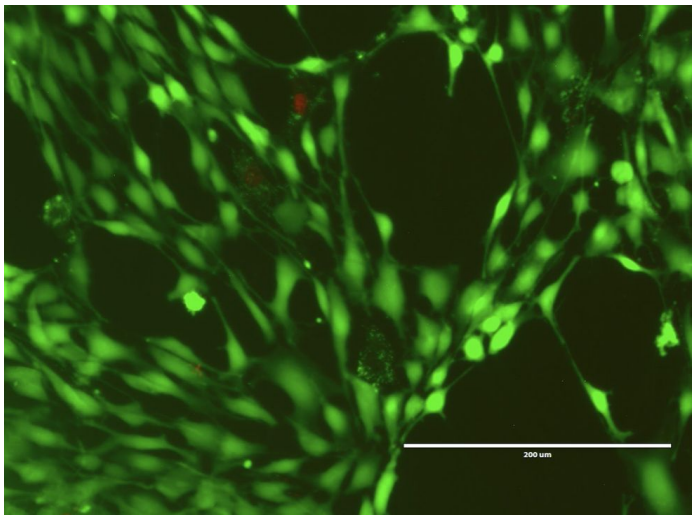
Protocol Discussion 3: Cell Viability Analysis

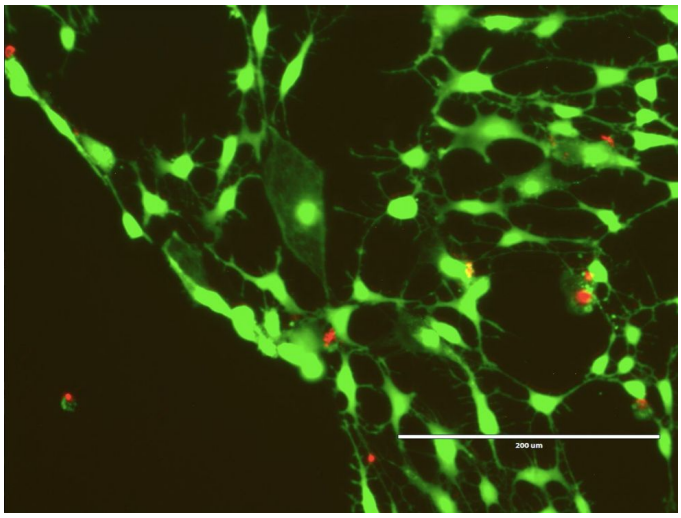
In Tuesday's protocol walkthrough, we discussed a cell viability experiment where 3T3 cells were incubated with different concentrations of DMSO and stained with calcein and ethidium homodimer (Live-Dead Stain). In this protocol discussion, we will be utilizing ImageJ to quantify the number of live and dead cells and differences in their morphology.

The figure below depicts 3T3 cells treated with 2%, 5%, 10%, and 25% DMSO under the GFP and RFP fluorescent microscope filters.

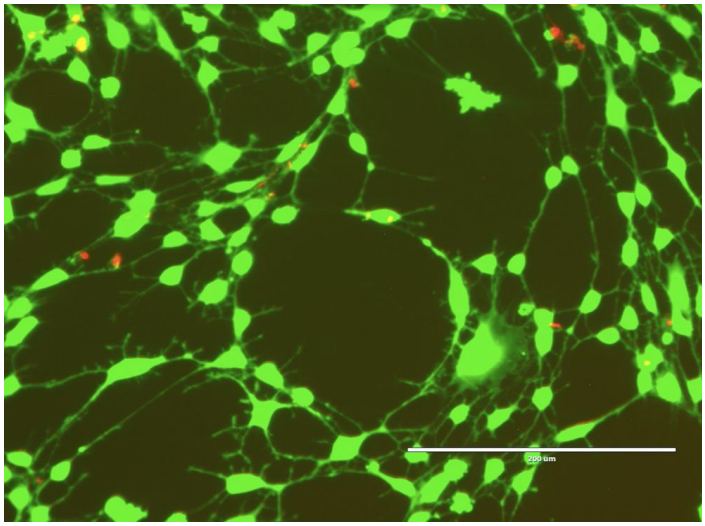
2% DMSO



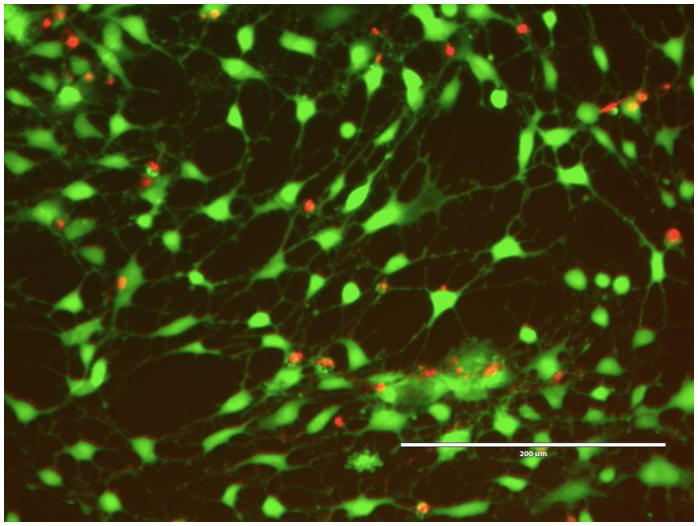
5% DMSO



10% DMSO



25% DMSO



Overview of Manual Cell Counting

1. Download the .jpg files from the Cell Team site
2. Open the “2% DMSO Treatment” .jpg file in ImageJ
3. Resize the image to the area that you want to work with
4. Calibrate the pixel size to the 200 um bar in the image using the “Set Scale” feature
5. Use the multipoint tool to mark live cells in yellow and record the live cell number in the table
6. To count the dead cells, you can change the multipoint counter color, re-open the image and use the multipoint counter, or just count them without the multipoint tool (recommended method for this set of data, as there are so few dead cells)
7. Repeat for the other three images (you can divide up this work in breakout rooms)

	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%**

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).

Quantifying Cell Morphology

1. Open the “2% DMSO Treatment” .jpg file in ImageJ
2. Select the area and perimeter measurements
3. Zoom into the image using the magnifying glass tool
4. Select the freehand tool and trace one of the live cells
5. Click Analyze -> Measure to record the area and perimeter of that cell
6. Click Process -> Noise -> Add Noise. This will help you keep track of the cells you have already counted
7. Repeat Steps 5-6 for all the live cells in the image
8. Copy-paste the live cell data into an Excel Sheet
9. Compute the circularity of all the cells, average circularity, average area, and average cell perimeter, and record you values below.
    - Note: Circularity = $4*pi*(area/perimeter^2)$
    - A circularity of one means that the cell is a perfect circle
10. Repeat Steps 5-9 for all the dead cells in the image
    - Note: this process is very tedious, so divide up the work in your breakout rooms!

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**

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.