Nikon TIRF

Cell Imaging Shared Resource (CISR)

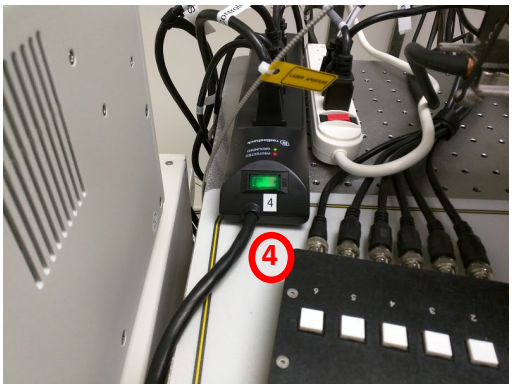
742B Light Hall

Quick Guide

I. Starting the Nikon TIRF

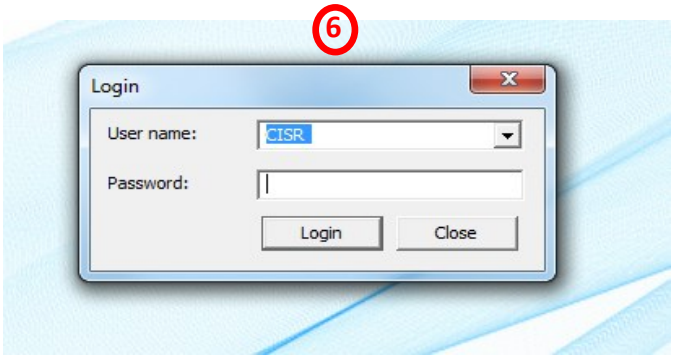
1. Sign in to the log book.
2. Turn on switches 1-4 in numerical order. The computer must be OFF before starting 1-4.

- **1** is the power strip on the left wall. (1)
- **2** is the key on the top laser box. (2)
- **3** is the key on the bottom laser box. (3)
- **4** is the green button on the power strip on the left and to the back of the microscope. (4)



3. The computer should start up. (5)
4. Log in to the computer using your VUNetID and password. (6)
5. Start **NIS-Elements** software.
6. **Login** to NIS-Elements using your first name and no password.

5

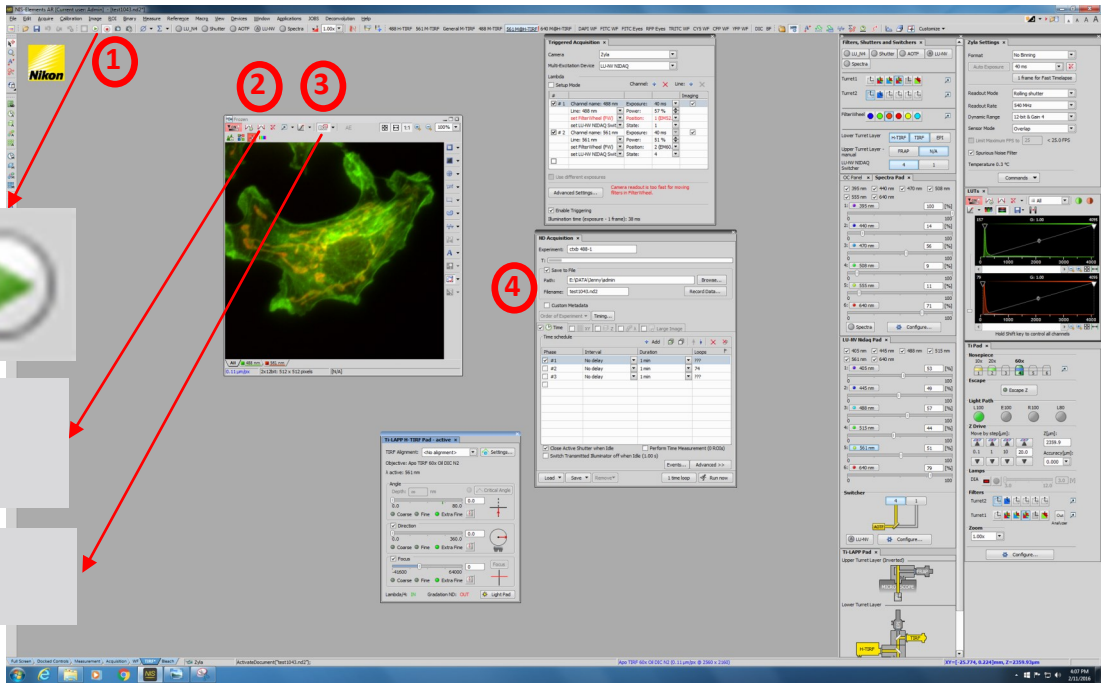


Nikon Elements Layout

1. There are preset layouts along the bottom of the screen for Widefield (WF), TIRF, and Bleaching. These will open the appropriate windows for each type of imaging.

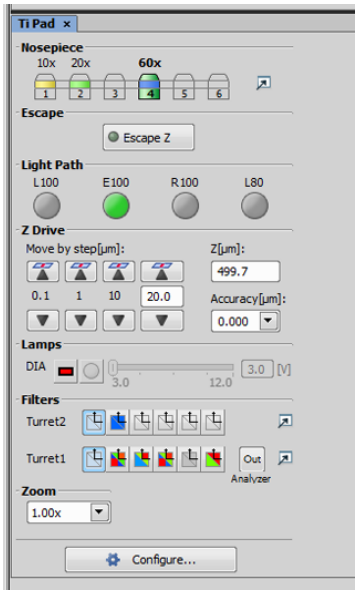
2. Along the top of the screen are preset **Optical Configurations**. You will see all the default configurations. You may duplicate a configuration by right clicking. Rename for yourself, and now you are able to change this new duplicated configuration to fit your imaging needs. This new configuration will only be visible under your named login.

3. To reuse settings from a previous image, open the image and right click within the image. You will have choice for “reuse camera settings”, “reuse acquisition settings”, or “reuse XYZ”.
4. To view your image on the screen, use the green arrow “live” (1) button on the top left. Stop with the red circle. Capture a single time point with the camera button.
5. Autoscale (2) can be useful for viewing images when setting exposure time and laser power.
6. For fast imaging, you may wish to adjust the frame size. In a live image, choose ROI. (3) You may choose a preset size or define an ROI.
7. Images may be saved as either .nd2 (Nikon format) or TIF. Movies will save automatically according to your settings in the ND Acquisition (4) window.



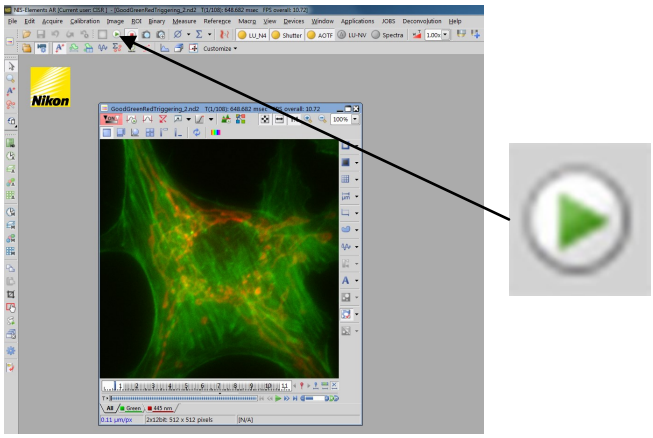
II. Mounting Sample and Focusing

1. Choose objective (10x dry, 20x dry, or 60x Oil immersion) on left side (1) of scope or in software. Use Nikon oil.
2. Add oil to objective if using 60x TIRF lens.
3. Loosen screws on stage to adjust for sample. Place sample in holder and tighten screws.
4. On either side of scope, choose Coarse, Fine, or Extra Fine (2) to move focus with focus knobs. Turn knob toward user to bring objective up.
5. XY joystick also has Coarse, Fine, and Extra Fine (3) for movement. Twist joystick to toggle between choices.



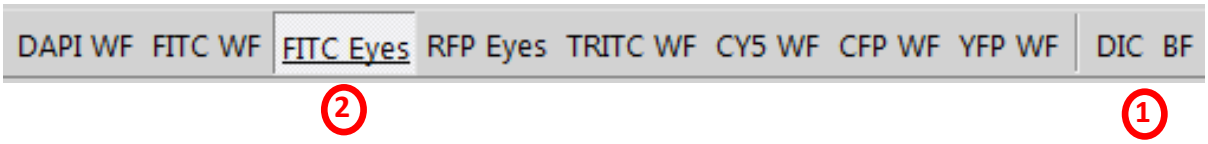
3

Joystick

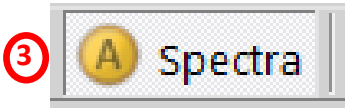


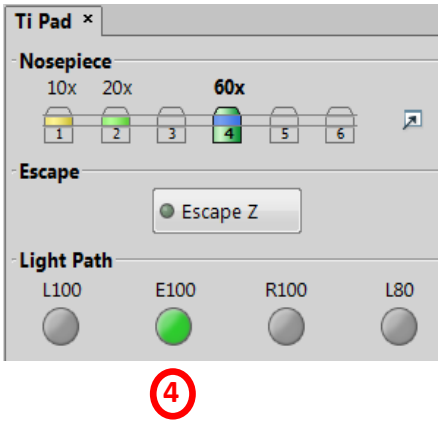
Perfect Focus (PFS)

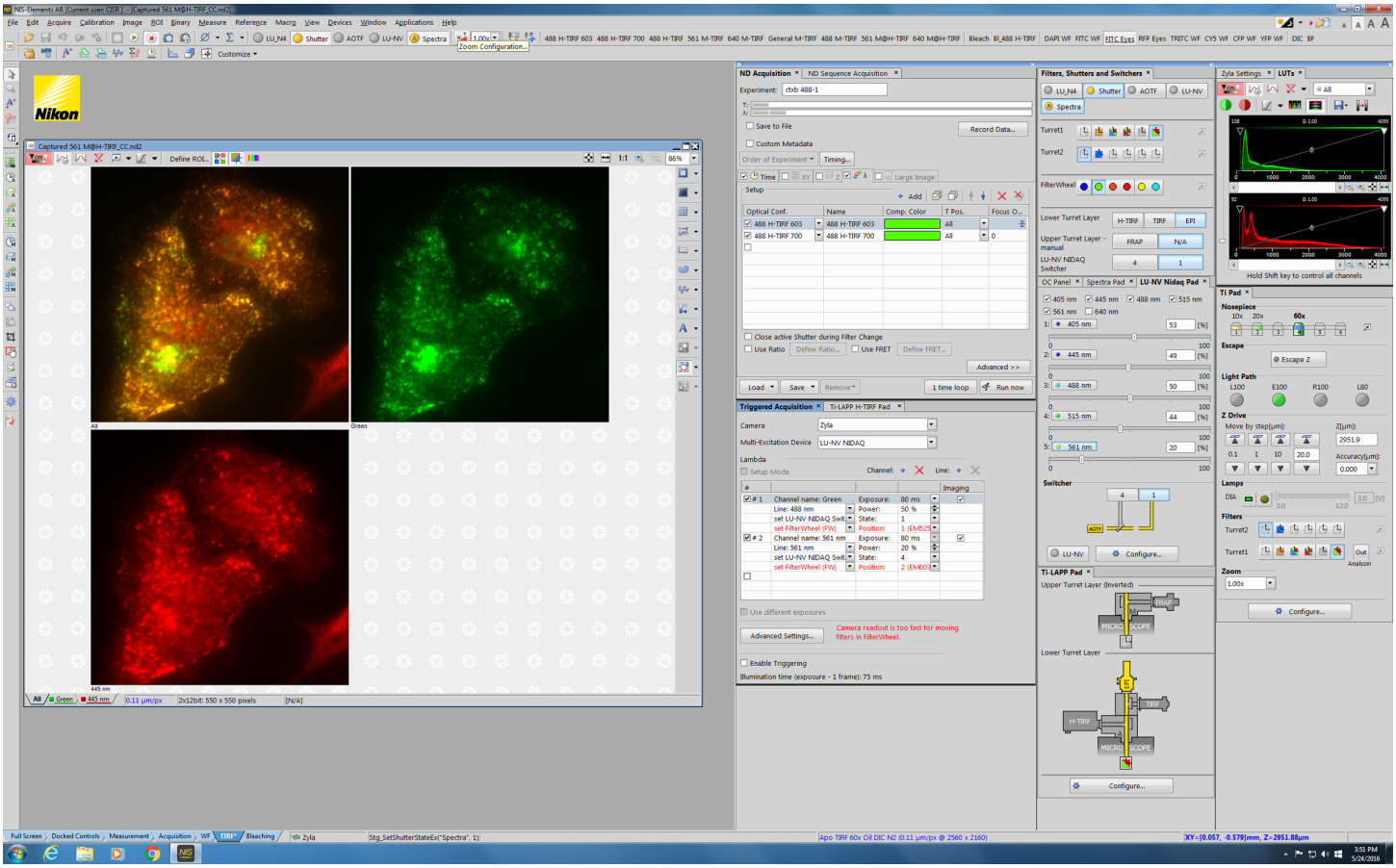
III. Finding Sample (Oculars)



1. To find your sample by bright light through the oculars, choose either DIC or BF from the (1) top menu bar.
2. To find your sample by fluorescence through the oculars, choose "FITC Eyes" (2) configuration from top menu bar.
3. Click "Spectra" (3) to turn on widefield illumination to eyes. Ensure that E100 (4) is selected under Ti Pad under Light Path.
4. You should be able to see fluorescence through the oculars. This filter show both Green and Red excitation.

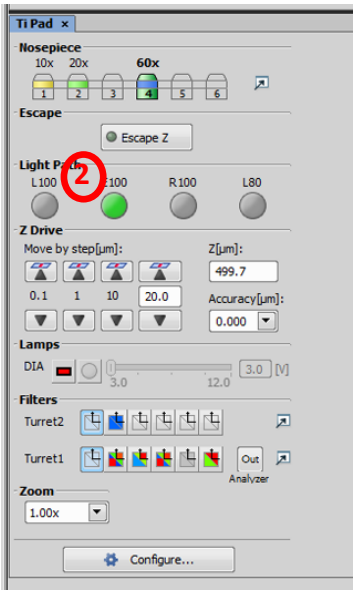




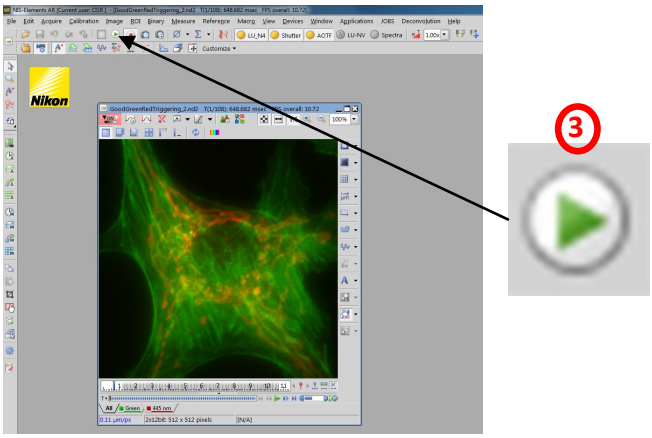


III. Finding Sample (Screen)

6. To visualize sample on screen, choose WF (1) tab at bottom of software, and choose WF optical configuration (2) at top (DAPI WF, FITC WF, TRITC WF, CY5 WF, CFP WF, YFP WF).
7. Start "**Live**" (3) with green arrow button.
8. Focus with focus knobs on scope. (4)
9. **Perfect Focus (PFS)** may be used to find and hold correct focal plane.
10. Choose PFS ON button (5) on front of scope. While this button is blinking, focus with the focus knob. When PFS stops blinking, focal plane is found. Now use the PFS wheel (6) for fine focusing.



Joystick



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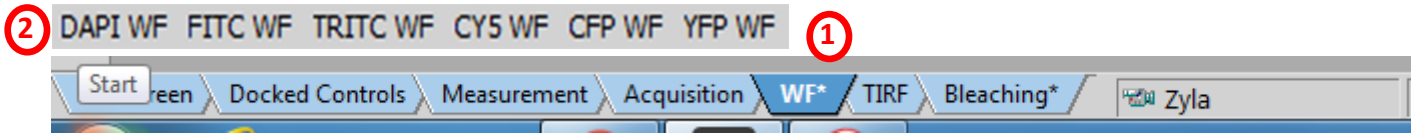
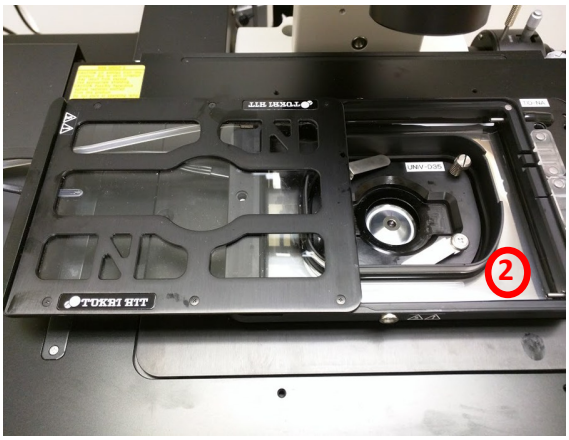
Perfect Focus (PFS)

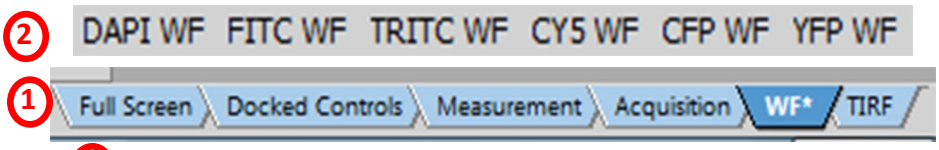


IV. Incubation (optional)

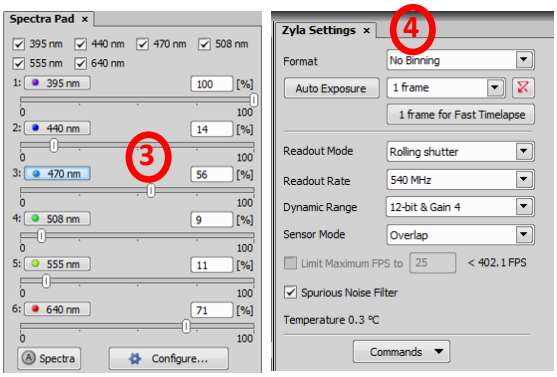
1. For live cell imaging, turn on orange power button ① on the incubator above the laser boxes.
2. Three heaters will come to their appropriate temperatures. Top Heater will reach 43, Stage Heater will reach 39, and Bath Heater will reach 41. The Lens Heater needs to be switched on separately. Dicuss your needs with CISR staff.
3. If not already in place, put the heated stage adaptor in place. Use lab tape to hold in place.
4. **Ensure there is sufficient water in the heated stage water bath. Use dI H2O.** ②
5. Turn on the CO2 tank on the wall by the main CISR door. ③ Turn on with the main silver knob.
6. Check the CO2 indicator on the front of the incubator box to ensure CO2 is on.
7. Although temperatures will be ready within 5-10 minutes, for optimal environmental conditions, allow temperature and CO2 to equilibrate for 30 minutes.

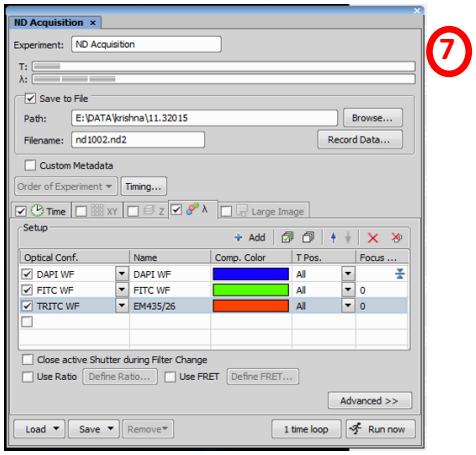


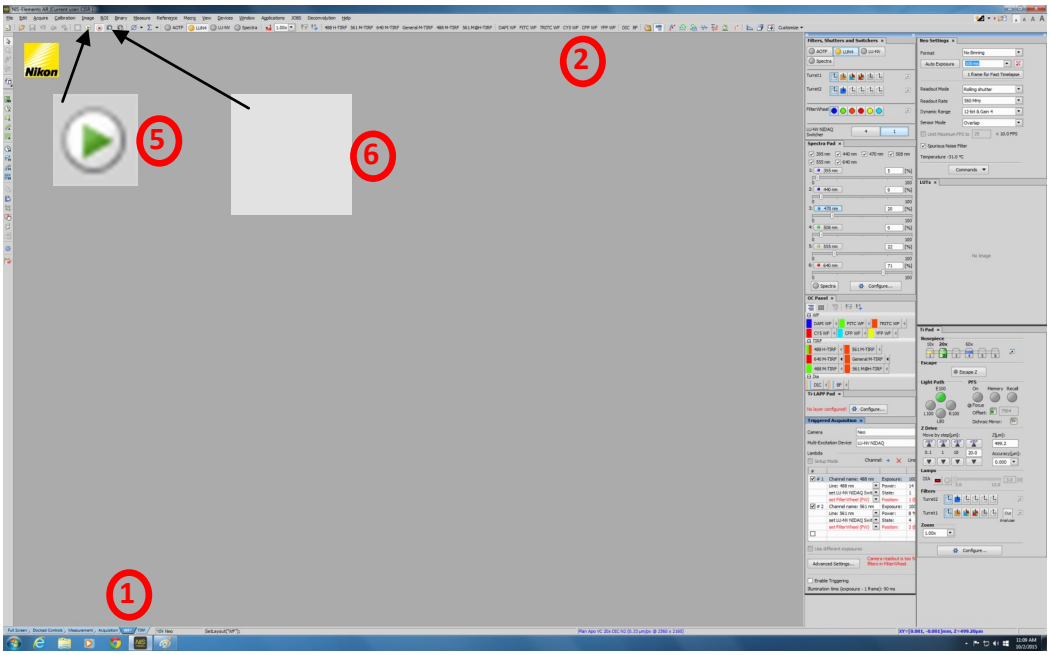
V. Widefield Acquisition



1. Choose WF tab (1) at the bottom of screen, and choose optical configuration (2) at the top of the screen to match your fluorophore of interest.
2. To adjust signal, adjust Spectra % (3) output as well as exposure time in Zyla camera window. (4)
3. Use PFS to focus sample.
4. Choose "**Live**" green arrow (5) to see image on screen.
5. For single time point, click "**Capture**". (6) Repeat for multiple channels, and merge to create multi-channel image. Merge can be found under File.
6. For time-lapse acquisition, use ND Acquisition (7) window.
7. Set-up multiple channels under Wavelength. Choose each channel under Optical Configuration.
8. Set-up time-lapse under Time. Choose Define. Interval is time between images, and Duration is total time.

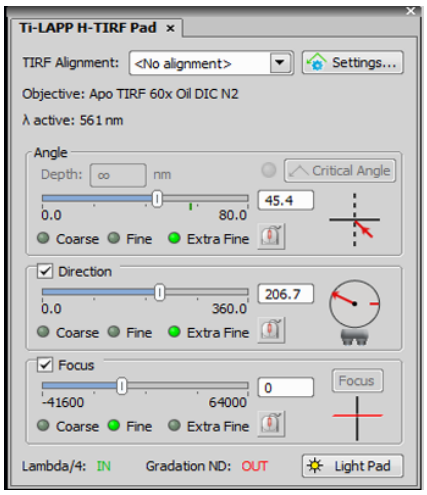






VI. TIRF Alignment

H-TIRF



Automated TIRF alignment in software

1. Raise microscope condenser to make visualizing light easier.
2. H-TIRF alignment is done in the software.
3. For H-TIRF, open Ti-LAPP H-TIRF Pad.
4. Adjust **Angle** until you see the laser spot on the wall.
5. Continue adjusting until the light is overhead.
6. **Focus** the spot to the smallest possible spot.
7. Set **Direction** to 180.
8. Adjust **Angle** again until you see TIRF signal on sample. You will see a bright signal and then nothing. Adjust **Angle** back until see image again.
9. Adjust **Direction** to fine tune across the best region of your sample.

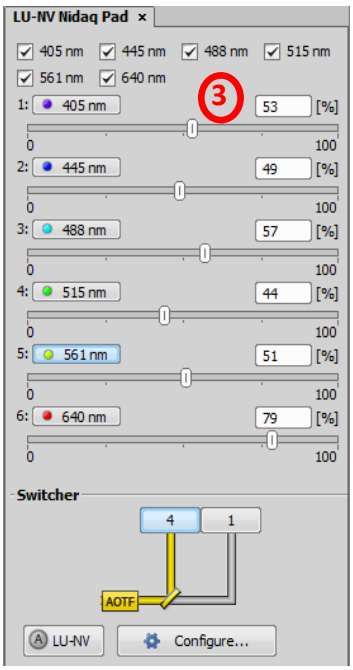
M-TIRF

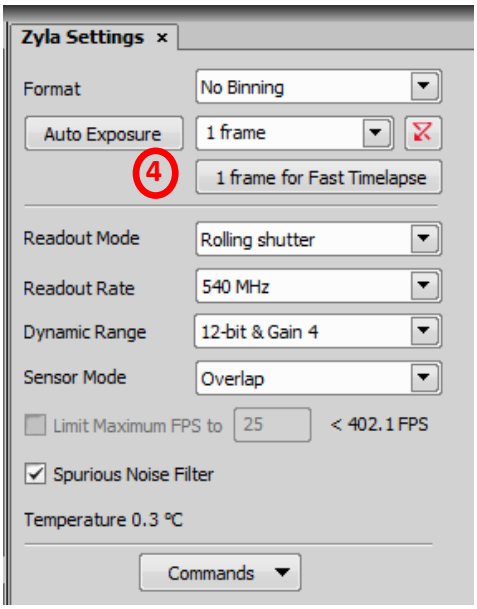
Manual TIRF Alignment

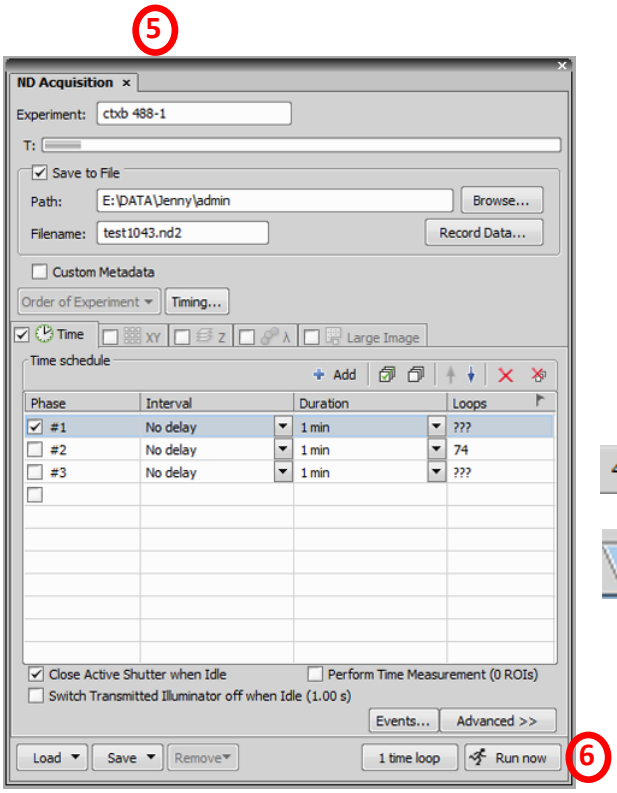
1. M-TIRF is done manually with adjustments on the microscope.
2. Angle
3. Focus
4. Direction

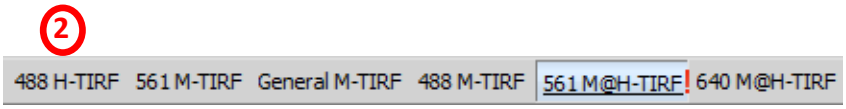
VII. Single Channel TIRF Acquisition

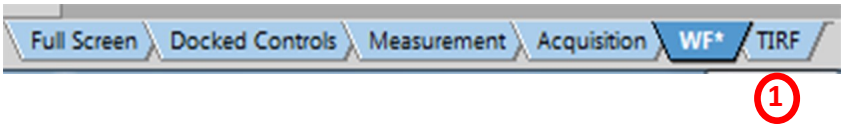
1. After finding an image by widefield, choose TIRF layout (1) at bottom of screen and TIRF optical configuration at top (2) of screen (488 H-TIRF, 561 M-TIRF, or General M-TIRF). In the previous sections you should have found cells and focused and adjusted TIRF angle.
2. Optimize signal by adjusting laser power in the LU-NV (3) Nidaq Pad window and exposure time in the Zyla (4) camera window.
3. For single time point, capture image with “capture” camera button along top of screen.
4. For time-lapse imaging, open ND Acquisition (5) window.
5. First tab in ND Acquisition is for time-lapse. Interval is delay between images. Duration is total time-lapse. For shortest possible interval, choose “no delay” for interval.
6. Choose **RUN NOW** in ND Acquisition window. (6)











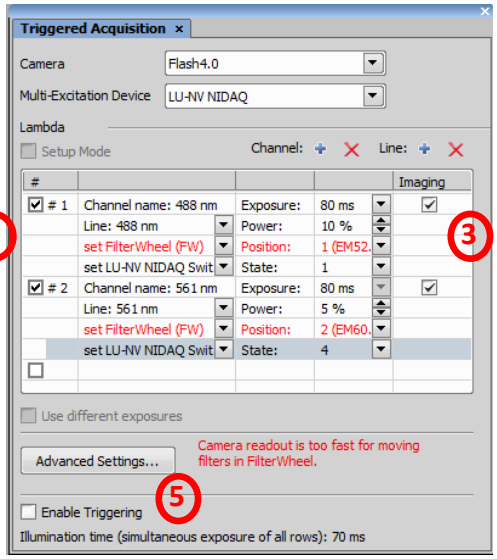
VIII. Multi-Channel TIRF Acquisition

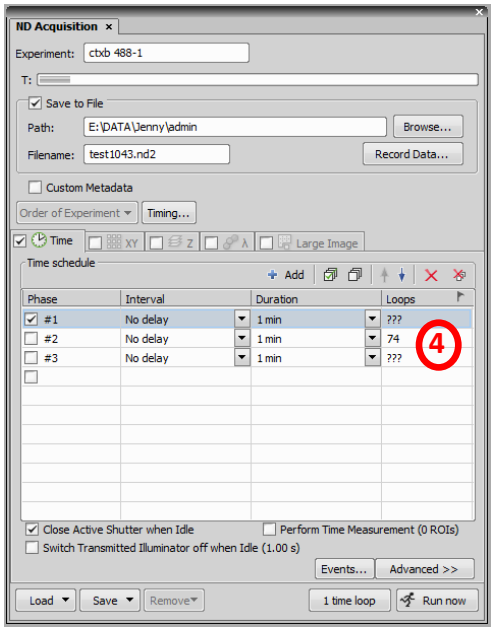
1. Switch configuration to 561M@H-TIRF (1) or 640M@H-TIRF on top of screen.
2. Open the following 3 windows — Triggered Acquisition, ND Acquisition, and Ti-LAPP H-TIRF Pad
3. For Triggered Acquisition add channel. For each channel add 3 lines — (2)

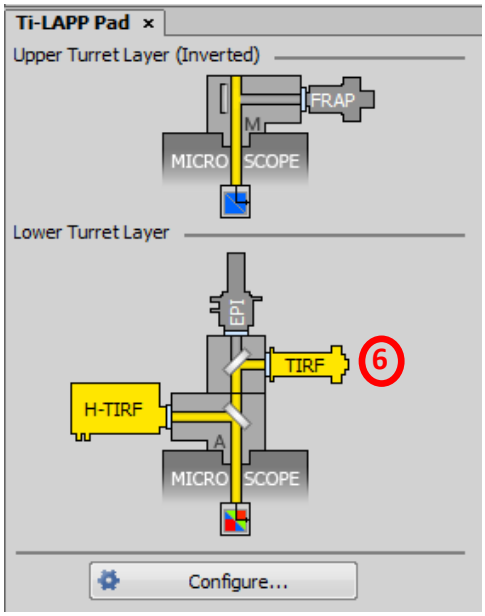
   Line

   Filter Wheel

   LU-NV NIDAQ Switcher.
4. Choose appropriate excitation wavelength for **Line**.
5. For 488, **FilterWheel**=1 and **Switcher**=1.
6. For 561, **FilterWheel**=2 and **Switcher**=4.
7. For 640, **FilterWheel**=3 and **Switcher**=4.
8. Set exposure time in Triggered Acquisition (3) window. Exposure time must be the same for both channels.
9. Set time-lapse parameters in ND Acquisition window. (4) Open Define window.
10. Check “Enable Triggering” (5) in Triggered Acquisition window.
11. Ensure that the Lower Turret Layer in the Ti-LAPP Pad shows both H-TIRF and TIRF highlighted . (6)
12. Click “RUN NOW” in ND Acquisition window.

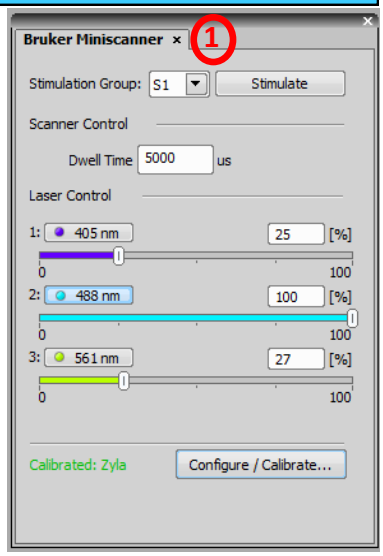


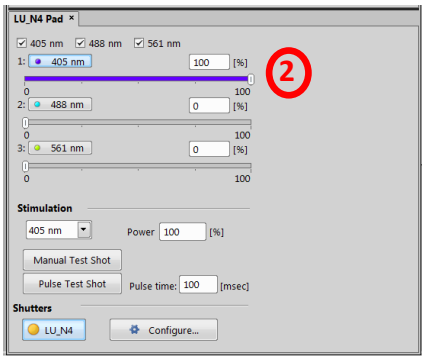


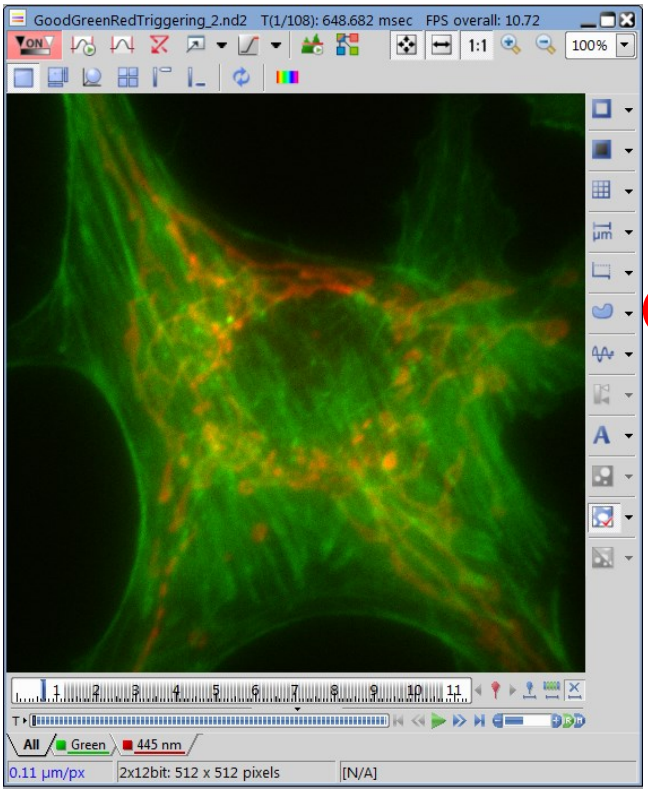


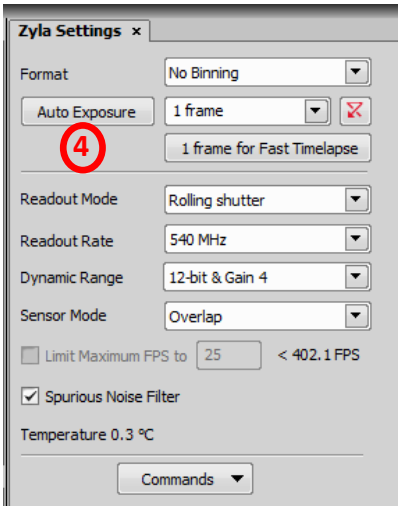
IX. Bleaching

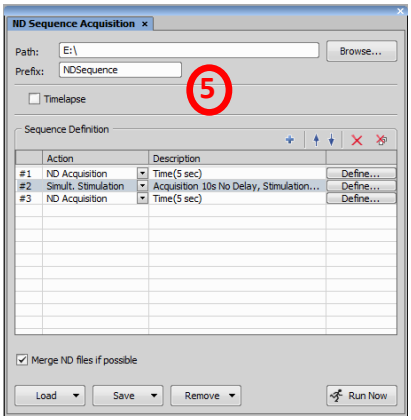
1. Choose the “Bleaching” tab at the bottom of the screen.
2. In addition to the TIRF set-up on the previous page, open the **Bruker Miniscanner** window. (1)
3. Choose laser for bleaching and set parameters (% and dwell time) in both the **Bruker Miniscanner** window and the **LU_N4 Pad** (2) window.
4. On right side of image window, right click on ROI (3) icon to choose ROI shape. Draw ROI.
5. Right click on ROI and choose “**Use as** (4) **Stimulation ROI**”.
6. Set exposure time in camera window.
7. Set bleaching and time-lapse in ND Sequence Acquisition (5) window. Bleaching can be Sequential or Simultaneous (next page).





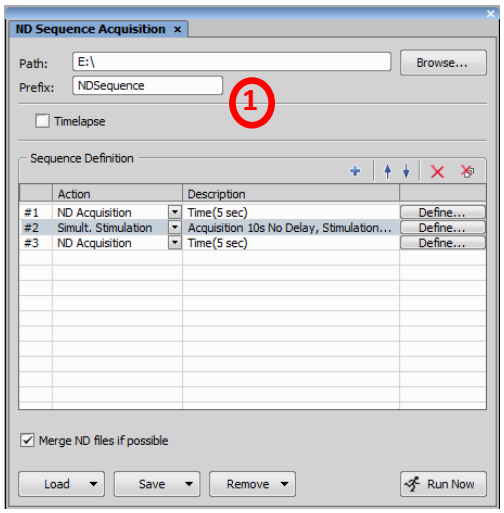


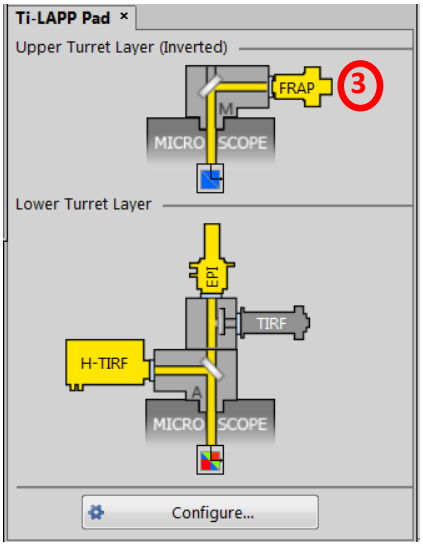


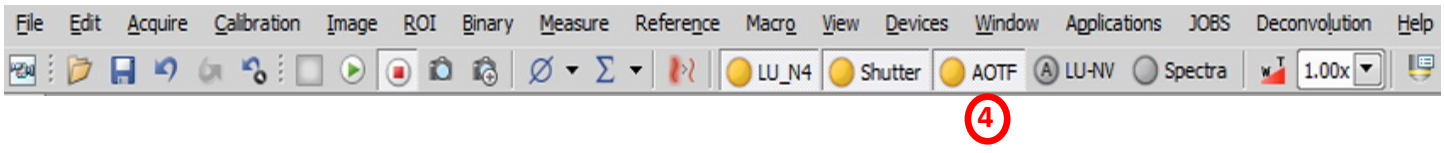


IX. Bleaching (continued)

7. For **Sequential** bleaching, set up actions in ND Sequence Acquisition window. (1) For example, add #1 ND Acquisition, #2 Stimulation, #3 ND Acquisition. Open Define window for each to set interval and delay.
8. For **Simultaneous** bleaching, set up actions in ND Sequence Acquisition using Simultaneous Stimulation. Open Define window to set interval and delay. Stimulation time will be set based on ROI size and dwell time in miniscanner window.
9. For bleaching, ensure that the following buttons are active:
   A. Under **Filters**, choose Galvo (2) on Turret 2 (blue box, second from left)
   B. In Ti-LAPP Pad window, choose **FRAP** (3) on Upper Turret Layer
   C. Under menu bar at top of screen, turn on **AOTF.** (4)
10. RUN NOW.







X. Shut Down

Check the CISR scheduling calendar to see if anyone is signed up after you. If another user is coming with 1 hour, please log out of the software, sign out in the log book, and leave the microscope and lasers ON.

If no one is coming after you, follow the next steps.

1. Close NIS software.
2. Shut down the computer.
3. Turn off power strip 4 (green)
4. Turn off laser box 3 (bottom)
5. Turn off laser box 2 (top).
6. Turn off power strip 1 (left wall).
7. Sign out in log book.
8. Come again soon!

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Please include acknowledgment of CISR use in publications.

Example:

Experiments/Data analysis/presentation [include what you use] were performed in part through the use of the VUMC Cell Imaging Shared Resource (supported by NIH grants CA68485, DK20593, DK58404, DK59637 and EY08126).

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