



Leica AM TIRF MC

Visualize Life's Secrets with True MultiColor TIRF

Living up to Life

Leica
MICROSYSTEMS

Flexibility Reproducibility Dynamics Integration

TIRF (Total Internal Reflection Fluorescence) microscopy is the ideal technique for the study of cellular membranes and their environment. TIRF uses the evanescent field generated by total reflection to excite fluorophores. Instead of illuminating an entire specimen with excitation light, as in widefield fluorescence microscopy, the evanescent field only penetrates the specimen to a depth of 70–300 nm. Fluorochromes at deeper levels of the specimen are not excited. This method enables a substantially improved signal-to-noise ratio, providing the highest quality results. The integration of four AOTF-controlled laser lines into the Leica TIRF system allows fast wavelength switching and a high image recording rate with multiple fluorophores.

Multiple contrast techniques

No more arduous searching for TIRF angles. The Leica AM TIRF MC system features an integrated sensor that does the searching for you and enables the evanescent field to be reproducibly introduced at a defined depth of the specimen. TIRF, widefield fluorescence, and transmitted light contrast methods can be used sequentially – simply by the push of a button.

True MultiColor Laser TIRF

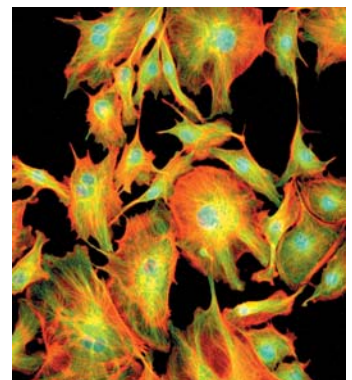
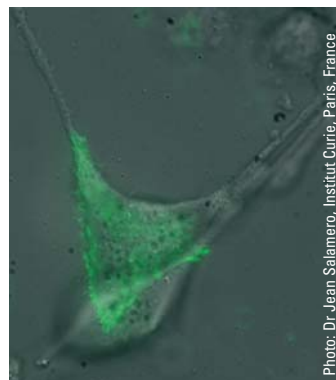
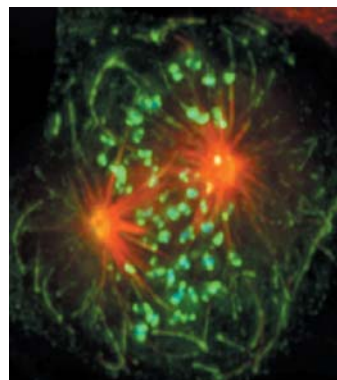
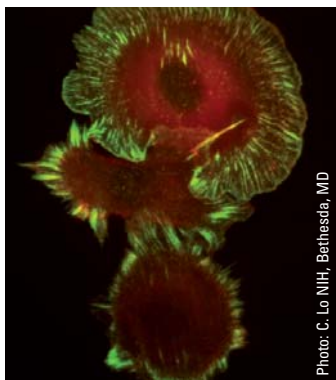
The new MultiColor TIRF from Leica Microsystems is an all-in-one system offering four integrated solid state lasers for excitation of fluorophores in all important wavelengths. The extremely short switching times, the automatically constant TIRF penetration depth when switching from one wavelength to another and the extremely high and synchronized image recording rate open up completely new horizons for researching dynamic processes in living cells.

The elegant solution for fluorescence microscopy

Leica's TIRF system features a dynamic scanner that can be used to precisely position the laser beam and determine the exact penetration depth of the evanescent field. The powerful Leica AF6000 fluorescence software offers full control of the TIRF system. All microscope functions and many analysis tools are included.

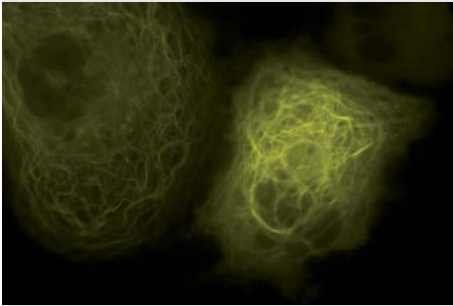
Superior imaging with superb optics

The Leica TIRF system combines superior imaging performance, high sensitivity and an optimum signal-to-noise ratio to ensure accurate results for all examinations of close-to-membrane structures.

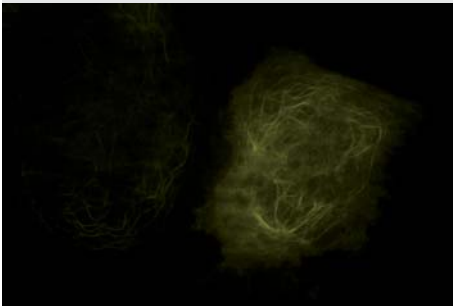




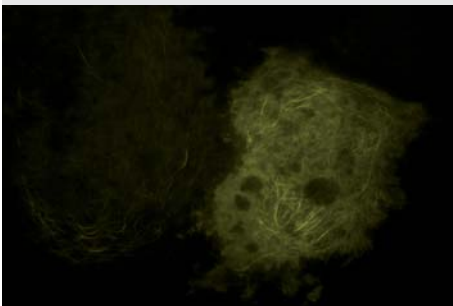
Leica Design by Christophe Apothéoz



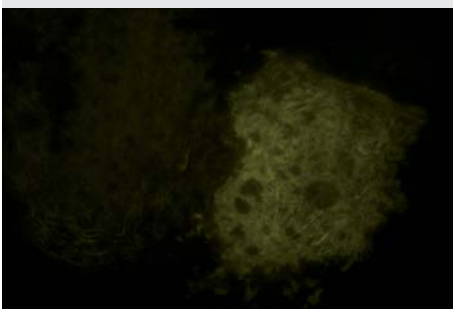
Tubulin YFP Widefield



Tubulin YFP TIRF 120 nm



Tubulin YFP TIRF 90 nm



Tubulin YFP TIRF 70 nm

Courtesy of: Prof. Dr. R. Jacob,
University Marburg, Department of Clinical
Cytobiology and Cytopathology,
Marburg, Germany

The ideal configuration for your application

TIRF microscopy is an excellent method for examining processes in live cells. Leica offers the best configuration for specific applications. Whether for vesicle transport, interactions between molecules, FRET (Fluorescence Resonance Energy Transfer), membrane research or single molecule examination, Leica has the right solution for every application – tailored precisely to your needs.

Your Personal TIRF System

Fluorescence microscopy of the future

The Leica TIRF module consists of a Leica HCX Plan Apo objective with a 1.46 numerical aperture, a high-performance 3D scanner, and a laser unit with four maintenance-free diode lasers. This module easily adapts to Leica's inverted microscopes and systems to suit user requirements and can be customized through a wide range of accessories including stages and objectives. For epifluorescence excitation, Leica offers the powerful EL6000 external light source.

Superlative performance for dynamic living cell research

Providing the wavelengths 405 nm, 488 nm, 561 nm and 635 nm for multicolor applications, the Leica AM TIRF MC offers maximum flexibility for TIRF applications with multiple fluorophores. The individual laser lines are coupled and controlled quickly and precisely via AOTF (Acousto-Optical Tunable Filters), achieving a switching time of only 1 ms. Also, the position of the laser is automatically adjusted when the wavelength is changed to ensure that the selected TIRF penetration depth remains constant. A



newly designed SyncBoard controls fast image recording of up to 30 frames per second. This high temporal resolution for exciting several fluorophores combined with the spatial TIRF resolution are key prerequisites for visualizing transport processes in cell membranes or interactions of proteins in real time.

Leica's new fluorescence axis and TIRF objective

To meet the exacting requirements of TIRF users, Leica has further improved the fluorescence axis of the microscope, while the high numerical aperture of 1.46 enables a specimen penetration depth of 70 to 300 nm to be achieved. Like the fluorescence axis, Leica's TIRF objective features excellent apochromatic correction and combines enhanced imaging performance with high sensitivity.

Reproducibility and improved technology

Through the coupling of the laser unit and TIRF module the light is precisely directed onto a scanning mirror. The positioning of this mirror, through the easy-to-use operating software, determines the penetration depth and direction of the evanescent field within the specimen. This software control ensures that these parameters can be precisely reproduced at any time.

Leica has not only eliminated the well-known TIRF operating difficulties, but has also made fundamental improvements to TIRF fluorescence technology. The fluorescence axis implements specifically corrected wavefronts for the TIRF method. And, for the first time, Leica offers a system without color aberration.

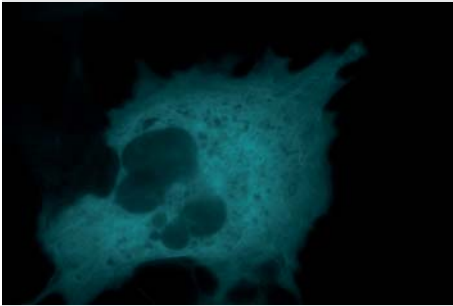
With TIRF microscopy techniques, cells are not directly irradiated with laser light. The method of exciting fluorochromes via an evanescent field is particularly gentle and allows examination of live cell functions to be conducted over longer periods of time.



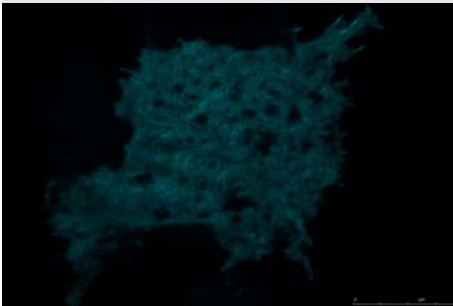
“Leica Microsystems offers superior optical quality with user-friendly control of practical techniques in quantitative biology and biomedicine. The revolutionary total internal reflection objective design gives fully automated TIRF control, precise penetration depth, and excitation light corrections without ambiguous optical handling.”

Prof. Gregory Harms

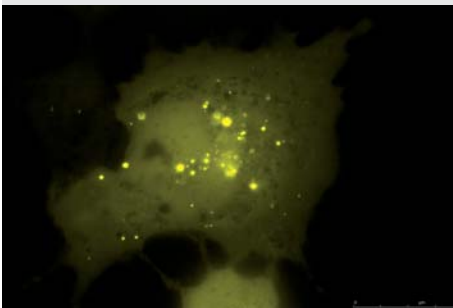
University Würzburg, Rudolf-Virchow-Zentrum,
DFG Research Center for Experimental
Biomedicine, Würzburg, Germany



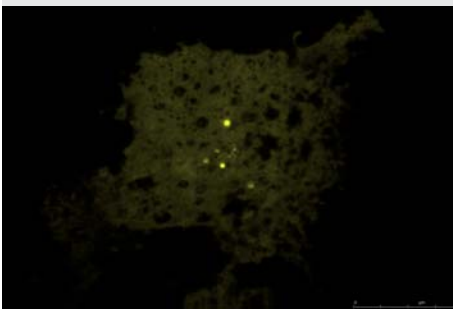
Tubulin CFP Widefield



Tubulin CFP TIRF



Gal3 YFP Widefield



Gal3 YFP TIRF

Courtesy of: Prof. Dr. R. Jacob,
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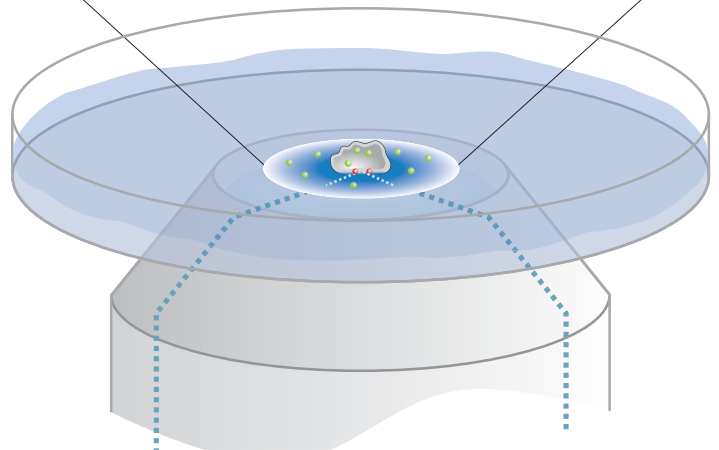
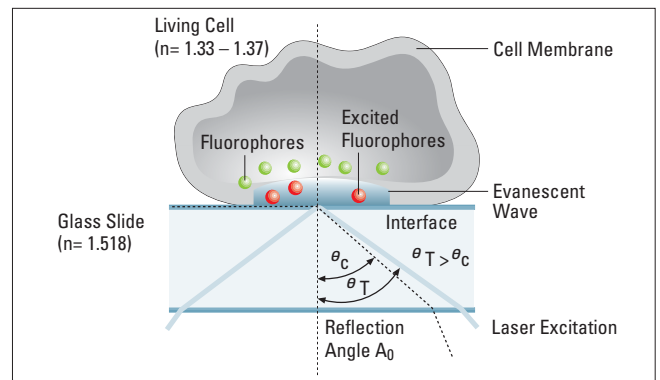
TIRF scanner and MultiColor laser control

The unique scanner-sensor technology in the Leica AM TIRF MC allows automatic setting of the TIRF angle and the adjustment of the required TIRF penetration depth to the various wavelengths. Thanks to the automatic AOTF control, it is possible to switch quickly between the laser lines at a constant penetration depth. This explains the superior performance, reproducibility and ultra high speed of the Leica AM TIRF MC system, which has the added benefit of being easy to use.

TIRF Membrane Research

View dynamic structures and events

Research of molecular interactions at cell membranes, of proteins and receptors involved in transport mechanisms, requires fast switching of the excitation wavelength. With Leica Multi-Color TIRF, wavelengths are switched in a few ms without alteration of the penetration depth. The system's fast control and high image recording rate of up to 30 frames per second is also an advantage for FRET analysis. Using the new FRET wizard, experiments can be conducted with ease and fully evaluated automatically. If alternating wavelengths are required in the widefield light path of the TIRF module, Leica's fast filter wheels can be used. Switching times of approximately 30–50 ms are easily achievable.



TIRF Single Molecule

Explore the world of single molecules

With the Leica TIRF system it is easy to image single molecules close to the cell membrane. Typically, a powerful objective's resolving power in the z axis is physically limited to approximately 400–500 nm by the wavelength of the light used. This physical restriction is eliminated by the high accuracy of the TIRF excitation of ~ 100 nm in the z axis. Even interactions of single molecules, the kinetics or co-localization of molecules can be visualized and measured with Leica's highly sensitive TIRF system. The constant penetration depth when switching the laser lines is a key prerequisite for using multiple fluorophores. Due to the excellent signal-to-noise ratio, the entire dynamic range of this high-performance camera can be exploited. Even with weak fluorescent signals, the EMCCD camera provides highest resolution images while ensuring the uncompromising sensitivity essential for the gentle treatment of light-sensitive specimens.

TIRF Vesicle Transport

High resolution and minimum light stress

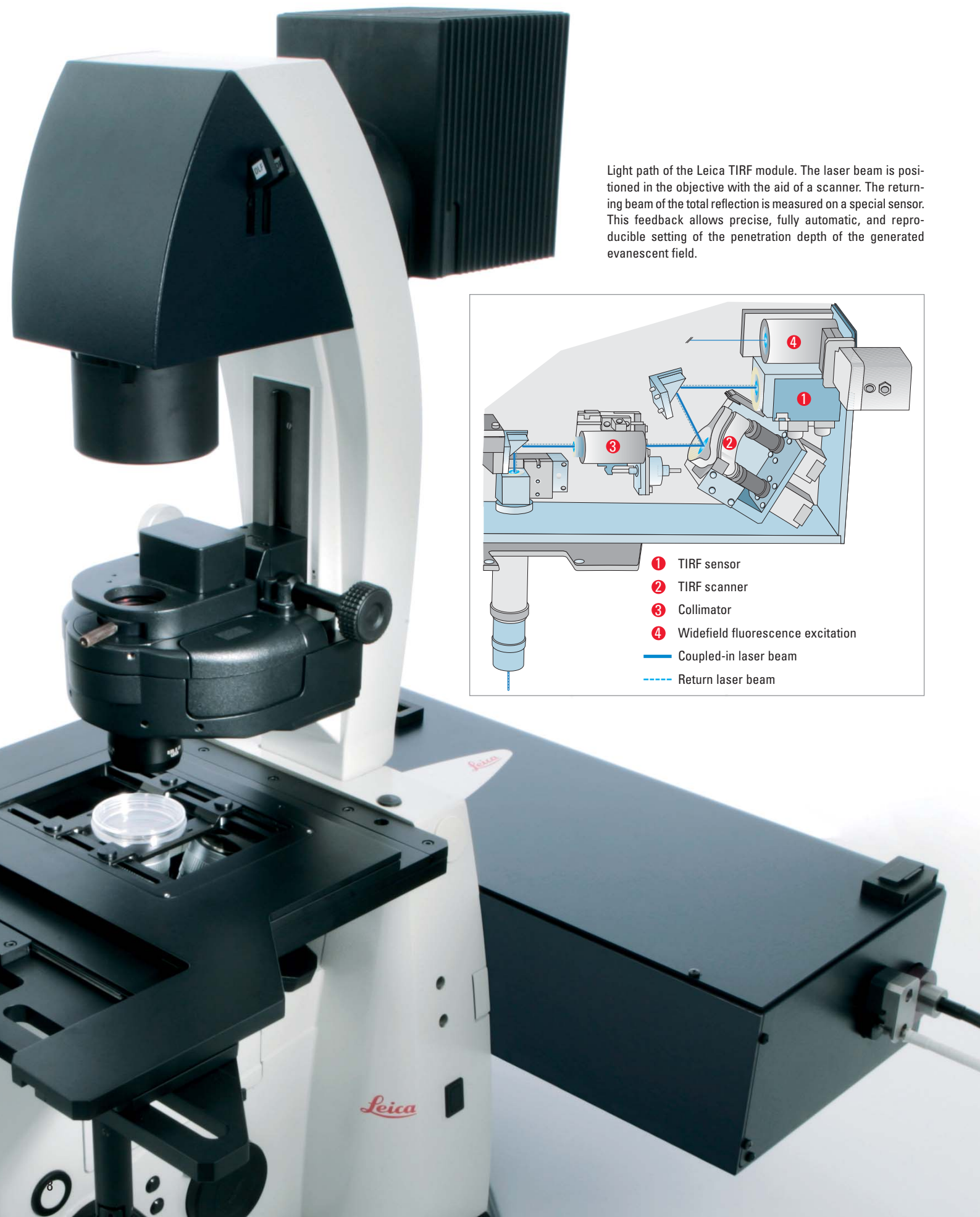
Due to strong background fluorescence, vesicle transport studies require an optimum signal-to-noise ratio. At the same time, stability for experiments lasting several hours or days is a must. Vesicle transport experiments are made simple with the Leica AM TIRF MC. With the addition of Leica's special climate chamber to the TIRF system, temperature and CO₂ levels remain constant. The Leica AF6000 multidimensional fluorescence workstation with intelligent application software and hardware provides accurate results. For brilliant photographs of vesicle transport investigations, a high-sensitive and high-speed EMCCD camera can be used.



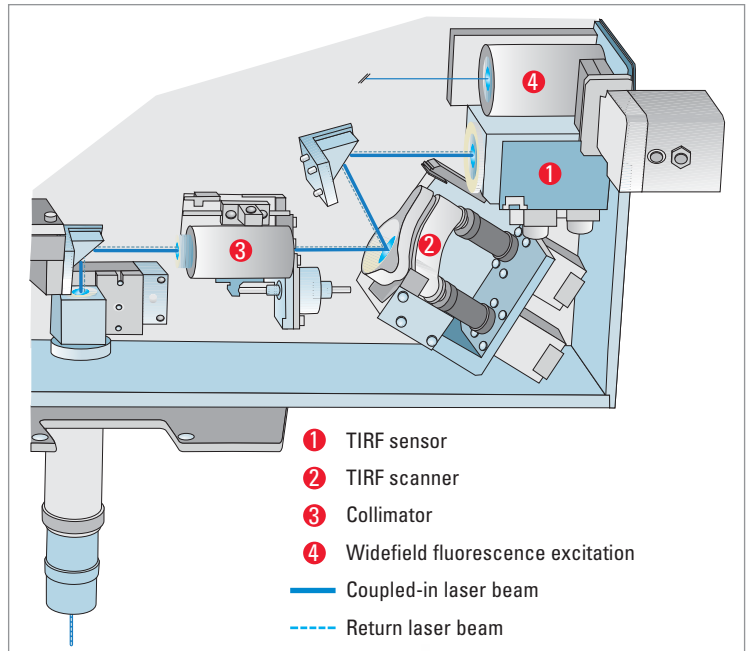
“Timing is particularly important for the understanding of cellular transport processes. Cells work quickly and precisely – just like our Leica AM TIRF MC system.”

Prof. Dr. R. Jacob

University Marburg, Department of Clinical Cytobiology and Cytopathology, Marburg, Germany



Light path of the Leica TIRF module. The laser beam is positioned in the objective with the aid of a scanner. The returning beam of the total reflection is measured on a special sensor. This feedback allows precise, fully automatic, and reproducible setting of the penetration depth of the generated evanescent field.



Automatic correlation determination

Leica's auto-alignment function automatically finds the correlation between the penetration depths of the evanescent field and the TIRF angles (to be set). An integrated TIRF sensor detects total reflection and enables the user to easily travel to specific penetration depths. The penetration depth of the evanescent field also depends on the wavelength of the laser, in addition to the refractive indices of the coverslip and specimen. The integrated refractometer function automatically determines the refractive indices of the specimens being examined. Intelligent mathematic algorithms precisely calculate the penetration depth of the TIRF field from the wavelength, the refractive index, and the incident angle of the laser beam. The selected contrast method is always TIRF and not a mixture of laser widefield excitation and TIRF. The user can start examining a specimen at the smallest penetration depth of the evanescent field and go continuously deeper. Penetration depth can be selected according to the specimen structure under examination and the orientation of the specimen. In this way the visualized structures or processes are assigned to an exact position close to the cell membrane. TIRF gives many structural and functional insights into a cell.

TIRF Auto-alignment

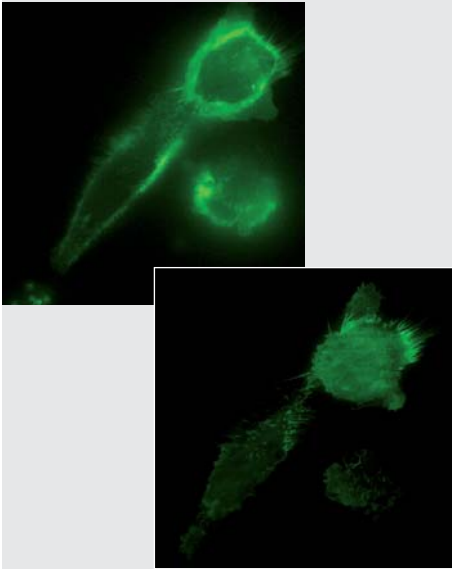
Reliable contrast methods and MultiColor TIRF

All contrast methods and colors are a click away

Leica's laser coupling technology allows both simultaneous and sequential use of TIRF, widefield fluorescence, and transmitted light contrast methods: the user can activate epifluorescence with a quick shutter mechanism to observe structures deeper than the evanescent field. All contrast methods are readily available and can be combined as required (for example transmitted light and fluorescence, or DIC and TIRF). If you use multiple fluorophores in your TIRF experiments, you can switch easily and quickly between the four laser lines, knowing that the penetration depth will automatically remain constant.

Leica AM TIRF MC at a glance:

- Integration of four wavelengths 405 nm, 488 nm, 561 nm and 635 nm with fast AOTF control
- Correction of the penetration depth when switching wavelength
- Combination of TIRF and fast FRET analysis
- Highest transmission of all optical TIRF components for maximum TIRF imaging speed and brilliance
- Fast image recording of up to 30 fps with simultaneous change of wavelength



Top: Widefield fluorescence image of breast carcinoma tumor cells expressing a GFP tagged cell adhesion Molecule CD44 that is expressed on the cell membrane.

Bottom: The same cells imaged in TIRF.

Courtesy of: Dr. Maria C. Montoya, CNIO, Spanish National Cancer Center, Madrid, Spain

Control all functions

Leica's powerful Leica AF6000 fluorescence software guides the operation of the TIRF system. TIRF and microscope functions are controlled via the clearly designed interface. Leica AF6000 also offers a wide range of analysis tools and software modules for FRET, time lapse, multi-dimensional imaging, and deconvolution, making light work of complex applications.

TIRF Scanner

The elegant solution for fluorescence microscopy

Make it personal

There are a number of options that tailor the Leica TIRF system to individual user needs.

- The TIRF module adapts to the Leica DMI 6000 B inverted, fully automated research microscope to deliver extremely detailed and reproducible results. This advanced solution is ideally suited to complex tasks in live cell microscopy.
- For multidimensional TIRF applications, the module can be fully integrated into the high-performance Leica AF6000 LX fluorescence platform.
- For Multicolor TIRF applications the module can be equipped with a four solid state laserbox (405, 488, 561, 635 nm) and AOTF control.
- Leica's made-to-measure TIRF solution for micromanipulation, the TIRF climate box, is available with micromanipulators.



TIRF Objective

High performance imaging through superb optics

Reliable, exact results

Leica's TIRF objective offers maximum apochromatic correction, an optimum signal-to-noise ratio and high performance imaging. The objective's correction ring compensates for spherical aberrations caused by temperature from 23–37°C, as well as coverslip thicknesses of 0.12–0.22 mm. Leica's TIRF objective meets all the requirements for consistent, high-quality imaging performance and exact analysis in TIRF microscopy.

TIRF MultiColor

Fluorescence analysis in the entire color spectrum

All colors automatically in focus

As a MultiColor TIRF system, the Leica AM TIRF MC offers wavelengths in the entire color spectrum from ultraviolet to red. Four longlife and maintenance-free diode lasers in the wavelengths 405 nm, 488 nm, 561 nm and 635 nm are integrated into the system. The automatic coupling and control via AOTF ensures extremely short switching times of 1 ms and adjusts the laser beam so that the penetration depths are not changed when the wavelength is switched. Key practical advantages are the compact design of the overall system with its small footprint and the optimized safety class I laser unit with quiet air cooling. All four wavelengths are guided via one single multi-mode fiber.

TIRF AFC

Long-term stability in TIRF with the Leica Adaptive Focus Control

Focus stability is the key to obtaining superior images with TIRF. Image quality is ensured when the distance between the objective interface and the coverslip remains constant. To achieve this, Leica has developed a powerful Adaptive Focus Control and fully integrated it into the Leica AM TIRF MC system. The Leica Adaptive Focus Control actively holds the focus over time – even under demanding environmental conditions. The principle is based on the surface reflection of the glass bottom of the Petri dish. Effective for all contrasting methods, it can easily be combined with a digital autofocus. This combination enables you to keep cells in focus, even if they change their shape over time.



Easily accessible specimen and high laser safety

Leica AM TIRF MC with Adaptive Focus Control:

- **Reliable:** holds focus position actively over time
- **Fast:** corrects for focus shifts in the blink of an eye
- **Flexible:** in combination with digital autofocus, corrects the focus to compensate for changes in cellular morphology

“With the user, for the user”

Leica Microsystems

Leica Microsystems operates globally in four divisions, where we rank with the market leaders.

● Life Science Division

The Leica Microsystems Life Science Division supports the imaging needs of the scientific community with advanced innovation and technical expertise for the visualization, measurement, and analysis of microstructures. Our strong focus on understanding scientific applications puts Leica Microsystems' customers at the leading edge of science.

● Industry Division

The Leica Microsystems Industry Division's focus is to support customers' pursuit of the highest quality end result. Leica Microsystems provide the best and most innovative imaging systems to see, measure, and analyze the microstructures in routine and research industrial applications, materials science, quality control, forensic science investigation, and educational applications.

● Biosystems Division

The Leica Microsystems Biosystems Division brings histopathology labs and researchers the highest-quality, most comprehensive product range. From patient to pathologist, the range includes the ideal product for each histology step and high-productivity workflow solutions for the entire lab. With complete histology systems featuring innovative automation and Novocastra™ reagents, Leica Microsystems creates better patient care through rapid turnaround, diagnostic confidence, and close customer collaboration.

● Medical Division

The Leica Microsystems Medical Division's focus is to partner with and support surgeons and their care of patients with the highest-quality, most innovative surgical microscope technology today and into the future.

The statement by Ernst Leitz in 1907, “with the user, for the user,” describes the fruitful collaboration with end users and driving force of innovation at Leica Microsystems. We have developed five brand values to live up to this tradition: Pioneering, High-end Quality, Team Spirit, Dedication to Science, and Continuous Improvement. For us, living up to these values means: **Living up to Life.**

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