TIRF Install and Alignment

Direct exposure to laser light can lead to an eye injury. During alignment, the laser beam may be unpredictable. Use low laser power, physical beam-stops and wear protective eye-wear.

When aligning a TIRF (Total Internal Reflection Fluorescence microscopy) system, one must be considerate of the tilt, and focus of the excitation light. The goal is to have the excitation beam be slightly off-center but perpendicular to the back focal plane of the objective. The TIRF system is aligned if the following are true.

1. The excitation beam is aligned through a single-mode fiber.
2. The excitation beam is straight as it passes through the iris and TIRF lens.
3. The excitation beam is straight before the beam splitter, but with a radial offset from center.
4. The excitation beam is centered on the non-adjustable axis in the objective mount.
5. The excitation beam is focused to the back focal plane of the objective with an offset, so that collimated light exits into the sample slide at an angle.

1) Fiber alignment: With the fiber attached to the laser source make sure that there is a uniform Gaussian distribution coming out of the fiber tip, projected on a flat surface. If this is not the case your fiber might be dirty, or the source is misaligned. If cleaning does not fix the issue, you will have to adjust the laser source. If the single-mode fiber cannot be aligned such that a uniform Gaussian spot projects from the output tip, the fiber may be scratched or cracked.

Printable square alignment target

2) Condenser alignment: Attach the fiber coupler to the condensing tube lens, then connect that assembly to the iris. Next, attach the aligned fibers output to the fiber coupler. With the laser at a low power but still visible (around 1mW), see if the laser light coming out the iris (with the iris open) is centered on the axis that the micrometer adjusts by placing a sighting target on the end of the iris, after the condenser lens. In most cases the micrometer adjusts the beam from left to right horizontally, so the spot should be vertically centered on a horizontal line in the center of the target which bisects the tube. For the following alignments, turn the micrometer counterclockwise until the Gaussian spot on the sighting target is perfectly centered. Once the spot is centered, then close the iris until the edges begin to disappear. If the beam is not centered, then you must adjust the micrometer for horizontal or the fiber coupler with shims for vertical. Repeat this process until you are able to close the iris as far as you can without blocking out the beam all together, so the center of the Gaussian spot is aligned to the center of the iris.

3) TIRF assembly alignment: Attach the assembled fiber coupled condenser lens and iris to the provided extender tubes. Next, attach the other end of the extender tubes to the TIRF lens cage holder, and from the TIRF lens cage to the offset rings on the C60 filter cube. With the condenser slightly loose but still connected to the iris, make sure that it is level to the RAMMs mounting surface using a bubble level such that the micrometer protrudes horizontally. Once the fiber launch and condenser assembly is level, carefully tighten the tube connections. Take the filter cube door off, and place a target on the opening. With the laser at a low power but still visible, adjust the micrometer

until the beam is centered on the target. The spot on the target should perfectly follow the central horizontal line on one half of the target. With the micrometer at the furthest counter-clockwise position, the spot on the target should be slightly off-center, ranging through the center to the other side of the target when the micrometer is adjusted clockwise. If the beam is not able to be centered by adjusting the micrometer, make sure your tube lenses are all connected properly to the cube. If the tube lenses are straight and the beam is still not centered, then the offset ring adapters between the lenses and the cube are off of the horizontal axis. You should email ASI or call us for help with this adjustment.

While rotating the micrometer counter-clockwise, there will be a point where it still rotates, but the position of the fiber ceases being adjusted. This is the furthest counter-clockwise position.

Printable round alignment target

4) Beam splitter alignment: Install your beam splitter, and your emission filter into your filter cube (Filter installation video tutorial: Installing Filters). Make sure that all three adjustment screws are engaged. Place a round target over the objective mount such that it is centered. With the laser still at a low power, manipulate the three adjustment screws on the cube until the beam is on the central horizontal line of the target. Once again adjusting the micrometer should only cause a horizontal translation of the excitation beam. If the beam was previously aligned, but does not travel solely across the central horizontal line of the round target, then the beam splitter requires further adjustment. The video Installing Mirrors should clarify the dichroic mirror installation and alignment into the filter cube body.

5) TIRF lens adjustment: Mount the objective on the scope. Place a light blocking object between your eyes and the tip of the objective, such as an open book, to act as a beam-stop for safety. Turn the laser on and open the iris. Adjust the micrometer until the beam is shining on the ceiling. If the beam makes an Airy disc pattern you are done. If not, adjust the TIRF lens back and forth until the laser spot on the ceiling makes an Airy pattern with a crisply focused black ring just outside of the bright central circle. TIRF Will occur when the beam coming out of the objective is at or near the critical angle of the sample-glass interface.

Your TIRF assembly alignment is now complete. For questions and clarifications email ASI at `info [at] asiimaging [dot] com`

For the old assembly and alignment procedures, see the old TIRF documentation.

manual, ramm, tirf