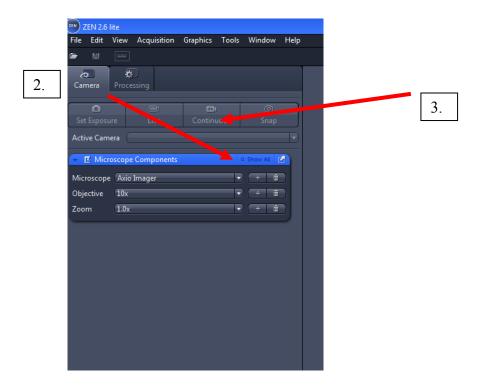
To operate the Zeiss axiocams (503 and 305 on the NikonE800 and the Olympus dissection scope, respectively) and the confocal LSM 800, use Zen Blue.

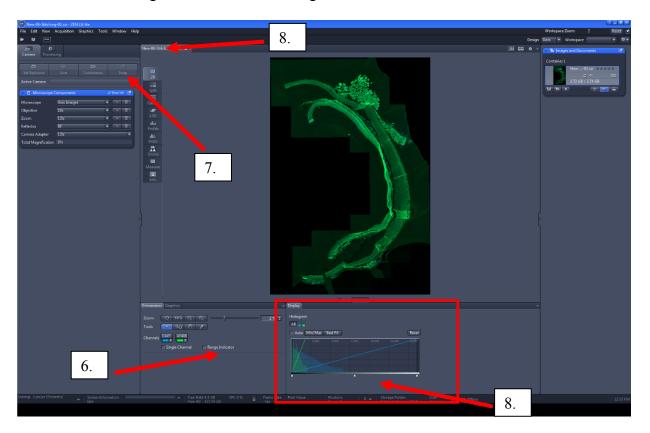


1. Click on the program to start. If using the Nikon or Olympus, a message may appear reminding you to move the manual slider to the camera view. If using the confocal, a message will appear to select operation for Image Analysis or for the Viewing System (i.e., the confocal). If you are using the confocal, select Viewing System. The program will open and appear. Locate the upper left part of the program. The menu system is designed with workflow from the top to down and left to right using a series of clicking on tabs.



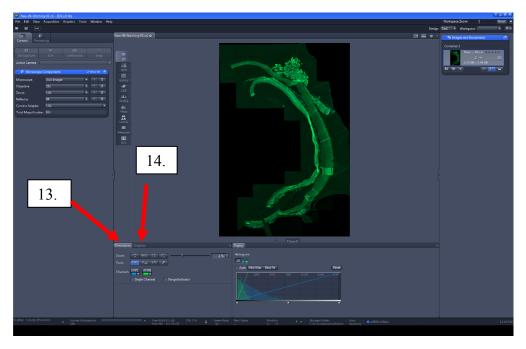
2. For example, click on the Mirosccope Components Tab. Confirm that correct light path (i.e., objective, filters, etc.). For example if usng the 20x objective select that option. If usng the FITC channel filter, select for that option.

- 3. Click on Live.
- 4. Hit Set Exposure (image of slide with specimen should appear).
- 5. Look at histogram at bottom middle right.



- 6. Check box for Range Indicator. If you see any red pixels decrease Exposure Time.
- 7. Click Snap.
- 8. Go to snapped image by clicking on the snapped image. Tab will appear tot right of Live image tab.
- 9. Adjust range values (triangles on x-axis of histogram) to fit the majority of the histogram.
- 10. Make gamma value = 1
- 11. In the camera tab, if quantifying fluorescence, be sure BW is selected for the Color Mode. If doing color, then RGB should be selected.
- 12. Save file using the format .czi (Carl Zeiss Image).

13. Go to the Dimensions tab. For C1 channel, if corresponding with FITC, choose color green. Since blue excitation emits green fluorescence for this filter.



- 14. Click the Graphics tab to add a graphic, e.g., scale bar. Click on the Format button to change properties of the scale bar, i.e., color, font, etc.
- 15. Make a measure of intensity and size of an object. Using the Draw Circle, Draw Rectangle, or Draw Spline Contour, draw a border around the object by holding down the left mouse button. Release buttoning will provide area and mean intensity values, etc. For the Draw Spline Contour, click on the right mouse button after drawing around region.

