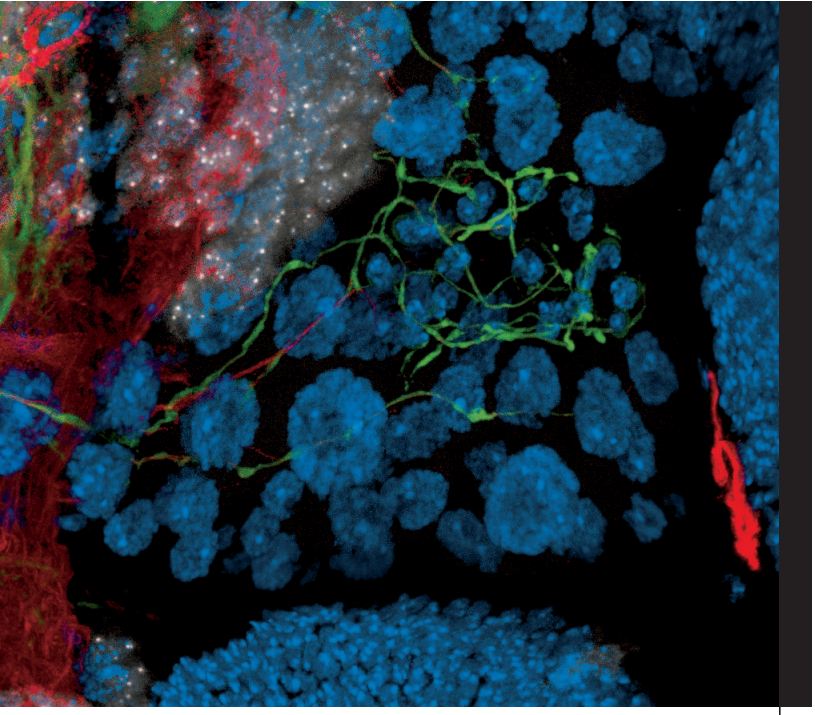
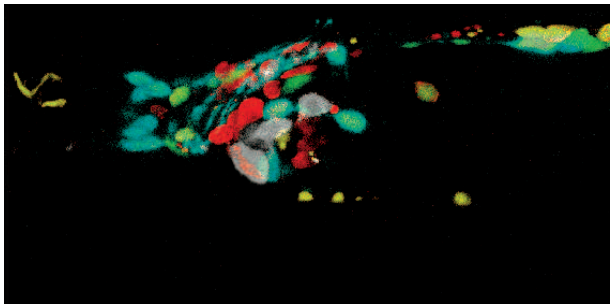


Spectral Detector SP®

Leica TCS SP5 – The Broadband Confocal

Leading in Multispectral Scanning

Leica
MICROSYSTEMS



Caenorrhabditis elegans, neurons.
5 channel image, overlay

1) Sensory and interneurons, CFP
2) Excitatory motoneurons, sensory neurons, YFP
3) GABAergic neurons, GFP
4) Glutamatergic interneurons, DsRed
5) Sensory neurons, DiD

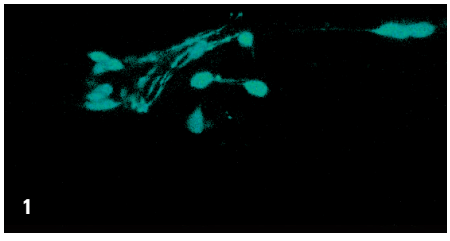
Courtesy of Dr. Harald Hutter, Max Planck Institute for Medical Research, Heidelberg, Germany

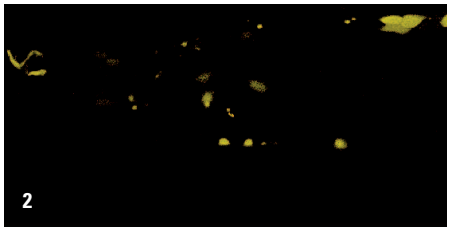
Multiparameter fluorescence

Fluorescence microscopy has become the major tool in modern biology. Nearly all disciplines in the biomedical field benefit from fluorescent staining. For structural analysis in morphology and anatomy, for kinetic measurements in physiology and biophysics, and for tracing gene-expression and dynamics in molecular biology – just to name the larger boundaries. Immunohistochemical fluorescence tags, fluorescence-in-situ hybridization, metabolite probes like Ca^{2+} or potential sensitive dyes and various specific stains for cell compartments and structural proteins fill the huge catalogues of fluorescence probes for current research tasks. And fashionable staining tools like fluorescent proteins and quantum dots, flood the laboratories.

Additional to the big variety of dyes and probes there is another common trend in research: multiparameter fluorescence – application of multiple dyes to the same sample. This is an essential technique when interaction of proteins is the target. It is indispensable, when communication between several individual cells is investigated and to find answers to questions in embryology – a classical example for identification of neuronal development by fluorescent proteins.

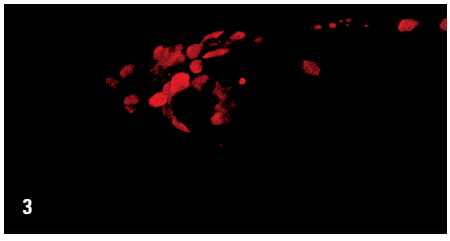
Colors count

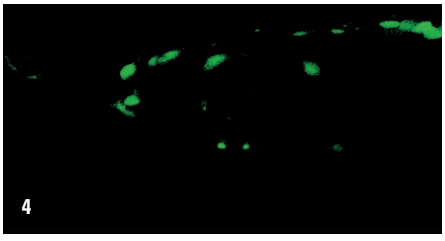


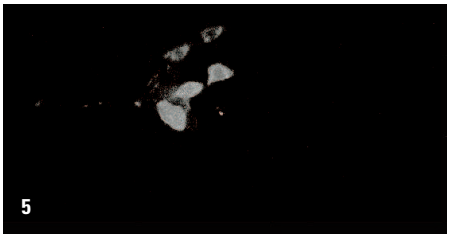


Past: cascade filtering – a nightmare for engineers and researchers

Of course, multi labeling is not a new invention. It was performed in the past by sequentially imaging the different channels. Or – if simultaneous recording was unavoidable – by cascading beam splitters and barrier filters. Whereas the first method is simply time consuming and prone to channel-misalignment, the latter suffers from bulky filter magazines and still very limited flexibility. As a rule: the appropriate filter was simply not available – or the installation required a service call, too late for the already set up experiment and quite expensive, above all.



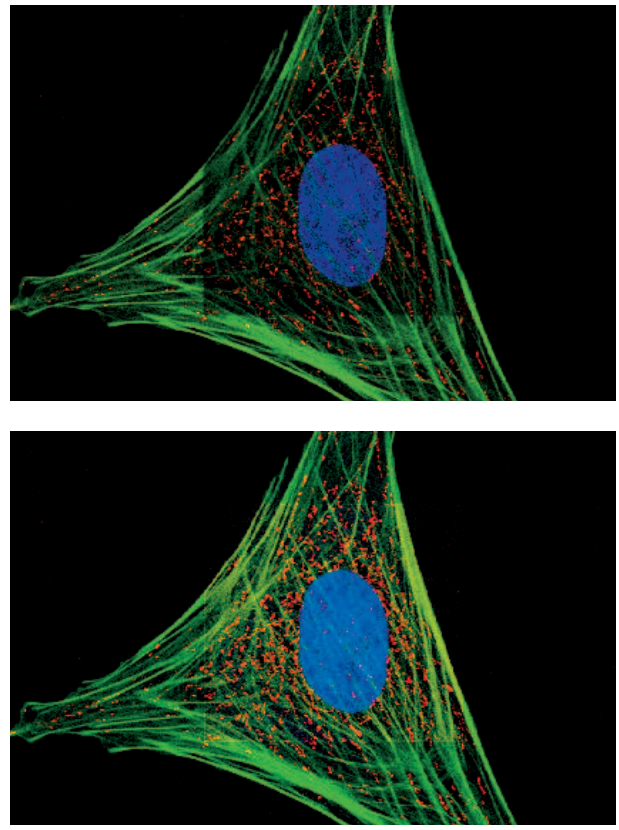




What is most unattractive with filter-cascades is the low efficiency. Imagine constructing a cascade offering five channels. This system would at least have one channel, where the light passes three different dichroic mirrors and a barrier filter. You can easily suspect that not too many photons would make their way through all these obstacles. As a result, the images are noisy – alternatively the sample bleaches, if you either average or increase the illumination intensity. Beside these efficiency-problems, filters have a very strong limitation: they are not tunable. To adapt the system to various dye-combinations, you are limited to the given characteristics of the filters and dichroic mirrors of your equipment. Although there might be combinations that "work", they will seldomly lead to optimal results due to loss in efficiency or unacceptable crosstalk – usually both.

Today: the Leica SP Detector

The Leica SP®-detector solves all these problems. The light from the sample, which is a sum of multiple emission spectra, first passes through the pinhole. Here, the optical sectioning occurs. Then, by means of a prism, the colors are spread into a spectrum. On its way to the first detector, the light passes a slit-photometer device consisting of two motorized barriers, which are controlled via software. The principle is simple but most effective: The device is steplessly adjustable: the barriers can be moved to any wavelength position. By means of this design, this single detector can make up any conventional filter specification in terms of center frequency and bandwidth.

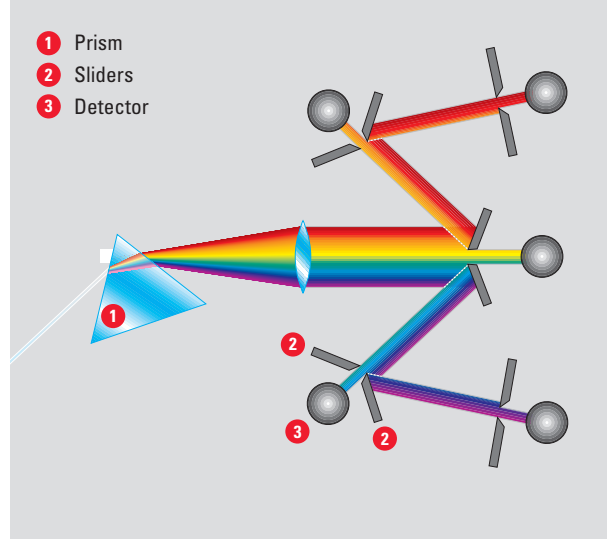


Sample protection by high-senstitivity detection.
Top: high laser power and rigorous averaging is necessary in conventional detection systems in order to generate noise-free images.
Bottom: low illumination intensity and few averages will not damage this extremely bleach-sensitive sample when imaged with SP(R) detector.

An ingenious invention

However, this is not a multi-parameter fluorimeter. How can we simultaneously collect multiple segments from the spectrum? The clever bits are the barriers: they are not made of low-reflection material as it is the case for standard photometers. Here, the barriers are in fact mirrors. So the residing parts of the spectrum on both sides of the band collected at sensor 1 are directed to sensors 2 and 3 with again motorized, reflective barriers – ready for the next sequence.

This is how the SP5 detector works. Five channels simultaneously. Full efficiency. No filters and dichroics. Just some high-efficiency coated mirrors. And all bands unrestrictedly tunable. Whether you need to cover the full spectrum for full efficiency or just small specific bands to the detector for unsurpassed crosstalk reduction. You decide! (Software for unmixing is, of course, available anyway.)



Better images in less time

Your benefit of Leica's SP® detector concept: maximum efficiency and full tunability which guarantees you best imaging quality and least crosstalk in multiparameter fluorescence. And as a side-effect: the SP® detector allows recording of lambda scans – spectral image stacks to analyze the in-situ emission characteristics of the dyes employed in your sample. There have been several attempts to top the versatility of this concept. But mainly targeting the issue of spectral imaging. Some approaches work with optical grids instead of prisms – by far too inefficient for imaging. There are attempts to overcome the grid problem, only by introducing even more optical elements, consequently eating photons. And cumbersome serial-filter systems can do basic spectral scanning, but at very limited accuracy.

FRET

After all. the real benefit of the SP® detector is not lambda scanning, but efficiently collecting specific emissions from different dyes in the sample. Most applications would not even need lambda scanning. For example if you want to monitor FRET it is sufficient to monitor just two channels, since the changes occur spectrally insensitive and only one species increases, whereas the other decreases. All you need is two channels – however, with the correct band-settings.

All those benefits

How the SP detector fits the future

Confocal microscopes – even when equipped with an SP® detector – still suffer from two main obstacles. Firstly, there is still the primary beam splitter: the element, which splits the excitation for illumination and the emission for detection. Classically, this is again a device, which is not tunable and as well not very efficient. Here, the Leica AOBS® overcomes these restrictions. The AOBS®, an acousto optical beam splitter, is a tunable element at very high transmission, which switches within a matter of microseconds to allow you to appropriately channel the illumination and emission bands.

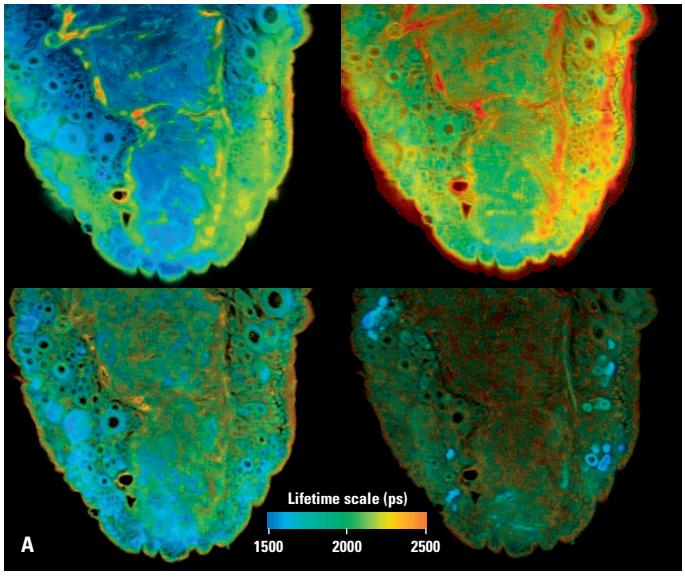
- **Spectral Detector, five confocal channels**
- True confocal point-scanning – real optical sectioning
- High dynamic sensors
- Low dark current PMTs
- High efficiency prism spectrometer
- Loss-free band selection
- Free tunable emission bands
- Any dye adaptable
- Five bands simultaneously
- Dye separation: sufficient for five channels simultaneously

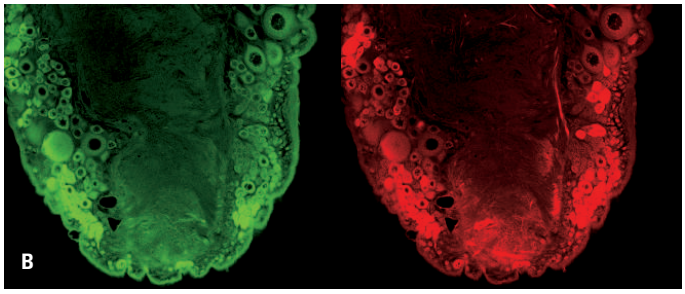
Optionally, Leica Microsystems offers two fast sensors connectable to detection equipment for fluorescence lifetime imaging within the SP® detector. This combination allows for performing FLIM measurements in any emission segment of the spectrum. A sequence of FLIM measurements at incremented wavelengths provides a new dimension in microscopy: fluorescence lifetime as a function of emission color "SP FLIM". A new area, which is still waiting for exploration. Completely new applications will be possible. More differentiated insights in FRET processes by simultaneous detection of acceptor and donor, and other quenching effects are now available for research. In addition, the SP-FLIM has better photon counting efficiency than filter-based concepts.

Spectral FLIM – a new dimension

Fluorescence Lifetime Imaging

To further increase the range of applications, it is possible to install two different sources for FLIM excitation in the same system: either a pulsed Titanium-Sapphire laser, which is the standard source for multiphoton excitation, or a pulsed 405 nm laser diode.





Schistocerca gregaria, nervous system, double staining Cy2, Cy3.
A) SP-FLIM images
left: emission 500-550 nm, right: emission 575-647 nm
top: D-FLIM, excitation 405 nm, bottom: MP-FLIM, excitation 780 nm
B) Intensity images for comparison
left: excitation 488 nm, emission 500-550 nm
right: excitation 561 nm, emission 575-647 nm

www.confocal-microscopy.com

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