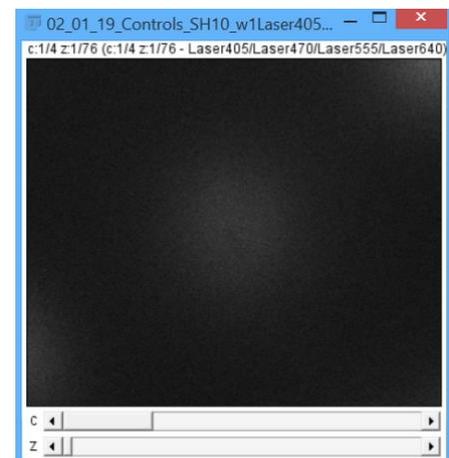
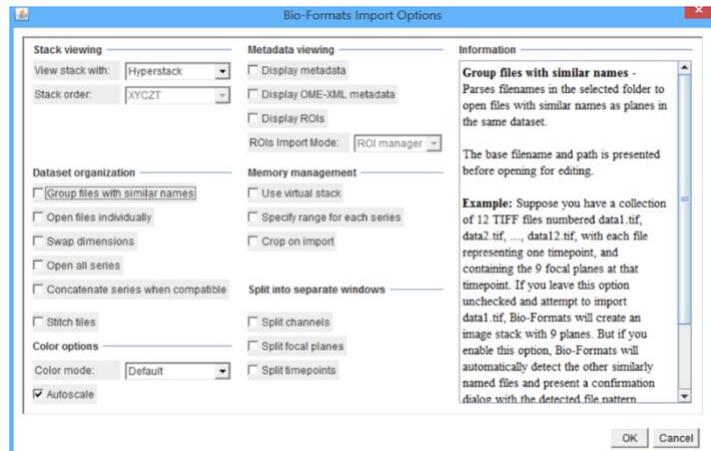


## Basic Image J/FIJI Use and Processing

1. Download ImageJ/FIJI for the respective operating system on the computer (follow link in resources on Blatt website to download)
2. Once downloaded open images up by dragging your saved .czi files onto your control panel for ImageJ/FIJI (Pictured Right)
3. If a screen pops up asking to update, select yes to update. Restart the application after it finishes and prompted to.
4. A Bio-Formats screen will pop up for FIJI/ImageJ 2.0 asking how you would like to open the file. Continue through this menu by selecting "OK" or select one of these other options for the data.
5. The image will open now and if there are multiple channels and multiple z slices these will open with a slider to scroll through each as shown.
6. These channels can be split if you have multiple by going to Image>Color>Split Channels
7. To Merge Channels into one image use Image>Color>Merge Channels
8. Set the look up table to appear as a desired color. Use the slider to navigate to that channel and then use the LUT function on your application bar or ribbon.
9. To adjust your histogram in channels. Select Image>Adjust>Brightness/Contrast. This will allow the histogram displayed to be adjusted. (Note: When doing quantifications, especially with fluorescent intensity it is important to set each image identical when comparing between samples. This can be achieved by using the "Set" function in this menu and then recording the min and max values and keeping this equal across all images)



10. To create a Z Projection of the image, use the ribbon to select Image>Stacks>Z Project. Select the starting and ending point of your stack and the method of projection (i.e. Max, Min, Average Projection, etc.). Click "OK" when ready to create the projection.
11. Regions of Interest (ROIs) can be drawn using the tools on the application panel such as a circle or a square. Draw on the image when tool is selected to create the ROI.
12. To take a general measurement in that ROI such as intensity or area select command M (for Macs) or Control M (for PCs)
13. To Duplicate this ROI for an inset, use command, Shift, and the 'D' Keys together (for Macs) and Control, Shift, and the letter 'D' (for PCs)
14. Smoothing Can be done by using Process>Smooth. This will apply a smoothing function to your image. Can help remove pixelation/noise.
15. Make sure to save images after every step because this will permanently save the images. Images should be saved in the original format (ie 8 or 16 bit images while working through processing and analysis). The original files should be left untouched and saved that were taken from the microscope. Use File>Save as>Tif to save working images.
16. After all processing is done, to save an image for publication, the image can be converted to an RGB to save the LUTs chosen. Use Image>Adjust>RGB Color. This RGB should now be saved separately from all other working files. This is not the image any data should be quantified from, and images should never be converted back to an 8 or 16 bit image to quantify. Always use the original images taken before the conversion to RGB.