

BIOLOGICAL IMAGING DEVELOPMENT COLAB



Zeiss Axio Imager Brightfield Microscope

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Contents

1	Introduction	2
2	Hardware Start-Up	2
3	Kohler	5
4	Software Set Up	5
5	Adjusting Image Brightness	6
6	Adjusting Image Contrast	7
7	Adjusting Image Color Correction	7
8	Flat Field Correction	8
9	Capture and Save Images	8

1 Introduction

The Axio Imager is an upright microscope with an objective turret that houses five lenses, listed in Figure 1.

Zeiss Axio Imager/ Brightfield	
Objectives	1.25x, 0.03 NA, Air
	5x, 0.15 NA, Air
	10x, 0.45 NA, Air
	20x, 0.75 NA, Air
	40x, 0.95 NA, Air

Figure 1: Objectives available on the Zeiss Brightfield Microscope

Please sign up for time using the MyCores microscope scheduler.

2 Hardware Start-Up

1. Turn on the computer.
2. Turn on the microscope. The power switch can be found on the left side of the scope behind the focus knob. See Figure 2



Figure 2: The image above shows the location of the power switch for the microscope.

3. Set the illumination/ color temperature by pressing the 3200K button found on the right side of the microscope. The on/off indicator LED will be green when 3200K has been properly activated. See Figure 3



Figure 3: The image above shows the location of the 3200K illumination button.

4. Place the slide on the microscope stage and find sample by looking through the eyepieces by adjusting the X and Y control knobs. See Figure 4. If nothing is visible through the eyepieces, check that the beamsplitter is set to have the light travel to the eyepieces, not the camera. See Figure 5.

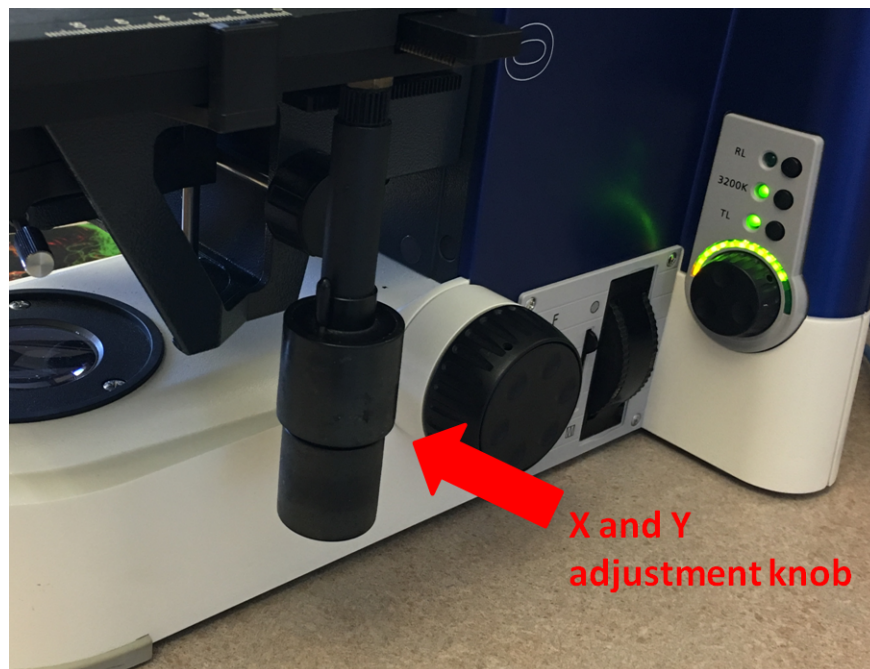


Figure 4: The image above shows the X and Y adjustment knob.



Figure 5: Beamsplitter to send light to eyes or camera.

5. Focus on the sample by adjusting the coarse and fine focus knobs. See Figure 6.



Figure 6: The image above shows the focus adjustment knob. The outer element of the knob adjusts the coarse focus, while the inner element adjusts the fine focus.

6. Kohler can be performed in order to optimize viewing, image brightness, contrast and resolution.

3 Kohler

To set up Kohler illumination:

1. Turn on microscope
2. Set inter-pupillary distance (Move eye pieces to a comfortable position)
3. Focus Specimen
4. Close down field stop
5. Focus blades of field stop with substage condenser
6. Center field stop with substage condenser
7. Open the field stop until the blades are just outside of the field of view
8. Set aperture diaphragm to balance contrast and resolution. To do so, take out one eyepiece and close down the aperture diaphragm until 3/4 open. This is the best compromise between contrast and resolution.

If you need help setting up Kohler illumination on the microscope, feel free to contact anyone within the BIDC. Setting up Kohler illumination is required for each individual objective.

4 Software Set Up

1. Open AxioVision software. See Figure 7.



Figure 7: The AxioVision software icon that can be found on the computer desktop.

2. A live feed of the sample can be viewed on the computer screen by clicking the 'Live' button. In order to view an image on the computer screen, the push-pull rod for camera path deflection must be set to camera (pulled out). This rod redirects the light from the eyepieces to the camera (See Figure 5). Adjust the focus of the live feed image as needed.

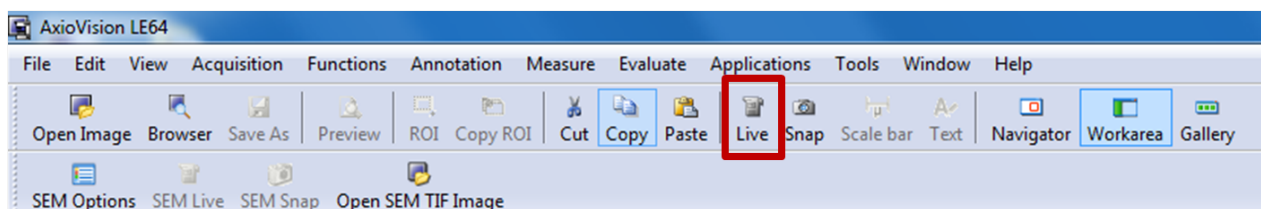


Figure 8: The 'Live' button in the image on the top can be seen highlighted in red.

3. Match the objective being used on the microscope to the objective being used in the software. This can be done by selecting the objective in the dropdown menu. The dropdown menu can be found in the toolbar at the bottom of the live view window. See Figure 9.

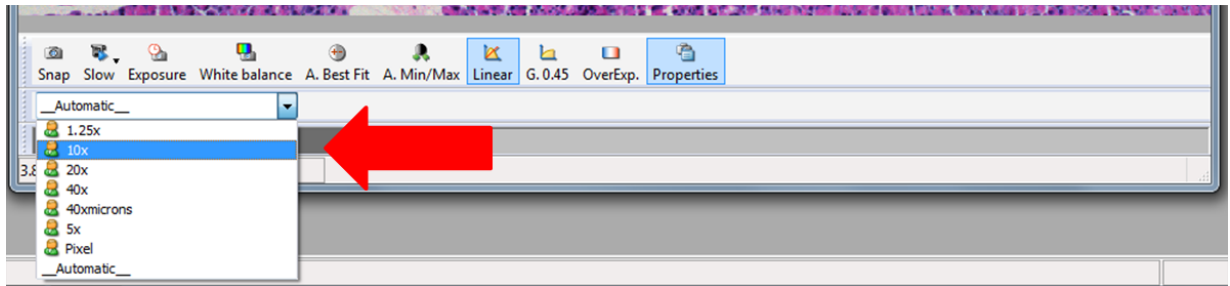


Figure 9: The dropdown menu where the objective can be chosen. Choosing an objective in the software now will help with the measurements and scale later.

5 Adjusting Image Brightness

Image brightness can be adjusted by clicking the 'Adjust' tab and clicking the 'Increase' or 'Decrease' arrows for exposure time. See Figure 10.

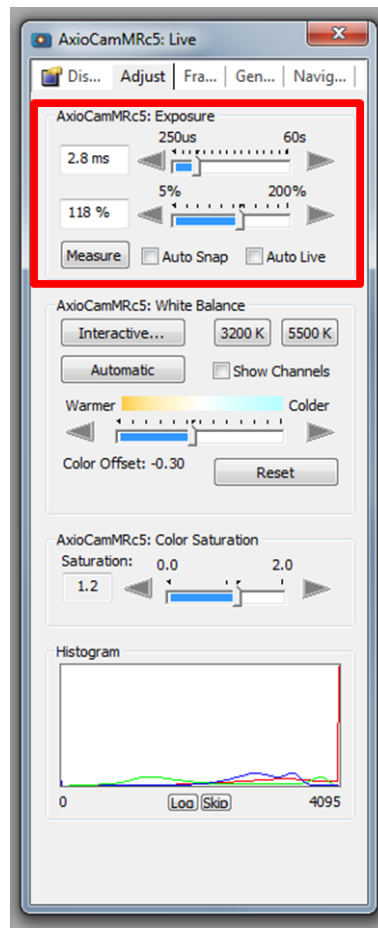


Figure 10: The image above shows the AxioVision exposure window. This is where exposure can be adjusted.

6 Adjusting Image Contrast

Image contrast can be adjusted by clicking the 'Linear' button in the tool bar at the bottom of the live view window. See Figure 11.

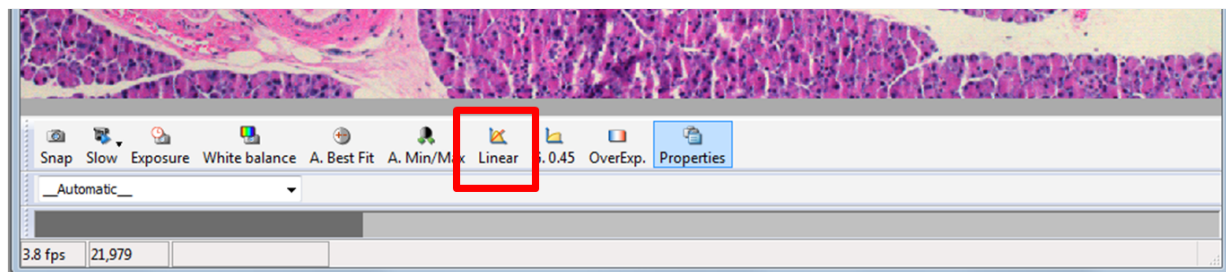


Figure 11: The image above shows the 'Linear' button highlighted in red.

7 Adjusting Image Color Correction

To adjust the color correction of the image, click the 'Adjust' tab, click 'Interactive' and then click on an area in your image that should be white. (Do not click on a part of the sample). See Figure 12.

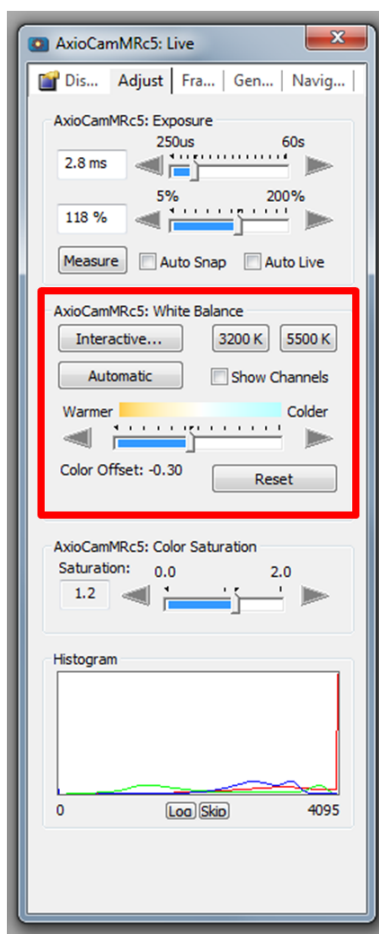


Figure 12: The image above shows the white balance adjustment area highlighted in red.

8 Flat Field Correction

Move to a clear area of the slide where there is no sample (as featureless as possible) and de-focus using the fine or coarse focus knob. Click the General tab then click Shading Correction. Go back to an area of the slide with the sample and re-focus. See Figure 13.

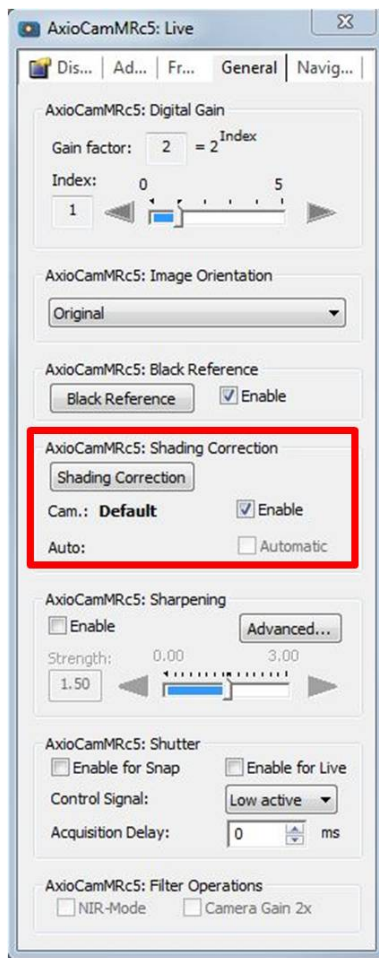


Figure 13: The image above shows the shading correction adjustment area highlighted in red. Flat Field Correction should be done every time the objective or sample are changed.

9 Capture and Save Images

1. When the live feed image has been optimized, an image can be taken by clicking the 'Snap' button. This button can be found in the tool bar at the top of the screen or in the tool bar at the bottom of the live feed window. See Figure 14.

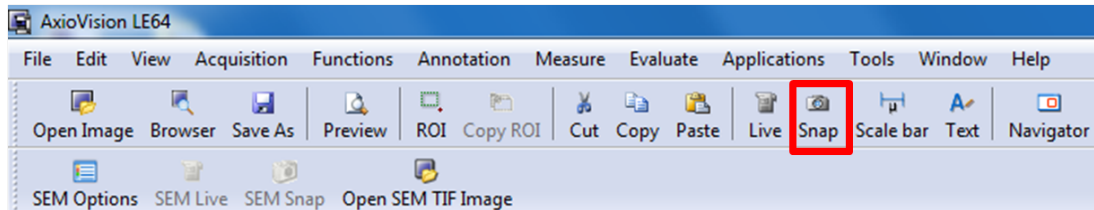


Figure 14: The image above shows the Snap icon.

2. When an image is captured, it will be bigger than the live feed view. The captured image can be viewed at a smaller or bigger size by clicking the minus or plus magnifying glass. See Figure 15.

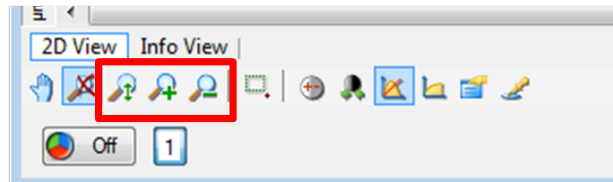


Figure 15: The image above shows the image magnification choices. The left option will fit the image to window size, the middle option will increase image size, and the right option will decrease the image size.

3. In order to save an image, click the 'Save As' button in the tool bar at the top of the window. Choose a folder to save the image to (Images should be saved to a personal hard drive or a box account. Do not save data to the microscope computer)
4. Name the captured image and choose a file type. The file type chosen will depend on how the image will be processed later.
5. When all save preferences have been selected, click the save button.

Contact the BIDC

The BIDC office is located in Medical Sciences Building Room S1109.

The BIDC office phone number is 415-476-4550.

If you need immediate assistance and no one is available in the office, or it is after business hours, please call the **BIDC Hotline** at 415-745-2432.