

## TIRF MC System Start Up



1. Turn on EL6000 Fluorescence supply
2. Switch on Main power switch on Laser
3. Turn on Camera, switch on back of camera
4. Turn on Computer and screens
5. Turn on Microscope at MICBOX (**Make sure condenser lens will clear stage**)
6. Next turn on the laser key switch and let laser warm up for 3 to 5 minutes
7. Make sure objective is clean and correction collar is set for proper temperature and cover glass thickness and at lowest point of Z travel
8. Place a drop of immersion oil on the objective
9. Place cover slip bottom Petri Dish in the stage insert making sure the dish is sitting flat and not sitting on the top of the objective. (Note: with dish cover off during alignment)
10. Start the LASAF software
11. Under the Acquire tab select the contrast method for TIRF, wavelength 488 and filter cube GFP
12. Add a second channel and select the second channel and configure it for Fluo using the GFP cube and keep this channel the active channel
13. Click the live button to illuminate the sample with fluorescence GFP and on the microscope press the eye button on the microscope so you can visualize the image through the eyepieces and focus to the bottom of the dish so you can see your sample just come into focus
14. Click the stop button
15. Select channel 1 for TIRF mode making sure you still have the GFP cube selected with wavelength 488
16. Under Acquire tab select Acquisition
17. Expand the TIRF window on the left
18. Select Auto Align
19. Follow the Auto Align instruction on the screen, focus laser on ceiling reference mark to smallest focus laser spot
20. Pull arm forward and hold arm with 1 hand while closing the chamber top covers to make sure the transmitted light arm switch remains closed after closing chamber top covers.
21. Make sure front chamber covers (upper ones) are closed
22. Click align and save and listen for the click in the TIRF assembly when the attenuator moves out of the laser path, LED on right side of module lights up.
23. Wait for the auto align to finish
24. Once completed close the alignment window and confirm the REF Index (Refractive Index) is between 1.28 and 1.4, anything outside of this range is invalid
25. You can replace the dish cover now to slow evaporation
26. If you do not plan to take fluo images along with the TIRF images before starting your experiments select channel 2 and click the – button to remove the fluo channel
27. Use software in live image mode to view sample and control TIRF operations.

### **On shut down**

1. Close LAS AF after saving your work
2. Turn off the microscope, computer, camera and EL6000
3. Switch off the laser and wait 5 minutes before turning off the main power to allow units to cool