OLYMPUS®

TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPE SYSTEM

TIRFM

Ultra-sensitive fluorescence microscopy

Two High N.A. Objective Lenses Designed for Evanescent Wave Illumination

100X N.A.: 1.65

- Exceptional fluorescence brightness due to high N.A.
- Maximum resolution
- Very shallow evanescent wave penetration possible due to high angle of incidence
- Special immersion oil and coverslips required

60X N.A.: 1.45

- Lower cost and ease of use
- Compatible with High Contrast or High Resolution DIC prisms
- 96X field of view possible with the IX71 or IX81's built-in 1.6X magnification changer
- Standard immersion oil and coverslips for convenience

Laser safety is Important

The laser is introduced directly into the microscope via a single mode optical fiber. Safety interlocks prevent laser light from exiting the microscope or being viewed through the eyepieces.

Introducing multiple lasers

Up to three lasers can be introduced simultaneously with the optional laser combiner unit.

Supported Lasers:

• Argon 488nm • HeNe-R 633nm • HeNe-G 543nm

Alignment-free laser coupling with simple adjustments

After initial alignment of the single mode fiber to the laser head and TIRFM illuminator, a simple micrometer adjustment is all that is required to move the laser position between TIRFM and widefield illumination.

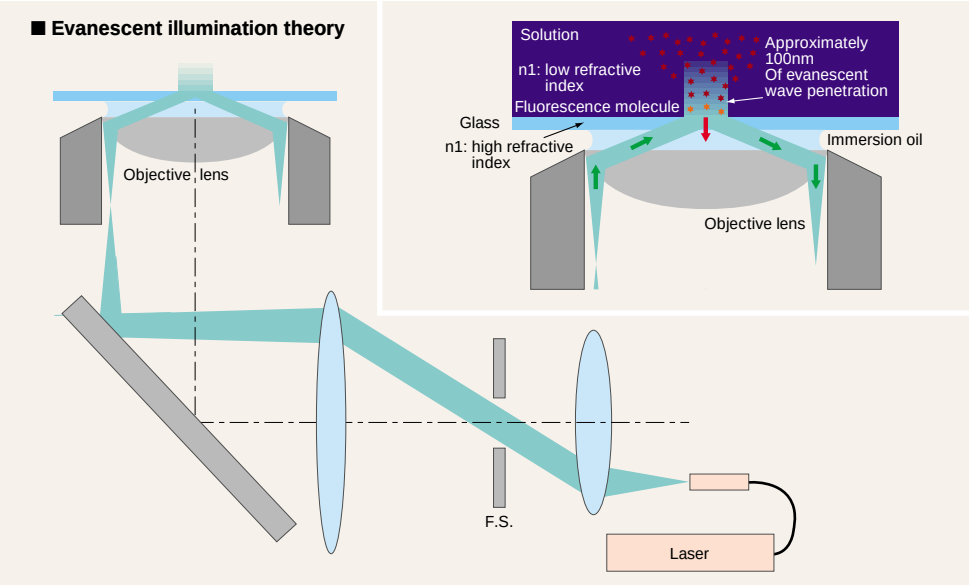
Arc Lamp fluorescence observation is available

TIRFM illuminator also accepts a standard arc lamp illuminator, allowing both evanescent wave and standard widefield fluorescence illumination.

Total Internal Reflection Fluorescence Microscopy

When light passes from a medium of higher refractive index to a medium of lower refractive index, the light is refracted (bent) away from the normal at the boundary. At higher angles of incidence there reaches a point where the light will not transmit into the lower refractive index medium and will instead be totally reflected. This phenomenon is known as Total Internal Reflection. We achieve this condition in the microscope with a laser illuminating from the periphery of the back focal plane of an objective lens. The immersion oil and coverslip are the higher refractive index media while the cell and aqueous media are of a lower refractive index. Even though the light is totally reflected according to traditional optical theory, a small amount of energy does pass through this interface into the lower refractive index media in the form of an evanescent wave. The energy wave is termed evanescent due to its penetration of only a few tens of nanometers from the boundary. The penetration depth for a TIRFM evanescent wave is typically 50-100nm. Only the fluorescence molecules within the shallow penetration of the evanescent wave will be visible, allowing very selective, high contrast fluorescence imaging. TIRFM is especially useful in studies of cellular membrane function and single molecule events without the interference of background fluorescence from other areas in the cell body. This results in high contrast images with a signal to noise ratio greatly improved over widefield and even confocal imaging.

Reference: Tokunaga M, et al: Biochem Biophys Res Commun (1997) 235: 47-53



The visible difference between widefield fluorescence and evanescent wave illumination

NeuroGlia cell: Core-PI, actin-Alexa Fluor546
Neuroglial cell with Alexa Fluor 546 labeled actin and Propidium Iodide labeled cytoplasm. On the left is the neuroglial cell under widefield fluorescence illumination. Note that the inner cytoplasmic core can be seen as a very bright central region of the image. On the right is the same cell under evanescent wave illumination. Note that only the actin filaments directly in contact with the coverslip are visible while the rest of the cell, including the cytoplasmic core, exhibit no fluorescence excitation.

Comparison between widefield fluorescence excitation and evanescent wave fluorescence excitation.

Widefield fluorescence observation with mercury arc lamp excitation

Total Internal Reflection Fluorescence observation with evanescent wave excitation

Super high N.A. objective lens Apo100XOHR (N.A.: 1.65) Molecule of the Tetramethyl rhodamine

Molecule of the Tetramethyl rhodamine
An aqueous solution of Tetramethyl rhodamine is put on a glass coverslip and observed with evanescent wave fluorescence excitation using the Apo100xOHR (N.A.: 1.65) objective to collect the following image. Note that the signal is very bright while the background is very dark resulting in a high contrast, high signal to noise ratio image allowing the observation of single molecule fluorescence.

Effect of the Apo100XOHR(N.A.: 1.65):
Single molecule fluorescence image of the Tetramethyl rhodamine

OLYMPUS®
www.olympus.com

OLYMPUS OPTICAL CO.,LTD.
San-Ei building, 22-2, Nishi Shinjuku 1-chome, Shinjuku-ku, Tokyo, Japan
OLYMPUS OPTICAL CO.(EUROPA) GMBH.
Postfach 10 49 08, 20034, Hamburg, Germany
OLYMPUS AMERICA INC.
2 Corporate Center Drive, Melville, NY 11747-3157, U.S.A.
OLYMPUS SINGAPORE PTE LTD.
491B River Valley Road, #12-01/04 Valley Point Office Tower, Singapore 248373
OLYMPUS OPTICAL CO.(U.K.) LTD.
2-8 Honduras Street, London EC1Y OTX, United Kingdom.
OLYMPUS AUSTRALIA PTY. LTD.
31 Gilby Road, Mt. Waverley, VIC 3149, Melbourne, Australia.
OLYMPUS LATIN AMERICA, INC.
6100 Blue Lagoon Drive, Suite 390 Miami, FL 33126-2087, U.S.A.

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