**TIRF microscope**

**Sample Prep**

* Use only glass bottom petri dishes (TIRF melts plastic)
* Warm up Tyrode’s solution to prevent cell shock
* Add 1uL fluorescent dye to 1mL Tyrode’s solution and wrap in foil
* In the hood, suck off cell media and gently add 500uL Tyrode’s + dye, wrap in foil and leave for 15-30 min
* Suck liquid out of dishes (make sure to get center circle) and add 2mL Tyrode’s solution

**Turning on microscope**

* Turn on the Olympus boxes 1-4 (the 4th is the touch screen) in the correct order, then the X-Cite, then the camera on the microscope. **Use a very short touch on the touchscreen button or the microscope will reset.**
* If using TIRF, push the power button on the black box (Lasers?) and turn the key (blue lights mean it is warming up and they turn to white when they are ready)
* **Always turn TIRF and x-cite off when not in use because they have a limited lifetime.**
* After the microscope is fully on, turn on the computer and let it boot up.
* Open the Metamorph software with the butterfly icon
* Remove circular stage and clean 60X objective by spraying cleaner on lens paper and wiping the objective
* Remove bubbles from oil and put a drop on the objective
* If using TIRF- turn on the white box after the sample is focused and ready to go.
* Open the TIRF software on the bottom menu bar and click connect.
* Turn everything off in the reverse order.

**General Information**

* Under illumination, select which wavelength you are using (cube allows you to switch quickly between wavelengths)
* The light on the x-cite box should be green if it recognizes the computer
* Acquisition tab- change exposure time to 10-20ms so you don’t bleach the cells (dyes are phototoxic so when excited they kill cells)
* Image scaling (acquisition button display tab)- select autoscale, low: 0.1%, high: 0.1%
* Focus in BF (bright field)
* Do not bend or kink the fiber optic cables going to the TIRF from the microscope
* Change the refractive index sample in TIRF to 1.368
* Electrodes are very fragile, changing the distance between them changes the electric field.

**Aligning the TIRF laser**

1. Select widefield in TIRF software
2. Select 488 and set intensity 4-10% in Metamorph software
3. Leave sample on and lift microscope condenser and allow beam to shine on ceiling- use screwdriver to loosen the focus screw and adjust beam until sharp.
4. Unscrew and remove the condenser
5. Hang the screwdriver from the aperture (?) and close the microscope so the beam is shining on the screwdriver handle hanging above the sample.
6. Center the beam to the middle of the screwdriver handle using the silver knobs. Once centered remove the screwdriver.

**Critical angle**

* Click critical angle in the TIRF software
* Use the horizontal silver positioning knob while holding a piece of paper to adjust the beam until the beam no longer reflects upwards.
* Set the PD to return to TIRF (Click enter or set PD)