Lab 2: Immunostaining

BMES Cell Team

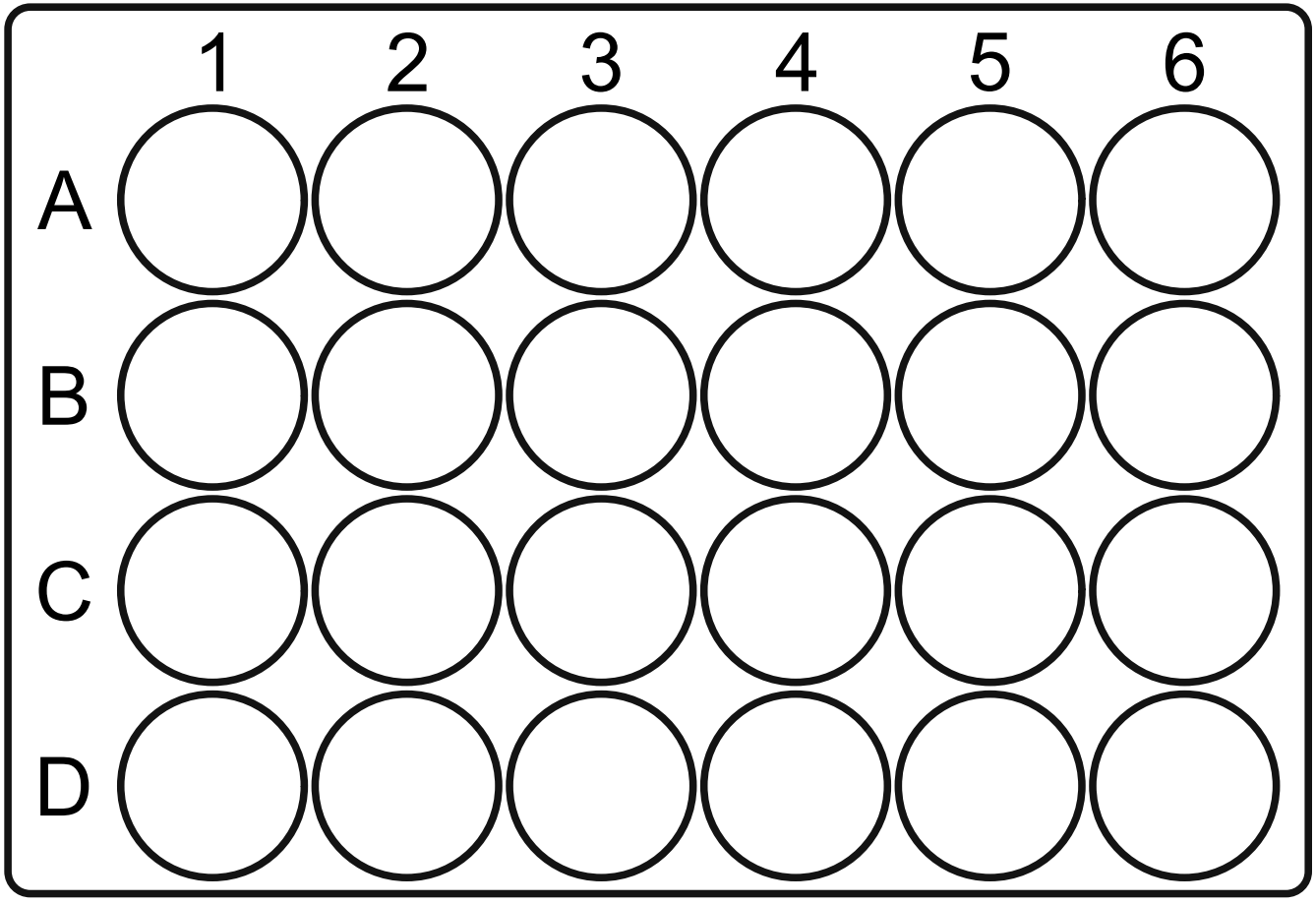
Winter 2021

Outline

- Experimental Protocol Walkthrough
- Data analysis activity with ImageJ and Python

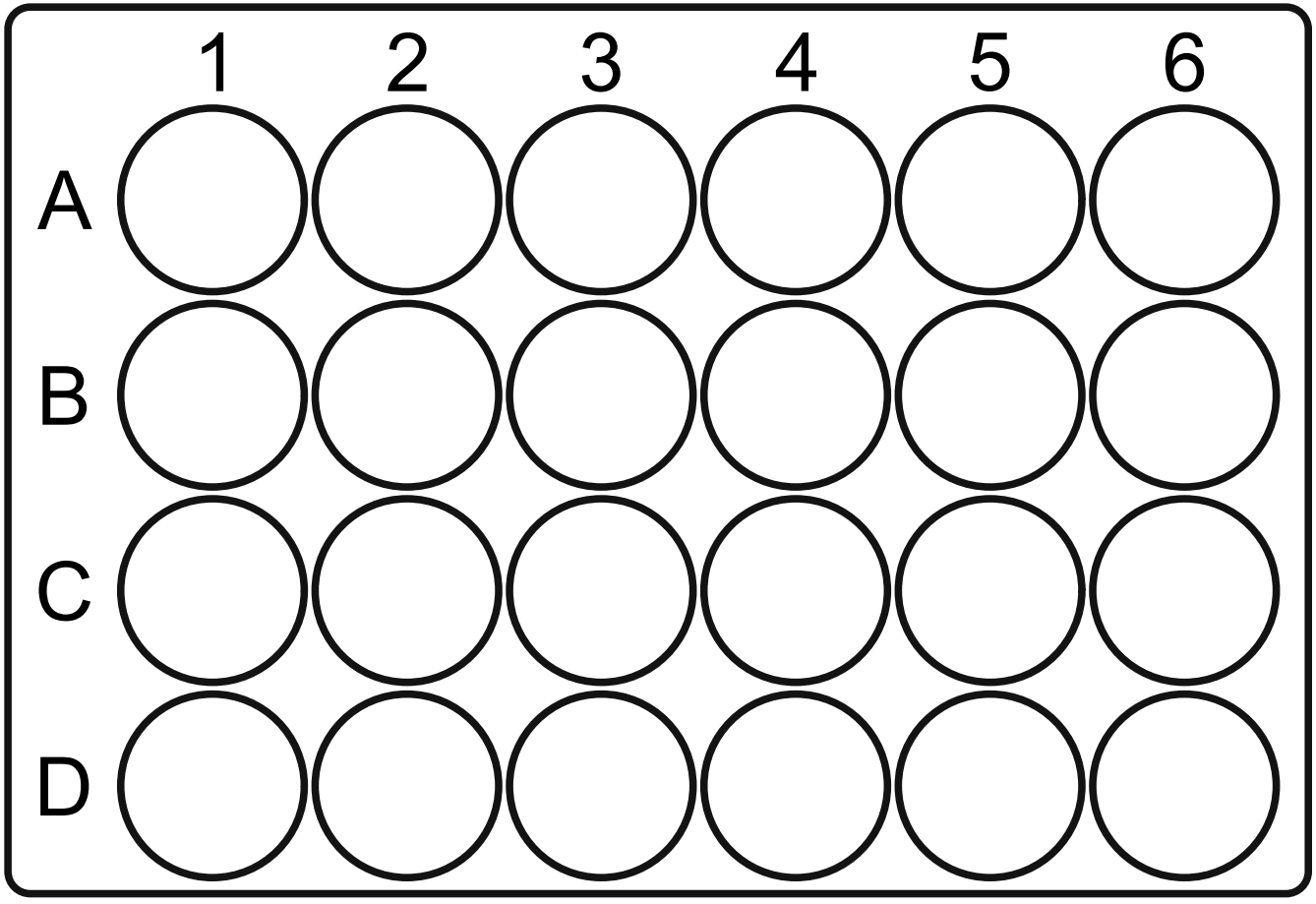
Experimental Setup

3T3 Cells



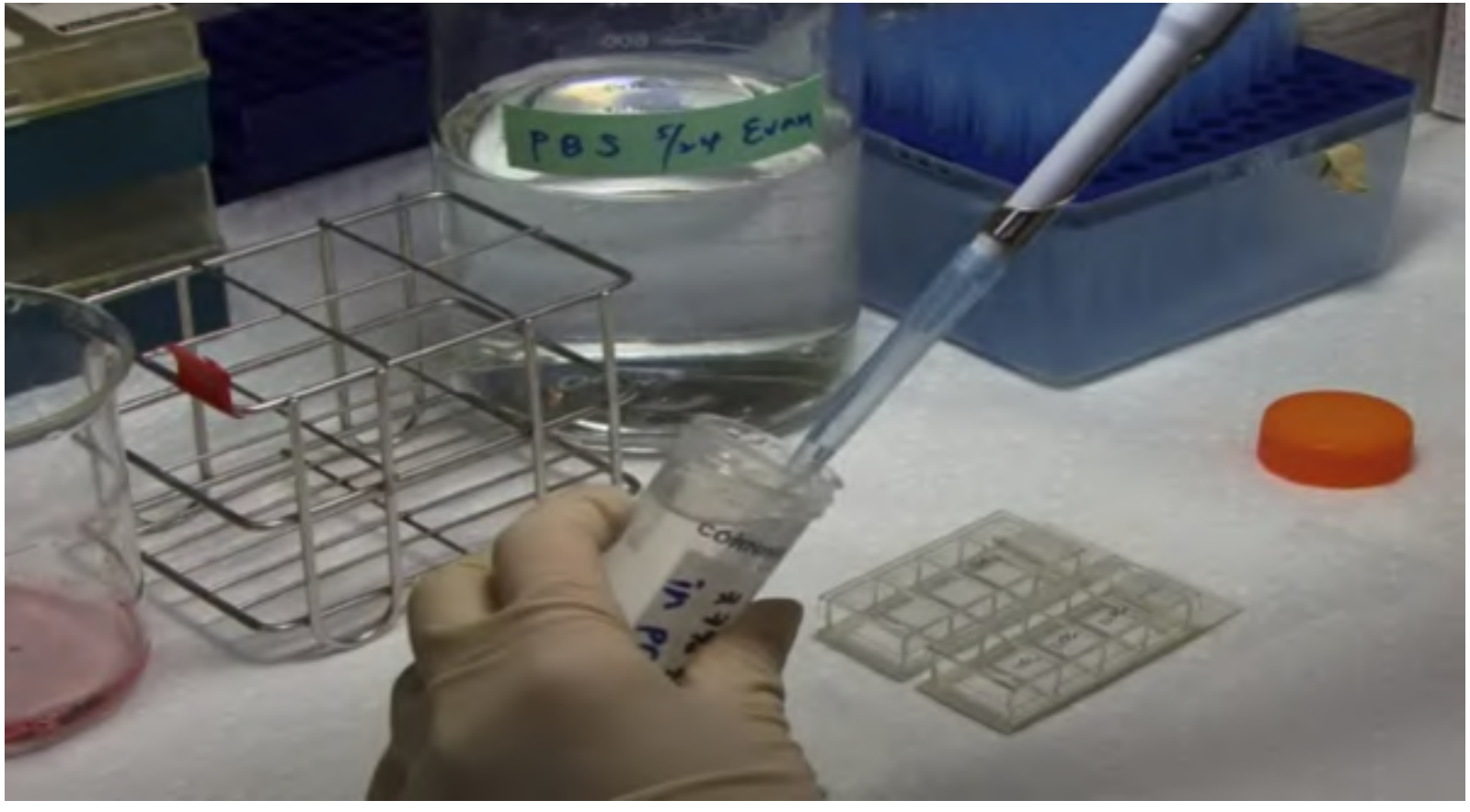
Experimental Setup

Neural Stem Cells



1. Fix Cells

Fix cells with 4% paraformaldehyde (PFA)



500µL per well

1. Fix Cells

Since PFA is toxic, we want to perform this in the fume hood

1. Fix Cells

Incubate for 15 minutes

2. Wash Cells

Add 500µL of PBS-Tween per well

Do this three times for 5 minutes each

2. Wash Cells

Add 500µL of PBS-Tween per well

Do this three times for 5 minutes each

2. Wash Cells

Add 500µL of PBS-Tween per well

Do this three times for 5 minutes each

2. Wash Cells

What is the purpose of PBST?

3. Permeabilization

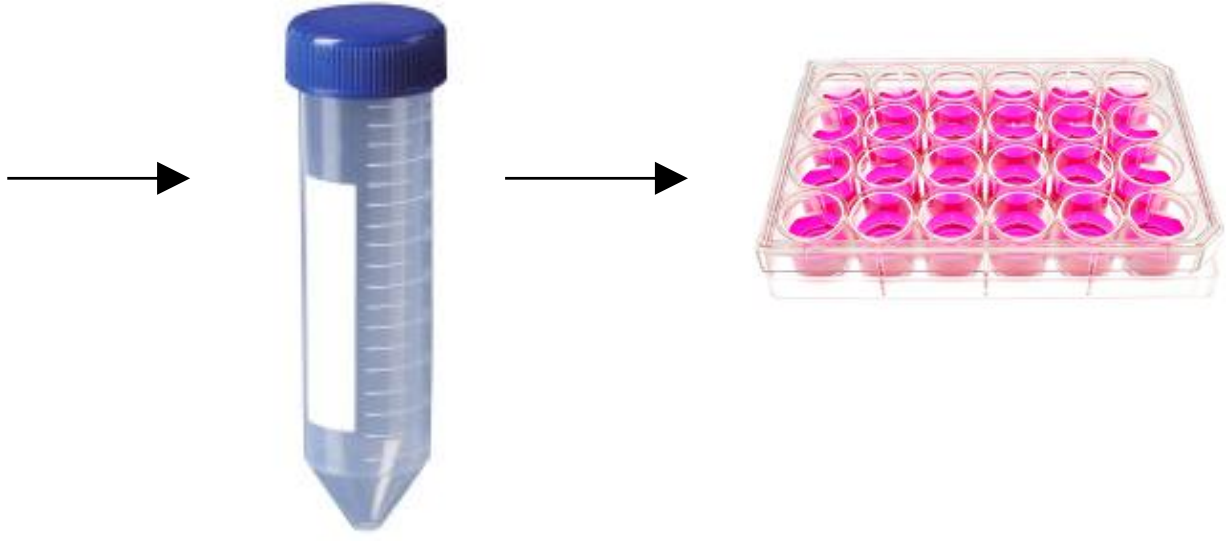
Add 500µL of Triton per well

3. Permeabilization

What is the purpose of Triton?

4. Wash Cells (again)

Add 500µL of PBS-Tween per well



Do this three times for 5 minutes each

5. Protein Blocking

Block with 500µL of 5% BSA-PBS-Tween for one hour at 25˚C

5. Protein Blocking

What is the purpose of an orbital shaker?

6. Primary Antibody Blocking

Dilute rabbit anti-SOX2 with PBS-Tween 20 at a 1:200 ratio

6. Primary Antibody Blocking

Add 500µL of the rabbit anti-SOX2 solution to all but one well

Incubate overnight at 4˚C

6. Primary Antibody Blocking

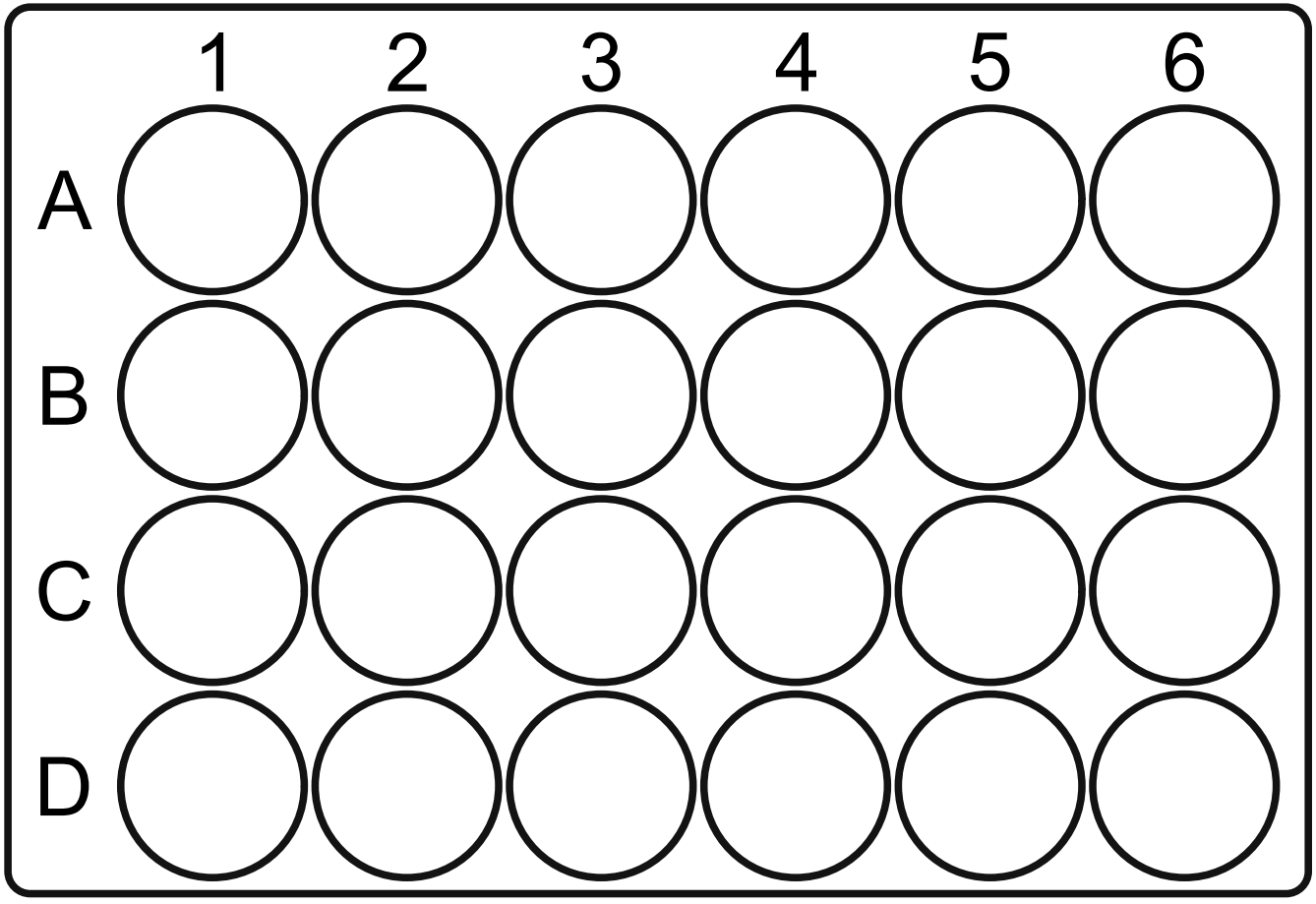
Math Review.

Let's say we want 500µL of solution per well and we have 10 wells. We also want a dilution factor of 1:200 Sox2 to PBS-Tween 20.

i. How much PBS-Tween 20 should we start with?
ii. How much Sox2 should we add to create the desired dilution factor?

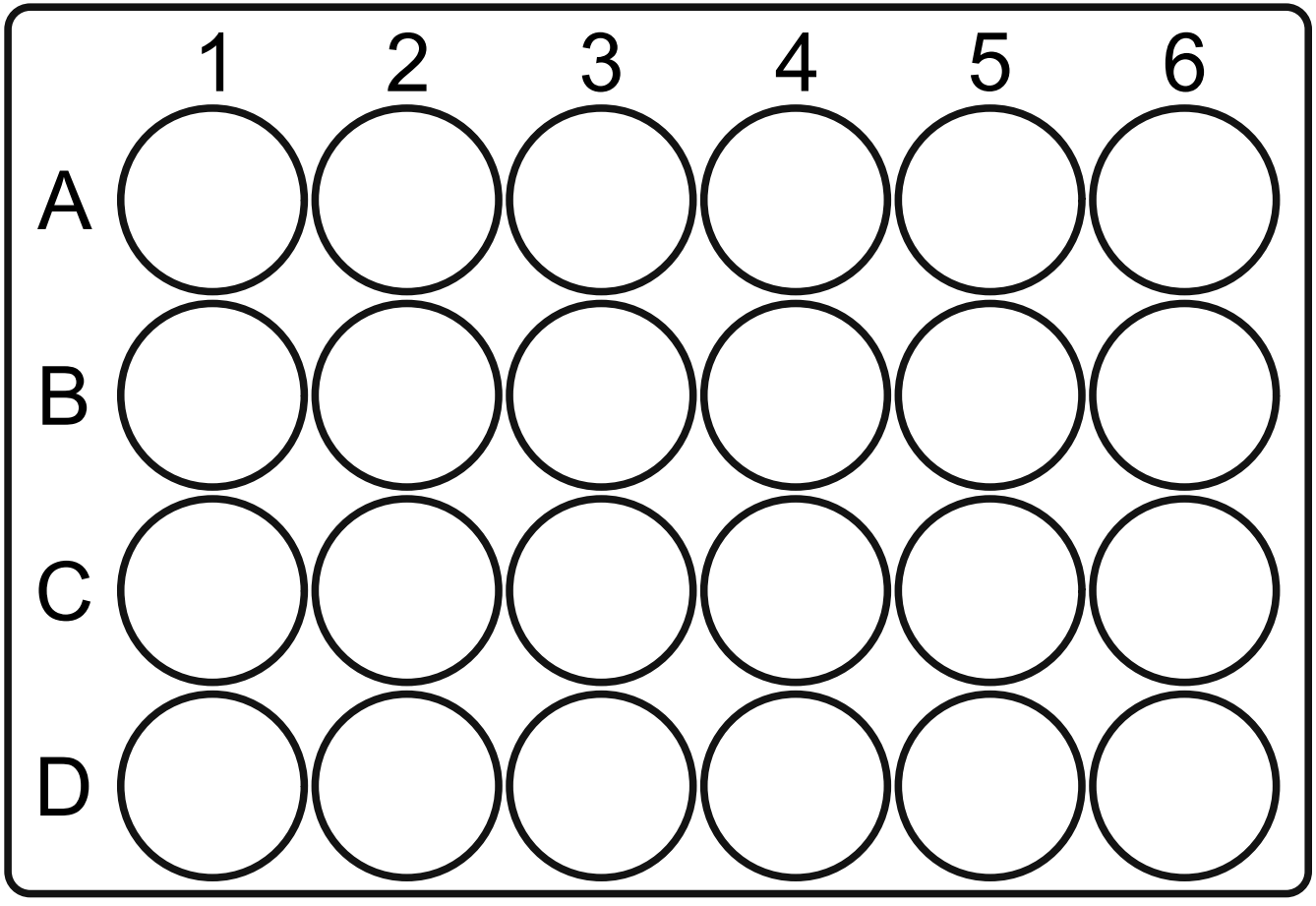
6. Primary Antibody Blocking

3T3 Cells



6. Primary Antibody Blocking

Neural Stem Cells



1. Wash Cells

Add 500µL of PBS-Tween per well

Do this three times for 5 minutes each

2. Secondary Antibody Blocking

Add 500µL of Hoechst and Goat anti-Rabbit solution to the **control**

Both diluted with PBS-T at 1:1000 ratio

2. Secondary Antibody Blocking

Add 500µL of Hoechst, Goat anti-Rabbit, AND Phalloidin solution to the **wells containing primary antibodies**

Hoechst and Goat anti-Rabbit diluted with PBS-T at 1:1000 ratio
Phalloidin diluted with PBS-T at 1:500 ratio

2. Secondary Antibody Blocking

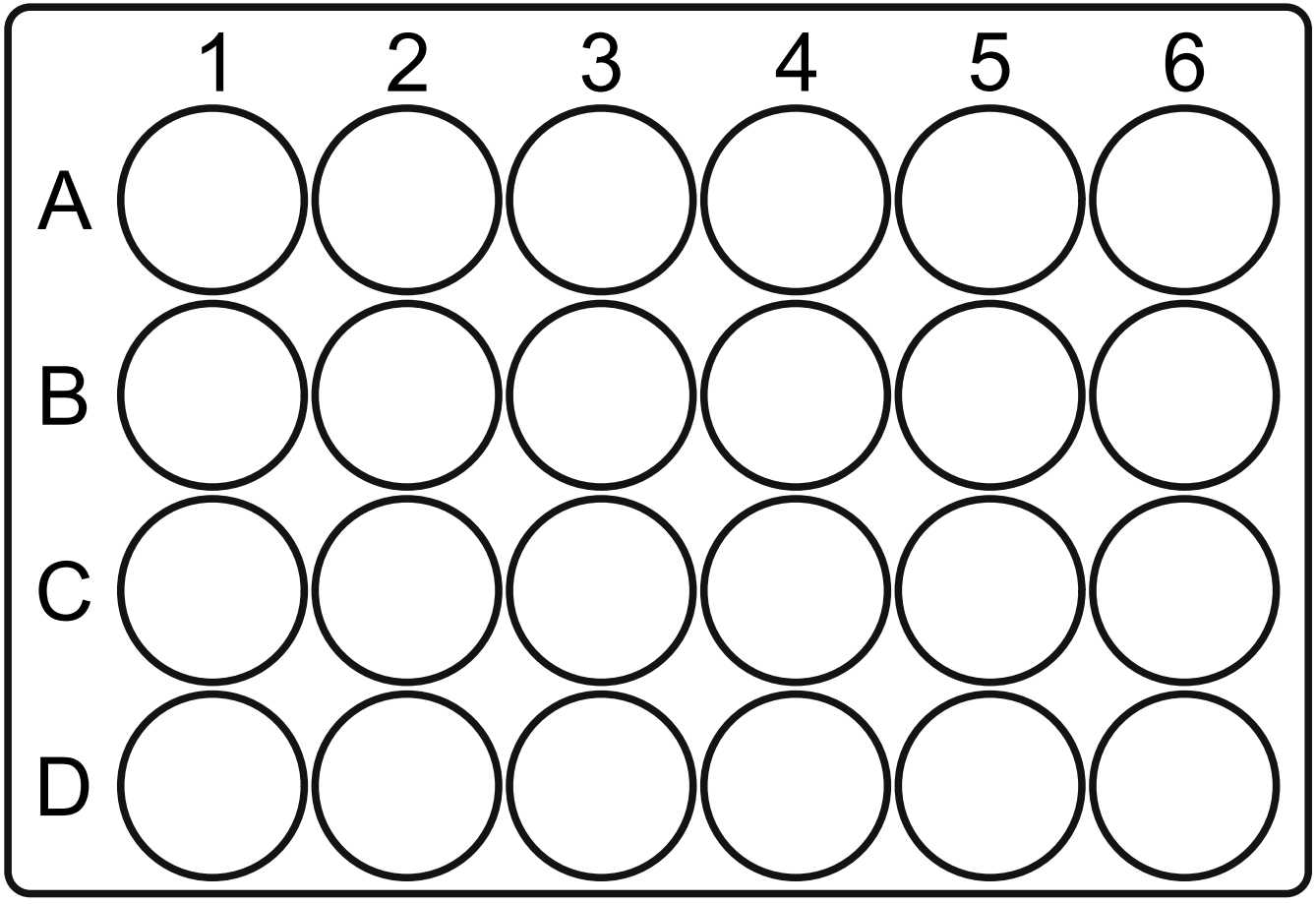
Math Review.

Let's say we want 500µL of solution per well and we have 10 wells. We also want a dilution factor of 1:1000 Hoechst to PBS-Tween 20, 1:1000 Goat anti-Rabbit to PBS-Tween 20, and 1:500 Phalloidin to PBS-Tween 20.

i. How much PBS-Tween 20 should we start with?
ii. How much Hoechst should we add to create the desired dilution factor?
iii. How much Goat anti-Rabbit should we add to create the desired dilution factor?
iv. How much Phalloidin should we add to create the desired dilution factor?

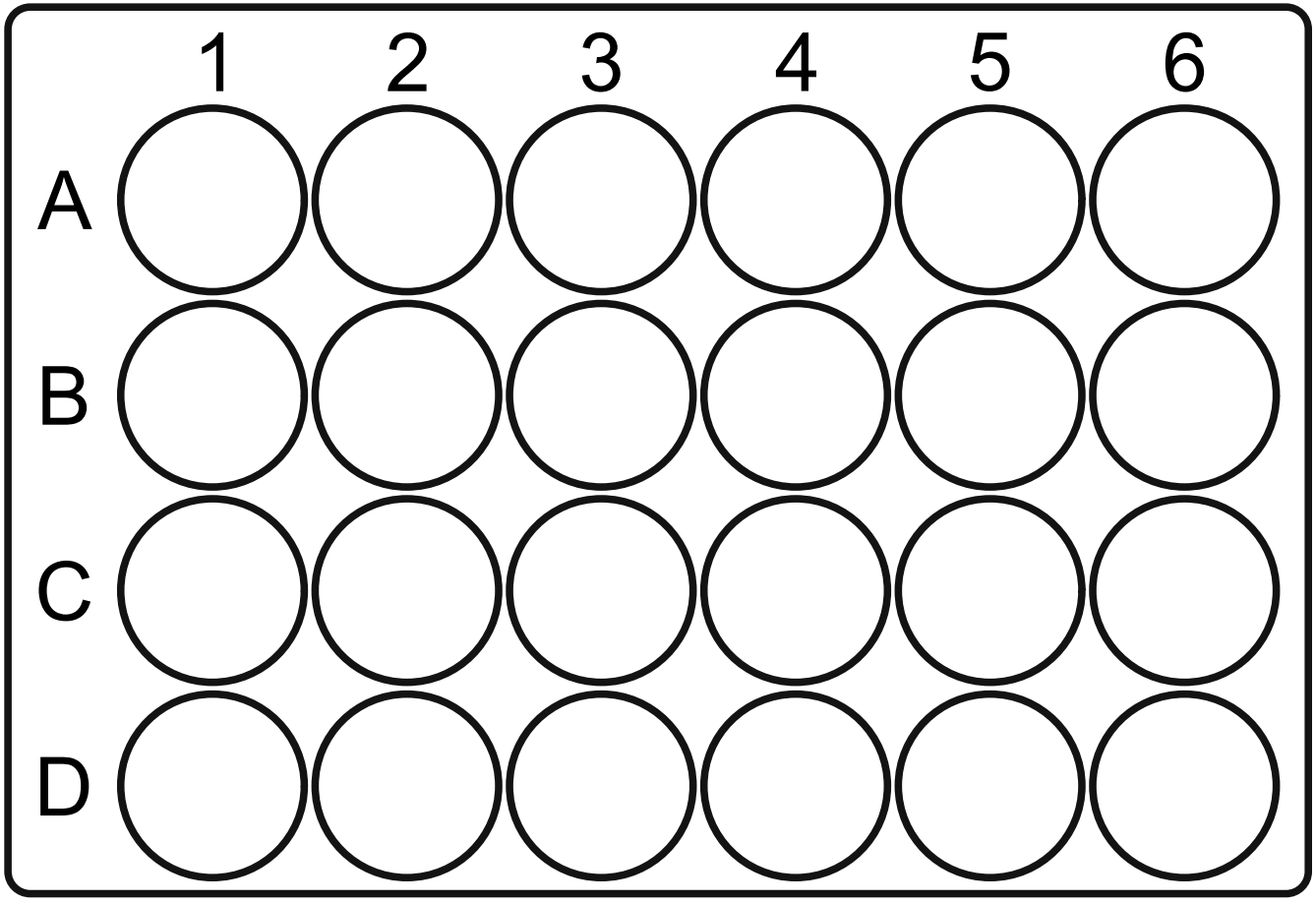
2. Secondary Antibody Blocking

3T3 Cells



2. Secondary Antibody Blocking

Neural Stem Cells



2. Secondary Antibody Blocking

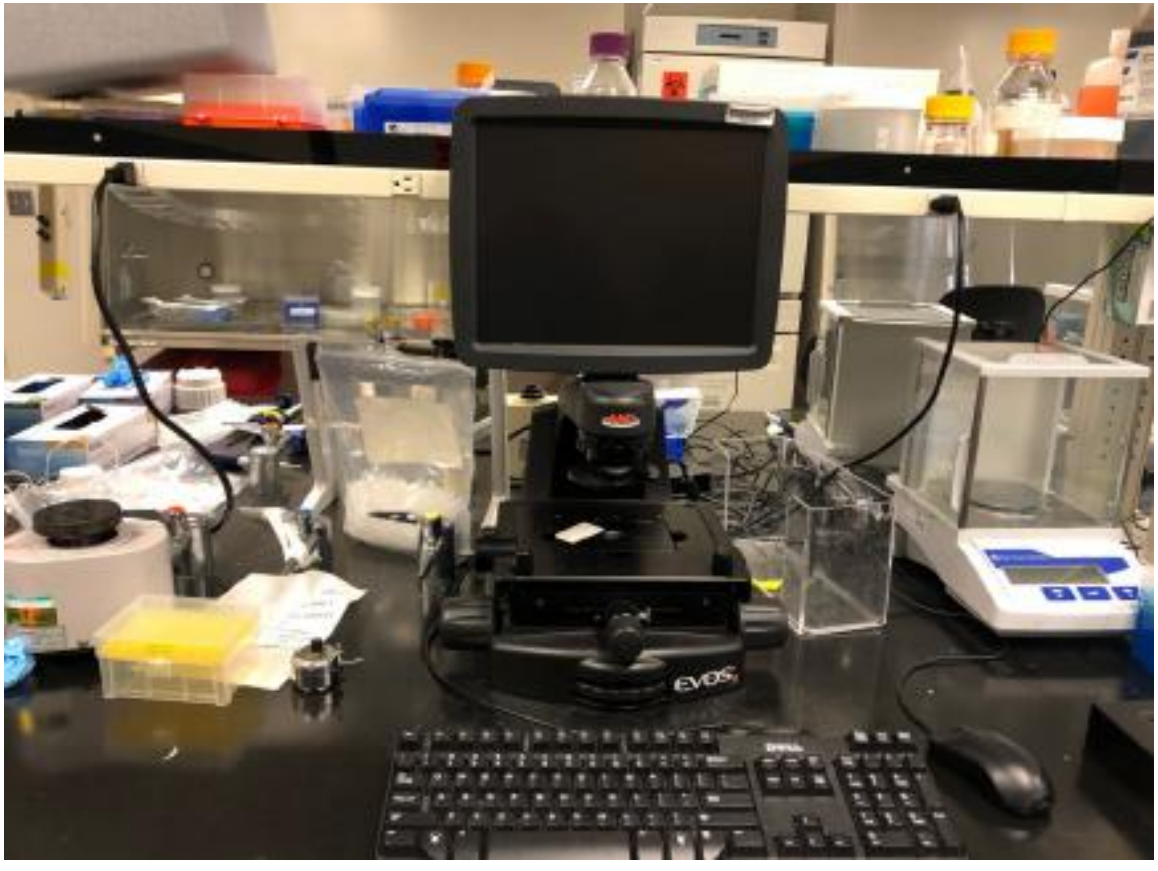
Wrap in foil and place in orbital shaker for 1 hour

2. Secondary Antibody Blocking

What is the purpose of wrapping the plate with foil?

3. Imaging

Use EVOS microscope to image

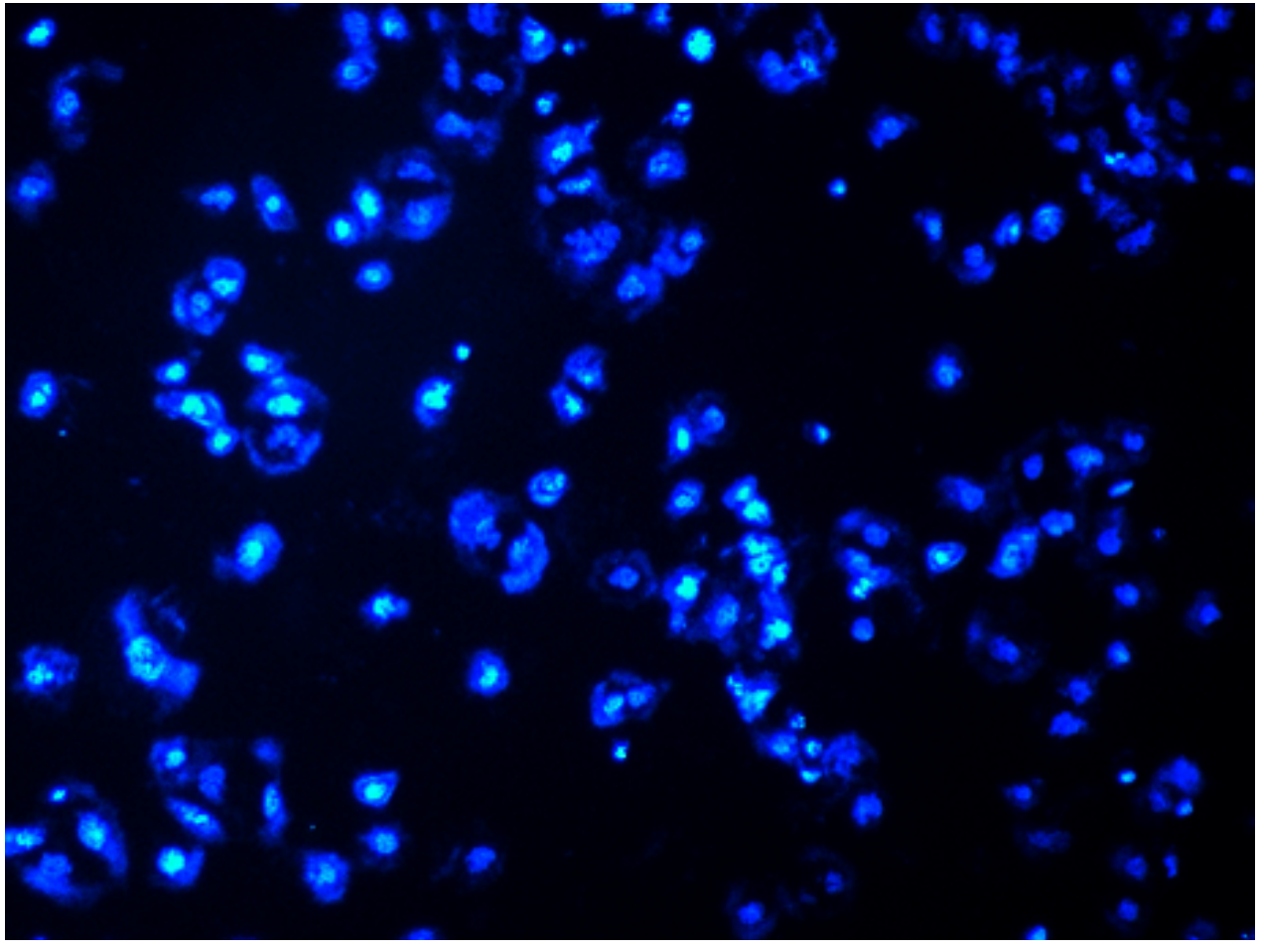


Important Remarks:

1. Turn off lights to prevent photobleaching
2. Look at cells under 4x objective

3. Imaging

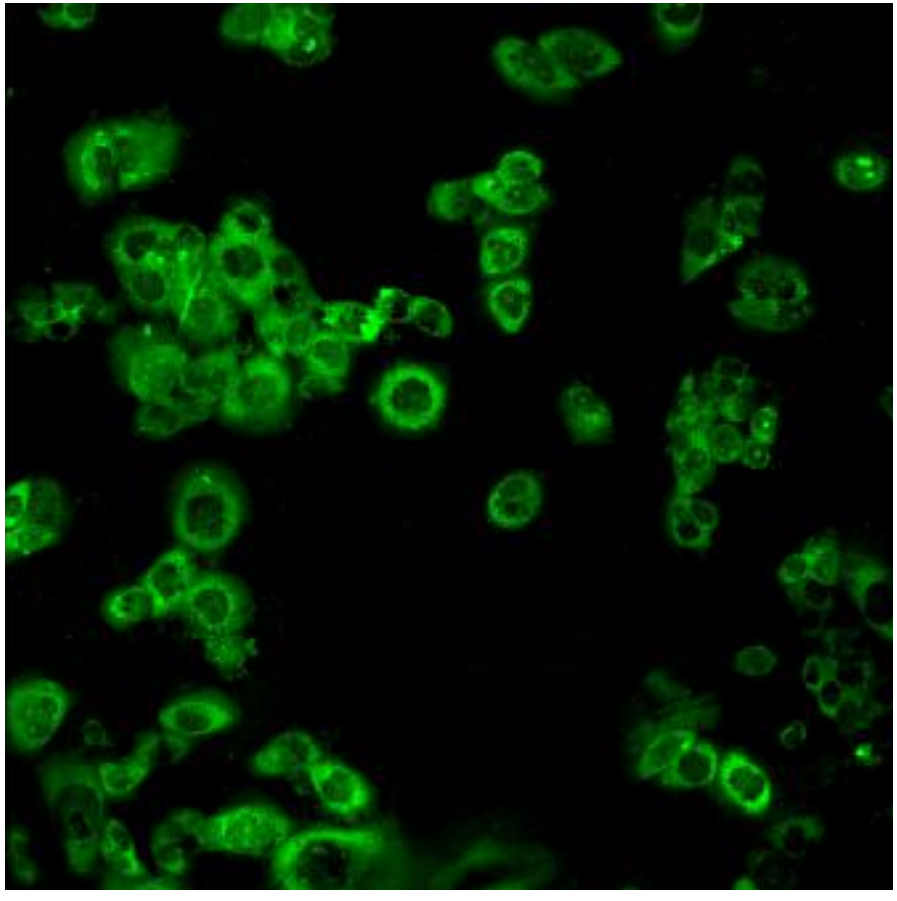
Change microscope setting to view fluorescence:



DAPI: Hoechst

3. Imaging

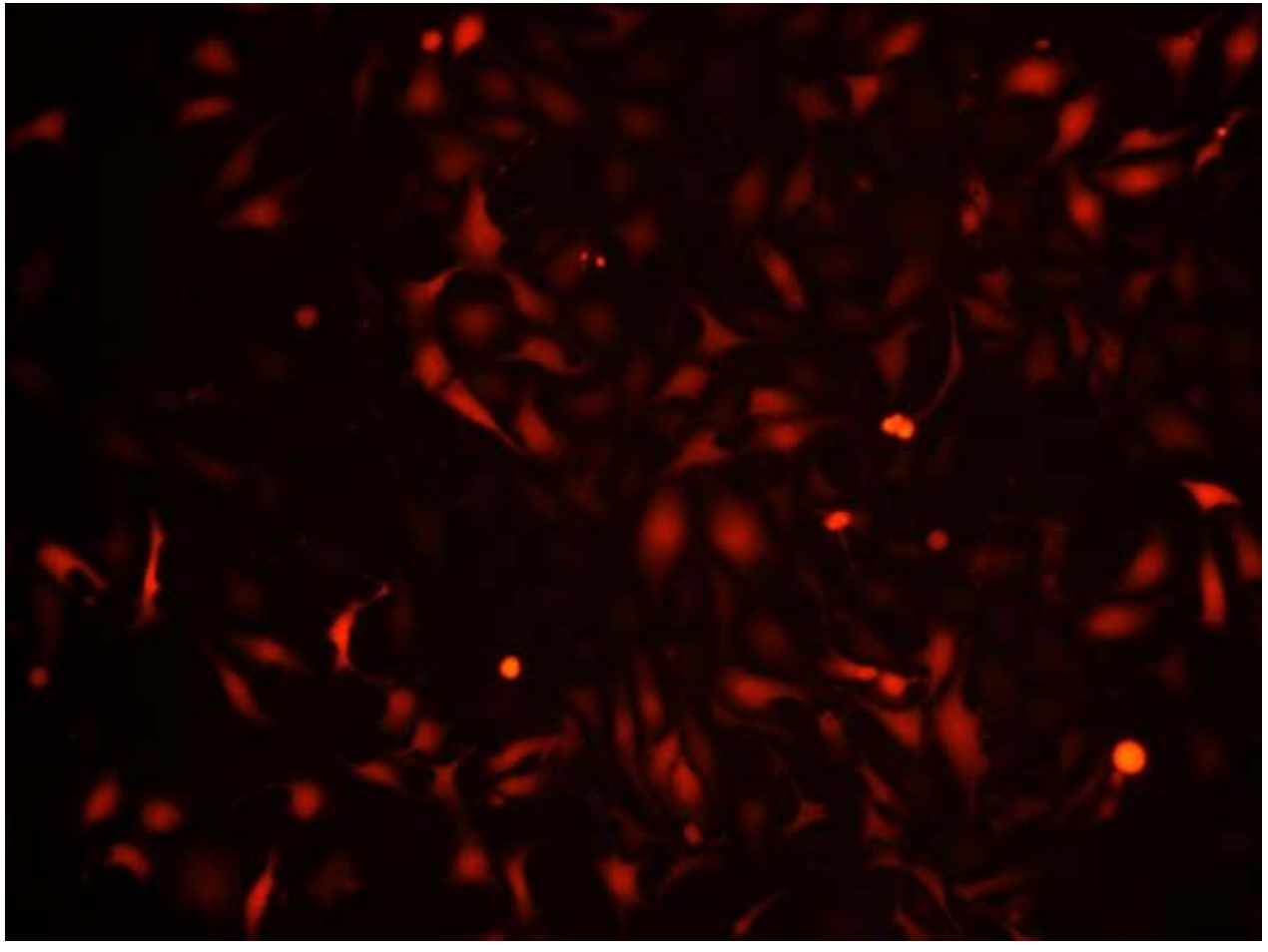
Change microscope setting to view fluorescence:



GFP: SOX2

3. Imaging

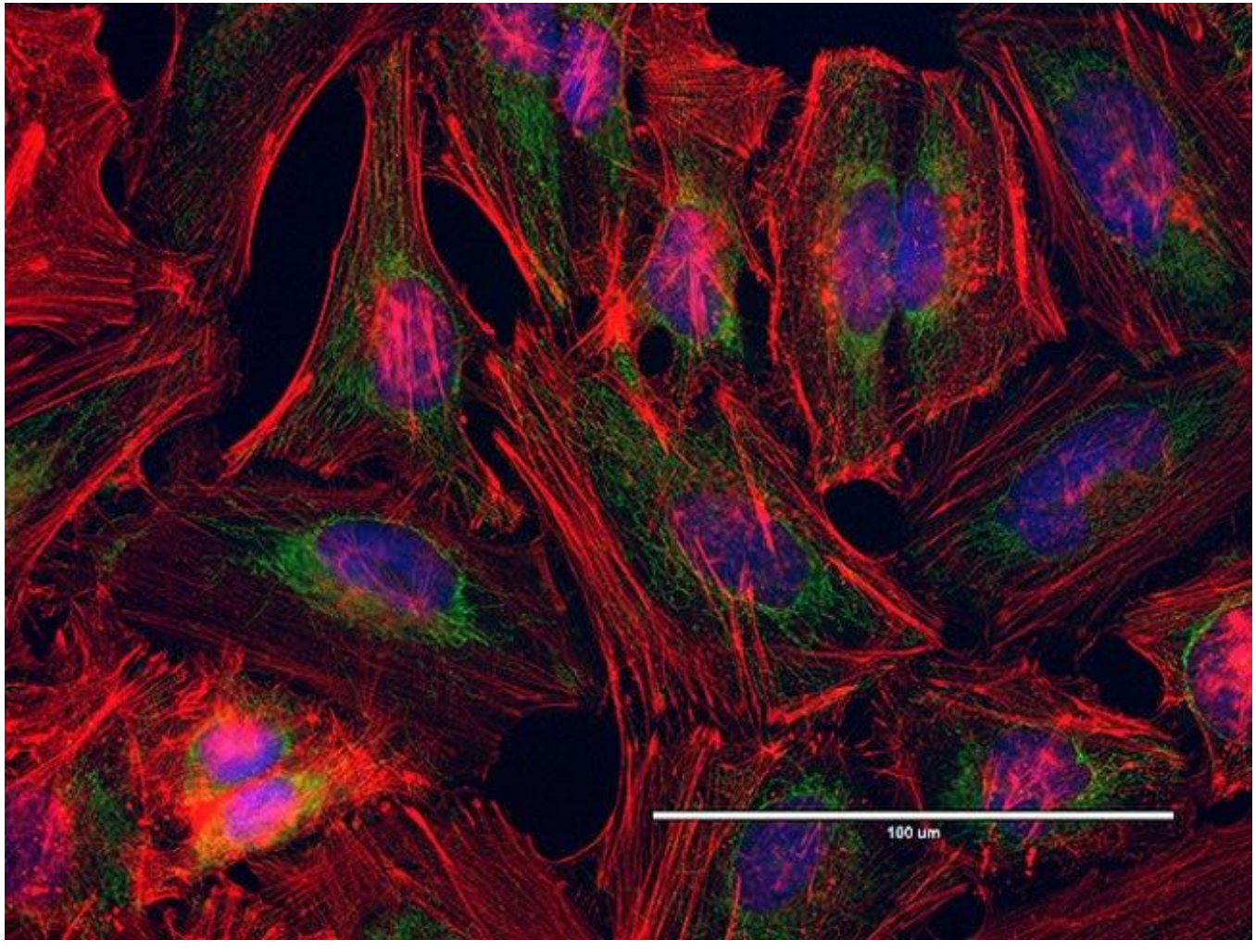
Change microscope setting to view fluorescence:



RFP: Actin (Phalloidin)

3. Imaging

Under 40x, you can view the cytoskeletal morphology



Video and Tips

On your own time, please find a video of immunostaining and watch it to get familiar with the protocol.

- Keep in mind that different labs have different protocols
- There may be some steps added or some steps taken out

Tips for success as a researcher:

- Your goal shouldn’t be to memorize each of these steps
- Instead, try to understand the purposes behind each step and what role each reagent plays