

OLYMPUS®

Your Vision, Our Future

cell[^]TIRF™ Illuminator

Motorized Total Internal Reflection Fluorescence

*Four individually aligned illumination beams
for simultaneous multi-color TIRF imaging*

UIS2
World-leading optics



Working Simultaneously
The Next Level of TIRF Microscopy



The Pioneers of Objective-Based TIRF Pioneering the Next Generation

In 1997, Olympus designed the first turn-key objective-based TIRF illuminator for commercial applications. As pioneers of TIRF, we've remained committed to the field and are continuously developing cutting-edge advancements to push the limits of science.

The **Olympus cell^TIRF** illuminator represents the very latest in TIRF technology and is complemented by the largest portfolio of specially designed TIRF optics.

The **cell^TIRF** illuminator is unique, offering independent and simultaneous control of the critical angle for four separate evanescent waves, allowing optimization of the angle adjustment for different wavelengths. Users can preset calculated penetration depths for all lasers with a single mouse click and the motors will individually adjust each laser's angle to simultaneously capture TIRF for all channels. Fine-tuning and adjusting the angle for each line is as simple as the scroll of the mouse wheel.

cell^{tirf} Illuminator Features and Benefits

Simultaneous Acquisition of 4 Lines

- Independent adjustment of incident angles
- Software confirmation of incident angles
- Seamless switch between widefield and TIRF imaging modes
- Motorized control of TIRF angle for accurate replication of parameters

Handle the Most Challenging TIRF Applications

- Compatible with our IX2® series of microscopes
- Simultaneous imaging of up to four channels
- Integrated point FRAP optics for the first laser line
- Zero Drift Compensation (ZDC) laser autofocus compatible
- 405-640nm integrated laser systems (additional systems may be available in the future)
- Superior optics: 4 dedicated TIRF objectives from 60X to 150X
- The world's highest NA with the unmatched APO 100X NA1.65
- Penetration depths less than 50nm
- New Type-F immersion oil: improved image quality and optical performance



celltirf
World-leading optics

cell^{tirf} Illuminator Product Specifications

TIRF Illuminator

- 4 laser inputs for TIRF
- Motorized and independent incident angle control
- TIRF/widefield or 100% widefield imaging with 340nm transmission
- Integrated point FRAP optics for the first laser line
- Adjustable and centerable control of field stop for each line
- Easily accepts 25mm laser cleanup filters

Advanced Laser Systems

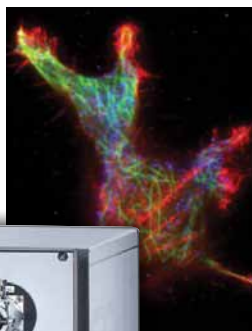
New Olympus laser systems from 405nm to 640nm, with up to 100mW of power, ensure optimal excitation with sufficient and controllable power to meet the demands of a wide variety of applications. Housed in compact, stackable units, our laser systems feature:

- Manual or software-based attenuation
- Wavelength matched single mode fibers
- High speed TTL imaging shutters
- Integrated laser safety interlocks

Laser lines:*

405nm	491nm	594nm
445nm	514nm	640nm
473nm	532nm	
488nm	561nm	

*Additional systems may be available.



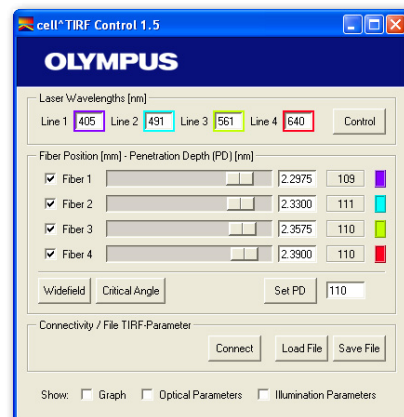
Compatibility

- Manual and motorized IX2 inverted microscopes can easily be upgraded
 - IX51 manual inverted platform
 - IX71 manual inverted platform
 - IX81 motorized inverted platform
 - IX81-ZDC2 motorized inverted platform with Zero Drift laser autofocus



Software Control

- Olympus software provides precise and independent control of TIRF angles for up to 4 laser lines
- Confirmation of TIRF angle is displayed along with other parameters such as:
 - Calculated penetration depth
 - Specific wavelength in nanometers for each laser line
 - Graphical display of incident angle/penetration depth relationship for each laser line
 - Save/load feature for TIRF software settings
 - Additional optical parameters such as laser spot position in the back focal plane
 - Mouse and keyboard control of TIRF angles
- One-button click to simultaneously set all wavelengths to the desired penetration depth
- One-button click to switch from widefield to TIRF imaging



The Largest Selection of TIRF Optics on the Market

Olympus offers six high numerical aperture objectives (available separately) that represent the most comprehensive line of TIRF optics including:

NEW! APON 60xO TIRF **with Correction Collar* (NA 1.49)**

This NEW high-performance UIS2 objective is optimized for TIRF imaging and other fluorescence applications requiring a high NA. Designed with a correction collar for adjustments at 23 and 37 degrees, this is one of the most popular lenses for TIRF microscopy. Compatible with DIC imaging.



NEW! UAPON 150xO TIRF **with Correction Collar* (NA 1.45)**

With its extremely high magnification, this UIS2 optic is the only TIRF objective of its kind on the market and was specifically developed for single molecule applications. It features a correction collar for temperature and coverslip thickness and is compatible with DIC imaging.



NEW! UAPON 100xO TIRF **with Correction Collar* (NA 1.49)**

This high-performance UIS2 objective with UV transmission and correction collar is ideal for high-magnification, high-resolution imaging. Market-leading performance for an objective using standard coverslips and immersion oil. Compatible with DIC imaging.



***Correction Collar**—Some aberrations are influenced by varying thickness of coverslips or oil refractive index changes due to temperature. High numerical aperture objective lenses are particularly susceptible to these effects. Olympus TIRF objective lenses have high NAs and the inclusion of the correction collar allows for compensation to ensure the sharpest possible image.

APO 100xOHR (NA 1.65)

This legendary objective has the world's highest NA with an unparalleled numerical aperture of 1.65. It allows extreme TIRF angles and adjustments over a wide-angle range. The penetration depth of the evanescent field can be reduced down to about 50nm, rendering exceptionally thin illumination. Ideal for high-magnification, high-resolution imaging this objective uses special, highly refractive coverslips and immersion oil to match the extreme NA.



Immersion Oils (Available Separately)

Type-F Low Auto-Fluorescence Immersion Oil

- Incredibly high tolerance within and across batches
- Guarantees improved optical quality for physiological temperature-based studies
- 1/10 the level of auto-fluorescence compared to standard oil
- Low odor—MSDS available

High-Refractive Index Oil

- Refractive index of 1.78 for use with the APO100XO-HR objective
- World's highest NA of 1.65
- Refractive index matched coverslips
- Low odor—MSDS available

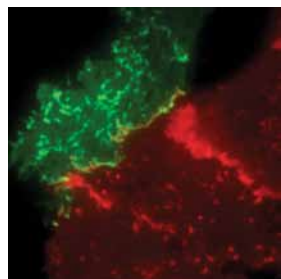


cell^{tirf} Illuminator Applications

Single Molecule • Membrane Research • Simultaneous Acquisition

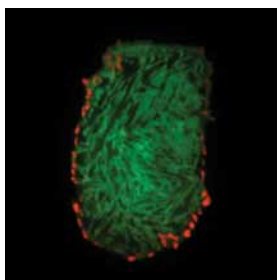
Total internal reflection fluorescence (TIRF) is employed to investigate events occurring at surfaces, an area that is of fundamental importance to a wide spectrum of disciplines in cell and molecular biology. TIRF has enabled scientists to realize significant discoveries in both intact cells and in solution.

cell[^]TIRF for imaging at the surface. In a majority of these studies, functionally relevant fluorophores bound to the surface and those in the surrounding medium exist in an equilibrium state. When these molecules are excited and detected with a conventional widefield fluorescence microscope, the resulting fluorescence from those fluorophores bound to the surface is often masked by the

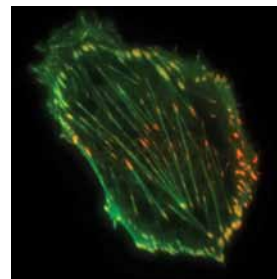


ambient fluorescence from a much larger population of nonbound molecules inhabiting the adjacent detection volume. The cell[^]TIRF illuminator and range of Olympus TIRF optics offer maximum flexibility for high signal-to-noise imaging of cell surface details.

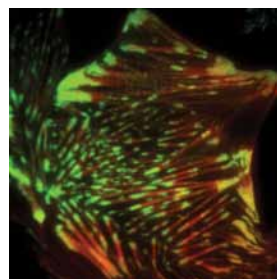
cell[^]TIRF for imaging living cells. Living cells in culture provide an excellent candidate for TIRF investigations. The technique enables selective visualization of contact regions between individual cells and the substrate, even in specimens where fluorescence from areas outside the surface would obscure important fluorescent information concerning adhesion points. Because illumination is restricted to the interface regions and does not penetrate through the entire depth of the cell, living cells tend to survive longer under fluorescence observation using TIRF techniques. This feature enables microscopists to increase the length of observations and to perform time-lapse imaging for extended periods, often ranging from many hours to one or more days.



cell[^]TIRF for studying co-localization. The 100X apochromatic objective with its 1.65 numerical aperture provides the maximum spatial resolution and optimal conditions for studying co-localization by producing the thinnest optical slice at the surface of the specimen. The dramatically larger numerical aperture allows a wider range of supercritical angles of incidence within which TIRF will occur, 48 to 68 degrees. The 1.65 NA 100X objective also allows users to set up evanescent waves that are more insensitive to refractive index changes within a cell than any other objective on the market.



cell[^]TIRF for molecular studies. TIRF can also be utilized on featureless non-microscopic specimens to measure fluorophore concentrations or to record binding/unbinding equilibria and kinetic rates at a biological surface.



Other applications include single molecule fluorescence experiments and model membranes, which have been constructed using the substrate for mechanical support. The technique is also useful for investigating the emission of fluorophores bound to surfaces.

These and other experiments are designed to examine the chemistry and physics of interfaces themselves, and should continue to be the focus of TIRF microscopy studies in many diverse fields. Single molecule studies in particular benefit from TIRF's enhanced signal-to-noise ratio of surface-bound molecules, without background emission from molecules in the adjacent solution. Olympus's 150X TIRF objective provides ideal magnification for Nyquist sampling when using large pixel, high-sensitivity detectors commonly used in single molecule detection. The high transmission of this lens down to 340nm makes it possible to perform TIRF microscopy and UV applications such as un-caging and/or photo-activation with one lens.

Examples include:

Cellular Signaling and Trafficking

- Binding and triggering of cells by hormones, neurotransmitters, and antigens
- Cellular secretion events
- Electron transport
- Intracellular metabolism

Cellular Adhesion and Motility

- Cytoskeletal and membrane dynamics
- Cell adhesion to surfaces

Molecular Studies

- Polymer translation near an interface
- Single molecule interactions using FRET
- Reaction and folding dynamics

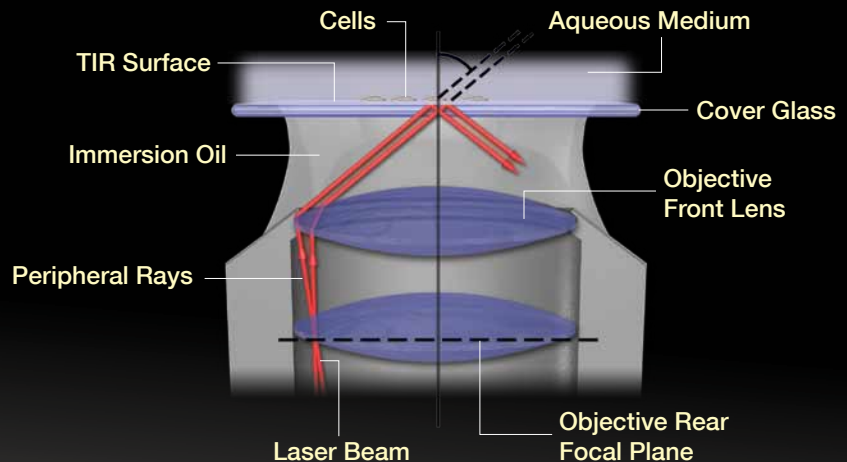
What is TIRF?

Total internal reflection fluorescence (TIRF) microscopy is an optical technique that uses an evanescent wave to illuminate a thin region immediately adjacent to the coverslip surface. The technique is utilized to observe single molecule fluorescence, biological events at cell membranes, and high-speed calcium events that are normally difficult to visualize by conventional widefield fluorescence.

TIRF microscopy is commonly employed to investigate the interaction of molecules with surfaces, an area that is of fundamental importance to a wide spectrum of disciplines in cell and molecular biology. The basic concept of TIRF microscopy is simple, requiring only an excitation light beam traveling at a high incident angle through the solid glass coverslip where the cells adhere. Refractive index differences between the glass and water phases regulate how light is refracted or reflected at the interface as a function of incident angle.

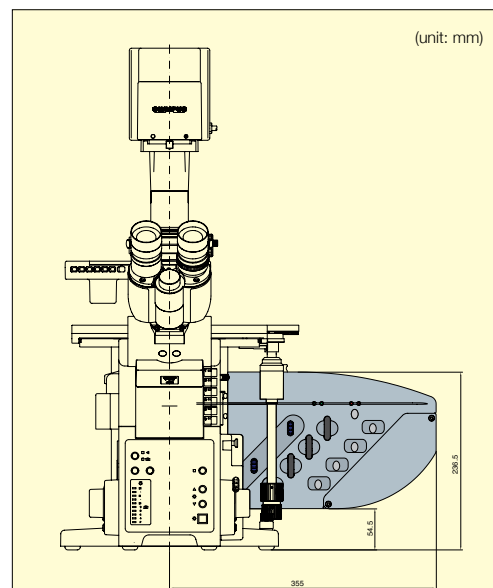
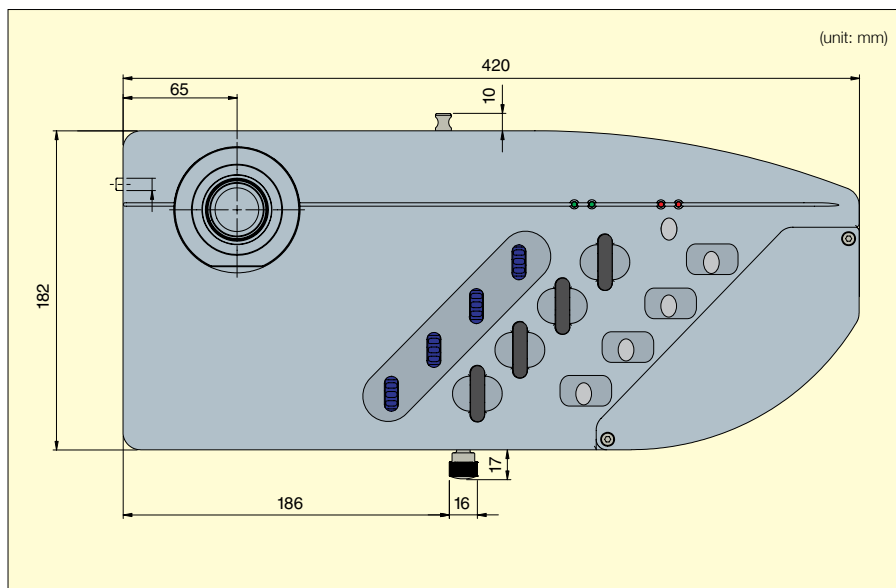
As the incident angle is increased, the refracted light passes through the sample in accordance with Snell's Law. Beyond a specific critical angle, the beam of light is totally internally reflected from the glass/water interface. The reflection generates a very thin electromagnetic field (usually less than 200nm) in the aqueous medium which behaves like light and can therefore be used to image events at the coverslip interface without widefield (out of focus) light contributing to the image. The results provide dramatic increases in signal-to-noise ratios and surface details.

High Numerical Aperture Objective TIRFM

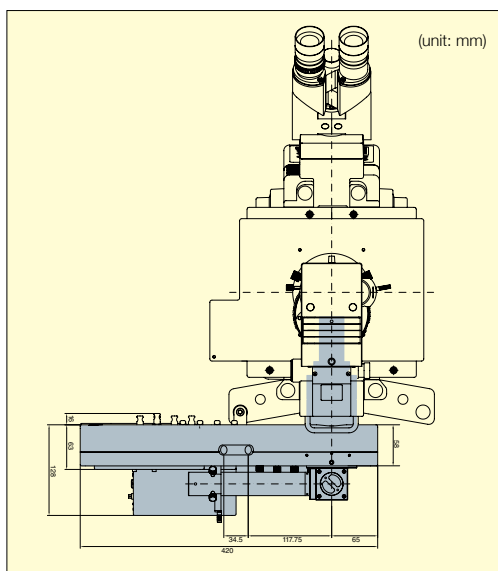


cell[^]TIRF[™] Illuminator Dimensions

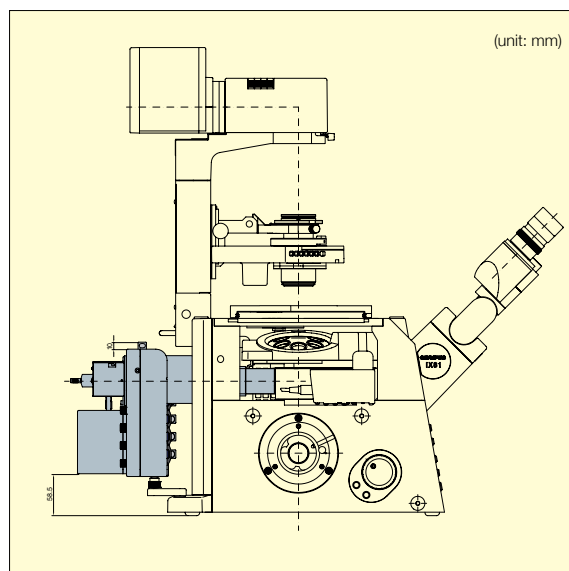
cell[^]TIRF Illuminator Dimensions Front



cell[^]TIRF Illuminator Dimensions Top



cell[^]TIRF Illuminator Dimensions Side



For more information on the cell[^]TIRF illuminator, call **800-446-5967** or visit olympusamerica.com/TIRF.

OLYMPUS[®]

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