

Leica AF6000

Modular Widefield Systems for Advanced Live Cell Research

Living up to Life

Leica
MICROSYSTEMS

Live Cell Imaging Solutions that Grow with Research Needs

Ease of use

- All experiment settings visible at a glance
- Full screen live image display
- Fast and intuitive setup of experiments
- Context sensitive online help
- Minimum training required

Flexibility and modularity

- Application-specific system configurations
- Available for upright, inverted, stereo microscopes and macroscope systems
- A wide choice of hardware components e.g. Water Immersion Micro Dispenser, high-speed filter wheels, EM CCD cameras, high precision stages, live cell accessories

Upgradability

- Systems grow with changing research needs
- From entry-level systems to high-end workstations for real-time studies
- Convenient, on-site upgrade process

Integration

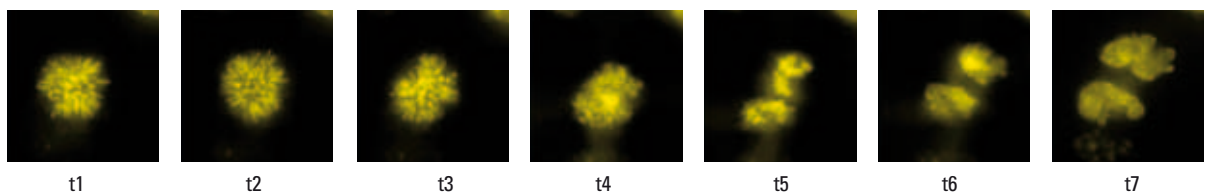
- Perfect synchronization between hardware and software
- Precise timing for high-speed experiments
- Maximum system reliability
- One software platform for all widefield research and confocal microscope systems
- Ideal for imaging centers and multi-user facilities

Biological and medical researchers are probing deeper into the secrets of life. Widefield fluorescence microscopy is a key technology for imaging living cells. Developmental and molecular processes of cells can be visualized directly by widefield fluorescence techniques shedding new light on the undiscovered. Leica Microsystems' advanced widefield systems provide leading innovations to advance your research and unlock secrets of life.

For many years, users all over the world have benefited from the speed, reliability, brilliant image quality and ease of use of the fluorescence research systems from Leica Microsystems. Together with leading scientists, Leica Microsystems has further developed its successful fluorescence solutions into the Leica AF6000 systems that can be customized to suit all live cell imaging requirements.

The Leica AF6000 systems offer the right tool for your research; from routine imaging and documentation to live cell imaging workstations for complex real-time experiments. The modular design affords you the freedom to expand the system, choosing appropriate hardware and software components as needed.

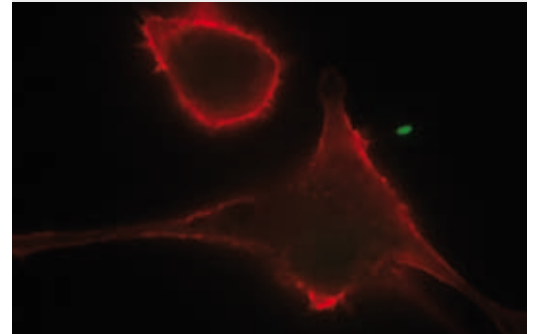
Regardless of the application, all Leica AF6000 systems utilize the same easy-to-use software platform, which is shared with the Leica Microsystems confocal systems.



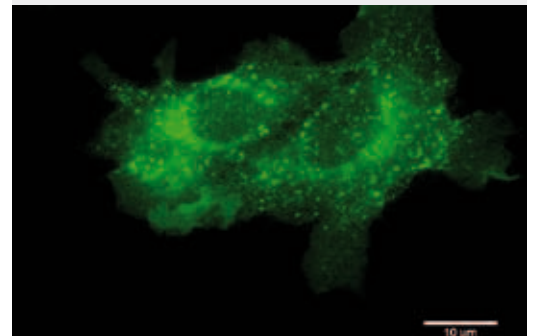
Mitosis of NIH 3T3 mouse fibroblast cells expressing H2A-YFP. Mark and Find time-lapse experiment. Courtesy of Dr. Constantin Kappel, DKFZ, Heidelberg, Germany

Application examples:

High-speed time-lapse experiments, see page 6
Developmental biology, see page 6
Studies of live cells up to whole organisms
Macro time-lapse experiments, see page 6
<i>In vivo</i> imaging
Cell cycle studies
Axon outgrowth
Intracellular transport, vesicle trafficking
Exocytosis, endocytosis
Calcium imaging, Fura2, see page 8
FRET ratio and FRET sensitized emission, see page 8
Screening, see page 9
Single molecule detection
Membrane research
Protein – protein interaction
Multi positioning experiments, Mark & Find, see page 7
Colocalization studies
Stitching, see page 7
Electrophysiology
Patch clamping
Deconvolution, 3D reconstruction
Imaging / documentation, see page 13
TIRF, see page 10
Super-resolution microscopy, see page 10



Dendritic cells infected with bacteria
Green: GFP expressing enteropathogenic *E. coli*
Red membrane staining: Cy3 labeled anti Cd44 antibody
Courtesy of Nadzeya Kramko, ZMBE,
Institute of Infectiology, University of Muenster and
Ivan Liashkovich, Institute for Physiology II, University of
Muenster, Germany



EGF stimulated endocytosis
Green: GFP tagged EGF-receptor
Courtesy of Vibor Laketa, Cell Biology and Biophysics
Unit, EMBL Heidelberg, Germany



5-day-old transgenic zebrafish larva
Green: GFP – actin
Courtesy of Prof. Dr. Stephan C. F. Neuhaus,
Institute for Brain Research, ETH Zurich, Switzerland

A System as Dynamic as Modern Research

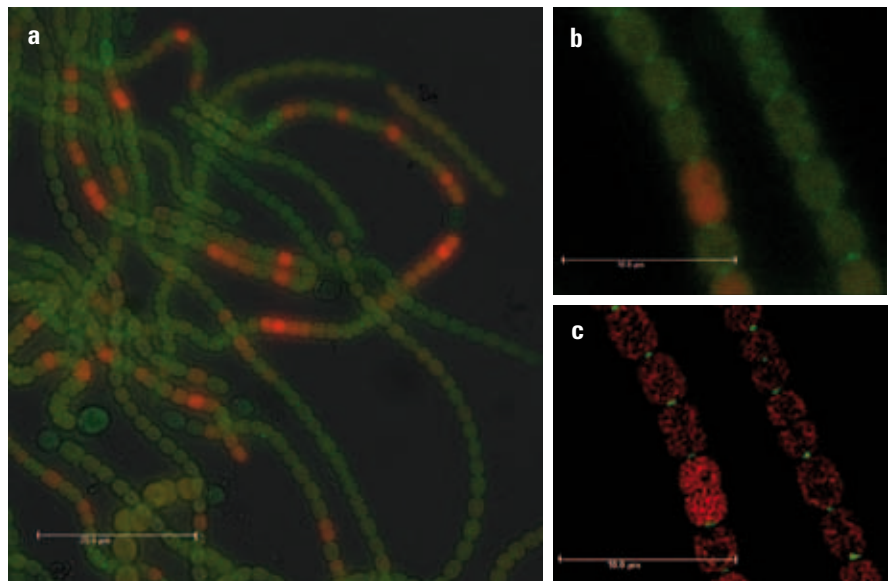


“I need to perform high-resolution 3D-deconvolution microscopy in time while keeping the cells in optimal conditions. Leica Microsystems provides the right tool for my live cell research.”

Dr. Eef Parthoens

Department for Molecular Biomedical Research (DMBR), VIB – Ghent University, Ghent, Belgium

The Leica AF6000 systems offer full flexibility to adapt to your changing needs. You will benefit from the utmost reliability and convenience, regardless of the modules and components you select to configure an individual system. As many of the system components are designed and developed by Leica Microsystems, we offer unparalleled automation control due to complete integration of our hardware within the software platform. Add a control unit to convert the system into a high-speed live cell imaging workstation with full real-time control. Leica Microsystems’ technical experts perform the upgrade on site at your facility, minimizing system downtime.



Subcellular localization of SepJ in the cyanobacterium *Nostoc punctiforme*. Green: GFP on C-terminus of SepJ, Red: Autofluorescence of chlorophyll a. **a** Maximum intensity projection of fluorescence Z-stacks. Overlay with a bright field image. **b** Cropped region of image a. Maximum intensity projection of Z-stack. Overlay of two fluorescence channels. **c** Overlay image of two fluorescence channels after 3D deconvolution. Displayed is one Z level. Courtesy of Susanne Berendt and Prof. Dr. Karl Forchhammer, Department of Microbiology/Organismic Interactions, University of Tuebingen, Germany

Leica AF6000 Software Modules

Acquisition modules

AF6000 Core, see page 13

- Basic software module for all Leica AF6000 modular systems
- Microscope control
- Processing tools
- Full image viewer
- Full review capability
- Annotations
- Quantification tools
- Driver for compatible Leica DFC cameras
- Configuration tool for Leica AF6000 systems

Multi Channel Acquisition
Experiments with several fluorescence channels

Z-control and SW autofocus
Z-stack acquisition includes software based autofocus

Time-Lapse
Define duration and frequency of image capture for time-lapse experiments, see page 6

Mark & Find
Multi positioning experiments, see page 7

Stitching
Acquire mosaic images
Create large overview images, see page 7

Advanced modules

Live Data Mode
Define acquisition jobs and macros. Send and receive trigger signals to peripherals, see page 9.

3D Visualization
3D rendering and dynamic user interaction of volume generation of multi-channel 3D time-lapse movies

2D Deconvolution
2D deconvolution offering no-neighbor and nearest-neighbor algorithm

3D Deconvolution
3D deconvolution with several algorithms using adaptive, calculated or supplied PSF

Dye Finder
Elimination of cross-talk between fluorochromes with a proprietary unmixing algorithm

Colocalization
Visualization and quantification of colocalized fluorochromes

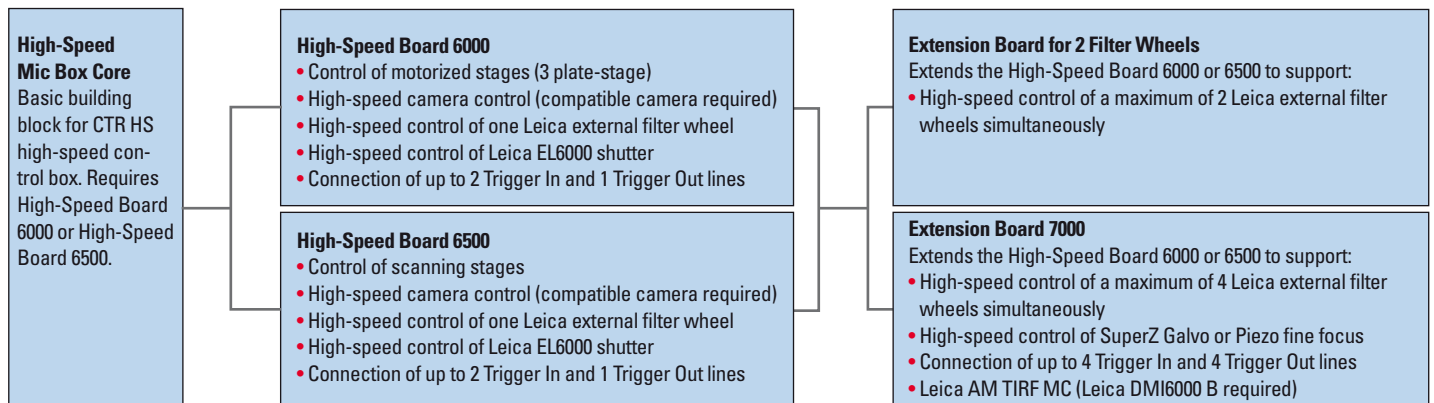
Calcium Imaging
Module for online ratio measurement, online display of ratio graphs and ratio image, see page 8

FRET Sensitized Emission
Powerful wizard for FRET SE acquisition and analysis, see page 8

Matrix M3
Wizard for fully automated high content screening experiments, see page 9

Well Plate Acquisition
Wizard for time-lapse experiments with micro plates, predefined and user-definable patterns

Leica AF6000 High-Speed Control Modules



Supported microscopes:

Inverted research microscopes	Upright research microscopes	Fixed stage microscopes	Stereos and macroscopes	Clinical routine microscopes
Leica DMI6000 B *	Leica DM6000 B *	Leica DM6000 FS *	Leica M205 FA	Leica DM3000
Leica DMI6000 B with AFC *	Leica DM5500 B *		Leica MacroFluo	Leica DM2500
Leica DMI4000 B *	Leica DM5000 B *			Leica DM2000
Leica DMI3000 B	Leica DM4000 B			Leica DM1000

* can be combined with modular high-speed control units

Leica AF6000

Time-Lapse Applications

Superior live cell imaging

- Stay in focus with hardware-based Adaptive Focus Control (AFC)
- Software-based autofocus
- Brilliant images under physiological conditions with dedicated 63x live cell objective
- High-speed acquisition
- Available on inverted, upright, fixed stage, stereo microscopes and macroscopes
- Extensive range of live cell accessories



Vascular anatomy of a zebrafish embryo as revealed by GFP expression driven by the Fl-1 promoter.

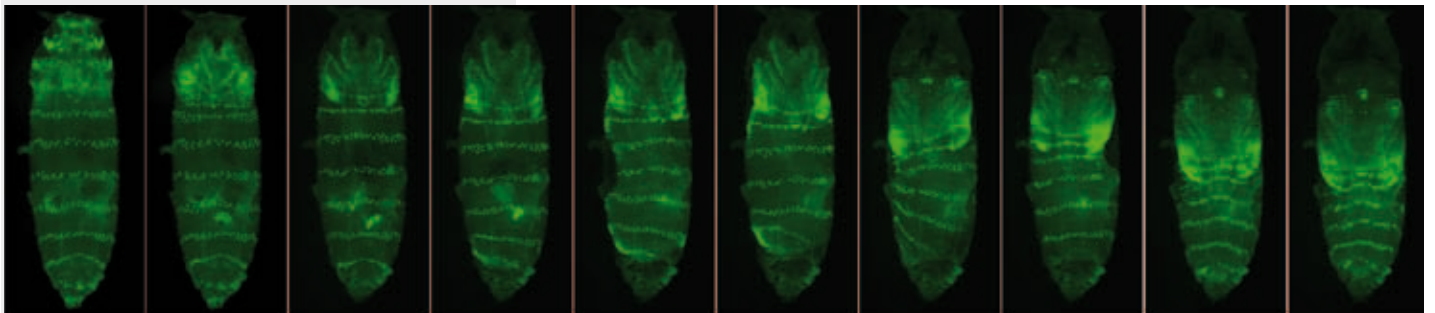
Courtesy of Dr. Brant Weinstein,
National Institutes of Health, Bethesda, MD, USA

2D and 3D time-lapse experiments over several hours or even days require the highest level of stability, reproducibility, acquisition speed and system reliability. With the AF6000, Leica Microsystems offers an integrated modular system solution to meet these requirements. Leica microscope stands are well known for their superior stability. A fast software-based autofocus keeps the sample in focus. In addition the hardware based optional Adaptive Focus Control (AFC) is offered on systems using the inverted research microscope Leica DMI6000 B for highest convenience and reliable results.

Achieve excellent results – fast!

Combined with a high-speed control unit, the system allows real-time control of all necessary hardware components for complex live cell studies, such as the camera, fine focus devices and up to four synchronized high-speed filter wheels. Each of the acquired images receives a precise time stamp.

The Leica EL6000 light source with its high-speed shutter reduces light exposure to the sample and therefore minimizes cell damage. This shutter can also be used between image acquisition to avoid light exposure during camera image transfer. Leica external filter wheels have a very short switching time of 27 ms for high-speed switching of fluorescence channels for simultaneous excitation and emission. Leica AF6000 is available on inverted, upright, fixed stage, stereomicroscopes and macroscopes.



Sample: 15 hour time-lapse imaging of *Drosophila* after pupation. A fluorescence protein (Venus) was expressed in the posterior compartment of each segment and the signal was clearly detected even under the presence of puparium. Courtesy of Dr. Erina Kuranaga, Laboratory for Histogenetic Dynamics, RIKEN Center for Developmental Biology (CDB), Japan

Multi Positioning and Stitching Experiments

The chances of observing a process of interest are much higher when several locations are observed. With the Mark & Find function, an unlimited number of user defined sample positions can be defined. This allows recording of many different cells within one time-lapse experiment, thus saving precious time and resources. At each stage position a cell specific Z-stack size can be defined. With the Pause and Relocate button, every single Mark & Find position can be corrected in its X, Y and Z location during the running experiment. The corrections can also be applied to all positions.

Such tools are perfectly suited to applications in developmental biology, for example to follow the development of the nervous system and transport systems of animal models. When the aim is to obtain quantitative parameters from the experiment (i.e. size, migration speed, particle tracking) increased throughput will effectively improve the statistical significance of the data.

Get the whole picture

The Tile Scan function creates large overview images by acquiring adjacent images and applying a subsequent stitching algorithm to merge the single images to one complete overview image. Stitching can be combined with different contrasting methods such as fluorescence and DIC and with other experimental parameters like time-lapse, Z-stack acquisition and the autofocus function. The overlap of the image tiles can be user defined. Stitching can either be done automatically during the experiment or offline after acquisition.

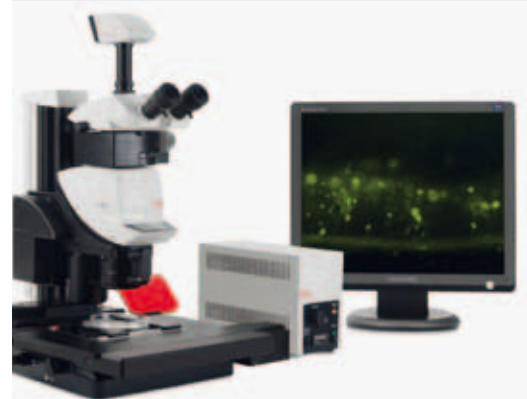
Overview images can serve as a reference for the whole experiment. Cells acquired in more detail can be viewed in their context. Overview images can also be used to obtain important quality parameters, for instance cell density, transfection efficiency or cell viability.

Wide range of samples and applications

- Define location-specific Z-stack size to minimize photo-bleaching and light stress
- Large specimens in focus: combine autofocus with Mark & Find and Stitching
- Change stage positions during a running long-term experiment
- Store and recall all stage positions
- Select from various stage options including high precision stage with encoder



Leica AF6000 with DMI6000 B inverted microscope and climate chamber

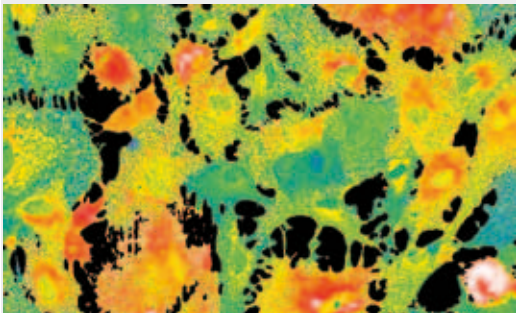


Leica AF6000 with M205 FA stereomicroscope and Leica EL6000 external light source

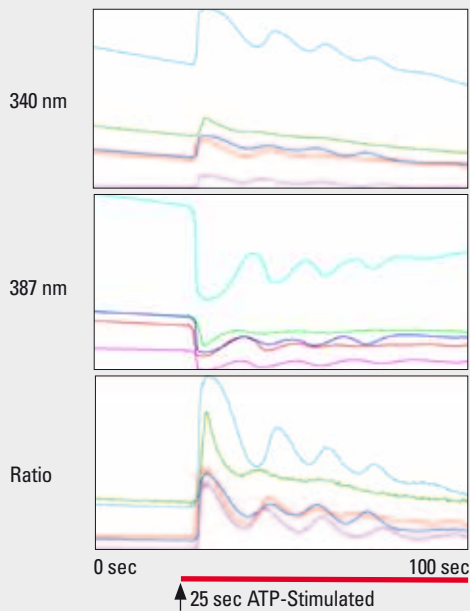
Get Instant Feedback with the Calcium and FRET SE Modules

Dedicated analysis modules

- FRET sensitized emission and FRET ratio
- Fura-2 calcium imaging
- Ca^{2+} quantification according to Grynkiewicz
- Subtraction of background fluorescence
- Stay in control of your experiment at any time:
 - online generation of measurement graph
 - online display of ratiometric image
 - online generation of image statistics



Fura-2 ratio image

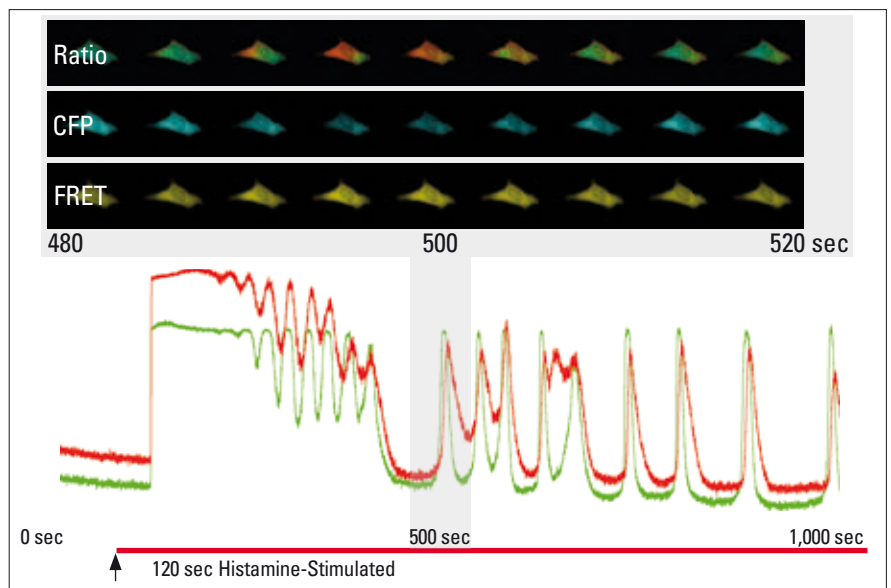


Cultured hippocampal astrocytes from 18-day-old embryos of Sprague-Dawley rats (Objective lens: Plan APO 20x0.7, 4 Ratio/sec) Courtesy of Drs. Kazunori Kanemaru and Masamitsu Iino, Department of Pharmacology, Graduate School of Medicine, The University of Tokyo, Japan

For Calcium and FRET experiments over time, an immediate feedback on location and intensity of the signal is crucial. The concentration of added reagents may need to be increased during the experiment or the experiment may need to be stopped due to a missing signal response. The Leica AF6000 Calcium Imaging module and the powerful FRET Sensitized Emission Wizard (FRET SE) give immediate feedback to the user with an online graph, online ratio result and the display of the resulting ratio image. Therefore, the user always knows the distribution of the signal within the image. Regions of interest can be added or removed during the course of the experiment.

Add the Live Data Mode module to the Calcium Imaging module and the experiments can be combined with TTL trigger signals sent and received from peripherals to start an experiment. An event manager can also be used to edit comments online during the experiment. Both the Calcium and the FRET SE module allow for removal of background. FRET SE also can correct for crosstalk from overlapping FRET spectra.

The FRET and Calcium modules are complemented by the high-speed controlled Leica external filter wheels and EM CCD and CCD cameras. With the dedicated high-speed electronics board, the time stamp on each image is down to exactly a 10th of a millisecond.



CFP-FRET images of a HeLa cell expressing Yellow Cameleon 3.6 Objective lens: PL APO 40x 1.25-0.75 Oil, 10 Ratio/sec, Exposure : 20 msec, 10,000 images, Courtesy of Drs. Takahiro Nakajima, Hideyuki Suzuki and Moritoshi Sato, Department of General Systems Studies, Graduate School of Arts and Sciences, The University of Tokyo, Japan

The Ultimate in Flexibility and Automation

Define acquisition macros with the Live Data Mode

The Live Data Mode module is designed to define acquisition jobs and macros within the easy-to-use interface of Leica AF6000. Within the macros, loops can be defined, a pause can be added and up to four trigger IN channels and four trigger OUT channels can be set. An Event Manager shows the start point of each job or macro. User defined events can also be generated during the experiment and can be edited online to add comments. The Live Data Mode module is therefore the ideal accompaniment to the Calcium Imaging module.

High content screening automation

For users requiring the ultimate in flexibility the Matrix M3 package is offered; a wizard for high content screening automation and much more. It combines the functionality of the Live Data Mode with a very flexible tool for defining any kind of scanning templates such as microplates, chambered coverslips, spotted arrays of samples, Tissue Micro Arrays, lab on a chip, petri dishes. The software package allows the assignment of different acquisition jobs to different locations. The course of a screening experiment can be changed by a feedback loop from an external analysis software (not included) via Computer Aided Microscopy (CAM) interface. The acquired images can either be stored in Leica's own .lif format or exported on the fly for online analysis. Optional features are drift compensation, the control of a Water Immersion Micro Dispenser or single object tracking, which lets the stage automatically follow cell movement.

- Define acquisition jobs and acquisition macros for complex experiments
- Export images during a running experiment
- Image analysis can be done by any software reading TIFF or OME TIFF format during the experiment
- CAM interface for feedback loop from analysis software to screening experiment
- Stage follows cell movement via single object tracking
- Water Immersion Micro Dispenser for long-term screening with high-NA water objectives



“We use the Matrix M3 screening software on both Leica widefield and confocal systems for rare event detection. The possibility to analyze the acquired data online with our own analysis software and change the course of the experiment via feedback loop allowed us to find new proteins which play crucial roles in basic cellular processes such as mitosis or membrane traffic and in record time!”

Dr. Rainer Pepperkok

Team Leader and Head of Advanced Light Microscopy Core Facility, EMBL, Heidelberg, Germany

Redefine the Limits of Microscopy



“Cell Biology is a fascinating field of research with a wide range of applications. My equipment needs to grow with the increasing number of demanding applications – just like the Leica Microsystems imaging solutions.”

Prof. Dr. R. Jacob

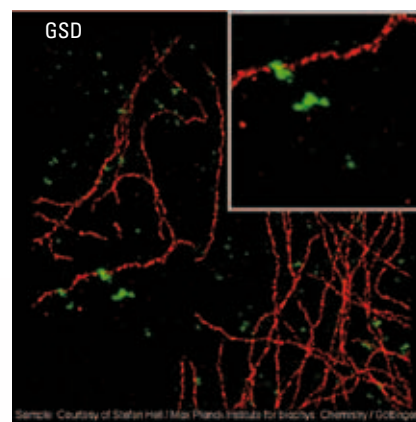
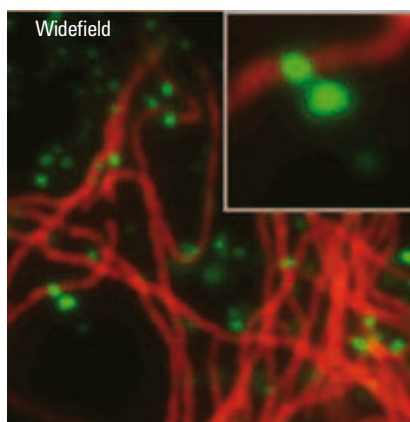
University of Marburg, Department of Clinical Cytobiology and Cytopathology, Marburg, Germany

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

Both the Leica SR GSD super-resolution system and the Leica AM TIRF MC are based on AF6000. 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.



Clathrin-coated pits and microtubules, red: Microtubules – Alexa Fluor 647, green: Clathrin – Alexa Fluor 488, Courtesy of Prof. Dr. Stefan Hell, Max-Planck-Institute, Goettingen, Germany

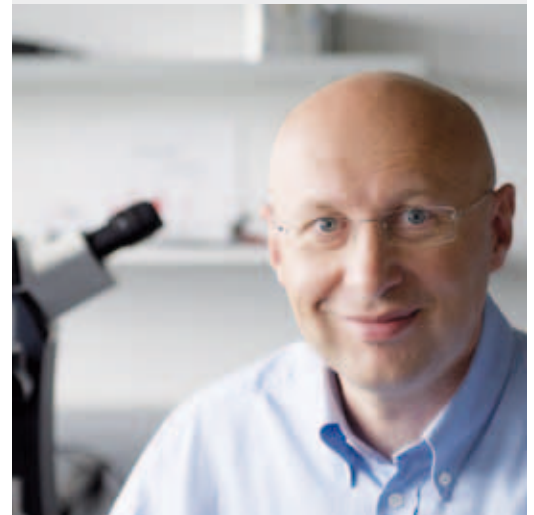


Leica SR GSD

– TIRF and Super-resolution

The Leica SR GSD offers the highest lateral resolution with fluorescence widefield and TIRF microscopy. It is a superb tool for determining the structure of fluorescently labeled samples in neurobiology, cell biology, virology and microbiology. The Leica SR GSD provides detailed insights into cellular structures and molecular sociology that cannot be seen with diffraction limited light microscopy. Using ground state depletion technology (GSDIM), it is possible to control the emission of fluorochromes and localize the position of single molecules down to a precision of 20 nm. This allows the reconstruction of fluorescent images with a resolution far beyond the diffraction limit.

The new Leica SR GSD system can be used for GSDIM, TIRF and widefield live cell imaging. The TIRF unit can also be used as an option on a Leica AF6000, making it the ideal tool for the study of cellular membranes and their environments.



“Leica Microsystems turned our idea of switching standard fluorophores by ground state depletion into a nanoscope delivering stunning images.”

Prof. Dr. Stefan Hell

Director at the Max-Planck-Institute for Biophysical Chemistry, Department of NanoBiophotonics, Goettingen, Germany

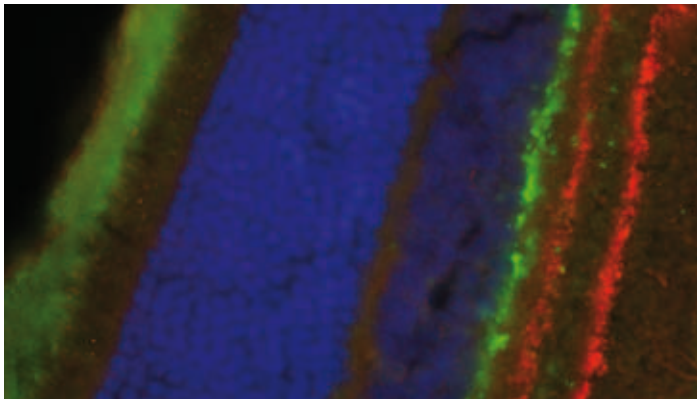
Leica SR GSD at a glance:

- Maximum resolution down to 20nm
- SuMo Stage with Suppressed Motion technology minimizes drift for accurate localization of molecules
- Online super-resolution image projection – see results as they are acquired
- Full application flexibility offered by combining super-resolution with TIRF and epifluorescence on a multi-purpose live cell imaging system
- Standard fluorochromes can be used – no need to change protocols
- Powerful lasers for the highest flexibility in fluorochrome selection
- Large set of powerful image processing tools

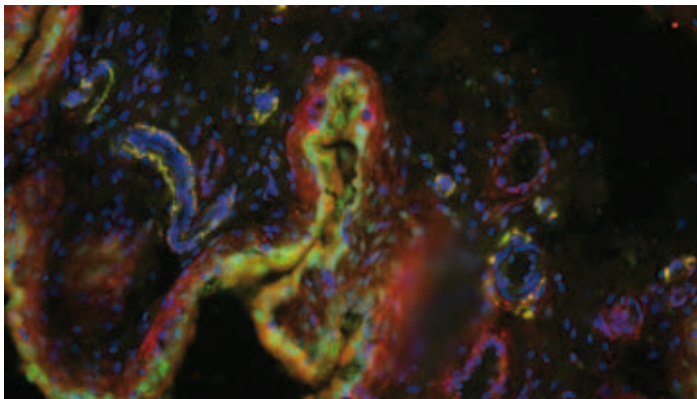
It's That Easy – Imaging and Documentation

Just press a button. The Leica AF6000 Core Module, a compatible microscope and a Leica camera already give you an imaging and documentation station offering great functionality for biological and cytomorphological examinations e.g. in histology or pharmacology. You can capture single images by simply pressing the Acquire button, combine single images to create a multi-color image and display it with the image overlay function, or just record images with a color camera.

Add comments with the annotations function, crop your image or quantify it with the quantify tool, all included in the Leica AF6000 Core Module. Add the time-lapse module and you can already acquire 2D time-lapse experiments in fluorescence or transmitted light mode.



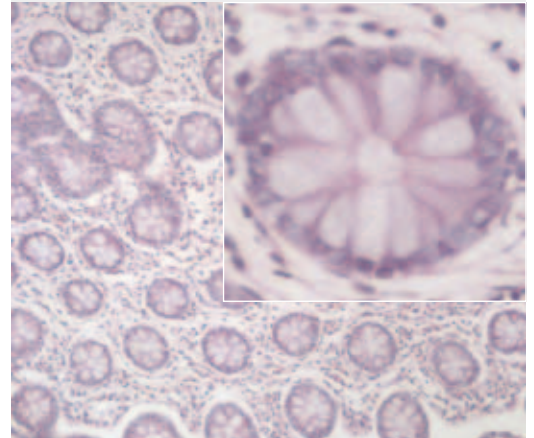
Retina of the rat. Double staining of Slc10a4 and VMAT2 in green and red. Nuclear stain with DAPI. Courtesy of Dr. Simone Burger and Prof. Dr. Joachim Geyer, Institute of Pharmacology and Toxicology, Justus-Liebig-University of Giessen, Germany



Urinary bladder of the rat with urothelium and submucosa. Double staining of Slc10a4 and VMAT2. Nuclear stain with DAPI. Courtesy of Dr. Simone Burger and Prof. Dr. Joachim Geyer, Institute of Pharmacology and Toxicology, Justus-Liebig-University of Giessen, Germany

Powerful Leica AF6000 core module including:

- Microscope control
- Processing tools
- Full image viewer
- Full review capability
- Annotations
- Quantification tools
- Driver for Leica DFC cameras
- Configuration tool for Leica AF6000 systems



Intestine H & E staining, captured with Leica DM3000 and DFC color camera using AF6000 Core



Leica AF6000 with DM2500 upright microscope and Leica EL6000 external light source

Optimum Conditions for Live Cells



Leica External Filter Wheels with extremely short switching time for high-speed excitation, attenuation and emission control



Leica SFL7000* – five different LED modules provide unlimited flexibility. Fully software integrated. Can be combined with Leica EL6000.

*Not available in the US



Water Immersion Micro Dispenser – Automatic supply of water immersion during experiments

Live cell experiments need optimized hardware components. Key requirements include fast or real-time image recording, temperature stability, minimum exposure of specimens to light and highly sensitive recording techniques for weak fluorescence. For successful studies and high-quality results, all parameters have to be optimized for the specimens being analyzed. Leica Microsystems offers a wide choice of hardware components and accessories for individual configuration of your research system such as the SuperZ fine focus, high-speed shutters or a high precision scanning stage with encoder. The modules can be combined in many different ways. The full integration of all automated functions with the software ensures perfect interaction of components in everyday use. The extensive range of accessories for live cell examinations include climate chamber, cell chambers, various manipulators, CO₂ controller and incubators.

Wide choice of monochrome, color and EM CCD cameras

Leica Microsystems supplies a wide choice of monochrome and color cameras. The flagship of Leica's fluorescence cameras is the Peltier-cooled Leica DFC365 FX, a very sensitive high-resolution monochrome camera recommended for the majority of Leica AF6000 applications. The DFC365 FX and many other monochrome and color cameras offer high-speed triggering.

For highest sensitivity and acquisition speed Leica Microsystems offers back- and front-illuminated EM CCD cameras from all major suppliers, perfectly integrated and high-speed controlled by Leica AF6000. For systems with more than one camera, for example a Leica color camera and a monochrome camera, the user can easily switch between the cameras within the Leica AF6000 user interface. All camera parameters such as the calibration parameters and the camera port are automatically taken over, ready for instantly starting a new experiment.



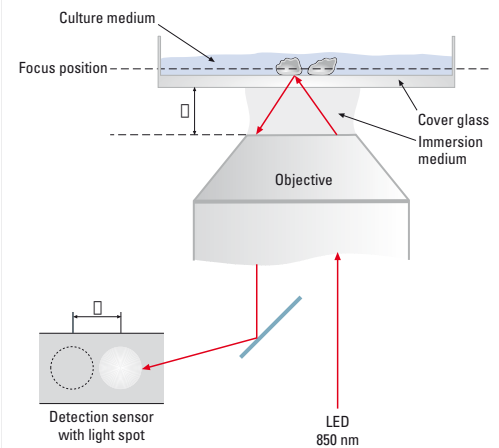
Leica DFC365 FX high-resolution camera for fast fluorescence and NIR imaging.

Stay in Focus

Explore life in all dimensions with the Leica Adaptive Focus Control

The inverted Leica DMI6000 B microscope, with its intrinsic temperature drift stability, is ideally suited for live cell imaging. But even the most robust microscope is susceptible to sudden temperature fluctuations, for instance if a climate chamber needs to be opened to add a solution to the sample during the course of an experiment. This can result in unsharp images in the time series, or even losing the sample out of focus completely. During such experiments, the Leica AFC actively holds the focus over time – even in the most demanding environmental conditions.

Its working principle is based on the reflection at the surface of the glass bottom of the petri dish. The Leica AFC is effective for all contrasting methods and actively works to protect cells from photo-bleaching. The user benefits from the Leica AFC's smart integration in all manner of live cell experiments such as time-lapse recordings, Mark & Find and Z-stacking.



Principle of Leica Adaptive Focus Control

Leica Adaptive Focus Control is based on the reflection of an LED light beam at an appropriate surface such as the bottom of a petri dish or multi well plate. The light is projected through the objective to the surface and reflected onto a light-sensitive sensor. The position of the light spot on the sensor is defined by the position of the sample in relation to the objective.



Leica DMI6000 B with Adaptive Focus Control

“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.**

Active worldwide

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Belgium:	Groot Bijgaarden	Tel. +32 2 790 98 50	Fax +32 2 790 98 68
Canada:	Concord/Ontario	Tel. +1 800 248 0123	Fax +1 847 236 3009
Denmark:	Ballerup	Tel. +45 4454 0101	Fax +45 4454 0111
France:	Nanterre Cedex	Tel. +33 811 000 664	Fax +33 1 56 05 23 23
Germany:	Wetzlar	Tel. +49 64 41 29 40 00	Fax +49 64 41 29 41 55
Italy:	Milan	Tel. +39 02 574 861	Fax +39 02 574 03392
Japan:	Tokyo	Tel. +81 3 5421 2800	Fax +81 3 5421 2896
Korea:	Seoul	Tel. +82 2 514 65 43	Fax +82 2 514 65 48
Netherlands:	Rijswijk	Tel. +31 70 4132 100	Fax +31 70 4132 109
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