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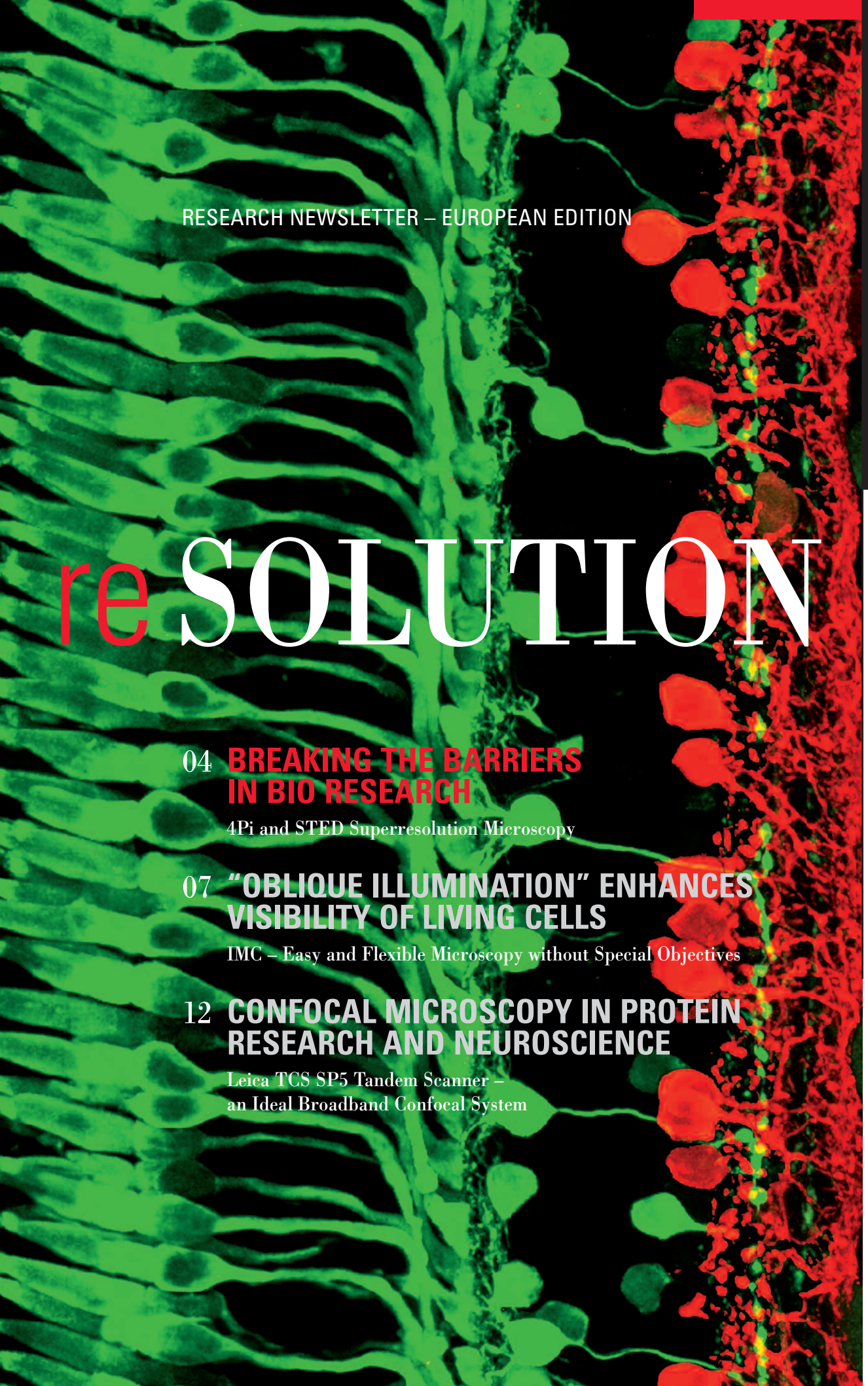


RESEARCH NEWSLETTER – EUROPEAN EDITION

reSOLUTION

Cover Picture

Photogenic Research

Nicolás Cuenca, Professor for Cell Biology at the University of Alicante, received no less than two prizes for an image of a retina that was generated using a Leica TCS SP2 confocal system. Last summer, the Spanish Foundation for Science and Technology (FECYT) in collaboration with the Spanish Scientific Research Council (CSIC) staged its third FOTCIENCIA, a national photography competition designed to increase public awareness of scientific research. The winners were announced in late 2006 – and Cuenca's image not only received the most votes from a panel of experts, but from the audience as well.

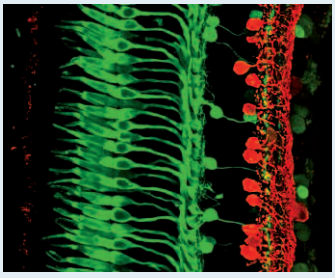
The image, entitled "La Retina: así vemos" (The Retina: How We See), shows the various neuron types of the retina at 63x magnification. "The difficult part of making a perfect photo was achieving a perfect tissue section and a good immunocytochemistry technique," explains Cuenca. "We then superimposed six individual images to ensure that enough 'photogenic' cells would be visible." These, and earlier awards, have inspired Cuenca with new ideas. Together with a neuroscientist friend, he is currently preparing an exhibition in Alicante, which may go on tour.

In his daily work at the Department for Physiology, Genetics, and Microbiology, Cuenca focuses on the neurobiological processes of sight. With the aid of the Leica TCS SP2, he analyses the condition of the connections between neurons of the retina. This involves using antibodies and fluorescent dyes to mark cells and receptor molecules that are modified in different experimental situations. For example, Cuenca is studying whether vision can be restored to rats using retinal transplants. Further research topics include changes to the retina due to the inherited condition retinitis pigmentosa and Parkinson's disease. > AS

The prize-winning images: **www.fotciencia.fecyt.es/premiados.htm**

Nicolás Cuenca, Professor for Cell Biology at the University of Alicante, is studying the neurobiological processes of sight.

Cover image: Cuenca's confocal image "La Retina: así vemos" (The Retina: How We See) won 1st prize at the Spanish FOTCIENCIA06 in the category Micro-Photographie. © FECYT



CONTENTS

Groundbreaking Innovations

For 130 years the validity of Ernst Abbe's law, which says that the resolution of light microscopes is physically limited to approximately half of the wavelength of light, remained unchallenged. Now, Professor Stefan Hell, Director at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, has proven that this law is not irrefutable.

He has developed 4Pi microscopy and the STED (STimulated Emission Depletion) fluorescence microscope which goes ten times beyond the Abbe limit. Both techniques are revolutionising the biomedical research environment by providing resolution beyond what is possible with the best light microscopes today, while maintaining all advantages of structure-specific fluorescence tagging. While the Leica TCS 4PI is already available, the Leica TCS STED system will be introduced to the market in 2007.

Please see page 4 for further details.

© German Future Award, photo: Ansgar Pudenz

EDITORIAL

Breaking Barriers and Bringing Great Ideas to Life

Dear Reader,

Since biological material has been dissected into its smallest bits and pieces, studied and analysed down to molecular level, there is still the need to see all these elements working together in their natural environment: LIFE. A fast growing family of fluorescence proteins compatible with life makes it possible to track them under the microscope while they move through living cells and tissue. Surfing on this unprecedented wave, research imaging facilities are taking full advantage of the improving technical performance in terms of sensitivity, space and time resolution. Scientists are starting to use new exciting tools to investigate the nano-world. On one hand there is a growing interest in the high resolution of electron microscopy when combined with live cell imaging aspects of GFP, giving birth to CLEM (Correlative Light Electron Microscopy), where freezing a crucial event in the cell life cycle becomes the challenge.

On the other hand, Abbe's law in optics has been shattered by the newly coined "superresolution microscopy". We saw in the last issue how 4Pi microscopy allows axial resolution to be improved. Today you can read on these pages how the STED technique (STimulated Emission Depletion) can improve lateral resolution by a factor of ten, breaking a new barrier in microscopy! Equally important in life science investigation is time resolution: how can a single system offer the best of two worlds, speed and optical sectioning? This will be illustrated by unveiling some of the secrets of the Leica TCS SP5 Tandem Scanner and with concrete practical results delivered by such a versatile system in protein research and neuroscience at Manchester University.

By developing innovative solutions dedicated to the life sciences community, we want to support the motto of the recently launched European Research Council "Bringing Great Ideas to Life". With the start of the Seventh Framework Program and a renewed EC Research Policy, 2007 will be an exciting year for Europe. With the breakthrough technologies such as those presented on these pages and the ones still to come, Leica Microsystems is determined to make its own contribution!

Looking forward to your feedback, we hope you enjoy reading this issue of reSOLUTION – European Research Newsletter

Didier Goor, European Marketing Manager Research

4Pi and STED Superresolution Microscopy

Breaking the Barriers in Bio Research

Dr. Tanjef Szellas and Dr. Martin Hoppe, Leica Microsystems CMS GmbH

The limits in optical resolution of light microscopes, as described by Ernst Abbe, have been overcome by two ingenious microscope technologies. While the Leica TCS 4PI increases axial resolution to form an almost isotropic spot, which is ideal for 3D reconstructions and spatial co-localisation analysis, the Leica TCS STED provides improved lateral resolution and therefore allows new insights in structural and functional analyses. Both new techniques in light microscopy will be able to answer numerous questions in biomedical research which are not yet resolved.

More than 130 years ago, Ernst Abbe set up the law that far-field optical microscopes are limited in resolution to approximately half of the wavelength of light. Today, these limits have been broken by two new concepts of microscopy: 4Pi and STED (STimulated Emission Depletion) microscopy. Both techniques are revolutionising the biomedical research environment by providing resolution beyond what is possible with best light microscopes today while maintaining all advantages of structure-specific fluorescence tagging. The principles have been invented by Stefan Hell of the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany and are commercialised by Leica Microsystems. While the Leica TCS 4PI is already available, the Leica TCS STED system will be introduced to the market in 2007.

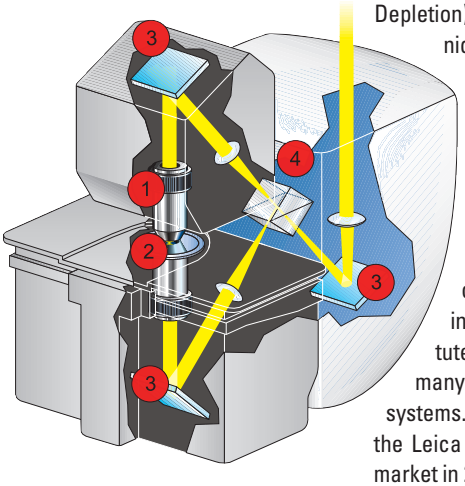


Fig. 1: Central element of the 4Pi system: the interferometer
1) Special High NA objectives
2) Sample holder
3) Ultra high quality surface mirror
4) Ultra high quality non-polarising beam splitter

4Pi microscopy

Even the newest confocal scanning microscopes which allow precise optical sectioning cannot overcome the disproportion in optical resolution of about 200–250 nm laterally (xy) compared to about 500 nm along the optical axis (z). A fundamental enhancement of z-resolution is strongly desired for accurate representation of three dimensional objects and structures and exact colocalisation analysis. Based on a fast beam scanning confocal microscope and combining the wavefronts from two opposing objectives through constructive interference, a 4Pi microscope achieves an axial resolution around 120 nm Full Width Half Maximum (FWHM) by creating a nearly full solid angle of 4π, giving the name to the microscope.

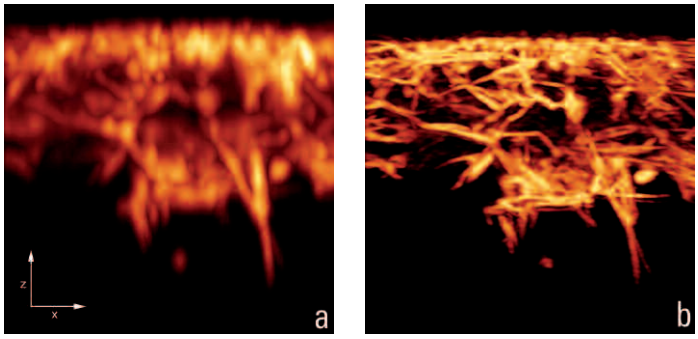


Fig. 2: Axial resolution improvement by 4Pi microscopy
Immunolabelled Actin fibers of HeLa cells in xzy maximum projection. Single filaments can be easily separated with Leica TCS 4PI.
a) Confocal image
b) 4Pi image

The value of 360° (4Pi) cannot entirely be obtained, the images are accompanied by interference "side lobes" above and below the main signal. For easy mathematical removal they are kept as low as possible. This is accomplished by the combined use of objectives with a high numerical aperture (1.2–1.46) and a two photon laser light source. The highly symmetrical assembly of the interferometer of the Leica TCS 4PI allows long term measurements of fixed and live cells. The system is equipped with up to 4 individual photomultiplier channels for multi-band spectral detection and two external Avalanche Photo Diodes (APD) for maximum sensitivity.

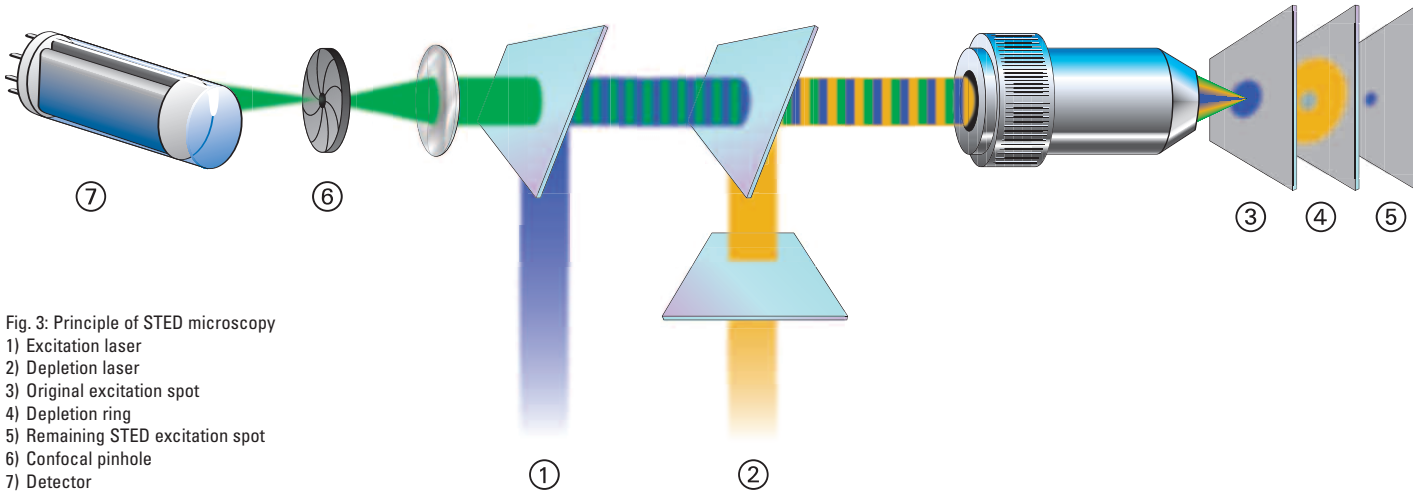


Fig. 3: Principle of STED microscopy
1) Excitation laser
2) Depletion laser
3) Original excitation spot
4) Depletion ring
5) Remaining STED excitation spot
6) Confocal pinhole
7) Detector

STED microscopy

The second approach to breaking the resolution barrier is taken by STED microscopy. Here, controlled localised saturated depletion of the fluorescent molecules leads to diffraction-unlimited resolution. The STED microscope is based on a Confocal Laser Scanning Microscope, with a pulsed fluorescence laser excitation beam focused on the specimen.

This beam is superimposed by a donut-shaped, tightly synchronised pulsed red-shifted laser beam which forces the excited fluorophores to move to an upper vibrational level of the ground state followed by ultra-fast vibrational decay.

As a result, fluorescence in the donut area is completely depleted before it is emitted and the remaining focal excitation spot is sharpened up to reach a diameter (FWH) of approx. 90 nm. Based on typical relaxation times of fluorophores, STED works best with pulse lengths in the picosecond range. The superresolution spot is moved across the specimen by a high-precision confocal scanner and images are collected by sensitive APDs or photomultipliers. Due to the fact that the Point Spread Function (PSF) of STED has a much sharper profile than for conventional confocal microscopy, structures even considerably smaller than 90 nm can be resolved. In the described practical implementation of STED, axial resolution is equal to confocal resolution (approx. 500–600 nm).

The platform of the Leica TCS STED system is the Spectral Confocal Microscope Leica TCS SP5 with multiphoton extension. In addition to providing a Superresolution STED channel, the system can be fully utilised as a universal confocal/multiphoton imaging system in a shared resource laboratory. Correlative localisation experiments can be performed between the STED channel and up to 4 confocal channels. The STED beam coupling and conditioning path is self-aligning to ensure superresolution performance, user comfort and long term stability.

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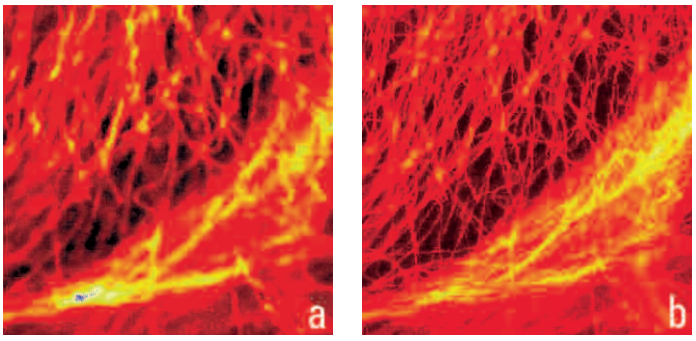

Fig. 4: Enhanced detail on microtubules in PTK2 cells, revealed by STED microscopy
a) Confocal image
b) STED image

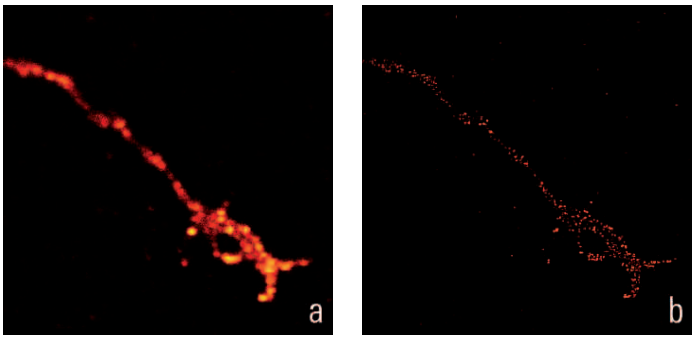

Fig. 5: Drosophila neuromuscular synapses. Liprin Protein, stained with ATTO 647N.
a) Confocal image
b) STED image, unfolded
The STED image resolves substructures in the presynaptic active zones. Courtesy of Stephan Sigrist, Würzburg, Germany

Professor Stefan Hell Wins German Future Award

Today, Professor Stefan Hell, Director at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany, has proven that Ernst Abbe's law is not irrefutable. He developed the STED fluorescence microscope which goes ten times beyond the Abbe limit.

For this invention, Hell won the 10th German Future Award, presented in November, 2006 by Federal President Horst Köhler. This Technology and Innovation award has a cash value of 250,000 Euros and is conferred annually in recognition of projects that not only have revolutionary implications for science but are also ready for application and marketing.

"STED microscopy breaks with previous rules," explained Stefan Hell in an interview on his nomination for the German Future Award. "Physicists had given up trying to solve the resolution problem and it was a closed book for biologists, who were confronted with it, as it is a problem of physics. On top of this, physics had developed in a completely different direction. In the nineties, optics sounded – how shall I put it? – rather old-fashioned. Physicists found solid state and high-energy physics more exciting. And every physicist knew: light is diffracted in waves. So there's simply no way of making this area of light smaller. And because it's impossible to make it smaller, nobody will find a solution, period! However, my sixth sense told me: there must be more to it than that. In fact, I was certain of it, at least as far as fluorescence microscopy was concerned."

Stefan Hell also invented 4Pi microscopy. Leica Microsystems won the 2005 Innovation Award of German Industry for developing the Leica TCS 4PI as a marketable instrument. The STED microscopy patented by the Max Planck Institute was licensed to Leica Microsystems, which is developing it into a user-friendly instrument that will be launched in 2007.

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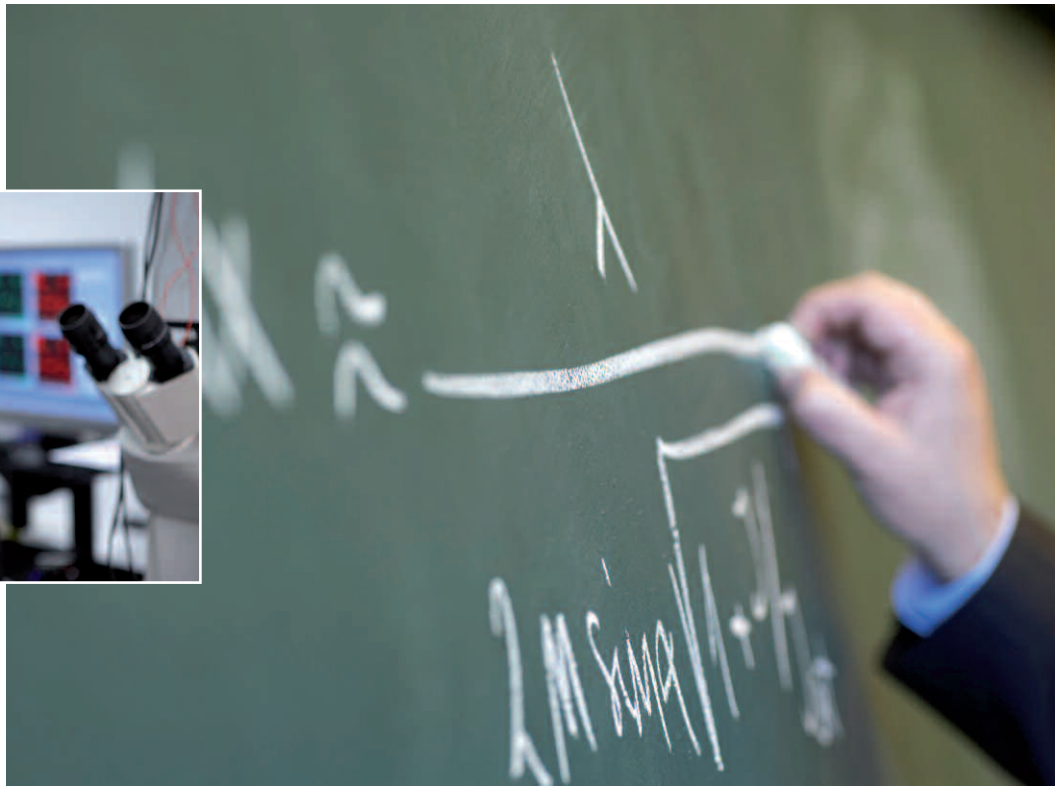


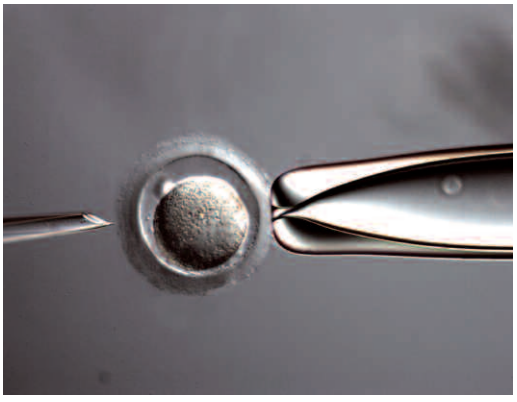
© German Future Award, photo: Ansgar Pudenz

Integrated Modulation Contrast (IMC) – Easy and Flexible Microscopy without Special Objectives

"Oblique Illumination" Enhances Visibility of Living Cells

Dipl.-Phys. Bernard Kleine, Leica Microsystems CMS GmbH; Anja Schué, Leica Microsystems GmbH

Hoffman modulation contrast has established itself as a standard for the observation of unstained, low-contrast biological specimens. Its innovative technical implementation by Leica Microsystems now permits significantly simpler handling and greater flexibility in deployment. The integration of the modulator in the beam path of the microscope allows a wide range of brightfield or phase objectives to be used, rather than a small selection of special objectives. For the first time, contrast can be modified and optimised individually using freely accessible modulators.



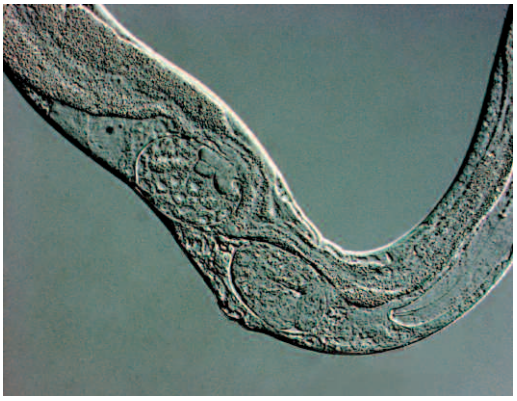


Fig. 1a, b: Modulation contrast visualises transparent, low-contrast specimens by converting phase gradients into brightness gradients. As a result, high-contrast grey levels are formed that create the impression of a three-dimensional image. Fig. 1a courtesy of C. Mehnert, In-Vitro Fertilisation Centre, Gießen, Germany.

Modulation contrast offers outstanding cell visualisation options, especially when using manipulation techniques such as in-vitro fertilisation. The combination of modulator and slit diaphragm converts density gradients of the specimen into brightness gradients with the aid of "oblique illumination". The objective-side modulator has areas of differing light transmission. The light falls obliquely onto the specimen plane via the illumination-side slit diaphragm and is diffracted at phase transitions. It is deflected toward the opaque or transparent part of the modulator depending on the operational sign of the slope. The result is a relief-like, three-dimensional image impression corresponding to the light and shadow surfaces of obliquely illuminated objects (Fig. 1a, b). However, the original technical implementation required special objectives because the modulator was permanently built into the objective. These objectives are neither optimised for brightfield nor for fluorescence. Also, every time the objective or magnification was changed, the slit diaphragm had to be manually aligned with the modulator.

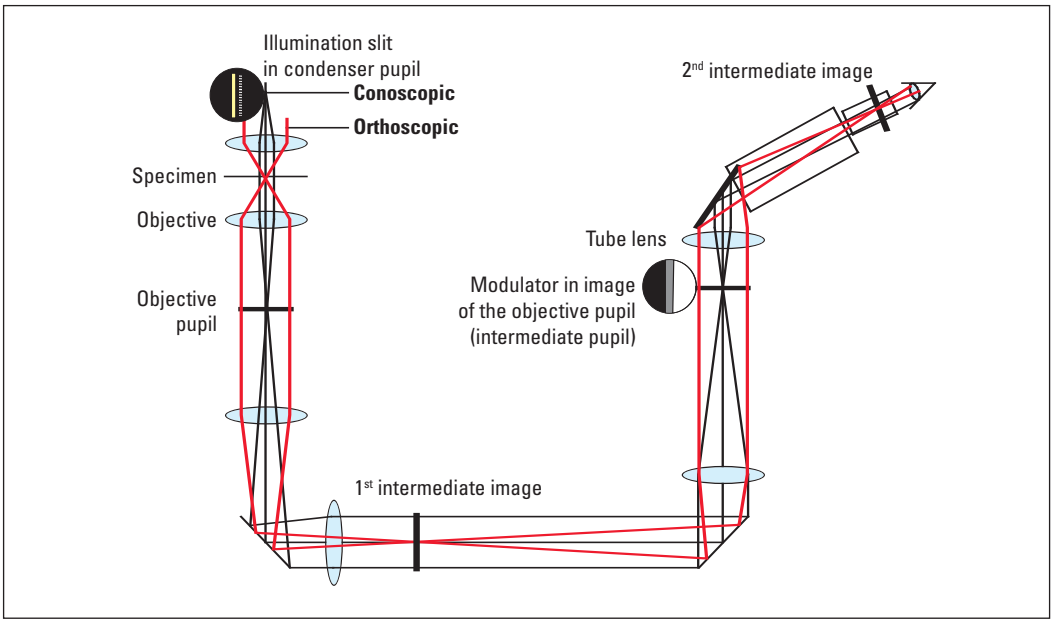


Fig. 2: IMC solution in the inverted digital microscopes of Leica Microsystems. The modulator is freely accessible outside the objective and positioned in the intermediate pupil for flexible adjustment. The slit diaphragm is positioned in the condenser pupil.

IMC – more freedom

In the case of Integrated Modulation Contrast (IMC) the modulator is integrated outside the objective in a focal plane conjugate to the condenser (intermediate pupil) in the beam path (Fig. 2). The modulators are mounted on sliders which are inserted into the interpupillary interface on the side of the stand (Fig. 3, 4). They are therefore freely accessible and allow the user to easily adjust the contrast as required. Beyond this, it is also possible to use modulators with different transmissions. The slider also features a brightfield position for unmodulated images. As with DIC, all Leica Microsystems microscopes that feature IMC have been set up to ensure that the orientation of the contrast (shadow direction of the 3D impression) is identical for all objectives. The spatial impression of the specimen thus remains unchanged when changing objectives or magnifications, so there is no need to adjust the slit diaphragm.

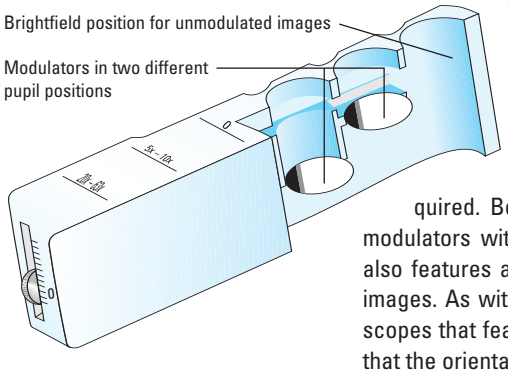


Fig. 3: The IMC slider with modulator discs in two different pupil positions are easy to use and permit individual adjustments.

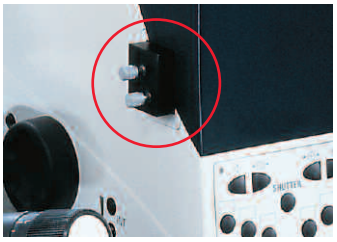


Fig. 4: The modulators are mounted on sliders, which are inserted into the so-called integrated interpupilary interface on the side of the stand.

A fundamental consideration when uncoupling the modulator was that the rear focal plane of the objective can be anywhere within the entire range of the 45 mm parfocality distance. A modulator outside of the objective must therefore be movable by at least that distance. Thanks to the design of the Leica objectives, which is defined by fixed focal planes, deployment in inverted microscopes and fixed magnification steps from 5x to 63x, it was possible to reduce the number of modulation planes to two pupil positions (Fig. 5). The beam path to the image of the pupil, the so-called intermediate pupil, is designed as a 1x telescope system. The infinite beam path behind the objective is reproduced in the intermediate pupil. As a result, plane-parallel optical modulators of any thickness can also be inserted into the beam path at the intermediate pupil, tilted or untilted, without impairing the position or quality of the image. Stray and false light are minimised by the optimal monochromatic and chromatic correction of the telescope system as well as the extremely low reflection properties of the vapour-deposited grey and dark metal coatings of the IMC modulator.

The advantages of IMC:
- additional special objectives not required
- one objective line for all contrast methods without transmission losses
- quick adaptation to new applications through modulators with varying transmission
- individually adjustable contrast properties

Individually variable parameters

The innovative and user-friendly concept of Leica Microsystems' IMC offers plenty of scope for individual adjustment of the modulation contrast. The modulator is adjustable vertically to provide a homogeneous image at all times. Varying stage or buffer solution levels and different positions of the cells within the specimen dish can thus be compensated. Moving the modulator sideways alters the resolution: The further the modulator and the slit diaphragm are moved outwards, the higher the resolution (max. resolution Res = λ\2NA).

Two modulator parameters affect the strength of the contrast: the transmission and the width of the grey

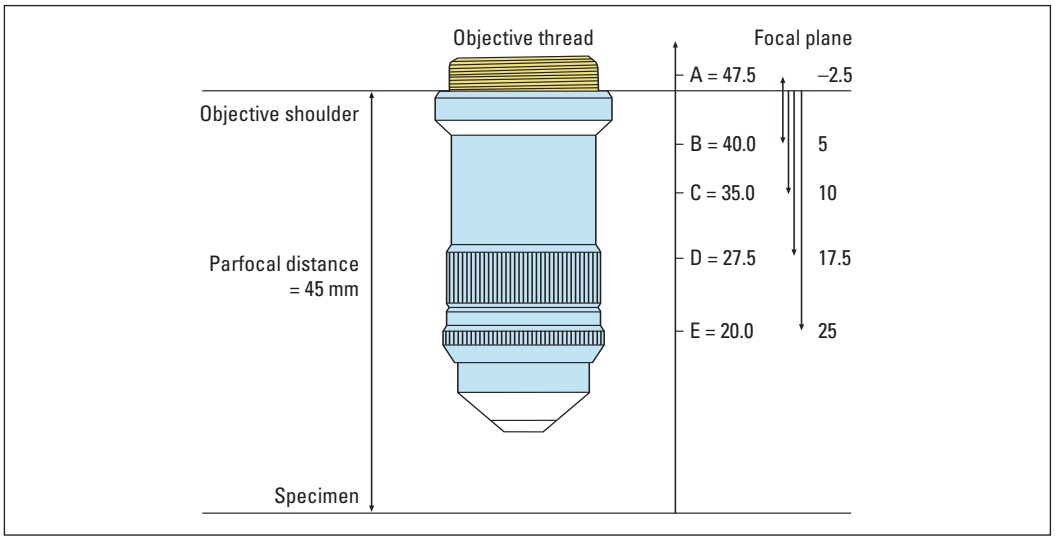


Fig. 5: Leica objectives are defined by fixed focal planes. Due to their use in inverted microscopes and due to fixed magnification steps of 5x-63x, it was possible to reduce the number of modulation planes to two pupil positions.

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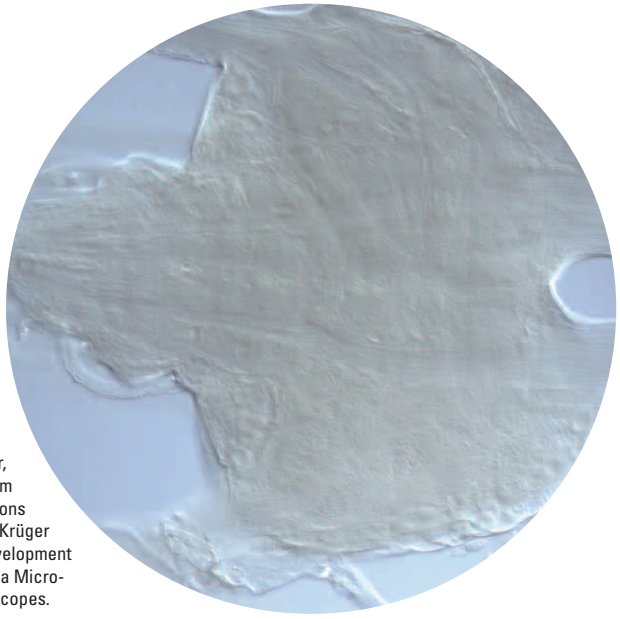
area. Reducing transmission increases contrast. Transmission of 25%, for example, results in smooth contrast. With a value of around 10%, the contrast becomes significantly crisper. Varying the modulator width is similarly effective (Fig. 6).

Narrowing the grey area results in crisper contrast, while wide modulators soften the overall image impression. The slit diaphragm of the IMC features two openings. The first slit is imaged on the grey area of the modulator, producing the oblique illumination for the modulation contrast. The second slit is covered by a polarisation foil (Fig. 6). A rotatable polariser positioned in front of the diaphragm can increase or decrease the 3D effect of the modulation contrast. The polariser does not affect image quality when working with plastic dishes, as the light is polarised only on the illumination side and no analyser is used on the objective side. The sizes of the slit diaphragms are matched to the most commonly used condensers.

Individually adjustable IMC parameters:
- homogeneity
- resolution
- contrast intensity
- relief effect

IMC is therefore not only suitable for routine examinations of biological material – it is also an excellent tool for demanding applications such as micromanipulation, microinjection or microdissection.



Acknowledgement

We would like to thank Ralf Krüger, Leica Microsystems, Optics System Planning, for the detailed discussions and background information. Ralf Krüger played a significant part in the development and implementation of IMC in Leica Microsystems inverted research microscopes.

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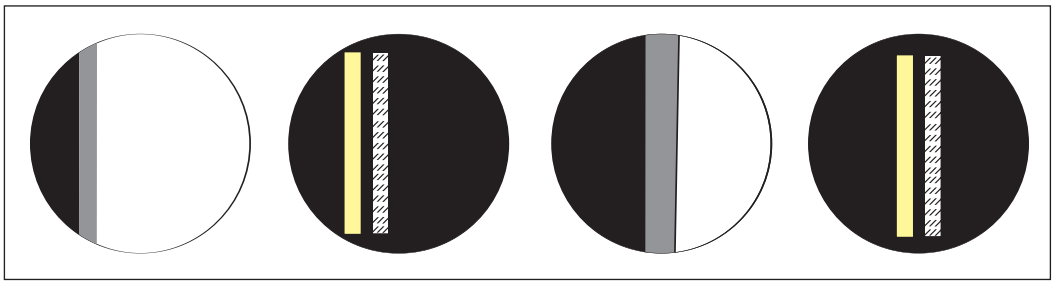
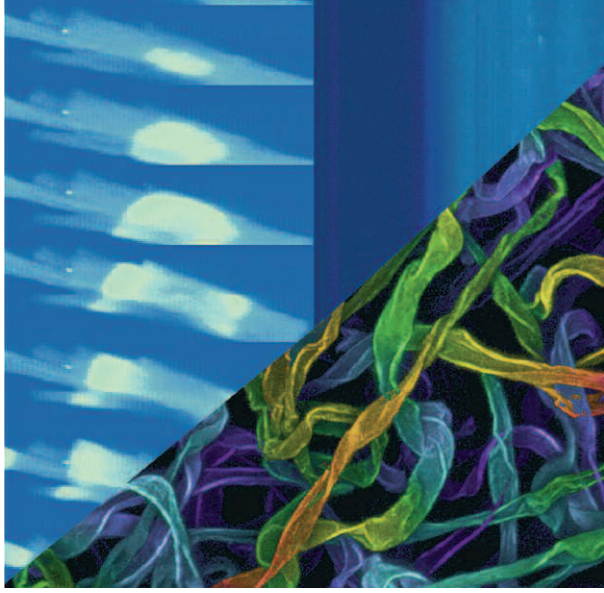


Fig. 6: Laterally adjustable modulators for variable resolution, slit diaphragm with additional polarisation slit for adjustable relief effect.

Really Confocal – True Optical Sectioning at All Speeds

Dr. Rolf Borlinghaus, Leica Microsystems CMS GmbH



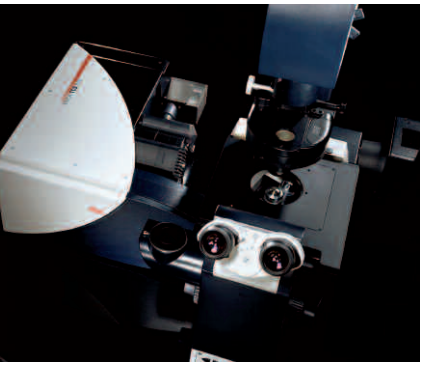


Fig. 1: Leica TCS SP5 – the broadband confocal

Two worlds …

Confocal microscopists today need appropriate equipment for numerous applications and the system requirements very often seem contradictory at first glance. Applications of confocal microscopes can be divided in two major areas: morphological studies, which typically represent multiparameter fluorescence in fixed samples, requiring high resolution imaging for 3D-reconstruction and data extraction by morphological measurements. Here, speed is welcome but it is not the major issue. Noise-free, high resolution imaging, however, is a must in order to see structural details, interconnections of organelles, proteins and so forth.

On the other hand, microscopes serve more and more as dynamic measuring machines: systems not primarily dedicated to creating nice images, but significant numbers. Physiological data such as Ca^{2+}-concentration changes, electrical potential differences and various probes for metabolites, proteins and genes are the target of biophysical research, preferably in living samples. Here, speed is a key for insight into dynamic processes and kinetic relationships.

… and one more

The latest advances in fluorescence microscopy combine these two worlds: fluorescent proteins reveal both structural and dynamic features in living material, including the dynamics of the structure itself. This complex application requires new approaches. Especially in multi-user laboratories, high resolution as well as high speed are often requested.

To meet the researcher's requirements, two different types of microscope are on the market: systems for high resolution imaging, and systems for high speed. As a user, one has to invest two times in order to have all options. And due to technological constraints and preferences, the fast systems on the market typically trade resolution, optical sectioning performance and multimode features against speed.

With the Leica TCS SP5 spectral true confocal microscopy system, these challenges have been solved.

High resolution and high speed – in one system

The Leica TCS SP5 combines two technologies in one single system. For extreme resolution and maximum signal-to-noise, a conventional scanner is available. It allows freely tunable speed anywhere from 1 Hz to 1,400 Hz, resulting in frame rates up to 5 Hz for full 512^2 pixel images. Conventional scanners allow direct signal averaging, spot data acquisition and spot-photobleaching or spot-photoactivation. The tunable slow speed also opens new approaches, like image correlation measurements. Besides variable speed, the conventional system also allows frame sizes of up to 8,192 x 8,192 pixels (64 Megapixels) – which is not just a high number, but required to transport the large optically resolved content of images acquired by high performance optics.

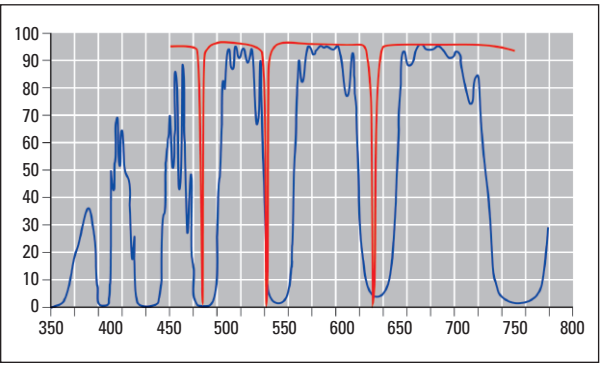
A resonant scanning system offers time resolution of up to 16,000 Hz line frequency corresponding to time resolution of 62.5 µs. Full frames of 512^2 pixels are available at 25 frames per second, and towel-formats allow up to 250 frames per second. Still it is truly confocal – different from line scanners or spinning disc systems, which sacrifice optical sectioning performance for speed – a questionable approach for confocal microscopes. The Leica TCS SP5 offers both scanning modes. By its unique Tandem Scanner both technologies are mechanically and electronically merged into one single instrument.

Fig. 2 at top of page: Left: Ca^{2+} waves in muscle cells shown by a fluorescent Calcium-indicator and recorded with a resonant scan system. Courtesy of Dr. Eisner, University of Manchester, UK. Right: Vitis vinifera. Hairs on young leaves. Color coded projection of 100 optical sections recorded over 200 µm; autofluorescence.

An absolute precision actuator provides the possibility to switch from conventional to resonant scanning during system initialisation. This means, the researcher is free to investigate one specimen through both approaches – a vital precondition for experiments with micropipettes inserted in the sample.

All those benefits

The advantages of favouring resonant scanning over other solutions are obvious. First, the sectioning is really confocal and does not suffer from spatial leakage, as multi-spot or line-scan approaches do. Furthermore, there is no need to accept compromises in multiparameter fluorescence, which is one of the other significant drawbacks of alternative solutions. The Leica TCS SP5 features five confocal channels in parallel. Emission selection is done for all channels and scanning techniques by Leica's SP® detectors, the only real spectral device for imaging, with tunable emission bands and maximum efficiency. Moreover, the high-transmission and tunable photon-gate, the Leica AOBS®, fits to both scanners, avoiding all disadvantages of dichroic mirrors – there is no better way to separate excitation from emission.



High transmission of Leica AOBS® shown in red. Higher white transmission, steeper slopes, wider bands and better excitation suppression. Comparison: dichroic beam splitting mirror (blue line)

Finally yet importantly, the resonant scanner serves for a field of view of 12.5 mm, the conventional scanner for 22 mm, and both allow zoom and pan functions as well as rotation without speed-compromises. Of course, UV, Vis and IR excitation is possible in all configurations.

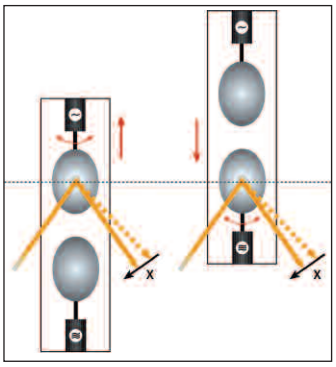
Superficial reasoning on photonic efficiency was unjustifiably casting doubt on resonant scanning as a solution for fast scanning. As it turned out, emission intensity was even increased and photobleaching was decreased. The first is because upon short excitation times, fewer fluorochromes will stay in triplets. Repeated short illumination therefore delivers more fluorescence per time as compared to a single (equivalent) long time exposure. As triplet incidence is lower, the probability for excited triplets also decreases – preventing the molecule from photo damage caused by these high-energy states.

A high achiever: Leica TCS SP5

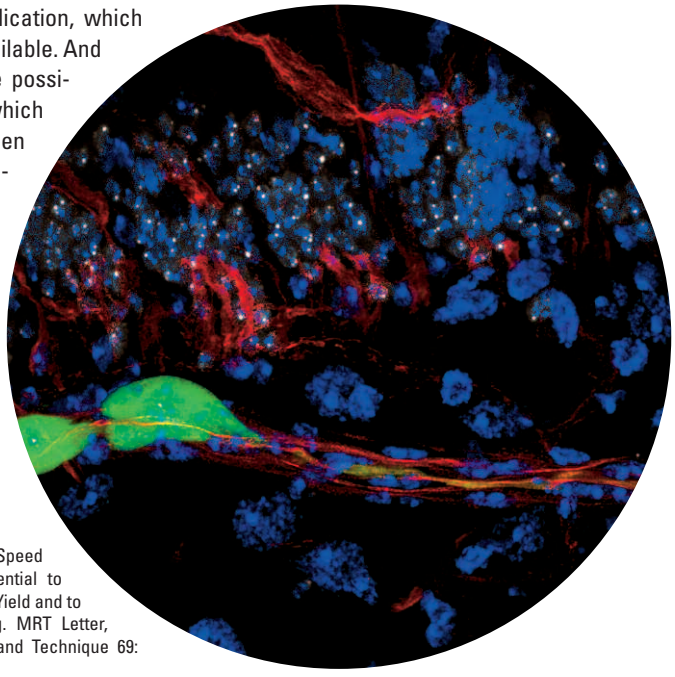
An additional advantage which comes with the new Leica TCS SP5: two out of the five spectral channels may be equipped for fluorescence lifetime imaging (FLIM). In conjunction with the spectral imaging performance, this setup opens a new dimension: spectral FLIM (SP-FLIM), Fluorescence lifetime imaging as a function of emission wave-length.

With the Resonant and the Tandem Scanner, the SP®-detector, AOBS® and SP-FLIM, Leica again sets new and very ambitious standards in confocal microscopy: Both high performance low noise imaging and high speed true confocal data acquisition – covered by the new Leica TCS SP5 – open up new areas of application, which were so far not available. And there will be more possibilities tomorrow which have not yet been thought of. The creativity of modern fluorescence scientists will surely challenge this and bring new applications to the surface.

> RB



Tandem Scanner:
By means of a motorised and computer controlled high precision device, a conventional and a resonant galvanometric driven scan mirror are exchanged into the proper position for scanning, while the scan electronics are switched simultaneously.



Drosophila menalogaster
Green: Feb211 positive neurons and their axons, Alexa 488;
Red: Fibrous part of the CNS (i.e. all axons), Cy3;
Blue: Nuclei of neurons, Alexa 594.

Reference

Borlinghaus, R. T.: High Speed Scanning has the Potential to Increase Fluorescence Yield and to Reduce Photobleaching. MRT Letter, Microscopy Research and Technique 69: 689–692 (2006).

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Leica TCS SP5 Tandem Scanner – an Ideal Broadband Confocal System

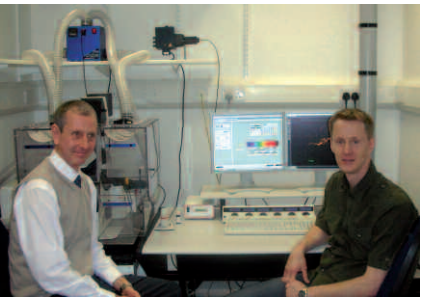
Confocal Microscopy in Protein Research and Neurosciences

Malcolm Lang, Leica Microsystems (UK) Limited

The Leica TCS SP5 Tandem Confocal Microscope in the Faculty of Life Sciences, University of Manchester, is enabling scientists with such diverse research interests as dynamic cellular imaging and high resolution morphological studies to gain an insight into the movement of proteins in relation to programmed cell death and how changes in neuronal growth cone behaviour are mediated through regulated changes in cytoskeletal components.

The University of Manchester, UK recently awarded its £ 1 million President fund to the Faculty of Life Sciences, Bioimaging Unit. With the funds available to double their imaging capacity and support staff, the Unit invested in two state-of-the-art confocal microscopes. One is a Leica TCS SP5 Multiphoton and the other a Leica TCS SP5 Tandem Scanning System. This increases their Leica Microsystems equipment to three confocal microscopes and two Leica AS MDW, dedicated workstations for multi-dimensional live cell imaging. The £ 39 million Michael Smith Building which opened in January 2004 houses a third of the faculty's academic staff, making it one of the most successful research units in Europe.

Dr. Peter March, Bioimaging Facilities Manager at the University of Manchester, UK (right) and Malcolm Lang, Leica Microsystems (UK) Ltd., (left) with the Leica TCS SP5 Tandem Scanning System.



University of Manchester, Bioimaging Facility: http://www.ls.manchester.ac.uk/research/facilities/bioimaging/

Dr. Peter March, Bioimaging Facilities Manager, explains why Leica Microsystems were the obvious choice for their investment. "This is a multi-user facility, available to the faculty of Life Sciences, the University and beyond. As such, it was essential that we purchased a confocal system that was very versatile. The Leica TCS SP5's are able to meet everyone's requirements and along with our other Leica systems are extremely user friendly". Initially, the unit had intended to purchase a spinning disc system, but the Leica TCS SP5 Tandem confocal microscope was able to meet the specifications for fast imaging of dynamic events, with high-resolution multi-channel imaging, all in one easy to use system.

Dr. March emphasises that the Leica system with twin scanners (high resolution and fast resonant) is easily adapted to a diverse range of applications, from dynamic plant and animal cell imaging to ultra fast diffusion rate studies, FRAP (Fluorescence Recovery After Photobleaching), FRET (Fluorescence Resonance Energy Transfer), Quantum dot spectral imaging, high resolution multichannel fluorescence imaging, multi-point time-lapse studies on brain slices to investigate circadian rhythms over 72 hour periods, to mention a few. "Essentially, the system can accommodate most current applications and those anticipated in the future. A particular advantage of the Leica TCS SP5 with resonant scanner is its low photo toxicity and photobleaching of live cells" says Dr. March. He commented that the amount of photobleaching due to the imaging process has been minimal, allowing investigation of drug induced apoptosis over long periods of time.

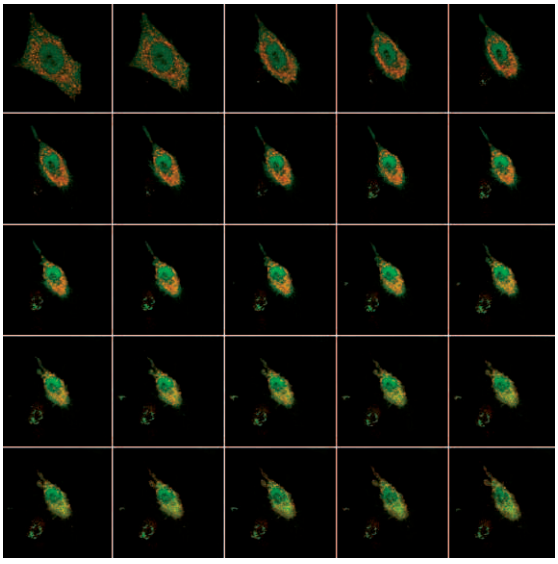


Fig. 1: Human HeLa Cervical Cells. Red Mitochondrial label DSRed-Mito. Green is EGFPBax. A truncated time series showing maximum intensity projected xyzt images, 512 x 512 pixels, 8 frames per second, 8 images per stack, over 86 minutes. The Z-stack at each time point was projected using the Leica LAS AF maximum intensity projection function.

Organelle functions in health and disease

The research interests of Dr. Viki Allan, Senior Lecturer in the Faculty of Life Sciences, are focussed around studies on protein trafficking, regulation, function and their disfunction in the diseased state. Dr. Allan has begun observing apoptosis, or programmed cell death, in human cervical HeLa cells over extended periods using the confocal system. Apoptosis causes dramatic changes in cellular architecture following the activation of specific proteases, called caspases. These changes are needed to allow the dead cell to be removed from the body by phagocytosis. Dr. Allan has worked with Drs. Philip Woodman and Martin Lowe to investigate what happens to the cytoskeleton and membrane trafficking pathways (Lane et al., 2002; Lane et al., 2005). Dr. Allan's group is now investigating the ways in which mitochondrial morphology changes during apoptosis. The apoptosis is induced in the cells using a recombinant TRAIL to target the TRAIL receptor-2 protein. The group have begun using the Leica TCS SP5 Resonant Scanner to acquire images of HeLa cells undergoing apoptosis. The system allows 512 x 512 pixel images to be acquired at a rapid rate (up to 25 frames per second at this format). In this experiment stacks of images were acquired at 8 fps for more than 80 minutes. The data are then replayed as a time series, providing a dynamic record of the cellular changes. The group can demonstrate that the translocation of EGFP to clusters next to the mitochondria only becomes visible after the execution phase (cell rounding phase) of apoptosis has commenced. This is shown in figure 1 as a truncated series of images from the original time series data.

Molecular mechanisms of axonal growth

Dr. Natalia Sanchez-Soriano is a scientist in Dr. Andreas Prokop's group within the Faculty of Life Sciences. The group is using the Leica TCS SP5 conventional scanner to research axonal growth cones in the development of the nervous system. In the developing nervous system, neurons form long axonal processes in an organised manner to form a functional nervous system. The key structures regulating this axonal growth are the so-called growth cones. Growth cones sense their environment and respond to it by modifying their behaviour through organised changes of their cytoskeletal components. "Our work aims at understanding the molecular mechanisms governing these cytoskeletal changes by using our newly established neuronal primary cell cultures obtained from Drosophila melanogaster embryos", explains Dr. Sanchez-Soriano.

The group is using antibodies on different cytoskeletal components and demonstrating these with the Leica TCS SP5 confocal. In figures 2 and 2a, they have used anti-tubulin primary with CY3-conjugated secondary antibodies to label the microtubules in the growth cones (green channel), with filamentous actin labelled with FITC-conjugated Phalloidin (red channel). The neuronal surfaces are demonstrated in the blue channel using CY5-conjugated anti-horseradish peroxidase (HRP) antibodies. The signals are generally of low light level, which means the combination of high resolution and sensitivity is an important prerequisite for their results.

Using Leica LAS AF software, these overlay colours can be optimised to facilitate interpretation. In figures 3 and 3a, the microtubules are now demonstrated with the blue channel, the filamentous actin with the red channel and the neuronal surfaces with the green channel (via targeted expression of moesin: GFP detected with anti-GFP antibodies and FITC-conjugated secondary antibodies).

Dr. Sanchez-Soriano explained that Drosophila neuronal growth cones can now be visualised with subcellular resolution, both in situ and primary cell cultures. "Via such analyses, we were able to show the role of the cell adhesion molecule Fasciclin II (Drosophila N-CAM homologue) in pioneer guidance of growth cones (Sanchez-Soriano and Prokop, 2005). We are currently investigating how changes in growth cone behaviour are mediated through regulated changes of their cytoskeletal components."

The Leica TCS SP5 broadband confocal enables scientists normally requiring two separate confocal systems for the investigation of dynamic cellular events and high-resolution morphological studies to use an all in one, cost effective point scanning confocal microscope.

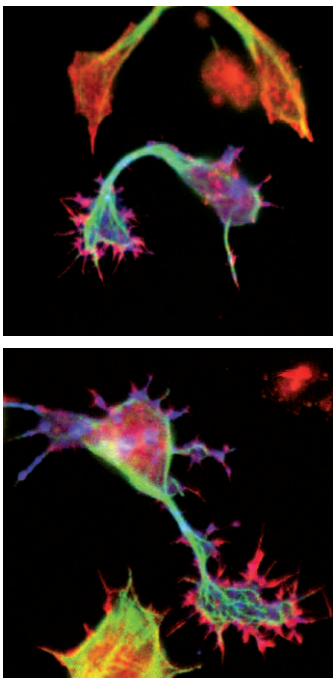


Fig. 2 and 2a: Cytoskeletal components of axonal growth cones. Green: microtubules; Red: filamentous actin; Blue: neuronal surfaces.

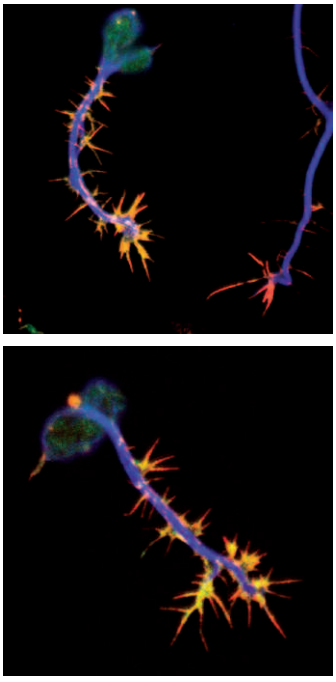


Fig. 3 and 3a: Cytoskeletal components of axonal growth cones. Blue: microtubules; Red: filamentous actin; Red: neuronal surface.

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HaloTag Interchangeable Technology & Confocal Microscopy

FRAP: Analysis of Protein Dynamics with a Non-fluorescent Protein

Dr. Jan Schröder, Leica Microsystems CMS GmbH; Dr. Truc N. Bui, Promega GmbH

Fluorescence Recovery after Photobleaching (FRAP) is often employed to analyse protein dynamics [1, 2]. All FRAP experiments discussed below were performed with a Leica TCS SP5 confocal microscope. Instead of commonly used fluorescent proteins (FPs) the HaloTag Interchangeable Labelling Technology, which is based on non-fluorescent HaloTag protein, was used. Signals were detected by incubation with various fluorescent ligands, which are able to covalently bind to the HaloTag protein.

In FRAP experiments fluorescent molecules are bleached by a laser pulse in a selected region of a specimen and the subsequent increase of fluorescence is measured in the same region. If fluorescent molecules diffuse through the specimen, kinetic parameters of a protein can be determined by the increase of fluorescence. For example, the diffusion constant, mobile fraction, transport rate or binding and dissociation rate of other proteins can be determined [3]. To investigate protein dynamics in living cells, genetic fusions of the examined proteins with fluorescent proteins (FPs) are mostly used [4]. In recent years, new imaging technologies have been developed which are based on the genetic mutation of non-fluorescent protein motives [5] or modified enzymes [6, 7]. They can be detected either by membrane permeable ligands attached to fluorescent dyes bearing a high affinity to the protein motive or by ligands which undergo a covalent binding with the modified enzyme. The HaloTag Interchangeable Technology (Promega, Mannheim, Germany) is based on the genetic mutation of a monomeric prokaryotic hydrolase enzyme expressed in cells. Due to its prokaryotic origin, endogenous activities in mammalian cell systems can be almost excluded. The HaloTag protein and the HaloTag ligands do not show any detectable cellular toxicity or morphological side effects with the applied experimental conditions [7]. To perform FRAP experiments with the Leica TCS SP5 confocal microscope, two HaloTag fusion proteins stained with HaloTag diAcFAM or TMR ligands were used. Both are easy to apply and are specific for FRAP.

Tab. 1: Hardware setting of Leica TCS SP5 laserscan microscope

	HaloTag dicFAM	HaloTAG TMR
Objective	HCX PL APO 63.0x1.20 WATER UV	HCX PL APO 63.0x1.20 WATER UV
Scan speed (Line frequency)	1400 Hz bidirectional scan	1400 Hz bidirectional scan
Excitation wavelength	488 nm	561 nm
Emission range	495–600 nm	568–672 nm
Format	256 x 256 pixels	256 x 256 pixels
Zoom	~ 6	~ 6
Laser power (Argon laser)	~ 100%	–
AOTF (imaging) 488 nm	5%	–
AOTF (imaging) 561 nm	–	10%
AOTF (bleaching) 488 nm	100%	–
AOTF (bleaching) 561 nm	–	100%
ROI geometry	circle (diameter 3 µm)	circle (diameter 3 µm)
Prebleach	10 x 118 ms	10 x 118 ms
Bleach	1 x 118 ms	1 x 118 ms
Postbleach 1	50 x 118 ms	50 x 118 ms
Postbleach 2	30 x 1 s	30 x 1 s

Material and Methods

HeLa cells were plated on 8-well chambered cover glass (Nunc, Wiesbaden) and incubated under conditions for standard cell cultures. The next day, cells were transiently transfected with TransIT® LT1 reagent – with or without HaloTag expression plasmids – (Mirus, Madison, USA). Plasmids were fused with human α-Tubulin or with the human p65 subunit of the transcription factor nuclear factor-κB (NF-κB). 16 hours after transfection, cells transfected with HaloTag α-Tubulin were stained with HaloTag-diAcFAM and cells transfected with p65-HaloTag were stained with TMR ligands according to the manufacturer's protocol (Promega). FRAP experiments and data analysis were done with the Leica FRAP application wizard. The experimental settings of the confocal microscope are displayed in table 1.

Results

For the FRAP experiments, cytoplasmic regions of stained cells were chosen and selectively bleached.

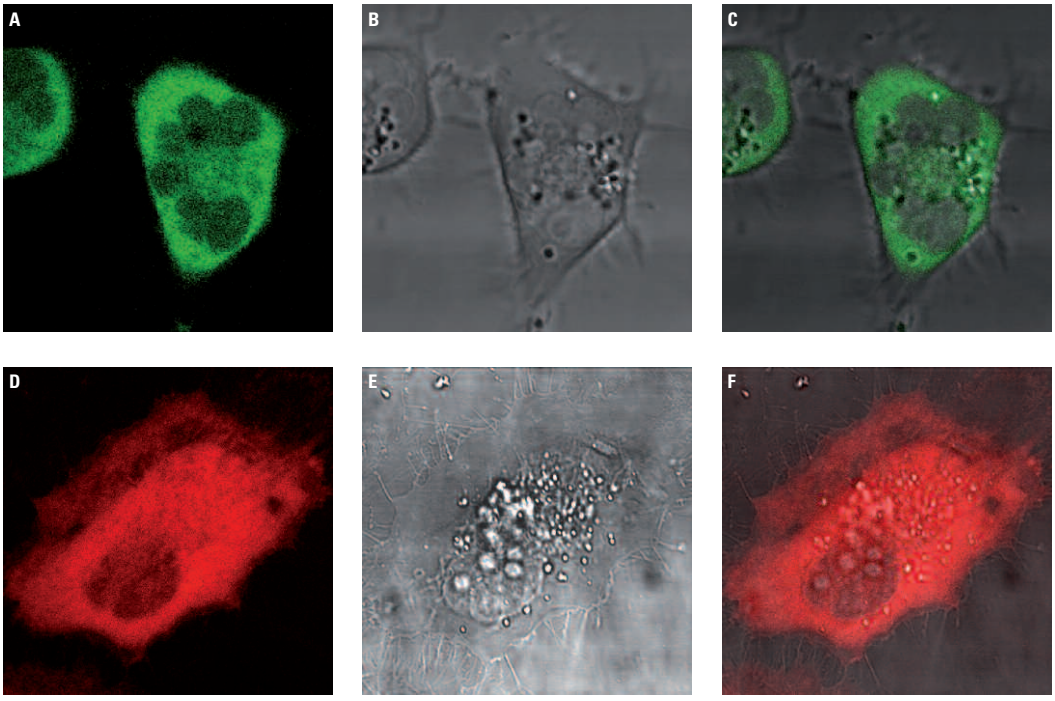


Fig. 1: A–C, α-Tubulin-HaloTag fusion protein stained with HaloTag-diAcFAM ligand. D–F, p65-HaloTag fusion protein stained with HaloTag-TMR ligand. (A, D) fluorescence, (B, E) transmitted light, (C, F) overlay of fluorescence and transmitted light image.

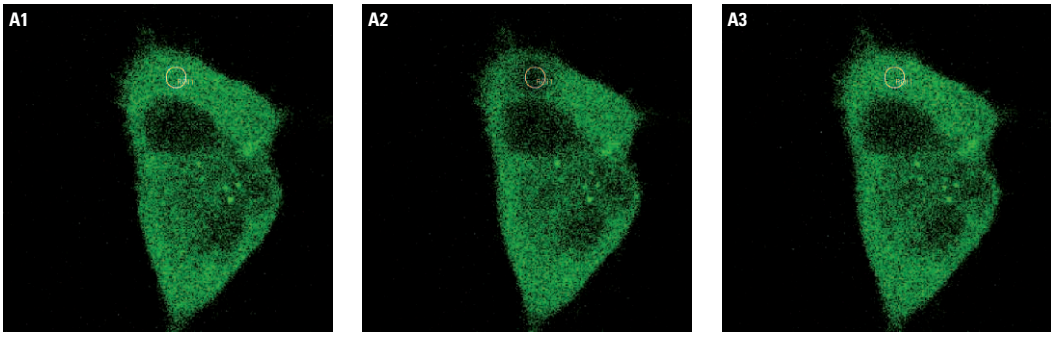
The loss of fluorescence caused by the image acquisition was corrected in the data and normalised with respect to the prebleach intensity. Transmitted light images were recorded in parallel to demonstrate the cell shape (Fig. 1). Cells stained with the HaloTag-diAcFAM ligand showed a decrease of fluorescence by 35% of the original intensity after the bleaching pulse. Approx. 30 seconds later, the original intensity was reached (Fig. 2).

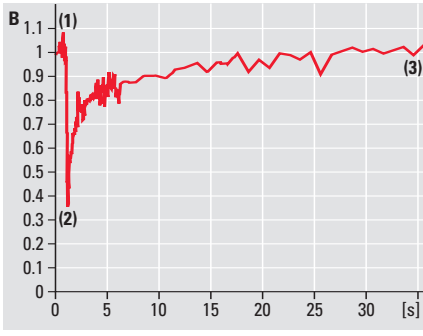
This experiment represents other experiments with HaloTag-diAcFAM stained cells. In all experiments, the half-time recovery of 1.14 ± 0.38 seconds was observed. Cells stained with the HaloTag-TMR ligand were investigated under the same conditions recovered with a half-time of 1.24 ± 0.15 seconds, a slightly slower kinetic (Fig. 3). For image acquisition before and after bleaching, the DPSS 561 nm laser was used. To achieve more effective bleaching, the 488 nm Argon laser was additionally applied only for bleaching. Here the initial fluorescence intensity decreased more than 80% and after 30 seconds the fluorescence reached 85% of the initial fluorescence (Fig. 3). Non-transfected cells were stained as control of specificity of the staining with both ligands. With the excitation wavelengths mentioned above the cells did not show any detectable fluorescence (data not shown) either for diAcFAM or for TMR.

Discussion

The use of new specific fluorescence ligands [5–7] with different spectral characteristics allows more flexibility to detect non-fluorescent proteins. Time-consuming and cumbersome sub-cloning work can be avoided with the described methods. However potential toxicity and specificity need always to be taken into account when applying these technologies. Since the HaloTag Interchangeable Technology is based on a prokaryotic protein, the non-specific staining in mammalian cells is significantly reduced.

Fig. 2: A, FRAP experiment with α-Tubulin-HaloTag fusion protein stained with HaloTag-diAcFAM-Ligand. A1) Last prebleach, A2) first postbleach, A3) last postbleach; B1) last prebleach, B2) bleach pulse with 488 nm argon-laser line, B3) last postbleach.





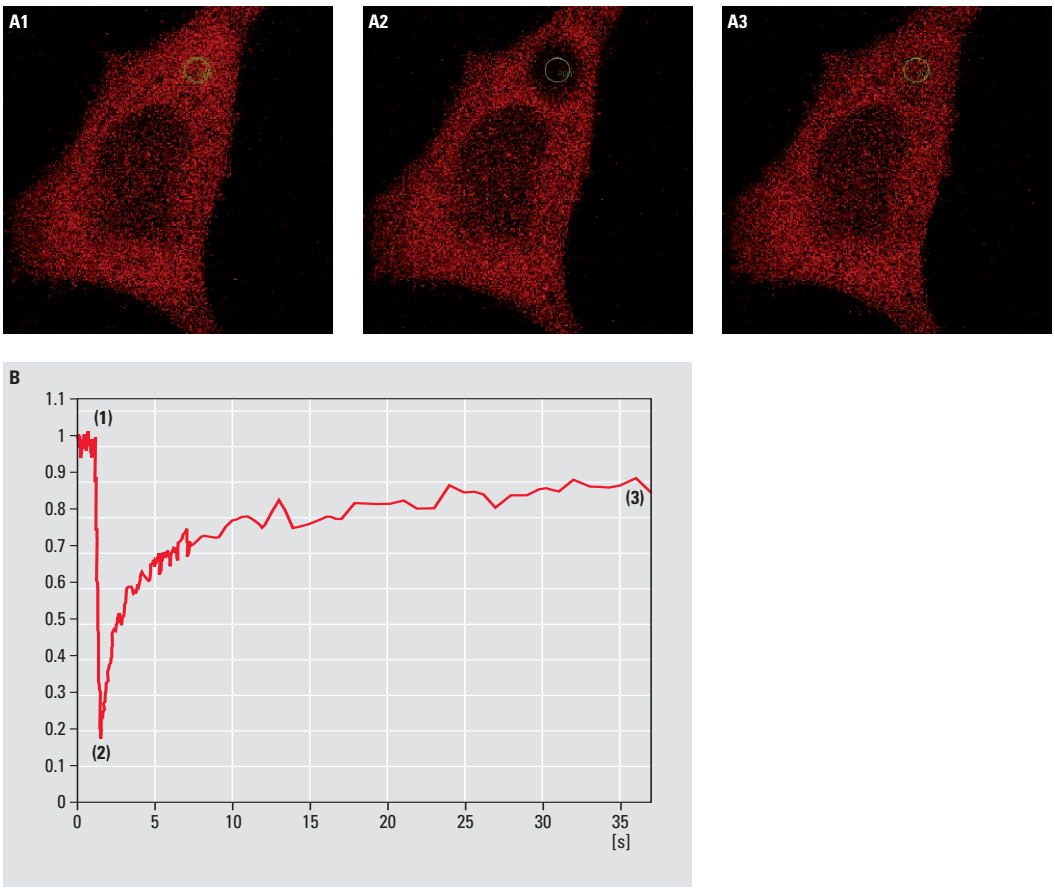


Fig. 3: A, FRAP experiment with p65-HaloTag fusion protein stained with HaloTag-TMR-digand, excitation with 561 nm DPSS laser. A1) Last prebleach, A2) first postbleach, A3) last postbleach; B1) last prebleach, B2) bleach pulse with 488 nm argon laser line together with 561 nm DPSS-laser, B3) last postbleach.

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Additionally, toxicity could not be observed during long-term investigations [7].

We used the Leica TCS SP5 confocal microscope for the FRAP analysis of human α-Tubulin and p65-HaloTag fusion proteins. Both HaloTag-diAcFAM and TMR-ligand bound specifically to the HaloTag fusion protein and could not be detected in control cells. The HaloTag α-Tubulin experiments showed nearly complete fluorescence recovery which implies that nearly all molecules are mobile. Thus, the immobile fraction is negligible. The kinetic of p65-HaloTag showed a higher immobile fraction which is likely due to the effect of its interaction with other NF-κB subunits and the cytoplasmatic inhibitor protein IκB [8]. Furthermore, it could be shown that FRAP experiments can also be successfully conducted with the excitation of the HaloTag-TMR ligand by 561 nm and with a high bleach efficiency. This enables the use of the "red" channel as an alternative for FRAP analysis in case the "green" channel is occupied.

These examples demonstrate that the HaloTag Interchangeable Technology (Promega) together with modern confocal microscopes like the Leica TCS SP5 is a new flexible and specific approach for analysing protein dynamics with FRAP experiments.

Acknowledgements

For making this publication possible we would like to thank Natasha Karassina, Chad Zimprich and Katharina Bohm from Promega.

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Leica MZ16 F and Leica S8 APO

Leica Microsystems Supports NASA's "Flies in Space"

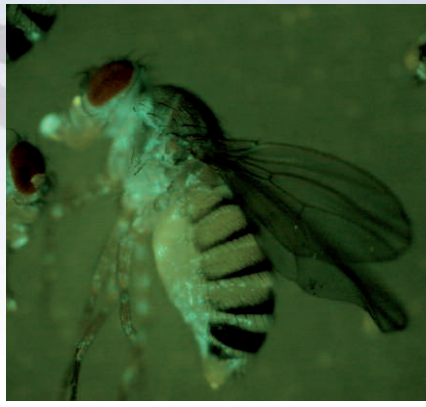
Craig Wollenschlager, Leica Microsystems Inc.

On July 14, 2006, NASA launched fruit flies into space aboard the Space Shuttle Columbia, destined for the International Space Station (ISS). The FIT Fungal Pathogenesis, Tumorigenesis, and Effects of Host Immunity in Space experiment is a comprehensive study to investigate susceptibility to fungal infection, progression of radiation-induced tumors, and changes in immune function in sensitised Drosophila. Leica Microsystems supported the study by providing top-of-the-line imaging systems to the NASA Kennedy Space Center's Life Sciences Laboratory for pre- and post-flight processing and analysis of the specimens.

Headed by Dr. Sharmila Bhattacharya, Principle Investigator; NASA Ames Research Center, Moffett Field, CA, and Dr. Deborah Kimbrell, Co-Investigator; University of California, Davis, the FIT experiment is primarily an examination of how immune system function is affected by the space flight environment. Other studies were coupled with this experiment as well, such as insect pathogen virulence in space. Drosophila melanogaster was selected as the model organism due to its well-known genetic makeup, molecular pathways that control immune functions (similar to humans), and phagocytic immune cell functions (also similar to humans).

Drosophila eggs arrived on the ISS in larval form and hatched while in orbit. The flies were housed in habitats equipped with video cameras to allow researchers to monitor their behaviour – courtship rituals, running speed, how they fly – on all clues to genetic activity. The flies grew and bred to produce the foundation of approximately nine generations of flies. Both stereo (dissection) and compound microscope systems were used during laboratory analysis.

Leica Microsystems provided Leica MZ16 F and Leica S8 APO stereomicroscopes with fluorescence and brightfield cameras for high-resolution imaging. These systems were used to conduct fly and larvae sorting; determination of sex; physiological examination; dissection of flies, eggs, embryos, and larvae; and examination of whole flies and organs for GFP fluorescence of target proteins or cells. High-resolution images were used to archive the data for later study. The compound microscope examinations involved immunohistochemistry and fungal sporulation processes.



Drosophila melanogaster – the model organism for studying how immune system function is affected by the space flight environment

The Leica MZ16 F and the motorised version, Leica MZ16 FA, are two of the leading fluorescence stereomicroscopes on the world market. Due to unmatched optical performance, fluorescence capabilities, and image quality, the Leica MZ16 F is the ideal research stereomicroscope. It is well suited for demanding fluorescence examination and for analysis and documentation in biology, medicine, pharmaceutical research, and many other fields. Equipped with a 2x planapochromatic objective, the Leica MZ16 F offers the best optical performance of all fluorescence stereomicroscopes: 920x magnification, resolution of 840 line pairs/mm, 600 nm visible structural width, and 0.282 NA (numerical aperture). Adding the optional FluoCombi III and 5x planapochromatic objective to the system can eliminate the process of moving sensitive specimens from the stereomicroscope to a compound microscope.

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For more information on this and other space research, including results, visit: http://spaceresearch.nasa.gov

Correlative Light Electron Microscopy Using High Pressure Freezing

CLEM: Combining the Strengths of Light and Electron Microscopy

Dr. Paul Verkade, School of Medical Sciences, University Walk, Bristol, UK

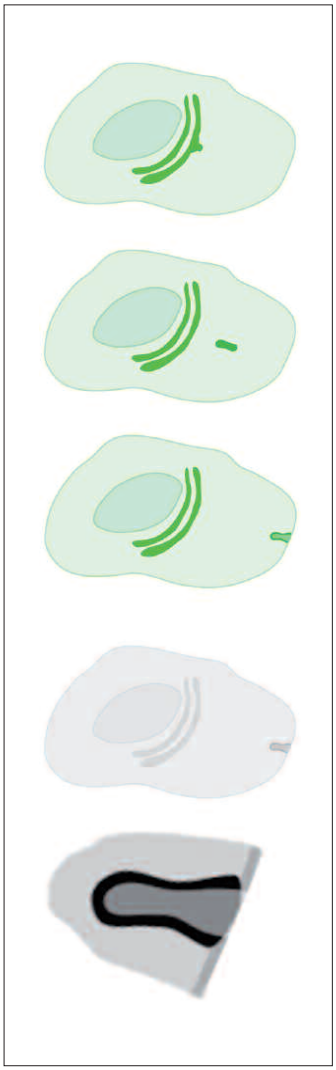


Fig. 1: Schematic model of a CLEM experiment. In a modern CLEM experiment a sample is first studied live under the light microscope (Green cells). A structure inside the cells can be followed (arrows). When an interesting event occurs the sample is fixed and processed for electron microscopy. The same cell is traced back in the EM (grey image) and the structure and event of interest can be studied at high resolution.

In recent years light microscopy studies have been dominated by live cell imaging while electron microscopy has been used for high-resolution studies. Latterly, there has been increasing interest in combining these techniques. This combination is called Correlative Light Electron Microscopy (CLEM). Due to the high resolution made possible by electron microscopy, artefacts induced during preparation of a sample can, however, also be clearly seen. The Leica EM PACT2 with RTS is a high pressure freezer designed for CLEM experiments to allow excellent preservation of ultrastructure, thus avoiding such artefacts.

With the emergence of Green Fluorescent Protein (GFP) in the 1990s the interest of live cell imaging studies has been tremendous. This, coupled with faster and more sensitive detection systems on microscopes provides even greater opportunities. Life science research has learned such an incredible amount from these live cell imaging studies that it is hard to imagine what research would be like without these tools.

The field of electron microscopy has not fully locked onto the momentum of the light microscopy wave, but in recent years it has become evident that the resolution of the light microscope is in some cases limiting in resolving the scientific question. Therefore, there has been increasing interest in developing techniques that combine the live cell imaging aspect of GFP with the high resolution of EM (Fig. 1). GFP is not directly visible in the EM (electron microscopy) but can be visualised using antibodies or by photo conversion. However, such methods are based on samples that are chemically fixed at room temperature. It is well documented that this can introduce artefacts. Such artefacts are not visualised at the LM (light microscopy) level since there is either no fixative present yet (live) or when fixed samples are studied the artefacts are below the resolution of the LM.

However, at the EM level they become apparent and that is exactly where we want to study structures at high resolution and in precise detail. There is an alternative fixation method that is based on physical fixation, namely, cryofixation. It fixes cells much faster and unlike chemical fixation is non-selective. High Pressure Freezing (HPF) is nowadays the most reliable method for cryofixation of cells and tissues. A sample is put under high pressure (2000 bar) and milliseconds afterwards the sample is sprayed with liquid nitrogen. This prevents the formation and growth of ice crystals and fixes samples instantly with up to about 200–300 µm depth of well-preserved, vitreous sample.

High Pressure Freezing with new time resolution

HPF machines were not originally designed for these types of experiments, the major constraint being the time it takes to study a sample under the LM, spot an interesting event and then transfer that sample into an HPF machine to fix it. This would take at least 30 seconds for a very experienced user. By that time the structure of interest would have already disappeared (Fig. 1).

So, Leica Microsystems, together with Dr. Paul Verkade (School of Medical Sciences, University Walk, Bristol, UK), set out to develop a tool that would

be able to carry out CLEM experiments with HPF but with a time resolution of less then 5 seconds. The end result is the Leica EM PACT2 with EM RTS (Fig. 2). It is mobile, so it can be moved to any light microscope. The RTS stands for Rapid Transfer System. This system consists of a rapid loader that contains the sample (Fig. 3) and the actual transfer system as an attachment to the HPF machine. After insertion of the rapid loader into the RTS, the sample is automatically enclosed and shoots along a rail into the Leica EM PACT2 to be frozen. This sequence only takes 2.4 seconds. This leaves enough time for the scientist to take the rapid loader from under a light microscope and put it into the RTS. Such a movement can easily be done within 1–1.5 seconds and thus results in an overall time resolution of about 4 seconds. The frozen sample can now be processed for electron microscopy.

New standard of freeze substitution

In most instances this will involve freeze substitution, where the frozen water is removed from the sample with solvents such as acetone prior to resin infiltration and polymerisation at low temperature. Here too, Leica Microsystems has recently made it much easier for the scientist with the introduction of the Leica EM AFS2 with EM FSP that allows for automatic exchange of all the freeze substitution chemicals. Figure 4 shows an example of such an experiment. Epidermal Growth Factor (EGF) was coupled to quantum dots. Quantum dots are both fluorescent and electron dense and therefore excellent markers for CLEM studies. The EGF has been internalised for 30 minutes and is present in multi-vesicular bodies (MVB). The MVB appear as a dynamic structure with lots of extensions appearing and disappearing over time (Fig. 4c).

When another extension was seen appearing the sample was taken and immediately frozen. The same cell is retraced and the structure of interest can now be visualised at high resolution, showing the connections. In conclusion, the Leica EM PACT2 plus EM RTS coupled with Leica Microsystems' confocal microscopes offers life scientists an excellent solution that serves both to extend their toolbox and knowledge.

Fig. 2 and 3: The Leica EM PACT2 + RTS high-pressure freezer, suitable for standard high pressure freezing and CLEM experiments. Its operation is very easy: All you need to do is insert the rapid loader, the machine will do the rest.

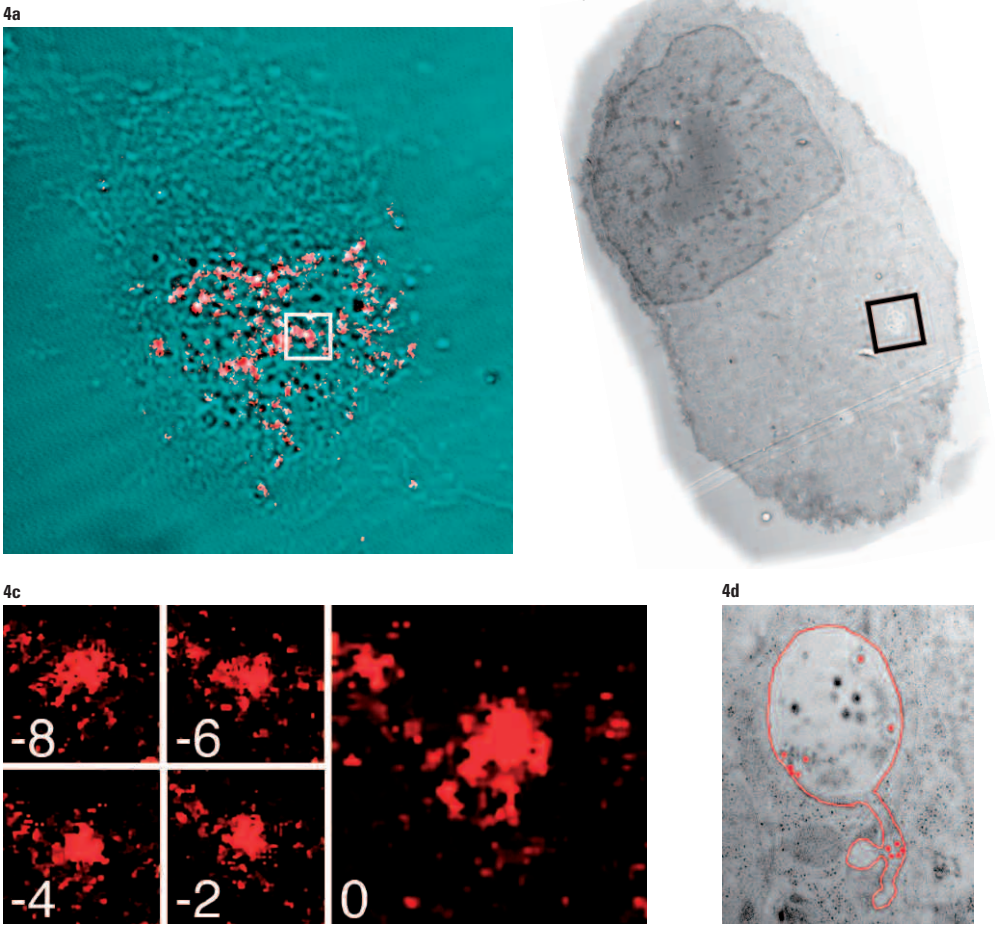


Fig. 4: Example of a CLEM experiment using the Leica EM PACT2 + RTS. A cell of interest is first studied at the light microscopy level. The DIC image of a cell of interest is overlaid with the red fluorescence of the quantum dots (Fig. 4a). The structure of interest is boxed and followed live. Fluorescence images are taken and the sequence of the last 10 seconds is shown (Fig. 4c). At time 0 seconds the rapid loader is taken and placed in the RTS. After it has been frozen and processed, the same cell is traced back (compare Figs. 4a and 4b). Note: The area of the nucleus as can be seen in the EM image is devoid from fluorescent label as would be expected. Now the structure of interest can be studied at high resolution and we see (Fig. 4d) a connection (membrane is false-coloured red) between the main MVB and the tubular extension. Both the main MVB and the extension contain Quantum dots (red dots false coloured).

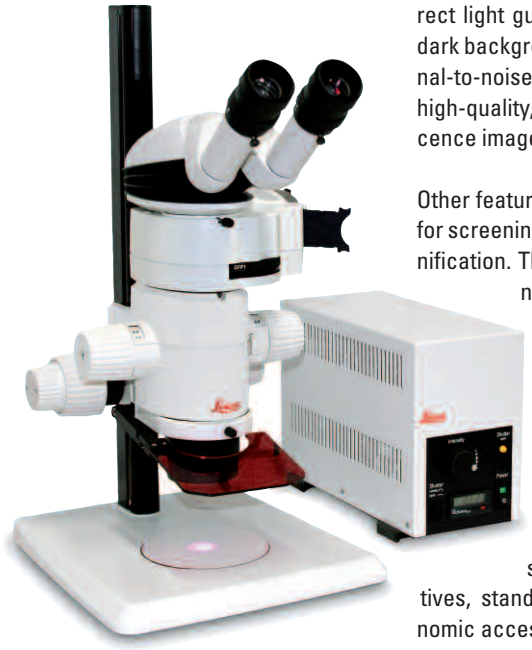
Leica MZ10 F and the New Leica Cross-Stage

Perfect Screening

Stereomicroscopy is an invaluable technique for inspecting, sorting and selecting specimens for further analysis in a wide variety of life sciences. Leica Microsystems has now developed two new products that speed up these routine jobs while enhancing precision and offering a consistently ergonomic design to allow strain-free, relaxed working.

Screening specialist with a "third eye"

To discover the special secret of the new Leica MZ10 F fluorescence microscope, just switch to continuous routine operation. The outstanding feature of the Leica MZ10 F is the TripleBeam, the third light path patented by Leica Microsystems. This separate fluorescence illumination ensures precise and correct light guidance at all zoom settings to provide a dark background within the field of view. The high signal-to-noise ratio guarantees optimum contrast and high-quality, non-reflecting, high-definition fluorescence images with a jet black background.



Other features that make the stereomicroscope ideal for screening are its 10:1 zoom range and 8 – 80x magnification. The high resolution of 375 Lp/mm and the numerical aperture of 0.125 (1.0 PlanAPO) produce stunningly clear and precise images of fine structures. UV filters are permanently built in to protect the user's eyes. The generous range of accessories includes the FluoCombi III, the quick-change four-position filter system patented by Leica Microsystems. In connection with the large selection of illumination systems, objectives, standard and special filters and other ergonomic accessories, the Leica MZ10 F offers an excellent and highly flexible solution for laboratory fluorescence.

True ergonomics is a matter of detail

The new fully motorised and automated x/y stage IsoPro combines the very best standards of mechanical engineering and precision with state-of-the-art ergonomics to ease the workload for the user and enable him or her to concentrate fully on the task in hand. The innovative SlideOn technology guarantees a constant focal plane for the entire working area even at high magnifications so there's no time wasted on refocusing. Specimens can be positioned under the microscope fully automatically, quickly and reliably with an accuracy of 2 µm. The new IsoPro is also fully compatible with all stereomicroscopes from Leica Microsystems.

> AS

www.leica-microsystems.com/MZ10_F.html | www.leica-microsystems.com/IsoPro.html

Leica DFC490 and Leica DFC420 C

High Performance Digital Camera Systems

Excellent picture quality is essential for precise image analysis, documentation, and reporting. The Leica DFC490 and Leica DFC420 C digital camera systems provide images for the highest colour fidelity, resolution and detail. The Leica DFC490 is ideal for the most indicate documentation in research and life science. Real time speeds can be achieved using an array of innovative read-out modes.

High resolution and real-time readout

The innovative Leica DFC490 integrates a 2/3 inch size 8-megapixel CCD, which offers superior quality, ultra high resolution images that were previously only possible with piezo stepping multi-shot cameras. High resolution CCD's are especially beneficial for low magnification imaging on microscopes as the amount of information provided by the optical system is here much larger than in high magnification conditions. Innovative data readout modes allow the free selection of image transfer, speed and scan method. The full frame readout mode, for example, utilises the full camera resolution (3264 x 2448 pixels) with 8 or 12 bit signal processing while the progressive scan readout mode offers an optimised live image resolution (1088 x 816) at near real time speeds (17 frames per second). Both the Leica DFC490 and DFC420 C incorporate an active Peltier cooling system designed to effectively reduce thermal noise buildup in the camera which draws heat away from the sensor to produce stunning images with less noise.

Excellent picture quality

Exceptional picture quality and ease of use make the Leica DFC420 C the perfect choice for precise, fast imaging for documentation and analysis. The Leica DFC420 C is based on a 1/1.8 inch 5-megapixel sensor with switchable resolutions between 2592 x 1944 pixels down to 576 x 432 pixels in 2x2 binning mode. Leica's true colour calibration takes care of the natural colour reproduction, which produces excellent picture quality.

Easy to use

The Leica DFC490 and the Leica DFC420 C make imaging easy. Both cameras operate automatically, and shutter and filter changes can be done without disturbing the work at the microscope. Leica's digital technology simplifies all operations, from image capture through image archiving, and allows digital retouching and analysis.

The cameras are equipped with a C-Mount interface for the widest range of microscope applications. The cameras' real-time live preview speed allows a sample to be adjusted and focused directly on a computer monitor. Focusing can take place conveniently without having to readjust the microscope's eyepieces.

System integration

Creating crisp, sharp images was never easier with the new Leica Application Suite software which features automatic microscope setup and calibration, annotation and measurement functions. If an automated microscope is used you can now store and recall the same camera and microscope parameters to exactly reproduce any previously made pictures. Thanks to the unique workflow approach of the Leica software, processing and organising of these high resolution images has become just a snap.

Intuitive solutions for PCs and MACs

The cameras' software makes digital recording on the screen quick and easy, using either a PC or MAC. The easy-to-use interface is specifically designed for microscopy applications. Numerous intuitive image capture and editing functions ensure that the recorded images are immediately available for viewing and further processing, offering the highest quality and full use of all the benefits of digital technology.

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Microscopists in Venice

Since 1810 the Istituto Veneto di Scienze Lettere e Arti, Venice, has played a major role in Italy in promoting culture, research and knowledge. The 11th School of Pure and Applied Biophysics was held in the prestigious institute at Palazzo Franchetti with the theme "Advanced Optical Microscopy Methods in Biophysics". Among the speakers was Prof. Erwin Neher, Nobel Prize winner in 1991.

Biophysics is a molecular science rapidly moving to the nanoscale. It seeks to explain biological function in terms of the molecular structures and properties of specific molecules. The size of these molecules varies dramatically, from small fatty acids and sugars (~1 nm = 10^{-9} m), to macromolecules like proteins (5–10 nm), starches (> 1000 nm), and the enormously elongated DNA molecules. A great deal of effort in biophysics is directed towards determining the structure of specific biological molecules and of the larger formations into which they assemble.

Bearing these considerations in mind, the school focussed for a week at the beginning of this year on all the latest optical microscopy methods, such as: CARS, TIRF, SHG-THG, Correlative microscopy, Multiphoton microscopy, Confocal Microscopy, FLIM, FRET, FRAP, Digital Deconvolution and others. Attendees had the opportunity to listen to such prestigious lecturers as Prof. Erwin Neher, Nobel Prize winner for Medicine in 1991, Tullio Pozzan, Italian member of the US National Academy of Science in US, Carlos Bustamante, pioneer in nanomanipulation, Tony Wilson and Fred Brakenhoff, inventors of modern three-dimensional microscopy.

Promoting young researchers

An enthusiastic young audience of 35 graduates from various European countries, selected by competition on qualification were involved for a week in a wide range of advanced topics from nucleic acid manipulation to innovative optical methods, from luminescent proteins unveiling cellular activities to methods of analysis for studying neurodegenerative diseases and tumour cell behaviour. Of equally high interest were the talks on nanomaterial like quantum dots and nanotubes as well as the new opportunities offered by nanoscopy. > DG, AD

Courtesy of Apple

Contest

Your Opinion is Valuable!

Win an iPod nano Red Special Edition* or other nice prizes!

Dear Reader:

Please give us your comments on this European Research Edition of reSOLUTION magazine. Send us your complete name and address of the institute where you work, along with your comments by going to the following link by 31 August, 2007:

www.leica-microsystems.com/EU-Research

Winners will be drawn from all completed entries.

Burkhard Alt, Sales Representative Research Germany, presents a Leica C-LUX 1 digital camera to the winner of our last contest, Prof. Dr. Reinhard Lakes-Harlan (left), Institute for Animal Physiology, Justus Liebig University of Gießen, Germany.

* Apple contributes $ 10 from the sale of each iPod nano RED to the Global Fund to help fight HIV/AIDS in Africa.

European Research Events

Here is just a compendium of some of the events Leica Microsystems will participate in or organise. For further information, please visit our website:
www.leica-microsystems.com/events

Events 2007

FOM (Focus on Microscopy)
April 10–13
Valencia, Spain
http://www.focusonmicroscopy.org/

International Chick Meeting
April 11–14
Barcelona, Spain
http://www.chicknet.es/barcelona.htm

7th ELMI meeting
April 17–20
York, UK
http://www.york.ac.uk/depts/biol/tf/ELMI/

AFM BioMed Conference
April 19–21
Barcelona, Spain
www.afmbiomed.org

1st Meeting of the Italian C.elegans Research Community
April 20–22
Naples, Italy
http://libra.igb.cnr.it/~bazzical/meeting/index.htm

Portuguese Society for Stem Cells and Cell Therapies (SPCE-TC)
April 27–28
Coimbra, Portugal
http://www.spce-tc.org/

Neurosciences 2007
May 22–25
Montpellier, France
http://www.neurosciences.asso.fr/Activites/colloques/SN07/

SFMU 2007 – Société Française des Microscopies
June 5–8
Grenoble, France
http://sfmu2007.free.fr/

NanoBioEurope
June 13–15
Münster, Germany
www.nanobio-europe.com

Light Microscopy in Live Cell Course
June 14–22
Lisbon, Portugal
http://cwp.embo.org/pc07-09/

European Human Genetics Conference
June 16–19
Nice, France
http://www.eshg.org/eshg2007

Cancer Research UK Beatson International Cancer Conference 2007
June 19
Glasgow, Scotland
http://www.beatson.gla.ac.uk/conf/

Leica VIII Confocal Seminar
June 20–22
Rome, Italy
www.leica-microsystems.com/events

Innovative Mouse Models (IMM)
June 21–22
Leiden, Netherlands
http://research.nki.nl/jonkerslab/immworkshop/immworkshop.htm

Mouse Neurological & Behavioural Forum (MNBF) 2007
June 28–29
Oxfordshire, UK

XII Congreso de la Sociedad Española de Biología Celular (SEBC) 2007
July 2–5
Pamplona, Spain

XXIII Reunión Bienal de la Sociedad de Microscopía de España (SME) 2007
July 3–6
Leioa, Spain

XXXIV Congreso Soc. Esp. Ciencias Fisiológicas
July 3–6
Valladolid, Spain

FEBS
July 7–12
Vienna, Austria
http://www.febs2007.org

Zebrafish Meeting 2007
July 12–15
Amsterdam, Netherlands
http://www.zebrafish2007.org/

International Chromosome Conference (ICC) 2007
August 25–29
Amsterdam, Netherlands
www.icc-2007.com

ELSO Meeting
September 1–4
Dresden, Germany
www.elso.org

The Tissue Engineering & Regenerative Medicine International Society (TERMIS) 2007
September 5–8
London, UK
http://www.termis.org/eu2007/

XII Congreso Bienal de la Sociedad Española de Neurociencias (SENC) 2007
September 5–9
Valencia, Spain
http://valencia2007.senc.es/

European Working Group Of Cardiac Cellular Electrophysiology (EWGCCE) 2007
September 6–7
Manchester, UK
http://www.physoc.org/meetings/man2007.asp

FEBS workshop on invadopodia, podosomes and tissue invasion
September 8–13
Ortona, Italy
www.invadosomes.org

Fluorescence Conference MAF 2007
September 9–12
Salzburg, Austria
http://www.maf-sip.com/maf10/

Zoologentagung 2007
September 22–26
Cologne, Germany

Scanlab 2007
September 24–26
Stockholm, Sweden
http://www.scanlab.nu/

ILMAC 2007
September 25–28
Basle, Switzerland
www.ilmac.ch/

Congresso Nazionale SINS (Soc. Italiana Neuroscienze)
September 27–30
Verona, Italy
http://www.sins2007.it/

RICH-MAC 07
October 2–5
Milano, Italy
www.richmac.it

III Advanced Living Cell Microscopy Workshop
October 3–5
Madrid, Spain
www.leica-microsystems.com/events

Biotechnica
October 9–11
Hanover, Germany
http://www.biotechnica.de/homepage_e

Capri Science Conferences, a NATURE Conference
"Cancer Therapeutics: The Road Ahead"
October 8–10
"Discovery tools in cardiovascular disease"
October 14–16
Capri, Italy

World Congress for Regenerative Medicine/Life Science Symposium 2007
October 17–19
Leipzig, Germany
http://www.regmed.org/

Bioceramics 20 – 20th International Symposium of Ceramics in Medicine
October 24–26
Nantes, France
http://bioceramics20.sante.univ-nantes.fr/

SBCF (Société de Biologie Cellulaire de France)
October 28–31
Grenoble, France

Medica 2007
November 14–17
Düsseldorf, Germany
http://www.medica.de

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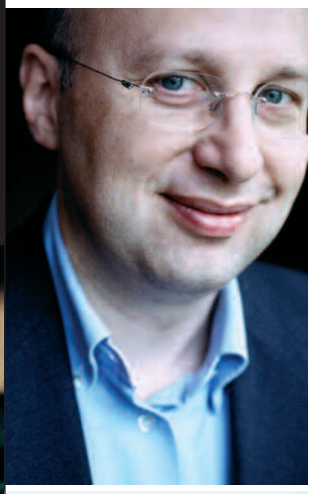

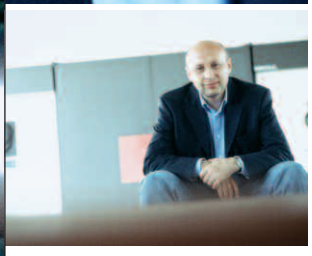

"To Break a Barrier it's Good to Have Leica Microsystems as a Partner."

In 1873, Ernst Abbe discovered that diffraction limits the resolution of a light microscope. Since then nobody thought that optical microscopes would ever image details much smaller than the wavelength of light.

Stefan Hell has changed this view.

Prof. Hell, today Director at the Max Planck Institute for Biophysical Chemistry in Göttingen, came up with the first physical concepts breaking the diffraction barrier. He and his team have verified nanoscale resolution in fluorescence microscopy using conventional lenses.

For his pioneering experiments, Stefan Hell has continuously relied on lenses from Leica.

www.leica-microsystems.com

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