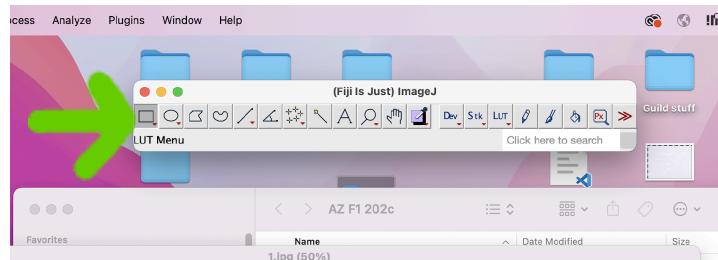
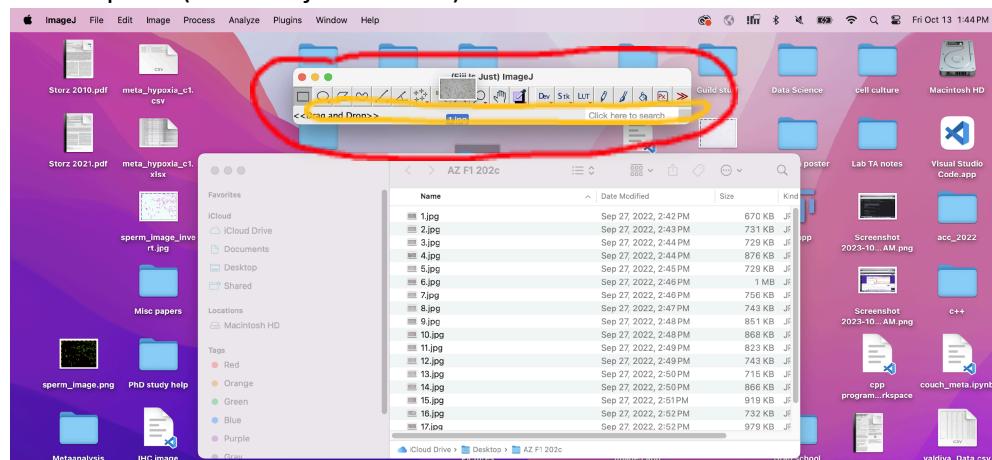


Measuring staining area on a microscope slide image using Fiji (Fiji is just ImageJ)

1. Install Fiji at <https://imagej.net/software/fiji/downloads>
2. Open the Fiji app.
 - a. The following bar will appear:

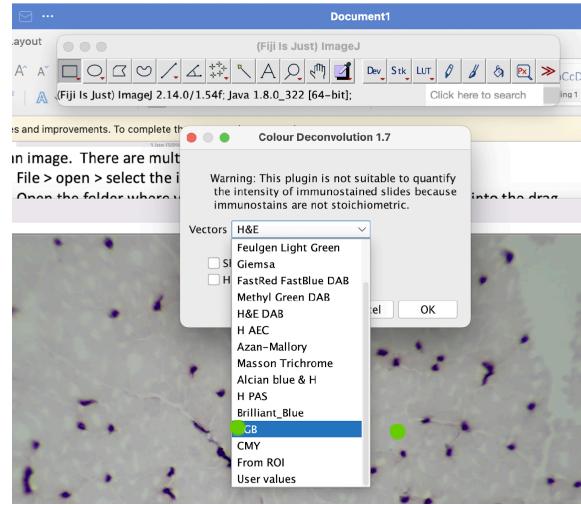


3. Open an image. There are multiple ways to do this:
 - a. File > Open > select the image you want to analyze.
 - b. Open the folder where your images are stored. Drag the image file into the drag and drop bar (on the Fiji menu bar).

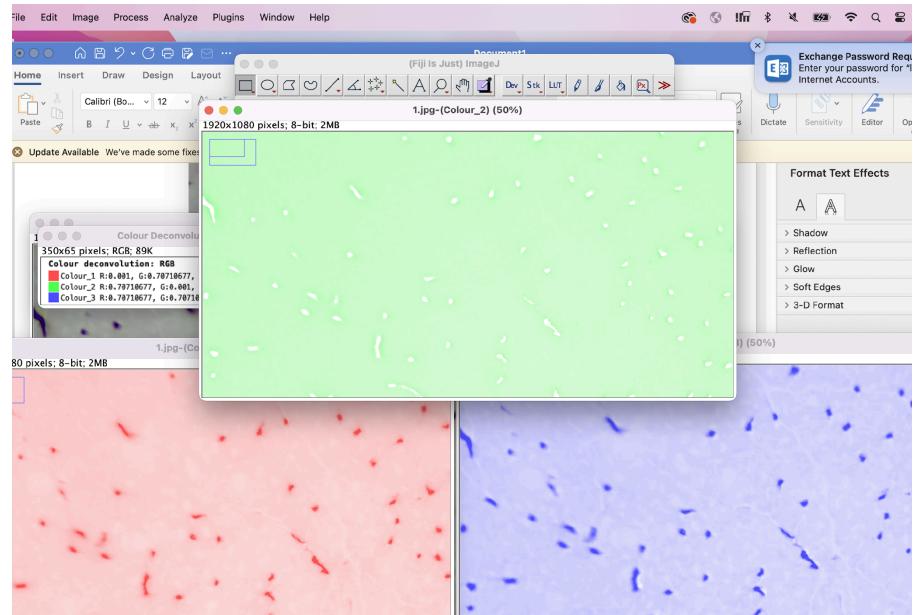


4. Once you have an image pulled up in Fiji, click on Image > Color > Color Deconvolution

- a. You will see this drop down appear:



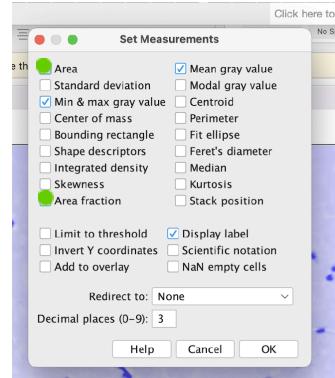
- b. Select RGB (or, if the stain you are quantifying is listed, select that stain).
 c. This process will create separate channels (i.e., images) for each color of the stain (in this case, since I used the RGB setting, it separated the original image into its red, green, and blue counterparts).



- d.
- e. Usually, one of these channels/images captures the positive staining in a way that is representative of what we want to quantify biologically. Since the stain I used as an example is purple, we have two channels (the red and blue) that look almost identical. Pick one of these channels to proceed to the next step.

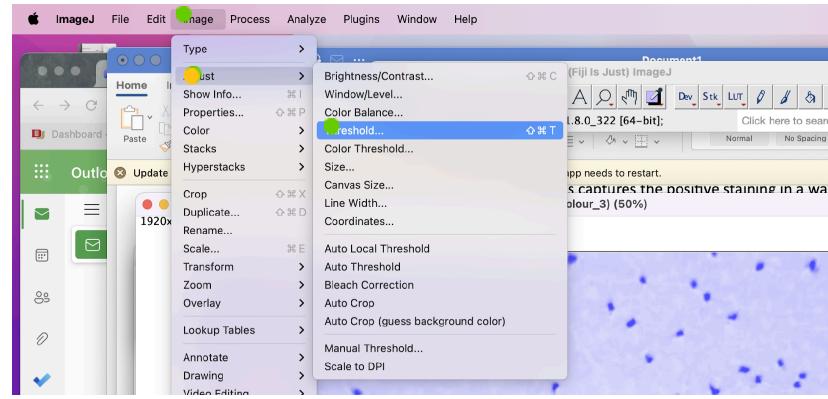
5. To calculate the area of positively stained cells:

- a. Click on Analyze > Set Measurements. Make sure all measurements you wish to collect from the image are selected. Area fraction will need to be checked (this is a value representing the overall percentage of the image area that stained positive).



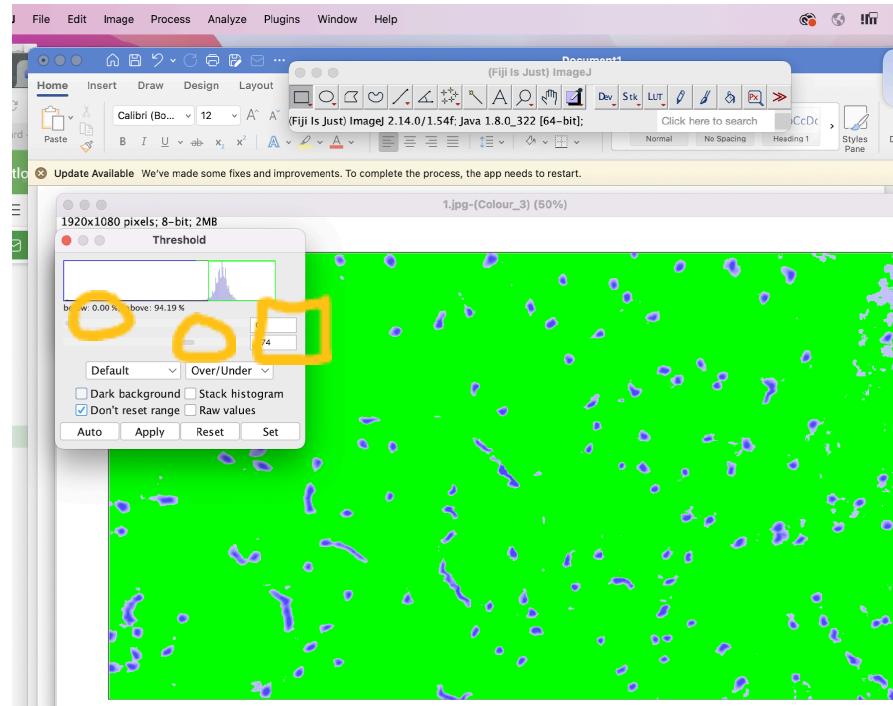
b.

c. Go to Image > Adjust > Threshold.



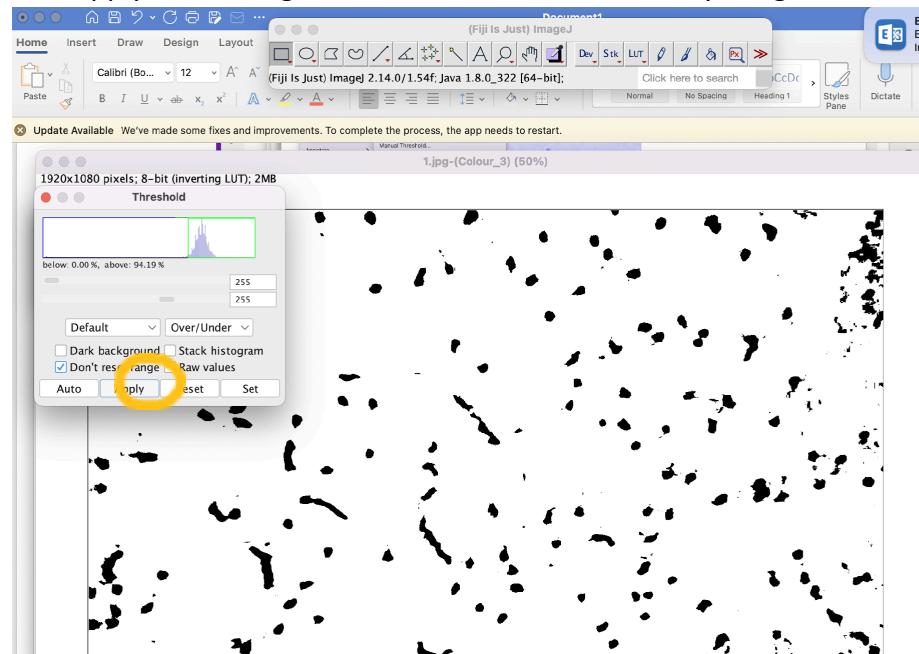
d.

e. Leave the top slider completely to the left. Move the bottom slide to the point where (in this case) the green encompasses everything that is not positive stain (=negative staining).



f.

- g. The values to the right of the sliders can be used across all images you are analyzing (assuming the lighting isn't significantly different).
- h. Click apply. Your image will be transformed to a binary image:



- i.
- j. Click on Analyze > Measure. A pop-up will display whatever measurements you selected for in 5a.

