Zeiss AxioImager.Z2 and AxioVision Imaging Software

Microscope's User Manual

Managed by:

Shared Equipment Authority

For detail information about this instrument, please contact
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Zeiss AxioImager.Z2
Operations Guide

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Please note that this guide is meant to reinforce your training session, and only contains the minimum steps required for collecting basic images. Consult the instrument manuals for more information on the more advanced features of this unit.

All manuals must be left by the instrument after use.

Serial #:
Zeiss tech support:
Field Service Engineer: Percell Halley

Issues with the instrument: A. Budi Utama, 713-348-8232 (office); 713-576-9614 (cell) or Johnny Lam (Superuser) 408-655-6829 (cell) or Daniel Harrington, 713-348-8751 (office); 847-323-5093 (cell).

1. Instrument Specifications

Manufacturer/Model: Zeiss AxioImager.Z2
Software: AxioVision (free LE analysis version available from Zeiss)

Upright Zeiss AxioImager microscope, purchased in 2010.

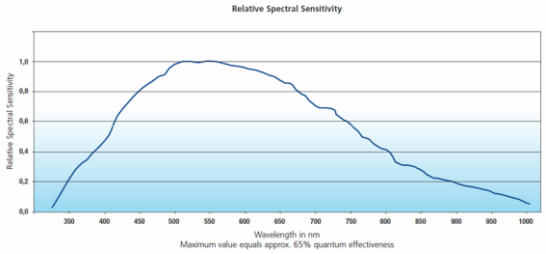
Cameras:

AxioCam MRc5 color camera

- 12-bit cooled CCD, 2/3" chip size, 6.45 µm x 6.45 µm pixel size
- 1388 x 1040 resolution
- 14 image/sec frame rate at highest resolution, up to 48 images/sec at lowest resolution

AxioCam MRm monochrome camera

- same specs as MRc5, but with no RGB filter mask
- camera wavelength sensitivity shown in figure at right



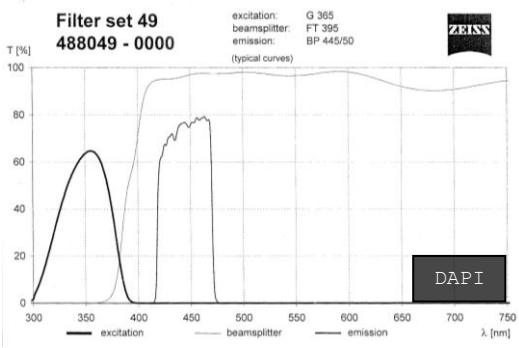
Illumination:

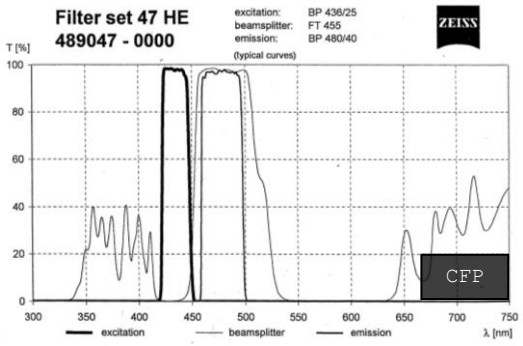
- XCite 120W self-centering HBO fluorescence source
- conventional halogen lamp

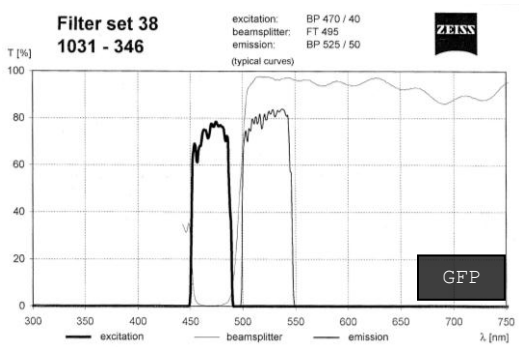
Microscope Hardware:

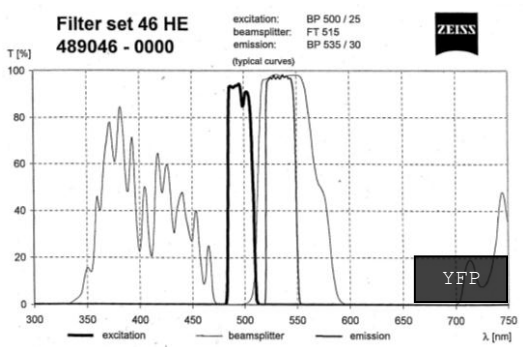
- automated filter cube turret
- DIC imaging

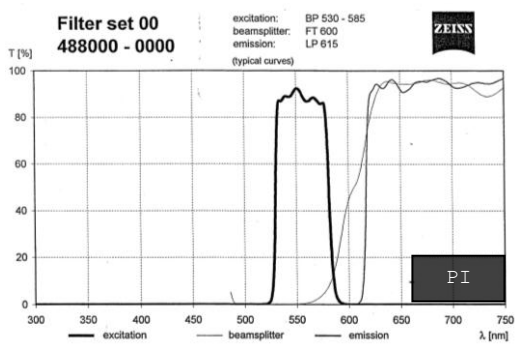
1.1 Fluorescence Filter Excitation/Emission Spectra

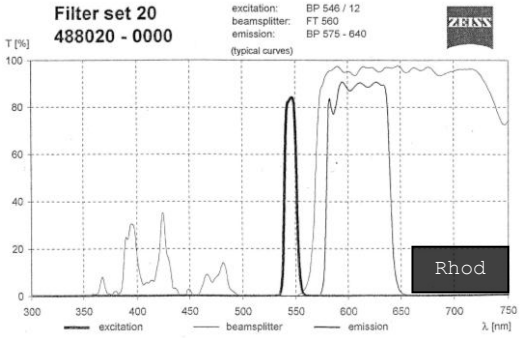


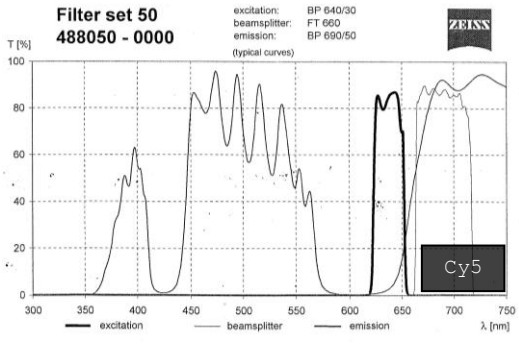












1.2 Microscope objective specifications

Objective Magnification	Imaging Medium	NA	Working Distance	Zeiss Catalog #:	Name
2.5x*	Air	0.06	15.1	422320996	EC Epiplan-Neofluar
5x	Air	0.16	13.6	4203309901	EC Plan-Neofluar
10x	Air	0.30	5.2	4203409901	EC Plan-Neofluar
20x	Air	0.80	0.55	4206509901	Plan-Apochromat
40x	Air	0.90	0.41	4203639901	EC Plan-Neofluar
63x	Oil	1.40		4207829900	Plan-Apochromat
100x	Oil	1.40	0.17	4207929900	Plan-Apochromat

* Note that the 2.5x objective is usually not installed. Interested users should contact the instrument administrator for information on using this objective.

2. Instrument Access

This instrument is available as shared equipment through the Superuser model. Researchers can become users of the instrument by following these guidelines:

- All users must complete the Shared Equipment Authority (SEA) paperwork through SEA website (http://sea.rice.edu/) followed by a training session from their lab's assigned Superuser or the instrument manager. After this training, new users should send an email to the instrument manager (A. Budi Utama, budiutama@rice.edu, 713-348-8232) with their Rice ID# to request the following necessary permissions: (a) access to BRC 640B, (b) a user account on the microscope computer, (c) access to the microscope reservations calendar through SEA website.
- Users must sign in to the paper login sheets at the beginning and end of their session, and log in to the microscope computer using the login and password provided to them during training. Anyone found using the instrument under another user's login will have their usage privileges revoked. Users must log off at the end of their session.
- Instrument time must be reserved using the Shared Equipment Authority (SEA). Any user reserving time on this calendar will take priority over a walk-on user.
- Safety guidelines must be followed at all times. Users must remove gloves before touching the keyboard, mouse, or computer.
- All login attempts and program usage will be logged. User privileges may be revoked for any unauthorized website access. Software may not be installed on the instrument computer without manager approval.

3. Supplies provided for the instrument

- Lens paper and lens cleaner for cleaning objectives
- Zeiss oil for objectives which use oil

4. Supplies to bring for your experiment

- your slides or any other object to be imaged
- pipettors, tips, or other disposables for adding liquid to samples if needed
- gloves if needed for handling your samples
- any additional Kimwipes if needed for cleaning oil off of your slides
- your USB drive for data retrieval

**Please do not rely on the host lab to provide gloves, tips, reagents, or other consumables for your experiment!

5. Things to consider in advance

5.1 General

- The brightfield condenser (small lever on the right hand side, under the stage) *needs to be pushed back for 2.5x and 5x objectives, and pulled forward for 10x and higher objectives.*
- The front condenser slider will usually yield optimal results when set between 0.3 – 0.5. Other settings will trade off between higher resolution or higher contrast. e.g. opening the condenser wider will improve resolution but decrease contrast; closing the condenser will improve contrast but decrease resolution.

5.2 Fluorescence Usage

The X-Cite 120Q light source functions similarly to a traditional mercury arc lamp, but uses a liquid-filled cable to deliver a pre-aligned source to the microscope with more uniform illumination across the field of view. Please follow these guidelines when imaging fluorescence on this microscope.

Using the X-Cite 120Q Light Source

1. Always turn the X-Cite source on before any other components in the system, due to the potential for an electronic pulse from the lamp startup.

2. For the most consistent lamp intensity, allow **5 minutes** for the lamp to warm up fully before using. The lamp icon on the X-Cite unit blinks until it is ready for use.

3. The liquid-filled light guide cable should always remain loose at the back of the X-Cite source and at its entry into the microscope. **Do not bend or crimp this cable, as this may result in severe damage.**

4. Use the intensity wheel on the front of the lamp housing to adjust light intensity. **Keep this dial at its lowest setting when not in active use, to prevent excess UV damage to the liquid in the light cable.**

5. Although the lamp has 10x the lifetime of a traditional mercury bulb, it still has a finite number of hours. Always remember to turn off the X-Cite source at the end of your session, according to the shutdown instructions.

6. There is no minimum duration for use on the X-Cite source, but **it must be completely cooled (20 minutes) after shutdown before another user restarts the lamp.** If another user is following after you, call that user before powering down the lamp.

5.3 Saving Files

Files will be saved by default in ZVI format. As well as the optimized ZVI format, AxioVision allows the image to be saved as a Tagged Image File (*.tif), a Microsoft Windows Bitmap (*.bmp) or as a JPEG compressed file (*.jpg) – you can choose the level of compression required in the JPEG here. You can also choose to compress the ZVI and TIFF image formats using the compression factor setting in the Save As window. Very high values generate very small files. However, in this case image quality may be significantly reduced. There is no compression available for the BMP file format.

If you annotate your image with a scale bar and text you need to burn this information into the image when it is stored as a TIFF file, thus the annotations can't be removed or changed at a later date. The new ZVI format keeps all graphic information separately so that it can always be changed and the underlying image is not destroyed. The ZVI format can also handle the full range of bit depths, multichannel images and image sequences.

6. How to operate the Zeiss AxioImager.Z2

IMPORTANT: ALWAYS REMOVE GLOVES BEFORE TOUCHING THE KEYBOARD, MOUSE, OR COMPUTER!

6.1 Startup procedures

1. Reserve time for a maximum 3 hour block (9-5, weekdays) on the online SEA instrument's Calendar. Nights and weekends are available for unlimited reservation.

2. Enter your name and user information in the log book by the microscope.

3. **If you will be using fluorescence imaging**, **turn on the X-Cite source before turning on any other components.**

4. Turn on the following remaining components:

    a. Turn on the Power Supply 232 (above microscope).

    b. Press the on/off switch on the rear left side of the microscope to turn it on.

    c. Turn on the computer. The LCD monitor should have a glowing light in its lower right corner (amber or green) if it is powered on. The monitor may take several minutes to sync with the PC during startup, so please be patient. If no light is visible in the bottom right corner, then press the monitor power button to turn it on.

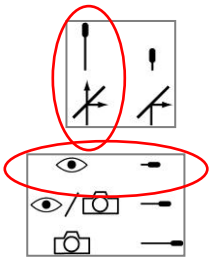
5. Log in to Windows XP using your assigned login and password. ***Users may only log in with their individually assigned login and password. Using another user's credentials for the microscope will result in an immediate withdrawal of usage privileges.***

6. Double-click the AxioVision icon on the computer to start the software.

6.2 Default Startup Positions

Users are recommended to reset the microscope to its default positions at the start of their sessions, to make image acquisition easier. Press the “Default” button in the upper right hand side of the AxioVision software to return the motorized portions of the microscope to their default positions (10x, brightfield transmitted illumination, color camera).

From top to bottom, manually return the microscope to these Default positions:

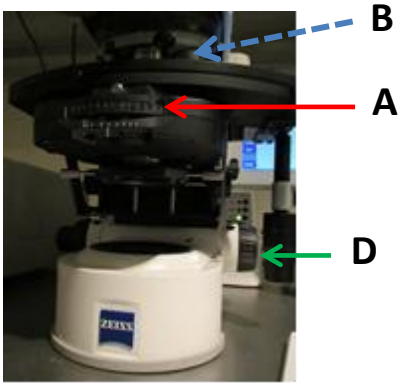


- Pull out the camera selection rod, sending light to both cameras.
- Push in the binocular selection rod (from the right hand side) to send all light to the eyepieces.
- Pull the condenser lens lever forward (default setting for 10x and higher objectives).
- Set the condenser turret to “H” for brightfield.

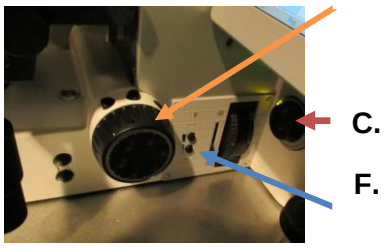
6.3 Kohler Alignment

This step is necessary to fully exploit the optical performance of brightfield, phase contrast and DIC imaging by setting objective, condenser, luminous-field diaphragm and aperture diaphragm according to the rules of the KÖHLER illumination principle.

1. Follow Startup Instructions to initialize the microscope, computer, and initial settings.

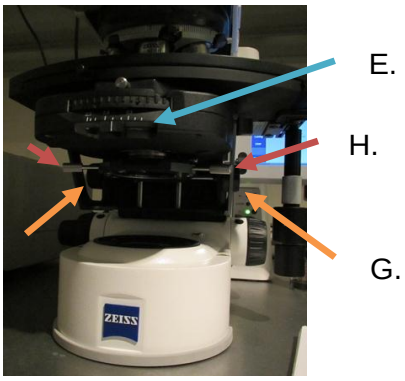


2. Start at 10X, Set the front condenser turret to the “H” position **(A)** with side brightfield condenser lens **(B)** pulled forward.
3. Install a sample on the microscope, adjust the transmitted-light brightness of light **(C)** and bring it into focus using the focusing drive **(D).**

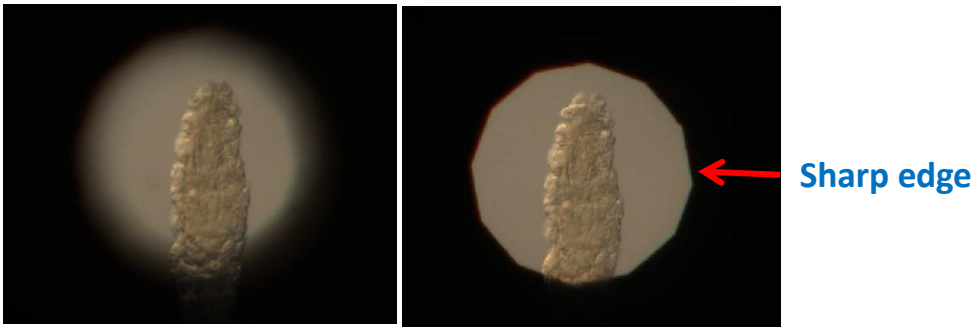


4. Close the field aperture **(F)** using the lower button on the back right hand side. You should see a hexagon somewhere in the field, and it may be out of focus (Image below - Before). You may also consider closing the front slider condenser **(E)** to the right to allow a better observation.

5. Loosen the screw on right side under the stage. Use the condenser height adjustment knob (**G**- black mid-size knob under the slide stage on the left and right hand sides) to get a crisp edge on the hexagon.



6. Use the two condenser adjustment knobs (**H** - small silver knobs on the front of the unit) to bring the hexagon to the precise center of the field. Repeat these two steps until ***the hexagon edge is focused and perfectly centered*** (NOT the sample focus!)



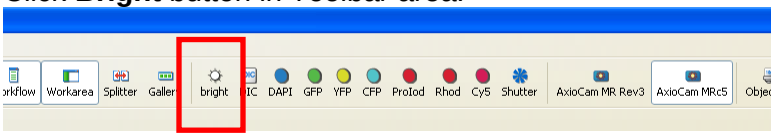
7. Re-open the field aperture fully (by pressing upper button of **F**) so that the hexagon is larger than the field (i.e. the hexagon is no longer visible).

8. Remove one eye-piece (ocular lens) and open/close the Condenser Aperture (**E**) until it just disappears from the ocular (or you can adjust this while looking at your sample until the image has the best contrast). Put the eyepiece back.

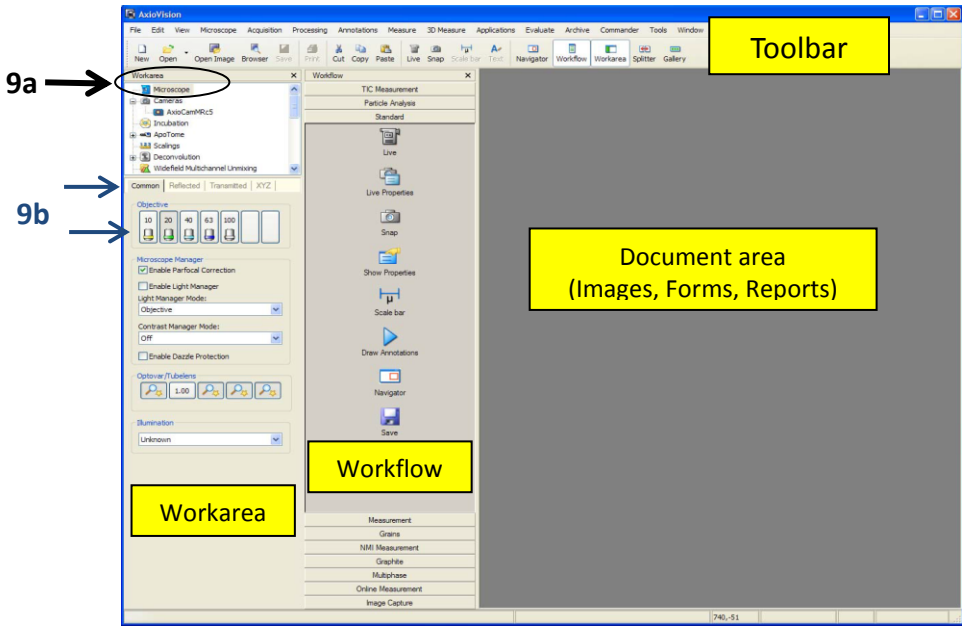
6.4 Taking a Basic Brightfield Image (e.g. H&E stained histology slides)

1. Follow Startup Instructions to initialize the microscope, computer, and initial settings. If necessary, conduct Kohler alignment. Install your slide with its coverslip facing up toward the objectives.

2. Click **Bright** button in Toolbar area.

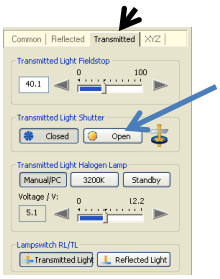


3. In the Axiovision software, select **Microscope** (9a) in the Workarea.



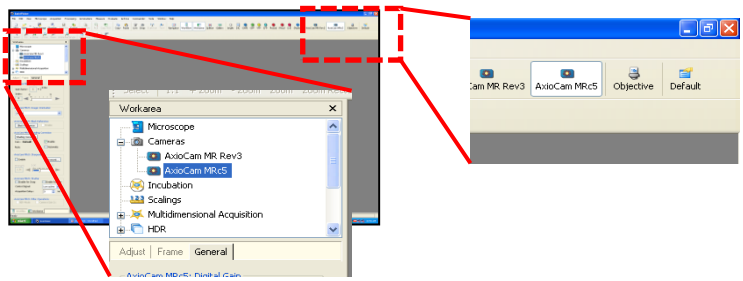
Select the **Common** tab and click on a low-power objective (e.g. 10x- 9b). Set the front condenser to the "**H**" position (see 6.3.1).

4. Click the **Transmitted** tab in Workarea and **Open** the transmitted light shutter. View the slide in the binoculars and adjust the on-screen Voltage slider to a suitable level for visualizing.

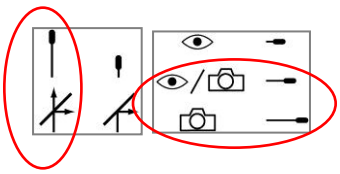


5. Focus on the sample as needed, and move to the area of interest using the xy adjustment knob. If a higher-power objective is needed, return to the Common tab, select the objective, and refocus as needed.

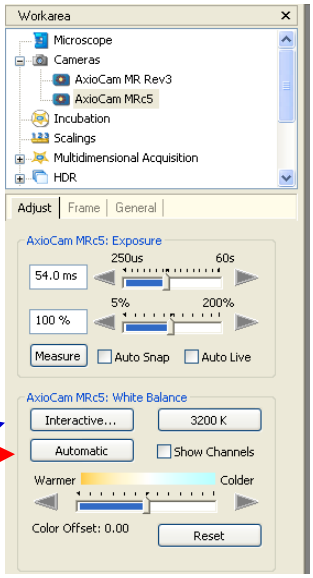
6. Select the color AxioCam MRc5 camera by clicking the on-screen "**AxioCam**



Mrc5" icon either in Toolbar area or by selecting it from Workarea. Adjust the push/pull rod on the camera selection box to be sure that the light is being sent to the appropriate camera. Adjust the push/pull rod on the binocular phototube to either 50/50 eyepiece/camera or 100% camera.



7. After selecting "AxioCam MRc5" from the Workarea, click **Adjust** tab. Ensure that the **Auto Snap** box is NOT checked and then click on the **Measure** button to let the camera find the optimum exposure level. Adjust the exposure manually using the top slider control. If you find that image brightness after using Measure is always too dark or too bright for your purposes you can adjust the level e.g. set to 90% for a darker image (underexposed) or 110% to overexpose using the lower slider control. ***If you have Auto Snap box checked*** the exposure will be adjusted automatically before each single snap. Checking the Auto Live box enables continuous exposure time adjustment in the live image.



8. **To set the white balance**, move to an open area of your slide and click the ***Interactive***... button. Button changes to read ***"...picking."*** Click on an open area of the slide in the Live box to identify what true white should look like.

9. Click the on-screen Live icon from Toolbar to begin live imaging. Click the ***Exposure*** button in the Live window to reach the appropriate exposure level. Many routine controls are accessible from these buttons on the toolbar in the live window or click ***Properties*** icon to do camera adjustment





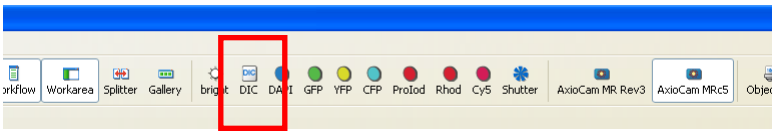
10. Click the on-screen ***Snap*** icon to capture the image.

11. Save your images in your folder on the E: drive (E:/images/yourfolder). ZVI is the recommended native format for saving all of the instrument's associated settings with your image.

6.5 Using Differential Interference Contrast (DIC)

The transmitted-light DIC method is a contrast method alternative to polarization allowing a contrast 3D presentation of transparent specimen details ***(pseudo-3D).***
The light linearly polarized by a polarizer is split into two partial beams by a birefringent prism. The beams pass two closely adjacent places of the specimen and thus are subject to different path differences caused by differences in refractive index and thickness of the specimen. Afterwards, both partial beams are recombined in a second birefringent prism and, after having passed the analyzer, they have the same vibration direction. Thus, both partial beams can interfere with each other in the intermediate image with the different path differences being converted to different gray values (intensities). A compensator λ (fullwave plate) then converts the gray values to colors.

1. Follow Startup Instructions to initialize the microscope, computer, and initial settings. Install your slide with its coverslip facing up toward the objectives. If necessary, conduct Kohler alignment.
2. Click ***"DIC"*** on the toolbar.



3. Set the front condenser wheel to match the objective:
    a. "I" for 10x
    b. "II" for 20x
    c. "III" for 40x or higher

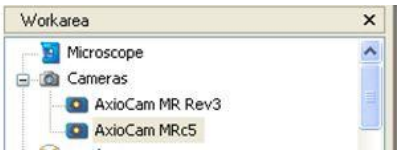
6.6 Darkfield

The incident light darkfield technique is used to examine specimens that do not only have reflective surfaces of different reflectivity (ideal brightfield objects), but feature scratches, cracks, pores, or in a nutshell, deviations in plane surfaces. All these light-scattering details shine brightly in the darkfield whilst the reflective plane surfaces stay dark.

6.7 Taking a Basic Fluorescence Image

1. Follow Startup Instructions to initialize the microscope, computer, and initial settings. Install your slide with its coverslip facing up toward the objectives. If necessary, conduct Kohler alignment.

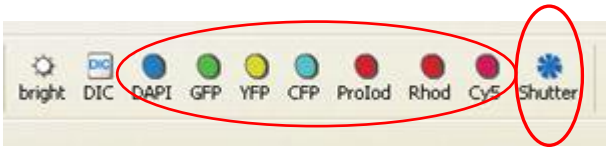
2. In the Axiovision software, select ***Microscope*** in the Workarea. Select the ***Common*** tab and click on a low-power objective (e.g. 10x).



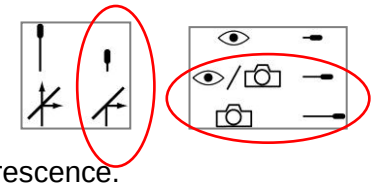
3. Click the ***Transmitted*** tab and Open the transmitted light shutter. View the slide in the binoculars and adjust the on-screen Voltage slider to a suitable level for visualizing.

4. Focus on the sample as needed, and move to the area of interest using the xy adjustment knob. If a higher-power objective is needed, return to the Common tab, select the objective, and refocus as needed.

5. Click ***the appropriate filter set*** in the icon bar (this turns off the transmitted light, selects the appropriate fluorescence filter, and ***opens the Shutter*** to the fluorescence light source). If necessary, change the dial on the X-Cite source to allow more light onto the slide. Click the on-screen ***Shutter*** Closed button when the image looks appropriate, to avoid bleaching.



6. Select the monochrome AxioCam MR rev3 camera by clicking the on-screen ***"AxioCam MR rev3"*** icon. Adjust the push/pull rod on the camera selection box to be sure that the light is being sent to the appropriate camera. Adjust the push/pull rod on the binocular phototube to 100% camera to capture the maximum amount of fluorescence.



7. Click the on-screen filter icon again, and then the ***Live*** icon to begin live imaging. Click the ***Exposure*** button in the Live window to reach an appropriate exposure level. Click the ***Properties*** button and use the ***Adjust*** tab to make manual adjustments to the signal intensity (by adjusting exposure time). Click the ***OverExp*** button in the Live window to identify any areas that are over-exposed beyond the camera's threshold.

8. Select **"AxioCam MR rev3**" from the on-screen list of cameras on the LHS. Under the ***Adjust*** tab, adjust the exposure as necessary using the sliders.

9. Click the on-screen ***Snap*** icon to capture the image.

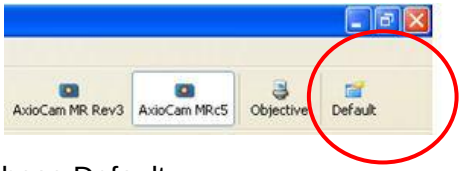
10. Save images in your folder on the E: drive (E:\images\yourfolder) **in ZVI format.**

6.8 Shutdown Procedures

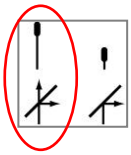
1. Clean off oil from any oil objectives (63x, 100x) using the cotton swabs and lens cleaner provided.

2. Save all of your files as needed into your directory on the E: drive, and transfer onto a USB drive.

3. Press the ***Default*** button on the upper right hand corner of AxioVision to return the automated portions of the microscope to their default states.

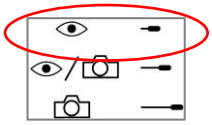


4. From top to bottom, manually return the microscope to these Default positions:



   a. Pull out the camera selection rod, sending light to both cameras.

   b. Push in the binocular selection rod to send all light to the eyepieces.



   c. Pull the condenser lens lever forward (default setting for 10x and higher objectives).

   d. Set the condenser turret to **"H"** for brightfield.

5. Quit the AxioVision software.

6. Check the login to see if anyone is following after you. Shutdown the PC.

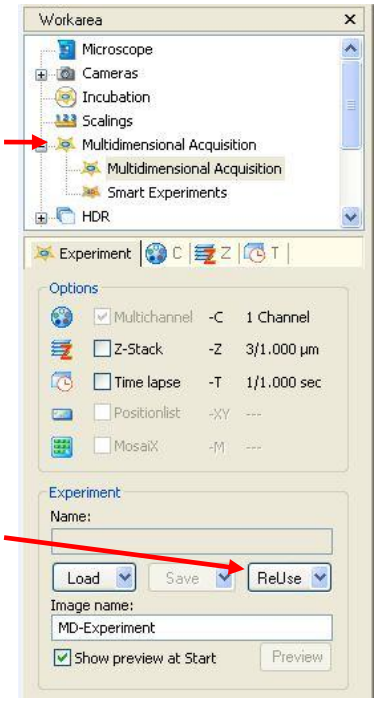
7. Turn off the microscope (power button is at the rear left side) and the Power Supply 232. If someone will be using fluorescence immediately after you, leave the X-Cite unit on. If not, close the X-Cite shutter and turn the unit off. Cover on the scope with blue dust cover.

8. Sign the log book with your finishing time.

7. Multidimensional Acquisition

7.1 Multi-Channel Imaging

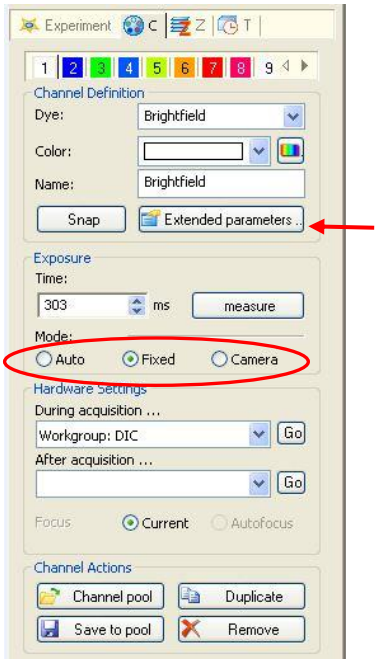
Up to 32 images can be captured and overlaid with a separate pseudo-color assigned to each channel.

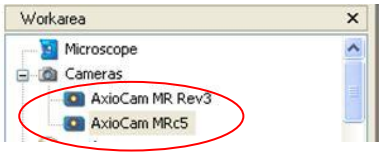


1. Go to Workarea window, click on **"+"** in the front of **Multidimensional Acquisition** and you will see another **Multidimensional Acquisition** option below. Click on it and you will have a new window underneath with options for Experiment, (Multi)Channel (C), Z-stack (Z) and Time Lapse (T). Make sure you have chosen the correct camera.

2. Enter an **Image name** in the box. Here image name is MD-Experiment and the first image will be called MD-Experiment_1 and so on. ***You do not need to load the Experiment File as it is already saved as a default*** unless you want to specifically use your own experiment setting file. You can use setting from a saved files or images as well by clicking **ReUse** pulldown menu.

3. Click on **"C"** (Color) tab to select the pre-set Channel controls. Select/unselect your channel(s) by right clicking on their tabs. You can use this option for both single and multi-channel or in combination of fluorescence and transmitted light (BF, Phase or DIC).

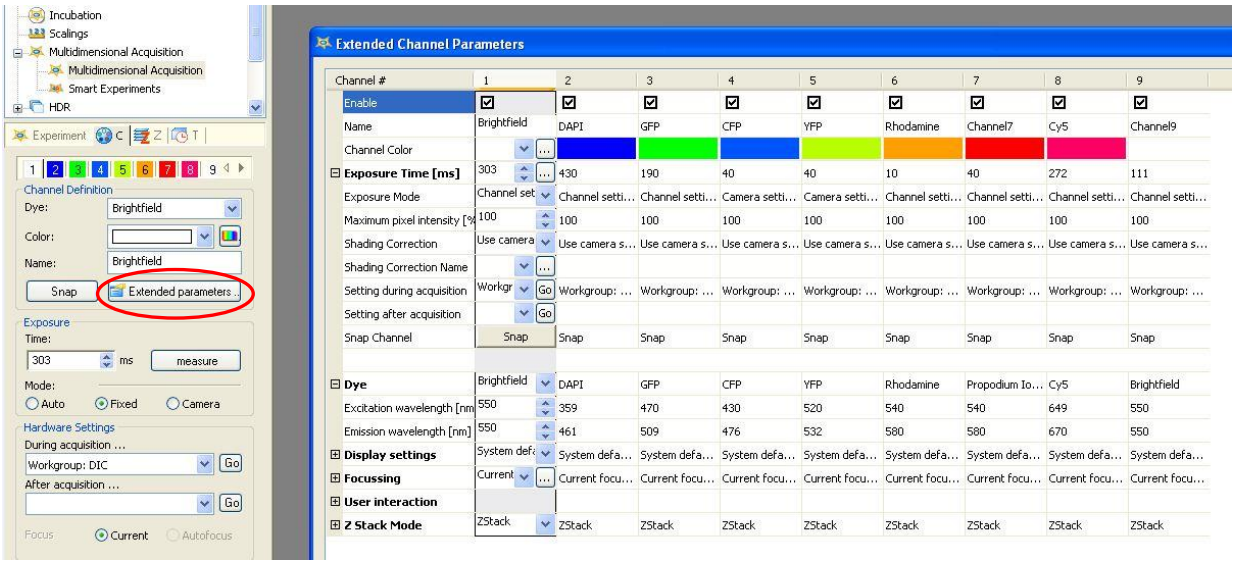




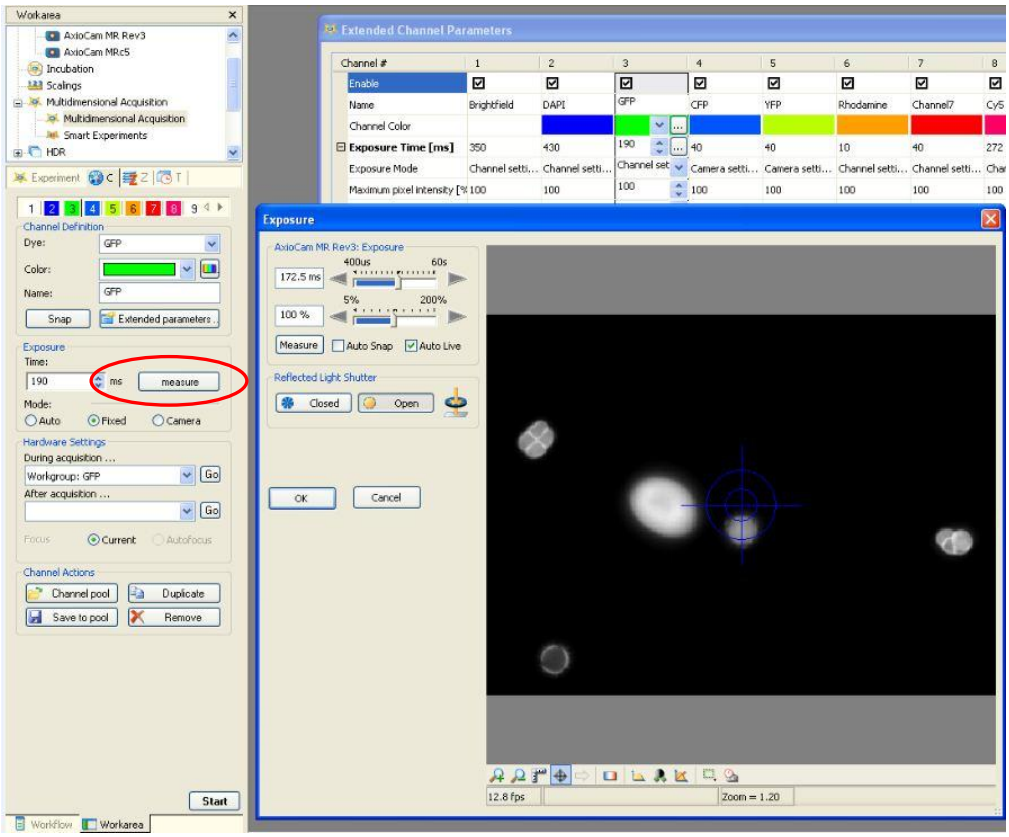
4. Choose the correct camera.

5. Three different set of camera exposure are available. **Auto-** using auto exposure automatically adjusted before the image is acquired for every channel (this may increase sample photo-bleaching). **Fixed-** the value in the Time: box is used. You should click on Measure button for every different sample. **Camera-** the current exposure value set in the camera control dialog.

6. Once you select the right dye(s), you can see the details of more parameter available by clicking on **Extended parameters**. Details for every single dye(s) are shown in the new window. You can further check/uncheck the dyes of interest from the window.

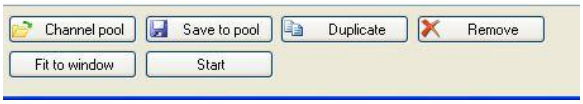


7. Once you choose the **Fixed** option of exposure, pick the right dye(s) and click on **Measure** button. A new Exposure window will open.



8. Readjust the focus if needed and adjust the exposure manually or using Auto (Snap/Live) option. Click on **OK** if done. The exposure parameter(s) would be saved and can be seen from Extended Channel Parameters window.

9. You can select more parameter setting for other dye(s). We might want to use these channels again in another experiment so before you close this window click on **Save to pool** to add each channel to the **Channel pool**. If you now click on Channel pool you will open the Channel pool and you can add any channel from the pool into your current experiment by clicking on **Add to Experiment.**

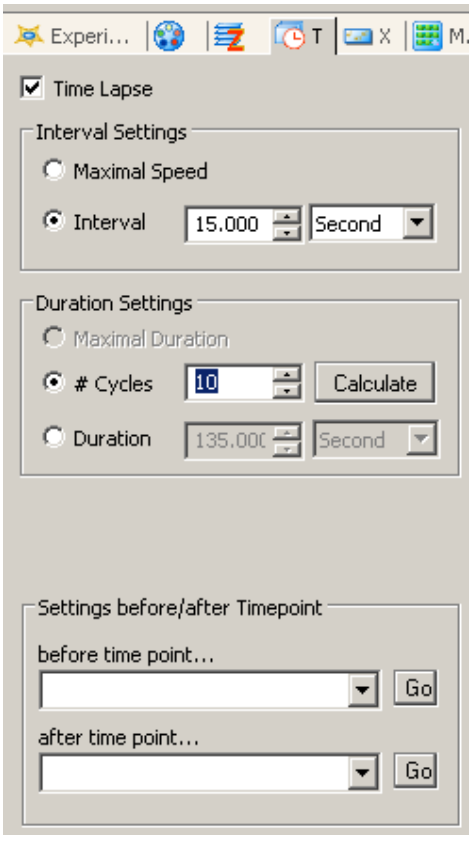


10. Once you have set up your channels you should save your experiment settings so that you can always repeat this type of image capture. Go back to the Experiment tab. Click on Save and enter a name for your experiment e.g. DAPI-FITC. Now you can click on the Load button to reload this image acquisition setup.

11. Taking image based on the parameter set up can be done automatically (using motorized channel) or manually. Simply click on **Start** button to do automatically. To do manually, set the first channel and click on **Snap** button. Repeat and overwrite channels if necessary. You can continue to acquire images and they will be automatically named for you.

7.2 Time Lapse

Click on the T tab to see the Time Lapse settings.



1. In our example shown here, we have chosen to acquire images from 10 time points, one every 15 seconds. Therefore, the total duration of the experiment will be 135 seconds (the first time point is at t=0).

2. Alternatively we could click on **Cycles** in which case we would enter the interval and total duration and then the number of cycles would be automatically calculated.

3. The interval is measured from start of image acquisition to the start of acquisition of the subsequent image. If you are acquiring multichannel, Z-Stack or long exposure images at each cycle you must ensure that the time required to acquire the images does not exceed the interval between acquisitions.

4. Click on **Start** button to initiate acquiring image.

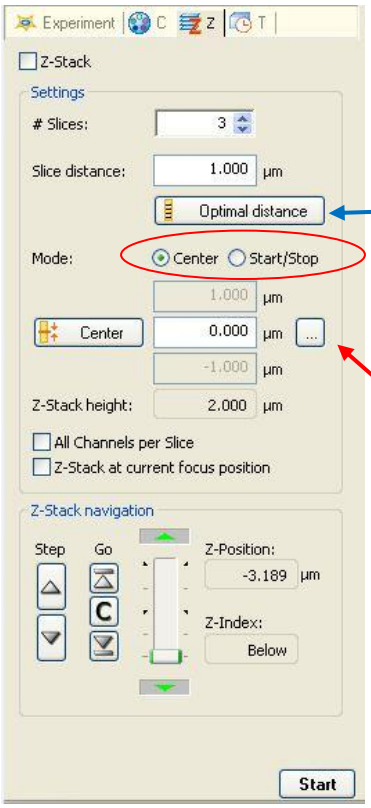
5. Additional display controls can be found at the bottom of the image window. To view a selection of the channels click on the corresponding colored button e.g. click on button 3 to switch it off. To



change the pseudo color for any channel, right click on the corresponding button and choose your color. If you click **"Circle Colored On"** button you can view each channel separately without pseudo-color. You must switch to this mode before you can adjust the display settings.

7.3 Z-stack

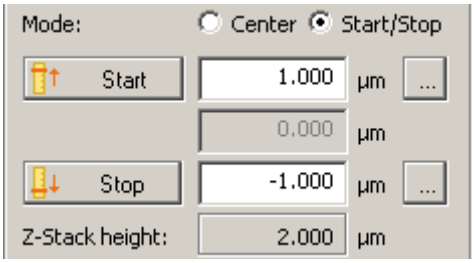
Click on the Z tab to see the Z-Stack settings. There are two ways to define the focus range:



1. Click on "**Center"** button to enter the current position.
2. Decide how many slices you want to acquire and the spacing between them and enter the values. For example if you will acquire 13 slices spaced at 0.5 um. The center slice will be number 7 and you will acquire 6 slices above and 6 slices below.
3. You can use the Z-stack navigation controls at the bottom of the control panel to step the microscope to each position.
4. Click "three dots" button next to "Center" to see a live image for focusing

Alternatively you can define the focus range using **Start/Stop** option.

1. Move the focus to the top of the range (see in live image) and click on **Start** button. Move focus to the bottom of the range and click **Stop** button.
2. Enter the number for the space between slices.



The **Optimal distance** button will set the ideal slice spacing (based on Nyquist criteria) to match the z resolution of the optics you are using. You may not need to use this unless if you subsequently want to use deconvolution module.