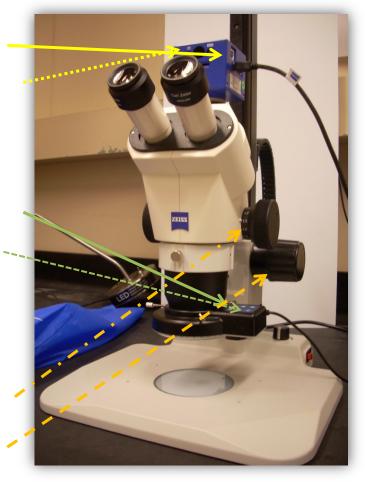
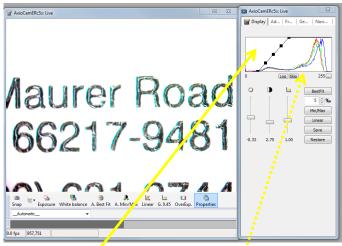
User guide for Zeiss 2000CS stereomicroscope

Andy Elwood Madden, v.2

- 1. Remove dust cover, set aside nearby
- 2. Plug in the AxioCam Erc 5s with the mini-USB cable
- 3. Wait until the light at the top of the camera cycles from red to green
- 4. Lunch AxioVision Rel. 4.8 from the desktop or start menu
- 5. Wait for the software to boot
- 6. Click "Live" on the main toolbar
- Turn on illumination from the ringlight with the on/off button.
- Cycle the "mode" button on the ringlight until all four quadrants of LEDs are light. You have to look underneath the light source to see this.
- Find something to use (white paper, for example) to perform a white balance correction. It helps for starters if there is text you can use for the initial focusing.
- 10. Change the zoom to the setting 1.25 (1.25 on the **zoom dial** x 1.25 objective x 10x ocular = 15.625x.
- Bring the item into focus with the **focus knob**. It should look something like this:

Notice how the intensity distribution over the red, green, and blue channels are seen in the "Live" side window. You must have this window open while collecting images in order to optimize the settings each time. For a purely "white" object, these channels should lie on top of each other. If you don't see them at all, then adjust the light intensity on the ring light until you do.

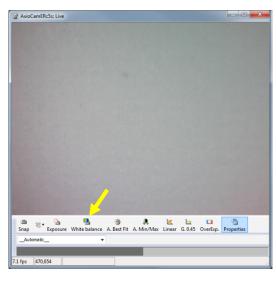




Also notice that the range of data the software is using to form the image, as given by the **black line with black squares**, doesn't correspond to the range of data values for each channel. Click the "**BestFit**" button and it should adjust. Now the image should look more 'natural'.

- 12. Now find a place on your chosen item where only white is showing.
- 13. Click the "White balance" button underneath the Live capture window (see photo)

If you change the lighting conditions or if the colors in the image don't seem to match what you see in the oculars, you should reset the white balance.



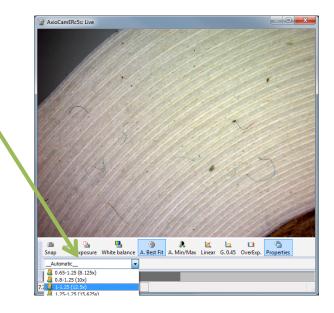
- Place the object you would like to view on the baseplate. Consider putting something underneath it to get the best contrast with your sample without having too much glare.
- 15. Adjust the zoom, then focus. If you change the zoom level, you will have to reset the focus. Generally, start first at the lowest zoom and move upward in zoom as you identify features of interest.
- 16. Use the "live" sidebar window to set the lighting.

In the photo below, the intensity distribution (histogram) for each of the 256 red, blue, and green channels is shown. Because "BestFit" is selected, the brightness and contrast automatically adjust to make the best fit image, and the black line (with black squares) matches the range of data from the color channels. If the data in the histogram is skewed off of the scale to the right, reduce the light intensity on the ring light. If the histograms are skewed off to the left (the "zero" side), then increase the light intensity to collect a good image. You can also use the 'Exposure' controls and tab to work to get the best lighting for your sample.



 Set the scale by choosing the zoom number from the microscope (zoom dial) from the pull down menu at the bottom of the live window.

You **must** check this **every** time you collect an image to make sure that the image scale is properly recorded. If this value is correct, the software will store the correct, calibrated pixel/length conversion so you can add scale bars and make measurements. If this value is not correct, all scale bars and measurements will be wrong!



- 18. Click the "Snap" button to record the image.
- 19. Collect as many images as you like this way.
- 20. Save the images as ".zvi" files on the desktop → Zeiss → YourFolder. Saving them in this format will preserve the measurement scale. Exporting or saving as jpg/tiff formats will not include the scale metadata, so you can't go back later and change the scale bar or make measurements.

When you are finished

1. Close AxioVision. It will ask you if you want to save any previously unsaved images.

- 2. Turn off baseplate light, if used.
- 3. Turn off ring light (on/off button)
- 4. Unplug USB cable from camera
- 5. Replace dust cover
- 6. Report any problems/ issues to Andy by email
- 7. Retrieve data by USB flash drive or email.

Note: AxioVision can do a lot of annotation and image processing. See the AxioVision manual/documentation on the PC (start menu \rightarrow Programs \rightarrow Carl Zeiss Vision).

Note2: AxioVision is a stand-alone software program that you can install on your own PC, then you can edit .zvi files on your own machine.

SOME ADDITIONAL FREQUENTLY USED FEATURES (tip of the AxioVision iceberg):

Adding a scale bar

- 1. Right-click on the image, select "Autozoom" to have the image fill the window
- 2. Select the "Scale bar" button from the AxioVision main toolbar.
- 3. Left-click and drag where on the image you want the scale bar and how long you want it to be.
- 4. You can edit or delete the scale bar as long as the image is saved as a .zvi file. Once you export jpg/tiff, you can't edit this anymore.

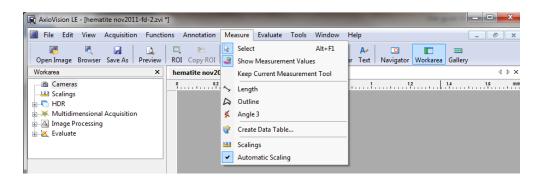
Changing a scale bar color / text size

- 1. Right-click on the scale bar. At the bottom of the pull-down menu, select "Properties".
- Tab over to the "Attributes" tab. Here you can edit the color of the scale bar, thickness, and text size/color (photo to the right).

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Measurement of features

In the main window of AxioVision, "Measure" is one of the choices at the bar on the top.



For example, to measure the length of a feature, select "Length", then left-click and drag the feature directly on the image. Click again to accept the feature selection and display the length measurement.

<u>Remember this is only valid for images that were collected with the correct scale originally, and</u> <u>cannot be performed on tiff or jpg files.</u>

Batch conversion of zvi to jpg or tiff

coming later.