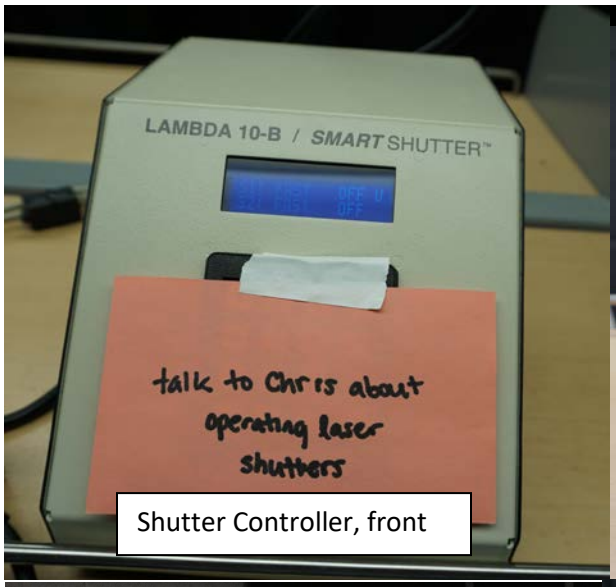
Start Up:

Upon entering the laser room turn on the wall mounted Laser Power Button by pulling it away from the wall.

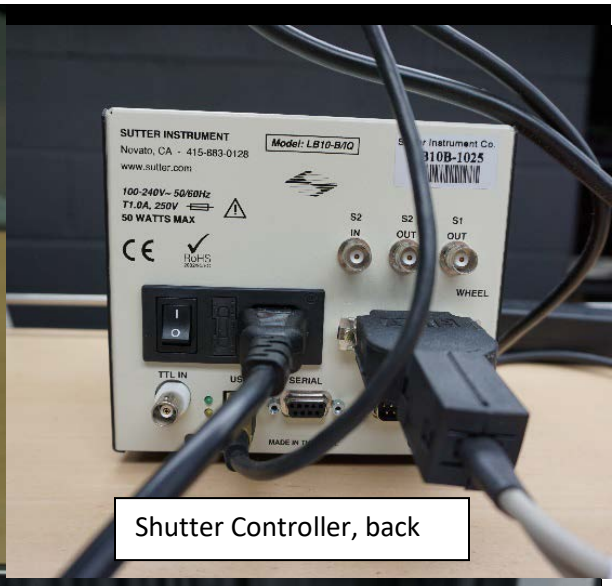
Turn on Shutter controllers (toggle switch on back of unit). There should be a "**U**" in the top right corner of the LCD window – this denotes that the shutter controller is taking commands from the computer via the **U**SB cable input.

Turn on Lasers and allow to warm up for 15 min – 1hr. (lasers are not connected to the computer in any way):

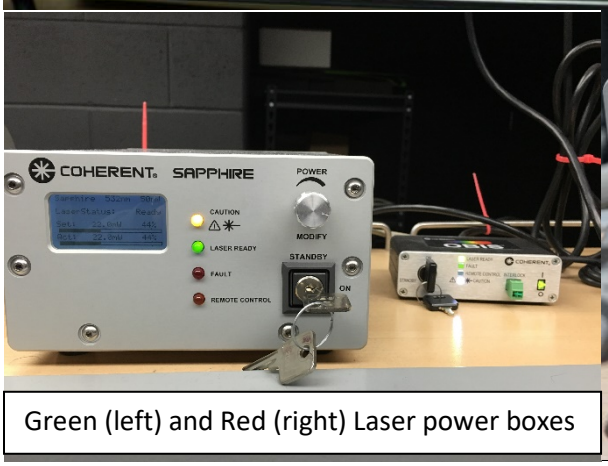
633 nm (red): Flip switch for power on front of box, wait 5-10 min turn key to on position.
532 nm (green): Flip switch for power on back of box, wait a few min, screen will tell you when it is ready, then turn key to "on" position. The power of the green laser can be set between 5-50mWatts by turning the "power" dial and then pressing the dial in to actuate when at the desired setting.



Shutter Controller, front



Shutter Controller, back



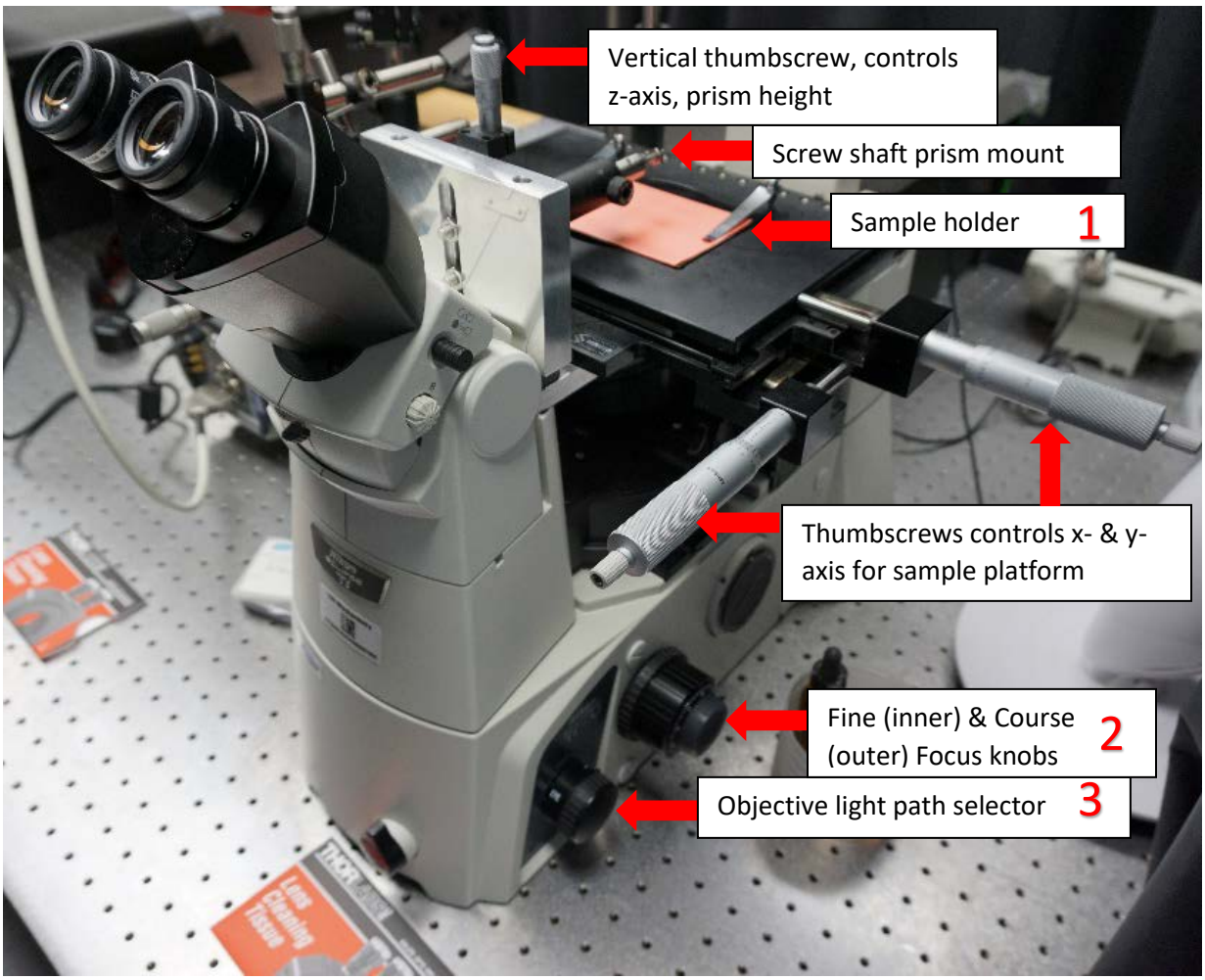
Green (left) and Red (right) Laser power boxes

Green Laser power box, back

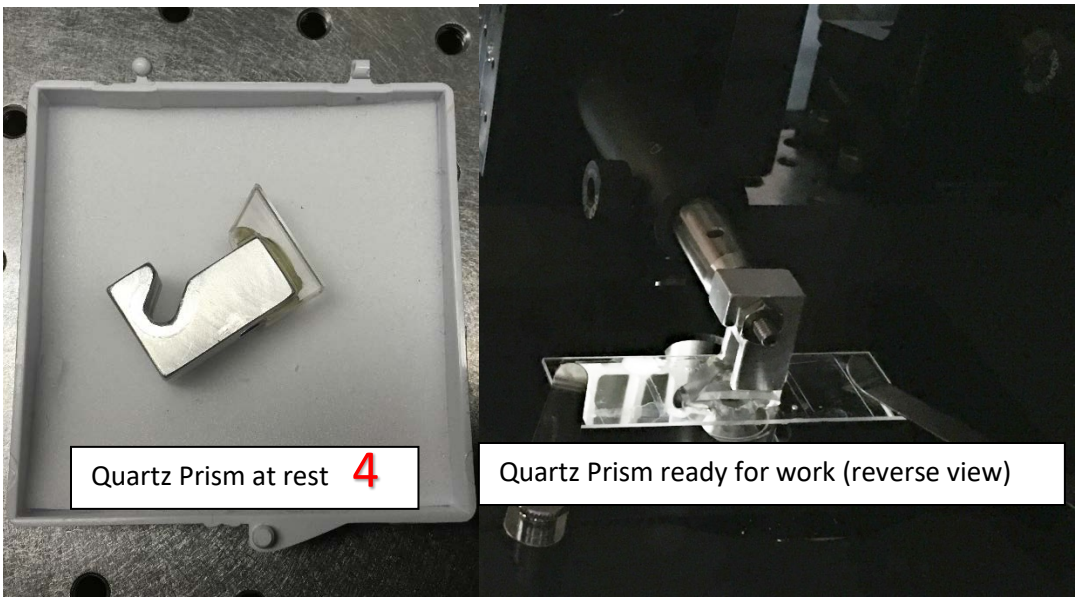
Turn on Computer, log in and launch Metamorph (only open program if shutter control box is already powered on)

The Prism microscope should be found the same way you should leave it when your experiments are completed (see photos below for assistance):

1. There should be an index card covering the sample stage
2. The objective should be moved to its lowest position (course focus turned to max clockwise position)
3. The Objective light path selector should be set to “EYE”
4. The quartz prism should be clean and stored on its side in its little white box



Slide Positioning:

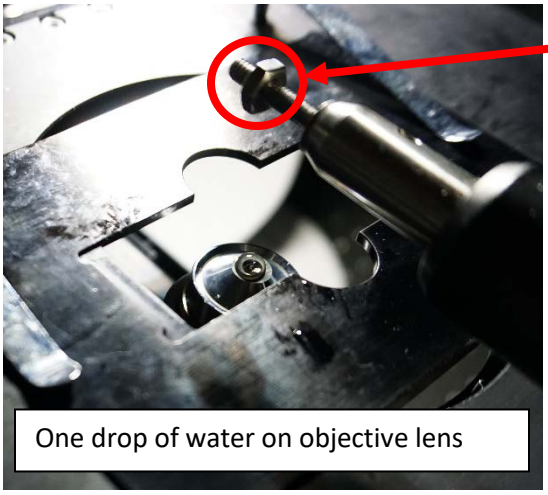


Quartz Prism at rest 4

Quartz Prism ready for work (reverse view)

Prepare your slides per experimental protocol and in accordance with "*Slide Cleaning and Preparation Protocol" and "Immobilization of Samples on Slide*"

Remove the index card from the stage covering the objective lens.
Place a drop of water on the objective lens.



One drop of water on objective lens

Note: this is a good view of the nut that can be tightened to hold the prism in place once optimal positioning is achieved.

Place your sample slide, coverslip down, into the recessed mount of the slide holder, and hold in place with the built-in spring-clips. The predrilled holes in the quartz slide should be facing up.

Raise the objective lens, using the course focus knob, until the water droplet on the objective touches the bottom of the cover slip and starts to spread.

Adjust the x & y positioning of the stage and slide using the horizontal thumbscrews on the right side of the stage. By eye try to get the center of the objective aperture to be aligned with the tape edge of your first sample lane.

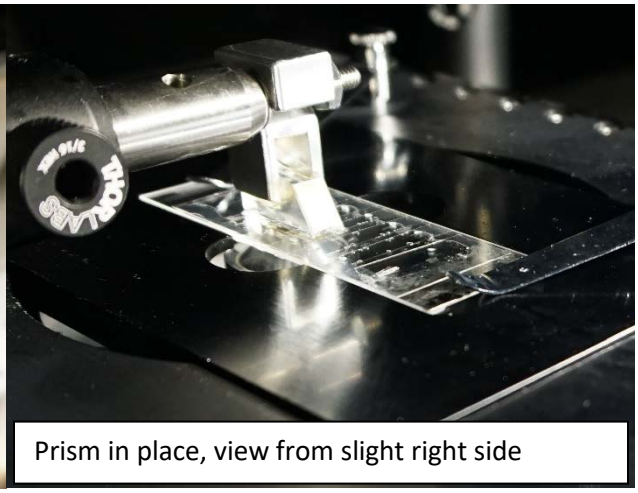
Viewing through the eye pieces under ambient light from the table lamp you should be able to use the fine focus knob and x & y stage adjustment thumbscrews to focus on the bottom edge of one side of the tape walls in your first sample lane.

Quartz Prism Positioning:

Carefully inspect the quartz prism to ensure that it is clean, having no remnants or smudges from previous uses. To clean the prism: apply a few drops of acetone to a piece of Thor lens cleaning tissue. Use the acetone saturated tissue to carefully wipe the prism clean. Repeat with clean tissue until prism is satisfactorily clean.

Apply one drop of Immersion Oil (type FF) to the bottom surface of the prism. Gently tip the inverted quartz prism to help the oil coat it's full bottom surface area.

One drop of oil on quartz prism



Prism in place, view from slight right side

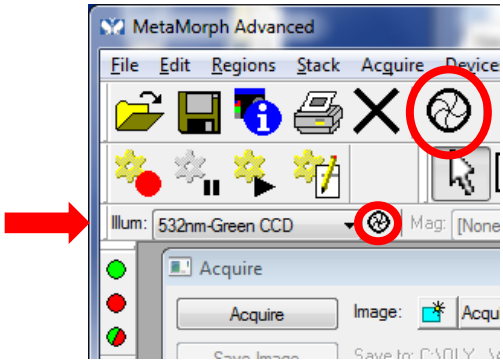
Carefully place the aluminum hook end of the prism holder over the screw shaft of the prism mount above the slide surface. The hook should approach from the left side as you are facing the microscope from the eye-piece-viewing side of the microscope. This will result in the right angled ("squared") side of the prism to face to your left, this is the side of the prism that the laser beam will be entering from.

At this point, the prism should be hanging from its support and may tilt freely towards one side. For proper alignment for TIRF the bottom surface of the prism must be parallel to the quartz slide surface. One way to ensure this is to use the vertical thumb screw to lower the prism until it first makes contact with the slide, continue gently lowering the prism such that it rotates itself and lies flat on the slide surface. As you are doing this the immersion oil should spread, fully coating the contact surface between the prism and slide. Always be careful not to lower the prism so far that it distorts or risks breaking the slide. Once the prism is in position, with its bottom surface parallel to the slide, the nut on the prism support screw can be tightened to lock the prism in this correct orientation.

If enough immersion oil is between the prism and slide you will be able to slightly raise and lower the prism without pulling the slide away from the objective. If there is less than adequate immersion oil there may be some suction/adhesion between the prism and slide, such that when the prism is raised, it pulls the slide with it. The danger is that as the prism is raised further it will release the slide allowing it to snap downward with enough force that it could scratch or damage the objective lens.

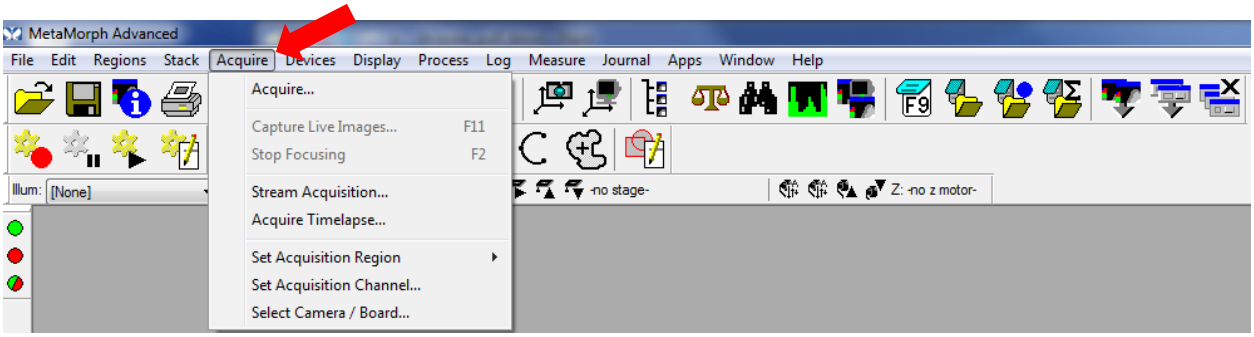
Launch MetaMorph Advanced from your Desktop. Launching Metamorph will automatically start cooling the camera to -80C.

Open the shutter for the Red Laser. From top ribbon of the Metamorph screen select the red laser, 633nm, from the "Illum:" pull down menu (see picture below for assistance). This "Illum:" pull down menu can be used throughout setup to change the shutter for each laser of interest. Once you have selected a laser you can toggle open and closed the shutter by sequentially clicking on the aperture icon immediately to the right of the "Illum:" pull down menu or in the upper ribbon menu (circled in red.) Alternatively, the Red and Green circles on the left border directly below this menu (and below the red arrow in the figure) can also be clicked on to toggle their corresponding shutter open/closed.

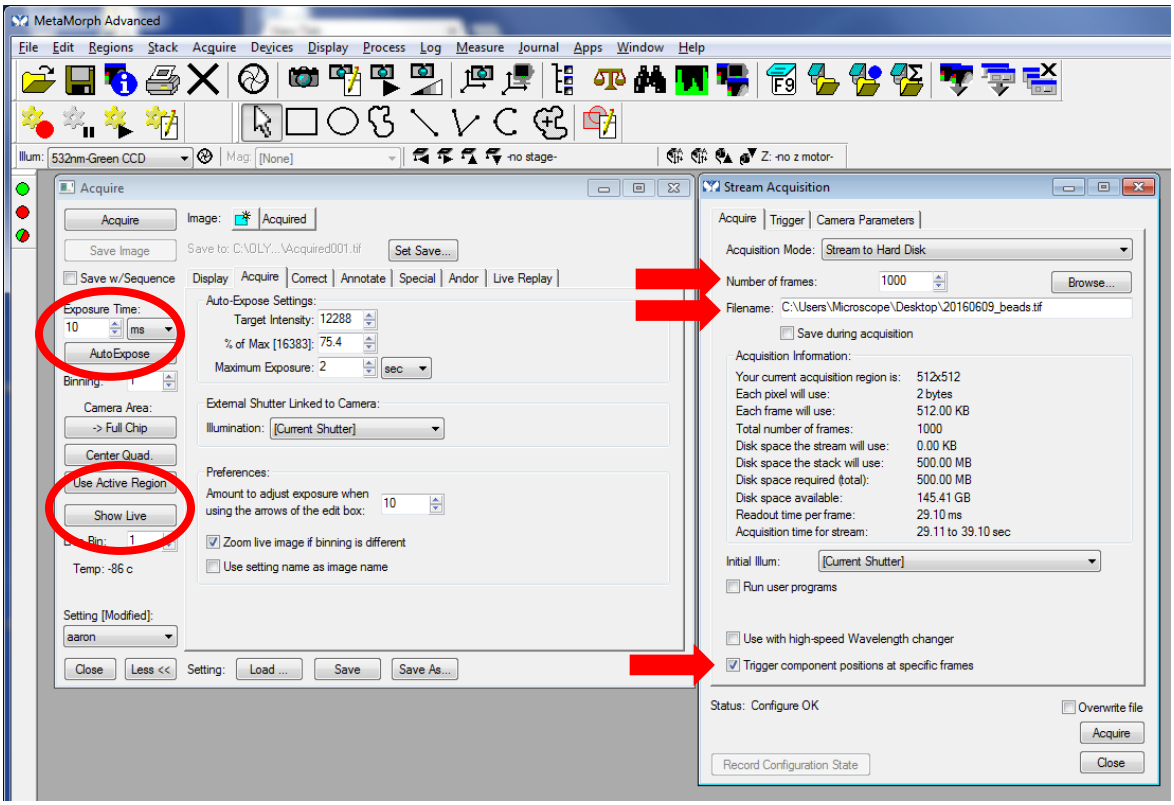


Acquiring Movies:

To set parameters for your movies select "Acquire" from the top menu (red arrow in figure below) and then select "Acquire…" and "Stream Acquisition…" from the menu that appears.



The “Acquire” and “Stream Acquisition” windows will open and should look basically like they do in the figure below.

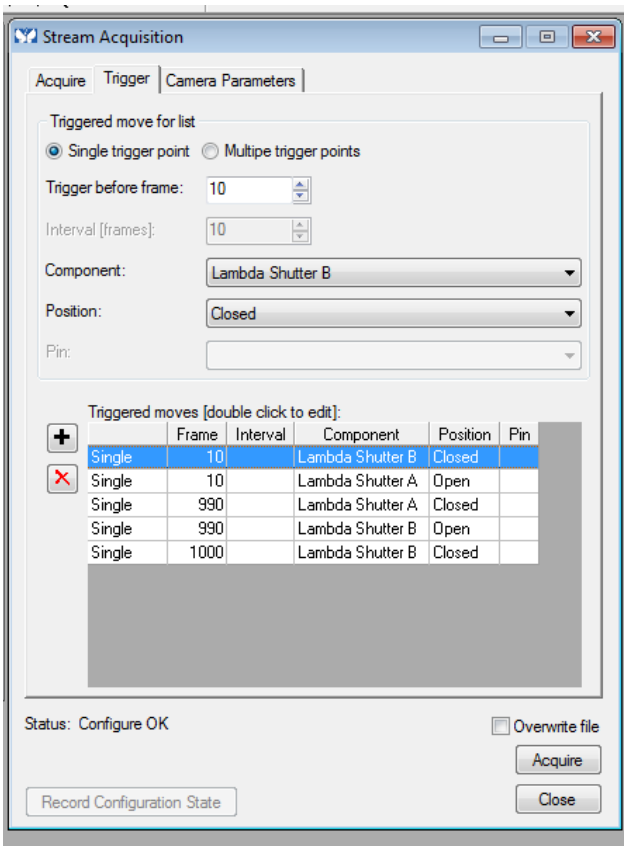


In the “Acquire” window, usually only the “Exposure Time” value is changed (usually from 10ms to 200ms,) most other setting should not be adjusted.

Click on the “Show Live” button to view a live stream of your sample. If you focused on the edge of a grease lane as described previously, you should move your field of view to be more towards the center of your lane or else the laser will reflect extremely brightly off the grease. With the red laser shutter open you should be able to focus on your sample with the fine focus know on the microscope. You can observe through the eye piece first if you wish, but the “Live” view is going to be more representative of what the movie will capture.

In the “Stream Acquisition” window you can set the total number of frames you want to collect in your movie, 1000 is common. Select a target directory and filename (be descriptive, but do not use spaces) in the “Filename” box. “Acquisition Mode:” should always be set to “Stream to Hard Disk”

In “Stream Acquisition” you can select to have shutters open and close at set frame numbers during your acquisition. Select the “Trigger” tab from near the top of the window. The “Stream Acquisition” window will change to look like the next figure:



“Lambda Shutter B” controls the shutter for the Red Laser.

“Lambda Shutter A” controls the shutter for the Green Laser.

This table of “Triggered moves” lists when the two shutters for the lasers will be open and closed throughout the movie. New lines can be added to the end of the table with the “+” button on the left. Any highlighted line can be deleted by pressing the red “X” button on the left.

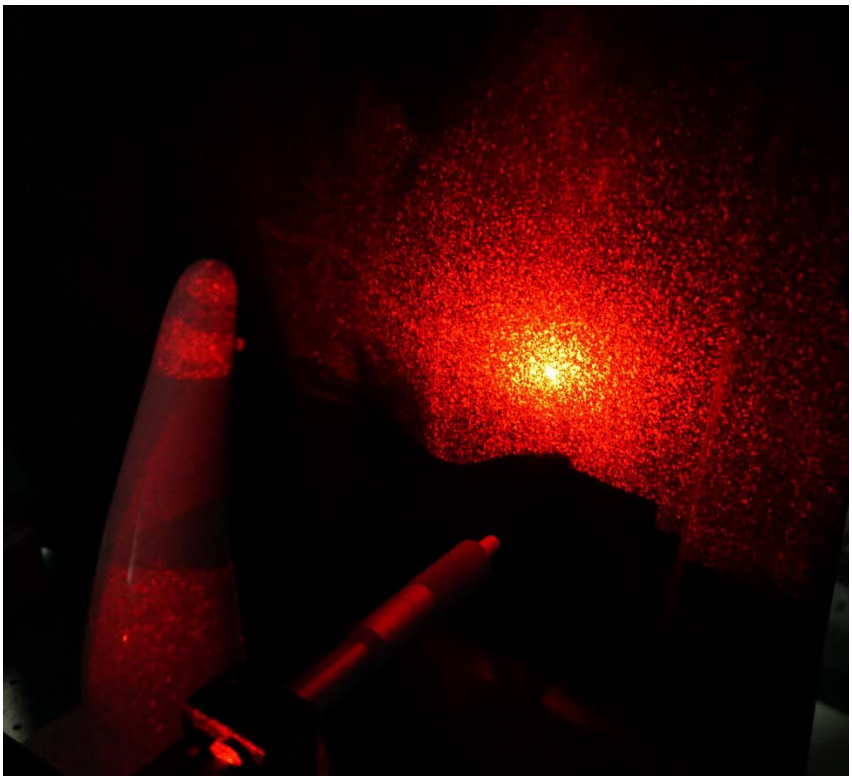
When the sample is in focus and all of the parameters are set and you are ready to start saving a movie click on the “Acquire” button.

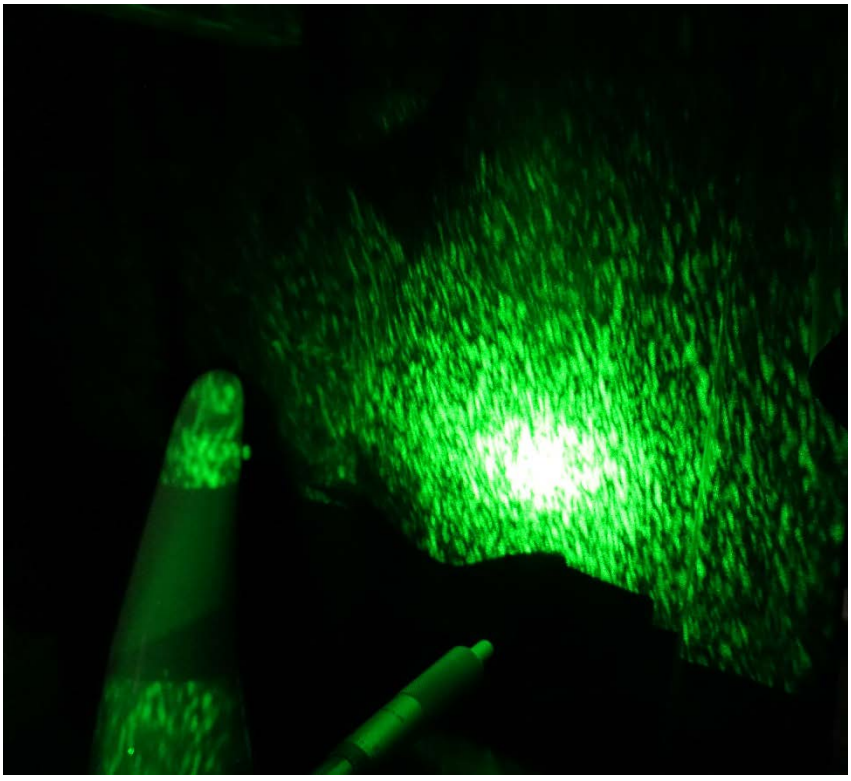
A display window with the sample view being captured to the movie file will appear on the screen.

Be sure to change the name of the file to which you are saving after each acquisition or you may overwrite your own data. Also be sure they are being saved into a valid directory, or else data may be lost. It pays to check you have a viable file after your first acquisition.

When you have completed collecting all movies. Move your files from the C: drive to a network drive or to another external drive. Once you have confirmed transfer please delete old files off of the C: drive, since space is severely limited.

Characteristic scatter patterns of Red and Green lasers as they exit out of the quartz prism, when at proper TIRF angle, and illuminate the black curtains between the microscope and computer:





Laser Power reading from Prism Microscope – collected July 14, 2016

set mW	read mW		July 14, 2016
5	3.56		Red Laser (640nM) was measured to be 15.67mW output
10	6.53		
15	10.02		
20	13.56		
25	16.6		
30	18.9		
35	20.6		
40	21.9		
45	22.95		
50	23.77