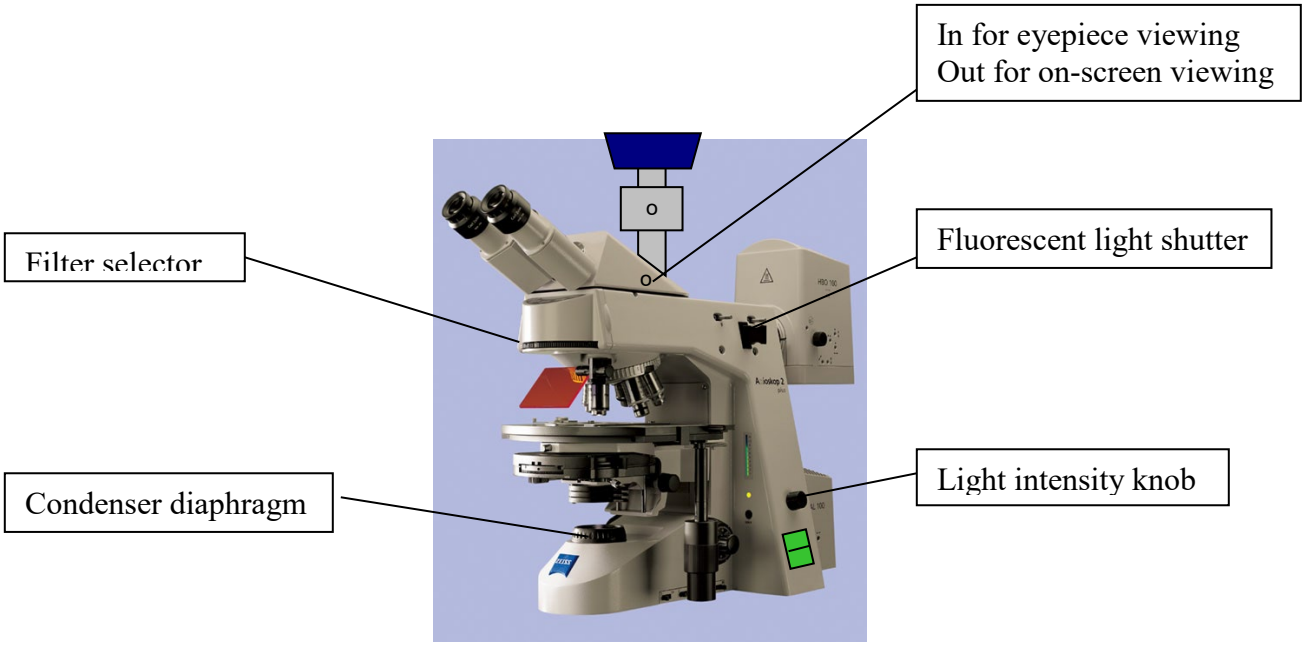
Zeiss Axioskop 2 Plus

Powering on:

1. Mercury lamp (if using fluorescence; remember to record lamp hours in the log book!)
2. Power strip
3. Microscope power switch
4. Stage controls
5. Computer

Open software: AxioVision Rel 4.7

Microscope controls:



Setting Up Köhler Illumination

1. Focus on the specimen.

2. Close the field diaphragm to its most closed state so that you can see the edges of the diaphragm (may be blurry) in the field of view.

3. Use the condenser focus knobs to bring the edges of the field diaphragm into the best focus possible.

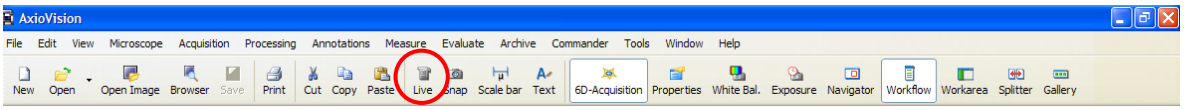
4. Use the condenser-centering screws to center the image of the closed field diaphragm in the field of view.

5. Open the field diaphragm just enough so that its edges are just beyond the field of view.

6. Adjust the condenser diaphragm to introduce the proper amount of contrast into your sample. The amount of contrast added will depend on the sample, however too much contrast can introduce artifacts into your images.

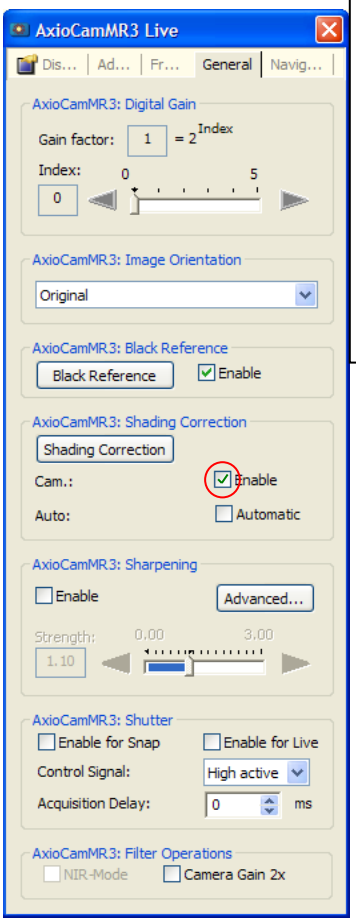
7. Adjust the light intensity as necessary. To adjust light intensity it is best to use a neutral density filter rather than increasing or reducing the supply of power to the light source. Neutral density filters block all wavelengths of light equally, while changing the power to the light source will alter the balance in the spectrum of incident light giving a yellow/brown appearance to the image.

Image capture – Transmitted Light:



If you are using transmitted light only, simply click on Live once you have found and focused your specimen under the scope. An AxioCamMR3 Live window will appear on screen. Make sure that the lower sliding bar on the microscope is in the OUT position to see image on screen (IN lets you see your specimen through the eyepieces).

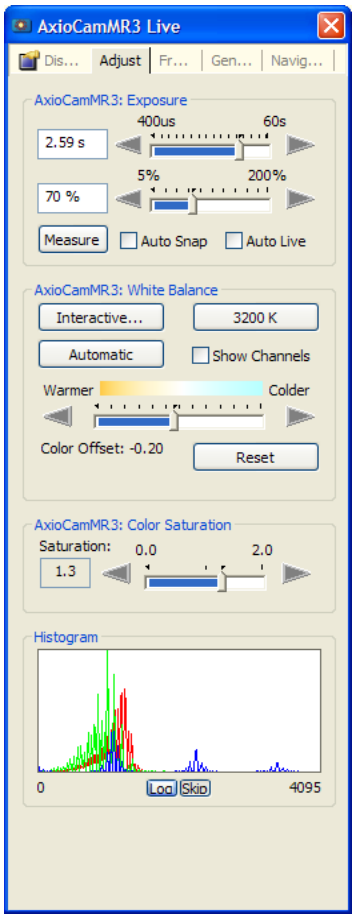
The live properties window has multiple tabs. The following settings are most important:



General tab:
Shading correction – click on this while achieving your desired white balance. This adjusts for any spots or inconsistent shading you may see on screen that are due to the objective and not to the specimen. Use prior to placing a slide in view, and use in conjunction with white balance. Make sure you check Enable under Shading Correction.

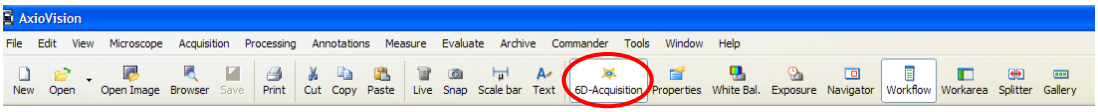
Adjust tab:
Exposure time – click Measure to automatically adjust, then manually adjust it as needed with the arrows/sliding bar.
Set exposure time weight to 100% for transmitted light.

White balance - Automatic usually does the trick. If colors are very screwy, hit 3200K first, then Automatic. You can also use Interactive… to manually click on white/light pixels within your image.

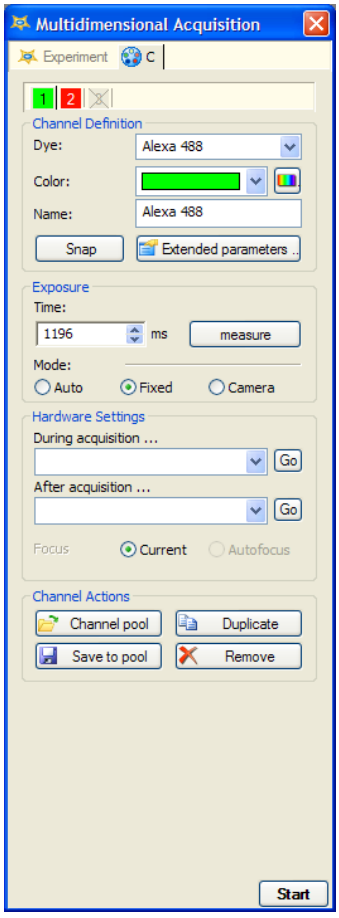


Remember to reset these three settings – exposure time, white balance, and shading correction – each time you change objectives if you plan to take a picture with that objective. Perform the latter two with no slide in place.

Image capture - Fluorescence:



If you are using fluorescence, open 6D(Multidimensional)-Acquisition.

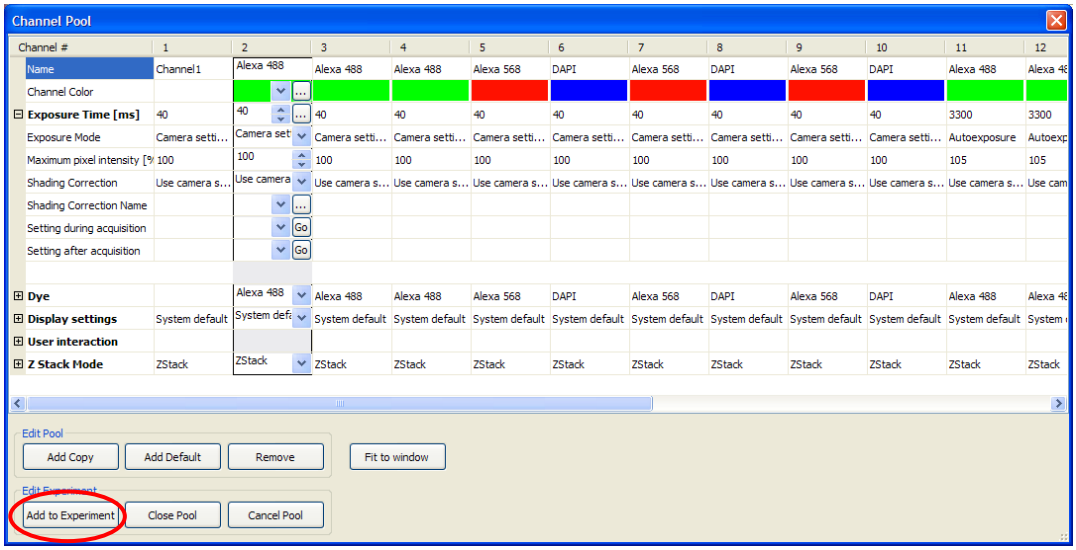


Under the C tab, you can adjust channel settings.

You will need to measure the exposure time for each channel by clicking on "measure." Be sure that you have selected the corresponding filter and are allowing excitation light to reach the specimen. A live window will open displaying the image with the currently selected exposure setting. Set the exposure time weight to 70% for fluorescence. Adjust exposure time manually as needed. Repeat with each channel.

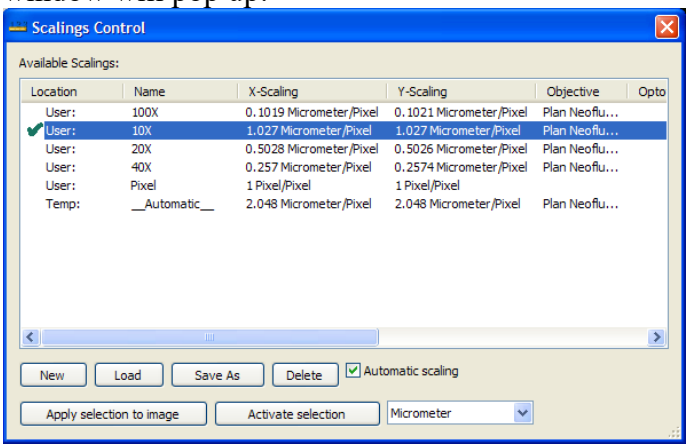
To take your image, manually set the proper filter settings for one channel, then hit Snap on that channel's tab under C. Adjust the filter setting for your next channel, open that channel's tab, and click Snap. A merged image will be created.

If you wish to add additional channels to your experiment, click on Channel pool under Channel Actions. The window below will appear. Select a channel you wan to add, then click Add to Experiment. A new tab for that channel will now be available.

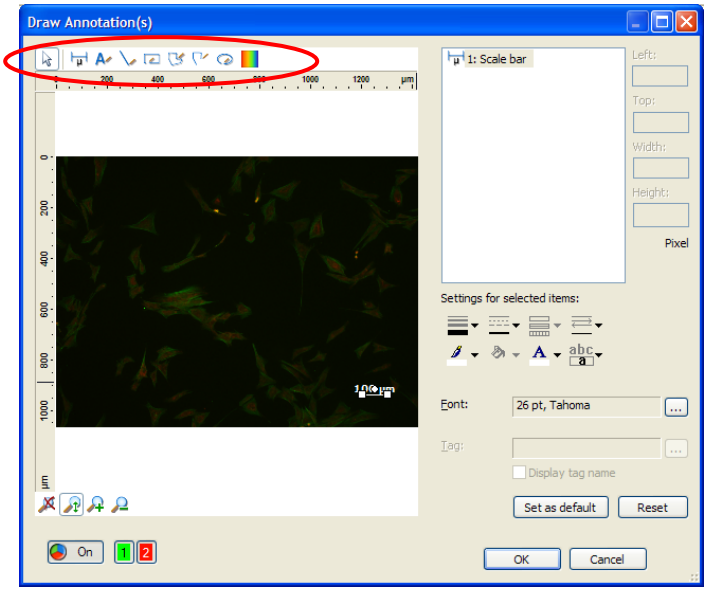


Annotations:

Before adding a scale bar, make sure that the software knows which objective was used during image acquisition. From the toolbar, click on Measure, and go to the subheading Scalings. This window will pop up:



A green check mark appears next to the objective that the software believes was used when capturing the active image. If this is incorrect, select the proper objective and click "Apply selection to image."
"Activate selection" will apply that objective setting to any subsequent images captured.



To bring up a window to draw annotations, click on Annotations from the toolbar, and select Draw Annotations. There are also shortcut markers for a scale bar elsewhere.

Any annotations you make will appear in a white box on the right side of the window. To alter (e.g. change line/text color, font, width, etc.) or delete a given annotation, highlight it in this box or click on it within the preview window and make changes as needed.

Saving and Exporting:

Zeiss has a proprietary image format with the extension .zvi. By saving files in this format, you preserve information such as annotations and objective used within the file. These can only be read with the AxioVision software. As a note, Zeiss does have a free reader for .zvi files for Windows that can be downloaded from the Zeiss website. It is not critical to save your files in this format if you do not wish to do so.

You will also need to save your images in another readable format such as .jpg or .tiff. This can be done from the Save As… option, or by exporting your already saved .zvi files to one of these formats. Exporting can be done as a batch process.