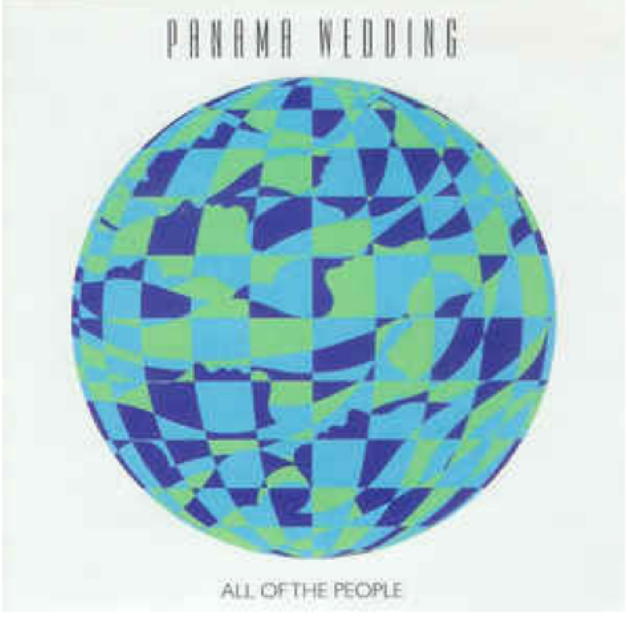
Welcome to Protocol Discussion 3!

- **Today's Pre-Module Playlist**
  - Little Wonders – Rob Thomas
  - All of the People – Panama Wedding
  - Out of My League – Fitz and The Tantrums
- **Team Check Ins**
  - Checking in with Groups 3 today
- **Upcoming BMES Events**
  - Week 6
    - Esports Day tomorrow at 7 PM
  - Week 7
    - Mini-GM Wednesday at 6 PM
  - Week 9
    - Medical Devices Forum
  - Next Quarter
    - BMES Hackathon



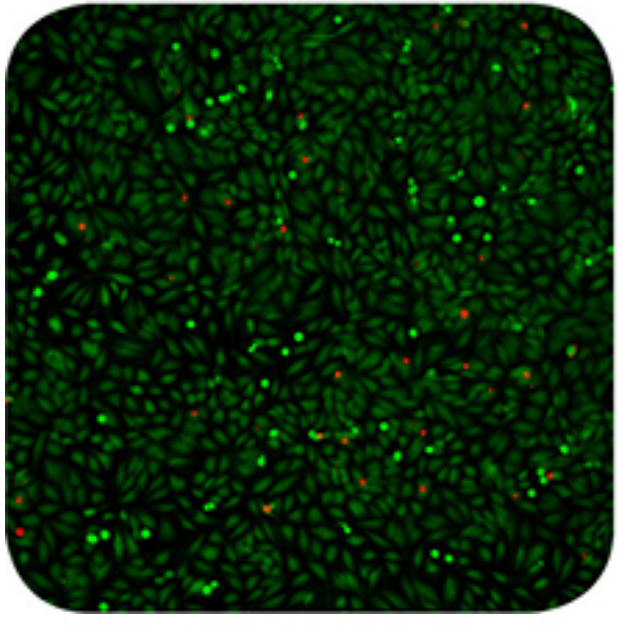
Protocol Discussion 3: Analyzing Cell Images

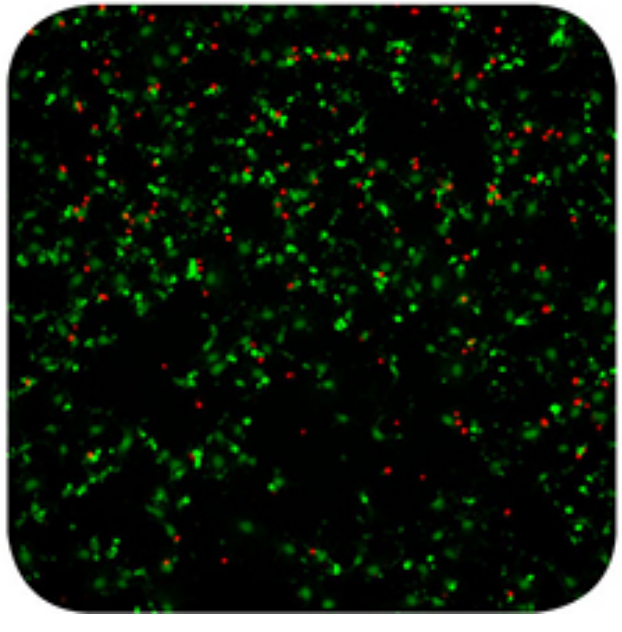
BMES Cell Team

Winter 2021

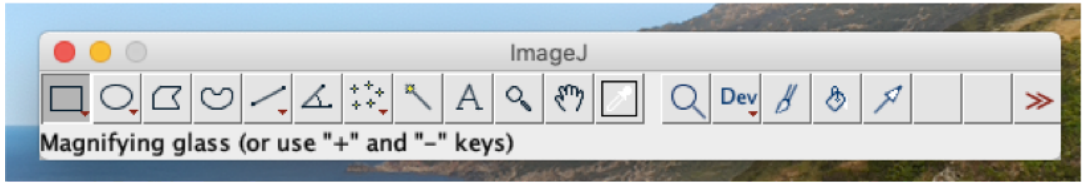
Outline

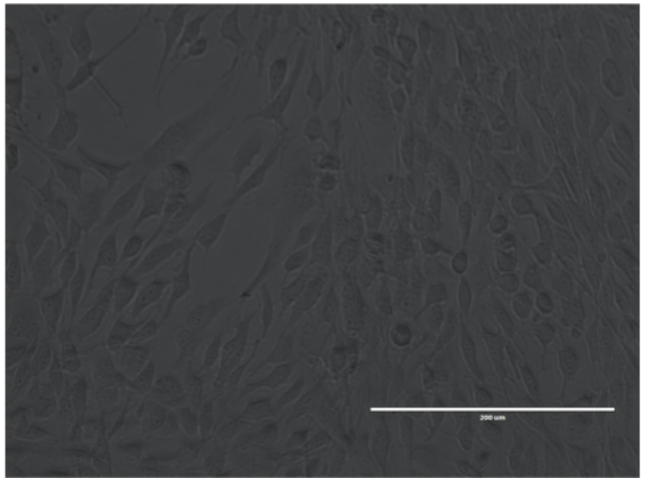
- ImageJ Walkthrough
    - Cell Counting
    - Cell Morphology
- Breakout Rooms
    - Complete cell count and morphology analysis for all 4 files
- Group 3 Check In





What is ImageJ?

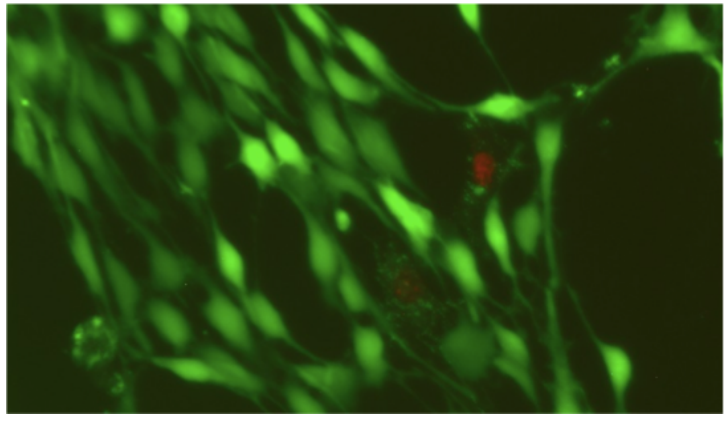




- Java-based image processing software
- Developed by the NIH
- Free to use!
- Will likely use it in 167L to analyze your imaging data
- Useful tool to know for Capstone

Protocol Discussion: Setup

- Download these 4 files from the Cell Team Site
    - Labs → Protocol Walkthrough 3
- Export the files as “2”, “5”, “10”, and “25”



2% DMSO file

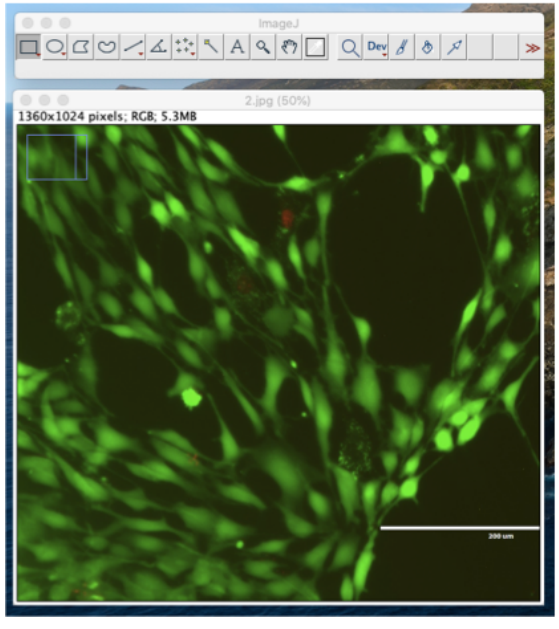
PROTOCOL WALKTHROUGH 3 – CELL PASSAGING, COUNTING, AND VIABILITY ASSAYS

- Cell Passaging, Counting, and Viability Protocol
- Protocol Walkthrough 3 Slides
- 2% DMSO Treatment
- 5% DMSO Treatment
- 10% DMSO Treatment
- 25% DMSO Treatment

Protocol Discussion: Setup

- Open ImageJ

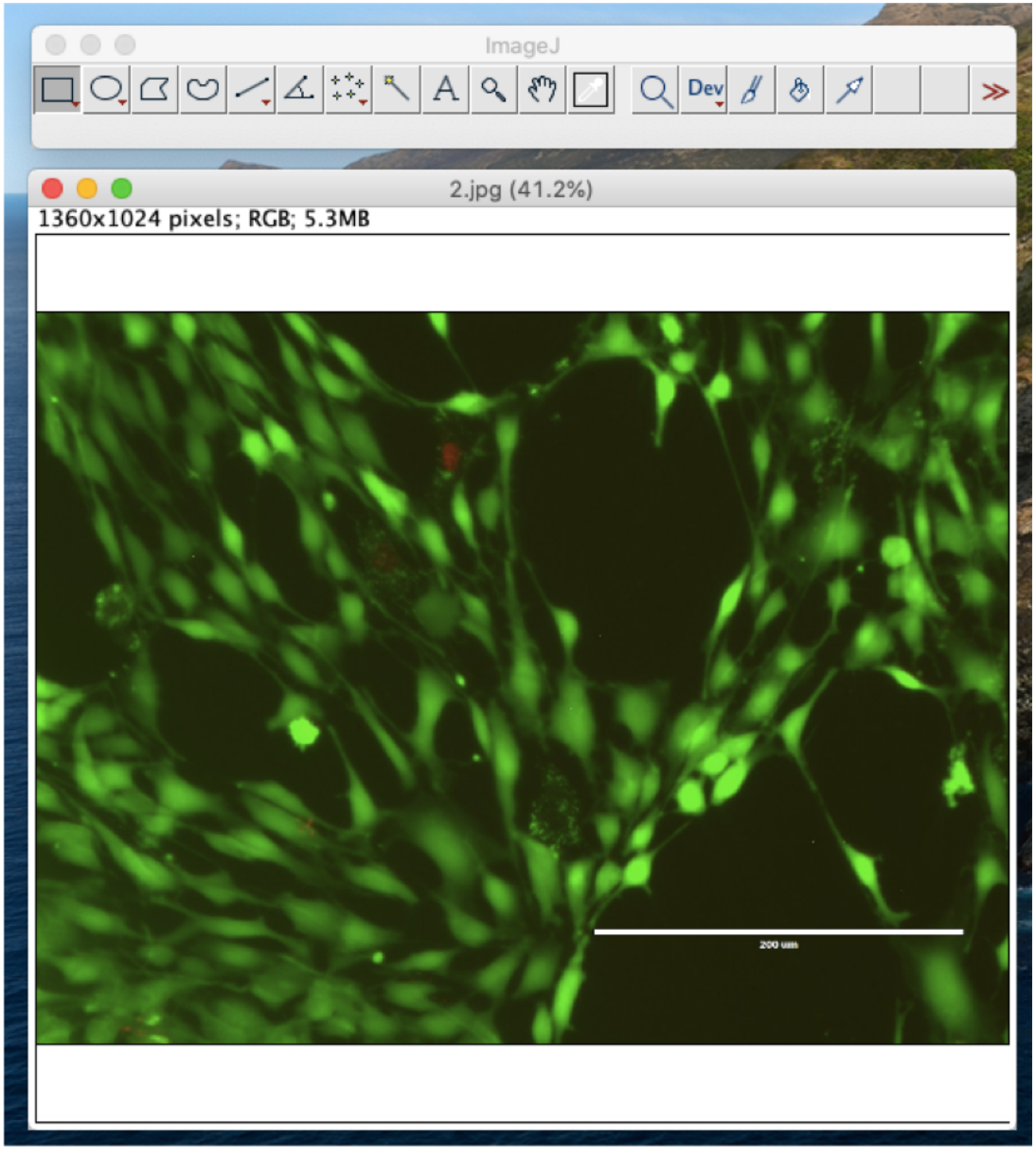
- Open the 2% DMSO Image
    - File → Open → 2.jpg



2% DMSO file in ImageJ

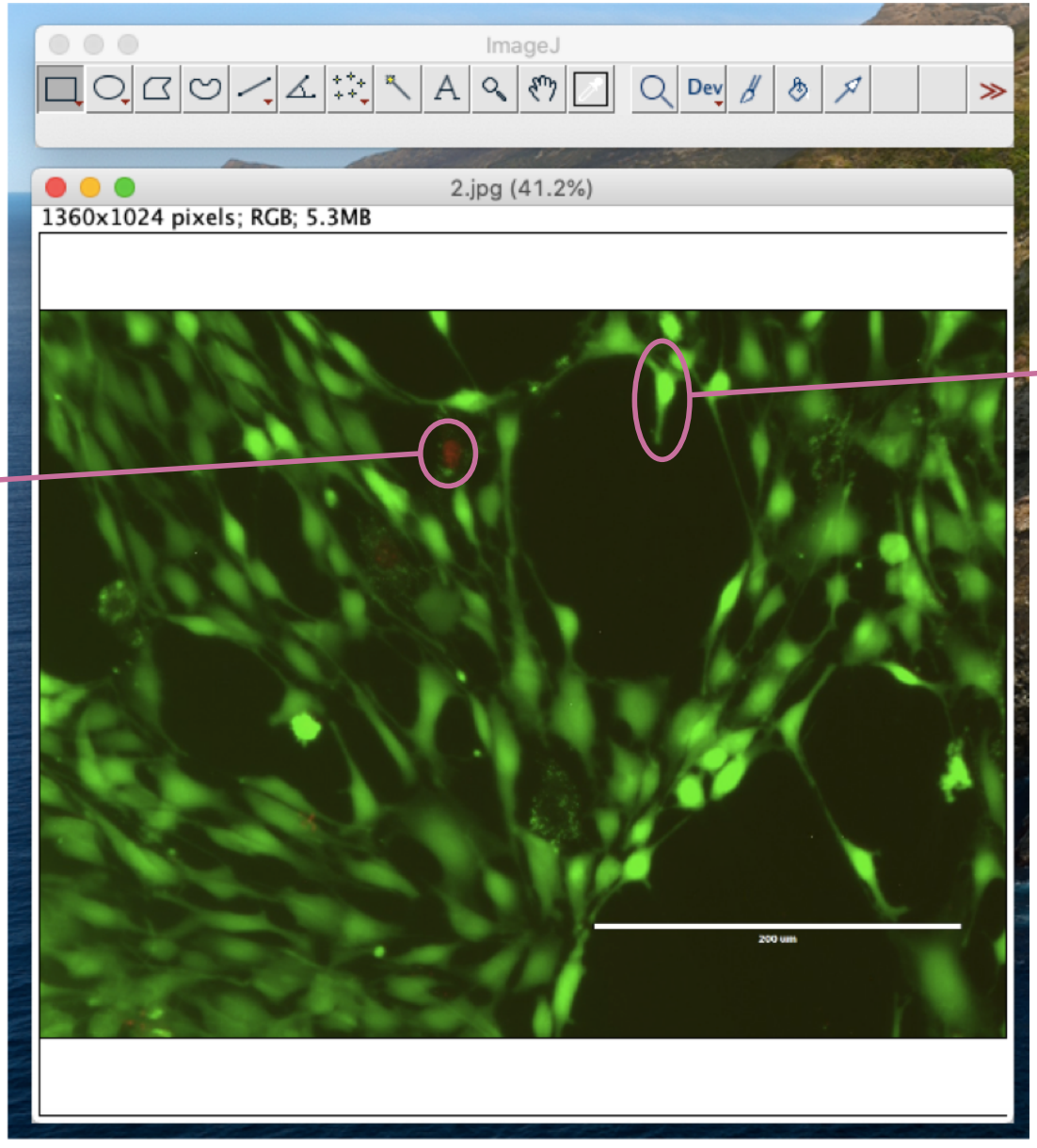
- Scale so that you can see the whole image
    - Change the image window to the desired size
    - Image → Zoom → Scale to fit

Protocol Discussion: Setup



2% DMSO file

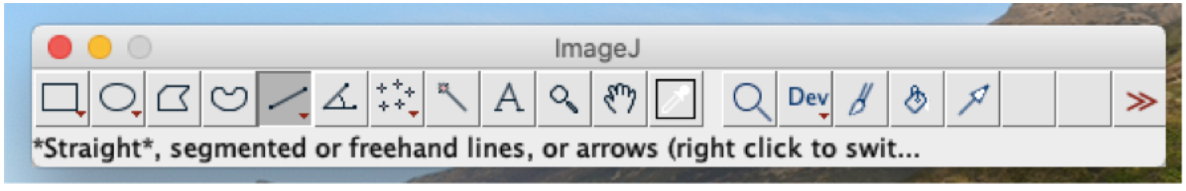
Protocol Discussion: Setup



2% DMSO file

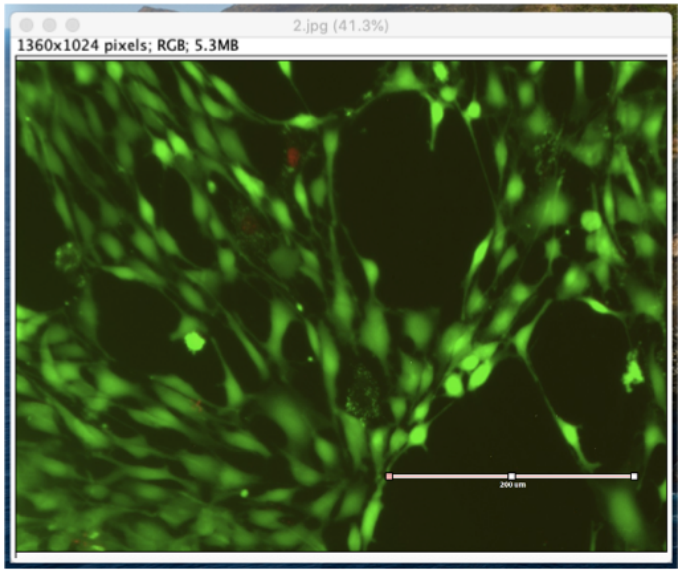
Protocol Discussion: Setup

- Now we need to specify the size of our image features
  - Click on the line tool



  - Measure the 200 um scale bar
  - Click Analyze → Measure
    - Yields results table with the

    length of the line in pixels

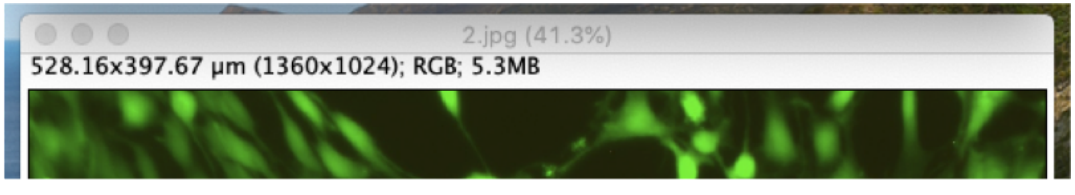


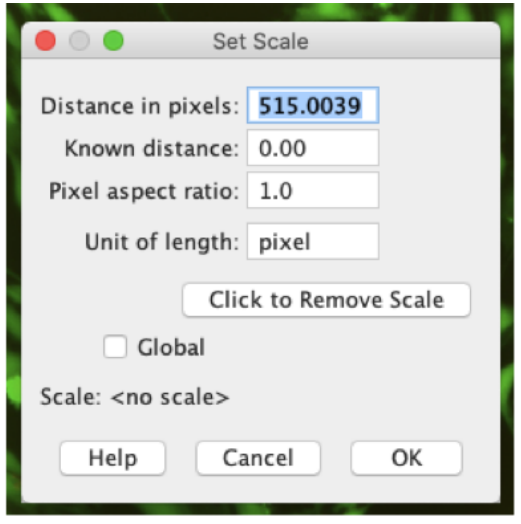
Results

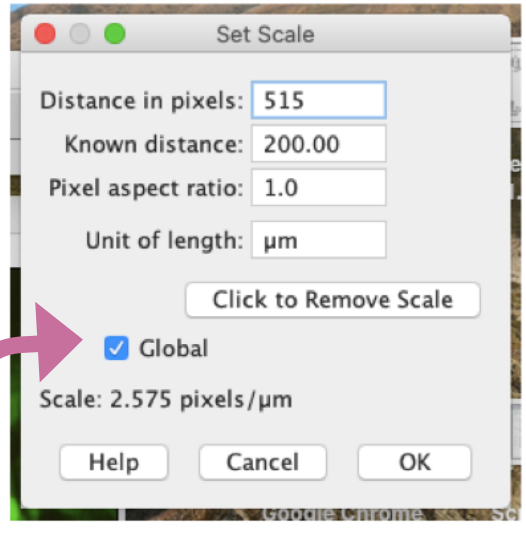
	Area	Mean	Angle	IntDen	RawIntDen	Length
1	516	253.733	-0.223	130926.273	130926.273	515.004

Protocol Discussion: Setup

- Now we need to convert from pixels to this line length
    - Analyze → Set Scale
- The length of the line you just measured should be highlighted in blue
    - Change the pixel distance to a round number (ex: 515) and the unit of length to “um”
- Image should now have um units in top left corner



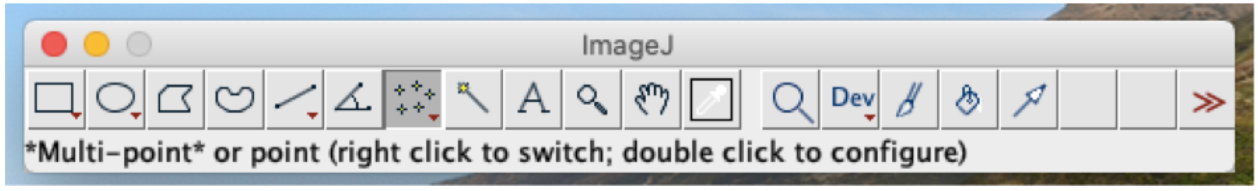




Select “global” to save this measurement

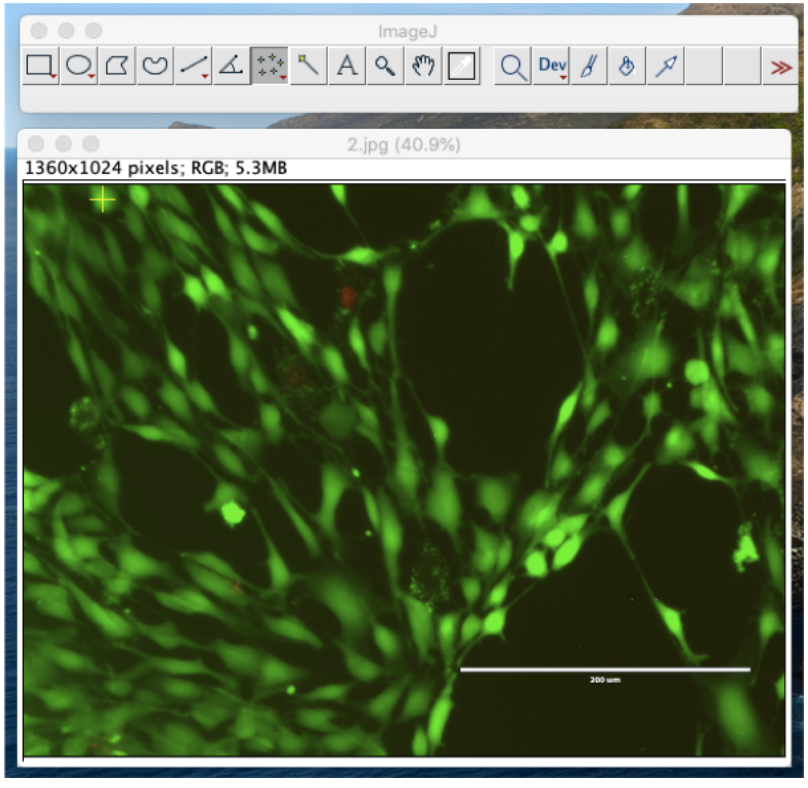
Protocol Discussion: Cell Counting

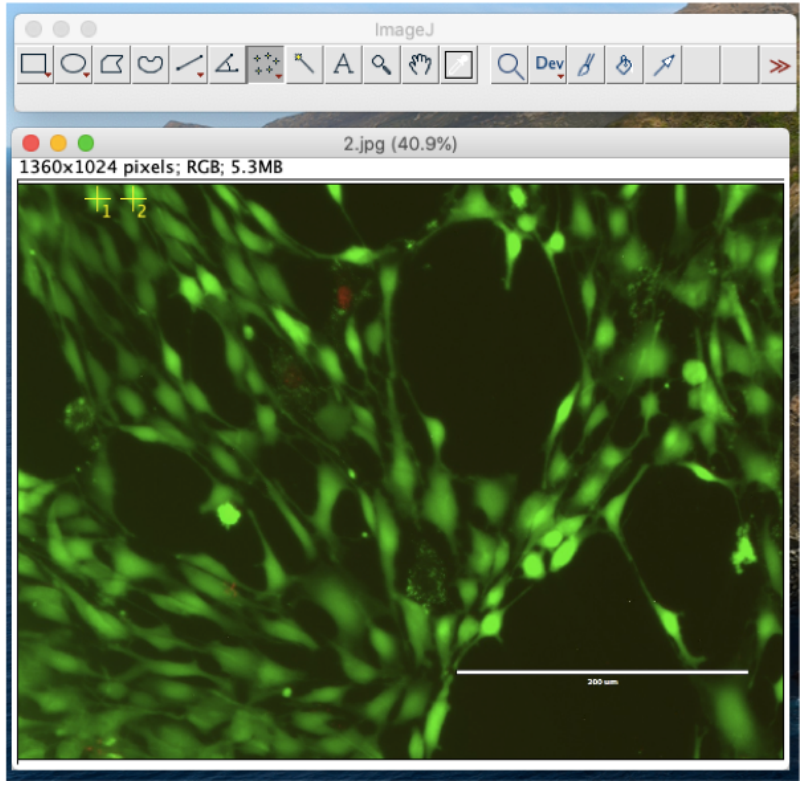
- Select the multipoint tool



  - This will allow us to count live cells using a yellow crosshare

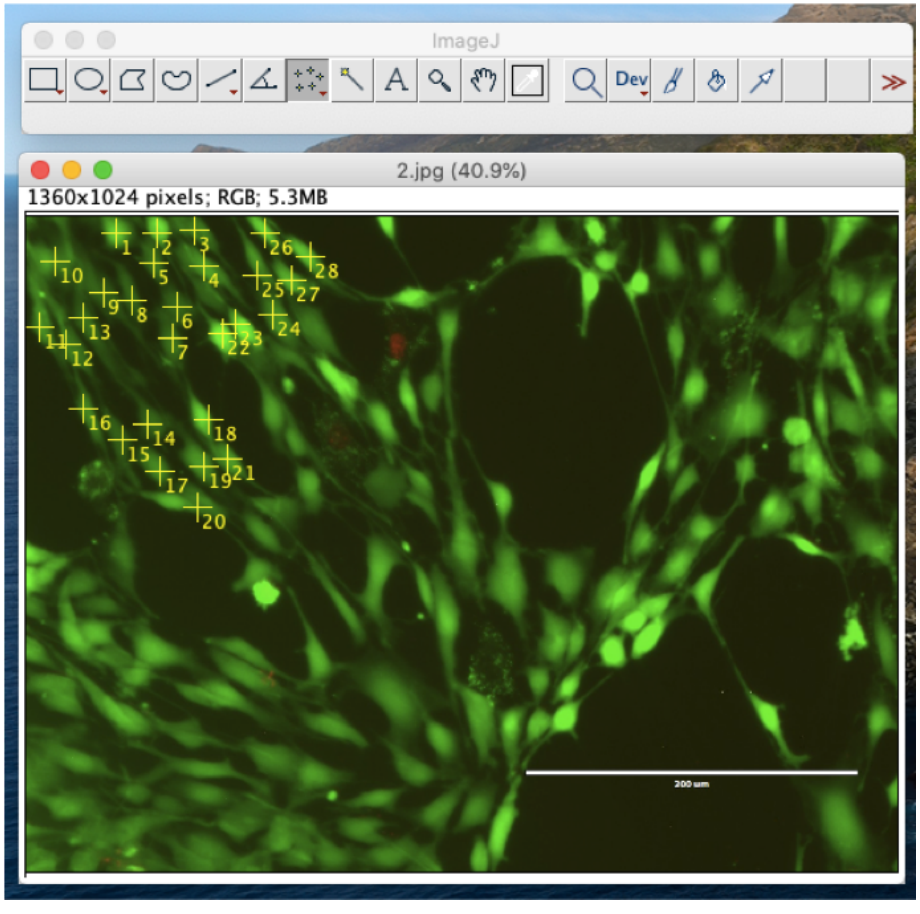
- Click on one of the live, green cells, then click on another live, green cell

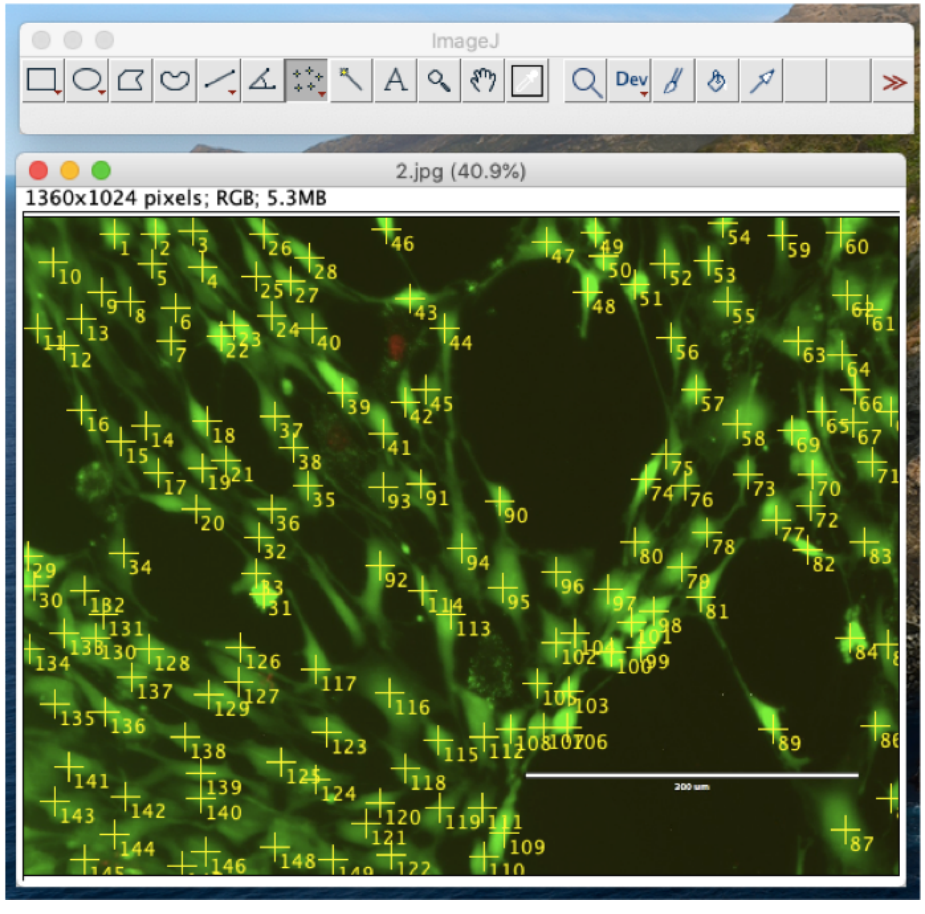




Protocol Discussion: Cell Counting

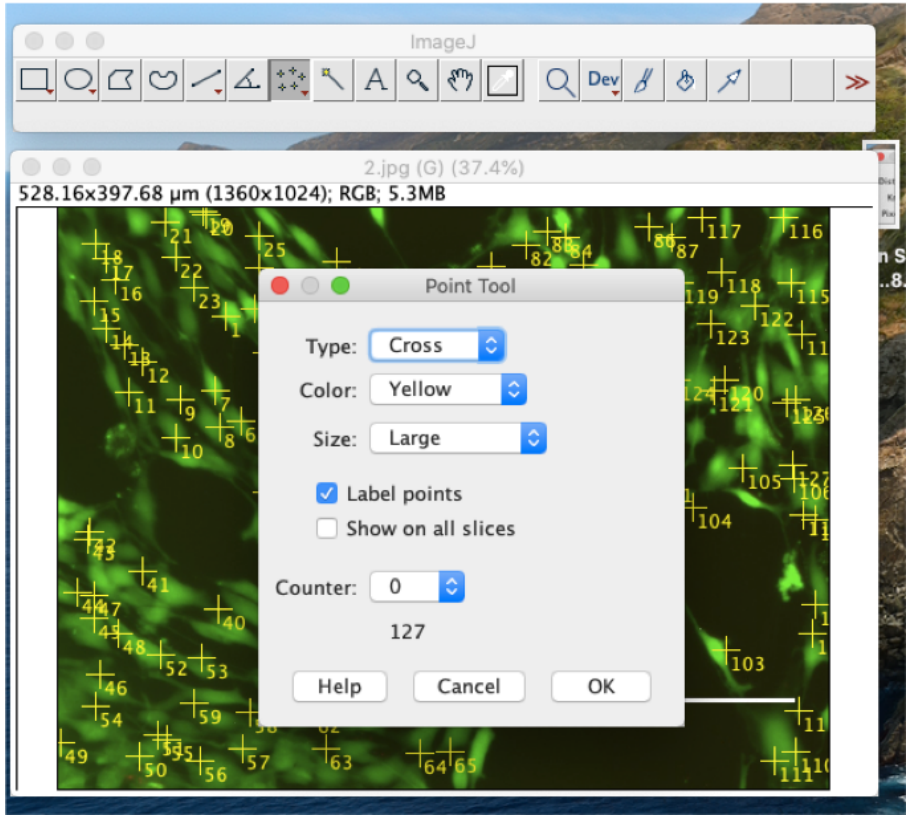
- Keep using the counter tool until you have selected all the live cells
- This will yield a total cell count for the field of view

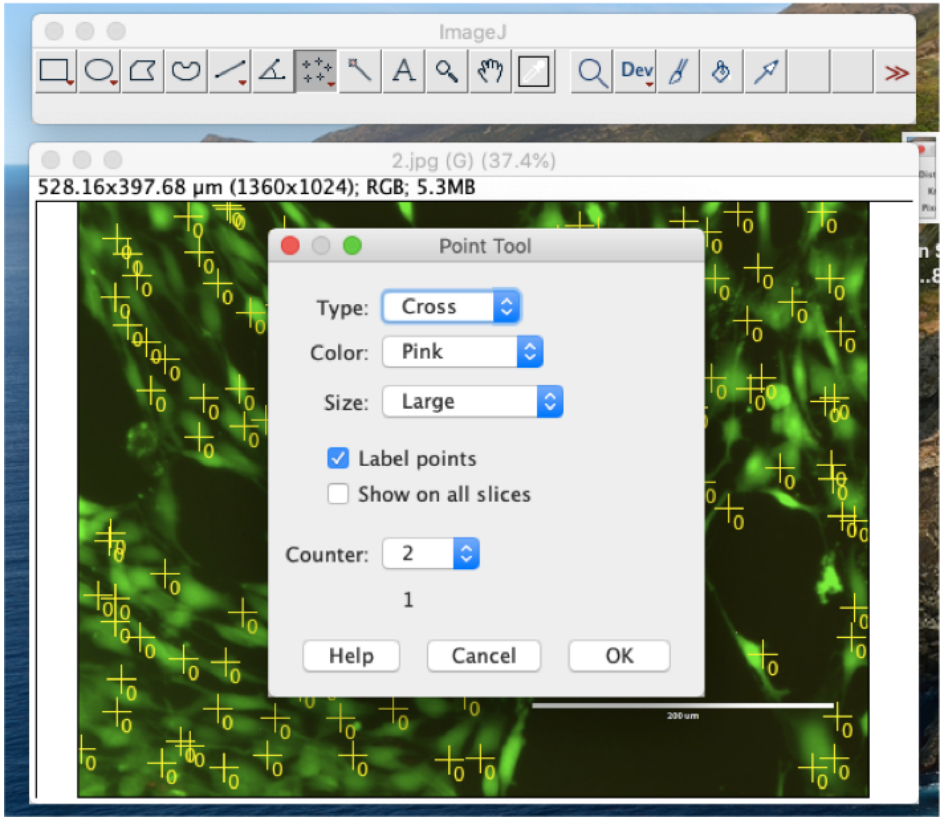




Protocol Discussion: Cell Counting

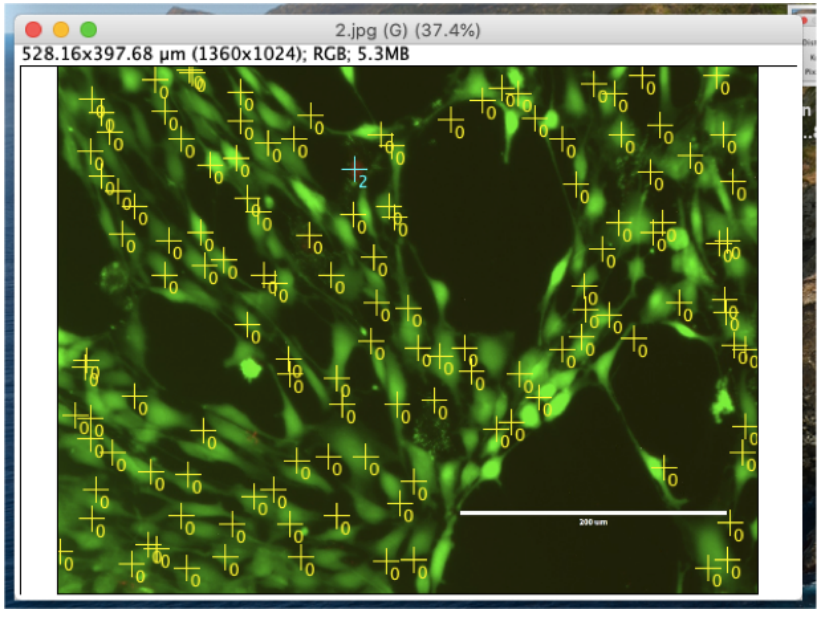
- Switch the counter color to count the dead cells
  - Edit → Options → Point Tool → Switch from Yellow to Pink





Protocol Discussion: Cell Counting

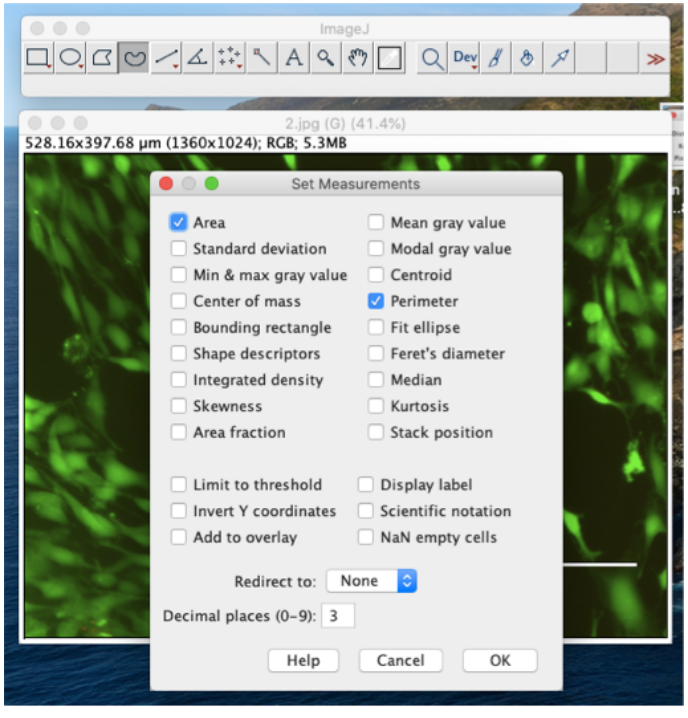
- Will now mark dead cells with this crosshair
  - You can also just do this manually (recommend for this example)



  - Record the # Live Cells (~150) and # Dead Cells (~1) on the worksheet and calculate the % Live Cells

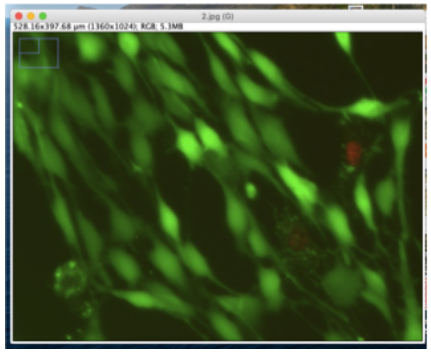
Protocol Discussion: Cell Morphology

- Now, we will collect the data necessary to determine average cell area, average cell perimeter, and average circularity
    - Analyze → Select Measurements → Check Area and Perimeter and uncheck everything else
    - We will use this information to calculate the circulatory in Excel later

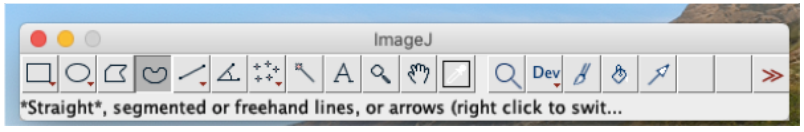


Protocol Discussion: Cell Morphology

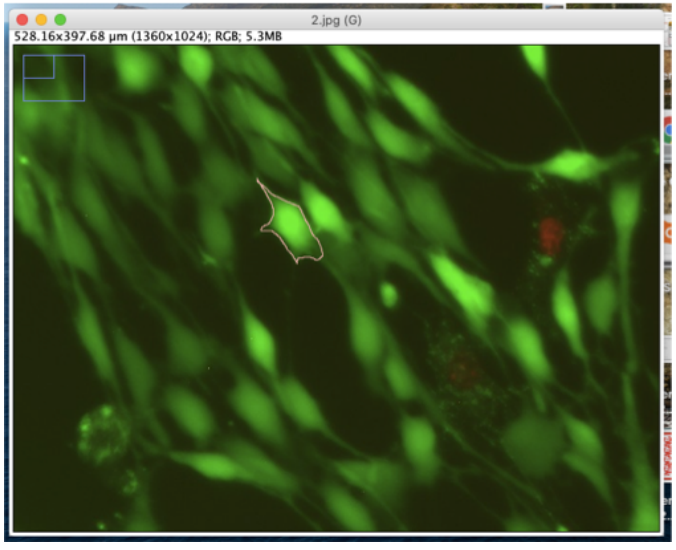
- Use the zoom tool to zoom into a cell area



- Click on the freehand selections tool

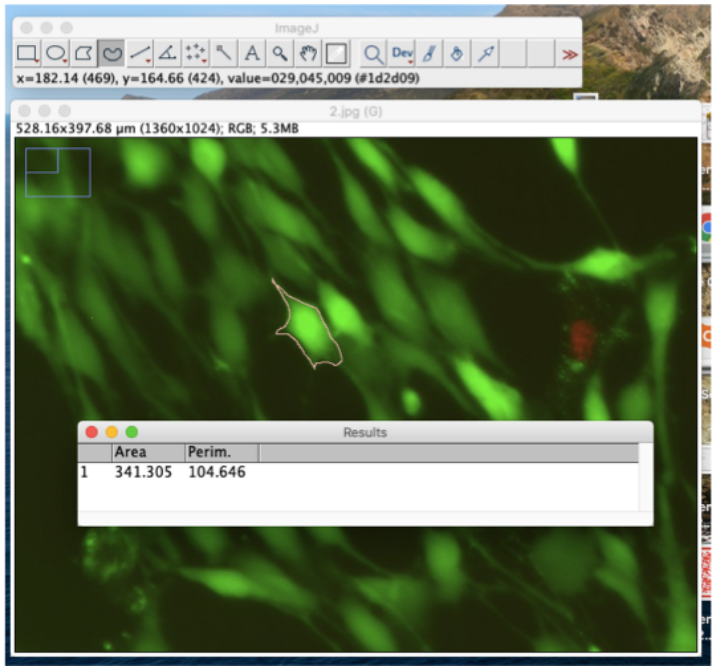


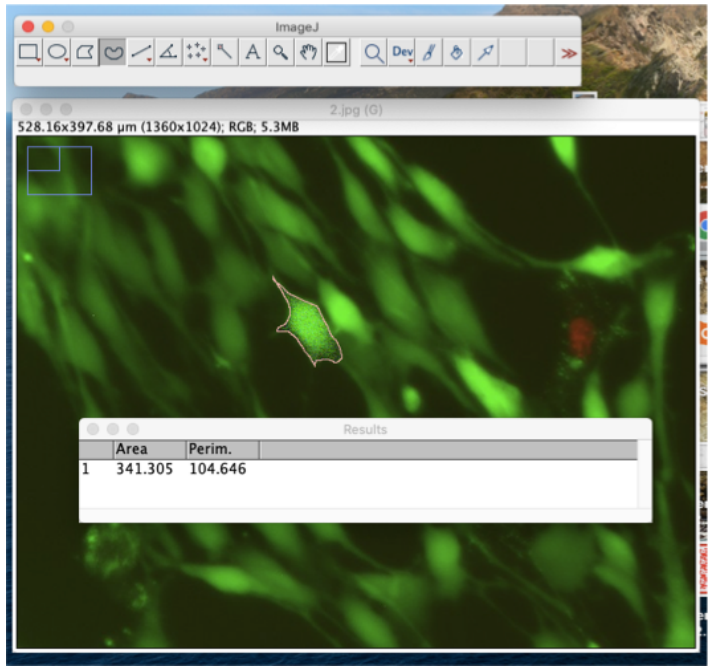
  - This will allow you to trace the cell perimeter



Protocol Discussion: Cell Morphology

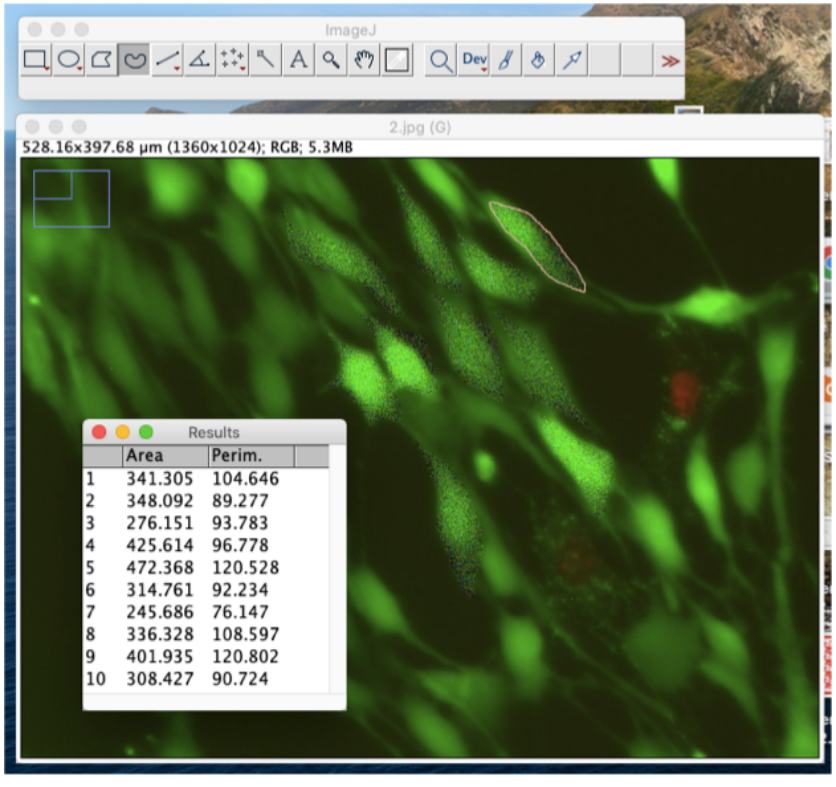
- Now, we will measure the area and the perimeter of the cell
    - Analyze → Measure
- To mark that we have counted the area of this cell, we will add noise to it
    - Process → Noise → Add Noise





Protocol Discussion: Cell Morphology

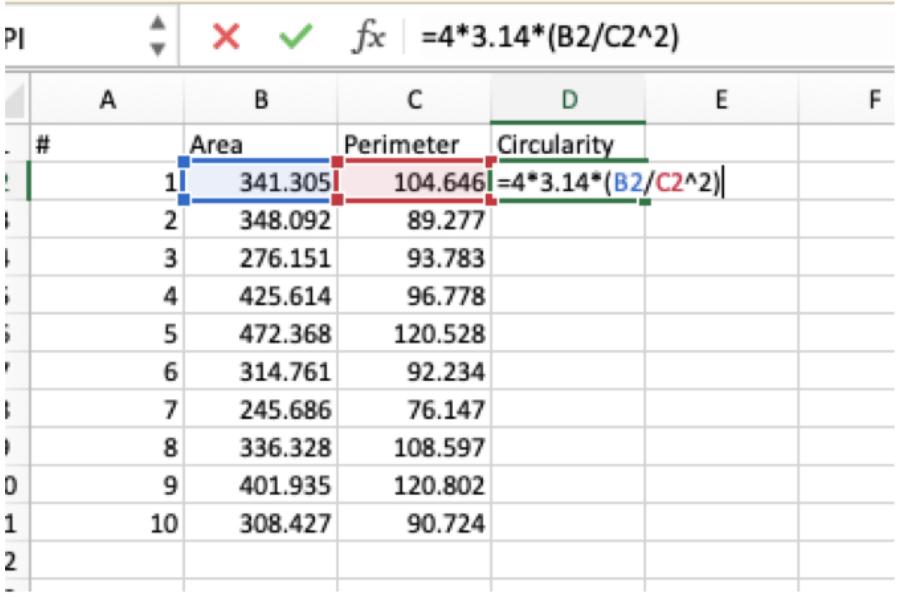
- Once you have done this for all the cells in the image, copy-paste the results table into an Excel or Google docs spreadsheet
- Label the columns as Cell Number, Area, Perimeter, and Circularity

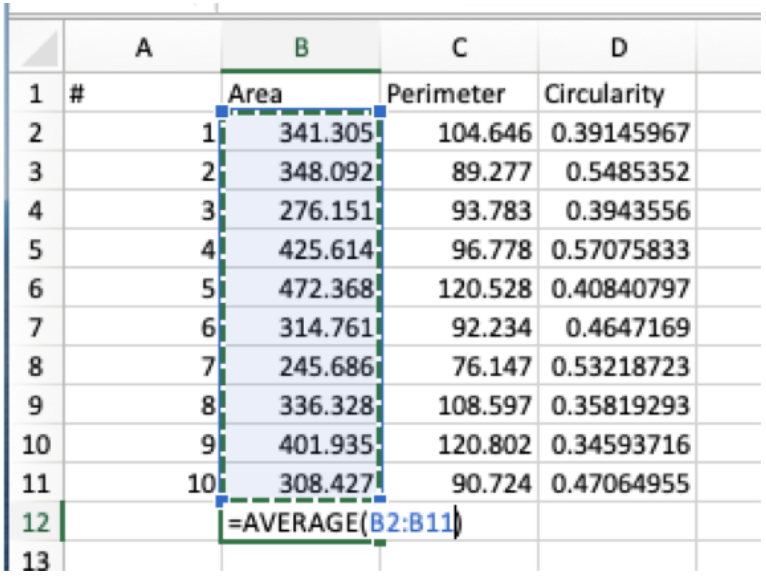


	A	B	C	D	E
1	#	Area	Perimeter	Circularity	
2	1	341.305	104.646		
3	2	348.092	89.277		
4	3	276.151	93.783		
5	4	425.614	96.778		
6	5	472.368	120.528		
7	6	314.761	92.234		
8	7	245.686	76.147		
9	8	336.328	108.597		
10	9	401.935	120.802		
11	10	308.427	90.724		
12					

Protocol Discussion: Cell Morphology

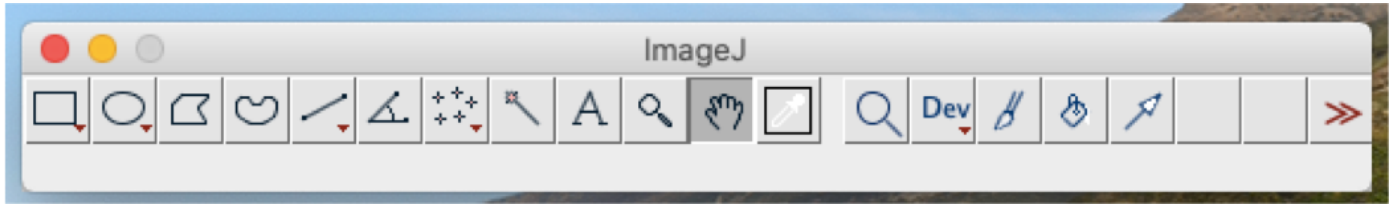
- Compute the circularity for each cell using the formula:
  - Circularity = $4\pi \frac{area}{perimeter^2}$
- Compute the average area, perimeter, and circularity using the AVERAGE function and record these valves on your worksheet





Breakout Rooms

- **Some Additional Notes**
  - Use the hand tool to move around your zoomed in image



- **Divide Up the Work!**
  - If you split up cell counting and cell tracing among members of your group, it will go a lot faster

Cell Counting Results

	Number of Live Cells	Number of Dead Cells	% Live Cells
2% DMSO Treatment	~150	~1	~99.3%
5% DMSO Treatment	~130	~9	~93.5%
10% DMSO Treatment	~120	~9	~93%
25% DMSO Treatment	~150	~30	83.3%

Cell Counting Results

How did increasing concentration of DMSO impact cell viability?

Researchers have studied cancer cells in low concentrations (<1%) of DMSO to study its effect as a chemotherapeutic. Why do you think researchers have been exploring the use of DMSO? Based on your finding above, what are some of your concerns with using DMSO as a chemotherapeutic?

Cell Counting Results

How did increasing concentration of DMSO impact cell viability?

Generally, increasing DMSO concentration decreases the percent of living cells, therefore decreasing the cell viability. It should be noted that 2% DMSO Treatment had little impact on the cell population, whereas 25% DMSO treatment resulted in the death of almost a fifth of the cell population.

Researchers have studied cancer cells in low concentrations (<1%) of DMSO to study its effect as a chemotherapeutic. Why do you think researchers have been exploring the use of DMSO? Based on your finding above, what are some of your concerns with using DMSO as a chemotherapeutic?

As briefly discussed in Module 5 and in Protocol Walkthrough 3, DMSO reacts with the plasma membrane of cells, creating pores in the membrane that disrupt the electrochemical barrier and lead to apoptosis. These researchers could wish to employ DMSO as a chemotherapeutic that disrupts the cell membranes of cancer cells, thereby inducing apoptosis and killing the cells. At low concentrations, DMSO kills few of the native 3T3 cells. However, at higher concentrations, DMSO damages a significant portion of the cell population. Researchers would need to conduct a pilot study examining if the benefits of DMSO treatment (killing cancer cells) outweigh the potential harm of treatment (killing host cells).

Cell Morphology Results

LIVE CELLS	Average Area	Average Perimeter	Average Circularity
2% DMSO Treatment	~350	~100	~0.45
5% DMSO Treatment	~330	~95	~0.50
10% DMSO Treatment	~340	~90	~0.55
25% DMSO Treatment	~260	~80	~0.55

DEAD CELLS	Average Area	Average Perimeter	Average Circularity
2% DMSO Treatment	~95	~39	~0.75
5% DMSO Treatment	~100	~40	~0.85
10% DMSO Treatment	~35	~25	~0.72
25% DMSO Treatment	~55	~28	~0.85

Cell Morphology Results

Compare your live cell and dead cell average area, perimeter, and circularity measurements. Why do you think the circularities are different? (Note: more circular cells have a circularity measurement closer to 1, and less circular cells have a circularity measurement closer to zero).

What are the limitations of cell counting for this particular sample using this method? What modifications can be made to the procedure to enhance the data acquisition process and quality of the data?

Cell Morphology Results

Compare your live cell and dead cell average area, perimeter, and circularity measurements. Why do you think the circularities are different? (Note: more circular cells have a circularity measurement closer to 1, and less circular cells have a circularity measurement closer to zero).

On average, live cell circularity should be lower than dead cell circularity (dead cells should be more circular). This is because the dead cells are no longer carrying out their function (migration toward wound sites) and thus no longer uphold the elongated fibroblast shape.

What are the limitations of cell counting for this particular sample using this method? What modifications can be made to the procedure to enhance the data acquisition process and quality of the data?

This method of cell counting is very tedious, requiring researchers to spend many hours examining images to collect meaningful data. In addition, this manual method of cell counting is highly variable researcher to researcher, as the recorded area and perimeter are a function of individual mouse movement and accuracy in cell tracing. This makes it easy to manipulate the final values to get to a favorable result (ex: greater circularity in dead cells than live cells. Furthermore, for this particular sample, the relatively low number of dead cells makes it difficult to extrapolate this data to a larger cell population.

This process can be enhanced by using a plugin that can use color threshold valves to count and measure different cell types. In addition, these images can be processed by a specialized MATLAB code and output the desired average area, perimeter, and circularity values, allowing researchers to process large quantities of image information using the same code.