TIRF Labs, Inc., 106 Grendon Pl., Cary, NC 27519, USA; ph: 919-463-9545; info@tirf-labs.com; www.tirf-labs.com

Total Internal Reflection Fluorescence

TIRF Microscopy, TIRF Spectroscopy, Fluorescence Illuminators, Digital Fluidics, Turnkey TIRF Microscopy and Biosensors, Cell Phone Based Molecular Diagnostics, TIRFM for Single Molecule Detection, Chemically Modified Slides

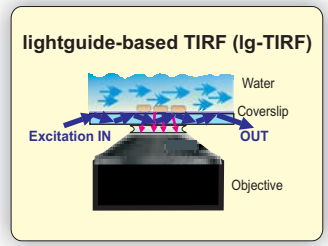
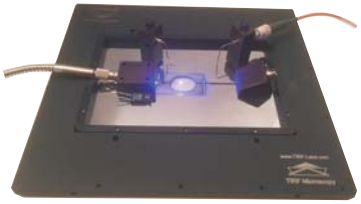
TIRF Principles. TIRF has become a method of choice for Single Molecule Detection (SMD) and found numerous applications in other areas of life sciences due to the fact of its exceptional spatial selectivity. Detection of single molecules requires excitation confined in space to minimize the background of irrelevant fluorescence. TIRF is well-suited for this purpose - it excites only ~0.1micron layer of solution, which is a spatial selectivity ~10-fold better than that in confocal microscopy. TIRF generates an evanescent electromagnetic wave, which provides maximum excitation at the interface and exponential decay with the distance from the glass surface. The depth of penetration is ~100 nm.

TIRF Geometries. There are several geometries that can be used for TIRF: (i) objective-, (ii) prims-, and (iii) lightguide-based. Each of the geometries features its own set of advantages and limitations. See for details the brochure **Compare TIRF Geometries** at *www.tirf-labs.com.* TIRF Labs offers prism- (pTIRF), lightguide- (lgTIRF), and objective-based (oTIRF) geometries. pTIRF flow system is known for the "cleanest" TIRF effect and the best signal-to-background ratio. pTIRF is the best-suited for SMD studies with molecular assemblies surrounded by a closed flow chamber. For experiments with live cells, when open perfusion chamber is necessary, lgTIRF is the most flexible and well-suited geometry; its signal-to-background is larger than that for pTIRF, but ~100-fold smaller than for oTIRF. oTIRF geometry uses high numerical aperture objectives (NA>1.4) to direct the excitation light to the specimen at angles larger than critical. High efficiency of collecting fluorescence due to the large NA represents the advantage of oTIRF. However, in oTIRF geometry the excitation light and emitted fluorescence share the same optical elements, which causes large intensity of stray light. Undesirable interference of stray light in oTIRF have been reported in the literature [1-3].

Selecting TIRF products. If you perform *in vitro* studies that require closed flow cell surrounding the TIRF area, pTIRF is the geometry, which will provide the best signal-to-background ratio and the cleanest TIRF effect. For experiments with live cells that require open perfusion chambers, lgTIRF will be the best geometry from the standpoint of signal-to-backgound ratio. lgTIRF is well-suited for SMD FRET and other multicolor TIRF experiments and can be used for studies that require UV excitation. lgTIRF is a flexible geometry well-suited for combining TIRF with AFM, electrophysiology, and electric field control. TIRF Labs supplies pTIRF and lgTIRF systems as factory-aligned add-on accessories. It takes no time to install/uninstall p- and lg-TIRF; they fit to virtually all microscopes. See Application Notes and references to articles at www.tirf-labs.com for typical studies performed with lg- and p-TIRF.

Compatibility with objectives, coverslips, illuminators, and XYZ microscope stages. pTIRF and lgTIRF systems are compatible with dry, water-, and oil-immersion objectives and can be used with regular glass coverslip. pTIRF also can be used with glass or silica 1-mm slides. For SMD experiments TIRF Labs offers low autofluorescence coverslips and slides; they also can be acquired from other sources. pTIRF and lgTIRF systems use single color and multicolor illuminators coupled into a 100-micron silica fiber. pTIRF and lgTIRF systems are mounted on core units (~85 mm x 127 mm) with the footprint of a 96-well SBS plate. The core unit is inserted into a K-frame (110 mm x 160 mm) , found in motorized XY translation stages. In turn, the K-frame nests into universal 200 mm x 220 mm platform, which is well-suited for manual translation stages. >>> more: ***www.tirf-labs.com.***

[1. Ambrose W, et al. Cytometry 1999, 36(3), 224] [2..Brunstein M, et al Part I: Biophys J. 2014; 106(5): 1020] [3. Part II. ibid, page 1044] [4. Simon S. Trends Cell Biol, 2009, 19: 661]

Product description	Notes
1. **lgTIRF - lightguide-based Total Internal Reflection Fluorescence microscopy** is a geometry, which provides superior signal-to-background ratio and exceptional flexibility. In lgTIRF, the excitation and emission channels are independent. Excitation light enters one of the ends of the lightguide as shown at the diagram and escapes from the opposite end. lgTIRF is a factory-aligned system with fixed angles, which provides reproducible intensity of the evanescent wave and is well-suited for multicolor TIRF. lgTIRF is compatible with dry, water-, and oil-immersion objectives. It uses 0.13-0.17mm glass or silica coverslips as TIRF lightguide. lgTIRF can also be used with 1-mm microscopy slides or Petri dishes with optical bottom. lgTIRF system is available with open perfusion chambers and closed flow cells. It takes no time to install lgTIRF at XY-translation stage of an inverted microscope. The core unit is mounted at a plate with the footprint of 96-well SBS plate, which can be inserted into K-frame (110 mm x 160 mm) found in motorized XY translation stages. In turn, the K-frame nests into a 200 mm x 220 mm platform, which is well-suited for manual XY translation stages. Base model is supplied with one of the single-color illuminators: 405, 465, 532, or 637 nm coupled into a fiber optics cable. Multicolor illuminators are available as options. lgTIRF also implements Shallow Angle Fluorescence Microscopy (SAFM) - novel powerful method for cell biology studies. See application note Multicolor lg-TIRFM and SAFM for Cell Biology Studies for more information.  	

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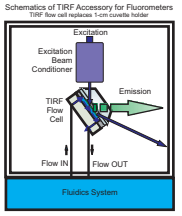
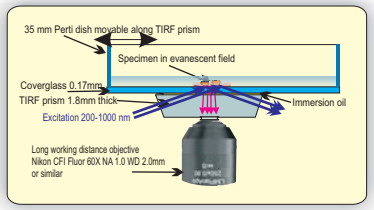
2. TIRF Flow System Accessory for Fluorometers TA1004

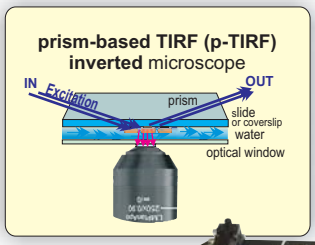
TIRF Flow System accessory TA-1004 is designed to replace standard 1-cm cuvette holders that are common to fluorometers. TA1004 accessory transforms a fluorometer into super sensitive TIRF biosensor instrument. Supported fluorometers include Horiba-JY-SPEX Fluorolog and Fluoromax, PTI, ISS, Varian Eclipse, SLM, Shimadzu, and Hitachi fluorescence spectrophotometers. TIRF system TA1004 is supplied as a factory-aligned accessory. It takes no time to install/uninstall TA1004. In TIRF mode, the bulk of solution is not excited, which reduces the background and allows for super-sensitive detection - down to single molecules. No other technique exists that can monitor fluorescence lifetime, polarization, anisotropy decay, quenching, resonance energy transfer (FRET), recovery after photobleaching (FRAP), and correlation spectroscopy (FCS) in real-time and with limit of detection at the level of single molecules. Base model of TA1004 is supplied with gravity-driven fluidics. TA1004 also can be used with external pumps. TIRF Labs' digital fluidics SmartFlow transforms a fluorometer into a computer-controlled TIRF biosensor capable of performing unattended TIRF sensogram experiments. Electrochemical, dielectrophoresis and temperature control are available as options. Chemically modified TIRF slides and reagent kits for surface immobilization of biomolecules are available as consumables. See TIRF Flow System Accessory brochure at www.tirf-labs.com for additional info.

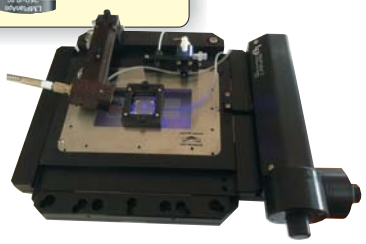


3. pdTIRF - prism-down Total Internal Reflection Fluorescence microscopy with open perfusion chamber and imaging through thin 2-mm prism**.** Prism-based TIRF is a geometry, which provides the best signal-to-background ratio. TIRF Labs offers imaging-through-the-prism - pdTIRF system, which uses 1.8 mm prism and 0.17mm thick coverslips, including Petri dishes with 0.2mm or thinner optical windows at the bottom. pdTIRF system is well-suited for live cell studies. It can be used with 36-mm Petri dishes. The dish can be XY translated over the prism. pdTIRF is compatible with microscope objectives with working distance 2 mm and larger. Such objectives with NA 1.0 and greater are available from Nikon, Olympus, Zeiss, and Leica. For details visit webpage: tirf-labs.com/ptirfmtl3p.pdf.



4. **puTIRF - prism-up Total Internal Reflection Fluorescence** microscopy with closed flow cell and TIRF imaging through the optical window. puTIRF system is well-suited for biomolecular studies that require closed flow cell for arranging flow of solutions along the TIRF surface. In puTIRF system a 6-mm prism and 1-mm slide are brought in contact by optically matching solution and are incorporated into a scheme with excitation in total internal reflection mode, as shown in the diagram. Imaging is performed through thin layer of solution and optical window. puTIRF system is supplied with microfluidic system, which is well-suited for handling small liquid aliquots, down to sub-microliter volumes. puTIRF is compatible with dry, water-, and oil-immersion objectives; it uses silica prism and silica or glass slides. It is factory aligned arrangement that can be used as an add-on accessories for rapid installation on XY translation stages of different microscopes.





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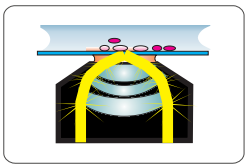
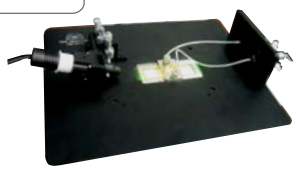
5. oTIRF - objective-based Total Internal Reflection Fluorescence microscopy flow system. TIRF Labs offers o-TIRF flow systems with open perfusion and closed flow cell chambers. Closed flow cells are equipped with advanced fluidics, which is well-suited for handling sub-microliter aliquots of solutions. Base model is supplied with one of the single-color illuminators: 405, 450, 532, or 630 nm and 60X 1.49 NA TIRF objective. Two- and three-color illuminators and 100X 1.49 NA TIRF objective are available as options. TIRF Labs offers o-TIRF systems for Nikon Eclipse TE2000, Olympus IX Series, and Zeiss Axiovert microscopes.  	
6. Turnkey TIRF Microscopy station **Turnkey TIRF Microscopy station** is **created on** Nikon, Olympus, Zeiss, and Leica inverted microscopes. The station includes**:** Prism-based pTIRF microscopy flow system, and/or Lightguide-based lgTIRF microscopy flow system, and/or Objective-based oTIRF microscopy flow system Multi-color computer-controlled illuminator Low light EMCCD camera Andor iXon or similar Optional digital fluidics SmartFlow Optional temperature control system TC-40 25-40°C Optional filter-wheel EW-6 at emission channel Optional electrochemical control unit EC-1070 TIRF Labs supplies turnkey TIRF microscopy station with TIRF Studio software package, which controls MCI-7000 illuminator, Andor EMCCD cameras, digital fluidics SmartFlow, temperature control TC-40, filter-wheel EW-6, and electrochemical unit EC-1070. Microscope is not included. TIRF Labs will collaborate with microscope supplier of your choice to provide seamless integration of TIRF microscopy complex with the microscope. 	**Request specifications and price quotation for specific configuration**
7. **Digital Fluidics SmartFlow** Computer-controlled TIRF Fluidics System SmartFlow® is equipped with precision digital syringe pump, computer-controlled valves, manifolds, containers for buffer solutions, and disposable plastic containers for bioanalyte samples. SmartFlow® fluidics is designed for delivery of bioanalyte and buffer solutions into TIRF flow cell or open perfusion chambers with profiled flow rates to minimize the effect of slow mass transfer and facilitate the measurement of true kinetics of association and dissociation and determination of rate constants k-on and k-off with minimum amount of bioanalyte solution. SmartFlow® fluidics is supplied with versatile software, which allows for configuring TIRF experiments for standard biosensor applications, as well as providing sophisticated flow profiles for injecting bioanalyte, buffer, and regeneration solutions for custom-defined experiments. The software contains flow profile wizard and set of fluidic programs for standard TIRF experiments. 	

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8. **TIRF BIOSENSOR INSTRUMENT FLUOROGAZER**

TIRF Biosensor FluoroGazer® is a TIRF flow system equipped with integrated digital fluidics, multicolor illuminator, filter wheels, photon-counting PMT, or low light EMCCD camera. The detection limit of FluoroGazer is at the level of single molecules. FluoroGazer is a cost-effective, upgradable system, with manual docking of TIRF sensor chips and automated delivery of up to six bioanalyte solutions. FluoroGazer is interfaceable with auto samplers for unattended analyses. Base model of FluoroGazer includes dual LED 480 and 530-nm illuminator and single-channel photon-counting PMT. High-end configurations includes 7-color illuminator covering the range from near UV to near IR - 280-1064 nm. Optional imaging photodetectors include low-light EMCCD cameras Andor iXon, Clara, or Luca. The software package TIRF Studio supports photon-counting PMT and Andor EMCCD cameras. The software acquires the kinetics of TIRF response and derives k-on and k-off rate constants. Optional electrochemical polarization system, electric field control, dielectrophoresis, and temperature control can be used in FluoroGazer flow cell for manipulating with biomolecules and live cells, stimulating association and dissociation, or regenerating the TIRF sensor surface for next cycle of analysis.

9. **PORTABLE TIRF BIOSENSOR**

Portable Biosensor TIRF Sense® is totally autonomous (small-shoe-box-size) device for point-of-care molecular diagnostics. TIRF Sense® is capable of performing highly multiplexed analysis - simultaneously detect from a few to several hundred DNA, RNA, protein, and metabolite markers. Rate of response is in the range from several seconds to a few minutes. TIRF Sense® biosensor requires no or minimal sample preparation. Whole blood, lysate of fine needle aspiration biopsy, or homogenated specimen of a tissue can be analyzed after minimal preparation procedures. TIRF Sense® measures real-time kinetics of microarray response, builds sensograms and derives k-on and k-off rate constants. The sensor is equipped with internal standards, positive and negative controls that are printed at the surface of TIRF sensor chips. TIRF Sense® uses silica, glass, and plastic microscopy slides (1 inch x 3 inches x 1 mm) as TIRF lightguides. TIRF Sense® also supports Electro-Chemi-Luminescence (ECL), bioluminescence, bead-based, and solution-phase fluorescence detection methods. The sensor is well-suited for real-time kinetic analysis of surface-immobilized gel-encapsulated bioassays. Custom-defined configurations of TIRF Sense® biosensor include multicolor illuminator, temperature, and electrochemical control.

10. ***i*Diagnostics - Cell Phone Based Molecular Diagnostics (*i*TIRF arrays)**

Molecular diagnostic device *i*Diagnostics (*i*TIRF arrays) employs CCD cameras of smartphones to acquire the dynamic response of real-time TIRF microarrays that simultaneously detect protein, nucleic acid, and metabolite biomarkers. *i*Diagnostics requires no or minimal sample preparation and is capable of detecting from a single to several thousands of molecular markers in a 50-microliter sample of biological fluids, including whole blood. Limit of Detection (LOD) for micro-RNA is 10^{-18}M. For proteins and metabolites LOD depends on the assay; for certain antibody-based assays LOD is at the level of ~10^{-15}M. The broad dynamic range of *i*TIRF sensor covers the entire spectrum of clinically significant concentrations. Classical TIRF microarrays operate with small, sub-monolayer amounts of antibodies and DNA probes immobilized on the surface. Classical TIRF signal is small; a low light EMCCD camera is necessary. *i*TIRF arrays are enhanced with silk fibroin that allows for immobilizing larger amounts of antibodies per unit area of bioassay spots. The *i*TIRF signal is a thousand-fold greater than that in classical TIRF. CCD cameras of cell phones are sensitive enough to detect the signal from *i*TIRF arrays. Because of several advantages, *i*TIRF biosensors will become popular devices for hundreds of applications, including MDx. If you are developing an MDx system based on luminescent assays, we invite you to collaborate to make your assays available to future users of *i*Diagnostics sensors.


iTIRF cradle with iPhone and cartridges

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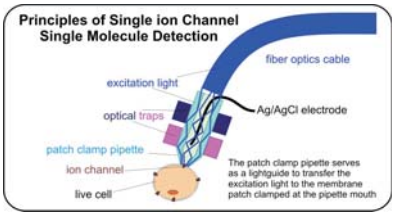
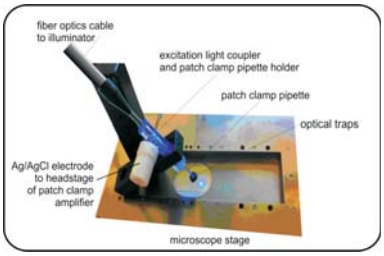
11. **Single ion Channel Single Molecule Detection.** SC-SMD system combines the patch clamp method with fluorescence imaging of single molecules. It allows for parallel electrophysiological study of a single ion channel and simultaneous fluorescence imaging of single molecules that comprise the same ion channel. In SC-SMD the tip of the patch clamp pipette serves as a probe, which delivers spatially confined excitation of fluorescence to the micron-size area at the mouth of the pipette. The patch clamp pipette functions for electrophysiology (as usual) and also as a lightguide, which transmits the excitation light to the tip, where the area of interest is located. This area is imaged by fluorescence microscopy and simultaneously probed by the patch clamp technique. Thus, the system allows simultaneously acquiring single channel electrophysiological recordings and detecting single molecule fluorescence within the same membrane patch. The SC-SMD system can be seamlessly integrated into existing patch clamp systems. Because the optical power is concentrated in a very small (1-micron diameter) area, the intensity of the excitation light is sufficient for single molecule detection experiments. Base model of the SC-SMD system consists of a modified patch clamp pipette holder, a fiber optics cable, and a fiber-coupled blue (465 nm), green (532 nm), or red (637 nm) fluorescence illuminator.





12. **Single Color Fluorescence Illuminators**

TIRF Labs offers broad range of fluorescence illuminators to meet the requirements for sophisticated multicolor fluorescence experiments, as well as simple single color tasks. The price of an illuminator depends on your choice of colors and optical power. Contact us to request a price quotation for your set of colors and optical power. Tell us about your applications - we will help you to determine the best configuration of the illuminator. We offer fiber-coupled and free beam illuminators. Our base models feature SMA 905 of FC-PC output for coupling with single 100-micron fiber or with fiber bundle ~1.5 mm diameter. Optical adaptors for microscopes enable use of TIRF Labs' illuminators with virtually all fluorescence methods. Our base models are equipped with ports for TTL/analog modulation and 0-100% manual adjustment of power. USB, Ethernet, Wi-Fi, and BlueTooth com ports and embedded processors are available as options.

13. **Multi Color Fluorescence Illuminators**

UV, Visible, Near IR: LED and laser light sources

Optical power: 100 mW - 5,000 mW

LEDs: 350, 405, 455, 465, 470, 505, 520, 530, 627, 800, 1270 nm

Lasers: 405, 445, 465, 473, 532, 637, 671, 1064 nm

Fiber-coupled and free-beam. Optical adaptors for microscopes

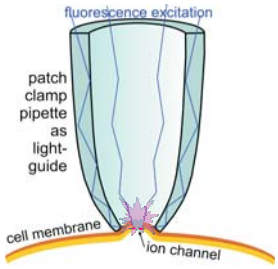
Manually adjustable optical power 0-100%

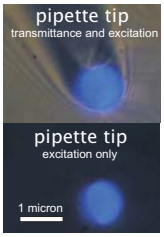
TTL, USB, Ethernet, Wi-Fi, BlueTooth com ports, embedded processors

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

TIRF Labs
Total Internal Reflection Fluorescence

Single ion Channel Single Molecule Detection





Patch clamp technique combined with fluorescence single molecule detection

*i*Diagnostics
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 TIRF systems include:

- Fluorescence microscope
- lg-, p-, or/and o-TIRF microscopy flow systems
- Low light EM CCD camera
- Multi-color computer-controlled illuminator
- Computer-controlled fluidics system
- Potentiostat and/or wave-function generator
- Software for instrument control and data analysis

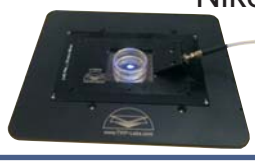
Lightguide- and Prism-based TIRF Microscopy

- Use YOUR microscope and YOUR objectives
- lg-and p-TIRF work with dry, water-, and oil-imm. lenses
- Use Xenon lamp, LED, or laser illuminators
- Open perfusion or closed flow chambers
- Install/uninstall in less than one minute
- Optional electrochemical control and computer-controlled fluidics

Olympus

Nikon

Zeiss



Leica

TIRF Accessories for Fluorometers

- **TIRF Accessory** transforms your 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}
- Novel microfluidics allows for handling nanoliter volumes