TIRF (Total Internal Reflection Fluorescence) Microscope

Evanescent Wave Imaging Systems
TIRF (Total Internal Reflection Fluorescence) microscopy facilitates extremely high-sensitivity and high-contrast visualization of single molecules near the coverglass, 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 TIR 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.

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.

*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 membrane.

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 multimode timelapse imaging. Actin was labeled with a low concentration of fluorophor (Speckle fluorescence) in cultured Aplysia neurons.

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.

Visualization of microtubules 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 imaged in TIRF (shown in green). Time lapse montage showing actin filaments (red) and microtubules (green) dynamically interacting in a live growth cone. (Top and bottom)

White-light TIRF
Reveals vesicle fusion with high S/N ratios

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 junction, in MDCK cells. Epi-fluorescence microscopy revealed staining fluorescent signals from cell-cell contact areas and weak, spotted fluorescent signals from other areas. While white-light TIRF microscopy meanwhile showed 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 coverglass 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.

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Nikon’s precision optics takes S/N ratios to new heights

**Evanescent Wave Illumination method (TIRF microscopy) for High-sensitivity Fluorescent Imaging at the molecular level**

Nikon’s high N.A. TIRF objectives make it possible to introduce laser illumination at incident angles greater than the critical angle (θc) resulting in TIRF that creates an evanescent wave immediately adjacent to the coverglass-specimen interface. The evanescent wave reaches maximally a few hundred nanometers into the specimen and its energy drops off exponentially.

Nikon’s laser TIRF system utilizes this evanescent wave to excite single molecules in the thin section in contact with the coverglass. Because the specimen is not excited beyond the evanescent wave, this imaging system can produce fluorescence images with an extremely high signal-to-noise (SN) ratio.

**Direct, stable, and cost-effective imaging**

Nikon’s TIRF objectives feature NA 1.49, the highest ever. 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**

The world’s first oil-immersion lenses, these TIRF objectives incorporate a correction ring for temperature changes and coverglass thickness. By rotating the correction collar, you can easily eliminate spherical aberrations and 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. The lenses have been calibrated for a range from 22°C (room temperature) to 37°C (physiological temperature). Additionally, these objectives also provide spectroscopic images under DAPI, epit fluorescence, confocal, and deconvolution imaging, while providing a strong trapping power during applications using laser tweezers.

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Integration of a laser TIRF system with epi-fluorescence system

The newly developed 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 shuttering 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.

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.

* Increasing the angle of incident light to slightly more than that of total internal reflection allows a deeper range of observation in the area near the coverslip.

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 TIRF system into an 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.

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Images courtesy of Dr. Yasushi Okada, Cell Biology, Graduate School Medical Department, University of Tokyo.

Images courtesy of Dr. Masataka Kinjo, Supermolecular Spectrum Research, Electronics Science Laboratory, Hokkaido University.

Images courtesy of Dr. Richard Cheney Ph.D., UNC Chapel Hill.
**TIRF-C1 System (Multimode Imaging System)**

**Multimode, multangle viewing of the same field**
The TIRF-C1 System allows 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.

**Multimode 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 antipaxillin antibodies and TRITC-phalloidin.

**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

**TIRF-C1 System (Multimode Imaging System)**

**Specifications**

| Laser TIRF-2 System | TIRF illuminator | Useful lasers | 405nm, 440nm, 470nm, 532nm, 561nm, 635nm, 640nm |
| Laser introduction | Fiber method (FC connector) |
| Brightness control | ND filter (ND2/8/16) |
| Field number | φ11mm optional |
| Shutter | Provided with laser safety mechanism |

| Epi-fluorescence system | Light source | Mercury lamp 100W, Xenon lamp 75W, Halogen lamp 100W |
| Brightness control | ND filter (ND2/16) |
| Field number | φ22mm |

**Excitation light changer**
Via mirror attachment for switching TIRF and epi-fluorescence

**Epi-fluorescence filter block holder**
High-performance epi-fluorescence cassette holder (with empty filter cassette); Epi-fluorescence cassette holder

**TIRF objectives**
CFI Apochromat TIRF 60x Oil (NA 1.49) with temperature-correction mechanism
CFI Apochromat TIRF 100x Oil (NA 1.49) with temperature-correction mechanism

**Compatible microscopes**
TE2000-E, TE2000-U, TE2000-Perfect Focus System (recommended for demanding time lapse applications)

**White-light TIRF System**

**Excitation filter slider**
ND filter, Epi/TIRF changeover slider

| White-light TIRF illuminator | Light source | Mercury lamp 100W, Xenon lamp 75W, Halogen lamp 100W |
| Brightness control | ND Filter (ND2/16) |
| Field number | φ22mm |

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Images and explanations: Shuichi Obata, Ph.D., Kitasato University
Reference

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

Specifications and equipment are subject to change without any notice or obligation on the part of the manufacturer. March 2006 ©2005-6 NIKON CORPORATION

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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