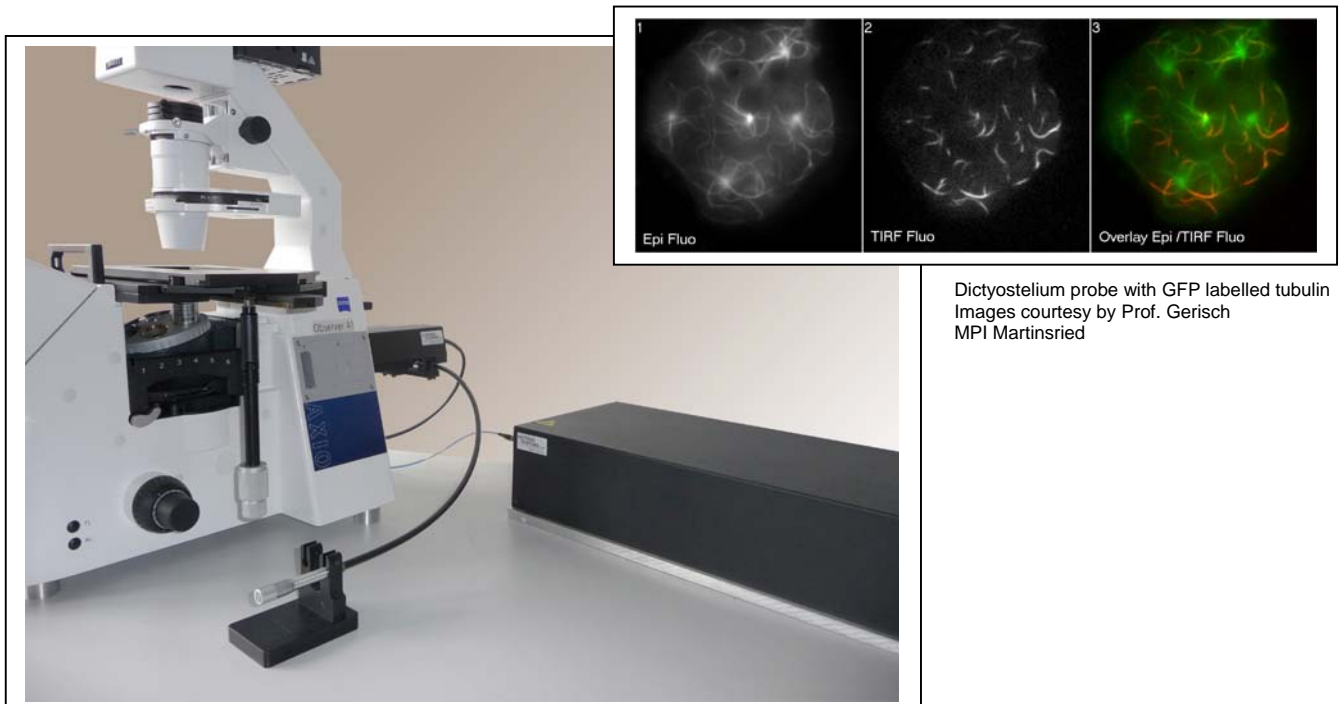


## VisiTIRF Imaging System

### VisiTIRF

#### Total-Internal-Reflection-Fluorescence Imaging System

The TIRF technique is a special kind of wide-field fluorescence technique for measuring cells or SMC in an evanescent field without haze. By total reflection of the exciting light (typically laser) at the coverslip / medium interface, the fluorescence excitation is limited to a very thin space in the vicinity of the glass surface. The resulting fluorescence images exhibit extremely high contrast and resolution with the possibility of realtime imaging of cell membrane or labelled single molecules SMC.



Dictyostelium probe with GFP labelled tubulin  
Images courtesy by Prof. Gerisch  
MPI Martinsried

The VisiTIRF Fluorescence Imaging System from Visitron Systems is the ideal solution for the analysis of finest structures or surfaces. The flexibility of the VisiTIRF system and the use of scientific digital CCD camera systems with optimal resolution, speed and sensitivity allows the adaptation of customized application and configuration. For fluorescence illumination we use different solid state laser or laser diodes. The coupling of the laser into the microscope is achieved by a flexible optical fibre. Our special own developed VisiTIRF condenser allows an easy adjustment of the laser beam to the correct illumination angle in the high aperture of the TIRF objective. A second optical fibre input allows the combination of TIRF and epi-fluorescence without moving any parts of the condenser. For epi fluorescence illumination we offer a standard HBO / HXP lamp or our VisiChrome polychromator system.

## The VisiTIRF Optics

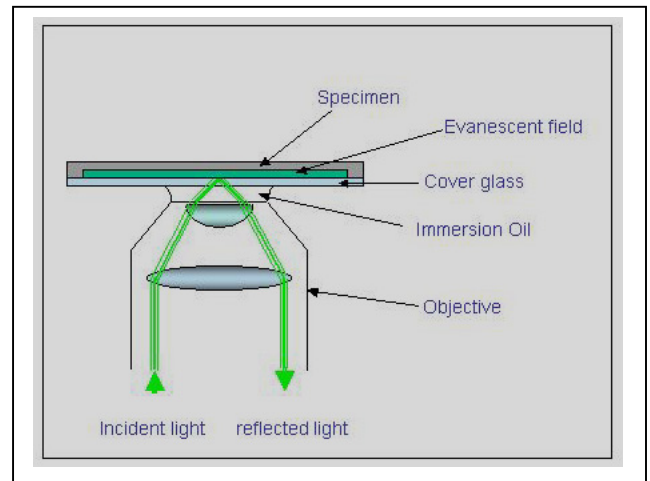
Typically we use the high aperture objectives from Olympus e.g. 60x NA.1,45; 100x NA.1,65 or from Zeiss e.g. alpha-Plan Fluor 100x NA.1,45. These objectives are specially designed for TIRF applications.

The laser beam is coupled to the epi fluorescence aperture with a defined angle. Due to that adjustable angle the laser beam will be totally reflected in the evanescent field located between the cover glass and specimen.

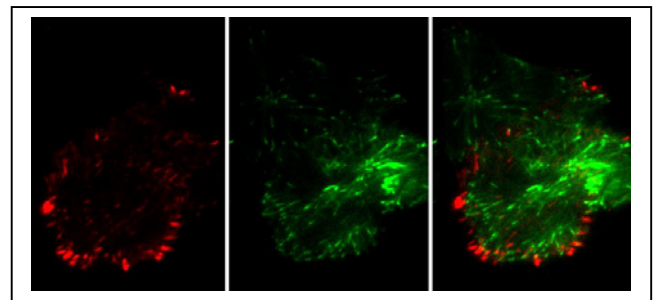
The total reflection in liquid solutions works only with objective apertures higher than NA.1,38.

Only the fluorescent particles near to the cover slip glass with an approximate distance of 200 nm will be excited by the angled laser beam.

The fluorescent particles of the specimen which are located in higher distance will not be excited and do not emit stray light. Therefore this will improve the signal to noise ratio of the current focus.



principle of light profile



EPIchEB3gZyx labeled cells  
Images courtesy by Prof. Small IMBA Vienna



## The VisiTIRF Illumination

For the TIRF Illumination typically a solid state laser or laser diodes will be used. Optionally VS-laser merge module (LMM's) are available to combine solid state or diode lasers. The selection of the laser lines will be handled in real time by high speed AOTF systems. Switching and synchronization are controlled by the powerful Visitron imaging software.

Epi fluorescence illumination with fiber coupled HBO lamp, filterwheel, HXP / XCite or polychromator could be activated and switched in realtime.

## VisiTIRF System Components Overview

- motorized microscopes with high-end optics
- high resolution scientific grade digital cameras with best sensitivity and cooling
- special coupling for lasers and epi fluorescence
- special TIRF objectives with high aperture  $>1.38$
- flexible laser setup e.g. solid-state 488; 561; 640nm; diode laser e.g. 405; 445; 457nm
- powerful Visitron imaging software with control of all hardware
- easy to upgrade existing Visitron Systems fluorescence imaging systems to TIRF
- optional: realtime piezo Z-focus upgrade