Fluorescence Analysis Activity

1 Calculating Fluorescence Area

1. Open up ImageJ and open `demo2_1.png` from the BMES Cell Team website
2. Click on Image > Adjust > Color threshold to separate the cells from the background
3. Go to Analyze > Analyze Particles > Display results
4. Highlight the entire table, click on File > Save as, and save your file as a comma separated value (csv).
   Feel free to name your file whatever you want (tacos.csv, screwthis.csv, etc.)
5. Open up Anaconda and click on Spyder
6. Follow along with the demo

2 Calculating the Fluorescence Intensity

1. Close ImageJ and reopen it again
2. Open `demo2_1.png` from the BMES Cell Team website
3. Click on Image > Type > 8-bit
4. Click on Analyze > Set Measurements. Make sure Area, Integrated Density, and Mean Grey Value are checked.
5. Under the toolbar, click on the freehand tool
6. With your mouse, trace around the outline of a particular cell
7. Click on Analyze > Measure. If you have a Mac, you can use Command + M. A window will pop up.
8. Repeat steps 5-7 three more times for different cells. You should have four different measurements.
9. Copy the information to your spreadsheet.
10. Now, find a small area of your image that has no fluorescence. This will be your background.
11. Click on the freehand tool and trace around this small area.
12. Click on Analyze > Measure (or Command + M if you have a Mac).
13. Repeat steps 10-12 three more times for different background areas. You should have a total of 4 background measurements.
14. Copy the information to your spreadsheet.

Your final spreadsheet should look like this, but with different values for Area, Mean, and Integrated Density:

Cell	Area	Mean	Integrated Density
1	385	39.681	15277
2	494	26.097	12892
3	226	41.615	9405
4	406	40.631	16496
Background			
1	277	0.152	42
2	183	0	0
3	72	0	0
4	74	0.243	18

15. Open up Anaconda and click on Spyder
16. Follow along with the demo