Life Science Software

Installation and User Instructions

Doc-It®LS Image Acquisition Software
Doc-It®LS Image Analysis Software

VisionWorks®LS Image Acquisition Software
VisionWorks®LS Image Acquisition and Analysis Software

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CHAPTER ONE: WELCOME TO LIFE SCIENCE (LS) SOFTWARE

- Introduction
- Minimum System Requirements
- Installation Instructions
- Secure User Accounts
- Usernames and Passwords
- Matrix of User rights

INTRODUCTION

Life Science (LS) software from UVP lets you acquire images, enhance them and analyze images in a simple and efficient way. There are two software packages in the series, with two sets of capabilities in each:

A. VisionWorks®LS

The package with advanced acquisition and analysis features. Available as:

- Image Acquisition (Capture) [symbol VC]
- Image Acquisition and Analysis [symbol VA]

B. Doc-It®LS

The package with basic acquisition and analysis features. Available as:

- Image Acquisition (Capture) [symbol DC]
- Image Analysis [symbol DA]

You can use the software to image electrophoresis gels (DNA, RNA, Protein), blots, membranes, TLC plates etc. using various kinds of cameras. Once you have captured an image, you can save it for your records, use various effects to show hidden detail, manipulate it to set it up for better analysis, annotate it to point out key features and perform various types of analysis.

This help manual is common for all four packages of the above. With every feature, there is a symbolic indication, as to in which software is the feature available.

VC VA

DC DA

MINIMUM SYSTEM REQUIREMENTS

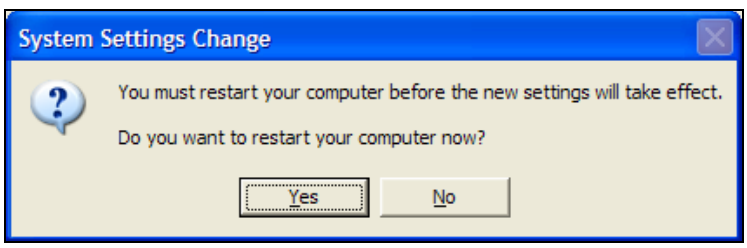
- Operating System:
  - Windows 2000 with Service Pack-4 or higher
  - Windows XP Professional with SP2

- Internet Explorer 6.0 or higher [To determine the version of Internet Explorer, open Internet Explorer and click on **Help** > **About**]
- Intel Pentium Processor or equivalent, 166mHz or higher
- 128 MB of RAM or greater
- 100 MB of available hard disk space for the program, more for data
- If you want to avail the functionality of 21 CFR Part 11, then the partition must be formatted with NTFS.
- CD-ROM drive
- Color monitor, supporting at least 1024 x 768 resolution and 16-bit or better colors; 24-bit or 32-bit color is strongly recommended
- Microsoft Outlook or Outlook Express, if you want to enable the email feature. Email feature lets you designate an administrator's email address. LS can initiate sending an email to that address when a user profile is being tampered with. If you do not have Outlook, LS will still work fine; only the email feature will not be available.

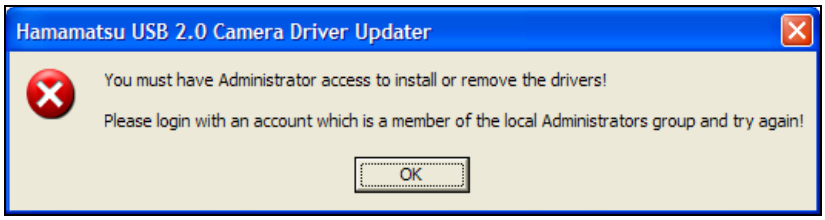
INSTALLATION INSTRUCTIONS

1. Installation requires your complete attention all through the process. It is not configured to be an unattended installation.
2. Log on to the computer with an account that has Administrative privileges. It is best if you are the actual Administrator, responsible for management of the software, so that you can make certain decisions right during installation process. **This description will assume you have this authority.**
3. Make sure that the computer meets minimum requirements as stated above.
4. Uninstall any previous version of Doc-It®LS software, Doc-It software, VisionWorks®LS software, and BioSpectrum®AC software from the computer. You can check the presence of existing software from **Control Panel > Add Remove Programs**. Use Windows Install Cleanup utility provided on the LS Software CD if there are problems with this un-installation process.
5. Insert the CD into the drive. Installation to start automatically. If it does not, then navigate to the folder **LS Main** on the CD and click on **setup.exe**.
6. Follow instructions to install the software.
7. Select the correct Darkrooms and Camera Drivers to install. Let the installation process begin.
8. Upon completion of the software installation, LS displays the Main Installation menu.
9. To install the correct camera drivers, click the **Cameras** button and select the appropriate camera.

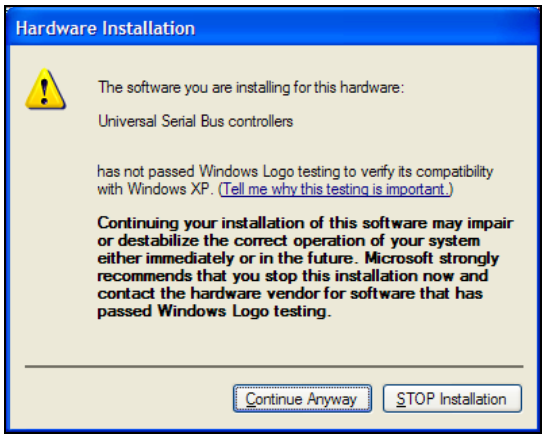
During the process, it may present you with dialog boxes that ask if you want to restart your computer. They look like the following. **This is not an error.** Click **No** to continue with installation.



Depending on the camera drivers that are selected, you may also get the following type of dialog-boxes. **This is not an error.** Click 'Ok' and move on.



Following window suggests that the camera drivers have not passed Windows Logo Testing suggested by Microsoft, which is not a problem. Click **Continue Anyway**. If you click on **Stop Installation**, you will not be able to use the selected camera.



During the installation, LS will provide an option to enable user account changes to be emailed to an Administrator. Click **Yes**, if you want to enable the software to send an email to the Administrator in case of possible tampering with user accounts (detailed instructions available in the chapter concerned with 21 CFR Part 11 support). You must have either Microsoft Outlook or Microsoft Outlook Express installed. If you clicked **Yes**, enter the email address of the Administrator in the following step. Finally, restart the computer when prompted.

Once the LS software is installed, it will operate in full-future trial mode for 14 days. Within the 14-day trial mode, the LS software must be registered with UVP. Otherwise the software will only operate in demonstration mode after the 14-day trail period. The demonstration mode limits the software to only open and use the demonstration images provided by UVP.

Refer to Chapter 2 for registration instructions.

SECURE USER ACCOUNTS

The concept of User Accounts for individual users is central to LS software by providing security of user's data from being tampered with by other users, accidentally or otherwise.

- This system of usernames and passwords is separate from the one that you use to login to the computer.

- Setting up user accounts is mandatory if you need 21 CFR part-11 support from LS software. Refer to the relevant chapter in this manual for more information.
- If, you decide not to setup individual accounts, you can create just one account for all users and give full permissions to that account.

VC VA DC DA

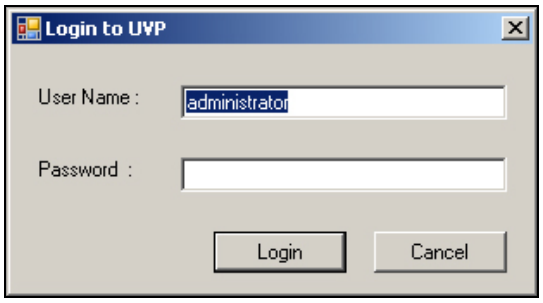
USERNAMES AND PASSWORDS

Start the software. It will bring up a Login window.

The administrator user name will show.

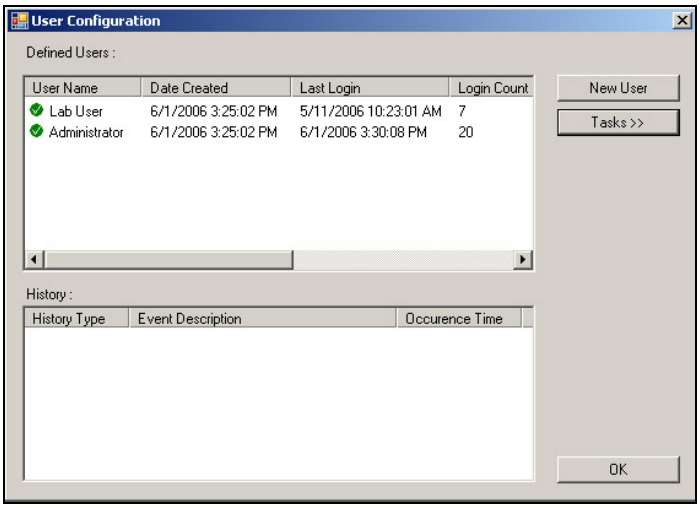
Enter a password. On initial installation, a screen will pop up to request a password and password confirmation. A password is required.

Click **Login**.



To Add a new user:

From the menus, click **Tools > User Administration** to open the User Configuration window.



Click the **New User** button, type in the user name and password. Then each time you login, use that new user name and password.

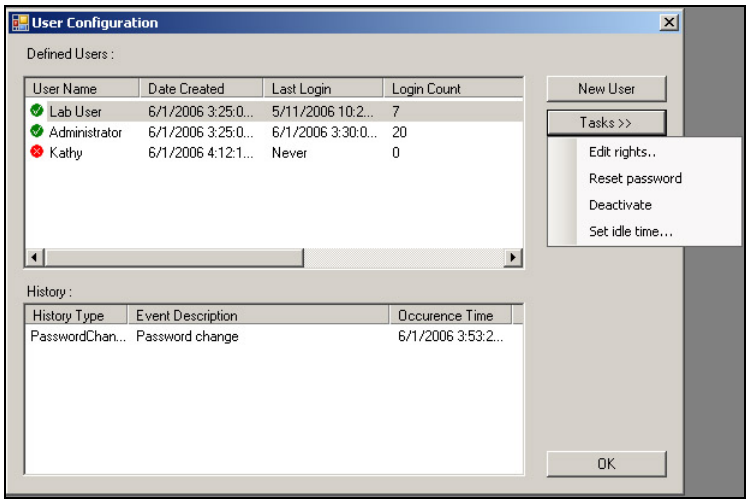
To Edit a new user:

Edit the New User by highlighting the user name in the User Configuration window, click the **Tasks > Edit Rights** and select an option shown below from the Define User Permissions screen.

Click **OK** when changes to the new user are complete.

To change a password and other settings:

To change a password, from the menu go to **Tools > User Administration** to open the User Configuration window. Click on the appropriate user to change. Select the **Tasks > Reset Password** and enter the new password. Enter the password again to confirm the change. Click **OK**. The change in password will be noted in the **User Configuration > History** box.

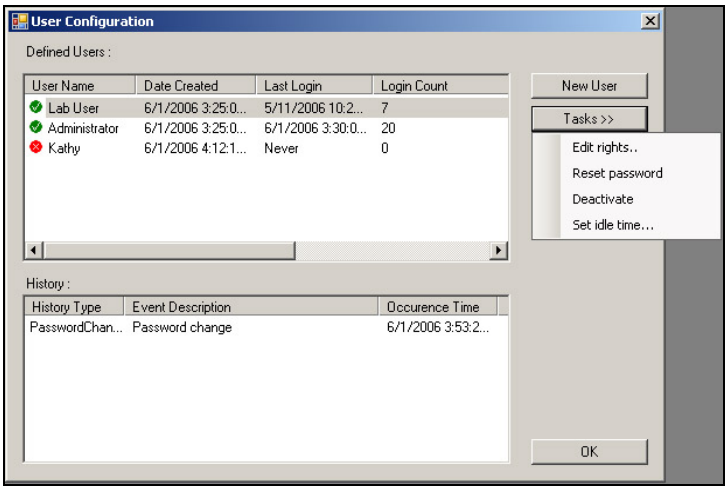


The description of the settings for each column of the defined user is shown below. To see all of the columns, move the scroll bar to right.

Column Heading	Description
User Name	Unique Identification name for a particular user. This could be a name or a word that makes it easier to identify the user.
Date Created	Date the user name was created.
Last Login	Date of last login.
Login Count	Number of times the user has logged in.
Idle Time Lock	Indicates the maximum idle time. To change the idle time, click **Tasks > Set Idle Time.** Zero means no idle time.
Password Expiration Date	Date the password expires.
View	Enables user to view images. To change this setting, click **Tasks > Edit Rights**. Click or unclick the View option.
Change	Enables user to change images. To change this setting, click **Tasks > Edit Rights**. Click or unclick the Change option.
Has Admin. Rights	Gives user administration permissions. To change this setting, click **Tasks > Edit Rights**. Click or unclick the Has Administration Rights option.

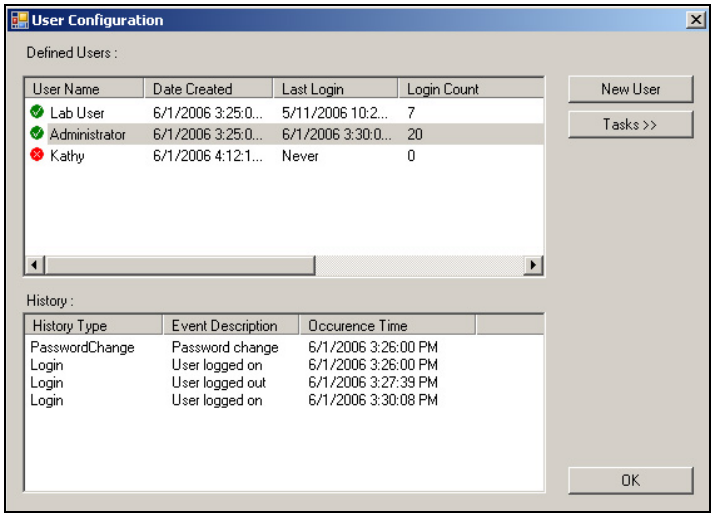
To deactivate or reactivate a user:

From the menu, select **Tools > User Administration** to open the User Configuration window. Select that user name and click **Tasks > Deactivate**. A red X will indicate the user is deactivated. To reactivate, click **Tasks > Activate.** Click **OK** to close the window.



To View the login history of a user:

Select a user name in the User Configuration table. The lower half of the window displays the login history associated with the selected user. Click OK to close the window.



MATRIX OF USER RIGHTS

Depending on the privileges for the user that has logged onto windows, the following rights are available to that user for LS software:

OS	Login Privileges	Rights				
		Install	Un-Install	Open and Run	User Administration	Use the Camera
Windows 2000 SP4 or Later	Restricted			X		X
	Standard/Power			X		X
	Admin	X	X	X	X	X
Windows XP Pro SP2 or Later	Restricted			X		X
	Standard/Power			X		X
	Admin	X	X	X	X	X

X = Supported rights.

Note that even though you may be able to do things with LS software which are outside of this matrix, UVP neither recommends it, nor supports it. For example, you may try (successfully or otherwise) to uninstall LS software as a Power User, but UVP does not provide support for problems arising during or due to that action.

CHAPTER TWO: SOFTWARE REGISTRATION AND ACTIVATION

OVERVIEW

Use of LS software requires activation of a security code from UVP. Registration and activation of the software can be accomplished by email or by phone. Two types of licenses are available, single-user or network-user with a five-user license.

SINGLE-USER

Single-user license allows the software to be used on a single computer.

NETWORK-USER LICENSE CONFIGURATION

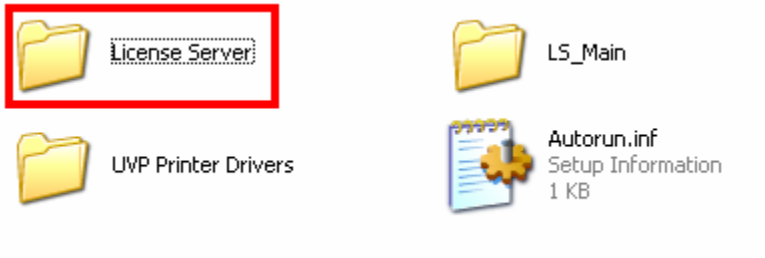
1.

A network license allows multiple users on the same network to operate VisionWorks LS simultaneously, with only one PC (server) having to register the software.

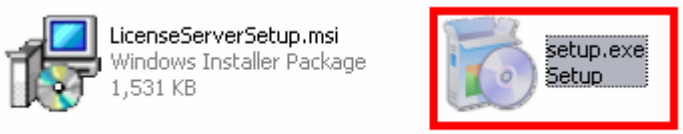
Follow these steps to install and use the network license:

1. Identify one computer on your network to hold the network license. This computer will be called the “Server”. Also, it must be running Windows XP (SP2) OR Windows 2000 (SP4).

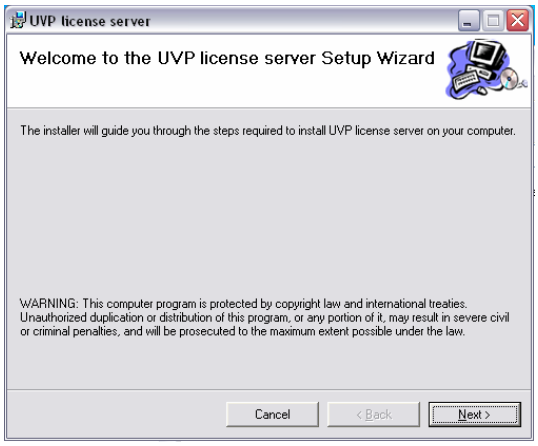
2. From the VisionWorks LS Disk 1, navigate to the License Server folder.



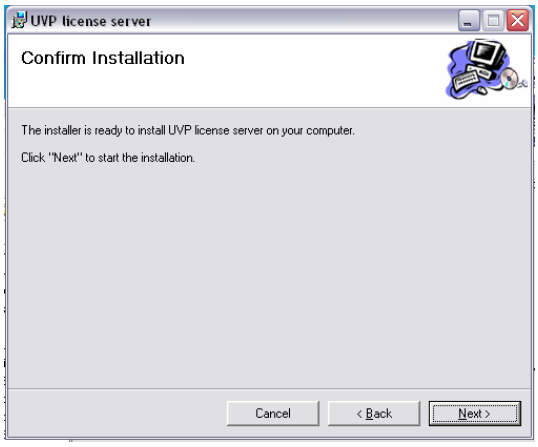
3. In the License Server folder, select the Setup.exe to begin the Network License configuration.



4. After selecting the Setup.exe, you will be presented with a simple Installation Wizard to assist with the installation. Select Next to continue.

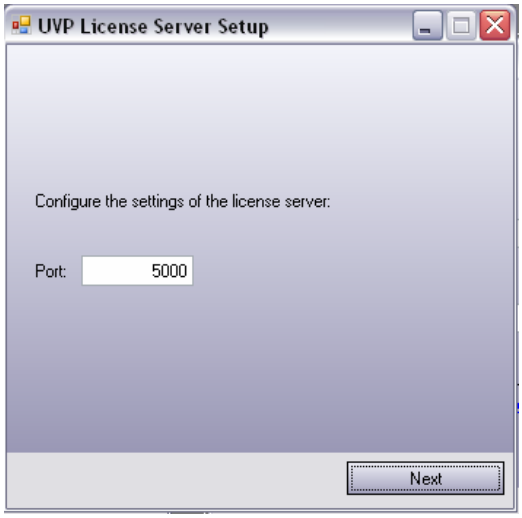


5. Click Next to confirm you would like to install the License Server.

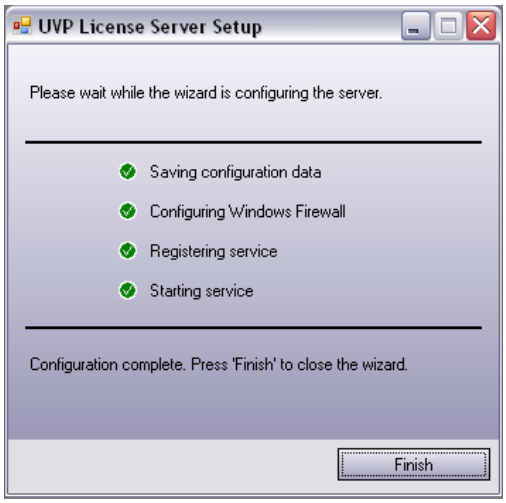


6. The next screen will require you to enter the Activation Number that was previously used for your VisionWorks LS installation. Enter the provided Activation Number, and click Next.

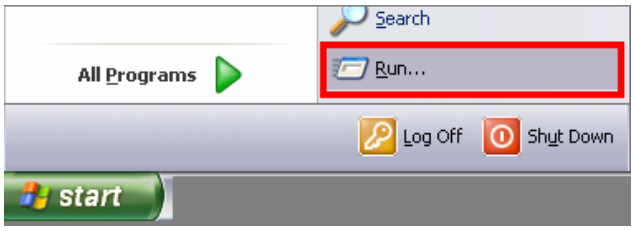
7. The next screen will verify the “Port” number to be used. It will be necessary to note this port number, as this is the port that the other/client PC’s will use to access the software license. Generally, the port number automatically provided will not need to be altered. Click next after noting the Port number being used.



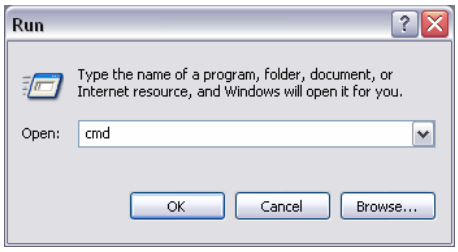
8. The next screen will display the configuration status as it is applied. When the configuration is complete you will see text on the bottom portion letting you know the configuration has completed. Click finish.



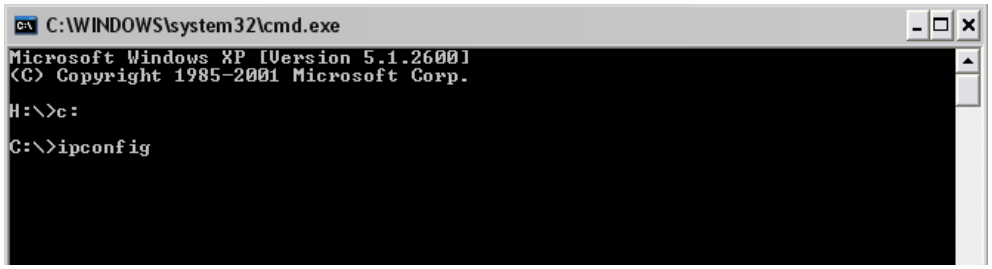
9. Next it will be necessary to obtain the "Server" computer's IP Address. This address will be used to set-up the other "client" computers, so that they will know where to locate the VisionWorks LS Network License. Click the Windows Start button and select the Run option.



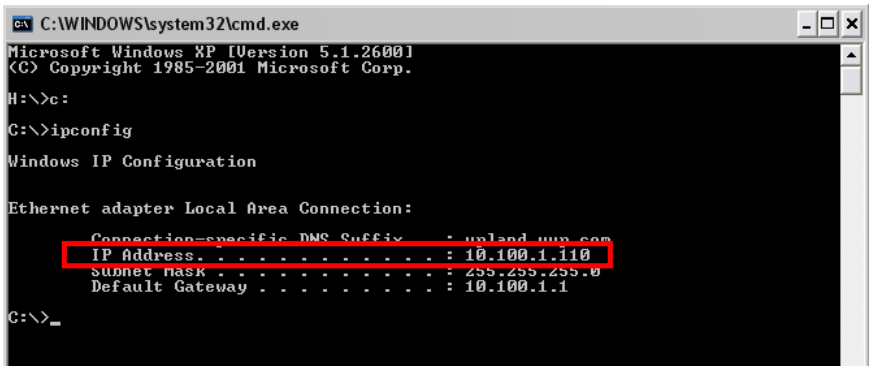
10. In the Run dialog box, type "cmd" (as pictured). Then click OK.



11. Next we will type the command to display the IP Address of this PC. Type "ipconfig" (as pictured), and click Enter.

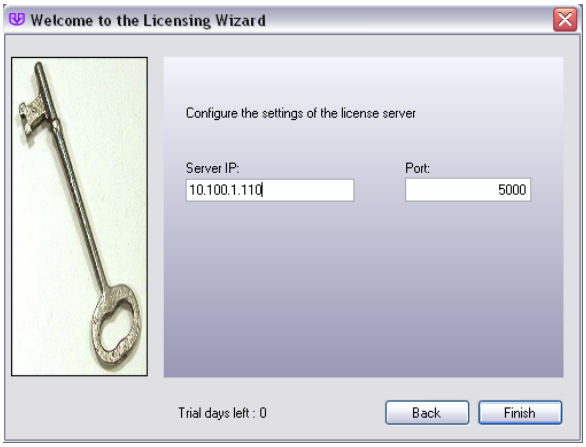


12. You will now see the IP Address listed. Please note this down, as it will be used on the other “client” computer’s License Manager setup. After noting the IP Address, you may close this Command Dialog.



13. On the other “client” computers, open VisionWorks LS. You should be presented with a Licensing Wizard screen. Select the Server Based Licensing option, and select Next.

14. This screen is where you will enter the previously noted IP Address and Port Number form the “Server” computer. Click Finish once you have entered those two values.

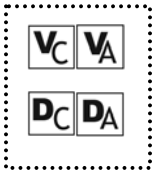


15. Next you will see a message letting you know the License Server installation/configuration was successful. Click OK, and close any other dialogs still open.

16. Repeat steps 13-15 for any other "client" computers to be configured.

If you did not receive the message letting you know the installation was successful, or you have any question, please contact UVP Technical Support.

HOW TO REGISTER THE SOFTWARE



LS applications will need to be activated by entering an activation code provided by UVP in order to gain full access rights to the software. There are three ways to retrieve an activation code: phone, email or Internet.

Register by Phone or Email

Call UVP in the US at (800) 452-6788 or (909) 946-3197 or email to softwareactivation@uvp.com. Provide the following information:

- Configuration number: this number is displayed at **Help > License Wizard**
- Serial number: this number is provided with the installation package
- User contact name
- Company/institution name
- Department
- Company address
- Email address
- Phone number

UVP will verify the serial number and provide an activation code.

Register by Internet

To register your software and obtain an activation code, go to UVP's web site at reg.uvp.com and be prepared to supply the information requested above.

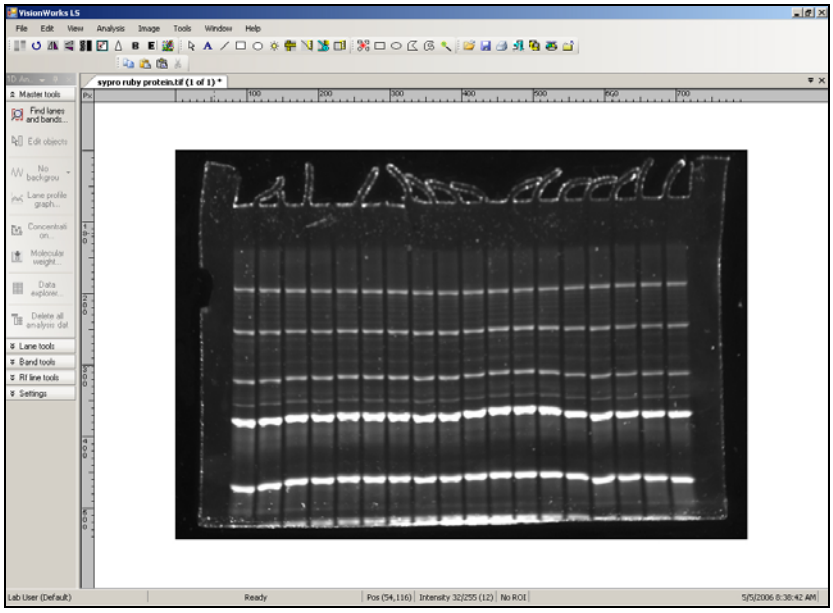
Activate your software

1. Open **Help > License Wizard**.
2. Select either Single client access license or Server based licensing (network). Click **Next**.
3. Enter the Activation code provided by UVP. Click **Finish**.

CHAPTER THREE: WORKSPACE NAVIGATION

- Main Window
- Menus
- Toolbars
- Plug-in Modules
- Image Windows
- Status Bar

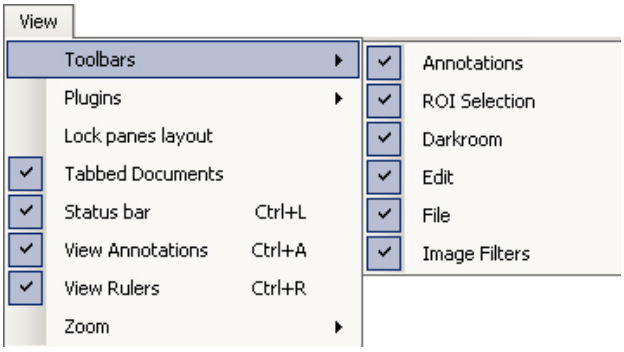
MAIN WINDOW



The LS Main Window contains the application's menu bar, toolbars, image windows, plug-in modules and status bar. Some parts of the window, such as the toolbars, plug-in modules and status bar, can be hidden or shown as you prefer.

To Show or Hide the Toolbar

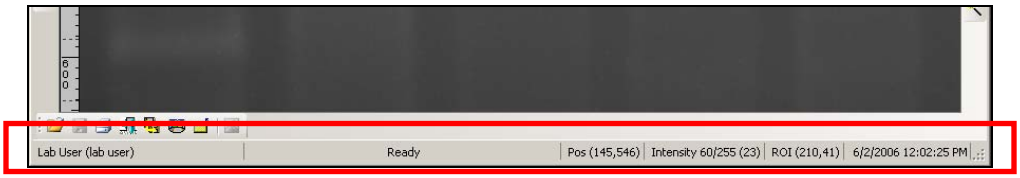
On the **View** Menu, choose **Toolbars**. All of the default toolbars and the space they occupy just below the menus will be hidden or shown. Click the tool buttons shown below to select or hide.



Customizing the toolbars is explained later in the toolbars section of this chapter.

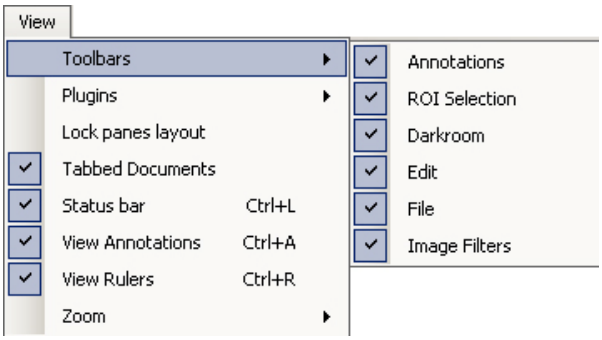
To Show or Hide the Status Bar

On the **View** Menu, click **Status Bar**. The status bar will be hidden or shown.



To Show or Hide the Plug-In Modules

On the **View** Menu, choose **Plugins**. Click the plug-in name to select or hide.



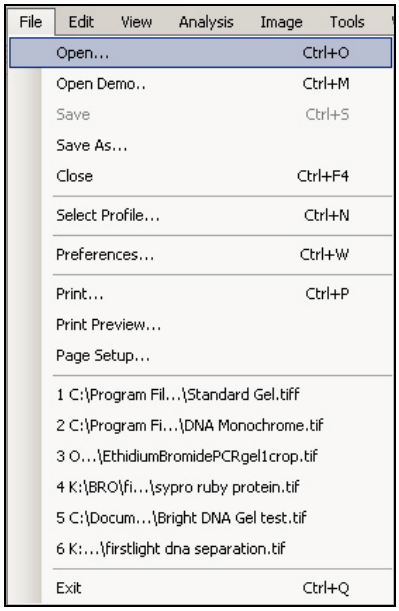
When a camera plugin is hidden, you cannot control the camera or change the exposure settings.

MENUS

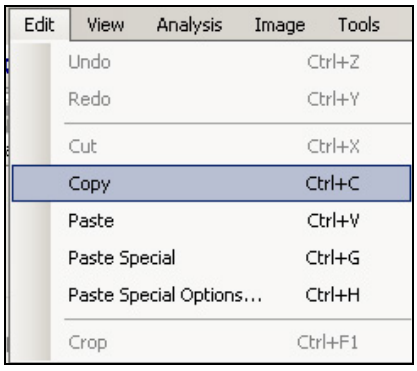
LS Software offers the following menus:

Although most commands appear on the menus, some features are only available through the Plug-in Modules. If you hide the Plug-in Modules, you can show them with selecting the models from **View > Plugins** whenever you need them.

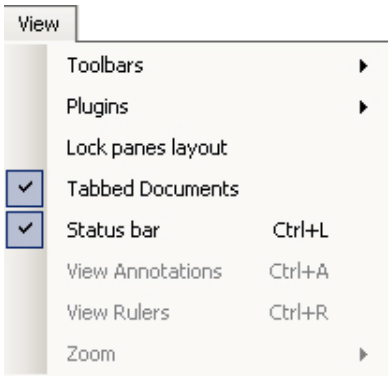
- ***File Menu***: Contains commands to load and to save files, to select profiles, adjust preferences and to print reports.



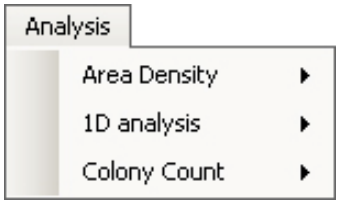
- ***Edit Menu***: Contains the Undo/Redo commands and the clipboard commands: Cut, Copy, Paste, Paste Special and Paste Special Options, plus the Crop function.



- ***View Menu***: Contains commands that show and hide various main-window features and commands that affect how the current sub window is displayed.

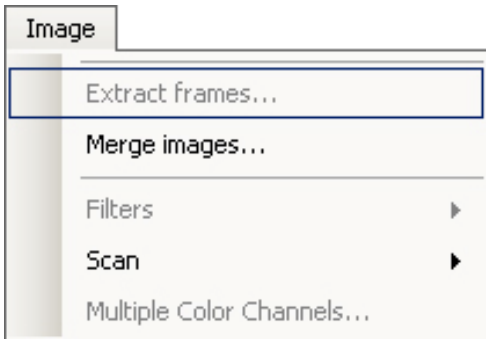


- ***Analysis Menu*****:** Contains commands to perform various types of analysis. Availability in different packages is as follows:

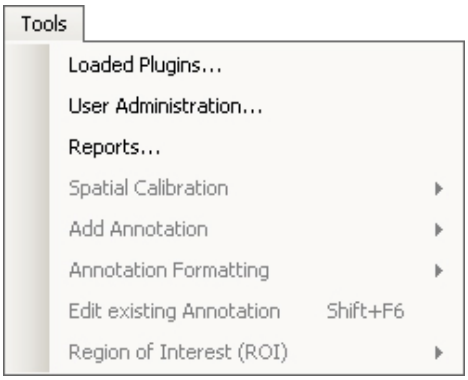


	VisionWorksLS Acquisition + Analysis	**Doc-ItLS Analysis**
1D Analysis	Available	Available
Colony Count	Available	Available
Area Density	Available	-

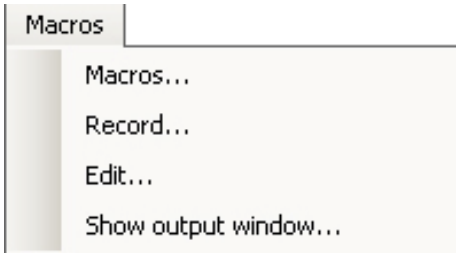
- ***Image Menu***: Contains commands that show the Scan options, extract frames, merge images, multiple color channels and commands for using different image filters.



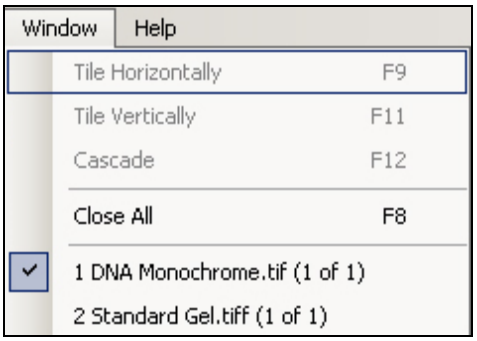
- ***Tools Menu***: Contains a list of the tools you can use to interact the software configurations with an image, including seeing Loaded Plugins, User Administration, pre-configured image Reports, plus Region of Interest (ROI), Spatial Calibration and Annotation tools.



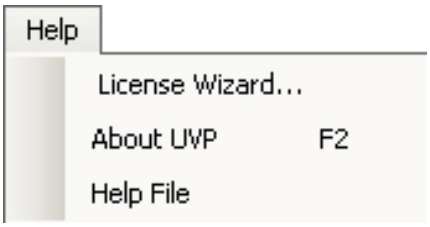
- ***Macros Menu*****:** Contains commands to work with Macros. A Macro is a collection of commands that can be run at one go.



- ***Window Menu***: Contains commands to organize Image windows. Shows the arrangement of the image window and shows the name of the images currently open.



- ***Help Menu***: Contains access to the software help, user license wizard and software version.



TOOLBARS

The toolbars in LS allow you to select most commands with a single button click. The toolbars are customizable, so you can include the commands you use most and remove commands you rarely use.

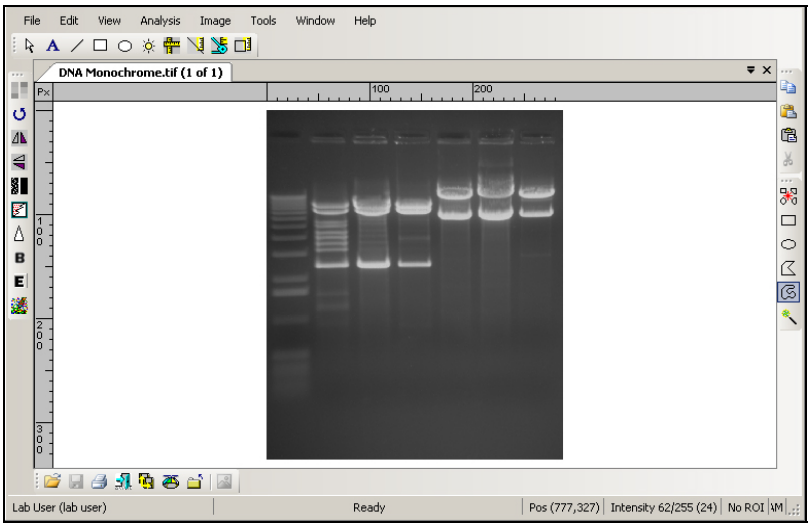
Toolbar Buttons

The initial default buttons include:

- ***Reduce to Mono***: Converts the image to a single-channel (monochrome) image from a multi-channel (color) image
- ***Rotate***: Brings up the dialog box to rotate image by a specific degree or by manual rotation
- ***Flip Horizontal***: Flip image horizontally
- ***Flip Vertical***: Flip image vertically
- ***DeSpeckle:*** Applies the despeckle filter to the image
- ***Remove Noise:*** Applies the the remove noise filter to the image
- ***Sharpen:*** Applies the sharpen filter to the image
- ***Blur:*** Applies the blur filter to the image
- ***Emboss:*** Emboss the image with selection of the direction of the emboss filer
- ***Starfield Subtraction:*** Applies the starfield subtraction filter to the image
- ***Copy:*** Copies selected text, selected portions of the current image or the entire image to the clipboard
- ***Paste:*** Pastes the current clipboard item onto the screen
- ***Paste Special:*** Pastes an overlay of the current item in the clipboard
- ***Cut:*** Cut a specific area
- ***Undo***: Undo last command
- ***Redo***: Redo last command
- ***Arrow:*** Select an annotation to edit
- ***Text Tool:*** Text annotation
- ***Line, Rectangle, Ellipse and Highlighter Tools:*** Annotation tools
- ***Define Image Scale:***
- ***Measure length:*** Lets you measure the distance between any two points on the image, units depend on spatial calibration
- ***Measure Angle:*** Lets you measure an angle
- ***Measure Area:*** Lets you measure an area on the image
- ***New ROI:*** Removes the active Region of Interest and prepares for a new one of the current type
- ***Rectangular ROI***: Changes the current mouse tool to the select Rectangular Region of Interest and brings up one if already present on the current image
- ***Elliptical ROI***: Changes the current mouse tool to the select Rectangular Region of Interest and brings up one if already present on the current image
- ***Polygonal ROI:*** Changes the current mouse tool to the select Rectangular Region of Interest and brings up one if already present on the current image
- ***Freeform ROI:*** Changes the current mouse tool to the select Rectangular Region of Interest and brings up one if already present on the current image
- ***Magic Wand ROI:*** Lets you select a consistently colored area (for example, a red flower) without having to trace its outline
- ***Open***: Opens an image file on disk
- ***Save***: Saves the current image to disk
- ***Print***: Prints the current image
- ***Exit***: Closes the software program
- ***Save As***: Saves the current image to a different name
- ***Preferences***: Opens the preferences window to set defaults in the following tabs: Main Settings, Analysis, Cameras, Miscellaneous and Hardware
- ***Close***: Close the software program
- ***External Application***: Launches a Qwik-Link external application selected in Preferences.
- ***Start Darkroom Hardware***: Interface for the BioSpectrum darkroom.

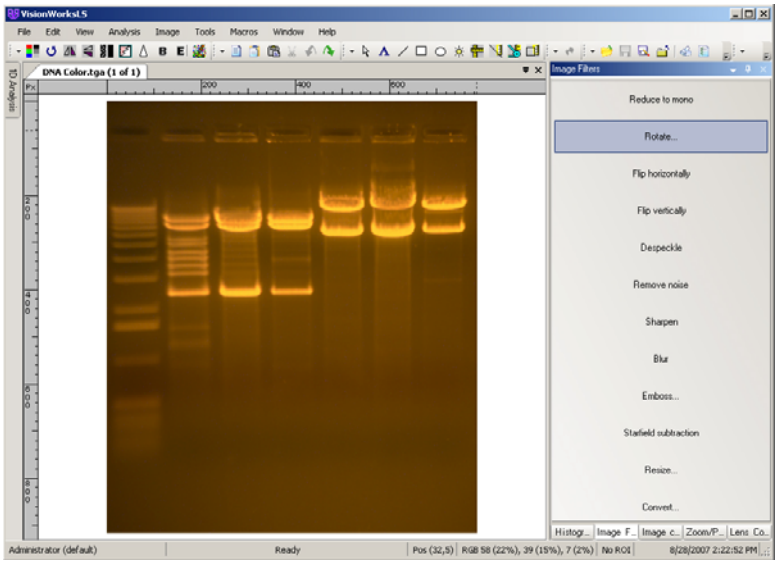
Rearranging Toolbars

You can rearrange the order of the individual toolbar sections by placing the mouse arrow over the dotted line at the edge of each toolbar section and moving the toolbar. The default position of the toolbars is located horizontally below the menus. The toolbars may be moved to the bottom of the screen or vertically on the left or right side. Each section can be deleted or added back by clicking **View > Toolbars** and select the toolbars to display on the screen.



PLUG-IN MODULES

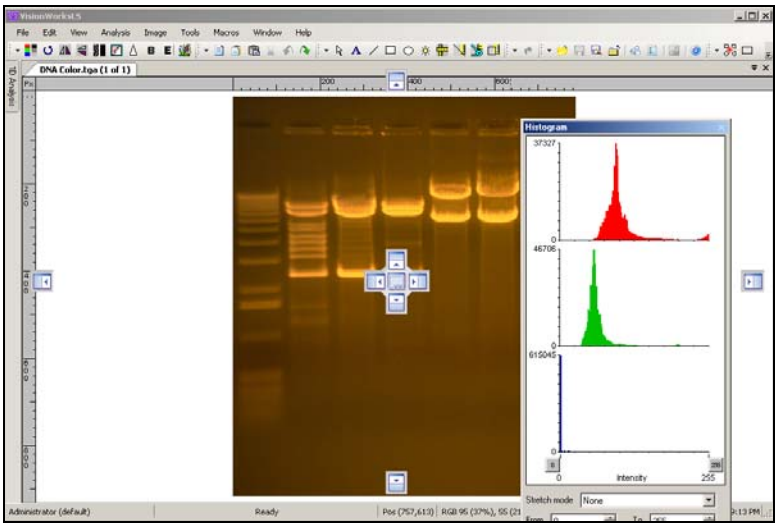
The plug-in modules in LS allow you to position modules on your work area for easy access to the plug-in functions. Position of the modules is customizable, so you can include the plugins you use most and remove plugins you rarely use. When multiple modules are open, the modules will show as tabs at the bottom of the plugin or as tabs along the side of window.



Rearranging Plug-in Modules

You can position the modules anywhere on your screen and several ways you can select visibility of these modules: Dockable, Hide, Floating, Auto Hide, Lock Panes Layout.

- ***Dockable***: Sets the module in a specific position
- ***Hide***: Closes the module. To reopen, go to **View > Plugins** and select the plug-in module
- ***Floating***: Allows module to be moved around on the screen. To move a module, click the top bar of the module and hold the left mouse button drag the module. Arrows will display. Move the module to one of the arrows to dock the module.
- ***Auto Hide***: Automatically hides the module when not in use. These tabs will be displayed in the same order as they were selected to Auto Hide. To show the full module, roll your mouse over the tab. To disable the auto hide function, unclick Auto Hide from the module drop down menu or click the push pin icon
- ***Lock Panes Layout***: Locks the plugins into the selected position.



Moving a Floating Plug-in Module

When a plug-in module is floating, it can be docked to the top, bottom, left or right on the workspace. A floating plug-in module can be placed into another plug-in module's window.

To move a floating plug-in module, click on the title bar of the module and drag it to any of the plug-in module position icons of choice. The following are descriptions of the plug-in module position icons.

	Position the plug-in module at the ***top*** of the workspace
	Position the plug-in module at the ***bottom*** of the workspace

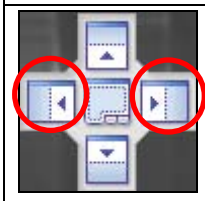
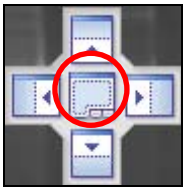
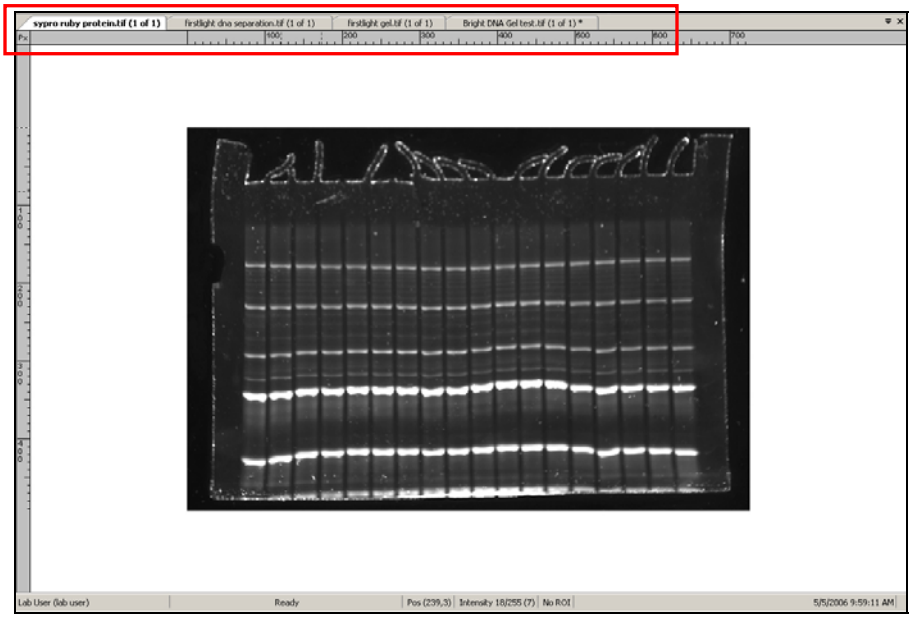
	Position the plug-in module at the ***left*** of the workspace
	Position the plug-in module at the ***right*** of the workspace
	The center arrows allow positioning of the plug-in module at the ***top, bottom, left or right of an empty*** workspace
	Position the plug-in module at the ***inside*** of another plug-in module

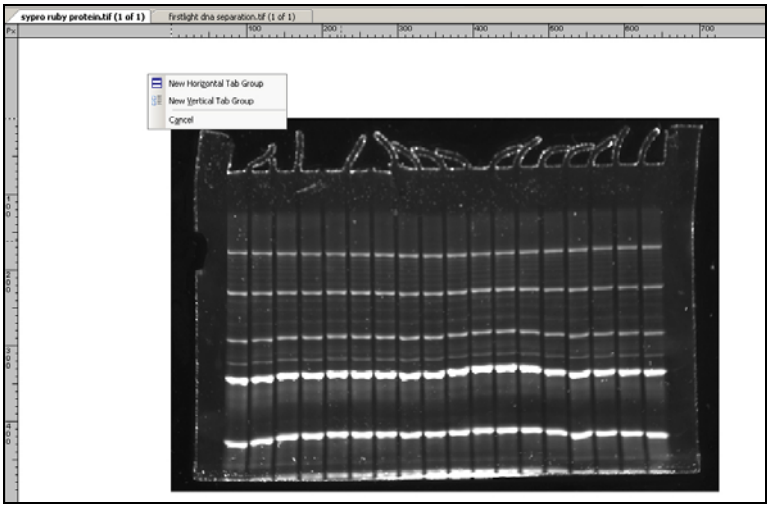
IMAGE WINDOWS

Each image that you generate or open in LS workspace will appear in a separate Image window. You can have several Image windows open at one time. The window below shows several images open, layered with tabs (tabbed interface turned ON in Preferences) for selection of images.



Organizing Image Windows

- Images can be visible in work area with either tabbed layout, shown above or cascaded images. To change from tabbed layout to cascaded layout, on the menu, go to **File > Preferences > Main Settings**.
- To *bring an Image window to the forefront*, either click on the window's title bar or find and select the image's filename from the list in the **Windows** menu.
- To *move a cascaded Image window*, drag the window's title bar. To move a tabbed image, drag the tabbed title bar down slightly. To tiles the images, right mouse click on the tabbed bar. Select either New Horizontal Tab Group or New Vertical Tab Group.



- To *resize a Cascaded Image window*, drag the lower right corner (or an edge) to the desired size.

Information Provided by the Image Window

Besides displaying an image, the Image tab tells you the filename of the image.

A caption of "Untitled" means the image has not yet been saved.

Showing the Image in Actual Size

To show the image in actual size (no scaling), choose **View > Zoom**. Set the zoom factor to 100%. Or click the right mouse button on the image and select View Original Size.

Fitting the Image to the Window

To show the entire image in the window (scaled up or down as required to make it fit),

click the right mouse button and click **View Best Fit**.

Context Menu Commands

A context (shortcut) menu appears when you click on the image itself with the right mouse button. It is a shortcut menu that lets you sidestep using the MENUS OR TOOLBARS. Once you bring it up, treat it as a regular menu by selecting features from the list.

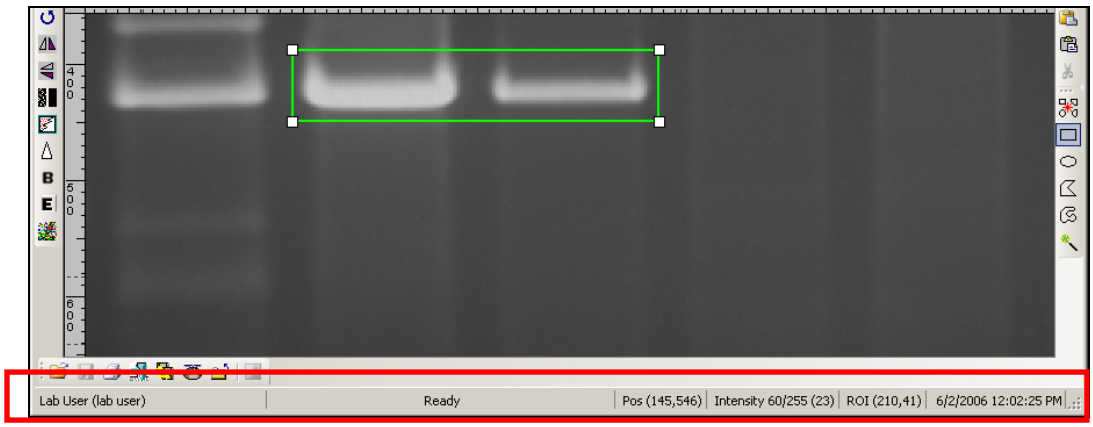
Click on the image with the right mouse button, a menu with the following commands opens:

- **Copy**
- **Paste**
- **Paste Special**
- **Print Selection**
- **View Best Fit**
- **View Original Size**
- **Print**
- **Image Information**

Both the **View Best Fit** and **View Original Size** commands are also available from a shortcut menu on the Image window itself. The shortcut menu can be displayed by right clicking on the image.

STATUS BAR

The Status Bar shows the User Name (Profile), current mouse position in an image, the intensity of the image at that position and status messages during operations. Current date and time is display in the right corner.



The mouse position (POS) is displayed in pixels (X and Y). The Intensity is displayed as a single value if the image is monochrome and has three values (Red, Green and Blue) if the image is colored. In both cases, the value is reported as a percentage value of the maximum intensity.

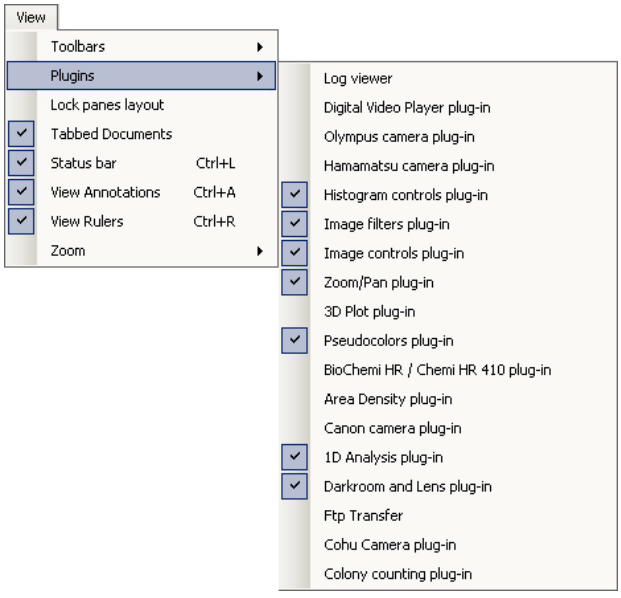
CHAPTER FOUR: PLUG-IN MODULES

- Log Viewer
- Digital Video Player
- Olympus Camera
- Hamamatsu Camera
- Histogram Controls
- Image Filters
- Image Controls
- Zoom/Pan
- 3D Plot
- Pseudocolors
- BioChemi / Chemi HR 410
- Area Density
- Canon Camera
- 1D Analysis
- Darkroom and Lens
- Ftp Transfer
- Cohu Camera
- Colony Counting

OVERVIEW

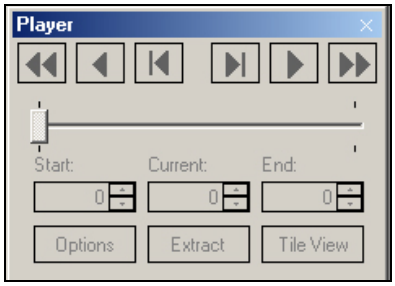
The LS software Image Control plug-in modules are essentially tool boxes for many of the commonly used features. Plug-in modules open individual windows can be viewed and placed in virtually any position on the screen and offer various functions. As the LS software continues to offer more features and options, plug-in modules can be installed to provide added functionality.

To open the plug-in modules, select **View > Plugins** and select the plugin you wish to show in the LS software workspace.



DIGITAL VIDEO PLAYER (DVP)

The Digital Video Player (DVP) clubs multiple images in a single file and lets you go thru the images in a video clip. The LS software serializes the images into a single file with an .sqv extension. Refer to Chapter Five for instructions on how to use the Digital Video Player.



3 DIMENSIONAL SURFACE PLOTS

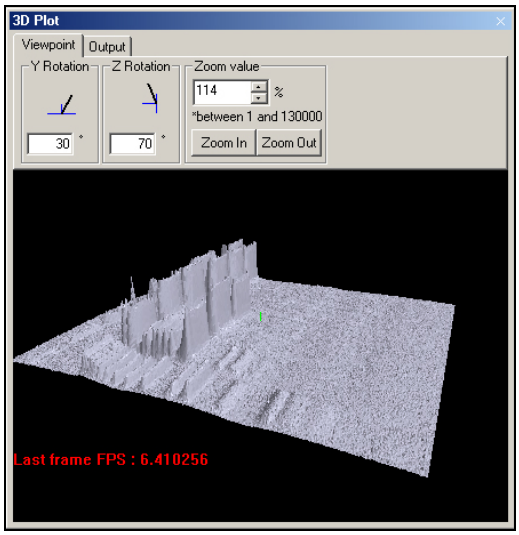
Purpose

1. Often times, it is useful to be able to see a three dimensional view of the sample. For example, if two bands look equally bright in an image, or with naked eye, a 3D plot can actually show if there is a quantitative difference in intensity, if any.
2. A 3D plot can also be used as a great presentation tool.
3. You can check the uniformity of your light source in conjunction with your camera response using 3D plots.

How to use

LS software lets you use 3D plots for static images as well as live preview and integration.

1. Load an image into LS workspace. (It could be an image captured from the camera or scanner or could be loaded from the disk.)
2. Click on **View > Plugins > 3D Plot Plug-in**. This will bring up a separate window that shows the 3D plot.



Controls on the 3D plot window

Viewpoint tab

Controls on this tab let the user set the correct angle of view.

Rotation Controls:

One can rotate the image in Y as well as Z axes, using the 'Y' rotation and 'Z' rotation handles. The axes are as follows:

Z axis: The vertical axis.

Y axis: The horizontal axis.

X axis: The axis coming out of the plot, towards you.

The plot can also be rotated by dragging it with the mouse in desired direction.

Zoom controls:

Zooming in and out of the plot is possible in two ways:

a) Using the buttons labeled 'Zoom In' and 'Zoom Out'

b) Using the spin-box and adjusting the percentage.

Output tab

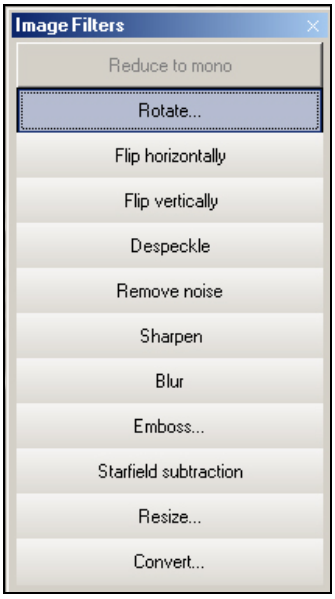
Three controls in this tab let you export the 3D plot information for various uses:

New Image: Pressing this button creates a new image in the LS workspace with what is visible on the surface plot. This new image must be saved.

Clipboard: Pressing this button copies the 3D plot onto clipboard, so that it can be pasted to any other software (eg. MSWord, Excel, Paint, Photoshop etc.)

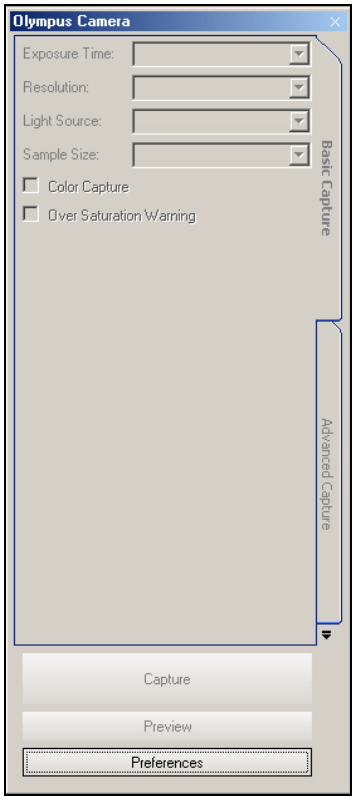
Printer: This button lets you print the 3D plot on a desired printer. Pressing it brings up the list of available printers.

IMAGE FILTERS



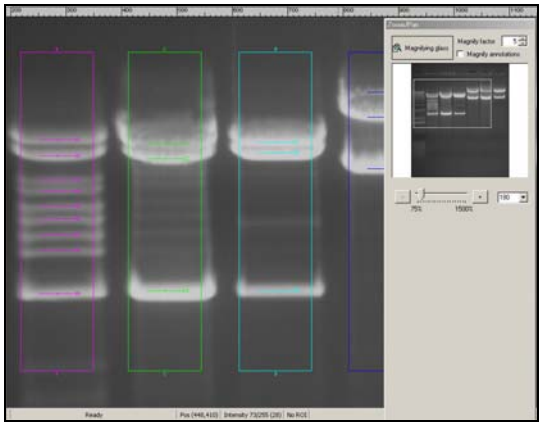
This plugin offers several types of image filters to make changes to an image. Filters can be used to adjust the image for problems in preparing for and acquiring images. Refer to Chapter 9 for instructions on using Image Filters. In addition to accessing the filters from the plugin module, select filters from the **Image > Filters** menu.

OLYMPUS CAMERA (DIGI CAMERA)



If using the Olympus digital camera (Digi Camera), open the Olympus Camera plug in module. This plugin allows selection of various imaging settings including preview and capture functions.

ZOOM/PAN



By **Zooming** you can magnify part of the image making small details more visible. Once you have zoomed in on the image the entire image will no longer be visible in the window. You can change the portion of the image that is visible easily by **Panning**.

To Zoom In or Out

1. Open **View > Plugins > Zoom/Pan**.
2. Click on **Zoom In** or **Zoom Out** (located below the thumbnail version of the image on the Effects tab, on either side of the slider).

   OR

   Slide the **zoom slider** to the left to zoom out or to the right to zoom in.

   OR

   Select the desired zoom factor from the drop-down list to the right of the slider and buttons.

There is no need to "turn off" the Magnify tool -- it will be turned off automatically by selecting any other tool (such as a selection tool or an annotation tool).

You can also magnify your image by selecting **View > Zoom** and clicking on the desired zoom percentage.

You can also type a number into the drop-down box and press TAB. This is particularly useful if you desire a zoom factor between choices in the list.

To Pan to a Different Part of the Image

1. Open **View > Plugins > Zoom/Pan**.
2. In the thumbnail image, drag the **Pan** rectangle to the desired location. If the desired location is outside the **Pan** rectangle, you can simply click the desired location to "jump" the pan rectangle there.

To Magnify Part of an Image

3. Open **View > Plugins > Zoom/Pan**.

4. Click the **Magnifying Glass** button.
5. The mouse becomes a magnifying glass. Click anywhere on the image to magnify an area. Adjust the **Magnify factor** number to increase or decrease the magnification.
6. To show annotations under the magnifying glass, click the **Magnifying Annotations** option.

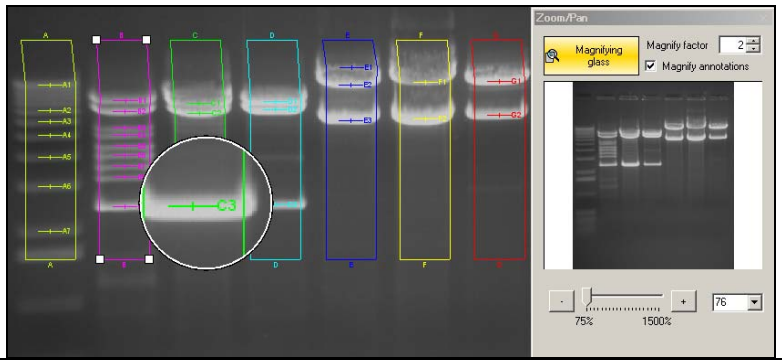
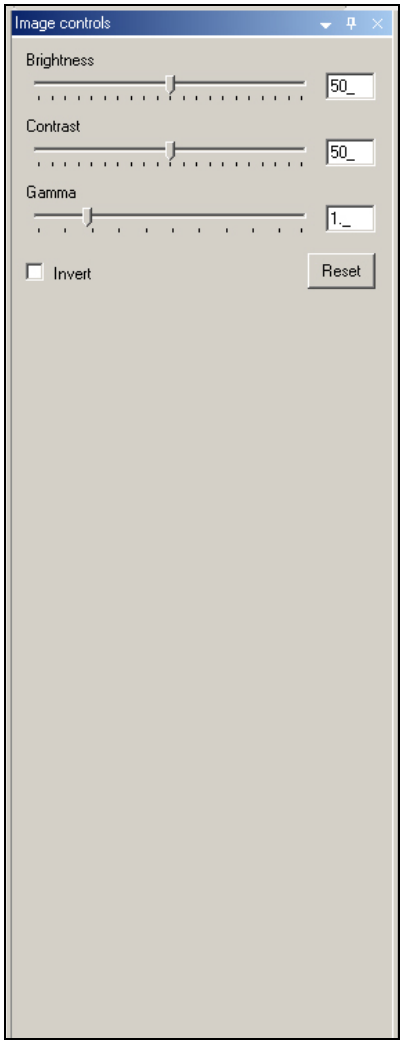


IMAGE CONTROLS



This plugin offers features to control how an image looks. None of the Effects makes permanent changes to the image. You can reverse Brightness, Contrast and Gamma with the Reset button. Specific features available on the Image Control module are:

- ***Brightness***: affects the overall brightness or dimness of the image. Brightness level of 50 means the image is displayed in its original brightness (i.e. unchanged). Changing the brightness level can make features near the top or the bottom of the intensity scale easier to see.
- ***Contrast***: affects the difference between light and dark parts of the image. A contrast level of 50 means that the image is displayed in its original contrast. A level higher than 50 means that contrast has been increased (lights are lighter, darks are darker). A level lower than 50 means that contrast has been decreased (lights and darks are both closer to middle values). *Increasing* the contrast tends to highlight differences in intensity level; *decreasing* it can make patterns that cross intensities more clear.
- ***Gamma***: also affects the difference between light and dark parts of the image, but it does so by using a "gamma correction curve." The gamma correction curve affects middle values more quickly than values at either the darkest or the lightest ends of the spectrum. Gamma contrast values range from 0.1 to 5.0. A value of 1.0 means that no gamma correction curve is in effect (the image is displayed at its original levels). Gamma contrast changes have similar results to regular contrast changes.
- ***Invert***: reverses all intensities, light for dark and dark for light. This also will have the effect of complementing colors (e.g. red to turquoise, yellow to blue). Inverting the image can make certain features easier to see.

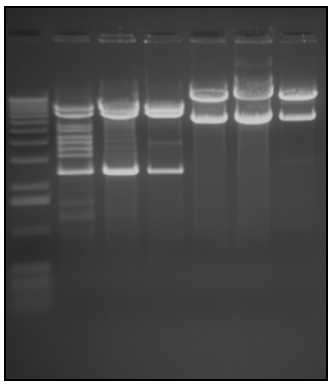
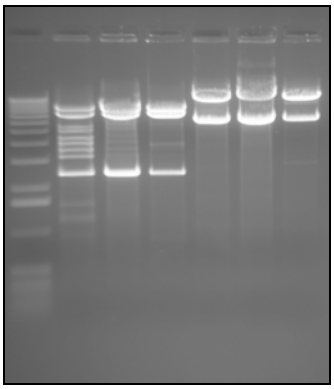
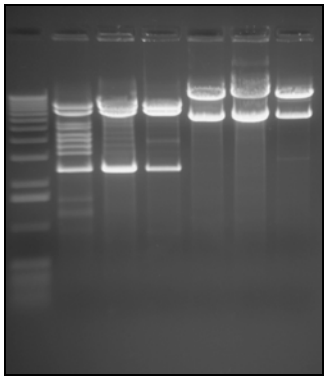


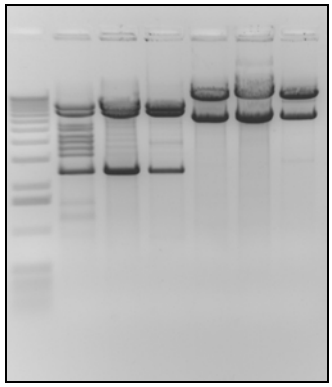
Image Before Effects Were Applied



Brightened



Contrast Enhanced



Inverted

To Change Brightness, Contrast, Gamma Or Invert

1.	If the **Image Controls** plugin is not showing, choose **View > Plugins > Image controls** plugin.
2.	*To change **Brightness***: slide the Brightness control either left or right, or type a desired brightness value into the Brightness text box to the right of the slider.
3.	*To change **Contrast***: slide the Contrast control either left or right, or type a desired contrast value into the Contrast text box to the right of the slider.
4.	*To change **Gamma***: slide the Gamma control either left or right, or type a desired Gamma value into the Gamma text box to the right of the slider.
5.	*To invert the image*: select the **Invert** check box.
6.	*To stop inverting the image*: clear the **Invert** check box.

To Return to Default Values

After changing any of the Image Control options, click **Reset** to return all settings to the default settings.

PSEUDOCOLOR

This applies a false-color spectrum to a monochrome or colored image. This process is sometimes called "colorizing." There are two primary reasons for using pseudocolor:

1. To make the image look more like what might be seen under a microscope with various kinds of lighting, primarily for comparison purposes.
2. To highlight specific intensities for analysis purposes. For example, one of the pseudocolor spectrums highlights black (intensity 0) pixels with blue and white (maximum intensity) pixels with red. This identifies the undersaturated and oversaturated parts of the image.

LS package supplies built-in pseudocolor spectrums:

- *Over-Saturation*: Colors pixels in top 5% of dynamic range red and next 5% as yellow. For example, in a 12-bit image, pixels with intensities 4095 to 3891 (5% range) will be colored red and the next 5% range 3890 to 3685 will be colored yellow. There is no indication for undersaturated pixels.
- *Inverted Over Saturation:* This tool is the same as Over Saturation, but applied to an inverted image.
- *Ethidium Bromide*: Mimics the colors used in Ethidium Bromide gel preparation.
- *Fluorescein*: Mimics the colors used in Fluorescein gel preparation.
- *Green Fluorescent Protein*: Mimics the colors used in green fluorescent protein gel preparation.
- *Texas Red*: Mimics the colors that appear with a Texas Red stain.
- *SYBR Gold*: Mimics the colors that appear with a SYBR Gold stain.
- *SYBR Green*: Mimics the colors that appear with a SYBR Green stain.
- *SYPRO Orange*: Mimics the colors that appear with a SYPRO Orange stain.
- *SYPRO Red*: Mimics the colors that appear with a SYPRO Red stain.
- *Coomassie Blue*: Mimics the colors that appear with a Coomassie Blue stain.
- *Silver*: Mimics the colors that appear with a Silver stain.
- *Blue to Red*: Colors all intensities from blue at the low end to red at the high end using a natural light spectrum.
- *Red to Blue*: Colors all intensities from red at the low end to blue at the high end using a natural light spectrum.
- *Blue*: Colors all intensities from black to bright blue.
- *Cyan*: Colors all intensities from black to bright cyan.
- *Green*: Colors all intensities from black to bright green.
- *Magenta*: Colors all intensities from black to bright magenta.
- *Red*: Colors all intensities from black to bright red.
- *Yellow*: Colors all intensities from black to bright yellow.

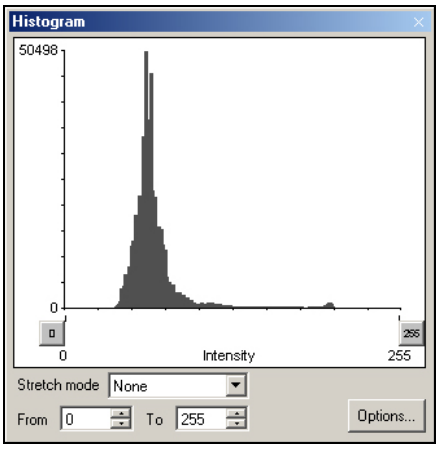
To Apply a Pseudocolor

1. If the Pseudocolor plugin is not showing, choose **Plugins > Pseudocolor plugin**.
2. From the **Pseudocolor** drop-down list, select the desired pseudocolor. The image will be colorized as desired.

To Remove a Pseudocolor

1. If the Pseudocolor plugin is not showing, choose **Plugins > Pseudocolor plugin**.
2. From the **Pseudocolor** drop-down list, select **None**. The image will no longer be colorized.

HISTOGRAM CONTROLS

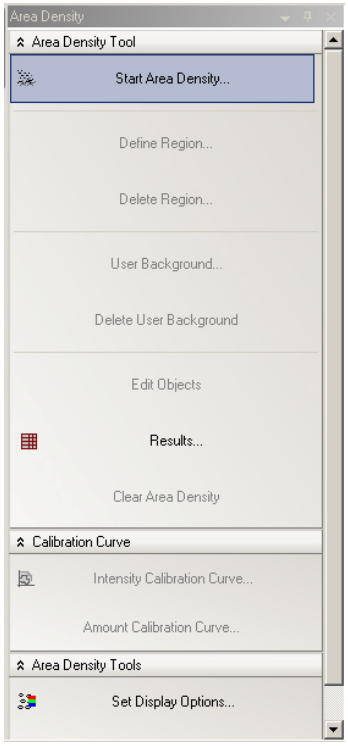


The Histogram plugin offers options for viewing tonal and color information about an image. By default, the histogram displays the tonal range of the entire image. To display histogram data for a portion of the image, first select that portion.

To Apply a Histogram

1. If the Histogram plugin is not showing, choose **Plugins > Histogram plugin**.
2. From the stretch mode drop-down list, select None, Automatic or Manual.
3. From the **Options** button, you can select Y-axis, reset zoom or copy graph.

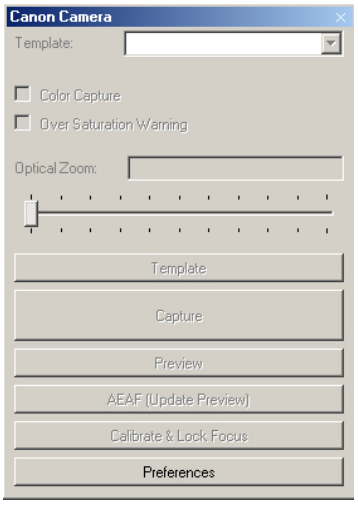
AREA DENSITY



The Area Density plugin can be used to carry out precise quantitative calculations on the spots of interest on your image. It gives you the flexibility to carry out calculations based on Optical Density as well as Grey Levels. Additionally, one can also calibrate the amount of sample loaded in each spot.

For more information on using the Area Density tools, refer to Chapter 18.

CANON CAMERA

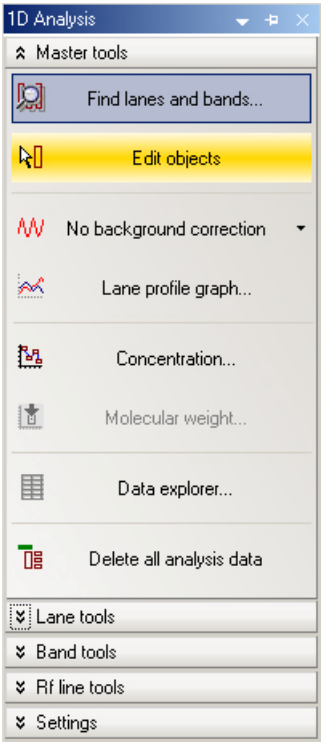


If using the Canon digital camera (Digi Camera), open the Olympus Camera plug-in module. This plugin allows selection of various imaging settings including preview and capture functions.

For additional instructions on capture functions using the Canon Camera, refer to the appropriate section in Chapter 5 Acquiring and Managing Images.

* VA
* DA

1D ANALYSIS

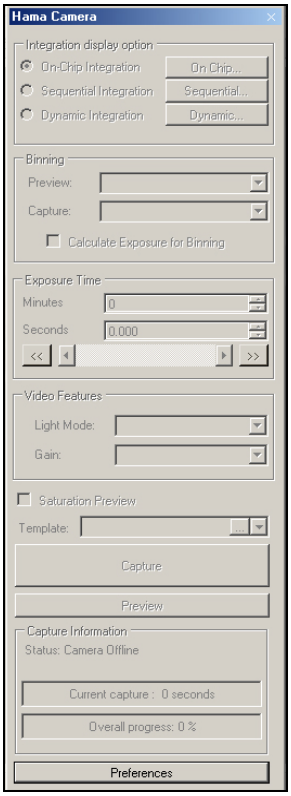


This plugin offers easy access to the analysis functions instead of using the menus. The module is separated into sections that can be expanded or minimized. These sections are:

- ***Master tools***: To find lanes and bands, edit objects, adjust the background correction, lane profile graphs, concentration, molecular weight, data explorer plus delete all analysis data options.
- ***Lane tools***: Add or modify lanes.
- ***Band tools***: Add or modify bands.
- ***Rf line tools***: Add or delete Rf lines.
- ***Settings***: Allows you to set the analysis defaults.

Refer to Chapter 13 for instructions on using the 1D Analysis tools. Chapter 14 discusses use of Rf lines when calibrating Molecular Weight standards.

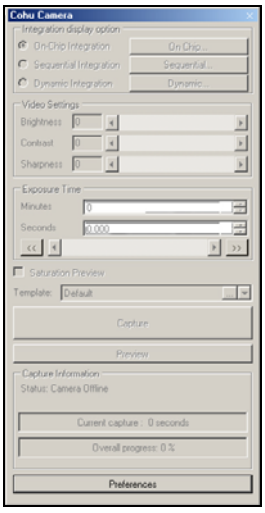
HAMAMATSU CAMERA



If using the Hamamatsu camera, open the Hamamatsu Camera plug-in module. This plugin allows selection of various imaging settings including preview and capture functions.

For additional instructions on capture functions using the camera,, refer to the appropriate section in Chapter 5 Acquiring and Managing Images.

COHU CAMERA



If using the Cohu camera (Fluor or Chemi Cameras), open the Cohu Camera plug-in module. This plugin allows selection of various imaging settings including preview and capture functions.

For additional instructions on capture functions using the Cohu Camera, refer to the appropriate section in Chapter 5 Acquiring and Managing Images.

COLONY COUNTING

* VA

* DA

This plugin offers easy access to the analysis instead of using the menus. The module is separated into sections that can be expanded or minimized. These sections are:

- ***Start Colony Count***
- ***Set Display Options***
- ***Show Results Window***

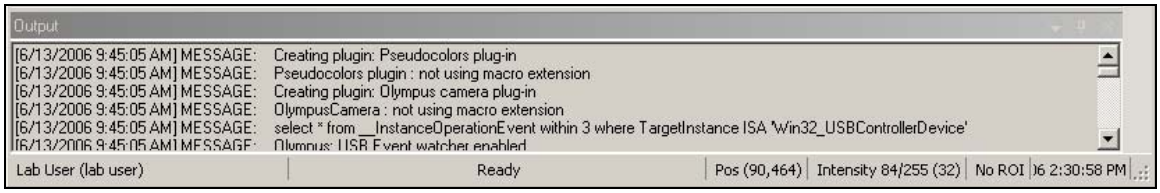
For instructions on performing colony counting, refer to Chapter 17.

VC VA

DC DA

LOG VIEWER

The Log Viewer displays output data of recorded events in the LS Software window. This data is most useful for troubleshooting and feature tracking.



VC VA

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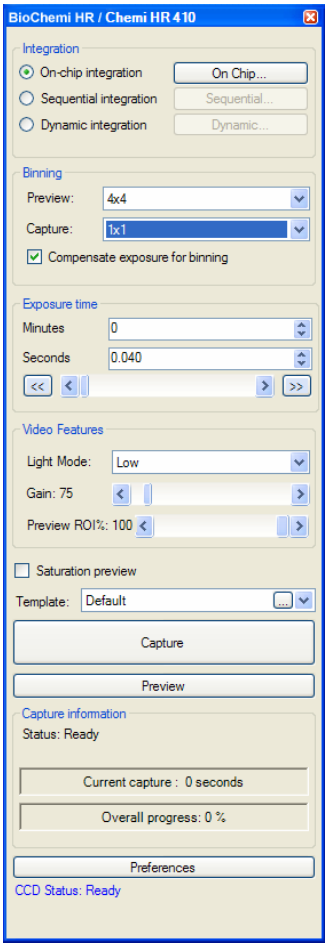
BIOCHEMI HR/CHEMI HR 410 CAMERAS



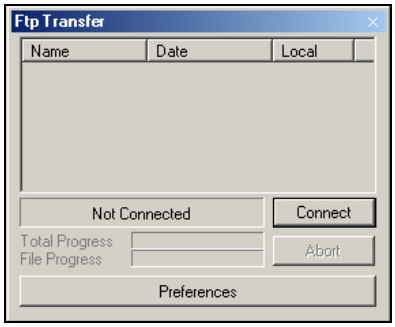
If using the BioChemi HR or Chemi HR 410 Cameras), open the Chemi HR Camera plug-in module. This plugin allows selection of various imaging settings including integration, binning, exposure time, preview and capture functions.

For additional instructions on capture functions using these cameras, refer to the appropriate section in Chapter 5 Acquiring and Managing Images.

VC VA DC DA

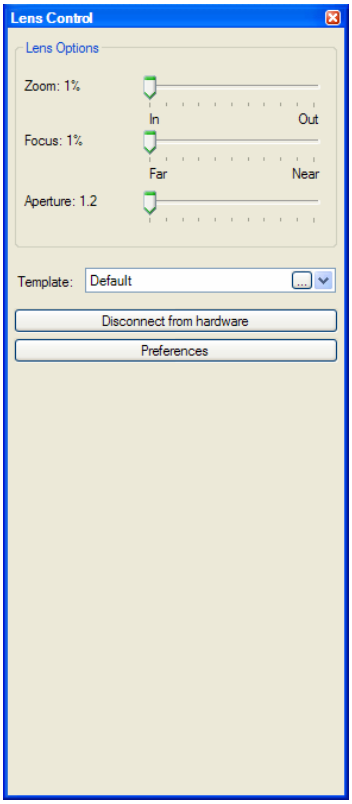
FTP TRANSFER

The FTP Transfer menu displays files that can be transferred via ftp to another computer.



* *

DARKROOM AND LENS



If using the motorized lens, open the Darkroom and Lens plug-in module. This plugin applies to systems other than the BioSpectrum system. The lens control allows selection of settings including zoom, focus, aperture as well as access to templates and preferences.

For additional instructions on the darkroom/lens functions, refer to the appropriate section in Chapter 5.

CHAPTER FIVE: ACQUIRING AND MANAGING IMAGES

OVERVIEW

LS Series packages allow you to acquire images in three ways:

- You can ***capture*** *images* from the supplied camera.
- You can ***scan*** *images* from most scanners.
- You can ***load*** *images* from disk. This allows you to return to images saved during a prior session and to import images either produced through some other software package or transported from an LS software installation on another computer. Loading and saving are discussed Chapter 7.

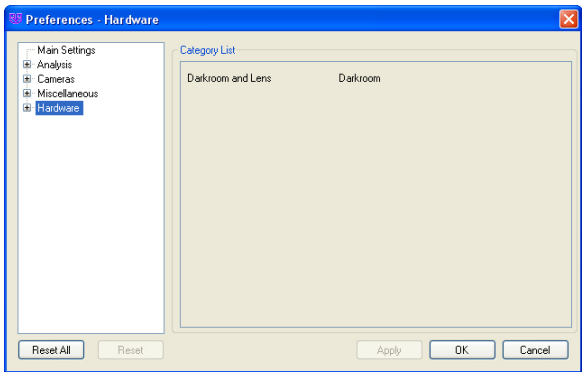
Many different UVP approved cameras can be used for image-acquisition using LS series software. An image acquired in any of these fashions will appear in its own Image window inside the workspace.

DARKROOM CONNECTIVITY

LS Software works with the BioSpectrum automated darkroom offered by UVP. Interface software will be installed along with the LS software, in a single installation. Detailed instructions on how to use those software interfaces are provided in the manuals that come with the systems.

To check if the Darkroom is connected

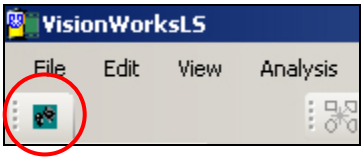
Click on **File > Preferences > Hardware > Darkroom**.

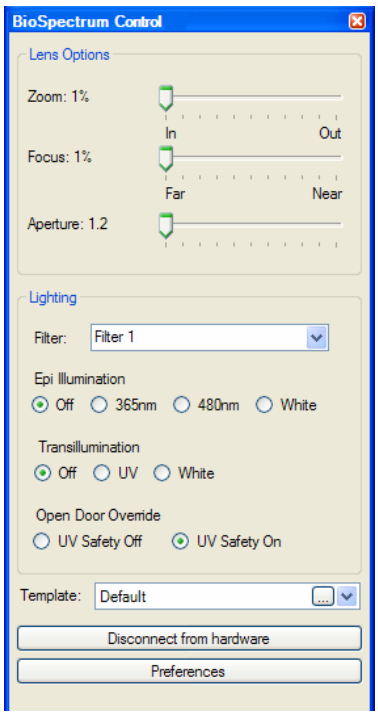


You will see the status of connectivity of the darkroom.If it says “BioSpectrum Hardware Detected” or “BioSpectrum Hardware Detected”, then it means that LS software is prepared to launch the said darkroom software successfully.

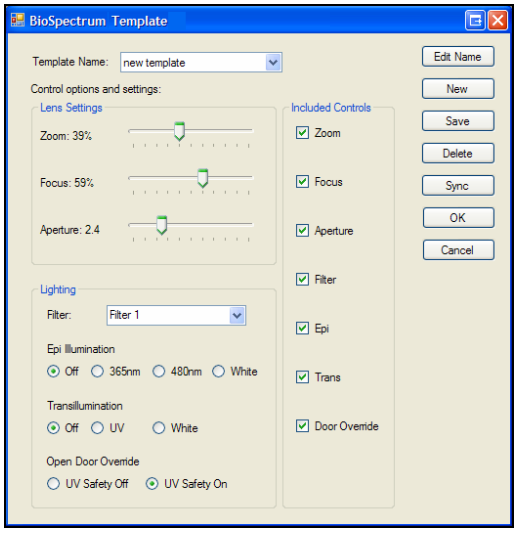
To Launch the Darkroom Software

Click on **Darkroom** toolbar icon, to launch the Darkroom interface software.





Darkroom Template



To Create a New Template

1.	Click **New** from the BioSpectrum Template window. The New Template window will appear. (If you don't have any capture templates yet, the New Template window will appear automatically.)
2.	Type a name for the new template and click **OK**. A new template will be created and then defaulted to the current settings in the Templates window.
3.	Set each lens setting to the desired value.
4.	Under included controls, clear the check box for functions to be excluded.
5.	Click **Save** (or **OK**) to save your new template.

To Sync a Template

When you have selected settings in the **Lens Control** window, you can click **Sync** in the BioSpectrum to save the new settings in the BioSpectrum templates window.

VC VA

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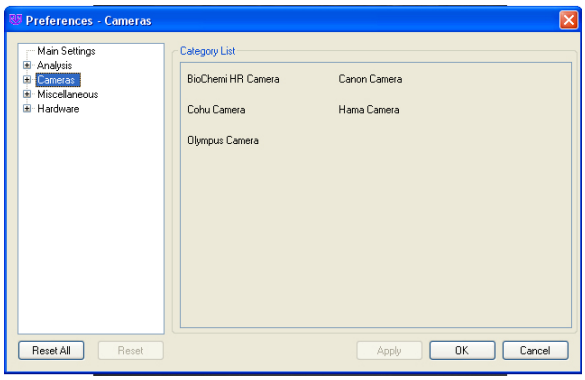
SELECTING A CAMERA

When you configure your system for the first time, or if you upgrade your camera or darkroom, you will need to select which camera to use.

Click on **File > Preferences > Cameras**. The type of camera you have will depend on the system you have purchased from UVP. You can find your system-name from the label on the darkroom. If in doubt, please call UVP Tech Support.

Click on the camera name to display the camera settings and template settings.



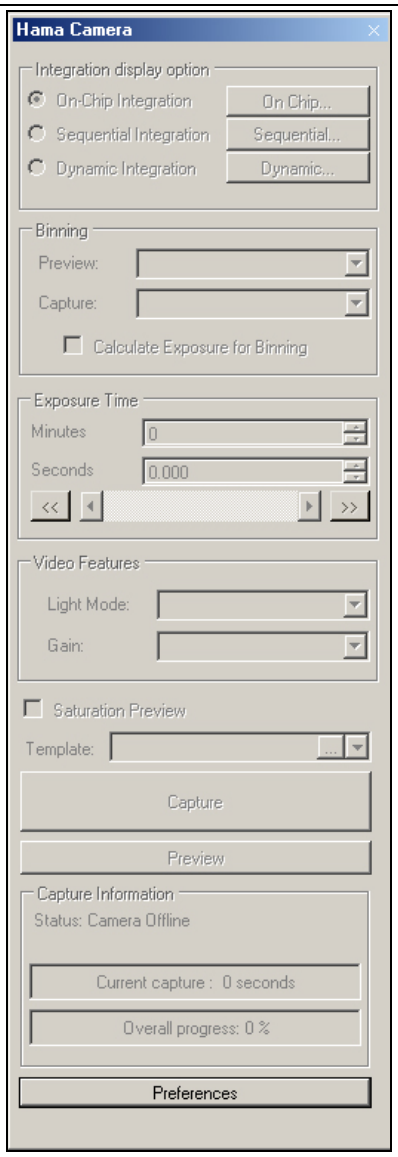
OPTICHEMI

This section pertains to several UVP cameras:

OptiChemi Camera

To view the plug-in for any of the above camera, select **View > Plugins > Hamamatsu Camera Plug-in.**

Hamamatsu Camera Plug-In



Integration display option

- **On-Chip Integration:** When this radio-button is selected before pressing **Capture**, the software takes only ONE picture, with the current exposure time (below) set.
- **Sequential Integration:** When this button is selected before clicking **Capture**, multiple pictures are taken at a uniformly increasing exposure time.
- **Dynamic Integration:** With this option selected before capture, multiple pictures are taken at set uniform intervals.

Binning:

- **Preview**: Sets the binning mode to be used when you press **Preview**.
- **Capture**: Sets the binning mode to be used when you press **Capture**.
- **Calculate Exposure for Binning:** When you change the binning mode (above), it automatically adjusts the exposure time to compensate for the new binning.

Exposure Time: Lets you adjust the time for how long the camera should expose to and collect light from the sample. Various arrows increment the time in a steady manner.

Video Features:

- **Light Mode**: When set to 'low', the Anti-Blooming feature of camera is turned off. When set to 'high', the said feature is on.
- **Gain**: Set a high value for gain to get increased sensitivity. That also increases background noise.

Saturation Preview: Click this checkbox during Preview to see if any part of the image is over exposed to light. Over exposed pixels are shown in Red color.

Template: A template is a group of camera settings, which can be saved under a common name. Select the correct Template for your sample, to apply from the drop-down box. Press the Template button below to define a new template.

Capture: Click on this button to take the picture.

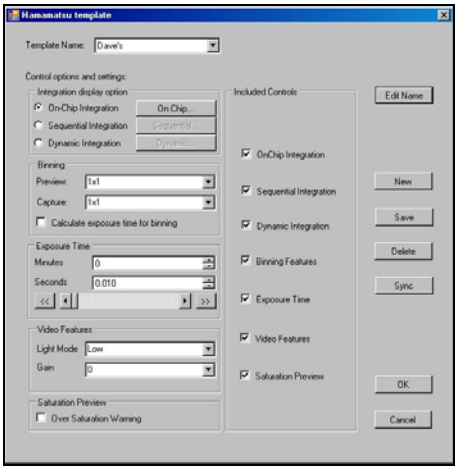
Preview: Click on this button to see a preview of your sample, in order to ensure that the camera sees the sample clearly before taking the actual

	picture. **Capture Information:** Shows camera status and capture progress. **Preferences**: Shows the Hamamatsu camera and template settings. These settings can also be accessed from **Files > Preferences > Camera > Hama Camera**

Hamamatsu Capture Templates

Templates are a great time-saver, regardless of the type of user you are – novice or an expert. A template is simply a group of all configurable camera settings (which present on the interface) grouped under a single name. So after you decide the right combination of settings for your sample the first time (by trial and error), you can ***save*** them as a Template and simply ***apply*** the template next time you want to image a similar sample.

To Save a Template, click on Template button. That brings up a window asking you to give a name to the template e.g BSA_Exposure. Once the name is entered, the window looks like this:



On the left side of this window, you can select specific values for all the available options. E.g. you can set a specific exposure time that gives you a sharp image.

On the right hand side, there is a list of features which can be included in the view of capture-panel. Check the features (controls) which you want to use and see. Only those checked will appear in the main capture-panel. Explanation of main buttons:

Edit Name: Click this button to change the existing name of the template.

New: Create a new Template here.

Save: Save this template and continue working.

Delete: Delete this template and create a new one.

Sync: Synchronize the settings from capture panel. This could be a very useful button if a Template changes. Press it once after you have changed your settings in the capture panel. In one click, all settings get copied (or synchronized) from the Capture Panel to the Template window.

Binning Modes

Binning is an advanced feature provided by these UVP cameras. “Binning” literally means to “bin” or “combine” pixel values. E.g. A camera set to the binning of 4 x 4 (read 4 by 4) means, that it “combines” the values from 4 pixels across and 4 pixels down – 16 pixels in all – into one single pixel on the image. Binning is also referred to as creating a “Super Pixel”.

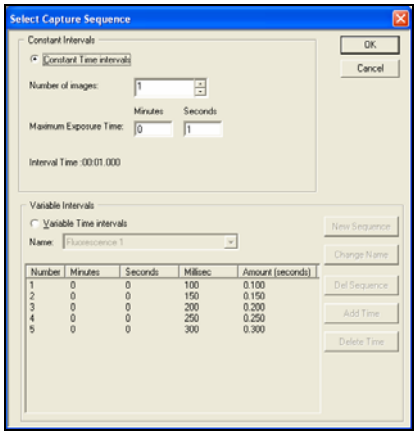
Higher binning, hence, increases the sensitivity of the images, at the cost of resolution and image-size (due to combining of pixels). A good strategy is to Focus at higher binning (say 2x2) so that the refresh rate is higher and then to snap at a lower binning, to capture full resolution in the resultant image.

Exposure Time Integration

Integration is the term used to mean the length of time camera is exposed to incoming light. Integration time and Exposure time are sometimes used interchangeably. There are various different types of Integration modes provided by LS software, based on your application needs:

Sequential Integration: Used when you do not know what is the ideal exposure time for your sample e.g. a low light Chemiluminescence sample. This option allows you to take multiple images at either constant or irregular time-intervals and then you can go ahead and pick the one you think gives best results.

Switch to ‘Sequential Integration’ radio button and click on the ‘Sequential…’ button, which brings up a window that looks like the following:



Constant Time Intervals: Use this when you have an idea of how many images you might need and what is the longest you want to integrate. LS software will then calculate the integration time to be used for the first image and use the same as an increment to capture subsequent images e.g. if you entered 5 images to be taken and you want to have the maximum integration time of 20s, the exposure times calculated will be as follows:

First: 4s

Second: 8s

Third: 12s

Fourth: 16s

Fifth: 20s

These five pictures will be placed inside a sequence file, and you can use the Digital Video Player to go through them. Use the player tools to extract the image that you think makes your sample look acceptably sharp.

Variable Time Intervals: Use this setting if you want to take multiple images, each with a different exposure time. No addition of exposures is done as in the 'Constant Time Intervals' case. A large number of images can be captured in sequence. Each image needs a separate line of entry in the table shown.

New Sequence: Click this to add a new sequence of images.

Del Sequence: Deletes the currently displayed sequence.

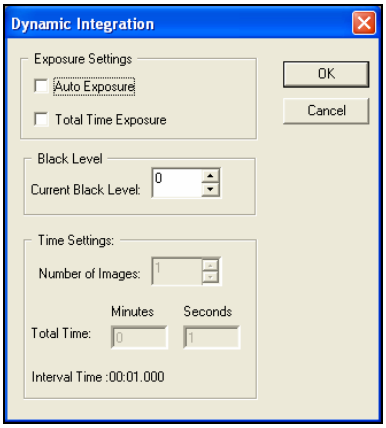
Change Name: Lets you enter a new name for the currently displayed sequence.

Add Time: Adds a new entry to the sequence. Enter time in Minutes, seconds and milliseconds. 'Amount' shows the total time in milliseconds.

Delete Time: Deletes the currently active entry of the sequence.

Dynamic Integration: Used when the integration time offered by the camera is not enough for a long exposure. In this mode, LS software does "stacking" of frames i.e. it adds the corresponding pixel values of first image-frame to the next. This compensates for a low light limitation, by making dim areas brighter with increasing number of images. [Stacking replaces the first image after the second is captured and pixel values added to it from the first.] UVP cameras typically have a long exposure time capability, which would be more than enough for most of your samples. If however you need to go beyond what is available, then this feature offers a software solution.

Switch to 'Dynamic Integration' option and click on 'Dynamic Integration' button to bring up the following window:



With no checkboxes checked (like the one shown above): If you click 'Snap' with no options checked in this window, it will keep stacking images (explained earlier in this chapter) until you click 'Stop Dynamic Snap'. Exposure time used will be used form what is entered on the capture-panel.

Auto Exposure: With this box checked, the stacking automatically stops once the image gets saturated.

Total Time Exposure: Check this box to have a greater control over how many images you want snapped, and for how long each one should be exposed. Interval Time is simply the division of total time by number of images. Uniformly exposed images are stacked.

Current Black Level: Stacking frames also stacks noise along with it. To clip noise from each image before stacking, it is important to set the black-level. Value of the darkest pixel in the image (which will typically be noise) should be set inside the spin-box. Pixel values less than this value are not recorded or clipped during acquisition and before stacking.

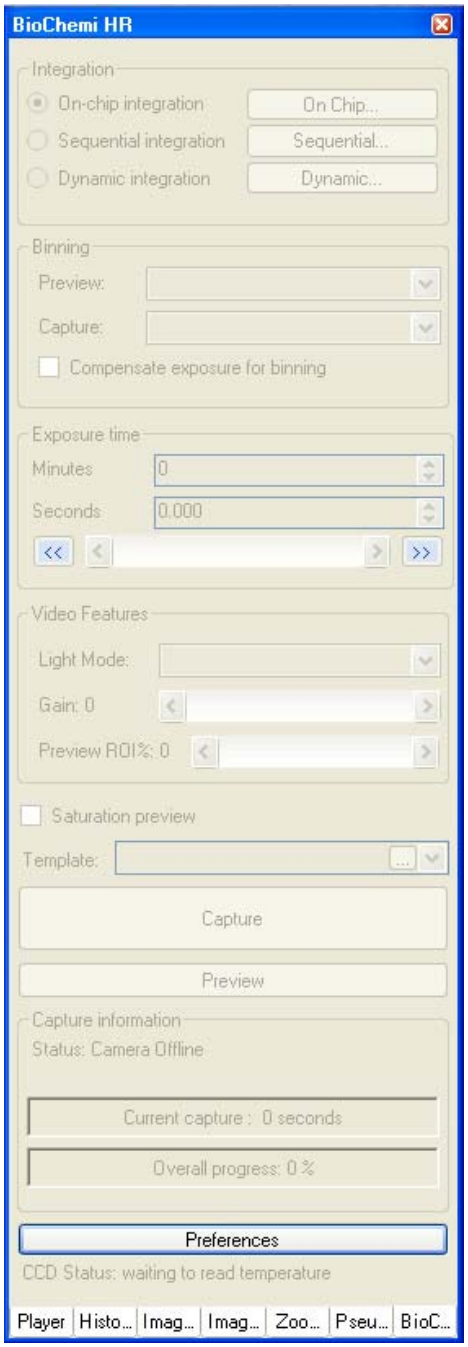
BIOCHEMI HR/CHEMI HR 410

This section pertains to several UVP cameras:

BioChemi HR Camera
Chemi HR 410 Camera

To view the plug-in for any of the above cameras, select **View > Plugins > BioChemi HR/Chemi 410 Camera Plug-in.**

BioChemi HR/Chemi HR 410 Camera Plug-In



Integration display option

- **On-Chip Integration:** When this radio-button is selected before pressing **Capture**, the software takes only ONE picture, with the current exposure time (below) set.
- **Sequential Integration:** When this button is selected before clicking **Capture**, multiple pictures are taken at a uniformly increasing exposure time.
- **Dynamic Integration:** With this option selected before capture, multiple pictures are taken at set uniform intervals.

Binning:

- **Preview**: Sets the binning mode to be used when you press **Preview**.
- **Capture**: Sets the binning mode to be used when you press **Capture**.
- **Calculate Exposure for Binning:** When you change the binning mode (above), it automatically adjusts the exposure time to compensate for the new binning.

Exposure Time: Lets you adjust the time for how long the camera should expose to and collect light from the sample. Various arrows increment the time in a steady manner.

Video Features:

- **Light Mode**: When set to 'low', the Anti-Blooming feature of camera is turned off. When set to 'high', the said feature is on.
- **Gain**: Set a high value for gain to get increased sensitivity. That also increases background noise.
- **Preview ROI**%: The percentage of the overall image area. Useful to speed up previewing image or zooming in on a particular area during preview.

Saturation Preview: Click this checkbox during Preview to see if any part of the image is over exposed to light. Over exposed pixels are shown in Red color.

Template: A template is a group of camera settings, which can be saved under a common name. Select the correct Template for your sample, to apply from the drop-down box. Press the Template button below to define a new template.

Capture: Click on this button to take the picture.

	Preview: Click on this button to see a preview of your sample, in order to ensure that the camera sees the sample clearly before taking the actual picture. **Capture Information:** Shows camera status and capture progress. **Preferences**: Shows the camera and template settings. These settings can also be accessed from **Files > Preferences > Cameras > BioChemi HR Camera** **CCD Status**: The software monitors the temperature of the camera. Status may say **cooling** while the camera is in the cooling process

Tip: To capture the best images, allow approximately 15 minutes cooling time for the camera to cool to the appropriate temperature.

BioChemi HR/Chemi HR 410 Capture Templates

Refer to the Hamamatsu Capture Templates for descriptions and usage of templates.

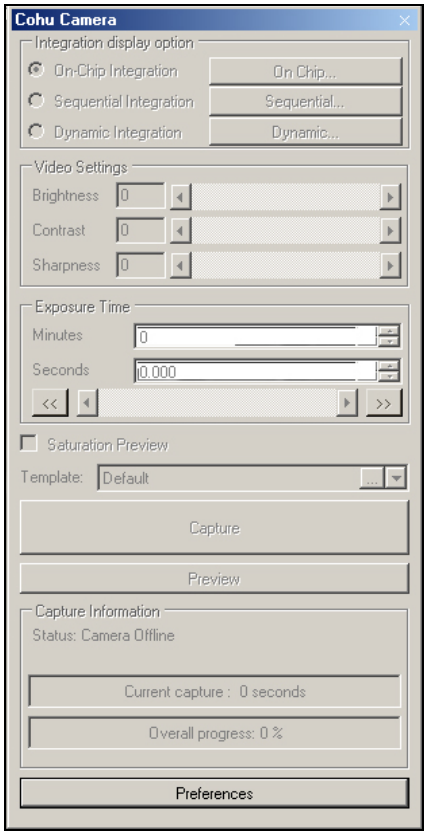
FLUOR / CHEMI CAMERAS

This section pertains to two types of cameras:

1. Fluor Camera
2. Chemi Camera

Both these cameras use a Frame-grabber card that goes into a PCI slot on your computer. They share an identical user-interface in LS software.

Cohu Camera Plugin



Integration display option:

- **On-Chip Integration:** When this radio-button is selected before pressing **Capture**, the software takes only ONE picture, with the current exposure time (below) set.
- **Sequential Integration:** When this button is selected before clicking **Capture**, multiple pictures are taken at a uniformly increasing exposure time.
- **Dynamic Integration:** With this option selected before capture, multiple pictures are taken at regular intervals.

Video Settings: The slider bars in this section, Brightness, Contrast and Sharpness, control the quality of images being acquired.

Exposure Time: Lets you adjust the time for how long the camera should expose to and collect light from the sample. Various arrows increment the time in a steady manner.

Saturation Preview: Click this checkbox during 'Focus' to see if any part of the image is over exposed to light. Over exposed pixels are shown in Red color.

Template: A template is a group of camera settings, which can be saved under a common name. Select the correct Template for your sample, to apply from the drop-down box. Press the 'Template' button to define a new template. More explanation on templates is provided later in this chapter.

Capture: Click on this button to take the picture.

Preview: Click on this button to see a preview of your sample, in order to ensure that the camera sees the sample clearly before taking the actual picture.

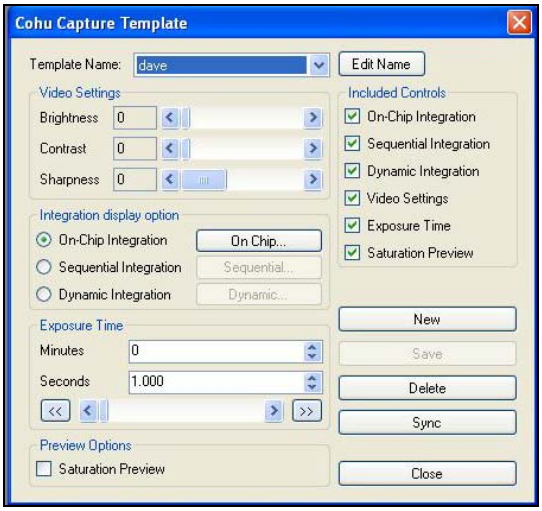
Capture Information: Shows camera status and capture progress.

Preferences: Shows the Cohu camera and template settings. These settings can also be accessed from **Files > Preferences > Cameras > Cohu Camera**.

Cohu Capture Template

Templates are a great time-saver, regardless of the type of user you are – novice or an expert. A template is simply a group of all configurable camera settings (which present on the interface) grouped under a single name. So after you decide the right combination of settings for your sample the first time (by trial and error), you can ***save*** them as a Template and simply ***apply*** the template next time you want to image a similar sample.

To ‘Save’ a Template, click on ‘Template’ button. That brings up a window asking you to give a name to the template e.g BSA_Exposure. Once the name is entered, the window looks like this:



On the left side of this window, you can select specific values for all the available options. E.g. you can set a specific exposure time that gives you a sharp image.

On the right hand side, there is a list of features which can be included in the view of capture-panel. Check the features (controls) which you want to use and see. Only those checked will appear in the main capture-panel. Explanation of main buttons:

Edit Name: Click this button to change the existing name of the template.

New: Create a new Template here.

Save: Save this template and continue working.

Delete: Delete this template and create a new one.

Sync: Synchronize the settings from capture panel. This could be a very useful button if a Template changes. Press it once after you have changed your settings in the capture panel. In one click, all settings get copied (or synchronized) from the Capture Panel to the Template window.

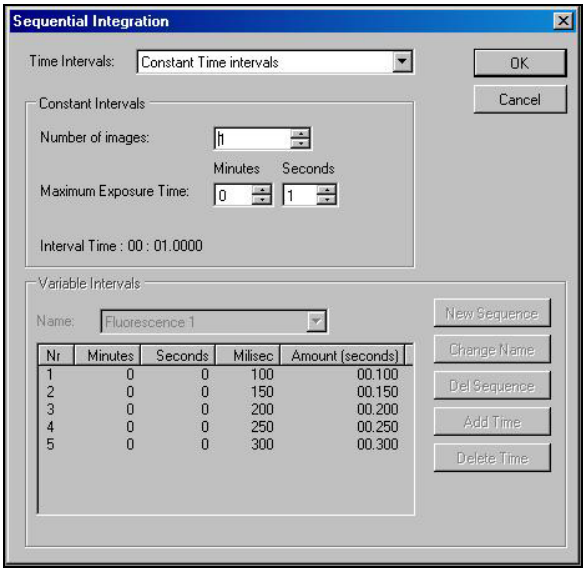
Exposure Time Integration

Integration is the term used to mean the length of time camera is exposed to incoming light. “Integration time” and “Exposure time” are sometimes used interchangeably. There are various different types of Integration modes provided by LS software, based on your application needs:

Sequential Integration: Used when you do not know what is the ideal exposure time for your sample e.g. a low light Chemiluminescence sample. This option allows you to take multiple images at either constant or irregular time-intervals and then you can go ahead and pick the one you think gives best results.

Switch to ‘Sequential Integration’ radio button and click on the ‘Sequential…’ button,

which brings up a window that looks like the following:



Constant Time Intervals: Use this when you have an idea of how many images you might need and what is the longest you want to integrate. LS software will then calculate the integration time to be used for the first image and use the same as an increment to capture subsequent images e.g. if you entered 5 images to be taken and you want to have the maximum integration time of 20s, the exposure times calculated will be as follows:

First: 4s

Second: 8s

Third: 12s

Fourth: 16s

Fifth: 20s

These five pictures will be placed inside a sequence file, and you can use the Digital Video Player to go through them. Use the player tools to extract the image that you think makes your sample look acceptably sharp.

Variable Time Intervals: Use this setting if you want to take multiple images, each with a different exposure time. No addition of exposures is done as in the 'Constant Time Intervals' case. A large number of images can be captured in sequence. Each image needs a separate line of entry in the table shown.

New Sequence: Click this to add a new sequence of images.

Del Sequence: Deletes the currently displayed sequence.

Change Name: Lets you enter a new name for the currently displayed sequence.

Add Time: Adds a new entry to the sequence. Enter time in Minutes, seconds and milliseconds. 'Amount' shows the total time in milliseconds.

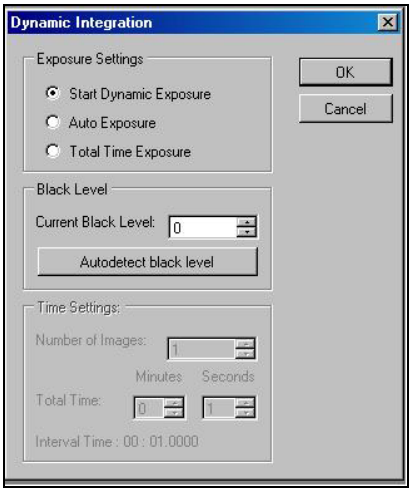
Delete Time: Deletes the currently active entry of the sequence.

Dynamic Integration: Used when the integration time offered by the camera is not enough for a

long exposure. In this mode, LS software does “stacking” of frames i.e. it adds the corresponding pixel values of first image-frame to the next. This compensates for a low light limitation, by making dim areas brighter with increasing number of images. [Stacking replaces the first image after the second is captured and pixel values added to it from the first.]

UVP cameras typically have a long exposure time capability, which would be more than enough for most of your samples. If however you need to go beyond what is available, then this feature offers a software solution.

Switch to ‘Dynamic Integration’ option and click on ‘Dynamic Integration’ button to bring up the following window:



With no checkboxes checked (like the one shown above): If you click ‘Snap’ with no options checked in this window, it will keep stacking images (explained earlier in this chapter) until you click ‘Stop Dynamic Snap’. Exposure time used will be used form what is entered on the capture-panel.

Auto Exposure: With this box checked, the stacking automatically stops once the image gets saturated.

Total Time Exposure: Check this box to have a greater control over how many images you want snapped, and for how long each one should be exposed. Interval Time is simply the division of total time by number of images. Uniformly exposed images are stacked.

Current Black Level: Stacking frames also stacks noise along with it. To clip noise from each image before stacking, it is important to set the black-level. Value of the darkest pixel in the image (which will typically be noise) should be set inside the spin-box. Pixel values less than this value are not recorded or clipped during acquisition and before stacking.

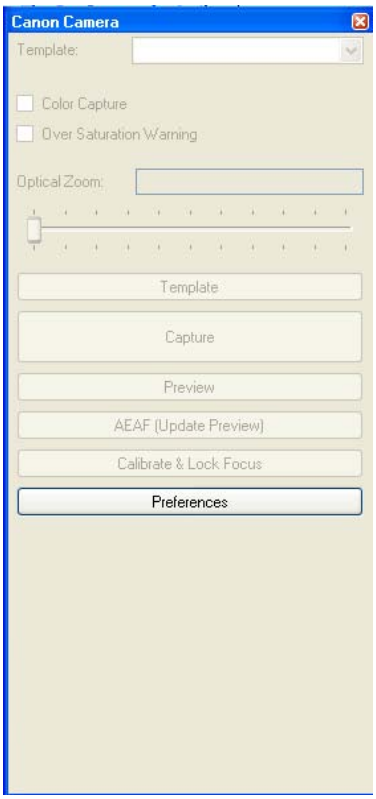
DIGI CAMERA

UVP provides Digital Cameras to use on UVP basic imaging systems. This section pertains to the Canon Digital Camera.

Note: See Appendix 3 for Olympus Camera plug-in descriptions.

Canon Capture Plug-in

The UVP Canon digital camera capture plugin provides simple camera controls that produce good images under most conditions. To open the capture tab, go to **View > Plugins > Canon Capture Plug-in.** The Canon Capture tab offers the following settings and controls:



- ***Template***: A template is a group of camera settings, which can be saved under a common name. Select the Template from the drop-down box to apply to for your sample, .
- ***Color Capture***: Determines whether the camera captures in color or monochrome.
- ***Over Saturation Warning***: If turned on, this will apply an over saturation pseudocolor to the image after it has been snapped and passed back from the camera.
- ***Optical Zoom***: The zoom setting of the camera's lens adjusts the lens to show the largest region of interest.
- ***Templates***: This button allows users to define templates to save customized setting and displayed features.
- ***Capture:*** To capture an image.
- ***Preview*****:** Preview a live image.
- ***AEAF:*** When changing any settings while in the **Preview** mode, click the **AEAF (auto exposure/auto focus) button** to update the live preview.
- ***Calibrate & Lock Focus***: Sets the focus of the lens and locks it in. Once locked, the focus will remain the same for all subsequent images captured.
- ***Preferences:*** Allows you to save modified template settings when disconnecting. Select from: Ask, Always, Never.

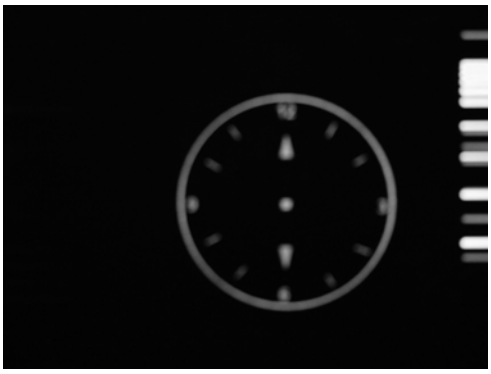
To Capture an Image Using the Camera Capture Plugin

1.	If the Canon Capture plugin is not showing, select **View > Plugins > UVP Canon Camera Plug-in**.
2.	Set the exposure time, sample size, color and resolution to the desired settings.
3.	Decide if you would like to see the degree of over saturation in the image. If so, select the **Over Saturation Warning** check box; if not, clear the check box.
4.	Click **Capture**.

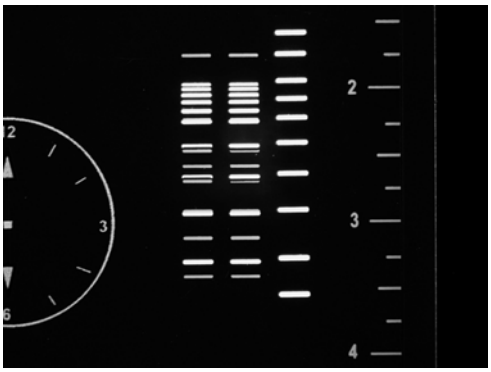
To Adjust the Focus Calibration and Lock

When taking pictures of gels and other samples inside the darkroom, it may be difficult to focus if there is little contrast toward the center of the image area. To enable easy image capture, users can preset the auto focus using the **Calibrate & Lock Focus.**

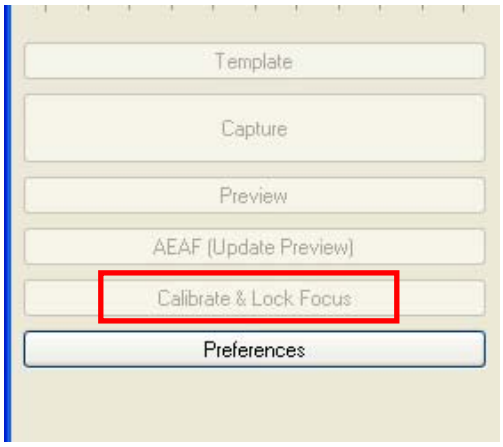
1. Place a UVP Focus Target on the transilluminator. If there are no bright or contrasting areas of the target in the center of the imaging area the resulting image may be out of focus.



2. Move the UVP Focus Target so that a bright area (fluorescent printed bands work well) are located in the center of the image area.



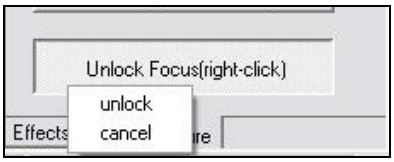
3. Click the **Calibrate & Lock Focus** on the Canon Capture plugin to lock the auto focus. This provides easy to use, consistent focus results on subsequent gels.



After clicking the Calibrate & Lock Focus, the camera will capture an image and lock the focus. Note that the **Calibrate & Lock Focus** button now reads **Unlock Focus (Right Click). NOTE:** If the captured image is still not clear, check to be sure there is a bright contrasting print in the center of the imaging area. Repeat step 2.

To Unlock the Focus Lock

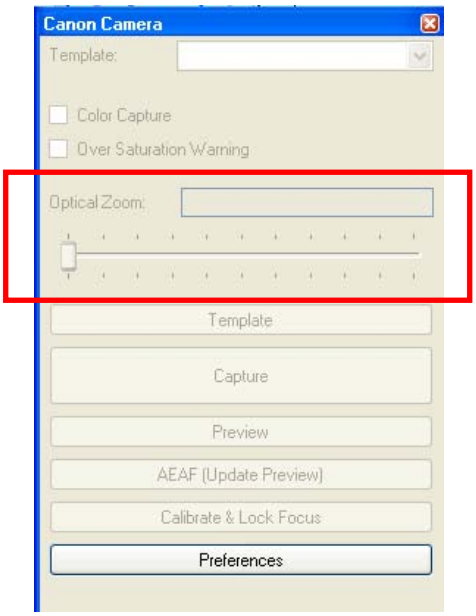
To unlock the calibrated focus lock, right click on the **Unlock Focus (Right Click)** button on the bottom of the capture plugin.



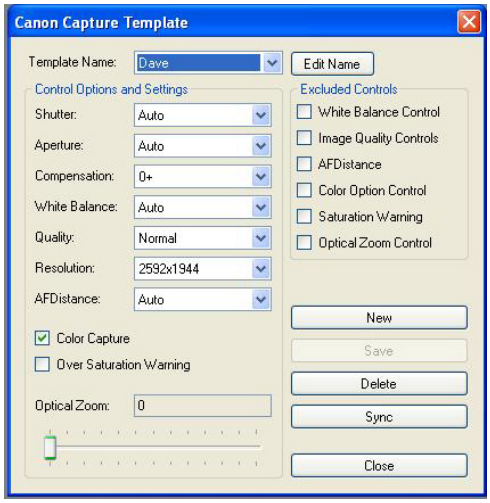
Zoom Adjustment with the Calibration and Lock Focus

While imaging with the focus locked, the **Optical Zoom** value is also locked. To enable zoom adjustment, you must unlock the focus lock.

1. To unlock the focus lock calibration, right click on the **Unlock Focus (Right Click)** button on the bottom of the capture plugin.
2. Press the **Preview** button to view the current image area.
3. Adjust the **Optical Zoom** to the desired magnification.
4. To calibrate the focus at the new zoom level, select **Calibrate and Lock Focus**. Note: See Focus Calibration and Lock section, steps 3 and 4



Canon Capture Templates



Capture Templates are groups of preset camera settings. They are used on the Canon Camera Plug-in either to return the camera quickly to a group of settings that you use often or to default some settings while making others available for alteration.

Each template has a name, a default value for every camera setting and a list of flags indicating which settings will be excluded and which will be shown from the Canon Camera Plug-in when this template is selected. If a setting (or group of settings) is not excluded, it can be overridden on the Canon Camera plugin before each capture.

To Create a New Template

6. On the Canon Camera Plugin click the drop-down **Templates** menu. The Capture Templates window will appear.
7. Click **New**. The New Template window will appear. (If you don't have any capture templates yet, the New Template window will appear automatically.)
8. Type a name for the new template and click **OK**. A new template will be created and then defaulted to the current settings in the Capture Templates window.
9. Set each camera setting to the desired value.
10. Determine for each setting or group of settings whether you want to override the default before each capture. If you do not want to override it, select the **Excluded Controls** check box next to the setting or group of settings. If you do want to override it, clear the check box.
11. Click **Save** (or **OK**) to save your new template.

To copy a template, select the template you wish to copy first, then click **New**. The new template will use the one you selected for initial values.

To Edit a Template

1. On the Canon Camera Plugin click **Templates** drop down menu. The Capture Templates window will appear.
2. Select the template you wish to edit from the drop-down list. The default values and excluded controls check boxes will be set to the values used for that template.
3. To edit the name, click **Edit Name** to the right of the template drop-down list. Type a new name and then click **OK**.
4. If you made changes in the Canon Camera Plugin to the same template you are editing, you can make the template take the changed settings by clicking **Sync**. This allows you to experiment with changes to settings and then save those changes.
5. To change any setting default, select the new value for that setting.
6. To exclude or to show a setting or a group of settings, select or clear the related **Excluded Controls** check box.
7. Click **Save** (or **OK**) to save your changes.

To Delete a Template

1. On the the Canon Camera Plugin click **Templates** drop down menu. The Advanced Capture Templates window will appear.
2. Select the template to be deleted from the drop-down list.
3. Click **Delete**.
4. Confirm that you wish to delete this template by clicking **Yes**.

You can make many template changes at one time by using **Save** instead of **OK**. **OK** will close the Advanced Capture Templates window, saving your latest changes.

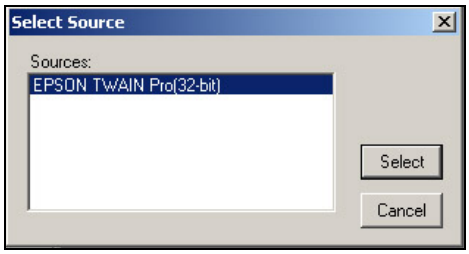
SCANNING IMAGES

LS software can acquire images from scanners that supply a TWAIN interface. (Almost all

scanners support TWAIN.) TWAIN helps scanners provide their own software interface during scanning.

To Select the Desired Scanner

1. Choose **Image > Scan > Select Scanning Source**. The Select Source window will appear.



2. Select the desired device from the list.
3. Click **Select**.

The device you select will remain the default until it is changed, even after rebooting. Generally, if you have only one TWAIN device, it will already be the default.

To Scan an Image from a Scanner

1. Select **Image > Scan > Start Scanning**.
2. Change any settings offered by the device as desired. Consult Help for the scanner to learn about the **Scan** dialog window for your device.
3. Click **Scan** (this button may have different names, including OK or Acquire, for different devices). The image will appear in a new Image window inside the workspace.

Most scanners offer a "fast preview" mode. You can use this to adjust brightness, contrast and other settings appropriately before scanning.

CHAPTER SIX: DIGITAL VIDEO PLAYER

- Purpose
- How to access the player
- Player Features
- Player Options
- Extract or Delete Individual Files (Frames)
- Create Sequence (.sqv) files by merging images
- .AVI files

PURPOSE

Digital Video Player (DVP) in LS software clubs multiple images in a single file and lets you go thru them in a very easy way.

If you need to observe your sample over a period of time, (e.g. Chemiluminiscence blots), it is required to snap multiple pictures at regular and definite time intervals, maybe with progressively higher integration times.

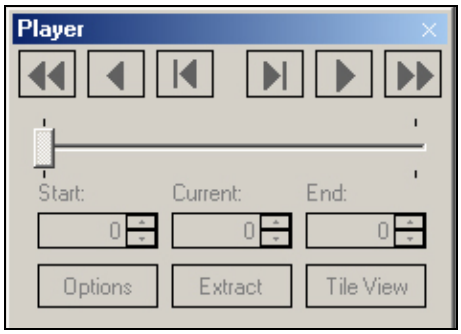
Depending on the camera used, LS software provides two features - Sequential Integration and Dynamic Integration. (For detailed explanation of this features, please see relevant chapters in this manual). Both these features produce a series of images as output. LS software serializes these images into a single file in the workspace, with extension .sqv). DVP lets you work with such .sqv files.

HOW TO ACCESS THE PLAYER

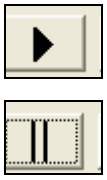
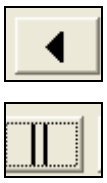
There are following ways to bring up the DVP.

1. The DVP comes up automatically, as soon as the image-capture process for Sequential and Dynamic Integration is complete.
2. When you load a .sqv file or a .avi file in the LS workspace, it can be accessed from **Acquire > Digital Video Player > Player.** Exception: if the .sqv or .avi has only one file (as opposed to multiple files) embedded.
3. When multiple image files are merged into a single file, this creates a .sqv file and the player is brought up.

PLAYER FEATURES

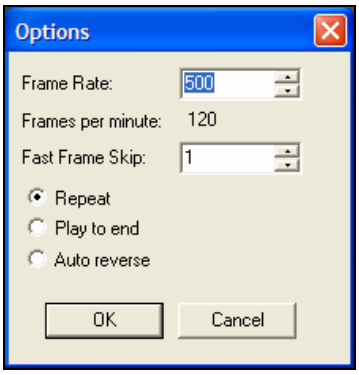


Below is the description for each of the Player's buttons:

BUTTON	FUNCTIONALITY
	This button allows viewing the **next image**.
	This button allows viewing the **previous image**.
	Press this button to **play** the sequence of images. Playing will show the images one after another. This button changes to a **Pause**/**Stop** button, which stops any kind of playing.
	Press this button for **playing** the sequence of images in the **reverse order**. This button changes to a **Pause**/**Stop** button, which stops any kind of playing.
	This button plays the sequence with a number of images to **skip**. The number of images to skip can be set in the Options dialog.
	This button does the same as the previous with the difference that he plays in **reverse order**. It uses the same number of images to **skip**.
	The Options button shows the Options dialog where can be set some properties of the Player. A detailed description follows in this chapter.
	This option lets the user work with individual frames (images) from a single .sqv file. You can extract a single frame if required and also delete unwanted ones. A detailed description follows in this chapter.
Tile view	Clicking this checkbox displays thumbnails of images in a tiled fashion. This can be useful in extracting or deleting specific images. A detailed description on how to delete/extract images follows. When the player is active, this option also shows active scrolling and the currently active frame is highlighted red.

PLAYER OPTIONS

Click the **Options** button in the player to set individual options for going through frames in an .sqv or .avi file. It brings up the following window:



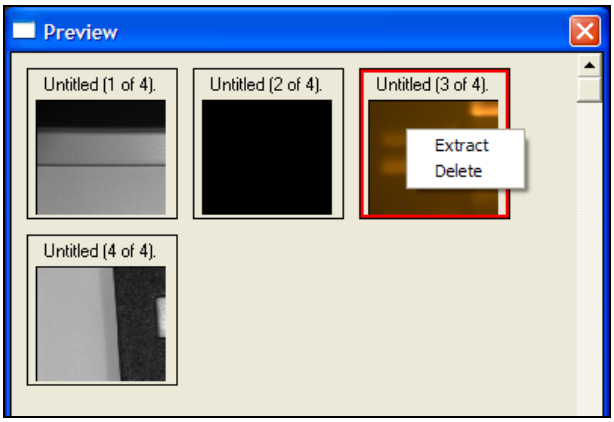
Frame Rate	This is the interval (in milliseconds) between two consecutive images that are displayed. For example, a value of 500 means that when you press the Play button a different image will be displayed each half of second. 'Frames per minute' is automatically calculated based on this value.
Fast Frame Skip	This is the number of images to be skipped when playing with  or  button.
Repeat	When the player reaches the end of the sequence of images it starts again with the first image.
Play to end	When this option is selected, the player will stop when it reaches the end of the sequence of images.
Auto reverse	When this option is selected, the player goes back in reverse order after it reaches the end of the sequence of images.

EXTRACT OR DELETE INDIVIDUAL IMAGE FILES (FRAMES)

It is possible to remove or extract individual frames from a single .sqv or .avi file.

Extraction creates new images in the workspace and **DOES NOT** remove them from the sequence. Deletion simply deletes them from the sequence.

The easiest way to initiate this action is by clicking on 'Tile View' checkbox in the player, which brings up the following window. Just right click on any image to extract or delete.

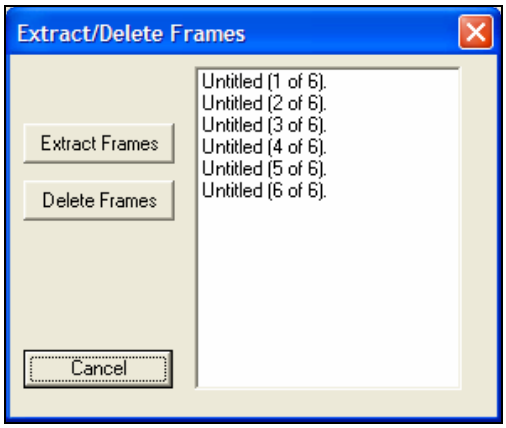


The other two ways this action can be initiated is:

If the player is open, click on **Extract/Delete** from the player.

If the player is not open, but the .sqv file is open, click on **Acquire > Digital Video Player > Extract/Delete…**

The following window is brought up, that lists all the frames inside the .sqv in question:



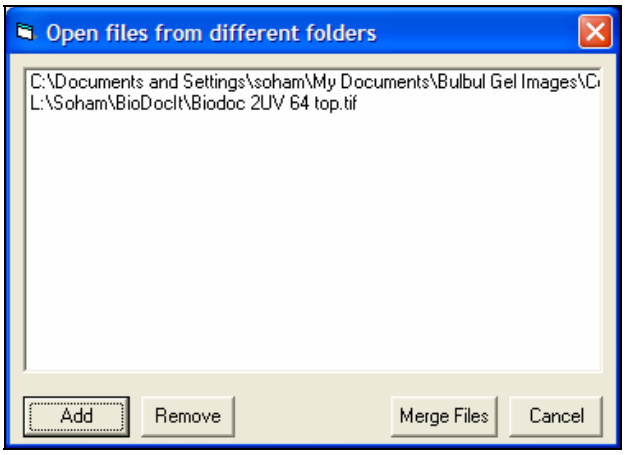
All the images contained in the active sequence are in the list. The user can extract (open the specified image in a new window) images or remove images from the active sequence.

CREATE A SEQUENCE (.SQV) FILE BY MERGING IMAGES

It is possible to merge existing files (or open images) and create a single .sqv file. This is a good way to keep all your images in one single file.

There are three different ways of doing this in LS software:

1. **Acquire > Digital Video Player > Merge** images. This feature can be used to merge images open in the LS workspace.
2. **Acquire > Digital Video Player > Merge** files. This feature can be used to merge files placed in a single folder.
3. **Acquire > Digital Video Player > Special Merge** files. This feature can be used to merge files present in different folders. See window below:



Controls:

Add button: Click on 'Add' button to find files from the hard-disk to merge. Existing files in the workspace are automatically listed.

Remove button: Select a file and click on 'Remove' button to remove it from the list.

Merge Files: Click this button to actually merge the listed files into a single .sqv .

.AVI FILES

Often times, it is required to gather all your files and share them with another researcher or open them in another place on another computer. .SQV being a custom format by UVP Inc., you will need to export .SQV files into a standard universal format, to accomplish this task.

Audio Video Interleaved (AVI) is one such broadly acceptable format. (It is a simplistic Windows format that caters for needs of slow animation that includes audio and video.)

To import an .AVI file, no special steps are required to be taken since it is one of the standard formats supported by LS Software.

To export a .SQV file to .AVI, use the 'Save As..' functionality provided by UVP Software.

[Depending on the requirement of the target player, AVI files may need to be compressed / encoded using a specific codec. Before exporting, LS software lets you choose from one of the pre-installed codecs on your computer, if you check the checkbox "Show Codecs Dialog When Exporting to AVI".

CHAPTER SEVEN: LOADING, SAVING AND TRANSFERRING IMAGES

- Loading Images
- Saving Images
- Image Files
- Transferring Images Using FTP

LOADING IMAGES

LS Software will load images in most popular formats, including JPEG, TIFF, GIF, PNG, TGA and BMP. If the image was previously saved using one of the LS software packages, then other image details such as the image's scale, history and annotations will be loaded as well.

Many demo images are included with LS software, so that you can experiment with different types of images.

For a discussion of exactly how image information is stored and how to move image files around on disk, or to send them to another LS user, see "Image Files."

To Load an Image

1. From the **File** menu, choose **Open**.

You can also use the **Open** button on the Toolbar. The software opens TIFF, JPEG, AVI, BMP, TARGA, GIF, PNG and SQV files.

2. Select the type of file you wish to open. If you're not sure of the file type, select "All Files."
3. Navigate through your disk drives to the file folder in which the image is stored. LS software keeps a folder structure of its own that it will show by default. Most images will probably be in this folder.
4. Select the desired image file.
5. Click **Open**. An Image window containing the desired image will appear.

To Load a Demo Image

1. Go to **File > Open Demo**.
2. Select the desired demo image file from the list of available files.
3. Click **Open**. An Image window containing the desired image will appear.

SAVING IMAGES

You can save images acquired in LS so that you can continue to work with them in later sessions, keep them as records or load them in other software packages. To Save a New Image

1. From the **File** menu choose **Save**. The Save window will appear.

You can also use the **Save** button on the Toolbar.

2. Select the file type that you would like to use from the drop-down list near the bottom of the window.
3. Navigate through the drive and folder structure to the location where you would like to save the image.
4. Enter a filename for the image.
5. Click **Save**.

To Save Changes to an Image

1. From the **File** menu choose **Save** (or use the **Save** button on the Toolbar). Changes will be saved to the existing file, overwriting the previous image data.

To Save Using a Different File Folder, Name or Type

1. From the **File** menu choose **Save As**. The Save window will appear.
2. Select the file type that you would like to use from the drop-down list near the bottom of the window.
3. Navigate through the drive and folder structure to the location where you would like to save the image.
4. Enter a filename for the image.
5. Click **Save**.

IMAGE FILES

Images are saved as two separate files. While the two files have the same name and appear in the same file folder, they have different extensions.

The Image File

The more important of the two files is the Image File, as it contains the actual image. It is saved in the image file format (file type) selected when saving, which is JPEG by default. The file extension will reflect the file format (e.g. ".JPG" for a JPEG file).

LS Software supports the following formats:

JPEG: Joint Photographic Experts Group. A common lossy compression image format used to store images on disk. JPEG files generally have JPG or JPEG extensions.

TIFF: Tagged Image File Format, a common image format. Depending on settings, TIFF can be either a lossy or a lossless compression format. In LS software, it is used in the lossless mode to reduce image file size without losing integrity. TIFF files generally have TIF or TIFF extensions.

TGA: Truevision Targa image format. TGA is a lossless compression format that reduces file size somewhat. TGA files generally have a TGA extension.

BMP: Microsoft Bitmap image file format. BMP is a lossless format which provides some compression to reduce file size. BMP files generally have a BMP extension.

PNG: Portable Network Graphics, a common image format. PNG is a lossy compression format that results in very small files. Files stored in PNG usually have a PNG extension.

GIF: Graphic Interchange Format, a proprietary Xerox image compression format. GIF is a lossy compression format that results in very small files. Files stored in GIF usually have a GIF extension.

JPEG, PNG and GIF are lossy compression formats. TIFF, TGA and BMP are lossless compression formats (at least, as used by this software; TIFF can actually be either lossy or lossless). Lossy compression makes small, usually non-visible changes to an image in order to make it store more compactly on disk. Typically, formats that use lossy compression store in much less space than lossless compression formats. By comparison, a lossless format does not store as compactly, but also does not change the image in any way.

The Extended Attributes File

The image will also have a second file that ends with the extension ".EXT." This file contains the image's effect settings, history, scale and annotations. It will appear in the same folder as the image file and will have the same name with the image's extension added to the end. For example, if the Image File is named "MyImage.JPG," the matching extension file would be "MyImageJPG.EXT".

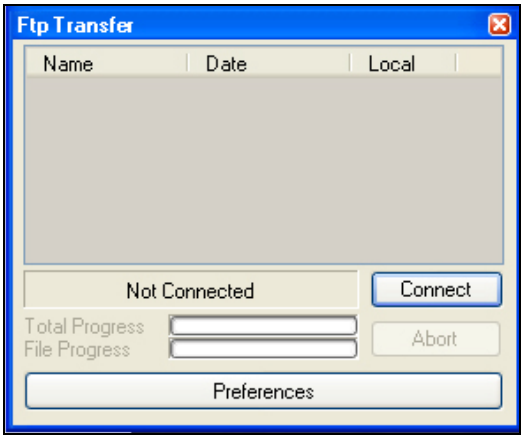
If you wish to copy an image, move an image or send an image to another LS software user, you should copy, move or send both files.

TRANSFERRING IMAGES USING FTP

You can transfer images acquired in LS so that you can continue to work with them on other computers. The ftp plugin automatically looks at the imaging system computer for new files and transfers them to a selected location on another computer.

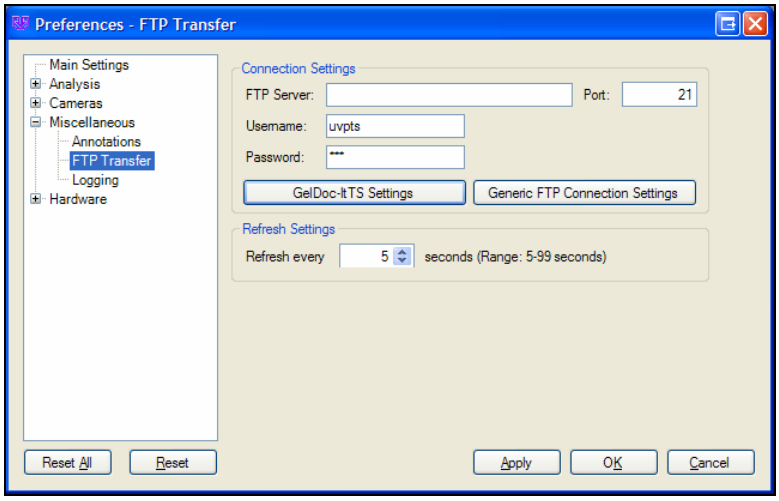
To Save an Image for Transfer

1. With an image open, from the **File** menu, choose **Save as** and save the file to VisionWorksLS/ftptransfer directory.
2. To see the images in the FTP Transfer window, select **View > Plugins > Ftp Transfer.**
3. The file will display in the FTP Transfer window



Configure the FTP Connection Settings

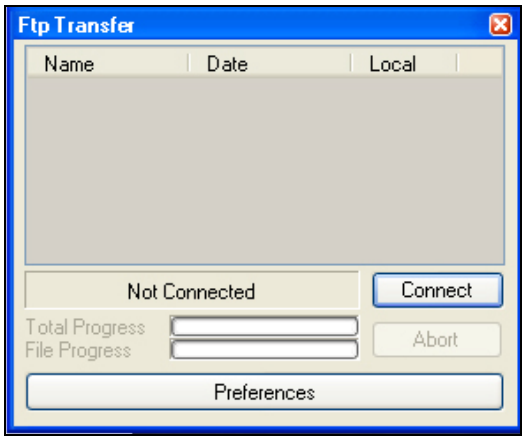
1. From the **File > Preferences > Miscellaneous**, click on **FTP Transfer**. The connection settings window will display.



2. Enter the ftp server location, username and password obtained from your system administration, if applicable.
3. Set the Refresh time for the files to automatically transfer.

To Transfer an Image

1. Click the **Connect** button if the FTP is not connected.
2. The file transfer progress will show in the FTP transfer window.



CHAPTER EIGHT: IMAGE EDITING

- Overview
- Undo and Redo
- Using Selection Tools
- Copy
- Paste
- Paste Special

OVERVIEW

The image editing features provided in LS Software allow you to undo changes to images, to copy images or parts of images, to paste images from the clipboard as new images and to merge clipboard images with existing images using the **Paste Special** feature.

Most editing features are found on the **Edit** menu. However, to be able to use all editing features, you will also need to use the **Selection Rectangle** and **Selection Ellipse** tools from the **Tools** menu.

The **Edit** menu also offers a **Cut** feature that can be used while editing text in LS Software. Cut does not work on images.

UNDO AND REDO

The **Undo** command will undo the last material change made to an image. Material changes include all manipulations and use of the **Paste Special** command. Changes made through the Effects tab (e.g., zooming, panning or changing brightness or contrast) cannot be undone with the Undo command. Changes to annotations also cannot be undone.

The **Redo** command reverses the last **Undo**. To see what the last material change did in detail, you can alternate between **Undo** and **Redo**.

Changes made on the Effects tab do not permanently change the image. To reverse these changes, click **Reset** on the Effects tab.

To Undo the Last Change to an Image

1. If the image is not the foremost image, select it by clicking the window title bar or by selecting its title from the Windows menu.
2. From the **Edit** menu, click **Undo**. The former version of the image will be restored.

If you have made several changes to an image that was saved, you can return to the saved copy using the **Revert** command. If you decide that you want the last change after all, you can reinstate it with the **Redo** command.

To Redo the Last Change to an Image

1. If the image is not the foremost image, select it by clicking the window title bar or

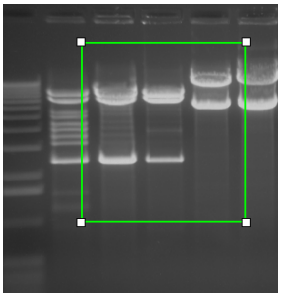
	by selecting its title from the Windows menu.
2.	From the **Edit** menu, click **Redo**. The last change will reappear.

The **Redo** command will be unavailable if you have not yet used **Undo**.

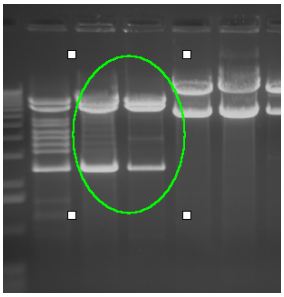
USING SELECTION TOOLS

These tools allow you to mark part of the image for use in other operations. LS software provides several Region of Interest (ROI) selection tools:

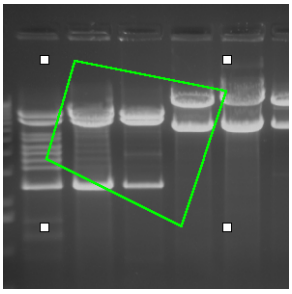
ROI tools (left to right): New ROI, Rectangular ROI, Elliptical ROI, Polygonal ROI, FreeForm ROI and Magic Wand ROI. Select the tools from the toolbar or from **Tools > Region of Interest (ROI).**



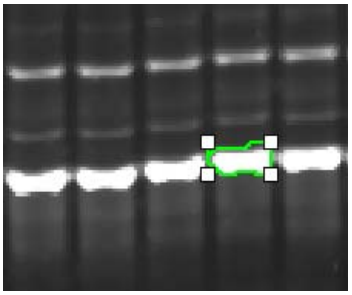
View Rectangular ROI selects rectangular regions.



View Elliptical ROI selects elliptical (oval) regions.



View Polygon ROI selects Polygonal regions.



View FreeForm ROI selects irregular regions.

Some notes on FreeForm ROI:

Tracing: Lets you trace the Region of Interest with your mouse pointer. Keep the left mouse-button pressed and draw around the region you are interested in. Lift the button to automatically complete and enclose the area.

Magic Wand: Marks the area automatically on the image. Click just once ***inside*** the region you are interested and the software tries to mark that area by identifying the edges. The spin-box 'Range' is the sensitivity of finding the edges, and is dependent on the dynamic range of the image. Lower the number, smaller is the area.

Apart from Analysis features, two operations in the software use an Region of Interest: **Copy** and **Crop**. **Copy** will copy the selected region to the clipboard. If there is no selected region, the entire image will be copied. **Crop** will remove (crop away) all parts of the image outside of the selected region. Either operation will work with either selection tool.

To Select a Region

1. Choose the desired selection tool either from the **Tools > Region of Interest (ROI)** or from the toolbar.
2. Starting with the upper-left corner of the desired region, drag the mouse downward and to the right until the desired area is marked.

You can actually begin at any corner. You must end at the corner opposite to where you started.

To Adjust the Selection

If the selection is not quite right, you can move it or resize it without having to start over:

1. *To move the selection*: Drag the interior of the selection to the new location.
2. *To make the selection wider or narrower*: Drag the left or right dotted lines that bind the selection to the desired size.
3. *To make the selection taller or shorter*: Drag the top or bottom dotted lines that bind the selection to the desired size.
4. *To change both height and width at one time*: Drag any corner control point to the desired size.

To Cancel the Selection

1. Click once anywhere on the image away from the current selection. The selection markers will disappear.

You can also cancel a selection by pressing the ESC key.

COPY

Used on an image, the **Copy** command copies all or part of the image to the clipboard. If the image currently has a selected region, only that region is copied. If the image currently has no selection, the entire image will be copied.

Copy can also be used on text, in which case it acts in the standard Windows fashion.

Once an image (or part of an image) has been copied, you can paste it into other software

packages that support images. You can also paste the clipboard contents back on an image using either the **Paste** or **Paste Special** commands.

Images pasted into other software packages will use the display settings from the Effects tab (*brightness*, *contrast*, *gamma*, *invert* and *pseudocolor*). They will include annotations if the annotations were displayed when **Copy** was used. If annotations were hidden, they will not be included.

Images copied from LS software and then pasted back using either **Paste** or **Paste Special** will always include annotations and will be affected by display settings only on a temporary basis, just like all other images.

Copy does not affect the image in any way.

To Copy an Entire Image

1. If there is a selection on the image, click away from the selection once to cancel it.
2. Choose **Copy** from the **Edit** menu.

To Copy a Selected Region Within an Image

1. Select the desired region using one of the selection tools.
2. Choose **Copy** from the **Edit** menu.

Whether you copy an entire image or a selected region of an image, **Copy** also is available by using the Ctrl-C accelerator key, by using the **Copy** button on the Toolbar and by using the shortcut menu on the Image window itself.

PASTE

This command takes an image from the clipboard and imports it into LS software, displaying it in a new Image window.

Paste can also be used on text, in which case it acts in the standard Windows fashion.

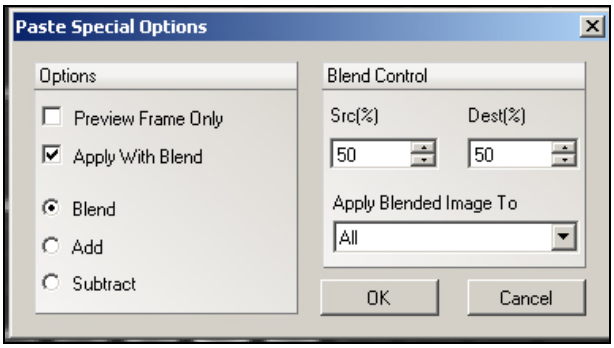
To Paste an Image

1. From the **Edit** menu choose **Paste**. The image will be displayed in a new Image window.

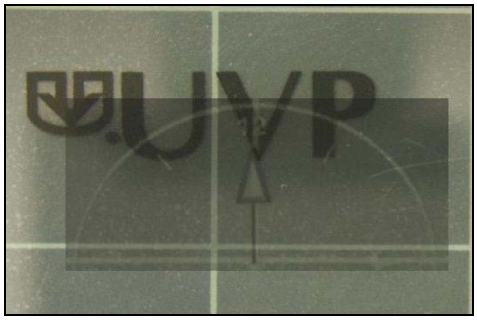
Paste is only available if there is an image on the clipboard.

PASTE SPECIAL

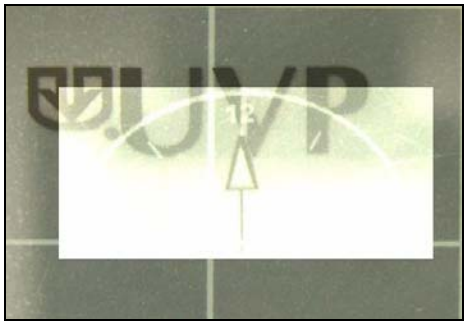
This command allows an image on the clipboard to be merged into the current image. It is useful for adding comparison or reference information into an image, for making composite images and for testing two images against one another for motion. To modify the Paste Special options, go to **Edit > Paste Special Options**:



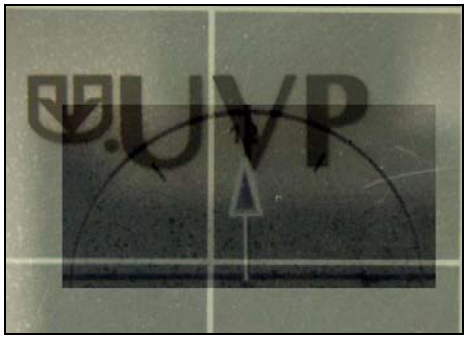
The following merge modes are available in all LS Software:



Blend: mixes the incoming image with the current image in a selected proportion. If the Source proportion (Src%) is set to 100%, pixels in the incoming image replace those in the existing image without mixing (i.e. the incoming image is copied entirely over the existing image wherever it lands). Blend is used primarily to place comparison information into an existing image, especially when using high proportions.



Add: adds pixels in the incoming image to those in the existing image up to maximum intensity. Add is used primarily to build composite images with little or no overlap. This feature requires no additional settings.



Subtract: subtracts pixels in the incoming image from those in the existing image. Subtract is used primarily to test for differences in or motion between two otherwise similar images. This feature requires no additional settings.

To Merge Two Images

Merging results in a new image that includes the existing image plus all of the area of the

incoming image, even if that area is off of the edge of the existing image. This allows you to compose "mosaic" images (usually with **Add**) by placing the incoming image at the edge of the existing image.

1. Place one of the images on the clipboard using the **Copy** command. If desired, a sub-region of the image can be used. See "Copy" for more details.
2. From the **Edit** menu, choose **Paste Special**. The Paste Special window will appear.

Paste Special also is available from the Toolbar and the Image window's shortcut menu.

3. If the incoming image is large, select the **Preview Frame Only** check box. This will allow you to position the incoming image without the lag caused by redrawing a large image. Otherwise, clear the check box so that you can preview the result.
4. Select the desired merge operation: **Blend**, **Add** or **Subtract**.
5. If using Blend, set the desired blend percentage.
6. Click **OK**.
7. Moving the mouse over the existing image, position the merge as desired. The merge results (or positioning frame, for **Preview Frame Only**) will be previewed as you move the mouse. You will be positioning the upper-left corner of the incoming image. Once positioned, the selection can be moved.
8. When you reach the desired position, click the right mouse button once. Merges that involve large images may take a few seconds to complete.

CHAPTER NINE: IMAGE FILTERS

- Overview
- Rotate
- Flip Horizontally or Vertically
- Resize
- Reduce to Mono
- Convert
- Remove Noise
- Despeckle
- Sharpen
- Blur
- Emboss
- Starfield Subtraction
- Duplicating Image
- Burn Changes onto New Image
- Background Correction
- Background Subtraction

Several Image Filters can be selected from the toolbar:

Left to right: Reduce to Mono, Rotate, Flip Horizontal, Flip Vertical, Despeckle, Remove Noise, Sharpen, Blur, Emboss, Starfield Subtraction

OVERVIEW

LS Software offers several types image filters. Each filter makes a substantial and material change to the image itself. In general, the results of an applied filter are *not* reversible. Image filters can be used to correct for problems in preparing for and in acquiring the image. They also can be used to expose new information in an image by removing or de-emphasizing other information. Any changes to the image are logged in the image history data, which also offers a notes sections.

To get the most from image filters, it is important to understand the following features first:

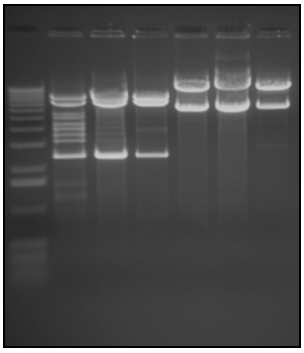
- ***Undo***: Undo reverses the last filter performed on an image.
- ***Saving***: Saving stores an image on disk. It allows you to return to a former version of an image if you dislike the results of one or more image filters or would like to try different options.

A record of image filters applied to an image is kept in the image's Image History.

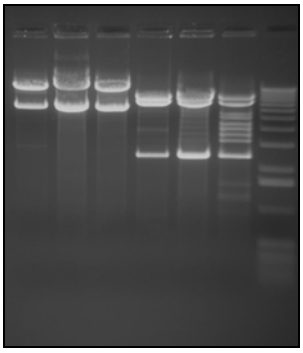
The types of image filters supplied are:

- ***Rotate***: Rotates the image around its center, useful for aligning images taken with a crooked gel.
- ***Flip Horizontally***: Mirrors the image right for left, correcting for an upside-down gel.
- ***Flip Vertically***: Mirrors the image top for bottom, also correcting for upside-down gels in the other direction.
- ***Resize***: Enlarges or reduces the image in size. You can use this to reduce an image's size so that you can work with it more easily. Reducing an image also takes less memory and disk space.
- ***Reduce To Mono***: Removes color from the image, making it monochrome. This is primarily used either to remove distracting color or to prepare an image for certain types of automated analyses outside the scope of this software.
- ***Convert***: Allows user to change the bit depth of the image. Bit depth options will show the affect the change will have on the image and compatibility with other software applications.
- ***Remove Noise***: Removes periodic (patterned) noise in the image.
- ***Despeckle***: Removes single-pixel flaws in the image (called artifacts).
- ***Sharpen***: Enhances edges in the image, making them more pronounced. This can make small features easier for you to see.
- ***Blur***: Dulls edges in the image, making them less visible.
- ***Emboss***: Gives the image a "chiseled in stone" look. The resulting 3D appearance makes certain edges and features appear more obvious.
- ***Starfield Subtraction***: This filter eliminates starfield interference.
- ***Duplicating Images***: Creates a duplicate image with filters, annotation, etc. applied to the image.
- ***Burn Changes onto New Image***: Creates a new image with the all enhancements and analysis applied to the image now burned into the image.
- ***Background Correction***: Adjusts the black level to either the current image or a to a new image.
- ***Background Subtraction***: This command burns the changes onto a new image instead of the changes being an overlay.

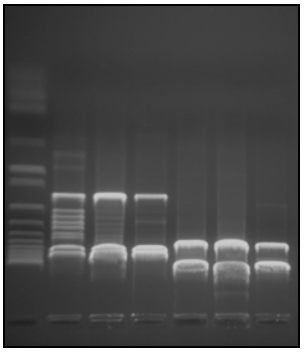
Examples of Some Image Filters



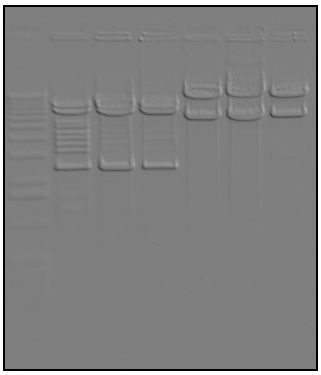
Before Manipulation



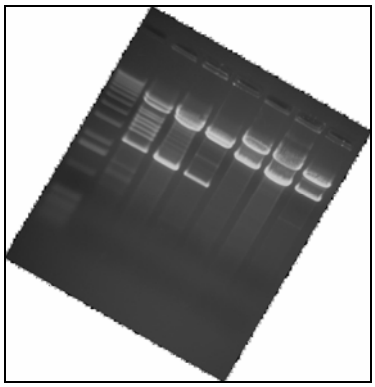
Flipped Horizontally



Flipped Vertically



Embossed

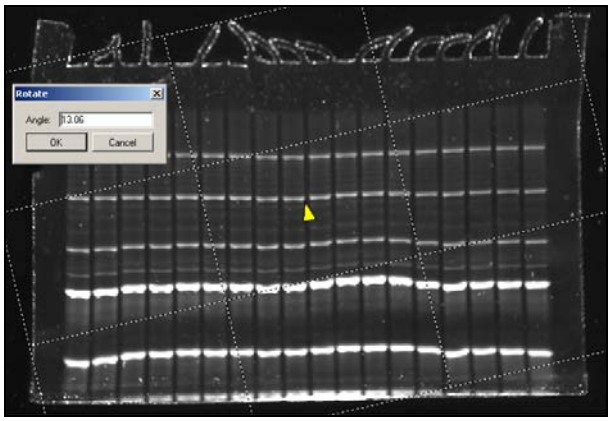


Rotated

ROTATE

Rotate an image by an arbitrary number of degrees. You might use it to correct for a misaligned gel. Rotate allows you to rotate the image easily by a graphically-selected degree to align the image based on an internal image feature.

To rotate an image, go to **Image > Filters > Rotate.**



All instructions below assume the desired image is foremost. If it is not, select it from the **Windows** menu or click its title bar.

To Rotate an Image to a Desired Orientation

If you have a desired orientation for the image, you can use the **Rotate**.

1.	From the **Image > Filters > Rotate**. The Rotate window will appear and a grid will be overlaid on the image.
2.	Drag the grid so the yellow arrow moves in the direction you would like the image rotated.
3.	Once the grid is oriented to your satisfaction, click **OK** on the **Rotate** window.

To Rotate an Image by an Exact Number of Degrees

You can also rotate the image by an exact number of degrees. For example, you can correct for an upside-down gel through rotating by 180 degrees.

1. Choose **Image > Filters > Rotate**. The Rotate window will appear and a grid will be overlaid on the image. For this operation, you will ignore the grid.
2. On the Rotate window, type the desired number of degrees into the **Angle** text box.
3. Click **OK**.

Rotations by 90, 180 or 270 degrees do not degrade the image. These operations can be completely reversed by a rotation of the same amount in the opposite direction.

FLIP HORIZONTALLY

This filter mirror-images an image, right for left. Image filters, it does not degrade the image and may be used repeatedly with no ill effect to the image. Two uses of the filter will return the image to its starting orientation.

To Flip an Image Horizontally

1. If the image is not the foremost image, select it from the **Windows** menu or click its title bar.
2. Choose **Image > Filters > Flip Horizontally**.

FLIP VERTICALLY

This image filter mirror-images an image, top for bottom. Unlike most image filters, it does not degrade the image and may be used repeatedly with no ill effect. Two uses of the filter will return the image to its starting orientation.

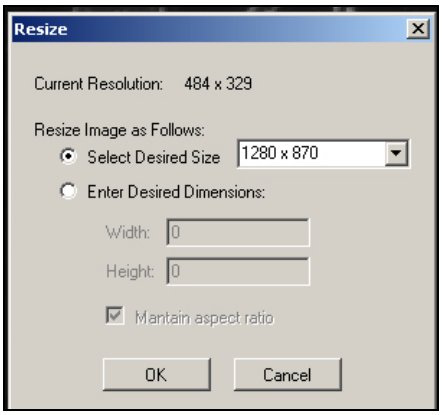
To Flip an Image Vertically

1. If the image is not the foremost image, select it from the **Windows** menu or click its title bar.
2. Choose **Image > Filters > Flip Vertically**.

RESIZE

The Resize filter allows the image to be changed in size. It replicates or merges pixels as appropriate to arrive at the new size. Resize most commonly would be used to create a smaller version of a very large image to allow you to increase response time when applying filters or to import the image into another software package that does not accept large images.

There is little point to increasing an image's size, although the filter does support it. Such an image would have more physical pixels after the operation, but it does not gain any new information content.



To Resize an Image

1. Choose **Image > Filters > Resize**. The Resize window will appear.
2. Select the desired new size from the drop-down list of suggested sizes.

   OR

   Select the **Enter Desired Dimensions** option and type either the desired new width or the desired new height.

If you wish to distort the image, you can clear the **Maintain Aspect Ratio** check box and type the new width and height. This should be used only to reverse a similar distortion created in the image capture process.

3. Click **OK**. Resizing a large image may take a few seconds.

REDUCE TO MONO

This filter reduces a color image to monochrome. This is primarily useful when colors in an image are distracting rather than informative. For example, if light strikes certain surfaces from some angles, a "rainbow effect" (prism) will appear. Another use is to adapt for some software packages and techniques that require monochrome images or which are less reliable on color images.

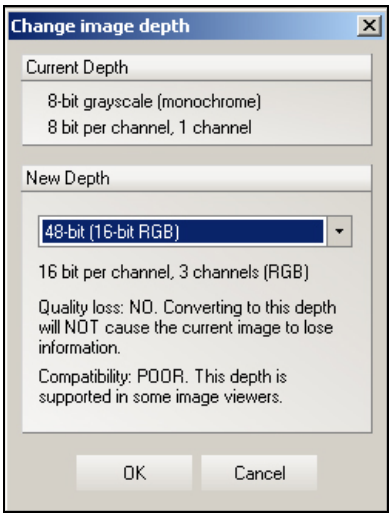
The Reduce To Mono filter uses a weighted mix of colors to arrive at each pixel's monochrome intensity. Green is very heavily weighted while blue is almost disregarded.

To Reduce a Color Image to Monochrome

1. If the image is not the foremost image, select it from the **Windows** menu or click its title bar.
2. Choose **Filters > Reduce To Mono**. Reducing a large image to monochrome may take a few seconds.

CONVERT

This filter converts an image bit and color depth when needed.



To Convert an Image Bit and/or Color Depth

1.	If the image is not the foremost image, select it from the **Windows** menu or click its title bar.
2.	Choose **Filters > Convert**. Reducing a large image to monochrome may take a few seconds. Select new depth and click **OK**.

REMOVE NOISE

This filter removes periodic (patterned) noise from an image. Patterned noise is removed by creating a frequency-space mapping (Fourier transform) of an image and removing frequency spikes away from the graph's origin.

There are two issues to be aware of with noise removal. First, if your image has actual (desired) pattern information, the operation will not be able tell these from noise and it will remove them. Second, the mathematics of the operation can cause some edges pixels to be identified as patterns, resulting in blurring on some images.

To Remove Noise from an Image

1.	If the image is not the foremost image, select it from the **Windows** menu or click its title bar.
2.	Choose **Image > Filters > Remove Noise**. Removing noise a large image may take ten or fifteen seconds.

DESPECKLE

This filter removes single-pixel flaws (called artifacts) from an image. For many reasons, the image capturing process and hardware might misread isolated individual pixels as either white or black. The Despeckle filter mathematically identifies and removes these "loner" pixels.

There are two issues to be aware of with the Despeckle filter. First, if your image has actual (desired) single-pixel bright or dark areas, the operation will not be able tell these from artifacts

and it will remove them. Second, the mathematics of the operation can cause some edge pixels to be identified as "speckles," resulting in slight blurring on some images.

To Despeckle an Image

1. If the image is not the foremost image, select it from the **Windows** menu or click its title bar.
2. Choose **Image > Filters > Despeckle**. Despeckling a large image may take a few seconds.

SHARPEN

This filter enhances edges in an image, making them more visible. It is easier to see fine detail after an image has been sharpened.

To Sharpen an Image

1. If the image is not the foremost image, select it from the **Windows** menu or click its title bar.
2. Choose **Image > Filters > Sharpen**. Sharpening a large image may take a few seconds.

BLUR

This filter blurs edges in an image, making them less prominent. You can see gross (large-scale) detail more easily after the edges have been blurred because details that may have been obscuring it are removed.

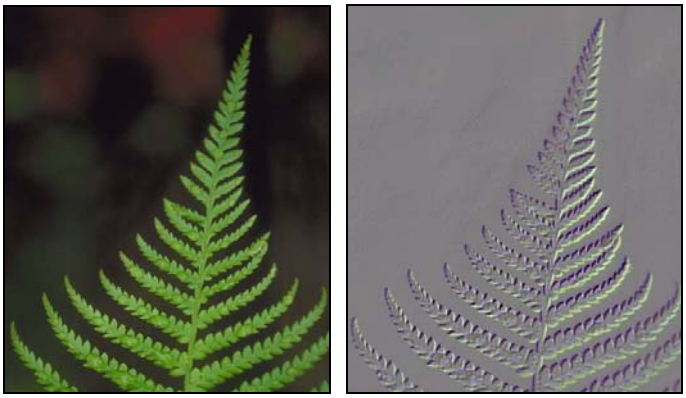
To Blur an Image

1. If the image is not the foremost image, select it from the **Windows** menu or click its title bar.
2. Choose **Image > Filters > Blur**. Blurring a large image may take a few seconds.

EMBOSS

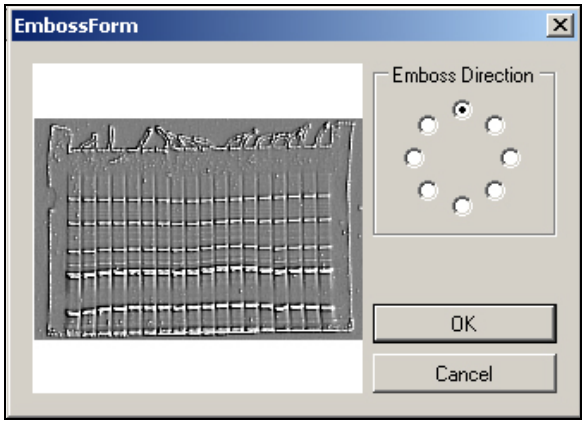
This filter gives an image a "chiseled in stone" look. Edges take on a three-dimensional (3D) appearance, making them stand out vividly.

Example: Before and After Embossing



Emboss can be performed from any of eight cardinal directions. Conventionally, these are referred to as North (up), Northeast, East (right), Southeast, South (down), Southwest, West (left) and Northwest.

The easiest way to think of the "direction" of the embossment is as the location of a strong light source around the image. For example, if you select North, the image will appear as if it is illuminated with a strong light from the top. Therefore, horizontal edges will be strongly lighted on the top edge and shadowed on the bottom, and vertical edges will tend to disappear. Diagonal edges will be shadowed in direct proportion to how closely they are horizontal. Alternatively, if you select East, vertical lines will be shadowed on the left side and lighted on the right side. Horizontal lines would become harder to make out.



To Emboss an Image

1. If the image is not the foremost image, select it from the **Windows** menu or click its title bar.
2. Choose **Image > Filters > Emboss**. The Emboss window will appear.
3. Select the desired **Emboss Direction**. The Preview window will show a thumbnail sample of what your image would look like embossed from this direction.

You can use the arrow keys to cycle through all eight directions while watching the Preview.

4. Click **OK**.

STARFIELD SUBTRACTION

This filter eliminates starfield subtraction

To eliminate starfield subtraction

1. Choose **Filters > Starfield Subtraction**. Reducing a large image to monochrome may take a few seconds.

DUPLICATING IMAGES

This command creates an identical copy of the foremost image. The new copy will appear in its own Image window.

If you previously have saved the image being duplicated, the new copy will *not* inherit the old copy's filename. Instead, the new copy will be an unsaved image.

To Duplicate an Image

1. From the **Image > Filters > Duplicate Image**. A second copy of the image will appear in a new Image window as an untitled image.

BURN CHANGES ON NEW IMAGES

This command burns the changes onto a new image instead of the changes being an overlay. This is for use with annotations that you want to show in an image placed in another document or for presentation.

BACKGROUND CORRECTION

This command burns the changes onto a new image instead of the changes being an overlay. This is for use with annotations that you want to show in an image placed in another document or for presentation.

BACKGROUND SUBTRACTION

This command burns the changes onto a new image instead of the changes being an overlay. This is for use with annotations that you want to show in an image placed in another document or for presentation.

CHAPTER TEN: ANNOTATIONS

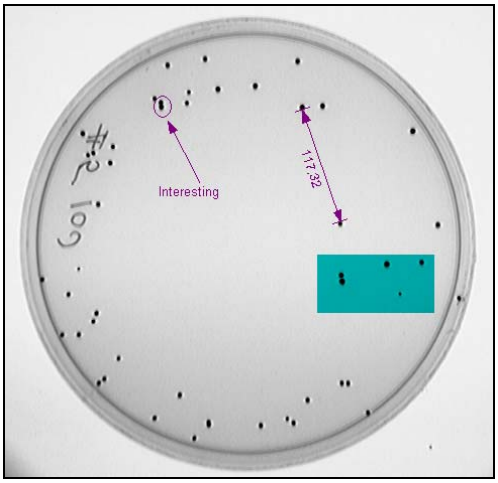
- Overview
- Viewing and Hiding Annotations
- Types of Annotation
- Spatial Calibration
- View Rulers
- Creating Annotations
- Selecting Annotations
- Moving and Resizing Annotations
- Editing Text Annotations
- Formatting Annotations
- Deleting Annotations

Many of the annotation tools can be accessed through the toolbar:

OVERVIEW

Annotations allow you to mark areas of an image without changing the image itself. This means that you can indicate areas that need more study, that are particularly interesting or that support a particular scientific interpretation.

When using annotations, imagine that you have a transparency over your image on which you can write or draw. At any time, you can remove or replace the annotation layer to see the image with or without annotations.



Gel with Annotations

Topics explain the annotation system in more detail:

- *Spatial Calibration*: Allows the user to spatially calibrate to a known measurement

- *Image Rulers*: Default to pixel units and can be spatially calibrated for image measurements.
- *Creating Annotations*: Explains how to create annotations.
- *Deleting Annotations*: Explains how to remove annotations you no longer want.
- *Formatting Annotations*: Explains how to use the Formatting menu when creating or editing annotations.
- *Moving and Resizing Annotations*: Explains how to move and resize annotations graphically.
- *Selecting Annotations*: Explains how to select annotations.
- *The Text Edit Window*: Explains how to use the Text Edit window for entering and editing text annotations.
- *Types of Annotations*: Explains the annotation tools provided in LS Software.
- *Viewing and Hiding Annotations*: Explains how to show or to hide all annotations on an image.

VIEWING AND HIDING ANNOTATIONS

Although annotations are useful for drawing attention to important features, sometimes they cover up features you need to see. You can view or hide all annotations on an image with a single command.

Each Image window keeps track of whether the annotations are hidden or shown for that image. So you can show annotations on one image while hiding them on another image.

Annotations are shown automatically when you select any annotation tool so that you can see what you're doing.

To Show or Hide Annotations for an Image

1.	If the image is not the foremost image, select it from the **Windows** menu or click its title bar.
2.	Choose **Toolbars > Annotations**. If this menu option is checked but no annotations appear, you have not yet put any annotations on the image.

TYPES OF ANNOTATION

LS software offers five different types of annotation:

- ***Text***: Text annotations consist of written information. You can use them to label a particular part of an image. You are able to select the *font size*, *color* and *formatting* (bold, italic or underline) of a Text annotation.

Text annotations display in a constant size, no matter what the zoom factor is, to ensure that you can always read them.

- ***Line***: Line annotations permit you to draw lines with optional arrowheads at one or both ends. You can use them to associate other annotations such as text with a particular image feature or to draw attention to an image feature. You can select the *color*, *line thickness, line style* (solid, dotted or dashed) and *arrowheads* (none, at start, at end, or both) of a Line annotation.

- ***Rectangle***: Rectangle annotations allow you to draw a rectangular frame around part of the image. You can use them to show the boundaries of an image feature. You can select the *color*, *line thickness* and *line style* (solid, dotted or dashed) of a Rectangle annotation.
- ***Ellipse***: Ellipse annotations are very similar to Rectangle annotations except that they are oval rather than rectangular. You can select the *color*, *line thickness* and *line style* (solid, dotted or dashed) of an Ellipse annotation.
- ***Highlighter****:* Highlighter annotations work like a highlighting pen by altering the color of the underlying image to draw attention to an area. You can select the *color* of a Highlighter annotation.

To **edit an annotation**, select the annotation, right mouse click on the annotation, then select from the menu options to change the annotation. Also refer to the Formatting Annotations section of this chapter for more information.

SPATIAL CALIBRATION

Special types of annotations are available from **Tools > Spatial Calibration** option:

- ***Define Image Scale***: Allows the user to spatially calibrate to a known measurement.
- ***Measure Length***: Length Measure annotations look somewhat like Line annotations except that they show the length of the line in *image scale units* (see "Rulers"). You can position Length Measure annotations from one point to another point to see how long any image feature is in real units. You can select the *colo*r, *line thickness*, *line style* (solid, dotted or dashed), *font size* and *formatting* (bold, italic or underline) of a Length Measure annotation.
- ***Measure Angle***: Angle Measure annotations consist of three points. They display the angle between the line formed by the first and second points and the line formed by the second and third points. You can use Angle Measure annotations to determine slants in lanes or in related bands across lanes in an electrophoresis gel. You can select the *color*, *line thickness*, *line style* (solid, dotted or dashed), *font size* and *formatting* (bold, italic or underline) of an Angle Measure annotation.
- ***Measure Area***: Area Measure annotations look somewhat like Rectangle annotations except that they show the area of the rectangle in *image scale units* (see "Rulers"). You can select the *color*, *line thickness, line style* (solid, dotted or dashed), *font size* and *formatting* (bold, italic or underline) of an Area Measure annotation.

Length Measure and **Area Measure** annotations use the image's scale in calculations. If these annotations display information in pixels ("px"), no scale has been set. See "Changing Sample Width" for instructions on setting the image's scale.

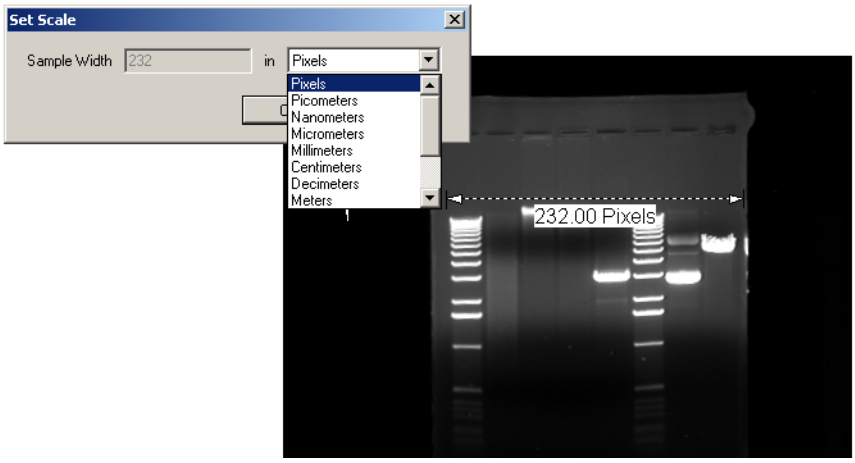
There are two ways to set the image's scale:

1. If you know the size of a single feature anywhere in the image, you can use the Scale Tool to set the scale of the entire image from that one feature.
2. If you know the size of the entire image in metric units, you will find it easier to change the Sample Width through the Image Information window.

To Calibrate the Image Using the Scale Tool

1. Choose **Tools > Spatial Calibration > Define Image Scale**.

2. Find the feature in the image for which you know the metric size. Click one edge of the feature.
3. Move to the other edge of the feature. As you move the mouse, the scale tool will show you how many pixels you are marking with the tool.
4. Click the other edge of the feature. The Set Scale window will appear.



5. In the Set Scale window, select the metric unit for the marked area (e.g. centimeters) and enter the number of units (e.g. 12).
6. Click **OK**. The rulers will reflect the new scale.

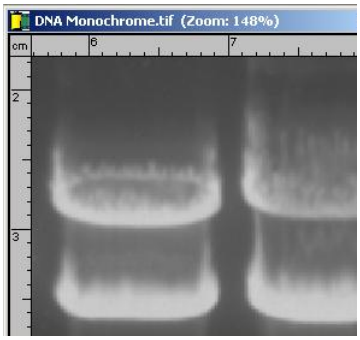
To Change Sample Width for an Image

Sample Width is calculated from the width of the image, which is usually an image of a sample or gel. It is the metric distance of the width of the scene in the image.

For example, if you removed the camera from the darkroom and carefully focused it on a 1-meter ruler, fitting the ruler exactly from left to right, the width of the scene in the image would be 1 m. If you focused the shot so that exactly half of the ruler was visible from left to right, the width of the scene would be 500 cm (1/2 m.).

1. Display the **Image Information** for the image. (See "Viewing Image Information" for instructions.)
2. In the **Overall Sample Width** area, select the metric unit (the right-most drop-down list) first. For example, if the sample width of the image is 500 cm, select centimeters.
3. Type the number of metric units in the text box immediately following **Overall Sample Width**. For example, if the sample width of the image is 500 cm, type "500."
4. Click **OK**.

RULERS



You will find rulers at the top and left of each Image window.

The rulers show the width and height of the visible portion of the image either in metric units (if calibrated) or in pixels (if un-calibrated). Images captured through the camera and Darkroom Hood are calibrated automatically in centimeters. As you zoom in or out and pan around the image, software updates the rulers to show the actual size and position of the visible portion of the image. As you move the mouse over the image, markers show your position on each ruler.

The units are shown in the upper-left corner (see the image above). Pixels (uncalibrated) are abbreviated "px;" all other values use metric standard abbreviations. When the rulers are calibrated to a metric measure, they may change units as you zoom in. For example, an image calibrated in centimeters may switch to millimeters when you zoom in by a large percentage. The measurements are still completely accurate; the rulers switch units because they are designed to show you a useful number of units at every point.

The scale information used by the rulers also is used by measurement annotations. Length measure annotations can be used to see the length of a feature that is not square to the rulers.

To Show or Hide the Rulers

You can show or hide rulers individually for each Image window. Hiding the rulers provides slightly more space in which to view the image.

1. On the **View** menu, click **View Rulers**.

CREATING ANNOTATIONS

You can create annotations using one of the annotation creation tools -- one for each type of annotation. The tools, which appear on the **Tools > Add Annotation Layers**, are **Text**, **Line**, **Rectangle**, **Ellipse**, **Highlighter**, and **Tools > Spatial Calibration** are **Define Image Scale, Measure Length**, **Measure Angle** and **Measure Area**.

All instructions below assume the desired image is foremost. If it is not, select it from the **Windows** menu or click its title bar.

To Create a Text Annotation

1. From **Tools** > **Add Annotation layers > Text**.
2. Click the position on the image where you would like the text annotation to be. The Text Edit window will appear.
3. Type the desired text in the text box.
4. Click **OK**. The Text annotation will be displayed at the position you indicated.

5. To change the color, font size and formatting (bold, italic, underline), click once on the text to change, from **Tools > Annotation Formatting**, select the appropriate settings.

To Create a Line or Length Measure Annotation

1. From **Tools** > **Add Annotation Layers > select Line** or **Spatial Calibration > Measure Length** as appropriate.
2. Click the position on the image where you would like the annotation to begin. A line will follow the mouse as you move it, showing you what the new annotation would look like. If you change your mind about adding the annotation at this point, simply press the ESC key.
3. Click the position on the image where you would like the annotation to end. The new annotation will be drawn.
4. If desired, change the color, line style and line thickness. Click on the annotation and from **Tools > Annotation Formatting** change the format..

To Create a Rectangle, Ellipse, Highlighter, or Area Measure Annotation

1. From **Tools > Add Annotation Layers** choose the appropriate tool **(Rectangle**, **Ellipse**, **Highlighter** or **Measure Area**).
2. Click the position on the image where you would like to place the upper-left corner of the annotation. A view of the new annotation will follow the mouse as you move, showing you what it would look like. If you change your mind about adding the annotation at this point, simply press the ESC key.
3. Click the position on the image where you would like the lower-right corner of the annotation to be. The annotation will be drawn.
4. If desired, change the color, line style and line thickness from **Tools > Annotation Formatting**. (With **Highlighter** annotations, you can only select color.) For an **Area Measure** annotation, you can also select formatting (bold, italic, underline).

To Create an Angle Measure Annotation

1. Choose **Tools > Add Annotation Layers > Measure Angle**.
2. Click the first point. A line will follow your mouse until you click a second point. If you change your mind about adding the annotation at this point, simply select a different tool from the **Tools** menu.
3. Click the second point. Again, you may cancel the new annotation by pressing the ESC key. A second line and the number of degrees will follow the mouse as you move it until you select the third point.
4. Click the third point. The annotation will be drawn.
5. If desired, change the color, font size, and formatting (bold, italic, underline) from the **Tools > Annotation Formatting**.

THE TEXT ANNOTATION WINDOW

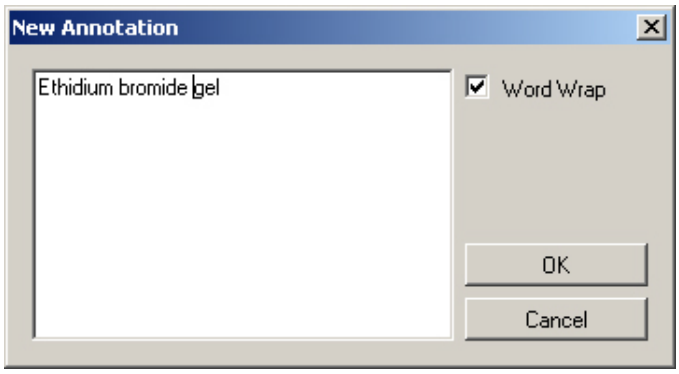
Text annotations allow you to place labels on an image to describe or to name a feature of the image. Use the New Annotation window to enter the text.

The New Annotation window appears whenever you add a new **Text** annotation. It also appears when you edit a **Text** annotation, which you can do by double-clicking the **Text** annotation or by choosing **Edit** from the shortcut menu associated with it.

In order to speed up your work, the Text Edit window saves the last text you entered, making it

the default for the next **Text** annotation you create. To enter new text, simply start typing and the existing text will be replaced.

You can enter multiple lines of text as a text annotation. To have the text automatically word-wrapped, paragraph style, select the **Word Wrap** check box. To control when a new line is created manually, clear the check box.



To Edit the Text of a Text Annotation

1. Double-click the **Text** annotation.
2. In the New Annotation window, make any changes to text.
3. Click **OK**.

SELECTING ANNOTATIONS

After you have created an annotation, you can select it either to change formatting properties or to graphically move, stretch or resize it. Since selecting and editing an annotation are different from selecting part of the image, there is a specific tool -- the **Edit Existing Annotation** tool -- that you use to select and edit annotations. Every time you add an annotation, the tool switches to the **Edit Existing Annotation** tool automatically so that you can immediately edit the new annotation.

To Select an Existing Annotation

1. Choose **Tools** > **Edit Existing Annotation**. This step is unnecessary if you just finished adding an annotation.
2. Click on any part of the annotation. Control handles will appear to mark the selected annotation. If there are several annotations in the same area, click on a portion of the annotation you wish to reformat that does not intersect any other annotation. The control handles will show you if you have the wrong one.

The cursor will change from an arrow to a hand when you are over an annotation.

MOVING AND RESIZING ANNOTATIONS

Once you have selected an annotation, you easily can move it and resize it with the mouse.

To Move an Existing Annotation

1. To move an annotation, select **Tools > Edit Existing Annotation** if not already

checked; then click on any part of the annotation.

If the annotation is not near any other annotations, you can drag its interior immediately. You do not need to select it first.

2. Dragging from the interior of the annotation or on a line, move it to the new position.

To Change the Points of an Existing Line, Length Measure or Angle Measure Annotation

1. Select the annotation as described above.
2. Drag the control handle for the point you wish to change to the new position. The annotation will stretch appropriately as you drag.

To Resize an Existing Rectangle, Ellipse, Highlighter or Area Measure Annotation

1. Select the annotation as described above.
2. Drag a control handle inward or outward to define the new size. The point on the opposite corner will remain fixed and the annotation will resize as you drag.

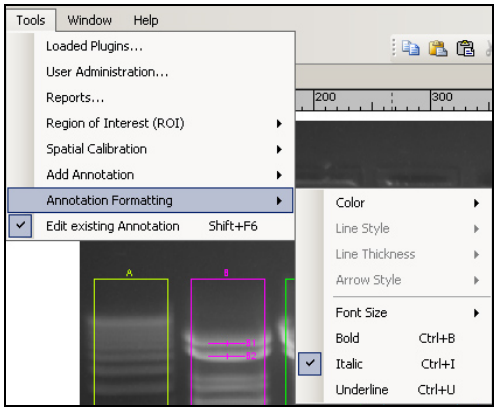
To Rotate a Text Annotation

1. Select the annotation as described above.
2. Drag any control point around the center of the annotation until you reach the desired rotation.

If you have a hard time aligning the text exactly as you want it, move the mouse away from the center of the text annotation while dragging.

FORMATTING ANNOTATIONS

The **Annotation Formatting** menu contains options to format a selected annotation.



When you are using the **Edit Existing Annotation** tool and have selected an annotation, you can change its formatting with the menu.

The **Formatting** menu contains the following formatting options:

- *Color*: You can either pick a color from the list or choose Custom Color at the bottom to pick any existing color. All annotation types support color options.
- *Line Style*: You can pick from solid, dashed or dotted. Only the following line annotations support line style: Line, Rectangle, Ellipse, Length Measure, Angle Measure and Area Measure.
- *Line Thickness*: You can pick a line thickness from the offered choices. You can only select Line Thickness if the line style is Solid. Otherwise the line thickness must be 1 and the Line Thickness menu will be unavailable. The same annotation types that support Line Style support Line Thickness.
- *Arrow Style*: You can choose whether a Line annotation (only) has:
  - No arrowheads.
  - An arrow at the start (first point) of the line.
  - An arrow at the end (second point) of the line.
  - Arrows at both ends of the line.
- *Font Size*: You can choose the font size of Text, Measure Length, Measure Angle and Measure Area annotations from among the listed values.
- *Bold*: You can choose whether Text, Measure Length, Measure Angle and Area Measure annotations should be boldfaced.
- *Italic*: You can choose whether Text, Measure Length, Measure Angle and Measure Area annotations should be italicized.
- *Underline*: You can choose whether Text, Measure Length, Measure Angle and Measure Area annotations should be underlined.

To Change the Format of an Annotation

1. Select the annotation by choosing **Tools > Edit Existing Annotation** and then click on any part of the annotation.
2. Change the formatting options using the **Tools > Annotation Formatting** menu.

DELETING ANNOTATIONS

Deleting an annotation removes it permanently from the image.

To Remove an Annotation

1. Select the annotation by choosing **Tools > Edit Existing Annotation**; then click on any part of the annotation.
2. Press the DELETE key.

You can also select **Delete Annotation** from the Shortcut menu that pops up by right-clicking on the annotation.

CHAPTER ELEVEN: IMAGE INFORMATION

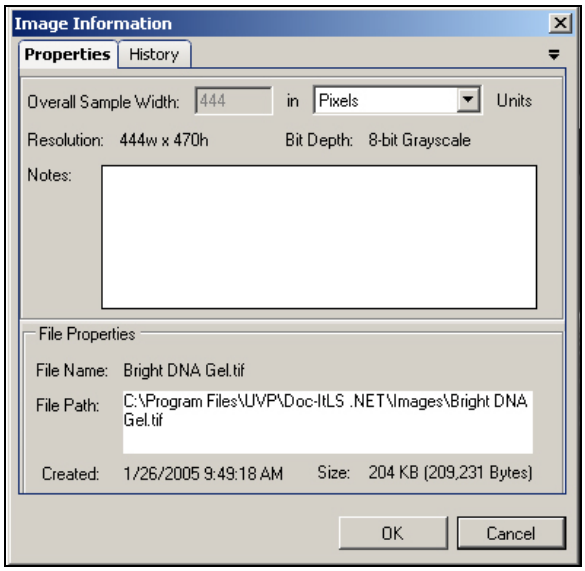
- Overview
- Calibrating Image Scale
- Image History
- Understanding a Pixel

OVERVIEW

LS Software keeps various sorts of information about an image. This Image Information includes:

- ***Image Scale***: Described as the number of metric units in the image's width, this information is used to calibrate Rulers and Measurement Annotations. Image Scale is also described under Ch. 10.
- ***Image Resolution***: The width and height of the image in pixels.
- ***Image Depth***: The number of bits used to represent intensity. LS Software supports 8-bit, 12-bit and 16-bit image depth.
- ***Notes***: Anything you wish to enter about an image.
- ***File Properties***: The file name, path, create date and size. All will be "N/A" if the image has not yet been saved.
- ***Image History***: A list of material changes to the image, when they occurred and any notes that you would like to add about why or how the change was made.

To be more compact, the Image Information window is organized into two tabs. All information except Image History is on the first tab; Image History is on the second tab.



To Display Image Information

1.	If the image is not the foremost image, select it from the **Windows** menu or click its title bar.
2.	Use the shortcut key and chick **> Image Information**. The Image Information window will appear.
3.	To switch between **Image History** and **Properties**, click the appropriate tab at the top of the window.

Image Information is also available on the toolbar and through a shortcut menu on the image itself.

To Enter Notes

1.	Display the Image Information window as described above.
2.	In the Properties tab, type your information into the **Notes** text box.
3.	Click **OK**.

CALIBRATING IMAGE SCALE

Each image in LS software has a scale associated with it. Scaling information is used to display rulers, measure length and measure area annotations. Refer to Chapter 11 under Spatial Calibration for information on using this tool.

Images scanned into the system from a scanner or imported from another program are not calibrated. In these two cases, therefore, you may wish to adjust or to set the image's scale.

An uncalibrated image will have "Pixels" as the unit type. If the unit type is Pixels, the number of units is the number of pixels in the image width and cannot be changed.

IMAGE HISTORY

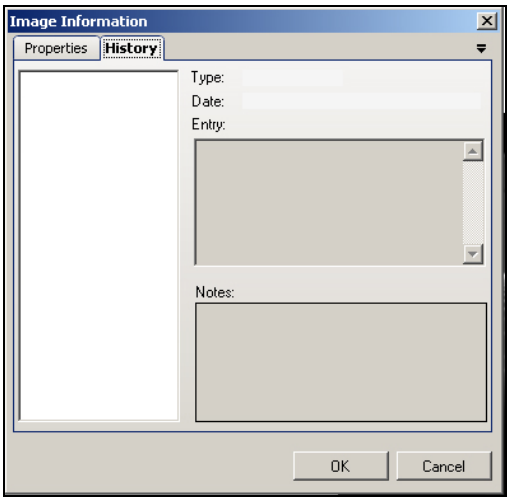
Each material change to an image is tracked in LS software's Image History feature. Material changes include use of any filters applied and use of the Paste Special feature. Changes to Effects and Annotations are not tracked in the Image History.

Entries in the Image History may be of three types:

- ***Creation***: Describes how an image was created (captured or scanned) and provides some details.
- ***Change***: Describes use of image filters or Paste Special.
- ***Error***: Describes an error that occurred while reloading the image from a saved file. The main use of an Error entry is to track the times when the image was changed in another software package, which is important for some kinds of laboratory practice.

The Image History includes information on:

- What type of entry it is, from among the types described above.
- When the change occurred.
- What the change was and details about it.
- Any notes that you add to explain the entry.



To View Image History

1.	If the image is not the foremost image, select it from the **Windows** menu or click its title bar.
2.	From the shortcut menu, choose **Image Information**. The Image Information window will be displayed.
3.	Click the Image History tab at the top of the window.
4.	Click on any history entry in the list on the left side to display details about the entry.

To Add Notes to a History Entry

1.	Display **Image History** as described above.
2.	Click on the history entry to which you wish to add notes.
3.	Type the notes in the **Notes** field.
4.	Repeat steps 2 and 3 for any other history entries.
5.	Click **OK**.

UNDERSTAND A PIXEL

A computer image is made up of a rectangular grid of dots called pixels. Each pixel is a single color -- bright red, dark blue, etc. In a monochrome image, each pixel is a shade of gray -- light gray, dark gray, black (the ultimate in dark gray), etc. Internally, the shade is represented by some number of intensity values.

Monochrome and Color Pixel Mechanics

Monochrome images are fairly simple. A monochrome pixel has a single intensity value, ranging between 0 (black) and some maximum intensity which is white (the ultimate in light gray). Colored images are slightly more complex. Computer color images typically are stored in "RGB," or "Red, Green, Blue." Each color pixel therefore has three intensities -- the intensities of the red, green and blue making up the pixel's color.

Some combinations result in different colors than one might expect. For instance, a color pixel with high red and green values but low blue values appears yellow. On the other hand, it is probably not very surprising that a pixel with high red values and low green and blue values appears red, or that a pixel with high red and blue values but low green values appears purple.

The Meaning of Image Depth

Intensity numbers are not fixed in size. Different images and different image file formats support different sizes of intensity number. The size of the intensity number is referred to as image depth. LS Software packages support 8-bit, 12-bit and 16-bit depth images. This means that, in the LS workspace, an image may have either an intensity range from 0 to 255 (8-bit), 0-4095 (12-bit) or from 0 to 65535 (16-bit). 16-bit images have considerably more granularity than 8-bit images. Either way, the intensity number range is per color channel. Thus a 8-bit color image requires 24 bits (or 3 bytes) per pixel to store an 8-bit red, an 8-bit green and an 8-bit blue value. A 16-bit color image requires twice as much storage per pixel.

Displaying Depth

Most color monitors and video cards today support 24-bit color (there is also a 32-bit color mode, but this is actually the same as 24-bit; it just has been padded out to make processing more efficient). The 24-bit color mode has three 8-bit color channels. Computer monitors are not manufactured to show greater depth ranges (although they could be) because the human eye really cannot perceive gradations finer than those within the 8-bit range.

Depths Greater than 8-bit in LS Software

LS Software will display and manipulate 12-bit and 16-bit depth images correctly. (However, the camera provided with Doc-ItLS does not capture more than 8-bit depth.) Most scanners also do not capture greater depth.

CHAPTER TWELVE: PRINTING IMAGE REPORTS

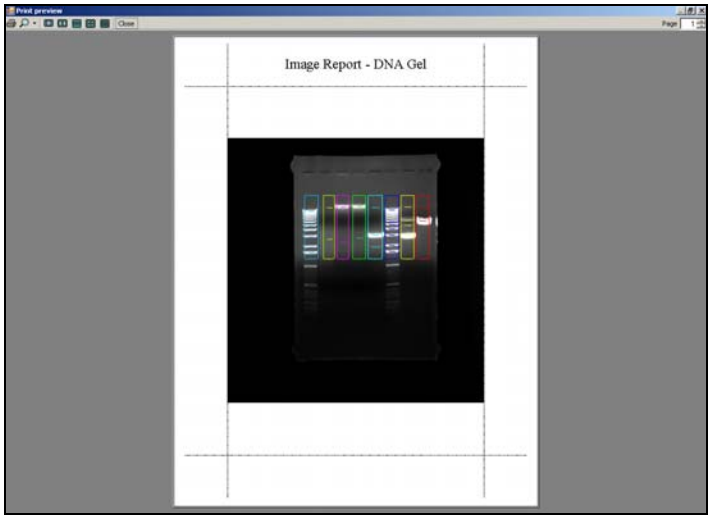
- Reports
  - Image Reports
  - Image Effects
  - Image History
  - Image Notes
  - Image Properties
- Print Command

REPORTS

LS Software provides several types of reports:

- ***Image Report***: Prints the image, using as much of the page as possible while preserving the image's aspect ratio.
- ***Image Effects***: Prints the setting values from the Effects tab.
- ***Image History***: Prints the image history, as reported in the Image Information window.
- ***Image Notes***: Prints the image notes, as entered in the Image Information window.
- ***Image Properties***: Prints the image's resolution (width and height), depth, scale and file information.

All reports include a header and footer that you can create.

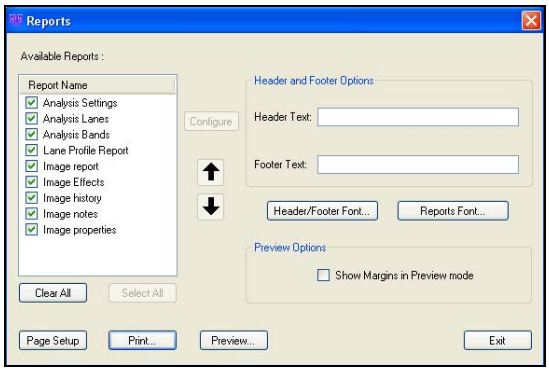


The Report Preview window shows the layout of the data on each page.

To View and Print a Report

1. If the image is not the foremost image, select it from the **Windows** menu or click on its title bar.

2. Choose **Tools > Reports** and then choose the report names from the Reports window.



3. To change the target printer, paper, paper source (tray) or page layout, click **Page Setup** and make the desired changes.
4. To enter the header or footer text, type new text in the text box and click **Preview** the pages. There are some special character combinations you can use in the header and footer:
   - "%p" is the current page number.
   - "%d" is the current date.
   - "%t" is the current time.
   - "%c" is the total count of pages for printing
5. To change the margins, choose an alternate margin setting in the Page Setup window . Click **OK** after selection.
6. In the **Reports > Preview Options**, click Show Margins in Preview Mode to see the margins graphically.
7. When the preview looks correct, click **Print**.

PRINT

With LS Software, you can print your images to any Windows-supported printer, regardless of it being a file-printer, local printer or a network printer.

The **Print** command uses exactly the same format as the Image Report in Reports.

To Print an Image

1. If the image is not the foremost image, either choose it from the **Windows** menu or click its title bar.

The **Print** command is also available on the Files toolbar, but does not show the Page Setup dialog window. Instead, it prints an Image Report directly to the default printer.

2. Choose **File > Print**. If necessary, choose the target printer, paper size, paper source (tray) and page layout. Your printer may offer additional configuration options.
3. Click **OK**.

CHAPTER THIRTEEN: FINDING LANES AND BANDS

OVERVIEW

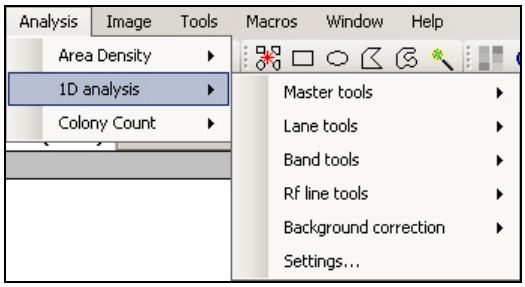
As you use the 1D Gel Analysis tools, you will find that LS software offers several ways to work with the image and to make calculations. In this basic section, you will learn the following:

- What *features* appear on the 1D Gel Menus, the 1D Gel toolbar and the Image Window which differ from before;
- How to find *lanes and bands* both automatically and manually, and how to find bands within existing lanes;
- How to *modify and add lanes* by moving, resizing, and curving or straightening them;
- How to *modify and add bands*; and
- How to *clear the image* of all lane and band information.

NAVIGATION

1D Analysis Menus

LS Software offers the following 1D Analysis Menu from the main menu:



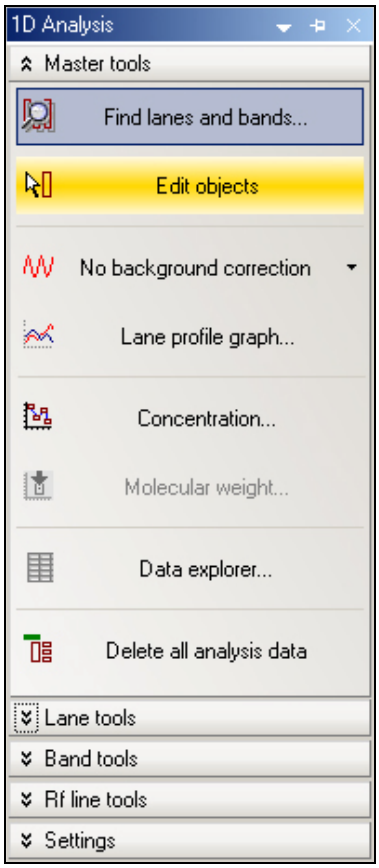
The main 1D Analysis menu: Contains the following features:

- ***Master Tools***: Allows you find and edit lanes and bands.
- ***Lane Tools***: Allows you to change the lanes in multiple ways.
- ***Band Tools***: Allows you to change bands in multiple ways.
- ***Rf Line Tools***: Allows you to create and change Retardation factor (Rf) Lines in multiple ways.
- ***Settings***: Opens the analysis settings window

1D Analysis Toolbar

Toolbars in LS allow you to select most commands with a single button click. The toolbar views, positioning and behaviors are configurable so you can show or hide the toolbars you use the most.

1D Analysis Plugin Buttons



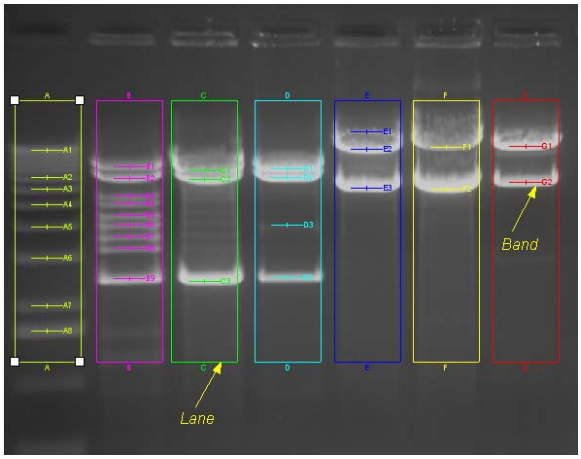
- ***Find Lanes and Bands***: Searches for lanes and bands in the image.
- ***Edit Objects***: Allows you to select, move and resize lanes, bands, Rf lines, and the Region of interest (ROI).
- ***No Background Correction***: Corrects the background with your choice of methods: No Correction, Straight Line, Joined Valleys, Rolling Disc and Area Between Lines.
- ***Lane Profile Graph***: Displays a line graph of intensity or concentration value verses position in the lane for the lane or lanes you select.
- ***Molecular Weight***: Calibrates molecular weight with Retardation factor (Rf) lines and by applying standards to lanes.
- ***Data Explorer***: Brings up datasheets showing the results of calculations on lanes and bands.
- ***Delete all analysis data:***
- ***Lane Tools***
- ***Band Tools***
- ***Rf Line Tools***
- ***Settings***

How to View and Position the 1D Analysis Plugin

1.	To view the toolbar, select the **View** menu, **Plugins > 1D Analysis plug-in**. The toolbar, shown above will appear in the workspace.
2.	The 1D Analysis toolbar can be positioned as **Floating** or **Dockable**. For a detailed description of how these toolbars can be positioned or hidden, see appropriate chapter.

1D Analysis Image Window Features

In addition to displaying the image, the Image Window also displays various 1D gel objects and allows you to manipulate them using the mouse.



The Image Window may display any or all of:

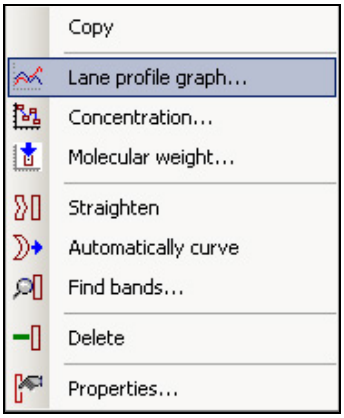
- The Region of Interest (ROI)
- Lanes (marked by colored rectangles)
- Lane Curve Lines (vertical lines down the center of each lane that allow you to control lane curving)
- Bands (marked by horizontal lines at the band peak)
- Rf Lines (white lines interrupted by small white circles at each intersection of the Rf line and a lane)

In addition to the objects above, there are also some 1D gel text labels that help you identify data in the image. The labels cannot be directly manipulated. These labels are:

- Lane IDs: Letter codes at the top of each lane. Calibrated lanes are indicated by showing the lane ID in brackets (e.g. "[A]" as opposed to "A").
- Lane Names: Names you entered in the Lane Information window for each lane, also shown at the top of the lane.
- Band IDs: Letter-and-number combinations showing the lane (letter) and band position (number) that uniquely identifies each band.

1D Analysis Context Menu Commands

The 1D Analysis \menu appears when you click on any 1D Analysis objects (lane, band, etc) with the right button of the mouse. It is a shortcut menu that lets you sidestep using the menus or the toolbars. Once it opens, you can select any features from the list.



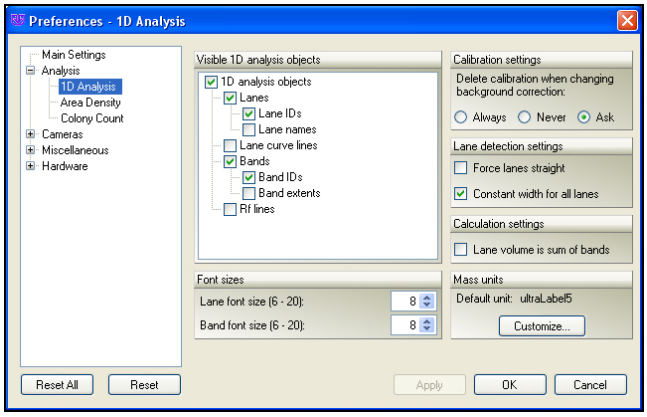
The 1D Analysis context menu contains the following functions which also can be selected from the 1D Analysis toolbar

- ***Copy***
- ***Lane profile graph***
- ***Concentration***
- ***Molecular weight***
- ***Straighten***

- ***Automatically curve***
- ***Find Bands***
- ***Delete***
- ***Properties***

1D Analysis Settings

To access analysis settings, go to **Analysis > 1D Analysis > Settings**.



This tab allows you to set the following defaults:

- **Visible 1D analysis objects**
- **Calibration settings**
- **Lane detection settings**: Use this command to make lane width constant across all lanes; to force all lanes straight;
- **Calculation settings: Run the lane volume of bands**
- **Font size**: By clicking the buttons for **Font Size Lane** and **Font Size Band**, you may choose the font size for the Lane ID, Lane Name and Band ID labels. The font range is from 6 - 20.
- **Mass Units**: This feature is for concentration units. The unit of mass that appears is "ng" (nanograms). Note that by clicking **Customize**, you may add further units of your own. Mass units are weight numbers, relative units or any relational units you define.
- **Font sizes**

You also can access this feature from **1D Analysis plugin > Settings > Analysis Settings**.

FINDING 1D GEL LANES AND BANDS

In this section, you will find step-by-step processes for finding lanes and bands within your image. You also will learn how to identify your Region of Interest and how to perform both automatic and manual searches for lanes and bands as you progress in your analyses.

Identifying Region of Interest (ROI)

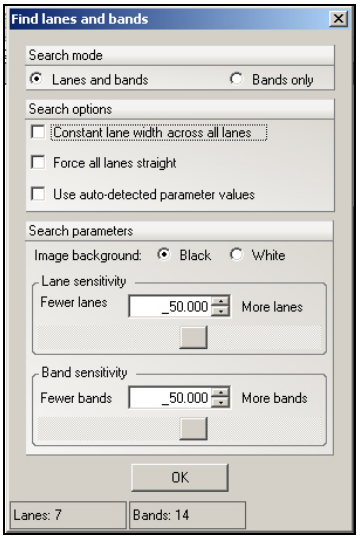
By selecting a Region of Interest, you are telling the software to analyze lane and band information only within that area. This typically improves the accuracy of the automatic lane and band finding when the image background and gel background intensities vary.

Selecting the Region of Interest

1. From the **Tools** menu, select **Region of Interest**. Select the rectangular ROI. In the Image Window, the selected shape is reflected.
2. Outline the lanes of interest with the ROI tool. Position the mouse at one of the corners to move that corner of the ROI. Then drag the corner to where you would like it to be.

Searching for Lanes and Bands

To Perform a Basic Search



1. From the **1D Analysis** toolbar menu, click on **Find Lanes and Bands**. The Lanes/Bands dialog window appears and a basic search is automatically performed using determined parameters.

2. If the results are not satisfactory, you can keep the window open and adjust the search parameters as described in the following section, or click **OK** and use the manual **Add Lane** and **Add Band** functions to identify all lanes and bands correctly. Clicking **Cancel** returns the image to the state it was in before you opened the Lanes/Bands dialog (for instance, if you had no lanes or bands before opening the window, **Cancel** will return to an image with no lanes or bands.)

Typically the options for “Constant lane width across all lanes: and “Force all lanes straight” are turned on for 1D lane/band analysis.

To Adjust Search Parameters

If the basic search did not find all lanes, you can adjust the parameters until the results are satisfactory. To adjust the parameters:

1. If you reopened the Find Lanes and Bands dialog window or manually adjusted lanes, ensure that **Lanes and Bands** is selected in the search mode at the top of the window. Otherwise, the system will only search for bands within the existing

lanes you have defined.

2. Under Search **Options**, select whether you want lane width to be constant across all lanes, force all lanes to be straight or use auto-detected parameter values.

3. Under **Search Parameters**, check that the **Image Background** is correctly set. If not, change it to match the background.

Having the wrong background setting is the cause of most problems in finding lanes and bands. Images with extraneous effects (writing, light leaks) on the edges of the image sometimes cause background color to be detected incorrectly due to poor band to background contrast.

4. Adjust the lane sensitivity using the slider control. In general, to detect more lanes, drag the slider to the right; to detect fewer lanes, drag it to the left. Sometimes, however, if the sensitivity is very low, you may find more lanes and better-defined ones by moving the slider to the right. As you are changing sensitivity, the image adjusts automatically. You may also type in a sensitivity value in the text box next to the Lane Sensitivity slider.

The up and down arrows on the can also be used for incremental adjustment of the lane and band sensitivity slider bars. The LS Software will automatically search after any adjustments are made.

5. You may also adjust the band sensitivity in the same manner as the lane sensitivity. However, it is usually premature to adjust band sensitivity if lanes are not correctly detected.

6. Directly below the **OK** button you will see how many lanes and bands the software found. Your image now reflects these lanes and bands with colored lines and rectangles.

To Return to the Automatically Detected Parameters

After changing lane and band sensitivity values, you may wish to return to the values selected originally by LS software for the Basic Search.

To return to the automatically-detected parameters:

1. On the Find Lanes and Bands window click **Use auto-detected parameter values**. The original parameters will be restored and the image will display lanes and bands as originally detected in Basic Search.

If the search mode is Bands Only, the lane sensitivity value will be reset but the system will not search for lanes with the new value. To search for lanes and bands both, ensure that the search mode at the top of the window is Lanes and Bands.

Searching Existing Lanes for Bands

LS software allows you to make band-searching more or less sensitive, resulting in more or fewer bands detected.

To Search for Bands in a Lane

1. Choose **1D Analysis > Master Tools > Find Lanes and Bands**.
2. Ensure that the search mode at the top of the window is for **Bands Only**.
3. You may now choose the sensitivity for bands. The fewer the bands that you want, the farther to the left you will drag the slider. To find more bands, move the slider to the right.
4. Click **OK.** Your image now reflects the new information.

You can also right click on any lane and select set the band sensitivity.

MODIFYING BANDS

If the software did not find all the bands in a lane, you can add bands manually. Just as with lanes, you can also move, resize, and delete bands. In this section, you will learn how to do so.

Adding Bands Manually

LS software allows you to add bands manually.

To Add Bands Manually

1.	On the **Analysis > 1D Analysis > Bands**. Select **Add Band**.
2.	Move the cursor over the image. A movable horizontal line will appear whenever you move the cursor over a lane. Simply move the mouse over the image to the spot where you would like the new band to be. If the color of the horizontal line is green, you can place the new band where the cursor is. If, however, the color is red, there is already a band at this position and you cannot place another band there. Move the mouse until the line appears green or adjust the other bands extents before adding the band.
3.	Click the left mouse button to place the new band. Note that you can place as many bands as you like as long as this feature is on.
4.	When you are finished placing your band(s), click on **1D Analysis >** Master **Tools > Edit Objects** to disable the Add Bands tool.

Moving and Resizing Bands

Just as with lanes, LS software allows you to move and resize bands according to where you want them.

To Move Bands

To Move Bands on the Image Window:

1.	Click **Analysis > 1D Analysis > Master Tools > Edit Objects**.
2.	Select the band you wish to move by clicking on it. Controls handles will appear at the four corners of the band as red boxes.
3.	Drag the box up or down within the lane until it appears where you want it. Note that you can only move a band between the bands above and below it, not beyond other bands.

To Move Bands Using the Lane Profile Graph:

1.	Click **Analysis > 1D Analysis > Master Tools > Lane Profile Graph**. The graph appears in a new window.
2.	Ensure that **Band Peaks** and **Band Extents** are both selected. Under the graph find the band markers. Drag the peak marker of the band (the large marker in the middle of two smaller markers) to the place you desire that band. Note that the markers on the graph change position as well. Note that you can only move a band between the bands next to it not beyond them.

3. Click on **OK**. The Lane Profile window closes and bands are now in their new positions.

When editing bands or lanes, you may need to select **1D Analysis > Master Tools > Edit Objects** is selected prior to clicking on a band or lane.

To Resize Band Extents

To Resize Bands using the Image Window:

1. To turn on and view the Band Extents, go to **Analysis > 1D Analysis > Settings** to open the Preferences Window. Select the 1D Analysis tab and click the **Band Extents** check box. Click **Apply**. Close the **Preferences** Window.
2. Click **Edit Objects** from the **1D Analysis** plugin. Place the mouse hand pointer over the band you wish to resize, and click on the band. Control handles will appear at the four corners of the band as red boxes.
3. Place the mouse hand over the top or bottom of the band's extents and click and drag the box border to increase or to decrease the size of the band until the size matches the band seen on the image

To Resize Bands Using the Lane Profile Graph:

1. Click **Analysis > 1D Analysis > Master Tools > Lane Profile Graph**, the graph appears in a new window.
2. Ensure that **Band Extents** is turned on. Under the graph, find the band markers. Drag the markers to the left or the right to the place you desire them. Note that the markers on the graph change position as well.
3. Click on **OK**. The Lane Profile Graph closes and the bands are now in their new positions.

To Place Bands Exactly

1. Click **Analysis > 1D Analysis > Master Tools > Edit Objects**.
2. Select the band you wish to resize. Controls handles will appear at the four corners of the band as red boxes.
3. Under the **Analysis > 1D Analysis > Bands** and then **Band Properties**. The Band Information window appears.
4. In the section of the window labeled **Geometry**, you may change the numerical values of the top, peak and bottom of the band.
5. After you enter the new numbers, click on **OK**. Now the location and dimensions of the band reflect precisely the values you entered.

Deleting Bands

You may choose to delete bands. To do so:

1. Click **Analysis > 1D Analysis > Master Tools > Edit Objects**.
2. Click on the band you wish to delete. A box will appear around the band with red boxes at the corners.
3. On the **Analysis > 1D Analysis** menu, select **Bands** and **Delete Band** (or press the delete key on your keyboard). A window will pop up to request confirmation. Click **Yes** to delete the band. The band you selected is now deleted.

CLEARING ALL LANE AND BAND INFORMATION

If you wish to begin a new analysis of your image, save your existing image with its lane and band information (unless you want to start over again). Then do a **Save As** for your new image, filing it under a new name, and proceed as follows:

To Clear All Lane and Band Information

1. From the **Analysis > 1D Analysis > Master Tools**, click on **Delete All Analysis Data**.
2. All lane and band information will be deleted. If you wish to restore information back in to the image click the **Undo** button on the **Edit Menu**. You may also return to the cleared image by pressing **Redo**. Once you have saved the file, however, you cannot undo or redo any changes you made.

CHAPTER FOURTEEN: MOLECULAR WEIGHT CALIBRATION

- Overview
- Retardation factor Lines
- Molecular Weight Calibration
- Applying a Standard to a Lane
- Removing a Standard from a Lane

OVERVIEW

Molecules in an electric field migrate through a gel matrix at rates inversely proportional to the log10 of the number of base pairs. Large molecules migrate more slowly due to large frictional force from the pore of the matrix while small molecules migrate faster due to less frictional force.

There are many experimental conditions affecting the migration rate: gel concentration; conformation of the DNA; applied voltage; direction of electric field; base composition and temperature; presence of intercalating dyes; and electrophoresis buffer. It is therefore desirable to use a known molecular weight standard as a reference to unknown samples. This marker is used to calibrate the resulting molecular weight for each unknown bands.

Using a molecular weight marker results in a band encompassing the whole gel horizontally. This band can be thought of as the distance traveled of a band relative to its front (Retardation factor - Rf) or starting position. This Rf line exists for each band in the molecular weight standard. Any bands in the unknown samples that migrate to any of these Rf lines are then compared to the Rf lines.

In this section, you will learn how to manage molecular weight standards and calibrate lanes to these standards. This section also explains how to determine Rf values automatically, how to add Rf lines manually, and how to move and delete Rf lines. In general, Rf line functions are only required if you have less than two calibrated lanes.

RETARDATION FACTOR (RF) LINES

Automatic Rf Line Determination

When you calibrate two or more lines with molecular weight standards, LS Software creates Retardation factor (Rf) Lines for you automatically. These lines express any differences in horizontal alignment between bands (or points on a lane) of equal molecular weight. Ideally, there will be one Rf line for each distinct molecular weight used in a calibration. Since you may use more than one standard on a single image, and each standard may contain several weights, automatic generation can result in a large number of Rf lines.

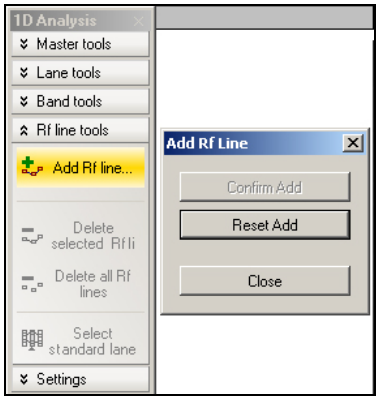
You can remove an automatic Rf line by re-calibrating the lanes. You can adjust an automatic Rf line by dragging the lane-intercept marker up or down, but only in lanes that are not calibrated.

You also can access these features using the 1D Analysis Plugin.

To Adjust an Automatic Rf Line

1.	If Rf lines are not visible, turn them on through **Analysis > 1D Analysis > Settings.** When the **Preferences** window opens, click on the **Rf line** box in the Visible 1D analysis objects area.
2.	Select the Rf line you wish to adjust. Control handles will appear.
3.	Any lane that has a white control handle *without* an "X" is an uncalibrated lane, and it is possible to move the intercept point up or down by dragging it. If lane has a gray control handle *with* an "X," it is calibrated and cannot be moved.
4.	After you have adjusted Rf lines, you may find it convenient to hide them so you can see the other 1D objects more easily. To hide the Rf lines, go to **Analysis > 1D Analysis > Settings.** When the **Preferences** window opens, unclick **Rf line** in the Visible 1D analysis objects area.

Adding Rf Lines Manually



LS Software allows you to add Rf lines to an image with less than two lanes that are calibrated to molecular weight standards. (On images with two or more calibrated lanes, Rf lines are created automatically and the software will not allow you to add new ones, although you can adjust the automatically added ones as described in the Automatic Rf Line Determination topic.)

To add an Rf line manually:

1.	On the **Analysis > 1D Analysis > Rf lines**, click **Add Rf Line**. Rf lines will be made visible if they were formerly hidden, and a window will pop up entitled **Add Rf Line**.
2.	The cursor will now appear as a square cross, and you can select the first band that is part of the Rf relationship. Then click on a second and subsequent bands in other different lanes. You may click on as many bands as you like to draw the line (up to one per lane), but note you must choose at least two.  To place an Rf line anchor (circle) on a non-band location, hold down the CONTROL (CTRL)key as you place the circles.
3.	When you are finished selecting the points that will make up the line, click **Confirm Add** in the **Add Rf Line** dialog window. The green line will now appear

	white, and you have created a new Rf Line.
4.	To place more Rf lines, follow the same process. Note, however, that you only will be able to place Rf lines as long as they do not cross another Rf line. If you can place the Rf line, the line will appear green. If there are any red marks at all on the Rf line you create, you will not be able to create the Rf line when you click **Confirm Add**. Ensure that the entire line is green before clicking **Confirm Add**.
5.	Click **Close** when you are finished adding Rf lines.

Moving Rf Lines

You can adjust existing Rf lines, whether they were created automatically or manually. To move Rf lines to match bands of the same molecular weight:

1.	Click **Analysis > 1D Analysis > Master Tools > Edit Objects**.
2.	Select the Rf line you wish to adjust. Control handles will appear.
3.	Any lane that has a white control handle *without* an "X" is an uncalibrated lane, and it is possible to move the intercept point up or down by dragging it. If the lane has a gray control handle *with* an "X," it is already calibrated and cannot be moved.
4.	After you have adjusted Rf lines, you may find it convenient to hide them so you can see the other 1D objects more easily. From the **Analysis** menu, select **1D Analysis Settings**, then turn off **Rf Lines**.

Deleting Rf Lines

Rf lines that were added manually can also be removed (automatic Rf lines cannot be removed).

To Remove One Rf Line

1.	Click **Analysis > 1D Analysis > Master Tools > Edit Objects**.
2.	Select the Rf line to delete.
3.	From the **Analysis > 1D Analysis > Rf lines** and click **Delete Selected Rf Line**.

You can also delete an Rf line by selecting it and pressing the DELETE key.

To Remove All Rf Lines

1.	From the **Analysis > 1D Analysis > Rf lines** and click **Delete All Rf Lines**.

You also can access these features using the 1D Analysis Plugin.

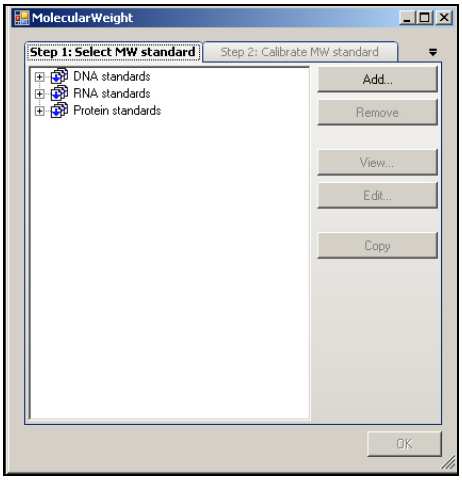
MOLECULAR WEIGHT CALIBRATION

Managing Weight Standards

Calibration of molecular weight involves associating a known standard with one of more lanes in the image. This allows Rf values to be calibrated to molecular weight values. LS Software allows you to use several different standards per gel.

To help you in your analysis, LS provides a library of molecular weight standards. You can add, edit, and delete standards from the library using the following instructions.

Adding a Molecular Weight Standard to the Library



Select the Lane to calibrate and click on it.

Under the **Analysis > 1D Analysis > Master Tools > Molecular Weight**. A new window appears.

In the **Step 1** tab, click **Add**. **Step 2 Create MW Standard** tab opens. In this window, select the Name of the standard you are using and the **Group** name. Select the **Unit Type**.

Click **Add**. This allows you to enter the numerical value of the standard. After entering the numerical value, click on **Add** again for as many values as you wish to enter.

5. Click **OK** on the right side. The first window appears again with the new standard entered.

Editing a Molecular Weight Standard to the Library

1.	Select the Lane to calibrate and click on it
2.	Under the **Analysis > ID Analysis > Master Tools > Molecular Weight, Step 1: Select MW standard** window will appear.
3.	In the Step 1 window, select the standard you wish to edit. (NOTE: This applies only to standards created by users. The MW Standards included with the software cannot be edited or deleted.) Then click on **Edit**.
4.	Now you can change any of the information in this window, including group, name, units, or most commonly, weight.
5.	Click **Edit** to reflect the new change, and then click **OK**.

Deleting a Molecular Weight Standard to the Library

1.	Select the Lane to calibrate and click on it.
2.	Under the **Analysis > 1D Analysis > Master Tools > Molecular Weight**, **Step 1: Select MW standard** window will appear.
3.	In the Step 1 window, select the standard you wish to delete. (NOTE: This applies only to standards created by users. The MW Standards included with the software cannot be edited or deleted.) Then click on **Delete**. LS software then asks you in a pop-up window if you want to delete that standard. Select **Yes** if it is correct, **No** if it is not.
4.	Click on **Cancel** to return to the image window.

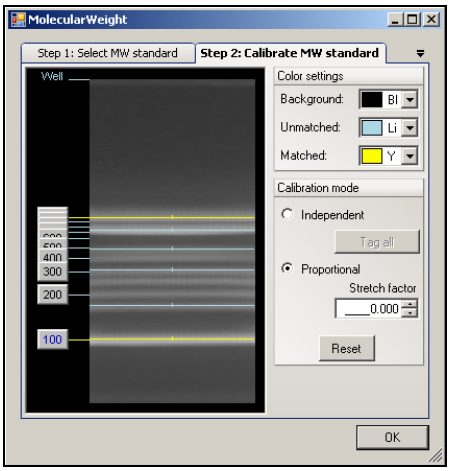
Copying a Molecular Weight Standard in the Library

1.	Select the Lane to calibrate and click on it.
2.	Under the **Analysis >1D Analysis > Master Tools > Molecular Weight, Step 1: Select MW standard** window will appear.

3.	In the new window, select the standard you wish to copy. Then click on **Copy**.
4.	The **Edit** window will appear. Change the Name of the new MW Standard. Click on **Add to enter a new Value** or click on a value and click **Edit** to edit what you wish in the copy. Then click on **OK**.

APPLYING A STANDARD TO A LANE

Calibrating a Lane



1. Select the lane you wish to calibrate.
2. From the **Analysis > 1D Analysis > Master Tools > Molecular Weight, Step 1: Select MW standard** tab window will appear. From the list of standards, choose the standard you wish to use. If the standard is not on the list, add the standard to the list (see Managing Weight Standards). Click **Next**. A new screen appears **Step 2: Calibrate MW standard**.
3. In the **Step 2** screen, you can adjust the weights to match the bands that actually appear. Complete instructions for this appear below.
4. Click **OK** to save the calibration.

You also can access this feature using the **1D Analysis > Rf lines** tool plugin.

Using the Stretch Factor

The stretch factor establishes a mathematical relationship between the weights to describe their relative movement. The larger the stretch factor, the lighter weights move in relationship to heavy ones. The smaller the stretch factor, the less the lighter weights move. A stretch factor of 1.0 indicates linear movement (weights move in direct proportion to their relative weights).

To adjust the weights to match the bands, you may choose to use the stretch factor:

1.	In the **Step 2** screen of the **Molecular Weight** tool, ensure that **Proportional** calibration mode is selected.
2.	Drag a known weight up or down with the mouse until it matches the appropriate band. Alternatively, you can select the weight with the mouse (or TAB key) and move the weight up and down with the UP and DOWN arrows on the keyboard (this helps adjust the weight by small amounts). When you have a match of the weight with the band, the line color changes from blue to yellow (default colors).
3.	Adjust the stretch factor (scaling) between weights until the other weights match their appropriate bands. You can adjust it using the mouse wheel (rolling up increases the value, rolling down decreases it), or you can enter a new value into the text box and click **Set**.
4.	Click **OK** to save the calibration.

Using the Stretch Factor gives you a weight match on the band up to 0.5% of the weight you assigned. To obtain *exact* placement on the band, use the manual mode, described below.

Using Manual Placement of Weights

If you do not want to use the Stretch Factor, you can also adjust weights individually using manual mode:

1.	In the **Step 2** screen of the **Molecular Weight** tool, select **Independent** calibration mode.
2.	Move each weight separately, with either the mouse or the keyboard arrows, to position them exactly on the band. Weights cannot pass one another, so it is usually best to start with either the lightest or heaviest weight and work toward the other end.

Exact Placement of Bands

In the **Independent** calibration mode, as soon as the weight is exactly on the band, the color of the line changes from blue to yellow (default colors). This means you now have *exact* placement -- the weight will be exactly on the band peak. Exact placement only occurs when the color changes from unmatched to matched; further movement of the weight may alter the exact positioning. When in doubt, move the weight completely away from the band and reposition it with the arrow keys until the color changes.

Tag All button reduces some manual work by aligning the weights of the ladder starting with first band in the lane. Stretch factor is not taken into account – only simple matching is done.

On the second window of the calibration operation (where you adjust weights to bands), you can change the colors of both the unmatched and the matched lines by using the controls in the upper right corner.

After calibration of two or more lanes, Retardation factor lines will be automatically calculated and will replace any previous Rf line work.

REMOVING A STANDARD FROM A LANE

If you have already calibrated a MW standard to a lane, the calibration can be removed from want a lane by doing the following:

1.	From the **Analysis > 1D Analysis > Master Tools > Molecular Weight**
2.	The **Step 2** tab appears. Click on the Step 1 tab.
3.	Choose **Uncalibrate lane**.
4.	Click **Yes** to confirm the removal of the MW calibrated lane.

CHAPTER FIFTEEN: QUANTITATION CALCULATIONS

OVERVIEW

Having sized and moved lanes and bands, you are now able to ask LS software for concentration calculations. In this chapter, you will learn how to do the following:

- Correct background for overexposure or uneven exposure of light or chemicals;
- Show the concentration graph for calibration;
- Select data points to plot on the graph;
- Select the type of curve to fit to the data points; and
- Remove all concentration calibration data.

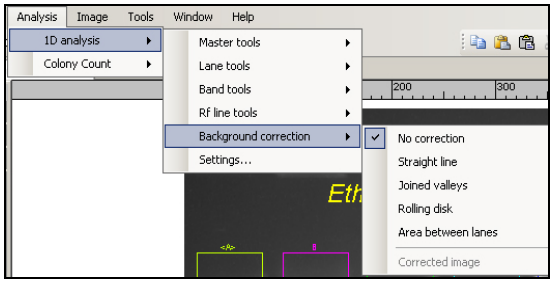
BACKGROUND CORRECTION

Background Correction Options

To account for possible variable illumination or overexposure during image capturing, LS software offers options to apply mathematical background correction. These options generally remove background "noise" and elevated levels of pixel intensity due to excess exposure, highlighting data.

General Procedure for Background Correction Selection

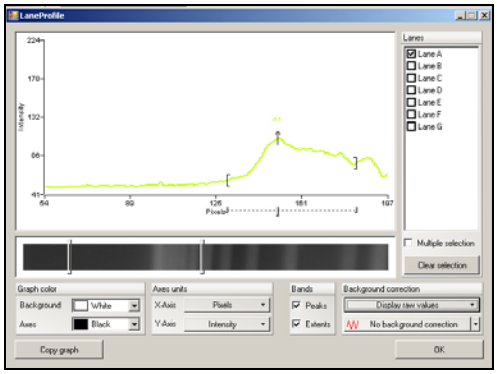
1. From the **Analysis > 1D Analysis > Master Tools > Background Correction**. A new list appears.



2. From the list select which type of background correction, if any, that the software should perform.

Viewing Background Correction in the Lane Profile Graph

1. Choose the **Analysis > 1D Analysis > Master Tools > Lane Profile Graph**.



2. On the graph, in the second box below **Background Correction,** click the down arrow to show the drop-down menu of the background correction options. Click the appropriate selection. The graph will now display the background correction in the selected lane as a dotted line.

Specific Correction Options

LS Software offers five background correction options.

No Background Correction

Selecting this option on the menu leaves the image uncorrected for overexposure, "as is."

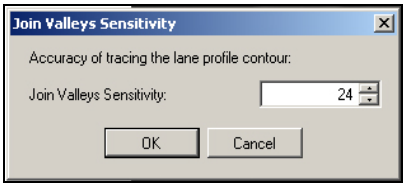
Straight Line

Selecting this option tells LS Software to place a straight (but not necessarily horizontal) line under the lowest points at the beginning and end of each lane. LS removes the area of the graph under the straight line, so that all remaining values are emphasized. Straight line correction tends to correct well for overexposure, and for variable illumination that is focused on an edge or corner of an image.

Straighten lines by using right-clicking the mouse button to open the shortcut menu.

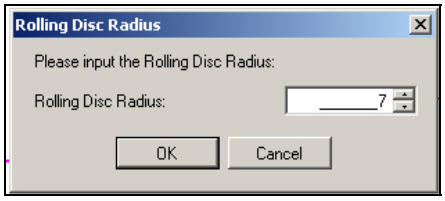
Joined Valleys

Selecting this option accentuates the data by telling LS software to join lines between the lowest point, or "valley", before the first band, between each pair of bands, and after the last band. Intensities above the valleys (band data) are emphasized. Joined valleys can perform well in a variable illumination condition where the "bright spot" is somewhere in the middle of the image, and where bands are sharply defined and quite distinct. Joined Valleys depends on the sensitivity – a higher value of sensitivity starts “eating” into the bands, which may not be accurate.



Rolling Disc

Picture turning the lane profile graph upside and then rolling a ball over the new top. Everything the ball is able to roll over is eliminated by the software. Whatever the ball cannot fit into remains in the graph for your analysis. Rolling disc performs well in all background conditions providing the size of the disc is carefully chosen. An excessively small disc will "roll into" bands, eliminating the band data almost entirely. An excessively large disc rolls across the lowest valleys, acting much like Straight Line correction.



To Use Rolling Disc:

1. Select **Analysis > 1D Analysis > Background Correction > Rolling Disc.**
2. A pop-up window appears, asking you to set the size of the radius of the disc. You can choose a radius size between 1 and 1000. Change the size either by typing in the number you want, or by using the up and down arrow signs to the right of the number box. Click **OK** after entering the radius.

Area Between Lanes

Part of the image may be overexposed, and there may be patterns of deformity between the lanes. This correction takes cross-sections between lanes and subtracts those "inter-lane" profiles. Area Between Lanes performs well in all variable illumination situations, providing lanes are distinct and there are clear gaps between them. It performs badly if bands in different lanes "bleed together" or touch, because it will tend to eliminate almost all band data at such a point.

You also can access background correction using the 1D toolbar or directly on the Lane Profile Graph.

For better concentration calibration accuracy, it is recommended the band boundaries/ extents be reviewed and adjusted if necessary. See Chapter 13 for band adjustment.

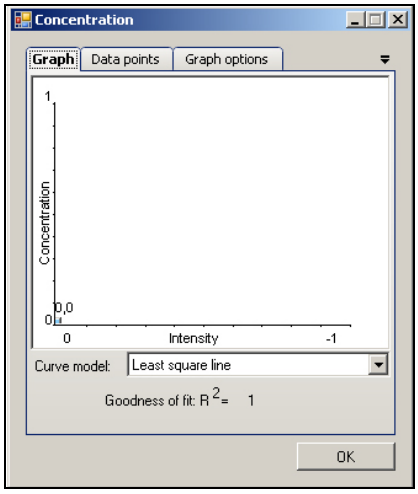
CONCENTRATION CALIBRATION

Showing the Concentration Graph

With the background of the image corrected, LS software now is ready to graph intensity versus concentration and to fit curves or lines on the graph. It also allows you to change the Unit Type plotted on the y-axis.

To Show the Concentration Graph

1. From the **Analysis > 1D Analysis > Master Tools > Concentration**. A new window appears with a blank graph.



You also can access this feature using the 1D Analysis Plugin.

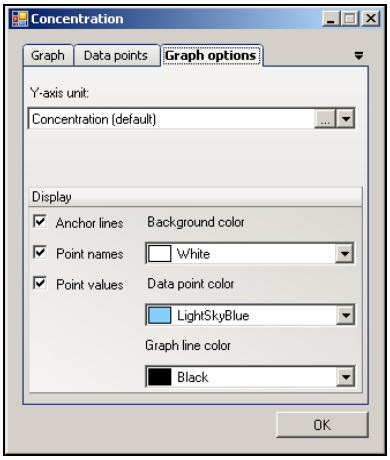
Changing Unit Type

When you bring up the Calibration Graph, the Unit Type plotted along the y-axis is given as Concentration.

Selecting Unit Type

If you wish to plot a different type of unit along the y-axis, do the following:

1. Select the **Graph Options** tab in the Concentration window. Click on the **Y-axis unit** drop down menu.

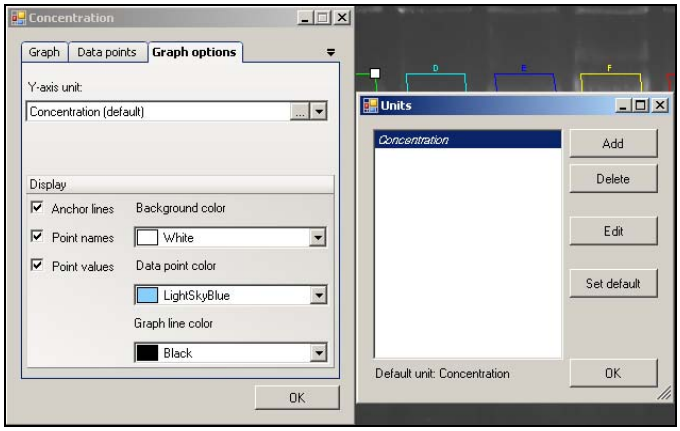


2. Select the unit type you wish to see displayed. The y-axis reflects the new unit name.

If you do not see the unit type you want in the drop-down menu, LS software allows you to add it in. See "Adding a New Unit Type" below.

Adding, Editing or Deleting a Unit Type

1. To open the Y-axis unit menu, click the **"…"** button under the **Graph options** tab **Y-axis unit**. Click a button as appropriate to add, delete, edit or set default.



2. To add a unit type, click the **Add** button. A New Unit field will appear and be highlighted. Type in the name of the unit you wish to see appear in the Y-axis unit drop down menu.
3. Click **OK**.

Editing Unit Type

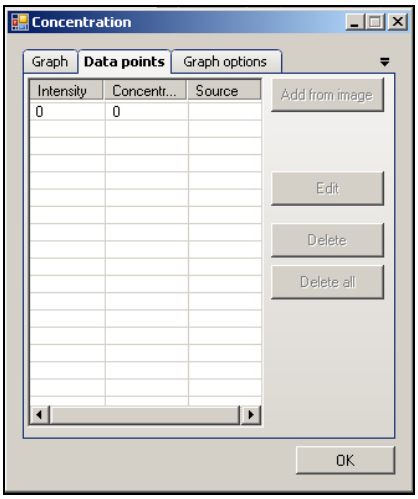
1. To edit a unit type, click on the unit name you wish to edit (you cannot edit **Concentration**). Edit the name of the unit as you wish to see it changed.
2. Click **OK**.

Deleting Unit Type

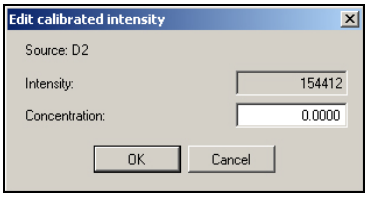
1. To delete the unit type, click on the unit name you wish to delete (you cannot delete **Concentration**). Then click on **Delete**. The unit name is removed.
2. Click **OK**.

SELECTING DATA POINTS

Once you have brought up the Concentration Graph, LS asks you to select data points to plot on the graph.



Selecting Data Points



In the Concentration Window, note that there are three tabs, **Graph, Data Points and Graph Options**. Any of these tabs may be selected when clicking on bands to calibrate to data points on the graph

1. Click on a band in the image that has a known amount to calibrate. The **Edit calibrated intensity** window opens. Enter the "known" amount (standard) in the **Concentration** field.
2. Click **OK**.
3. Continue to select the remainder (individually) of the "known" concentrations and enter the "known" concentration as you go. The data point you entered is now plotted on the graph under the **Graph** tab. Under the **Data Points** tab, LS shows the exact position of the data points and where they will be plotted.

Select as many data points as you wish following the steps above. Note that as you add in data points, LS will fit a curve to the points using the method selected above the graph, plotting this on the graph.

Editing Data Points

To edit the data points that you already selected:

1. Click on the **Data Points** tab of the Concentration window
2. Click on the value that you wish to edit.
3. Click **Edit.** The **Edit** window pops up.
4. Change the concentration value to the number you wish to see plotted.
5. Click **OK.**

Deleting Data Points

To delete data points from the graph:

1. Click on the **Data Points** tab of the Concentration window.
2. Click on the concentration that you wish to delete.

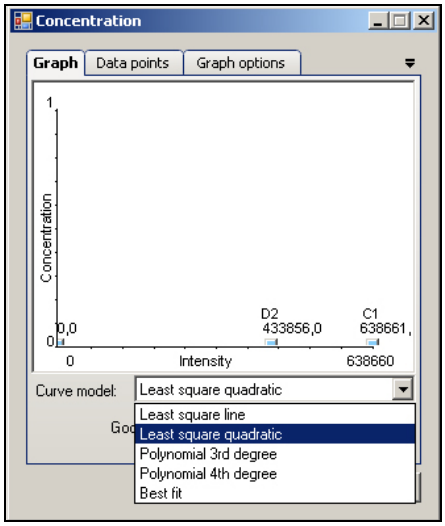
3.	Click **Delete**.
4.	Click **OK**.

You can access the Concentration window from the 1D Analysis Toolbar.

SELECTING CURVE TYPE

Once you select the data points to graph, the software allows you to select the type of curve or line to fit to the data points.

In the **Graph** tab of the Concentration window, select the curve model drop down menu. Select type of curve you would like to see on the graph.



LS software has several possibilities for curve models:

- ***Least square line****:* a straight line (polynomial degree 1);
- ***Least square quadratic***: a binomial curve (polynomial degree 2);
- ***Polynomial 3rd degree***: a polynomial curve of degree 3;
- ***Polynomial 4th degree***: a polynomial curve of degree 4;
- ***Best Fit:*** Selects the curve with the highest 'Goodness of Fit' value.

In using polynomial curve types, make sure that you have at least one more data point selected than the degree of the curve e.g., if you select a Polynomial 3rd degree, you need at least four data points.

LS software automatically and immediately fits the curve model you choose to the data points as you select the models. In the **curve model** list, it shows the **Best Fit** for the curves as they are graphed. The goodness of fit is found from the coefficient of determination (also known as "r-squared"). The goodness-of-fit value ranges between 0.0 and 1.0. A value of 1.0 for the goodness of fit indicates a perfect fit.

LS software also allows you to choose to see the line or curve graphed either on its own, or with additional information. In the **Display** section, you can choose to display **Point Values**, **Anchor Lines** from the data points down to the x-axis, and the **Point Name** assigned from the band IDs. Note that you can select to see one or all of these three **Display** options.

REMOVING CONCENTRATION CALIBRATION

1. To remove all calibration information including data points plotted on the graph and curve lines, simply go to **Analysis > 1D Analysis > Master Tools > Concentration** and then select **Delete All**. A window will pop up asking you to confirm that you wish to remove all calibration data.
2. Click **Yes** or **No**. By clicking on **Yes** all calibration data is removed and you can start a new analysis.

Changing the background correction method changes net intensity values and therefore invalidates concentration calibration. LS software will automatically ask you if you wish to remove all concentration data if you change the background correction method. Answering **Yes** is the same as selecting **Delete All** in the **Data Points** tab.

Moving the bands or resizing them also changes their net intensity values. As a result, you will see the word “Custom” appear in the data-source column (Concentration Calibration Window), instead of the name of specific band.

On the same lines, when all lanes and bands information is deleted, you will be asked if you want to remove all corresponding Concentration Curve data.

CHAPTER SIXTEEN: VIEWING & PRINTING 1D GEL DATA

OVERVIEW

LS software simplifies viewing and printing information about the image, lanes, bands and analyses. In this chapter, you will learn the following:

- How to view lane and band information;
- How to use the Lane Profile Graph, including displaying multiple lanes in a graph, changing the variables on the axes, and changing the display options;
- Managing and printing tabular reports;
- Using the Data Explorer;
- Exporting data; and
- Viewing and printing fixed reports of analysis settings, analysis lanes, analysis bands and the lane profile.

LANE INFORMATION

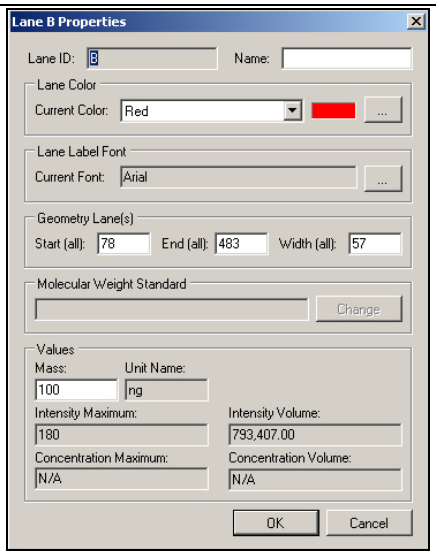
In LS software, you have several ways of viewing lane information:

- **In the *Lane Profile Graph***;
- **In the *Data Explorer***;
- **In the *Tabular Reports*,**
- **In *Analysis Settings***; and
- **In *Lane Properties***.

When editing bands or lanes, you may need to select **1D Analysis > Master Tools > Edit Objects** plugin is selected prior to clicking on a band or lane.

To View and Use Lane Properties

Once you have a lane selected, from **Analysis > 1D Analysis > Lanes,** select **Lane Properties**. A new window appears.



In this window, the software allows you to perform various changes to the lane:

- You can *name* the lane;
- You can *alter the color* of the lane;
- You can *change the geometric proportions* of the lane (or of all of the lanes, if you have selected to make all lanes the same width); and
- You can *change the molecular weight standard* of the lane (if you have performed a molecular weight calculation).
- You can change the mass assigned to the lane.

Lane Properties also offers you the following information:

- The *Lane ID*;
- The *unit of mass*;
- The *intensity maximum*;
- The *intensity volume*;
- The *concentration maximum*; and
- The *concentration volume*.

You may also access this feature by right-clicking on the lane and selecting **Lane Information** in the shortcut menu, or by using the **1D Analysis Plugin**.

BAND INFORMATION

In LS software, you have several ways of viewing band information:

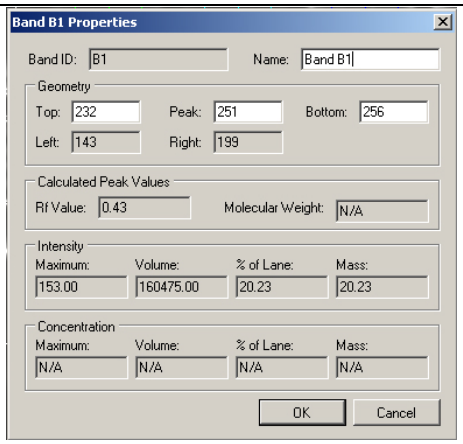
- **In the *Lane Profile Graph***;
- **In the *Data Explorer***;
- **In *Tabular Reports***;
- **In *Analysis Settings***; and
- **In *Band Properties***.

To View and Use Band Properties

Once you have a lane selected, from the **Analysis > 1D Analysis > Bands** and then select **Band Properties**. A new window appears.

In this window, the software allows you to perform two changes to the band:

- You can *name* the band; and
- You can *change the geometric*



proportions of the band.

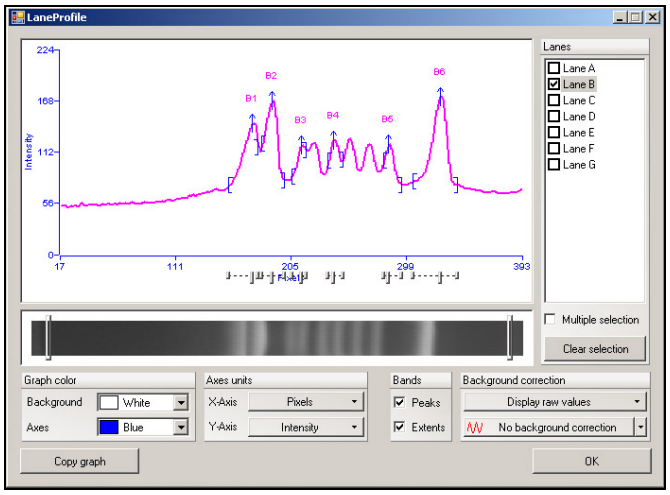
Band Properties also offers you the following information:

- The *Band ID*;
- The *calculated peak values* (*Rf value* and *molecular weight*);
- The *intensity* of the band, including its *maximum*, *volume*, *percentage of the lane*, and *mass*; and
- The concentration of the band, including its maximum, volume, percentage of the lane, and mass.

You also can access this feature using the 1D toolbar.

LANE PROFILE GRAPH

LS software allows you to view profile graphs (intensity vs. position) of one or more of the lanes in your image in the Lane Profile Graph. To access this function, select **Lane Profile Graph** from the **1D Analysis** Menu. A new window appears with the graph itself and with several options.



Underneath the graph, the software displays an image of the graphed lane (or of the lane you last selected to be graphed).

In this section, the following topics are presented:

- How to display multiple lanes at one time;
- How to change variables for the x-axis and the y-axis; and
- How to change colors of the graph and how to display specifics such as band extents, band peaks, and background correction.

You also can access the Lane Profile Graph using the 1D Analysis Plugin, or using the context menu for Lanes or Bands..

Displaying Multiple Lanes

LS software allows you to display one or more lanes at a time.

To Display a Single Lane

It has two options for displaying a single lane.

To Display a Single Lane by Selecting a Lane

1. Select **Edit Objects** from the **Analysis > 1D Analysis > Master Tools**.
2. Click on the lane you wish to see graphed.
3. From the **Analysis > 1D Analysis > Master Tools** select **Lane Profile Graph**. A new window appears with the lane you selected graphed.

To Display a Single Lane from the Lane Profile Graph

1. Once you have a lane selected, from the **Analysis > 1D Analysis > Master Tools**, select **Lane Profile Graph**. A new window appears with an empty graph.
2. From the **Lanes** section to the right of the graph select the lane you wish to see graphed. The software automatically displays the graph of that lane.

To Display Multiple Lanes

The software also has two options for displaying a multiple lanes.

To Display Multiple Lanes by Selecting Lanes

1. Select **Edit Objects** from **Analysis >1D Analysis > Master Tools**.
2. Click on the lanes you wish to see graphed as you hold down the **Control** key.
3. From the **Analysis > 1D Analysis > Master Tools**, select **Lane Profile Graph**. A new window appears with the lanes you selected graphed.

To Display Multiple Lanes from the Lane Profile Graph

1. Once you have a lane selected, from the **Analysis > 1D Analysis > Master Tools**, select **Lane Profile Graph**. A new window appears with an empty graph.
2. From the **Lanes** section to the right of the graph, click the Multiple Selection box and select the lanes you wish to see graphed. LS software automatically displays the graphs of those lanes.

To Change the Selected Lane:

1. Click on **Lane** in the graph.
2. De-select or re-select the desired lane in the Lanes list.

Axis Options

Depending upon what type of analysis you wish to perform, you may change what variables appear on the lane profile graph's axes.

By default, the x-axis displays **Pixels** and the y-axis displays **Intensity**. However, after calibrating molecular weight, you may select to view **Pixels**, **Rf** values or **Molecular Weights (MW Standard)** on the x-axis. Similarly, after calibrating concentration, you may select to view either **Concentration** or **Intensity** on the y-axis.

To Change Axis Variables

To change axis variables after you have performed either molecular weight calculations or concentration calibrations, simply go to the **Y Axis** and **X Axis** options under the bottom left of the graph. Select the variable you wish to see displayed.

Effects of Selecting Other Axis Values

If you select Retardation factor (Rf) or Molecular Weight (MW) to be displayed on the x-axis, then the graph takes into account Rf effects. This means that other lanes may appear to be stretched or compressed horizontally relative to the selected lane.

If you select Concentration to be displayed on the y-axis, then the curve adjusts the intensities of the lane, and relative differences in the graph may change.

Display Options

In the Lane Profile graph, LS software allows you to choose what details you would like to see in the graph. The program also allows you to change the colors of the background of the graph and of its axes.

Display Options for Details

Underneath the graph and the lane image, in the **Display** options section, you can select to view various details. The following are available:

- ***Band Peaks***: Selecting this option means LS software will display an arrow labeled with the band's position at the top of the band on the graph, and a small rectangular control under the graph that can be used to move the band peak.
- ***Band Extents***: Selecting this option means it will display parentheses showing the width of each band, and two small rectangular controls under the graph that can be used to adjust the band's extent.
- ***Raw or Corrected Values***: If you have asked the software to perform a background correction, selecting this option means it will change the graph to reflect the new values after the correction.
- ***Background Correction***: If you have asked the software to perform a background correction, selecting this option means it also will place on the *original* graph the graphed line of whatever background correction you chose.

If you have chosen a background correction, you can display *either* the graph with corrected values *or* the original graph with the correction line. It is not possible to display both at once.

Color Options

Depending on what color the lane lines are, you may wish to change the background color of the graph for easier viewing. To do so:

1. Underneath the Graph color options in **Lane Profile Graph**, click on the down arrow of **Background Color**.
2. Select the color you wish to see. The software automatically changes the color.

The software also allows you to choose the color of the graph's axes. To do so:

1. Underneath the Graph color options in **Lane Profile Graph**, click on the down arrow of **Axis Color**.
2. Select the color you wish to see. The software automatically changes the color.

Background Correction Options

If you wish to change the background correction option from the Lane Profile Graph:

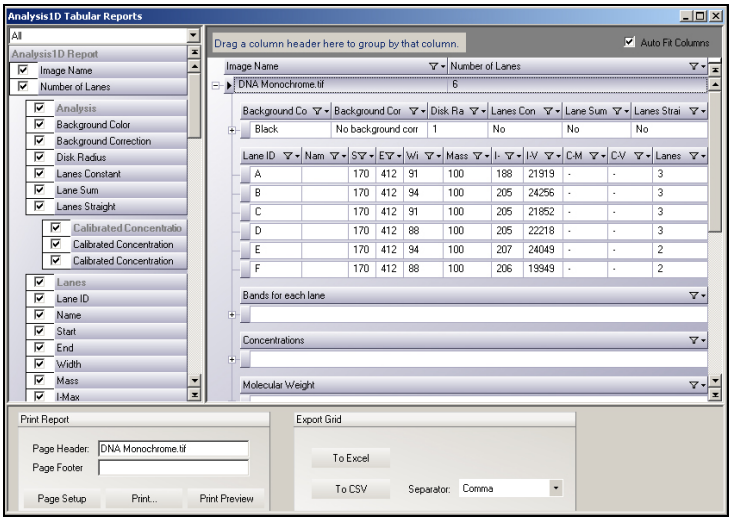
1. Underneath the Background Correction options in **Lane Profile Graph**, click on the down arrow of No **Background Correction**.
2. Select the correction you wish to see. The software automatically changes the graph. You can also select to see a graph that takes into account the corrected values.

If you select **Rolling Disc**, a new dialog box appears asking you to enter the desired radius size of the disc.

DATA EXPLORER

Viewing Data Explorer Tabular Results

Aside from viewing graphs and information windows about the lanes and bands, the software also offers you the option of seeing the data in a spreadsheet format that is user configurable.



To access this **Analysis > 1D Analysis > Master Tools**, select **Data Explorer.**
Data Explorer opens a tabular format with the ability to include or exclude various data fields from the Data Explorer Report. Predefined report configurations are included to quickly select/deselect data fields appropriate to certain experiments.

The Data Explorer window also offers Report Printing and Data Export options.

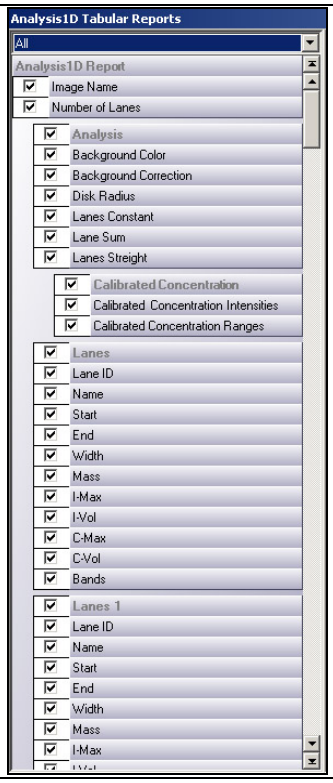
The top left corner of the Data Explorer window offers a drop-down menu for quickly selected preconfigured reports rather than having to manually filter report data for commonly reported analysis data. When selecting theses reports you will notice the various fields being selected/deselected from the list of data fields.

You also can access this feature using the **1D Analysis** Plugin.

Filtering Data

Accessible when you create tabular reports or export data, the drop-down menu allows you to choose what specific data you would like to appear in reports and in files you export.

To Select Filter Data Fields



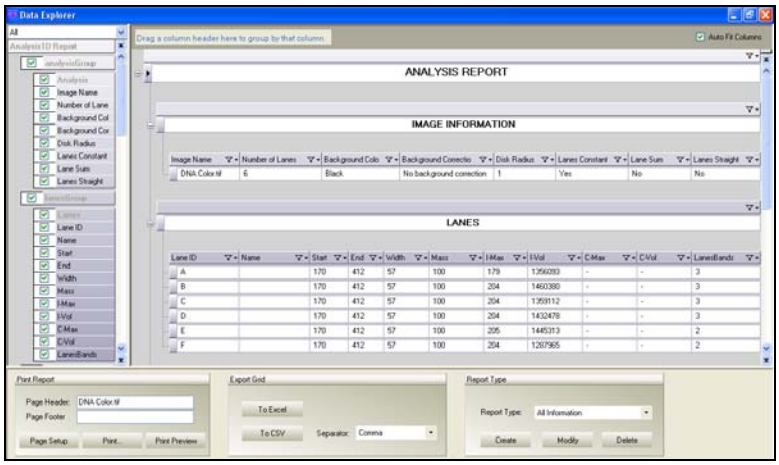
The top left corner of the Data Explorer window offers a drop-down menu for quickly selected preconfigured reports rather than having to manually filter report data for commonly reported analysis data.

When selecting theses reports you will notice the various fields being selected/deselected from the list of data fields. Select from:

- Image Name
- Number of lanes
- Background information
- Disk radius
- Lane and band information
- Concentrations
- Molecular weights

To Show Filtered Data

To the right of the data fields is the actual analysis data which includes the fields currently selected. Next to the image name there is a + symbol which indicates all of the analysis data is minimized under the image name.

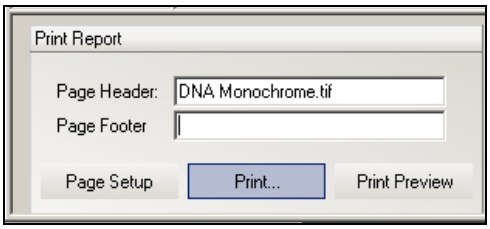


1. To show the analysis data, click the + symbol.
2. To further expand the data views for the individual categories, select the + symbol to the left of each category.
3. If you wish to filter the data further, select/deselect the appropriate data fields on the left side of the Data Explorer window.

Any data column can be removed from the report by clicking and dragging that data column back to the data field on the left side.

Printing Data Explorer Tabular Reports

In Tabular Reports, you can print whatever data you select about the image and the analyses you have performed on the bands and lanes.



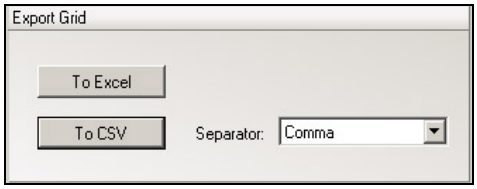
To Print Data Explorer Tabular Reports

1. In the bottom left corner of the Data Explorer Tabular Reports window there are several print options:
    a. Page header: Displays as the page title on the top of the report
    b. Page footer: Displays as the page information at the bottom of the report
    c. Page setup: Displays the page setup options as offered by your specific printer
    d. Print preview: Displays a preview of what will be print on the report.
2. Enter the header and footer information, set the page format, and click **Print**. When the print window opens, click **OK**.

Export Data Explorer Reports

LS software allows you to export data to Microsoft Excel® or to other software packages for further analysis or documentation.

To Export Data Explorer Tabular Reports



To export data:

1. Under **Analysis > 1D Analysis > Master Tools**, select **Data Explorer**. The **Tabular** Reports window appears.
2. Select the data fields you wish to export.
3. In the bottom right corner of the Data Explorer Tabular window, you can select from two options and select whether you wish to export the data by **Comma**, **Semicolon**, **Space**, **Tab** (where you type in the delimiting character or characters):
    - To Excel
    - To CSV
4. Click the **To Excel** or **To CSV** button.

	5. Name the file and click **Save**.

FIXED IMAGE AND ANALYSIS REPORTS

Fixed Reports

LS software offers several standard reports that you cannot alter but that provide you valuable reference information:

- **The Analysis Settings Report**: Gives you information on Background Color and Correction, lane width and volume, and whether the lanes are straight**;**
- **The Analysis Lanes Report**: Gives you information on Lane ID and name, band count in each lane, concentration, intensity and mass;
- **The Analysis Bands Report**: Gives you information on Band ID and name, calculated peak values, intensity and concentration; and
- **The Lane Profile Report**: A graphical representation of lane data.

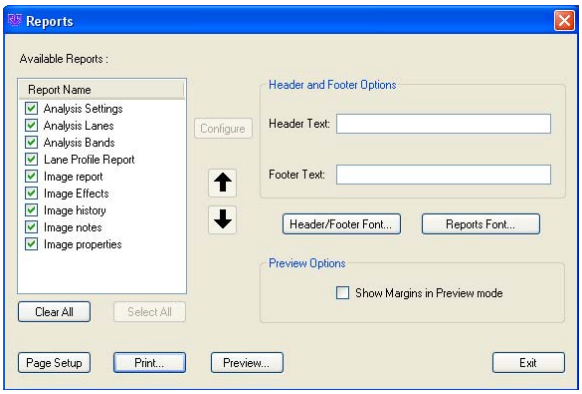
Analysis Settings

One fixed report that the software offers is the Analysis Settings Report. This report gives you information on the following 1D Gel Analysis Settings:

- Background Color;
- Background Correction;
- Disc Radius (If you used Rolling Disc);
- Whether Lane Width is Constant Across All Lanes;
- Whether all lanes were forced straight; and
- Whether the lane volume is the sum of all of its bands.

To Print the Analysis Settings Fixed Report

1. Under the **Tools** Menu, select **Reports.** Select only the **Analysis Settings** check box.
2. Select **Print**, **OK**. Click **Exit** to close the **Reports** window.



To Change General Layout Before Printing

1. To change the margins, click on the **Page Setup**. You can make the margins larger or smaller by varying degrees. To view the margin lines, select **Show Margins in the Preview Mode** in the **Reports** window.
2. Enter information in **Header Text** or **Footer Text**. Note the following abbreviations for header and footer text:

   %p puts the page number at the top or bottom of the page;

   %c puts the page count at the top or bottom of the page;

   %d puts the date at the top or bottom of the page; and

   %t puts the time at the top or bottom of the page.

Analysis Lanes

The software will print a fixed report of the lane analyses for you. In this report, you will find the following information:

- The *Lane ID* and *name*;
- Where the lane *starts* and *ends*;
- The *band count*;
- The *mass* of the lane;
- The *unit of mass*;
- The *intensity maximum*;
- The *intensity volume*;
- *Molecular weight* information;
- The *concentration maximum*; and
- The *concentration volume*.

To Print the Analysis Lanes Fixed Report

1. Under the **Tools** Menu, select **Reports.** Select only the **Analysis Lanes** check box.
2. Select **Print, OK**. Click **Exit** to close the **Reports** window.

To Change General Layout Before Printing

1. To change the margins, click on the **Page Setup**. You can make the margins larger or smaller by varying degrees. To view the margin lines, select **Show Margins in the Preview Mode** in the **Reports** window.
2. Enter information in **Header Text** or **Footer Text**. Note the following abbreviations for header and footer text:

   %p puts the page number at the top or bottom of the page;

   %c puts the page count at the top or bottom of the page;

   %d puts the date at the top or bottom of the page; and

   %t puts the time at the top or bottom of the page.

Analysis Bands

The software prints reports detailing band analysis. The Analysis Bands Fixed Report offers the following information:

- The *Band Name* and *ID*;
- The *calculated peak values* (*Rf value* and *molecular weight*);
- The *intensity* of the band, including its *maximum*, *volume*, *percentage of the lane*, and *mass*; and
- The concentration of the band, including its maximum, volume, percentage of the lane, and mass.

To Print the Analysis Bands Fixed Report

1. Under the **Tools** Menu, select **Reports.** Select only the **Analysis Bands** check box.
2. Select **Print**.

To Change General Layout Before Printing

1. To change the margins, click on the **Page Setup**. You can make the margins larger or smaller by varying degrees. To view the margin lines, select **Show Margins in the Preview Mode** in the **Reports** window.
2. Enter information in **Header Text** or **Footer Text**. Note the following abbreviations for header and footer text:
   - %p puts the page number at the top or bottom of the page
   - %c puts the page count at the top or bottom of the page
   - %d puts the date at the top or bottom of the page
   - %t puts the time at the top or bottom of the page

Lane Profile Report

To print a graphical representation of the lanes, you can select the Lane Profile Fixed Report.

To Print the Lane Profile Fixed Report

1. Under the **Tools** Menu, select **Reports.** Select only the **Lane Profile Report** check box.
2. Select **Print**, **OK**. Click **Exit** to close the **Reports** window.

To Change General Layout Before Printing

1. To change the margins, click on the **Page Setup**. You can make the margins larger or smaller by varying degrees. To view the margin lines, select **Show Margins in the Preview Mode** in the **Reports** window.
2. Enter the **Header Text** or **Footer Text**, note the following abbreviations for header and footer text:
   - %p puts the page number at the top or bottom of the page
   - %c puts the page count at the top or bottom of the page
   - %d puts the date at the top or bottom of the page
   - %t puts the time at the top or bottom of the page

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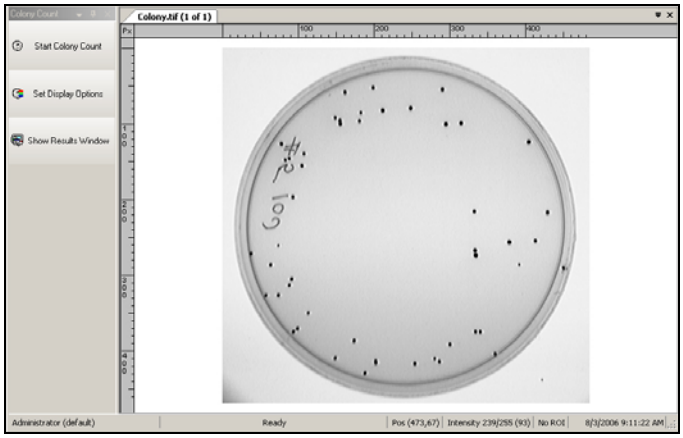
CHAPTER SEVENTEEN: COLONY COUNTING

OVERVIEW

Colony Counting in LS packages is an advanced object recognition tool, primarily suited for identifying bacterial colonies in a Petri dish. Circular/Elliptical objects (or colonies) with peak at the center are recognized best with the help of this tool.



The software provides two different ways to count objects:

1. Automatic Counting
2. Wizard assisted Manual Counting

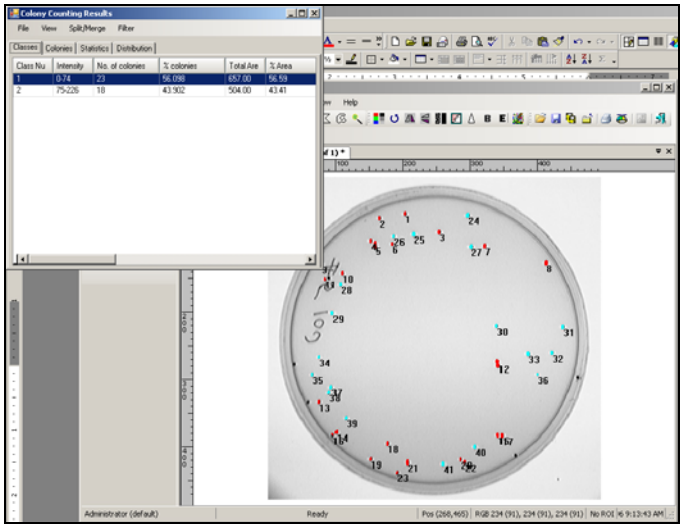
Counting is always done on a duplicate image in order to preserve any analysis which may have been present in the original image.

AUTOMATIC COUNTING

Automatic colony counting is intended to automatically separate and count light and dark colonies. The result is a population count of the two intensity ranges. Due to the speed of this tool, many users that want to only count the total population of colonies (not separated by intensity) rely on the automatic count method.

1. Open the image that contains colonies. (Software considers the colonies as objects embedded in the image.)
2. Click on **Analysis > Colony Count > Start Colony Count**.
3. Click **Yes** to start the automatic colony counting

Without any more input, the software will open a new image and recognize the colonies (or objects) present in the image of interest and bring up a chart with relevant measurements, statistics and distribution. Refer to the section below on how to interpret the results.



Colony counting can be started by clicking the **Start Colony Count** button on the **Colony Count** plug-in.

MANUAL COLONY COUNTING

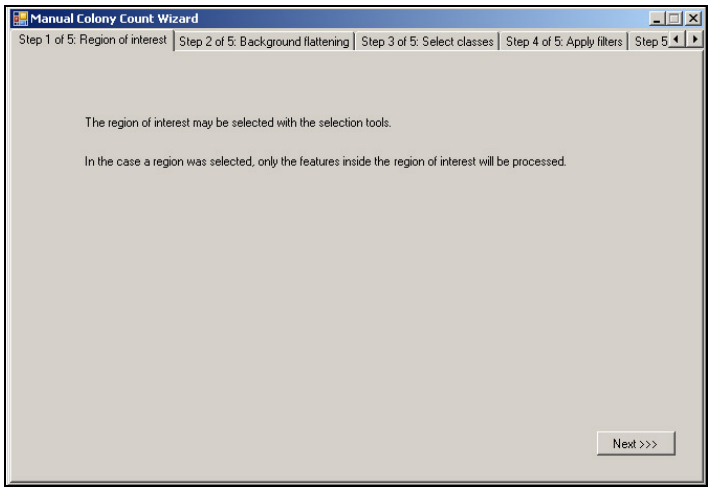
Manual counting process has a few more steps, but it gives you much greater control over where and how you want colonies to be identified on the image:

1. Open the image of the container (usually a Petri dish) that contains colonies. (Software considers the colonies as objects embedded in the image.)
2. Click on **Analysis > Colony Count > Start Colony Counting**. The software asks if you want to perform Automatic Colony counting.
3. Click **No**. This starts the **Manual Colony Counting Wizard** that takes you through the steps involved in recognizing objects in images.

Region of Interest (ROI) – Step 1 of 5

The Wizard automatically enters a circular Region of Interest to mark the area within which the colonies are present and need to be counted.

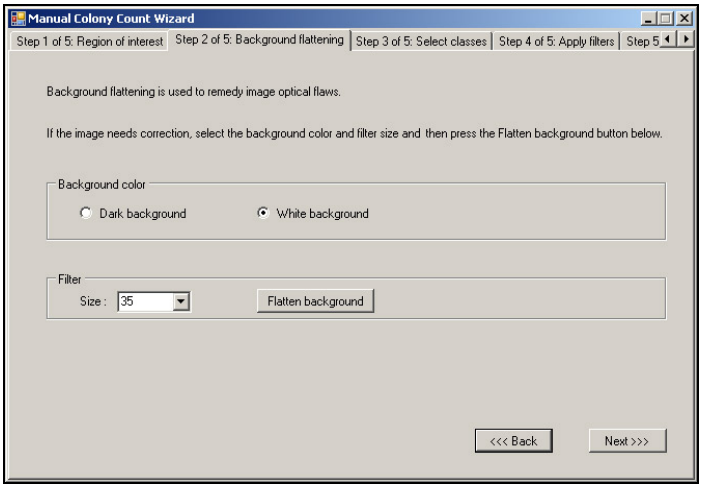
Drag the corners to adjust the size of the ROI if necessary. For round Petri dishes, the circular tool is recommended. To fine-tune a specific area which is irregular in shape, use the irregular ROI (tracing option preferred) tool. Only one ROI can be active at one time.



Once the ROI is adjusted, click **Next** to proceed.

Background flattening – Step 2 of 5

This is a very important step. Some noise is often present in the background, due to nuances in optics, camera and lighting conditions. Removal of such background noise is likely to increase the quality of analysis significantly.



The **Background Color** radio button is for selection of the color of background – white or dark. Usually, bacterial colonies are dark, and the background is white when the container is illuminated with white light.

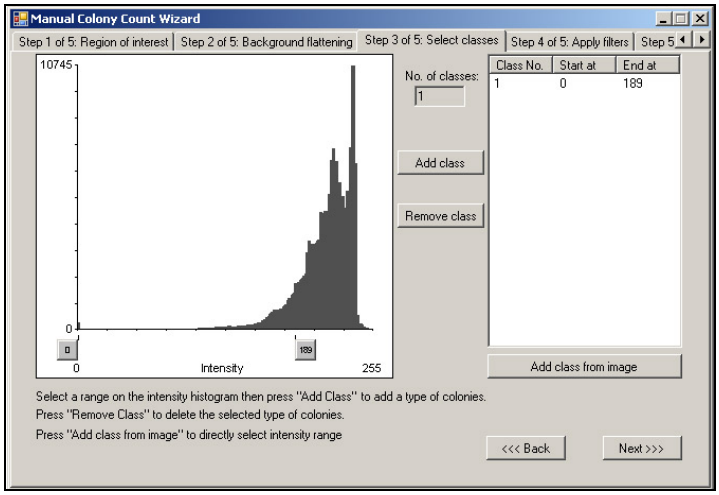
The **Filter** option allows selection of the size of what is called the 'kernel'. It is recommended that you use one of the values already listed. Lesser the number, more blurry the borders of the colonies will get. Higher the number, more the sharp the borders will be, up to a certain extent.

The filter-size may vary greatly from image to image, though for most images, the size of about 35 should provide a crisp image. Adjusting the number helps to sharply distinguish most of the colonies. For example, an image with a lot of colonies clumped together may need a lower number to distinguish the peaks of one colony from the other by blurring the

boundaries. Change the filter-size to verify how the image looks by pressing the **Flatten Background** button right next to it. Zoom into the image, especially to the area where colonies are clubbed together, to get a more realistic idea of what the right size will be for your image.

Select classes – Step 3 of 5

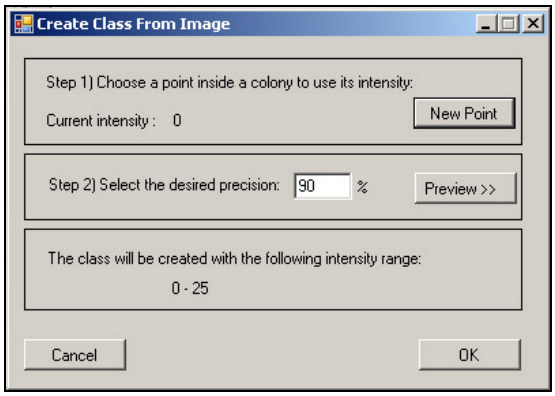
The software classifies colonies based on their levels of darkness (or brightness). For a given dynamic-range, the darkness can be classified into various classes. A class is simply a defined interval of grey-levels. The screenshot below shows an instance of a class (0-243) in an image with 8 bits of information.



For example, for a 12-bit image which has 4096 levels of grey-scale, [0-300] would be an instance of a dark class. Colonies, all the pixels of which fall into this range, would be classified into this class. A range on the higher end e.g. [3000-3500] could be an example of light and bright colonies.

This step lets you specify the classes precisely. For a colony to be classified in a given class, ALL pixels of that colony must have intensity falling within the boundaries of that class. Hence essentially, specification of a class determines the sizes and boundaries of colonies. Wider the class, more pixels in and around the colony are likely to be included in the range, which means that the colonies may get bigger in size. Typically, 2 or more classes would be required for correct identification of all most of the colonies. Colonies not falling into any range are discarded.

If detailed results are required for every object (colony) and if specific colonies need to be excluded / included, then defining precise ranges becomes important. Click on the button **Add class from image** to do the same. That brings up the following dialog box:



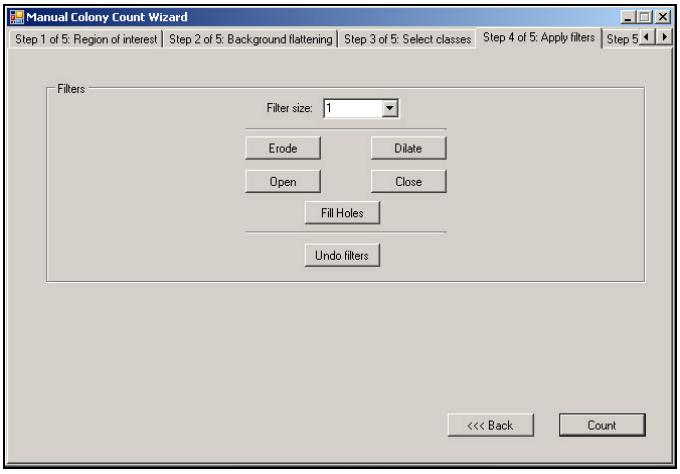
This lets you choose the class based on an existing object (colony) in the image.

First, click on a colony of interest. Then, depending on how wide you want the range, you could vary the percentage precision. Higher the precision, smaller is the class. As the percentage changes, the changes show in the preview window.

Repeat this step for each class that needs to be added.

Apply filters – Step 4 of 5

Now that the colonies are identified by the software (but not yet counted and statistics not done) there is a chance to apply filters to colonies, as opposed to the entire image which we did in Step-2. Four types of filters are available. Preview on the original image is available on application of each filter. One can even undo the application of filters.



Each filter constitutes an application of a specific standard algorithm in. A brief explanation of each follows:

Erode: This has the effect of “erosion” or “eating away” of the colonies (also called objects or blobs) from the surroundings. This makes colonies smaller to look at and provides distinction among clumped up colonies.

Dilate: This has the effect of “dilating” or “expanding” the colonies. This can be useful if the colonies formed are too small and spread apart.

Open: This filter has the effect of “opening” up the colonies or simply disconnecting the colonies from each other and erasing connections.

Close: This filter fills up spaces between adjacent colonies.

Some facts about filters:

'Size' signifies how strong the filter is. Larger the size, stronger the effect.

Second application of a filter works on the result of the first one, giving even stronger effects.

There is no way to apply these filters to individual colonies.

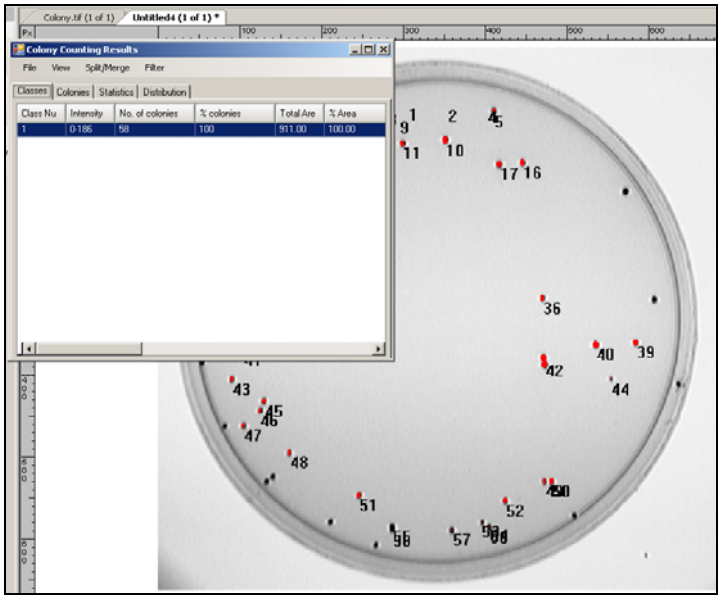
Changing the filters affects the statistical data and resultant count of colonies.

If the colonies are well-formed, you may not need to apply any filters at all. But these filters come in handy when the colonies have not particularly grown well and still have to be counted.

Click the **Count** button to count the colonies.

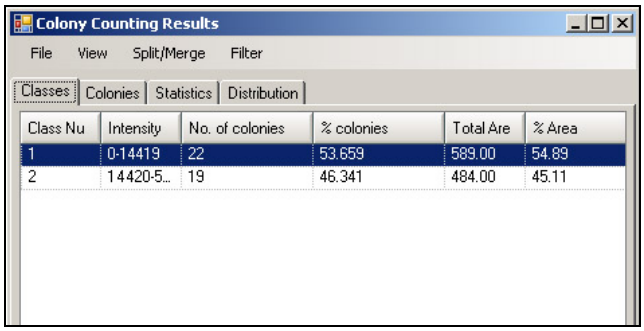
Finish – Step 5 of 5

Click **Finish** when to show the **Results** window.



RESULTS WINDOW AND EXPLANATION OF STATISTICS

After colony counting is completed, a Colony Counting Results window will automatically display showing various data.



The result window

The result window has four **tabs**:

- **Classes:** Provides statistics at the level of classes.

Sr. No.	Column	Description
1	Intensity Range	Lower and upper limits of the range of intensity of the class
2	No. of Colonies	Total colonies found in this class
3	% Colonies	Percentage of colonies found in this class, among overall number of colonies
4	Total Area	Total area (in pixels) covered by the colonies detected
5	% Area	Percentage of area (in pixels) covered by colonies of this class
6	Mean Area	Average area of a colony (in pixels)
7	Std. Dev. Area	Standard deviation of Area (in pixels) of colonies which belong to this class
8	Min. Area	Minimum area among all colonies of this class
9	Max. Area	Maximum area among all colonies of this class
10	Total Density Lum	Average pixel intensity (aka Density Luminance) totaled over all colonies found
11	% Density Lum	Percentage of “Total Density Lum” (above) present in this particular class, over all classes.
12	Mean Density Lum	Average Density Lum for this given class
13	Std. Dev. Density Lum	Standard Deviation of Density Lums of all colonies
14	Min. Density Lum	Minimum Density Lum found for this class

15	Max. Density Lum	Maximum Density Lum found for this class

- **Colonies:** This section lists all colonies found with its individual statistics

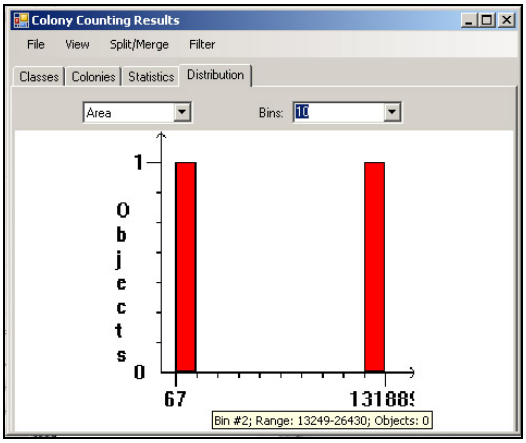
1	Colony Number	Unique number (ID) given to each colony
2	Class	Class identifier for the colony
3	Area	Area of the colony (in pixels)
4	Perimeter	Perimeter (in pixels) of the colony
5	Average Diameter	Average diameter of the colony
6	Density Luminance	Average intensity of pixel values included in the colony
7	Circularity	Measure of roundness of the colony – Minimum value, showing ideal circularity is 1.00. Higher the value, less circular the colony is
8	Rectangularity	This is a measure of how rectangular the colonies are. Values are between 0 and 1. Lesser the value, less rectangular and slightly more circular is the colony
9	Hole Count	If the colony has holes, this shows how many it has
10	Major Axis	For an elliptical (or oblong) colony, this measure is the length of Major Axis in pixels
11	Minor Axis	For an elliptical (or oblong) colony, this measure is the length of Minor Axis in pixels
12	Aspect Ratio	Ratio of Major Axis to Minor Axis
13	Angle	Angle between Major Axis and Horizontal Axis

- **Statistics:** This section provides overall statistics regardless of the class and connects max and min values to specific colonies.

Property	**Area**	**Density Lum**
Min.	Minimum Area (pixels)	Minimum Density Luminance
Obj. No.	Colony id, that has minimum area	Colony id, that has minimum Density Lum
Max.	Maximum Area	Maximum Density Lum
Obj. No.	Colony id, that has maximum area	Colony id, that has maximum Density Lum
Range	Max area minus Min area	Max Density Lum – Min Density

		Lum
Mean	Average area of all colonies	Average Density Lum over all colonies
Std. Dev.	Standard Deviation across areas of all colonies found	Standard Deviation across Density Luminance of all colonies found
Sum	Total area covered by all colonies found (in pixels)	Total Density Luminance of all Colonies
Samples	= Sum / Mean	= Sum / Mean

- **Distribution**: Area and Density Luminance are the two main characteristics reported with colonies. This tab shows a histogram of how the colonies are distributed over the range of each of these quantities.

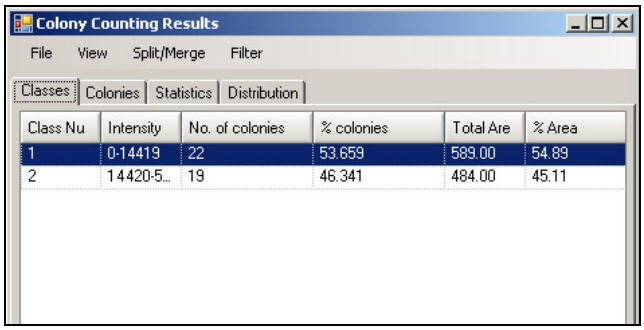


The X axis shows the number of bins, or number of segments between min and max of areas. The Y axis shows the count of objects in each bin.

There are two drop-down type boxes right below the tab: 1) Area and Density Luminance; 2) number of bins in the histogram for the characteristic selected. More the bins, more spread the histogram will be.

Colony Counting Menu Bar

The result window has four **drop-down menus**:



- **File Menu**

Save Results to Excel: Clicking this option saves the results of first three tabs (classes, colonies and statistics) into three separate sheets. Microsoft Excel must already be installed to avail this functionality. If you do not have Microsoft Excel, please contact your IT staff.

Save Results to File: Results can also be saved in three separate text files (which can then be opened in any text editor or Excel) using this option.

- **View Menu:** Lets you chose what columns (characteristics or attributes) you want seen in the second tab – Colonies.
  - **Split / Merge:** This menu lets you do important things with the colonies:

    Split Colonies –

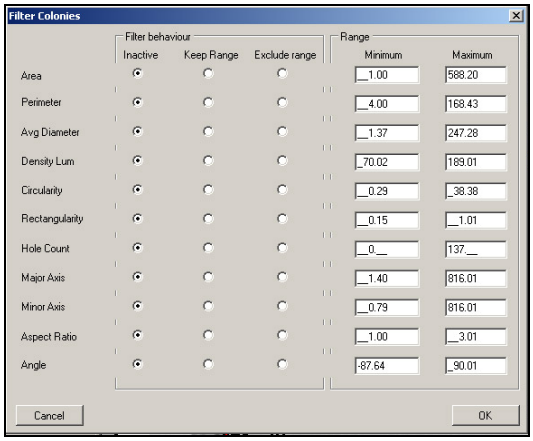
    - Click on this option, which will pop a dialog box.
    - Zoom into the colony you want split.
    - Follow the instructions in the box and draw an ROI around the desired colony.
    - Draw a line through the colony where you need the separation and click OK.

    Merge Colonies –

    - Click on this option, which will pop a dialog box.
    - Zoom into the colonies you want merged.
    - Follow the instructions in the box and draw an ROI around the desired colonies.
    - Click OK.

    Remove/Add Colony –

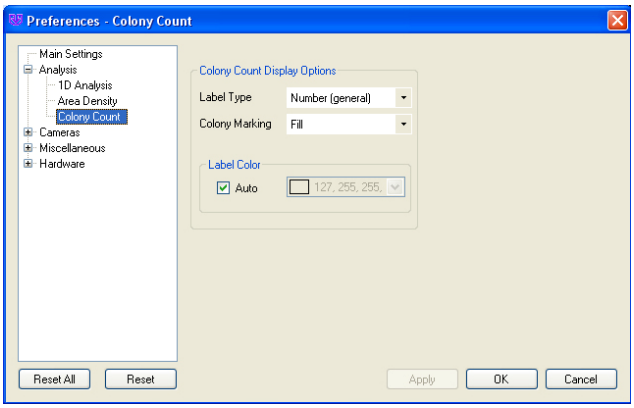
    - Click on this option, which will pop a dialog box.
    - Zoom into the place you want the colony added/removed.
    - Follow the instructions in the box and draw an ROI around the desired places.
    - Click OK.
  - **Filter: Filter Class** - After the colonies have been found, you may no longer want to have all of the colonies in your view. To use this option to filter the colonies based on all characteristics, click **Filter > Filter Class > 1** (or other class to be filtered).



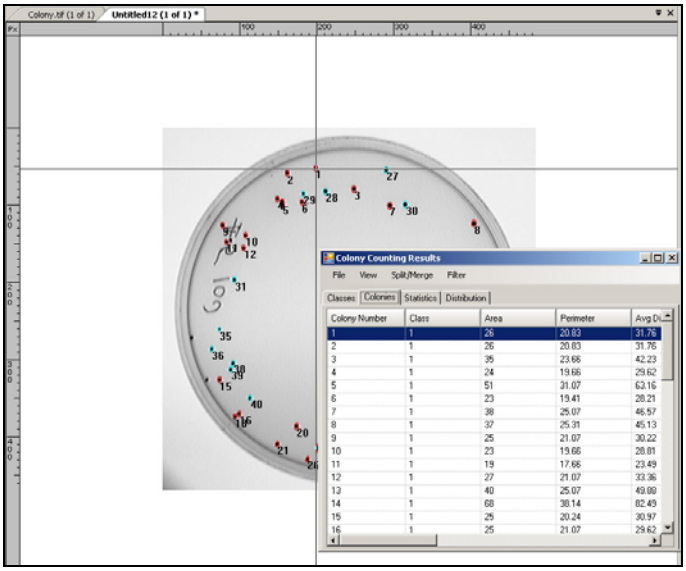
For each row (or characteristic), specify the range you want to see. Select the **Inactive** radio button to ignore a particular characteristic. Select the **Keep Range** radio button to include that parameter in the results. Select the **Exclude** radio button to specifically exclude a parameter range.

TIPS

- Use the **Zoom/Pan** tool to work with smaller areas for more accurate assessment.
- Colonies can be labeled in various ways. Click on **Analysis > Colony Count > Set Display Options** (or from **File > Preferences > Analysis > Colony Count**) to choose the label identification.



- To locate the colony on the image, click on the Colony Number in the **Colony** tab **(Analysis > Colony Count > Show Results Window).** Corresponding colony will be identified with crosshairs on the image.



- Multiple images can be opened at the same time to execute colony-counting.
- As long as the image with Analysis is open, its results' are preserved. Results window can be brought up anytime using 'Show Results Window' button on the toolbar.

CHAPTER EIGHTEEN: AREA DENSITY

- Purpose
- Area Density Options
- Steps to Follow
- Saving the Results
- Intensity Calibration Curves
- Spatial Calibration

PURPOSE

This tool can be used to carry out precise quantitative calculations on the regions of interest on your image. It gives you the flexibility to carry out calculations based on Optical Density as well as Grey Levels. Additionally, one can also calibrate the amount of sample loaded in each spot.

AREA DENSITY OPTIONS

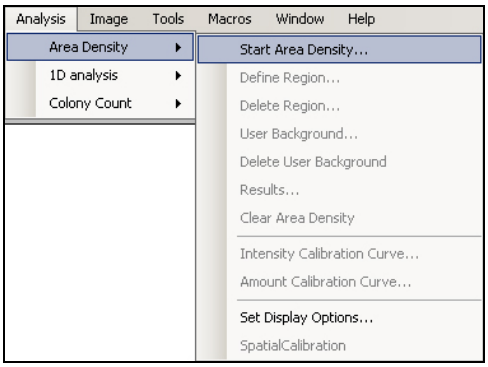
The software provides two different ways to access the Area Density tool:

1. **Analysis > Area Density** drop-down menu
2. **View > Plugins > Area Density plugin**

The **Area Density Results** window is also discussed in this section.

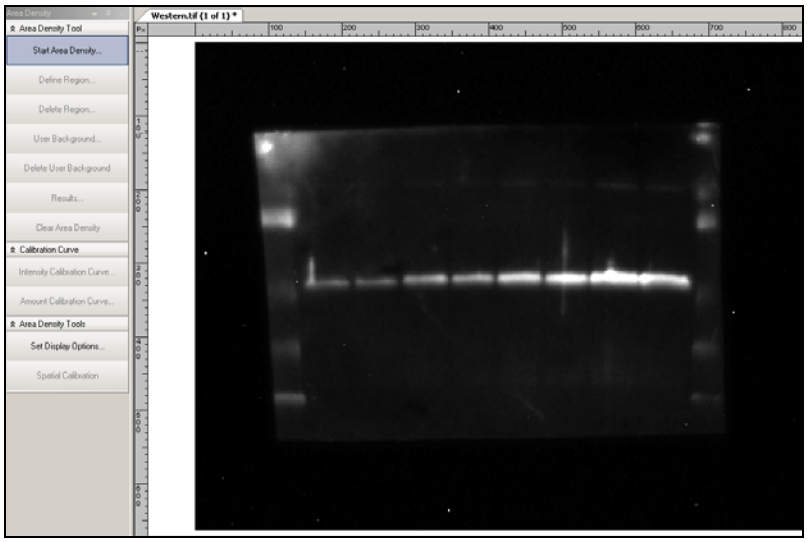
Area Density Menu

The **Analysis > Area Density** menu, allows you start the area density function, define the region and obtain results. Calibration curves, spatial calibration and area density display options can also be selected from the Area Density menu.



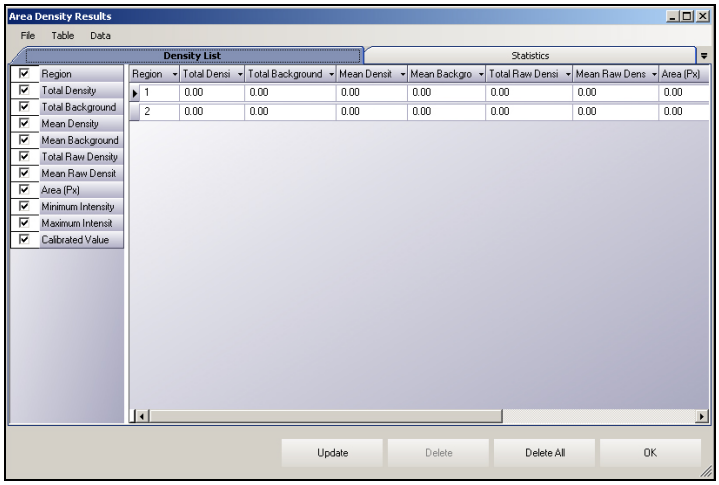
Area Density Plug-in

To access the Area Density Plugin, select **View > Plugins > Area Density**. The plugin will display with the following functions available:



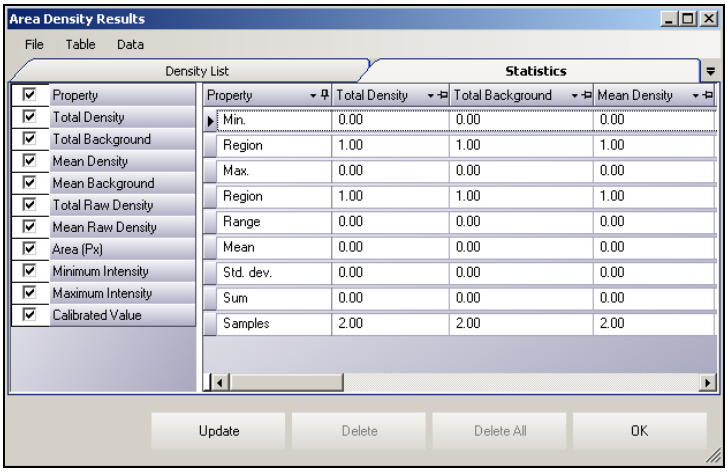
Start Area Density	Click this button to start the area density procedure. This will prompt the user to define a region. If a region has been defined, this button will change to read **Restart Area Density**.
Define Region	Select the region of interest (ROI) on the active images with the ROI tools.
Delete Region	Select a region in the list and press this button to delete it from the image as well as this list.
User Background	This button lets you define your own background on the image for calculations.
Delete User Background	This button lets you delete your own background on the image for calculations.
Results	Refer to the Area Density Results discussed in this chapter.
Clear Area Density	
Intensity Calibration Curve	This button lets you choose between **Standard Optical Density** calculations and **Freeform** calculations. One can define a custom intensity calibration curve for each type.
Amount Calibration Curve	This button lets you assign the quantity of sample loaded in each region and create a curve thereof. Unknown amounts are calibrated using that curve.
Set Display Options	This option lets you choose which colors to use while marking and displaying areas on the image.
Spatial Calibration	
Delete All	Deletes all regions marked.

Area Density Results – Density List (Tab)



Region	
Total Density	Sum total of intensities of all pixels within the region, minus the background. Intensity is either in terms of Grey-Levels (GL) or Optical Density (OD), depending on the Intensity Calibration curve.
Total Background	Sum total of intensities of all pixels within the region marked as background. Background is calculated in two different ways. Explanation below.
Mean Density	Average of intensities of all pixels of the region, minus average intensity of background pixels.
Mean Background	Average intensity of background pixels.
Total Raw Density	Sum total of intensities of all pixels within the region. No background is subtracted.
Mean Raw Density	Average intensity of intensities of all pixels within the region.
Area (Px)	Total number of pixels in the region.
Minimum Intensity	Minimum intensity value among all pixels.
Maximum Intensity	Maximum intensity value among all pixels.
Calibrated Value	If an Amount Calibration curve exists, this displays the calibrated value based off that curve.

Area Density Results Statistics (Tab)



Min	Minimum value of attribute listed in corresponding columns, among all regions. **Region** row below it tells the region for which that value was recorded.
Max	Maximum value of the attribute listed in the corresponding columns, among all regions. **Region** row below it tells you the region for which that value was recorded.
Range	Difference between Maximum and Minimum values, among all regions
Mean	Average of the corresponding attribute, among all regions
Std. Dev.	Standard Deviation of corresponding attribute in columns, among all regions
Sum	Total of the particular attribute across all regions
Samples	Total number of regions

STEPS TO FOLLOW

Open the image of interest in the LS workspace.

Open **View > Plugins > Area Density Plugin**.

If desired, you can select the default colors for boundaries and labels of regions. To select, under **Area Density Tools plugin**, click on **Set Display Options**. You can also set the defaults for:

a. **Label color:** Select the region's label color from the drop down menu.

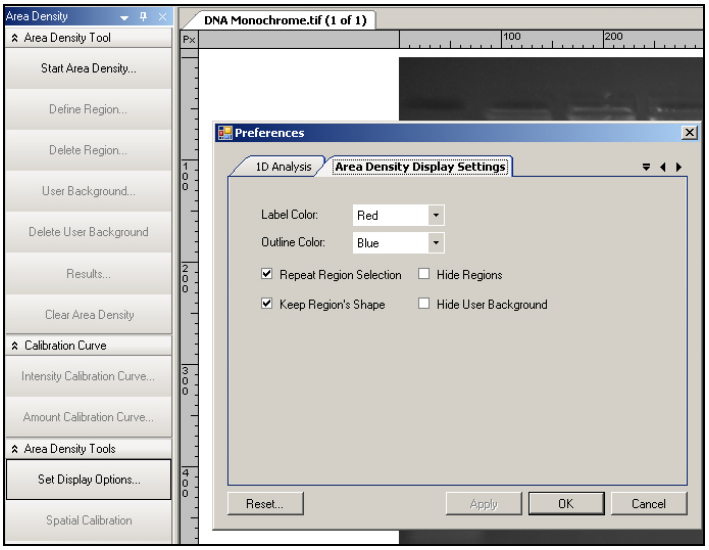
b. **Outline color:** Select the region's outline color from the drop down menu.

c. **Repeat region selection**: When checked, it lets you add multiple regions at a time. If most of you experiments only need one region to be analyzed, keep this unchecked

d. Hide regions

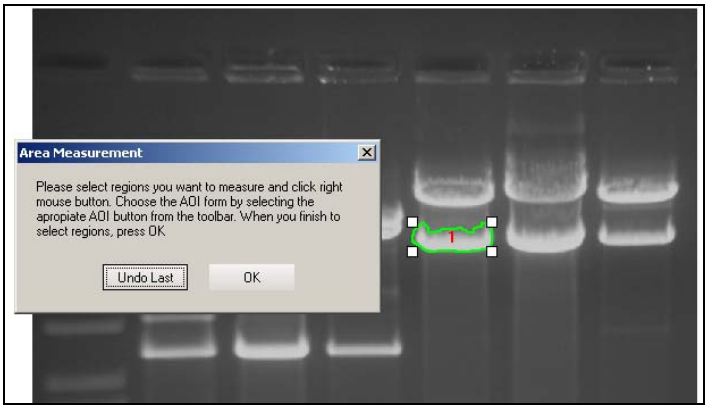
e. **Keep region's shape**: When checked, this saves time by preserving the shape (ROI type) of the region across new regions. So if most of your regions are of the same shape and size, it is beneficial to use this option

f. **Hide user background**



Start Area Density and Define the Region

1. From the **Area Density plugin**, click **Start Area Density** button.

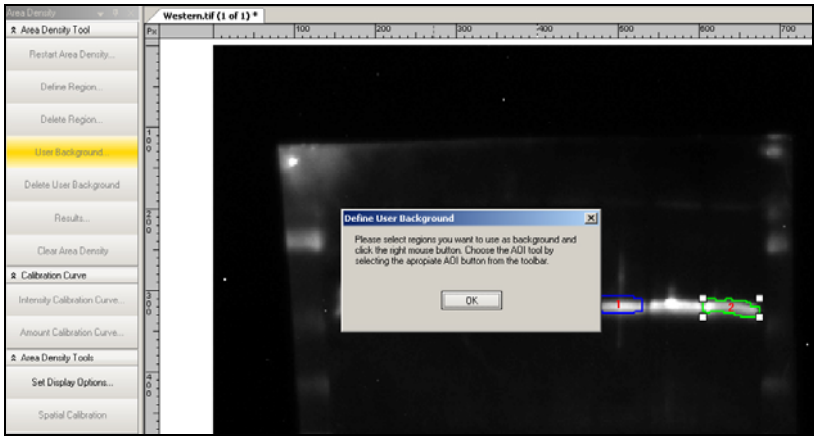


2. An **Area Measurement** window will display requesting you to **Define the Region** you want to measure. **Define Regions**: Double click on the ROI tool from the toolbar that helps to mark your spots or regions in the most inclusive way (i.e. covers all pixels). Adjust the size of the ROI with white bounding boxes. Right-click on each area to set. Once set, a number will display in the ROI and the bounding box will turn a different color.
3. Click **OK** when done. This will show all the regions/areas marked, in a list form in the **Area Density plugin > Results** window.

Use Magic wand from freeform ROI tool to mark the area in the best way using minimum number of clicks.

Define Background

By default, each area has a separate background. It is equal to the sum total of the perimeter around the region marked, three pixels wide.



If you want to mark a common background for entire image, from the **Area Density plugin**, click on **User Background** and mark an area just the same way you would mark a spot or region. If you want to move the background, follow the same process i.e. click on **User Background**.

Define Intensity Calibration Curve

Using the **Intensity Calibration Curve** button, two types of curves options can be defined:

g. Standard Optical Density

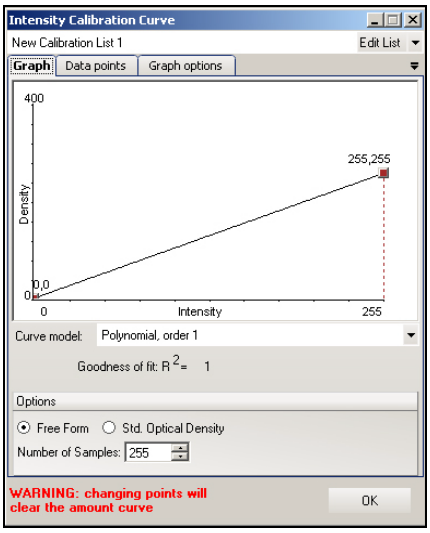
h. Freeform

Use Standard Optical Density curve if your sample is excited through transmitted light (such as FirstLight, world's first uniform UV Illuminator by UVP) and you need to understand the optical density of your sample.

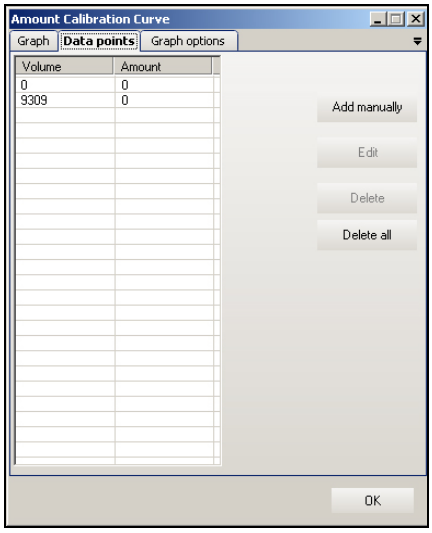
Use a Freeform calibration curve, if you need to fine tune input and output intensity values.

1. Click the **Intensity Calibration Curve** button from the **Area Density Plugin**.
2. To create a new calibration, click **Add**. Rename the calibration name if desired.
3. Click **OK**. You can now select the Standard Optical Density or Freeform options.

A detailed explanation on how to calibrate the curves is given in a later section of this chapter.



4. (Optional) Create an **Amount Calibration Curve** by clicking on the corresponding button on the **Area Density Plugin**. You can assign the amount of material loaded for known regions. Unknown regions get calibrated from the resultant curve. Click 'Update' after creating the curve, to update these unknown values.



Clicking Amount Calibration button brings up a window like the following. Click on **Data Points** tab to show all the areas defined in the main window. You can assign the concentration values to each one of them, inside the box **Amount**. You cannot change **Volume** since it is automatically calculated from the given area, and depends on the type of Intensity curve calibrated (Freeform or Standard Optical Density).

Switch to **Graph** tab, which lets view the points/regions with the curve model on the graph and set the curve model to be applied.

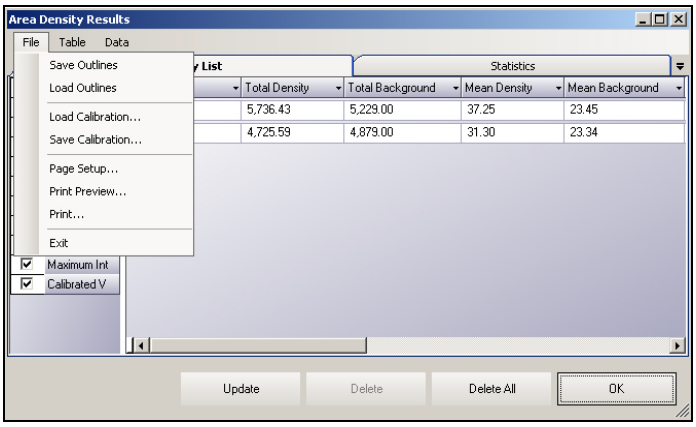
The **Graph Options** tab lets you specify units, results and graph label options.

Update the Results

Click the **Results** button from the **Area Density Plugin**. Click the **Update** button to calculate correct statistics.

You can also **Update** the results from the **Table** menu on the **Area Density Results** window.

SAVING AND PRINTING THE RESULTS AND DATA



Save the Results

From the **Area Density Results window > File** menu you can select from multiple options if you need to save the analysis data:

Save Outlines: Lets you save the file

Load Outlines

Print the Results

From the **Area Density Results window > File** menu you can select from multiple options if you need to save the analysis data:

- Page Setup
- Print Preview
- Print

Export the Results

From the **Area Density Results window > Data** menu you can select from multiple options if you need to save the analysis data:

- Copy to Clipboard
- Export to CVS
- Export to Excel

INTENSITY CALIBRATION CURVES

Intensity Calibration is the method of creating a mapping (and subsequently a curve) of input intensities to output intensities. Ideally, such a mapping would be linear. However, if you require finely tuned results, you may need to take into account, various errors introduced in the process

of lighting and imaging. These errors may change the mapping of original input intensity value to higher or lower than that. E.g. a pixel value of 100 might look slightly brighter, say 120. Or possibly the camera being used has a noise step of 40, which will make all values below 40 look as dark as 0.

One can use Area Density tool in LS Software to carry out analysis based on two different metrics of light intensity:

- Optical Density
- Grey Levels

LS software lets you create curves for both types of calibrations.

Optical Density

Standard Optical Density (OD) is used when the sample of interest is imaged with transmitted light. (i.e. light going thru the sample, into the camera for imaging.) OD value of an area gives an idea of how much light can pass through that area. If the area belongs to a sample in question, it simply how much of sample might be present in that area. Higher OD means less light can get through, suggesting presence of higher quantity of sample.

Following Beer's Law, the Optical Density of a given pixel P (say at position x,y) is calculated by LS software in the following way:

OD = - Log [(P(x,y) – Black) / (Incident – Black)], if P(x,y) < Black

= - Log [1/ (Incident - Black)], otherwise

Where

White = value of brightest white pixel in the imaging environment

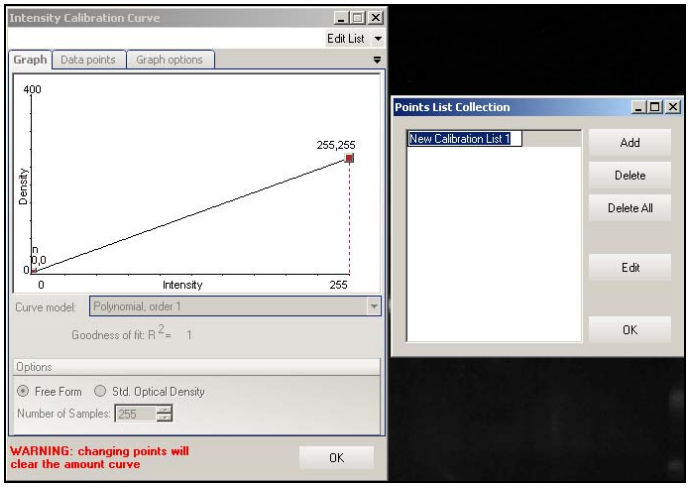
Black = value of darkest black pixel in the imaging environment

Total Optical Density of an area is simply the sum total of OD values of all pixels.

Grey Levels

Grey Level calculations are used when the sample is imaged using reflective light. Grey level of an area is simply the sum of grey levels of all pixels in the area.

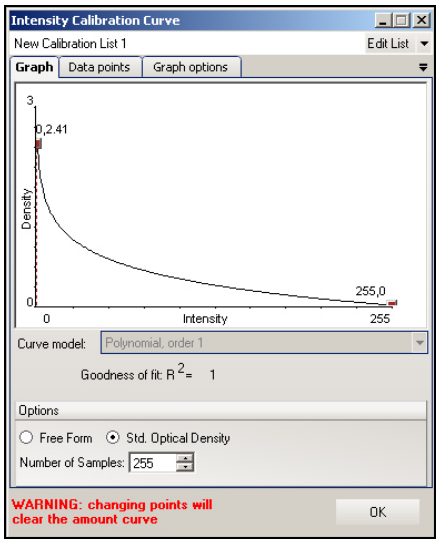
To Calibrate the Intensity



To add points on the **Intensity Calibration Curve**, click **Add** on the **Points List Collection** window. Click **OK**. You can also add points by clicking the Edit List button on **Points List Collection** window.

Select the **Free Form** curve for use on fluorescent/luminescent samples to produce results in Grey Levels.

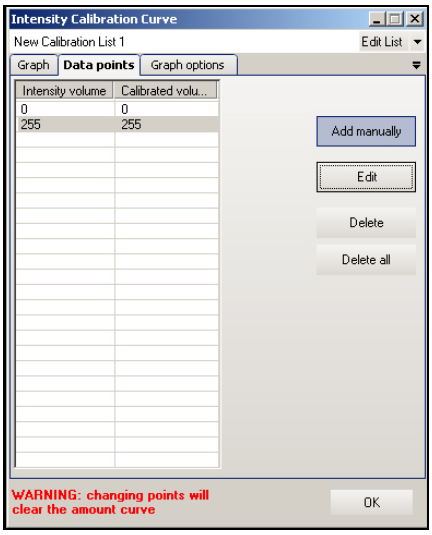
Select the **Std. Optical Density** for colorimetric samples imaged using transmitted white light to produce results in Optical Density units.



The scroll-box **Number of Samples** lets you select the total samples you want displayed on the X-axis of the curve. By default, it is set to the highest value of the dynamic range of the image (8 bit=>256, 16bit =>65536).

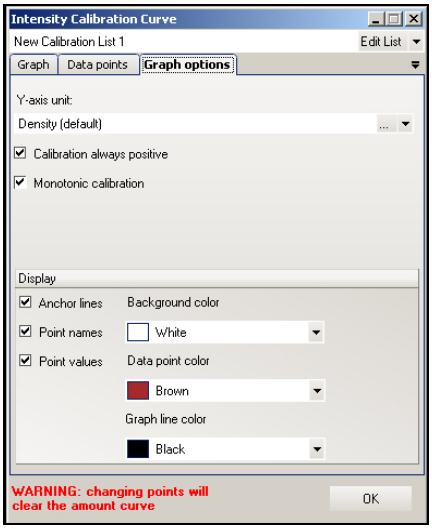
To Calibrate Free Form Intensity Curve

Click on the **Data Points** tab in the **Intensity Calibration Curve** window. This window lets you manually add or edit each point on the curve (hence the name Free Form). For each intensity volume there is a calibrated volume value that can be assigned. You can also fit a proper curve (in the **Graph** tab **> Curve Model**).



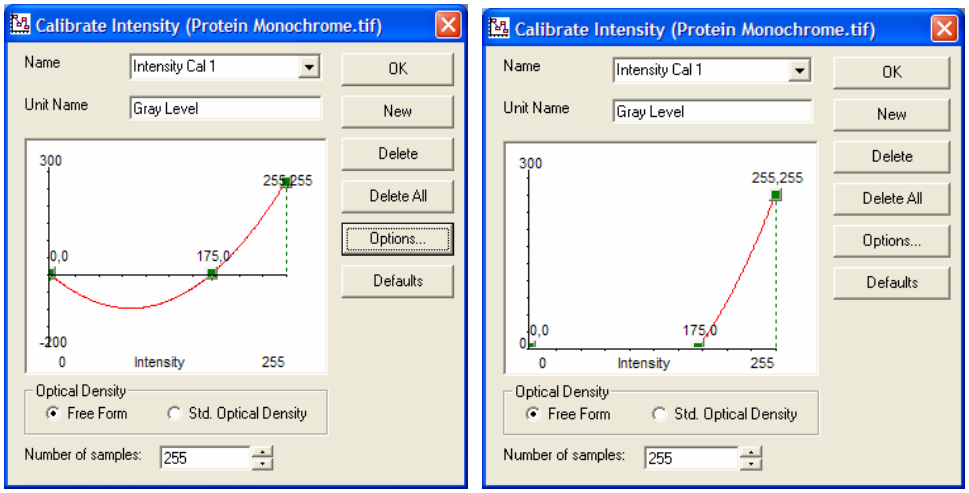
Changes are reflected on the curve after the window is closed.

Special Cases of Free Form Curves



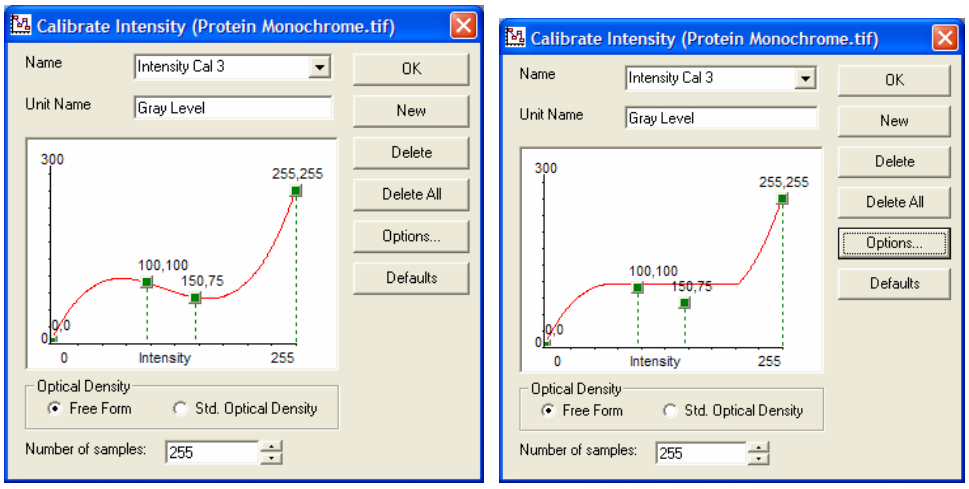
Calibration Always Positive

A curve of the following type (left) has a significant section in the negative 'Y'. If you may not want such a curve, click on **Calibration Always Positive** checkbox shown above in the **Intensity Calibration Curve > Graph Options** tab. Result is the curve on the right.



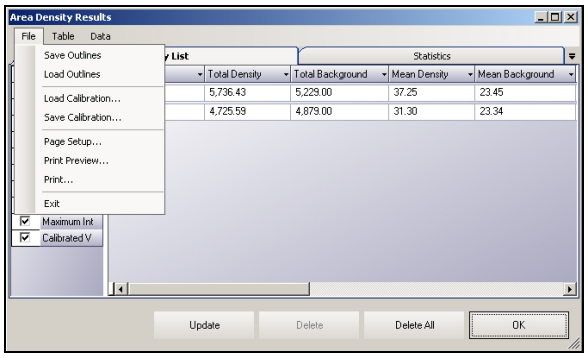
Monotonic Calibration

A curve of the following type (left) can have two values of input (X) for some values of output (Y). In order to avoid that, check the box **Monotonic Calibration** from the **Intensity Calibration Curve > Graph Options** tab to turn the slope of the curve all positive (or negative). Corrected curve looks like the one on the right below.



Saving and Loading calibration

In LS software, Intensity Calibration can be saved as well as loaded back when required. The calibration is stored in a .cal file. It stores information about all calibration curves created.



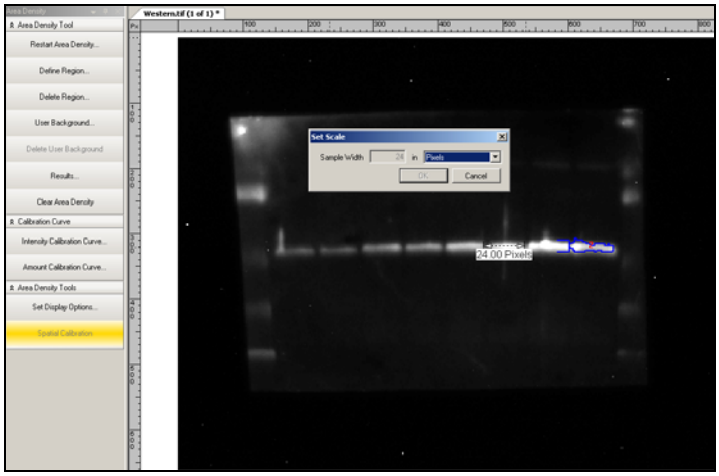
To Save Calibration: From the **Area Density Results** window, click on **File > Save Calibration** and save the calibration to disk as a .cal file.

To Load Calibration: From the **Area Density Results** window, Click on **File > Load Calibration** and find the .cal file.

SPATIAL CALIBRATION

This feature lets you create a mapping (or a scale) between pixels on the image and actual units of the area being imaged.

From the LS software main menus, click on **Tools > Spatial Calibration > Define Image Scale**. Click one point on the image and then second point at a distance. This brings up a window that lets you choose the units and corresponding distance.



Save and Load Outlines:

These options let you save and retrieve the regions marked on the image and can be accessed from File Menu on the main Area Density window.

Save Outlines...: Lets you save the region information (just the regions how they look on the image) to a file, with an extension .out. .OUT file is a custom file-format.

Load Outlines…: Lets you load the outlines' file .OUT. The outline is applied to the current image. This helps to transfer exact regions' data from one image to the second, without having to go through adding regions on the second image.

Chapter Nineteen: Towards 21 CFR Part-11 Compliance

- Purpose
- Features
- Usage

Purpose

US - Food and Drug Administration (US-FDA) created and released Part 11 of Title 21 of Code of Federal Regulations (CFR) in August 1997.

The rules delineate the conditions under which the US-FDA considers electronic records and electronic signatures equivalent to paper records and paper signatures. The instructions for compliance really span the entire organization and its practices. LS software by UVP is one piece that rightly fits into the bigger picture and *supports* compliance.

Note: *While LS software from UVP Inc. is an essential tool for assisting your organization to maintain CFR compliance, UVP cannot claim that this is the only tool you will need to achieve overall CFR compliance. Your organization must establish policies and procedures that work in conjunction with such efficient tools, to ensure total compliance with 21 CFR Part 11 regulations.*

Features

UVP provides software support for the following two sections of CFR regulations:

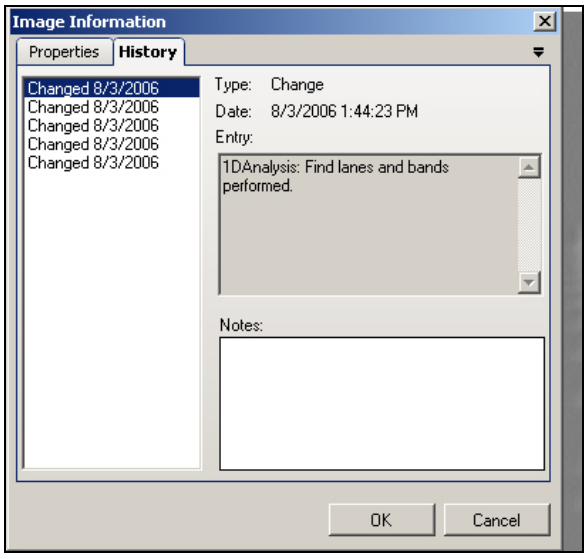
1. Section 11.10 (e) – For electronic records, this section requires the use of computer-generated, time-stamped audit-trails to track changes.
   LS software keeps track of all changes that affect image-data. Any action in the software that modifies the original data of an image open in the LS workspace, is logged. The log of such changes is individually maintained for each image and is referred to as 'History' in the software.
2. Section 11.3 (b) (4) – This section mandates that the system be controlled by users responsible overall for contents of electronic records required to track.

LS software provides an elaborate system of maintaining secure user accounts. Once can assign unique usernames and passwords to all the users who will be using the software. Each account can also be configured to provide read or modify access to other users' data. Events generated in the audit trail (above) are logged with the username.

Usage

To view an Audit Trail (History)

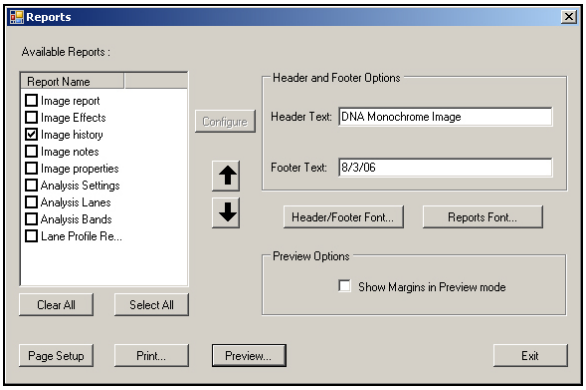
1. Open the image in question.
2. Right click on the image and select **Image Information**. Open the **History** tab.



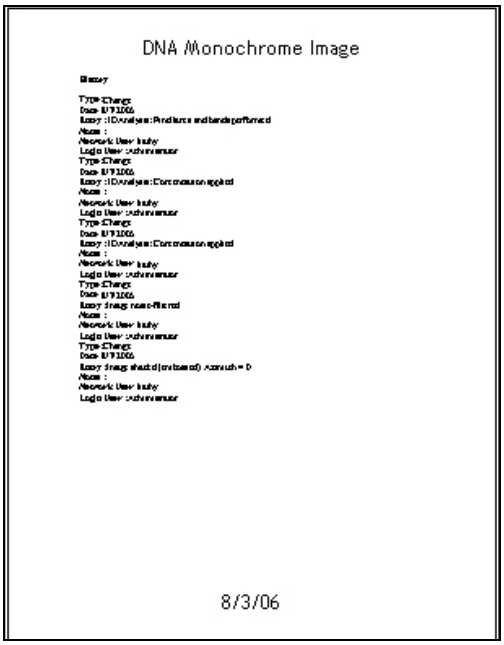
3. Events are listed in the left column. Click on each event to view the entry details on the right.
4. You can add notes to each event.

To print an Audit Trail (History)

1. Open the image for which you want to print the Audit Trail.
2. Click on **Tools > Reports**. (This option is disabled, if no printer is available). A window opens with various types of reports available.



3. If you want only the Audit Trail, click the **Image History** item. If you also want the image to be printed along with the trail, click on **Image Report and Image History**. Adjust the header and footer settings or printer settings if necessary, and print the trail.



SECURE USER ACCOUNTS

Refer to Chapter 1 discussing **Secure User Accounts** for detailed information on how to manage secure user accounts.

Appendix 1: 3rd Party Software Agreements

Copyright and License agreements of third-party components used in LS software

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```
printf("%s",png_get_copyright(NULL));
```

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September 1, 2001

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APPENDIX 2: GLOSSARY

Artifact: In imaging, a flaw caused either by the imaging process or by the hardware itself. For example, dust on the camera lens could cause small bright or dark spots in an image.

Aspect Ratio: The ratio between an image's width and its height. If the aspect ratio is not preserved, the image will appear stretched or squashed.

Bits: The smallest units of computer measurement. A bit is a single binary value (i.e. it can be "on" or "off" only). Bits typically are combined into units of eight, called "bytes." Modern computer processors work with groups of 4 ("32-bit processor") or 8 ("64-bit processor") bytes at a time.

BMP: Microsoft Bitmap image file format. BMP is a lossless format which provides some compression to reduce file size. BMP files generally have a BMP extension.

Control Handle: A small square at the corner (or similar point) of a graphical object that marks its extent and indicates that the object is selected. Usually the object can be resized by dragging the control handle; in some cases, different behavior results.

Electrophoresis: The movement of suspended particles through a fluid or gel through the application of electrical current to the suspension medium.

Fidelity: The degree to which an image is true (i.e. accurate and uncorrupted) to the original scene it represents. Also used in audio technology with the same meaning.

GIF: Graphic Interchange Format, a proprietary Xerox image compression format. GIF is a lossy compression format that results in very small files. Files stored in GIF usually have a GIF extension.

Image Depth: The size (and thus range) of intensity numbers supported per pixel in an image. Doc-It supports two depths: 8-bit (in which intensity numbers range from 0 to 255) and 16-bit (in which intensity number range from 0 to 65535). For a more detailed explanation, see Inside a Pixel.

Intensity: The measure of brightness of a pixel. In a monochrome image, each pixel has a single intensity. In a colored image, each pixel has three intensities: one for red; one for green; and one for blue. The actual intensity values depend on an image's depth.

JPEG: A common lossy compression image format used to store images on disk. JPEG files generally have JPG or JPEG extensions.

Lossless Compression: Compression schemes that preserve the image's integrity in full. Generally, lossless compression results in much larger files than lossy compression on the same image.

Lossy Compression: Compression schemes that tolerate some pixel value changes to make the image compress to a smaller size. Because the changes are irreversible, the image has "lost" some of its original detail after such an operation.

Macro Mode: Close-up mode for a digital camera or web-camera. Macro mode is usually appropriate for imaging microbiology slides.

Microbiology: The branch of biology dealing with microscopic forms of life.

Microscopy: The use of or investigation with a microscope.

Monochrome: Black-and-white, with shades of gray. Doc-It cameras capture 256 shades of gray in monochrome mode.

Pixel: Short for "picture element." A pixel is a single dot in a computer image. The dot has a certain color (for a color image) or an intensity (for a monochrome image). For a more detailed explanation, see Inside a Pixel.

PNG: Portable Network Graphics, a common image format. PNG is a lossy compression format that results in very small files. Files stored in PNG usually have a PNG extension.

Pseudocolor: Artificial application of color to a non-color (monochrome) image, or artificial re-tinting of a colored image. Doc-It provides several built-in pseudocolor sets that mimic certain lighting conditions and reveal specific information in the image.

Resolution: The number of total pixels (width of the image in pixels multiplied by height of the image in pixels). Higher resolution produces a smoother image (especially when zoomed in) but requires more RAM and disk space.

TGA: Truevision Targa image format. TGA is a lossless compression format that reduces file size somewhat. TGA files generally have a TGA extension.

Thumbnail: A reduced-size version of an image. From "thumbnail sketch."

TIFF: Tagged Image File Format, a common image format. Depending on settings, TIFF can be either a lossy or a lossless compression format. In Doc-It, it is used in the lossless mode to reduce image file size without losing integrity. TIFF files generally have TIF or TIFF extensions.

Zoom Factor: The percentage by which the image is scaled. A zoom factor of 100% (1.0) means that each pixel is not scaled; it is its original size. Zoom factors greater than 100% indicate that the image has been scaled up (meaning that several screen pixels are used to show one actual pixel). This generally makes detail easier to see. Zoom factors less than 100% mean that the image has been scaled down. This makes it possible to see more of the image in the Image window.

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