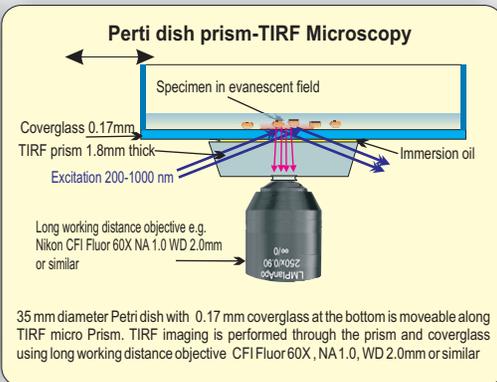
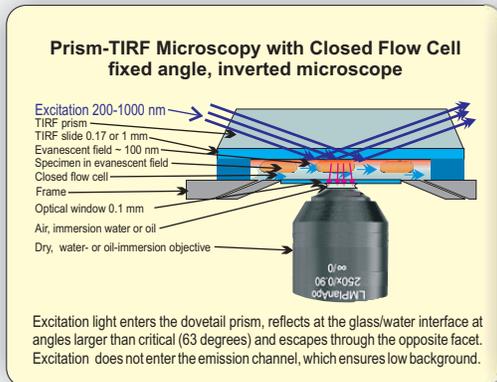




# Prism-based TIRF Microscopy



## Selected geometries of prism-based TIRF Microscopy

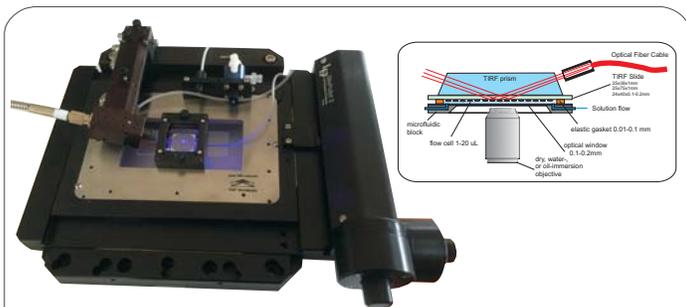
Total Internal Reflection Fluorescence (TIRF) has become a method of choice for single molecule detection and other studies that require confined in space excitation of fluorescence. TIRF provides submicron optical slicing - it excites only ~100 nm of the specimen with maximum intensity of the evanescent wave at the surface. Prism-based geometry ensures the “cleanest” TIRF effect and the best signal-to-background ratio, which has been documented in the literature [1-4]. TIRF Labs’ pTIRF systems are well-suited for working with closed flow cells or open perfusion chambers either on upright or inverted microscopes. We offer state-of-the-art pTIRF microscopy with different schemes; three of them are shown in the figures above. TIRF Labs also offers pTIRF with semisphere prism for variable angle TIRF, as well as lightguide- and objective-based TIRF. Contact TIRF Labs to better determine which geometry is better suited for your applications.

pTIRF provides the lowest background fluorescence, the lowest scatter, and hence the best signal-to-background ratio. See the brochure Compare TIRF Geometries for details. If your application permits, prism-TIRF is the geometry to consider at the first place. pTIRF can be used for a variety of applications, including single molecule detection, analysis of biomolecular interactions, characterizing of antibody- and nucleic acid-based assays, real-time microarrays, membrane biophysics, and other studies.

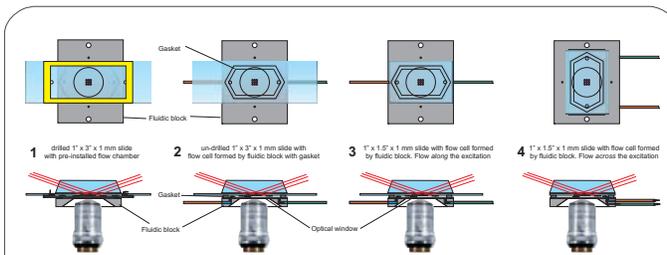
pTIRF systems are compatible with dry, water-, and oil immersion objectives; they are available from TIRF Labs for upright and inverted microscopes. 1-mm thick slides or 0.12-0.24 mm coverslips made of glass or silica can be used with pTIRF. Our pTIRF systems are equipped with advanced microfluidics, which allows for operating with sub-microliter amounts of solutions. We also offer a pTIRF system for TIRFing specimens in Petri dishes, as shown in the figure above. Most of our pTIRF accessories are factory aligned systems: the angles of incidence of the excitation light are fixed to provide highly reproducible intensity of the evanescent wave. If decreased depth of penetration is necessary, TIRF Labs offers optical traps for this purpose. TIRF Labs also offers broad range of computer-controlled and manually-operated illuminators that cover broad range of applications and budgets. For more information visit [www.tirf-labs.com](http://www.tirf-labs.com).

### Literature:

1. Ambrose W, Goodwin P, Nolan J. *Single-molecule detection with TIRF: comparing signal-to-background in different geometries*. Cytometry 1999, 36(3), 224.
2. Brunstein M, Teremetz M, Héroult K, Tourain C, Oheim M. *Eliminating unwanted far-field excitation in objective-type TIRF*. Part I. Biophys J. 2014; 106(5): 1020.
3. Brunstein M, Héroult K, Oheim M. *Eliminating unwanted far-field excitation in objective-type TIRF*. Part II. Biophys J. 2014; 106(5): 1044.
4. Simon S. *Partial internal reflections on total internal reflection fluorescent microscopy*. Trends Cell Biol, 2009, 19: 661.



**pu7TIRF Prism-based TIRF Microscopy Flow Cell System**  
for inverted microscopes installed on the motorized XY translation stage. pu7TIRF is well suited for single molecule detection FRET experiments and other multicolor TIRF applications. pu7TIRF is designed as add-on accessory for inverted microscopes



**Four versions of arranging fluidic chamber for pu7TIRF**  
1-Use drilled 1"x 3" slide with pre-installed flow chamber.  
2- Use fluidic block with optical window and elastic gasket. The flow cell is formed by the gasket between slide and optical window.  
3- Arrangement similar to version 2, but using 1" x 1.5" slide.  
4- Similar to version 3, but using shorter tubing.

**iDiagnostics (iTIRF Arrays)**  
**TIRF Spectroscopy**  
**TIRF Microscopy**



**TIRF Labs**

Total Internal Reflection Fluorescence

**Single ion Channel Single Molecule Detection**

fluorescence excitation  
 patch clamp pipette as light guide  
 cell membrane  
 ion channel

pipette tip transmittance and excitation  
 pipette tip excitation only  
 1 micron

SC-SMD on microscope stage

Patch clamp technique combined with fluorescence single molecule detection  
*Cell Calcium 57 (2015) 1-13.*

**iDiagnostics**

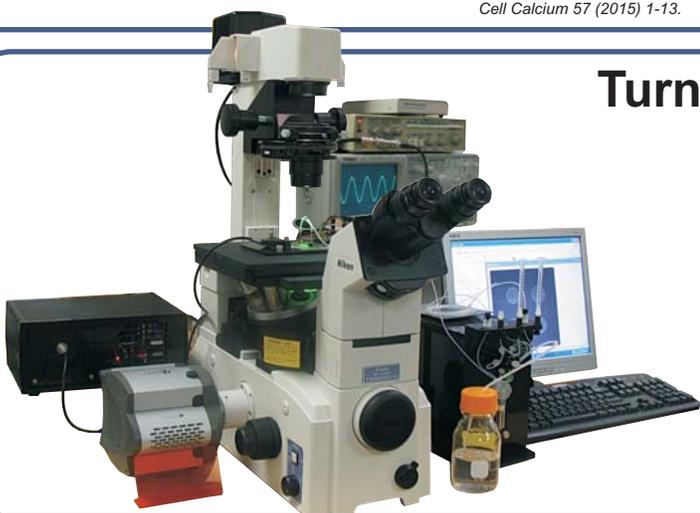
*cellphone based molecular diagnostics*



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

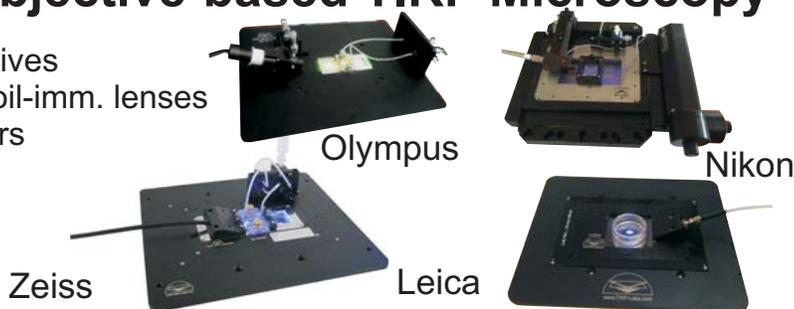
**Turnkey Single Molecule Detection TIRF Microscopy System**

- Modular TIRF systems include:
- Fluorescence microscope
  - Ig-, 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
- Ig- 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



**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

