



Inverted Research Microscope ECLIPSE Ti
Live-cell Imaging Accessories

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Live-cell Imaging Accessories



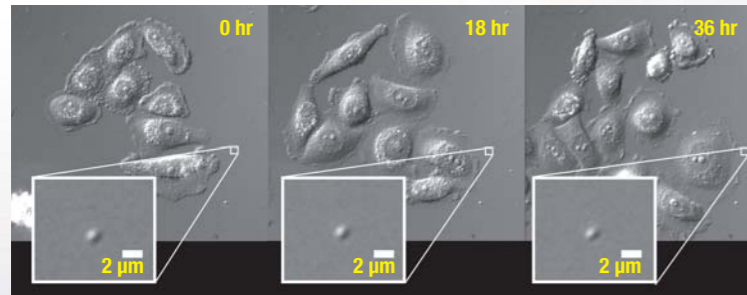
Cutting-edge Functions to Meet the Needs of Advanced Live-cell Imaging

Nikon provides a diverse choice of accessories such as illuminators and focus maintenance systems to customize Ti series inverted microscopes as long-term observation and imaging systems that capture the dynamics of living cells and support cutting-edge research.

Multi-dimensional Time-lapse Imaging

Nikon's exclusive Perfect Focus System (PFS) corrects focus drift in real time during long-term multipoint observation and when reagents are added. Focus is maintained over hours and days. Integrated control of the microscope and motorized accessories with NIS-Elements imaging software facilitates long-term multi-dimensional time-lapse imaging.

Focus maintained during long-term time-lapse observation



Specimen: HeLa cell proliferation over 0-36 hours
Objective: CFI Plan Fluor 40x dry (NA 0.6)

Photo Activation

The photo activation illuminator allows excitation of a desired point with a specified wavelength. Spot excitation with UV or near UV wavelengths allows photo conversion or photo activation at specific parts of nerve cells or intracellular molecules labeled by fluorescence proteins such as Kaede or PA-GFP. This visualizes changes in living molecule dynamics and promotes analysis of the intracellular communication process.

Photo activation of PA-GFP in a living mammalian cell



Photos courtesy of Tomoki Matsuda and Takeharu Nagai, Research Institute for Electronic Science, Hokkaido University

Laser TIRF

Nikon's TIRF objectives make it possible to introduce laser illumination at incident angles greater than the critical angle because of their high NAs. This enables Total Internal Reflection Fluorescence (TIRF) that creates an evanescent wave immediately adjacent to the coverslip-specimen interface, allowing visualization of a thin section that is within approximately 100 nm of the coverslip surface. Because there is no noise caused by fluorescence from areas other than the coverslip-specimen interface, fluorescence images of single molecules near cell membranes can be captured with an extremely high signal-to-noise (S/N) ratio. The newly developed motorized laser TIRF illumination unit allows storage and reproduction of laser incident angles using NIS-Elements software.

TIRF image of coverslip-specimen interface, epi-fluorescence image of entire cell

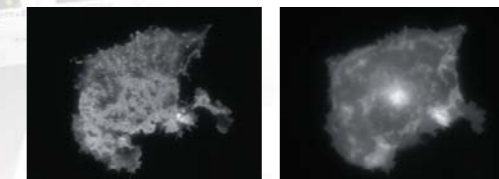


Laser TIRF image Epi-fluorescence image TIRF/epi-fluorescence image overlay (pseudo color)
Photos courtesy of Dr. Gregg G. Gundersen, Columbia University

White-light TIRF

Nikon's unique white-light TIRF system enables multiple methods of fluorescence observation such as white-light TIRF, oblique light fluorescence and epi-fluorescence with white light, including mercury illumination. By exciting a limited depth, white-light TIRF enables images with a much higher S/N ratio than is possible using the epi-fluorescence method. With oblique light fluorescence, increasing the angle of incident light to slightly more than that of TIRF allows a deeper range of observation in the area near the coverslip.

White-light TIRF and epi-fluorescence images using the same light source

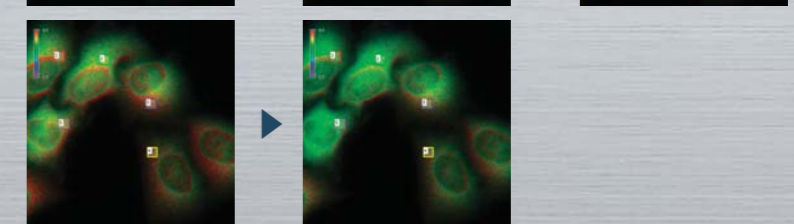
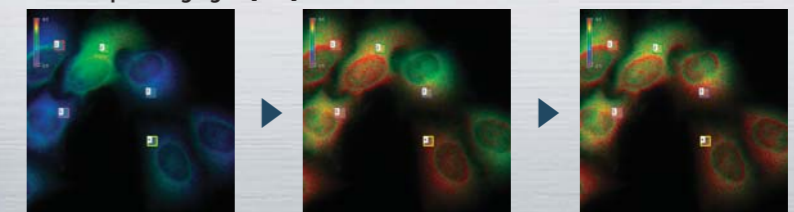


White-light TIRF image Epi-fluorescence image
Photos courtesy of Dr. Yasushi Okada, Cell Biology, Graduate School Medical Department, the University of Tokyo

FRET

The Ti's multi-level stratum structure allows the use of two fluorescence filter turrets in tiers. By capturing different wavelength images simultaneously with cameras attached respectively to the back port and side port, highly accurate measuring of intensity ratios can be achieved. This facilitates research applying the Förster Resonance Energy Transfer (FRET) technique that supports analysis of intermolecular interactions and the development of molecular sensors to detect intracellular calcium concentration ($[Ca^{2+}]_i$) changes.

Time-lapse imaging of $[Ca^{2+}]_i$ release



Photos courtesy of Kenta Saito and Takeharu Nagai, Research Institute for Electronic Science, Hokkaido University

Multi-dimensional Time-lapse Imaging with Highly Reliable Data

Ti-E PFS

Nikon's exclusive automatic focus maintenance system PFS (Perfect Focus System) enables stress-free acquisition of highly accurate and repeatable data during multi-dimensional time-lapse imaging to obtain wavelength information and spatial information of live cells. In-focus images are always captured during TIRF observation using high NA TIRF objectives. PFS allows system configuration to acquire more accurate and reliable time-lapse data than ever before.

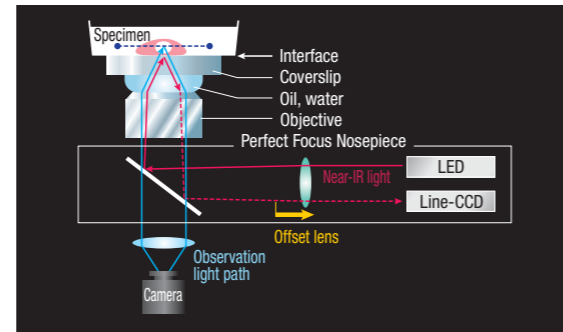


*PFS is only compatible with Ti-E

Real-time Focus Correction

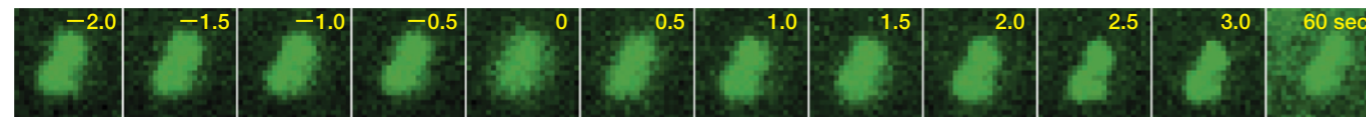
Nikon's PFS eliminates focus drift, one of the biggest obstacles in long-term live-cell observation. It automatically detects the surface of the coverslip and constantly corrects focus with reference to that position to compensate for even the most infinitesimal changes. Focus drift resulting from long-term observation, stage shake during multipoint observation and sudden temperature changes when reagents are added are immediately corrected, meaning cell changes are never overlooked and fluorescence intensity measurements are accurate and repeatable. Focus can be maintained on a desired plane over a number of days, while multiple Z-positions can be saved and accurately reproduced.

Perfect Focus System concept

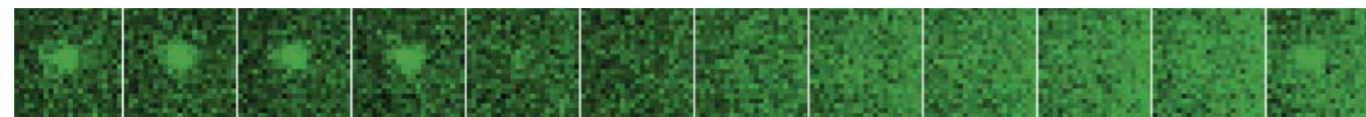


The diagram shows a configuration using an immersion type objective. A dry type objective can also be used.

Focus maintained even when reagents are added



Reagent added with PFS on



Reagent added with PFS off

Numbers indicate the time in seconds before and after the addition of the reagent. With PFS on, focus maintained even with sudden temperature drop when reagents added. With PFS off, focus drift present when reagents added.

Specimen: Rapid Ca^{2+} imaging of HeLa cells with Fluo4 load using white-light TIRF
Objective: CFI Apo TIRF 100x Oil, NA 1.49

Efficient Use of Stratum Structure

PFS is now incorporated in the Ti-E's motorized nosepiece unit. This saves space and allows focus to be maintained by PFS even when two components such as the TIRF attachment and laser tweezers are simultaneously mounted.



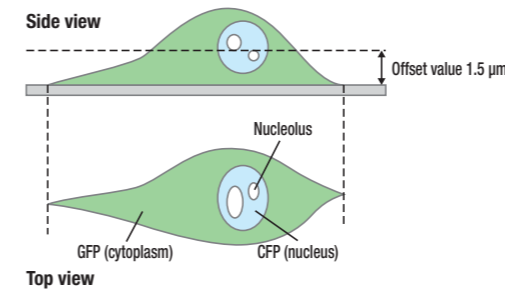
Motorized nosepiece incorporating PFS

Excellent Focus Reproducibility even with Time-lapse Imaging of Multiple Focus Planes

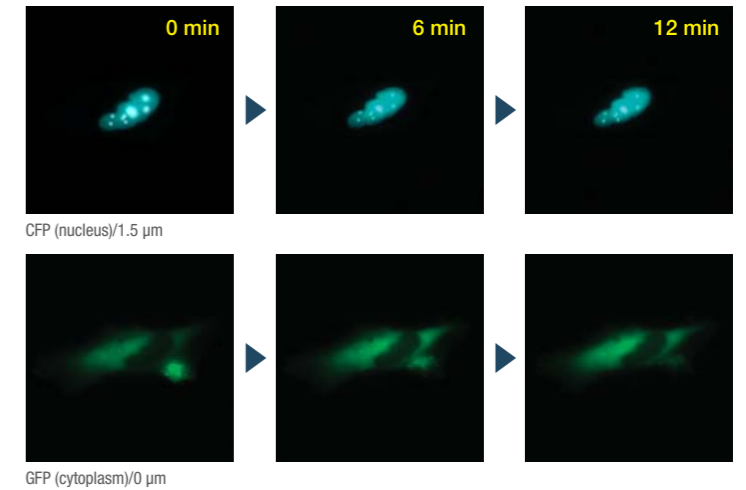
Offset observations of a desired Z-axis plane can be conducted. This allows focus to be maintained during ZT series acquisition under confocal microscopy or the optimal focal plane to be accurately tracked during TIRF microscopy. The Memory function can be used to record offset planes and the Recall function to reproduce them for powerful multi-plane, multi-point imaging.

Multi-plane GFP imaging

Time-lapse imaging acquired while changing the Z-axis point using the offset functionality. Offset value: 1.5 μm for nucleus, 0 μm for cytoplasm.

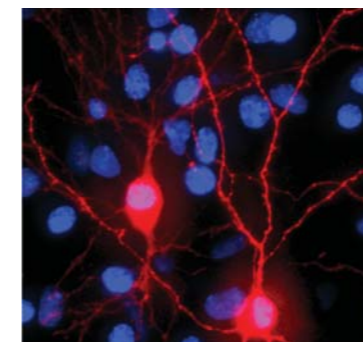


Specimen: HeLa cells expressed CFP and GFP.
Objective: CFI Plan Apo TIRF 60x Oil, NA 1.45

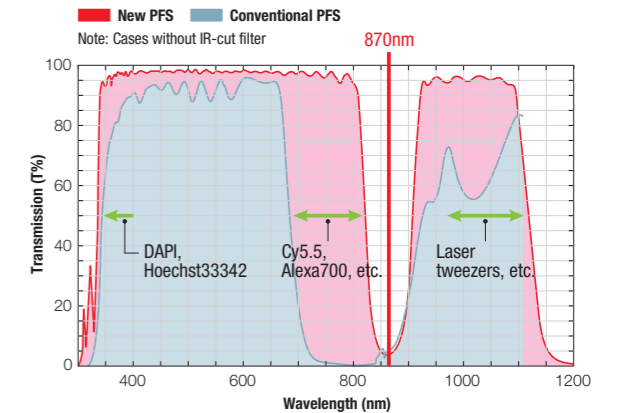


Compatible with Diverse Illumination Techniques and Improved Performance in Broader Wavelength Ranges

PFS can be used at both high and low magnifications for all illumination techniques, including brightfield, fluorescence, DIC, TIRF and phase contrast. PFS is compatible with dry objectives. Because it employs near-infrared light with an 870 nm wavelength for the coverslip interface detection, and because the optical characteristics from ultraviolet to infrared range have been improved, observation of bright fluorescence images across a broad wavelength range is possible.



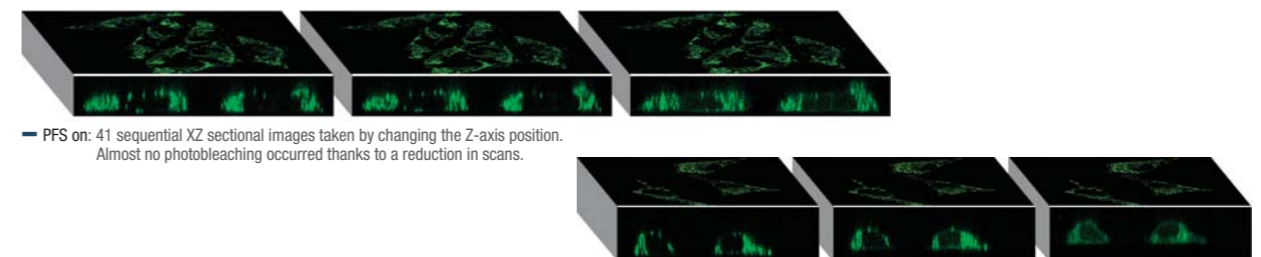
Live imaging of primary rat cortical neurons stained with Hoechst33342 and DiR
Photo courtesy of Ippei Kotera, Shinya Hosaka and Takeharu Nagai, Research Institute for Electronic Science, Hokkaido University



Phototoxic Damage is Minimized

PFS automatically maintains focus position once it is set, eliminating the need to take extra images of different planes in anticipation of focus drift; so the exposure time and the number of images to be acquired can be minimized. Excessive optical exposure to the specimen is eliminated and photobleaching and phototoxic damage is reduced.

Extra imaging of different planes is eliminated, reducing photobleaching



PFS on: 41 sequential XZ sectional images taken by changing the Z-axis position. Almost no photobleaching occurred thanks to a reduction in scans.

PFS off: 65 sequential XZ sectional images taken by changing the Z-axis position. 24 extra pictures were taken to cover focus drift. Strong photobleaching occurred due to frequent scans.

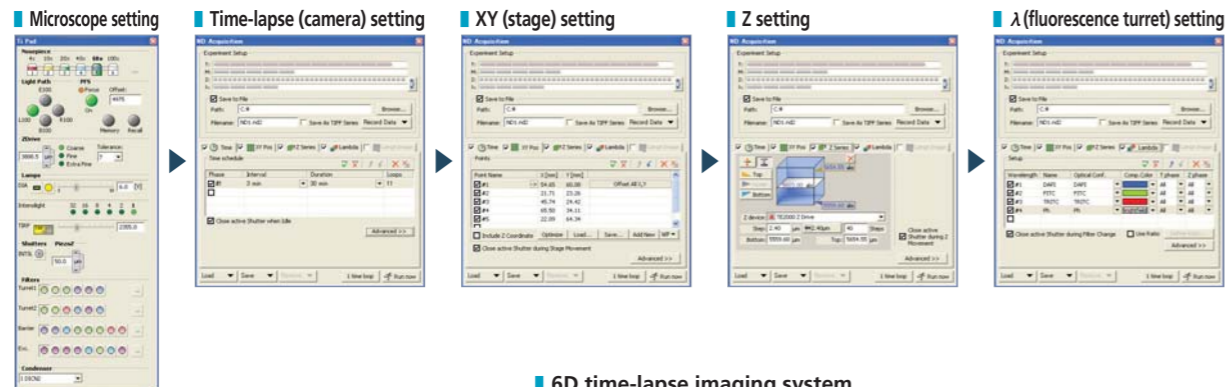
Specimen: Confocal images of mitochondria in HeLa cells stained with Rhodamine 123
Objective: CFI Plan Apo VC 60x water dipping NA 1.20

Comprehensive Software Realizes Stable and Reliable Time-lapse Imaging

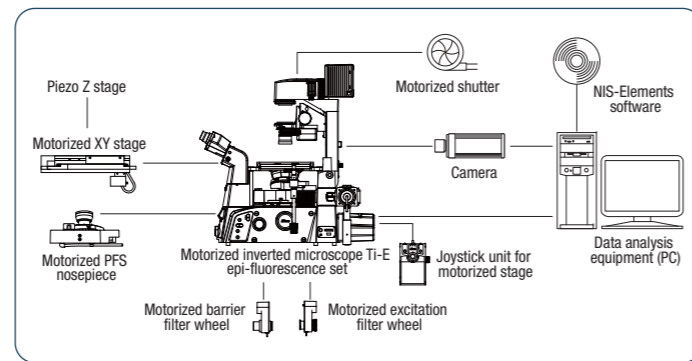
Imaging Software NIS-Elements



Nikon's original imaging software NIS-Elements provides integrated control of the microscope, cameras, components and peripherals, and allows the programming of automated sequences for up to 6D (X, Y, Z, t (time), Lambda (wavelength), multipoint) multi-dimensional image acquisition. The intuitive GUI facilitates complex settings while a diverse suite of analysis tools supports measurement, documentation and databasing.



6D time-lapse imaging system



Total Solution for Image Acquisition

By combining the Nikon motorized stage, motorized filter turret and specified "smart" shutters, acquisition of multipoint, multi-channel time-lapse images and Z-axis information of each of these points is possible.

Nikon motorized XY stage



Fast and precise positioning is possible. Suitable for multipoint time-lapse observation. (Photo shows encoded type)

Piezo Z stage



High-speed, precise Z-axis control is possible. (Manufactured by Mad City Labs, Inc.)

Motorized nosepiece



With six objective positions (Photo shows motorized PFS nosepiece)

Motorized filter rotating turret



Position of filter cubes can be changed in 0.3 sec. per position (Photo shows high-performance type)

Motorized barrier filter wheel



Barrier filter positions (8 positions—using 25 mm filters) can be changed at a high speed of 0.15 sec. per position.

Motorized "Smart shutter"



High-speed shutter for fluorescence excitation and brightfield illumination (Manufactured by Sutter Instrument Company)

Motorized excitation filter wheel



Excitation filters (8 positions—using 25 mm filters) can be changed at a high speed of 0.15 sec. per position.

Digital cameras for microscopes



Various digital cameras for microscopes are available. (Photo shows DS-Q11)

Photo Activation of PA-GFP/Kaede for Cell Marking and Observation of Cell Dynamics

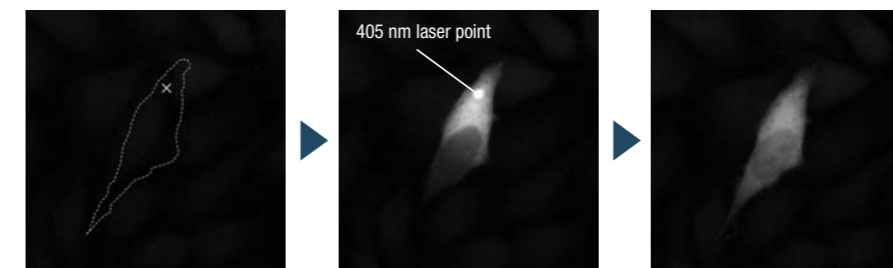
Photo activation
Epi-fluorescence

Ti-E Ti-U

The Ti-E/Ti-U is compatible with a specialized photo activation illuminator using a laser.

The excitation with a specific wavelength such as 405 nm allows fluorescent time-lapse observation of dynamic behavior of living cells by causing photo activation or photo conversion. For example, by marking cells with PA-GFP photo-convertible protein, which increases fluorescence intensity 100 times, or Kaede, which changes fluorescence colors from green to red, fluorescent time-lapse observation of localization of intercellular proteins and dynamic changes is possible.

Photo activation of PA-GFP in a living mammalian cell by 405 nm laser irradiation



Photos courtesy of Tomoki Matsuda and Takeharu Nagai, Research Institute for Electronic Science, Hokkaido University

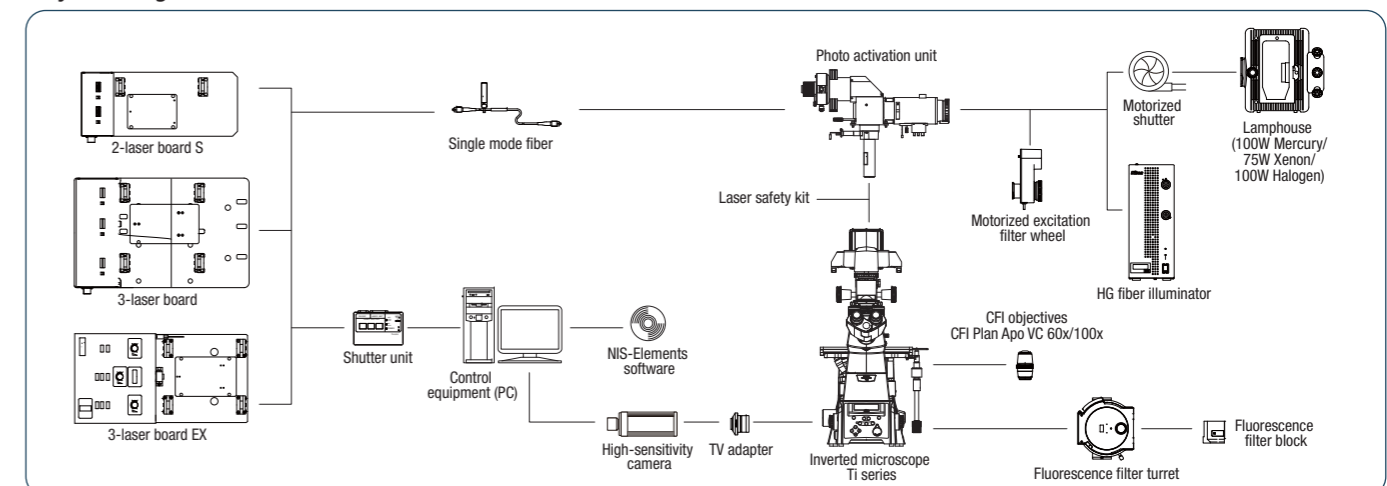


Photo activation unit



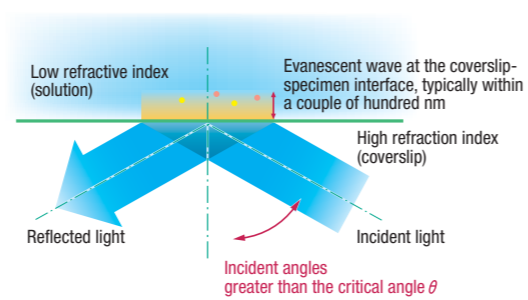
Configuration with the Ti-E

System diagram



TIRF for Observation of Single-molecule Dynamics

TIRF (Total Internal Reflection Fluorescence) allows high-sensitivity, high-contrast dynamic imaging of living molecules while keeping them active. When a coverslip is exposed to laser illumination at an incident angle greater than the critical angle, total internal reflection occurs. Under these conditions, an evanescent wave is only generated within a couple of hundred nm from the coverslip-specimen interface. By using this light to excite the coverslip-specimen interface, fluorescence images with extremely high S/N ratios can be acquired. Nikon's TIRF systems allow the use of TIRF methods most suitable to research purposes and supports observation of live cell dynamics and the study of live cell functions.



Overview of TIRF

When a laser illumination incident angle is greater than a critical angle, total internal reflection occurs due to refractive index differences between glass and solution. An evanescent wave created at the time reaches a maximum of a few hundred nanometers into the specimen. Using this evanescent wave for excitation, the thin section in contact with the coverslip can be observed.

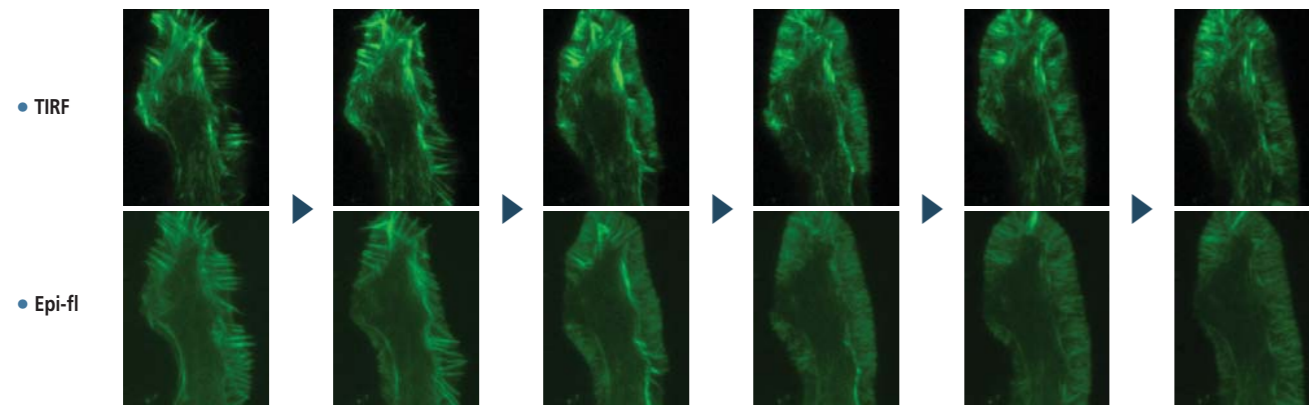
Motorized TIRF Attachment

The newly developed motorized laser TIRF illumination unit allows laser incident angle adjustment, shutter control and the switch to mercury illumination with the control pad or a PC. The laser incident angle can be saved with a single touch of the control pad button. Saved laser incident angles can be easily reproduced. This enables alternate time-lapse recording between fluorescence and TIRF images using NIS-Elements software and supports temporal and spatial dynamic analysis of intracellular protein molecules.



Remote controller

Time-lapse imaging by switching TIRF and epi-fluorescence observation



NG108 cell: Growth cone stained with EGFP-fascin

Photos courtesy of Satoe Ebihara, Kaoru Katoh, The National Institute of Advanced Industrial Science and Technology (AIST)

TIRF Objectives Feature an Unprecedented NA 1.49

Nikon has developed TIRF objectives with a super high NA of 1.49. With correction of all optical aberrations throughout the visible spectrum, the objectives are the optimum for multi-wavelength observations. These lenses can be used with standard coverslips and immersion oils. Moreover, these objectives incorporate a correction ring for temperature changes and coverslip thickness. Negative influences on image quality resulting from temperature-induced changes in the refractive index of the immersion oil within the temperature range of 23°C (room temperature) and 37°C (physiological temperature) are eliminated. Additionally, the elimination of influences from variations in coverslip thickness allows high-resolution images of minute structures to be captured at diffraction limited resolutions.



CFI Apo TIRF 60x Oil, NA 1.49 (left)
CFI Apo TIRF 100x Oil, NA 1.49 (right)

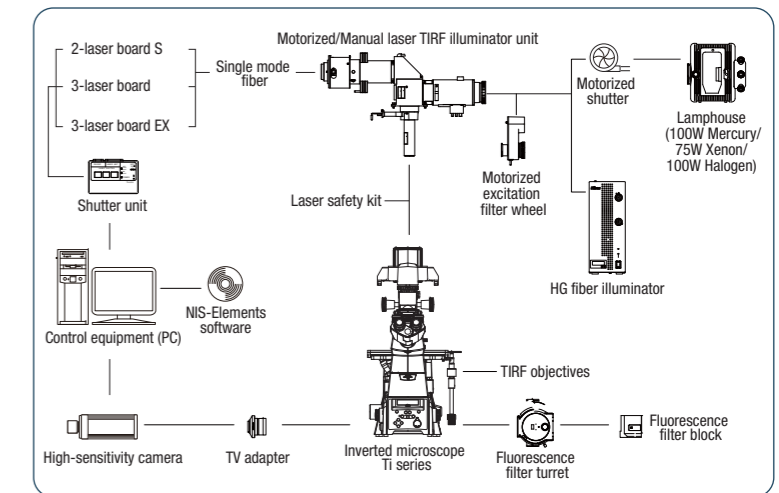
Motorized/Manual Laser TIRF Illuminator Unit

This unit allows TIRF observation to capture ultra-high S/N ratio images of single fluorescent molecules localized at near cell membranes using laser illumination. The motorized illuminator enables control and saving of laser incident angles.



Configuration with the Ti-E and motorized laser TIRF illuminator unit

System diagram



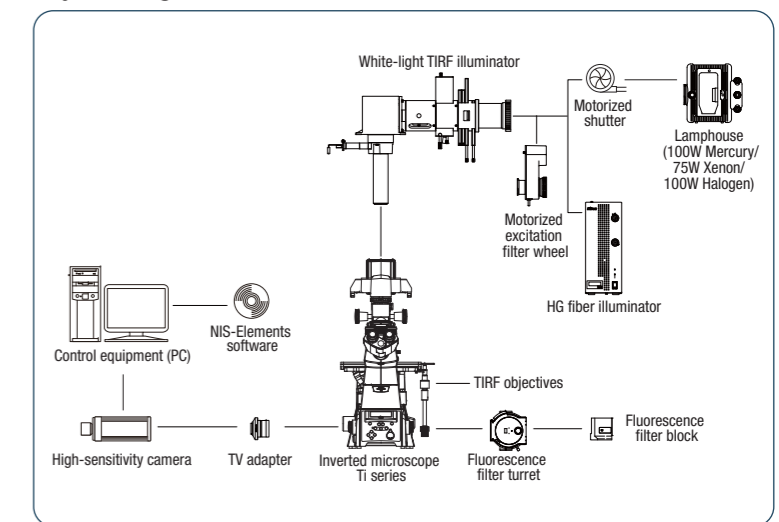
Epi-fl Illuminator Unit with White Light TIRF

This unit allows affordable TIRF observation using a white light such as a mercury lamp. White light TIRF, epi-fluorescence, oblique light fluorescence and surface reflection interference contrast techniques are all possible using a single mercury illumination. As mercury illumination has a broad wavelength range, the wavelength of the TIRF excitation can be selected by changing fluorescence filters.



Configuration with the Ti-E

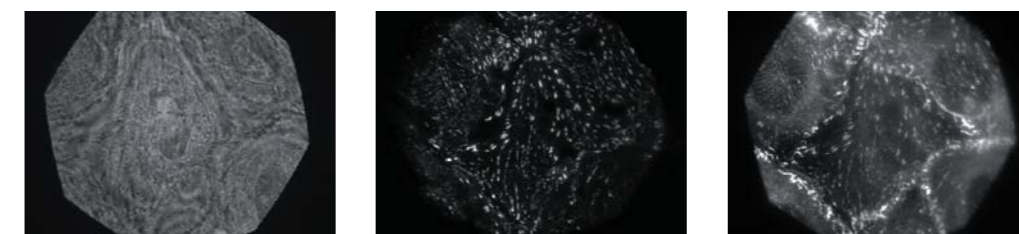
System diagram



Surface Reflection Interference Contrast (SRIC)

TIRF observations require specimens to be in contact with the coverslip, otherwise no TIRF image is obtained. The SRIC technique makes all parts of the cell in contact with glass coverslip appear black, allowing the user 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 more time can be spent on focusing. Switching from laser TIRF and white-light TIRF to SRIC is as simple as switching to the special filter cube.

Visualization of cell contact areas in MDCK cells



SRIC image

White-light TIRF image

Epi-fluorescence image

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

Multi-camera Imaging for FRET Analysis Using the Unique Stratum Structure

Ti-E Ti-U Ti-S

The Ti employs Nikon's original multi-level stratum structure. By using the "stage up position set," two illuminator units can be mounted simultaneously in addition to PFS*, which automatically maintains long-term focus. Because two fluorescence filter turrets can be layered, simultaneous image capture of FRET in two wavelengths is possible with the use of two cameras, one for each optical wavelength-path, separated by specific filter blocks.

*Can be attached to the Ti-E only

Simultaneous Mounting of Two Components

Two arbitrary components, including illuminators, can be mounted on Ti series microscopes. Depending on research purposes, different systems, such as the simultaneous use of laser tweezers and the photo activation unit, can be easily configured.

Moreover, the Ti-E allows simultaneous use of PFS in addition to two optical components.



Example: In addition to PFS, a photo activation module (upper tier) and a back port (lower tier) are attached.

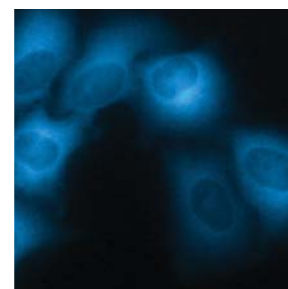
Simultaneous Two-wavelength Image Acquisition with Two Cameras

Use of an optional back port enables simultaneous image acquisition for different wavelengths.

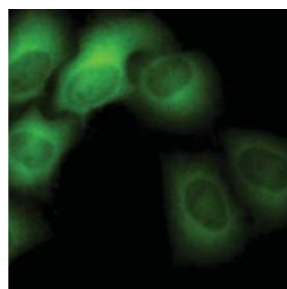
Because the position of the filter cubes in each of the two-tier motorized fluorescence filter turrets can be changed individually, simultaneous two-wavelength image acquisition for FRET analysis is possible with two cameras attached respectively to the back port and side port.

It is also possible to capture high-resolution images using the full field of view for each wavelength and detector. Because individual camera sensitivity can be adjusted when the intensity difference between wavelengths is considerable, high-sensitivity, high-contrast images can be captured.

ECFP image from YC3.60



cp173Venus image from YC3.60



When the intensity difference between CFP and YFP is considerable, individual camera sensitivity can be adjusted. Photos courtesy of Kenta Saito and Takeharu Nagai, Research Institute for Electronic Science, Hokkaido University

Back port unit



Simultaneous image acquisition for two wavelengths with two cameras is possible.

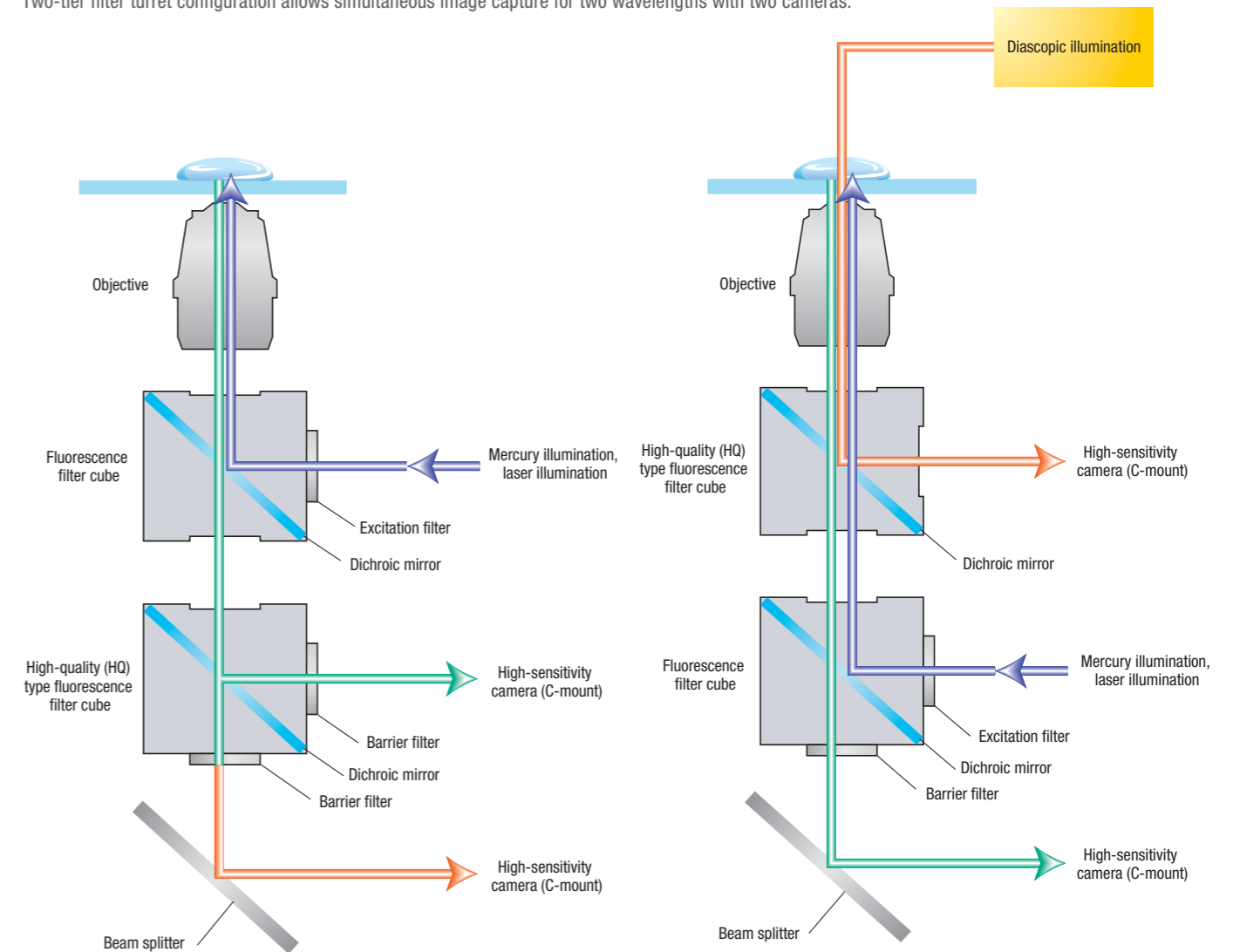
Motorized barrier filter wheel



Changing barrier filters allows image acquisition for multiple wavelengths.

Configuration Examples of Stratum Structure

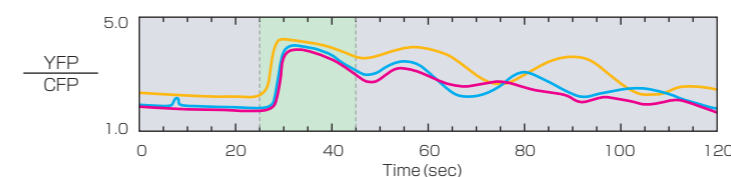
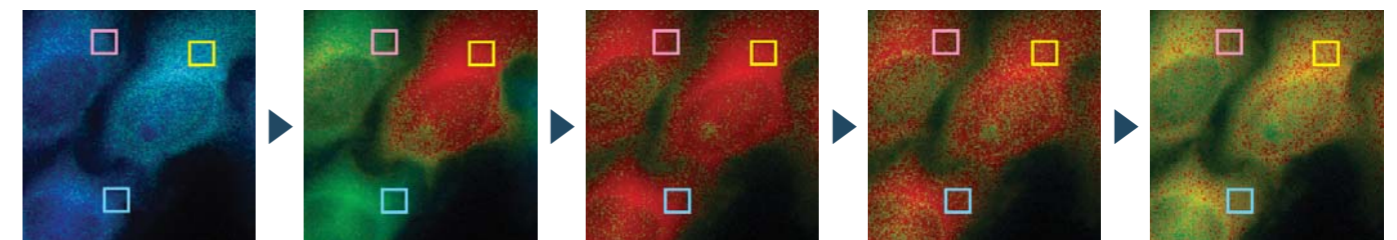
Two-tier filter turret configuration allows simultaneous image capture for two wavelengths with two cameras.



Simultaneous acquisition of two-color fluorescence images

Simultaneous acquisition of fluorescence and brightfield images

FRET Analysis of Intracellular Calcium Concentration ([Ca²⁺]_i)



Imaging histamine-evoked Ca²⁺ release in mammalian cells reported by a FRET-based Ca²⁺ indicator, YC3.60

Photos courtesy of Kenta Saito and Takeharu Nagai, Research Institute for Electronic Science, Hokkaido University

Ti-E configured with back port and two-tier fluorescence filter turrets

Specifications and equipment are subject to change without any notice or obligation on the part of the manufacturer. December 2008 © 2008 NIKON CORPORATION



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NIKON CORPORATION

6-3, Nishiohi 1-chome, Shinagawa-ku, Tokyo 140-8601, Japan
phone: +81-3-3773-8973 fax: +81-3-3773-8986
<http://www.nikon.com/products/instruments/>

NIKON INSTRUMENTS INC.

1300 Walt Whitman Road, Melville, N.Y. 11747-3064, U.S.A.
phone: +1-631-547-8500; +1-800-52-NIKON (within the U.S.A. only)
fax: +1-631-547-0306
<http://www.nikoninstruments.com/>

NIKON INSTRUMENTS EUROPE B.V.

Laan van Kronenburg 2, 1183 AS Amstelveen, The Netherlands
phone: +31-20-44-96-222 fax: +31-20-44-96-298
<http://www.nikoninstruments.eu/>

NIKON INSTRUMENTS (SHANGHAI) CO., LTD.

CHINA phone: +86-21-5836-0050 fax: +86-21-5836-0030
(Beijing branch) phone: +86-10-5869-2255 fax: +86-10-5869-2277
(Guangzhou branch) phone: +86-20-3882-0552 fax: +86-20-3882-0580

NIKON SINGAPORE PTE LTD

SINGAPORE phone: +65-6559-3618 fax: +65-6559-3668

NIKON MALAYSIA SDN. BHD.

MALAYSIA phone: +60-3-7809-3688 fax: +60-3-7809-3633

NIKON INSTRUMENTS KOREA CO., LTD.

KOREA phone: +82-2-2186-8410 fax: +82-2-555-4415

NIKON CANADA INC.

CANADA phone: +1-905-602-9676 fax: +1-905-602-9953

NIKON FRANCE S.A.S.

FRANCE phone: +33-1-4516-45-16 fax: +33-1-4516-45-55

NIKON GMBH

GERMANY phone: +49-211-941-42-20 fax: +49-211-941-43-22

NIKON INSTRUMENTS S.p.A.

ITALY phone: +39-055-300-96-01 fax: +39-055-30-09-93

NIKON AG

SWITZERLAND phone: +41-43-277-28-67 fax: +41-43-277-28-61

NIKON UK LTD.

UNITED KINGDOM phone: +44-208-247-1717 fax: +44-208-541-4584

NIKON GMBH AUSTRIA

AUSTRIA phone: +43-1-972-6111-0 fax: +43-1-972-6111-40

NIKON BELUX

BELGIUM phone: +32-2-705-56-65 fax: +32-2-726-66-45