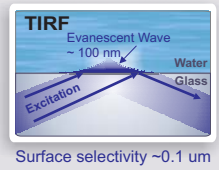
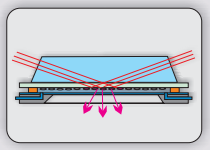
*i*Diagnostics (*i*TIRF Arrays)
TIRF Spectroscopy
TIRF Microscopy

TIRF Labs

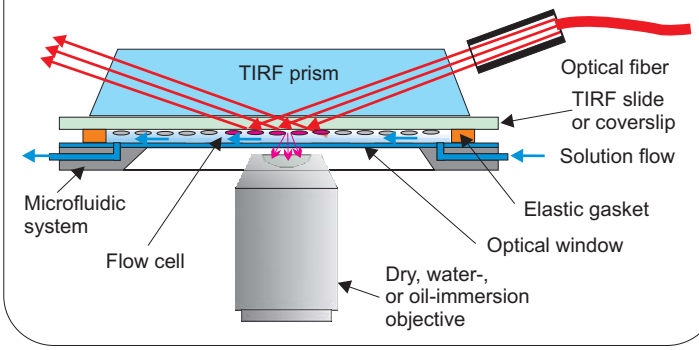
Prism-up TIRF Microscopy Flow System *puTIRF*
for Single Molecule Detection
Analysis of Biomolecular Interactions
Cell Membrane and other studies

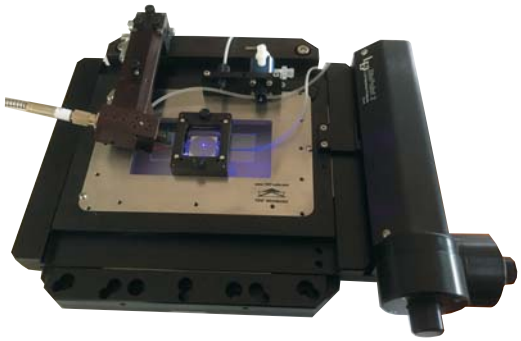


Surface selectivity ~0.1 um



Prism-TIRF - the best signal-to-background ratio
The cleanest TIRF effect
Excitation channel is independent from emission
UV-vis excitation. Use with dry, water-, or oil-immersion lenses





TIRF Microscopy Flow System *puTIRF*
Shown installed on motorized XY translation stage of inverted microscope
Well-suited for single molecule detection sm-FRET experiments
and other multicolor and single color TIRF applications
Designed as add-on accessory for inverted microscopes

Prism-based TIRF Flow System for Inverted Microscopes - *puTIRF*

Total Internal Reflection Fluorescence (TIRF) has become a method of choice for single molecule detection (SMD) and other studies that require the excitation of fluorescence confined in space [1,2]. In SMD, the spatial confinement is necessary for minimizing the background signal and obtaining contrast sufficient for detecting single molecules. TIRF provides a superior spatial confinement - it excites only a ~100 nm layer of the specimen (in comparison, the confocal scheme excites ~1,000 nm). The intensity of excitation is maximal at the TIRF surface and exponentially decays with distance. Only molecules that are at the surface and in a ~100 nm proximity to the surface are excited and fluoresce; the bulk of the specimen is not excited. The surface selectivity of TIRF allows for detecting single molecules, and is also well-suited for cell membrane studies, analysis of biomolecular interactions, and other areas.

Record-high Signal-to-Background Ratio. Among TIRF geometries that include through-objective and lightguide-based TIRF, prism-based scheme ensures the cleanest TIRF effect and provides the best signal-to-background ratio. In the case of through-objective TIRF, excitation and emission channels share the same optical elements; the intensity of undesirable stray light is large, and the TIRF effect is compromised [1]. In prism-TIRF, the excitation is independent from the emission channel. This fact and the absence of additional light-scattering and reflecting surfaces ensures the best signal-to-background ratio to the prism-TIRF [2].

XY Translation Stages. TIRF Labs offers prism-based TIRF systems configured for inverted and upright microscopes, with fixed and variable angles of incidence. (Visit www.tirf-labs.com and contact TIRF Labs for more information.) This brochure describes the most popular prism-up TIRF system (puTIRF). puTIRF is designed as an add-on accessory for inverted microscopes. The schematics and the photo above show the puTIRF system installed into a K-frame window of a motorized XY translation stage. puTIRF is supplied on a platform of nested design, which also can be used with manual XY translation stages, round 4-inch diameter windows of microscopes or Gibraltar platforms, or rectangular windows with the footprint of 96-well SBS plate.

puTIRF System is Compatible with Dry, Water- and Oil-immersion Objectives. In puTIRF total internal reflection occurs at the interface between a slide (or a coverslip) and water or aqueous solution, as shown in the scheme above. The TIRF prism and slide are brought in optical contact by a droplet of refractive-index-matching fluid. For excitation light, the prism and the slide represent continuous optical medium. A thin layer of aqueous solution and an optical window separate the TIRF surface from the objective.

Microfluidic Channels Create Planar Low-volumeTIRF Flow Cell. An advanced microfluidic system embedded into puTIRF provides high share rates at small volumetric rates, which allows for measuring k-on and k-off rate constants with *minimal* amount of bioanalyte solution. Typically, 20-40 uL of bioanalyte is sufficient for measuring a kinetic sensogram. An external pump, or gravity flow, which is always by hand, can be used witha puTIRF system for kinetic experiments.

Precision Optical-Mechanical Design of puTIRF provides high reproducibility of TIRF measurements within one experiment and between different TIRF sessions. Undergraduate students are comfortable to operate puTIRF after minimal training.

Silica Optics includes an adjustable collimator, TIRF prism, TIRF slides, and an optical window. The range of excitation wavelengths encompasses UV-vis-near IR 190-1000 nm. The size of the excitation spot can be adjusted in the range 0.1mm - 20 mm. For more information visit www.tirf-labs.com and contact TIRF Labs..

Literature cited: **1**. Brunstein M. et al. Biophys Journal 2014; 106(5): Part 1: p.1020; Part 2: p.1044. **2**. Ambrose W, et al. Cytometry 1999, 36(3), 224.

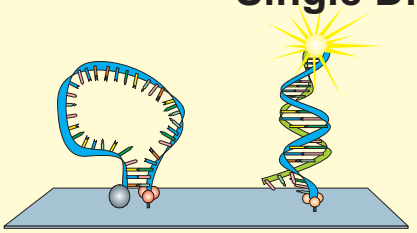
www.TIRF-Labs.com info@tirf-labs.com

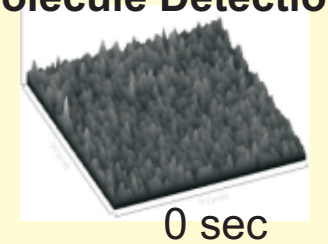
Prism-up TIRF Microscopy for Single Molecule Detection

TIRF Labs
Application Note SMD

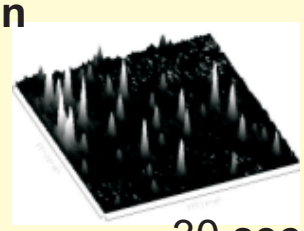
puTIRF and IgTIRF Microscopy for Single Molecule Detection

Single DNA Molecule Detection





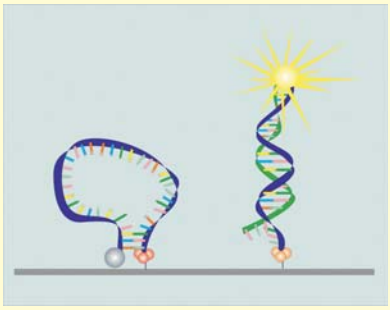
0 sec

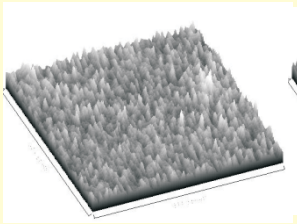


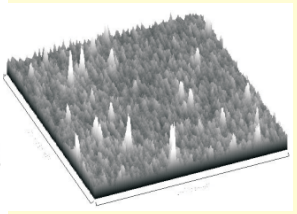
30 sec

Real-time monitoring of DNA hybridization at single molecule level using puTIRF with Nikon Eclipse TE2000 microscope. Excitation - 532 nm, emission - 560-600 nm, objective - Nikon X100 NA1.25 oil, EMCCD camera iXon Andor, subframes 100x100 pxl. 32-nt molecular beacon assay was immobilized at the TIRF slide. The beacon was equipped with TAMRA fluorophore and DABSYL quencher. Hybridization with target DNA resulted in separation of TAMRA and DABSYL and dequenching of fluorescence. Fluorescent sites with single molecules were irreversibly photobleached in a single step.

Single RNA Molecule Detection



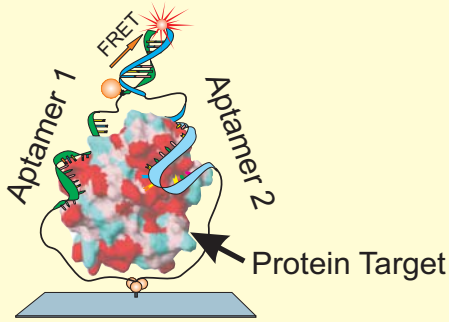


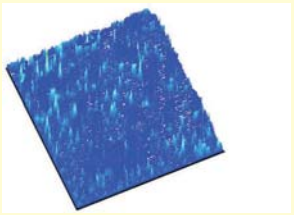


0 sec **30 sec**

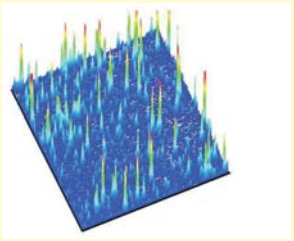
Real-time monitoring of RNA hybridization at single molecule level using puTIRF with Nikon Eclipse TE2000 microscope. Hybridization of target p53 RNA with surface-immobilized DNA molecular beacon 24-nt equipped with HEX fluorophore and BH1 quencher. Excitation - 532 nm, emission band - 550-590 nm, objective - Nikon X100 NA1.25 oil, EMCCD camera iXon Andor, subframes 80x80 pixel. Hybridization with target RNA resulted in dequenching of HEX fluorescence. Fluorescent sites with single molecules were irreversibly photobleached in a single step.

Single Protein Molecule Detection





0 sec



60 sec

Detection of single thrombin molecules using IgTIRF with Nikon Eclipse TE2000 microscope. Thrombin was binding to two-chain FRET aptamer assay immobilized at the surface of TIRF slide. Binding resulted in FRET between Cy3 and Cy5 fluorophores conjugated to aptamer 1 and aptamer 2. Excitation - 532 nm, emission band - 650-680 nm, objective - Nikon X100 NA1.25 oil, EMCCD camera iXon Andor, subframes 100x100 pixel.