method does not work well if the distance above the bottom of the well changes in your sample.<br><br>• **Image-based**: Uses a specified algorithm to identify the best focus image. Best for experiments using low power objectives and when the sample distance above the bottom of the plate varies. This method can be slower than laser-based and can fail if there is out-of-focus debris present.<br><br>• **Both Laser- and Image-based**: Uses the laser to get to a specified position above the bottom of the well and image-based focusing to fine tune. This method works best when |

there is some variation in the distance above the plate bottom. It is especially useful at very high magnifications.

**3** Select *Perform shading correction* to enable shading correction.

**Note**: In order for shading correction to be applied during acquisition, correction images must exist for each wavelength used. The correction images must be named in the following format:: **shading_<magnification setting>_<wavelength>_.tif**. For example — **C:\shading_10x_DAPI.tif**.

**4** To change the location where MetaXpress looks for shading correction images, click *Directory* and selecting a new location using the Browse dialog box. The default location is the root C:\ directory.

**5** Click *Next* to move to the next tab.

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## Plate Acquisition Setup Tutorial - Autofocus Tab

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The Autofocus tab enables you to configure autofocus options for your acquisition. The choices available on this tab will vary depending on both the hardware platform you are using and what is selected in the *Autofocus Options* field in the *Acquisition Loop* tab.

**Note**: For detailed instructions on configuring laser auto focus, refer to the Acquisition Guide for you hardware platform.

To configure the *Autofocus* tab, complete one of the following procedures that matches your Autofocus Options selection:

If you enabled both Laser and Image-based focusing, complete both of these procedures before moving to the Wavelength tab(s).

**Configuring Autofocus Tab—Laser Auto Focus only**

The Laser Auto Focus settings are accessed through the *Configure Laser Sensor* button on the Autofocus Tab. These settings usually do not need to be modified. Try testing the sensor as described below and only make changes if needed:

**Note:** The options available in the Configure Laser Sensor dialog box vary depending on your hardware platform. The procedure below is specific to the Discovery-1 platform. For more information on the options available to other platforms, refer to the Configure Laser Sensor Dialog Box Options help.

**Note:** *Enable laser-based focusing* must be selected in the *Acquisition Loop* tab before starting this procedure.

| Step | Action |
|---|---|
| **1** | Click *Configure Laser Sensor*. The Configure |

Laser Sensor dialog box opens.

**Note:** The default values for each magnification setting do not need to be changed under most circumstances.

**Note:** The *Acquisition Settings for Mag: XX* field contains the settings for the active Magnification setting, which was set in the *Objective/Camera* tab.

**2** Click *Test Sensor* to test the current settings. a live window opens using the current auto focus settings.

Ensure that the laser focus spot is in view. If it is not, manually focus until you see the laser focus spot. When the window is in focus and the laser focus spot is in view, click *Stop Test* to close the live window and go to step 3.

> **Note**: If you cannot see the laser focus spot after manually focusing, the laser sensor region may have been changed (for example, if you are using a different camera). If you are using the Discovery-1 hardware platform, select *User Defined* from the *Region* field and manually enter the region to use when autofocusing.

**3** Click *Focus Snap* to acquire a new image using the current auto focus settings. The acquired test image should have maximum grey level intensity values between 40-75% of the camera's dynamic range (approximately 3:1 signal to noise ratio).

**4** If the focus snap image was focused and had the correct grey level intensity values (see step 3), you do not need to make any changes to the settings. Click *Close* to close the Configure Focus box and skip to Step 12.

**OR**

If your test images were not in focus, continue with this procedure and change settings as needed. You can test your changes at any time using the *Test Sensor* and *Focus Snap* commands.

**5** To change the Well Reflectivity setting, select *Dim* or *Bright*.

> **Note**: Select *Dim* for wells with little reflectivity, such as those with plastic plates or those using media. Select *Bright* for wells with more reflectivity, such as certain types of glass plates, and wells without any media.
>
> **Note:** The *Well Reflectivity* setting

has a greater impact on the grey level intensity value of the test image than other intensity settings such as exposure, binning, and gain.

**6** To change the exposure time used during auto-focusing, ensure that *Use Bottom of Well Settings* is not selected and type or select a value in the *Exposure [ms]: [plate bottom]* box.

**Note**: For best results, the exposure time should be kept in the range of 2-50 ms.

**7** To change the binning used for auto-focusing, type or select a value in the *Binning* box.

**Note**: This is only enabled when using the Discovery-1 hardware platform.

**Note**: Binning values greater than 3 are not recommended.

**8** To change the gain used during auto-focusing, type or select a value in the *Gain* box.

**9** To change the laser power intensity, type or select a value in the *Laser Power* field.

**Note**: This is only enabled if you are using the ImageXpress.

**10** To change the maximum allowable distance for a single Z-motor move, type or select a value (in ums) in the *Max Step* field.

**Note**: The greater the Max Step size, the faster the focus can occur. However, if the value is too high, the focus may be missed. The default value is recommended.

**Note**: To restore all the default settings for the selected objective, click *Set to Defaults*.

**11** To save your settings, click *Save Settings*. This saves the settings to the current plate settings configured in the Plate tab.

**Note**: You must have a custom plate configuration open in the Plate tab for the settings to be saved. The default 96- or 346-well settings files will not save your laser focus settings.

**12** Click *Close* to close the Configure Laser Sensor dialog box and return to the Autofocus tab.

**13** If you are using a glass bottom plate and a liquid medium, select *Focus on plate bottom only, then offset by bottom thickness* to increase the laser's accuracy.

**14** If you enabled *Visit multiple sites per well* in the Wells to Visit tab, select the site autofocus setting in the Site Autofocus field. The following choices are available:

**First site only** — auto focuses in the top left site in the well.

**Center of well** — auto focuses in the center of the well. This is the recommended method.

**All sites** — auto focuses for each site.

**Note**: This option is only available if you have already configured the use of multiple sites in the Sites tab.

**15** To view and/or copy the current autofocus parameters, click *View Focusing Details*.

**16** Click *Next* to move to the next tab.

### Configuring Autofocus Tab—Image-based Focus only

Complete this procure if you selected *Enable image-based focusing* in the Acquisition Loop tab:

| Step | Action |
|---|---|

**1** Select an algorithm to use from the Algorithm drop-down list. Select one of the following algorithms:

***Standard*** – Algorithm based on a standard group of settings including a normal camera signal level. (Default)

***Low Signal*** – Algorithm based on a set of values selected to compensate for a low signal level from the camera. This setting can compensate for situations in which some pixel intensities are somewhat brighter when slightly out of focus.

**Note:** MDC recommends using the Low Signal setting whenever you are using a 20x Apo lens.

**2** Select the binning the camera will use during image-based auto focus in the *Binning* field. Horizontal and vertical binning are always set the same and should be set to three or less.

**3** If you do not want to use a calculated exposure time for each wavelength during autofocus**,** select *Allow custom exposure time*. This will enable you to set an exposure

time for each wavelength in the Wavelength tab.

**4** To disable the initial find sample auto focus routine when starting a plate, select *Skip Find Sample*. Select this option if your sample is already in focus.

**5** If Find Sample is enabled, select the first well to be used when performing the initial find sample auto focus by setting the *Initial Well for Finding Samples* fields. A1 is the default and should not need to be changed if you are acquiring the entire plate.

**6** In the *Wavelength Offsets form W1* field, Select how the wavelength offset from the first wavelength is determined when auto focusing. Valid choices include the following:

- **None** – No offsets are calculated.
- **Calculate at start** — Offsets between each of the wavelengths are calculated at the start of acquisition.
- **Define for each wavelength** – Enables you to configure the offset for each wavelength in the *Wavelength* tabs.

**7** If you enabled *Visit multiple sites per well* in the Wells to Visit tab, select the site autofocus setting in the Site Autofocus field. The following choices are available:

**First site only** — auto focuses in the top left site in the well.

**Center of well** — auto focuses in the center of the well. This is the recommend method. This occurs even if the center of the well location is not configured to be acquired.

**All sites** — auto focuses at each site.

**Note**: This option is only available if you have already configured the use of multiple sites in the *Sites* tab.

**8** To view and/or copy the current autofocus parameters, click *View Focusing Details*.

**9** Click *Next* to move to the next tab.

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## Plate Acquisition Setup Tutorial – Wavelength Tab(s)

Use the Wavelength tab(s) to configure exposure, autofocus, and time-lapse settings for each wavelength. The total number of wavelength tabs depends on the number of wavelengths selected in the Acquisition Loop tab. To configure the Wavelength tab(s), complete the following procedure:

**Note:** The options available in the wavelength tab(s) vary depending on the selection made in the Acquisition Loop and Autofocus tabs.

| Step | Action |
|---|---|
| **1** | In the Illumination box, select the illumination setting for this wavelength. The illumination settings are defined in the Configure Illumination dialog box. |
| **2** | In the Exposure box, type or select an exposure time in milliseconds or, if you have an appropriate sample in view, click *Auto Expose* to set this value automatically. |
| **3** | Enter a value for the Target Intensity in the *Target Intensity* field or use the default value. This value sets the intensity that auto exposure should attempt to attain for the brightest pixel in the image. When Auto Expose is selected, the target intensity value default is 75 percent of the maximum gray level that the camera driver reports as possible to obtain. |
| **4** | Select the type of Autofocusing to perform for the wavelength in the Autofocus drop-down list.<br><br>**Note:** Laser-based autofocusing is recommended under most circumstances. It is faster than image-based, less sensitive to the sample, and does not cause photo bleaching damage to the sample.<br><br>**Note:** The number of options available in the drop-down list vary depending on selections made in the *Acquisition Loop* and *Autofocus* tabs. Step 5 explains each option. |
| **5** | • ***None*** – no autofocusing will be performed on this wavelength.<br><br>• ***Laser with z-offset*** – This option is available if *Enable laser-based focusing* is selected in the Acquisition Loop tab *and* this is the first wavelength. Select an offset to use for the first wavelength in the *Offset* field. No image-based focusing will be performed on this |

wavelength.

- ***Image Only*** – This option is available if *Enable image-based focusing* is selected in the Acquisition Loop tab. It enables the *Range, Max. Step, Exposure, and Gain* (*Exposure, and Gain* are only available if Allow custom exposure time is selected in the Autofocus tab*).* This option is best used for complex samples with variations in distance between the surface of the plate and the sample. It is also the best choice for experiments using magnification settings of less than 10x.
- **Laser and image** – This option uses the laser to focus, then uses image-based focusing to fine-tune the image. It is only available if both *Enable image-based focusing* and *Enable laser-based focusing* are selected in the *Acquisition Loop* tab. It enables the *Image-based range*, *Max. Step*, *Exposure*, and *Gain* fields(*Exposure, and Gain* are only available if Allow custom exposure time is selected in the Autofocus tab*)*.
- **Z-offset from W1** — This option moves the specified offset from the wavelength 1 focus position and is only available for the second and subsequent wavelengths. Enter the Z-offset value (in um) in the *Offset* field. This option is only available if *Enable laser-based focusing* is selected in the *Acquisition Loop* tab.

**6** If you enabled multiple timepoints in the *Timelapse* tab, use the *Timelapse Acquisition* drop-down box to set the image collection intervals to use for each wavelength. The following choices are available:

- **All time points** – Acquires an image for each timepoint for this wavelength.
- **At start** – Acquires an image at this wavelength for the first timepoint only.
- **At start and end** – Acquires images at this wavelength for the first and last timepoints only.

- **Every nth timepoint** – Acquires an image at this wavelength at the selected timepoint interval beginning at the first timepoint.

**7** Click *Next* to move to the next tab.

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## Plate Acquisition Setup Tutorial - Journals Tab

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Use the Journals tab to configure specific journals to run during different stages of acquisition. For more detailed descriptions of each Acquisition step, refer to the Plate Acquisition Setup Dialog Box - Journals help page. To configure the Journals tab, complete the following procedure:

**Note:** If you do not need to run any journals during the acquisition, click *Next* to move to the next tab.

| Step | Action |
|---|---|
| **1** | Click the checkbox next to the Acquisition Step where you want to run a journal to select it. |
| **2** | Click the folder icon next to the selected acquisition step. The Select Screen Acquisition Journal dialog box opens with the contents of the Journals folder displayed by default. |
| **3** | Choose the journal that you want to run at the selected acquisition step, and click *Open*. If the journal is not located in the Journals folder, browse to the folder it is in, select it and click *Open*. |
| **4** | Repeat steps 1-3 for as needed to assign journals to run at additional acquisition points. |
| **5** | If any of the journals you run move hardware (change shutters, move focus, etc.), select *Prevent asynchronous hardware moves*. This ensures that the journals will run correctly. |
| **6** | Click *Next* to move to the next tab. |

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## Plate Acquisition Setup Tutorial - Display Settings Tab

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Use the Display Settings tab to configure the MetaXpress desktop appearance during acquisition. You can choose to use the default display settings or create custom settings. The default display settings tile and autoscale all acquired images, and ensure that the status dialog box is unobstructed. To change the default display settings, complete the following procedure:

**Note:** If you do not need to change the display settings, ensure that *Use default display settings* is selected and click *Next* to move to the next tab.

| Step | Action |
|---|---|
| **1** | Select *Manually set image display settings*.<br><br>Click *Display Images*. The Screening Status dialog box opens and an image is acquired for each configured wavelength. |
| **2** | Change the configuration of the display by arranging the image windows and dialog boxes; you can change the location, size, zoom, scaling, gamma, and LUT of images. These new settings will be saved and used during acquisition. |
| **3** | To display images acquired during auto focus, select *Display images during auto focus*. |
| **4** | To display images acquired acquisition, select *Display Images During acquisition*. |
| **5** | Click *Next* to move to the next tab. |

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## Plate Acquisition Setup Tutorial - Post Acquisition Tab

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Use the Post Acquisition tab to choose a specific analysis to run on a data set after the acquisition is complete. The data set will added to the Auto Run queue for analysis by a system set to Auto Run mode. You can select from a list of saved settings from any application modules or journal analyses saved to the database. To select an analysis to automatically run after acquisition, complete the following procedure:

**Notes:**

- If you do not want to automatically run post-acquisition analysis, ensure that *Auto Run analysis after acquisition* is not selected and click *Next* to move to the next tab.
- The list of available analyses and settings is the same list that is in the *Run Analysis* tab of the Review Plate Data (DB) dialog box.

| Step | Action |
|---|---|
| 1 | Select *Auto Run analysis after acquisition*. |
| 2 | Select the analysis (Application module or Journal) to run after acquisition from the *Analysis* drop-down list. |
| 3 | Select a setting file from the *Settings* drop-down list. The Field below the Settings drop-down box displays a description of the settings if one exists.<br><br>**Note**: You can configure and save settings in the Review Plate Data (DB) dialog box. |
| 4 | Select a base folder in which to store measurement results from the *Base Folder* drop-down list. To select a new location, pick <Select> and click the  button to open the Measurements Sets dialog box. This enables you to select another base folder within the database to store measurements results. |
| 5 | Click *Next* to move to the next tab. |

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## Plate Acquisition Setup Tutorial - Summary Tab

**Previous** **Plate Acquisition Setup Home**

Use the Summary tab to view a list of all current settings for the acquisition, save the settings to a file, and start acquiring images. Use the following procedure to save your settings file and start acquisition:

**Notes:**

- The information in the summary tab is identical to the information displayed when you click the Summary button on the bottom of the dialog box.
- If you want to make any changes to the stage or Z Position, or snap an image to test the current settings before starting the acquisition, use the Plate Acquisition and Control dialog box or the Plate Acquisition Toolbar to perform these and other tasks.
- If an error dialog box opens after you click *Acquire*, the most likely cause is a configuration error. Read the text in the dialog box to determine the error.
- After your acquisition is complete and the images have been saved to the database, you can use the Review Plate Data (DB) dialog box to view the images and setup analysis.

| Step | Action |
|---|---|
| 1 | Click *Save Settings* to open the Save Plate Acquisition Settings dialog box and save the current settings to a file on the local hard drive or to the database. |
| 2 | If you want to print the settings summary, click *Print* to open the Print Setup dialog box and then print the settings. |
| 3 | Click *Acquire Plate* to acquire images from a plate based on the settings made in the Plate Acquisition Setup dialog box. The expected behavior after clicking *Acquire* is outlined below:<br>▪ The Plate Acquisition Setup dialog box closes and the Screen Status dialog box opens<br>▪ Each image appears briefly on the MetaXpress desktop as it is acquired and saved to the database.<br>▪ After the last image is acquired and saved, the Screen Status dialog box closes and the Plate Acquisition Setup dialog box reopens. |
| 4 | Click *Close* to exit the Plate Acquisition Setup dialog box. |

**Previous** **Plate Acquisition Setup Home**

## Plate Acquisition Tutorial - Creating and Saving a New Plate Layout

**Plate Acquisition Setup Home**

This procedure explains how to create and save a new plate configuration file. After creating and saving the plate configuration, you will be able to select it from the *Plate name* drop-down list as needed when you set up acquisition.

To create and save plate settings, complete the following procedure:

**Notes:**

- 
- You must know the optical thickness and bottom variation for your plate. The optical thickness is a value in microns that is the average thickness for the well bottom. The bottom variation is a value in microns that is the maximum variation in Z direction between adjacent well bottoms. For instructions on determining the optical thickness and bottom variation for your plate, refer to the following PDF Application Notes:

**PDF Application Notes**

Application Note — Defining Plate Types in MetaXpress: ImageXpress$^{MICRO}$ hardware platform

Application Note — Defining Plate Types in MetaXpress: ImageXpress 5000A hardware platform

Application Note — Defining Plate Types in MetaXpress: Discovery-1 hardware platform

- The correct *Well depth* and *Plate height* values are needed in order for the autofocus *Find Sample* command to work correctly.

| Step | Action |
|---|---|
| **1** | From the Screening menu, select Plate Acquisition Setup, Plate Acquisition Setup dialog box opens with the settings that were last entered. |
| **2** | Click the *Plate* tab to open the *Plate* tab. |
| **3** | Select a plate that corresponds with the type you want to use in the *Plate name* drop-down list.<br><br>If you select *96 Wells (8x12)* or *384 Wells (16x24)*, the following options will be available for configuration: Well Shape, Well depth, Plate height, Bottom thickness, and Bottom tolerance.<br><br>If you select *Custom*, all options will be available for configuration. |
| **4** | Select your well shape (round or square) from the *Well shape* drop-down list. The graphics of the plate are updated to reflect your selection. |
| **5** | Enter values in the *Optical Thickness* and *Bottom Variation* fields (see note above). |
| **6** | If needed, change the values in the *Well depth* and *Plate height* fields (see note above). |
| **7** | If you selected *Custom*, enter other values as needed. |
| **8** | After you have configured the plate settings, click *Save Configuration* to enter a name for your setting and save the plate configuration. Settings that have been saved are then available to select in the *Plate Name* drop-down list. |
| **9** | If you want to begin acquiring using the new plate setting, use the Plate Acquisition and Control dialog box and the Plate Acquisition |

Toolbar to start acquisition.

**OR**

Click *Close* to close the dialog box.

**Plate Acquisition Setup Home**

## Plate Acquisition Tutorial - Using a Saved Setting on a New Plate

**Plate Acquisition Setup Home**

This procedure explains how to use a previously saved settings file to rerun an experiment on a new plate. After settings files are created and saved using the Plate Acquisition Setup dialog box, they can be loaded in several ways. This procedure uses the Plate Acquisition and Control dialog box to load the settings file and start the acquisition. See the note below for more options when loading a settings file.

**Notes:**

- This procedure uses the Plate Acquisition and Control dialog box to load the settings file and start the acquisition. You can also use either the Plate Acquisition dialog box or the Plate Acquisition toolbar to perform these tasks.
- This procedure assumes that the settings file you load is configured correctly for the plate you will use. If you need to make changes to the settings file before acquiring, use the Plate Acquisition Setup dialog box.

To load a saved plate setting and begin an acquisition, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Screening menu, select Plate Acquisition and Control. The Plate Acquisition and Control dialog box opens. |
| **2** | Select *Load existing settings file*, then click *Load Settings*. The Load Plate Acquisition Settings dialog box opens. |
| **3** | If you are loading a settings file that was saved to the database, select the settings file to load from the Settings File drop-down list. Then select the individual settings to load from the file using the check boxes next to each settings group.<br>**OR**<br>If you are loading a settings file that was saved outside the database, select *From File* in the drop-down list and then select the |

individual settings to load from the file using the check boxes next to each settings group.

**4** If you are using a settings file from the database, click *OK*. The file will load from the database and the Load Plate Acquisition State dialog box will close.

**OR**

If you are loading a settings file saved outside the database, click *OK*. The Load Screen state dialog box opens. Navigate to the state file (.HTS) you want to open and click *Open*. The state file you selected will load and the Load Screen Acquisition dialog box will close.

**5** Click *Summary* to open the Screen Summary dialog box and review your settings. Close the dialog box when finished.

**6** If you need to make any changes to your settings, click *Setup* to open the Plate Acquisition Setup dialog box and make the changes.

**7** Click *Acquire* to begin the acquisition with the loaded settings.

**Plate Acquisition Setup Home**

## Plate Acquisition Setup - Dialog Box Options

Main

Experiment

Names and Descriptions

Objective/Camera

Plate

A1 Center

Wells to Visit

Sites to Visit

Time Lapse

Fluidics

Acquisition Loop

Autofocus

W1. . . W8 (Wavelengths)

Journals

Display Settings

Post-acquisition

Summary

**Note**: When configuring the Plate Acquisition Setup dialog box, you will encounter settings highlighted either in yellow or red. A yellow highlight can mean that an optional field is not filled in or could indicate another minor error. A red highlight means that a required field is either not filled in or contains invalid data that should be changed. These visual reminders help when configuring an experiment

## Plate Acquisition Setup Dialog Box Options - Main

### Tree Tab

Provides a way to view and navigate each tab in the Plate Acquisition Setup dialog box. You can click any tab to view and edit the corresponding pane. The tabs are set up sequentially from the top down with the steps needed to configure a new acquisition.

### Save Settings

Opens the Save Settings dialog box.

### Summary

Opens a summary dialog that contains the current parameters. These parameters update in real-time as they are changed in the Plate Acquisition dialog box.

### Previous

Moves to the previous tab in the dialog box.

### Next

Moves to the next tab in the dialog box.

### Close

Closes the dialog box.

## Plate Acquisition Setup Dialog Box Options - Experiments Tab

### Create New Settings

Use this option to begin creating new acquisition settings. Enabled by default.

### Load existing settings file

Enables the Load settings button.

### Load Settings

Opens the Load Settings dialog box and enables you to select a previously saved settings file.

## Plate Acquisition Setup Dialog Box Options - Names and Descriptions Tab

### Experiment Set

Enables you to add an additional field in the database associated with the experiment. This label can be used to help sort and group experiments when view them using the Review Plate Data command.

### Experiment base name

Defines the base file name.

### Storage Location

Enables you to select a location where screening images are saved.

**Note:** You must configure the image locations using the Meta Imaging Series Administrator command Database Utilities command.

### Description

Enables you to type an experiment description to be stored with the image information in the database.

## Plate Acquisition Setup Dialog Box Options - Objective/Camera Tab

### Magnification

Selects the magnification setting that you want to use in your experiment. Magnification settings are made using the Configure Magnification dialog box. Magnification settings assign X and Y offset values and a Z escape distance to a specific objective.

**Note:** You must assign a calibration to the magnification setting using the Calibrate Distances dialog box.

### Camera binning

Specifies the binning value to be applied to the camera. Binning combines the output of adjacent pixels in square multiples. For example, a camera binning value of 1 is only one pixel, a binning value of 2 combines 2x2 or four pixels in a square, a binning value of 3 combines 3x3 or nine pixels in a square, and so on.

### Camera gain

Specifies the amplification to be applied to the camera output. The higher the gain, the greater the potential for background noise in the signal.

## Plate Acquisition Setup Dialog Box Options - Plate Tab

### Plate type

Specifies the plate type you are using. Choices include generic configurations such as Generic 96 well, custom configurations, or a previously defined configuration. Select the plate size that corresponds to your plate, or select *Custom* to specify a non-standard plate configuration. The values in the remaining boxes are pre-filled according to what is selected here.

### Save Configuration

Opens the Save Configuration dialog box and enables you to name and save a custom configuration based on the current values.

### Number of columns

Contain the number of columns for the selected Plate type. This value can be changed to create a custom configuration. This option is only available if a custom plate type is selected.

### Number of rows

Contain the number of rows for the selected Plate type. This value can be changed to create a custom configuration. This option is only available if a custom plate type is selected.

### Well Shape

Selects the shape of the well – either Circle or Square.

**Note:** each of the following fields has a graphic that illustrates the measurement defined:

### Well diameter

Contains a value in microns that is the diameter of the well. This value can be changed to create a custom configuration. This option is only available if a custom plate type is selected.

### Column spacing

Specifies the spacing in microns between each well on the X axis. Normally this value should be the same for both the X and Y axis. However, you can specify different values for X and Y for plates that use different spacing between well on the X and Y axis. This option is only available if a custom plate type is selected.

### Plate Length

Specifies the plate length in microns. This option is only available if a custom plate type is selected.

### Column Offset

Specifies distance in microns between the center of a well in the first column of a plate and the left edge of the plate. This option is only available if a custom plate type is selected.

### Row spacing

Specifies the spacing in microns between each well on the Y axis. Normally this value should be the same for both the X and Y axis. However, you can specify different values for X and Y for plates that use different spacing between well on the X and Y axis. This option is only available if a custom plate type is selected.

### Plate Width

Specifies the plate width in microns. This option is only available if a custom plate type is selected.

### Row Offset

Specifies distance in microns between the center of a well on the top row of a plate and the top edge of the plate. This option is only available if a custom plate type is selected.

### Well Depth

Specifies the plate width in microns.

### Plate Height

Specifies the plate height in microns.

### Optical Thickness

Contains a value in microns that is the average thickness for the well bottom. This value can be changed to create a custom configuration.

### Bottom Variation

Contains a value in microns that is the maximum variation in Z direction between adjacent well bottoms. This value can be changed to create a custom configuration.

## Plate Acquisition Setup Dialog Box Options - A1 Center Tab

**Note**: This dialog box is only applicable to the Discovery-1 hardware platform and does not apply to the ImageXpress.

### Go To A1

Moves the stage to the set A1center position. You must set this position for each plate type. See the A1 Center tutorial page for more information.

### Set A1 Center to Current Position

Sets the current position as the center position of well A1 for each specific plate type. This position may vary for each plate type.

**Warning:** Do not look into the microscope objective or look into the well over the objective as damage to eyes can occur. This lamp produces concentrated, high-intensity Ultraviolet (UV) light, which can permanently damage eyes. Use appropriate safety precautions in the presence of UV light.

### Center of well A1

Shows the current X and Y values (in mm) for A1 center.

## Plate Acquisition Setup Dialog Box Options - Wells to Visit Tab

### Wells to visit

Select the wells to sample during the experiment. You can select a single well up to all the wells in the plate. Click individual well positions to toggle wells off and on separately. Right-click a well to move the stage to that well.

Click the column or row buttons to activate or deactivate an entire row. Click the *All* button in the upper left corner to toggle all wells on the plate simultaneously.

### Visit multiple sites per well

Enables the Sites to Visit tab. When selected, there is a *Sites to Visit* tab in the treetab view and clicking *Next* opens the Sites to Visit dialog box where you can configure multiple sites per well.

## Plate Acquisition Setup Dialog Box Options - Sites to Visit Tab

### Site arrangement in well (NxN)

Specifies the number of sites in the array, from 2 X 2 to a maximum of 127 X 127. Within the selected array of sites, you can left-click on any site to toggle the site on and off and right-click a site to move the stage to that site. You can have as few as one site or as many as 16, 129 sites. Practical usage suggests that the maximum number of sites that you select and use would be significantly less than 127 X 127.

### Sites to visit

Select the sites to sample during the experiment. Click individual site positions to toggle sites on and off separately. Right-click a site to move the stage to that site.

### Spacing between images

Specifies the distance between adjacent sites for both X and Y spacing. A negative value will overlap a portion of the data between adjacent sites images.

## Plate Acquisition Setup Dialog Box Options - Timelapse Tab

### Number of timepoints

Specifies the total number of time points to be acquired. When you change this field, the duration field is automatically updated by calculating the duration from the number of time points and the time interval.

### Perform time series for

Selects the loop order to be used when acquiring images at multiple time points and determines the set of images to be acquired at each time point.

Valid acquisition sequences include the following:

- One well, then next – A set of wavelength images is acquired at each site in the well at each time point. No refocusing is done.
- One row, then next – All the images in one row's worth of wells are collect at each time point. Once the series is collected the next row is acquired. No refocusing done.
- One column, then next – All the images in one column's worth of wells are collected at each time point. Once the series is collected, the next column is acquired. No refocusing done.
- All selected wells – Every well selected for acquisition is acquired at each time points. The well selection is determined at the start of the first acquisition. No state file will be acquired. No refocusing is done.

### Interval

Specifies the amount of time between the start of acquisition at one time point to the start of acquisition at the next time point. If the time interval is shorter than the length of time required to actually acquire the images, the next acquisition will occur as soon as possible once the first acquisition is complete. No warning notice will be given if the acquisition time is longer than the specified interval. Use the drop-down box to select a time unit. Units can be ms, sec, min, or hr. Changing the interval field updates the duration field by calculating the duration from the number of time points and the time interval. The interval field can be set to 0 to acquire images as fast as possible. If the interval is set to 0, the duration will be set according to the approximate minimum time, and the duration field will be inactive.

### Duration

Specifies the time it will take to acquire the number of times points based on the interval. Changing this field updates the number of time points field by calculating the number of time points from the time interval and the duration. Use the drop-down box to select a time unit. Units can be ms, sec, min, or hr.

### Perform fluidics experiment

Activates the Fluidics tab. To set up fluidics and schedule fluidic events, click this checkbox and the Fluidics tab will appear.

## Plate Acquisition Setup Dialog Box Options - Fluidics Tab

### Scheduled Events

Displays a list of scheduled Fluidic events. Events are scheduled using the Add New Event dialog box.

### Configure Stations

Opens the Configure Fluidic Stations dialog box. After selecting the Fluidics tab, you should click this button first to make all physical settings for the Tips, Compound Plates, and Sample Plate. Follow the procedure for the Configure Fluidic Stations dialog box.

### Reset Tips

Opens the Reset Tips dialog box. Use this dialog box to reset the tip count values for the Tip Trays and to reset the fluid values for the Sample and Compound Plates. These values must be reset whenever you replace a tip tray with a full tip tray. Also, use this dialog box to reset the sample plate whenever a new sample plate is put in place, and reset a compound plate whenever it is replaced with a full compound plate.

### Add New Event

Opens the Fluidic Event dialog box. After you have configured your fluidic stations, click Add New Event. The Fluidic Event dialog box opens.

### Delete Event

Deletes one or more selected events. Select the event that you want to delete, and click Delete Event.
**Note:** No verification message is displayed – the event is immediately deleted.

### Edit Event

Opens a scheduled event in the Fluidic Event dialog box. Use this button to revise settings for an already scheduled event.

## Plate Acquisition Setup Dialog Box Options - Acquisition Loop Tab

### Number of wavelengths

The total number of wavelengths that you have configured for use during your experiment. Set from one to eight wavelengths in this box.

### Autofocus options

#### Enable laser-based focusing

Selects laser-based focusing for the acquisition. You will be able to configuring laser auto focusing in the Auto Focus tab.

#### Enable image-based focusing

Selects image-based focusing for the acquisition. You will be able to configuring image-based focusing in the Auto Focus tab.

### Perform shading correction

Enables shading correction for the acquisition. If a correction image exists for a wavelength, correction is performed. Correction images must be named in the following format: **shading_<magnification setting>_<wavelength>_.tif**. For example — **C:\shading_10x_DAPI.tif**.

### Directory

Opens the Browse dialog box. Use this command to change the location where MetaXpress looks for shading correction images. The default location is the root C:\ directory.

# Plate Acquisition Setup Dialog Box Options - Autofocus Tab

## Laser-based Focusing

### Laser Configuration

Opens the Configure Laser Sensor dialog box.

## Focus on plate bottom only, then offset by bottom thickness

Offsets the laser by the bottom thickness of the plate, as defined in the Plate tab. Select this option if you are using a glass bottom plate and a liquid medium to increase the laser's accuracy.

## Image-based Focusing

## Algorithm

Enables you to select the algorithm to use when focusing. Valid choices include the following:

- Standard – Algorithm based on a standard group of settings including a normal camera signal level. (Default)
- Low Signal – Algorithm based on a set of values selected to compensate of a low signal level from the camera. This setting can compensate for situations in which some pixel intensities are somewhat brighter when slightly out of focus.

## Camera settings

### Binning

Sets the binning used by the camera during the Auto Focus command. Horizontal and vertical binning are always set the same and should be set to less than four.

### Allow custom exposure times

Enables setting custom exposure times for individual wavelengths during autofocus. If this is not selected, the exposure time is calculated based on autofocus binning and acquisition exposure time.

## Find Sample

### Skip Find Sample (select if sample is already in focus)

Disables initial find sample auto focus when starting a plate. Select this option if your sample is already in focus.

### Initial Well for Finding Samples

Select the well to use when performing the initial find sample auto focus.

### Wavelength offsets from W1

Defines how the wavelength offset from the first wavelength is determined when auto focusing. Valid choices include the following:

- None – No offsets are calculated.
- Calculate at start — Offsets are calculated at the start of acquisition.
- Define for each wavelength – Enables you to configure the offset for each wavelength in the Wavelength tabs.

### Site Autofocus

Determines how autofocusing is done for each wavelength when sites are configured. The options are:

**First site only** — auto focuses in the top left site in the well.

**Center of well** — auto focuses in the center of the well. This is the recommended method.

**All sites** — auto focuses for each site.

This option is only available if you have already configured the use of multiple sites in the Sites tab and enabled image-based focusing in the Acquisition Loop tab.

### View Focusing Details

Opens the Auto Focus Details dialog box. This dialog box contains the current autofocus values, as well as descriptions for each parameter. Use the *Copy* button to copy the entire table to the Windows clipboard. The table can then be pasted into a spreadsheet application or Word document and used to help troubleshoot focusing.

## Plate Acquisition Setup Dialog Box Options - Wavelength Tab(s)

**Note:** There is one tab for each wavelength. The number of wavelengths is configured in the Acquisition Loop tab.

### Exposure

#### Illumination setting

Selects an illumination setting to be used with the active wavelength. Illumination settings are defined in the Configure Illumination dialog box.

#### Exposure

Specifies the exposure time in milliseconds to be associated with the active wavelength. Type a value in this box or click Auto Expose to automatically determine an exposure time.

#### Auto Expose

Automatically determines the exposure time for the currently loaded sample, and applies it as the exposure value.

#### Target max. intensity

Sets the intensity that auto exposure should attempt to attain for the brightest pixel in the image. The target intensity value default is 75 percent of the maximum gray level that the camera driver reports as possible to obtain.

### Autofocus

Selects the type of autofocus to be used when acquiring images. The options available here depend on how the autofocus tab was configured. Valid autofocus choices include the following:

- None – No autofocusing is done. This selection enables no other options.
- Laser with Z-offset – Uses the laser autofocus as configured in the Configure Laser Sensor dialog box. This selection enables the Offset (um) setting and is only available for the first wavelength. This option is only available if *Enable laser-based focusing* is selected in the Acquisition Loop tab.
- Z-offset from W1 – This option moves the specified offset from the W1 focus position and is only available for the second and subsequent wavelengths. Enter the Z-offset value (in um) in the Offset field. This option is only available if *Enable laser-based focusing* is selected in the Acquisition Loop tab.
- Image Only – This selection enables the *Range, Max. Step, Exposure,* and *Gain* fields. This option is only available if *Enable image-based focusing* is selected in the Acquisition Loop tab.
- Laser and image – This selection enables using both laser and image based autofocusing as configured in the Autofocus tab. It enables the Image-based range, Max. Step, Exposure, and Gain fields. This option is only available if both *Enable laser-based focusing* and *Enable image-based focusing* are selected in the Acquisition Loop tab.

### Offset (um)

Laser offset to use for either the Laser with *Z-offset* option or the *Z-offset from W1* option.

#### Range

Specifies the total focus range that the focus operation is permitted to occur. This is a plus or minus value from the current or previous focus position. Thus, if the range is +/- 500, the Z motor can move a maximum

of 500 microns in either direction from the current or previous focus position.

### Image-based range

Specifies the range to use for the image-based portions of auto-focusing. Laser auto-focusing is performed to an accuracy equal to this range before image-based auto-focusing begins. This option is only available if both *Enable laser-based focusing* and *Enable image-based focusing* is selected in the Acquisition Loop tab.

### Max step (um)

The maximum step size in microns of a single Z move to be used in attaining the correct focus position. This setting is dependent on the objective used. Use a smaller step size with higher NA objectives because the focus peak is narrower.

**Note:** Smaller step sizes typically require more steps to arrive at the final focus position.

### Exposure (ms)

Specifies the exposure time in milliseconds to be used when auto-focusing.

### Gain

Sets the sensitivity of the camera when used with the Auto Focus command.

### Timelapse acquisition

Specifies the image collection intervals to use for each wavelength. The following choices are available:

**All time points** – Acquires an image for each timepoint for this wavelength.

**At start** – Acquires an image at this wavelength for the first timepoint only.

**At start and end** – Acquires images at this wavelength for the first and last timepoints only.

**Every nth timepoint** – Acquires an image at this wavelength at the selected timepoint interval beginning at the first timepoint.

### Image Shading Correction

Displays the current status of shading correction. This text is only visible if shading correction is enabled on the Acquisition Loop tab.

## Plate Acquisition Setup Dialog Box Options - Journals Tab

### Acquisition step

Specifies that a journal should be run for the selected step. Only one journal can be assigned to each step. Click the check box next to the step that you want to use, then click *File Open* to assign the journal to the step. After you have assigned a journal to a step, you can temporarily deactivate the running of the journal by deselecting (unchecking) the check box for the step. To reactive a pre-assigned journal, simply click the check box.

***Before each Image*** – Runs only during the acquisition loop, after the illumination is set and focusing is done.

***After each image*** – Runs only during the acquisition loop, after the shutter is closed and before images are saved.

***Before focusing*** – Runs only during the acquisition loop, just before focus evaluation begins.

***Start of site*** – Runs only during the acquisition loop, before any images are acquired from each site.

***End of site*** – Runs only during the acquisition loop, after all images have been acquired from each site.

***Start of well*** – Runs only during the acquisition loop, at the beginning of each well, before any images are acquired from a well.

***End of well*** – Runs only during the acquisition loop, at the end of each well, after all images have been acquired from a well.

***Start of plate*** – Runs after the stage is moved to the find sample position, but before the find sample action is performed.

***End of plate*** – Runs after the last acquisition for a plate is complete.

***Start of time point*** – Runs only during the acquisition loop, at the beginning of each time point, before any images are acquired for a time point.

***End of time point*** – Runs only during the acquisition loop, at the end of each time point, after all images have been acquired for a time point.

### File Open 

Opens the Select Screen Acquisition Journal dialog box. Use this dialog box to select and assign a journal to a step. Also use this dialog box to deselect or unassign a journal to a step. To assign a journal, click the checkbox for the acquisition step and click the *File Open* button.

#### Journal

Lists the names of the journals that you have assigned to each step.

#### Prevent asynchronous hardware moves

Select this option if any of the journals you run move hardware (change shutters, move focus, etc.). This ensures that the journals will run correctly.

## Plate Acquisition Setup Dialog Box Options - Display Settings Tab

Use this tab to configure the MetaXpress desktop appearance during acquisition.

#### Use default display settings

Uses the MetaXpress default settings for displaying images and dialog boxed during acquisition. Images are tiled and autoscaled and the status dialog box is unobstructed.

#### Manually set image displays settings

Makes the *Display Images* button visible, which enables you to change the configuration of the MetaXpress desktop during acquisition.

#### Display Images

Previews the current display settings by opening the Screening Status dialog box and acquiring an image for each wavelength. Once all images have been acquired, you can change the configuration of the display by arranging the image windows and dialog box and changing the size, scaling, and LUT of images. These new settings will be saved and used during acquisition.

#### Display images during Auto Focus

Displays the images acquired during autofocus. Disabled by default.

#### Display images during acquisition

Causes each image to be displayed as it is acquired. Disabled by default

## Plate Acquisition Setup Dialog Box Options - Post Acquisition Tab

#### Auto-Run analysis after acquisition

Activates the Analysis drop-down list that enables you to select an analysis to auto-run on a separate MetaXpress computer after each plate is acquired. Refer to the Auto Run Mode help file for more information.

#### Analysis

Selects the analysis to run. This list includes any application modules or journal analyses saved to the database.

#### Settings

Chooses the settings file to use in conjunction with the selected analysis. The list included all settings previously saved to the database. The list of available analyses and settings is the same list that is on the Run Analysis tab of the Review Plate Data dialog box.

### Base Folder

Opens the Measurements Sets dialog box which enables you to select the base folder within the database to store measurements results.

**Note:** You can configure and save settings in the Review Plate Data dialog box.

## Plate Acquisition Setup Dialog Box Options - Summary Tab

### Summary

Lists the current settings selected for your acquisition, the number of selected wells, the number of sites in each well, the distance between images, the number of wavelengths, the total number of images, the amount of storage required, and the specified type of focusing for each wavelength for both the first and the remaining sites in the well.

### Print

Opens the Print Setup dialog box and enables you to print the current settings.

### Acquire Plate

Starts the sequential acquisition of images from a plate based on the current settings.

# Plate Acquisition and Control

**Acquires images from a multi-well plate using Discovery-1 or ImageXpress hardware.**

**Drop-in: PLATEACQUIRE**

*Availability: Exclusive to MetaXpress*

Use this dialog box to acquire plates using the settings defined in the Plate Acquisition Setup command. You can also control the stage and Z-motor from this dialog box, as well as change the current wavelength and save and load settings.

**Note:** Most of the tools available in the Plate Acquisition and Control dialog box are also available in the Plate Acquisition toolbar. To display the Plate Acquisition toolbar, select Window>Toolbars> Plate Acquisition from the MetaXpress menu bar.

There are several possible workflows available for your acquisition. One typical workflow for multi-well plate acquisition is as follows:

- Configure and save your settings file using the Plate Acquisition Setup dialog box.
- Use the Plate Acquisition and Control dialog box or toolbar to do the following:
    - Load your settings file and review the settings using the Summary button.
    - Confirm your settings if needed using the available tools.
    - Enter an experiment base name.
    - Start the acquisition. During acquisition, the acquired images are saved into the database.
- Perform any post-acquisition analysis using the Review Plate Data or Plate Data Utilities dialog boxes. This can be configured to start automatically from the Plate Acquisition Setup dialog box if desired.

## Using the Plate Acquisition and Control Dialog Box

The controls on the Plate Acquisition and Control dialog box allow you to manually control certain microscope functions to enable you to test settings and conditions and acquire preliminary or test images of samples. The *Acquire* button is used to begin the automated acquisition process configured in the Plate Acquisition Setup dialog box. Use the following procedure to familiarize yourself with the controls on the Plate Acquisition and Control dialog box:

Note: Refer to the Plate Acquisition Setup Step by Step Tutorial for a sample setup.

| Step | Action |
|---|---|
| **1** | From the Screening menu, select Plate Acquisition and Control, the Plate Acquisition and Control dialog box opens. |
| **2** | Ensure that a plate is in place on the microscope stage. |
| **3** | To move the plate to A1, click *Go To A1*.<br>**OR**<br>Type the well number for a specific well that you want to view in the *Go to well* box, and click **Go to well**. The plate moves to the desired location. |
| **4** | To change the Z-focus motor position, use the Z-control arrows. |
| **5** | To change the increment the arrows use in the *Z Current position* field, enter a value in the *Step size* field. Set a large value for course movement or a small value for fine movement. |
| **6** | Click *Go to origin* to move the Z focus motor to its origin position as defined in Focus dialog box. |
| **7** | Click *Find Sample* to initiate the Find Sample focusing routine on the current well. |
| **8** | Click *Autofocus* to perform auto focus on the current well using the wavelength selected in the *Wavelength* field. |
| **9** | Click *Load Settings* to open the Load Plate Acquisition Settings dialog box and load a previously saved Settings file. |
| **10** | Click *Save Settings* to open the Save Plate Acquisition Settings dialog box and save the current settings to a file on the local hard drive or to the database. |
| **11** | Click *Summary* to open the Plate Acquisition Summary dialog box and view your current settings. |
| **12** | Click *Setup* to open the Plate Acquisition Setup dialog box and change your acquisition settings. |
| **13** | Click the *Wavelength* drop-down box to select a wavelength that has been defined for the current setting in the Plate Acquisition Setup dialog box. |

**14** Click *Snap Current* to acquire a single image with the current settings.

**15** Click *Show Live* to acquire images so you can manually focus the microscope.

**16** Click *Preview* to open the Plate Acquisition Status dialog box and an image view dialog box for each wavelength. During this time, you can adjust the display of images and windows so that they will be appropriately sized and positioned for acquisition.

**17** Click *Acquire Plate* to acquire images from a plate based on the settings made in Plate Acquisition Setup dialog box.

**18** Click *Close* to exit the dialog box.

## Plate Acquisition and Control - Dialog Box Options

### Plate Navigation

### X, Y Controls

Moves the stage in one well increments in the direction of the selected arrow button.

#### Well

#### Indicates the well currently in position for image acquisition.

#### Site

Indicates a site within a specific well that is currently in position for image acquisition.

#### Go to well

Moves the stage to the well number that you type into the Go to well box.

#### Go To A1

Moves the stage to the A1 position.

#### Go To Stage Load

Moves the stage to Stage Load position. This option is only available for ImageXpress systems.

#### Z Controls

Moves the Z-motor in one-step increments in the direction of the selected arrow button.

#### Go to Origin

Moves the Z-motor to the focus position as defined in the Focus dialog box.

#### Step size

Sets the size of the individual focus increments used by the *Z* control arrows.

### Find Sample

Performs a very coarse auto focus on the current well position. The range covered in Find Sample is the same as the initial Find Sample when starting an Acquire.

### Auto Focus

Performs auto focus on the current well as configured for the current wavelength in the Autofocus plane of the Plate Acquisition Setup tool.

## Acquisition Control

### Load Settings

Loads the selected settings from an existing screening settings file. Settings files are stored either in the database or on the file system. When you click *Load Settings*, the Load Screen Acquisition dialog box opens. Check the boxes for the conditions and groups of settings that you want to load from the settings file, uncheck the ones that you do not want to load. Click *Select All* to load all conditions; click *Clear all* to clear all selections. Click *Load* to load your selected conditions. The Load Settings function is identical to the Load Settings option in the *Experiment* tab of the Plate Acquisition Setup dialog box.

### Summary

Lists the current settings selected for your acquisition, the number of selected wells, the number of sites in each well, the distance between images, the number of wavelengths, the total number of images, the amount of storage required, and the specified type of focusing for each wavelength for both the first and the remaining sites in the well. The Summary function is identical to the *Summary* tab in the Plate Acquisition Setup dialog box.

### Save Settings

Saves the current settings to a file on the local hard drive or to the database. When you click *Save Settings*, the Save Acquisition dialog box opens. Type the name of a new settings file that you want to create, or select a listed settings file name to overwrite an existing settings file.

### Setup

Opens the Plate Acquisition Setup dialog box and enables you to change acquisition settings.

## Experiment base name

Defines the base file name.

### Wavelength

Selects the wavelength to use for your snap or live image.

### Snap Current

Acquires a single image of the currently in place well at the current settings for stage (xy-position), focus (z-position), wavelength, well, site, and exposure.

### Show Live

Continuously acquires images based on the current settings, and updates the image as settings are changed.

### Preview

Previews the current display and exposure settings by opening the Plate Acquisition Status dialog box and autofocusing and acquiring an image for each wavelength. Once all images have been acquired, you can change the configuration of the display by repositioning the image windows and dialog box and changing the size, scaling, and LUT of images. These new settings will be saved and used during acquisition.

### Acquire Plate

Starts the sequential acquisition of images from a plate based on the settings made in Plate Acquisition Settings dialog box.

**Close**

Closes the dialog box.

# Configure Fluidic Stations

**Provides access to all ImageXpress Plate and Tip Tray settings**

**Drop-in: PLATEACQUIRE**

*Availability: Exclusive to MetaXpress*

The Configure Fluidic Stations dialog box provides a graphical representation of the relative position of the plates and tip trays on the ImageXpress 5000 Live Cell/Fluidics Worktable. This arrangement enables you to easily associate specific settings with the physical plate or tip tray to which they belong.

When you begin to configure a fluidics experiment, you should configure this dialog box and the three different dialog boxes that you can access from this one. Even before you begin to make settings in this Configure Fluidic Stations dialog box, you should first click *Define Tips* to open the Define Tips dialog box and define as many different tip types as necessary for your experiment. Then click *System Properties* to set the fluidics properties that will apply to the entire system.

***Configuring Fluidics Stations***

***Configure Fluidic Stations - Dialog Box Options***

## Configuring Fluidics Stations

To configure fluidic stations, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Plate Acquisition Setup – Fluidics dialog box, click Configure Stations. The Configure Fluidic Stations dialog box opens. |
| **2** | In the Configure Fluidic Stations dialog box, click Define Tips. The Define Tips dialog box opens. |
| **3** | Complete the procedure to define tips in the Define Tips dialog box, then return to the Configure Fluidic Stations dialog box. |
| **4** | If you will be tracking the volume of the wells in both the sample plate and the compound plates, click Track Volume.<br><br>**Note:** You must select *Track Volume* if you select *Track Liquid Surface*. |
| **5** | If you will be tracking the liquid surface when aspirating, click *Track Liquid Surface*. Note : If *Track Liquid Surface* is selected, then *Track Volume* must also be selected. |
| **6** | Complete the settings for your sample plate. |
| **7** | In the boxes for *Offset from well center*, type or select appropriate values for X and Y if you need to have the pipette tip enter the well without disturbing the cells within the field of view of the camera. |
| **8** | In the *Volume* box, type or select a value to specify the initial liquid volume for your |

sample plate.

**9** In the *Liquid height* box type or select a value to specify the initial liquid height for your sample plate.

**10** For each tip type that you will use, select the Tip Type from the Tip Type drop down list.

**11** Complete the settings for the Compound Plates according to the instructions in steps 12 through 14.

**12** In the Plate Type box, choose the name of the plate definition for the plate type that you are using. Plates types that appear on this list are defined on the Plate tab of the Plate Acquisition Setup dialog box.

**Note:** Plates used for fluidic events must have either 96 or 384 Wells. However, all defined plates are shown in this drop down list box. If you choose a plate that does not have 96 or 384 wells, an error message is displayed.

**13** In the *Volume* box, type or select the initial volume of the wells in microliters for the specific compound plate associated with the setting.

**14** In the *Liquid height* box, type or select the initial liquid height in micrometers for the specific compound plate associated with the setting.

**15** After you have completed all settings, click *Close* to close the dialog box and return to the *Plate Acquisition Setup – Fluidics tab*.

## Configure Fluidic Stations - Dialog Box Options

### Define Tips

Opens the Define Tips dialog box. Use the Define Tips dialog box to create and save definitions of the various types of disposable pipette tips that you are using. Saved definitions can be recalled at any time for future use, however the definition cannot be modified or deleted and the definition settings cannot be viewed in this or any dialog box. You can only reference the definitions by name.

### Reset Tips/Liquid Levels

Opens the Reset Tips/Liquid Levels dialog box. Use the Reset Tips/Liquid Levels dialog box to reset the count to full for each individual tip tray and reset the liquid levels to their initial values. As tips are used, the system keeps track of the remaining number of tips. When the supply of tips in a tray is exhausted and you replace the tray with a full tray of tips, you must click the associated button on the Reset Tips dialog box to reset the count to full. Liquid levels are also reset using this dialog box. When you click the associated *Reset Liquid Levels* buttons for compound plates and/or the sample plate, levels are reset to their initial specified levels.

### Track Volume

Tracks available volumes. If this option is selected, you must specify the initial volume information for the wells. As volume levels change the values for available volumes are updated.

**Note**: You must select *Track Volume* if you select *Track Liquid Surface*.

**Caution:** An error message will be displayed if Track Volume is selected and the requested draw volume exceeds the recorded available volume.

**Track Liquid Surface**

Determines whether the draw will take place from the top of the liquid, moving the tip downward as the liquid is drawn. If this is selected, you will need to specify the initial fluid level in the wells.

**Note :** If *Track Liquid Surface* is selected, then *Track Volume* must also be selected.

**System Properties**

Opens the Fluidics System Properties dialog box. The System Properties dialog box contains settings used to control the rates at which fluids are drawn and dispensed. It also specifies the amount of air that needs to be drawn into the disposal pipette tips to create a transport air gap to prevent the tip from dripping during movement. This dialog also contains a Settle Time value that is used to allow the tubing to settle after a draw has occurred.

**Sample Plate**

Indicates the relative position of the Sample Plate.

**Offset from well center (µm)**

Specifies X and Y offset values from the center of the well. Offset values enable you specify a location where the tip can enter the sample well in which it is less likely to disturb the cells.

**Caution:** Avoid specifying too great a value for X and/or Y to ensure that the tip does not contact the Environment Chamber Cover or well edge.

**X**

Specifies the X-axis offset value from the center of the well.

**Y**

Specifies the Y-axis offset value from the center of the well.

**Volume (µl)**

Specifies the initial volume in microliters of an individual well in the sample plate. This value is for the syringe and related liquid system to help ensure that wells are not overfilled. Note: The capacity of the well is calculated based on the specified well parameters.

**Liquid Height (µm)**

Specifies the initial liquid height in micrometers the sample plate wells. This value ensures that tip will enter the well correctly relative to the height of the fluid in the well.

**Tip Tray (n)**

There are four possible Tip Tray locations. Each tray can be loaded with a different tip type.

**Tip Type**

Specifies the type of tip that is loaded into the associated tray. The Tip Types shown in the dropdown list result from the Tip Types that you define in the Define Tips dialog box.

**Note:** If two or more tip trays of the same tip type are loaded on the worktable, they are considered to be a single tray or pool of tips. Therefore, when the supply of tips in a tray is exhausted, the program automatically proceeds to the next tip tray.

**Compound Plate (n)**

There are two possible compound plate locations – Compound Plate 1, and Compound Plate 2. Each has

the following three settings:

**Plate Type**

Specifies whether the plate is a 96 (8x12) well plate or a 384 (16x24) well plate. Plates types that appear in this drop down list box are defined on the Plate tab of the Plate Acquisition Setup dialog box. Choose the name of the plate definition for the plate type that you are using.

**Notes:**

- Plates used for fluidic events must have either 96 or 384 Wells. However, all defined plates are shown in this drop down list box. If you choose a plate that does not have 96 or 384 wells, an error message is displayed.
- It is important that you accurately define the dimensions of the plate(s) that you are using for your fluidics event on the Plate tab. Inaccurate definitions can cause the pipette tips and other components of the IX 5000 liquid handling system to be damaged.

**Volume (µl)**

Specifies the initial volume in microliters for the wells in the associated compound plate.

**Liquid height (µm)**

Specifies the initial height in micrometers for the wells in the associated compound plate.

**Close**

Closes the Configure Fluidics Stations dialog box.

# Define Tips

**Defines the dimensional characteristics of the disposable pipette tips that you will be using for your experiments.**

**Drop-in: PLATEACQUIRE**

*Availability: Exclusive to MetaXpress*

There are four tip trays and each tip tray can be loaded with a different type of tip. However, within a single tip tray, all of the tips must be of the same type. This dialog box is used to define each specific type of tip that you will be using and to store the definition. You can assign a name of your choice to each tip specification or use the default name, which is the microliter capacity of the tip. When you make the settings in the Configure Fluidics Stations dialog box, for each tip tray that you will be using you select the name of the type of tip that you previously defined.

***Defining Tips***

***Define Tips - Dialog Box Options***

## Defining Tips

To define a tip definition, complete the following procedure:

**Note:** To accurately define the dimensions of the tip(s) that you will use, refer to the tip manufacturer's published specifications for the tip. If the manufacturer's specifications are not available, use a caliper to obtain the tip measurements.

| Step | Action |
|---|---|
| 1 | In the *Tip Properties* area, select the tip volume that matches the volume of the tip for which you want to create a definition. If the volume of the tip that you want to define |

is not shown, click *Other*, then type or select the tip volume in microliters in the Volume box.

**2** In the *Length* box, type or select the length (Dimension 1) of the tip in micrometers.

**3** In the *Overlap* box, type or select the Overlap distance (Dimension 2) of the tip in micrometers. This dimension specifies the distance that the disposable tip overlaps the robot tip.

**4** In the *Pickup* box, type or select the Pickup distance (Dimension 3) of the tip in micrometers. This dimension specifies the distance that the robot tip needs to move downward in order to pickup the disposable pipette tip.

**5** Click *Save* to save your tip definition. The *Plate Acquisition – Save Configuration* dialog box opens. If you have selected a predefined tip volume, this dialog box field will be prefilled with the selected value followed by µl.

For example, if you selected 200 µl, the default name of your definition will be 200 µl. You can add to or modify this name in any way. If the name that you chose already exists, the following message is displayed: *A tip file 200 ul already exists, OK to replace it?* If you click OK you will overwrite to existing definition with the new definition.

**6** While this dialog box is open, you can create as many tip definitions as needed. When you have finished creating your definitions, click *Close*.

## Define Tips - Dialog Box Options

The Define Tips dialog box contains the following settings:

### Tip Properties

Specifies the tip capacity and three dimensional measurements that are essential for enabling the defined tip to function correctly.

### [Maximum tip capacity]

Defines that maximum fluid capacity of your tip. Four preset values are available. If your tip is not one of these capacities, click *Other* and then type or select the corresponding capacity in the *Volume (µl)* box.

- 20 µl
- 50 µl
- 100 µl
- 200 µl
- Other

### Volume (µl) (optional)

A custom volume capacity value that you can specify for tips that are not one of the preset values.

### Length (µm)

Specifies the total length of the pipette tip.

> **Note:** This value needs to be accurate to ensure that plates and sample material will not be damaged.

### Overlap (µm)

Specifies the distance that the disposable tip overlaps the robot tip.

### Pickup (µm)

Specifies the distance that the robot tip needs to move downward in order to pickup the disposable pipette tip.

### Save

Opens the Plate Acquisition-Save Configuration dialog box. Use this dialog box to specify the name of the tip definition that you want to save. The dialog box edit field is prefilled with the microliter value of the tip. To specify a new name, select the edit field and type the name that you want to use. Your saved tip definitions will appear in the drop down lists for Tip Type in the *Configure Fluidic Stations* dialog box.

### Close

Closes the Define Tips dialog box.

# Reset Tips

**Resets tip counts for tip trays and resets fluid levels for compound sample plates.**

**Drop-in: PLATEACQUIRE**

*Availability: Exclusive to MetaXpress*

Use the Reset Tips/Liquid Levels dialog box to reset the count to full for each individual tip tray and reset the liquid levels to their initial values. As tips are used, the system keeps track of the remaining number of tips. When the supply of tips in a tray is exhausted and you replace the tray with a full tray of tips, you must click the associated button on the Reset Tips dialog box to reset the count to full. Liquid levels are also reset using this dialog box. When you click the associated *Reset Liquid Levels* buttons for compound plates and/or the sample plate, levels are reset to their initial specified levels.

***Reset Tips - Dialog Box Options***

## Reset Tips - Dialog Box Options

### Reset tips

Provides four separate reset buttons to separately reset individual tip trays.

#### Reset tip tray 1

Resets tip tray 1 to a full count of tips.

#### Reset tip tray 2

Resets tip tray 2 to a full count of tips.

#### Reset tip tray 3

Resets tip tray 3 to a full count of tips.

#### Reset tip tray 4

Resets tip tray 4 to a full count of tips.

#### Count

Displays individual counts of the remaining tips for each separate tip tray.

**Reset liquid levels**

Provides three separate reset buttons to separately reset individual plates.

**Reset compound plate 1**

Resets compound plate 1 to its initial level

**Reset compound plate 2**

Resets compound plate 2 to its initial level

**Reset sample plate**

Resets the sample plate to its initial level

# Fluidics System Properties

**Defines the global properties of the MetaXpress/ImageXpress Fluidics System.**

**Drop-in: PLATEACQUIRE**

*Availability: Exclusive to MetaXpress*

Use this dialog box to specify the global parameters and settings for MetaXpress Fluidics running on your ImageXpress system. Typically, these parameters need to be changed only as necessary to match the accuracy requirements of your experiment.

**Note:** If you make and apply any changes to the Fluidics System Properties dialog box, the program will automatically reinitialize the robotic system when you click *OK*.

***Configuring Fluidics System Properties***

***Fluidics System Properties - Dialog Box Options***

## Configuring Fluidics System Properties

To configure the Fluidic System Properties, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Configure Fluidic Stations dialog box, click *System Properties*, the Fluidics System Properties dialog box opens. |
| **2** | In the *Draw Rate* box, type or select an appropriate draw rate for your experiment. The default draw rate is 25 microliters per second.<br><br>**Note:** *Draw Rates* are typically slower, while *Dispense Rates* are typically faster. |
| **3** | In the *Dispense Rate* box, type or select an appropriate Dispense rate for your experiment. The default Dispense rate is 250 microliters per second.<br><br>**Note:** *Dispense Rates* are typically faster, while *Draw Rates* are typically slower. |
| **4** | In the *Extra draw of air transport* box, type or select a value in microliters for an amount of air to serve as a transport air gap to prevent fluid from dripping from the tip during robot movement. |

5 In the *Draw Overfill* box, type or select a value in microliters that represents a small additional amount of compound fluid added to the specified draw volume. This additional amount of fluid is used to overcome surface tension in wells to provide better dispensing accuracy.

6 In the *Pump Settle Time* box, type or select a value in milliseconds as a settle time between dispensing or drawing and movement of the robot.

**Note:** Increasing *Pump Settle Time* helps to provide better drawing and dispensing accuracy; decreasing pump settle time reduces the time between dispensing and the start of image acquisition.

7 Click *OK* to apply any setting changes and return to the Configure Fluidic Stations dialog box, or click *Cancel* to disregard all setting changes and return to the Configure Fluidic Stations dialog box.

## Fluidics System Properties - Dialog Box Options

### Draw Rate

Specifies the rate at which fluid will be drawn from wells.

### Dispense Rate

Specifies the rate at which fluid will be dispensed into wells.

### Extra draw of air transport

Specifies the amount of air that you want to draw into the disposable pipette tip after each fluid draw. This create a transport "air gap" which helps to ensure that leakage does not occur when the robot is moving the tip.

### Draw Overfill

Specifies the amount of compound or fluid that you want to draw as a buffer margin over and above the specified draw volume.

### Pump Settle Time

Specifies the amount of time that the program should wait after drawing or dispensing liquid before activating the robot.

### OK

Applies the settings that you made and closes the dialog box. This also causes the fluidics robot to reinitialize.

### Cancel

Disregards any settings changes that you made and closes the dialog box.

# Fluidic Event

**Enables you to define and schedule fluidic events, and edit previously scheduled fluidic events.**

**Drop-in: PLATEACQUIRE**

*Availability: Exclusive to MetaXpress*

Use this dialog box to define and schedule a fluidic event. In addition, when you click *Edit Event*, this same dialog box is used to edit an already scheduled event. All fluidic events are synchronized with a MetaXpress time point. Relative to the time point with which you choose to associate your fluidic event, you can schedule the event to occur either before imaging for that time point has occurred, or after it has occurred. You can schedule one of three types of events: a compound addition event, a washout event, or an event that initiates the running of a journal of fluidic-based activity.

You can choose from one of two compound plates from which to draw the compound that you will use. For every well on your sample plate to which you want to add compound, the corresponding well on the compound plate is the one from which compound will be drawn to add to the sample plate. Compound plates must have the same number of wells as the sample plate, and in the same configuration. Compounds drawn from a particular well are usually placed in a well labeled with the same number. If a different compound plate with a different number of wells from the sample plate must be used, the fluidic event must be controlled by one or more journals.

For Example, a compound addition event occurring in conjunction with an image acquisition time point for well A1 will draw compound fluid from well A1 in the compound plate and dispense it into well A1 of the sample plate.

> **Note:** When each image acquisition at a different well represents a single time point, there are two opportunities for a fluidic event to occur in conjunction with the time point: a fluidic event before the image acquisition time point and a fluidic event after the image acquisition time point.

For example, the order of events would occur as follows:

1. Fluidics event before the time point for well A1
2. Image acquisition at the time point for Well A1
3. Fluidics event after the time point for well A1
4. Fluidics event before the time point for well A2
5. Image acquisition at the time point of Well A2
6. Fluidics event after the time point for well A2

And so on…

Therefore, ***fluidic events*** scheduled for before the time point and after the time point will both be completed before proceeding to the next time point.

***Fluidic Event Steps***

***Scheduling a Fluidic Event***

***Fluidic Event - Dialog Box Options***

# Fluidic Event Steps

The following paragraphs list the steps that the system completes when running a compound addition event and a washout event. These steps are for informational purposes only; they are not steps for you to complete.

### Compound Addition Event Steps

The following are the steps that the system and robot completes when performing a compound addition event. **Note:** These steps are for informational purposes only; they are not steps for you to complete.

1. Load the first/next new Tip from the tip tray.
   **Note:** When two or more tip trays contain the same type of tip, the system considers all identical trays as a single, extended tray, and will automatically proceeded to the next available tray when the current tray is exhausted of tips.
2. Move to the first/next well on the compound plate.
3. Draw the specified amount of compound from the compound plate.
4. Move the tip to the associated well in the sample plate.
5. Dispense compound fluid into the sample well.
6. Run the specified number of mix cycles. **Note:** If *Mix while imaging* is specified, image acquisition will initiate between steps 5 and 6.
7. Move the tip to the waste station and eject the tip.

### Washout Event Steps

The following are the steps that the system and robot completes when performing a washout event.
**Note:** These steps are for informational purposes only; they are not steps for you to complete.

1. Load the first/next new Tip from the tip tray.
   **Note:** When two or more tip trays contain the same type of tip, the system considers all identical trays as a single, extended tray, and will automatically proceeded to the next available tray when the current tray is exhausted of tips.
2. Move to the first/next well on the sample plate.
3. Draw the specified amount of fluid from the well in the sample plate.
4. Move the tip to the waste station and dispense all the fluid in the tip. If a new tip is specified, eject the tip.
5. Move the robot to the next available tip in the tip tray and load a new tip.
6. Move the robot to first/next well in the compound/buffer plate and draw the specified amount of fluid.
7. Move the tip back to the well in the sample plate that you are washing out.
8. Dispense compound fluid into the sample well.
9. If another washout exchange is scheduled, repeat steps 2 through 8.
10. Move the tip to the waste station and eject the tip.

## Scheduling a Fluidic Event

To schedule or edit a fluidic event, complete the following procedure:

| Step | Action |
|---|---|
| **1** | In the *Time point* box, type or select the time point with which you want to synchronize this event. |
| **2** | Click either *Before imaging* or *After imaging*, depending on whether you want your fluidic event to run before or after image acquisition has occurred. |
| **3** | In the *Event Type* area, choose the type of |

fluidic event that you want to run. Click Compound addition, Washout or Journal. The dialog box options are different for each of these, and the options shown in the dialog box will change when you choose a different selection.

**Note:** If you are scheduling a Journal-based event, click the file selector folder, and select the journal you want to run in the *Select Fluidic Event Journal* dialog box, then skip to Step 10.

**4** In the *Compound plate* box, select the compound plate that you want to use for this event.

**5** In the *Tip* box select the type of tip that you want to use. The tips on this dropdown list are the ones that you previously defined in the ***Define Tips*** dialog box.

**Note:** You can define additional tip types in the ***Define Tips*** dialog box whenever necessary.

**6** In the *Volume* box, type or select the volume of compound fluid that you want to add to the corresponding sample well.

**7** In the *Number of Mixes* box, type or select the number of mix cycles that you want to run. Each mix cycle consists of fluid draw followed by re-dispensing.
***OR***
If you are scheduling a *Washout* event, this setting will be *Number of exchanges*. Type or select the number of exchanges required. If you need maximum cross-contamination prevention, click *New tip each exchange*.

**Notes:**

- Washouts typically use a buffer solution from a different plate.
- If you are scheduling a *Washout* event, skip to Step 10.

**8** In the *Mix Volume* box, type or select the volume of fluid that you want to include in the mixing process. If you want to perform the mix while you are acquiring images, click *Mix while imaging*.

**9** In the *Mix Dead Volume* box, for mixes that incorporate more than one mix cycle, type or select the volume of fluid that you want to remain in the tip during the intermediate cycles of the mixing process. The *Mix Dead Volume* helps ensure that air is not injected into the well during multiple mix cycles. Note: This setting does not apply to single mix cycles.

**10** In the Wells Affected area, click *All wells* if you want to apply this fluidic event to all

wells for which you are acquiring images at the selected time point. Click *Select Wells* to open the *Select Wells for Fluidic Event* dialog box, which is a well selection grid. Click individual wells to choose individual wells for the fluidic event; click a row or column to choose an entire row or column. Click *All* to activate all wells. Note: If the well selection for a specific well on this grid does not coincide with the wells selected for acquisition on the *Wells to visit* tab, then no fluidic event will occur for that well.

**11** After you have completed making all settings in this dialog box, click *Test at A1*. The settings that you made will be used to run the IX 5000 fluidics system through the event that you have defined without adding it to the event schedule. This will enable you to test your settings and verify that they are correct.

**12** After you have run a successful test on well A1, click *OK* to schedule the event.

## Fluidic Event - Dialog Box Options

### Time point

Selects the time point with which you want to synchronize your fluidic event.

### Before Imaging

Specifies that the event will occur at the start of the imaging cycle for the time point.

### After Imaging

Specifies that the event will occur at the end of the imaging cycle after all images have been acquired for the selected time point.

### Event Type

Defines the type of event that you want to take place. There are three types of events:

- ***Compound Addition*** – Schedules the addition of a compound.
- ***Washout*** – Schedules a washout event.
- ***Journal*** – Enables you to use a Journal to control this fluidic event.

**Note:** The dialog box options are different for each of these choices; therefore, the options shown in the dialog box will change when you choose a different selection.

### Compound Plate

Selects the compound plate that will be used for this fluidic event.

### Tip

Selects the type of disposable pipette tip to be used from the list of tip definitions that you created.

### Volume

Specifies the volume of liquid in microliters to add to the selected well. If *Track Volume* is turned on, the system will ensure that the volume capacity of the well is not exceeded.

**Number of Mixes** (Compound addition only)

Specifies the number of mix cycles to run during this fluidic event.

**Mix Volume** (Compound addition only)

Specifies the amount of fluid to aspirate during a mix cycle. During the mix cycle, the tip remains in the draw position, near the bottom of the well, for both drawing and dispensing.

**Mix while imaging** (Compound addition only)

Initiates image acquisition while mixing is still occurring .

**Mix Dead Volume** (Compound addition only)

Specifies an additional volume of fluid in the tip that is not included in the mixing process. When you are running mixes that consist of more than a single cycle, the *Dead Volume* specifies an amount of fluid that is to remain in the tip and not be returned to the well during intermediate repeated cycles of draw and dispense. On the final dispense cycle, all of the fluid in the tip including the dead volume is returned to the well. This helps to ensure that air is not dispensed into the well during the intermediate dispense cycles.

**Note:** This setting applies only if *Number of Mixes* is greater than 1.

**Number of Exchanges** (Washout only)

Specifies the number of times that the washout should be performed.

**New tip each exchange** (Washout only)

Specifies that a new tip must be loaded for each exchange.

**Wells Affected**

- ***All Wells*** – All wells in the plate will be processed with this fluidic event.
- ***Selected Wells*** – Only wells selected on the *Select Wells for Fluidic Event* dialog box will be processed with this fluidic event.

**Select Wells**

Opens the *Select Wells for Fluidic Event* dialog box. Click the Wells or Well Rows and/or Columns to select wells. **Hint:** To selectively exclude only certain wells, click *All* to select the entire plate, then click the wells that you want to exclude from processing in this fluidic event.

**OK**

Applies the settings and schedules the event. Click *OK* to accept the settings for this event and to place the event on the event schedule.

**Test at A1**

Applies this fluidic event process settings to well A1, but does not schedule the event. Use this option to perform an initial test of the settings that you made.

**Cancel**

Disregards all settings and closes the dialog box. Does not schedule the event.

# Fluidic Control

**Provides a fluidics interface to enable you to manually run and test individual commands and to include individually configured commands in journals.**

**Drop-in: PLATEACQUIRE**

*Availability: Exclusive to MetaXpress*

The fluidic control dialog box can be used in one of the following two ways:

- Operating the fluidics robot manually to develop and test your planned automated experiment procedure.
- Recording a journal that you will run later from the Fluidic Event dialog box.

**Note:** Refer to the *ImageXpress 5000A Hardware User's Guide*, Chapter 6, *Live Cell Addition,* for procedures on preparing the IX 5000 fluidics system for Live Cell operation.

## Fluidic Control - Dialog Box Options

### Action

Defines the type of action that you want to complete. Open the drop-down list to choose one of the following actions:

**Note:** Depending on the action that you choose, the options that are displayed can vary.

- **Move** – Moves the tip robot to the location that you choose from the *Station* drop-down list.
- **Draw** – Aspirates the specified amount of fluid at the specified position in the well.
- **Dispense** – Dispenses the specified amount of fluid at the specified position in the well or at the waste stations.
- **Mix** – Runs the specified number of mix cycles during which the system aspirates and dispenses the specified amount of fluid at the specified positions in the well.
- **Reset Liquid Levels** – Allows you to reset the liquid level for a plate.
- **Get Next Tip** – Retrieves the next available tip of the tip type that you have selected from your list of available tip definitions.
- **Eject Tip** – Ejects the tip that is currently in place.
- **Reset Tips** – Resets the tip count for the selected tip tray to either 96 or 384, according to which one is currently selected.
- **Prime** – Primes the fluid system between the tip transport mechanism and the syringe.
- **Flush Tip** – Flushes compound or fluid from tip in preparation for the next fluid draw.

### Station

Choose from following list of locations

- Tip Tray 1
- Tip Tray 2
- Tip Tray 3

- Tip Tray 4
- Compound Plate 1
- Compound Plate 2
- Sample Plate
- Waste
- Prime
- Home

**Row** – A-H – Select the Row Location

**Column** – Type or select the Column number.

**Volume** (µl) – Type or select the amount of fluid in microliters that you want to draw.

**Mixes** – Type or select the number of mix cycles that you want to run.

**Dead Volume** (µl) – When you are running mixes that consist of more than a single cycle, the *Dead Volume* specifies an amount of fluid that is to remain in the tip and not be returned to the well during intermediate repeated cycles of draw and dispense. On the final dispense cycle, all of the fluid in the tip including the dead volume is returned to the well. This helps to ensure that air is not dispensed into the well during the intermediate dispense cycles. **Note:** this setting applies only if *Mixes* is greater than 1.

**Tip Type** – Selects the appropriate tip configuration.

**Prime %** – Type or select the percentage of priming that you want to apply. The default value is 110%. You need to prime your fluidics system whenever air bubbles have formed in the system tubing. When you prime the system, The entire pathway is flushed with distilled water. Then, at the completion of the flushing process, the syringe draws back 100 microliters of air to serve as a buffer between the sample fluids drawn into the disposable pipette tip, and the distilled water with which the remainder of the system is filled.

# Plate Acquisition Toolbar

**The Plate Acquisition toolbar contains tools used to control the hardware on the MetaXpress screening system.**

**Drop-in: PLATEACQUIRE**

*Availability: Exclusive to MetaXpress*

If the Plate Acquisition toolbar is not currently loaded, you can enable it by going to the Window menu and selecting Toolbars>Plate Acquisition. The following tools are available on the Plate Acquisition toolbar:

| Tool | Description |
|---|---|
|  | Moves the stage up in one-well increments. |
|  | Moves the stage down in one-well increments. |
|  | Moves the stage forward in one-well increments. |
|  | Moves the stage backward in one-well increments. |
|  | Moves the stage forward in one-site increments. |
|  | Moves the stage backward in one-site increments. |
|  | Moves the stage to the load/eject position. This option is only available with the ImageXpress. |
| Well: A02, Site: 1 | Current well and/or site position. |
|  | Moves the Z position (focus) upward in single step increments. |
|  | Moves the Z position (focus) downward in single step increments. |
|  | Performs a very coarse auto focus on the current well position. The range covered in Find Sample is the same as the initial Find Sample when starting an Acquire. |
|  | Performs auto focus on the current well as configured for the current wavelength in the Autofocus plane of the Plate Acquisition Setup tool. |
| Wavelength: | Selects the wavelength to use for your snap or live image. |
|  | Acquires a single image of the currently in place well at the current settings for stage (xy-position), focus (z-position), wavelength, well, site, and exposure. |
|  | Show Live continuously acquires images based on the current settings, and updates the image as settings are changed. |
|  | Loads the selected settings from an existing screening settings file. Settings files are stored either in the database or on the file system. When you click Load Settings, the Load Screen Acquisition dialog box opens. Check the boxes for the conditions and groups of settings that you want to load from the settings file, uncheck the ones that you do not want to load. |
|  | Screen Summery lists the current settings selected for your acquisition, the number of selected wells, the |

number of sites in each well, the distance between images, the number of wavelengths, the total number of images, the amount of storage required, and the specified type of focusing for each wavelength for both the first and the remaining sites in the well.

Previews the current display and exposure settings by opening the Plate Acquisition Status dialog box and autofocusing and acquiring an image for each wavelength. Once all images have been acquired, you can change the configuration of the display by repositioning the image windows and dialog box and changing the size, scaling, and LUT of images. These new settings will be saved and used during acquisition.

Starts the sequential acquisition of images from a plate based on the settings made in Plate Acquisition Settings dialog box.

# Plate Acquisition

**Acquires images from a multi-well plate using MetaXpress.**

**Drop-in: PLATEACQUIRE**

*Availability: Exclusive to MetaXpress*

Use this dialog box to quickly start acquiring plates using any of the settings defined in the Plate Acquisition Setup command. You can also view a summary of the current settings file and change the base name of the experiment from this dialog box.

**Note:** To perform additional configuration of the experiment before starting the acquisition, use the Plate Acquisition and Control dialog box.

There are several possible workflows available for your acquisition. One typical workflow for multi-well plate acquisition is as follows:

- Configure and save your settings file using the Plate Acquisition Setup dialog box.
- Use the Plate Acquisition and Control dialog box or toolbar to do the following:
  - Load your settings file and review the settings using the Summary button.
  - Confirm your settings if needed using the available tools.
  - Enter an experiment base name.
  - Start the acquisition. During acquisition, the acquired images are saved into the database.
- Perform any post-acquisition analysis using the Review Plate Data or Plate Data Utilities dialog boxes. This can be configured to start automatically from the Plate Acquisition Setup dialog box if desired.

## Using Plate Acquisition

Use the following procedure to familiarize yourself with the controls on the Plate dialog box:

| Step | Action |
|---|---|
| **1** | From the Screening menu, select Plate Acquisition, the Plate Acquisition dialog box opens. |
| **2** | Ensure that a plate is in place on the microscope stage. |
| **3** | Select a Settings file to use from the *Settings* drop-down list. |
| **4** | To change the experiment name, enter a name in the *Experiment Base Name* field. |
| **5** | Click *Summary* to view details about the current settings. |
| **6** | Click *Load* to open the Load Screen Acquisition dialog box opens. Check the boxes for the conditions and groups of settings that you want to load from the settings file, uncheck the ones that you do not want to load. Click *Select All* to load all conditions; click *Clear all* to clear all selections. Click *Load* to load your selected conditions. |
| **7** | Click *Acquire Plate* to acquire images from a plate based on the current settings. |
| **8** | Click *Close* to exit the dialog box. |

## Plate Acquisition - Dialog Box Options

### Settings

Contains a list of all settings currently available. These settings are configured in the Plate Acquisition Setup dialog box.

### Experiment Base Name

Defines the base file name.

### Summary

Lists the current settings selected for your acquisition; the number of selected wells, the number of sites in each well, the distance between images, the number of wavelengths, the total number of images, the amount of storage required, and the specified type of focusing for each wavelength for both the first and the remaining sites in the well.

### Load

Loads the selected settings from an existing screening settings file. When you click Load Settings, the Load Screen Acquisition dialog box opens. Check the boxes for the conditions and groups of settings that you want to load from the settings file, uncheck the ones that you do not want to load. Click *Select All* to load all conditions; click *Clear all* to clear all selections. Click *Load* to load your selected conditions.

### Acquire Plate

Starts the sequential acquisition of images from a plate based on the settings made in Plate Acquisition Settings dialog box.

**Close**
Closes the dialog box.

# Shading Correction

Shading correction images are useful for low magnification image acquisition, but are usually not needed for higher magnification image acquisition. A shading correction image should be captured from a uniform target. The fluorescent plastic plate included with your MetaXpress system can be used as a target. Locate a clean area on the plate without scratches, or acquire multiple images, and then average them. It is also recommended that you then run a smoothing operation such as Low Pass or FFT, then subtract the camera black level.

**Caution:** During acquisition, do not allow the shading image to reach saturation. The shading image should have as great a dynamic range as possible without attaining saturation.

***Go to*** the ***Correct Shading*** dialog box topic for more information.

***Return to Acquisition Loop Tutorial Page***

# Load Plate Acquisition Settings

**Loads saved acquisition settings files to use in an experiment.**

*Availability: Exclusive to MetaXpress*

**Drop-in: PLATEACQUIRE**

Loads the selected settings from an existing MetaXpress acquisition settings file. You can load all the settings from a saved file, or use the check boxes to select specific conditions or groups of settings to load.

After settings files are created and saved using the Plate Acquisition Setup dialog box, they can be loaded using the following dialog boxes, all found under the Screening menu of MetaXpress:

- Plate Acquisition Setup
- Plate Acquisition and Control
- Plate Acquisition

You can also use the Load Settings button on the Plate Acquisition toolbar to access the Load Plate Acquisition Settings dialog box.

## Loading the Plate Acquisition Settings File

To load a saved screen acquisition state file from one of the Plate Acquisition dialog boxes, complete the following steps:

| Step | Action |
|---|---|
| **1** | From the *Screening* menu, open one of the following:<br>o Plate Acquisition and Control<br>o Plate Acquisition |
| **2** | Click *Load Settings*. The Load Plate Acquisition Settings dialog box opens. |

3 If you are loading a settings file that was saved to the database, select the settings file to load from the Settings File drop-down list. Then select the individual settings to load from the file using the check boxes next to each settings group.

**OR**

If you are loading a settings file that was saved to your hard drive, select *From File* in the drop-down list and then select the individual settings to load from the file using the check boxes next to each settings group.

4 If you are using a settings file from the database, click *Load*. The file will load from the database and the Load Plate Acquisition Settings dialog box will close.

**OR**

If you are loading a settings file saved to your hard drive, click *Load*. The Load Screen state dialog box opens. Navigate to the state file (.HTS) you want to open and click *Open*. The state file you selected will load and the Load Screen Acquisition Settings dialog box will close.

## Load Plate Acquisition Setting - Dialog Box Options

### Settings File List

Selects a saved settings file from the database or from a local file on your hard drive.

These checkboxes enable or disable the loading of specific settings from a settings file. The settings listed in the Load Plate Acquisition Settings dialog box are configured on the various tabs of the Plate Acquisition Setup dialog box.

### Settings

Select the checkbox next to each setting to load it with your settings file.

### Select All

Selects all the settings options.

### Clear All

Clears all the settings options.

### OK

Loads the settings file selected in the Settings File drop-down list or opens the Load Plate Setting dialog box, enabling you to select a saved settings file stored outside the database.

> **Note**: You can only selected a settings file stored outside your database if *From File* is selected in the Settings drop-down list.

### Cancel

Cancels the command and closes the dialog box.

# Save Acquisition Setting

**Saves Acquisition settings files to the database or a local file.**

*Availability: Exclusive to MetaXpress*

**Drop-in: HTACQUIR**

Saves the current settings in the Screen Acquisition dialog box to a settings file stored either in the database or locally on your machine.

## Saving the Plate Acquisition Settings

To save the current Plate Acquisition settings, complete the following steps:

| Step | Action |
|---|---|
| | From the *Screening* menu, open one of the following:<br>o Plate Acquisition and Control<br>o Plate Acquisition<br>o Plate Acquisition Setup |
| **1** | Click *Save Settings*. The Save Acquisition Settings dialog box opens. |
| **2** | To save the settings file on the local computer, select *Save to file rather than database* and proceed to Step 3. To save the settings file to the database, skip to Step 5. |
| **3** | Click *Save*. The Plate Acquisition Setting dialog box opens. |
| **4** | Type the name of a new setting file that you want to create in the *File name* field, or select a listed settings file name to overwrite an existing state file and click *Save*. |
| **5** | To save the settings file to the database, ensure that *Save to file rather than database* is not selected and type the name of a new settings file that you want to create in the *Setting Name* field, or select a listed settings file from the Stored Settings field to overwrite an existing settings file in the database and click *Save*. |

## Save Acquisition Setting- Dialog Box Options

### Save to file rather than database

Enables the state file to be saved to a file on the local computer instead of in the database.

### Setting Name

Enter a setting file name in this field.

### Stored Settings

Contains a list setting files saved in the screening database.

**Save**

Saves the current acquisition setting to the database using the name selected in the Setting Name field. If *Save to file rather than database* is selected, the *Save* button opens the Plate Acquisition Setting dialog box.

**Cancel**

Closes the dialog box without taking any action.

# Configure Laser Sensor

**Configures the MetaXpress laser focus system settings.**

*Availability: Exclusive to MetaXpress*

**Drop-in: PLATEACQUIRE**

Use this dialog box, in conjunction with the Plate Acquisition Setup Autofocus tab, to configure the settings for the MetaXpress laser focus system. From this dialog box you can make settings that define the operating parameters of the focus sensor for each objective and plate type.

**Note:** For instructions on how to configure this dialog box, refer to the Plate Acquisition Setup Tutorial - Autofocus Tab.

**Note:** Once configured correctly, the settings in this dialog box usually do not need to be modified.

**Plate Acquisition Setup Home**

**Configure Laser Sensor Dialog Box Options**

## Configure Laser Sensor - Dialog Box Options

The options available in the Configure Laser Sensor dialog box vary depending on your hardware platform:

**ImageXpress 5000 Hardware Platform**

**ImageXpress Micro Hardware Platform**

**Discovery-1 Hardware Platform**

## Configure Laser Sensor - Discovery-1 Dialog Box Options

**Dialog Box Options for Discovery-1 Hardware Platform**

**Acquisition Settings for Mag: xx**

Lists the laser sensor settings for the active magnification setting. The active magnification setting is the one currently selected in the Objective/Camera tab. Changes made to each setting are saved in a state file and used going forward for laser auto focusing unless the defaults are restored.

**Note:** Change these settings only if the default values do not produce the best results. To get the best results, set these values so that the resulting image, acquired using the Focus Snap command, has maximum grey level intensity values of ~1000 when the laser is focused on the bottom surface of the well.

**Note:** These settings are only used when auto-focusing using the Laser Auto Focus and are not used when during image acquisition. They are independent of the acquisition settings set in the in the Objective/Camera tab.

**Set to Defaults**

Restores the default settings to the active magnification setting.

**Save Settings**

Saves the settings to the current plate settings configured in the Plate tab.

**Note**: You must have a custom plate configuration open in the Plate tab for the settings to be saved. The default 96- or 346-well settings files will not save your laser focus settings.

### Well Reflectivity

#### Dim

Sets an illumination setting used during laser auto focus suited for wells with little reflectivity, such as those with plastic plates or those using media.

#### Bright

Sets an illumination setting used during laser auto focus suited for wells with higher reflectivity This is useful when using some types of glass plates, or wells without any media, that are highly reflective. This setting uses a filter in the emission path to reduce the intensity.

### Binning

Sets the binning used by the camera during the Auto Focus command. Horizontal and vertical binning are always set the same and should be set to less than four.

### Gain

Sets the sensitivity of the camera when used with the Auto Focus command.

### Exposure; [plate bottom]

Specifies the exposure time in milliseconds to be used when the laser is looking for the bottom of the plate.

### Exposure; [well bottom]

Specifies the exposure time in milliseconds to be used when the laser is looking for the bottom of the well. This value should be higher than the plate bottom exposure time.

### Max Step

The maximum step size in microns of a single Z move to be used in attaining the correct focus position. This setting is dependant on the objective used. Use a smaller step size with higher NA objectives because the focus peak is narrower.

**Note:** Smaller step sizes typically require more steps to arrive at the final focus position.

## Region

**Note:** The Region settings are only available when using the Discovery-1 hardware platform. ImageXpress region settings can be adjusted using the ImageXpress camera dialog box in the Meta Imaging Series Administrator.

### Full Chip

Uses the full CCD chip when the sensor is auto focusing.

### User Defined

Enables you to define a region of interest to use when auto-focusing. Choosing a smaller region then the default center quadrant can reduce the time needed for auto-focusing; however, you must ensure that the region is large enough to contain the sensor spot when it is in focus at each objective.

### X

Sets the X position of the user-defined auto focus region of interest. User Defined must be selected to enable this setting.

### Y

Sets the Y position of the user-defined auto focus region of interest. User Defined must be selected to enable this setting.

#### Width

Sets the width of the user-defined auto focus region of interest. User Defined must be selected to enable this setting.

#### Height

Sets the height of the user-defined auto focus region of interest. User Defined must be selected to enable this setting.

#### Set to Active Region

Sets the auto focus region of interest to the active region on the desktop.

### Use Bottom of Well Settings

Uses the bottom of well exposure setting when acquiring images using *Test Sensor* or *Focus Snap*.

### Test Sensor

Acquires a live image window showing the current auto focus settings image. Press F2 or *Stop Test* to stop updating the image.

### Focus Snap

Acquires an image using the current auto focus settings.

### Close

Closes the dialog box and saves the most recent settings.

## Configure Laser Sensor - ImageXpress 5000 Dialog Box Options

### Dialog Box Options for ImageXpress 5000 Hardware Platform:

### Acquisition Settings for Mag: xx

Lists the laser sensor settings for the active magnification setting. The active magnification setting is the one currently selected in the Objective/Camera tab. Changes made to each setting are saved in a state file and used going forward for laser auto focusing unless the defaults are restored.

**Note:** Change these settings only if the default values do not produce the best results. To get the best results, set these values so that the resulting image, acquired using the Focus Snap command, has maximum grey level intensity values of ~1000 when the laser is focused on the bottom surface of the well.

**Note:** These settings are only used when auto-focusing using the Laser Auto Focus and are not used when during image acquisition. They are independent of the acquisition settings set in the in the Objective/Camera tab.

#### Set to Defaults

Restores the default settings to the active magnification setting.

### Save Settings

Saves the settings to the current plate settings configured in the Plate tab.

**Note**: You must have a custom plate configuration open in the Plate tab for the settings to be saved. The default 96- or 346-well settings files will not save your laser focus settings.

#### Well Reflectivity

##### Dim

Sets an illumination setting used during laser auto focus suited for wells with little reflectivity, such as those with plastic plates or those using media.

##### Bright

Sets an illumination setting used during laser auto focus suited for wells with higher reflectivity This is useful when using some types of glass plates, or wells without any media, that are highly reflective. This setting uses a filter in the emission path to reduce the intensity.

**Bottom of Plate**

**Exposure**

Specifies the exposure time in milliseconds to be used when the laser is looking for the bottom of the plate.

**Max. Step**

The maximum step size in microns of a single Z move to be used in attaining the correct focus position at the bottom of the plate.

**Laser Power**

Sets the laser intensity when the laser is looking for the bottom of the plate. The Laser power setting is only available when using the ImageXpress hardware platform.

**Bottom of Well**

**Exposure**

Specifies the exposure time in milliseconds to be used when the laser is looking for the bottom of the well.

**Max. Step**

The maximum step size in microns of a single Z move to be used in attaining the correct focus position at the bottom of the well.

**Laser Power**

Sets the laser intensity when the laser is looking for the bottom of the well. The Laser power setting is only available when using the ImageXpress hardware platform.

**Use Bottom of Well Settings**

Uses the bottom of well exposure setting when acquiring images using *Test Sensor* or *Focus Snap*.

**Test Sensor**

Acquires a live image window showing the current auto focus settings image. Press F2 or *Stop Test* to stop updating the image.

**Focus Snap**

Acquires an image using the current auto focus settings.

**Close**

Closes the dialog box and saves the most recent settings.

## Configure Laser Sensor - ImageXpress Micro Dialog Box Options

**Acquisition Settings for Mag: xx**

Lists the laser sensor settings for the active magnification setting. The active magnification setting is the one currently selected in the Objective/Camera tab. Changes made to each setting are saved in a state file and used going forward for laser auto focusing unless the defaults are restored.

**Note:** The default values are for guidelines only and vary depending on the active objective. To get the best results, set these values so that the peaks on the Preview Pass graph are as sharp as possible.

**Note:** These settings are only used when auto-focusing using the Laser Auto Focus and are not used when during image acquisition. They are independent of the acquisition settings set in the in the Objective/Camera tab.

**Set to Defaults**

Restores the default settings to the active magnification setting.

**Save Settings**

Saves the settings to the current plate settings configured in the Plate tab.

**Note**: You must have a custom plate configuration open in the Plate tab for the settings to be saved. The default 96- or 348-well settings files will not save your laser focus settings.

**Exposure (plate bottom)**

Specifies the exposure time in micro-seconds to be used when the laser is looking for the bottom of the plate.

**Exposure (well bottom)**

Specifies the exposure time in micro-seconds to be used when the laser is looking for the bottom of the well.

**Coarse step**

Z-motor step size used during the initial stage of autofocusing. The default value varies for each objective.

**Fine step**

Z-motor step size used during the second stage of autofocusing. The default value varies for each objective.

**Laser Power**

Sets the laser intensity. Valid values are 1-100. 100 is the default.

**Use Bottom of Well Settings**

Uses the bottom of well exposure setting when using *Preview Pass* or *Focus Snap*.

**Start preview pass from current position**

Use this option to run the *Preview Pass* function from the current Z position. This is useful in checking the current exposure values.

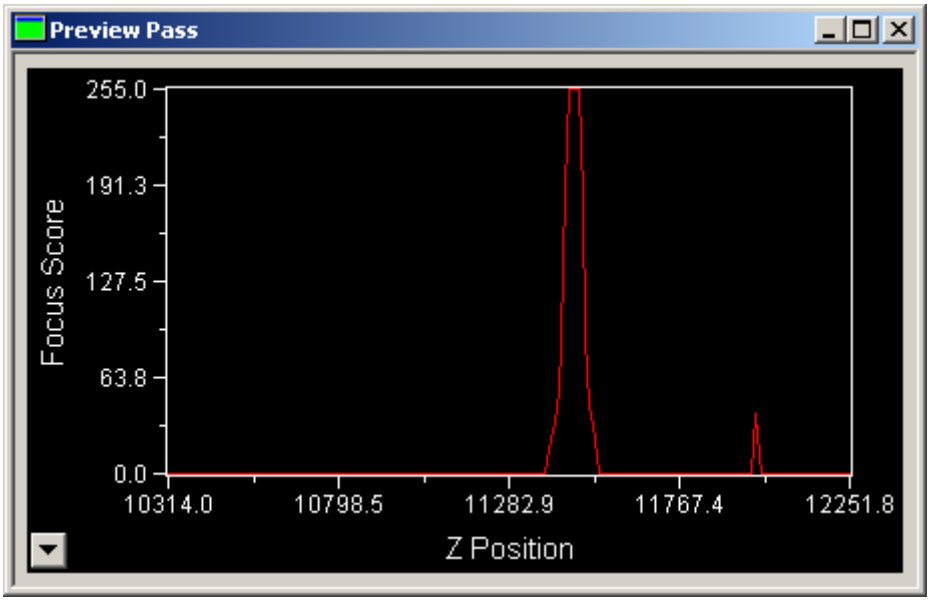
**Find Sample**

Performs a wide range auto focus on the current well position. Find Sample attempts to find the bottom of the plate, and then the bottom of the well (if configured on *Autofocus* tab). Displays a message box containing the results of the autofocus.

**Preview Pass**

Run the Z-motor through a range calculated from the current plate file (unless *Start preview pass from current position* is selected). Opens a graph that charts the focus values of each Z position. If *Use Bottom of Well settings* is checked, the well bottom exposure value, along with the *Fine Step* value, are used in the preview pass. If *Use Bottom of Well settings* is not checked, the plate bottom exposure and *Coarse Stop* values are used.

Use the Preview pass function to determine the optimal exposure settings to use during autofocus. Ideally, the graph will show two sharp peaks that represents the focus values at the bottom of the plate and the bottom of the well, as shown below:




**Autofocus**

Performs auto focus on the current well position using the current settings. Autofocus attempts to find bottom of well only. If unable to search for bottom of well only, a full Find Sample search is done. Displays a message box containing the results of the autofocus.

**Focus Snap**

Acquires an image using the current auto focus settings.

**Close**

Closes the dialog box and saves the most recent settings.

# Plate Data Utilities

**Enables you to import images into the database, run analyses on selected images, and delete plates, images, and measurements.**

*Availability: Exclusive to MetaXpress*

Use the Plate Data Utilities command to manage images and data acquired during plate acquisition. You can perform the following actions from this dialog:

- Run analysis on plates
- Import images into the database or the file server
- Export images to a local or networked drive
- Delete plates
- Delete measurements associated with plates
- Delete images associated with plates
- Permanently remove data from the database/fileserver

## Using Plate Data Utilities

To use the Plate Data Utilities dialog box, complete all or part of the following procedure:

| Step | Action |
|---|---|
| 1 | From the Screening menu, choose Plate Data Utilities. The Plate Data Utilities dialog box opens. |
| 2 | To run analysis on a selected plate, click *Run Analysis*. The Plate dialog box opens. |
| 3 | Expand the plates folder in the top pane of the dialog to view folders containing plates saved to the database. |
| 4 | Double-click a folder to view its name, date created, creator, and barcode (if applicable) on the bottom pane. |
| 5 | Select the plate to run analysis on and click *OK*. The Run Analysis on Plates dialog box opens. Follow the instructions in the Run Analysis on Plates help file to complete importing the images. |
| 6 | Click *Close* to close the Plate Data Utilities dialog box. |
| 7 | To import a new data set into the database or file system, click *Import Images.* The Import Images dialog box opens. Follow the instructions in the Import Images help file to complete importing the images. |
| 8 | To export images to a local or networked server, click Export Images. The Plate dialog box opens. |
| 9 | Expand the plate's folder in the top pane of the dialog to view folders containing plates saved to the database. |
| 10 | Double-click a folder to view its name, date created, creator, and barcode (if applicable) on the bottom pane. |
| 11 | Select the plate to import from the bottom pane and click *OK*. |
| 12 | To delete measurements associated with a plate, click *Delete Measurements*. The Plate dialog box opens. |
| 13 | Expand the plate's folder in the top pane of the dialog to view folders containing plates saved to the database. |
| 14 | Double-click a folder to view its name, date created, creator, and barcode (if applicable) on the bottom pane. |
| 15 | Select the plate containing measurement data to delete from the bottom pane and click *OK*.<br>**Note**: This step removes the data from the Plate dialog box but not from the database/fileserver. To remove the data from the database/fileserver, you must click |

*Remove Deleted Data* after using the Plate dialog box. Once the data has been deleted it cannot be recovered.

**16** To delete images associated with a plate, click *Delete Images*. The Plate dialog box opens.

**17** Expand the plate's folder in the top pane of the dialog to view folders containing plates saved to the database.

**18** Double-click a folder to view its name, date created, creator, and barcode (if applicable) on the bottom pane.

**19** Select the plate containing images to delete from the bottom pane and click *OK*.

**Note**: This step removes the data from the Plate dialog box but not from the database/fileserver. To remove the data from the database/fileserver, you must click *Remove Deleted Data* after using the Plate dialog box. Once the data has been deleted it cannot be recovered.

**20** To delete a plate, click *Delete Plates*. The Plate dialog box opens.

**21** Expand the plate's folder in the top pane of the dialog to view folders containing plates saved to the database.

**22** Double-click a folder to view its name, date created, creator, and barcode (if applicable) on the bottom pane.

**23** Select the plate to delete from the bottom pane and click *OK*.

**Note**: This step removes the data from the Plate dialog box but not from the database/fileserver. To remove the data from the database/fileserver, you must click *Remove Deleted Data* after using the Plate dialog box. Once the data has been deleted it cannot be recovered.

**24** To permanently remove data from the database after performing a *Delete Measurements*, *Delete Images*, or *Delete Plates* command, click *Remove Deleted Data*.

**25** Click *Close* to close the Plate Data Utilities dialog box.

## Plate Data Utilities - Dialog Box Options

### Run Analysis

Opens the Plate Dialog box. Use the top pane to select a plate from the tree view. Then select the plate from the bottom pane and click *OK*. This opens the Run Analysis on Plates dialog box for the selected plate. Select the setting to use and the images to open and click *OK* to run the analysis. For more information, refer to the Run Analysis on Plates help topic. The Run Analysis on Plates dialog box closes, and the Review Plate Data dialog box opens.

### Import Images

Opens the Import Images dialog box. Use this dialog box to import a new data set into the database or to another defined local or network location. You can also access the Import Cellomics Data dialog box from here.

### Export Images

Copies images from a selected plate from the database/fileserver to another local or networked location.

### Delete Measurements

Opens the Plate dialog, which enables you to select the plate(s). The measurements associated with the selected plates will be deleted from the database.

> **Note**: This step removes the data from the Plate dialog box but not from the database/fileserver. To remove the data from the database/fileserver, you must click *Remove Deleted Data* after using the Plate dialog box. Once the data has been deleted it cannot be recovered.

### Delete Images

Deletes individual images from plates stored in the database.

> **Note**: This step removes the data from the Plate dialog box but not from the database/fileserver. To remove the data from the database/fileserver, you must click *Remove Deleted Data* after using the Plate dialog box. Once the data has been deleted it cannot be recovered.

### Delete Plates

Opens the Plate dialog, which enables you to select the plate(s) to delete from the database.

> **Note**: This step removes the data from the Plate dialog box but not from the database/fileserver. To remove the data from the database/fileserver, you must click *Remove Deleted Data* after using the Plate dialog box. Once the data has been deleted it cannot be recovered.

### Remove Deleted Data

Commits the deletions performed in the Plate dialog box. See above for more information.

### Close

Closes the Plate Data Utilities dialog box.

# Add Analysis to Database

**Adds analysis developed from one or more journals to the Screening Database.**

*Availability: Exclusive to MetaXpress*

Use this dialog box to add preexisting Analysis journals to the Screening Database. You can also use this dialog box to update existing analysis in the database with updated journals or other files included in the analysis.

To save analysis to the Screening database, you must first save it in a subdirectory of C:\Assay. Create the subdirectory and save all journals and other analysis files to it. The name of this directory (folder) will be used as the name of the analysis in MetaXpress.

## Adding Analysis to the Database

Use the following procedure to add analysis to the Screening Database:

| Step | Action |
|---|---|
| **1** | Create the directory **C:/Assay**. |

| | |
|---|---|
| **2** | Create a subdirectory with the same name as the main journal that contains the journals used in the analysis that you want to add to the database.<br><br>For example, a journal called count.jnl should be place in the folder c:assay\count\count,jnl. |
| **3** | From the Screening Menu, click *Add Analysis to Database*; the Add Analysis to Database dialog box opens. |
| **4** | Click *Select Directory*. The Browse for Folder dialog box opens. |
| **5** | Navigate to the subdirectory created in step 2 using the Browse for Folder dialog box, then click *OK*. The path is displayed next to the *Select Directory* button. |
| **6** | Select *Add new* from the *Settings* field. |
| **6** | Enter a setting name in the *Name* field.<br><br>Enter a description in the *Analysis description* field. |
| **7** | Click *Add* to add the analysis to the screening database. |
| **8** | Click *Close* to exit the dialog box. |

## Updating Analysis in the Database

Use the following procedure to update an existing analysis in the Screening Database:

| Step | Action |
|---|---|
| **1** | Ensure that the updated journal(s) or other files are saved in the subdirectory of **C:/Assay** that contains the analysis to update. |
| **2** | From the Screening Menu, click *Add Analysis to Database*; the Add Analysis to Database dialog box opens. |
| **3** | Click *Select Directory*. The Browse for Folder dialog box opens. |
| **4** | Navigate to the subdirectory containing the analysis to update and click *OK*. The path is displayed next to the *Select Directory* button. |
| **5** | Select *Overwrite existing* from the *Settings* field. |
| **6** | Use the *Name* drop-down box to select the setting to update. |
| **7** | Click *Overwrite* to update the analysis in the database |
| **8** | Click *Close* to close the dialog box. |

## Add Analysis to Database - Dialog Box Options

### Select Directory

Opens the Browse for Folder dialog box. Use this to browse to the subdirectory of C:\Assay that contains the analysis to add or overwrite.

### Settings

#### Add New

Adds a new analysis to the database. Use the *Name* and *Analysis description* fields to enter the name and a description of the analysis.

#### Overwrite existing

Overwrites the existing analysis selected in the *Select Directory* field. Selecting this option enables a drop-down list in the *Name* field containing the existing settings for the analysis in the selected folder.

### Name

Use to enter a setting name for the selected analysis when *Add new* is selected. Use to select an analysis to overwrite when *Overwrite existing* is selected.

### Analysis Description

Use to enter a description of the analysis you are adding if *Add new* is selected. Use to view a description of the selected analysis if *Overwrite existing* is used.

### Add

Adds the analysis selected in the *Select Directory* field to the Screening Database. This option is only available if Add new is selected.

### Overwrite

Overwrites the selected analysis in the database with the modified analysis selected in the *Select Directory* field. This option is only available if *Overwrite existing* is selected.

### Close

Closes the dialog box.

# Import Images

**Imports HTD files into the selected local, network, or database location.**

*Availability: Exclusive to MetaXpress*

Use the Import Images command to import one or more data sets created by the non-database version of Screen Acquisition into the screening database or to another defined local or network location. The data will then be accessible using either the Screen Data Utilities (DB) command or the Review Screen Data (DB) command. You can also access the Import Cellomics Data dialog box from here, which enables you to import a plate of Cellomics DIB files into the screening database or a local or network folder.

## Importing Images

Use the following procedure to import data sets into the screening database or a local or network folder:

| Step | Action |
|---|---|

1. From the Plate Data Utilities (DB) dialog box, click *Import Images*. The Import Images dialog box opens.
2. Click *Select Directory*. The Browse for Folder dialog box opens.
3. Navigate to the local or network folder containing the data set(s) to import using the Browse for Folder dialog box, then click *OK*. The path is displayed next to the *Select Directory* button and the HTD files are displayed in the *HTD Files* field.
4. Chose the data set(s) to be imported by checking the check boxes next to the HTD files to import in the *HTD Files* field.
5. Select a location (either the database or an available network folder) to import the data sets to from the *Move images to* drop-down box.

   Note: The locations in this list were configured using the Screen Database: Database Utilities command in the Meta Imaging Series Administrator.
6. Click *Import*. The files are imported and will be available for review in the Review Screen Data (DB) dialog box.

   **Note:** Depending on the number of files to import, this step may take several minutes to complete.
7. If you need to import Cellomics data, click *Import Cellomics Data*. The Import Cellomics Data dialog box opens.
8. To close the dialog box, click *Cancel*.

## Import Images - Dialog Box Options

### Select Directory

Opens the Browse for Folder dialog box and enables you to select a local or networked location for the folder containing the data sets (HTD files) to import.

### Move Images to

Displays a drop-down list of available local or network location(s) where the data set should be imported. You can also import directly into the database. The locations in this list were configured using the Screen Database: Database Utilities command in the Meta Imaging Series Administrator.

### HTD Files

Displays a list of valid HTD files (plates) contained in the folder selected in the *Select Directory* field. Use the checkboxes to select which files to import.

### Description

Displays a description of the experiment. This is only available if the *Description* field in the *Main* tab of the Screen Acquisition dialog box was filled in when the plate was acquired.

**Import**

Imports the selected HTD files into the database or directory selected in the *Move Images to* field.

**Import Cellomics Data**

Opens the Import Cellomics Data dialog box.

**Cancel**

Cancels the command.

# Import Cellomics Data

**Imports Cellomics DIB files into the selected local, network, or database location.**

*Availability: Exclusive to MetaXpress*

**Drop-in: HTDB_PLAYER**

Use the Import Cellomics Data command to convert sets of Cellomics DIB files into data sets in the database that can be accessed using either the Plate Data Utilities (DB) command or the Review Plate Data (DB) command. The command creates MetaXpress HTD files based on the data in the DIB files and converts the images to the TIFF format. It then moves the data into your MetaXpress screening database or a selected local or network folder.

**Note:** Cellomics DIB files do not include image calibration data. Unless you enter a specific calibration when you import the data, a default calibration of 1 um = 1 pixel will applied to the imported images.

## Importing Cellomics Data

Use the following procedure to import Cellomics data into MetaXpress:

| Step | Action |
|---|---|
| **1** | From the Plate Data Utilities (DB) Import Images dialog box, click *Import Cellomics Data*. The Import Cellomics Data dialog box opens. |
| **2** | Click *Select Directory*. The Browse for Folder dialog box opens. |
| **3** | Navigate to the local or network folder containing the Cellomics data to import using the Browse for Folder dialog box, then click *OK*. The path is displayed next to the *Select Directory* button and the Cellomics files are displayed in the *Cellomics Data Files* field. |
| **4** | Select the check boxes next to the Cellomics file sets to import in the *Cellomics Data Files* field. |
| **5** | Select a location (either the database or an available network folder) to import the Cellomics data to from the *Store images in* drop-down box. |

6 Enter a calibration ratio (ums per pixel) for the x and y values of the imported images in the *Image calibration* fields

7 Click *Import*. The files are imported and will be available for review in the Review Plate Data (DB) dialog box.

8 Click *Close* to close the dialog box.

## Import Cellomics Data - Dialog Box Options

### Select Directory

Opens the Browse for Folder dialog box and enables you to select a local or networked location for the folder containing the Cellomics (.dib) files to import.

### Cellomics data files

Displays a list of valid Cellomics file sets contained in the folder selected in the *Select Directory* field. Use the checkboxes to select which files to import.

### Move images to

Displays a drop-down list of available local or network location(s) where the data set should be imported. You can also import directly into the database. The locations in this list were configured using the Screen Database: Database Utilities command in the Meta Imaging Series Administrator.

### Image Calibration

Sets a calibration ratio for the x an y values of the imported images. The default calibration is 1 um = 1 pixel.

### Import

Imports the selected Cellomics files into the database or directory selected in the *Move Images to* field.

### Close

Closes the dialog box.

# Monopole Detection

**Detects, analyzes, and quantifies mitotic cells with monopolar and bipolar spindles.**

*Availability: Exclusive to MetaMorph and MetaXpress*

### Dropin: Monopole

Use this application module to detect and count the number of mitotic cells with monopolar spindles and count the number of mitotic cells with bipolar spindles. Monopolar spindles can appear when spindle assembly is disrupted. Bipolar spindles occur during the normal cell cycle.

Analyzing your images with this application module produces measurements for both the entire image and for individual cells.

Depending on the classification cutoff values that you choose, cells are appropriately classified as Interphase, Bipole, or Monopole. Classification is based on the correlation between intensity in the DNA stained image and the Microtubule stained image. For interphase cells, the two stains are not colocalized and produce a low correlation coefficient. For bipoles, the stains are partially overlapping and the correlation is increased, and for monopoles the greatest correlation is exhibited.

The default correlation coefficient value for the Interphase to Bipole cutoff is 0.3, and the default correlation coefficient value for the Bipole to Monopole cutoff is 0.6. You can use these values as a starting point, and adjust the values according to the measurement results produced.

After the application module has processed the image, you can use the ***Cellular Results*** table to interactively view data belonging to an individual cell or group of cells by clicking the cell(s) in the image.

**Application Note — *MetaMorph Application Module Overview* (PDF)**

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Detecting Monopoles

To run the Monopole detection application module in conjunction with an appropriate analysis, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click Monopole Detection. The Monopole Detection dialog box opens. |
| **2** | To be able to view your Monopole Detection results immediately after processing is complete, in the *Results legend* box, click *Display result image*. The Results legend image selector will become active. The result image name by default is *Segmentation*. |
| **3** | To change the name of the result image, click *Segmentation*, then click *Specified(Segmentation)*. The *Specify Image Name* dialog box will open. Type the new file name that you want to use, then click *Close*. |
| **4** | In the DNA structures box, click the DNA source image button to select the DNA source image that you will use. |
| **5** | Under DNA structures in the *Approximate min width box*, type or select a value in microns for the minimum width of the Stained area. The value should be small (typically between 2 and 20 microns). Determine which cell appears to be the smallest in the field, then determine the approximate width of the smallest dimension.<br><br>***Hint:*** You can use the Caliper or Line Region tools to help determine the width. |
| **6** | Under DNA structures in the *Approximate max width* box, type or select a value in microns for the maximum width of the Stained area. The value should be small (typically between 5 and 30 microns). Determine which cell appears to be the largest in the field, then determine the approximate width of the widest dimension.<br><br>***Hint:*** You can use the Caliper tool or Line Region tool to help determine the width. |

7 Under DNA structures in the Intensity above local background box, type or select a value that represents the minimum nucleus intensity minus the intensity of the background near the nucleus. *Hint:* You can determine these values by placing your mouse pointer on pixels just inside and outside the nucleus that you want to detect. The gray level values are shown on the status bar below the image area. *Note:* The lower the value selected, the more sensitive the detection. The software will estimate the background for each nucleus locally to correct for cases of images with uneven backgrounds.

8 In the *Microtubules* area, set the appropriate correlation coefficient values for Interphase to Bipole and Bipole to Monopole cutoffs.

9 Configure the Summary Log. Click *Configure Summary Log*. The Configure Log dialog box opens.

10 Double-click the measurements that you want to include in the Summary Log file. Summary Log measurements apply to the entire image.

11 Configure the Data Log. Click *Configure Data Log*. The Configure Log dialog box opens.

12 Double-click the measurements that you want to include in the Data Log file. Data Log measurements apply to individual cells.

13 Click *Apply* to run the Monopole Detection application module analysis on your sample.

14 Click *Close* to close the Monopole Detection dialog box. Settings that you make in this dialog box are not saved.

## Monopole Detection - Dialog Box Options

### Display result image

Specifies that you want to create a separate final result image identical to the overlay. Click the check box to optionally display the DNA segmentation and classification result image.

### DNA structures

Provides the settings used to achieve effective DNA image segmentation.

### DNA source image

Image selector for 16-bit source of DNA stain.

**Note:** This setting is required.

### Approximate min width

Specifies the minimum object width in microns (µm) of all the nuclei in your image that you expect to detect. Patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to

the right of the edit box.

#### Approximate max width

Specifies the maximum object width in microns (µm) of all the nuclei in your image that you expect to detect (floating point number format allowed). This width determines which intensity fluctuations are potential cells as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

#### Intensity above local background

Specifies the intensity sensitivity used to isolate the objects that you want to identify during processing.

### Microtubules

Provides the settings used to correctly detect specific cell cycle phases in the microtubules (MT) image.

#### MT source image

Opens the Image Selector for the 16-bit source image of microtubule (MT) or tubulin stain. This source image is required.

#### Classification by correlation with DNA image

Specifies the correlation coefficient limits for the three categories. Correlation is between the two source images' pixels occurring within a fixed distance (a percentage of the approximate width parameters) surrounding and including the detected DNA structures.

#### (Classification names)

Shows the three possible classification names and their corresponding color-coded arrows, indicating the classes for the three ranges of correlation coefficients:

#### Interphase

-1.0 to Interphase/Bipole cutoff for Interphase classification

#### Bipole

Interphase/Bipole cutoff to Bipole/Monopole cutoff for Bipole classification

#### Monopole

Bipole/Monopole cutoff to +1.0 for Monopole classification.

**Note:** The range arrows are color-coded identical to the legend for the result segmentation.

#### Interphase/Bipole cutoff

Set maximum interphase correlation coefficient/minimum bipole correlation coefficient (always between -1.0 and +1.0).

#### Bipole/Monopole cutoff

Set maximum bipole correlation coefficient/minimum monopole correlation coefficient (always between -1.0 and +1.0).

### Configure Summary Log

**DNA Structures:** Total number of DNA structures detected in the image segmentation.

**Monopoles:** Number of structures classified as Monopole.

**Bipoles:** Number of structures classified as Bipole.

**Interphase Cells:** Number of structures classified as neither monopole nor bipole.

**% Monopoles:** (Monopoles ÷ Total Cells) x 100.

**% Bipoles:** (Bipoles ÷ Total Cells) x 100.

**% Interphase:** (Interphase Cells ÷ Total Cells) x 100.

**DNA Structures Total Area:** Total µm²s of all DNA structures.

**DNA Structures Mean Area:** DNA Structures Total Area ÷ DNA Structures.

**DNA Structures DNA Average Intensity:** DNA structures image integrated intensity values within all structures ÷ DNA Structures Total Area (in pixels).

**DNA Structures MT Average Intensity:** Microtubule image integrated intensity values within all structures ÷ DNA Structures Total Area (in pixels).

**Monopoles Mean Area:** Total µm²s of all DNA structures among monopoles ÷ Monopoles.

**Monopoles DNA Average Intensity:** DNA structures image integrated intensity values within monopole structures ÷ monopole structures total area (in pixels).

**Monopoles MT Average Intensity:** Microtubule image integrated intensity values within monopole structures ÷ monopole structures total area (in pixels).

**Bipoles Mean Area:** Total µm²s of all DNA structures among Bipoles ÷ Bipoles.

**Bipoles DNA Average Intensity:** DNA structures image integrated intensity values within bipole structures ÷ bipole structures total area (in pixels).

**Bipoles MT Average Intensity:** Microtubule image integrated intensity values within bipole structures ÷ bipole structures total area (in pixels).

**Interphase Cells Mean Area:** Total µm²s of all DNA structures among interphase nuclei ÷ Interphase Cells.

**Interphase Cells DNA Average Intensity:** DNA structures image integrated intensity values within interphase nuclei ÷ interphase nuclei total area (in pixels).

**Interphase Cells MT Average Intensity:** Microtubule image integrated intensity values within interphase nuclei ÷ interphase nuclei total area (in pixels).

### Configure Data Log (Cells)

**Cell: Assigned Label #:** unique label number (1 through number of structures).

**Cell: Classification**: Interphase Cell, Bipole, or Monopole.

**Cell: Correlation Coefficient:** Value from -1.0 (anti-correlated) to 1.0 (correlated) of DNA structure image and Microtubule image both masked by the dilated segmentation.

**Cell: DNA Structure Area:** Total µm²s in the structure.

**Cell: DNA Integrated Intensity:** Summed grayscale values in the structure from wavelength 1 (DNA structures).

**Cell: DNA Average Intensity:** DNA Integrated Intensity ÷ DNA Structure Area (in pixels).

**Cell: MT Integrated Intensity:** Summed grayscale values in the DNA structure from wavelength 2 (Microtubules).

**Cell: MT Average Intensity:** MT Integrated Intensity ÷ DNA Structure Area (in pixels).

### Load Settings (from Review Screen Data only)

Opens the Load Settings dialog box. Use this dialog box to select and load a Monopole Detection settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the Monopole Detection settings file. Select the name of the setting that you want to use, then click OK.

### Save Settings

Opens the Save Settings dialog box. Use this dialog box to create and save a Monopole Detection settings file. You can store all the settings currently specified in the Monopole Detection dialog box. Use Load Settings to retrieve and use these settings at a later time. Type a setting name and an optional description, then click OK.

### Set to Defaults

Resets all options and settings to their default values.

**Test Run** (from Review Plate Data only)

Runs one pass of the Monopole Detection application module on the images currently open and selected. Measurements will be logged if the Summary and/or Data Logs are open.

**Apply** (from the Apps menu only)

Applies the settings that you made and runs the application module.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

**Close**

Closes the dialog box.

# Multi Wavelength Cell Scoring (Apps Menu)

**Detects, counts, and logs measurements derived from a maximum of seven wavelengths associated with seven different stains for the following cell areas: nuclei, cytoplasm, or the entire cell.**

*Availability: Exclusive to MetaMorph and MetaXpress*

**Dropin: MULTIWAVESCORING**

Use this application module to detect, count, and log measurements of cells detected by a secondary wavelength in samples using from two to seven stains in conjunction with two to seven wavelengths.

Multi Wavelength Cell Scoring is a general purpose application module that can be used in conjunction with a user-defined analysis to identify from one to six, secondary markers in a sample in which all nuclei are identified with a primary marker. The first marker should stain and identify all nuclei. The second through seventh marker(s) should stain either nuclei, cytoplasm, or both. The module identifies a variety of useful measurements including the number and percent of cells scored positive for each marker and individual cell scoring profiles across the set of all markers.

**Note:** Multi Wavelength Cell Scoring can be used with only a single wavelength.

## General Procedures

1. Prepare samples using two to seven different stains; where the first stain is for All Nuclei and the remaining stains are for scoring.
2. Collect your dataset using two to seven different wavelengths (All Nuclei and from one to six Positive Markers).
3. Open the Review Plate Data dialog box and choose Multi Wavelength Cell Scoring.
4. Select either a Multi Wavelength Cell Scoring stored configuration setting or set dialog box parameters to correctly detect each wavelength.
5. Open log files for both the Summary Log and the Data Log.
6. Select the measurements that you want to log for both the Summary Log and the Data Log.
7. Run the analysis for a single well, selected wells, the entire plate, or multiple plates.

After the application module has processed the image, you can use the ***Cellular Results*** table to

interactively view data belonging to an individual cell or group of cells by clicking the cell(s) in the image.

**Application Note — *MetaMorph Application Module Overview* (PDF)**

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Running Multi Wavelength Cell Scoring

To run the Multi Wavelength Cell Scoring Application Module, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the *Apps* menu, click Multiwavelength Cell Scoring. The Multi Wavelength Cell Scoring dialog box opens. |
| **2** | From the File menu, click Open. The Open dialog box is displayed. |
| **3** | From the *Open dialog* box, choose the images for Wavelengths 1, 2, and any additional wavelengths, then click *Open*. |
| **4** | In the *Number of Wavelengths* box, type or select the total number of wavelengths in your experiment that you want to analyze. |
| **5** | To display a combined segmentation result image for all wavelengths, click *Display Result Image*. The result image will be named *Segmentation* by default. To change the name of the result image, click the button labeled *Segmentation* to open the image selector. |
| **6** | If is not currently displayed, click the *All Nuclei* tab. |
| **7** | In the W1 Source Image Selector for *All Nuclei*, select the appropriate image for wavelength number 1. |
| **8** | On the *All nuclei* tab, in the *Approximate min width* box, type or select a value in microns for the minimum nuclei width. This dimension should be based on the smallest width of the smallest nucleus that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings.*** |
| **9** | On the *All nuclei* tab, in the *Approximate max width* box, type or select a value in microns for the maximum nuclei width. This dimension should be based on the largest width of the largest nucleus that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings.*** |

**10** In the *All nuclei* area, in the *Intensity above local background* box, type or select a value to define the difference in intensity between the dimmest nuclei and the background adjacent to it. For details on making this setting, refer to the procedure for determining Intensity above local background under ***Determining Segmentation Settings.***

**11** Click *Preview* to display an image of the result segmentation overlaid on the original image for this wavelength. ***Note:*** The following steps apply to *all* of the Wavelengths. Repeat these steps for each additional wavelength that you are analyzing.

**12** Click the *W2-W7* Wavelength tab. The *W2-W7* wavelength tab is displayed.

**13** In the *Name* box, type the name that you want to assign to the selected wavelength.

**14** In the *W2-W7* Source Image Selector for *W2-W7* wavelengths, select the appropriate image for the wavelength.

**15** In the *Legend Color* box, select the color that you want to assign to this wavelength.

**16** In the *Stained area* box, select the area to which the stain is applied in the image. Select either Nucleus, Cytoplasm, or Nucleus and Cytoplasm.

**17** In the *Approximate min width* box, type or select a value in microns for the minimum object width. This dimension should be based on the smallest width of the smallest object that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings.***

**18** In the *Approximate max width* box, type or select a value in microns for the maximum object width. This dimension should be based on the largest width of the largest object that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings.***

**19** In the *Intensity above local background* box, type or select a value to define the difference in intensity between the dimmest object and the background adjacent to it. For details on making this setting, refer to the procedure for determining Intensity above local background under ***Determining Segmentation Settings.***

**20** In the *Minimum stained area* box, type or

select the minimum area for a cell that should be scored positive for this wavelength.

**21** Click *Preview* to view the segmentation derived from the image staining. The preview image is displayed as a graphic overlay on the original image of the appropriate staining type.

**22** Click *Configure Summary Log* to choose the measurements that you want to include in your Summary Log.

**23** Click *Configure Data Log* to choose the measurements that you want to include in your Data Log.

**24** Click *Show Legend* to show a list of the current color assignments.

**25** Click *Apply* to run the Multi Wavelength Cell Scoring application module.

**26** To interactively view data belonging to an individual cell or group of cells, select the cell(s) in the image and view the data in the ***Cellular Results*** table.

**27** Click *Close* to close the Multi Wavelength Cell Scoring dialog box.

## Determining Segmentation Settings

Use the following general procedures to make segmentation settings for each wavelength.

| Step | Action |
|---|---|
| **1** | If applicable, in the Stained Area box choose the area of the cell in which you expect the stain associated with this wavelength to be detected. Choose one of the following:<br>• *Nucleus* if you expect the stain to be detected only in the nucleus.<br>• *Cytoplasm* if you expect the stain to be detected only in the cytoplasm.<br>• *Nucleus* and *Cytoplasm* if the stain will be detected in both areas. |
| **2** | In the Approximate min width box, type or select a value in microns for the minimum width of the Stained area. If the stained area is the nucleus, the value should be small (typically between 2 and 20 microns ). If the stained area is the cytoplasm or both, the value should be larger (between 15 and 200 microns). Determine which cell appears to be the smallest in the field, then determine the approximate width of the widest dimension.<br>**Hint:** You can use the Caliper or Line |

Region tools to help determine the width.

**3** In the Approximate max width box, type or select a value in microns for the maximum width of the Stained area. If the stained area is the nucleus, the value should be small (between 5 and 30 microns). If the stained area is the cytoplasm or both, the value should be larger (between 20 and 300 microns). Determine which cell appears to be the smallest in the field, then determine the approximate width of the widest dimension.

**Hint:** You can use the Caliper tool or Line Region tool to help determine the width.

**4** In the Intensity above local background box, type or select a value that represents the minimum nucleus or cytoplasm intensity minus the background intensity near the nucleus or cytoplasm. The lower the value, the more sensitive the detection. The software will estimate the background for each nucleus or cytoplasm locally to correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the blob that you want to detect. The graylevel values are shown on the status bar below the image area. To obtain the Intensity above local background value, subtract these two values.

**5** Click *Preview* to open a preview image of the segmentation for this wavelength overlaid on the original image. This preview image can be used to separate the effects of the individual wavelength detections interacting so that each wavelength's proper settings can be determined independently.

**Note:** To turn on or off the overlay image, click the overlay button on the image window.

## Multi Wavelength Cell Scoring - Dialog Box Options

### Number of wavelengths

Specifies the total number of wavelengths that are present in your images that you want to analyze. For each wavelength, a separate wavelength tab is displayed.

### Display result image

Activates the display of the of the segmentation result image (optional).

### [Result Image Selector]

Opens the Image Selector for the destination image. This selection is enabled only if Display result image is checked. This setting shows segmentation of the cell or nucleus according to your selection. This image is displayed as an autoscaled, 16-bit image for easy color combine, arithmetic, or overlay with the original.

**Notes:**

- When processing is complete, the source images are automatically overlaid with the segmentation results. If you do not re-save the source image, the original source image is not affected. However, if you re-save the source image the overlay information will be saved with it.
- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

### All Nuclei tab

The following settings and options are located on the *All nuclei* tab.

### Name

Specifies the name that you want to assign to the image and wavelength that you will be processing. For the All Nuclei, the name is constant as All nuclei.

### W1 Source image

Specifies the name of the All nuclei source image.

### W[2-7] Source image

Specifies the name of the source image assigned to the selected wavelength(s).

### Legend Color

Indicates the color that will be assigned to the wavelength and segmentation. Specifies Gray as the legend color assignment for the *All nuclei* stain.

### Stained area

Specifies the Nucleus as the stained area for the All nuclei stain.

### Approximate min width

Specifies the minimum object width in microns (µm) that you expect to detect. Patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

### Approximate max width

Specifies the maximum object width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential cells as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of the selected stained area compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

### Preview

Shows a color preview image of the segmentation for the *All nuclei* wavelength overlaid on the original image.

### W2 through W7 tabs

The following settings and options apply to wavelengths 2 (W2) through 7 (W7). **Note:** Using the *Name* option, you can assign a custom name to each wavelength tab.

### Name

Specifies the name that you want to assign to the image and wavelength that you will be processing. The default name for each wavelength 2 through 7 is W2 through W7. You can rename this displayed identifier. If you rename this identifier, the corresponding name on the associated tab also changes.

### W[2-7] Source image

Specifies the name of the source image assigned to the selected wavelength(s).

### Legend Color

Indicates the color that will be assigned to the wavelength and segmentation. The colors that you assign will interact to determine the legend colors for the scoring profile.

### Stained area

Use this drop down list to specify the cell area stained with a positive marker. Choose one of the following cell areas:

- Nucleus
- Cytoplasm
- Nucleus and cytoplasm

### Approximate min width

Specifies the minimum object width in microns (µm) that you expect to detect. Patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

### Approximate max width

Specifies the maximum object width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential cells as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of the selected stained area compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

### Preview

Shows a color preview image of the segmentation for the all wavelengths overlaid on the original image. In this preview, the segmentation of all identified objects is shown indicated by the appropriate assigned color for each type of object. Click the Overlay Image button on the image window to turn on and off the overlay image. If you resave the original image after the overlay has been created, the overlay is saved with the original image, even if the overlay view is turned off when you save the image. After the overlay has been saved with the original image, you can turn it off and on as needed. If you resave the image with a new overlay, it will replace the current overlay image.

### Scoring

Specifies the scoring criterion to be applied to the associated wavelength.

### Minimum stained area

Specifies the minimum positive stained area required to score the cell positive for the associated stain and wavelength.

### Configure Summary Log

- Total Cells
- Positive W*n* <*name*>
- % Positive W*n* <*name*>
- Scoring Profile 1*nnnnnnn*<*user-selected name*> where *n* is an available wavelength number.

  The following is an example of the available scoring profiles when you are analyzing three additional wavelengths in addition to wavelength 1. The numbers that are present indicate a

wavelength that scored positive. Cells that meet the criteria of the various scoring profiles are assigned the resulting color that is shown in the legend.

Scoring Profile 1--- <*user-selected name*>

Scoring Profile 12-- <*user-selected name*>

Scoring Profile 1-3- <*user-selected name*>

Scoring Profile 123- <*user-selected name*>

Scoring Profile 1--4 <*user-selected name*>

Scoring Profile 12-4 <*user-selected name*>

Scoring Profile 1-34 <*user-selected name*>

Scoring Profile 1234 <*user-selected name*>

**Note:** The total number of Scoring Profile entries depends on the total number of analyzed wavelengths.

### Configure Data Log (Cells)

- Cell: Assigned Label #
- Cell: Scoring Profile (see above format, for example, "1-3-")
- Cell: Custom Profile Name
- Cell: Total Area
- Cell: W1 Stained Area
- Cell: W1 Integrated Intensity
- Cell: W1 Average Intensity
- Cell: Positive for W2 <*user-selected name*>
- Cell: W2 Stained Area
- Cell: W2 Integrated Intensity
- Cell: W2 Average Intensity

### Save Settings

Opens the Save Settings dialog box. Use this dialog box to create and save a Multi Wavelength Cell Scoring settings file. You can store all the settings currently specified in the Multi Wavelength Cell Scoring dialog box. Use Load Settings to retrieve and use these settings at a later time. Type a setting name and an optional description, then click OK.

### Load Settings

Opens the Load Settings dialog box. Use this dialog box to select and load a Multi Wavelength Cell Scoring settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the Multi Wavelength Cell Scoring settings file. Select the name of the setting that you want to use, then click OK.

### Show Legend

Opens the *Legend* dialog box. The Legend dialog box is used to assign custom name designations to each

instance of a positive wavelength scoring indicator. Click Show Legend to open the dialog box. This dialog box is divided into two areas. The Wavelengths area shows the wavelength number (W1 through W7), the color that you assigned to the wavelength, and the name that you assigned to the wavelength. This area of the dialog box cannot be edited. Changes to the color and name assignment are made in the main dialog box.

The lower area displays the color legend for the colors that represents each possible positive-scored wavelength combination. The colors in this area are not assignable, as each color is a result of the colors of the combined positive-scored wavelengths. However, you can specify the name that you want to assign to each positive –scored profile. Cells that are scored positive by specific combinations of wavelengths are assigned that color. The color assigned to Wavelength 1 (W1) cannot be changed – it is always Gray. The colors assigned to the various scoring wavelength combinations are typically the result of what you might anticipate when colors are additively combined.

**Test Run** (from Review Plate Data only)

Runs one pass of the Multi Wavelength Cell Scoring application module on the images currently open and selected. Measurements will be logged if the Summary and/or Data Logs are open.

**Apply** (from the Apps menu only)

Applies the settings that you made and runs the application module.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

**Close**

Closes the dialog box.

# Neurite Outgrowth (Apps Menu)

**Locates and identifies cell bodies and their neurites; associates neurites with the correct cell body; analyzes the measurable characteristics of each cell body and its associated neurites, and logs the acquired data into associated tables.**

*Availability: Available for MetaMorph and MetaXpress*

**Drop-in: NEURITE**

Use this dialog box to identify, analyze, and report the measurable physical characteristics of cell bodies and associated neurites in previously acquired cell images containing visible neurites.

**Application Note — *MetaMorph Application Module Overview* (PDF)**

**Note:** Images processed using Neurite Outgrowth should be 16-bit, calibrated images.

The Neurite Outgrowth command provides options that, depending on your image content and acquired wavelengths, enables you to isolate cell bodies and identify their attached neurites with different degrees of sensitivity. Image sets of two wavelengths that include stained nucleus in one image typically will provide the greatest degree of accuracy for isolating closely-grouped cell bodies. The use of a nuclear stain insures a one-to-one correlation between nuclei and cell bodies.

Cell bodies and associated neurites can still be isolated and identified when only a single wavelength of your cell body samples are available. Options enable you to customize settings to obtain the best possible results.

Default settings that are optimized for typical imaging conditions can be used for a wide range of samples. It is recommended that you use the default values for the settings as a starting point, even if you are reasonably certain that you need to modify the settings. The settings are used to specify threshold values to separate cell bodies from the image background and to specify the anticipated minimum and maximum dimensions of both cell bodies and neurites. The more accurate the settings

are, the greater the likelihood of correctly isolating cell bodies and neurites.

Results are available as both segmentation result images and numerical results that can define the physical characteristics of both the cell bodies and the neurites. The results are placed into two different configurable log tables, and include the following measurements:

- ***Cells –*** Number of cells in a field.
- ***Cell Body Area –*** Total µm² of the cell body (excluding outgrowths).
- ***Mean Cell Body Area –*** Total µm² of pixels in the cell body divided by the number of cells.
- ***Total Outgrowth –*** Total length of skeletonized outgrowth ums (corrected for diagonal lengths).
- ***Mean Outgrowth –*** Average skeletonized outgrowth in µm corrected for diagonal lengths divided by the number of cells.
- ***Mean Straightness –*** Ratio varying between 0 (not straight) and 1 (perfectly straight) defined as end-to-end Euclidean distance between segment junctions divided by corresponding actual neurite curve length – the sum of end-to-end lengths divided by the sum of curve lengths.
- ***Total Processes –*** Number of outgrowths that connect to the cell body.
- ***Total Branches*** – Number of branching junctions.
- ***Mean Processes –*** Number of processes divided by number of cells.
- ***Mean Branches*** – Number of branching junctions divided by number of cells.
- ***Cells Significant Growth –*** Number of cells with outgrowth greater than the threshold length specified by the user.
- ***%Cells Significant Growth –*** % of cells with outgrowth greater than the threshold length specified by the user.

In addition, for *each* cell body, the following measurements are also available on a cell by cell basis:

- ***Cell: Label Number –*** Unique label corresponding to the cell's (pseudocolor) intensity value in the result image and also to its ordered entry in the Data Log. (These result image intensities can be distinguished by placing the mouse over a cell of interest or by applying appropriate image thresholds.)
- ***Cell: Outgrowth –*** Total number of skeletonized outgrowth µm (corrected for diagonal lengths) associated with the cell.
- ***Cell: Processes –*** Number of outgrowths that connect to the cell body.
- ***Cell: Mean Process Length –*** Total outgrowth (µm) divided by number of processes of the cell.
- ***Cell: Median Process Length –*** Median value of the outgrowth lengths (µm) associated with the cell's various processes.
- ***Cell: Max Process Length –*** Maximum value of the outgrowth lengths (µm) associated with the cell's various processes.
- ***Cell: Cell Body Area –*** Total µm² of the cell body (excluding outgrowths).
- ***Cell: Processes –*** Number of outgrowths that connect to the cell body.
- ***Cell: Branches –*** Number of branching junctions of all the processes connected to the cell.

- ***Cell: Straightness –*** Ratio varying between 0 (not straight) and 1 (perfectly straight) defined as end-to-end Euclidean distance between the cell's segment junctions divided by corresponding actual neurite curve length – the sum of end-to-end lengths divided by the sum of curve lengths.

The available measurements can be selected or deselected by clicking *Configure Summary Log* to select or deselect measurements made within a specific field, or clicking *Configure Data Log (Cells)* to select or deselect measurements made for each cell within the field.

After the application module has processed the image, you can use the ***Cellular Results*** table to interactively view data belonging to an individual cell or group of cells by clicking the cell(s) in the image.

Neurite Outgrowth dialog box settings are used to define the measurement criteria used to identify and isolate cell bodies, neurites, and the associated physical characteristics of each. This dialog box can optionally produce separate images for the Neurite result and the Nuclear result.

To provide an effective starting point, default values have been assigned to all Neurite Outgrowth dialog box settings. Modify these settings based on your knowledge of the sample image characteristics and trial-and-error results. If you want to return all settings to default values, click *Set to Defaults*.

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.***. This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Defining and Analyzing Neurite Outgrowth Data

To process and analyze images for Neurite Outgrowth data, you have several options available to you, from simple to complex. In its most basic usage, you can use a single image from a single wavelength, and use the default values for this command. In its most complex usage, you use a second wavelength, nuclear stained image of cell nuclei. Adjusting settings for cell and outgrowth width, nuclear diameter, area, intensity, and signal-to-noise ratio typically will yield better results.

**Processing of Neurite Outgrowth Images using Default Settings**

**Advanced Processing of Neurite Outgrowth Images**

**Processing Neurite Outgrowth Images from Nuclear Stained Samples**

**Configuring the Log Files**

## Processing of Neurite Outgrowth Images using Default Settings

To complete basic processing of Neurite Outgrowth Images using Default Settings use the following procedure:

| Step | Action |
|---|---|
| **1** | From the File Menu, open the neurite image that you want to process. |
| **2** | From the Apps Menu, click Neurite Outgrowth; the Neurite Outgrowth dialog box opens. |
| **3** | Click *Display result image* to turn this setting either on or off. If you want to see a segmentation image showing the cells and neurites that have been identified, leave this setting on. However, if you are processing a large number of images using one set of settings, and you simply need to build a table of data, you can turn this setting off. |

When this is on, no additional processing overhead is created.

**4** If you want to use a different name than Neurite for the result image, open the *Neurite result* image selector and specify a new image name.

**5** In the Illumination box, click *Fluorescence* if your images were acquired with a fluorescence light source or click *Transmission* if they were acquired with a transmitted light source.

**6** If you are processing only a single image and do not have a Nuclear stain image, click *Nuclear stain* to turn off this option.

**7** Click *Configure Summary Log*; the Configure Log dialog box opens.

**8** Deselect (uncheck) the last two entries in the list: *Cells significant growth* and *% cells significant growth,* then click OK. These two entries require the association of outgrowths with particular cells, which requires slightly more processing time.

**9** Click *Configure Data Log (Cells)*; the Configure Log dialog box opens.

**10** Click *Disable All*, then click *OK*. Again, this disables measurements that require the association of outgrowths with particular cells, which requires slightly more processing time.

**11** Click *Set to Defaults* to ensure that all default values are set.

**12** Click *Apply* to process your image using the default settings. The Neurite result image is displayed.

**13** To interactively view data belonging to an individual cell or group of cells, select the cell(s) in the image and view the data in the Cellular Results table.

## Advanced Processing of Neurite Outgrowth Images

To use the advanced features of the Neurite Outgrowth command, combine the following steps with steps 1-6 of the preceding procedure, ***Processing of Neurite Outgrowth Images using Default Settings***.

| Step | Action |
|---|---|
| **1** | Complete Steps 1-6 in the preceding procedure, *Processing of Neurite Outgrowth Images using Default Settings.* |
| **2** | Visually locate the widest cell body in your image. |
| **3** | Increase the magnification of the image using the zoom tool so that you can identify individual pixels.. |

**4** On either a vertical or horizontal axis, as appropriate, use the mouse pointer to measure the width of the cell body.

- Place the pointer on the left side of the cell.
- Read and record the horizontal (or vertical) (as appropriate) pixel location from the status bar.
- Move the pointer to the opposite side of the cell, and read and record the pixel position value.
- To determine the cell width in pixels, subtract the smaller value from the greater value.

**5** Convert the Cell Width in pixels to cell width in microns, add an appropriate additional amount to the calculated value to compensate for cell irregularities, and type or select the total amount in the **Cell bodies** *Maximum width* box.

**6** Repeat a similar process for Outgrowths Maximum width:

- Visually locate the widest outgrowth, giving particular attention to the bases of the outgrowths.
- Zoom in until you can clearly define individual pixels.
- Place the pointer on one side of the outgrowth, and note the pixel location.
- Place the pointer on the other side of the outgrowth, and also note the pixel location.
- For the axis that applies to your measurement, subtract the smaller value from the larger value.
- Convert the neurite width in pixels to width in microns.
- Type or select the calculated width in pixels in the Outgrowths *Maximum width* box.

**7** Calculate the *Intensity above local background* for both Cell bodies and Outgrowths. This procedure is similar for both, but can yield different results. Be sure to make separate calculations for both.

- Locate a cell body that is difficult to distinguish from the background.
- Zoom in until you can clearly define

individual pixels.

- Place your mouse pointer on the least brightest pixel within the cell body, and note the intensity level as indicated on the status bar.
- Locate a pixel of low intensity in the background area adjacent to the cell body.
- Place your mouse pointer on the selected pixel, and note the intensity of the pixel as indicated on the status bar.
- Subtract the smaller value from the larger value.
- Type or select the value in the Cell bodies *Intensity above local background* box.
- Repeat the preceding steps for Outgrowths *Intensity above local background,* and enter the gray level value.

**8** Type or select the Cell body Minimum area in the Cell bodies Minimum area box.

- Locate the smallest cell body in the image.
- Determine the number of pixels across the center of the cell.
- Divide by two to obtain the radius, then square the result and multiply by 3.141592 (pi) for the area.

**9** In the *Min cell growth to log as significant* box, type or select the length in microns of the shortest outgrowth that you want to include in the log.

(**Hint**: Visually locate an example of the shortest outgrowth to log, then use the mouse pointer to measure its length in pixels. Then convert the pixels to microns.)

**10** Configure the Summary and Data logs.

**11** Click Apply to process the images and transfer the data to the log files.

**12** Retry the above steps to obtain optimal settings for your set of images. The intensity values are likely to have the greatest effect and vary the most from the default settings due to different samples and imaging situations. However, one optimal values are obtained for a typical image, the settings should remain appropriate to the entire image set.

## Processing Neurite Outgrowth Images from Nuclear Stained Samples

When you acquire images from samples containing an appropriate nuclear stain, you increase the probability of accurately isolating individual cells and their associated neurites. Using this capability adds some complexity to the process, and therefore slightly increases the length of time to process an image.

To correctly set up the Neurite Outgrowth dialog box to process nuclear stained samples, complete either the preceding first procedure only, or both the first and second preceding procedures, then complete the following procedure before applying the settings to your image(s):

| Step | Action |
|---|---|
| **1** | Click *Use nuclear stain* to activate the **Nuclear stain** area. |
| **2** | Click *Display result image* to have the result image displayed after processing is complete. |
| **3** | Locate the cell in the image with the largest nucleus. |
| **4** | Zoom in until you can differentiate individual pixels yet have the entire nucleus visible in the window. |
| **5** | Place your mouse pointer on one edge of the nucleus, and note the pixel location. |
| **6** | Move the pointer to the opposite side of the cell, and note the pixel location. |
| **7** | Subtract the smaller value from the larger value, then convert the number of pixels to microns. |
| **8** | Type or select the number of microns in the Nuclear diameter box. |
| **9** | Use the default value for the Signal to Noise Ratio or enter an alternative value in the Signal to noise ratio box. Estimating this value is easiest by trial and error, rather than by making intensity measurements, and is typically not very sensitive as you adjust the setting. |
| **10** | Configure the Summary and Data logs. |
| **11** | Click Apply to process the images and transfer the data to the log files. |
| **12** | To interactively view data belonging to an individual cell or group of cells, select the cell(s) in the image and view the data in the Cellular Results table. |

## Configuring the Log Files

The two logs accessible in the Neurite Outgrowth dialog box, the Summary Log and Data Log, provide the capability for selecting, accumulating, and reporting image data.

**Configuring the Data Log (Cells)**

**Configuring the Summary Log**

## Configuring the Summary Log

The Summary Log, with two exceptions, contains measurements that are not cell-specific; for example, they do not require associating the outgrowths with particular individual cells. It also contains two cell-specific parameters, *Cells Significant Growth*, and *%Cells Significant Growth.* (The inclusion of cell-specific measurements requires slightly longer processing time.) This log also contains entries for a standard, general set of image information for use in all MetaMorph and MetaXpress log files. Complete the following steps to configure the Summary Log.

| Step | Action |
|---|---|
| **1** | Click Configure Summary Log. The Configure Log dialog box opens. |
| **2** | Select or deselect individual measurements as appropriate, or select enable all or disable all. |
| **3** | Select or deselect the appropriate logging options. |
| **4** | Click OK when your selections are complete, or click Cancel to disregard your selections. |

## Configuring the Data Log (Cells)

The Data Log contains a set of cell-specific measurements along with a standard, general set of image information for use in all MetaMorph and MetaXpress log files. If any of the cell-specific measurements are selected in this dialog box, the image result will contain the physical and visual characteristics that delineate each cell and its attributed outgrowths from the other cells in the image. (An intensity level is assigned to each cell in the result image, corresponding to the label number in the Data Log.) However, if no cell-specific measurements are selected, and *Cells Significant Growth*, and *%Cells Significant Growth* were not selected in the Summary Log parameters, the resulting image will not show cells and their associated outgrowths delineated from the other cells in the image, and only a monochromatic image will be produced.

| Step | Action |
|---|---|
| **1** | Click Configure Data Log. The Configure Log dialog box opens. |
| **2** | Select or deselect individual measurements as appropriate, or select enable all or disable all. |
| **3** | Select or deselect the appropriate logging options. |
| **4** | Click OK when your selections are complete, or click Cancel to disregard your selections. |

## Neurite Outgrowth - Dialog Box Options

### Neurite Image

Opens the Source Image selector. This image selector works on the active plane only, and allows only 16-bit images.

**Notes:**

- When processing is complete, this image is automatically overlaid with the segmentation result image. If you do not re-save the source image, the original source image is not affected. However, if you re-save the source image the overlay information will be saved with it.
- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

### Display Result Image

Selects an optional output image of the resulting neurite segmentation.

### Neurite result

Opens the Destination Image selector. This image selector is enabled only when Display Result Image is checked. *Overwrite source* and *Add to* are not permitted. Each neuron is drawn in a unique gray level over a black background. The intensity values then correspond to the cell labels in the Data Log. This image is displayed as an autoscaled pseudocolor 16-bit image. When played back from Review Screen Data, the result image will not be stored in the database.

### Illumination

Selects the appropriate illumination to match the type of illumination used during image acquisition. For images acquired with Fluorescence illumination, click *Fluorescence*, for images acquired with transmitted light illumination, click *Transmission*.

### Cell Bodies

Defines the parameters for detecting cell bodies.

### Maximum Width

Specifies the approximate maximum cell body width. This width aids in estimating what intensity fluctuations are potential cell bodies compared to background fluctuations. This value is entered in µm units. The width, in integer pixel units, is displayed on the right side of the edit box.

### Intensity Above Local Background

Specifies the estimated intensity threshold of cell bodies compared to neighboring background values. This setting determines the gray level sensitivity of cell body detection.

### Minimum Area

Specifies the minimum individual cell body area. This setting is optional and does not affect initial cell body detection, but rather serves to exclude isolated small bright areas initially detected as cells. This aides in distinguishing foreign particles that may occur in certain samples from legitimate cell bodies. This value is entered in $um^2$ units. The area, in integer pixel units, is displayed on the right side of the edit box.

**Note:** To exclude nuclei without cell bodies, adjust the ***Minimum Area*** of cell bodies parameter to an appropriate value. The only exception to this minimum area selection is for the cases of cells with multiple nuclei and multiple cells stuck together. These cases are indistinguishable in processing. The area of the entire stuck-together cell body mass is the area that is included or excluded by an "area open" filter. So in this case, small cells (one per nucleus is assumed, but may not be the case) below minimum area *are* allowed to remain if they are stuck together in a continuous mass that exceeds min area.

### Nuclear Stain

Defines the parameters for detecting cell nuclei.

### Use Nuclear Stain

Specifies generation of an additional nuclear stained image. This image can help to mark cell bodies, improving the ability to distinguish them from dirt and from each other when in close proximity.

### Nuclear Image

Opens the Image selector for the nuclear stained image. To use this option, *Use Nuclear Stained Image* must be checked .

### Display Result

Specifies generation of an optional output image of the resulting nuclear segmentation.

### Nuclear Result

Opens the image selector for the nuclear stained result image. This is active only when *Use Nuclear Stain* is checked.

### Nuclear Diameter

Specifies the approximate diameter of stained nuclei. This parameter determines the scale of processing functions that detect the nuclei and label the cells. This value is entered in µm units. The width in integer pixel units is displayed to the right of edit box.

### Signal to Noise Ratio

Specifies the estimated signal to noise ratio (approximate intensity of nuclear stains to approximate fluctuations in background intensity).

### Outgrowths

Defines the parameters for detecting neurites.

### Maximum Width

Specifies the approximate maximum outgrowth width. This width aids in the differentiation between cell body deformations and actual outgrowths. It also helps to estimate the range of outgrowth widths that can be distinguished from random noise patterns and background. This value is entered in integer pixel units. The width in user units (such as microns) is displayed to the right of edit box.

### Intensity Above Local Background

Specifies the estimated intensity threshold of neurites as compared to neighboring background values. This setting determines the gray level sensitivity of outgrowth detection.

### Min cell growth to log as significant

Specifies the required minimum length of a Neurite Outgrowth in order for the outgrowth data to be logged.

### Set to Defaults

Resets all dialog box option parameters to the default values.

### Configure Summary Log

Opens the Configure Log dialog box. The Summary Log records results related to the entire image area. Use this dialog box to choose the values to include. When you select *Cells significant growth* and/or *% cells significant growt*h, cell-specific data are accumulated. For accumulating site-specific data only, ensure that neither *Cells significant growth* nor *% cells significant growth* are selected.

### Configure Data Log (Cells)

Opens the Configure Log dialog box. The Data Log for Cells records cell-specific results. When any measurement item on this list is checked, the program accumulates results that define physical characteristics and measurements corresponding to each individual cell and its associated neurites.

### Set to Defaults

Resets all options and settings to their default values.

### **Test Run** (from Review Plate Data only)

Runs one pass of the Neurite Outgrowth application module on the images currently open and selected. Measurements will be logged if the Summary and/or Data Logs are open.

### **Apply** (from the Apps menu only)

Applies the settings that you made and runs the application module.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

**Close**

Closes the Neurite Outgrowth dialog box.

# Angiogenesis Tube Formation (Apps Menu)

**Analyzes Angiogenesis Tube Formation experiments through image segmentation, differentiation between tubules and nodes, tubule growth measurement, and measurement data logging.**

*Availability: Exclusive to MetaMorph and MetaXpress*

### Drop-in: Angiogenesis

Use this application module to segment images for the purpose of identifying, differentiating, and measuring tubules and nodes. The segmentation labels each pixel as one of the following: background (black), tubule growth (white), or node region (green). Segmented images are used to measure tubule length, tubule thickness, tubule area, the total area of tubules as a fraction of the image area (excluding nodes), segments, branch points, and nodes. All spatial measurements are given in microns, or where appropriate, squared-microns.

Source images must be 16-bit images. This application module produces 16-bit result images. Default settings enable you to produce a result from a typical image without changing settings. Three settings are available to you: Approximate minimum tubule width, Approximate maximum tubule width, and the average graylevel intensity of the tubule above the background.

Any tubule-like pattern of the image less than the specified minimum width will be considered to be noise, and will be excluded from the segmentation. The value you specify for the maximum width is used to differentiate tubules from nodes. When tubule growths become thicker than the maximum width, they are labeled as nodes in the segmentation and are excluded from all tubule measurements, such as length. If you want to include nodes in your tubule measurements, set the maximum width to a value equal to or greater than the widest node in the image. In this case, the results will indicate that zero nodes were found, and the entire growth will contribute to the tubule measurements.

After the application module has processed the image, you can use the ***Cellular Results*** table to interactively view data belonging to an individual cell or group of cells by clicking the cell(s) in the image.

**Application Note PDF — *Using the Angiogenesis Tube Formation Application Module in MetaMorph***

**Application Note PDF — *MetaMorph Application Module Overview***

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Analyzing Angiogenesis Tube Formation Images

To run the Angiogenesis Tube Formation application module, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click *Angiogenesis Tube Formation*, the Angiogenesis Tube Formation dialog box opens. |
| **2** | From the File menu, click *Open*, and open an appropriate 16-bit image. |
| **3** | In the Angiogenesis Tube Formation dialog box, click the *Source image* selector button, and choose the image that you want to |

segment.

**4** Click *Display result image* if you want the result image to open after it is created.

**5** In the *Approximate min width* box, type or select the appropriate minimum tubule width. Tubule-like patterns in the image less than this width will be considered to be noise, and will be excluded from the segmentation. **Note:** If you specify 0 or 1 pixels as the minimum width, no detected growths will be excluded from detection.

**6** In the *Approximate max width* box, type or select the appropriate maximum tubule width. This value is used to differentiate tubules from nodes and exclude nodes from the segmentation. If you want to include node regions in tubule measurements to prevent nodes from being differentiated from tubules and excluded from measurement, set this to a value that is greater than the widest node.

**7** In the *Intensity above local background* box, type or select a value that represents the minimum tubule intensity minus the background intensity near the tubules. The lower the value, the more sensitive the detection of dim or subtle growth The software will estimate the background for each tubule locally to correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the most subtle tubule that you want to detect. The graylevel values are shown on the status bar below the image area.

**8** Click *Configure Summary Log*. The Configure Log dialog box opens. Check and/or uncheck individual parameter configuration settings. Refer to the description of these settings under *Dialog Box Options: Configure Summary Log.*

**9** To restore default values the available settings, click *Set to Defaults* at any time.

**10** Click *Apply* to run the application module and generate the result image.

**11** Click *Close* to close the Angiogenesis Tube Formation dialog box.

## Angiogenesis Tube Formation Dialog Box Options

### Source Image

Opens the source Image Selector. This selector works on the active plane only, and allows 16-bit images only. A value of [None] is allowed for cases in which images for a specific settings file cannot be found in the database. Source images opened with this image selector should be calibrated in microns in order to correctly process the image.

**Notes:**

- When processing is complete, this image is automatically overlaid with the segmentation result image. If you do not re-save the source image, the original source image is not affected. However, if you re-save the source image the overlay information will be saved with it.
- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

### Display Result

Activates the display of the of the segmentation result image (optional).

### Result image

Opens the Image Selector for the Destination Image. This selection is enabled only if Create Result Image checked. This setting shows segmentation of the vessels drawn as a value of 2 (white) with nodes as a value of 1 (green) over a value of 0 (black) background. This image will be displayed as an autoscaled, 16-bit image for easy color combine, arithmetic, or overlay with the original. When this image is opened using Review Screen Data, the result image will not be stored in the database.

### Parameters

Defines the parameters for detecting vessels.

### Approximate minimum width

Specifies the minimum tubule width in microns (µm) that you expect to detect. Tubule-like patterns in the image less than this width will be considered to be noise patterns unless they are part of a longer continuous growth. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

> **Note**: If you specify 0 or 1 pixels as the minimum width, no detected growths will be excluded from detection.

### Approximate maximum width

Specifies the maximum tubule width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential tubules as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box. If you want to include node regions in tubule measurements to prevent nodes from being detected and excluded from measurement, set this value to one equal to or greater than the widest node.

### Intensity above local background

Specifies a value for the intensity threshold of vessels compared to the neighboring background values. This setting is for controlling the sensitivity of the detection/segmentation.

### Configure Summary Log

Opens the configure log dialog box. Use this dialog box to select the measurements that you want to include in your summary log. The following measurements can be selected:

**Total Tubule Length –** Total microns of vessel length (excluding nodes)

**Mean Tubule Length –** Total length divided by the number of segments.

**Total Tubule Area –** Total square microns of vessel area (excluding nodes)

**Mean Tubule Area –** Total square microns of vessel area divided by the number of segments.

**Tubule % Area Covered –** Total vessel area (excluding nodes) divided by total image area (width times height).

**Average Tubule thickness –** Average thickness of vessels computed as total area (excluding nodes) divided by total length (excluding nodes), and indicated in microns.

**Segments –** Total number of vessel segments connecting branch points and/or ends.

**Branch points –** Total number of junctions connecting segments (Nodes are not considered branches).

**Nodes –** number of connected blobs with thickness exceeding maximum width. Excluded from length and area measures. Nodes are shown as green in the segmentation image; tubules are shown as white.

**Total Node Area –** Total square microns of node area.

**Mean Node Area –** Total square microns of node area divided by the number of nodes.

**Node % Area Covered –** Total node area divided by total image area (width times height).

**Connected Sets –** Number of distinct objects detected in the image not connected to one another (no path of connected pixels of tubules or nodes connects the objects). This measures the overall connectivity of the growth network (a completely connected network would have just one connected set of pixels).

**Tubule Length Per Set –** Total tubule length in microns divided by the number of connected sets.

### Save Settings

Opens the Save Settings dialog box. Use this dialog box to create and save a Angiogenisis settings file. You can store all the settings currently specified in the Angiogenisis dialog box. Use Load Settings to retrieve and use these settings at a later time.

### Load Settings

Opens the Load Settings dialog box. Use this dialog box to select and load a Angiogenisis settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the Angiogenisis settings file. Select the name of the setting that you want to use, then click OK.

### Set to Defaults

Resets all options and settings to their default values.

### **Test Run** (from Review Plate Data only)

Runs one pass of the Angiogenisis application module on the images currently open and selected. Measurements will be logged if the Summary and/or Data Logs are open.

### **Apply** (from the Apps menu only)

Applies the settings that you made and runs the application module.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

### Close

Closes the dialog box.

# Cell Proliferation HT

**Identifies and isolates cell nuclei through image segmentation and logs image data for Cell Proliferation to an associated log file.**

*Availability: Exclusive to MetaXpress*

**Drop-in: CellProliferationHT**

Use this application module to segment images that are used to identify and differentiate cell nuclei. The segmentation labels each isolated and identified nucleus asas white. Source images must be 16-bit images. This application module produces 16-bit result images. Default settings enable you to produce a result from a typical image without changing settings. Three settings are available to you: Approximate minimum nuclei width, Approximate maximum nuclei width, and the minimum gray level intensity of the nuclei above the local background.

Any nuclei-like pattern in the image less than the specified minimum width will be considered to be noise, and will be excluded from the segmentation. The value you specify for the maximum width can be used to exclude any blobs larger than the specified size and to control the locality of background intensity estimates near each nucleus. This application module is similar to Count Nuclei, but it is optimized for high throughput.

**Application Note — *MetaMorph Application Module Overview* (PDF)**

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Counting Nuclei

To run the Cell Proliferation application module, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click *Cell proliferation*; the Cell proliferation dialog box opens. |
| **2** | From the File menu, click *Open*, and open an appropriate 16-bit image. |
| **3** | In the *Cell proliferation* dialog box, click the *Source image* selector button, and choose the image that you want to segment. |
| **4** | Click *Display result image* if you want the result image to open after it is created. |
| **5** | In the *Approximate min width* box, type or select the appropriate minimum cell nuclei width.<br><br>***Hint:*** You can use the Caliper tool or the Line Region tool to help you approximate the minimum nuclei width. The caliper tool measures directly in microns; the line region tool measures in pixels, which you must convert to microns. |
| **6** | In the *Approximate max width* box, type or select the appropriate maximum nuclei width. This value is used to differentiate nuclei from non-nuclear material and exclude nuclei greater than the specified size from the segmentation.<br><br>***Hint:*** You can use the Caliper tool or the Line Region tool to help you approximate the maximum nuclei width. The caliper tool measures directly in microns; the line region tool measures in pixels, which you must convert to microns. |
| **7** | In the *Intensity above local background* box, type or select a value that represents the minimum nucleus intensity minus the background intensity near the nucleus. The lower the value, the more sensitive the detection. The software will estimate the background for each nucleus locally to |

correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the most subtle nuclei that you want to detect. The graylevel values are shown on the status bar below the image area. To obtain the *Intensity above local background* value, subtract the value of the background from the value of the nucleus.

**8** Click *Configure Summary Log*. The Configure Log dialog box opens. Check and/or uncheck individual parameter configuration settings. Refer to the description of these settings under *Dialog Box Options: Configure Summary Log.*

**9** To restore default values for the available settings, click *Set to Defaults* at any time.

**10** Click *Apply* to run the application module and generate the result image.

**11** Click *Close* to close the Cell Proliferation dialog box.

## Cell Proliferation HT - Dialog Box Options

### Source Image

Opens the source Image Selector. This selector works on the active plane only, and allows 16-bit images only. A value of [None] appears for cases in which images for a specific settings file cannot be found in the database. Source images opened with this image selector should be calibrated in microns in order to correctly process the image.

### Notes:

- When processing is complete, this image is automatically overlaid with the segmentation result image. If you do not re-save the source image, the original source image is not affected. However, if you re-save the source image the overlay information will be saved with it.
- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

### Display Result

Activates the display of the of the segmentation result image (optional).

### Result image

Opens the Image Selector for the Destination Image. This selection is enabled only if Create Result Image checked. This image will be displayed as an autoscaled, 16-bit image for easy color combine, arithmetic, or overlay with the original. When this image is opened using Review Screen Data, the result image will not be stored in the database.

### Parameters

Defines the parameters for detecting nuclei.

### Approximate minimum width

Specifies the minimum nuclear width in microns (µm) that you expect to detect. Nuclei patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

### Approximate maximum width

Specifies the maximum nuclei width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential nuclei as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of nuclei compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

### Configure Summary Log

Opens the configure log dialog box. Use this dialog box to select the measurements that you want to include in your summary log. In addition to the standard log measurements, you can also choose the **Total Nuclei** measurement, which indicates the total number of nuclei within the image.

### Save Settings

Opens the Save Settings dialog box. Use this dialog box to create and save a Cell Proliferation HT settings file. You can store all the settings currently specified in the Cell Proliferation HT dialog box. Use Load Settings to retrieve and use these settings at a later time.

### Load Settings

Opens the Load Settings dialog box. Use this dialog box to select and load a Cell Proliferation HT settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the Cell Proliferation HT settings file. Select the name of the setting that you want to use, then click OK.

### Set to Defaults

Resets all options and settings to their default values.

### **Test Run** (from Review Plate Data only)

Runs one pass of the Cell Proliferation HT application module on the images currently open and selected. Measurements will be logged if the Summary and/or Data Logs are open.

### **Apply** (from the Apps menu only)

Applies the settings that you made and runs the application module.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

### Close

Closes the dialog box.

# Cell Scoring (Apps Menu)

**Detects, counts, and logs measurements derived from a user-designated positive marker applied to cell nucleus, cytoplasm, or both.**

*Availability: Exclusive to MetaMorph and MetaXpress*

**Dropin: CELLSCORING**

Use this application module to count and log measurements of cells detected by a second wavelength in samples using two stains and two wavelengths.

Cell Scoring is a general purpose application module that can be used in conjunction with a user-defined analysis to identify a single, secondary type of marker in a sample in which all nuclei are identified and segmented with a primary marker. This application module uses two wavelengths in conjunction with two markers. The first marker should stain and identify all nuclei. The second marker should stain either nuclei, cytoplasm, or both. The module identifies a number of useful measurements including the number of cells scored positive and the percent of cells scored positive as detected by the marker for the

second wavelength.

## General Procedures

1. Prepare samples using two different stains; one for All Nuclei and one for Positive Marker images.
2. Collect your dataset using two different wavelengths (All Nuclei and Positive Marker).
3. Open the Review Screen Data dialog box and choose Cell Scoring.
4. Select either a Cell Scoring stored configuration setting or set dialog box parameters to correctly detect each wavelength.
5. Open log files for both the Summary Log and the Data Log.
6. Select the measurements that you want to log for both the Summary Log and the Data Log.
7. Run the analysis for a single well, selected wells, the entire plate, or multiple plates.

After the application module has processed the image, you can use the ***Cellular Results*** table to interactively view data belonging to an individual cell or group of cells by clicking the cell(s) in the image.

**Application Note — *MetaMorph Application Module Overview* (PDF)**

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Running the Cell Scoring Application Module

To run the Cell Scoring Application Module, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click Cell Scoring. The Cell Scoring dialog box opens. |
| **2** | From the File menu, click Open. The Open dialog box is displayed. |
| **3** | From the Open dialog box, choose the images for Wavelengths 1 and 2, then click *Open*. |
| **4** | To display a combined segmentation result image for all three wavelengths, click *Display Result Image,* then open the Image selector for Display result Image, and choose "*Segmentation."* |
| **5** | In the Source Image Selector for *All Nuclei*, select the appropriate image for wavelength number 1. |
| **6** | In the Source Image Selector for *Positive Marker*, select the appropriate image for wavelength number 2. |
| **7** | In the *All nuclei* area, in the *Approximate min width* box, type or select a value in microns for the minimum nuclei width. This dimension should be based on the smallest |

width of the smallest nucleus that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings***.

**8** In the *All nuclei* area, in the *Approximate max width* box, type or select a value in microns for the maximum nuclei width. This dimension should be based on the largest width of the largest nucleus that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings***.

**9** In the *All nuclei* area, in the *Intensity above local background* box, type or select a value to define the difference in intensity between the dimmest nuclei and the background adjacent to it. For details on making this setting, refer to the procedure for determining Intensity above local background under ***Determining Segmentation Settings***.

**10** In the *Positive Marker* area, in the *Stained area* box, select the area to which the stain is applied in the image. Select either Nucleus, Cytoplasm, or Nucleus and Cytoplasm.

**11** In the *Positive Marker* area, in the *Approximate min width* box, type or select a value in microns for the minimum object width. This dimension should be based on the smallest width of the smallest object that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings***.

**12** In the *Positive Marker* area, in the *Approximate max width* box, type or select a value in microns for the maximum object width. This dimension should be based on the largest width of the largest object that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings***.

**13** In the *Positive Marker* area, in the *Intensity above local background* box, type or select a value to define the difference in intensity between the dimmest object and the background adjacent to it. For details on making this setting, refer to the procedure for determining Intensity above local background under ***Determining Segmentation Settings***.

**14** For both of the staining types, All Nuclei, and

Positive marker, click *Preview* to view the segmentation derived from the image staining. The preview image is displayed as a graphic overlay on the original image of the appropriate staining type.

**15** Click *Configure Summary Log* to choose the measurements that you want to include in your Summary Log.

**16** Click *Configure Data Log* to choose the measurements that you want to include in your Data Log.

**17** Click *Apply* to run the Cell Scoring application module.

**18** To interactively view data belonging to an individual cell or group of cells, select the cell(s) in the image and view the data in the ***Cellular Results*** table.

**19** Click *Set to Defaults* to reset all Cell Scoring settings to their default values.

**20** Click *Close* to close the Cell Scoring dialog box.

## Determining Segmentation Settings

Use the following general procedures to make segmentation settings for each wavelength.

| Step | Action |
|---|---|
| **1** | If applicable, in the Stained Area box choose the area of the cell in which you expect the stain associated with this wavelength to be detected. Choose one of the following:<br>• *Nucleus* if you expect the stain to be detected only in the nucleus.<br>• *Cytoplasm* if you expect the stain to be detected only in the cytoplasm.<br>• *Nucleus* and *Cytoplasm* if the stain will be detected in both areas. |
| **2** | In the Approximate min width box, type or select a value in microns for the minimum width of the Stained area. If the stained area is the nucleus, the value should be small (typically between 2 and 20 microns ). If the stained area is the cytoplasm or both, the value should be larger (between 15 and 200 microns). Determine which cell appears to be the smallest in the field, then determine the approximate width of the widest dimension.<br>**Hint:** You can use the Caliper or Line Region tools to help determine the width. |
| **3** | In the Approximate max width box, type or select a value in microns for the maximum width of the Stained area. If the stained area is the nucleus, the value should be small |

(between 5 and 30 microns). If the stained area is the cytoplasm or both, the value should be larger (between 20 and 300 microns). Determine which cell appears to be the smallest in the field, then determine the approximate width of the widest dimension.

**Hint:** You can use the Caliper tool or Line Region tool to help determine the width.

**4** In the Intensity above local background box, type or select a value that represents the minimum nucleus or cytoplasm intensity minus the background intensity near the nucleus or cytoplasm. The lower the value, the more sensitive the detection. The software will estimate the background for each nucleus or cytoplasm locally to correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the blob that you want to detect. The graylevel values are shown on the status bar below the image area. To obtain the Intensity above local background value, subtract these two values.

**5** Click *Preview* to open a preview image of the segmentation for this wavelength overlaid on the original image. This preview image can be used to separate the effects of the individual wavelength detections interacting so that each wavelength's proper settings can be determined independently.

**Note:** To turn on or off the overlay image, click the overlay button on the image window.

## Cell Scoring - Dialog Box Options

### All nuclei

This area defines the settings that apply to the Wavelength 1 source image for all nuclei in the image.

#### W1 Source Image [<image name>]

Opens the source Image Selector for Wavelength 1. This selector works on the active plane only, and allows 16-bit images only. A value of [None] is shown for cases in which images for a specific settings file cannot be found in the database. Source images opened with this image selector should be calibrated in microns in order to correctly process the image.

#### Display result image

Activates the display of the of the segmentation result image (optional).

#### [Result Image Selector]

Opens the Image Selector for the destination image. This selection is enabled only if Display result image is checked. This setting shows segmentation of the cell or nucleus according to your selection. This image is displayed as an autoscaled, 16-bit image for easy color combine, arithmetic, or overlay with the original. When this image is opened using Review Screen Data, the result image will not be stored in the database.

**Notes:**

- When processing is complete, this image is automatically overlaid with the segmentation result image. If you do not re-save the source image, the original source image is not affected. However, if you re-save the source image the overlay information will be saved with it.
- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

#### Approximate min width

Specifies the minimum object width in microns (µm) that you expect to detect. Patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

#### Approximate max width

Specifies the maximum object width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential cells as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

#### Intensity above local background

Specifies a value for the intensity threshold of either nuclei or cytoplasm compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

#### Preview

Shows a color preview image of the segmentation for the associated wavelength overlaid on the original image. Click the Overlay Image button on the image window to turn on and off the overlay image. If you resave the original image after the overlay has been created, the overlay is saved with the original image, even if the overlay view is turned off when you save the image. After the overlay has been saved with the original image, you can turn it off and on as needed. If you resave the image with a new overlay, it will replace the current overlay image.

### Positive Marker

This area contains the settings used to isolate and identify cells or cell nuclei stained with an appropriate positive marker.

#### W2 Source image [<image name>]

Opens the source Image Selector for Wavelength 2. This selector works on the active plane only, and allows 16-bit images only. A value of [None] is shown for cases in which images for a specific settings file cannot be found in the database. Source images opened with this image selector should be calibrated in microns in order to correctly process the image.

#### Stained area

Use this drop down list to specify the cell area stained with a positive marker. Choose one of the following cell areas:

- **Nucleus**
- **Cytoplasm**
- **Nucleus and cytoplasm**

#### Approximate min width

Specifies the minimum object width in microns (µm) of the objects in your image that you expect to detect as positive using wavelength 2. Depending on the Stained Area that you selected, you can detect Nucleus, Cytoplasm, or both. Patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

### Approximate max width

Specifies the maximum object width in microns (µm) of the objects in your image that you expect to detect as positive using wavelength 2. Depending on the Stained Area that you selected, you can detect Nucleus, Cytoplasm, or both. This width determines which intensity fluctuations are potential cells as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of the selected stained area compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

### Preview

Shows a color preview image of the segmentation for the all wavelengths overlaid on the original image. In this *Positive Marker* preview, the segmentation of all identified objects is shown indicated by the appropriate assigned color for each type of object. Click the Overlay Image button on the image window to turn on and off the overlay image. If you resave the original image after the overlay has been created, the overlay is saved with the original image, even if the overlay view is turned off when you save the image. After the overlay has been saved with the original image, you can turn it off and on as needed. If you resave the image with a new overlay, it will replace the current overlay image.

## Configure Summary Log (Field Measurements)

Opens the Configure Log dialog box for the Summary Log. Use this dialog box to select the measurements that you want to include in your summary log. The following measurements can be selected:

**Total Cells:** Total number of cells (number of nuclei as determined from All nuclei image).

**Positive Cells:** Total number of cells that were positive for the Positive marker staining.

**Negative Cells:** Total number of cells that were negative for the Positive marker staining.

**% Positive Cells:** 100 * Positive Cells / Total Cells.

**% Negative Cells:** 100 * Negative Cells / Total Cells.

**All Nuclei Total Area:** Total µm²s (as determined from the All nuclei stain) of all nuclei.

**All Nuclei Mean Area:** All Nuclei Total Area / Total Cells.

**All Nuclei W1 Integrated Intensity:** Summed grayscale values from W1 of the detected nuclei pixels (as determined from the All nuclei stain).

**All Nuclei W1 Average Intensity:** All Nuclei W1 Integrated Intensity / All Nuclei Total (pixel) Area.

**All Nuclei W2 Integrated Intensity:** Summed grayscale values from W2 of the detected nuclei pixels (as determined from the All nuclei stain).

**All Nuclei W2 Average Intensity:** All Nuclei W2 Integrated Intensity / All Nuclei Total (pixel) Area.

**Positive Cells Total Area:** Total µm$^2$s of positive cells' stained area (nucleus, cytoplasm, or both as selected by the user for W2).

**Positive Cells Mean Area:** Positive Cells Total Area / Positive Cells.

**Positive Cells W2 Integrated Intensity:** Summed grayscale values from W2 of positive cells' stained area (nucleus, cytoplasm, or both as selected by the user for W2).

**Positive Cells W2 Average Intensity:** Positive Cells W2 Integrated Intensity / Positive Cells Total (Pixel) Area.

**Positive Nuclei Total Area:** Total µm²s (as determined from the All nuclei stain) of positive nuclei.

**Positive Nuclei Mean Area:** Positive Nuclei Total Area / Positive Cells.

**Positive Nuclei W1 Integrated Intensity:** Summed grayscale values from W1 of the detected nuclei pixels (as determined from the All nuclei stain) of positive cells.

**Positive Nuclei W1 Average Intensity:** Positive Cells W1 Integrated Intensity / Positive Nuclei Total (Pixel) Area.

**Positive Nuclei W2 Integrated Intensity:** Summed grayscale values from W2 of the detected nuclei pixels (as determined from the All nuclei stain) of positive cells.

**Positive Nuclei W2 Average Intensity:** Positive Nuclei W2 Integrated Intensity / Positive Nuclei Total (Pixel) Area.

**Negative Nuclei Total Area:** Total µm²s (as determined from the All nuclei stain) of negative nuclei.

**Negative Nuclei Mean Area:** Negative Nuclei Total Area/Negative Cells.

**Negative Nuclei W1 Integrated Intensity:** Summed grayscale values from W1 image of nuclear pixels (as determined from the All nuclei stain) of negative cells.

**Negative Nuclei W1 Average Intensity:** Negative Cells W1 Integrated Intensity / Negative Nuclei Total (Pixel) Area.

**Negative Nuclei W2 Integrated Intensity:** Summed grayscale values from W2 image of nuclear pixels (as determined from the All nuclei stain) of negative cells.

**Negative Nuclei W2 Average Intensity:** Negative Cells W2 Integrated Intensity / Negative Nuclei Total (Pixel) Area.

### Data Log (Cells)

Opens the Configure Log dialog box for cell-specific measurements data logging. Use this dialog box to select the measurements that you want to include in your cell-specific data log file log. This log file records cell-by-cell measurements. In addition to the standard log measurements, the following measurements can be selected:

**Cell: Assigned Label #**

**Cell: Classification:** Positive or negative.

**Cell: Nuclear Area:** Total µm²s in the all nucleus.

**Cell: Cell Area:** Total µm²s in the cell's stained area (nucleus, cytoplasm, or both as selected by the user for W2).

**Cell: W1 Integrated Nuclear Intensity:** Summed grayscale values from W1 within the detected nuclear pixels (as determined from the All nuclei stain).

**Cell: W1 Average Nuclear Intensity:** Total Cells Integrated Intensity / Nuclear Area.

**Cell: W2 Integrated Nuclear Intensity:** Summed grayscale values from W2 of the detected nuclear pixels (as determined from the All nuclei stain).

**Cell: W2 Average Nuclear Intensity:** W2 Integrated Nuclear Intensity / Nuclear Area.

**Cell: W2 Integrated Cell Intensity:** Summed grayscale values from W2 of the cell's stained area (nucleus, cytoplasm, or both as selected by the user for W2).

**Cell: W2 Average Cell Intensity:** W2 Integrated Cell Intensity / Cell Area.

### Save Settings

Opens the Save Settings dialog box. Use this dialog box to create and save a *Cell Scoring* settings file. You can store all the settings currently specified in the *Cell Scoring* dialog box. Use Load Settings to retrieve and use these settings at a later time. Type a setting name and an optional description, then click OK.

### Load Settings

Opens the Load Settings dialog box. Use this dialog box to select and load a *Cell Scoring* settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the *Cell Scoring* settings file. Select the name of the setting that you want to use, then click OK.

### Set to Defaults

Resets all options and settings to their default values.

**Test Run** (from Review Plate Data only)
Runs one pass of the *Cell Scoring* application module on the images currently open and selected.

**Apply** (from the Apps menu only)
Applies the settings that you made and runs the application module.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

**Close**
Closes the dialog box.

# Cell Cycle

**Detects the cell cycle phase of appropriately stained cells in one, two, or three wavelengths and analyzes cells for DNA content, mitosis, and optionally detects apoptosis.**

*Availability: Exclusive to MetaMorph and MetaXpress*

**Drop-in: Cellcycle**

Use this application module to determine the cell cycle phase of each cell in your image. Proper application of this module assumes that the cells in the image have been correctly stained. You can analyze images that have been acquired from samples using one, two, or three different wavelengths with appropriately applied staining. One wavelength is required for labeling the DNA in the sample. A second wavelength can be used to provide a marker to indicate Mitotic (M) phases. If a mitotic-specific stain is not used, mitosis is detected by the average intensity of the DNA stain. A third wavelength can optionally be used as an apoptotic marker.

DNA-containing structures, depending on their cell cycle phase, can be classified as G0/G1, S, G2, early M, late M, or apoptotic. Each DNA-containing structure can also be classified as either viable or apoptotic.

This application module provides both a Summary Log and a Data Log. The Summary Log shows results for the entire site. The Data Log shows cell-based results for each individual cell in the site.

After the application module has processed the image, you can use the ***Cellular Results*** table to interactively view data belonging to an individual cell or group of cells by clicking the cell(s) in the image.

**Application Note — *MetaMorph Application Module Overview* (PDF)**

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Analyzing Cell Cycles

To Analyze cell cycles, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click Cell Cycle. The Cell Cycle dialog box opens. |
| **2** | In the Results Legend Area, check *Display result image* if you want to create a result |

image in addition to the graphic overlays that show detection and classification results on the original images. The default name of the result image is Segmentation. To change the name of the result image, click the button marked Segmentation; the image selector opens. Use the standard Meta Imaging series image selector conventions to change the name of the result image.

**3** In the *DNA content* area, click the Source image selector to choose the image that you want to process.

**4** In the *Approximate min width* box, type or select the appropriate minimum width of the smallest cell nucleus in the image. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings***.

**5** In the *Approximate max width* box, type or select the appropriate maximum width of the largest cell nucleus in the image. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings***.

**6** In the *Intensity above local background* box, type or select an appropriate graylevel value for the cell nucleus. For details on making this setting, refer to the procedure for determining Intensity above local background under ***Determining Segmentation Settings***.

**7** In the *Background subtraction* box, choose *Auto Constant* (default), *Constant*, or *None*. Refer to the *Background subtraction* description. Choose *Auto Constant* if you want to automatically correct for varying amount of background intensity in the DNA content images within a dataset; choose *Constant* if you want to subtract the same specified amount of background intensity from all DNA content images within a dataset; choose *None* if the background levels in all images are close in intensity value and the background intensity is low.

**8** Click the DNA content *Preview* button to show a color-coded segmentation overlay on the original image along with a DNA content histogram.

**9** Move the left vertical slider in the histogram to visually designate the cut-off points between G0/G1 (2N) and S phase. The numerical intensity level value is indicated in the G0/G1 (2N) to S phase edit box. The slider and the edit box are linked to allow cutoff selection using either control.

**10** Move the right vertical slider in the histogram to visually designate the cut-off points

between S phase and G2 (4N). The numerical intensity level value is indicated in the S phase to G2 (4N) edit box. The slider and the edit box are linked to allow cutoff selection using either control. *Hint:* The histogram indicates the total number of pixels for each range of integrated intensity values. The highest counts typically appear in two clusters corresponding to 2N and 4N DNA content.

**11** For Mitotic classification, click *Mitotic-specific staining* if you have a separate Mitotic stained image, or click *DNA average intensity* if you are using the DNA stained image to classify mitotic cells.

**12** If you chose *Mitotic specific staining*, in the *intensity above local background* box, type or select the appropriate graylevel value for the stained cells.

**13** If you chose *DNA average intensity*, in the *Minimum average intensity* box, type or select an appropriate graylevel value.

**14** Click the Mitotic classification*Preview* button to see an overlay of the Mitotic classification segmentation on the original image. If you are using DNA average intensity for mitotic classification, a scatterplot of average intensity vs. DNA content also appears. The dashed vertical slider lines are linked to the DNA Content cutoff values (see steps 9 and 10), while the dashed horizontal slider line is linked to the mitotic classification cutoff value.

**15** Optionally, click *Apoptotic classification* to isolate, view, and analyze any cells that are apoptotic.

**Note:**You must have an apoptotic-stained image to use this classifier.

**16** Click the *Source image* selector to choose the image to use for apoptotic classification.

**17** Under Apoptotic staining In the *Stained area* box, select *Nucleus*, *Cytoplasm*, or *Nucleus and Cytoplasm*, depending on the area(s) that are stained in the image.

**19** Under *Apoptotic staining*, in the *Approximate min width* box, type or select a value in microns for the minimum object width. This dimension should be based on the smallest width of the smallest object that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings***.

**20** Under *Apoptotic staining*, in the *Approximate max width* box, type or select a value in microns for the maximum object width. This

| | |
|---|---|
| | dimension should be based on the largest width of the largest object that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings***. |
| **21** | Under *Apoptotic staining*, in the *Intensity above local background* box, type or select a value to define the difference in intensity between the dimmest object and the background adjacent to it. For details on making this setting, refer to the procedure for determining Intensity above local background under ***Determining Segmentation Settings***. |

## Determining Segmentation Settings

Use the following procedures to guide you in making the appropriate segmentation settings for each type of stain. *Mitotic classification* requires only the setting for *Intensity above local background*, and uses the same approximate widths used for DNA content detection.

| Step | Action |
|---|---|
| **1** | In the Approximate min width box, type or select a value in microns for the minimum width of the Stained area. If the stained area is the nucleus, the value should be small (typically between 2 and 20 microns ). If the stained area is the cytoplasm or both, the value should be larger (between 15 and 200 microns). Determine which cell appears to be the smallest in the field, then determine the approximate width of the widest dimension.<br><br>**Hint:** You can use the Caliper or Line Region tool to help determine the width. |
| **2** | In the Approximate max width box, type or select a value in microns for the maximum width of the Stained area. If the stained area is the nucleus, the value should be small (between 5 and 30 microns). If the stained area is the cytoplasm or both, the value should be larger (between 20 and 300 microns). Determine which cell appears to be the largest in the field, then determine the approximate width of the widest dimension.<br><br>**Hint:** You can use the Caliper tool or Line Region tool to help determine the width. |
| **3** | In the Intensity above local background box, type or select a value that represents the minimum nucleus or cytoplasm intensity minus the background intensity near the nucleus or cytoplasm. The lower the value, the more sensitive the detection. The software will estimate the background for |

each nucleus or cytoplasm locally to correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the object that you want to detect. The graylevel values are shown on the status bar below the image area. To obtain the Intensity above local background value, subtract these two values.

4 Click Preview to view the segmentation for an individual wavelength overlaid on the original image. This preview image can be used to separate the effects of the individual wavelength detections interacting so that each wavelength's proper settings can be determined independently.

**Note:** To turn on or off the overlay image, click the overlay button on the image window.

5 Click Apply/Test Run to see the complete segmentation using all wavelengths. Note: Preview results are only approximate. The complete segmentation may utilize information between the multiple wavelengths to give a more accurate segmentation than seen in the preview results alone.

## Cell Cycle - Dialog Box Options

### Results legend

Provides a color legend used to classify cells according to their current cell cycle state. These cell classifications are displayed as a graphic overlay on the source image.

#### (Results Legend)

Defines the color that is associated with a particular cell cycle state. The color definition assignments are as follows:

- ***G0/G1*** **– Blue**
- ***S Phase*** **– Turquoise**
- ***G2*** **– Green**
- ***Early M*** **– Orange**
- ***Late M*** **– Red**
- ***Apoptotic*** **– Violet**

#### Display result image

Specifies that you want to create a separate final result image identical to the overlay.

### DNA content

Provides the settings that you need to configure to correctly analyze the DNA staining image.

#### Source image

Opens the source Image Selector for the DNA staining image. This selector works on the active plane only, and allows 16-bit images only. Source images opened with this image selector should

be calibrated in microns in order to correctly process the image. This image/wavelength is required.

**Approximate min width**

Specifies the minimum width of the stained cell nuclei in microns (µm) that you expect to detect. Patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

**Approximate max width**

Specifies the maximum width of the stained cell nuclei in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential cells as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

**Intensity above local background**

Specifies a value for the intensity threshold of the stained compartments compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

**Preview**

Shows preview segmentation of DNA staining image as a graphic overlay of the source image and displays the DNA Content Histogram. The histogram graph information is color-coded according to the cell cycle phase that the data represents. The histogram contains two vertical dashed lines that act as "sliders" linked to the G0/G1 (2N) to S phase and S phase to G2 (4N) classification cutoff values. When you move (drag) the sliders side to side, the value in the corresponding cutoff value box changes accordingly.

**Background subtraction**

Specifies an overall constant value to remove from all pixel intensities in the DNA content image before measuring the integrated and average intensities. This setting improves the ability of the integrated intensity value to differentiate GO/G1, S, and G2 phases.

Choose ***Auto Constant*** to automatically estimate the constant background intensity. With Auto Constant selected, the background intensity is recalculated for each new image.

Choose ***Constant*** to manually specify a constant background intensity. When you choose Constant, a graylevels box opens. Type or select the gray level value that you want to use as the estimated background intensity.

Choose ***None*** to leave the background intensity unchanged. This setting is recommended for samples that have low level background intensities and where the background intensity varies only slightly from image to image.

**Classification by integrated intensity (x1000)**

Specifies the cutoff points within the three classifications of DNA content: G0/G1(2N), S phase, and G2 (4N).

**G0/G1 (2N) to S phase cutoff**

Specifies the integrated intensity multiplied by 1000. For example, if the user input is 3, the integrated intensity value would be 3,000. This setting is interactively linked to the DNA Content Histogram sliders.

**S phase to G2 (4N) cutoff**

Specifies the integrated intensity multiplied by 1000. For example, if the user input is 3, the integrated intensity value would be 3,000. This setting is interactively linked to the DNA Content Histogram sliders.

**Mitotic classification**

Provides settings used to determine the detection of mitosis (Early M (4N) and Late M (2N)). Mitotic classification can be determined either from samples using a mitotic stain or by measuring the DNA average intensity.

**Mitotic-specific staining**

Specifies that the image is acquired from a sample stained with a mitotic-specific stain. Mitotic cells will be classified as either Early M or Late M.

**DNA average intensity**

Specifies that mitotic cells will be identified based on the DNA average intensity value. DNA-stained mitotic cells will have the highest intensity value.

**Source image**

Image selector for 16-bit source of M phase marker. Disabled if DNA average intensity is used rather than Mitotic-specific staining.

**Intensity above local background**

Specifies a value for the intensity threshold of either nuclei or cytoplasm compared to the neighboring background values. This setting controls the sensitivity of the detection and segmentation.
**Note:** This setting is active if *Mitotic-specific staining* is selected.

**Minimum average intensity**

This setting is active if DNA average intensity is selected. Interactively linked to DNA Content Scatterplot vertical slider.
**Note:** This setting is active if *DNA average intensity* is selected.

**Preview**

Shows a color preview image of the segmentation for the associated wavelength overlaid on the original image. Click the Overlay Image button on the image window to turn on and off the overlay image. If you resave the original image after the overlay has been created, the overlay is saved with the original image, even if the overlay view is turned off when you save the image. After the overlay has been saved with the original image, you can turn it off and on as needed. If you resave the image with a new overlay, it will replace the current overlay image.

**Note:** If *DNA average intensity* is selected, this result image shows the DNA Content Scatterplot in addition to the color preview overlay. If *Mitotic-specific staining* is used, only the color preview overlay is shown.

**Apoptotic classification** (Optional)

Defines the settings and options used to configure the dialog box for detection of apoptosis. Apoptosis classification of a cell overrides all other cell cycle classification based on DNA content and/or the detection of mitosis. The options in this area are not active if apoptotic classification is not selected. If this box is checked, the following related settings and options are shown.

**Source image**

Image selector for 16-bit source of apoptotic marker.

**Stained area**

Use this drop down list to identify the stained cell area in your image to be assigned as apoptotic. Choose one of the following cell areas:

- Nucleus
- Cytoplasm
- Nucleus and cytoplasm

**Approximate min width**

Specifies the minimum object width in microns (µm) of all apoptotic cells in your image that you expect to detect. Patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

**Approximate max width**

Specifies the maximum object width in microns (µm) of the selected stained area of all the apoptotic cells in your image that you expect to detect. This width determines which intensity fluctuations are potential cells as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

**Intensity above local background**

Specifies a value for the intensity threshold of the selected stained area compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

**Preview**

Shows a color preview image of the segmentation for the all wavelengths overlaid on the original image. In this apoptotic staining preview, the segmentation of all identified objects is shown indicated by the appropriate assigned color for each type of object. Click the Overlay Image button on the image window to turn on and off the overlay image. If you resave the original image after the overlay has been created, the overlay is saved with the original image, even if the overlay view is turned off when you save the image. After the overlay has been saved with the original image, you can turn it off and on as needed. If you resave the image with a new overlay, it will replace the current overlay image.

**Configure Summary Log**

**DNA Structures:** Number of DNA structures detected in the image segmentation.

**DNA Background Value:** The constant background value of the DNA content image as determined by the "Background subtraction" method, the number entered by the user for the method "Constant," 0 for the method "None", or the computed value for the method "Auto Constant."

**G0/G1 Cells:** Number of DNA structures classified as G0/G1.

**% G0/G1: G0/G1 Cells** / **DNA Structures** multiplied by 100.

**S Phase Cells:** Number of DNA compartments classified as S phase.

**% S: S Phase Cells** / **DNA Structures** multiplied by 100.

**Classified G2:** Number of DNA structures classified as G2.

**% G2: G2 Cells** / **DNA Structures** multiplied by 100.

**Early M Cells:** Number of DNA structures classified as early M.

**% Early M: Early M Cells** / **DNA Structures** multiplied by 100.

**Late M Cells:** Number of DNA structures classified as late M.

**% Late M: Late M Cells** / **DNA Structures** multiplied by 100.

**Apoptotic Cells:** Number of DNA structures classified as positive for apoptosis.

**% Apoptotic: Apoptotic Cells** / **DNA Structures** multiplied by 100.

**Configure Data Log**

**Cell: Label:** unique label number (1 through number of DNA structures).

**Cell: Classification:** G1, S phase, G2, Early M, Late M, or Apoptotic.

**Cell: Area:** Total µm²s in the DNA structure.

**Cell: DNA Integrated Intensity:** Summed grayscale values in the DNA structure**.**

**Cell: DNA Average Intensity:** DNA Integrated Intensity / Area (in pixels).

**Cell: Mitotic Integrated Intensity:** Summed grayscale values in the Mitotic-specific staining image overlapping the DNA structure. *Note: appears only if Mitotic-specific staining used.*

**Cell: Mitotic Average Intensity:** Mitotic Integrated Intensity / Area (in pixels). *Note: appears only if Mitotic-specific staining used.*

**Cell: Apoptotic Integrated Intensity:** Summed grayscale values in the Apoptotic staining image overlapping the DNA structure. *Note: appears only if Apoptotic classification used.*

**Cell: Apoptotic Average Intensity:** Apoptotic Integrated Intensity / Area (in pixels). *Note: appears only if Apoptotic classification used.*

**Save Settings**

Opens the Save Settings dialog box. Use this dialog box to create and save a Cell Cycle settings file. You can store all the settings currently specified in the Cell Cycle dialog box. Use Load Settings to retrieve and use these settings at a later time.

**Load Settings**

Opens the Load Settings dialog box. Use this dialog box to select and load a Cell Cycle settings file. When this file is loaded, it replaces all current settings

with the saved settings stored in the Cell Cycle settings file. Select the name of the setting that you want to use, then click OK.

**Set to Defaults**

Resets all options and settings to their default values.

**Test Run** (from Review Plate Data only)

Runs one pass of the Cell Cycle application module on the images currently open and selected. Measurements will be logged if the Summary and/or Data Logs are open.

**Apply** (from the Apps menu only)

Applies the settings that you made and runs the application module. Measurements will be logged if the Summary and/or Data Logs are open.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

**Close**

Closes the dialog box.

# Cell Health (Apps Menu)

**Detects and quantitatively analyzes both apoptotic and necrotic cells in samples using appropriately applied fluorescent markers.**

*Availability: Exclusive to MetaMorph and MetaXpress*

**Drop-in: CELLHEALTH**

Use this application module to count cells in images acquired from samples that contain three separate stains detected by three separate wavelengths.

This application module enables you to analyze images in which staining of nuclei, cytoplasm, or both is being detected by three wavelengths that identify markers for All Nuclei, Apoptotic cells, and Dead cells. The All Nuclei marker is detected in the All Nuclei wavelength (W1) to determine the total number of cells. The Apoptotic cell marker and the Dead cell marker are detected in separate images corresponding to the associated wavelengths. The combined detection data from the Apoptotic wavelength (W2) and the Dead wavelength (W3) are used to determine the following cell health conditions:

- ***Viable –*** Indicates a normal living cell; no evidence of Apoptosis or Necrosis is detected. In this instance, both the apoptotic marker and the dead marker are negative.
- ***Early Apoptotic*** – Indicates that beginning signs of Apoptosis have been detected in the cell. In this instance, the apoptotic marker is positive and the dead marker is negative.
- ***Late Apoptotic*** – Indicates that advanced signs of Apoptosis have been detected in the cell. In this instance, both the apoptotic marker and the dead marker are positive.
- ***Necrotic*** – Indicates that cell death has not been through the apoptotic pathway. In this instance, the apoptotic marker is negative and the dead marker is positive.

The measurement results from running this module are stored in two different logs. The Summary Log records "Field Measurements" that pertain to the entire image that you are analyzing. The Data Log records "Cell-by-cell" Measurements specific to each individual cell.

Whenever you run this application module, if you have selected *Display result image,* three image windows open. Each window dialog box provides a unique overlay that shows only the color coded

segments relevant to the type of cell classifications present.

After the application module has processed the image, you can use the ***Cellular Results*** table to interactively view data belonging to an individual cell or group of cells by clicking the cell(s) in the image.

**Application Note — *MetaMorph Application Module Overview* (PDF)**

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Running the Cell Health Application Module

To run the Cell Health Application Module, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click Cell Health. The Cell Health dialog box opens. |
| **2** | In the Source Image Selector for *All Nuclei*, select the appropriate image for wavelength number 1. |
| **3** | In the Source Image Selector for *Apoptotic staining*, select the appropriate image for wavelength number 2. |
| **4** | In the Source Image Selector for *Necrotic staining*, select the appropriate image for wavelength number 3. |
| **5** | In the *All nuclei* area, in the *Approximate min width* box, type or select a value in microns for the minimum nuclei width. This dimension should be based on the smallest width of the smallest nucleus that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings.*** |
| **6** | In the *All nuclei* area, in the *Approximate max width* box, type or select a value in microns for the maximum nuclei width. This dimension should be based on the largest width of the largest nucleus that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings.*** |
| **7** | In the *All nuclei* area, in the *Intensity above local background* box, type or select a value to define the difference in intensity between the dimmest nuclei and the background adjacent to it. For details on making this setting, refer to the procedure for determining Intensity above local background under ***Determining Segmentation Settings.*** |
| **8** | In the *Apoptotic staining* area, in the *Stained area* box, select the area to which the stain is applied in the image. Select either |

Nucleus, Cytoplasm, or Nucleus and Cytoplasm.

**9** In the *Apoptotic staining* area, in the *Approximate min width* box, type or select a value in microns for the minimum object width. This dimension should be based on the smallest width of the smallest object that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings.***

**10** In the *Apoptotic staining* area, in the *Approximate max width* box, type or select a value in microns for the maximum object width. This dimension should be based on the largest width of the largest object that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under *Determining Segmentation Settings*.

**11** In the *Apoptotic staining* area, in the *Intensity above local background* box, type or select a value to define the difference in intensity between the dimmest object and the background adjacent to it. For details on making this setting, refer to the procedure for determining Intensity above local background under ***Determining Segmentation Settings.***

**12** In the *Dead staining* area, in the *Stained area* box, select the area to which the stain is applied in the image. Select either Nucleus, Cytoplasm, or Nucleus and Cytoplasm.

**13** In the *Dead staining* area, in the *Approximate min width* box, type or select a value in microns for the minimum object width. This dimension should be based on the smallest width of the smallest object that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings.***

**14** In the *Dead staining* area, in the *Approximate max width* box, type or select a value in microns for the maximum object width. This dimension should be based on the largest width of the largest object that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings.***

**15** In the *Dead staining* area, in the *Intensity above local background* box, type or select a value to define the difference in intensity

between the dimmest object and the background adjacent to it. For details on making this setting, refer to the procedure for determining Intensity above local background under ***Determining Segmentation Settings.***

**16** For each of the staining types, (All Nuclei, Apoptotic, and Dead), click *Preview* to view the segmentation derived from the image staining. The preview images is displayed as a graphic overlay on the original image of the appropriate staining type.

**17** Click *Configure Summary Log* to choose the measurements that you want to include in your Summary Log.

**18** Click *Configure Data Log* to choose the measurements that you want to include in your Data Log.

**19** To create a combined segmentation result image for all three wavelengths, click *Display Result Image,* then open the Image selector for Display result Image, and choose "*Segmentation."*

**20** Open the Summary or Data Log, *if needed.*

**21** To interactively view data belonging to an individual cell or group of cells, select the cell(s) in the image and view the data in the ***Cellular Results*** table.

**22** Click *Apply* to run the Cell Health application module.

**23** Click *Close* to close the Cell Health dialog box.

## Determining Segmentation Settings

Use the following general procedures to determine segmentation settings for each wavelength.

| Step | Action |
|---|---|
| **1** | In the Stained Area box choose the area of the cell in which you expect the stain associated with this wavelength to be detected. Choose one of the following:· <br>• Nucleus if you expect the stain to be detected only in the nucleus. <br>• Cytoplasm if you expect the stain to be detected only in the cytoplasm. <br>• Nucleus and Cytoplasm if the stain will be detected in both areas. |
| **2** | In the Approximate min width box, type or select a value in microns for the minimum width of the Stained area. If the stained area is the nucleus, the value should be small |

(typically between 2 and 20 microns ). If the stained area is the cytoplasm or both, the value should be larger (between 15 and 200 microns). Determine which cell appears to be the smallest in the field, then determine the approximate width of the smallest dimension.

**Hint:** You can use the Caliper or Line Region tools to help determine the width.

**3** In the Approximate max width box, type or select a value in microns for the maximum width of the Stained area. If the stained area is the nucleus, the value should be small (between 5 and 30 microns). If the stained area is the cytoplasm or both, the value should be larger (between 20 and 300 microns). Determine which cell appears to be the largest in the field, then determine the approximate width of the widest dimension.

**Hint:** You can use the Caliper tool or Line Region tool to help determine the width.

**4** In the Intensity above local background box, type or select a value that represents the minimum nucleus or cytoplasm intensity minus the intensity of the background near the nucleus or cytoplasm. (Hint: You can determine these values by placing your mouse pointer on pixels just inside and outside the object (nucleus or cytoplasm) that you want to detect. The gray level values are shown on the status bar below the image area) The lower the value selected, the more sensitive the detection. The software will estimate the background for each nucleus or cytoplasm locally to correct for cases of images with uneven backgrounds.

**5** Click Preview to overlay a preview image of the segmentation for this wavelength on the original image. This preview image can be used to determine each wavelength's proper settings independently.

**Note:** To turn on or off the segmentation overlay image, click the overlay button on the image window.

## Cell Health - Dialog Box Options

### Results legend

Provides a four-color legend that indicates Viable, Early apoptotic, Late apoptotic, and Necrotic. These cell classifications are displayed as a graphic overlay on the source image.

### Notes:

- When processing is complete, this image is automatically overlaid with the segmentation result image. If you do not re-save the source image, the original source image is not

affected. However, if you re-save the source image the overlay information will be saved with it.

- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

**Display result image** – specifies that you want to create a separate final result image identical to the overlay.

**Display result image:** image selector control for separate image result identical to the overlay.

**Adaptive Background Correction**™ -- Backgrounds of images analyzed using this or any MetaXpress application module are processed using the Adaptive Background Correction™ system. This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

### All nuclei

The following settings are applied to Wavelength 1 which is used to locate, identify, and measure all nuclei in the image.

#### W1 Source image [<image name>]

Selects the source image that you want to use from the available open source images.

**Note:** In the All nuclei image, the stain is expected to label the nuclei from all cells.

#### Approximate min width

Specifies the minimum object width in microns (µm) of all the nuclei in your image that you expect to detect. Patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

#### Approximate max width

Specifies the maximum object width in microns (µm) of all the nuclei in your image that you expect to detect. This width determines which intensity fluctuations are potential cells as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

#### Intensity above local background

Specifies a value for the intensity threshold of both nuclei or cytoplasm compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

#### Preview

Shows an overlay preview of the segmentation for the all nuclei image overlaid on the original W1 (All nuclei) image. Click the Overlay Image button on the image window to turn on and off the overlay image. If you resave the original image after the overlay has been created, the overlay is saved with the original image, even if the overlay view is turned off when you save the image. After the overlay has been saved with the original image, you can turn it off and on as needed. If you resave the image with a new overlay, it will replace the current overlay image.

### Apoptotic staining

The following settings are applied to Wavelength 2 which is used to locate, identify, and measure apoptotic cells in the image. The segmentation overlay for this image identifies both early apoptotic cells, (shown as blue) and late apoptotic cells, (shown as violet).

#### W2 Source image [<image name>]

Selects the source image that you want to use from the available open source images.

#### Stained area

Use this drop down list to identify the stained cell area in your image to be assigned as Apoptotic. Choose one of the following cell areas:

- **Nucleus**
- **Cytoplasm**
- **Nucleus and cytoplasm**

### Approximate min width

Specifies the minimum object width in microns (µm) of apoptotic cells in your image that you expect to detect. Patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

### Approximate max width

Specifies the maximum object width in microns (µm) of apoptotic cells in your image that you expect to detect. This width determines which intensity fluctuations are potential cells as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of the selected stained area compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

### Preview

Shows an overlay preview of the segmentation for the selected stained area of all of the apoptotic cells overlaid on the original W2 (Apoptotic staining) image Click the *Overlay Image* button on the image window to turn on and off the overlay image. If you resave the original image after the overlay has been created, the overlay is saved with the original image, even if the overlay view is turned off when you save the image. After the overlay has been saved with the original image, you can turn it off and on as needed. If you resave the image with a new overlay, it will replace the current overlay image.

## Dead staining

The segmentation for Dead Staining, wavelength 3, takes into account both Late Apoptotic cells and Necrotic Cells. The segmentation overlay image in the preview for this classification will show both Late Apoptotic cells (shown as Violet) and Necrotic Cells (shown as Red)

### W3 Source image [<image name>]

Selects the source image that you want to use for your dead stained wavelength from the available open source images.

### Stained area

Use this drop down list to identify the stained cell area in your image to be assigned as dead. Choose one of the following cell areas:

- **Nucleus**
- **Cytoplasm**
- **Nucleus and cytoplasm**

### Approximate min width

Specifies the minimum object width in microns (µm) of all Dead cells in your image that you expect to detect. Patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

### Approximate max width

Specifies the maximum object width in microns (µm) of the selected stained area of all the Dead cells in your image that you expect to detect. This width determines which intensity fluctuations are potential cells as compared to the background fluctuations or remaining shading errors. The value

for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of the selected stained area compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

### Preview

Shows a color preview image of the segmentation for the all wavelengths overlaid on the original image. In this *Dead staining* preview, the segmentation of all identified objects is shown indicated by the appropriate assigned color for each type of object. Click the Overlay Image button on the image window to turn on and off the overlay image. If you resave the original image after the overlay has been created, the overlay is saved with the original image, even if the overlay view is turned off when you save the image. After the overlay has been saved with the original image, you can turn it off and on as needed. If you resave the image with a new overlay, it will replace the current overlay image.

## Configure Summary Log

Opens the configure log dialog box. Use this dialog box to select the measurements that you want to include in your summary log. In addition to the standard log measurements, the following measurements can be selected:

**Total Cells:** Total number cells determined from the All nuclei image.

**Viable Cells:** Total number cells determined from the All nuclei image that were negative in Apoptotic staining image and negative in the Dead staining image.

**Early Apoptotic Cells:** Total number cells determined from the All nuclei image that were positive in Apoptotic staining image, but negative in the Dead staining image.

**Late Apoptotic Cells:** Total number cells determined from the All nuclei image that were positive in Apoptotic staining image and positive in the Dead staining image.

**Necrotic Cells:** Total number cells determined from the All nuclei image that were positive in Dead staining image, but negative in the Apoptotic staining image.

**% Viable Cells:** 100 * Viable Cells / Total Cells.

**% Early Apoptotic Cells:** 100 * Early Apoptotic Cells / Total Cells.

**% Late Apoptotic Cells:** 100 * Late Apoptotic Cells / Total Cells.

**% Necrotic Cells:** 100 * Necrotic Cells / Total Cells.

**All Cells Total Area:** Total $um^2$s for all nuclei in all cells.

**All Cells Mean Area:** Total $um^2$s for all nuclei image in all cells / Total Cells.

**All Cells W1 Integrated Intensity:** Summed grayscale values for all nuclei measured in W1 (All nuclei) image.

**All Cells W1 Average Intensity: All Cells W1 Integrated Intensity** / **All Cells Total Area** measured in pixels.

**All Cells W2 Integrated Intensity:** Summed grayscale values for all nuclei measured in W2 (Apoptotic staining) image.

**All Cells W2 Average Intensity: All Cells W2 Integrated Intensity** / **All Cells Total Area** measured in pixels.

**All Cells W3 Integrated Intensity:** Summed grayscale values for all nuclei measured in W3(Dead staining) image.

**All Cells W3 Average Intensity: All Cells W3 Integrated Intensity** / **All Cells Total Area** measured in pixels.

## Configure Data Log (Cells)

Opens the Configure Log dialog box for cell-specific measurements data logging. Use this dialog box to select the measurements that you want to include in your cell-specific data log file log. This log file records cell-by-cell measurements. In addition to the standard log measurements, the following measurements can be selected:

**Cell: Assigned Label #**

**Cell: Health Classification:** Viable, Early Apoptotic, Late Apoptotic, Necrotic

**Cell: Nuclear Area:** Total $um^2$s in the all of the nuclei detected..

**Cell: W1 Integrated Intensity:** Summed grayscale values from W1 within the detected nuclear pixels. (as determined from the All nuclei stain).

**Cell: W1 Average Intensity: Cell: W1 Integrated Intensity** / **Cell: Nuclear Area** converted to pixels.

**Cell: W2 Integrated Intensity:** Summed grayscale values from W2 within the detected nuclear pixels (as determined from the All nuclei stain).

**Cell: W2 Average Intensity: Cell: W2 Integrated Intensity** / **Cell: Nuclear Area** converted to pixels.

**Cell: W3 Integrated Intensity:** Summed grayscale values from W3 within the detected nuclear pixels (as determined from the All nuclei stain).

**Cell: W3 Average Intensity: Cell: W3 Integrated Intensity** / **Cell: Nuclear Area** converted to pixels.

### Save Settings

Opens the Save Settings dialog box. Use this dialog box to create and save a *Cell Health* settings file. You can store all the settings currently specified in the *Cell Health* dialog box. Use Load Settings to retrieve and use these settings at a later time. Type a setting name and an optional description, then click OK.

### Load Settings

Opens the Load Settings dialog box. Use this dialog box to select and load a *Cell Health* settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the *Cell Health* settings file. Select the name of the setting that you want to use, then click OK.

### Set to Defaults

Resets all options and settings to their default values.

### **Test Run** (from Review Plate Data only)

Runs one pass of the *Cell Health* application module on the images currently open and selected. Measurements will be logged if the Summary and/or Data Logs are open.

### **Apply** (from the Apps menu only)

Applies the settings that you made and runs the application module. Note: This button will not appear when configuring the settings within Review Screen Data.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

### Close

Closes the dialog box.

# Mitotic Index (Apps Menu)

**Analyzes cellular images to determine both Mitotic and Interphase cells for live cells in a normal cell cycle.**

*Availability: Exclusive to MetaXpress*

### Drop-in: MITOTICINDEX

Using image segmentation in conjunction with cell staining, the MetaXpress Mitotic Index application module differentiates between mitotic cells and interphase cells in the normal cell cycle, and quantifies the various data extracted during image analysis.

Cells should be labeled with a DNA stain and a mitosis-specific stain such as Histone 3 S10 phosphorylation. The DNA stain labels all of the cells and only the mitotic cells are labeled with the second stain.

The DNA stain and the associated wavelength are used to differentiate all cells in the image from non-cell material and the background. The Mitotic stain differentiates Mitotic cells from the background and Interphase cells. Typically, when properly stained, the Mitotic cells will appear to be significantly brighter than the Interphase cells in the Mitotic staining image.

## General Procedures

To use the Mitotic Index application module to analyze your images, review and complete the following general procedures when you are acquiring your images and analyzing your image data.

1. Acquire your data sets using two different wavelengths of light. One wavelength should be for your selected DNA marker; the second wavelength should be for your selected Mitotic marker.

2. When acquisition is complete, open the Review Screen Data Dialog box and choose Mitotic Index.

3. Set up the Mitotic Index dialog box. Either use a prepared Mitotic Index state file or complete the appropriate settings in the Mitotic Index dialog box.

    a. Set appropriate values for All nuclei that correctly isolate all nuclei in the Wavelength 1 image. Setting the correct values for min and max width helps to distinguish cells from possible non-cell material and to separate touching cells. Setting the correct value for Intensity above local background ensures that all cells are included.

    b. In the Mitotic staining area for Wavelength 2, set the graylevel value for *Intensity above local background* to be high enough to include only cells that are mitotic and exclude all Interphase cells.

4. Open log files for both the summary log and the data log.

5. In both the summary log and the data log, select the appropriate measurements to log and display.

6. Run the analysis for an individual well, multiple selected wells, a single plate, or multiple plates.

After the application module has processed the image, you can use the ***Cellular Results*** table to interactively view data belonging to an individual cell or group of cells by clicking the cell(s) in the image.

**Application Note — *MetaMorph Application Module Overview* (PDF)**

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Running the Mitotic Index Application Module

To run the Mitotic Index Application Module, complete the following procedure:

| Step | Action |
|---|---|

**1** From the Apps menu, click Mitotic Index. The Mitotic Index dialog box opens.

**2** From the File menu, click *Open*. The Open dialog box is displayed.

**3** From the Open dialog box, choose the images for Wavelength 1 (All nuclei) and Wavelength 2 (Mitotic staining), then click *Open*.

**4** To display a combined segmentation result image for both wavelengths, click *Display Result Image,* then open the Image selector for Display result Image, and choose "*Segmentation."*

**5** In the W1 Source Image Selector for *All Nuclei*, select the appropriate image for wavelength number 1.

**6** In the W2 Source Image Selector for *Mitotic staining*, select the appropriate image for wavelength number 2.

**7** In the *All nuclei* area, in the *Approximate min width* box, type or select a value in microns for the minimum nuclear width. This dimension should be based on the smallest width of the smallest nucleus that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings***.

**8** In the *All nuclei* area, in the *Approximate max width* box, type or select a value in microns for the maximum nuclear width. This dimension should be based on the largest width of the largest nucleus that you want to include in your measurements. For details on making this setting, refer to the procedure for determining object width under ***Determining Segmentation Settings***.

**9** In the *All nuclei* area, in the *Intensity above local background* box, type or select a value to define the difference in intensity between the dimmest nucleus and the background adjacent to it. For details on making this setting, refer to the procedure for determining Intensity above local background under ***Determining Segmentation Settings***.

**10** In the *All nuclei* area, click *Preview* to view a test image showing the segmentation derived from the image staining. Before continuing to the settings in the Mitotic staining area, be sure that the segmentation you see when you click Preview indicates that you are correctly identifying all nuclei in the image. To better visualize the result, click the *Overlay* button in the image window controls to toggle between the original image and the segmentation overlaid on the

original image.

**11** In the *Mitotic staining* area, in the *Intensity above local background* box, type or select a value to define the difference in intensity between the dimmest object and the background adjacent to it. For details on making this setting, refer to the procedure for determining Intensity above local background under ***Determining Segmentation Settings***.

**12** In the *Mitotic staining* area, click *Preview* to view a test image showing the segmentation derived from the image staining.

**13** Click *Configure Summary Log* to choose the measurements that you want to include in your Summary Log.

**14** Click *Configure Data Log* to choose the measurements that you want to include in your Data Log.

**15** Click *Apply* to run the Mitotic Index application module.

**16** To interactively view data belonging to an individual cell or group of cells, select the cell(s) in the image and view the data in the ***Cellular Results*** table.

**17** Click *Set to Defaults* to reset all Mitotic Index settings to their default values.

**18** Click *Close* to close the Mitotic Index dialog box.

## Determining Segmentation Settings

Use the following general procedures to make segmentation settings for each wavelength. Wavelength 2 (W2) requires only the setting for *Intensity above local background*.

| Step | Action |
|---|---|
| **1** | In the *Approximate min width box*, type or select a value in microns for the minimum width of the Stained area. If the stained area is the nucleus, the value should be small (typically between 2 and 20 microns ). If the stained area is the cytoplasm or both, the value should be larger (between 15 and 200 microns). Determine which cell appears to be the smallest in the field, then determine the approximate width of the widest dimension.<br><br>**Hint:** You can use the Caliper or Line Region tool to help determine the width. |
| **2** | In the *Approximate max width* box, type or select a value in microns for the maximum width of the Stained area. If the stained area is the nucleus, the value should be small (between 5 and 30 microns). If the stained |

area is the cytoplasm or both, the value should be larger (between 20 and 300 microns). Determine which cell appears to be the largest in the field, then determine the approximate width of the widest dimension.

**Hint:** You can use the Caliper tool or Line Region tool to help determine the width.

**3** In the *Intensity above local background* box, type or select a value that represents the minimum nucleus or cytoplasm intensity minus the background intensity near the nucleus or cytoplasm. The lower the value, the more sensitive the detection. The software will estimate the background for each nucleus or cytoplasm locally to correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the object that you want to detect. The graylevel values are shown on the status bar below the image area. To obtain the Intensity above local background value, subtract these two values.

**4** Click Preview to view the segmentation for an individual wavelength overlaid on the original image. This preview image can be used to separate the effects of the individual wavelength detections interacting so that each wavelength's proper settings can be determined independently.

**Note:**To turn on or off the overlay image, click the overlay button on the image window.

**5** Click *Apply/Test Run* to see the complete segmentation using all wavelengths.

**Note:** Preview results are only approximate. The complete segmentation may utilize information between the multiple wavelengths to give a more accurate segmentation than seen in the preview results alone.

## Mitotic Index - Dialog Box Options

### All nuclei

This area defines the settings that apply to the Wavelength 1 source image for all nuclei in the image.

#### W1 Source Image

Opens the source Image Selector for Wavelength 1. This selector works on the active plane only, and allows 16-bit images only. (Note: the software automatically converts images from 10, 12, and 14-bit cameras to 16-bit format.) A value of [None] is shown for cases in which images for a specific settings file cannot be found in the database. Source images opened with this image selector should be calibrated in microns in order to correctly process the image.

#### Display result image

Activates the display of the of the segmentation result image (optional).

**[Result Image Selector]**

Opens the Image Selector for the destination image. This selection is enabled only if Display result image is checked. This setting shows segmentation of the cell or nucleus according to your selection. This image is displayed as an autoscaled color coded 16-bit image. When this image is opened using Review Screen Data, the result image will not be stored in the database.

**Notes:**

- When processing is complete, this image is automatically overlaid with the segmentation result image. If you do not re-save the source image, the original source image is not affected. However, if you re-save the source image the overlay information will be saved with it.
- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

**Approximate min width**

Specifies the minimum object width in microns (µm) that you expect to detect. Patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

**Approximate max width**

Specifies the maximum object width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential cells as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

**Intensity above local background**

Specifies a value for the intensity threshold of either nuclei or cytoplasm compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

**Preview**

Shows a color preview image of the segmentation for the associated wavelength overlaid on the original image. Click the Overlay Image button on the image window to turn on and off the overlay image. If you resave the original image after the overlay has been created, the overlay is saved with the original image, even if the overlay view is turned off when you save the image. After the overlay has been saved with the original image, you can turn it off and on as needed. If you resave the image with a new overlay, it will replace the current overlay image.

**Mitotic Staining**

This area defines the settings that apply to the Wavelength 2 source image for any mitotic cells in the image.

**W2 Source Image**

Opens the source Image Selector for Wavelength 2. This selector works on the active plane only, and allows 16-bit images only. A value of [None] is shown for cases in which images for a specific settings file cannot be found in the database. Source images opened with this image selector should be calibrated in microns in order to correctly process the image.

**Intensity above local background**

Specifies a value for the intensity threshold of either nuclei or cytoplasm compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

**Preview**

Shows a color preview image of the segmentation for the associated wavelength overlaid on the original image. Click the Overlay Image button on the image window to turn on and off the overlay

image. If you resave the original image after the overlay has been created, the overlay is saved with the original image, even if the overlay view is turned off when you save the image. After the overlay has been saved with the original image, you can turn it off and on as needed. If you resave the image with a new overlay, it will replace the current overlay image.

### Configure Summary Log

Opens the configure log dialog box. Use this dialog box to select the measurements that you want to include in your summary log. The measurements recorded in this log file are for the entire image. In addition to the standard log measurements, the following measurements can be selected:

**Summary Log (Field Measurement) –** For all labeled nuclei, the following measurements are included in the Summary Log.

**Total Nuclei:** Total number cells determined from wavelength 1.

**Mitotic Nuclei:** Total number nuclei determined from wavelength 1 that were positive in wavelength 2.

**Interphase Nuclei:** Total number nuclei determined from wavelength 1 that were negative in wavelength 2.

**% Mitotic Nuclei:** 100 * Mitotic Nuclei/Total Nuclei.

**% Interphase Nuclei:** 100 * Interphase Nuclei/Total Nuclei.

**All Nuclei Total Area:** Total µm²s in wavelength 1 in all nuclei.

**All Nuclei Mean Area:** All Nuclei Total Area/Total Nuclei.

**All Nuclei W1 Integrated Intensity:** Summed grayscale values in wavelength 1 in all nuclei.

**All Nuclei W1 Average Intensity:** All Nuclei Integrated Intensity/All Nuclei Total (Pixel) Area.

**All Nuclei W2 Integrated Intensity:** Summed grayscale values in wavelength 2 in all nuclei.

**All Nuclei W2 Average Intensity:** All Nuclei W2 Integrated Intensity/All Nuclei Total (Pixel) Area.

**Mitotic Total Area:** Total µm²s in wavelength 1 in mitotic nuclei.

**Mitotic Mean Area:** Mitotic Total Area/Mitotic Nuclei.

**Mitotic W1 Integrated Intensity:** Summed grayscale values in wavelength 1 in mitotic nuclei.

**Mitotic W1 Average Intensity:** Mitotic Nuclei W1 Integrated Intensity/Mitotic Nuclei Total (Pixel) Area.

**Mitotic W2 Integrated Intensity:** Summed grayscale values in wavelength 2 in mitotic nuclei.

**Mitotic W2 Average Intensity:** Mitotic W2 Integrated Intensity/Mitotic Total (Pixel) Area.

**Interphase Total Area:** Total µm$^2$s in wavelength 1 in interphase nuclei.

**Interphase Mean Area:** Interphase Total Area/Interphase Nuclei.

**Interphase W1 Integrated Intensity:** Summed grayscale values in wavelength 1 in interphase nuclei.

**Interphase W1 Average Intensity:** Interphase W1 Integrated Intensity/Interphase Total (Pixel) Area.

**Interphase W2 Integrated Intensity:** Summed grayscale values in wavelength 2 in interphase nuclei.

**Interphase W2 Average Intensity:** Interphase W2 Integrated Intensity/Interphase Total (Pixel) Area.

### Configure Data Log (Cells)

Opens the Configure Log dialog box for cell-specific measurements data logging. Use this dialog box to select the measurements that you want to include in your cell-specific data log file log. This log file records cell-by-cell measurements. In addition to the standard log measurements, the following measurements can be selected:

**Data Log (Cell-by-Cell Measurement)** - For each nucleus, the following measurements are included in the Data Log:

**Cell: Label #**

**Cell: Classification:** Interphase, Mitotic

**Cell: Total Area:** Total µm²s in the nucleus.

**Cell: W1 Integrated Intensity:** Summed grayscale values in wavelength 1 for this nucleus.

**Cell: W1 Average Intensity:** W1 Integrated Intensity/Total (Pixel) Area

**Cell: W2 Integrated Intensity:** Summed grayscale values in wavelength 2 for this nucleus.

**Cell: W2 Average Intensity:** W2 Integrated Intensity/Total (Pixel) Area

### Save Settings

Opens the Save Settings dialog box. Use this dialog box to create and save a *Mitotic Index* settings file. You can store all the settings currently specified in the *Mitotic Index* dialog box. Use Load Settings to retrieve and use these settings at a later time. Type a setting name and an optional description, then click OK.

### Load Settings

Opens the Load Settings dialog box. Use this dialog box to select and load a *Mitotic Index* settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the *Mitotic Index* settings file. Select the name of the setting that you want to use, then click OK.

### Set to Defaults

Resets all options and settings to their default values.

**Test Run** (from Review Plate Data only)

Runs one pass of the *Mitotic Index* application module on the images currently open and selected. Measurements will be logged if the Summary and/or Data Logs are open.

**Apply** (from the Apps menu only)

Applies the settings that you made and runs the application module.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

### Close

Closes the dialog box.

# Live Dead (Apps Menu)

**Identifies both live and dead cells in an image and counts the number of live cells, number of dead cells, and the total number of cells.**

*Availability: Exclusive to MetaMorph and MetaXpress*

**Drop-in: LIVEDEAD**

Use this application module to count both live and dead cells in appropriately prepared live/dead assays. This application module enables you to use two separate wavelengths and two separate stains. Typically, one stain identifies all cells and the other stain identifies dead cells. In assays that apply one stain to all cells and a second stain to either all live cells or all dead cells, the live or dead cell count (respectively) is subtracted from the "all cells" stain to obtain the count of the remaining cells.

The dialog box is divided into two equal parts; the upper part for *Wavelength 1 Parameters*, the lower part for *Wavelength 2 Parameters.* Each wavelength has the same parameters and options. You

differentiate the way in which they work by selecting different settings. By choosing parameters that are unique to the staining and the part of the cell that is stained, you can effectively separate live cells from dead cells.

Select the correct identifier for the stained area, then set both the minimum and maximum width. Objects that are significantly larger or smaller than the specified range are excluded. The object's intensity above the background is an additional factor that can determine if the object will be included or considered to be background and excluded.

After the application module has processed the image, you can use the ***Cellular Results*** table to interactively view data belonging to an individual cell or group of cells by clicking the cell(s) in the image.

**Application Note — *MetaMorph Application Module Overview* (PDF)**

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Identifying and Counting Live and Dead Cells

For assays that will use this application module, you need two stains to differentiate between live and dead cells. There are several different methods that this application module can accommodate. One of the more typical methods is to use one stain to label all of the nuclei for wavelength 1, and a second stain to be used in conjunction with wavelength 2 to identify either live or dead cells.

To identify and count live and dead cells, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click *Live Dead*. The Live Dead dialog box opens. |
| **2** | From the File menu, click *Open*, the Open dialog box is displayed. |
| **3** | From the Open dialog box, choose the images for Wavelengths 1 and 2 on which you want to run this application module. |
| **4** | Using the *Wavelength 1 Source* image selector, select the image that you want to use for Wavelength 1. |
| **5** | Check *Display result image* to display a segmentation result image for this wavelength when processing is complete. Use the assigned name, W1-Segmentation, or click the file name button to open the *Specify Image Name* dialog box and rename the image. |
| **6** | In the *Stained cell type* box, choose the type of cell that you want to count with Wavelength 1. If this wavelength will be used to count all cells, choose *All Cells*. If this wavelength will be used in conjunction with a stain absorbed by live cells, choose *Live*. If this wavelength will be used in conjunction with a stain absorbed by dead cells, choose *Dead*. |

**7** In the *Stained Area* box choose the area of the cell in which you expect the stain associated with this wavelength to be detected.

Choose one of the following:

- *Nucleus* if you expect the stain to be detected only in the nucleus.
- *Cytoplasm* if you expect the stain to be detected only in the cytoplasm.
- *Both* if the stain will be detected in both areas.

**8** In the *Approximate min width* box, type or select a value in microns for the minimum width of the *Stained area*. If the stained area is the nucleus, the value should be small (typically between 2 and 20 microns ). If the stained area is the cytoplasm or both, the value should be larger (between 15 and 200 microns). Determine which cell appears to be the smallest in the field, then determine the approximate width of the widest dimension.

***Hint:*** You can use the Caliper or Line Region tools to help determine the width.

**9** In the *Approximate max width* box, type or select a value in microns for the maximum width of the *Stained area*. If the stained area is the nucleus, the value should be small (between 5 and 30 microns). If the stained area is the cytoplasm or both, the value should be larger (between 20 and 300 microns). Determine which cell appears to be the smallest in the field, then determine the approximate width of the widest dimension.

***Hint:*** You can use the Caliper tool or Line Region tool to help determine the width.

**10** In the *Intensity above local background* box, type or select a value that represents the minimum nucleus or cytoplasm intensity minus the background intensity near the nucleus or cytoplasm. The lower the value, the more sensitive the detection. The software will estimate the background for each nucleus or cytoplasm locally to correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the blob that you want to detect. The graylevel values are shown on the status bar below the image area. To obtain the *Intensity above local background* value, subtract these two values.

**11** Click *Split touching objects* to segment two or more cells (objects) that are touching. If you do not check this box, touching objects

will be considered to be one object.

**12** Click *Preview* to open a preview image of the segmentation for this wavelength overlaid on the original image. This preview image can be used to separate the effects of the two wavelength detections interacting so that each wavelength's proper settings can be determined independently.

**Note:** To turn on or off the overlay image, click the overlay button on the image window.

**13** Repeat steps 4 through 12 for Wavelength 2.

**14** Click *Configure Summary Log*. The Configure Log dialog box opens. Check and/or uncheck individual parameter configuration settings. Refer to the description of these settings under *Dialog Box Options: Configure Summary Log.*

**15** To restore default values for the available settings, click *Set to Defaults* at any time.

**16** Click *Apply* to run the application module and generate the result image.

**17** To interactively view data belonging to an individual cell or group of cells, select the cell(s) in the image and view the data in the ***Cellular Results*** table.

**18** Click *Close* to close the Live Dead dialog box.

## Live Dead Dialog Box Options

**Note:** This dialog box contains settings for both Wavelength 1 and Wavelength 2. These settings are identical. Therefore, only the settings for Wavelength 1 are shown.

### Wavelength 1 Parameters

Defines the dialog box parameters and options assigned to Wavelength 1.

### Wavelength 1 Source

Opens the source Image Selector for Wavelength 1. This selector works on the active plane only, and allows 16-bit images only. A value of [None] is shown for cases in which images for a specific settings file cannot be found in the database. Source images opened with this image selector should be calibrated in microns in order to correctly process the image.

**Notes:**

- When processing is complete, this image is automatically overlaid with the segmentation result image. If you do not re-save the source image, the original source image is not affected. However, if you re-save the source image the overlay information will be saved with it.
- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

### Display result image

Activates the display of the of the segmentation result image (optional).

### [Result Image Selector]

Opens the Image Selector for the destination image. This selection is enabled only if *Display result image* is checked. This setting shows segmentation of the cell or nucleus according to your selection. This image is displayed as an autoscaled, 16-bit image for easy color combine, arithmetic, or overlay with the original. When this image is opened using Review Screen Data, the result image will not be stored in the database. Live cells are shown in green; dead cells are shown in red.

### Stained cell type

Specifies the type of cell to which the stain has been applied. Choices are Live, Dead, and All Cells. The selection that you make in this box controls the math formula that will be used to determine the number of live and dead cells and the total number of cells. For example, if you choose *All Cells* for Wavelength 1, and *Live* for Wavelength 2, then the number of cells counted as Wavelength 1 are the total number of cells. Wavelength 2, which is the number of *Live* cells, is subtracted from Wavelength 1 to determine the number of dead cells.

### Stained area

Specifies the area of the cell to which the stain has been applied.

### Approximate min width

Specifies the minimum object width in microns (µm) that you expect to detect. Patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

### Approximate max width

Specifies the maximum object width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential cells as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of either nuclei or cytoplasm compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

### Split touching objects

Identifies touching objects or blobs and divides them in the segmentation when you check this box.

### Preview

Shows a color preview image of the segmentation for the associated wavelength overlaid on the original image. Live cells are shown in green; dead cells are shown in red. Click the Overlay Image button on the image window to turn on and off the overlay image. If you resave the original image after the overlay has been created, the overlay is saved with the original image, even if the overlay view is turned off when you save the image. After the overlay has been saved with the original image, you can turn it off and on as needed. If you resave the image with a new overlay, it will replace the current overlay image.

### Configure Summary Log

Opens the configure log dialog box. Use this dialog box to select the measurements that you want to include in your summary log. The measurements recorded in this log file are for the entire image. In addition to the standard log measurements, the following measurements can be selected:

- ***Total Cells*** – The total number of cells in the image as defined by the applied wavelength labeling.
- ***Live Cells*** – The total number of live cells in the image as defined by the applied wavelength labeling.
- ***Dead Cells*** – The total number of dead cells in the image as defined by the applied wavelength labeling.
- ***%Live Cells*** – The percentage of live cells in the image as defined by the applied wavelength labeling.

- ***% Dead Cells*** – The percentage of dead cells in the image as defined by the applied wavelength labeling.
- ***W1 Total Area*** – Total area in square microns of cells detected in the Wavelength 1 image.
- ***W1 Area Per Cell*** – W1 Total Area divided by the number of cells detected in the Wavelength 1 image.
- ***W1 Integrated Intensity*** – Sum of the intensity values of all cell pixels detected in the Wavelength 1 image.
- ***W1 Average Intensity*** – W1 Integrated Intensity divided by the number of pixels detected in the Wavelength 1 image.
- ***W2 Total Area*** – Total area in square microns of cells detected in the Wavelength 2 image.
- ***W2 Area Per Cell*** – W2 Total Area divided by the number of cells detected in the Wavelength 2 image.
- ***W2 Integrated Intensity*** – Sum of the intensity values of all cell pixels detected in the Wavelength 2 image.
- ***W2 Average Intensity*** – W2 Integrated Intensity divided by the number of pixels detected in the Wavelength 2 image.

### Configure Data Log (Cells)

Opens the Configure Log dialog box for cell-specific measurements data logging. Use this dialog box to select the measurements that you want to include in your cell-specific data log file log. This log file records cell-by-cell measurements. In addition to the standard log measurements, the following measurements can be selected:

- ***Cell: Assigned Label #*** – Provides a number label to identify each individual cell in the image.
- ***Cell: Live Classification*** – Specifies whether the cell is classified as live (1) or dead (0).
- ***Cell: W1 Total Area*** – The total area in square microns of the part of the cell detected in the Wavelength 1 image.
- ***Cell: W1 Integrated Intensity*** – The integrated intensity for a specific cell detected in the Wavelength 1 image.
- ***Cell: W1 Average Intensity*** – The average intensity for a specific cell detected in the Wavelength 1 image.
- ***Cell: W2 Total Area*** – The total area in square microns of the part of the cell detected in the Wavelength 2 image.
- ***Cell: W2 Integrated Intensity*** – The integrated intensity for a specific cell detected in the Wavelength 2 image.
- ***Cell: W2 Average Intensity*** – The average intensity for a specific cell detected in the Wavelength 2 image.

### Save Settings

Opens the Save Settings dialog box. Use this dialog box to create and save a <AppName> settings file. You can store all the settings currently specified in the <AppName> dialog box. Use Load Settings to retrieve and use these settings at a later time.

### Load Settings

Opens the Load Settings dialog box. Use this dialog box to select and load a <AppName> settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the <AppName> settings file. Select the name of the setting that you want to use, then click OK.

### Set to Defaults

Resets all options and settings to their default values.

**Test Run** (from Review Plate Data only)

Runs one pass of the <AppName> application module on the images currently open and selected. Measurements will be logged if the Summary and/or Data Logs are open.

**Apply** (from the Apps menu only)

Applies the settings that you made and runs the application module.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

**Close**

Closes the dialog box.

# Granularity (Apps Menu)

**Detects, counts, measures, and logs granularity characteristics and optionally detects, counts, measures, and logs nuclei characteristics.**

*Availability: Exclusive to MetaMorph and MetaXpress*

**Drop-in: Granularity**

Use this application module to detect and count granules in cells and measure the physical characteristics of granules. Optionally, you can detect and count nuclei and measure the physical characteristics of nuclei within the cells in which you are detecting granules. All measurements are logged according to the log configuration that you specify.

This application module detects, measures, and logs the following granularity characteristics:

- Granules (count)
- Granules Per Cell (average)
- Total Granule Area
- Mean Granule Area
- Integrated Granule Intensity
- Average Granule Intensity

This application module also detects, measures, and logs the following nuclei characteristics:

- Nuclei (count)
- Total Nuclear Area
- Mean Nuclear Area
- Integrated Nuclear Intensity
- Average Nuclear Intensity

In addition, this application module can determine and log the following six Granularity Indexes.

- Texture Index
- Cellular Texture Index
- Gradient Index
- Cellular Gradient Index
- Laplacian Index
- Cellular Laplacian Index

Each Granularity Index is based on or derived from a formula that measures the distribution

characteristics of the granules relative to the nucleus.

After the application module has processed the image, you can use the ***Cellular Results*** table to interactively view data belonging to an individual cell or group of cells by clicking the cell(s) in the image.

**Application Note — *MetaMorph Application Module Overview* (PDF)**

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Measuring Granularity

To run the Granularity application module, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click *Granularity*, the Granularity dialog box opens. |
| **2** | From the File menu, click *Open*, and open an appropriate 16-bit granularity image. |
| **3** | In the Granularity dialog box, click the *Granule image* selector button, and choose the image that you want to use. |
| **4** | Click *Display result image* if you want the result image to open after it is created. |
| **5** | In the *Approximate min width* box, type or select the appropriate minimum granule width.<br><br>***Hint:*** You can use either the Caliper tool or Line Region tool to help you approximate the minimum granule width. |
| **6** | In the *Approximate max width* box, type or select the appropriate maximum granule width. This value is used to differentiate nuclei from non-nuclear material and exclude nuclei greater than the specified size from the segmentation. Hint: You can use the Caliper tool to help you approximate the maximum granule width. |
| **7** | In the *Intensity above local background* box, type or select a gray level value that represents the least bright granule minus the background intensity near the granule. The lower the value, the more sensitive the detection. The software will estimate the background for each granule locally to correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the most subtle granule that you want to detect. The gray level values are shown on the status bar below the image area. To obtain the intensity value, subtract the value of the background from the value of the granule. |

**8** If your assay images include nuclear stained images, and you want to include Nuclei counts and measurements in your logged data, click *Nuclear stain*.

**9** In the *Nuclear stain* area, click the *Nuclear image* selector button, and choose the nuclear image that you want to use.

**10** In the *Approximate min width* box, type or select the appropriate minimum cell nuclei width.

***Hint:*** You can use the Caliper tool to help you approximate the minimum nuclei width.

**11** In the *Approximate max width* box, type or select the appropriate maximum nuclei width. This value is used to differentiate nuclei from non-nuclear material and exclude nuclei greater than the specified size from the segmentation.

***Hint:*** You can use the Caliper tool to help you approximate the maximum nuclei width.

**12** In the *Intensity above local background* box, type or select a value that represents the minimum nucleus intensity minus the background intensity near the nucleus. The lower the value, the more sensitive the detection. The software will estimate the background for each nuclei locally to correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the most subtle nuclei that you want to detect. The gray level values are shown on the status bar below the image area. To obtain the intensity value, subtract the value of the background from the value of the nucleus.

**13** Click *Configure Summary Log*. The Configure Log dialog box opens. Check and/or uncheck individual parameter configuration settings. Refer to the description of these settings under *Dialog Box Options: Configure Summary Log.*

**14** To restore default values for the available settings, click *Set to Defaults* at any time.

**15** Click *Apply* to run the analysis and generate the result image.

**16** Click *Close* to close the Granularity dialog box.

## Granularity - Dialog Box Options

### Granules

Dialog box area that contains settings used for detecting granules within your image.

### Granule Image

Opens the source Image Selector for the Granule image. This selector works on the active plane only, and allows 16-bit images only. A value of [None] appears for cases in which images for a specific settings file cannot be found in the database. Source images opened with this image selector should be calibrated in microns in order to correctly process the image.

**Notes:**

- When processing is complete, this image is automatically overlaid with the segmentation result image. If you do not re-save the source image, the original source image is not affected. However, if you re-save the source image the overlay information will be saved with it.
- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

### Display Result image

Activates the display of the of the granularity result image (optional).

### (Result image)

Opens the Image Selector for the destination image. This selection is enabled only if *Display result image* checked. This image will be displayed as an autoscaled, 16-bit image for easy color combine, arithmetic, or overlay with the original. When this image is opened using Review Screen Data, the result image will not be stored in the database.

### Approximate min width

Specifies the minimum granule width in microns (µm) that you expect to detect. Granule patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

> **Note:** Even though you enter values in microns, the measurement occurs in the image, and as a result, is made in pixels. Therefore in images acquired at low magnifications, a greater range in microns will constitute a single pixel. For example, at a low magnification, any granules within the range of 0.3 microns to 0.7 microns will each be considered to be one pixel in width.

### Approximate max width

Specifies the maximum granule width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential granules as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of granules compared to the neighboring background values. This setting is for controlling the sensitivity of the detection.

### Nuclear stain (optional)

Specifies that a second wavelength image of stained nuclei is included in the screen.

### Approximate min width

Specifies the minimum nuclei width in microns (µm) that you expect to detect. Nuclei patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

### Approximate max width

Specifies the maximum nuclei width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential nuclei as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of nuclei compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

### Configure Summary Log

Opens the Configure Log dialog box. Use this dialog box to select the measurements that you want to include in your summary log. In addition to the standard log measurements, the following measurements can be selected:

- Granules (count)
- Granules Per Cell (average)
- Total Granule Area
- Mean Granule Area
- Integrated Granule Intensity
- Average Granule Intensity
- Nuclei (count)
- Total Nuclear Area
- Mean Nuclear Area
- Integrated Nuclear Intensity
- Average Nuclear Intensity

In addition to the preceding counts of granules and nuclei, this application module provides granularity measurements which you can use to define the graininess or texture characteristics of images. . Each index measures grayscale intensity fluctuation and is in units of graylevels per pixel.

- ***Texture Index*** – Standard deviation of intensity values in the image.
- ***Cellular Texture Index*** -- Cell-by-cell standard deviation of intensity values near the nuclei. (Requires use of nuclear stain).
- ***Gradient Index*** – A texture-dependent measurement that reflects the amount of local intensity contrast. Measures the difference between the maximum and minimum intensity within a local neighborhood.
- ***Cellular Gradient Index*** -- Cell-by-cell Gradient Index measured near the nuclei. (Requires use of nuclear stain).
- ***Laplacian Index*** – Similar to the morphological gradient, however this morphological measurement reflects fluctuations in the gradient.
- ***Cellular Laplacian Index*** – Cell-by-cell Laplacian Index measured near the nuclei. (Requires use of nuclear stain).

### Save Settings

Opens the Save Settings dialog box. Use this dialog box to create and save a Granularity settings file. You can store all the settings currently specified in the Granularity dialog box. Use Load Settings to retrieve and use these settings at a later time.

### Load Settings

Opens the Load Settings dialog box. Use this dialog box to select and load a Granularity settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the Granularity settings file. Select the name of the setting that you want to use, then click OK.

### Set to Defaults

Resets all options and settings to their default values.

**Test Run** (from Review Plate Data only)

Runs one pass of the Granularity application module on the images currently open and selected. Measurements will be logged if the Summary and/or Data Logs are open.

**Apply** (from the Apps menu only)

Applies the settings that you made and runs the application module.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

**Close**

Closes the dialog box.

# Nuclear Translocation HT

**Determines whether a probe that is present outside of a nucleus or group of nuclei can be detected inside any nucleus within the image.**

*Availability: Exclusive to MetaXpress*

**Drop-in: NuclearTranslocationHT**

Use this application module to determine if a specific fluorescent probe can be detected both outside and inside a nucleus or nuclei.

The resulting segmentation image is overlaid on the original Nuclear-stained image. The overlaid image displays positive nuclei as green and negative nuclei as red. Using the Threshold Image command in conjunction with Nuclear Translocation HT, you can use either the negative population or the positive population to create a binary mask. This application module is similar to Translocation, but it is optimized for high throughput.

**Application Note — *MetaMorph Application Module Overview* (PDF)**

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Using Nuclear Translocation HT

The following general steps are intended to guide you through the process of acquiring images appropriate for use with the Nuclear Translocation HT application module and using the Nuclear Translocation HT application module to complete your analysis:

1. Collect your data set consisting of both a nuclear and a probe image using two different wavelengths of light.
2. Collect background images and bleed through correction samples either as part of your microplate acquisition or in a preliminary experiment using the standard MetaMorph acquisition dialog box.
3. Open the Review Screen Data dialog box, and choose the *Nuclear Translocation HT* application module.
4. Choose a *Nuclear Translocation HT settings file* (*.snt).

   or

   Create a new settings file by setting parameters in the *Configure Settings* dialog box as follows:

a. Select two images appropriate for the nuclear stain and translocation probe stain (the goal is to measure whether the probe gets in or out of the nucleus)

b. Define the approximate width and intensity above the local background for the nuclei to be detected. For this standard version, automatic blob-separation is assumed and only width and minimum intensity are controlled.

c. Specify the correlation coefficient value (between the two stains) of all pixels in the nucleus and extending out through the outer region. The value must be between minus one (-1) and one (1). The detected value must be greater than or equal to the specified value in order to classify the nucleus as positive. A recommended starting value is 0.6.

5. Run the analysis for a specific well, a group of selected wells, the entire microplate, or multiple microplates.

## Analyzing Nuclear Translocation HT Images

Complete the following procedure to apply the Nuclear Translocation HT analysis to images:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click *Nuclear Translocation HT*.<br><br>**Or**<br><br>From the *Review Screen Data* dialog box, choose the *Nuclear Translocation HT* application module, then click *Configure Settings*. The Nuclear Translocation HT dialog box opens. |
| **2** | From the File menu, click *Open*, and open an image that qualifies as a Nuclear Translocation image.<br><br>**Or**<br><br>Select one or more wells in the well arrangement grid of the *Review Screen Data* dialog box. |
| **3** | From the File menu, click *Open*, and open an image that qualifies as a Translocation probe image for the preceding nuclear-stained image. |
| **4** | In the *Nuclear Translocation HT* dialog box, click *Display result image*, then use the image selector to assign a name to the result image. |
| **5** | Under *All Nuclei*, in the *Approximate width box,* type or select a value for the approximate width of the nuclear boundary.<br><br>**Hint:** Magnify a boundary region and use the *Line Region* tool to measure the width of the boundary. |
| **6** | Under *All Nuclei*, in the *Intensity above local background* box, type or select a value for the approximate intensity of the nuclear boundary.<br><br>**Hint:** Magnify a boundary region and place |

your pointer on pixels on either side of the boundary. Read the pixel intensity values from the status bar, and subtract the smaller value from the larger value.

**7** In the *Translocation probe* area, in the *Classify positive if correlation coefficient is* box, type or select an evaluation criterion value between -1 and 1 to be used to classify each nucleus as either positive or negative. If the correlation coefficient for a nucleus is equal to or greater than the specified value, the nucleus will be classified as positive. A recommended starting value is 0.6.

**8** Click *Apply* to run the application module.

**9** To interactively view data belonging to an individual cell or group of cells, select the cell(s) in the image and view the data in the Cellular Results table.

## Nuclear Translocation HT - Dialog Box Options

### All Nuclei image

Selects and opens the 16-bit nuclear-stain image. This image file is required. This image should be calibrated in microns. If the image is not calibrated, a red warning rectangle is displayed.

**Notes:**

- When processing is complete, this image is automatically overlaid with the segmentation result image. If you do not re-save the source image, the original source image is not affected. However, if you re-save the source image the overlay information will be saved with it.
- The resulting overlay segmentation image displays positive nuclei as green and negative nuclei as red.
- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

### Translocation probe image

Selects and opens the 16-bit translocation probe stain image. This image file is required.

### Display result image

Activates the image selector for the result image. Check this box to activate or deactivate the image selector for destination image of the nuclear segmentation.

### All Nuclei

Defines the options used to specify your nuclear segmentation parameters.

### Approximate width

Specifies the approximate scale in microns of the nuclei to be segmented. Type or select the approximate nuclei width in Microns. The value that you enter in microns is converted to pixel units and displayed on the right.

### Intensity above local background

Specifies the intensity range of the nuclear segmentation. This value is the threshold after automatic noise and background removal.

### Translocation probe

Defines the criteria for probe image measurement.

### Classify positive if correlation coefficient is

Specifies the criterion used to classify each nucleus as positive. The resulting overlay segmentation image displays positive nuclei as green and negative nuclei as red. If the nuclear image resembles the probe image, the correlation will be classified as positive if the result satisfies the criterion of your selected result evaluation value. The value you can enter must be between minus one (-1) and one (1). A recommend starting value is 0.6.

### Configure Summary Log

Opens the Configure Log dialog box. The Summary Log stores and records settings and results related to the entire image, and include the following:

***Total Cells –*** Number of cells detected in the *All Nuclei* image.

***Mean Nuclear Area –*** Total square microns in the nucleus divided by the total number of nuclei.

***Integrated Inner Intensity –*** Summed grayscale values in all inner regions.

***Integrated Outer Int*ensity –** Summed grayscale values in all outer regions.

***Average Inner Intensity –*** Total inner intensity divided by total inner area in square microns.

***Average Outer Intensity –*** Total outer intensity divided by total outer area in square microns.

***Probe Background Intensity –*** Constant background value of probe image in gray levels.

***Correlation Coefficient –*** Value from -1 (anti-correlated) to 1 (correlated) of nuclear-stained image and probe image both masked by: the regions defined by the inner surfaces of the inner rings expanded outward to the outer surfaces of the outer rings.

***Classified Positive –*** Number of nuclei classified as positive for translocation.

***% Classified Positive –*** Number of nuclei classified as positive divided by the total number of nuclei.

### Save Settings

Opens the *Save Settings* dialog box. Use this dialog box to create and save a *Nuclear Translocation HT* settings file. You can store all the settings currently specified in the *Nuclear Translocation HT* dialog box. Use *Load Settings* to retrieve and use these settings at a later time. Type a setting name and an optional description, then click *OK*.

### Load Settings

Opens the *Load Settings* dialog box. Use this dialog box to select and load a *Nuclear Translocation HT* settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the *Nuclear Translocation HT* settings file. Select the name of the setting that you want to use, then click *OK*.

### Set to Defaults

Resets all options and settings to their default values.

### **Test Run** (from Review Plate Data only)

Runs one pass of the *Nuclear Translocation HT* application module on the Nuclear and Probe images currently open and selected.

### **Apply** (from the Apps menu only)

Applies the settings that you have made and runs the translocation.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

### Close

Closes the Nuclear Translocation HT dialog box.

# Transfluor®

**Detects, counts, measures, and logs G-Protein Coupled Receptor (GPCR) cycling characteristics and optionally detects, counts, measures, and logs related nuclei characteristics.**

*Availability: Exclusive to MetaXpress*

**Drop-in: GPCR**

Use this application module to detect, label, measure, and count Pits, Vesicles, or both Pits and Vesicles. In addition, use this application module to detect, label, and count nuclei in correctly stained Transfluor® assays.

GPCR images analyzed with this application module typically contain two stains. One stain is typically a nuclear stain used to isolate and identify individual cell compartments, the other stain is used to identify either pits or vesicles or both pits and vesicles. The nuclear stain can be used to detect and count the total number of cell nuclei and associated nuclear characteristics.

This dialog box is divided into three distinct areas: Pits, Vesicles, and Nuclear stain. The settings in each of these areas determine the effectiveness of the programs ability to detect the designated image element. The settings create "filters" that are used to detect a specific element based on its minimum width, maximum width, and its graylevel intensity above the image background.

After the application module has processed the image, you can use the ***Cellular Results*** table to interactively view data belonging to an individual cell or group of cells by clicking the cell(s) in the image.

**Application Note — *MetaMorph Application Module Overview* (PDF)**

Backgrounds of images analyzed using theTransfluor® application module or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Running Transfluor®

To run the Transfluor® application module, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click *Transfluor®*, the Transfluor® dialog box opens. |
| **2** | From the File menu, click *Open*, and open an appropriate 16-bit GPCR image. |
| **3** | In the Transfluor® dialog box, click the *Pits and Vesicles image* selector button, and choose the image that you want to use. |
| **4** | Click *Display result image* if you want the result image to open after it is created. |
| **5** | To count and measure pits, be sure that the Pits box is checked. |
| **6** | In the *Approximate min width* box, type or select the appropriate minimum pit width.<br>***Hint:*** You can use either the Caliper tool or Line Region tool to help you approximate the minimum pit width. |

**7** In the *Approximate max width* box, type or select the appropriate maximum pit width. ***Hint:*** You can use the Caliper tool to help you approximate the maximum pit width.

**8** In the *Intensity above local background* box, type or select a gray level value that represents the least bright pit minus the background intensity near the pit. The lower the value, the more sensitive the detection. The software will estimate the background for each pit locally to correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the most subtle pit that you want to detect. The gray level values are shown on the status bar below the image area. To obtain the intensity value, subtract the value of the background from the value of the pit.

**9** To count and measure vesicles, be sure that the Vesicles box is checked.

**10** In the *Approximate min width* box, type or select the appropriate minimum vesicle width.

***Hint:*** You can use either the Caliper tool or Line Region tool to help you approximate the minimum vesicle width.

**11** In the *Approximate max width* box, type or select the appropriate maximum vesicle width. ***Hint:*** You can use the Caliper tool to help you approximate the maximum vesicle width.

**12** In the *Intensity above local background* box, type or select a gray level value that represents the least bright vesicle minus the background intensity near the vesicle. The lower the value, the more sensitive the detection. The software will estimate the background for each vesicle locally to correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the most subtle vesicle that you want to detect. The gray level values are shown on the status bar below the image area. To obtain the intensity value, subtract the value of the background from the value of the vesicle.

**13** If your assay images include nuclear stained images, and you want to include Nuclear counts and measurements in your logged data, click *Nuclear stain*.

**14** In the *Nuclear stain* area, click the *Nuclear image* selector button, and choose the nuclear image that you want to use.

**15** In the *Approximate min width* box, type or select the appropriate minimum cell nuclei

width.

***Hint:*** You can use the Caliper tool to help you approximate the minimum nuclei width.

**16** In the *Approximate max width* box, type or select the appropriate maximum nuclei width. This value is used to differentiate nuclei from non-nuclear material and exclude nuclei greater than the specified size from the segmentation.

***Hint:*** You can use the Caliper tool to help you approximate the maximum nuclei width.

**17** In the *Intensity above local background* box, type or select a value that represents the minimum nucleus intensity minus the background intensity near the nucleus. The lower the value, the more sensitive the detection. The software will estimate the background for each nuclei locally to correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the most subtle nuclei that you want to detect. The gray level values are shown on the status bar below the image area. To obtain the intensity value, subtract the value of the background from the value of the nucleus.

**18** Click *Configure Summary Log*. The Configure Log dialog box opens. Check and/or uncheck individual parameter configuration settings. Refer to the description of these settings under *Dialog Box Options: Configure Summary Log.*

**19** To restore default values for the available settings, click *Set to Defaults* at any time.

**20** Click *Apply* to run the application module and generate the result image.

**21** To interactively view data belonging to an individual cell or group of cells, select the cell(s) in the image and view the data in the Cellular Results table.

**22** Click *Close* to close theTransfluor® dialog box.

## Transfluor® - Dialog Box Options

### Pits and Vesicles image

Opens the source Image Selector for the Pits and Vesicles image. This selector works on the active plane only, and allows 16-bit images only. A value of [None] appears for cases in which images for a specific settings file cannot be found in the database. Source images opened with this image selector should be calibrated in microns in order to correctly process the image.

**Notes:**

- When processing is complete, this image is automatically overlaid with the segmentation result image. If you do not re-save the source image, the original source image is not affected. However, if you re-save the source image the overlay information will be saved with it.
- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

### Display Result image

Activates the display of the of the Transfluor® result image (optional).

### [Result image]

Opens the Image Selector for the destination image. This selection is enabled only if *Display result image* checked. This image will be displayed as an autoscaled, 16-bit image for easy color combine, arithmetic, or overlaid with the original. When this image is opened using Review Screen Data, the result image will not be stored in the database.

### Pits

Specifies that you want to detect pits in your image. This area of the dialog box contains the settings you need to make to detect pits within your image.

### Approximate min width

Specifies the minimum pit width in microns (µm) that you expect to detect. Pit patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

> **Note:** Even though you enter values in microns, the measurement occurs in the image, and as a result, is made in pixels. Therefore in images acquired at low magnifications, a greater range in microns will constitute a single pixel. For example, at a low magnification, any pits within the range of 0.3 microns to 0.7 microns will each be considered to be one pixel in width.

### Approximate max width

Specifies the maximum pit width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential pits as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of pits compared to the neighboring background values. This setting is for controlling the sensitivity of the detection.

### Vesicles

Specifies that you want to detect vesicles in your image. This area of the dialog box contains the settings you need to make to detect vesicles within your image.

### Approximate min width

Specifies the minimum vesicle width in microns (µm) that you expect to detect. Vesicle patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

> **Note:** Even though you enter values in microns, the measurement occurs in the image, and as a result, is made in pixels. Therefore in images acquired at low magnifications, a greater range in microns will constitute a single pixel. For example, at a low magnification, any vesicles within the range of 0.3 microns to 0.7 microns will each be considered to be one pixel in width.

### Approximate max width

Specifies the maximum vesicle width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential vesicles as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown

to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of vesicles compared to the neighboring background values. This setting is for controlling the sensitivity of the detection.

### Nuclear stain (optional)

Specifies that a second wavelength image of stained nuclei is included in the screen.

### Approximate min width

Specifies the minimum nuclei width in microns (µm) that you expect to detect. Nuclei patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

### Approximate max width

Specifies the maximum nuclei width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential nuclei as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of nuclei compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

### Configure Summary Log

Opens the Configure Log dialog box. Use this dialog box to select the measurements that you want to include in your summary log. In addition to the standard log measurements, the following measurements can be selected:

- Pit Count
- Pit Count Per Cell
- Pit Total Area
- Pit Area Per Cell
- Pit Integrated Intensity
- Pit Average Intensity
- Vesicle Count
- Vesicle Count Per Cell
- Vesicle Total Area
- Vesicle Area Per Cell
- Vesicle Integrated Intensity
- Vesicle Average Intensity
- Nuclear Count
- Nuclear Total Area
- Nuclear Area Per Cell
- Nuclear Integrated Intensity
- Nuclear Average Intensity

In addition to the preceding counts of pits, vesicles and nuclei, this application module provides measurements that you can use to define the graininess or texture characteristics of images. Each index measures grayscale intensity fluctuation and is in units of graylevels per pixel.

- ***Texture Index*** – Standard deviation of intensity values in the image.
- ***Cellular Texture Index*** -- Cell-by-cell standard deviation of intensity values near the nuclei. (Requires use of nuclear stain).
- ***Gradient Index*** – A texture-dependent measurement that reflects the amount of local intensity contrast. Measures the difference between the maximum and minimum intensity within a local neighborhood.
- ***Cellular Gradient Index*** -- Cell-by-cell Gradient Index measured near the nuclei. (Requires use of nuclear stain).
- ***Laplacian Index*** – Similar to the morphological gradient, however this morphological measurement reflects fluctuations in the gradient.
- ***Cellular Laplacian Index*** – Cell-by-cell Laplacian Index measured near the nuclei. (Requires use of nuclear stain).

### Configure Data Log (Cells)

The following data can be logged for each labeled cell:

- Cell: Assigned Label #
- Cell: Pit Count
- Cell: Pit Total Area
- Cell: Pit Integrated Intensity
- Cell: Pit Average Intensity
- Cell: Vesicle Count
- Cell: Vesicle Total Area
- Cell: Vesicle Integrated Intensity
- Cell: Vesicle Average Intensity
- Cell: Nuclear Total Area
- Cell: Nuclear Integrated Intensity
- Cell: Nuclear Average Intensity
- Cell: Texture Index
- Cell: Gradient Index
- Cell: Laplacian Index

### Save Settings

Opens the Save Settings dialog box. Use this dialog box to create and save a Transfluor settings file. You can store all the settings currently specified in the Transfluor dialog box. Use Load Settings to retrieve and use these settings at a later time.

### Load Settings

Opens the Load Settings dialog box. Use this dialog box to select and load a Transfluor settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the Transfluor settings file. Select the name of the setting that you want to use, then click OK.

### Set to Defaults

Resets all options and settings to their default values.

### **Test Run** (from Review Plate Data only)

Runs one pass of the Transfluor application module on the images currently open and selected. Measurements will be logged if the Summary and/or Data Logs are open.

**Apply** (from the Apps menu only)
Applies the settings that you made and runs the application module.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

**Close**
Closes the dialog box.

# Cellular Results

**Enables you to select individual cells in an image and quickly view its data in a table**

*Availability: Included in MetaXpress; available for MetaMorph Premier*

The Cellular Results table is used by the MetaXpress group of application modules (it is also used by the stand alone Neurite Outgrowth command in MetaMorph Premier) and opens after an application module finishes processing an image. You can also configure it not to open each time an application module is run.

Use this feature to interactively view data belonging to an individual cell by clicking the cell in the image. This causes the data for the selected cell to be highlighted in the Cellular Results table. You can also click a value in the Cellular Results table and the corresponding cell will become highlighted in the image. When selecting cells from the image or the Cellular Results table, you can select multiple cells by using the **Ctrl+Click** combination. You can also select a range of cells from the Cellular Results table using the **Shift+Click** combination.

**Note:** The data displayed in the Cellular Results table is the same data configured using the *Configure Data Log (Cells)* command for the application module you are using. However, you do not have to have a log open to view the Cellular Results table.

***Using the Cellular Results Table***

## Using the Cellular Results Table

Complete the following procedure to use the Cellular Results table with application modules:

| Step | Action |
|---|---|
| **1** | From the Apps menu, select an application module to run. |
| **2** | Configure the module (see the help file for the module for more information), and configure the data log using the *Configure Data Log (Cells)* command. You do not have to open a data log in order to view the Cellular Results table. |
| **3** | Click *Apply* to run the application module. The source image is processed and the Cellular Results table opens. |
| **4** | Select one of the segmented cells in the source image; the corresponding data for the cell will highlight in the Cellular Results table.<br>**OR**<br>Select a row in the Cellular Results table; the corresponding cell will highlight in the source |

image.

**Note:** When selecting cells from the image or the Cellular Results table, you can select multiple cells by using the **Ctrl+Click** combination. You can also select a range of cells from the Cellular Results table using the **Shift+Click** combination.

**5** If you do not want the Cellular Results table to open each time an application module is run, uncheck *Show Cellular Results*.

**Note:** If you uncheck this and later want to view the Cellular Results table when running an application module, click *Show Cellular Results* from the Windows menu on the MetaXpress or MetaMorph menu bar.

**6** Click *Close* to exit the Cellular Results table.

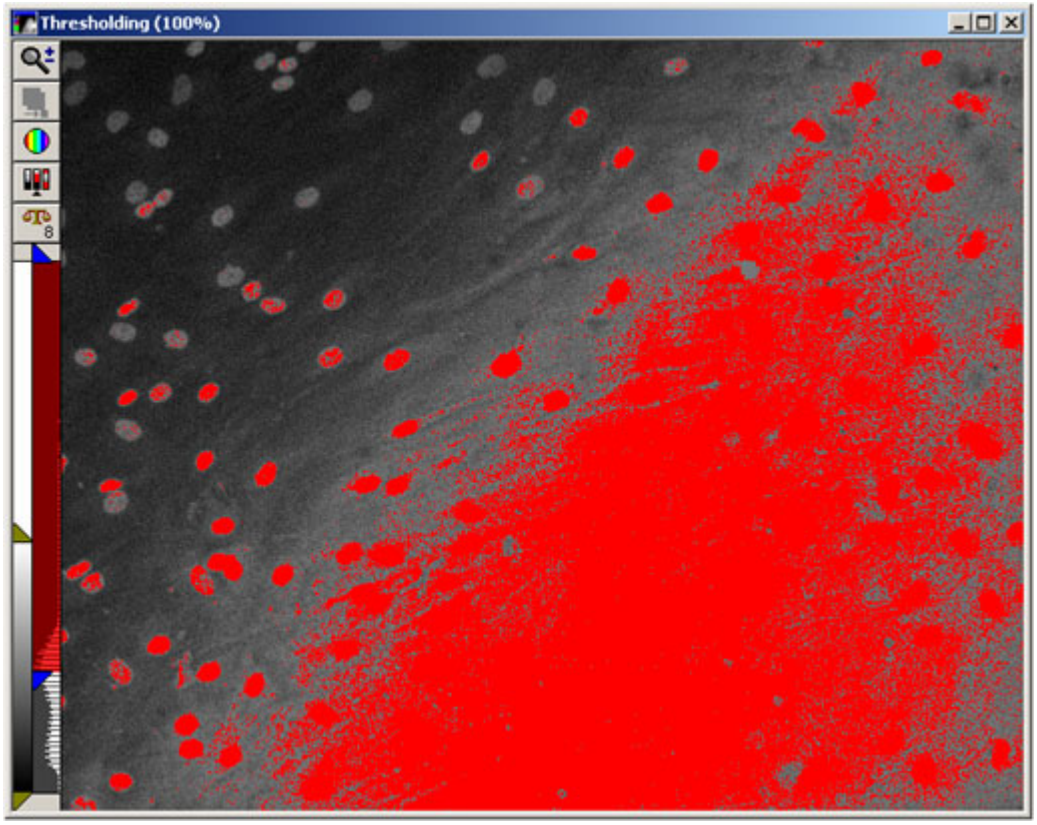
# Adaptive Background Correction™ System

This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

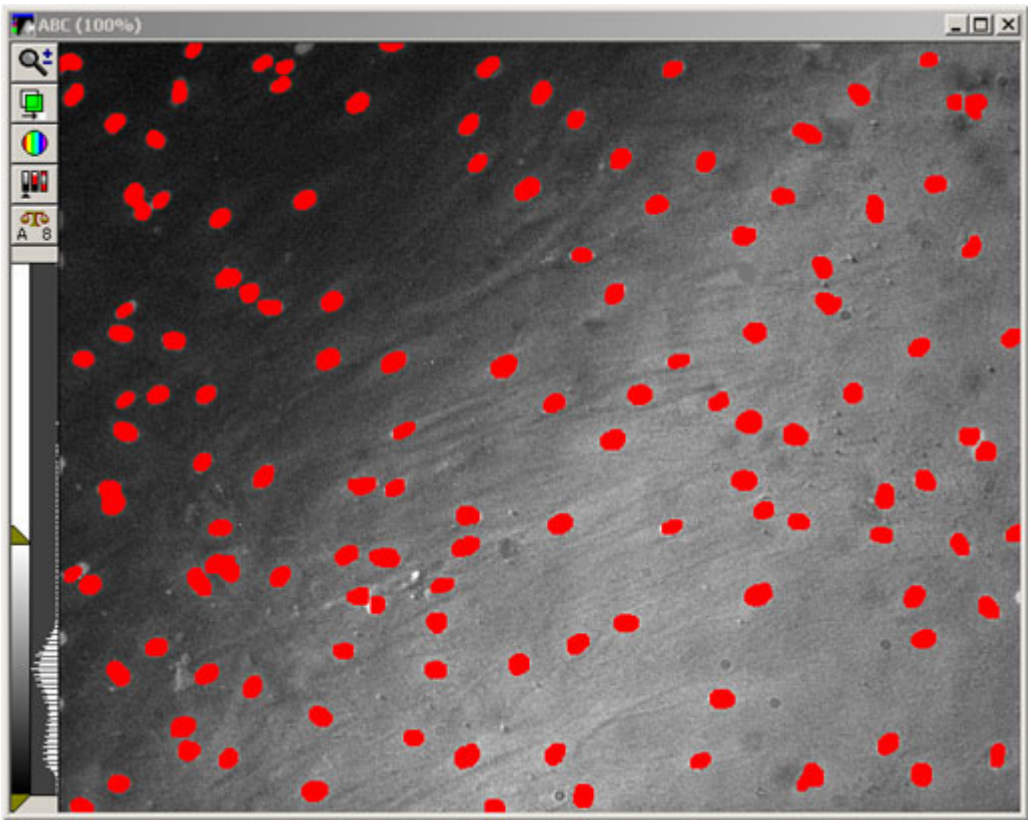
Uneven background levels that vary between images or within a single image are a common difficulty in automated fluorescence image analysis. For example, the following image has a significantly brighter background in the lower right quadrant than in the upper left quadrant.

{bmct UnevenBackgroundScreenshot_c75p.bmp}

As a result of uneven background, intensity thresholding cannot extract the cells for counting and measuring. Too high a threshold misses cells in the dark background regions, while too low a threshold erroneously detects the bright background region as a large object. In the following example, the thresholding result is shown as a red graphic overlay on the original image.



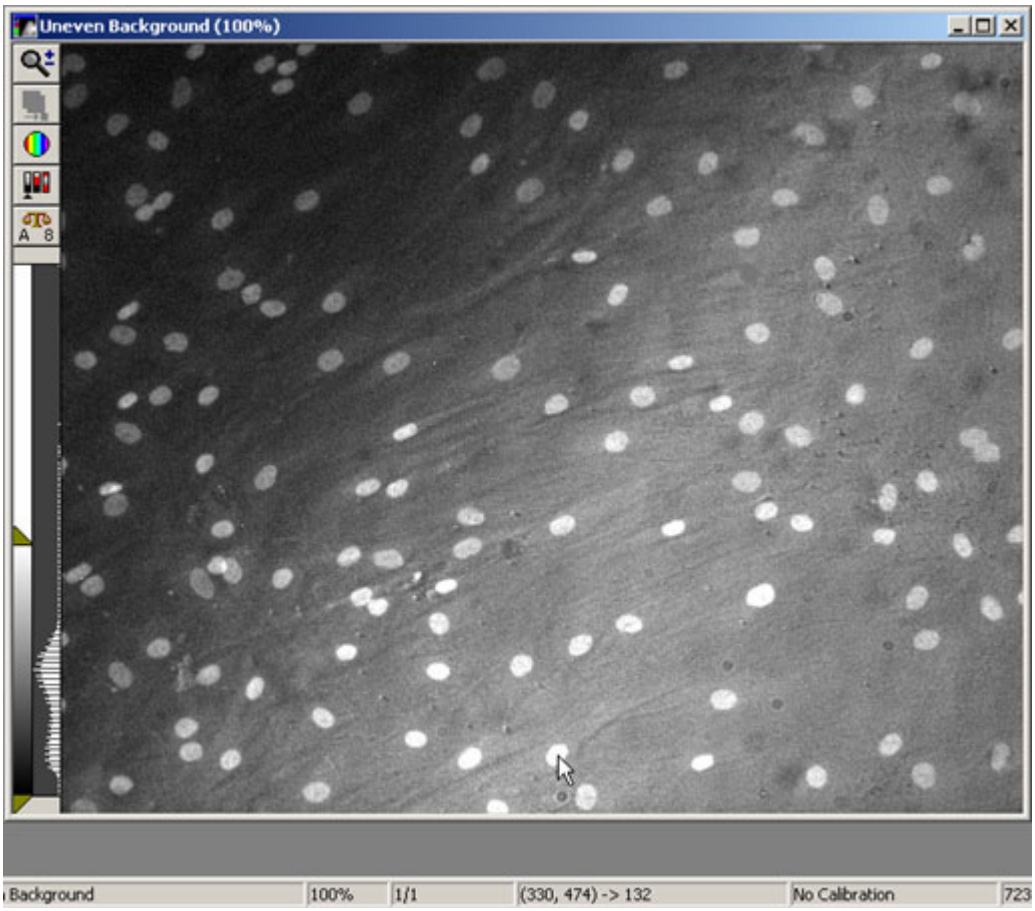

The Adaptive Background Correction™ system automatically estimates and compensates for uneven background fluctuations, resulting in robust object segmentation for more accurate counting and measurement. For example, in the following image, the segmentation result is shown as a red graphic overlay on the original image—with far superior detection than the non-adaptive thresholding method.




***See Also*: *Making the Best Use of the Adaptive Background Correction™ System***

# Making the Best Use of the Adaptive Background Correction™ System

Application modules that use the Adaptive Background Correction™ system enable you to specify the detection sensitivity as ***Intensity above local background***. To estimate this parameter, place your mouse pointer on the most subtle object that you want to detect (inside the object, but close to the margin between the object and the background). On the *Status Bar* at the bottom of the MetaMorph window, note the intensity value shown. This is the intensity value of the image pixel directly under the tip of the mouse pointer. For example, refer to the following figure in which the intensity value for the pixel directly under the tip of the mouse pointer is 132. Note this value, then move your mouse pointer to a pixel in the background area near the same object. The second pixel should be as close as possible to the first pixel that you measured, but distinctly part of the background, and not within the object. The difference in intensity between the object and its nearby background is the *Intensity above local background* parameter value. Lowering this parameter value results in more sensitive detection, while raising this parameter value decreases sensitivity.




***Hint:*** To enable you to measure the object and background pixel intensity values more easily, you can zoom in on the area where you want to make your measurements. After you zoom in, you should notice an almost subtle difference between the object and the background close to where the object and background meet. You should also notice a line of pixels that define a distinct boundary between the object and the background. It is within the object and background pixels that are closest to the boundary line of pixels in which you want to make your measurements.

***See Also*: *The Adaptive Background Correction™ system***

# Count Nuclei (Apps Menu)

**Identifies and isolates cell nuclei through image segmentation and logs image data for count nuclei to an associated log file.**

*Availability: Exclusive to MetaMorph and MetaXpress*

### Drop-in: Countnuclei

Use this application module to segment images that are used to identify and differentiate cell nuclei. The segmentation labels each isolated and identified nucleus as a different color index to enable you to see a visual separation between nuclei that are close or touching. Source images must be 16-bit images. This application module produces 16-bit result images. Default settings enable you to produce a result from a typical image without changing settings. Three settings are available to you: Approximate minimum nuclei width, Approximate maximum nuclei width, and the minimum gray level intensity of the nuclei above the local background.

Any nuclei-like pattern in the image less than the specified minimum width will be considered to be noise, and will be excluded from the segmentation. The value you specify for the maximum width can be used

to exclude any blobs larger than the specified size and to control the locality of background intensity estimates near each nucleus.

After the application module has processed the image, you can use the ***Cellular Results*** table to interactively view data belonging to an individual cell or group of cells by clicking the cell(s) in the image.

**Application Note PDF —***Using the Count Nuclei Application Module in MetaMorph*

**Application Note PDF —** *MetaMorph Application Module Overview*

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Counting Nuclei

To run the Count Nuclei application module, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click *Count Nuclei*; the Count Nuclei dialog box opens. |
| **2** | From the File menu, click *Open*, and open an appropriate 16-bit image. |
| **3** | In the count Nuclei dialog box, click the *Source image* selector button, and choose the image that you want to segment. |
| **4** | Click *Display result image* if you want the result image to open after it is created. |
| **5** | In the *Approximate min width* box, type or select the appropriate minimum cell nuclei width.<br><br>***Hint:*** You can use the Caliper tool or the Line Region tool to help you approximate the minimum nuclei width. The caliper tool measures directly in microns; the line region tool measures in pixels, which you must convert to microns. |
| **6** | In the *Approximate max width* box, type or select the appropriate maximum nuclei width. This value is used to differentiate nuclei from non-nuclear material and exclude nuclei greater than the specified size from the segmentation.<br><br>***Hint:*** You can use the Caliper tool or the Line Region tool to help you approximate the maximum nuclei width. The caliper tool measures directly in microns; the line region tool measures in pixels, which you must convert to microns. |
| **7** | In the *Intensity above local background* box, type or select a value that represents the minimum nucleus intensity minus the background intensity near the nucleus. The lower the value, the more sensitive the |

detection. The software will estimate the background for each nucleus locally to correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the most subtle nuclei that you want to detect. The graylevel values are shown on the status bar below the image area. To obtain the *Intensity above local background* value, subtract the value of the background from the value of the nucleus.

**8** Click *Configure Summary Log*. The Configure Log dialog box opens. Check and/or uncheck individual parameter configuration settings. Refer to the description of these settings under *Dialog Box Options: Configure Summary Log.*

**9** To restore default values for the available settings, click *Set to Defaults* at any time.

**10** Click *Apply* to run the application module and generate the result image.

**11** To interactively view data belonging to an individual cell or group of cells, select the cell(s) in the image and view the data in the Cellular Results table.

**12** Click *Close* to close the Count Nuclei dialog box.

## Count Nuclei - Dialog Box Options

### Source Image

Opens the source Image Selector. This selector works on the active plane only, and allows 16-bit images only. A value of [None] appears for cases in which images for a specific settings file cannot be found in the database. Source images opened with this image selector should be calibrated in microns in order to correctly process the image.

### Notes:

- When processing is complete, this image is automatically overlaid with the segmentation result image. If you do not re-save the source image, the original source image is not affected. However, if you re-save the source image the overlay information will be saved with it.
- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

### Display Result

Activates the display of the of the segmentation result image (optional).

### Result image

Opens the Image Selector for the Destination Image. This selection is enabled only if Create Result Image checked. This setting shows segmentation of the nuclei drawn as different indexed colors according to nucleus size on a black background. This image will be displayed as an autoscaled, 16-bit image for easy color combine, arithmetic, or overlay with the original. When this image is opened using Review Screen Data, the result image will not be stored in the database.

### Parameters

Defines the parameters for detecting nuclei.

### Approximate minimum width

Specifies the minimum nuclear width in microns (µm) that you expect to detect. Nuclei patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

### Approximate maximum width

Specifies the maximum nuclei width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential nuclei as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of nuclei compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

### Configure Summary Log

Opens the configure log dialog box. Use this dialog box to select the measurements that you want to include in your summary log. In addition to the standard log measurements, the following measurements can be selected:

**Total Nuclei –** Total number of nuclei within the image.

**Total Area –** Total nuclei area in square microns.

**Mean Area –** Total nuclei area in square microns divided by number of nuclei.

**Integrated Intensity –** Raw sum of the intensity values of all pixels in the object or image.

**Average Intensity –** Sum of all intensity values of all pixels in the image divided by the number of pixels.

### Configure Data Log (Cells)

Opens the Configure Data Log (Cells) dialog box. Use this dialog box to select the measurements that you want to include in your data log. In addition to the standard log measurements, the following cell-by-cell measurements can be selected:

**Cell Assigned Label # –** Provides a number label to identify each individual cell in the image.

**Cell: Area –** Total area for a specific cell.

**Cell: Integrated Intensity –** The integrated intensity for a specific cell.

**Cell: Average Intensity –** The average intensity for a specific cell.

### Save Settings

Opens the Save Settings dialog box. Use this dialog box to create and save a Coumt Nuclei settings file. You can store all the settings currently specified in the Coumt Nuclei dialog box. Use Load Settings to retrieve and use these settings at a later time.

### Load Settings

Opens the Load Settings dialog box. Use this dialog box to select and load a Coumt Nuclei settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the Coumt Nuclei settings file. Select the name of the setting that you want to use, then click OK.

### Set to Defaults

Resets all options and settings to their default values.

### **Test Run** (from Review Plate Data only)

Runs one pass of the Coumt Nuclei application module on the images currently open and selected. Measurements will be logged if the Summary and/or Data Logs are open.

**Apply** (from the Apps menu only)
Applies the settings that you made and runs the application module.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

**Close**
Closes the dialog box.

# Transfluor® HT

**Detects, counts, measures, and logs granularity characteristics and optionally detects, counts, measures, and logs nuclei characteristics.**

*Availability: Exclusive to MetaXpress*

**Drop-in: TransfluorHT**

Use this application module to detect and count granules in cells and measure the physical characteristics of granules. Optionally, you can detect and count nuclei and measure the physical characteristics of nuclei within the cells in which you are detecting granules. All measurements are logged according to the log configuration that you specify. This application module is similar to Granularity, but optimized for high throughput.

This application module detects, measures, and logs the following granularity characteristics:

- Granules (count)
- Granules Per Cell (average)
- Total Granule Area
- Mean Granule Area
- Integrated Granule Intensity
- Average Granule Intensity

This application module also detects, measures, and logs the following nuclei characteristics:

- Nuclei (count)
- Total Nuclear Area
- Mean Nuclear Area

**Application Note — *MetaMorph Application Module Overview* (PDF)**

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Measuring Granularity with Transfluor® HT

To run the Transfluor® HT application module, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click *Transfluor® HT*, the Transfluor® HT dialog box opens. |
| **2** | From the File menu, click *Open*, and open |

an appropriate 16-bit granularity image.

**3** In the *Transfluor® HT* dialog box, click the *Granule image* selector button, and choose the image that you want to use.

**4** Click *Display result image* if you want the result image to open after it is created.

**5** In the *Approximate min width* box, type or select the appropriate minimum granule width.

***Hint:*** You can use either the Caliper tool or Line Region tool to help you approximate the minimum granule width.

**6** In the *Approximate max width* box, type or select the appropriate maximum granule width. This value is used to differentiate nuclei from non-nuclear material and exclude nuclei greater than the specified size from the segmentation. Hint: You can use the Caliper tool to help you approximate the maximum granule width.

**7** In the *Intensity above local background* box, type or select a gray level value that represents the least bright granule minus the background intensity near the granule. The lower the value, the more sensitive the detection. The software will estimate the background for each granule locally to correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the most subtle granule that you want to detect. The gray level values are shown on the status bar below the image area. To obtain the intensity value, subtract the value of the background from the value of the granule.

**8** If your assay images include nuclear stained images, and you want to include Nuclei counts and measurements in your logged data, click *Nuclear stain*.

**9** In the *Nuclear stain* area, click the *Nuclear image* selector button, and choose the nuclear image that you want to use.

**10** In the *Approximate min width* box, type or select the appropriate minimum cell nuclei width.

***Hint:*** You can use the Caliper tool to help you approximate the minimum nuclei width.

**11** In the *Approximate max width* box, type or select the appropriate maximum nuclei width. This value is used to differentiate nuclei from non-nuclear material and exclude nuclei greater than the specified size from the segmentation.

***Hint:*** You can use the Caliper tool to help

you approximate the maximum nuclei width.

**12** In the *Intensity above local background* box, type or select a value that represents the minimum nucleus intensity minus the background intensity near the nucleus. The lower the value, the more sensitive the detection. The software will estimate the background for each nuclei locally to correct for cases of images with uneven backgrounds. You can determine this value by placing your mouse pointer on pixels just inside and outside the most subtle nuclei that you want to detect. The gray level values are shown on the status bar below the image area. To obtain the intensity value, subtract the value of the background from the value of the nucleus.

**13** Click *Configure Summary Log*. The Configure Log dialog box opens. Check and/or uncheck individual parameter configuration settings. Refer to the description of these settings under *Dialog Box Options: Configure Summary Log.*

**14** To restore default values for the available settings, click *Set to Defaults* at any time.

**15** Click *Apply* to run the application module and generate the result image.

**16** Click *Close* to close the Transfluor® HT dialog box.

## Transfluor® HT - Dialog Box Options

### Granules

Dialog box area that contains settings used for detecting granules within your image.

### Granule Image

Opens the source Image Selector for the Granule image. This selector works on the active plane only, and allows 16-bit images only. A value of [None] appears for cases in which images for a specific settings file cannot be found in the database. Source images opened with this image selector should be calibrated in microns in order to correctly process the image.

### Notes:

- When processing is complete, this image is automatically overlaid with the segmentation result image. If you do not re-save the source image, the original source image is not affected. However, if you re-save the source image the overlay information will be saved with it.
- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

### Display Result image

Activates the display of the of the granularity result image (optional).

### (Result image)

Opens the Image Selector for the destination image. This selection is enabled only if *Display result image*

checked. This image will be displayed as an autoscaled, 16-bit image for easy color combine, arithmetic, or overlay with the original. When this image is opened using Review Screen Data, the result image will not be stored in the database.

### Approximate min width

Specifies the minimum granule width in microns (µm) that you expect to detect. Granule patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

> **Note:** Even though you enter values in microns, the measurement occurs in the image, and as a result, is made in pixels. Therefore in images acquired at low magnifications, a greater range in microns will constitute a single pixel. For example, at a low magnification, any granules within the range of 0.3 microns to 0.7 microns will each be considered to be one pixel in width.

### Approximate max width

Specifies the maximum granule width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential granules as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of granules compared to the neighboring background values. This setting is for controlling the sensitivity of the detection.

### Nuclear stain (optional)

Specifies that a second wavelength image of stained nuclei is included in the screen.

### Approximate min width

Specifies the minimum nuclei width in microns (µm) that you expect to detect. Nuclei patterns in the image less than this width will be considered to be noise patterns. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of edit box.

### Approximate max width

Specifies the maximum nuclei width in microns (µm) that you expect to detect. This width determines which intensity fluctuations are potential nuclei as compared to the background fluctuations or remaining shading errors. The value for the width that you entered in microns is converted to integer pixel units, and is shown to the right of the edit box.

### Intensity above local background

Specifies a value for the intensity threshold of nuclei compared to the neighboring background values. This setting is for controlling the sensitivity of the detection and segmentation.

### Configure Summary Log

Opens the Configure Log dialog box. Use this dialog box to select the measurements that you want to include in your summary log. In addition to the standard log measurements, the following measurements can be selected:

- Granules (count)
- Granules Per Cell (average)
- Total Granule Area
- Mean Granule Area
- Integrated Granule Intensity
- Average Granule Intensity
- Nuclei (count)

- Total Nuclear Area
- Mean Nuclear Area

**Save Settings**

Opens the Save Settings dialog box. Use this dialog box to create and save a Transfluor HT settings file. You can store all the settings currently specified in the Transfluor HT dialog box. Use Load Settings to retrieve and use these settings at a later time.

**Load Settings**

Opens the Load Settings dialog box. Use this dialog box to select and load a Transfluor HT settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the Transfluor HT settings file. Select the name of the setting that you want to use, then click OK.

**Set to Defaults**

Resets all options and settings to their default values.

**Test Run** (from Review Plate Data only)

Runs one pass of the Transfluor HT application module on the images currently open and selected. Measurements will be logged if the Summary and/or Data Logs are open.

**Apply** (from the Apps menu only)

Applies the settings that you made and runs the application module.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

**Close**

Closes the dialog box.

# Translocation (Apps Menu)

**Determines whether a probe that is present outside of a compartment or group of compartments can be detected inside any compartments within the image.**

*Availability: Exclusive to MetaXpress*

**Drop-in: Translocation**

Use this application module to determine if a specific fluorescent probe can be detected both outside and inside one or more compartments. This application module is provided in two versions, *Translocation – Enhanced*, a comprehensive dialog box that includes settings to compensate for images that cannot be analyzed by the standard Translocation command, and this dialog box, *Translocation*.

Use this standard *Translocation* command for images that conform to a basic set of criteria, and need a minimum amount of adjustments made to their default settings. Use *Translocation – Enhanced* for images that require manual adjustments.

The resulting segmentation image is overlaid on the original compartment image. The overlaid image displays positive compartments as green and negative compartments as red. Using the Threshold Image command in conjunction with Translocation, you can use either the negative population or the positive population to create a binary mask.

After the application module has processed the image, you can use the ***Cellular Results*** table to interactively view data belonging to an individual cell or group of cells by clicking the cell(s) in the image.

**Application Note — *MetaMorph Application Module Overview* (PDF)**

Backgrounds of images analyzed using this or any MetaXpress application module are processed using

the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Using Translocation

The following general steps are intended to guide you through the process of acquiring images appropriate for use with the Translocation application module and using the Translocation application module to complete your analysis:

5. Collect your data set consisting of both a compartment and a probe image using two different wavelengths of light.
6. Collect background images and bleed through correction samples either as part of your microplate acquisition or in a preliminary experiment using the standard MetaMorph acquisition dialog box.
7. Open the Review Screen Data dialog box, and choose the *Translocation* application module.
8. Choose a *Translocation settings file* (*.str).

   or

   Create a new settings file by setting parameters in the *Configure Settings* dialog box as follows:

   d. Select two images appropriate for the compartment stain and translocation probe stain (the goal is to measure whether the probe gets in or out of the compartment)

   e. Define the approximate width and intensity above the local background for the compartments to be detected. For this standard version, automatic blob-separation is assumed and only width and minimum intensity are controlled.

   f. Specify the correlation coefficient value (between the two stains) of all pixels in the compartment and extending out through the outer region. The value must be between minus one (-1) and one (1). The detected value must be greater than or equal to the specified value in order to classify the compartment as positive. A recommended starting value is 0.6.

6. Run the Analysis for a specific well, a group of selected wells, the entire microplate, or multiple microplates.

## Analyzing Translocation Images

Complete the following procedure to apply the Translocation Analysis to images:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click *Translocation*.<br>Or<br>From the *Review Screen Data* dialog box, choose the *Translocation* application module, then click *Configure Settings*. The Translocation dialog box opens. |
| **2** | From the File menu, click *Open*, and open an image that qualifies as a compartment image.<br>Or<br>Select one or more wells in the well arrangement grid of the *Review Screen Data* dialog box. |

3 From the File menu, click *Open*, and open an image that qualifies as a Translocation probe image for the preceding compartment image.

4 In the *Translocation* dialog box, click *Display result image*, then use the image selector to assign a name to the result image.

5 Under *Compartments*, in the *Approximate width box,* type or select a value for the approximate width of the compartment boundary.

**Hint:** Magnify a boundary region and use the *Line Region* tool to measure the width of the boundary.

6 Under *Compartments*, in the *Intensity above local background* box, type or select a value for the approximate intensity of the compartment boundary.

**Hint:** Magnify a boundary region and place your pointer on pixels on either side of the boundary. Read the pixel intensity values from the status bar, and subtract the smaller value from the larger value.

7 In the *Translocation probe* area, in the *Classify positive if correlation coefficient is* box, type or select an evaluation criterion value between -1 and 1 to be used to classify each compartment as either positive or negative. If the correlation coefficient for a compartment is equal to or greater than the specified value, the compartment will be classified as positive. A recommended starting value is 0.6.

8 Click *Apply* to run the application module.

9 To interactively view data belonging to an individual cell or group of cells, select the cell(s) in the image and view the data in the Cellular Results table.

## Translocation - Dialog Box Options

### Compartment image

Selects and opens the 16-bit compartment stain image. This image file is required. This image should be calibrated in microns. If the image is not calibrated, a red warning rectangle is displayed.

### Notes:

- When processing is complete, this image is automatically overlaid with the segmentation result image. If you do not re-save the source image, the original source image is not affected. However, if you re-save the source image the overlay information will be saved with it.
- The resulting overlay segmentation image displays positive compartments as green and negative compartments as red.

- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

### Translocation probe image

Selects and opens the 16-bit translocation probe stain image. This image file is required.

### Display result image

Activates the image selector for the result image. Check this box to activate or deactivate the image selector for destination image of the compartment segmentation.

### Compartments

Defines the options used to specify your compartment segmentation parameters.

### Approximate width

Specifies the approximate scale in microns of the compartments to be segmented. Type or select the approximate compartment width in Microns. The value that you enter in microns is converted to pixel units and displayed on the right.

### Intensity above local background

Specifies the intensity range of the compartment segmentation. This value is the threshold after automatic noise and background removal.

### Translocation probe

Defines the criteria for probe image measurement.

### Classify positive if correlation coefficient is

Specifies the criterion used to classify each compartment as positive. The resulting overlay segmentation image displays positive compartments as green and negative compartments as red. If the compartment image resembles the probe image, the correlation will be classified as positive if the result satisfies the criterion of your selected result evaluation value. The value you can enter must be between minus one (-1) and one (1). A recommend starting value is 0.6.

### Configure Summary Log

Opens the Configure Log dialog box. The Summary Log stores and records settings and results related to the entire image, and include the following:

***Compartments –*** Number of compartments detected in the compartment image segmentation.

***Mean Compartment Area –*** Total square microns in the compartments divided by number of compartments.

***Integrated Inner Intensity –*** Summed grayscale values in all inner regions.

***Integrated Outer Int*****ensity –** Summed grayscale values in all outer regions.

***Average Inner Intensity –*** Total inner intensity divided by total inner area in square microns.

***Average Outer Intensity –*** Total outer intensity divided by total outer area in square microns.

***Probe Background Intensity –*** Constant background value of probe image in gray levels.

***Correlation Coefficient –*** Value from -1 (anti-correlated) to 1 (correlated) of compartment image and probe image both masked by: the regions defined by the inner surfaces of the inner rings expanded outward to the outer surfaces of the outer rings.

***Classified Positive –*** Number of compartments classified as positive for translocation.

***% Classified Positive –*** Number of compartments classified as positive divided by the total number of compartments.

### Configure Data Log

Opens the Configure Log dialog box. The Data Log stores and records settings and results related to an individual compartment, and include the following:

***Cell: Assigned Label # –*** unique label number (1 through number of compartments).

***Cell: Inner Area –*** Total square microns in the inner region.

***Cell: Outer Area –*** Total square microns in the outer region.

***Cell: Integrated Inner Intensity –*** Summed grayscale values in inner region.

***Cell: Integrated Outer Intensity –*** Summed grayscale values in outer region.

***Cell: Mean Inner Intensity –*** Total inner intensity divided by inner area in square microns.

***Cell: Median Inner Intensity –*** The median intensity value for pixels within the inner region.

***Cell: Mean Outer Intensity –*** Total outer intensity divided by total outer area in square microns.

***Cell: Median Outer Intensity –*** The median intensity value for pixels within the outer region.

***Cell: Correlation Coefficient –*** Correlation coefficient value from -1 (anti-correlated) to 1 (correlated) of compartment image and probe image both masked by: the region defined by the inner surface of the inner ring expanded outward to the outer surface of the outer ring.

***Cell: Classification*** – 1 for positive, 0 for negative translocation classification.

### Save Settings

Opens the *Save Settings* dialog box. Use this dialog box to create and save a *Translocation* settings file. You can store all the settings currently specified in the *Translocation* dialog box. Use *Load Settings* to retrieve and use these settings at a later time. Type a setting name and an optional description, then click *OK*.

### Load Settings

Opens the *Load Settings* dialog box. Use this dialog box to select and load a *Translocation* settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the *Translocation* settings file. Select the name of the setting that you want to use, then click *OK*.

### Set to Defaults

Resets all options and settings to their default values.

### **Test Run** (from Review Screen Data only)

Runs one pass of the *Translocation* application module on the Compartment and Probe images currently open and selected.

### **Apply** (from the Apps menu only)

Applies the settings that you have made and runs the translocation.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

### Close

Closes the Translocation dialog box.

# Translocation-Enhanced (Apps Menu)

**Determines whether a probe that is present outside of a compartment or group of compartments can be detected inside any compartments within the image.**

*Availability: Exclusive to MetaXpress*

**Drop-in: Translocationenhanced**

Use this application module to determine if a specific fluorescent probe can be detected both outside and inside one or more compartments. This application module is provided in two versions, a standard Translocation dialog box, and this dialog box, *Translocation – Enhanced*. Use the standard Translocation command for images that conform to a basic set of criteria, and need a minimum amount

of adjustments made to their default settings. Use *Translocation – Enhanced* for images that require manual adjustments.

The Translocation and Translocation-enhanced commands derive from your supplied compartment and probe grayscale images a segmentation image that, based on your dialog box option settings, defines the areas of both the compartment image and the probe image that will be compared to each other on a compartment-by-compartment basis. The results of this comparison are logged in an associated log file.

The resulting segmentation image is overlaid on the original compartment image. The overlaid image displays positive compartments as green and negative compartments as red. Using the Threshold Image command in conjunction with Translocation, you can use either the negative population or the positive population to create a binary mask.

After the application module has processed the image, you can use the ***Cellular Results*** table to interactively view data belonging to an individual cell or group of cells by clicking the cell(s) in the image.

**Application Note — *MetaMorph Application Module Overview* (PDF)**

Backgrounds of images analyzed using this or any MetaXpress application module are processed using the ***Adaptive Background Correction™ system.*** This system automatically corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

## Using Translocation-Enhanced

The following general steps are intended to guide you through the process of acquiring images appropriate for use with the Translocation application module and using the Translocation application module to complete your analysis:

1. Collect your data set consisting of both a compartment and a probe image using two different wavelengths of light.
2. Collect background images and bleed through correction samples either as part of your microplate acquisition or in a preliminary experiment using the standard MetaMorph acquisition dialog box.
3. Open the Review Screen Data dialog box, and choose *Translocation-Enhanced*.
4. Choose the appropriate *Translocation-Enhanced settings file* (*.ste).

   ***or***

5. Create a new settings file by setting parameters in the *Configure Settings* dialog box as follows:
   a. Select a pair of images; one image appropriate for the compartment stain, and one translocation probe stain image (the goal is to measure whether the probe gets into or out of the compartment)
   b. Define the approximate widths, intensity ranges, and area ranges for the compartments to be detected. Additionally, you should specify whether touching compartments should be automatically separated (assuming they are round, blob-like shapes). In combination, these parameters help to exclude unwanted compartments including overly bright, large, or small compartments, clusters of overlapping or touching compartments, and so on. For the basic version, automatic blob-separation is assumed and only width and minimum intensity are controlled, so specific control over which compartments to exclude is not an option.

**c.** Define the inner and out regions associated with each compartment by specifying their widths and distances from the compartment boundary. Inner regions do not need a specified width since they are contained within the compartment. Outer regions do need a defined width to indicate how far out from the compartment they extend. Both regions allow the specification of an offset away from the boundary to adjust for imperfections in segmentation accuracy or finite-width membranes. For the standard version, 1-pixel offsets are assumed and a fixed percentage of the compartment width is used for the outer region width.

**d.** Specify the method of background correction for the probe image: auto-constant, constant, or none. All methods end up using a single constant gray level value which is subtracted from all probe image intensities before measurement and logging of data. Auto-constant uses a special algorithm to determine the best constant value; this is the choice for the standard method.

**e.** Specify the method of compartment classification (positive/negative) by selecting a on of the following threshold on a selected compartment measurement: Outer/Inner Mean Intensity, Outer/Inner Median Intensity, or Correlation Coefficient. The chosen parameter can be classified by a choice of >, <, =, >=, or <= a user specified number. For the basic version, the choice is fixed as Correlation Coefficient >= a user-specified number.

**6.** Choose which parameters to log and display. The summary log contains site-specific measures, while the data log includes compartment-specific measures.

**7.** Run the Analysis for a specific well, a group of selected wells, the entire microplate, or multiple microplates.

**8.** To interactively view data belonging to an individual cell or group of cells, select the cell(s) in the image and view the data in the ***Cellular Results*** table.

**9.** Verify your collected analysis data in the respective log file.

## Analyzing Translocation Images using Translocation-Enhanced

Complete the following procedure to apply the Translocation Analysis to images:

| Step | Action |
|---|---|
| **1** | From the Apps menu, click *Translocation-Enhanced*. The Translocation-Enhanced dialog box opens. |
| **2** | From the File menu, click *Open*, and open an image that qualifies as a compartment image. |
| **3** | From the File menu, click *Open*, and open an image that qualifies as a Translocation probe image for the preceding compartment image. |
| **4** | In the *Translocation-Enhanced* dialog box, using the two image selectors, select the appropriate Compartment image and Translocation probe image. |
| **5** | In the *Translocation-Enhanced* dialog box, click *Display result image*, then use the image selector to assign a name to the result image. |
| **6** | Under *Compartments*, in the *Approximate* |

*width* box, type or select a value in microns for the approximate width of the compartment.

***HINT*:** You can use the Caliper tool on the Measure menu to measure the width in microns of a typical compartment that you want to include in your image segmentation.

**7** Under *Compartments*, in the *Intensity above local background* boxes, type or select two values to specify the range in gray levels for the intensity of the compartment boundary relative to the nearby background intensity.

***HINT*:** In the compartment image, zoom in on the area where you want to determine the difference in intensity value between the edge of the compartment and the background. The lightest value you should see will be a perimeter of pixels that represent the edge of the compartment. Moving away from the center of the compartment, the next lightest border of pixels visible should belong to the background. Place the mouse pointer on a pixel belonging to the edge, and note the intensity value. Then, place the mouse pointer on a pixel adjacent to the one you measured and that you would consider to be part of the background. Subtract the background value from the edge value, and type that value into the *Intensity above local background* box.

**8** Under Compartments, in the *Minimum area* box, type or select a value that represents the smallest compartment area that you want to include. Areas smaller than the one that you specify are excluded. A recommended value for this setting is zero (0) if you do not want to eliminate any small compartments.

**9** Under Compartments, in the *Maximum area* box, type or select a value that represents the largest compartment area that you want to include. Areas greater than the one that you specify are excluded. The recommended value for this setting is a value greater than the area of the largest compartment, if you do not want to eliminate any compartments from the image.

**10** Check or uncheck the *Auto separate touching compartments* box as appropriate. If you check this box, touching compartments are separated into individual compartments. If this box is not checked, touching compartments are considered to be a single compartment.

***Note:*** Separation is intended for round, blob-like shapes only. By unchecking *Autoseparate*, and adjusting the Maximum

area box, you can exclude large, touching clusters of compartments.

**11** In the *Define regions for measurement* area in the *Inner region distance from edge* box, type or select a value in microns to specify the offset distance from the detected compartment edge to the edge of the inner region segmentation. This area will be excluded from your comparison between the Compartment image and the Probe image.

***Note:*** The combined *Inner region distance from edge* and *Outer region distance from edge* defines a buffer zone between the outer and inner regions used for measurements. This buffer enables you to avoid ambiguous regions near the boundary of the compartment segmentation.

**12** In the *Define regions for measurement* area in the *Outer region distance from edge* box, type or select the value in microns to specify the offset distance from the detected compartment edge to the inside edge of the outer region segmentation. This area will be excluded from your comparison between the Compartment image and the Probe image.

***Note:*** The combined *Inner region distance from edge* and *Outer region distance from edge* defines a buffer zone between the outer and inner regions used for measurements. This buffer enables you to avoid ambiguous regions near the boundary of the compartment segmentation.

**13** In the *Define regions for measurement area* in the *Outer region width* box, type or select a value in microns to specify the width of the outer region. This will be the area of the Probe image used to compare to the Inner region of the Compartment Image as defined by the outer region segmentation.

**14** Under *Translocation probe*, in the *Background Estimation Method* box, select the method that you want to use to estimate and calculate the overall constant background intensity. The calculated background intensity will then be subtracted from all probe image intensities before measurements are made and data logging occurs.

**15** Under Translocation probe, in the Classify positive if box select *Outer/Inner Mean*, *Outer/Inner Median*, or *Correlation Coefficient* to choose the type of evaluation criterion that you want to use to classify each compartment as positive or negative, then choose the evaluation operator and result comparison value that you want to use.

**16** Click *Apply* to apply the settings and create

a segmentation image.

**17** To interactively view data belonging to an individual cell or group of cells, select the cell(s) in the image and view the data in the ***Cellular Results*** table.

## Translocation-Enhanced Dialog Box Options

### Compartment image

Selects and opens the 16-bit compartment stain image. This image file is required. This image should be calibrated in microns. If the image is not calibrated, a red warning rectangle is displayed.

**Notes:**

- When processing is complete, this image is automatically overlaid with the segmentation result image. If you do not re-save the source image, the original source image is not affected. However, if you re-save the source image the overlay information will be saved with it.
- The resulting overlay segmentation image displays positive compartments as green and negative compartments as red.
- If the image calibration cannot be converted to microns, a warning message will be shown. The warning message does not prevent you from continuing to process your image.

### Probe image

Selects and opens the 16-bit translocation probe stain image. This image file is required.

### Display result image

Activates the image selector for the result image. Check this box to activate or deactivate the image selector for destination image of the compartment segmentation.

### Compartments

Defines the options used to specify your compartment segmentation parameters.

### Approximate width

Specifies the approximate scale in microns of the compartments to be segmented. Type or select the approximate compartment width in microns. The value that you enter in microns is converted to pixel units and displayed on the right.

### Intensity above local background

Specifies the intensity range of the compartment segmentation. This value is the threshold after automatic noise and background removal.

### Minimum area

Specifies the minimum compartment area in square microns needed to include the compartment in the segmentation. Compartments with areas less than the specified value are discarded from the segmentation. The value that you enter as microns is converted to pixel units and displayed on the right.

### Maximum area

Specifies the area in square microns above which the compartment is discarded from the segmentation. The value that you enter as microns is converted to pixel units and displayed on the right.

### Auto separate touching compartments

Activates or deactivates separation of touching compartments. Use this checkbox to automatically separate blobs that are touching each other.

### Define regions for measurement

Defines settings used to specify the inner and outer regions surrounding a compartment boundary

### Inner region distance in from edge

Specifies in microns the inner region offset width from the detected compartment boundary. The value that you enter as microns is converted to pixel units and displayed on the right. This option creates a boundary between the detected edge of the compartment and the compartment area as defined in the segmentation image, within which measurements are considered valid. Objects and object values that are located between the Inner Region and the Outer Region are ignored.

### Outer region distance in from edge

Specifies in microns the outer region offset width from the detected compartment boundary. The value that you enter as microns is converted to pixel units and displayed on the right. This option creates a boundary between the detected edge of the compartment and the outer region area as defined in the segmentation image, within which measurements are considered valid. Objects and object values that are located between the Inner Region and the Outer Region are ignored.

### Outer region width

Specifies in microns the outer region segmentation width, which defines the limit of the outer region. Objects and object values that are located beyond the boundary of the Outer Region segmentation are ignored.

### Translocation probe

Specifies the parameters related to the probe image measurements.

### Background estimation method

Selects from one of two methods for estimating the background intensity. The calculated background intensity in subtracted from the background.

Choose *Auto Constant* to automatically estimate the constant background intensity. With *Auto Constant* selected, the background intensity is recalculated for each new image.

Choose *Constant* to manually specify a constant and calculate an estimated background intensity. When you choose *Constant*, a *graylevels* box opens. Type or select the gray level value that you want to use to calculate the estimated background intensity.

### Classify positive if

Specifies the criteria used to classify each compartment as positive. The resulting overlay segmentation image displays positive compartments as green and negative compartments as red.

***Outer/Inner Mean*** - Outer/Inner Mean is defined as the ratio of the average pixel intensity of the outer to the average pixel intensity of the inner.

***Outer/Inner Median*** - Outer/Inner Median is defined as the ratio of the middle pixel intensity value of the outer to the middle pixel intensity value of the inner. Middle, meaning that half the pixels are less than this value and half the pixels are greater than this value.

***Correlation Coefficient*** - Correlation Coefficient is defined as the Pearson's correlation coefficient between the intensities of the two stains for all pixels in the compartment and extending out through the outer region. The value ranges from -1 (anti-correlated) to 1 (correlated).

### Configure Summary Log

Opens the Configure Log dialog box. The Summary Log stores and records settings and results related to the entire image, and include the following:

***Compartments*** – Number of compartments detected in the compartment image segmentation.

***Mean Compartment Area*** – Total square microns in the compartments divided by number of compartments.

***Integrated Inner Intensity*** – Summed grayscale values in all inner regions.

***Integrated Outer Intensity*** – Summed grayscale values in all outer regions.

***Average Inner Intensity*** – Total inner intensity divided by total inner area in square microns.

***Average Outer Intensity*** – Total outer intensity divided by total outer area in square microns.

***Probe Background Intensity*** – Constant background value of probe image in gray levels.

***Outer/Inner Intensity Ratio*** – Mean outer intensity less background divided by mean inner intensity less background.

***Correlation Coefficient*** – The Pearson's correlation coefficient between the intensities of the two stains for all pixels in all compartments and extending out through the outer regions. The value ranges from -1 (anti-correlated) to 1 (correlated).

***Classified Positive*** – Number of compartments classified as positive for translocation.

***% Classified Positive*** – Number of compartments classified as positive divided by the total number of compartments.

### Configure Data Log

Opens the Configure Log dialog box. The Data Log stores and records settings and results related to an individual compartment, and include the following:

***Cell: Assigned Label #*** – unique label number (1 through number of compartments).

***Cell: Inner Area*** – Total square microns in the inner region.

***Cell: Outer Area*** – Total square microns in the outer region.

***Cell: Integrated Inner Intensity*** – Summed grayscale values in inner region.

***Cell: Integrated Outer Intensity*** – Summed grayscale values in outer region.

***Cell: Mean Inner Intensity*** – Total inner intensity divided by inner area in square microns.

***Cell: Median Inner Intensity*** – The median intensity value for pixels within the inner region.

***Cell: Mean Outer Intensity*** – Total outer intensity divided by total outer area in square microns.

***Cell: Median Outer Intensity*** – The median intensity value for pixels within the outer region.

***Cell: Outer/Inner Mean Intensity*** – Mean outer intensity minus background value divided by mean inner intensity minus background value.

***Cell: Outer/Inner Median Intensity*** – Median outer intensity minus background value divided by median inner intensity minus background value.

***Cell: Correlation Coefficient*** – The Pearson's correlation coefficient between the intensities of the two stains for all pixels in the compartment and extending out through the outer region. The value ranges from -1 (anti-correlated) to 1 (correlated).

***Cell: Classification*** – 1 for positive, 0 for negative translocation classification.

### Save Settings

Opens the *Save Settings* dialog box. Use this dialog box to create and save a *Translocation-Enhanced* settings file. You can store all the settings currently specified in the *Translocation-Enhanced* dialog box. Use *Load Settings* to retrieve and use these settings at a later time. Type a setting name and an optional description, then click *OK*.

### Load Settings

Opens the *Load Settings* dialog box. Use this dialog box to select and load a *Translocation-Enhanced* settings file. When this file is loaded, it replaces all current settings with the saved settings stored in the *Translocation-Enhanced* settings file. Select the name of the setting that you want to use, then click *OK*.

### Set to Defaults

Resets all options and settings to their default values.

### **Test Run** (from Review Screen Data only)

Runs one pass of the *Translocation-Enhanced* application module on the Compartment and Probe images currently open and selected.

**Apply** (from the Apps menu only)

Applies the settings that you have made and runs the Translocation-Enhanced.

> **Note:** The *Apply* button is replaced by the *Test Run* button when configuring your settings for this dialog box from the *Review Plate Data* dialog box.

**Close**

Closes the Translocation-Enhanced dialog box.

# Review Plate Data (DB)

**Displays and analyzes screen data currently stored in the Screening database that was previously acquired using the Plate Acquisition tools; Runs analyses on images stored in the database.**

**Drop-in: HTDB_PLAYER**

Use this dialog box to view and analyze screen data in the Screening database that was acquired with Plate Acquisition tools, such as Plate Acquisition and Control and the Plate Acquisition Toolbar. The Review Plate Data (DB) dialog box enables you to load, combine, arrange, view, and analyze screen data according to review settings made in this dialog box. Also, use this dialog box to run analyses from the available screening analyses that you create from journals that you write.

## Reviewing Plate Data (DB)

To view and analyze images in a HCS screen data set, complete the following procedure.

| Step | Action |
|---|---|
| **1** | From the Screening menu, click Review Plate Data. The Review Plate Data dialog box opens. |
| **2** | Click *Select Plate*. The Plate dialog box opens, and the Review Screen Data dialog box temporarily closes. |
| **4** | Expand the plates folder in the top pane of the dialog to view folders containing plates saved to the database. |
| **5** | Double-click a folder to view its contents on the bottom pane.<br><br>Select the plate containing the data to view and click *OK*. The Plate dialog box closes, and the Review Screen Data dialog box reopens |
| **6** | In the *Wavelengths* box, click the check box next to each wavelength that you want to view. |
| **7** | In the *Data view* box, choose the type of view that you want to use.<br><br>*Well arrangement* – Arranges your viewable images in the montage in the order in which the wells are presented in the microwell plate.<br><br>*Time vs well* – Compares timepoints against wells or well selections.<br><br>*Well vs measurement* – Compares wells |

against the available measurements.

**8** Using the table features, define the wells that you want to view. You can view "thumbnails" in a montage of images and select the size of the thumbnail images. You can select images from the Montage and view each image separately in an image window. You can select images from anywhere in the table, and load the images into a stack.

- To define the images that you want to include in the thumbnail view, type or select an area of number of wells in the *Montage* boxes.
- Click Apply to implement the # of Wells and Timepoint settings that you made.
- Click either the left or right arrow to move thumbnail selector left or right in the table;

  OR

  Click anywhere on the table to place the first selection box at that location.
- To load images into a stack, right click on the squares for the images that you want to include in the stack, then click Load Image(s) in the Selections [In Green] area.
- Click either the left or right arrow in the Selection area to open a full image view and/or to change the view to the next or previous selected image. The image currently displayed in the image view is indicated by yellow highlight.
- Click on an image in the montage to see full resolution of the displayed image. You can make measurements on the images as they are displayed.
- Click Clear to remove all Selections [In Green] from the table.

**9** Click the ***Display*** tab to set display options applicable to the analysis you will run.

**10** Click the ***Measurements*** tab to specify measurement criteria for your selected analysis, as required.

**11** Click the ***Run Analysis*** tab to specify settings for running your selected analysis.

**12** Click the ***Graph*** tab to configure a graph for the data.

**13** Click *Reset Image Displays* to reset any open image displays to the default values.

**14** Click *Close* to close the Review Plate Data dialog box.

## Reviewing Plate Data (DB) - Display Tab

To configure the display settings for the Review Plate Data dialog box, complete the following procedure.

| Step | Action |
|---|---|
| **1** | Click *Well Number on Images* to include the well number in the upper left corner of the image. |
| **2** | Click the arrow in the *Col:* box to select the text color for the Well Number and the Values that appear in the image. |
| **3** | Click *Intensity Profile* to transform the image displayed into a three-dimensional intensity profile graph. |
| **4** | Click *Color Composite* to combine images for two or three wavelengths into a single image using the color assignments in the *Source R/G/B* boxes. |
| **5** | Click *Auto Scale* to turn on auto scaling for 16-bit images, or click to turn off auto scaling and manually specify the scaling range |
| **6** | When Auto Scaling is off, click the arrow in the range box to select the appropriate scaling range for your image. |

## Reviewing Plate Data (DB) - Measurements Tab

To specify well selection based on your specified measurements query criteria, complete the following procedure.

| Step | Action |
|---|---|
| **1** | From the Review Plate Data dialog box, click the *Measurements* tab. The measurements tab moves to the front. |
| **2** | In the *Analysis* drop-down box, choose the analysis that contains the measurements to query. |
| **3** | In the *Measurement* drop-down box, choose the name of the measurement that you want to use to query your images in the database. |
| **4** | In the Display Format box. Select the number of decimal places that you want to display for your data in the grid. |
| **5** | In the Value is box, select the variable range limit specifier, and the numerical value(s) of the range in the adjacent box(es). |
| **6** | Click *Open Log* to open either a Dynamic Data Exchange or a text log file, then click *OK*. |
| **7** | Click *Configure Log* to choose either Column and Row labels, Plate info, or both, then click *OK*. |
| **8** | Click *Select* to query the image database |

using your selected measurement and variable range limits. The selected wells or sites will be highlighted in green.

**9** Click *Log Data* or press the F9 key to log your query data.

## Reviewing Plate Data (DB) - Run Analysis Tab

To run either an application module or an analysis created from a journal, complete the following procedure:

**Note:** The following procedure uses the Neurite Outgrowth application module as an example of how to use this tab.

| Step | Action |
|---|---|
| **1** | From the Review Plate Data dialog box, click the *Run Analysis* tab. The *Run Analysis* tab moves to the front. |
| **2** | In the *Analysis* drop-down box, choose the analysis that you want to run, or use the prefilled selection. |
| **3** | If you are using the default settings of a MetaXpress application module, such as Neurite Outgrowth, or a custom analysis derived from a journal, skip to step 10. |
| **4** | In the *Settings* box, choose the setting that you want to apply to the analysis, if more than one selection is available. |
| **5** | If you need to modify the configuration of an application module such as Neurite Outgrowth, click *Configure Setting*. The dialog box for the application module opens. |
| **6** | If you need to add another setting to an existing application module, click *Edit List*. The Edit List of Settings for <Neurite Outgrowth> opens. |
| **7** | Click *New Settings*. The New Settings for <Neurite Outgrowth> dialog box opens. |
| **8** | Type a name for the new Settings in the Name box, then click *OK*. The Configure Settings for Neurite Outgrowth dialog box opens. This is a special version of the Neurite Outgrowth dialog box specifically for defining new settings for your Neurite Outgrowth application module. |
| **9** | Using the help information for ***Neurite Outgrowth***, make the appropriate settings changes, then click *Close*. |
| **10** | To log the measurement data from your analysis into the database, click *Log into the database*. |
| **11** | If you are running a custom analysis derived from a journal and have created a setup journal for it, click *Run Setup for Analysis* to run the setup journal. |

To select a database location for your analysis data, click the box next *to Base folder for results*. The Measurements Sets dialog box open. Select the Measurements Sets folder and click *OK*. All measurement data from your analysis will be stored as a subfolder under this folder.

**Note:** This step is necessary only the first time you run the analysis.

**12** If you are running the analysis for all wells and all defined sites in the database, click *Run Analysis for All Positions*.

**13** To run your analysis for only the selected wells, click *Run Analysis for Selections.*

**14** To run your analysis for a specific site, select the site, then click *Run Analysis for Site*.

## Reviewing Plate Data (DB) - Graph Tab

For a graph to display your screen data, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the Review Plate Data dialog box, click the *Graph* tab. The Graph tab moves to the front. |
| **2** | Select the analysis containing the data you wish to graph from the *Analysis* field. |
| **3** | In the *Graph View* field, select what the source location for the data you want graphed. |
| **4** | Select a graph type from the *Graph Type* drop-down box. The options available for each graph type vary depending on the *Graph View* setting. |
| **5** | Select the measurement(s) to be graphed from the *Measurement* and *Measurement2* (if applicable) field(s). |
| **6** | If you selected *Histograms* from the *Graph Type* field, select the number of bins to display in the resulting histogram in the *Number of Bins* field. |
| **7** | Check the *Auto Scale* checkbox to automatically scale the bin(s) based on the range of data from the selected *Measurement*. This option is only available when *Histogram* is selected from the *Graph Type* field. |
| **8** | Click *Show Graph* to open the graph based on the current settings. |

**Note:** If the data on the graph is not

displaying properly, click and drag one of the corners of the graph window to resize it.

**9** To configure the graph settings, double-click inside the graph or click the Show Graph Menu arrow on the bottom left corner of the graph and select ***Graph Settings***.

**10** To set the display parameters for the current graph to the default view, click *Set Display to Default*. There are separate graph defaults for each combination of Graph View and Graph Type.

## Review Plate Data (DB) Dialog Box Options - Main

### Select Plate

Opens the ***Plate Dialog*** box. Use this dialog box to select Plate Data Sets of stored microwell plates from the database for viewing. To perform other operations, such as deleting plates or individual images, use the ***Plate Data Utilities*** dialog box.

### Data view

Selects and indicates the well arrangement as shown in the Microwell Plate Selection Grid and the Image Montage window.

***Well arrangement*** – Arranges your viewable images in the montage in the order in which the wells are presented in the microwell plate.

***Time vs well*** – Compares timepoints against wells or well selections.

***Well vs measurement*** – Compares wells against the available measurements.

### Print

Prints the data in the table on the selected windows printer.

### Wavelengths

Selects one or more wavelengths of the images in your data set to view.

### Sites

Selects display of one or all the sites in each selected well in your experiment. Available sites are indicated by a dash. Click on any available site to view only that site for all selected wells in the thumbnail view. Click *All Sites* to view all sites for all selected wells. If you run an Analysis on wells with sites, the values displayed in the table refer to the selected sites or if "All Sites" is selected, the average for the total number of sites in the well.

### All Sites

Specifies that all sites for all wells are to be included in each thumbnail image, and that each image in the thumbnail will be represented as an individual image in the image selection grid for the Montage. However, the number of sites shown in both the thumbnails in the Montage and in the image viewer also depends on the Montage dimensions that you specify. When this box is checked, the sites in the well are included in the Montage dimensions. For example, if each well has four sites, and the Montage dimension is 1X1, only the upper left site in well A01 is shown; if you change the dimension to 2X1, the two upper sites are shown. Only when the dimension is set to 4X4 are all four sites in A01 shown.

If this box is not checked, only a single, selected site is shown in the Montage, and each selection box in the image selection grid represents all sites for each well. Therefore, when not checked, when you select a different site in the Sites box, both the thumbnail in the montage and the image in the image window will be updated.

### [Microwell Plate Selection Grid]

Indicates the wells containing image data, the images included in the montage area, and the images

selected for display.

### Montage

Specifies the dimension in image thumbnails of the Image Montage window.

### Time point

Specifies the number of timepoints that you want to display and/or include in the image montage.

### Apply

Applies any new settings that you made to the image montage.

### Selections (In Green)

Controls the selection and loading of images that you selected in the table. Select images by right-clicking on an image selection box, or by right-clicking on the associated image in the thumbnail view.

### Load Image(s)

Loads the images you selected in the table into a stack for each wavelength, or a single stack if Color Composite View is selected.

### (Arrow Buttons)

Changes the displayed selected image to the previous or next selection (Selections [In Green]).

### Clear

Clears all Selections from the table.

### Reset Image Displays

Resets the view settings in all image displays.

### Cellular Results

Opens the Cellular Results dialog box. Use this dialog box to view and browse available analysis measurements. These measurements are configured in the *Configure Settings>Configure Data Log (Cells)* dialog box.

### Close

Closes the Review Plate Data (DB) dialog box.

## Review Plate Data (DB) Dialog Box Options - Display Tab

### Well Number on Images

Displays the Well Number in the upper left corner of the image.

### Show Values

Displays average Analysis values on the table and in the upper right corner of each image in the montage. For each site, the values shown both on the table grid and the montage display are the average of all the values for all defined objects in the site. To see individual values for identified objects, click *Cellular Results*. The Cellular Results dialog box will open and display a table of values for all objects in the selected well or site.

### Col

Selects text color to apply to well numbers and values displayed in the image.

### Intensity Profile

Transforms the image into a three-dimensional intensity profile graph, using the colors assigned to the image and assigning the highest intensities to the highest peaks in the graph. This enable the Scale 16-bit Image fields.

### Color Composite

Creates a single color composite of two or three wavelengths based on the colors (R/G/B) that you assign to

the wavelengths in the Source boxes.

### Source (R, G, B)

Assigns one or more of the primary colors to one or more of the wavelengths that you are using in your experiment.

### Scale 16-bit Images

Enables you to apply scaling to 16-bit images either automatically or manually when a color composite of intensity profile images are created. If source images are displayed, then their scaling can be set through the scale image dialog.

### Range

Assigns the upper limit of the 16-bit scaling range when using manual scaling for 16-bit images. This box is inactive when Auto Scale is checked.

## Review Plate Data (DB) Dialog Box Options - Measurements Tab

The Review Plate Data (DB) Measurements Tab enables you to query the database for specific measurements that fall within your specified limiting range.

### Analysis

Selects the completed analysis that contains the measurements to extract.

### Measurement

Selects a single measurement to use for querying measurement data stored in the screening database. Click the down arrow to open the list of available measurements. The list of measurements can vary for each image or for each experiment's group of images.

### Display Format

Specifies the number of decimal places to be included in the result data. The display format selection can vary depending on the type of measurement selected.

### Select Wells Based on Variable Range

Specifies the range within which you want to run the query for the measurement that you selected.

### Value is

Selects and specifies the query qualifier. Qualifiers are equals (=), greater than (>), less than (<), between, outside, or like.

### Select

Runs the specified query and selects all wells or sites that fall within the query requirements.

### Configure Log

Configures the open log file. Opens the Configure Log dialog box. Select Column and Row labels, and /or Plate info, or neither.

### Open Log

Opens the Data Log file. Opens the Open Data Log dialog box. Choose to log measurements to either Dynamic data Exchange or to a text file or both.

## Review Plate Data (DB) Dialog Box Options - Run Analysis Tab

### Analysis

Selects and indicates the name of the analysis that you are running. This can be either a prepared analysis, such as Neurite Outgrowth, or analyses created from journals.

### Settings

Selects and indicates the settings name from the available settings associated with the analysis that you are using. Use the *Configure Settings* button on this tab to make different settings easily available for use with your selected Analysis.

### Edit List

Opens the *Edit List of Settings* for <Neurite Outgrowth> dialog box. The *Edit list of Settings for* dialog box is used to edit settings applied to application modules included in MetaMorph, such as Neurite Outgrowth. Analyses that you create from journals must have all possible settings stored in the database at the time the journal is created and stored in the database C:\Assay folder.

### <Description>

Displays one of the following types of descriptions. For application modules that are MetaMorph Drop-ins, such as Neurite Outgrowth, this area displays the same description as the Meta Imaging Series Administrator Configure Drop-in dialog box displays for the module. For analyses defined by journals, it displays the description information typed into the Analysis Description area when the analysis was added to the database.

### Log into the database

Opens the database login dialog box if you are not logged in when you run the analysis.

### Base Result Folder

Enables you to select the location within the database where the results data from the application module is stored.

### Configure Settings

Opens the Configure settings for <Neurite Outgrowth> dialog box or the associated dialog box for the selected application module. This option is only available for application modules. Analyses created from journals must be configured in the originating journal.

### Run Setup for Analysis

Runs the setup journal for the selected analysis if it exists. The setup journal must be in the same folder as the main analysis and must be named in the format EXAMPLEJOURNAL_SETUP.JNL.

> **Note**: This option is only available if the selected analysis is created from a journal and not a prepared analysis like Neurite Outgrowth.

### Run Analysis for All Positions

Runs the analysis for all wells that are indicated to contain a sample. Wells containing valid samples are indicated by a dash in the grid location for the well.

### Run Analysis for Selections

Runs the analysis for all selected wells. Right-click on a well to select it. Selected wells are indicated by a green rectangle.

### Run Analysis for Site

Runs the analysis for just the selected and displayed image site.

## Review Plate Data (DB) Dialog Box Options - Graph Tab

### Analysis

Selects the completed analysis that contains the data to graph

### Graph View

Determines the source of the data plotted in the graph. Valid options include the following:

### Plate

Graphs the summary well measurement for all the wells in the plate. Select this option to view the data for

an entire well.

### Multiple graphs of displayed wells

Displays separate graphs for each well displayed in the montage. If you change the appearance of the graph using the Graph Settings dialog box, each mini-graph is updated.

### Single Well

Graphs all values of a measurement within a well.

### Graph Type

Determines the type of data and how it is displayed for the graph. The choices available depend on what is selected in the *Graph View* field. The following options are available for each Graph View option:

### Plate:

**Histogram**— Displays a bar chart, in the format of Measurement (Area, Correlation, Coefficient, etc.) vs. Count.

**Measurements vs Well Column** — Displays a graph with one trace for each column of the plate, in the format of Measurement (Area, Correlation Coefficient, etc.) vs. Column. You can view the data as one trace with error bars by clicking the Down Arrow button directly below the left side of the graph and selecting *Show Mean with Error Bars* from the drop-down menu.

**Measurements vs Well Row** — Displays a graph with one trace for each row of the plate, in the format of Measurement (Area, Correlation Coefficient, etc.) vs. Row. You can view the data as one trace with error bars by clicking the Down Arrow button directly below the left side of the graph and selecting *Show Mean with Error Bars* from the drop-down menu.

**Measurements vs Well Number** — Displays a graph with one trace for each well number in the format of Measurement (Area, Correlation Coefficient, etc.) vs. well number. The well number is calculated as follows:

Well number = Current Column + (Current Row * Number of Columns)

**Scatter Plot**— Displays a scatter plot graph containing two measurement variables for the plate.

### Multiple Graphs of displayed wells:

**Time**— Displays multiple graphs of the selected wells in the format of Measurement (Area, Correlation Coefficient, etc.) vs. time point number. If the selected measurement is not recorded at specific time points, but applies to all data, the trace will be flat. If the value is only recorded at some time points, only those time points will be used in the graph.

**Single Measurement**—Displays a bar graph for each of the well in the montage view. If the *Data View* field is set to *Measurement vs Well* or *Well Arrangement*, then the measurement shown is taken from the time point indicated in the Time Point field. If the D*ata View* field is set to *Time vs Well*, then the graph shows the measurement for each time point that is shown in the montage.

**Measurement Pair**—Displays a bar graph for each of the selected wells that contains data for two Measurement selections.

### Single Well:

**Time**— Displays a graph with one trace for each row of the plate, in the format of Measurement (Area, Correlation Coefficient, etc.) vs. Time Point.

**Histogram**— Displays a bar graph of the counts of values sorted into bins.

**Scatter Plot**— Displays a scatter plot graph containing two measurement variables (Area, Correlation Coefficient, etc.) for the plate.

### Measurement

Selects the measurement to be graphed. The options available are extracted from the active experiment and include all numerical data measurements configured in the application module or analysis. The y-axis is autoscaled to contain the measurement data selected in this field.

### Measurement2

Selects the second measurement option when available. The range of this data is used to scale the x-axis in both scatter plots and measurement pair graphs.

### Number of Bins

Selects the number of bins use when creating the histogram. This option is only available when *Histogram* is selected from the Graph Type field.

### Auto Scale

Automatically scales the bins based on the range of data from the selected *Measurement*. This option is only available when *Histogram* is selected from the Graph Type field.

### Scale Min/Max

Manually selects the minimum and maximum range for histogram based on the data in the Measurement field. This option is only available when *Histogram* is selected from the Graph Type field and *Auto Scale* is not checked.

### Set Display to Default

Sets the display parameters for the current graph to its default. There are separate graph defaults for each combination of Graph View and Graph Type.

### Show Graph

Opens the graph using the current settings. If a graph is already open the and the settings are changed, Show Graph will update the open graph.

# Plate Dialog Box

**Interfaces with the MDCStore database and enables you to perform a number of functions on acquired plates.**

The Plate dialog box is a front-end to the MDCStore database that contains your screening images and data. The dialog box performs different actions depending on how it was called. For example, if you select the Select Plate command from the Review Plate Data dialog box, the Plate dialog box will open and enable you to select a plate to review. The following commands open the Plate dialog box:

### Review Plate Data Dialog Box:

**Select Plate —** Selects a plate to open in the Review Plate Data dialog box.

### Plate Data Utilities Dialog Box:

**Run Analysis** — Enables you to select one or more plates to open in the Run Analysis on Plates dialog box.

**Export Images**— Copies images from one or more selected plates from the database/fileserver to another local or networked location.

**Delete Measurements** — Deletes the measurements associated with a selected plate(s) from the database.

**Delete Images** — Deletes individual images from one or more selected plates stored in the database/fileserver.

**Delete Plates** — Deletes one or more selected plates from the database.

**Note**: For more information on the above commands, refer to the Review Plate Data and Plate Data Utilities help files.

## Plate Dialog Box Procedure

The action of the Plate dialog box depends on the command that called it (see the main Plate dialog box help file for a list of commands that open the Plate dialog box). For more information on the action taken for each command, refer to the Plate Data Utilities help file. The following procedure is specific to the Select Plate command in the Review Plate Data dialog box:

| Step | Action |
|---|---|
| **1** | From the Review Plate Data dialog box, select *Select Plate*. The Plate dialog box opens, and the Review Plate Data dialog box temporarily closes. |
| **2** | Double click the Plates folder in the top pane of the dialog to view folders containing plates saved to the database. |
| **3** | Expand the plate's folder in the top pane of the dialog to view folders containing plates saved to the database. |
| **4** | Double-click a folder to view information about it on the bottom pane. |
| **5** | Select the plate to view from the bottom pane and click *OK* to close the Plate dialog box and view the selected plate.<br>**OR** |
| **6** | To configure the tree structure in the Plates field (the top half of the dialog box), click the Plate Query Attributes icon  or right click the dialog box and choose Configure Plate Attributes to open the Plate Query Attributes dialog box. To add conditions to sort plates by, select a condition from the *Column Selection* field, click the  icon, and then click *OK*. You can also sort the order of the columns using the up and down arrows. |
| **7** | To configure what columns are available in the Details field (the bottom half of the dialog box), click the Attribute Columns icon, or right click the dialog and choose Select columns to open the Attribute Columns dialog box. To add columns of plate details select a column from the Column Selection field and click the right arrow icon, and then click *OK*. You can also change the left to right ordering of the columns using the up and down arrows. |
| **8** | To refresh the Plate dialog (requery the database), click the Retrieve Data icon |

## Plate Dialog Box Options

**Plate Query Attributes** 

Opens the Plate Query Attributes dialog box. Use this dialog box to configure how the plates tree structure in the Plates Field (the top half of the dialog box) is organized.

**Attribute Columns** 

Opens the Attribute Columns dialog box. Use this dialog box to specify which columns are available in the Details field (the bottom half of the dialog box). You can also sort the left to right order of the columns.

**Retrieve Data** 

Requeries the database and updates the dialog box.

**Plate Security** 

Opens the Sharing and Security dialog box. Use this dialog box to set up sharing of plates between groups.

### Plates Field

Contains a tree-view organization of all available plates. Left-click a folder to expand it and display the contents in the *Details* Field.

### Details Field

Displays the details of the available plates. Left-click a plate to make it active. Shift-click multiple plates or Control-click individual plates to select multiple plates (where applicable).

### OK

Performs the appropriate action on the selected plate(s). See the main Plate dialog box help file for a list of commands that use the Plate dialog box.

### Cancel

Cancels the command and closes the dialog box.

# Sharing and Security

**Enables you to set user and group permissions on a per plate basis.**

The Sharing and Security dialog box is accessed through the Plates dialog box. It lists the permissions that groups have for the selected plate. Use this dialog box to grant or restrict access to plates that you have acquired.

The following permissions can be granted to groups for a plate:

**Read Only** — Can view data only. Cannot import, modify, delete, or analyze data in place. Cannot copy or paste data. Cannot modify security permissions, users, or groups.

**Read-Write** — Can view, import, modify, delete, and analyze data in place. Can modify groups of which they are a member and can create new groups. Cannot modify security permissions or users.

**Lab Head** — Full control of all data, within the group. Lab Heads can not create groups or users if they do not have administrator privileges.

***Sharing Plates***

***Sharing and Security — Dialog Box Options***

## Sharing Plates

To view, add, or modify the current permissions of a selected plate, complete the following procedure.

| Step | Action |
|---|---|
| **1** | From the Plate dialog box, select a plate from the Details field and click the Sharing and Security icon  . The Sharing and Security dialog box opens with a list of all groups that currently have access to the plate. |
| **2** | To enable a new user or group to access the plate, click Add. The Select dialog box opens. |
| **3** | Select the name of the group to add and click Select. The Select dialog box closes and the group is added to the Sharing and Security list. |
| **4** | To change the permissions for a group, select the group from the list, select the permission from the Set the plate access for "*" drop-down list, then click Apply. The available permissions are:<br><br>**Read Only** — Can view data only. Cannot import, modify, delete, or analyze data in place. Cannot copy or paste data. Cannot modify security permissions, users, or groups.<br><br>**Read-Write** — Can view, import, modify, delete, and analyze data in place. Can modify groups of which they are a member and can create new groups. Cannot modify security permissions or users.<br><br>**Lab Head** — Full control of all data, within the group. Lab Heads can not create groups or users if they do not have administrator privileges. |
| **5** | Click Close to exit the dialog box. |

## Sharing and Security - Dialog Box Options

### Users and groups who can see the plate:

Displays the name of the current plate.

**Plate Table**

Displays the details of the selected plate. Each row represents a group that has access to the plate. The access type is also listed.

**Add**

Opens the Select dialog box. Use this dialog box to enable additional groups to access the plate.

**Remove**

Removes the selected group from the plate table.

**Set the plate access for "*"**

Sets or changes the permissions for the selected group. The available permissions are:

**Read Only** — Can view data only. Cannot import, modify, delete, or analyze data in place. Cannot copy or paste data. Cannot modify security permissions, users, or groups.

**Read-Write** — Can view, import, modify, delete, and analyze data in place. Can modify groups of which they are a member and can create new groups. Cannot modify security permissions or users.

**Lab Head** — Full control of all data, within the group. Lab Heads can not create groups or users if they do not have administrator privileges.

**Apply**

Applies the permissions selected in the Set the plate access for "*" drop-down list

**Close**

Closes the dialog box.

# Review Screen Data

**Displays and analyzes screen data acquired with the Screen Acquisition dialog box.**

**Drop-in: HTPLAYER**

**Note:** This is the non-database version of Review Screen Data used with the legacy Screen Acquisition command. If you are using the Plate Acquisition commends, refer to the Review Plate Data Help file.

Use this dialog box to view and analyze screen data acquired with the Screen Acquisition dialog box. This dialog box enables you to load, combine, arrange, view, and analyze screen data according to review settings made in this dialog box.

**Note:** If you are using the Assay tab of the command to run an assay on screen data and the folder containing the assay contains either setup or post journals, the setup and post journals will be automatically run before and after the assay is run.

## Reviewing Screen Data

To view and analyze images in a MetaXpress screening data set, complete the following procedure.

| Step | Action |
|---|---|
| 1 | From the Apps menu, click Review Screen Data. The Review Screen Data dialog box opens. |
| 2 | Click *Select Plate*. The Screen Data Utilities dialog box opens, and the Review Screen Data dialog box temporarily closes. |

**3** If no plate data sets are shown in the Plates (*.htd) box, click *Select Directory*. The Browse for Folder dialog box opens.

**4** Select the folder (directory) containing the screen data set that you want to view, and click *OK*. The names of the data sets contained in the folder are displayed in the Plates (*.htd) box.

**5** In the *Plates* box, click the check box next to the data set you want to view, then click *View*. The Screen Data Utilities dialog box closes, and the Review Screen Data dialog box reopens.

**6** In the *Wavelengths* box, click the check box next to each wavelength that you want to view.

**7** In the *Sites* box, click on any available individual site to view that site for all selected wells.

OR

Click *All Sites* to view all sites for all selected wells.

**8** Using the table features, define the wells that you want to view. You can view "thumbnails" in a montage of two or more images and select the size of the thumbnail images. You can select images from anywhere in the table, and load the images into a stack.

- To define the images that you want to include in the thumbnail view, in the *Montage* boxes, type or select the dimensions for the number of wells to be displayed in the montage view.
- To set the relative size of each thumbnail, type or select a size percentage in the Size box.
- Click Apply to implement the # of Wells and Size% settings that you made.
- Click either the left or right arrow to move thumbnail selector left or right in the table;

  **OR**

  Click anywhere on the table to place the first selection box at that location.
- To load images into a stack, right click on the squares for the images that you want to include in the stack, then click Load Image(s) in the Selections [X's] area.
- Click either the left or right arrow in the Selections [X's] area to open a full image view and/or to change the view to the

next or previous selected image. The image currently displayed in the image view is indicated by brackets [X].

- Click on an image in the montage to see full resolution of the displayed image. You can make measurements on the images as they are displayed.
- Click Clear to remove all Selections [X's] from the table.

**9** Click the ***Display*** tab to access and set display options.

**10** Click the ***Measurements*** tab to specify measurement criteria for your selected assay, as required.

**11** Click the ***Assay*** tab to access and set options for running an assay journal.

**12** Click the ***Colocalization*** tab to access and set options used to analyze colocalization of results of two different wavelengths of the same sample(s).

**13** Click *Reset Image Displays* to reset any open image displays to the default values.

**14** Click *Close* to close the Review Screen Data dialog box.

## Reviewing Screen Data - Display Tab

To configure the display settings for the Review Screen Data dialog box, complete the following procedure.

| Step | Action |
|---|---|
| **1** | Click *Well Number on Images* to include the well number in the upper left corner of the image. |
| **2** | Click *Show Values* to display the Assay or the Colocalization values in the upper right corner of the image. |
| **3** | Click the arrow in the *Col:* box to select the text color for the Well Number and the Values that appear in the image. |
| **3** | Click *Intensity Profile* to transform the image displayed into a three-dimensional intensity profile graph. |
| **4** | Click *Color Composite* to combine images for two or three wavelengths into a single image using the color assignments in the *Source R/G/B* boxes. |
| **5** | Click *Auto Scale* to turn on auto scaling for 16-bit images, or click to turn off auto scaling and manually specify the scaling range. (**Note:** Scale 16-bit Images is available only when *Color Composite* is selected.) |
| **6** | When Auto Scaling is off, click the arrow in the range box to select the appropriate |

scaling range for your image.

## Reviewing Screen Data - Measurements Tab

To specify well selection based on your specified measurements query criteria, complete the following procedure.

| Step | Action |
|---|---|
| **1** | From the Review Screen Data dialog box, click the Measurements tab. The measurements tab moves to the front. |
| **2** | In the Measurement box, choose the name of the measurement that you want to use to query your images in the database. |
| **3** | In the display format box. Select the number of decimal places that you want to display for your data in the grid. |
| **4** | In the *Value is* box, select the variable range limit specifier, and the numerical value(s) of the range in the adjacent box(es). |
| **5** | Click Select to query the image database using your selected measurement and variable range limits. The selected wells or sites will be highlighted in green. |

## Reviewing Screen Data - Assay Tab

To run a specific or pre-defined assay journal, complete the following procedure.

| Step | Action |
|---|---|
| **1** | From the Review Screen Data dialog box, click the Assay tab. The Assay tab moves to the front. |
| **2** | In the Assay box, choose the assay that you want to run, or use the prefilled selection. To change your assay selection, click *Location*. |
| **3** | If you are going to use the default setting in a application module such as Neurite Outgrowth or the custom assay derived from a journal, skip to step 10. |
| **4** | In the Settings box, choose the setting that you want to apply to the assay, if more than on selection is available. |
| **5** | If you need to modify the configuration of a prepared setting, such as Neurite Outgrowth, click Configure Setting. The dialog box for the Assay opens. |
| **6** | If you need to add another setting to an existing application module, click Edit List. The Edit List of Settings for <Neurite Outgrowth> opens. |
| **7** | Click New Settings. The New Settings for <Neurite Outgrowth> dialog box opens. |
| **8** | Type a name for the new Settings in the |

Name box, then click OK. The Configure Settings for Neurite Outgrowth dialog box opens. This is a special version of the Neurite Outgrowth dialog box specifically for defining new settings for your Neurite Outgrowth Assay.

**9** Using the help information for ***Neurite Outgrowth***, make the appropriate settings changes, then click *Close*.

**10** If you are going to run the assay for all wells and all defined sites in the database, click Run Assay for All Positions.

**11** To run your assay for only the selected wells, click Run Assays.

**12** To run your assay for a specific site, select the site, then click Run Assay for Site.

**Note:** If you are using the Assay tab of the command to run an assay on screen data and the folder containing the assay contains either setup or post journals, the setup and post journals will be automatically run before and after the assay is run.

## Review Screen Data - Colocalization Tab

To determine the percentage of colocalization of image data for any two wavelengths, complete the following procedure.

| Step | Action |
|---|---|
| **1** | Click the *(Probe) 1:* drop-down list arrow, and select a wavelength for Probe 1. |
| **2** | Click *Thresh: Auto* to automatically set the threshold for probe 1;<br>**OR**<br>Type or select a Low and a High threshold limit value in the Low and High threshold boxes. |
| **3** | Click the *(Probe) 2:* drop-down list arrow, and select a wavelength for Probe 2. |
| **4** | Click *Thresh: Auto* to automatically set the threshold for probe 2;<br>**OR**<br>Type or select a Low and a High threshold limit value in the Low and High threshold boxes. |
| **5** | Click *Run*. The colocalization function begins and enters a percentage of colocalization for each well into the boxes in the table. The colocalization value for each well will also appear in the upper right corner of the associated image. |
| **6** | Once colocalization has run, you can specify selection criteria for viewing and/or loading into a stack. In the *Value is* boxes, select a |

logical operator and a range of values for the selection criteria.

**7** Click *Select* to compare the colocalization percentage values for all wells to the specified range of values. The wells that meet the selection criteria are highlighted.

**8** Click *Load Image(s)* in the Selections [X's] box to load the selected images into a stack;

**OR**

Click either the left or right arrow in the Selections [X's] box to display the selected image as "Encoded."

**9** Click *Print Table* to print the information in the table exactly as it appears.

**10** Click *Clear* to clear all Selections [X's].

**11** Click *Close* to close the Review Screen Data dialog box.

## Review Screen Data - Dialog Box Options - Main

### Select Plate

Opens the Screen Data Utilities Dialog Box. Use this dialog box to select Screen Data Sets for viewing, to rename, copy, move, or delete a screen data set, or to initiate running a journal in conjunction with viewing the data set.

### Print Table

Prints the data in the table on the selected windows printer.

### Wavelengths

Selects one or more wavelengths of the images in your data set to view.

### Sites

Selects display of one or all the sites in each selected well in your experiment. Available sites are indicated by a dash. Click on any available site to view only that site for all selected wells in the thumbnail view. Click *All Sites* to view all sites for all selected wells. If you run an Assay or Colocalization on wells with sites, the values displayed in the table refer to the selected sites or if "All Sites" is selected, the average for the total number of sites in the well.

### All Sites

Specifies that the images all sites for all wells included within the Montage dimensions are to be included in each thumbnail image. Click on any individual site within a well to open the site image in the image viewer.

### Montage

Selects the number of wells to be included in the "thumbnail" view of your experiment images as defined by their two dimensional arrangement. For example, specifying 5 X 4 selects 5 rows horizontally by 4 rows vertically, and creates a thumbnail view of 20 images. Click any thumbnail to see a full-size view of the image. If there are two or more sites in the well, click an individual site to open the image for that site in the image viewer.

### Size %

Select the image size of the thumbnails in the thumbnail view.

### Apply

Applies the settings for the number of wells, the specific wells to show, and the thumbnail size.

### Selections [X's]

Controls the selection and loading of images that you selected in the table. Select images by right-clicking on an image selection box, or by right-clicking on the associated image in the thumbnail view.

### Load Image(s)

Loads the images you selected in the table into a stack for each wavelength, or a single stack if Color Composite View is selected.

### (Arrow Buttons)

Changes the displayed selected image to the previous or next selection (Selections [X's]).

### Clear

Clears all Selections [X's] from the table.

### Reset Image Displays

Resets the view settings in all image displays.

### Close

Closes the Review Screen Data dialog box.

## Review Screen Data Dialog Box - Display Tab

### Well Number on Images

Displays the Well Number in the upper left corner of the image.

### Show Values

Displays Assay or Colocalization values on the table and in the upper right corner of each image.

### Col:

Selects text color to apply to well numbers and values displayed in the image.

### Intensity Profile

Transforms the image into a three-dimensional intensity profile graph, using the colors assigned to the image and assigning the highest intensities to the highest peaks in the graph.

### Color Composite

Creates a single color composite of two or three wavelengths based on the colors (R/G/B) that you assign to the wavelengths in the Source boxes.

### Source R/G/B

Assigns one or more of the primary colors to one or more of the wavelengths that you are using in your experiment.

### Scale 16-bit Images

Enables you to apply scaling to 16-bit images either automatically or manually when a color composite of intensity profile images are created. If source images are displayed, then their scaling can be set through the scale image dialog.

### Auto Scale

Activates auto scaling for 16-bit images.

### Range

Assigns the upper limit of the 16-bit scaling range when using manual scaling for 16-bit images. This box is inactive when Auto Scale is checked.

## Review Screen Data Dialog Box - Measurements Tab

The Review Screen Data Measurements Tab enables you to query a single measurement type of image data for specific measurement values that fall within your specified limiting range.

### [Assay Measurements]

In this non-database version of the Review Screen Data dialog box, assay measurements generated by the available MetaXpress application modules are stored in the spreadsheet format that you selected. For each application module, the most relevant measurement is also shown in the well selection table in this dialog box inside the field for each well. This measurement can optionally be superimposed on the thumbnail image in the Montage and individual full-size images. Each application module is programatically configured to return a preselected single measurement value to the well selection table. The following table defines the measurement returned by each application module:

| Application Module | Measurement Returned |
|---|---|
| Count Nuclei | Total Nuclei |
| Neurite Outgrowth | Mean Outgrowth Per Cell |
| Translocation | Correlation Coefficient |
| Translocation-Enhanced | Correlation Coefficient |
| Angiogenisis Tube Formation | Total Tubule Length |
| Cell Scoring | Percent (%) Positive |
| Cell Health | Percent (%) Viable cells |
| Mitotic Index | Percent (%) Mitotic |
| Live Dead | Percent (%) Live Cells |
| Granularity | Granules |
| Transfluor® | Pit Count |

### Measurement

Selects a single measurement to use for querying measurement data stored in the screening database. Click the down arrow to open the list of available measurements. The list of measurements can vary for each image or for each experiment's group of images. (**Note:** This box is not shown when colocalization is the determining measurement.)

### Display Format

Specifies the number of decimal places to be included in the result data. The display format selection can vary depending on the type of measurement selected.

### Select Wells Based on Variable Range

Specifies the range within which you want to run the query for the measurement that you selected.

### Value is

Selects and specifies the query qualifier. Qualifiers are equals, greater than, less than, between, outside, or like.

### Select

Runs the specified query and selects all wells or sites that fall within the query requirements.

## Review Screen Data Dialog Box - Assay Tab

### Assay

Selects and indicates the name of the Assay that you are running. This can be either a application module, such as Neurite Outgrowth, or assays created from journals.

### Settings

Selects and indicates the settings name from the available settings associated with the assay that you are

using. Use the *Configure Settings* button on this tab to make different settings easily available for use with your selected Assay.

### Location

Opens the browse for folder dialog box. Use this setting to specify the location of the associated Assay folder. If you select a folder that contains a sub-folder with an assay in it, that assay will be available to select from the Assay drop-down list.

### Edit List

Opens the *Edit List of Settings* for <Neurite Outgrowth> dialog box. The *Edit list of Settings for…* dialog box is used to edit setting applied to application modules included in MetaMorph, such as Neurite Outgrowth. Assays that you create from journals must have any possible different settings stored in the database at the time the journal is created and stored in the database as an assay.

### <Description>

Displays one of the following types of descriptions. For application modules that are MetaMorph Drop-ins, such as Neurite Outgrowth, this area displays the description displayed by the Meta Imaging Series Administrator Configure Drop-in dialog box. For assays defined by journals, it displays the description information typed into the Assay Description area when the assay was added to the database.

### Configure Settings

Opens the Configure settings for <Neurite Outgrowth> dialog box or the associated dialog box for the selected application module. This option is used for application modules only. Assays created from journals must be configured in the originating journal.

### Run Assay for All Positions

Runs the assay for all wells that are indicated to contain a sample. Wells containing valid samples are indicated by a dash in the grid location for the well.

### Run Assay for Selections

Runs the assay for all selected wells. Right-click on a well to select it. Selected wells are indicated by a green rectangle.

### Run Assay for Site

Runs the Assay for one or more selected sites when each grid location represents a single site.

## Review Screen Data Dialog Box - Colocalization Tab

### 1:

Selects the wavelength that you want to designate as Colocalization Probe 1. This function measures the percentage of probe 1 that overlaps with probe 2.

### 2:

Selects the wavelength that you want to designate as Colocalization Probe 2. This function measures the percentage of probe 2 that overlaps with probe 1.

### Thresh

Selects either auto thresholding or manual thresholding for Colocalization. When manual thresholding is selected, the Low and High threshold boxes are active.

### Auto

Activates automatic thresholding for colocalization.

### Low

Specifies the lower threshold limit. This setting is inactive when Auto Threshold is selected.

### High

Specifies the upper threshold limit. This setting is inactive when Auto Threshold is selected.

### Run

Runs colocalization of the selected wavelengths.

### Select Wells Based On% of Probe 1 Colocalized with Probe 2

Selects wells to display based on the colocalization results. Using one of the available logical operators and the high and/or low percentage of colocalization limits, this option selects the images that meet the selection criteria.

### Value is:

Specifies the appropriate logical operator and sets the high and/or low colocalization percentage limits. Operators are Equal (=), Greater than (>), Less than (<), Between, Outside, and Like.

### Select

Applies the selection criteria that you typed or selected in the *Value is*: boxes, and indicates selected images on the table.

# Screen Data Utilities

**Enables you to select data sets for viewing, run a journal in conjunction with selected data sets and, perform file system functions on your data set including deleting and copying data sets and moving data sets to different directories.**

**Drop-in: HTPLAYER**

**Note:** This is the non-database version of Screen Data Utilities used with the legacy Screen Acquisition command. If you are using the Plate Acquisition commends, refer to the Plate Data Utilities Help file.

Use the Screen Data Utilities command to initiate screen data file system functions, including copying, moving and deleting Screen Data files. You can access the Review Screen Data dialog box from the Screen Data Utilities dialog box.If you are performing file system functions for one or more data sets, select the source directory of the data, then select the set(s) to be manipulated and click the appropriate function.

If you are preparing to view an existing data set, you can begin in this dialog box or in the Review Screen Data dialog box. If you begin here, click Select Directory to select the directory where your data set is located, select your data set in the Plates window, then click View to open the Review Screen Data dialog box.

## Using Screen Data Utilities

To delete, copy, or move data sets, use the following procedure:

| Step | Action |
|---|---|
| **1** | From the Apps menu, choose Screen Data Utilities. The Screen Data Utilities dialog box opens. |
| **2** | Click *Select Directory*. The Browse for Folder dialog box opens. |
| **3** | Click the folder that contains the data sets that you want to delete, copy, or move. |
| **4** | Click *OK*. The Browse for Folder dialog box closes and the list of data sets contained in the folder appears in the Plates (*.scr) box. |
| **5** | In the Plates (*.scr) box, double-click the names of the data set(s) that you want to |

delete, copy, or move. A check mark appears next to each data set file on the list that you checked.

**6** If you are deleting one or more data sets, click *Delete Set(s)*. The data set(s) are deleted. OR If you are copying or moving one or more data sets to a different folder, click *Copy Set(s)* or *Move Set(s)*. The Browse for Folder dialog box opens.

**7** Click the folder to which you want to copy or move your selected data sets or click *New* to create a new folder.

**8** Click *OK*. The selected data sets are moved to the selected folder.

## Screen Data Utilities - Dialog Box Options

### Select Directory

Opens the Browse for Folder dialog box to select an existing directory or create a new directory in which to locate Screen Data sets.

### Plates (*.htd)

Lists the screen data sets (files) located in the currently selected directory.

### Description

Displays the description associated with the highlighted data set in the Plates window.

### View

Opens the Review Screen Data dialog box. Use this dialog box to view your selected data set.

### Rename Set(s)

Opens the Rename Screen dialog box. Use this dialog box to assign a different name the an existing screen data set. In the Plates box, check the name of the dataset that you want to rename, then click Rename Set. Type the new name that you want to use in the *New Name* box and click *OK*.

### Import to Database

Imports a new data set into the database. In the Plates box, check the name of the Dataset that you want to import, then click *Import to Database*.

### Run Assay

Opens the ***Run Assay on Plates*** dialog box. Use this dialog box to select, define the operating parameters for, and run a predefined assay for your experiment data.

### Delete Set(s)

Removes the selected dataset file(s) from the directory.

### Copy Set(s)

Copies the selected data set file(s) to the location specified in the Browse for Folder dialog box.

### Move Set(s)

Moves the selected dataset file(s) to the location specified in the Browse for Folder dialog box.

### Close

Closes the Screen Data Utilities dialog box.

# Auto Run Mode

**Enables a networked system to run analysis on plates immediately after they are acquired.**

Use this command, along with the Post Acquisition tab of the Plate Acquisition Setup dialog box, to automatically start running analysis on plates as they are acquired. After each plate is acquired on the main MetaXpress system, the plate data is sent to the database. When other MetaXpress computers connected to the database are in Auto Run Mode, they check the database and run analysis on plates as the data becomes available.

Having separate computers acquire and analyze your screening data greatly reduces the overall screening time by freeing up the main MetaXpress computer to continue acquiring. You can also set up more than one MetaXpress computer to run in Auto Run Mode, making the time it takes to process multiple plates even shorter.

**Note:** You can monitor the status of the Auto Run by either clicking the Status button within the command or running the Auto Run Plate Status command from the Apps Menu.

**Note:** When the MetaXpress software is in Auto Run mode, the application cannot be used for any other purpose.

## Auto Run Mode - Dialog Box Options

### Machine Name

Lists the network name of the current MetaXpress computer.

### Analysis Running

Lists the name of the currently running analysis. The analysis is selected in the Post Acquisition tab of the Plate Acquisition Setup dialog box on the MetaXpress computer doing the acquisition.

### Plate

Lists the plate ID of the plate currently being analyzed.

### Leave auto run mode when plate is finished

Exits Auto Run Mode after the current plate is analyzed. Select this option if you want to use the system for other tasks and do not want MetaXpress to start analyzing the next plate once it is finished with the one it is currently analyzing. Note that while in Auto Run mode, all other MetaXpress functions are disabled.

### Status

Opens the Auto Run Status dialog box.

### Cancel

Cancels the analysis being run after the current site is completed and closes the dialog box. Data from sites already analyzed will remain in the database.

## Running in Auto Mode

Use the following procedure to set-up and use the Auto Run Mode command:

| Step | Action |
|---|---|
| 1 | On the computer acquiring the images, open MetaXpress and, from the Screening Menu, click Plate Acquisition Setup; the Plate Acquisition Setup dialog box opens. Click the Post Acquisition tab to enable it. |
| 2 | Check the *Auto run analysis* checkbox and use the drop-down menu to select an |

analysis to run.

**3** Continue to setup and run your acquisition as normal. After the first plate is acquired, the images are sent to the database.

**4** On the computer(s) running the analysis, open MetaXpress and, from the Screening Menu, click Start Auto Run Mode; the Auto Run Mode dialog box opens.

**5** If you want to run analysis on more than one plate, ensure that *Leave auto run mode* when plate is finished is not checked

**6** If you want to check the status of the analysis, On the computer running the analysis, click *Status* to open the Auto Run Status dialog box.

**OR**

On another computer running MetaXpress and logged into the database, select Start Auto Run Plate Status in the Screening Menu.

**7** After the analysis has completed for all plates, click *Cancel* to close the Auto Run Mode dialog box.

# Auto Run Status

**Enables you to view the status of an auto run analysis.**

*Availability: Exclusive to MetaXpress*

Use this command to view the status of analysis started with the Auto Run Mode command.

## Auto Run Plate Status - Dialog Box Options

### Plate ID

Lists the ID number of the plate.

### Analysis

Lists the name of the analysis in the database being run on the plate. The analysis is selected on the MetaXpress computer doing the acquisition in the Screen Acquisition dialog box.

### Setting

Lists the setting name in the database associated with the analysis. The analysis setting is selected on the MetaXpress computer doing the acquisition in the Screen Acquisition dialog box.

### Status

Lists the current status of the plate. The following statuses are possible:

***Running*** - Indicates that the analysis is currently running on the plate. Once the analysis is completed for the plate, the plate will be removed from the auto run plate status list.

***Timeout*** - Indicates that the analysis has not completed progress on a well or site in the expected time. The timeout value is selected in the Meta Imaging Series Administrator with the *Set Auto Run Timeout* command. A timeout is normally caused by an error on the machine running the analysis.

To diagnosis the cause of the timeout, inspect the machine that has timed out for error messages or other problems. In some cases the problem can be resolved and the analysis can continue. If this happens the status will return to *Running*. In other causes the auto run must be canceled and the analysis run again. Some analysis, particularly custom ones created through the journaling system, take a long time to complete. In this case the timeout value set in the *Set Auto Run Timeout* command should be increased to allow enough time to run the analysis.

***Pending*** - Indicates the analysis has not started to run for the plate.

### Machine ID

Lists the ID of the MetaXpress computer processing the analysis. The machine ID is the network name for the machine processing the analysis.

### Progress

Lists the current well and site the analysis is analyzing.

### Cancel selected plate

Stops the analysis from analyzing the selected plate. The computer running the analysis will not respond to this command until it completes analysis of the site it is currently processing.

### Cancel all plates

Stops the analyzing of all plates. The computer running the analysis will not respond to this command until it completes analysis of the site it is currently processing.

### Start Auto Run Mode

Starts running analysis on plates currently in the database and on new plates as they are acquired. This is the same command as Start Auto Run Mode [DB] on the Screening menu.

**Note:** When the MetaXpress software is in Auto Run mode, the application cannot be used for any other purpose.

### Close

Closes the dialog box.

## Viewing the Auto Run Status

Use the following procedure to view the Auto Run status:

| Step | Action |
|---|---|
| **1** | On the computer running the database version of MetaXpress select Auto Run Plate Status from the Screening Menu; the Auto Run Status dialog box opens.<br><br>Start MetaXpress on the computer running the database version of MetaXpress, open MetaXpress and, from the Screening Menu, click Start Auto Run Plate Status; the Auto Run Plate Status dialog box opens.<br><br>OR<br><br>Click *Status* in the Auto Run Mode dialog box. |
| **2** | To stop running an analysis on a plate, select the plate from the table and click *Cancel selected plate*. |
| **3** | To stop running the analysis on all plates, click *Cancel all plates*. |
| **4** | To close the dialog box, click *Close*. |

# Run Analysis on Plates

**Chooses the analysis that you want to run in order to process and/or analyze your selected microwell plate images.**

*Availability: Exclusive to MetaXpress*

Use this dialog box to run a specific analysis, select the appropriate settings for the analysis, and select from the available wavelengths for the analysis. Also, use this dialog box to locate and specify the analysis result folder location.

This dialog box opens automatically when you click *Run Analysis* in the *Plate Data Utilities* dialog box and choose a plate to run in the Plate Dialog.

## Running Analysis on Plates

To run a specific analysis on plate images, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From the *Plate Data Utilities* dialog box, click *Run Analysis*. The Plate Dialog opens. Choose one or more plates on which to run your selected analysis, then click OK. The *Run Analysis on Plates* dialog box opens. |
| **2** | In the *Analysis* box, choose the analysis that you want to run from the list of available analysis. |
| **3** | In the *Settings* box, choose the setting that you want to use from the list of available settings. |
| **4** | In the *Images to open for the analysis* box, click the wavelengths against which you want to run your selected analysis. |
| **5** | In the *Base Results Folder* drop-down list, select the name of the user into whose folder you want to store the results. |
| **6** | Click the Base Result Folder selector to open the *Measurements Sets* dialog box. Choose the appropriate measurement set folder from the *Measurement Sets* dialog box. |
| **7** | After all settings are complete, click *OK* to run your selected analysis. |
| **8** | Click *Cancel* to discontinue running the analysis and return to the Plate Data Utilities dialog box. |

## Run Analysis on Plates - Dialog Box Options

### Analysis

Chooses the analysis that you want to run from the list of available analysis.

### Settings

Chooses the setting that you want to use from the list of available settings.

### Description

Displays a description for the currently selected analysis.

### Images to open for the analysis

Chooses the images in your currently selected analysis folder that you want to run with the analysis. Click to check the checkbox of each wavelength that you want to process and analyze.

### Base Results Folder

Selects a specific user name and associated base results folder location for storing your analysis results.

### OK

Initiates running the selected analysis using the selected settings and wavelengths.

### Cancel

Closes the Run Analysis on Plates dialog box.

# Run Assay on Plates (Apps Menu)

**Chooses the assay that you want to run to process and/or analyze your selected microwell plate images.**

*Availability: Exclusive to Discovery-1*

**Drop-ins: HTPLAYER**

Use this dialog box to run specific assays, select the appropriate settings for the assay, and select from the available wavelengths for the assay. Also, use this dialog box to locate and specify the assay folder location.

This dialog box opens automatically when you click *Run Assay* from the *Screen Data Utilities* dialog box.

## Running Assays on Plates

To run a specific assay on plate images, complete the following procedure:

| Step | Action |
|---|---|
| **1** | From either the Database or Non-database versions of the *Screen Data Utilities* dialog box, Select one or more plate images on which to run your selected assay, then click *Run Assay*. The *Run Assay on Plates* dialog box opens. |
| **2** | In the *Assay* box, choose the assay that you want to run from the list of available assays. If the assay that you want to use is not shown on the list in the Assay box, click *Set Location* to specify the folder location where your assay is stored. |
| **3** | In the *Settings* box, choose the setting that you want to use from the list of available settings. |
| **4** | In the *Images to open for the assay* box, click the wavelengths against which you |

want to run your selected assay.

5 After all settings are complete, click *OK* to run your selected assay.

6 Click *Cancel* to discontinue running the assay and return to the Screen Data Utilities dialog box.

## Run Assay on Plates - Dialog Box Options

### Assay

Chooses the assay that you want to run from the list of available assays.

### Settings

Chooses the setting that you want to use from the list of available settings.

### Description

Displays a description for the currently selected assay.

### Assay location

Indicates the current folder location selected by *Set Location.*

### Set Location

Selects and/or specifies the folder location for your non-database assay images and data.

### Images to open for the assay

Chooses the images in your currently selected assay folder that you want to run with the assay. Click to check the checkbox of each wavelength that you want to process and analyze.

### OK

Initiates running the selected assay using the selected settings and wavelengths.

### Cancel

Closes the Run Assay on Plates dialog box.

# Screen Acquisition (Legacy)

**Defines Screen Acquisition settings and acquires Screening images from one or more multi-well plates using the Discovery-1 system.**

*Availability: Exclusive to Discovery-1*

**Drop-in: HTACQUIR**

**Note:** This help file is for the legacy version of Screen Acquisition. It does NOT apply to the current method of plate acquisition used by MetaXpress and does not support using a database to store experiments. Refer to the Plate Acquisition Setup Help file for documentation on using the current acquisition method.

Use this dialog box to configure the settings necessary to acquire images from one or more multi-well plates using the Discovery-1 system imager, and to initiate image acquisition.

Settings in this dialog box are used to specify plate size (number of wells), single or multiple plates, multiple wavelengths, and the wells from which you want to acquire images, including the location and arrangement of the samples within each well.

Eight separate tabs in this dialog box provide settings associated with specific elements and components of your image acquisition procedure. These tabs are identified as:

- **Main**
- **Plate**
- **Sites**
- **Wavelength(s)**
- **Time**
- **Auto Focus**
- **Journal**
- **Loader**

The ***Main*** tab provides settings for defining the name of the experiment, saving and/or loading the state of the Screen Acquisition dialog box, viewing a summary of the dialog box settings, and selecting the Magnification, Camera binning, and Gain settings. You can specify the use of multiple plates, multiple wavelengths, multiple sites-per-well, and whether you want to show the images during acquisition. In addition, if you are using the database version of Discovery-1, you can choose to use the Auto run assay feature and select a location to save your images to.

The ***Plate*** tab enables you to specify the plate size, the first well to visit, and the complete selection of wells to visit during the experiment. In addition, you can click Set A1 Center to designate the center reference for the first cell on the plate.

The ***Sites*** tab enables you to designate the number of sites in each well and the arrangement of the pattern and sequence of the sites. You can also designate the spacing between images and the range of auto-focusing to be applied before acquisition at a new site.

The ***Wavelength(s)*** tab enables you to specify the illumination setting, the exposure time, and target intensity. This tab also contains settings for auto focus and image alignment.

The ***Time*** Dialog tab enables you to acquire a series of images at multiple time points during specified time intervals. Acquisition sequences can be organized according to site, well, row, column, or plate, as well as by wavelength. You have the option of separately selecting the image interval at which you want to acquire each wavelength.

The ***Auto Focus*** tab enables you to configure each of the three different auto focus ranges, Find Sample, Wide Focus, and Narrow Focus. Settings on this tab enable you to choose the Z-motor that you want to assign to each focus range, and the range and accuracy in microns that you want applied to each motor. You can also specify the maximum single step distance in microns, the amount of time that you want to allow for the Z-motor to settle before acquiring an image, the Z origin, and whether to show images while auto focusing.

The ***Journal*** Dialog tab enables you to select specific journals to run at specific instances during image acquisition. These instances typically coincide with acquisition events such as the beginning or ending of acquiring images from a well, at a site, or at the beginning or end of a group of images acquired at a time point.

The ***Loader*** tab enables you to specify settings for the plate loading device.

## Acquiring Screen Images

**Acquiring Screen Images - Sidebar**

**Acquiring Screen Images - Main**

## Acquiring Screen Images - Sidebar

The controls on the Screen Acquisition dialog box "Sidebar" (the non-tabbed area) allow you to manually control certain microscope functions to enable you to test settings and conditions and acquire preliminary or test images of samples. The Acquire button used to begin the automated acquisition process is also located here.

In the process of making the necessary settings on the various tabs, you can activate individual functions from the sidebar to test the effectiveness of certain individual settings.

To effectively use the sidebar controls, complete the following procedure.

| Step | Action |
|---|---|
| **1** | From the Apps menu, click Screen Acquisition, the Screen Acquisition dialog box opens. |
| **2** | Ensure that a plate is in place on the microscope stage, or that the Plate Loader has moved the first plate into position. |
| **3** | Click *Go To A1*. If the stage is not positioned correctly, Go To A1 will move the stage to the first position;<br><br>OR<br><br>Type the well number for a specific well that you want to view in the Go To box, and click *Go To:* |
| **4** | In the *Wavelength* box, select the wavelength that you want to use for preliminary test images. |
| **5** | Click *Live* to Acquire images continuously to enable you to focus the Microscope. |
| **6** | Click *Snap Current* to Acquire a single image. |
| **7** | Click any of the four arrow buttons to move the stage to a new well. |
| **8** | Click *Find Sample* to initiate the Find Sample focusing routine. |
| **9** | Click *Wide Focus* to initiate the Wide Focus focusing routine. |
| **10** | Click *Preview*. The Screening Status dialog |

box opens and an image view dialog box for each wavelength opens. During this time, you can adjust the display of images and windows so that they will be appropriately sized and positioned for acquisition.

**11** Click *Acquire* to begin automated acquisition of fluorescence images.

**12** Click *Close* to close the Screen Acquisition dialog box.

## Acquiring Screen Images - Main

To set general options for acquiring images on the Main tab, complete the following procedure.

| Step | Action |
|---|---|
| **1** | In the Screen Acquisition dialog box, click the *Main* tab. The Main tab moves to the front. |
| **2** | If you need to load a previously saved Screen Acquisition state file, click *Load State*. The Load Screen Acquisition State dialog box opens. |
| **3** | Click the selections for the types and/or groups of settings that you want to load from the state file. The types of settings that are not checked will not be loaded. Click *Select All* if you want to load all saved settings. Click *Load* to load your selected settings. |
| **4** | Select the Magnification file to load from the *Magnification* drop-down list. |

> **Note:** The settings available in the Magnification drop-down list are read from the Configure Magnification dialog box in the Devices menu. Magnification settings assign X and Y offset values and a Z escape distance to a specific objective.

| | |
|---|---|
| **5** | In the *Camera Binning* box, type or select a camera binning value from 1 to 8. Camera binning enables you to use shorter exposures, but reduces the accuracy if the information in the image. |
| **6** | In the *Gain* box, select the level of camera gain you need. Higher gain yields shorter exposures, but also results in more background noise. |
| **7** | Click *Load Multiple Plates* if you are loading multiple plates from a plate loader. The *Loader* tab appears. |
| **8** | Click *Multiple Sites Per Well* if you have more than one site in each well that you need to acquire. The *Sites* tab appears. |

**9** Click *Multiple Wavelengths* if you are acquiring more than one wavelength. The Wavelength tab changes to Wavelengths.

**10** Click *Show Images During Acquisition* if you want each image to be displayed while it is being acquired.

**11** Click Auto Run Assay and use the drop-down list to select an assay to run on a separate Discovery-1 computer as each plate is acquired. (optional).

> **Note:** This option only applies to the database version of Discovery-1.

**12** Use the Image Storage drop-down list to select a location where screening images are saved. (optional).

Note: This option only applies to the database version of Discovery-1.

**13** In the *Description* box, type a brief description of your experiment (optional).

**14** Click *Directory* to assign a directory to your experiment images. The Browse for Folder dialog box opens. Select or create an appropriate directory, then click OK.

**15** In the *Base Name* box type an appropriate base name for your experiment, otherwise, a default base name of *Experiment(#)* will be assigned.

**16** Click *Increment base name if file exists* if you want MetaMorph to automatically assign an incremental numerical suffix to your file base name that you designated in the Base Name box.

## Acquiring Screen Images - Plate

To set the plate size you are using and the wells that you want to visit on the plate, complete the following procedure.

| Step | Action |
|---|---|
| **1** | In the Screen Acquisition dialog box, click the *Plate* tab. The Plate tab moves to the front. |
| **2** | In the *Plate Size* box, click the arrow to select the plate size you are using. |
| **3** | In the *First Well For Finding Samples* boxes, select the letter and number for the first well to visit. |
| **4** | In the *Wells To Visit* box, click to select the wells that you want to visit.<br>• Click individual wells to select or deselect each well. |

- Click lettered buttons to select or deselect an entire row.
- Click numbered buttons to select or deselect an entire column.
- Click the unlabeled button in the upper-left corner to select or deselect all wells on the plate.

**5** To move the stage position to a different well, right-click the well to which you want to move.

**6** Click *Set A1 Center* to set the center position for well A1. The *Set A1 Center* dialog box opens.

**Note:** *Complete this step only during initial installation or when a new plate size is first used.*

**7** Use the stage joystick or type or select values in the X and Y boxes for the current position, then click *Set A1 to Current* to define the current position as the center of well A1. The setting will be stored until it is changed or deleted.

For suggested procedures, go to ***Finding the Center of Well A1*** .

**Note:** *Complete this step only during initial installation or when a new plate size is first used.*

**8** Click *OK* to close the dialog box.

***Warning:*** ***Do not*** look into the microscope objective or look into the well over the objective as damage to eyes can occur. This lamp produces concentrated, high-intensity Ultraviolet (UV) light, which can permanently damage eyes. Use appropriate safety precautions in the presence of UV light.

## Finding the Center of Well A1

You can use one of the following three methods to help you to find the center of well A1:

# *WARNING*: *Do not* look into the *well* to find the well center.

### *Method A*

| Step | Action |
|---|---|
| **1** | Mark the center of the A1 well on a test plate. |
| **2** | With the microscope in "Live" mode, use the joystick to move the mark to the center position. |

| Step | Action |
|---|---|
| **3** | When you are sure that well A1 is centered over the objective and in the image area, click Set A1 Center. |

***Method B***

| Step | Action |
|---|---|
| **1** | Place a piece of white paper over the top of the plate, ensuring that it covers the A1 position. |
| **2** | Set the excitation wave to a visible wavelength (For example, 490), and activate "Live" mode. |
| **3** | Move the plate until the circle of light is uniformly round on the paper, then click Set A1 Center. |

***Method C***

| Step | Action |
|---|---|
| **1** | Set the excitation wave to a visible wavelength (For example, 490), and activate "Live" mode. |
| **2** | Darken the room, and allow the image of the light from the objective to project onto the ceiling (assuming that the ceiling is white, and not too high). |
| **3** | Move the plate until the circle of light is uniformly round, then click Set A1 Center. |

***Warning*: *Do not*** look down through the well into the objective at any time. The potential exists for bright light to pass through if the shutter is activated accidentally. Use one of the previously described methods to set A1 center.

## Acquiring Screen Images - Sites

To define the sites to acquire in each well, complete the following procedure.

| Step | Action |
|---|---|
| **1** | In the Screen Acquisition dialog box, click the *Sites* tab. The Sites tab moves to the front. |
| **2** | Under *Arrangement of Sites in each Well,* click the arrow in the drop-down menu box and select the number of sites that you want to acquire from each well.<br>• Select *2x2* to acquire four sites<br>• Select *3x3* to acquire nine sites<br>• Select *4x4* to acquire 16 sites, etc. |
| **3** | In the site array that you selected, click individual sites to turn off any sites that you |

do not want to acquire or to turn on any sites that are turned off.

**4** In the *Spacing Between Images* box, type or select the space in microns to place between each image.

**5** In the *Auto Focus Each Site* box click *None* for no auto focus on sites other than one; click *Narrow* to run the narrow auto focus procedure defined on the Auto Focus tab on each site; click *Wide* to run the wide auto focus procedure defined on the Auto Focus tab on each site.

**6** To change the current site selection to a specific site, in the *Current Site* selection box, select the number of the site to which you want to move, then click *Go To*.

## Acquiring Screen Images - Wavelength

The Wavelengths tab enables you to define a maximum of eight wavelengths. Each wavelength is defined in the same way. This procedure describes how to define one wavelength. To define additional wavelengths, move the Current Wavelength selector to the next wavelength, and repeat the steps.

To define a wavelength, complete the following procedure.

| Step | Action |
|---|---|
| **1** | In the Screen Acquisition dialog box, click the *Wavelength(s)* tab. The Wavelength(s) tab moves to the front. |
| **2** | In the *# of Waves* box set the maximum number of waves that you will use for this experiment. |
| **3** | Using the *Current Wavelength* selector, select the wavelength that you want to define. |
| **5** | In the *Illumination* box, select the illumination setting for this wavelength number. The illumination settings are defined in the ***Configure Illumination*** dialog box. |
| **6** | In the *Exposure* area, type or select a value for the Target Intensity in the *Target Intensity* box or use the default value. This value sets the intensity that auto exposure should attempt to attain for the brightest pixel in the image. When Auto Expose is selected, the target intensity value default is 75 percent of the maximum gray level that the camera driver reports as possible to obtain. |
| **7** | In the *Exposure* box, type or select an exposure time in milliseconds or, if you have an appropriate sample in view, click *Auto Expose* to set this value automatically. |
| **8** | In the first *Auto Focus Acquisition* box, choose whether to use the Wide, Narrow, or both auto focus procedures for this |

wavelength.

- Click *Wide* for the wide focus procedure as defined on the Auto Focus tab.
- Click *Narrow* for the narrow focus procedure as defined on the Auto Focus tab.

**Note:** Set **Wavelength 1** to *Wide Focus* since it is the first wavelength acquired when the stage moves to a new well.

**9** In the second *Auto Focus Acquisition* box, you can set different exposure parameters to use during auto focus procedures, or you can use the same exposure parameters as those used during image acquisition. Click *Same setting as wavelength* to use the acquisition parameters for auto focus.

OR

- Uncheck *Same setting as wavelength*.
- Check *Center Quad* to use only the center quadrant of pixels.
- Type or select an exposure time in milliseconds in the *Exposure* box.
- Type or select a binning value from 1 to 8 in the *Bin* box.
- Select a gain level in the *Gain* box.

**Note:** When *Same setting as wavelength* is off, ensure that the exposure settings for both the normal acquisition and focus acquisition are appropriate for the selected wavelength.

**10** In the *Alignment Cropping* area, type or select alignment cropping values for X and/or Y in the *X* and *Y* boxes.

OR

Click ***Set Alignment***. The Screen: Set Alignment dialog box opens. Complete the steps for setting image alignment.

**11** To calculate the offset for all wavelengths at the Find Sample well, Select *Calculate wavelength offsets at start.* The range of this focus is 15um. This calculates the offset between the current wavelength and the previous wavelength.

## Acquiring Screen Images - Time

To configure Screen Acquisition to acquire images at multiple time points, complete the following procedure:

| Step | Action |
|---|---|

1. In the Screen Acquisition dialog box, click the Time tab. The Time tab moves to the front.
2. In the *Loop Order, time series for* box, select the time series sequence that you want to use for each plate. The available time series provides different orders of acquisition from wells on a plate. Choose the acquisition order that best suits your experiment.
3. In the *Number of Time Points* box, type or select the number of time points that you want to acquire at each well or site in your image.
4. In the *Interval* box, type or select the appropriate time interval and associated unit of time.
5. In the *Duration of Time Lapse* box type or select the corresponding time lapse duration and the associated unit of time.
6. In the *Wavelength collection* area, for each wavelength, choose the appropriate timepoint acquisition pattern.

## Acquiring Screen Images - Auto Focus

The Auto Focus tab enables you to configure three separate types of auto focus procedures.

- ***Find Sample*** performs the initial focus operation for the plate. Find Sample uses the well selected in the *First Well For Finding Samples* setting on the Plate tab to determine a coarse focus setting for the entire plate.
- ***Wide Focus*** performs an auto focus within a medium range, but to a high level of accuracy. Typically, you use this focus selection to attain accurate focus at the "well" level for the first image in the well. The focus range for this selection should be wide enough to accommodate potential well-to-well range differences affected by differences in physical characteristics of the various samples.
- ***Narrow Focus*** performs a fine auto focus within a narrow range with a high level of accuracy. Use this focus selection for all subsequent images once a Wide Focus within the well has been attained. This focus operation is best suited for refocusing for each individual wavelength and/or site that is acquired.

The procedures for each these is the same, with only the setting values for each being different. Therefore, this procedure is described only once.

The following four settings on this tab are used by all three types of focus procedures.

- *Maximum Single Step*
- *Set Z Origin*
- *Show Images During Auto Focus*
- *Settle Time*

Two settings, *Go To Z Origin* and the *Z:* position up and down arrows are used to manually change the Z position during configuration.

To configure *Find Sample, Wide Focus, and Narrow Focus*, complete the following procedure.

| Step | Action |
|---|---|
| 1 | In the Screen Acquisition dialog box, click the *Auto Focus* tab. The Auto Focus tab moves to the front. |
| 2 | In the *Maximum Single Step* box, type or select a value for the maximum distance in microns that you want the Z-Motor to move in a single step.<br><br>**Note:** A smaller single step value ensures more accurate focus, but also increases the focus time. |
| 3 | To set the Z Origin, use the up or down *Z* position (focus) arrows to change the current Z position to the position that you want to use for the origin, then click *Set Z Origin*. |
| 4 | Click *Show Images During Auto Focus* to display the image of the sample currently being auto-focused.<br><br>**Note:** You can decrease the focus time by turning off this option. |
| 5 | Type or select a value in the *Settle Time* box to specify a waiting time between focus operations. |
| 6 | In the *ZMotor* box, select the Z-Motor that you want to use for this auto focus procedure. |
| 7 | In the *Range* box, type or select a range in microns that limits the range of this focus operation. |
| 8 | In the *Accuracy* box, type or select a value in microns that determines that the intended range of accuracy has been achieved. |
| 9 | Repeat steps 6 through 8 for each focus procedure that you need to configure. |
| 10 | To manually change the Z-position, click on either the up or down Z arrow at the bottom of the tab. |
| 11 | To move the Z-Motor to the Z-Origin, click *Go To Z Origin.* |

## Acquiring Screen Images - Journal

The Screen Acquisition Journal settings enable you to run specific journals at specific time points during acquisition, as indicated by the descriptions on the tab. For more detailed descriptions, refer to the Journal Tab Dialog Box Options.

To use specific journals in conjunction with acquiring screen images, complete the following procedure:

| Step | Action |
|---|---|
| 1 | From the Screen Acquisition dialog box, click the Journal tab. The Journal tab containing the journal selection settings moves to the |

front.

**2** For each journal that you want to run, determine the most appropriate time pint to run the journal, and click the time point checkbox to select it.

**3** Click the associated Select button. The Screen Acquisition Journal dialog box opens.

**4** Select the appropriate folder containing the journal(s) that you want to run.

**5** Choose the journal that you want to run at the selected time point, and click Open.

**6** Repeat Step 5 for each additional time point to which you want to assign a journal

**7** After all journals have been assigned, you can continue with other Screen Acquisition option selections on other tabs, then click Acquire to acquire the images you selected.

## Acquiring Screen Images - Loader

To Configure the plate Loader, you must configure the settings in the Configure Plate Loader dialog box in addition to the settings on the Loader tab. Configure settings in the Configure Plate Loader dialog box before making settings on the Loader tab.

To configure the Plate Loader, complete the following procedure.

| Step | Action |
|---|---|
| **1** | In the Screen Acquisition dialog box, click the *Loader* tab. The Loader tab moves to the front. |
| **2** | Click *Configure Loader*, the Configure Plate Loader Dialog box opens. |

## Screen Acquisition Dialog Box

### Snap Current

Acquires a single image of the currently in place well at the current settings for focus (z-position), wavelength, plate, well, site, and exposure.

### Live

Continuously acquires images based on the current settings, and updates the image as settings are changed.

### Well

Indicates the well currently in position for image acquisition.

### Site

Indicates a the site within a specific well that is currently in position for image acquisition.

### Go To

Moves the stage to the well number that you type into the Go To box.

### Go To A1

Moves the stage to the A1 position.

### Wavelength

Selects the wavelength that you want to use for your acquisition.

### Find Sample

Performs a very coarse auto focus on the current well position. The range covered in Find Sample is the same as the initial Find Sample when starting an Acquire.

### Wide Focus

Performs a coarse focus operation on the selected well based on the Wide Focus settings on the Auto Focus tab.

### Preview

Simultaneously opens the Screening Status dialog box and an Image Window, while closing the Screen Acquisition dialog box. The Screening Status dialog box indicates the wells selected for acquisition. It then acquires an image for each wavelength using the appropriate auto focus routine. Once all images have been acquired, the display of images and dialogs can be arranged in the MetaMorph window during the Preview operation so that they are appropriately configured for acquisition.

### Acquire

Starts the sequential acquisition of images from one or more plates based on the settings made in this dialog box.

### Close

Closes the Screen Acquisition dialog box and discontinues the acquisition of images, if it is in process.

## Screen Acquisition Dialog Box - Main Tab

**Note:** Some of these options are dependent on how your Discovery-1 system is configured and may not be available to all users.

### Save State

Saves the current settings in the Screen Acquisition dialog box to an State file. When you click Save State, the Save Acquisition dialog box opens. Type the name of a new state file that you want to create, or select a listed state file name to overwrite an existing state file.

### Load State

Loads the selected settings from an existing screening state file. When you click Load State, the Load Screen Acquisition State dialog box opens. Check the boxes for the conditions and groups of settings that you want to load from the state file, uncheck the ones that you do not want to load. Click *Select All* to load all conditions; click *Clear* all to clear all selections. Click *Load* to load your selected conditions.

### View Summary

Opens the Screen Summary dialog box. This dialog box indicates the current settings selected for your acquisition, the number of racks of plates, the number of selected wells, the number of sites in each well, the distance between images, the number of wavelengths, the total number of images, the amount of storage required, and the specified type of focusing for each wavelength for both the first and the remaining sites in the well.

### Magnification

Selects the magnification settings that you want to use in your experiment. Magnification settings are made using the Configure Magnification dialog box. Magnification settings assign X and Y offset values and a Z escape distance to a specific objective.

### Camera Binning

Specifies the binning value to be applied to the camera. Binning combines the output of adjacent pixels in square multiples. For example, a camera binning value of 1 is only one pixel, a binning value of 2 combines 2x2 or four pixels in a square, a binning value of 3 combines 3x3 or nine pixels in a square, and so on.

### Gain

Specifies the amplification to be applied to the camera output. The higher the gain, the greater the potential for background noise in the signal. Settings are Low, High, and Super High, with Low being the default.

### Load Multiple Plates

Activates the Loader tab for making settings to control a Hudson PlateCrane plate loading device. Click this checkbox to activate the loader tab. When the loader is not active, images are acquired from the plate currently on the stage; When active, plates are loaded onto the stage for acquisition.

### Multiple Time Points

Activates the Time tab. Use the settings on this tab to specify the number and time points, their interval, and their duration. This dialog box also enables you to select one of several possible time point acquisition patterns and associate it with a specific wavelength. **Note:** This option is available only with the database version of Discovery-1.

### Multiple Sites Per Well

Activates the Sites tab for specifying multiple sites per well. If inactive, only one site per well is acquired. If active, images from four to 16 sites per well can be acquired, depending on the settings you make on the Sites tab. Click this checkbox to activate the Sites tab.

### Multiple Wavelengths

Changes the state of the wavelength tab (to Wavelengths) and Adds settings for Alignment cropping to the dialog box.

### Show Images During Acquisition

Causes each image to be displayed as it is acquired.

### Auto Run Assay

Activates the drop-down list that enables you to select an assay to auto-run on a separate Discovery-1 computer after each plate is acquired. Refer to the Auto Run Mode helpfile for more information.

**Note:** This option is only available with the database version of Discovery-1.

### Image Shading Correction

Indicates whether a **shading correction** image for this magnification is present and being applied. The correction images must be stored in the root C:\ directory and named in the following format:

**C:\Shading_<Magnification Name>.tif**. For example — **C:\Shading_4X.tif**.

### Image Storage

Enables you to select a location where screening images are saved. Refer to the Screen Database: Configure Image Locations helpfile for more information. This option is only available with the database version of Discovery-1.

**Note:** You must configure the image locations using the Meta Imaging Series Administrator command Screen Database: Database Utilities command.

### Description

Enables you type an experiment description to be stored with the image information.

### Base Name

Defines the base file name to which an incremental suffix can be attached. Type a base file name (prefix) to assign to your experiment.

## Screen Acquisition Dialog Box - Plate Tab

### Plate Size

Specifies the plate size you are using according to the number of wells it contains and the number of rows and columns in which they are arranged. Select the plate size that corresponds to the size of the plate you are using, or select Custom to specify a non-standard plate size.

### Configure

Opens the **Configure Custom Plate** dialog box. Use this dialog box to make settings that define well positions for non-standard plates.

### First Well for Finding Samples

Specifies the well number to use for the Find Sample focusing routine. This well should contain an ideal sample that enables the Find Sample focusing routine to achieve the best focus for the entire plate.

> **Note:** The well must contain an appropriate sample to enable the images of this well to be correctly acquired.

### Wells to Visit

Indicates the wells that you need to sample during the experiment. Select from a single well to all wells in the plate. Click individual well positions to toggle wells separately. Click column or row buttons to activate or deactivate an entire row. Click the unlabeled gray button in the upper left corner to toggle all wells on the plate simultaneously. The well for finding samples is indicated as a button.

### Center of Well A1

Indicates the position within the well area that is designated as the center of the well. The center of well A1 is different for each different plate size. Each time a new plate is first used, you need to define the A1 Center position for the specific plate size.

### Set A1 Center

Sets the current position as the center position of well A1 for each specific plate size. This position varies for each plate size.

> ***Note:*** The A1 position is stored for each plate size. Set the A1 Center position only the first time a new plate size is used.

> ***Warning:*** ***Do not*** look into the microscope objective or look into the well over the objective as damage to eyes can occur. This lamp produces concentrated, high-intensity Ultraviolet (UV) light, which can permanently damage eyes. Use appropriate safety precautions in the presence of UV light.

## Configure Custom Plate

### Number of Wells

Specifies the number of wells in the X and Y axis of the plate. Type or select the total number of wells in a row in each axis. For the total number of wells on the plate, multiply the number of wells in X by the number of wells in Y.

### Well Spacing

Specifies the spacing in microns (micrometers) between each well. Normally this value should be the same for both the X and Y axis. However, you can specify different values for X and Y for plates that use different spacing between well on the X and Y axis. Type or select the correct values for X and Y.

### Set Interactively

Interactively determines and sets the spacing between wells by measuring and determining the spacing between the center of the first well in the plate (A1) and the center of the last well in the plate. Click *Set Interactively* and follow the instructions.

> ***Warning:*** Be sure to read the instructions on how to **Locate the Center of Well A1** (or any other well). ***NEVER*** look directly through the well into the objective. High-intensity Ultraviolet (UV) light is emitted from the objective and can cause serious eye damage.

### OK

Applies the configuration settings and closes the dialog box.

### Cancel

Disregards the configuration settings and closes the dialog box.

## Screen Acquisition Dialog Box - Sites Tab

### Current Site

Indicates the site in the currently selected well that is in position for acquisition.

### Go To

Specifies a site within a single well to which to move. For a 2x2 site arrangement, select from sites 1-4; for a 3x3 site arrangement, select from sites 1-9: for a 4x4 site arrangement, select from sites 1-16, and so on.

### Arrangement of Sites in each Well

Specifies the number of sites in the array, from 2 X 2 to a maximum of 127 X 127. Within the selected array of sites, you can right-click on any site to either exclude or include the site in the array. Therefore, you can have as few as one site or as many as 16, 129 sites. Practical usage suggests that the maximum number of sites that you select and use would be significantly less than 127 X 127.

### Total Sites

Indicates the total number of sites selected for each well.

### Spacing Between Images

Applies the specified edge-to-edge spacing value to calculate the locations in the well from where each image will be acquired. When you combine this parameter with your selected well array and your choice of the wells in the array from which you want to acquire images, you can achieve a high degree of sample selectability in the well.

### Calibration

Indicates the designated number of microns per pixel.

### Image Size

Indicates the acquired image size.

### Well Size

Indicates the well size.

### Image Spread

Indicates the combined dimensions of each acquired image at each site plus the spacing between each image.

### Auto Focus Each Site

Specifies whether auto focus is performed for each site in the well and whether the auto focus operation uses the Narrow procedure or the Wide procedure.

## Screen Acquisition Dialog Box - Wavelengths Tab

### # of Waves

The total number of wavelengths that you have configured for use during your experiment. Set from 1 to 8 wavelengths in this box.

### Current Wavelength

Selects and indicates the currently active wavelength. The settings on this tab are applied to the active wavelength. A maximum of eight individual sets of settings can be associated with eight different wavelengths. The settings can consist of values for illumination, exposure, auto focus acquisition, and

alignment cropping, and are applied uniquely to a specific wavelength.

### Illumination

Selects an illumination setting to be used with the active wavelength. Illumination settings are defined in the ***Configure Illumination*** dialog box.

### Exposure

Specifies the exposure time in milliseconds to be associated with the active wavelength. Type a value in this box or click Auto Expose to automatically determine an exposure time.

### Auto Exposure

Automatically determines the exposure time for the currently loaded sample, and applies it as the exposure value.

### Target Intensity

Sets the intensity that auto exposure should attempt to attain for the brightest pixel in the image. The target intensity value default is 75 percent of the maximum gray level that the camera driver reports as possible to obtain.

### Auto Focus Acquisition

Selects whether you want to perform Wide auto focus, Narrow auto focus, both, or neither during image acquisition. Complete auto focus settings on the Auto Focus tab.

***Wide*** – Selects the wide auto focus procedure as specified in the Wide Focus settings on the Auto Focus tab to run when an image is acquired for the associated wavelength. Use the Wide procedure for acquisition of the first wavelength in a new well.

***Narrow*** – Selects the narrow auto focus procedure as specified in the Narrow Focus settings on the Auto Focus tab to run when an image is acquired for the associated wavelength. Use the Narrow procedure for acquisition of all subsequent wavelengths and sites in a well after the first wavelength and/or site in the well has been acquired with the wide procedure.

### Auto Focus Acquisition

Applies alternate exposure settings to auto focus to improve acquisition speed during auto focus.

***Same setting as wavelength*** – Makes the acquisition exposure values during auto focus the same as those specified for normal image acquisition.

**Note:** If this box is checked, the following settings for Auto Focus Acquisition are not active.

***Center Quad*** – Uses only the pixels in the center area of the camera for auto focus.

***Exposure*** – Specifies the exposure time to be used during auto focus.

***Bin*** – Specifies the binning value to be used during auto focus.

***Gain*** – Specifies the gain setting to be used during auto focus.

### Alignment Cropping

Compensates for image shifting that occurs at different wavelengths. Type or select values for X and/or Y to realign an image at one wavelength with a similar image at another wavelength. Due to refractive color shifting, images of the same sample at different wavelengths might not precisely converge. Use these settings to correct for this. To apply this correction interactively, click Set Alignment.

**Note:** At installation, your system should have less than a 2-pixel shift in both X and Y axis.

***X*** – Crops the specified amount in microns from either the left side or the right side of the image, depending on whether the value is positive or negative. Negative crops the left side; positive crops the right side.

***Y*** – Crops the specified amount in microns from either the top or the bottom of the image, depending on whether the value is positive or negative. Negative crops the bottom; positive crops the top.

### Set Alignment

Opens the *Screen: Set Alignment* dialog box. Use this dialog box to interactively apply color shift correction (Alignment Cropping) to one or more images of the same sample acquired at different wavelengths. Set alignment creates a stack from the acquired images and applies horizontal and vertical shift values to each selected image.

### Calculate wavelength offsets at start

Performs a focus calculation for all wavelengths at the Find Sample well. The range of this focus is 15um. This calculates the offset between the current wavelength and the previous wavelength. At each site the offset is applied before focusing for the wavelength begins. This checkbox applies to all wavelengths.

### Auto Expose at start

Automatically calculates the exposure for the first image acquired for each individual plate and applies the exposure value to all subsequent images acquired from the same plate.

### Auto Expose plate #1 only

Automatically calculates the exposure for the first image from the first plate and applies the exposure value to all images acquired from all plates during a single acquisition session. Auto Expose at start must be selected in order to select this option.

## Screen Acquisition Dialog Box - Time Tab

### Loop Order, time series for

Selects the loop order to be used when acquiring images at multiple time points and determines the set of images to be acquired at each time point. At each time point all wavelengths must be acquired.

Select from one of the following acquisition sequences:

> ***One site then the next:*** Acquires all the wavelength images at the site and then collects the next set of wavelength images after the interval has elapsed. Once the series is collected, the next site is acquired. No refocusing is done.
>
> ***One well then the next:*** A set of wavelength images is acquired at each site in the well at each time point. No refocusing is done.
>
> ***One row, then the next:*** All the images in one row's worth of wells are collect at each time point. Once the series is collected the next row is acquired. No refocusing done.
>
> ***One column, then the next:*** All the images in one column's worth of wells are collected at each time point. Once the series is collected, the next column is acquired. No refocusing done.
>
> ***Full plate :*** Every well selected for acquisition is acquired at each time points. The well selection is determined at the start of the first acquisition. No state file will be acquired. No refocusing is done.
>
> ***A set of plates:*** As many plates as are available and specified for loading will be acquired. If necessary the plates will be resorted before the next time point is acquired.

### Approximate minimum time interval

Indicates the approximate length of time required to acquire all images for a single time point. This value is calculated based on the available information, including exposure times, number of wavelengths to acquire, focusing steps (z-positions), number of sites, wells, and plates. This value does not take into account stage travel time, plate transfer time, or image transfer time. Therefore, the amount of time indicated will typically be less than the actual time to acquire all images for a single time point.

### Number of Time Points

Specifies the total number of time points to be acquired. When you change this field, the duration field is automatically updated by calculating the duration from the number of time points and the time interval.

### Interval

Specifies the amount of time between the start of acquisition at one time point to the start of acquisition at the next time point. If the time interval is shorter than the length of time required to actually acquire the images, the next acquisition will occur as soon as possible once the first acquisition is complete. No warning

notice will be given if the acquisition time is longer than the specified interval. Use the adjacent drop-down box to set the time units-of-measure. Units can be ms, sec, min, or hr. Changing the interval field updates the duration field by calculating the duration from the number of time points and the time interval. The interval field can be set to 0 to acquire images as fast as possible. If the interval is set to 0, the duration will be set according to the approximate minimum time, and the duration field will be inactive.

### Duration of Time Lapse

Specifies the time it will take to acquire the number of times points based on the interval. Changing this fields update the number of time points field by calculating the number of time points from the time interval and the duration. The time units of the interval field are settable from a drop down. The available units will be ms, sec, min, and hr.

### Wavelength Collection

Specifies the image collection intervals that you want to use for each individual wavelength. This feature enables you to specify different image collection intervals separately for each individual wavelength.

### Wn

Specifies the image collection interval for the associated wavelength. Interval choices are the following:

***All time points*** – Acquires an image for each timepoint for this wavelength.

***At start*** – Acquires an image at this wavelength for the first timepoint only.

**At start and end** – Acquires images at this wavelength for the first and last timepoints only.

***Every nth timepoint*** – Acquires an image at this wavelength at the selected timepoint interval beginning at the first timepoint.

## Screen Acquisition Dialog Box - Auto Focus Tab

### Algorithm

Selects the algorithm that you want to use to complete the auto focus operation. Select one of the following algorithms.

***Standard*** – Algorithm based on a standard group of settings including a normal camera signal level. (Default)

***Low Signal*** – Algorithm based on a set of values selected to compensate of a low signal level from the camera. This setting can compensate for situations in which some pixel intensities are somewhat brighter when slightly out of focus.

***<Set by MMVar>*** – Reserved for future use.

### Maximum Single Step

Defines the maximum distance in microns that the Z-motor can move in a single step. The smaller the step (fewer microns per step) the greater the number of steps are required to find the sample or to perform wide or narrow focus operations. However, a smaller step size ensures that within the defined range, correct focus within the specified range of accuracy will be found.

### Set Z Origin

Sets the Z position from which you want all focus operations to begin. Change the Z position with the up and down Z position arrows at the bottom of the tab, then click *Set Z Origin.*

### Typical Accuracy for Objective

Indicates the typical degree of focus accuracy that you might expect for the selected objective based on the numerical aperture of the lens such that the message displayed indicates that the Z position achieved is within the depth of field range of the objective.

### Find Sample

Performs the initial, coarse focus operation for the plate using a reference well as designated by the well you specify as the *First Well For Finding Samples*. This focus operation determines the best focus to the specified level of accuracy over the entire specified range.

***Z-Motor*** – Specifies the name of the Z-Motor that will be used to perform this focus operation. This setting is specified during initial setup only.

***Max # Z Moves*** – Indicates the maximum number of Z moves that will be used to find the best focus. This value is based on the maximum single step size, the range, and the specified Accuracy value in microns.

***Range*** – Specifies the total focus range from the Z Origin in which the focus operation is permitted to occur. This is a plus or minus value from the Z Origin or the current Z-position. Thus, if the range is +/- 500, the Z motor can move a maximum of 500 microns in either direction from the Z Origin or the current Z-position.

**Note:** The range for Find Sample needs to be large enough to accommodate the focus variation that can occur from plate-to-plate.

***Accuracy*** – Specifies the degree of focus accuracy in microns needed to complete the operation. Therefore, if the accuracy value is specified as 20 microns, the operation is complete when a focus movement of less then 20 microns is completed.

**Note:** Accurate focus in not expected from the Find Sample operation. The Accuracy value for Find Sample needs only to be accurate enough to place the Z-position distance from true focus within the range of Wide Focus.

### Wide Focus

Performs a focus for the first image to be acquired from each well. This focus should be configured to achieve maximum accuracy within the range established by the Find Sample routine.

***Z-Motor*** – Specifies the name of the Z-Motor that will be used to perform this focus operation. This setting is specified during initial setup only.

***Max # Z Moves*** – Indicates the maximum number of Z moves that will be used to find the best focus. This value is based on the maximum single step size, the range, and the specified Accuracy value in microns.

***Range*** – Specifies the total focus range from the Z Origin in which the focus operation is permitted to occur. This is a plus or minus value from the Z Origin or the current Z-position. Thus, if the range is +/- 50, the Z motor can move a maximum of 50 microns in either direction from the Z Origin or the current Z-position.

***Accuracy*** – Specifies the degree of focus accuracy in microns needed to complete the operation. Therefore, if the accuracy value is specified as 5 microns, the operation is complete when a focus movement of less then 5 microns is completed.

### Narrow Focus

Performs focus operation in conjunction with each wavelength change and/or each site change within an individual well. This auto focus setting may or may not be used, at your discretion.

***Z-Motor*** – Specifies the name of the Z-Motor that will be used to perform this focus operation. This setting is specified during initial setup only.

***Max # Z Moves*** – Indicates the maximum number of Z moves that will be used to find the best focus. This value is based on the maximum single step size, the range, and the specified Accuracy value in microns.

***Range*** – Specifies the total focus range from the Z Origin in which the focus operation is permitted to occur. This is a plus or minus value from the Z Origin or the current Z-position. Thus, if the range is +/- 5, the Z motor can move a maximum of 5 microns in either direction from the Z Origin or the current Z-position.

***Accuracy*** – Specifies the degree of focus accuracy in microns needed to complete the operation. Therefore, if the accuracy value is specified as 1 microns, the operation is complete when a focus movement of less then 1 microns is completed.

### Show Images During Auto Focus

Specifies that MetaMorph display each sample's image as the auto focus operation is applied to the image.

**Note:** To reduce to time to auto focus, ensure that this setting is off.

### Settle Time

Specifies the amount of time to wait between each move of the Z motor and each image capture. The image capture could be either a focus or acquisition operation.

**Note:** This setting should be made during initial setup only.

### Configure Sensor

Opens the Configure Focus Sensor dialog box, which is used to configure sensor settings for the laser focus feature.

### Z

Manually moves the Z position (focus) upward or downward in single step increments.

### Go To Z Origin

Moves the Z position to the Z origin that was set using the Set Z Origin button.

# Load Screen Acquisition State

**Loads saved Screening State files to use in an experiment.**

*Availability: Exclusive to Discovery-1*

**Drop-in: HTACQUIR**

Loads the selected settings from an existing Discovery-1 screening state file. You can load all the settings from a state file, or use the check boxes to select specific conditions or groups of settings to load.

**Note:** The use of the Load Screen Acquisition dialog box varies depending on whether you are using the database or non-database version of Discovery-1. Ensure that you follow the procedure for the version you are using.

## Loading the Screen Acquisition State File

To load a saved screen acquisition state file using the non-database version of Discovery-1, complete the following steps:

| Step | Action |
|---|---|
| 1 | From the *Main* tab on the Screen Acquisition dialog box, click *Load State*. The Load Screen Acquisition State dialog box opens. |
| 2 | Select the settings to load from the state file using the check boxes next to each settings group. |
| 3 | Click *Load*. The Load Screen state dialog box opens. |
| 4 | Navigate to the state file (.HTS) you want to open and click Open. The state file you selected will load and the Load Screen Acquisition dialog box will close. |

## Load Screen Acquisition State - Dialog Box Options

### State File List

Selects a saved state file from the database.

**Note:** This option is only available with the database version of Discovery-1.

These checkboxes enable or disable the loading of specific settings from a state file. The Settings listed in the Load Screen Acquisition dialog box are configured on the various tabs of the Screen Acquisition dialog box. The following table lists each setting and where they are set in the Screen Acquisition tab:

| Setting | Tab | Fields/Comments |
|---|---|---|
| **Time Points** | Time | All fields<br>This setting is only available in the database version of Discovery-1 |
| **Image Displays and Binning** | Main | • *Camera Binning*<br>• *Show Images During Acquisition*<br>The location of the image window when you acquire a preview is also saved when this setting is checked. |
| **Main: Magnification Setting** | Main | *Magnification* |
| **Main: Acquisition Choices** | Main | • *Load Multiple Plates*<br>• *Multiple Time Points* (database version only)<br>• *Multiple Sites per Well*<br>• *Multiple Wavelengths* |
| **Main: File Name and Directory** | Main | • *Increment Base Name*<br>• *Base name*<br>The path to the file is also saved when this setting is checked. |
| **Main: Auto Run Assay** | Main | *Auto Run Assay* (check box and drop-down list)<br>This setting is only available in the database version of Discovery-1. |
| **Main: Image Storage** | Main | *Image Storage*<br>This setting is only available in the database version of Discovery-1. |
| **Plate: Configuration** | Plate | All settings except for *Set A-1 Center*. |
| **Plate: A1 Center** | Plate | *Set A-1 Center* |
| **Site: Configuration** | Site | All settings except for *Auto Focus Each Site*. |
| **Site: Auto Focus Choice** | Site | *Auto Focus Each Site* |
| **Wavelengths: Illumination and Alignment** | Wavelengths | • *# of Waves*<br>• *Illumination*<br>• *Alignment Cropping*<br>• *Set Alignment* |
| **Wavelengths: Exposures and Gain** | Main/ Wavelengths | • Main: *Gain*<br>• Wavelengths: *Exposure, Target Intensity* |

| | | |
|---|---|---|
| **Wavelengths: Auto Focus Choice** | Wavelengths | *Auto Focus Acquisition* (1) |
| **Wavelengths: Auto Focus Camera Settings** | Wavelengths | *Auto Focus Acquisition* (2) |
| **Illumination Sets: Configuration** | N/A | If you are loading a saved screening state file from Discovery-1, version 5.0, it may contain illumination settings. These settings are no longer stored in screening state files. Check this box to load these settings into the Configure Illumination dialog box. |
| **Auto Focus: Configuration** | Auto Focus | All fields except for the *Configure Sensor* settings. |
| **Loader: Controls on tab** | Loader | All fields except for *Configure Plate Loader* settings. |
| **Loader: Loader Configuration of Positions** | Loader | All fields from Configure Plate Loader dialog box. |
| **Focus Sensor Configuration** | Auto Focus | All fields from Configure Focus Sensor dialog box. |
| **Journals to be run** | Journal | All fields. |

### Select All

Selects all the settings options

### Clear All

Clears all the settings options.

### Load

Loads the state file selected in the State File drop-down list (database version only) or opens the Load Screen State dialog box, enabling you to select a saved state file (non-database version).

### Cancel

Cancels the command and closes the dialog box.

# Configure Focus Sensor (Version 1)

**Configures the Discovery-1 hardware laser focus system settings.**

*Availability: Exclusive to Discovery-1*

**Drop-in: HTACQUIR**

**Note:** This dialog box is only used with the legacy Screen Acquisition command. If you are using the Plate Acquisition and Setup commend, refer to the Configure Laser Sensor Help file.

Use this dialog box to configure the settings for the Discovery-1 laser focus system. From this dialog box you can enable or disable the laser focus feature and make settings that define the operating parameters of the focus sensor.

**Note:** Except for enabling or disabling the Auto Focus sensor, the settings in this dialog box usually do not need to be modified.

# Configure Focus Sensor (Version 2)

**Configures the Discovery-1 hardware laser focus system settings.**

*Availability: Exclusive to Discovery-1*

**Drop-in: HTACQUIR**

**Note:** This dialog box is only used with the legacy Screen Acquisition command. If you are using the Plate Acquisition and Setup commend, refer to the Configure Laser Sensor Help file.

Use this dialog box to configure the settings for the Discovery-1 laser focus system. From this dialog box you can enable or disable the laser focus feature and make settings that define the operating parameters of the focus sensor for each objective.

**Note:** Except for enabling or disabling the Auto Focus sensor, the settings in this dialog box usually do not need to be modified.

## Configuring the Focus Sensor - Version 1

To change the configuration of version 1 of the focus sensor, complete the following steps:

**Note:** The default values in the Configure Focus Sensor dialog box do not need to be changed under most circumstances. Only advanced users should change these settings.

| Step | Action |
|---|---|
| **1** | From the *Auto Focus* tab on the Screen Acquisition dialog box, click *Configure Sensor*. The Configure Focus Sensor dialog box opens. |
| **2** | To enable the laser focus sensor, select *Enable* from the *Sensor Version 1* drop-down list. |
| **3** | To override the values calibrated and saved in the driver configuration, click Override driver values.<br><br>**Note:** Use this setting only if the values stored in the driver settings do not produce the best results. |
| **4** | In the Accuracy box, type or select a value to specify the degree of accuracy to which you want the focus to complete. Smaller values produce greater accuracy. |
| **5** | In the Threshold box type or select the threshold value that you want to use. This value specifies the number of illuminated pixels that need to be detected as being above the sensor's fixed threshold level. |
| **6** | In the Max Step box, type or select the Max Step size. The Max Step size is the maximum allowable distance for a single Z-motor move. The smaller the Max Step size, the potentially greater the accuracy. |
| **7** | To activate and test the sensor, click Test Sensor. The results are displayed in the dialog box |

| | |
|---|---|
| **8** | Click *Close* to save the current settings and close the dialog box. |

## Configuring the Focus Sensor - Version 2

To configure version 2 of the focus sensor, complete the following steps:

**Note:** The default values in the Configure Focus Sensor dialog box do not need to be changed under most circumstances. Only advanced users should change these settings.

| Step | Action |
|---|---|
| **1** | From the Auto Focus tab on the Screen Acquisition dialog box, click *Configure Sensor*. The Configure Focus Sensor dialog box opens. |
| **2** | To enable the laser focus sensor, select *Enable* from the *Sensor Version 2* drop-down list. |
| **3** | To use the center quadrant of the CCD chip as the region of interest for auto-focusing, select *Center Quad* in the *Regions* section. |
| **4** | To define your own region of interest used during auto-focusing, select *User Defined* in the Regions section and select values for the *X*, *Y*, *Width*, and *Height* fields. |
| **5** | To use the active region on the desktop as the region of interest while auto-focusing, click *Set to Active Region*. |
| **6** | To specify different auto focus acquisition settings for the active objective, change the values for the settings listed in the *Acquisition Settings for Mag: xx* section. The default values for each setting are listed next to the setting name.<br><br>**Note:** Change these settings only if the default values do not produce the best results.<br><br>**Note:** To get the best results, set these values so that the resulting image, acquired using the *Focus Snap* command, has maximum grey level intensity values of ~2000-3000 when the laser is focused on the bottom surface of the well. |
| **7** | To enable the use of the laser to auto focus, select *Enabled* in the *Laser use* checkbox.<br><br>To change the Well Reflectivity setting, select *Dim* or *Bright*.<br><br>**Note:** Select *Dim* for wells with little reflectivity, such as those with plastic plates or those using media. Select *Bright* for wells with more reflectivity, such as certain types of glass plates, and wells without any media. |

**8** To change the exposure time (in milliseconds) used during auto-focusing, type or select a value in the *Exposure* box.

**9** To change the binning used for auto-focusing, type or select a value in the *Binning* box.

**10** To change the gain used during auto-focusing, type or select a value in the *Gain* box.

**11** In the *Accuracy* box, type or select a value to specify the degree of accuracy to which you want the auto focus to complete. Smaller values produce greater accuracy.

**12** In the Max Step box, type or select the Max Step size. The Max Step size is the maximum allowable distance for a single Z-motor move. The smaller the Max Step size, the potentially greater the accuracy.

**13** To restore the default settings for the selected objective, click *Set to Defaults*.

**14** To open a live window using the current auto focus settings, click *Test Sensor*.

**15** To acquire a new image using the current auto focus settings, click *Focus Snap*. The acquired test image should have maximum grey level intensity values of ~2000-3000.

**16** Click *Close* to close the dialog box.

## Configure Focus Sensor Version 1 - Dialog Box Options

### Sensor Version 1

Enables or disables the Laser Focus Sensor feature.

### Sensor Parameters

This setting specifies whether the driver settings or the settings on this dialog box will be used as sensor parameters.

#### Use values provided by driver

Specifies that the values set in the driver dialog boxes will be used for this Laser Focus feature. All settings boxes will be inactive.

#### Override driver values

Specifies that the values set in this dialog box will be used instead of the values stored in the driver configuration.

### Accuracy

Defines the degree of focus accuracy in microns that this focus feature should attempt to achieve. A lower value specifies a greater degree of accuracy; a higher value specifies a lesser degree of accuracy.

### Threshold

Value of measured laser focus light intensity as defined by the number of pixels illuminated that register on the sensor above a predefined threshold level.

### Max Step

The maximum step size in microns of a single Z move to be used in attaining the correct focus position. Use

a smaller maximum step size to attain a more precise focus position.

**Note:** Smaller step sizes typically require more steps to arrive at the final focus position.

### Last calculated offset

The last calculated Z-offset value. This offset is the Z-distance between the initial Z position calculated by the auto focus feature and the Z position determined by the laser focus feature.

### Test Sensor

Activates and tests the sensor. The results of the test are reported as follows:

***Last calculated offset*** – Displays the last calculated offset value.

***Sensor Value*** – Displays the current sensor value as the number of pixels above the threshold.

***Last 10 Avg*.** – Displays the average of the last ten calculated offset values.

### Close

Closes the dialog box and saves the most recent settings.

## Configure Focus Sensor Version 2 - Dialog Box Options

### Sensor Version 2

Enables or disables the Laser Focus Sensor feature for all magnifications.

### Region

Selects the region of interest used to auto focus.

#### Center Quad

Selects the center quadrant of the CCD chip for the sensor to use when auto focusing.

#### User Defined

Enables you to define a region of interest to use when auto-focusing. Choosing a smaller region then the default center quadrant can reduce the time needed for auto-focusing; however, you must ensure that the region is large enough to contain the sensor spot when it is in focus at each objective.

#### X

Sets the X position of the user-defined auto focus region of interest. *User Defined* must be selected to enable this setting.

#### Y

Sets the Y position of the user-defined auto focus region of interest. *User Defined* must be selected to enable this setting.

#### Width

Sets the width of the user-defined auto focus region of interest. *User Defined* must be selected to enable this setting.

#### Height

Sets the height of the user-defined auto focus region of interest. *User Defined* must be selected to enable this setting.

#### Set to Active Region

Sets the auto focus region of interest to the active region on the desktop.

### Acquisition Settings for Mag: *xx*

Lists the settings for the active magnification setting and enables you to change settings. The active magnification setting is the one selected in the *Magnification* drop-down list in the *Main* tab of the Screen Acquisition dialog box. Changes made to each setting are saved in a state file and used going forward

unless the defaults are restored.

**Note:** Change these settings only if the default values do not produce the best results. To get the best results, set these values so that the resulting image, acquired using the *Focus Snap* command, has maximum grey level intensity values of ~1000 when the laser is focused on the bottom surface of the well.

**Note:** These settings are only used when auto-focusing using the Laser Auto Focus and are not used when during image acquisition. They are independent of the acquisition settings set in the Screen Acquisition dialog box.

### Default

Lists the default values for the acquisition settings for the active objective. These setting are dependent on the NA of each objective and will update each time you change objectives.

### Set to Defaults

Restores the default settings to the active magnification settings.

### Laser use

#### Enabled

Enables the use of the laser when auto focusing.

**Note:** You may not wish to use the laser focus sensor for all magnification settings.

### Well Reflectivity

#### Dim

Sets an illumination setting suited for wells with little reflectivity, such as those with plastic plates or those using media.

#### Bright

Sets an illumination setting suited for wells with higher reflectivity This is useful when using some types of glass plates, or wells without any media, that are highly reflective. This setting uses a filter in the emission path to reduce the intensity.

### Exposure

Specifies the exposure time in milliseconds to be used when auto-focusing.

### Binning

Sets the binning used by the camera during the Auto Focus command. Horizontal and vertical binning are always set the same and should be set to less than four.

### Gain

Sets the sensitivity of the camera when used with the Auto Focus command.

### Accuracy

Defines the degree of focus accuracy in microns that this focus feature should attempt to achieve. A lower value specifies a greater degree of accuracy; a higher value specifies a lesser degree of accuracy.

### MaxStep

The maximum step size in microns of a single Z move to be used in attaining the correct focus position. This setting is dependant on the objective used. Use a smaller step size with higher NA lenses because the focus peak is narrower.

**Note:** Smaller step sizes typically require more steps to arrive at the final focus position.

### Test Sensor

Acquires a live image window showing the current auto focus settings image. Press F2 or Stop Test to stop updating the image.

### Focus Snap

Acquires an image using the current auto focus settings.

### Close

Closes the dialog box and saves the most recent settings.

## Screen Acquisition Dialog Box - Journal Tab

### Acquisition Step

Enables you to assign journals to run at specific time points during acquisition. Only one journal can be assigned to each time point. Click the check box next to the time point that you want to use, then click *Select* to assign the journal to the time point. After you have assigned a journal to a time point, you can temporarily deactivate the running of the journal by deselecting (unchecking) the check box for the time point. To reactive a pre-assigned journal, simply click the check box.

***Before each Image*** – Runs only during the acquisition loop, after the illumination is set and focusing is done.

***After each image*** – Runs only during the acquisition loop, after the shutter is closed and before images are saved.

***Before focusing*** – Runs only during the acquisition loop, just before focus evaluation begins.

***Focus evaluation*** – Runs whenever an image focus step occurs, through Find Sample or Wide focus button and during acquisition. It does not occur during laser focus steps.

***Start of site*** – Runs only during the acquisition loop, before any images are acquired from each site.

***End of site*** – Runs only during the acquisition loop, after all images have been acquired from each site.

***Start of well*** – Runs only during the acquisition loop, at the beginning of each well, before any images are acquired from a well.

***End of well*** – Runs only during the acquisition loop, at the end of each well, after all images have been acquired from a well.

***Start of plate –*** Runs after the stage is moved to the find sample position, but before the find sample action is performed.

***End of plate –*** Runs after the last acquisition for a plate is complete.

***Start of time point*** – Runs only during the acquisition loop, at the beginning of each time point, before any images are acquired for a time point.

***End of time point*** – Runs only during the acquisition loop, at the end of each time point, after all images have been acquired for a time point.

### Journal

Lists the names of the journals that you have assigned to each time point.

### Select

Opens the Select Screen Acquisition Journal dialog box. Use this dialog box to select and assign a journal to a time point. Also use this dialog box to deselect or unassign a journal to a time point. To assign a journal, click the checkbox for the acquisition step, click *Select*.

## Screen Acquisition Dialog Box - Loader Tab

### Configure Loader

Opens the ***Configure Plate Loader*** dialog box.

# Configure Plate Loader

**Defines the settings that provide the operational parameters and correct physical alignment between the Plate Crane and the Discovery-1 Imager.**

*Availability: Exclusive to Discovery-1*

**Drop-in: HTACQUIR**

Use this dialog box to make the necessary settings that enable the plate crane to accurately load and unload plates from the Discovery-1 imager.

***Note:*** These settings must be made correctly to enable the Plate Crane to load and unload plates from the Discovery-1 Imager without causing damage to the imager.

This dialog box contains controls that enable you to accurately determine and define for the Plate Crane the physical locations of the plate stacks and the stage-mounted plate holder, plus the horizontal and vertical limits of these locations.

***Caution:*** The settings you make for the plate crane are critical. An improperly made setting could cause the Plate Crane to come in contact the Imager body or objectives.

The Configure Plate Loader dialog box is divided into several areas associated with several different functions of the Plate Crane. The *Current Position* area is used to move the three separate plate crane motors that control the Vertical Position, Rotary Position, and Gripper Position. When calibrating the Plate Crane, all changes to the plate crane position from its present position to a new position are made using these settings.

***Caution:*** Do not physically/manually move the plate crane components such as the Arm, Turret, or Gripper to move the plate crane to a specific position for calibration. During calibration, control all Plate Crane movements using the Current Position settings.

See Also ***Configure Plate Loader dialog box options*** to configure the Plate Crane.

## Configure Plate Loader - Dialog Box Options

### Current Position

Indicates or sets the current vertical and rotational positions for the Plate Crane arm and the rotational position of the gripper. Also sets the vertical and rotary home positions. Use these settings boxes to change the position of the Plate Crane when making calibration settings.

#### *Vertical Position*

Indicates or sets the vertical position of the Plate Crane arm.

#### *Rotary Position*

Indicates or sets the rotary position of the Plate Crane arm.

#### *Gripper Position*

Indicates or sets the rotary position of the Gripper.

#### *Vertical Home*

Sets the current vertical position of the Plate Crane arm as the Vertical Home position.

#### *Rotary Home*

Sets the current rotary position of the Plate Crane arm as the Rotary Home position.

### Move Stage to Load

Moves the stage to the specified loading position

### Set Stage Load Position

Sets the stage loading position to the current stage position.

### Use stage load position for unloading

Specifies that the stage loading position will also be used for the unloading position. This box is checked as default. Uncheck this box to use a separate unloading position.

### Move Stage to Unload

Moves the stage to the specified unloading position.

### Set Stage Unload

Sets the stage unloading position to the current stage position.

### Rack Rotary Position

Provides a group of settings for calibrating the Plate Crane arm and tower to each available rack location. The gripper rotation angle that you set applies to all rack locations.

#### *Get Positions from Driver*

Loads a set of predefined rack position values that are stored in the driver.

#### *Gripper Rotation*

Specifies, in degrees, the gripper angle that should be used for accessing each rack location.

#### *Set to Current*

Sets the gripper location to the current gripper rotational position.

#### *Number of Racks*

Specifies the total number of racks that the Plate Crane is to access. **Note:** The minimum number or racks is 2; the maximum number of racks is 10.

#### *Go To Rack <#>*

Advances the tower rotational position to the position specified in the associated settings box.

#### *Set to Current*

Sets the rack location for the indicated rack to the current Plate Crane tower position.

### Lids

Specifies that all plates will have lids, and that each plate's lid must be removed and stored before the plate is loaded onto the stage.

### Bar Codes

Specifies that the bar code reader is installed and active.

### Plate Acquisition Position

Sets the location in which the Plate Crane deposits the plate onto the Stage. This position is a combination of values specified by vertical, rotational, and gripper rotational positions.

#### *Go To*

Moves the Plate Crane to the position specified by the combined values for Vertical, Rotation, and Gripper Rotation.

#### *Vert*

Specifies the vertical position for the Plate Crane arm. All locations below the home position are specified as negative values.

#### *Rot*

Specifies the rotational position of the Plate Crane tower.

***Gripper Rot***

Specifies the rotational angle of the gripper.

***Set to Current***

Sets the values for vertical, rotational, and gripper rotation to the current position values.

### Plate Acquisition Pickup Position

Specifies the position in which the Plate Crane must be in order to pick up the plate from the stage. This position is determined by the stage position and the relative position settings for Vertical, Rotational, and Gripper rotation. This can be a unique position or the same position as the plate acquisition.

***Use Plate Acquisition Position***

Specifies that the Plate Acquisition Position and the Plate Acquisition Pickup Position are the same. If the Plate Acquisition Position is different from the Plate Acquisition Pickup Position, make sure that the *Use Plate Acquisition Position* box is checked.

***Go To***

Moves the plate crane arm (vert) and tower (rot) to the specified location.

***Vert***

Specifies the vertical position for the Plate Crane arm. All locations below the home position are specified as negative values.

***Rot***

Specifies the rotational position of the Plate Crane tower.

***Set to current***

Sets the values for vertical and rotational to the current position values.

### Rest Position

Specifies the location of the Plate Crane rest position. This is the position in which the plate crane waits while acquisition is in progress. This position is usually a position between the rack locations and the Plate Acquisition position.

***Go To***

Moves the plate crane arm to the rest position.

***Vert***

Specifies the Plate Crane arm vertical location for the rest position.

***Rot***

Specifies the Plate Crane arm rotational location for the rest position.

***Set to Current***

Sets the values for the current vertical and rotational positions as the values for the Rest position.

### Lid Storage Position

Defines the location for plate lid storage. Specify values for both the vertical and rotational positions. Note: To use this option, *Lids* must be checked in the checkbox at the top of this dialog box.

***Go To***

Moves the Plate Crane to the vertical and rotational positions defined in the Vert and Rot boxes.

***Vert***

Specifies the vertical position to which to move the Plate Crane arm in order to store a lid.

***Rot***

Specifies the rotational position to which to move the Plate Crane arm in order to store a lid.

***Set to Current***

Sets the values in the Vertical and Rotational boxes to the values for the current vertical and rotational positions for the plate crane.

### Bar Code Reading Position

Specifies the location in which a plate must be in order to read a bar code label on the side of the plate.

***Go To***

Moves the Plate Crane to the Bar Code Reading Positions as specified by the associated settings for Vertical, Rotational, and Gripper Rotation.

***Vert***

Specifies the vertical position of the Plate Crane arm for the Bar Code Reader Position.

***Rot***

Specifies the rotational position of the Plate Crane arm for the Bar Code Reader Position.

***Gripper Rot***

Specifies the Gripper Rotation position of the Plate Crane arm for the Bar Code Reader Position.

***Set to Current***

Sets the Bar Code Reader Position to the current Plate Crane position.

***Test Bar Code***

Tests to see if the bar code reader can visualize and decode a bar code.

### Heights

Specifies the thickness of both plate and lid, plus the amount of vertical travel needed to locate the first or bottom plate on a rack.

***Plate***

Specifies the height or thickness of an individual plate without a lid.

***Lid***

Specifies the amount of additional thickness added to a plate when a lid is covering the plate. Using the combined values for Plate and Lid thickness, the program can determine the amount of total vertical travel needed when locating the next plate on the rack.

***Run and Set***

Runs a test to determine the heights of the plate and plate lid, and to set these values in the respective setting boxes.

***Vertical position of Bottom Plate***

Specifies the vertical location of the lowest plate for all rack locations. This value serves as a lower limit when attempting to find a plate in a rack.

***Set to Current***

Sets the current vertical position of the plate crane arm as the vertical position of the bottom plate.

### Find Plate

Initiates the *Find Plate* procedure to find the first available plate on the selected rack.

### Close Grippers

Manually closes the plate grippers.

### Open Grippers

Manually opens the plate grippers.

### Run Test

Runs the complete test procedure to test all positions. This includes removing and parking a plate lid and reading the bar code on the edge of the plate, loading a plate onto the stage, and removing the plate from the stage***. Note:*** This test runs the complete loading and unloading cycle from start to finish. Be sure that you have the appropriate plates on the racks to complete the test.

### OK

Applies the settings that you have made and closes the *Configure Plate Loader* dialog box.

# Screen: Set Alignment (Apps Menu)

**Aligns images from two or more wavelengths to compensate for the image shift that results from the variation in light transmission at different wavelengths.**

**Drop-in: HTACQUIR**

Use this dialog box to interactively apply color shift correction (Alignment Cropping) to one or more images of the same sample acquired at different wavelengths. Set alignment creates a stack from the acquired images and applies horizontal and/or vertical shift values to each selected image.

## Setting Image Alignment

To align the images of two or more wavelengths, complete the following procedure.

| Step | Action |
|---|---|
| **1** | From the Screen Acquisitions Wavelengths tab, click *Set Alignment*. The *Screen: Set Alignment* dialog box opens. |
| **2** | Click *Acquire Alignment Stack*. |
| **3** | In the Display group, choose *Subtract* or *Average*. |
| **4** | Move the horizontal and vertical sliders to set the Horizontal Shift and Vertical Shift values.· |
| **5** | Click *Zero Shift* to reset the horizontal and vertical values to Zero. |
| **6** | Click *Previous* or *Next* to change images. |
| **7** | Click *OK* when you are finished setting the alignment of your wavelengths. |

## Screen: Set Alignment - Dialog Box Options

### Acquire Alignment Stack

Acquires an image at each wavelength and builds a stack that can be used in adjusting alignment.

### Display

Selects the method to be used to display differences between the reference plane and the shifting plane:

***Subtract*** – uses subtraction to show the difference between the reference plane and the shifting plane. The planes will be aligned when there is nearly a uniform grayscale level throughout the entire image.

***Average*** – uses averaging to display the offset between the two planes. The aligned plane should look like the original plane with as little blurring as possible.

**Horizontal Shift (text box and slider)**
Adjusts the horizontal alignment of the plane in one-pixel increments

**Vertical Shift (text box and slider)**
Adjusts the vertical alignment of the plane in one-pixel increments.

**Zero Shift**
Resets the horizontal and vertical shift to zero.

**Previous**
Places the previous plane in the alignment image window.

**Next**
Places the next plane in the alignment image window.

**Cancel**
Cancels your adjustments and closes the dialog box.

**OK**
Applies the shift to all of the wavelengths.