



Zeiss Axio Imager manual

Power-up protocol

1. Mercury lamp
2. Power strip on shelf
3. Computer

The Mercury lamp should always be first-on and last-off. This prevents any electrical surges caused by ignition damaging other equipment on the same circuit.

Then log-in with your "Homer" password, double-click the MetaMorph icon on the desktop and choose your name from the drop down menu.



Make sure these two lights are both on (press buttons if not)

Transmitted light brightness

Both the TL and RL shutters on the microscope should be open (LEDs on). These switches are on the rightside at the back of the scope

(The light will now come through when the computer controlled shutters are activated)

The Microscope

- Binocular eye pieces - adjust the inter-ocular distance
- Ergotube height adjustment knob - the "periscope"
- Plunger controlling the light path - camera (out) or eyepieces (in)
- Stage - control xy position manually using hanging drive; stage clips for holding slide; stage can rotate by loosening thumbscrew on stage front
- Focus knobs - outer part is coarse; inner is fine focus.
- Z-stepper - this is attached to the left side focus knob. This controls the z-position allowing auto-focusing and 3D-imaging. The box to the left of the microscope controls the stepper motor, when it is disengaged manual focus is possible. When engaged, use ONLY the digipot controller knob on the box for focusing.

Objectives

Mag	NA	Oil?	DIC?
5x	0.15	NO	no
10x	0.30	NO	no
20x	0.50	NO	DIC
40x	0.75	NO	DIC
63x	1.4	Yes	DIC
100x	1.4	Yes	DIC

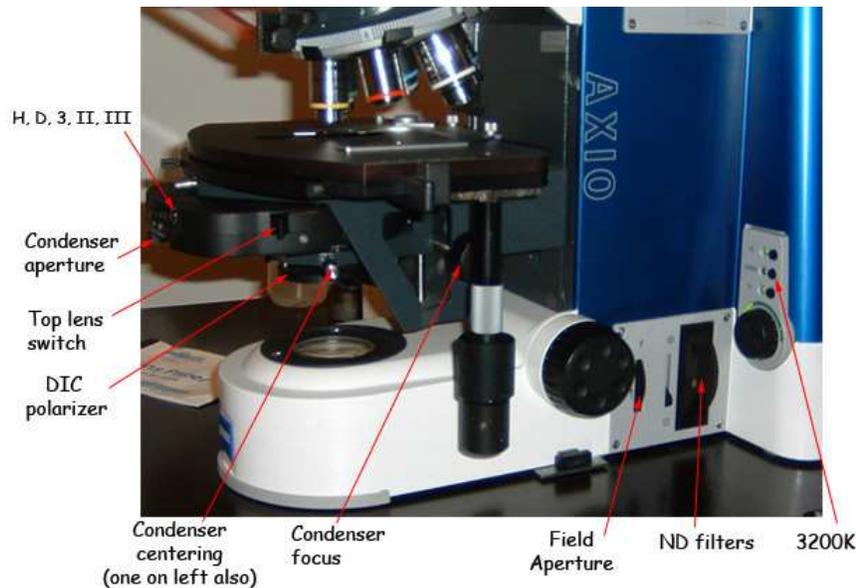
Please change the objectives when the stage is lowered by carefully turning the knurled ring (rather than pushing the actual objective).

Condenser

The condenser is beneath the stage and is only used for transmitted light imaging -

- H - Brightfield
- D - Dark field, works with low NA objectives (10x, 20x and 40x)
- 3 - Phase contrast
- II - DIC
- III - DIC

- The top lens is pushed in using a thumb switch
- The DIC polarizer swings in below the condenser
- The DIC analyzer is in the emission filterwheel or the manual slider



Transmitted light imaging

- Cube 1 (quad pass dichroic) is the best choice for transmitted images
- Choose "DIC" or "brightfield" from the drop-down illumination menu. Click the shutter icon to open/close the shutter.
- If you want to view your sample down the eyepieces as DIC you will need to have the condenser set appropriately and have the slider analyzer set to the left (move this back to the right afterwards or your fluorescence images will be poor).

Kohler illumination

If you want to accurately observe or capture transmitted light images, you will need to establish Kohler illumination *each time you change objective*. This ensures the illumination is even and is essential for optimal image quality.

- Focus on your specimen using transmitted light
- Close down the field aperture (dial labeled F on bottom right of stand) until it occupies about 2/3 of the field of view.
- Focus the condenser with the knobs underneath the stage that raise/lower entire condenser. Then center the field of view using the centering pins also located underneath the stage. Finally, open the field aperture until it is out of view.
- Remove eyepiece and close condenser aperture until 2/3 of field is illuminated (or you can adjust this while looking at your sample until the image has the best contrast)

Fluorescence imaging

The reflector turret contains filter cubes with six positions:

1. Quad pass dichroic
2. DAPI
3. CFP
4. GFP
5. YFP
6. Red

Cubes 2-6 are best for viewing your sample down the eyepieces. These are also the best choice for imaging most samples, especially if you have only one fluorophore.

- Choose "CubeXX" from the dropdown illumination menu in MetaMorph (where XX is the %power of the excitation)
- Click the toggle shutter icon to the right of the menu to open/close the shutter

Quad pass dichroic in position 1 is for taking multi-colour timelapse. It also has a far-red filter that is not present in any of the individual cubes. From the the dropdown illumination menu in MetaMorph:

- Choose "DapiXX" for DAPI (where XX is the %power of the excitation)
- Choose "FITCXX" for green fluorophores (eg GFP, FITC, Alexa488)
- Choose "TRITCXX" for red fluorophores (eg Alexa 568, Tritc)
- Choose "CY5XX" for far-red fluorophores (eg Alexa 647 and CY5)
- Click the toggle shutter icon to the right of the menu to open/close the shutter

Image acquisition using MetaMorph

When you have located and focused on your specimen manually, pull out the slider to allow the light to the cameras. Remember that the field of view of the camera is a smaller than the field of view down the eye pieces so make sure your sample is centered.

Fluorescence -

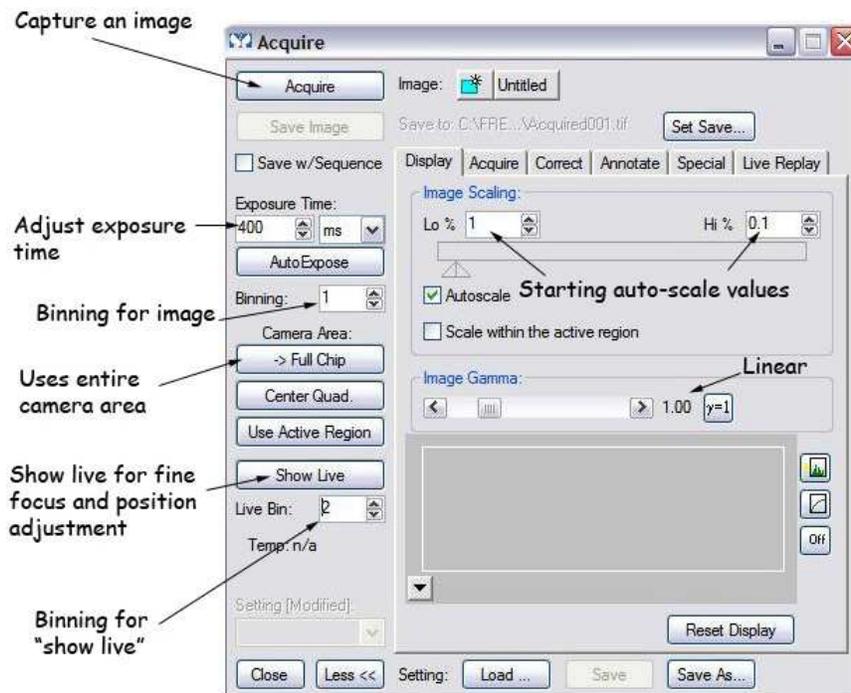
- Click "Fluo camera" on the task bar
- Make sure the little black dial underneath the camera = upwards arrow

Colour camera (eg histology) -

- Click "Colour camera" on the taskbar
- Make sure the little black dial underneath the camera = rightwards arrow

Open the acquire dialog box

This can be opened from the task bar Acquire or from the dropdown menu, **Acquire> Acquire**



Things to set in the Acquire window:

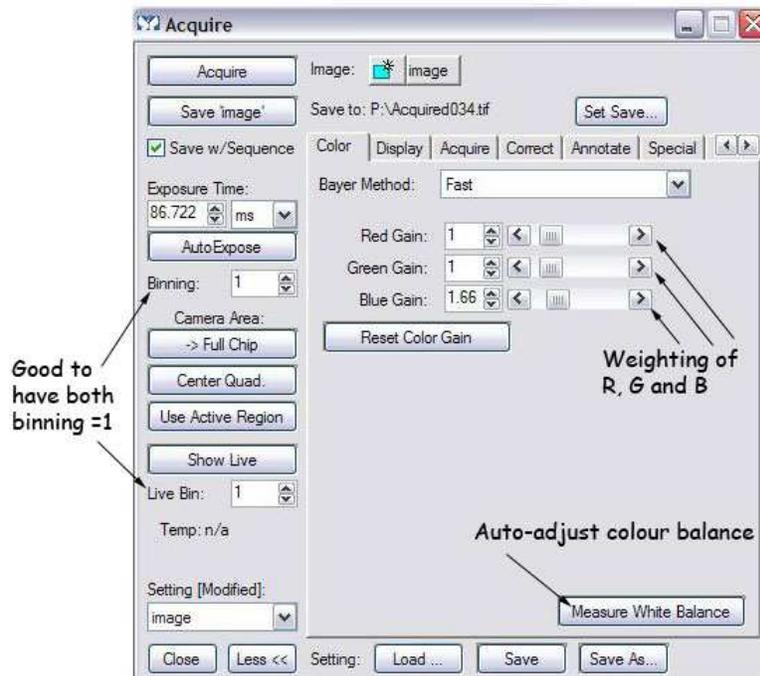
- Set the display to "Autoscale" - the triangles on the image histogram show the scale min and max.
- The image gamma - the relationship between measured and displayed brightness. 1=linear
- Exposure Time - Type in value (or choose AutoExpose) mouse-over image to give pixel intensity values (between 0 and 4095)
- Full chip uses entire CCD camera, sub-arrays produce smaller files and may be faster
- Binning - combines CCD pixels to form n x n super pixels - increases sensitivity but reduces resolution
- Press Show Live to see the specimen through the camera - it may be necessary to focus or move the stage slightly
- Clicking the folder icon allows you to set whether each image starts in a new window or overwrites the previous image (handy for optimizing your image, be careful not to lose your data though)
- External shutter linked to camera should be [Current Shutter]
- Gain and offset for the camera under the Special tab - Start as 0 and 255 respectively

Press the Acquire button to capture the image

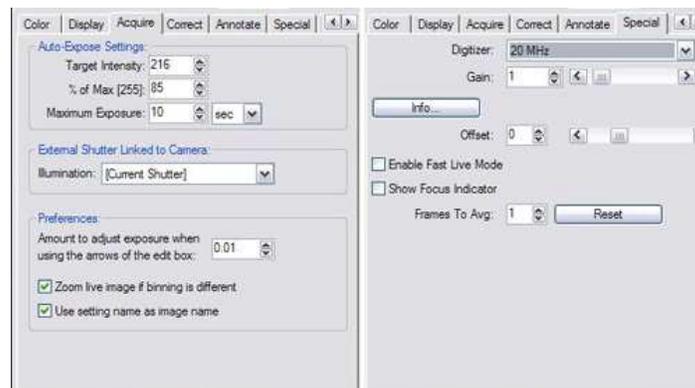
For colour images

Select "colour" from the illumination menu, this turns on the transmitted light (no need to toggle shutter). Make sure the microscope is in kohler illumination.

To take a colour image there is one extra tab that appears in the acquire dialog box when you click the colour camera button on the taskbar. . .



These are how the settings in the Acquire and Special tabs should look:



- Click Autoexpose to find a value that works (you might need to reduce the lamp brightness if it is too bright)
- Press show live, focus and adjust the xy fine position, then press measure white balance
- "Acquire" captures a 24bit RGB image. If that looks good you can save your image and move on to the next slide

If you are struggling with the colour balance:

- Press the 3200K button on the rightside of the scope (this makes the bulb very bright and white rather than orange)
- Adjust the ND (neutral density) filters so the intensity of light at the sample is reasonable
- Capture an image as above

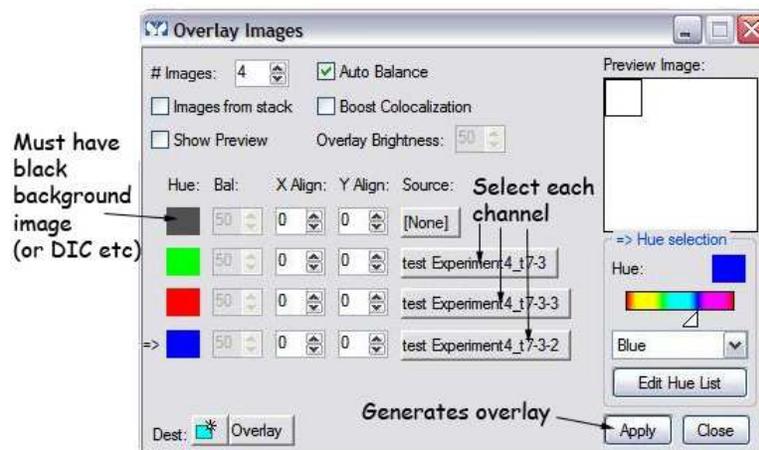
Saving images

Simply click save and name your file. Images will be saved as 12-bit TIFF images with all the original data. Not all programs will open these images but you are recommended to save the original images.

To save your individual images as 8-bit files (compatible with all programs but some information is lost)

- Press Copy to 8-bit button on the task bar
- Select the desired image from the "Image:" drop-down menu
- Press Copy and Save by going to **File > Save as** (make sure the file name is different otherwise you will overwrite the original)

If you have more than one colour, you can make an overlay of the images. Click "overlay images" on the task bar and follow the dialog box. The result is 24 bit RGB image compatible with all programs.



The colour camera produces 24 bit RGB images directly, which require no special treatment.

Multidimensional acquisition

Open this dialog box for timelapse and z-stacks for 3D acquisition.

Finishing and shutting down

- Clean any oil objective(s) using lens tissue and cotton tipped applicators
- Close MetaMorph
- If there is somebody booked after you, logoff the computer

If nobody is scheduled to use the instrument within 1 hour of finishing, please shut it down:

1. Computer
 2. Powerstrip
 3. Mercury lamp (Remember: First-on, last-off)
- Fill in log book, noting lamp hours
 - If you are the last user of the day, please cover the microscope making sure the cover doesn't touch the hot mercury lamp housing