Laser TIRF 3
Simply Excite More

The New Standard: Six Laser Lines for the Investigation of Processes Near the Cell Membrane
**TIRF Microscopy from Carl Zeiss.**

TIRF microscopy (Total Internal Reflection Fluorescence), has firmly established itself in life science research as a standard imaging technique for the investigation of processes near the cell membrane. Now with Laser TIRF 3, Carl Zeiss brings a new instrument generation onto the market, which allows even more advanced applications: e.g. synchronous observation of differently labeled molecules at high temporal resolution.

**More flexible, more economical, safer: Laser TIRF 3 from Carl Zeiss**

As a method for the investigation of molecular mechanisms in the vicinity of the cell membrane, TIRF microscopy has proven itself in cell biology and membrane biophysics for years. With the new product generation Laser TIRF 3 Carl Zeiss elevates this technique to a new performance level.

- New motorized slider for reproducible setting of the illumination angle
- New manual slider as an economic entry version
- New, flexible and upgradeable laser module with up to 4 solid state lasers
- Rapid switching between the excitation wavelengths and intensities via AOTF
- Maximum protection by means of intelligent laser safety
- Excellent optical performance: best contrast and highest resolution in x, y, and z

Based on the modular research platform of Axio Observer, the Imaging System Laser TIRF 3 can be adapted to changing requirements at any time. Handling and operation is simple and comfortable. TIRF sliders are merely inserted into the luminous field diaphragm plane without complex add-ons. In addition, different incubators from Carl Zeiss for long term observation under physiological conditions are available.

1. Epi-fluorescence green (excitation with 458 nm), α-Plan-FLUAR 100x/1.45 Oil Oberbanscheidt, van den Boom, Bähler, Institute of General Zoology and Genetics, University of Münster, Germany
2. Under TIRF illumination you only excite structures within the evanescent field. You can see structures that you cannot see in Epi-fluorescence
The sum of the advantages – Laser TIRF 3 in overview

Compact complete system
- The motorized/manual TIRF slider inserts in the field diaphragm, small footprint, all operating elements are easily accessible
- No complex external add-ons
- Available as complete system or for retrofitting on Axio Observer

Multi-color laser module
- New laser module houses up to four solid state lasers
- Laser lines with different wavelength and output power: 405 nm (50 mW), 488 nm (100 mW and 20 mW), 532 nm (75 mW / 20 mW) or 561 nm (40 mW / 20 mW) and 635 nm (30 mW), respectively
- For optimal excitation of more fluorophores combinations
- All laser lines are guided through one broad band fiber into the microscope

Excellent optics
- Enhanced brightness with the α Plan-FLUAR 100x/1.45 Oil (TIRF angle up to 72°) and α Plan-APOCHROMAT 100x/1.46 Oil (TIRF angles up to 73.2°)

- High transmission, very good contrast and color correction, homogenous illumination
- Apochromatic beam path results in one optimal setting for laser beam divergence for all used wavelengths

Standardized system software
- Seamless integration into AxioVision: from Fast Image Acquisition up to complex image analysis

Numerous combination options
- TIRF/Epi-fluorescence, TIRF/DIC, TIRF/Phase Contrast
- TIRF/incubation, TIRF with Time-lapse Imaging

Sophisticated laser safety system
- Maximum protection of the user in all applications: invisible, cannot be deactivated or demounted
- System also provides laser safety in TIRF/transmission and TIRF/incubation

Modular structure
- Modules can be expandable for new applications
Features of the New Laser TIRF Generation from Carl Zeiss.

Laser TIRF 3 is oriented toward the high requirements of live cell research in every detail. With a compact, easily operated system. Providing extremely fine control and automated reproducibility over setting the illumination angle with a motorized TIRF slider. With a new laser module, which is controlled by AOTF and which handle up to 6 laser lines. For significantly more quality in scientific practice.

**Advanced technology for complex research projects: the complete system**
Integrated in the Axio Observer research platform, Laser TIRF 3 combines a maximum of information and safety with extremely simple operation. The apochromatically corrected beam path ensures the best possible image quality. The software AxioVision can be operated intuitively and easily adapted to your individual requirements.

**More than just an option: the new TIRF sliders**
Optimized for even more application variety: the motorized TIRF slider for reproducible angle settings. The mechanical design of the slider allows a resolution of achievable angle difference of about 5 nm within the center range of TIRF angles.

- The TIRF angle is stored in the AxioVision system software and can be reset again at any time.
- All wavelengths available in the laser module are guided through the same broad band fiber and via the TIRF slider into the microscope’s beam path. The extended focusing range of the TIRF slider allows the use of a large number of stages as well as piezo focusing devices or the combination with AFM (Atomic Force Microscopy). Overall, this highly ergonomic system provides flexible handling and a broad range of applications with numerous combination options.

**Best optics for an optimum TIRF angle: the objectives**
In order to make the interaction of individual molecules or objects close to the interface from glass to medium...
visible, an objective must have a numerical aperture that achieves a TIRF angle of at least 61°. Carl Zeiss has developed or optimized two objectives especially for TIRF:

- $\alpha$ Plan-APOCHROMAT 100x/1.46 Oil achieves an angle of up to 73,2°
- $\alpha$ Plan-FLUAR 100x/1.45 Oil achieves an angle of up to 72°.

As a result, penetration depths of < 100 nm are realized depending on the wavelength. Together with filter sets which are optimized for TIRF and the newly conceived beam path in the TIRF slider, reflections and interferences in the image have been reduced to a minimum.

**Sophisticated and well-conceived: laser safety**
Often neglected, yet crucial for your safety: protection against laser light. For Laser TIRF Carl Zeiss developed its own laser safety device which allows you to work freely without impediments.

**New possibilities: four laser wavelengths for Multi-color TIRF**
With the new laser module in Laser TIRF 3, up to six laser wavelengths can be selected. Therewith even more fluorophores can be optimally excited if more than one fluorescent marker is used.

### Examples of dyes and markers for the available wavelengths

<table>
<thead>
<tr>
<th>Laser lines</th>
<th>405</th>
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<th>488</th>
<th>514</th>
<th>532</th>
<th>561</th>
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**α Plan-FLUAR 100x/1.45 Oil with DIC slider**  **α Plan-APOCHROMAT 100x/1.46 Oil with DIC slider**  **Setting the TIRF angle with the manual slider**
Possible Applications with Laser TIRF.

Exocytosis, endocytosis or the display of individual molecules in the cell membrane – anyone who conducts research at the limits of visibility needs technology which provides the best possible image quality and flexibility. Laser TIRF 3 now supplies even more information: with four laser lines and a wide variety of possible applications.

Modular and economical: the new laser module
With the new laser module in Laser TIRF 3 the user has even more applications at his disposal. A maximum of four solid-state lasers with different performance classes can be installed. An Argon laser (458 nm, 488 nm, 514 nm) can be incorporated in the new laser module instead of the 488 nm line by means of a coupling module. Thus, one has a maximum of 6 different excitation wavelengths available. Control via AOTF allows rapid switching between the different wavelengths as well as the regulation of the intensity.

Multi-color TIRF with up to three laser lines for synchronous excitation
Multi-color TIRF provides information on the interactions of several proteins in a specific process. An example is the observation of the molecular aspects of cell movement. It provides information on how the molecules operate, which the cell needs for movement. In this work, Multi-color TIRF is used to visualize the distribution of stress fibers showing which part of the cell is solid and which part is moving. With Laser TIRF 3 this application is substantially simplified: up to three differently labeled proteins can be excited and hence been detected synchronously. During sequential excitation of the different proteins, also the illumination angle and therefore the penetration depth can be kept constant for each wavelength.

TIRF/Epi-fluorescence
The combination of TIRF and Epi-fluorescence is used when it is also of interest where the molecules under observation orginate from. With TIRF/Epi-fluorescence, for example, the transport of vesicles from the Golgi apparatus in the depths of the cell can be followed to their secret location of the membrane. When using the motorized TIRF slider, only the illumination angle is changed to switch between TIRF and Epi-fluorescence illumination.

1. B16/F1 melanoma cells (mouse) – TIRF illumination, blue: CFP-actin. Excitation 458 nm, green: dRed-Clathrin Light, Chain A, Excitation 541 nm, α Plan-FLUAR 100x/1.45 Oil, Oberbanscheidt, van den Boom, Bähler, Institute of General Zoology a. Genetics, University of Münster, Germany
TIRF/Transmitted-light contrasting methods

With TIRF only the structures in closest proximity to the coverslip are visible, never the entire cell nor the entire supporting surface of the cell. Frequently, however, it is critical to compare the fluorescence signals from Epi-fluorescence and TIRF with the overall morphology to get a more comprehensive view of the cell. Carl Zeiss Laser TIRF is the only TIRF system which can combine TIRF with brightfield contrast techniques such as Differential Interference Contrast or Phase Contrast while simultaneously maintaining laser safety.

TIRF and High Speed Imaging

High time resolution is required where processes occur at high speed or when several different markers are to be detected as quickly as possible. The answer to the question of colocalisation of events is only possible when neither the switching of the excitation wavelength nor the adjustment of the illumination angle nor the exposure time of the camera limit the image acquisition rate. With the integration of Laser TIRF 3 into the concept of Fast Image Acquisition in AxioVision it is now possible to analyze near-membrane processes with this standard for the first time.

TIRF and FRET

With new filter sets and the complete integration into the AxioVision system software, Laser TIRF 3 now also allows the combination of TIRF with FRET. For high temporal and spatial resolution the new Dual Camera setup is recommended. Two cameras simultaneously detect the different fluorescence signals which are excited by two different laser wavelengths simultaneously in TIRF with full resolution. The subsequent image analysis can be done very conveniently using the physiology module of the AxioVision software.
Laser TIRF in System.

Carl Zeiss has developed components especially for Laser TIRF 3, which are at the cutting-edge of high performance technology. All of them are thoughtfully matched to one another. In the Cell Observer® system they provide an unrivaled performance spectrum and an exceptional variety of applications ranging from simple observation up to long-term experiments with incubation.

Cutting-edge microscopy: Axio Observer from Carl Zeiss
As a research platform with the acknowledged best fluorescence technology, unmatched stability and excellent optics, Axio Observer has established itself in research as the standard to be met. In combination with Laser TIRF 3, the semi-motorized variant Axio Observer.D1 or the fully motorized Axio Observer.Z1 stand is extremely appropriate for state-of-the-art research. It provides outstanding performance in all important aspects. Perfectly matched to the apochromatic reflected-light beam path of Axio Observer, the optics of the Laser TIRF 3 slider provides unprecedented image quality. The motorized z-Focus allows extremely fine focusing in 10 nm intervals.

Perfect conditions and maximum protection for the user: incubation with laser safety
For long-term observation under physiological conditions, several incubators are available for different applications: Incubator PM (laser safe), Incubator XL (multi-compatible: in red for all wavelengths up to 561 nm and black for all wavelengths up to 650 nm) as well as Incubator S TIRF. The control of the incubation equipment is completely integrated into AxioVision. The ingenious concept for laser safety assures laser safety when working with all laser lines and energies. At the push of a button it is possible to switch between laser operation and normal widefield illumination for examination of the sample through the eyepieces. Extremely convenient: this function can even be assigned to the control buttons on the microscope.

TIRF sample cover – laser spot can be seen through the opaque material. The laser beam can be adjusted either to the front or to the back

Incubator S Dark for TIRF
Ultrafast and high resolution:
cameras for high image acquisition rates
For the highest speeds, Carl Zeiss offers a range of highly sensitive detectors, including the EM CCD camera Quant EM from Roper Scientific. In the Fast Acquisition mode they are completely integrated in AxioVision. This camera with 13 µm pixel size and a 512 x 512 chip is particularly appropriate for low-light applications and for single molecule detection. For all other TIRF applications AxioCam MR monochrome from Carl Zeiss with 12 bit dynamic range and high resolution is the camera of choice.

Interference-free: the special filter sets for TIRF
Specially developed for TIRF microscopy and optimized for the most common fluorescent proteins and dyes. Double and triple band-pass filters, which allow the excitation waves to pass synchronously or sequentially. The new filters are coated on a special glass substrate which minimizes interference phenomena.

Simply intelligent:
control and calibration with AxioVision
AxioVision provides a variety of modules which can be combined with Laser TIRF 3. Multichannel Image Acquisition, Fast Image Acquisition, Mark&Find, Physiology, Colocalisation, etc.

- High safety: all calibration and adjustment processes are performed under laser safety conditions.
- Simple calibration: when using the motorized TIRF slider, the angle adjustment is calibrated with regard to the actual sample via a calibration routine in the software. The routine automatically determines at the sample location the critical angle for total internal reflection in two different directions. A possible offset is then calculated in the future angle adjustment. The actual TIRF angle and the refraction index of the sample or the penetration depth of the evanescent field, respectively, result from the measurement of the critical angle.
The Functional Principle of TIRF Microscopy

The analysis of images which have been acquired with conventional widefield fluorescence excitation near the cell membrane is difficult due to the background fluorescence from other planes which are not in focus. Only under TIRF conditions a resolution of < 200 nm in z is achieved by selective illumination.

The principle of total reflection
Total reflection occurs at the interface between an optically denser medium and an optically less dense one when a light beam is incident at an angle which, according to Snell’s law of refraction (1), is larger than the so-called critical angle \( \alpha_g \) (i.e. \( \alpha_g = 90^\circ \)).

\[
\begin{align*}
\text{(1)} & \quad n_1 \cdot \sin(\alpha_1) = n_2 \cdot \sin(\alpha_2) \\
\text{So the following applies:} & \quad n_1 \cdot \sin(\alpha_1) = n_2 \cdot 1
\end{align*}
\]

For the transition from coverslip (\( n_1 = 1.518 \)) to water (\( n_2 = 1.33 \)) a critical angle of 61° results. Under conditions of total reflection a standing evanescent (= ephemeral) wave whose intensity decays exponentially with respect to the distance from the interface is formed (2).

\[
\text{(2)} \quad I(z) = I(0) \cdot e^{-\frac{z}{d}}
\]

The penetration depth of the evanescent field is defined as the distance \( d \) from the interface in which the energy of light has fallen to 37 % of its original value. This depends on the angle of incidence and the wavelength of the light used (3).

\[
\text{(3)} \quad d = \frac{\lambda_g}{4 \pi n_2 \sqrt{\sin(\alpha_1)/ \sin(\alpha_g)^2 - 1}}
\]

(With \( \lambda_g = 488 \text{ nm}, n_1 = 1.518, n_1 = 1.33 \rightarrow \alpha_g = 61.2^\circ \))

Because of the energy distribution, fluorophores which are further away from the boundary are not excited. Thus, a thin optical section with a thickness of approximately 100 to 200 nm and with a very high signal/background ratio is formed. The image contrast and resolution in z are significantly improved.
Object guide 130x85 mm right 00000-1005-833

Universal mounting frame M 471718-0000-000
further mounting frames M see price list 40.19.01

Mounting Frame K for specimen slider 76x26 mm 451341-0000-000

Universal mounting frame K 451352-0000-000

Universal mounting frame K-Duo 000000-1116-078
further mounting frames K see price list 40.19.01

Filter sets for line selection and fluorescence
see price list Laser TIRF
Reflector module FL EC P&C 424931-0000-000
Reflector module FL EC ACR P&C 424933-0000-000
further modules see price list
Analyzer module DIC ACR P&C for transmitted light 424921-9901-000
Analyzer module DIC ACR P&C shift free for transmitted light 424932-9901-000

Specimen stage 250x230 mm 432017-9901-000
Heating stage S1 432049-9000-000
Temperable microscope stage 000000-1116-066

Mechanical stage 130x85 mm 432016-9901-000
Mechanical stage 130x85 mm R/L with short coaxial drive 432047-9901-000

Scanning Stage 130x85 mm P, CAN 432031-9902-000
Electronic Coaxial Drive, CAN 432904-9901-000

Scanning Stage 130x100 DC for Axio Observer 432028-9901-000
XY DC Stage Controller MCU 2008 432929-9000-000
Joystick XY for stage controller PIEZO / MCU 2008 432903-9902-000
or
Scanning Stage 130x100 STEP 432029-9901-000
with
Ludl MAC5000 XY Stage Controller Stepper incl. Joystick 000000-0431-478
Ludl Stepper Motor Cable with Right Angled Plug (2x) 000000-0445-551
or
Scanning stage XY DC 110x90 w. top plate Z Piezo/Rot. En. 000000-0495-117
Required accessories see price list 40.17.12

Laser safety incubator refl/transmitted light S1 433611-9901-000

Laser safety cover reflected light for mechanical/scanning stage 432319-9902-000

Incubator S TIRF S1 433612-9901-000
Incubator S TIRF DARK S1 433614-9000-000

Incubator XL TIRF S1 411857-9091-000
The technology of the TIRF slider

The decisive performance characteristic of the TIRF slider: linearly polarized laser light is introduced into the beam path via the TIRF slider, which is inserted into the luminous field diaphragm plane of the reflected-light beam path. A double polarization-maintaining prism in the slider ensures that

- the laser light coming from the mono mode fiber is again selected for the polarization direction and
- the module can simultaneously be combined with conventional HBO or other white light.