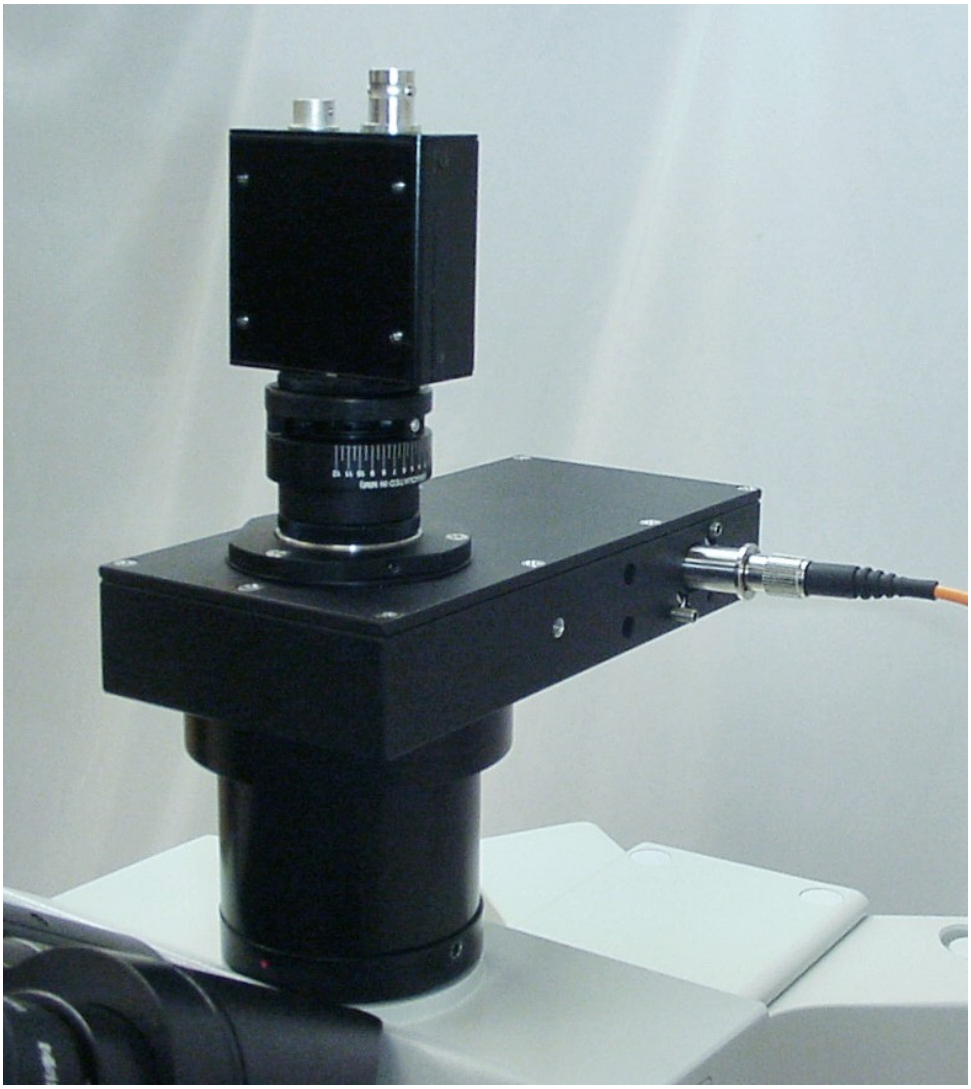
ASI Photoport TIRF Injector
Instruction Manual



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Introduction

ASI's Photoport TIRF Injector opens the possibility of TIRF illumination microscopy to any microscope that has a camera port. The injector incorporates the dichroic beam splitter and blocking filters required, and includes a C-mount adapter for a camera. The user's laser is coupled into the injector with a FC fiber connector. This manual will discuss the general theory of the TIRF injector and show you how to set up and align the system. We will also discuss the important issue of laser safety and how best to configure the system in a safe manner.

Figure 1 illustrates the main features of the injector. A steerable laser collimator is used to inject a fiber-coupled laser into the system.

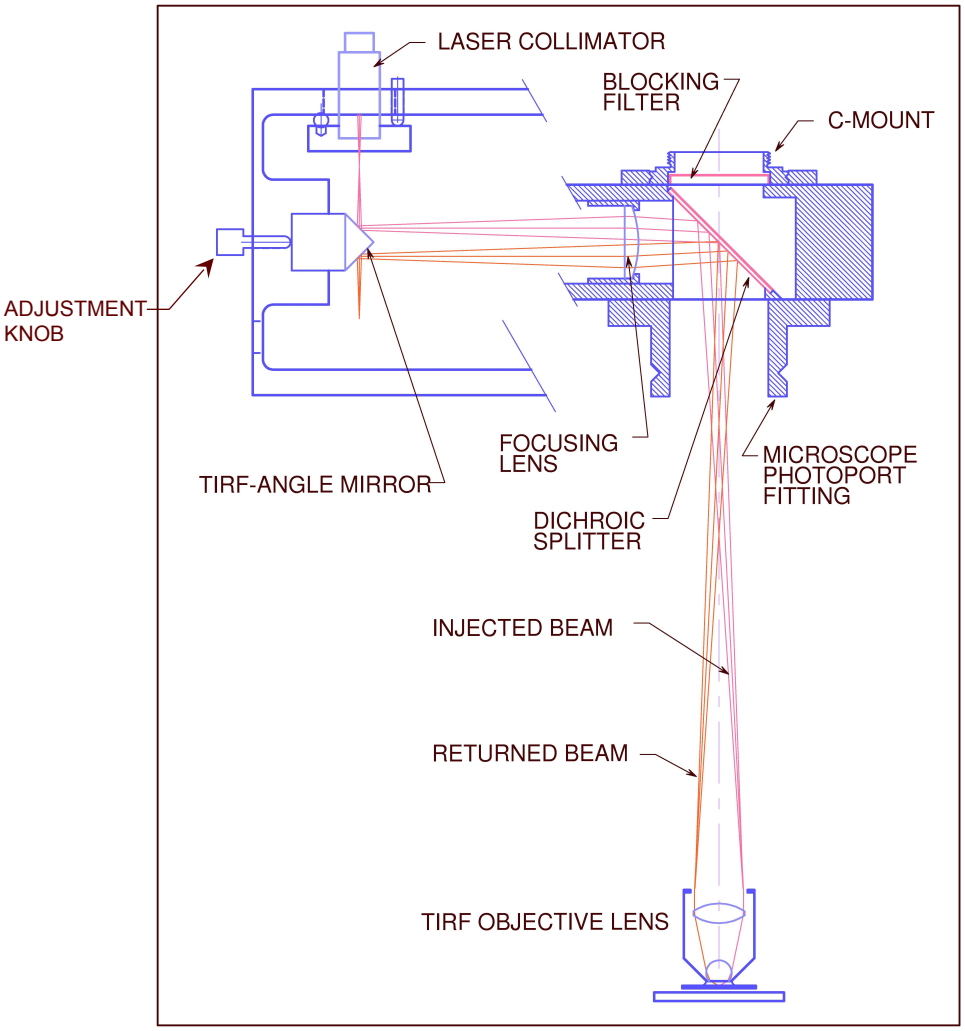


Figure 1: TIRF injector optical diagram

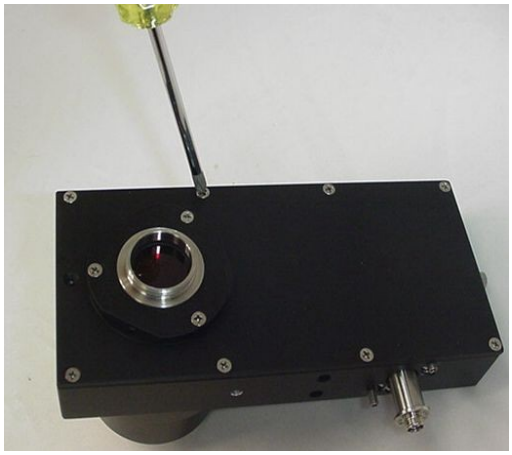
The adjustable TIRF-Angle mirror reflects the light from the laser collimator. A focusing lens images the tight spot from the collimator into the back aperture of the objective lens. Moving the TIRF-Angle mirror inward moves the focused beam to the edge of the objective lens aperture. Light focused at the very edge of the TIRF objective will be deflected sufficiently that total internal reflection of the light will occur at the cover-slip/water interface.

Installation

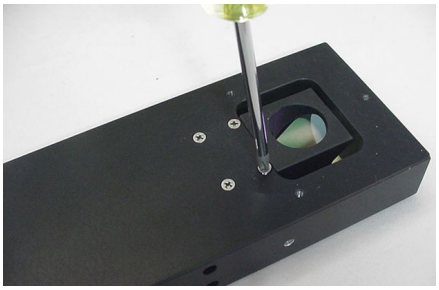
ASI will have already installed the dichroic splitter and blocking filter initially ordered. These first steps show how to change these components as required.

Begin by changing the dichroic beam splitter in the beam splitter holder if necessary. This is easiest if you first remove the holder from the main body. To reach the beam splitter holder screws, you must first remove the photoport adapter using a 3/32 Allen driver. You will also need to remove the cover plate using a Phillips screwdriver. The pictures below illustrate the steps involved.

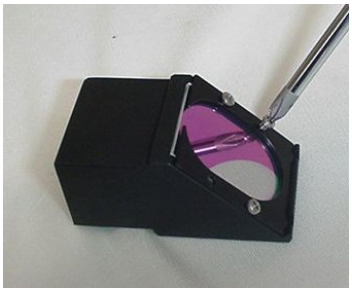
Remove photoport adapter



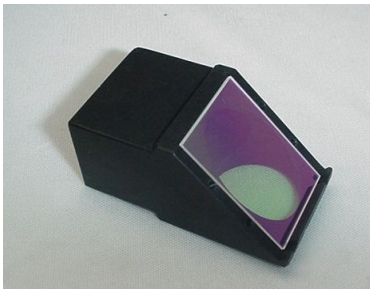
Remove cover plate



Remove beam splitter holder



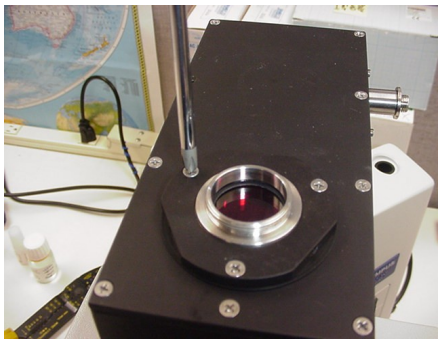
Remove keeper



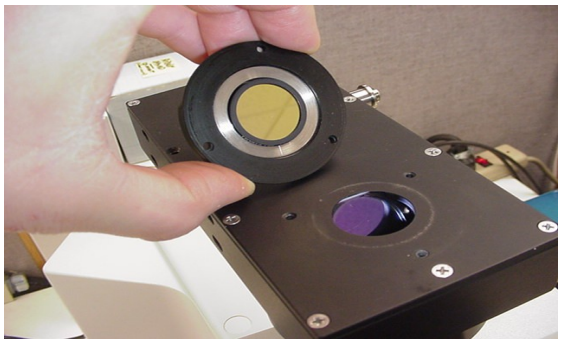
Change the dichroic beam splitter in holder

Re-attach keeper. Replace the beam splitter holder in the body with the four flat-head screws and reattach the photoport adapter. Reinstall the cover plate with nine flat-head screws.

Next, change the blocking filter behind the c-mount adapter if necessary. Remove the C-mount adapter, slip the filter out of the C-mount recess, and replace with the desired blocking filter. Reinstall the C-mount on the cover-plate. See the figures below.



Removing the C-mount



Recess, with filter installed

Next, install the TIRF injector assembly onto the photoport of the microscope. Orientation is not critical, but keep in mind how the optical fiber is routed, and allow for easy access to the adjustment screws. The cover photo shows the TIRF injector installed on a BX41 microscope.

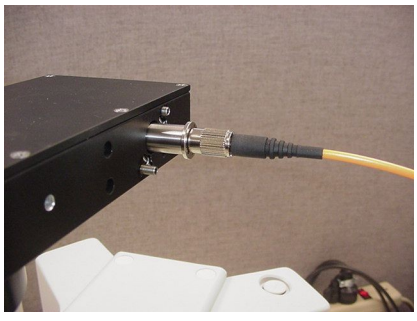
The Laser and Laser Safety

Best results will be obtained with a single-mode fiber-coupled laser. A multi-mode laser cannot be focused to a small spot in the back aperture of the objective lens, and when not fully in TIRF mode, the speckle pattern can be objectionable. The uniformity of the lighting will be much worse with a multi-mode fiber than with a single mode fiber.

Attach the fiber from the laser to the FC connector on the fiber collimator.

Laser safety must be taken into account in an overall system. The best approach is to interlock the laser to the eyepiece shutter. With the photoport injector, the eyepieces have no laser blocking filters in front of them (unless an extra pair of blocking filters is purchased for the expressed purpose of including them within the eyepieces themselves) so the eyepieces cannot be used when illuminating with the TIRF laser. The interlock system will vary with the specific microscope and laser used.

For the BX41 illustrated here, a Hall effect sensor and a small magnet are installed inside the eyepiece assembly. This is used to detect when the eyepiece slider is fully in the "camera" position and the eyepieces are shuttered. The interlock is connected to the laser power supply using miniature stereo phone plugs and cables. The interlock circuit is shown in Appendix A of this manual.



Fiber attached to collimator

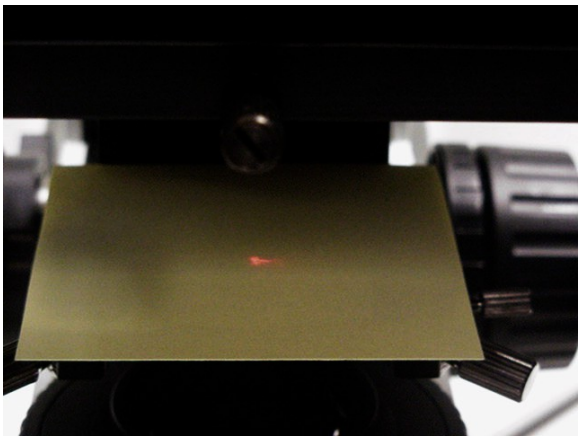
Interlock cable attached to BX41 eyepiece assembly

Alignment of the Laser

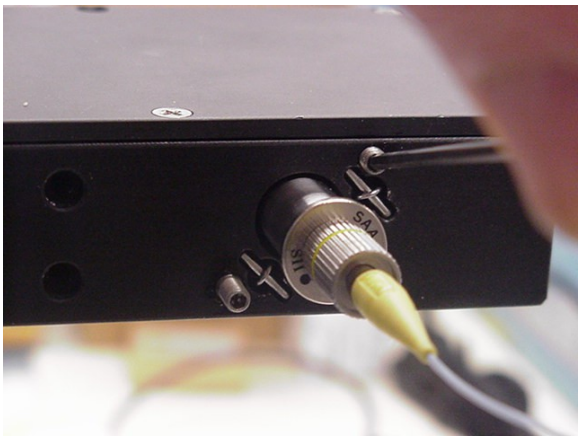
Begin the alignment process by first backing off the TIRF-Angle adjuster all the way counterclockwise (CCW) until no resistance is felt on the adjusting screw. This retracts the mirror and puts the laser on the very tip of the TIRF-Angle mirror.

TIRF-Angle mirror adjuster

Remove the condenser from the microscope and place a piece of paper as a target directly under the objective lens on an upright microscope, or directly above it on an inverted microscope. Turn the laser on and look for the laser spot on the target paper. With the TIRF-Angle mirror fully retracted, the laser should exit the microscope centered along the optical axis when adjusted correctly. Beam steering is accomplished with the two adjusters on the laser collimator. Use a 5/64 or 2 mm Allen driver to manipulate the adjusters.



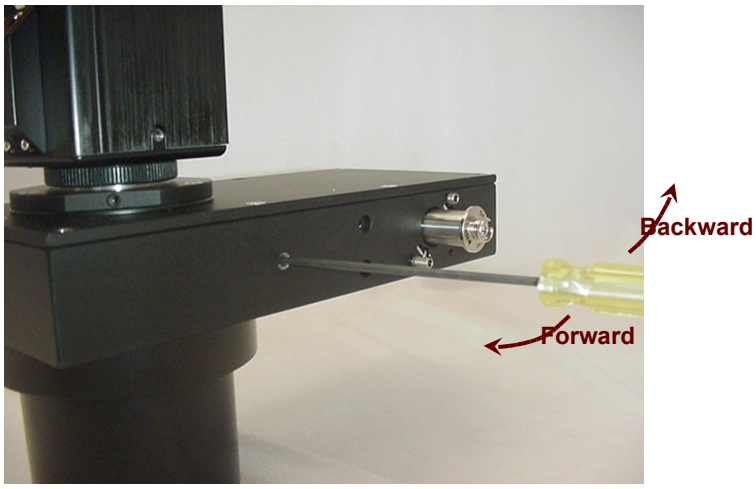
Laser spot on paper target



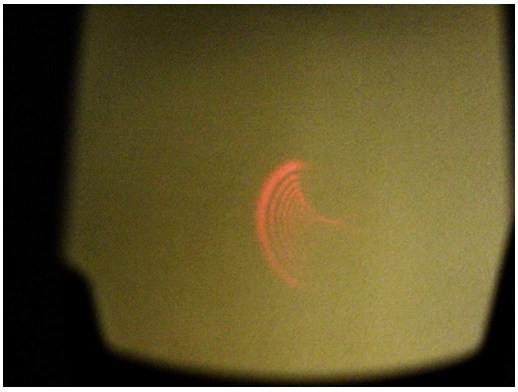
Adjusting the position of the beam axes

Adjust the steering screws until the laser is aimed directly along the optical axis. If the laser is too far out of focus, proceed to focus the laser, as describe below, before completing the steering alignment.

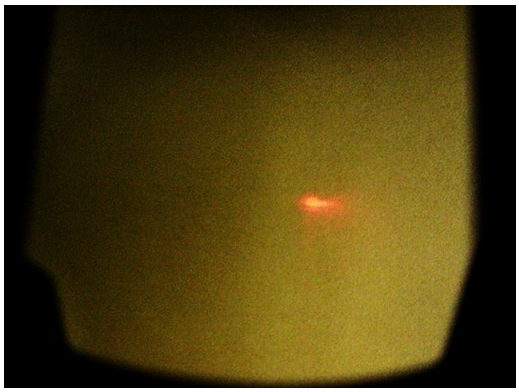
Laser focus is accomplished by loosening the Focusing Lens slider clamp screw with a 5/64 Allen wrench, and then using the Allen wrench as a lever to slide the lens either backwards or forwards. Once best focus is found, tighten the clamp screw to lock the Focusing Lens in place. The clamp screw is found through the hole or slot in the side of the housing as shown in the photo below.



Adjusting the laser focus



Poor focus



Better focus

Once the laser is focused and aligned to leave the objective along the optical axis, we are ready to illuminate a sample and set up the Total Internal Reflection (TIR) condition.

Viewing a sample in TIR and non-TIR modes

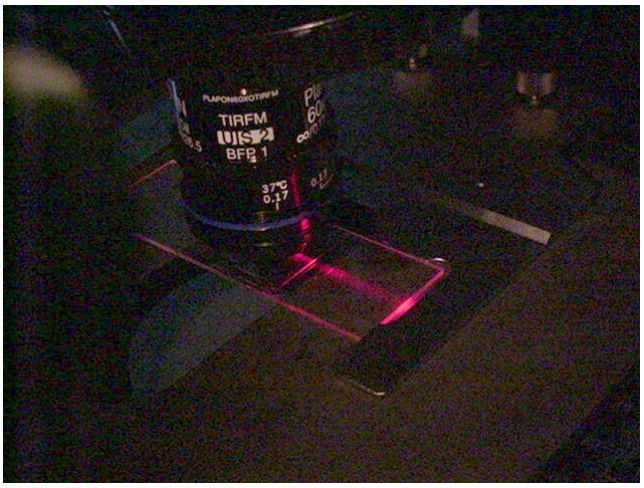
The ASI TIRF Injector works on both upright and inverted microscopes. Although the following references apply to an upright microscope, use appropriate slide and cover slip references when using an inverted microscope.

Fluorescent microspheres provide a good sample for testing the TIRF system. The following tests were done using Molecular Probes #F8789 FluoSpheres - dark red fluorescent (660/680) 0.04µm carboxylate-modified microspheres. The fluorescent sample was diluted in water, and a small drop placed on a slide with a cover slip. The slide was place under the objective and immersion oil was used between the TIRF objective lens and the cover slip.

The eyepieces are neither safe nor useful for observation, so a digital camera (TILL Imago) was attached to the C-mount coupler on the TIRF injector and used to observe the fluorescent sample.

Adjusting the TIR Angle

Best illumination of the sample occurs when the laser is tilted to near the TIR angle. Even if not in TIRF mode, direct reflections of the laser are minimized and the illumination is more uniform when the laser is coming in near the TIR angle. To tilt the beam over, turn the TIRF-Angle mirror adjuster screw clockwise (CW) on the injector. Watch the beam on the target as you do this. You should see the beam tilt over to one side. Continue moving the TIRF-Angle mirror inward until the beam just starts to be seen running along the cover slip. See the photo below.



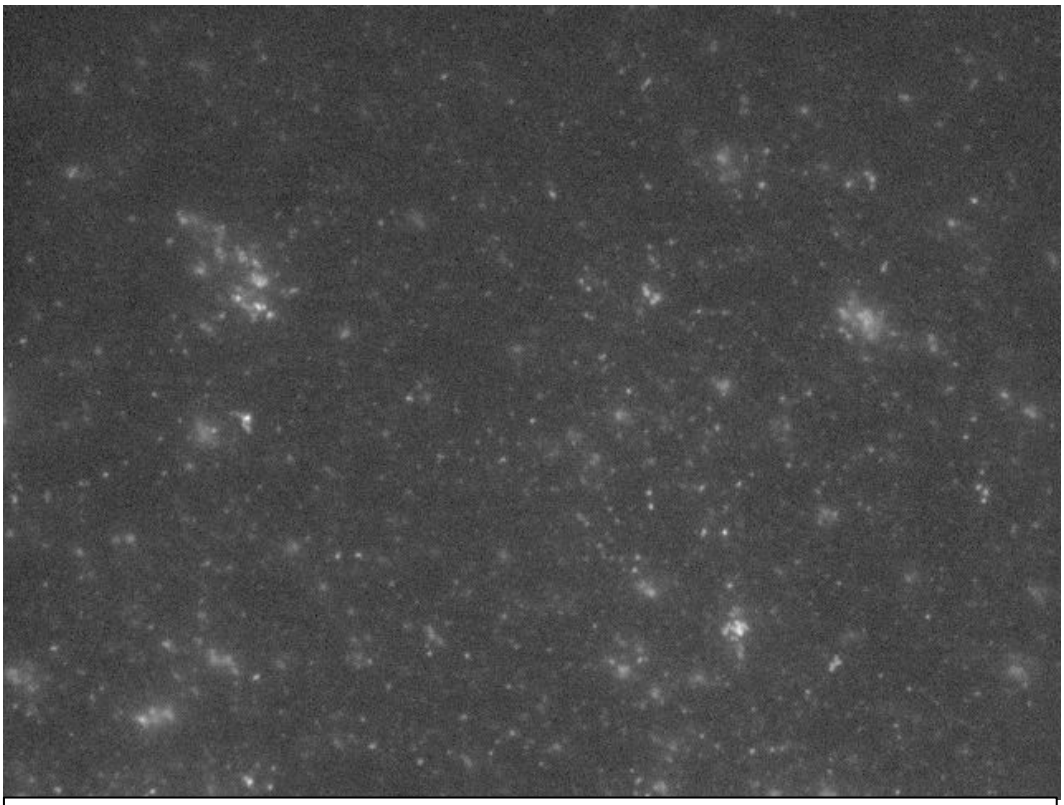
At this point you should be able to focus on the fluorescent sample. Once the sample is in focus, continue to increase the TIRF angle until the sample goes dark as the illumination disappears completely. Then back off the adjuster until the illumination just returns again. This is the TIRF region where only objects very near the cover slip are illuminated. Re-focus the microscope for best image.

Images of Bead Samples

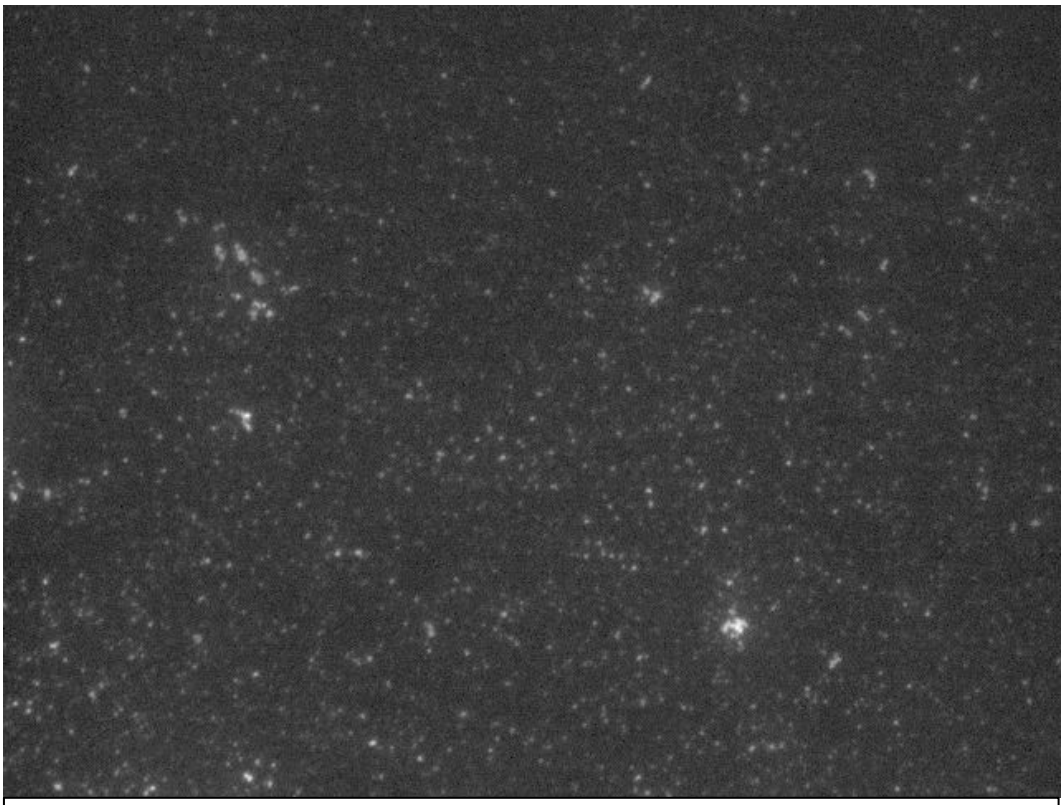
The photos on the next page show both TIRF and non-TIRF modes. The fluorescent beads tend to end up on the glass surface of either the cover slip or the slide. Note that these 40 nm beads are sub-diffraction-limited in size, and have a tendency to clump together. In TIRF mode (on an upright microscope) only the beads on the cover slip are illuminated. In the non-TIRF image, the beads on the slide surface are slightly out of focus, but definitely within the depth-of-field of the objective, which is focused on the cover slip surface. You will notice that most of the blurry beads are gone in the TIRF mode image.

The laser power was about 40 μW at the end of the single-mode fiber for the images shown. The exposure time was 200 ms.

A bead field provides a good sample to re-adjust the laser focus for best uniformity in TIRF mode. Tweak the focus adjustment while observing the uniformity of the illumination across the field of view.



Non-TIRF image of bead sample



TIRF-mode image of same bead field

Appendix A - Laser Safety Interlock Schematic