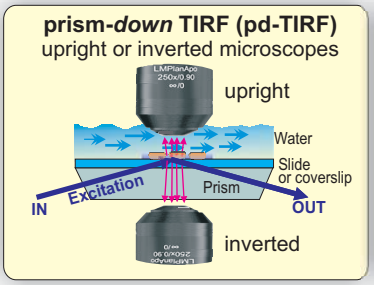
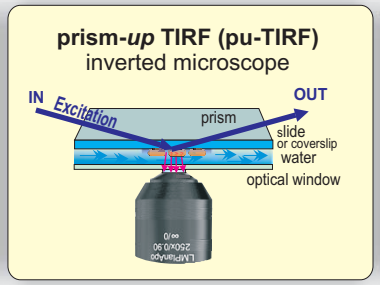
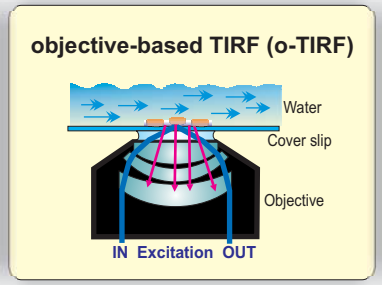
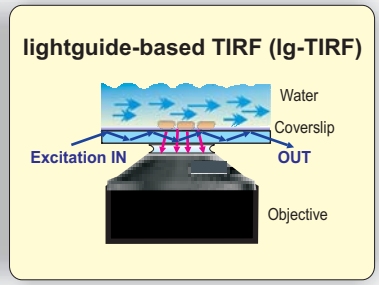
Compare TIRF Geometries

Total Internal Reflection Fluorescence Microscopy (TIRFM)









TIRF is a powerful analytical technique with numerous applications in the area of life sciences [1-3]. 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. *A real-time view of life within 100 nm of the plasma membrane.* Nat Rev Mol Cell Biol. 2001, 2(4), 268]. TIRF systems can be implemented in different ways. Prism-, objective-, and lightguide-based geometries produce the evanescent wave, which is well suited for analysis of biomolecular interactions, cell biology, and real-time microarray studies. Each geometry has its own set of advantages and limitations. A prism-based scheme provides the best signal-to-background ratio, but is difficult to implement with open perfusion chamber on an inverted microscope. An objective-based scheme is compatible with open perfusion chamber, but the background is larger, the intensity of evanescent wave is irreproducible, and one must use only specialized objectives with NA >1.43. A lightguide-based geometry yields exceptional flexibility - it can be used with dry, water-, and oil-immersion objectives, and is well-suited for multicolor TIRF, but requires larger optical power to obtain equal intensity of the evanescent wave. The table below compares the three most popular TIRF geometries. Contact TIRF Labs for details to better determine which geometry is best suited for your applications.

Property \ Geometry	p-TIRFM*	lg-TIRFM	o-TIRFM
Depth of penetration of the evanescent wave	~100 nm	~100 nm	~100 nm
Signal-to-background ratio	best	good	fair
Excitation wavelengths	200-900 nm	200-900 nm	380-800 nm
Well-suited for multicolor TIRF studies	✓	✓	–
Reproducibility of the evanescent wave intensity	good	excellent	poor
Can be used with **dry objectives**	✓	✓	–
Can be used with **water-immersion objectives**	✓	✓	–
Can be used with **oil-immersion objectives NA<1.4**	✓	✓	–
Can be used with **oil-immersion objectives NA>1.4**	✓	✓	✓
Compatible with laser illuminators	✓	✓	✓
Compatible with LED, Hg- and Xe-arc lamp illuminators	✓	✓	–
Can be used for live cell studies with open perfusion chamber	pd ✓ pu –	✓	✓
Can be used for single molecule detection studies	✓	✓	✓
Can be used for microarray studies (large area imaging)	✓	✓	–
Area of the evanescent wave	~0.1 - 5 mm	0.1 - 20 mm	D ~0.1-0.3 mm
Volume of closed flow chamber	1-100 uL	1-100 uL	1-100 uL

[1.] 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.
[2.] 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.
[3.] See TIRF Labs' Application Notes,Tech Note "Stray Light Causes Errors" for more information and additional literature

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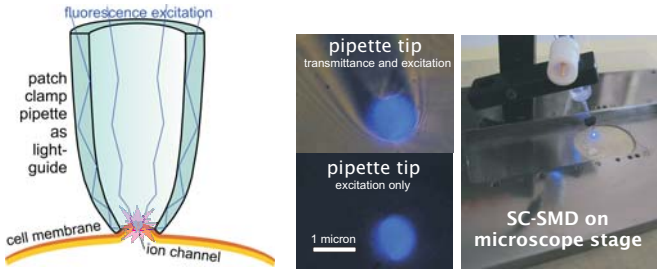
TIRF Labs

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 the 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

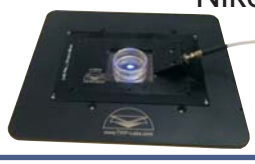
Lightguide-, Prism-, and Objective-based TIRF Microscopy

- 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