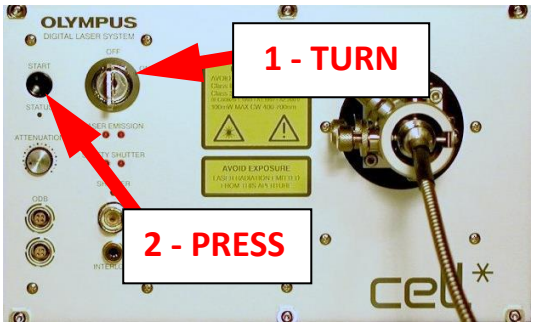
Olympus cellSens TIRF Guide

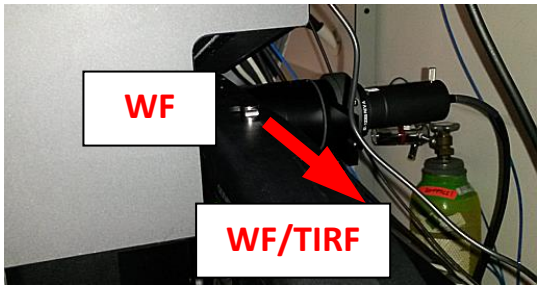
Startup

- Power up the system as described in the "Startup & Environmental Control" guide, including power strip #2 ("TIRF").

- For each laser line needed, turn the key on the control unit right and press the "Start" button.



- Push the light path selector (on the rear side) to the right (position "WF/TIRF").



- Push in the laser safety ocular shutter and close all openings of the microscope enclosure.

- Select the 100x TIRF objective and adjust the correction collar to the thickness of the cover glass. There are 2 marks and 2 graduations on the objective, corresponding to 23°C (white) and 37°C (yellow):

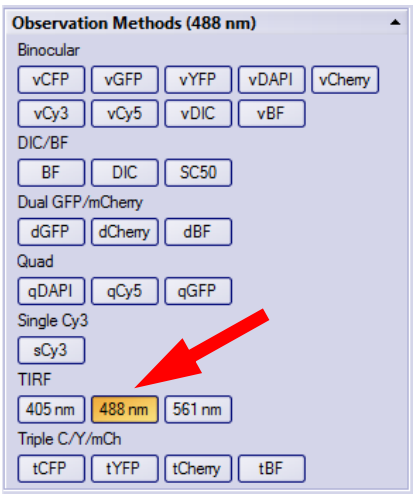
Cover glass types:
#1 0.15 mm
#1.5 0.17 mm (standard)

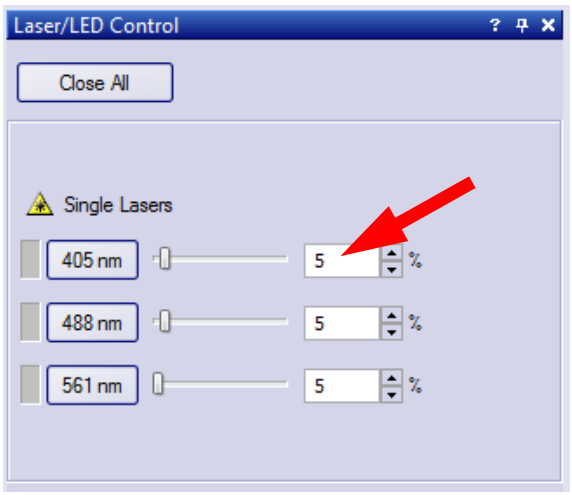
For 30°C, you can either use the 23°C graduation, subtracting ~0.03 mm from the true thickness, or use the 37°C graduation, adding ~0.03 mm:

Type	*Thickness*	*23°C scale*	*37°C scale*
#1	0.15 mm	~0.12 mm	~0.18 mm
#1.5	0.17 mm	~0.14 mm	~0.20 mm

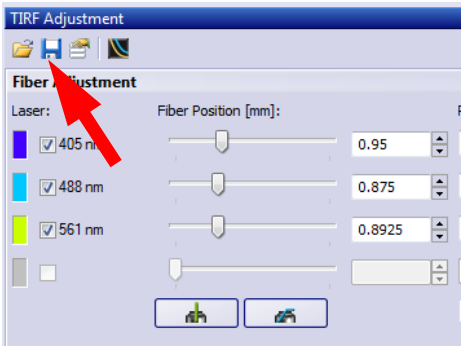
- Place a small drop of immersion oil on the objective, mount the sample and focus on the surface using a widefield illumination. Once the focus is found, remove the sample and clean the objective (first with dry lens cleaning paper, then with lens cleaning paper wetted with petroleum ether). Alternatively, you may focus using the 60x objective and swing in the 100x (still dry and clean) once the focus is found. *Do not change the focus position after this step*.

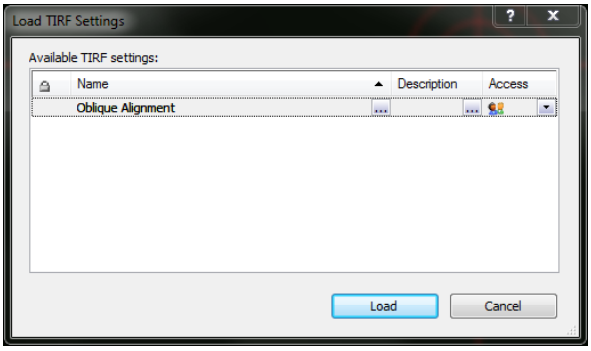
- Select a TIRF observation method ("405 nm", "488 nm" or "561 nm") and set the laser power ("Laser/LED Control" box) to a low value (5% or so):



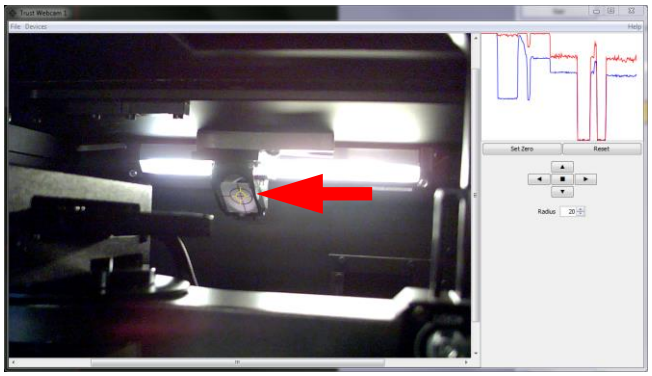


- In the "TIRF Adjustment" box, click the icon to load TIRF settings. In the dialog popping up, select "Oblique Alignment" and press "Load".

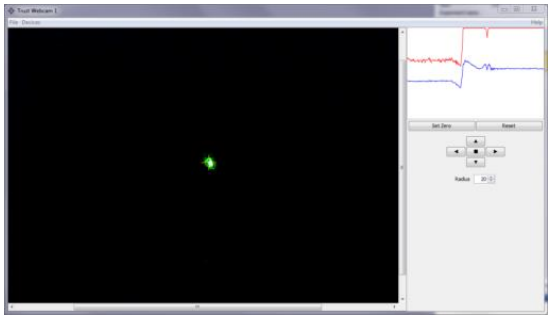


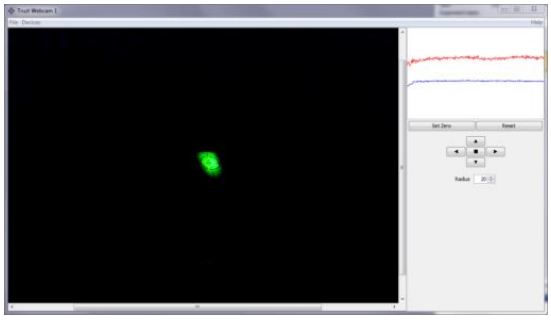


To observe the interior of the microscope enclosure, open the WebCam utility program (crosshair icon) on the toolbar. Turn on the light in the enclosure. You may have to lower the condenser turret to observe the laser target. Use the arrow buttons (or arrow keys on the keyboard) to move the yellow circle over the center of the laser target (it monitors the light intensity in the yellow circle and displays the mean signal over time as a blue line and the maximum pixel value as a red line).



- Dim the light in the enclosure and switch on "Live" mode. You should see the laser beam hitting the target. It may either sharp or diffuse:

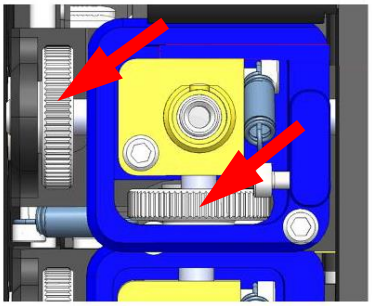




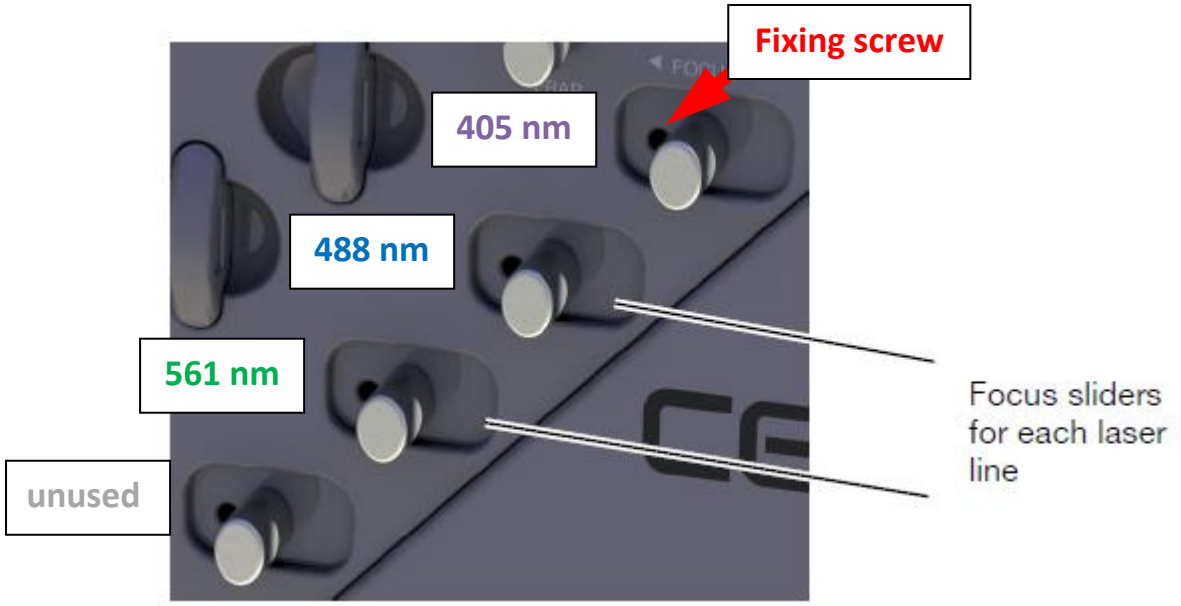
If the spot does not hit the center of the target or is diffuse, proceed with laser alignment.

Laser Alignment

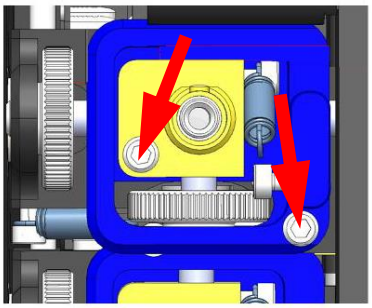
- The beam position can be adjusted with the knurled wheels ath the fiber couplings



- To focus the beam, loosen the fixing screw of the laser line (Allen wrench #3) and move the slider horizontally until the beam has maximum sharpness. Re-thighten the fixing screw.

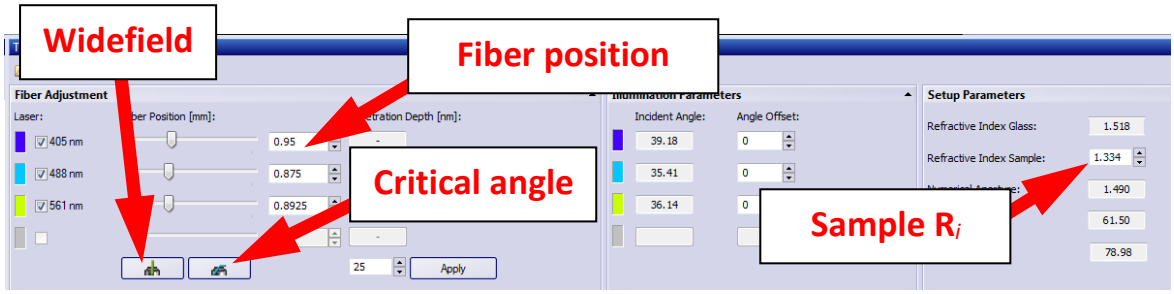


- If the field of view is not homogenously illuminated in widefield mode (upright laser beam), one can also adjust the tilt of the lasers (Allen wrench screws #2):

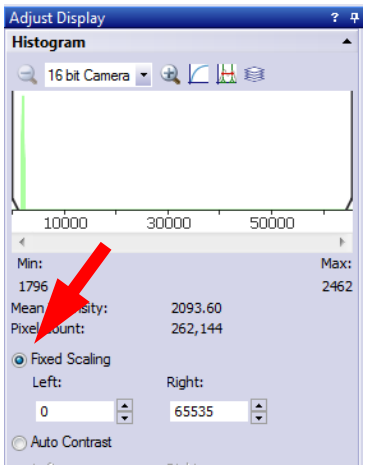


Finding and Adjusting the Critical Angle Position

- Mount the sample, select a laser illumination method ("405 nm", "488 nm" or "561 nm"), set the lasers to widefield mode (upright) by clicking the corresponding icon in the "TIRF Alignment" box. Focus on the surface.



- In the "Adjust Display" box, select "Fixed Scaling" (to observe the absolute signal intensity).



- Set the laser to the critical angle. Vary the angle by scrolling the fiber position spinner up and down a bit. The signal will be maximal at the critical angle and will decay somewhat above and below. If the true critical angle is very different from the theoretical value, adjust the sample refractive index field until a better match is obtained. Significant deviations are expected to occur at high salt, buffer or sugar solutions (the R_i of 1 M HEPES, for example, is 1.37).

Shutdown

- Set the lasers to widefield mode (upright)
- Turn the key(s) on the laser controller(s) to the left (the red LEDs should extinguish)
- Move the light path selection slider to the left (position "WF")
- Pull the ocular laser safety shutter out
- Proceed with lens cleaning and shutdown as described in the "Startup & Environmental Control" guide