TIRF (Total Internal Reflection Fluorescence) Microscope

Evanescent Wave Imaging Systems
Simple and fast switching between the epi-fluorescence and two types of TIRF methods—laser or white-light. Cellular focal adhesion images can be acquired with excellent S/N ratios.

TIRF (Total Internal Reflection Fluorescence) microscopy facilitates extremely high-sensitivity and high-contrast visualization of single molecules near the coverslip, without disturbing cellular activity, thereby enabling the tracking of biomolecules, and the study of their dynamic activity and interactions at the molecular level.

Nikon’s laser TIRF-2 system integrates a laser TIRF system and epi-fluorescence system, while the white-light TIRF system shares the mercury lamp of the epi-fluorescence system, and enables oblique and TIRF illumination. By combining a TIRF system with PFS (Perfect Focus System)—Nikon’s new focus maintaining system—you can continuously capture TIRF images of extraordinarily high S/N ratios and in perfect focus over an extended period of time. Furthermore, these TIRF systems can accommodate confocal, laser tweezers and other modules thanks to the expandable stratum structure of the TE2000 Inverted Microscope.

Nikon’s TIRF series dramatically expands the boundaries of what is possible in bioscience research, and is the perfect tool for scientists exploring molecular dynamics.

W-TIRF illumination system honored with “R&D 100 Award”

Nikon’s white light TIRF microscopy illumination system, which includes the 60x and 100x TIRF 1.49 NA objectives, has been honored with the “R&D 100 Award” of the year 2005 by the highly regarded R&D Magazine. Now in their 43rd year and globally recognized as a standard of excellence, the R&D 100 Awards are given to products that embody the most innovative ideas of the year. An independent panel of judges evaluate entries from every possible technology aspect to decide which products best improve the quality of life.
**Laser TIRF**

Enables single molecule visualization, allowing dynamic observation and functional analyses both in vitro and in living cells.

**Observation of single molecular dynamics**

YFP-tagged neurotransmitter receptors were expressed in dispersed hippocampal neurons in primary culture. TIRF microscopy enhances the cell surface image contrast, reducing the background signal from the cytoplasm (left). Under optimal conditions, TIRF microscopy allows observation of single receptor molecules moving rapidly, one by one, on the cell surface (right). This enables scientists to understand the elementary steps of signal transduction in the neuronal cell membranes.

**Visualization of microtubule structure near the coverglass**

Microtubules in fixed 3T3 fibroblasts were labeled with fluorescent conjugated antibodies. Most of the microtubules visible by epi-fluorescence imaging are not visible by laser TIRF imaging. However, the ends of the microtubules near the cell periphery and microtubules under the nucleus in the center of the cell are detected by TIRF.

**Dynamic observation of actin filaments in neuronal growth cone**

While DIC microscopy allows for observation of growth cone morphology, TIRF made it possible to study the underlying actin dynamics through multidimensional time-lapse imaging. Actin was labeled with a low concentration of fluorescent probe (Speckle fluorescence) in cultured Aplysia neurons.

**Visualization of clathrin coated vesicles at the cell membrane**

GFP-tagged clathrin was expressed in COS cells. Epi-fluorescence imaging shows clathrin expressed ubiquitously in the cells. Taking advantage of white-light TIRF illumination, that selectively excites the adjacent area to the coverglass, it is possible to visualize single clathrin coated vesicles undergoing exocytosis.

**Visualization of ZO-1 in cultured MDCK cells**

The immunofluorescent staining method was used to study the localization of ZO-1, a component of tight junctions, in MDCK cells. Epi-fluorescence microscopy revealed distinct fluorescent signals from cell-cell contact areas and weak, spotted fluorescent signals from other areas. White-light TIRF microscopy revealed only spotted signals, signals from cell-cell adhesion sites cannot be seen. This shows that the localization of ZO-1 at the cell-cell adhesion sites lies outside the evanescent wave range adjacent to the coverslip under the cells and that the spotted structures are inside the evanescent wave range. The distributions of spotted signals is confirmed in the SRIC (surface reflection interference contrast) image in which the spotted signals appear black.

**Visualization of microtubule and actin filaments**

Microtubules and actin filaments were visualized in a live Aplysia neuronal growth cone using multimode microscopy (Center). The image displays an overlay of DIC (shown in grey) and microtubules and actin filaments (green) dynamically interacting in a live growth cone. (Top and bottom)
Nikon has developed new TIRF objectives—the CFI Apochromat TIRF series—with a numerical aperture (NA) that is the highest (1.49) of all Nikon objectives. The higher NA results in a thinner evanescent field that increases the SN ratio. These breakthroughs, together with correction of all optical aberrations throughout the visible spectrum, make the new objectives optimum for multi-wavelength observations.

**World's first Temperature Correction ring**
These objectives incorporate a correction ring for temperature changes and coverglass thickness. By rotating the correction collar, you can easily eliminate spherical aberrations, negative influence on the image quality resulting from temperature-induced changes in the refractive index of the immersion oil, and influence from variation in the coverglass thickness. These objectives also provide spectacular images under DIC, confocal, and deconvolution imaging, while providing a strong trapping power during applications using laser tweezers.

**Evanescent Wave Illumination method (TIRF microscopy) for High-sensitivity Fluorescent imaging at the molecular level**
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**Surface Reflective Interference Contrast (SRIC) observations (patent pending)**
SRIC method can reveal focal contacts prior to switching to TIRF. As evanescent wave illumination excites within approximately 100 nm from the glass surface, TIRF observations require that specimens contact the coverglass, otherwise no TIRF image is obtained. SRIC makes all sections in contact with glass appear black, allowing users to confirm whether a specimen has adhered to the glass before proceeding with TIRF observation. As no excitation light is used in this process, specimen damage is minimized and users can take their time focusing. Nikon has developed an SRIC system that can be used with both laser TIRF-2 and white-light TIRF systems. Using SRIC is as simple as switching to the special filter cube.

**PPS enables TIRF images to retain high SN ratio**
In combination with PPS (Perfect Focus System), Nikon’s new real-time focus maintaining system, the TIRF system consistently delivers high SN images during long-term observations.
**Laser TIRF-2 System**

Integration of a laser TIRF system with epi-fluorescence system

The newly developed laser TIRF-2 system combines a laser TIRF system and epi-fluorescence system in a single unit. Researchers can observe the same field of view using both the TIRF and epi-fluorescence methods by simply switching light sources. Alignment is also extremely easy.

Ultra-high signal/noise ratio enables observations of single molecules

The extremely high S/N ratio created by Nikon's laser TIRF Imaging system makes it possible to observe single molecules. Thanks to Nikon's proprietary Noise Terminator mechanism, the system can also produce breathtaking epi-fluorescence images with a high S/N ratio.

**NEW**

Bright images over a wider range

Bright, clear images up to the edge of the field of view are obtained.

New high-performance fluorescence cassette holder

In the new filter cube holder, registration shift in the optical axis resulting from changing filters has been successfully eliminated for each laser wavelength.

Simultaneous mounting of other modules

The expandable stratum structure of the TE2000 Inverted Microscope allows other modules, such as laser tweezers, to be mounted on the microscope without altering the basic configuration.

Easy multiple-wavelength imaging

As this system uses a light source with a broad wavelength range, such as mercury illumination, by simply switching filters, TIRF observations are possible at a variety of wavelengths.

The extremely high S/N ratio created by Nikon's laser TIRF Imaging system makes it possible to observe single molecules.

**NEW**

One white-light illuminator supports various types of fluorescence observations (Patent pending)

The white-light TIRF system enables TIRF microscopy using mercury lamps. By exciting a confined depth, TIRF enables imaging of fluorescence images with a much higher S/N ratio than is possible using the epi-fluorescence method. The integration of a laser TIRF system and epi-fluorescence system enables the use of:

- **White-light TIRF**,
- Fluorescence with variable angle oblique illumination*,
- Epi-fluorescence, and
- SRIC methods.

All modes use the same light source and switching them is simple.

* Increasing the angle of incident light to slightly more than that of TIRF allows a deeper range of observation in the area near the coverglass.

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**NEW**
**Specifications**

<table>
<thead>
<tr>
<th>Laser TIRF-2 System</th>
<th>TIRF illuminator</th>
<th>Usable lasers</th>
<th>405nm, 440nm, 488nm, 532nm, 543nm, 561nm, 594nm, 633nm, 635nm, 640nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser introduction</td>
<td>Fiber method (FC connector)</td>
<td>Brightness control</td>
<td>ND filter (ND2/8/16)</td>
</tr>
<tr>
<td>Field number</td>
<td>φ18mm (φ11mm optional)</td>
<td>Shutter, provided with laser safety mechanism</td>
<td></td>
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<tr>
<td>Epi-fluorescence</td>
<td>Light source</td>
<td>Mercury lamp 100W, Xenon lamp 75W, Halogen lamp 100W</td>
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<tr>
<td>Brightness control</td>
<td>ND filter (ND2/8)</td>
<td>Field number</td>
<td>62mm</td>
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<tr>
<td>Epi-fluorescence changeover</td>
<td>Via mirror attach/detach for switching TIRF and epi</td>
<td></td>
<td></td>
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<tr>
<td>Epi-fluorescence filter block holder</td>
<td>High-performance epi-fl cassette holder (with empty filter cassette)</td>
<td></td>
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<tr>
<td>TIRF objectives</td>
<td>CR Immersion Oil 40X, 56X, 100X with temperature-correction mechanism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIRF objectives</td>
<td>CR Immersion Oil 100X-Oil (NA 1.49) with temperature-correction mechanism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compatible microscopes</td>
<td>TE2000-E, TE2000-U, TE2000-S, TE2000-Perfect Focus System (recommended for demanding time lapse applications)</td>
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</table>

**White-light TIRF System**

<table>
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<tr>
<th>White-light TIRF Illuminator</th>
<th>Excitation filter slider, ND filter, Epi/TIRF changeover slider</th>
</tr>
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<tbody>
<tr>
<td>Usable light source</td>
<td>Mercury lamp 100W, Xenon lamp 75W, Halogen lamp 100W</td>
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**TIRF-C1 System (Multimode Imaging System)**

**Multimode, multiangle viewing of the same field**

The TIRF-C1 System utilizes multimode imaging with laser TIRF, confocal and epi-fluorescence. This configuration can be a powerful tool for research, allowing the investigation of an event at the cell membrane, and the ability to follow the subsequent event cascade deep into the cell interior.

**Simultaneous mounting of other modules**

The TE2000 Inverted Microscope’s extensible stratum structure allows it to simultaneously accommodate laser tweezers* and other modules with the epi-fluorescence module, enabling tracking and measurement of single molecules.

* Option at the time of purchase

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**Confocal Image**

A clear F-actin belt (red) exists at the leading edge of the cell, which is migrating toward the right side. Paxillin molecules (green) are localized at the peripheral region. The localization of paxillin is shown in relation to actin stress fibers linking the focal adhesion at one end and the center of the cell at the other end.

**Epi-fluorescence image**

A clear F-actin belt (red) exists at the leading edge of the cell, which is migrating toward the right side. Paxillin molecules (green) are localized at the peripheral region. The localization of paxillin is shown in relation to actin stress fibers linking the focal adhesion at one end and the center of the cell at the other end.

**Surface reflection interference contrast (SRIC) image**

This SRIC image was observed using a conventional epi-fluorescence microscope with simple modification. The black area is the closest to the glass coverslip under the cell. The black area in the SRIC image coincides with the position of the focal adhesions. This method is available for identifying the basal surface of the cell before obtaining a TIRF image.

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**Multimode imaging with TIRF-C1 system**

ST2 cell (mouse bone marrow stromal cell line)

The ST2 cells were fixed with 4% formaldehyde, administered with 0.25% Triton X-100, then stained with anti-paxillin antibodies and TRITC phalloidin. The paxillin (green) is localized at the peripheral region, while the actin cytoskeleton (red) is clearly shown at the leading edge of the cell. The focal adhesions (green) are observed near the basal surface of the cell.
Reference

Front cover image (bottom right) courtesy of Dylan Burnette, Paul Forscher Laboratory, Yale University

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WARNING
TO ENSURE CORRECT USAGE, READ THE CORRESPONDING MANUALS CAREFULLY BEFORE USING YOUR EQUIPMENT.

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Nikon promotes the use of eco-glass that is free of toxic materials such as lead and arsenic.

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