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Leica MICROSYSTEMS

Exploring the Secrets of Cell Logistics: TIRF Microscopy Visualizes Intracellular Transport Paths

by Dominik Schneider and Prof. Ralf Jacob, Department of Cytobiology and Cytopathology
Philipps University, Marburg, Germany

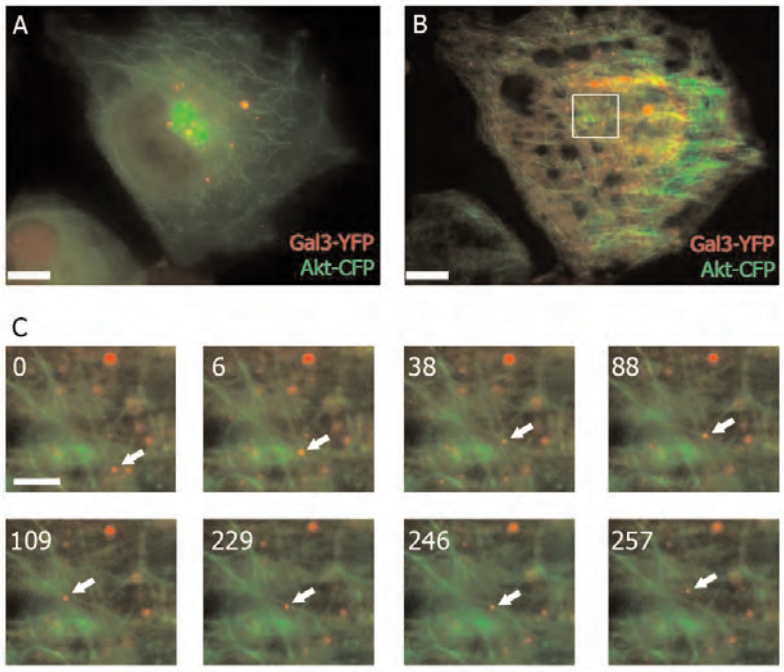

Figure: Transport of Gal-3 vesicles close to the membrane along actin filaments: **A** – Overview image with epifluorescence. **B** – Overview image with TIRF, labeled section is shown in image C. **C** – Time sequence of section of TIRF; time given in seconds

A Gal-3-YFP vesicle close to the membrane (arrow) is first transported on an actin filament (from the bottom upwards), switches to another filament (88 s), moves to the left (109 s), is transported to the right again, switches filament again, and is then transported uwards (246 s). YFP – red, CFP – green, scale of overview images: 20_m, section: 6_m. Penetration depth TIRF 110nm.

Epithelial cells, which control the exchange of substances between the organism and the outside world, are equipped with highly specific mechanisms for selective transport of substances to the apical or basolateral membrane. Using TIRF microscopy, scientists have been able to take a closer look at intracellular transport processes with the example of the galactose-binding protein Galectin-3, which has been identified as a potential apical sorting receptor.

As polarized cells, epithelial cells have clearly separated functional domains for selectively regulating the exchange of substances with the outside world and, at the same time, protecting the organism from harmful influences: the apical (facing the lumen) and the basolateral membrane. The enterocytes of the small intestine also have a highly complex system of sorting and transport mechanisms, which are used, for example, to channel digestive enzymes out of the cell and nutrients into the cell via the apical membrane.

Many details of polarized protein transport are still unclear. Malfunctioning of these processes may lead to intolerances or metabolic disorders. Cystic fibrosis, for example, is caused in some cases by a

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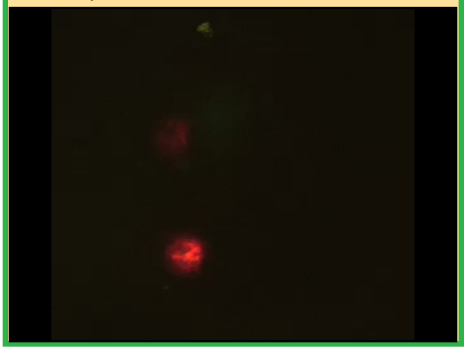
The Objective

In this section of our newsletter, we like to highlight application-oriented stories of value to our readers. The following movie, submitted by Dr. Thomas Nevian of the University of Bern, shows a cell filled with 200um Oregon Green Bapta 1 and its response to a single backpropagating action potential.



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genetic defect in the apical transport of osmotically effective chloride ions from epithelial cells. The consequence is the secretion of mucus in the lungs, pancreas, and small intestine, among other places.

The Cell's Sorting Machinery

The starting point of the intracellular transport is the trans-Golgi network (TGN), which supplies functional glycoproteins (e.g., hormones, digestive enzymes) in vesicles. The apical protein transport probably works in two main ways: through the association with specific membrane micro-domains called lipid rafts, and via a raft-independent form of transport. Sorting signals in the protein structure and corresponding receptor proteins could make sure, on the one hand, that vesicles are charged with apical proteins and, on the other hand, that the secretory vesicles are channeled into the right transport path.

Prof. Ralf Jacob and his study group at the Institute of Cytobiology and Cytopathology of the Philipps University in Marburg have identified the galactose-binding protein Galectin-3 (Gal- 3), which could act as sorting receptor [1, 2]. It seems to be important for apical transport that Gal-3 forms clusters of high molecular weight with apical-sorted proteins – packing its cargo into the largest parcels possible, so to speak. A current study recently examined COS cells to explore the intracellular localization of Gal-3 in the secretion pathway and the structures involved in its exo- and endocytosis.

Visualizing Transport Processes in Real Time with TIRF

To visualize these processes, the Marburg scientists use TIRF (Total Internal Reflection Fluorescence) microscopy. The combination of the high z resolution and fast frame rate offered by TIRF microscopy enables a far better image to be obtained of dynamic processes in or near to the cell membrane than with epifluorescence (see figure on page 1). TIRF is able to selectively excite fluorophores in an aqueous or cellular environment at a depth of approx. 70–300nm below the surface.

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Technology Fast Track

FusionOptics™

by Lon Nelson, Marketing Manager, Leica Microsystems

The Leica M205 C

The human brain is truly an amazing tool. Consider the ability to see the world around us. One of the most fascinating processes that occur inside the human head is localized in the visual cortex, which combines a monocular image from each eye into a stereoscopic impression of the world. The resultant 'stereo' (3D), view of our surroundings allows the brain to create a three dimensional map of our environment, complete with the necessary depth perception to drive a car, hit a baseball, or simply pick up a pencil.

The mind's visual cortex does not stop at simply combining two monocular images, but performs a much more complex process. For example, if a person views an image with both eyes, and a blurred region of that image is applied to just one eye, the brain compensates to suppress the blurred region seen by that eye and to replace it with the perfect image seen by the other eye. To take this concept a step further, when different blurred regions of the same image are seen by each eye separately, the visual cortex selects, for each location, the highest resolution impression for the brain's assembly of the final image. It is for this reason that presbyopic patients (those without the ability to clearly image objects close to their eyes due to decreased elasticity of the eye) often have their vision corrected so that one eye is used for distance vision and the other is used for viewing objects close up (known as monovision).

Not known until recently, was whether or not this 'selection' of the best resolution could be performed simultaneously with depth perception. In a study entitled "Spatial visual acuity at different depth planes: The role of eye of origin," C. Schulthess and D.C. Kiper of the Institute of Neuroinformatics at the University of Zurich and Swiss Federal Institute of Technology teamed with H. Schnitzler from Leica Microsystems AG to answer this question. The group found that "the human visual cortex seems capable to extract, at each retinal location and each perceived depth plane, the highest amount of information contained in either of the two monocular images."

The results of this study have been directly applied to a new and exciting stereomicroscope design by Leica Microsystems called FusionOptics™. Stereomicroscopes, often referred to as dissecting microscopes, have been used for decades to image microstructures while manipulating, sorting, polishing, and cutting specimens. In the past however, users of dissecting microscopes had to choose between higher image detail and better 3D depth perception, as these factors are inversely proportional to each other.

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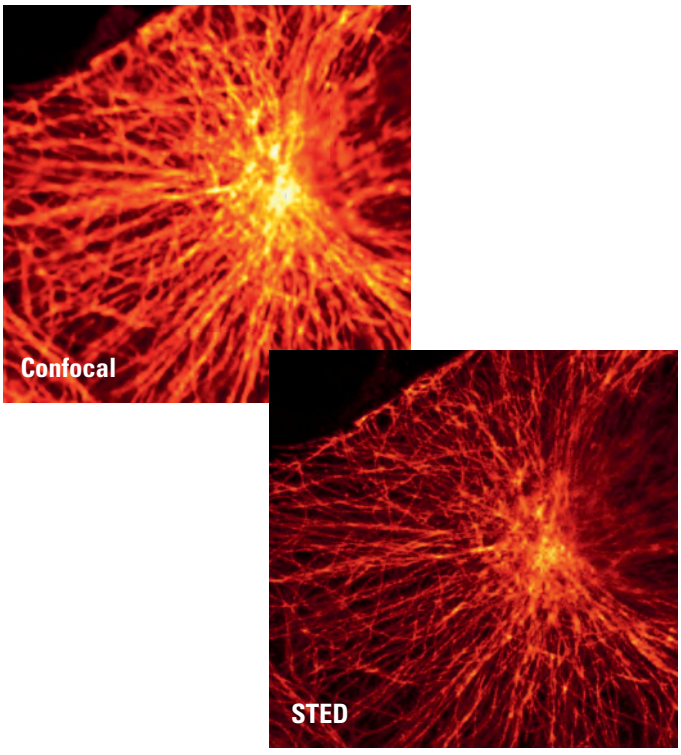
The Private Eye

The Diffraction Limit of Microscopy is Broken!

Leica Microsystems was excited to introduce the first super-resolution microscope with STED (STimulated Emission Depletion) technology this November in San Diego, CA. STED is capable of increasing fluorescence microscopy resolution by more than 2x over today's limit of 200nm. With resolution of 70 to 90nm, STED super-resolution allows the life science researcher to see details in the cell that were never before visible!

STED was developed by Professor Stefan Hell of the Max Planck Institute for Biophysical Chemistry. This invention won Dr. Hell the German Future Award in 2006.

Please visit us online to learn more about the STED innovation: **http://www.leica-microsystems.com/confocal_microscopes** and scroll to STED.



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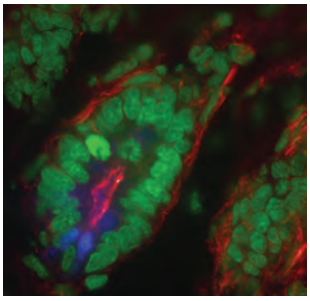
Open Forum

Spinning Disk Confocal

Question: Why is Spinning Disk Confocal Technology so popular for live cell studies?



Answer: In general, cellular events happen very quickly, in the order of milliseconds, and spinning disk technology provides a spectacular balance between speed and resolution to image these events. Typically, a rotating pinhole or slit simultaneously scans all image points in the focal plane multiple times per second. The combination of this multi-point scanning and highly sensitive EMCCD fluorescence camera technology allows images to be recorded at the ultra high speed required for live cell research.

By design, spinning disk confocals provide an instantly deblurred image on-screen as its pinholes or slits quickly scan the field-of-view. This technique is much quicker than deconvolving widefield fluorescence images and allows real time imaging of biological processes. Further, spinning disks minimize cell photo bleaching and photo toxicity, which enables biological processes to be observed over long periods of time. This is possible since fluorescence excitation via the spinning disk is a fraction of that used in widefield imaging, and typically not as intense as the illumination spot used in traditional laser point-scanning confocal.

Leica's new SD6000 Spinning Disk Confocal System is designed around a fully automated, inverted, research microscope. This system can utilize climate chambers and other live cell accessories to customize the solution to each laboratory's needs. In addition, the SD6000 can be retrofit to existing Leica AF6000 LX workstations and can be outfit new with Total Internal Reflection Fluorescence (TIRF) capability. All contrast techniques from confocal to DIC to widefield fluorescence can be controlled automatically and integrated with the same experiment for complete flexibility. The system's software guides users through experiments, which also makes this new tool very easy to use.

Answered by Lon Nelson.

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For You

Special product offers currently available from Leica Microsystems:

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Starting a new lab? Beginning a new research project? Contact your local Leica sales executive to see if you qualify for thousands of dollars in discounts!

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Demonstration Equipment Available at Significant Discounts

Contact your local Leica sales executive to find out about certain microscope accessories that are available from our demonstration stock; some receive up to a 60% discount.

Call 800-248-0123 and press 2 for more information.

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Your Educational Resource

Marine Biological Laboratory

The MBL offers advanced, graduate-level courses in embryology, physiology, neurobiology, microbiology, and parasitology for six to eight weeks each summer. Download the complete 2007 course schedule: **http://www.mbl.edu/education/courses/summer/index.html**

Cold Spring Harbor Laboratory

CSHL is a private, non-profit institution with research programs in cancer, neuroscience, plant genetics, genomics, and bioinformatics and a broad educational mission. Download the complete 2007 course schedule: **http://meetings.cshl.edu/courses.html**

Jackson Laboratory

The Jackson Laboratory's mission is to improve the quality of human life through discoveries arising from our own genetic research and by enabling the research and education of others. Download current and upcoming events: **http://www.jax.org/courses/events/current.do**

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Upcoming Events

Visit Leica Microsystems at the following exhibitions:

- ASCB, Washington, D.C.
  December 1-5, 2007

For more events, visit: **http://www.leica-microsystems.us**
(click on Company, then Events)

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TIRF Microscopy

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The quality of the results depends on the precise setting of the TIRF angle and the exact positioning of the laser beam. Leica Microsystems' scanner-sensor technology automatically matches the TIRF angle to the desired penetration depth at all wavelengths and positions the laser in such a way that the TIRF penetration depth automatically remains constant. Epithelial cells, which control the exchange of substances between the organism and the outside world, are equipped with highly specific mechanisms for selective transport of substances to the apical or basolateral membrane. Using TIRF microscopy, scientists have been able to take a closer look at intracellular transport processes with the example of the galactose-binding protein Galectin-3, which has been identified as a potential apical sorting receptor, even when the wavelength is changed. Selected settings can be reproduced at the press of a button.

The Intracellular Transport of Galectin-3

All in all, the results of the Gal-3 studies in COS cells paint a largely coherent picture of intracellular transport. The first contact between Gal-3 and the glycoproteins it sorts presumably takes place in a still unidentified post-Golgi compartment. By manipulating the pH, it was possible for the first time to localize YFP-labeled Gal-3 in endosomal compartments, which are probably EEs (early endosomes) or REs (recycling endosomes), and are discussed as the possible location of post-Golgi sorting. The vesicles formed by budding could then be transported via microtubules and then switch to actin microfilaments (see figure) in the vicinity of the membrane. After fusion of the vesicles with the membrane, the apical glycoproteins sorted by Gal-3 have reached their destination and are distributed together with Gal-3 in the membrane. However, a different mechanism seems to be responsible for transporting Gal-3 into the inside of the cell. Whereas Gal-3 is important for the apical transport of non-raft-associated proteins, the endocytosis of Gal-3 is probably raft-dependent. Gal-3 could then be transported into the inside of the cell via actin filaments in order to rejoin the raft-independent transport.

References

1. Delacour D. et al.: Traffic 8(4), 379–388 (2007)
2. Delacour D. et al.: Curr Biol 16(4), 408–414 (2006)
3. Dominik Schneider, diploma thesis, Philipps-University, Marburg, Germany, July 2007

Application Note first appeared in *BIOforum Europe* 9/2007.

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FusionOptics™

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No longer is sacrifice necessary with FusionOptics™. This new optical design provides a high-resolution image to one eye and a high depth image to the other eye. Two separate images of the same specimen are automatically fused by the visual cortex, and the result is stunning image detail with huge depth relief. The new Leica M205 C stereomicroscope employs this new optical design, which provides easy positioning of tools and manipulators in 3D as well as unprecedented optical resolution. For the life science researcher, this innovation translates to an easier, more comfortable-to-use dissecting microscope that is extremely versatile in that it provides brilliant images across the entire magnification range.

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Editor-in-Chief:	Molly Baker
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Contributors:	Dominick Schneider and Prof. Ralf Jacob, Phillips University

Note: *We are interested in your comments and thoughts about the newsletter. Please feel free to email your comments to:* ***microscience.imaging@leica-microsystems.com***