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Really Confocal

True Optical Sectioning at all Speeds

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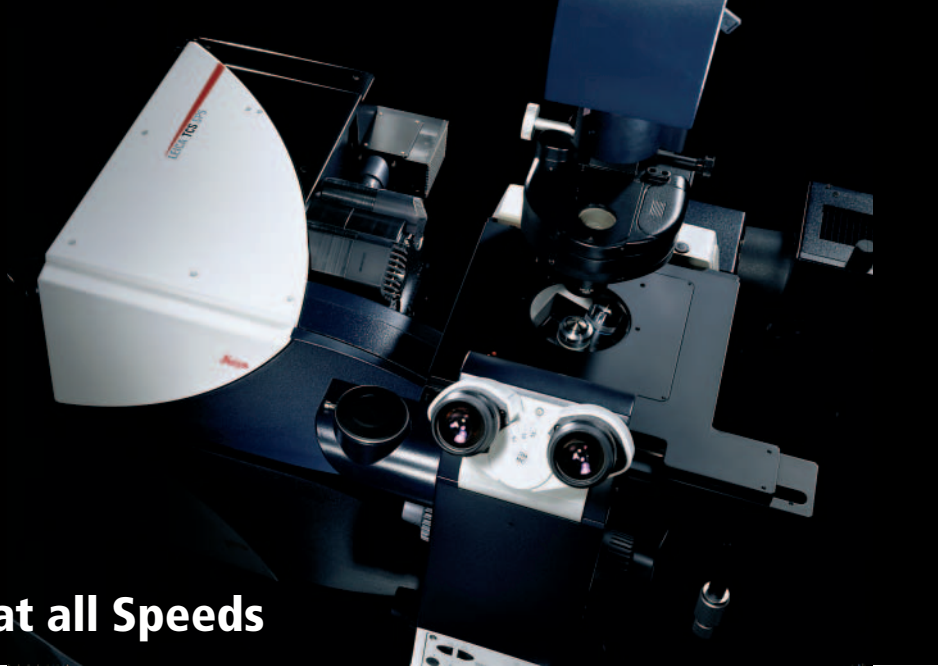
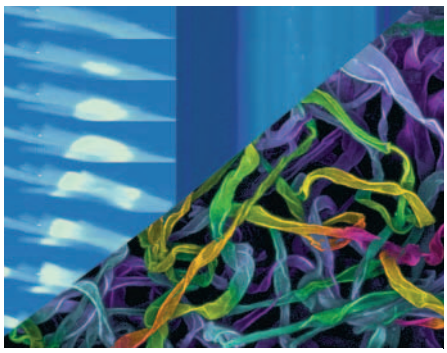


Really Confocal

– True Optical Sectioning at all Speeds

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 or



Two worlds
Upper left: Ca2+ waves and sparks (xt-scan) recorded with high speed resonant scanning. Lower right: colour coded 3D-reconstruction of leaf-hairs of Vitis vinifera

der 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 dedicated not to primarily create nice images, but significant numbers. Physiological data as Ca^{2+}-concentration changes, electrical potential differences and various probes for metabolites, proteins and genes are 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 a lot.

To meet the researcher's requirements, two different types of microscopes are on the market: systems for high resolution imaging, and systems for high speed. As 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.

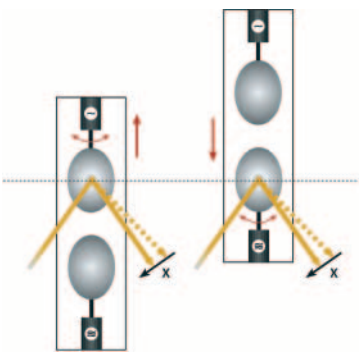
With the new Leica TCS SP5 spectral true confocal microscopy system by Leica Microsystems, these challenges have been solved.

High Resolution and High Speed – in one System

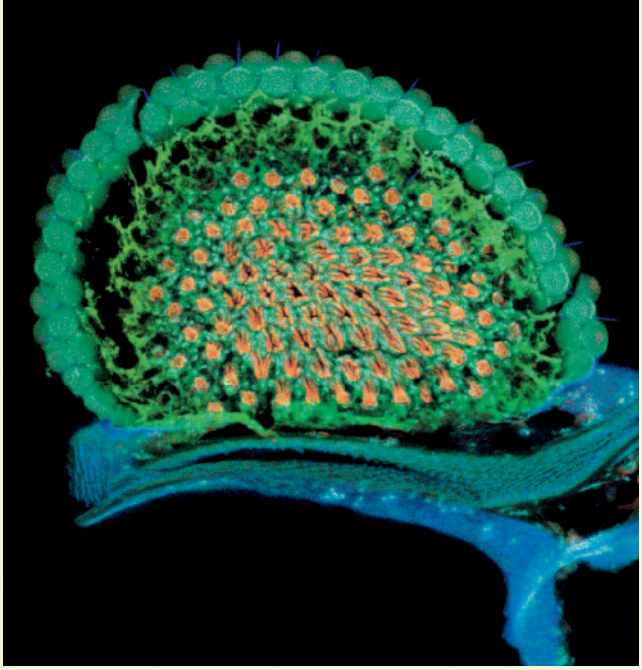
The Leica TCS SP5 combines two technologies in one single system. For extreme resolution and maximum signal-to noise, a conventional scanner is available. It allows freely tunable speed anywhere from 1 Hz to 1,400 Hz, resulting in frame rates up to 5 Hz for full 512^2 pixel images. Conventional scanners 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 new Leica TCS SP5 offers both scanning modes. By its unique Tandem Scanner, both technologies are mechanically and electronically merged into one single instrument.

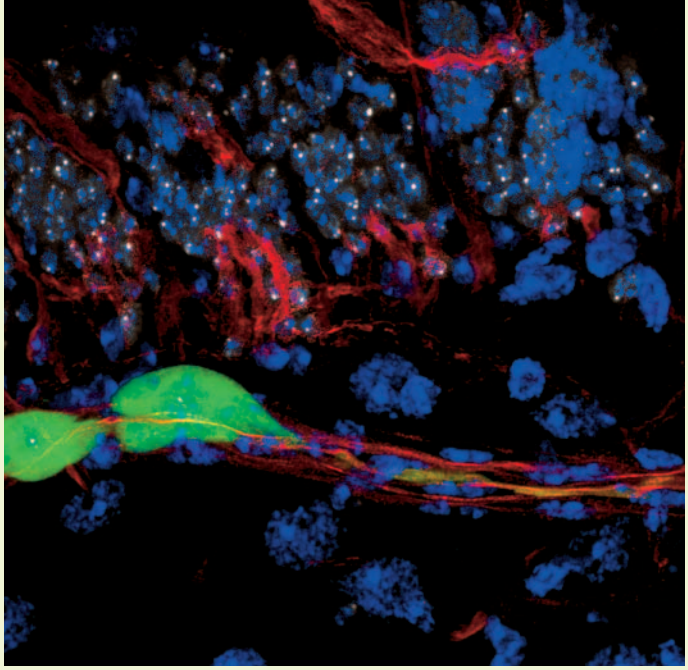


By means of a motorised and computer controlled high precision device, a conventional and a resonant galvanometric driven scan mirror are exchanged into the proper position for scanning, while the scan electronics is switched simultaneously.



Drosophila melangogaster (Eye section)
Red: F-Actin, Cy3; Blue: Nuclei DAPI; Green: pigmented cells, GFP

Courtesy of Anne Galy, IGBMC, Strasbourg-Illkirch, France



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.

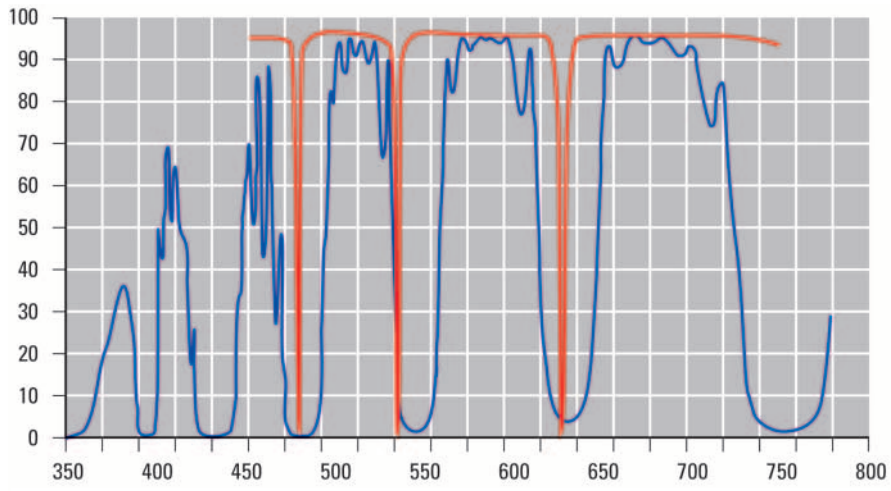
An absolute precision actuator provides the possibility to switch from conventional to resonant scanning during system initialisation. Thus the researcher is free to investigate one specimen through both approaches – a vital precondition for experiments with micropipettes inserted in the sample.

All those Benefits

The advantages of favoring 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 multi-parameter fluorescence, which is one of the other significant drawbacks of alternative solutions. The 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.

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.

Shortish reasoning on photonic efficiency was casting resonant scanning unjustifiably into doubt as solution for fast scanning. In reality, it turned out to even increase emission-intensity and decrease photobleaching. The first is because upon short excitation times, less fluorochrome 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 decreases also – preventing the molecule from photo damage caused by these high-energy states.



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

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 wavelength.

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 for new areas of application, which were not available so far . And for sure there will be more possibilities tomorrow, which yet have not been thought of. The creativity of modern fluorescence scientists will surely challenge this and bring new applications to the surface.

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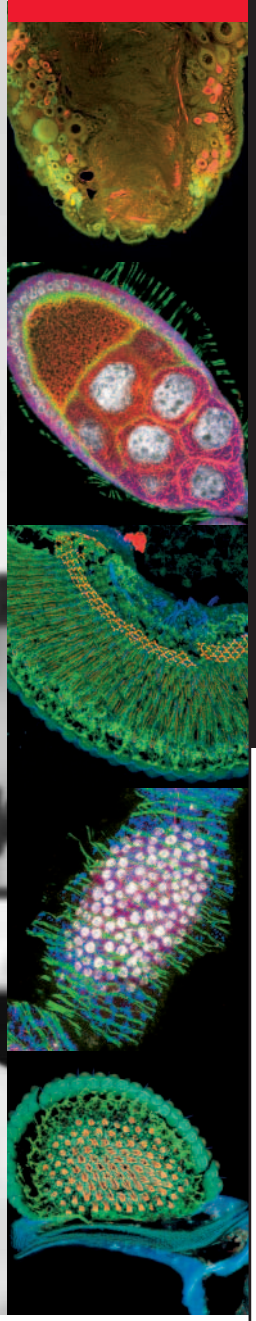

Reach High!

The New TCS SP5 – The Only Broadband Confocal!

- High-speed Live Cell Imaging and High Resolution Morphology – All in One
- The Fastest True Confocal System
- Leading in Multispectral Imaging
- Intelligent and Intuitive Interface

Learn more about the new Leica TCS SP5 at www.leica-microsystems.com/SP5

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