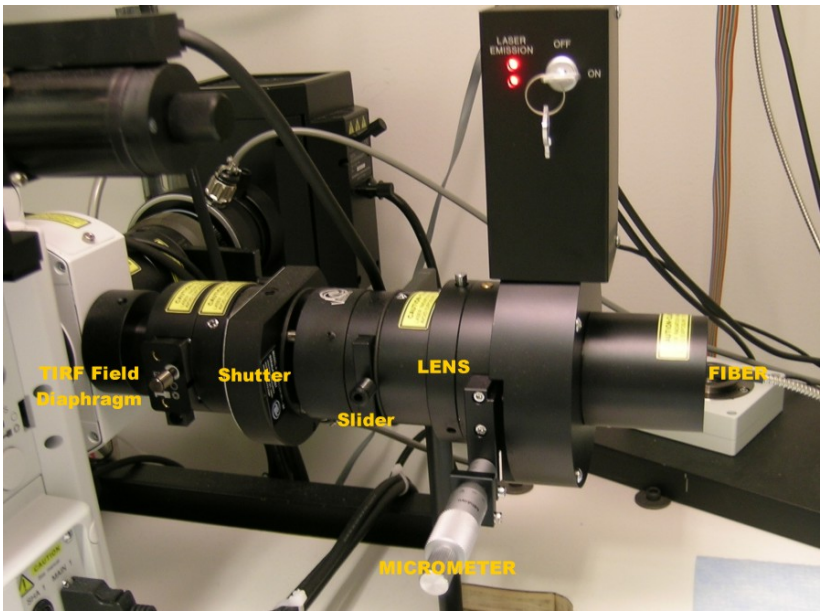
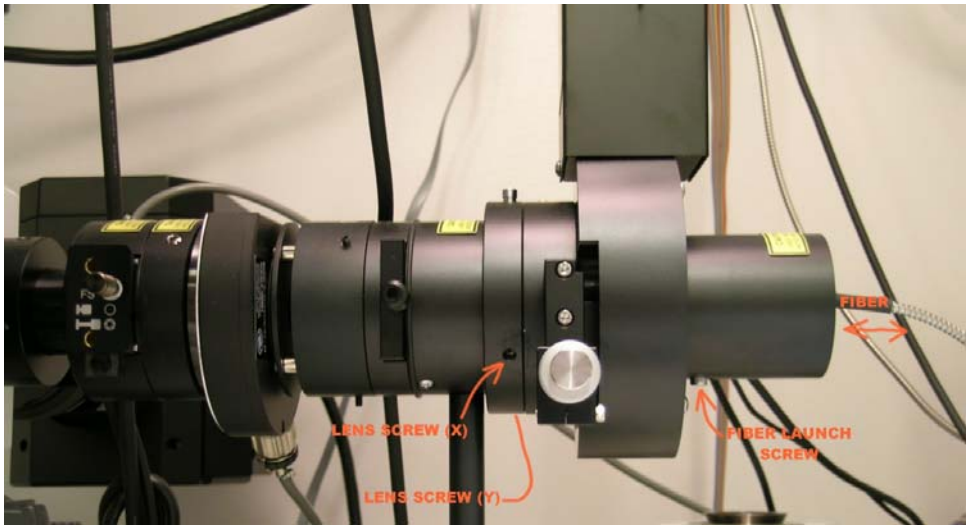
TIRF ALIGNMENT PROTOCOL

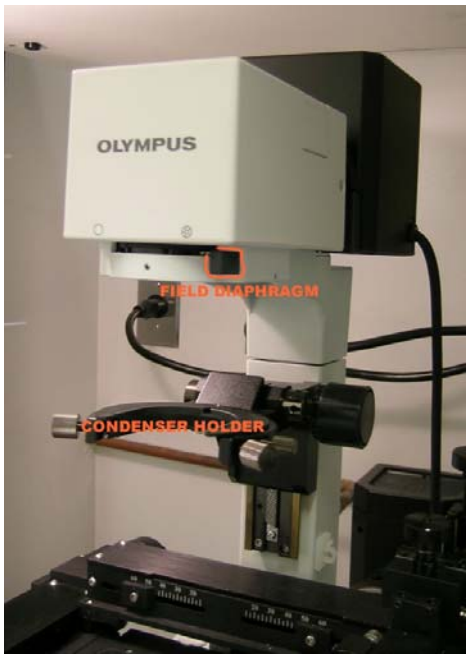


1) Check the fiber alignment
Remove one of the objective caps, tilt the condenser arm backward and, with the TIRF prism upright, project the laser beam onto the bottom of the overhead shelve. The aperture, limited by the TIRF field diaphragm, should be filled homogeneously. Look for left / right gradients. *Because of the current custom fiber launch manufactured by Olympus, realigning the fiber is a challenging task best done by Olympus representatives!*



2) Set focus plane
Put the specimen on the stage (*), select the proper objective (**) and focus on the TIRF plane, i.e. the glass / water interface to be imaged in TIRF. *Without changing focus*, remove the specimen and wipe the objective free of oil. Loosen the fiber assembly holding screw and slide it until the center beam is as sharp as possible. Lock the fiber in place (***).

3) Align TIRF lens
Remove the condenser if present and close the field diaphragm on the condenser arm. With the condenser arm in the upright position, adjust the centering screws on the TIRF lens to center the laser beam on the opening of the condenser field diaphragm (optical axis).



4) Check for proper TIRF
Put the specimen back on stage and increase the angle on the TIRF prism using the micrometer. The projection of the laser beam will move away from the operator. Once the critical angle is reached, check for proper TIRF by moving the focus up and down (the image disappears in every direction) and the TIRF micrometer (image disappears when increasing the angle, wide field fluorescence appears when decreasing it (****)

Notes:

(*) Olympus recommends using only Olympus immersion oil. While the refractive index is almost identical to Zeiss Immersol (1.516 vs 1.518), immersion oil has a significant impact on TIRF quality.

(**) Due to large differences in the back apertures, switching objectives requires a complete realignment of the system. The microscope is currently aligned for the 60x objective (April 07).

(***) Optimum alignment depends on the focus plane and is lost when switching specimen or chamber height.

(****) As of this writing (April 07), multi-channel TIRF imaging requires switching fluorescence cubes. Because of small differences in cube alignment, optimum dual-color TIRF is not possible. To alleviate this issue, the microscope will be outfitted with dual-lines dichroic cubes and an emission filter wheel.