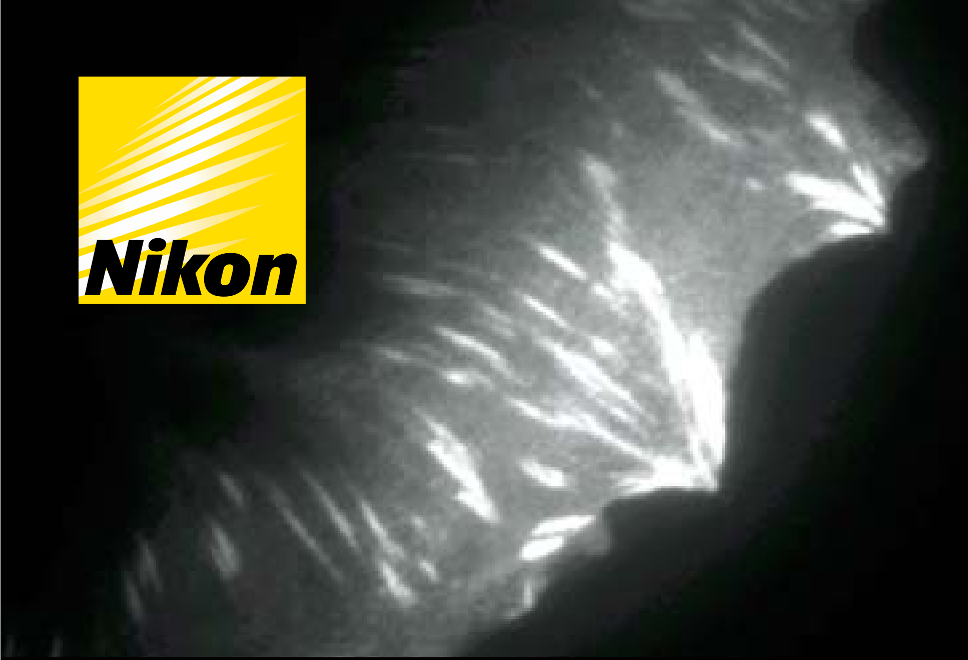
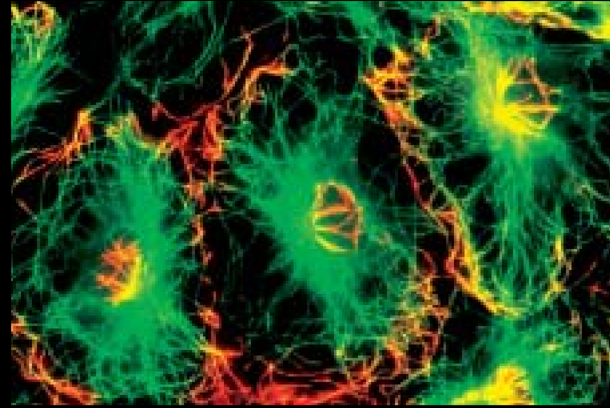
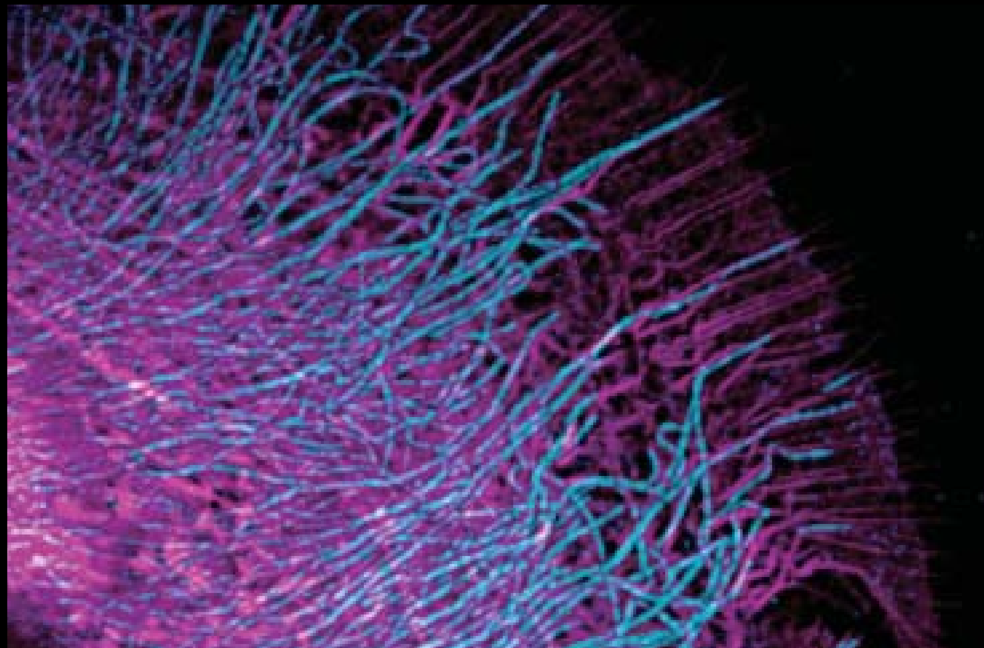




TIRF (Total Internal Reflection Fluorescence) Microscope

Evanescent Wave Imaging Systems



TIRF

Total Internal Reflection Fluorescence

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 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.

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.

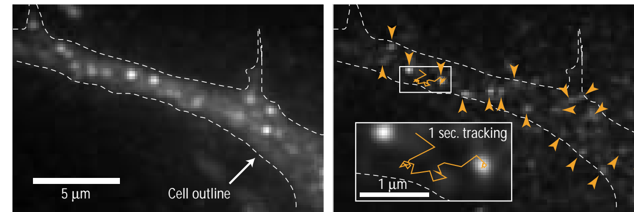
Laser TIRF

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.

Laser TIRF images

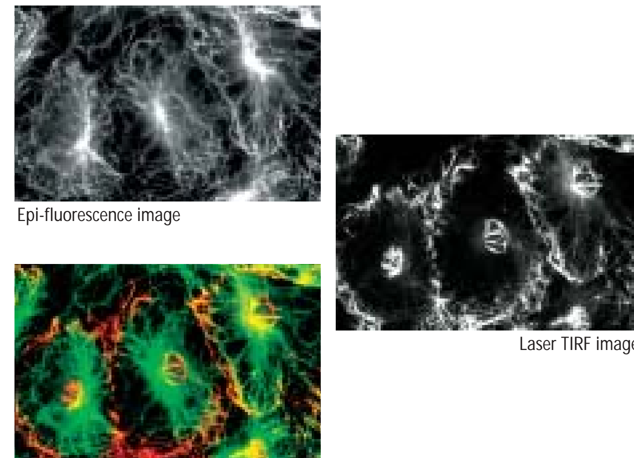


Fluorescent receptor image (normal distribution) Fluorescent receptor image (molecular dynamics)

Microscope: TE2000
Camera: Image Intensifier (Hamamatsu Photonics C8600-03) + EB-CCD (C7190-23)
Images courtesy of: Dr. Chieko Nakada, Kusumi Office, Institute for Frontier Medical Sciences, Kyoto University
Co-researcher: Professor Shigeo Okabe, Tokyo Medical and Dental University

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.



Epi-fluorescence image

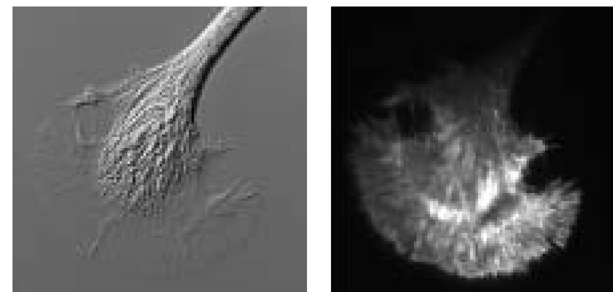
Laser TIRF image

TIRF/epi-fluorescence image overlay (pseudo color)

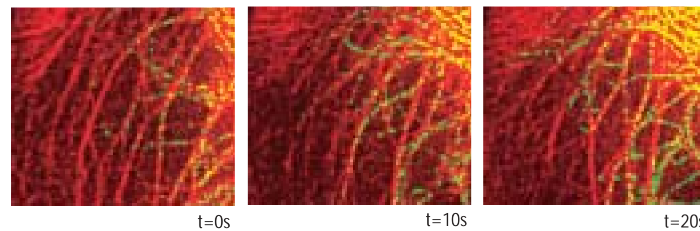
Images courtesy of Dr. Gregg G. Gundersen, Columbia University

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.



DIC image Laser TIRF image

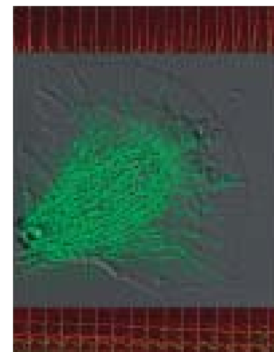


t=0s t=10s t=20s

Images courtesy of Dylan Burnette, Paul Forscher Laboratory, Yale University

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)



Laser TIRF image
Vertical field: 80µm

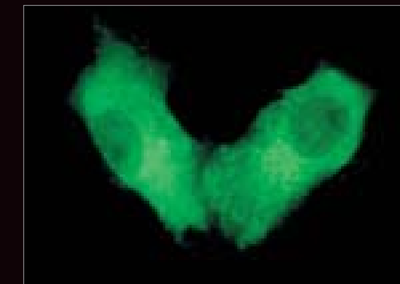
Image courtesy of Andrew Schaefer, Paul Forscher Laboratory, Yale University

White-light TIRF

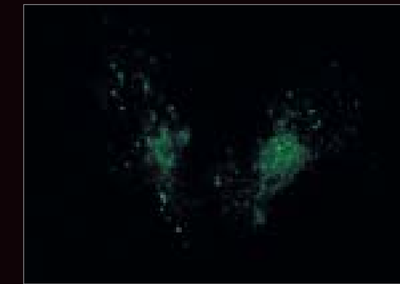
Reveals vesicle fusion with high S/N ratios

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.



Epi-fluorescence image

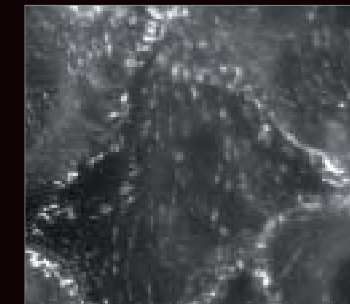


White-light TIRF image

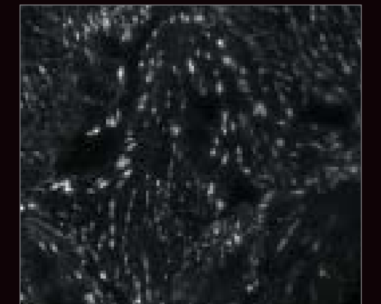
Images courtesy of Daniel Axelrod Ph.D. and Stephen Ross, Ph.D., taken at the "3D Microscopy in Living Cells" course 2004, UBC, Vancouver, Canada

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 strong fluorescent signals from cell-cell contact areas and weak, spotted fluorescent signals from other areas. 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 coverslip under the cells and that the spotted structures are inside the evanescent wave range. The distribution of spotted signals is confirmed in the SRIC (surface reflection interference contrast) image in which the spotted signals appear black.



Epi-fluorescence image



White-light TIRF image

SRIC image

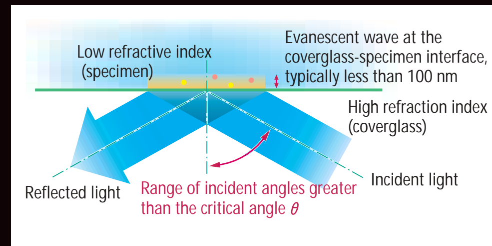
Images courtesy of Shuichi Obata, Ph.D., Kitasato University; Kei-ichiro Yoshida, Ph.D., Yokohama City University

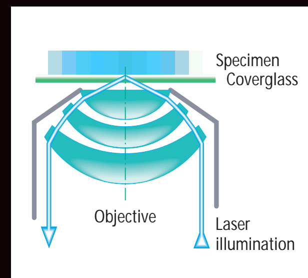
Nikon's precision optics takes S/N ratios to new heights

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 (S/N) ratio.

Overview of Evanescent Wave Illumination





TIRF objectives feature NA 1.49, the highest ever

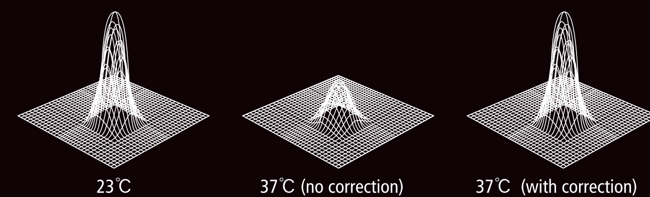
Nikon has developed new TIRF objectives—the CFI Apochromat TIRF series—with a numerical aperture (NA) that is the highest (1.49) of all Nikon objectives. The higher NA results in a thinner evanescent field that increases the S/N ratio. These breakthroughs, together with correction of all optical aberrations 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' 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 23°C (room temperature) to 37°C (physiological temperature). Additionally, these objectives also provide spectacular images under DIC, epi-fluorescence, confocal, and deconvolution imaging, while providing a strong trapping power during applications using laser tweezers.

CFI Apochromat TIRF 60x oil/NA 1.49 (left)
CFI Apochromat TIRF 100x oil/NA 1.49 (right)
Support regular coverglass and oil.

Correction Ring Effects (severity distribution)



PFS enables TIRF images to retain high S/N ratio

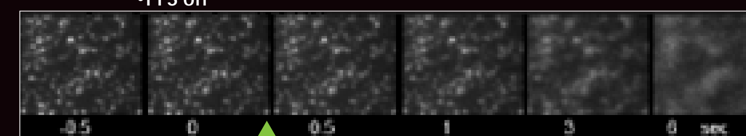
In combination with PFS (Perfect Focus System), Nikon's new real-time focus maintaining system, the TIRF system consistently delivers high S/N images during long-term observations.

Correction to focus drift caused by expansion/contraction of the plastic dish when reagents are added

•PFS on

Adding reagent

•PFS off



Adding reagent

Images courtesy of Profs. Akihiro Kusumi and Dr. Chieko Nakada, Kusumi Office, Institute for Frontier Medical Sciences, Kyoto University

High precision control

PFS detects the coverslip interface with infrared LED light and corrects focus drift due to reagent droplets or temperature changes during long-term observations with precision one third the focal length.

Documents reactions in real time

You will never miss any change in the cell, even immediately after reagent droplets, as PFS continuously corrects focus. Since you can keep a stable focus for long time, the system is a perfect choice for live-cell time-lapse recordings. The reduction in the amount of excitation reduces the chance of photobleaching.

Focus on the plane of interest

Focus correction with PFS is rapid and extraordinarily precise as it maintains focus directly on the plane of interest and does not require focusing on the coverslip interface.

No influence on fluorescent dyes

PFS uses 770nm LED light for interface detection. This practically eliminates the risk of photobleaching fluorescent dyes or adversely affecting live cells, resulting in observation of high S/N TIR images.

Surface Reflective Interference Contrast* (SRIC) observations (*patent pending)

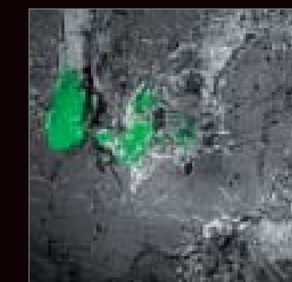
SRIC method can reveal focal contracts prior to switching to TIRF

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 the laser TIRF-2 and white-light TIRF systems. Using SRIC is as simple as switching to the special filter cube.

Cell adhesion to the glass surface is a prerequisite for TIRF microscopy. SRIC shows whether or not cell adhesion exists.

Laser TIRF image SRIC image



Overlay image

HeLa cell
Images courtesy of Dr. Masaya Hashido, Cellular and Molecular Pharmacology, Faculty of Medicine, the University of Tokyo

Laser TIRF-2 System

Laser TIRF | Epi-fluorescence | SRIC

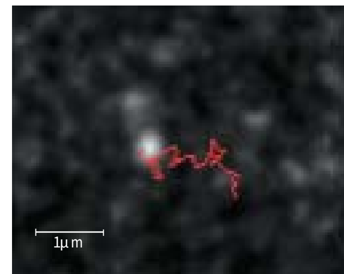
Integration of a laser TIRF system with epi-fluorescence system

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 shuttering light sources. Alignment is also extremely easy.

NEW

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.



The image shows the basal membrane of HeLa cells expressing GFP-tagged PKCβII. It is an image taken 10 minutes after phorbol ester, a carcinogenic promoter, was added. PKCβII is activated when stimulated by phorbol ester, and it translocates from the cytoplasm to cell membrane. Red dotted lines show the path of translational diffusion of single molecular GFP-tagged PKCβII in the cell membrane.

Image courtesy of Dr. Yu Ohsugi and Dr. Masataka Kinjo, Supermolecular Spectrum Research, Electronics Science Laboratory, Hokkaido University.

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

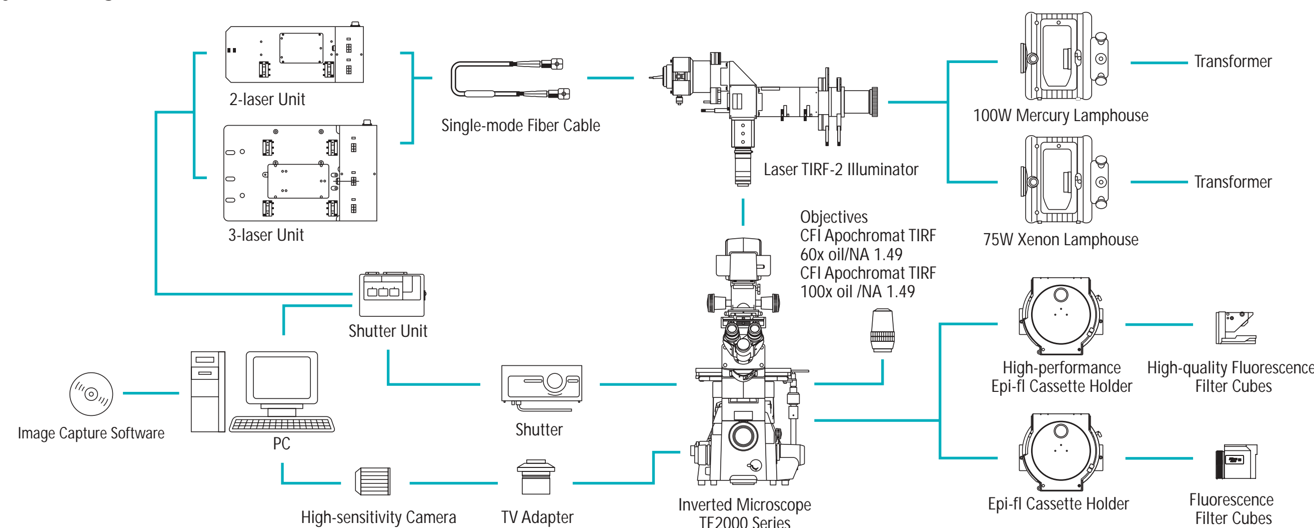
In the new filter cube holder, registration shift in the optical axis resulting from changing filters has been successfully eliminated for each laser wavelength.

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.

System Diagram

For usable lasers, see the specifications on page 11.



White-Light TIRF System (Multi-Fluorescence Imaging System)

White-Light TIRF | Oblique light fluorescence | Epi-fluorescence | SRIC

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 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.

NEW

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.

Epi-fluorescence image | White-light TIRF image | White-light TIRF image

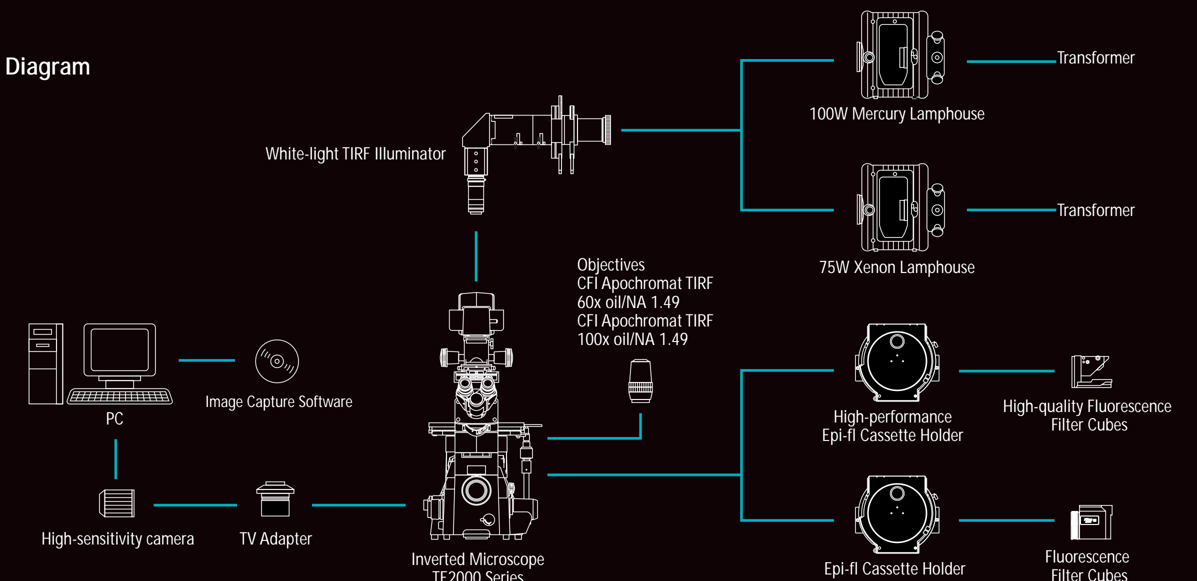
A YFP-fusion protein is targeted to the membrane of COS cells. As both modes use the same light source, switching between them is simple. Fluorescence emitted from various cell membranes, including the Golgi apparatus is seen in epi-fluorescence imaging, while white-light TIRF imaging allowed specific visualization of the cell membrane adjacent to the coverglass.

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

GFP-myosin X motor protein was expressed in cultured cells. Dynamic activities of myosin X can be observed in the cell's fine filopodia.

Image courtesy of Richard Cheney Ph.D., UNC Chapel Hill.

System Diagram



TIRF-C1 System (Multimode Imaging System)

Confocal | Laser TIRF | Epi-fluorescence | SRIC

Multimode, multiangle 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 cells interior.

Simultaneous mounting of other modules

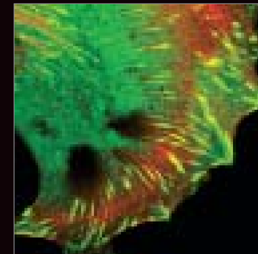
The TE2000 Inverted Microscope's extensible stratum structure allows it to simultaneously accommodate laser tweezers* and other modules with the epi-fluorescence module, enabling tracking and measurement of single molecules.

* Option at the time of purchase

Multimode imaging with TIRF-C1 system

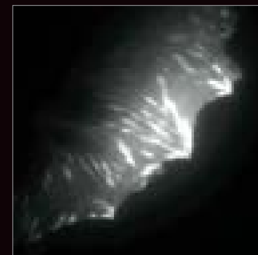
ST2 cell (mouse bone marrow stromal cell line)

The ST2 cells were fixed with 4% formaldehyde, administered with 0.25% Triton X-100, then stained with antipaxillin antibodies and TRITC-phalloidin.



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 shows focal adhesions. Stress fibers link the focal adhesion at one end and the center of the cell at the other end.



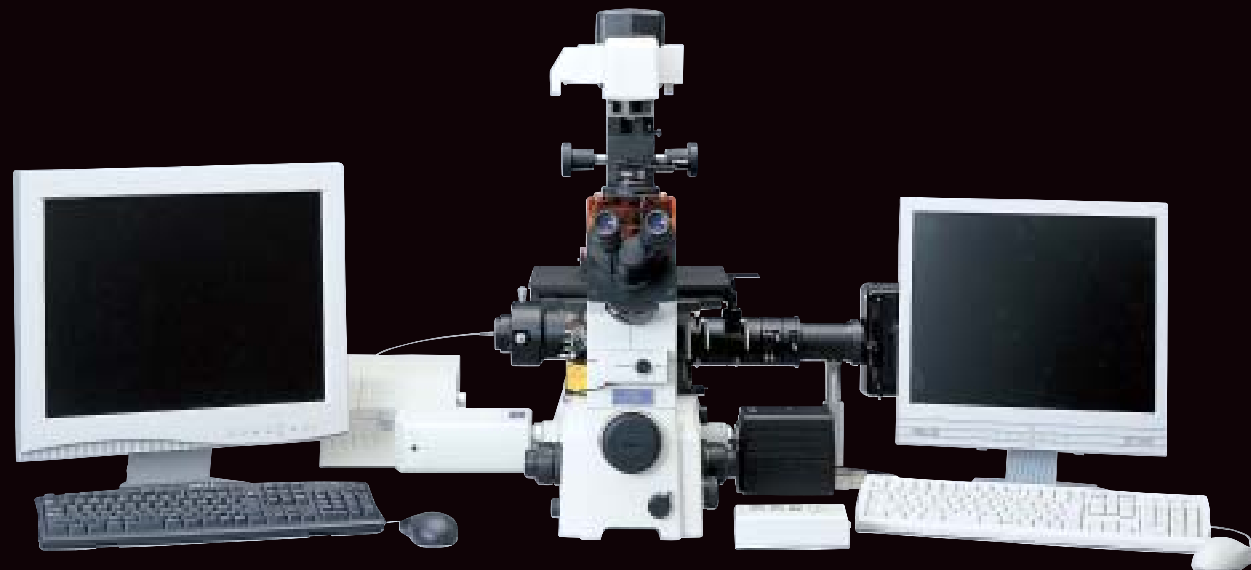
Total internal reflection fluorescence (TIRF) image

Strong and clear fluorescence derived from paxillin is observed in the evanescent field. The focal adhesions existing at the basal surface of the cell were clearly confirmed.

Surface reflection interference contrast (SRIC) image

This SRIC image was observed using a conventional epifluorescence 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 focal adhesions. This method is available for identifying the basal surface of the cell before observing a TIRF image.

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



Specifications

Laser TIRF-2 System

TIRF illuminator	Usable lasers	405nm, 440nm, 488nm, 532nm, 543nm, 561nm, 594nm, 633nm, 635nm, 640nm
	Laser introduction	Fiber method (FC connector)
	Brightness control	ND filter (ND2/8/16)
	Field number	ϕ18mm (ϕ11mm optional)
	Shutter, provided with laser safety mechanism	
Epi-fluorescence illuminator	Light source	Mercury lamp 100W, Xenon lamp 75W, Halogen lamp 100W
	Brightness control	ND filter (ND2/16)
	Field number	ϕ22mm
Excitation light changeover	Via mirror attach/detach for switching TIRF and epi	
Epi-fl filter block holder	High-performance epi-fl cassette holder (with empty filter cassette); Epi-fl 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)	

Laser TIRF-2 System

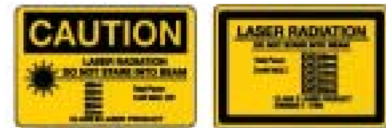
White-light TIRF System

White-light TIRF illuminator	Excitation filter slider, ND filter, Epi/TIRF changeover slider	
	Usable light source	Mercury lamp 100W, Xenon lamp 75W, Halogen lamp 100W
	Brightness control	ND filter (ND2/16)
	Field number	ϕ22mm
Epi-fl filter block holder	High-performance epi-fl cassette holder (with empty filter cassette); Epi-fl 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-S, TE2000-Perfect Focus System (recommended for demanding time lapse applications)	

White-light TIRF system

Reference
- Tokunaga, M. et al.: Single Molecule Imaging of Fluorophores and Enzymatic Reactions Achieved by Objective-type Total Internal Reflection Fluorescence Microscopy. Biochem.Biophys. Res. Commun., 235, 47-53 (1997)
- Kusumi, A. et al.: Single Molecule Cell Biology, Cell Engineering, 20, 656-703 (2001)

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



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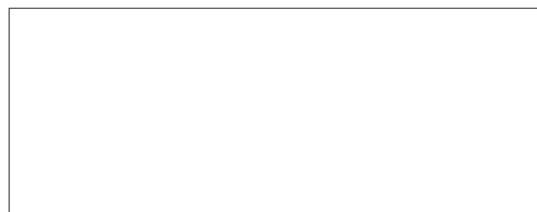

Nikon promotes the use of eco-glass that is free of toxic materials such as lead and arsenic.

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