OLYMPUS

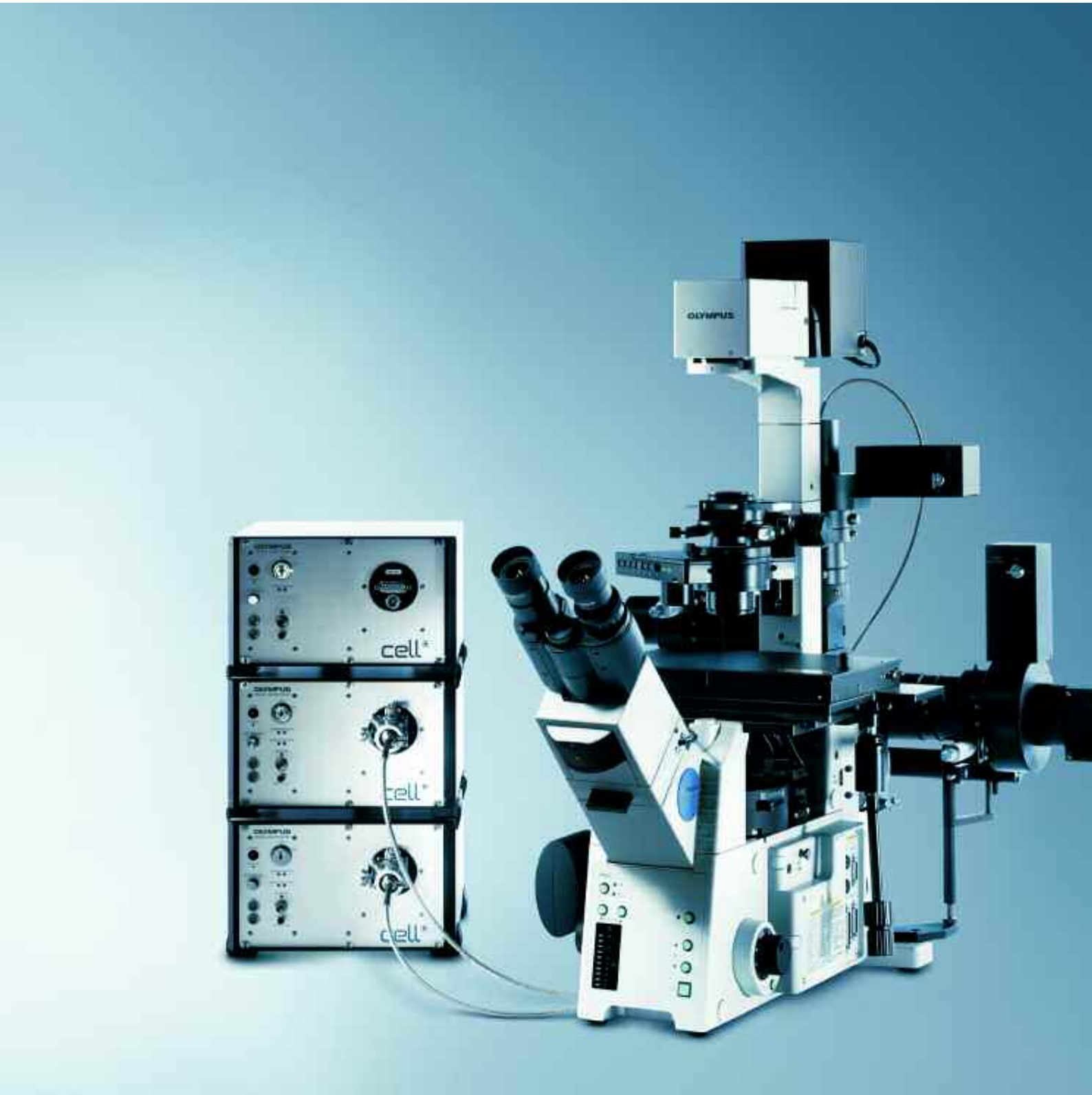
Your Vision, Our Future

TIRF Imaging Equipment

cell^tool

cell* Family Device

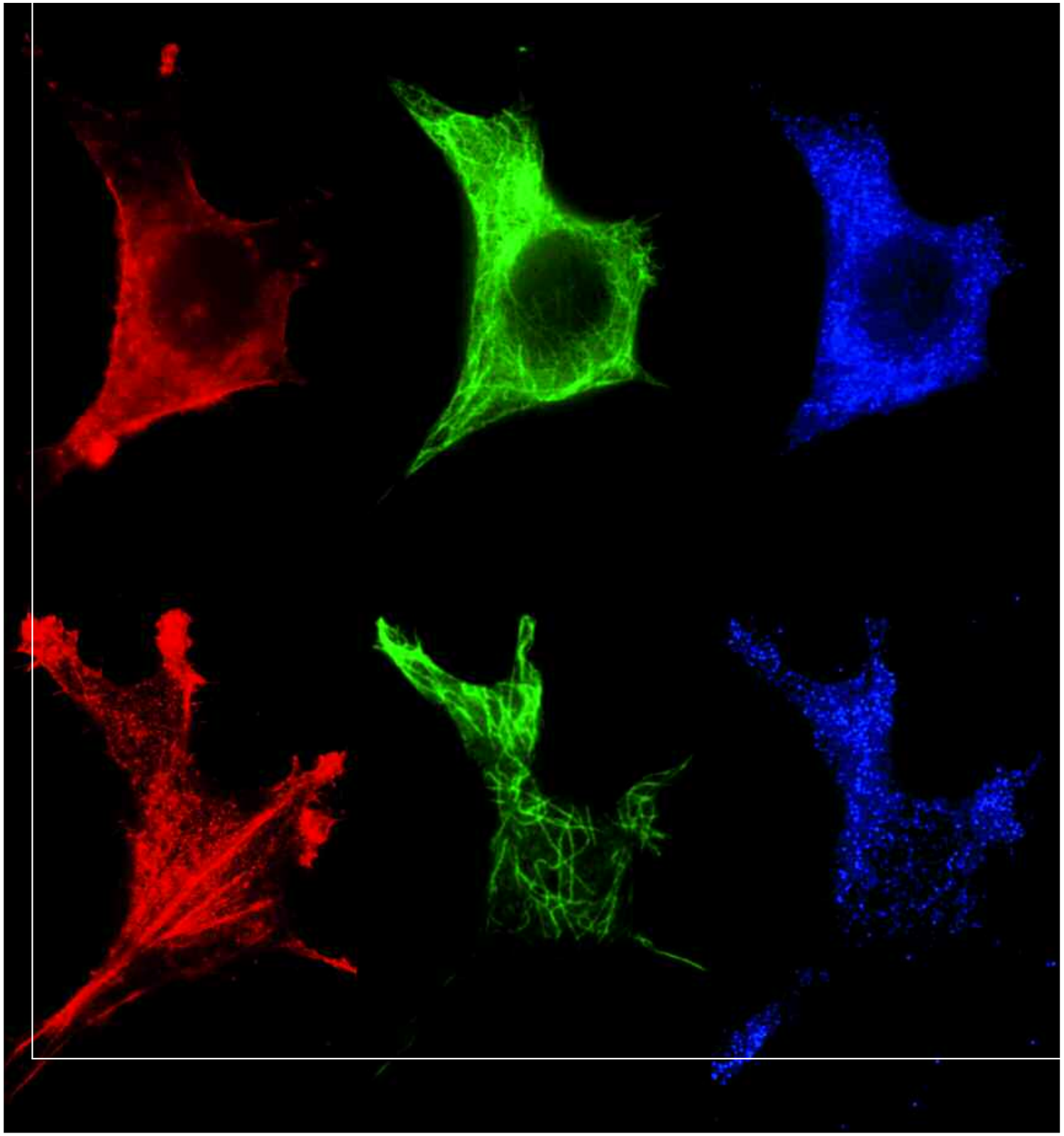
Advanced Solutions for Fluorescence Microscopy



TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY

Targeted imaging of the cell surface

Total internal reflection fluorescence microscopy (TIRFM) is an elegant optical technique for the selective visualisation of processes and structures of the cell membrane and pre-membrane space like vesicle release and transport, cell adhesion, secretion, membrane protein dynamics and distribution or receptor-ligand interactions. It can also be employed for molecular studies in cell-free preparations on the single molecule level. TIRF microscopy is based on the “evanescent” electro-magnetic field that is generated upon total reflection of the excitation light at the interface between coverslip and medium. It enables imaging of small structures at an unparalleled signal-to-background ratio and a z-resolution that is not even achieved by confocal microscopy.



The three colour channels of widefield (top) and TIRF (bottom) images; red (phalloidin): actin, green (FITC): tubulin, blue (Alexa405): SHC; courtesy of M. Faretta, Eur. Inst. Oncology (IEO-IFOM), Milan, Italy

OLYMPUS: THE PIONEER IN TIRF MICROSCOPY

Complete solutions with up to three lasers

Olympus has the longest-running experience in commercial solutions for TIRF microscopy. In 1998, we introduced the first TIRFM illuminator and an objective with a sufficiently high numerical aperture to enable objective-based TIRF illumination. With highly trained specialists and a modular approach to TIRFM imaging stations that encompasses all necessary components, we provide completely integrated systems. A series of TIRFM objectives, a range of lasers and several multi-port illuminators allow you to tailor your system for your specific applications.

Objective-based TIRFM – TIRFM Objectives and Illuminators 6–11

Objective-based TIRFM requires objectives with very high numerical aperture (NA) as well as a specially designed laser illumination system. The laser beam, after exciting the objective at a shallow angle, is totally reflected at the cover glass-to-specimen interface, where it generates a thin evanescent field that exclusively excites fluorophores in the vicinity. Olympus offers an entire family of objectives specifically dedicated to TIRFM with unmatched quality and performance. Researchers can now select from four different objectives with magnifications between 60x and 150x. A special illuminator directs the laser beam into the microscope to cause evanescent wave illumination. Olympus also offers a good-value evanescent wave imaging module based on a standard white-light source instead of relying on laser illumination.

cell^{M} and cell^{R} Imaging Stations 12–19

Multicolour TIRFM applications require the coupling of more than one laser to the microscope. Our modular illumination combiners provide an individual port for each laser to enable optimised optical alignment. Olympus offers a series of diode-pumped solid-state lasers specifically developed for TIRF microscopy. These include fast shutters, intensity control and laser safety equipment.

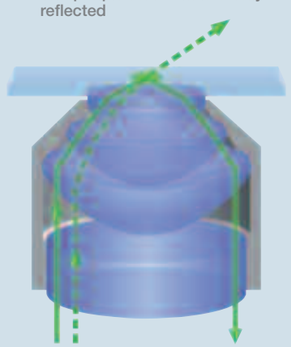
OBJECTIVE-BASED TIRFM – TIRFM OBJECTIVES AND ILLUMINATORS

The theoretical principle and the technical solution

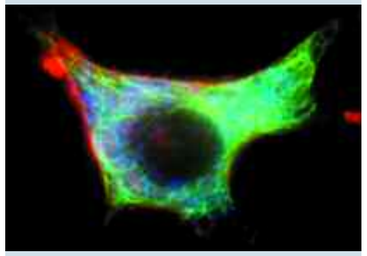
Spatial resolution, especially along the optical axis, is rather limited in conventional widefield microscopy. In fluorescence applications, additional limitations are caused by a relatively intense background signal originating from outside the focal plane. This out-of-focus blur results from the illumination cone of the focused light that penetrates the sample and excites all fluorochromes in its path. The objective captures the emitted fluorescence regardless of whether its origin is in focus or not (only the intensity decreases with the distance from the focal plane). The vastly superior z-resolution of TIRFM and the very high signal-to-background ratio are due to the total absence of any out-of-focus fluorescence. This is achieved because excitation only occurs in the plane of observation directly adjacent to the cover glass-to-sample interface.

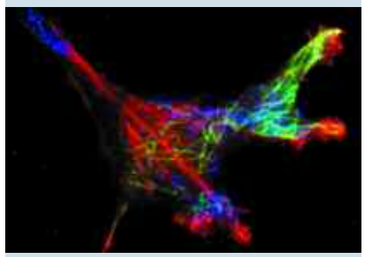
A Laser beam in the objective

The peripheral beam is totally reflected



Drawings based on graphics from http://micro.magnet.fsu.edu/primer; with kind permission of M. W. Davidson, Natl High Mag. Field Lab., FSU, Tallahassee, FL, USA





Widefield (top) and TIRF (bottom) image; blue (Alexa405): SHC, green (FITC): tubulin, red (phalloidin): actin; courtesy of M. Faretta, Eur. Inst. Oncology (IEO-IFOM), Milan, Italy)

THROUGH THE LENS: THE PRINCIPLE OF OBJECTIVE-BASED TIRFM

TIRFM is an ideal technique for the observation of dynamic processes associated with the cell membrane and the cell surface. Unlike standard widefield microscopy, excitation light does not travel through the specimen. Consequently, photodamage is noticeably reduced and living cells survive much longer. TIRFM is, for example, used to study the formation of focal adhesion, the release and receptor binding of neurotransmitters in the synapse, exocytosis and endocytosis, membrane dynamics and cell migration. The tremendous suppression of any out-of-focus fluorescence makes cellular structures visible that standard epi-illumination would never reveal and even enables imaging on the single-molecule level.

Olympus objective-based TIRFM systems also enable conventional reflected and transmitted widefield techniques, making the TIRF microscope a versatile platform. Illumination switches enable results comparison of TIRFM with widefield microscopy.

Total internal reflection

Several criteria must be met for the optical phenomenon of total internal reflection (TIR) to happen. Light has to travel through an interface between a medium of a high refractive index, for example glass, and reach the interface to a second medium of a lower refractive index, for example water. Then, if the angle of incidence is shallow enough, the light no longer penetrates into the aqueous phase, but is totally reflected back into the glass.

TIR is the physical principle behind fibre optics where the optical fibre guides light from the laser to the microscope. The “critical angle” at which TIR starts to occur is dependent on the refractive index difference between the two media and the light wavelength. The latter property, to give an everyday example, also causes the coloured twinkle of cut glass and the fire of diamonds.

In contrast to standard widefield illumination, incident photons in TIRFM do no travel through the specimen. Instead, an induced “evanescent field” caused by quantum effects associated with total internal reflection is employed for excitation of the fluorophores at the coverslip-to-water interface. This thin electromagnetic field fades exponentially with the distance from the surface and does not affect fluorophores in the bulk. The effective penetration depth of the evanescent field where most of the excitation occurs is usually adjustable between 100 and 700 nm for objective-based TIRFM. This short range is directly attributable to the superior z-resolution of this technique. It depends on wavelength, refractive index difference and incident angle.

Total reflection of peripheral laser beam

A Oil immersion objectives with very high numerical apertures are ideally suited for TIRF illumination. A laser beam travelling through the objective’s periphery exits the front lens at an angle that is limited by the numerical aperture. If the NA is larger than 1.38, the peripheral light enters the coverslip at a sufficiently shallow angle to be reflected totally at the interface to an aqueous medium and to induce the evanescent field that excites the fluorophores near the interface. The objective then quantitatively recaptures the beam and guides it back into the microscope.

GUIDING A LASER BEAM INTO THE TIRFM OBJECTIVE

In principle, TIRFM requires only a high NA objective and an illuminator that guides the light beam to the periphery of the objective’s back focal plane. To obtain the necessary narrow collimated beam of sufficient intensity for TIRFM, it is best to use laser illumination. The Olympus IX2-RFAEVA TIRFM illuminator is a perfect entry-level model to this cutting-edge technique.

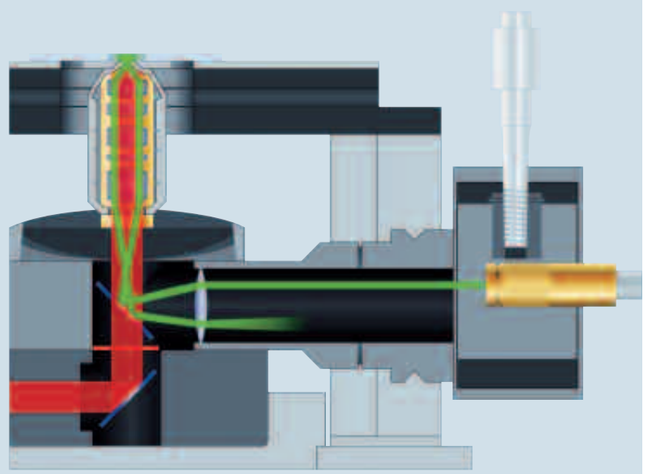
Off-axis illumination

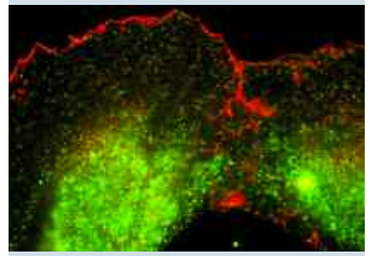
If a laser beam is focused on the periphery of the TIRFM objective’s back focal plane, evanescent wave illumination of the specimen surface is achieved. The more off-centre this beam is directed, the shallower the angle of exit at the front lens becomes. A micrometer screw in the illuminator changes the position of the laser beam in order to reach TIRF illumination and to adjust the depth of field to a certain degree. When in non-TIR mode, the laser beam simply penetrates the specimen – albeit vertically, only if positioned exactly on the optical axis – and causes widefield illumination.

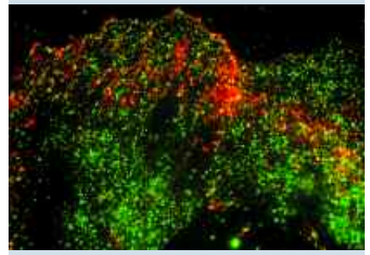
The Olympus TIRFM illuminator

B The IX2-RFAEVA is a basic stand-alone module for both laser TIRFM and standard widefield applications. The illuminator can be mounted on the reflected light port of Olympus’s inverted IX2 microscopes and coupled via light fibre to a variety of lasers. Additionally, it fits a standard fluorescence lamp housing with motorised filter wheel and shutter. This set-up allows the user to switch easily between the two illumination modes.

Laser light path in TIR mode



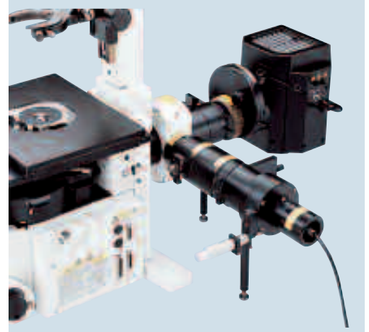




Widefield (top) and TIRF (bottom) image of FITC-labelled actin and DyeMer605-labelled EPS8; courtesy of M. Faretta, Eur. Inst. Oncology (IEO-IFOM), Milan, Italy

B IX2-RFAEVA

TIRFM illuminator with additional fluorescence lamp housing



A PLAPON 60xO TIRFM

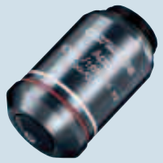
All-round TIRFM objective

B PLAPO 100xO TIRFM

High-magnification TIRFM objective

C APO 100xO HR

Extreme NA objective (NA 1.65)



D UAPO 150xO TIRFM

TIRFM objective with extraordinary magnification

TIRFM OBJECTIVES

Olympus was the first company to launch an objective with a numerical aperture that was significantly larger than the theoretical minimum of 1.38 required for objective-based TIRF microscopy. This was followed by an objective with the ultra high NA of 1.65, still the world record holder. Drawing on its long-standing advanced development experience, Olympus has just introduced the UIS2 series of objectives of unmatched quality and performance. This provides researchers with a choice of four different objectives specifically dedicated to TIRFM.

60x plan apochromat (NA 1.45)

A This new addition to the UIS2 series features UW multi-coating and unparalleled chromatic aberration correction. It combines very low autofluorescence with high transmission over a wide spectral range, reaching much further into the near infrared region then any other comparable objectives. It is designed for use with conventional immersion oil and coverslips, and contains a compensation collar for temperature and coverslip thickness.

100x plan apochromat (NA 1.45)

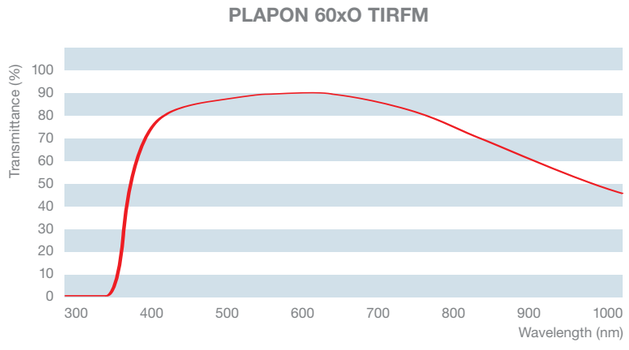
B The high magnification of this excellent plan apochromatic corrected objective for standard immersion oil guarantees high-resolution TIRF images.

100x apochromat (NA 1.65)

C This already legendary objective with the unsurpassed numerical aperture of 1.65 still holds the world record in resolution, depth of focus and light efficiency. It allows extreme TIR angles and adjustments over a wide angle range. The depth of excitation can thus be lowered to yield a z-section as narrow as 50 nm at short wavelengths. Special highly refractive coverslips and immersion oil have to be employed to match the extremely high NA.

150x universal apochromat (NA 1.45)

D With its extraordinarily high magnification, this is the only TIRFM objective of its kind on the market and was specially developed for single-molecule applications. It features a compensation collar for temperature and coverslip thickness.



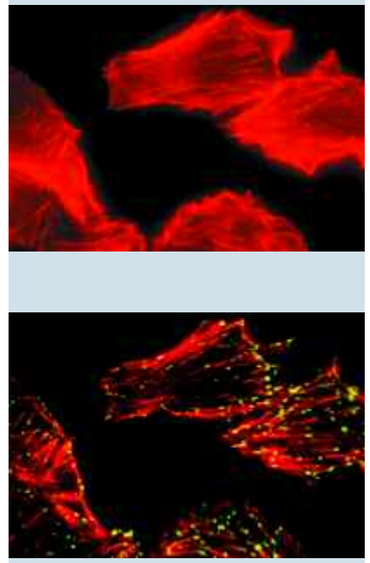
Belonging to the new UIS2 objectives, the PLAPON 60xO TIRFM transmittance extends into the near UV and far into the IR.

EVANESCENT WAVE IMAGING WITH WHITE-LIGHT ILLUMINATOR

With the development of the first TIRFM illumination system to be based on a standard white-light source instead of relying on laser illumination, Olympus has introduced a new basic approach in fluorescence microscopy. Evanescent wave imaging using a coventional light source is more favourably priced than using a laser – or several lasers in the case of multicolour applications. With a standard lamp house, the excitation wavelength can easily be switched by use of optical filters. This is a cost-effective add-on to standard widefield imaging systems for high-resolution cell surface imaging.

The optical principle of the IX2-ARCEVA white-light TIRFM system is rather simple. A crescent-shaped slit aperture is positioned off-centre in the excitation light path. A wedge prism directs the focused light of the arc burner onto the aperture to reduce the amount of blocked light and thus increases the illumination brightness. The crescent-shaped aperture is projected onto the periphery of the objective back aperture. Making use of the high numerical aperture of the PLAPON 60xO TIRFM objective, the light exits the objective front lens at the shallow angle required to obtain total internal reflection at the interface of the sample. The additional high-precision IX2-RFACEVA mirror unit cassette minimises any positional deviation of the laser beam at the objective pupil when switching the mirror unit in multi-line TIRFM experiments.

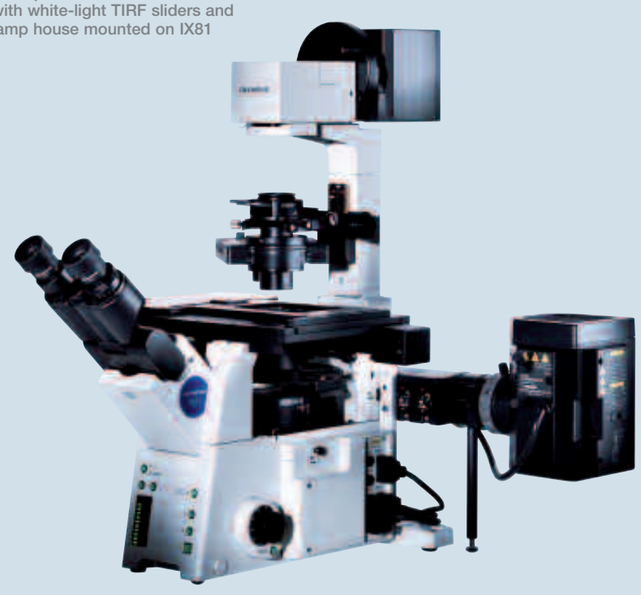
*Top: widefield image (red only); bottom: the dual-colour TIRFM image reveals that the actin fibres are spanned between the FAK-containing adhesion structures.
Courtesy of G. Pilarczyk, U. Joos, T. Biskup, O. Ernst, I. Westphal, Fraunhofer Institut für Biomedizinische Technik, Berlin; H. Kahl, Olympus Germany



Double labelling of the f-actin cytosceleton (red) and the focal adhesion kinase (FAK, green).*

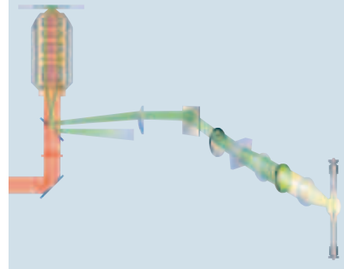
E IX2-ARCEVA

L-shape fluorescence illuminator with white-light TIRF sliders and lamp house mounted on IX81



E IX2-ARCEVA

Scheme of the illumination



OLYMPUS cell^{M} AND cell^{R} TIRFM IMAGING STATIONS

FULLY INTEGRATED TURNKEY SOLUTIONS

Olympus takes TIRF microscopy to a new level with complete solutions and upgrade modules for inverted microscopes. Always a step ahead, the four different Olympus TIRFM objectives with unprecedented optical features are setting a new standard. Up to three lasers can be directed into the microscope for TIRFM applications. This is possible using modular illumination combiners which also enable easy adjustment of the laser beam incident angle. A series of specially designed Olympus laser systems, with complete laser safety features, provides the optimal TIRF excitation source for the entire range of fluorophores. Additionally, an MT10 or MT20 illumination system can be added to permit a millisecond switch to standard widefield illumination. This enables the researcher to combine the observation of the cell surface with the imaging of the processes inside the cell in a single experiment.

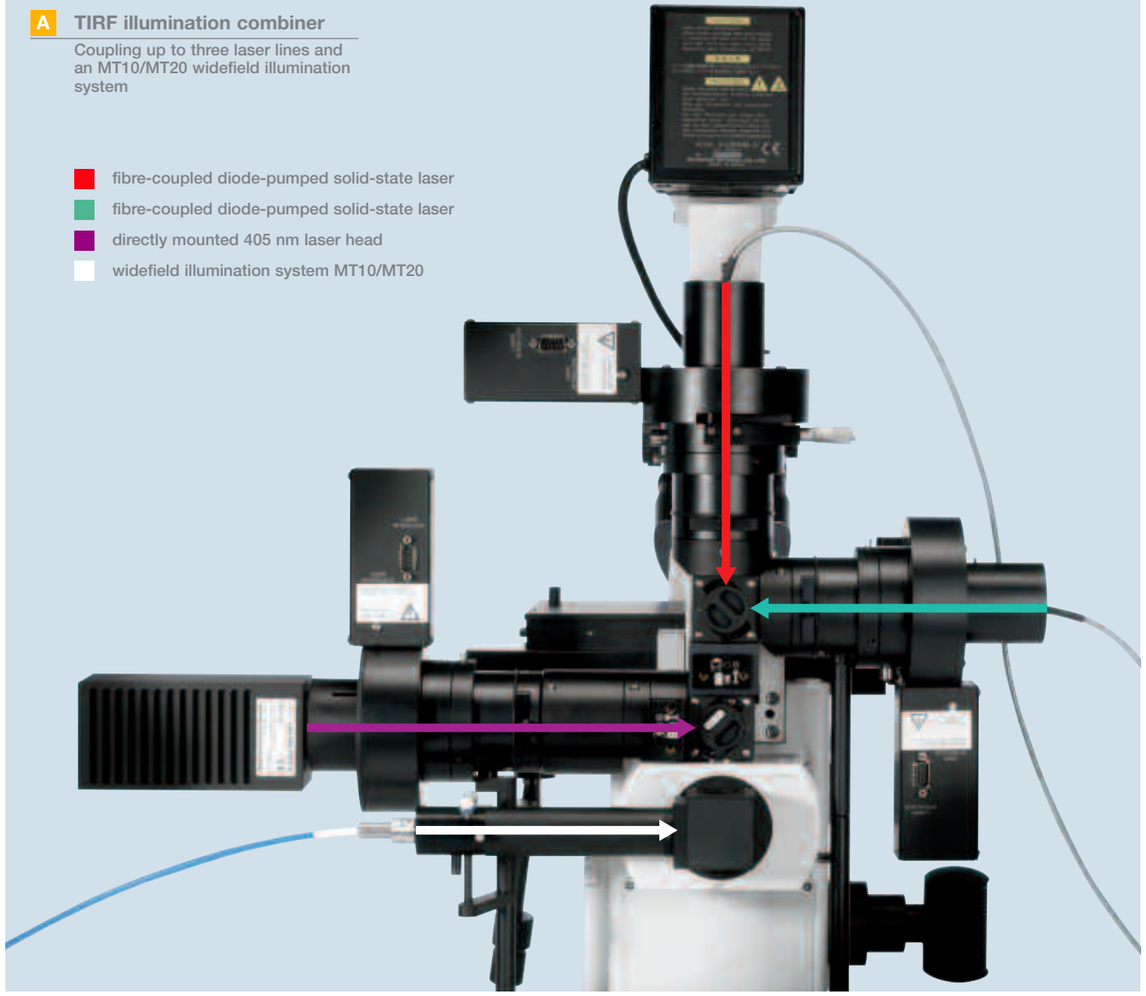
A functional system is much more than just an assembly of equipment. By integrating the TIRFM modules with full software support of the versatile cell^{M} and cell^{R} imaging stations, Olympus offers easy-to-use turnkey solutions for life science researchers to conveniently exploit the potential of the fascinating new technique of total internal reflection fluorescence microscopy.

MULTI-PORT ILLUMINATION COMBINERS

Multicolour TIRFM applications require a set-up of several lasers on the microscope. Where multi-band dichroic mirrors and emitters cannot be used, it is necessary to employ more than one filter cube to match different wavelengths. Since the orientation of the dichroic mirrors in the single filter cubes always differs slightly, the individual beam position of each laser must be adjusted to the corresponding filter cube. Otherwise the beam position will shift and TIR may not be achieved. Conventionally, multi-laser beams are combined externally and guided through a broadband fibre to the microscope, and this does not allow the individual adjustment of more than one beam. TIR may be achieved for one laser but not for a second, thus making multicolour TIRF imaging impossible. The Olympus multi-port illumination combiners are a convenient answer to avoid problems resulting from conventional laser coupling ensuring perfect TIRF illumination for all laser lines.

A TIRF illumination combiner

Coupling up to three laser lines and an MT10/MT20 widefield illumination system



One port for each laser in multi-line TIRFM

A Using a unique approach, the Olympus TIRFM illumination combiners provide individual ports for each laser to be coupled via a corresponding single-line fibre. Each laser beam position can thus be adjusted to match the corresponding filter cube. All laser ports feature a field stop to set the area of illumination. Wavelength-dependent variations of the TIR angle can also be compensated.

Modular design

The modular design of the combiners allows stepwise upgrading from a single laser solution to a system with up to three lasers as experimental demands increase. Laser combinations can be configured to individual requirements by choosing from a wide range of different lasers. Furthermore, all combiners for single, dual and triple-line set-ups contain an additional port for the widefield illumination systems MT10 or MT20 of the cell^{M} and cell^{R} imaging stations. Standard fluorescence and TIRFM applications can both be undertaken on the same system, even within the same experiment without any restrictions.

High-speed illumination switch

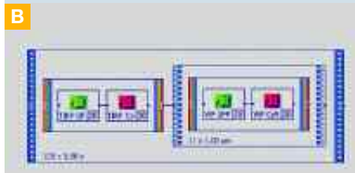
Very fast shutters guarantee a rapid illumination switch between all lasers and the widefield illumination systems to prevent loss in time resolution in high-speed experiments. Integration into the graphical Experiment Manager interface of the cell^{M} and cell^{R} imaging software makes setting up experiments easy and intuitive.

B The ability to switch to widefield illumination in a combined TIRFM experiment allows observation of the interplay between dynamic processes within the cell and in the cell membrane almost simultaneously – providing a fast focus switch device such as a piezoelectric objective mover is integrated. This complex hardware interplay is easily set up and executed using the cell^{M} and cell^{R} Experiment Manager.

Laser safety

C Special care has been taken of all laser safety issues for laser illumination. An interlock at the stage cover, a special ocular shutter and a safety shutter at each laser port prevent hazardous exposure to laser light during standard operation of the system (laser safety class 1).

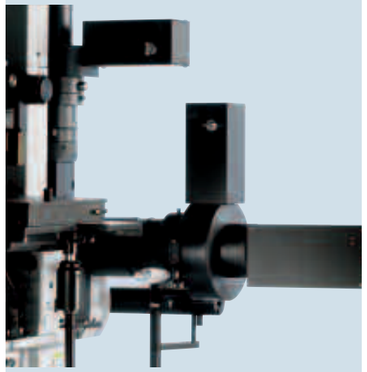
B

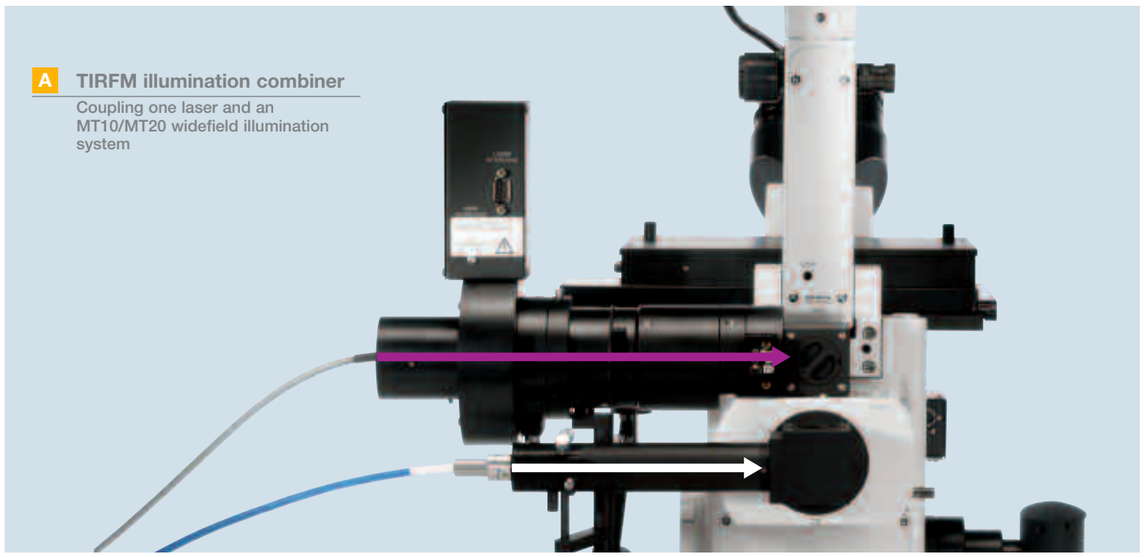


Screenshot of the Experiment Manager's intuitive graphical user interface. Combined widefield TIRFM time-lapse experiment.

C Laser safety shutters

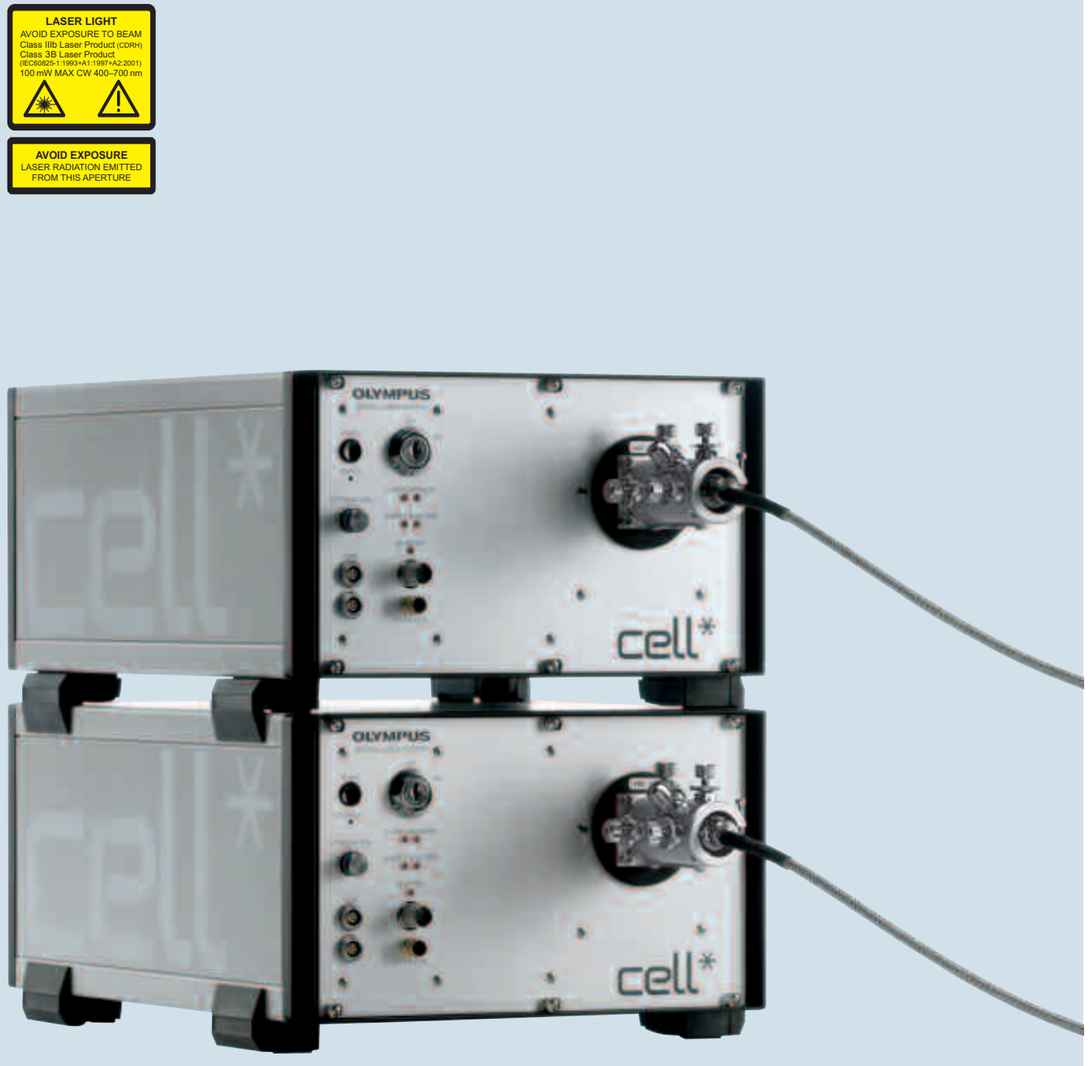
Each laser port features a safety shutter





TIRFM LASER SYSTEMS

A wide variety of different laser types with different specifications are on the market. However, not every laser can be used effectively for TIRFM. Certain specifications are important, for example line profile, mode and power. Olympus offers a series of laser systems that have been specifically developed to meet the requirements of TIRF microscopy.



Solid-state laser systems

All Olympus TIRFM laser systems are diode or diode-pumped solid-state lasers with a number of inherent advantages: they are small and rather silent, do not produce any ozone, have long lifetimes and a reduced energy consumption.

Fast shutters, fast wavelength switch

A fast integrated shutter controlled by the cell^{M} and cell^{R} imaging stations ensures optimised light management and precise synchronisation with image acquisition by the camera. Off-acquisition exposure of the specimen is thus avoided – always an important consideration in live cell studies. Also, for multi-line set-ups, near instantaneous wavelength switch (1 ms) can be achieved to provide utmost speed in multi-colour time-lapse experiments.

Laser intensity control

Olympus laser systems are equipped with 20-level attenuators that permit adjustment of the light intensity in reproducible steps between 0.2% and 100% according to the requirements of the experiment.

Laser safety shutter

To guarantee safe operation, an additional double-redundant laser safety shutter with an interlock connection to the TIRFM set-up is integrated.

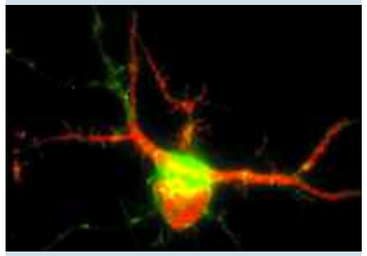
Fibre-coupled TIRFM laser systems

A The fibre-coupled Olympus TIRFM laser systems are of compact design and are available with four different wavelengths: 488 nm, 532 nm, 561 nm and 635 nm. They are suitable for a vast variety of fluorophores that emit in the green to near infrared region.

Directly mounted 405 nm laser head – a unique solution

B Light transmission of optical elements in the microscope light path becomes increasingly problematic at short wavelengths. Fibre-based illumination with a deep violet laser suffers from severe intensity losses.

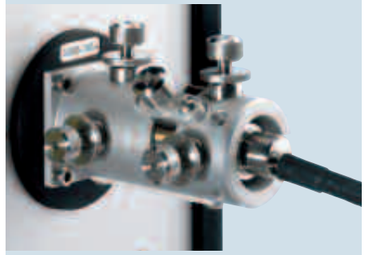
Olympus addresses the fibre transmission problem by providing a 405 nm diode laser system and mounting the small laser head directly on the modular TIRFM illumination combiners. In combination with the new PLAPON 60xO TIRFM objective that offers the UV-extended transmission range of the new UIS2 objectives series, this unique solution provides deep violet TIRFM illumination of unparalleled efficiency. Directly coupling the 405 nm laser is also a perfect solution for non-TIRFM applications that require high illuminative power, such as bleaching or photo-uncaging techniques.



EGFP-TrkB fusion transfected in hippocampal neurons; green colour coding: dendrite in widefield (490 nm), red colour coding: filopodia and spines in TIRF illumination (488 nm); courtesy of L. Khirug, Univ. of Helsinki, Finland

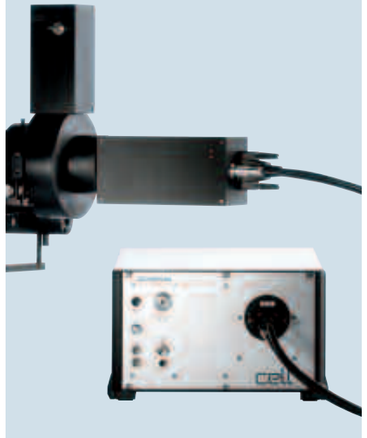
A Fibre coupling

Single-mode fibre delivery system for efficient and convenient beam coupling



B 405 nm laser

Control unit and directly coupled laser head



Widefield illumination systems

MT20_I
Illumination system
1

MT10_I/ODB
Illumination system
2

MT_FIB/2m
Single-quartz light fibre

ADD_FLASH/C2
UV flash illumination system

CTR_TRIGOUT_5V
TTL-to-5 V converter

MT_ARC/XE
Xenon arc burner, 150 W

MT_ARC/HG
Mercury-xenon arc burner, 150 W

cellM and cellR TIRFM illumination combiners

MT illumination coupling for IX2_MPITIR* illumination combiners

MT illumination coupling with field stop slider for IX2_MPITIR* illumination combiners

Two-port MT illumination coupling for IX2_MPITIR* illumination combiners

Two-port MT illumination coupling with field stop slider for IX2_MPITIR* illumination combiners

IX2_MPITIRTL
Triple-line TIRFM illumination combiner

IX2_MPITIRDL
Dual-line TIRFM illumination combiner

IX2_MPITIRSL
Single-line TIRFM illumination combiner

Stand-alone TIRFM illumination modules

IX2-SHA
Motorised shutter
9

U-FWR
Motorised reflected light filter wheel
8

U-LH100HGAPO
100 W mercury apo lamp housing (others possible)

Power supply unit

IX2-ARCEVA
Excitation filter slider
Wedge prism slider
Off-centre slit slider

FV5-LAAR
Argon ion laser
A

IX2-RFAL
L-shaped fluorescence illuminator

IX2-RFAEVA-2
TIRFM illumination combiner

FV5-FUR *
FVL-FS450-640*
LASFIB_PS*.

10

TIRFM laser systems

LAS/405/25DC
Directly coupled 405 nm, 25 mW laser head

LAS/405/25DC
Control unit

A

LASFIB_PS*
Laser fibre

LAS/488/20
488 nm, 20 mW laser

LAS/488/30
488 nm, 30 mW laser

LAS/532/20
532 nm, 20 mW laser

LAS/532/50
532 nm, 50 mW laser

LAS/561/20
561 nm, 20 mW laser

LAS/635/25
635 nm, 25 mW laser

System control

*** CTR_M_DIG**
Digital I/O interface required for cellM

COMP_CELL/E
cellM / cellR Imaging PC
7

1 3 4 5

CTR_R
cellR Real-time controller board

6

SW_CELLR
cellR imaging software

CTR_M/ODB
cellM System coordinator board

2 3 4 5

SW_CELLM and **SW_CELLM_PERICON**
cellR imaging software

Cameras and observation equipment

CCD and EMCCD cameras: different models of different manufacturers

3
Camera trigger connection

FireWire or camera link board

4

U_FFWO
Fast motorised observation filter wheel

Dual-view micro-imager
Beam splitting device

Stages

IX2-SFR
Cross stage with flexible handle

Motorised stage & controller: different models of different manufacturers

5

Mirror turrets

4

IX2_FRFACA
Fast motorised mirror unit turret

IIX2-RFACA
Motorised mirror unit turret

IX2-RFAC
Mirror unit turret

IX2_RFACEVA
High-precision mirror unit turret

ADD_PFC or
ADD_NPPFC/IX81/71
PIFOC objective or PIFOC nosepiece & controller
6

IX81
Motorised inverted microscope or
IX71
Inverted microscope

10

IX2-UCB2
Microscope controller for IX81
7
8
9

Mechanical connections
Communication lines

1 – 9 Communication connections
10 Coupling for TIRFM illumination combiners

* Please consult your local Olympus supplier

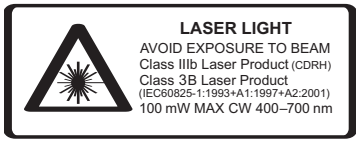
Specifications

Multi-port illumination combiners
Available for IX2 microscopes
Modular design for the combination of up to three lasers and an MT10/MT20 illumination system
Individual ports for each laser fibre guarantee optimised beam alignment
Field stop for each laser port to reduce the field of illumination
Fast shutters (1 ms) allow fast switch between all lasers and MT10/MT20
Easy experiment set-up by integration into the Experiment Manager of cell^M and cell^R
Incorporation of all safety features required by laser safety regulations
Class 1 laser product (normal operation), class 3B (maintenance mode), IEC60825:1

TIRFM laser systems
Five wavelengths available: 405 nm (50 mW), 488 nm (20 or 30 mW), 532 nm (20 or 50 mW), 561 nm (20 mW) and 635 nm (25 mW)
Polarisation-maintaining single-mode fibre (except for 405 nm laser)
Directly mounted 405 nm laser head
20 levels of attenuation between about 0.2% and 100%
Fast shutter with 1 ms switching time
Diode or diode-pumped solid-state lasers
Expected lifetime of up to 10,000 h
Low operating noise
Compact housing
Control via hand switch, and cell^M/cell^R or external TTL trigger
Full compliance with laser safety regulations
Class 3B laser product (IEC60825:1)

“White-light TIRFM” illuminator
Inverted Olympus IX71 microscope
IX2-ARCEVA illuminator unit with slit aperture slider, wedge prism slider, excitation filter slider and IX2-RFAL L-shaped fluorescence illuminator
High-precision IX2-RFACEVA fluorescence turret cassette (optional)
100 W mercury or 75 W xenon lamp house
PLAPON 60xO TIRFM objective, NA 1.45

CE



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