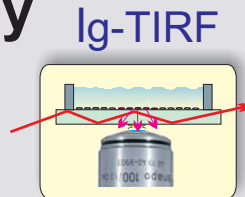
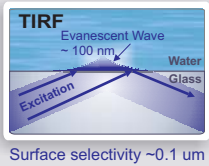




Lightguide-based TIRF Microscopy

for Cell Biology Studies Single Molecule Detection

Analysis of Biomolecular Interactions and other studies



IgTIRF - superior signal-to-background ratio

well-suited TIRF geometry for cell biology and SMD studies

Excitation path is independent from emission channel

UV-vis excitation. Use with dry, water-, or oil-immersion lenses

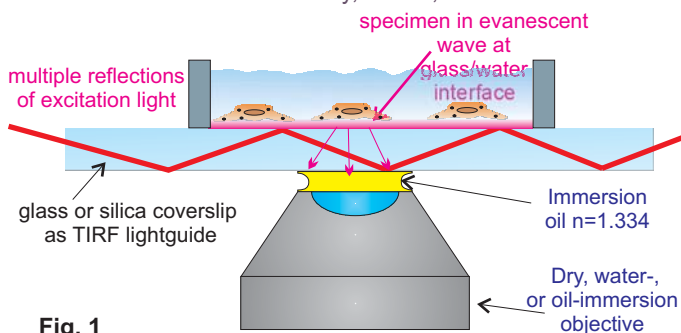
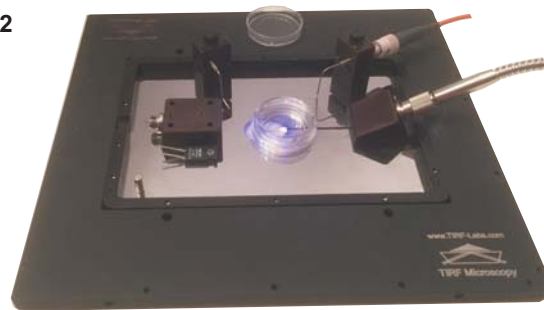


Fig. 1

Fig. 2



lightguide-based TIRF Microscopy System IgTIRF

Shown installed on K-frame for motorized XY-stages, nested into larger platform IgTIRF is well-suited for multicolor cell biology, single molecule detection, parallel use with optical tweezers, DEP control, AFM, and other applications. Designed as add-on accessory for inverted microscopes

Lightguide-based Total Internal Reflection Fluorescence Microscopy - IgTIRF

IgTIRF is a novel powerful tool for single molecule detection, cell membrane, real-time microarray, and other lifescience studies that requires the excitation of fluorescence confined in a space.

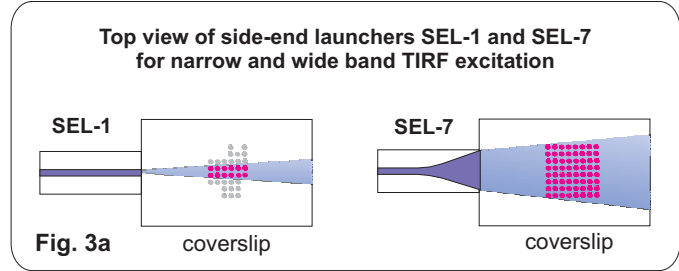
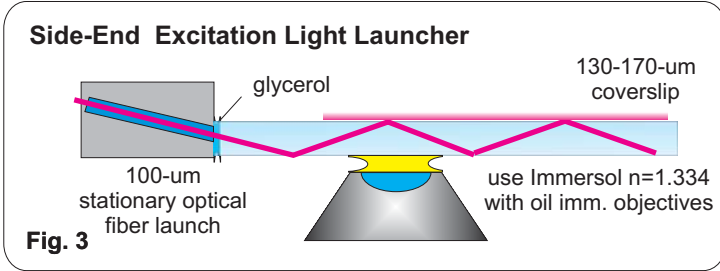
- <> IgTIRF features the excitation path, which is independent from the emission channel
- <> IgTIRF yields a superior signal-to-background ratio
- <> IgTIRF can be used with dry-, water-, and oil-immersion objectives
- <> IgTIRF is a factory-aligned system that is well-suited for multicolor TIRF experiments
- <> IgTIRF uses regular glass or silica coverslips, or Petri dishes with optical bottom
- <> IgTIRF platform also implements Shallow Angle Fluorescence Microscopy (SAFM)
- <> IgTIRF uses fiber-coupled illuminators connected by ~2-m optical fiber cable
- <> IgTIRF provides a reproducible intensity of the evanescent wave
- <> IgTIRF can be used with UV excitation, which is not available in objective-TIRF
- <> IgTIRF allows for precision control of the penetration depth by using optical traps
- <> It takes no time to install/uninstall IgTIRF, and to switch between TIRF, SAFM, micro-spot excitation, epi-fluorescence, transmittance, and other methods

This brochure describes the principles and features of the lightguide-based Total Internal Reflection Fluorescence (IgTIRF) system. The IgTIRF system is designed as an add-on accessory for inverted microscopes and can be reconfigured for upright stations. IgTIRF is a powerful and versatile analytical tool, which implements at the same platform TIRF effect, Shallow Angle Fluorescence Microscopy (SAFM), and Micro-Spot Excitation (MSE). The latter method, MSE, is well-suited for the probing of organelles, single ion channels, and other objects with sizes from 1 to 100 microns. There are three versions of TIRF excitation light launchers that differ by the geometry of coupling light: (i) from the end of the coverslip; (ii) from the top, and (iii) from the bottom of the coverslip. Different light launchers are used for TIRFing Petri dishes, rectangular coverslips, and other formats of specimen substrate.

TIRF has become a method of choice for single molecule detection (SMD) and other studies that require the excitation of fluorescence confined in space [1-4]. In SMD, spatial confinement is necessary for minimizing the



Lightguide-based TIRF Microscopy - Ig-TIRF

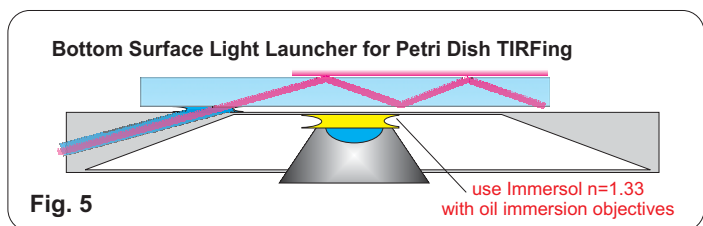
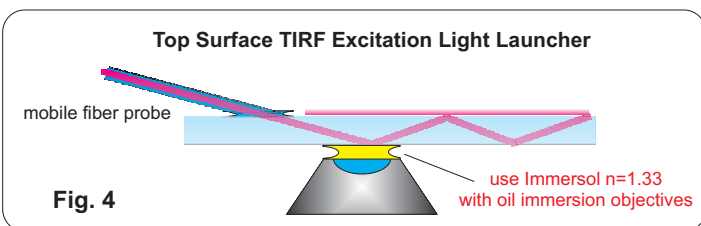


background signal and obtaining the contrast sufficient for detecting single molecules. TIRF provides a superior spatial confinement - it excites only a ~100 nm layer of the specimen. In comparison, the confocal scheme excites ~1,000 nm. In TIRF, the intensity of excitation is maximal at the surface and exponentially decays with the distance. Only molecules that are at the TIRF surface and in ~100 nm proximity to the surface are excited and fluoresce; the bulk of the specimen is not excited and does not fluoresce. The surface selectivity of TIRF allows for detecting single molecules, and is also well-suited for the analysis of biomolecular interactions, and other areas.

In particular, TIRF is “...a method uniquely suited to image the plasma membrane with its associated organelles and macromolecules in living cells. The method shows even the smallest vesicles made by cells, and can image the dynamics of single protein molecules.” [Steyer JA, Almers W., Ref. 5]. The TIRF effect can be achieved in different optical schemes, including prism-, objective-, and lightguide-based geometries. Each of the geometries has its own set of advantages and limitations. The prism-based scheme provides the best signal-to-background ratio [1], but is difficult to use with open perfusion chamber on inverted microscopes. The objective-based scheme collects the maximal amount of emitted fluorescence, but TIRF effect is compromised due to large intensity of stray light, which excites fluorophores located in the bulk of specimen, outside of the evanescent wave. Only in objective-TIRF the excitation light travels through the emission channel and generates large intensity of stray light. It has been documented in the literature that only in objective-TIRF geometry stray light deteriorates the TIRF effect by illuminating the bulk of specimen [1-3]. Lightguide-based geometry (IgTIRF) offers superior signal-to-background ratio and is exceptionally well-suited for multicolor TIRF, including FRET for single molecule detection, cell membrane, real-time microarray, and other studies. IgTIRF can be used with dry, water-, and oil-immersion objectives. It provides a reproducible intensity of the evanescent wave in one experiment and between experiments. IgTIRF can be used with UV excitation, a feature which is not available in objective-based TIRF.

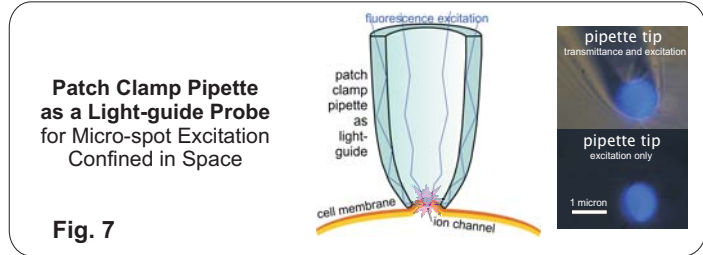
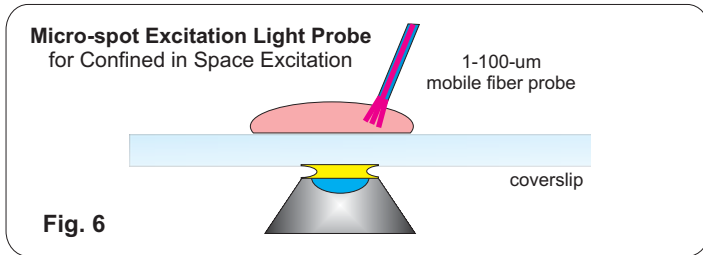
Fig. 1 illustrates the principles, and Fig. 2 shows a photo of the IgTIRF system mounted onto a 110x160mm K-frame, which is a standard for motorized XY translation stages. In Fig. 2, K-frame is shown nested into a larger platform suitable for manual XY stages. The base model of IgTIRF system is equipped with four TIRF excitation light launchers: two side-end launchers SEL-1 and SEL-7, a mobile launcher for coupling light from the top surface, and a stationary bottom-entrance launcher. The launchers are schematically shown in Figs. 3-5. Two versions of the side-end launchers SEL-1 and SEL-7 differ by the width of the TIRF area generated at the surface. SEL-1 produces a narrow band - up to 1-mm wide. SEL-1 is recommended for single molecule detection and other applications that require high intensity of the evanescent wave. SEL-7 launcher generates a wider band of the evanescent wave up to 20-mm wide, which is well-suited for real-time microarray applications that require measuring of the response of the entire microarray printed at ~20x20 mm area (Fig. 3a).

Fig. 4 shows the scheme of coupling light using a top-surface mobile fiber optics launcher. The latter can be used for coupling TIRF excitation from any site available at the top surface of the coverslip. Fig. 5 illustrates coupling from the bottom surface. This geometry is engineered for TIRFing Petri dishes equipped with optical bottoms. In this case, the light enters from the bottom of a Petri dish and undergoes multiple reflections as shown in Fig. 5. The fiber, which is embedded into the K-frame, and the coverslip are brought in optical contact by a droplet of immersion oil, so that the Petri dish can “float” along the XY axes.





Lightguide-based TIRF Microscopy - Ig-TIRF



Figs. 6 and 7 show optional Micro-spot Excitation (MSE) mobile probes. These probes are well-suited for confined in space excitation of live cell organelles and other objects of interest with sizes from 1 to 100 microns.

Superior Signal-to-Background Ratio. The principles of IgTIRF are close to that of prism-TIRF geometry. Similar to pTIRF, the excitation path in IgTIRF is naturally independent from the emission channel, which provides superior signal-to-background ratio - 3-4 orders of magnitude better than that for objective-TIRF.

Reproducible TIRF Measurements. IgTIRF is a geometry with the intensity of evanescent wave reproducible within one experiment and between different experiments. For this purpose, the angle of incidence is fixed. Switching between different excitation wavelengths does not require realignment. The depth of penetration can be changed by optical traps that extinguish light with small angles of incidence.

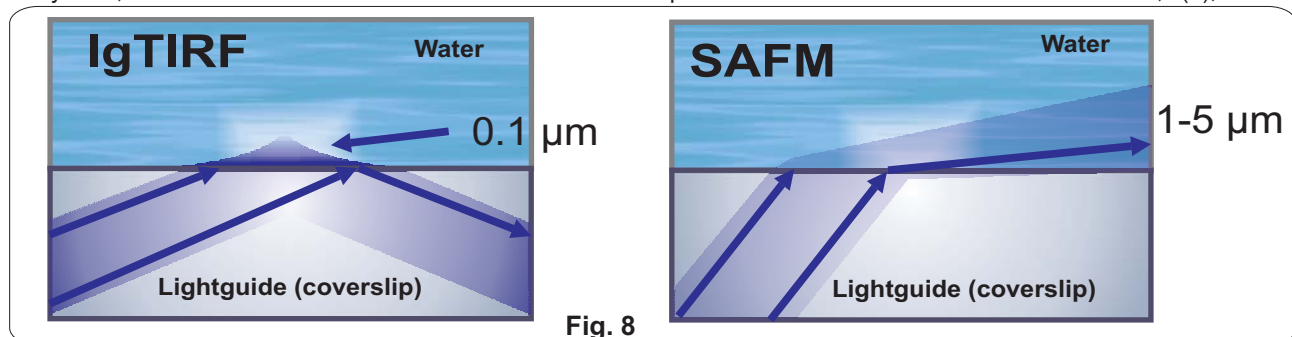
IgTIRF System is Compatible with Dry, Water- and Oil-immersion Objectives and, thus, can be used for broad range of studies - from TIRF imaging of cellular organelles to parallel detection of real-time response from microarrays and TIRF viewing of a group of cells. IgTIRF can be used with **Glass or silica coverslips** as TIRF lightguides. Rectangular or round coverslips, with mounted disposable or reusable temperature-controlled open perfusion chambers and closed flow cells are available as options for IgTIRF. **Silica Optics** of the excitation channel comprises fiber optics cable, collimators, and optics of the excitation light launchers. Respectively, the use of silica coverslips allows for TIRFing with UV excitation, a feature not available in objective-based TIRF.

Turnkey TIRF Station. TIRF Labs offers the entire range of instruments, supplies, and services for a turnkey TIRF installations, including: fluorescence illuminators, digital fluidics, electrochemical, dielectrophoretic, temperature control, filter wheels, XY and XYZ motorized stages, and software to control the station. Using of electrochemical or dielectrophoretic control permits not only to image the cells under the microscope, but also interact with them. Application Notes illustrate the use of IgTIRF for single molecule detection, cell biology, lipid rafts, and real-time microarray studies.

SAFM, MSE. The IgTIRF platform also implements Shallow Angle Fluorescence Microscopy (SAFM) - the mode of excitation schematically shown in Fig. 8. In SAFM mode, a portion of the excitation light propagates at shallow angles along the surface and excites fluorophores that are 1-5 microns away from the surface. IgTIRF is supplied with Micro-Spot Excitation (MSE) probes schematically shown in Figs. 6 and 7. In MSE mode, one uses a patch-clamp pipette as a lightguide which transmits the excitation light to the 1-micron tip of the pipette, or a fiber 5-50 um diameter to selectively excite an object with sizes 1-100 um. It takes no time to switch between TIRF, SAFM, and MSE mode, epi-fluorescence, and other methods. For more information visit www.tirf-labs.com/applications.html. Contact TIRF Labs for more details.

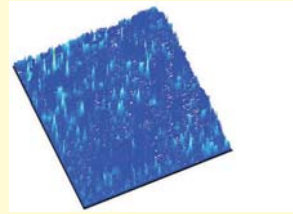
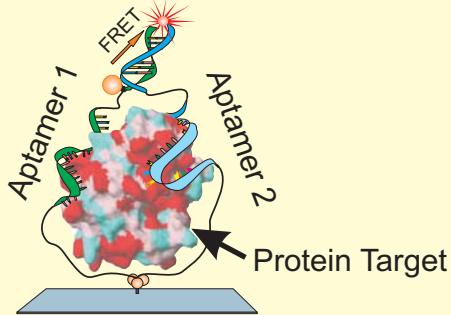
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3. Brunstein M, Héroult K, Oheim M. Eliminating unwanted far-field excitation in objective-type TIRF. Part II. *Biophys J.* 2014; 106(5): 1044.
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5. Steyer JA, Almers W. A real-time view of life within 100 nm of the plasma membrane. *Nat Rev Mol Cell Biol.* 2001, 2(4), 268.

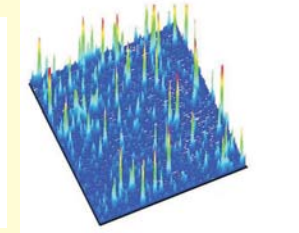




IgTIRF Microscopy for Single Molecule Detection



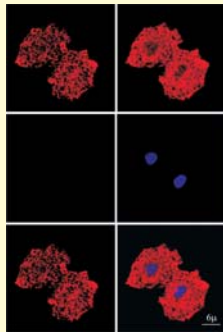
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Detection of single thrombin molecules binding to two-chain FRET aptamer assay using IgTIRF system with Nikon Eclipse TE2000 microscope. Binding of human thrombin to its aptamer assay resulted in FRET between Cy3 and Cy5 fluorophores conjugated to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

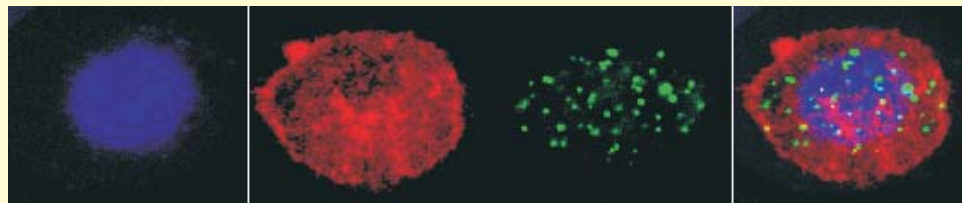
Multicolor IgTIRF Microscopy and SAFM for Cell Biology



IgTIRF SAFM

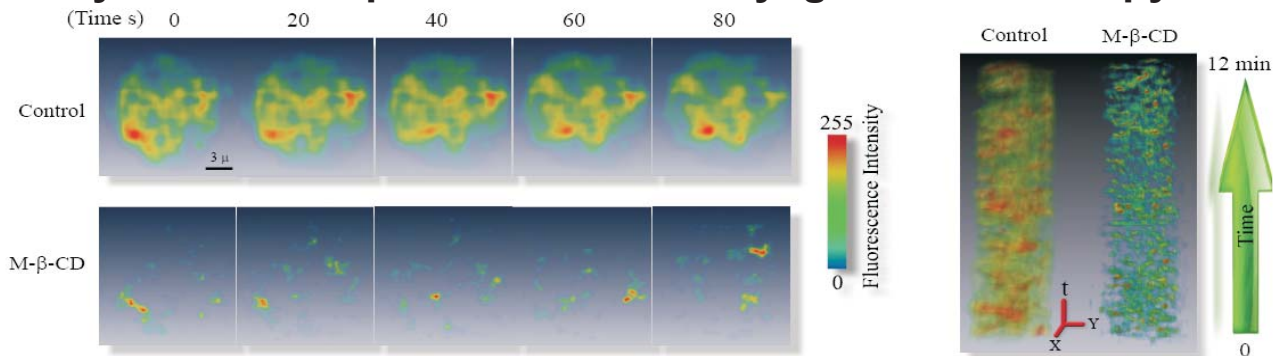
[Cell Calcium, 45 (2009) 439; BBA, 1801 (2010) 147.]

Nucleus (DAPI) Plasma Membrane FM-4-64 Caveolin-GFP Merge



SAFM images of human embryonic kidney cells HEK293 transfected with plasmid containing the cDNA for human caveolin fused to GFP, ex/em 488/510 nm; nuclei labeled with DAPI; 358/461 nm; plasma membranes - FM-4-64, 556/586 nm. Objective was Olympus Plan-Apo-N oil-immersion 60X NA 1.45, Olympus CCD camera XM10, integration time 1s, Cell-M software.

Dynamics of Lipid Rafts Studied by IgTIRF Microscopy



Displacement of beta-ChTx-Alexa on cell surface visualized by IgTIRF. Upper left panel shows cell under resting condition; lower panel- same cell 3 min after addition of M-beta-CD. [See for details: Asanov A, Zepeda A, Vaca L. Biochim Biophys Acta, 1801 (2010) 147-155]

iDiagnostics (iTIRF Arrays)

TIRF Microscopy

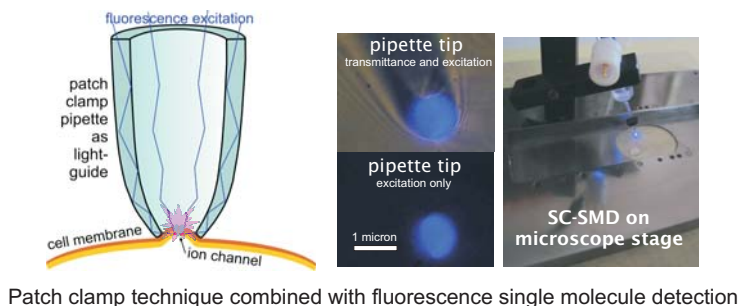
TIRF Spectroscopy



TIRF Labs

Total Internal Reflection Fluorescence

Single ion Channel Single Molecule Detection



iDiagnostics

cellphone based molecular diagnostics



We extended TIRF into 3rd dimension and invented iDiagnostics
Now you can hold a hospital laboratory in the palm of your hand

Turnkey Single Molecule Detection TIRF Microscopy System



Modular TIRFM systems include:

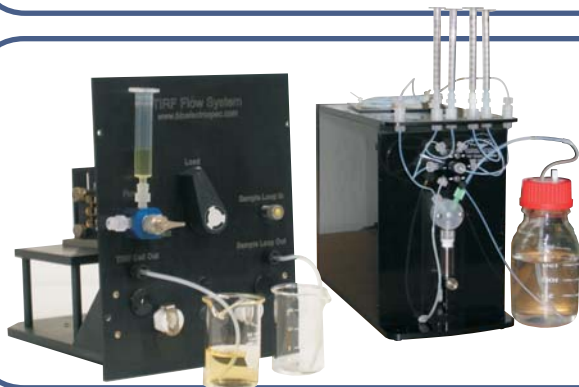
- Fluorescence microscope
- Ig-, p-, or/and o-TIRF microscopy flow systems
- Low light EM CCD camera
- Multi-color computer-controlled illuminator
- Digital fluidics SmartFlow
- Optional temperature and electric field control
- Software for instrument control and data analysis

Prism- and Lightguide-based TIRF Microscopy Accessories

- Single molecule detection, cell membrane studies
- Superior signal-to-background ratio
- Minimal stray light, crisp, high-contrast TIRF images
- Work with dry, water-, and oil-immersion objectives
- Use UV or visible excitation light 190-900 nm
- Use Petri-dish, open perfusion, or closed flow chamber
- Nested design - fits inside 96-well plate, K-frame. 4-inch round, or manual XY stage
- Optional temperature and electric field control



TIRF Accessories for Fluorometers



- **TIRF Accessory TA-1004** transforms a spectrofluorometer into a super-sensitive TIRF biosensor instrument
- Optional electrochemical, DEP and temperature control
- **SmartFlow** Fluidic System allows to run unattended TIRF experiments, measure sensograms to derive k_{on} and k_{off}
- Microfluidic system allows for handling nanoliter volumes