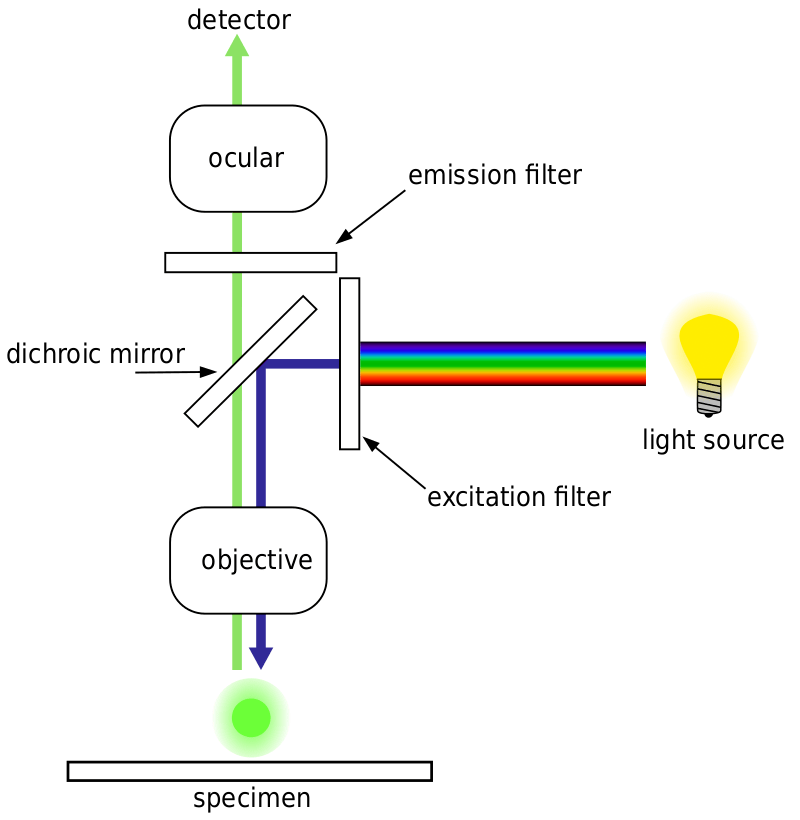
**CELL BIOLOGY AND PHYSIOLOGY LAB**

**Fluorescence Name:**

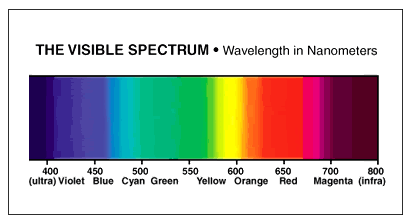
**Students must PRINT these worksheets and HANDWRITE your answers. Please keep all worksheets together in a binder.**

1. What is an emission filter? What is an excitation filter?



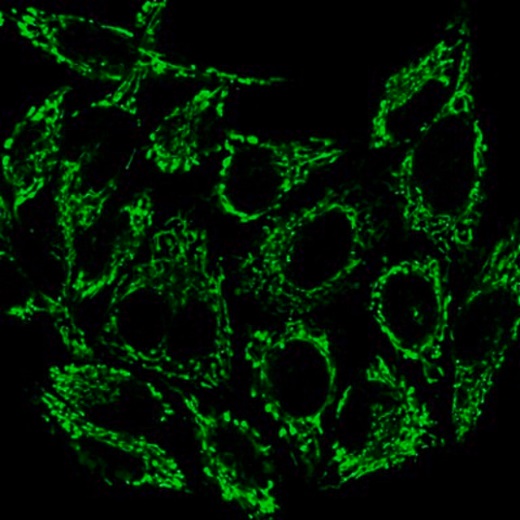
What are the steps to fluorescence microscopy?

1. What is immunofluorescence?
2. What is a fluorophore?



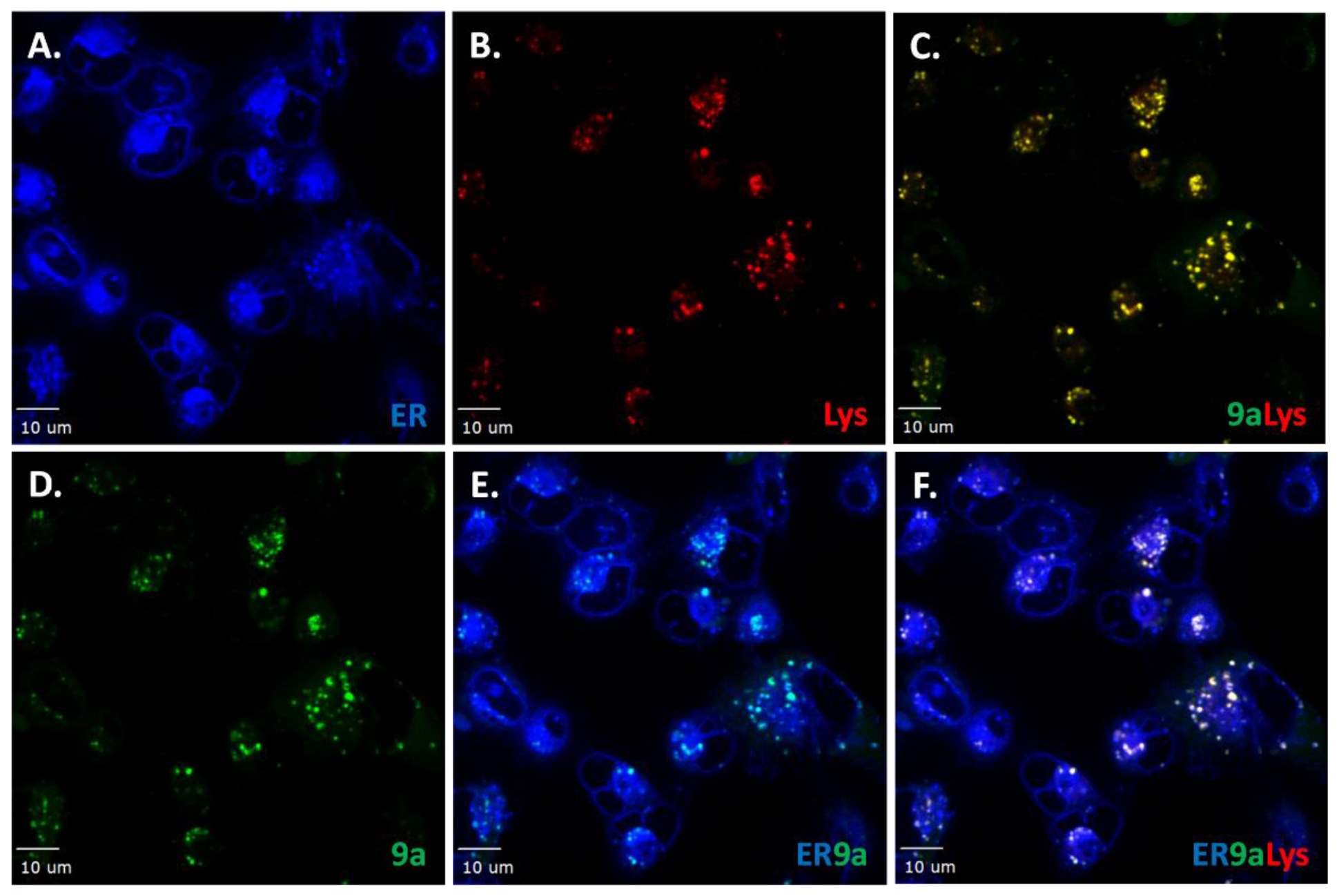
BioTracker 488 Green Mitochondria Dye

* Used to detect live cells, the dye accumulates in the mitochondrial membrane of live cells.
* Emission: 523nm
* Use GFP cube on Evos Microscope.



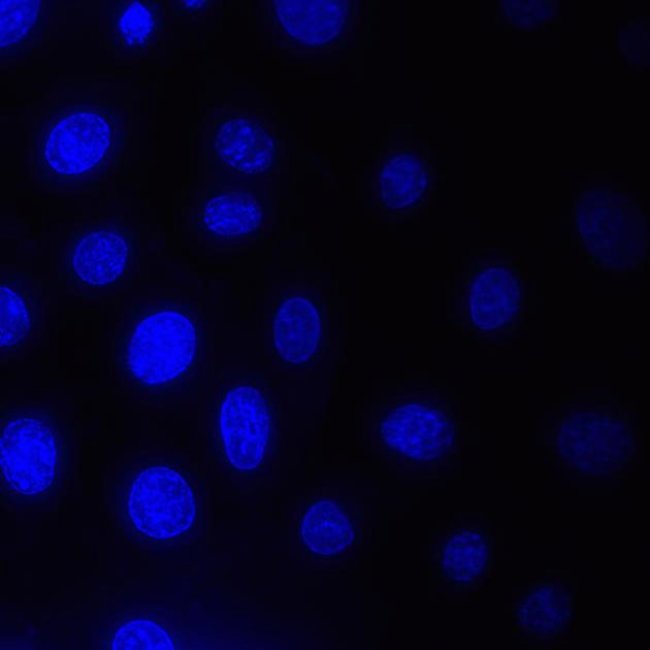
ER-Tracker Blue-White DPX

* Use to detect live cells, the dye is selective for the endoplasmic reticulum.
* Use DAPI cube on Evos Microscope.
* 1mM stock solution in DMSO.
* Warm the vial, then centrifuge to deposit DMSO at the bottom. Make the staining media:
  + Need 1µM solution – take 25µL and add to 25mL of fresh media.
* Remove old media from cells, add 5ml of staining media. Incubate at 37°C for 15-30 minutes.



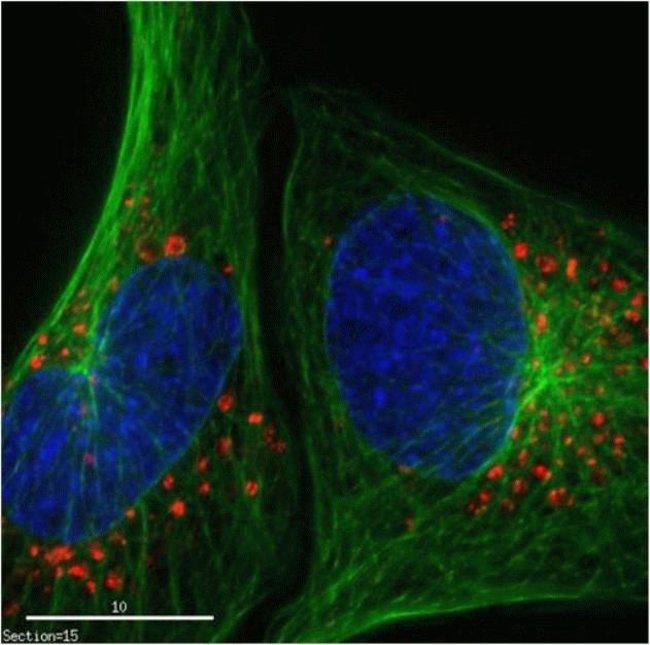
NucBlue Live Cell Stain

* Binds to DNA.
* Add 2 drops per mL media, incubate 15-30 minutes. I have found it works best to do one drop per flask.
* Emission: 460nm max
* Use DAPI cube on Evos Microscope.

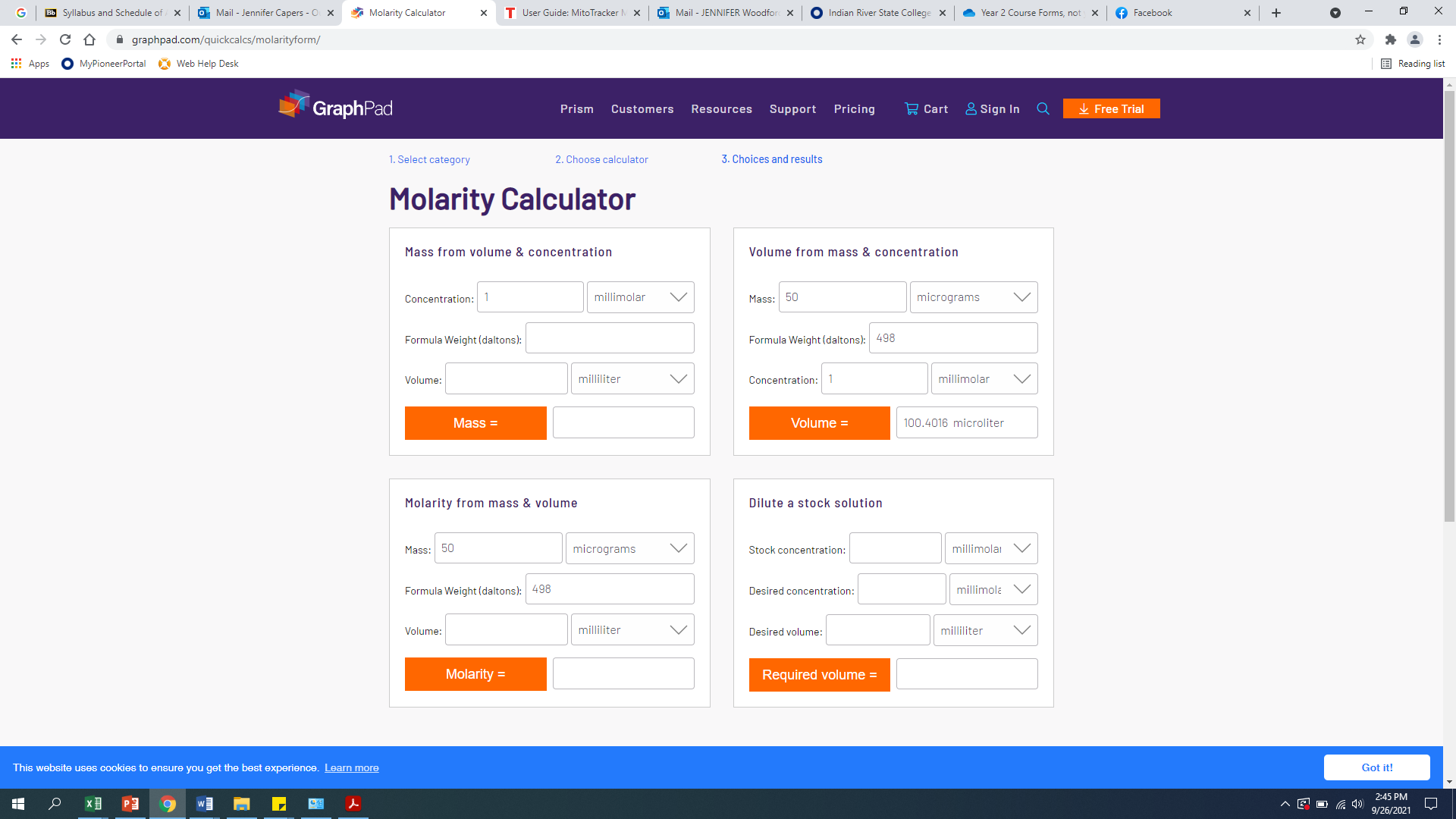


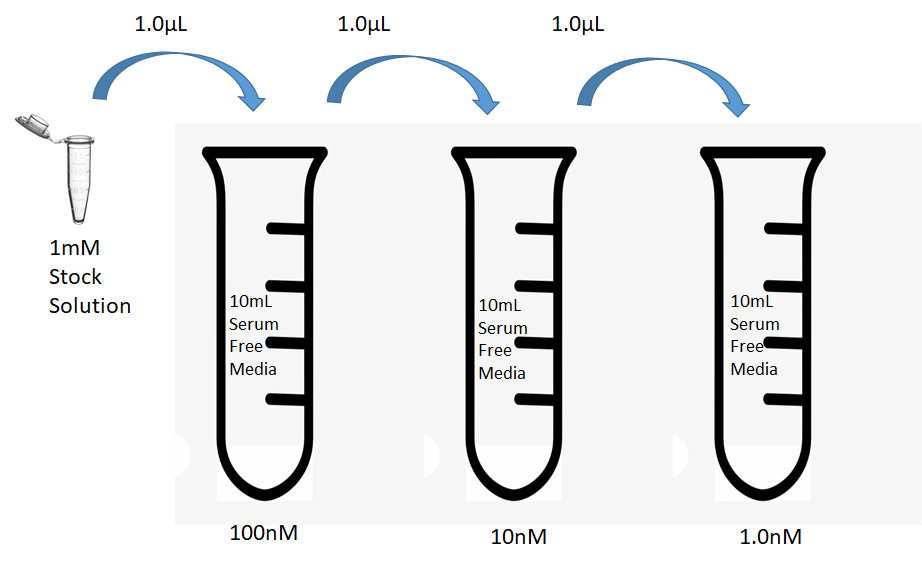
CellLight Tubulin GFP

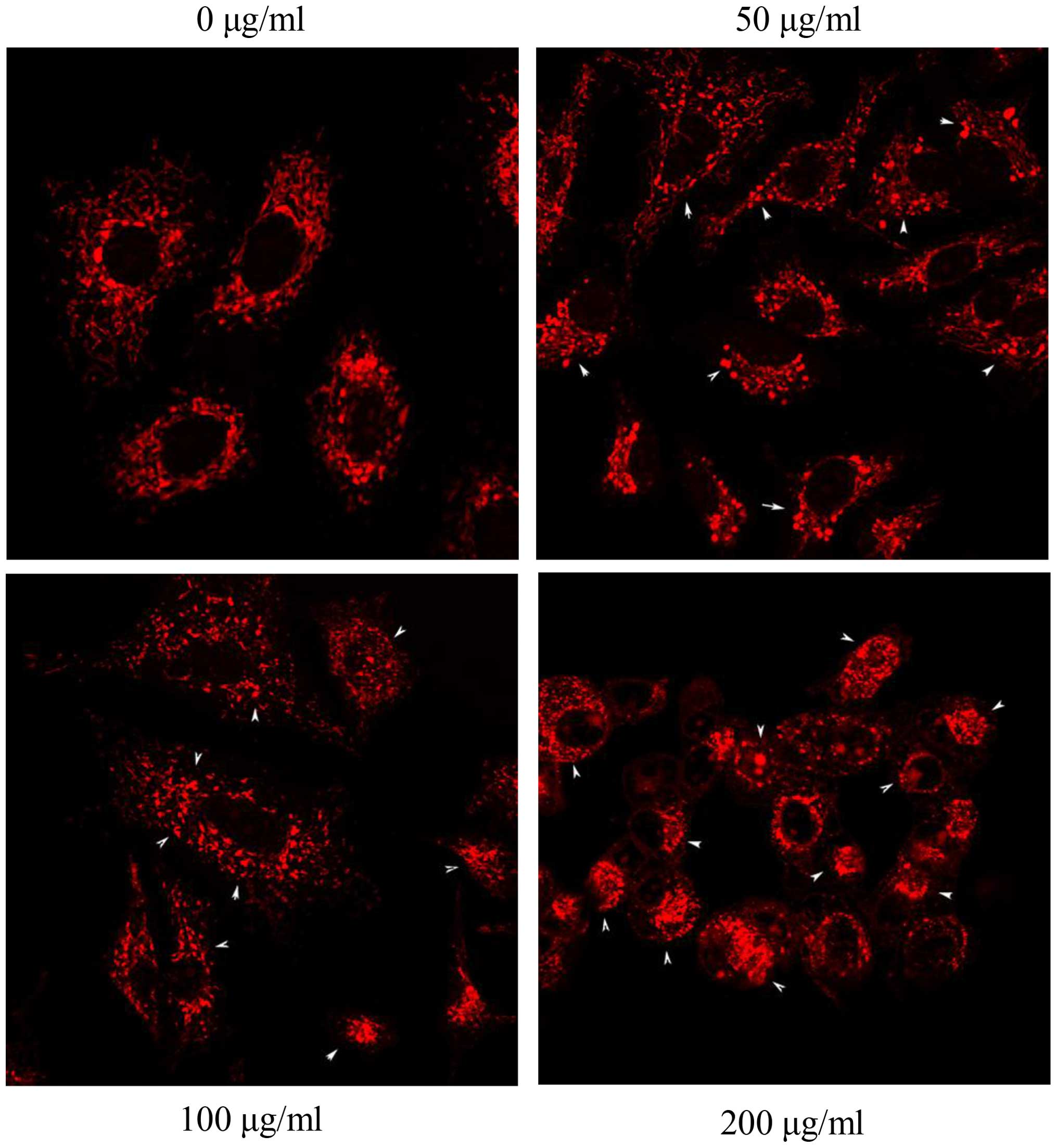
* Binds to tubulin (microtubules).
* Add 75uL to flask, incubate overnight.
* CellLight® Tubulin-GFP, BacMam 2.0, is a fusion construct of human tubulin and emGFP, providing accurate and specific targeting to cellular tubulin-GFP. This fusion construct is packaged in the insect virus baculovirus, which does not replicate in human cells and is designated as safe to use with biosafety level (BSL) 1 in most laboratories. BacMam technology ensures that most mammalian cell types are transduced/transfected with high efficiency and minimal toxicity.

 Notice the green microbutules.

MitoTracker Red CM

* Emission: 654nm
* Use Texas Red cube on Evos Microscope.
* Need to prepare 1mM stock solution in sterile DMSO.
  +  Add 100.4uL of sterile DMSO to vial to make the 1mM stock solution
  + Now prepare media without serum. Need 1.0nM so you will need to do serial dilutions.





Mixing Fluorescent Dyes

* Incubate cells over night with CellLight Tubulin GFP
* Remove media
* Add media with MItoTracker Red
* Add 2 drops of NucBlue