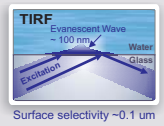
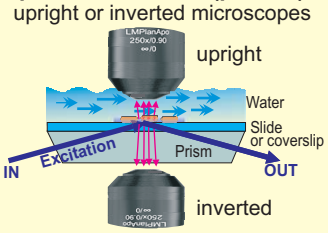


Surface selectivity ~0.1 um

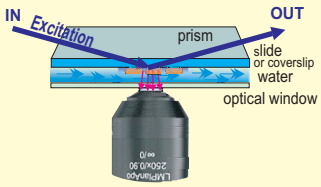
Compare TIRF Geometries

Total Internal Reflection Fluorescence Microscopy (TIRFM)

prism-*down* TIRF (pdTIRF)
upright or inverted microscopes

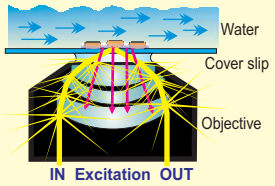


prism-*up* TIRF (puTIRF)
inverted microscope



lightguide-based TIRF (lgTIRF)

objective-based TIRF (oTIRF)



In pTIRF and lgTIRF, the excitation and emission channels are independent; the excitation light does not use and does not enter into the emission channel, which results in "clean" TIRF effect and superior signal-to-background ratio

In oTIRF, the excitation light uses the emission channel optics, which results in large intensity of stray light and poor signal/background ratio

TIRF microscopy has become a method of choice for single molecule detection and other areas of life sciences [1-4]. 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***" [1]. TIRF method can be realized by prism-, lightguide-, and objective-based geometries, as shown in the schemes above. Each geometry has its own set of advantages and limitations. Prism-based scheme provides the best signal-to-background ratio, but is difficult to implement with open perfusion chamber on an inverted microscope. Lightguide-based geometry yields superior signal-to-background ratio and exceptional flexibility - can be used with dry, water-, and oil-immersion objectives, but requires larger optical power to obtain equal intensity of the evanescent wave. Objective-based scheme is known for its high efficiency of collecting fluorescence by high numerical aperture TIRF objectives [1], but the background is large, signal-to-background ratio is poor, and the intensity of the evanescent wave is irreproducible. Table below compares three most popular TIRF geometries. Contact TIRF Labs for details to better determine which geometry is best suited for your applications.

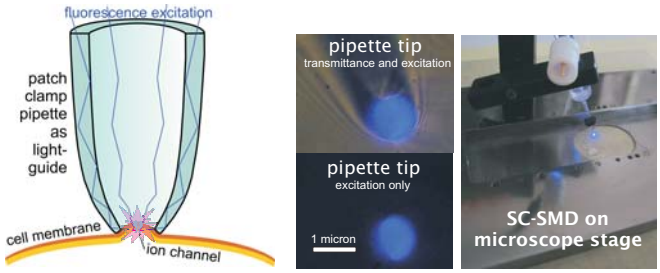
Property \ Geometry	pTIRF	lgTIRF	oTIRF
Depth of penetration of the evanescent wave	~100 nm	~100 nm	~100 nm
Signal-to-background ratio	best	excellent	poor
Efficiency of collecting fluorescence	Depends on objective	Depends on objective	Best
Excitation wavelengths	190-900 nm	190-900 nm	380-900 nm
Reproducibility of the evanescent wave intensity	good	excellent	poor
Can be used with dry objectives	Yes	Yes	No
Can be used with water-immersion objectives	Yes	Yes	No
Can be used with oil-immersion objectives NA<1.4	Yes	Yes	No
Can be used with oil-immersion objectives NA>1.4	Yes	Yes	Yes
Compatible with laser illuminators	Yes	Yes	Yes
Compatible with LED, Hg- and Xe-arc lamp illuminators	Yes	Yes	No
Can be used for live cell studies with open perfusion	No	Yes	Yes
Can be used for single molecule detection studies	Yes	Yes	Yes
Can be used for microarray studies (large area imaging)	Yes	Yes	No
Area of the evanescent wave	~0.1-10 mm	~0.1-20 mm	~0.1-0.3 mm
Volume of closed flow chamber	1-100 uL	1-100 uL	1-100 uL

[1]. 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.
[2]. Ambrose WP, Goodwin PM, Nolan JP. Single-molecule detection with TIRF: comparing signal-to-background and total signals in different geometries. Cytometry 1999, 36(3), 224.
[3]. Asanov A, Zepeda A, and Vaca L. A Platform for Combined DNA and Protein Microarrays Based on Total Internal Reflection Fluorescence. Sensors, 2012, 12, 1800.
[4]. See TIRF Labs' Application Notes and references to articles for more information and additional literature: http://www.tirf-labs.com/applications.html.

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 Station

Modular TIRF station includes:

- 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

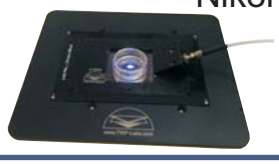
Lightguide-, Prism-, and Objective-based TIRF Microscopy

- 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