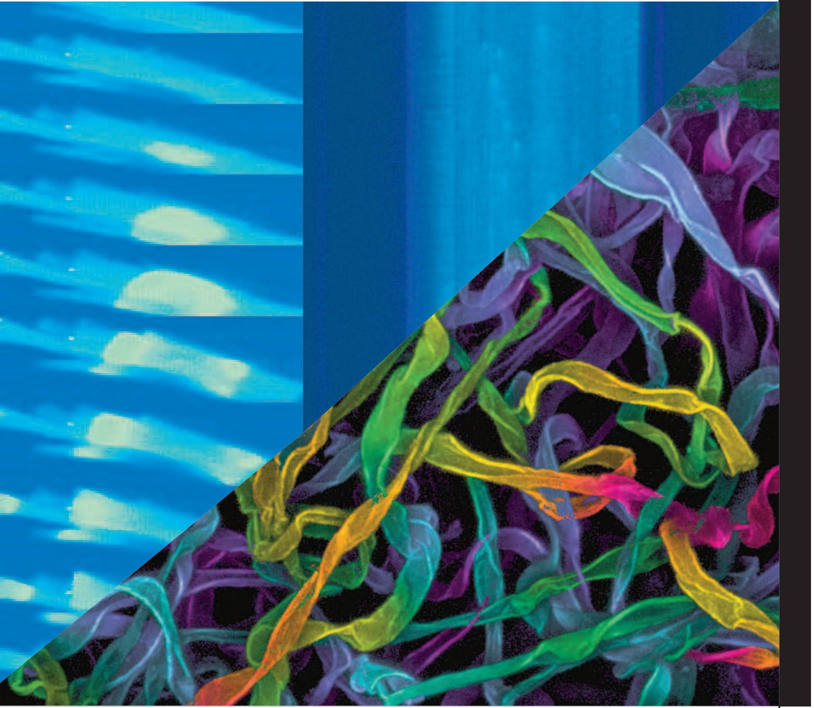
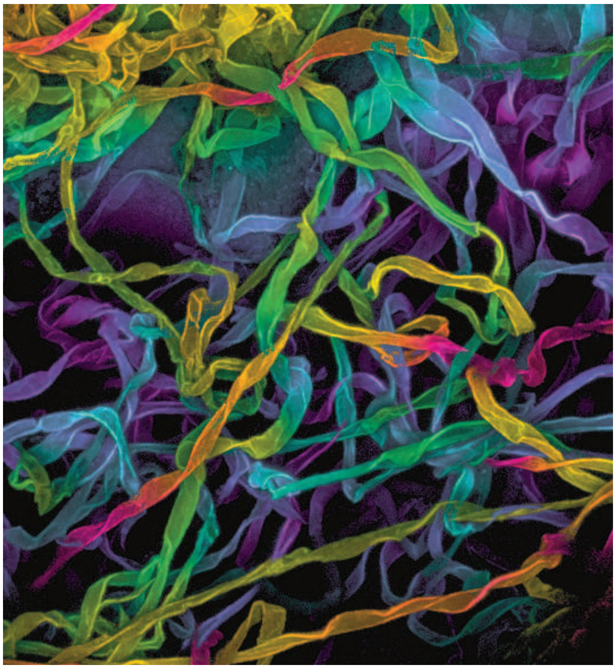


Tandem Scanner

Leica TCS SP5 – The Broadband Confocal

High Speed and High Resolution – All in One

Leica MICROSYSTEMS



Vitis vinifera.
Hairs on young leaves.
Color coded projection of 100 optical sections recorded over 200 µm; autofluorescence.

Modern microscopy comes in two versions. On one hand, the goal is to record brilliant images to illustrate clearly morphological features (and by the way create impressive pictures). On the other hand, microscopy systems have evolved into measuring instruments, extracting significant numbers from living samples. This discrepancy also translates into technological solutions.

Regarding confocal microscopy, the systems on the market, today, are either optimized for morphological image acquisition – where sufficient time is available for image restoration – or for rapid data acquisition – where speed is the main concern whereas beauty is not an issue. In the latter case the recording parameters are mostly set in a way to „just see something behind the noise".

This means one has to invest twice, if research shall be done in both categories. In central facilities, serving for institutions or whole universities, this is a common request nowadays.

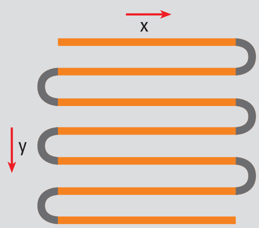
Two worlds

Spot-recording and real-time-imaging

The problem

To achieve good optical sections a true confocal system is the right choice. This system moves a diffraction limited illumination spot in a scanning mode line by line over the sample. Also the detection system must have a point-like sensitivity distribution. The detected signal is then again composed back into an image.

For high speed recording the line-wise data acquisition is the limiting bottleneck caused by physical movement of the scanning mirror which is responsible for line-acquisition. This mirror is by convention referred to as „x-mirror".



Scan process in a true-confocal laser scanning system. Data are recorded during the linear parts (orange). Fast movement is required for x-directed scanning.

To fulfill both requirements the x-mirror should provide the possibility of very fast and also very slow movement. Conventional scanning systems work satisfactorily up to 1000 Hz. For higher speeds the field of view is severely limited. This means a 512 x 512 standard image can be recorded in roughly half a second. For fixed samples this causes no problem at all. Here the goal is to acquire nice, very well resolved images at full contrast and without visible noise. A slow and feed back controlled movement of the scanning mirror will ensure these requirements.

Also living samples can benefit from conventional scanners as long as the movement or fluorescence-changes in the sample are slower than the frame frequency.

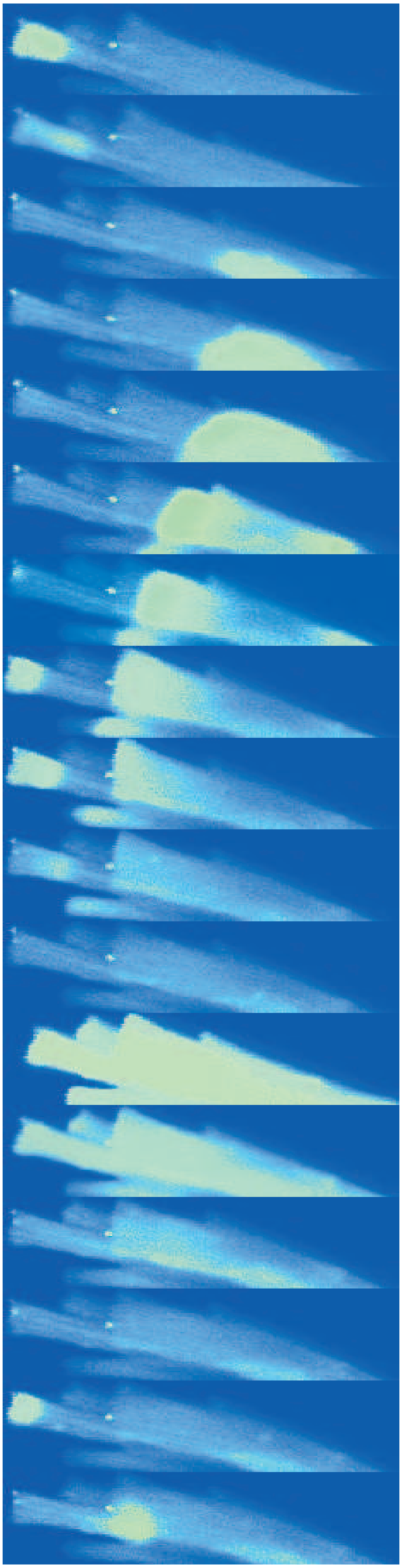
For the recording of e.g. Ca^{2+} waves in a living sample a conventional scanning system is far too slow. But „tuning“ it to higher speeds brings significant limitations (field of view). Here, a new solution by Leica Microsystems solves the problem:

Resonant Scanner

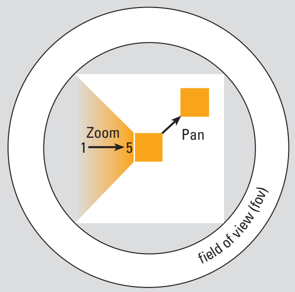
The resonant scanning system of the Leica TCS SP5 works at 8000 Hz frequency and enables to record data in both directions of the scan motion. It thus acquires about 25 images per second at a frame resolution of 512 x 512 pixels. This is about 10 times faster compared to the recording speed of conventional scanners. For even faster events, one can reduce the number of lines per image managing up to 200 frames per second. For extremely fast changes, data can be recorded by images containing only one single line. These xt-scans allow up to 16.000 Hz time resolution.

Large field of view

The application of resonant scanners has additional advances compared to other high-speed solutions. The field of view is very large (15 mm fov) compared to conventional scanners, where the field of view at 2000 Hz is restricted to some 3 mm fov. The field of view is tunable by resonant scanners in a large range. This does not work for systems that employ cameras (typically spinning-disk based systems or similar technologies with parallel illumination) since their field of view is fixed by the CCD chip size and the optical magnification.



Ca^{2+} waves in muscle cells shown by a fluorescenct Calcium-indicator and recorded with a resonant scan system.
Courtesy of D. Eisner
University of Manchester, UK



Zoom function by decreasing the scanning angle of galvanometer-mirror, pan function by adding a scan-offset in x and y to the zoom-image.

With the computer controlled shifting of the y-position, the Leica TCS SP5 has a panning function also for the resonant scanner which allows for moving the position in the optical field. This is important for adjusting to a small section of the image field without cumbersome adjustment of the stage. This saves time and avoids the early damage of the sample.

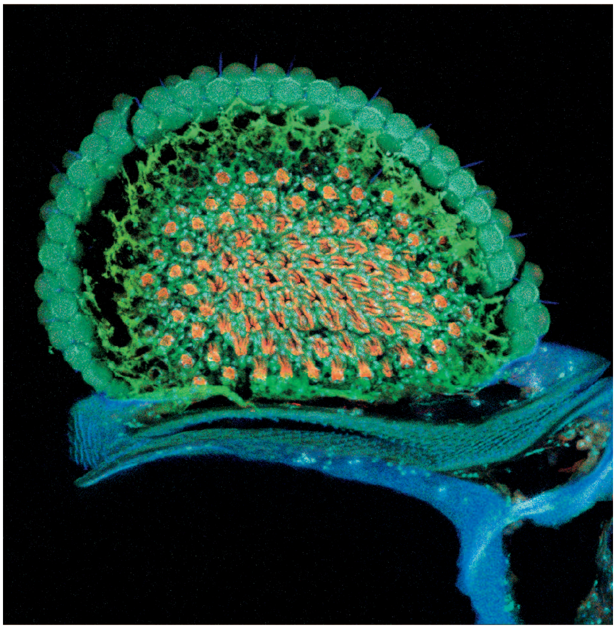
Multicolor Imaging

A further advantage of the resonant scanner is its integration into a confocal system with multiple channels and high efficient AOBS® and SP® detection for multi-parameter fluorescence experiments at high temporal resolution. Regarding this parallel beam systems (e.g. spinning-discs) have significant limitations, which can only be compensated by simultaneous recording with multiple cameras. A very expensive and complicated approach, where multiple in fact is restricted to two. The Leica TCS SP5 en passant offers effectively 5 channels simultaneously for high speed imaging.

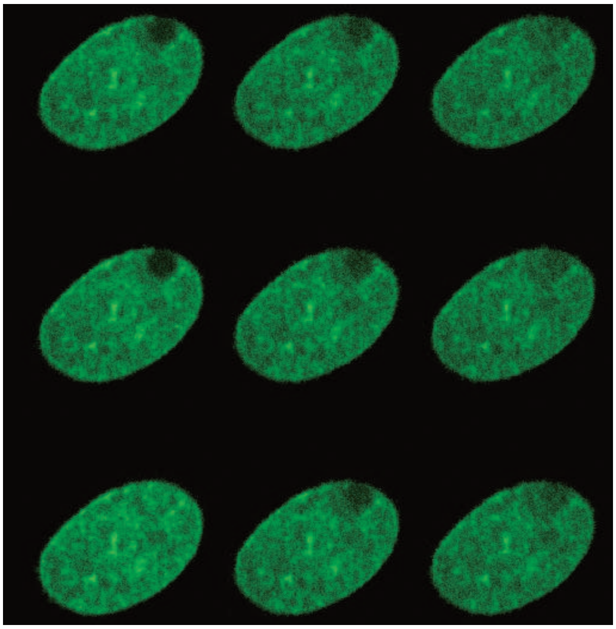
Beam Park

But a resonant scanner cannot stand still at a given coordinate. Here, conventional scanners have still advantages. A „beam park" function allows to select a position in the sample and bleach selectively fluorescence at that point, e.g. for FRAP analysis. Furthermore, it opens the possibility to record fluorescence at that spot at a very high time-resolution (up to 40 MHz).

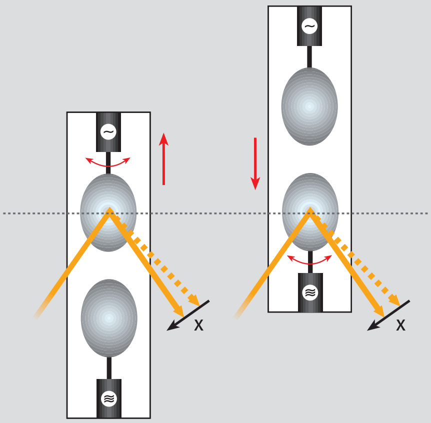
It is obvious that a system offering both technologies would be very advantageous for users.



Drosophila melanogaster (eye section)
Red: F-Actin, Cy3; Blue: Nuclei, DAPI; Green: pigmented cells, GFP
Courtesy of Anne Galy, IGBMC, Strasbourg-Illkirch, France



Fluorescence Recovery After Photobleaching (FRAP) upon spot-bleaching at the lower right corner of a nucleus using the beam park function.



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

Tandem Scanner

Here, Leica Microsystems sets new standards with its new TCS SP5: It works with both a conventional and a resonant scanner which can alternatively be employed. Both scanner systems are mounted on a high-precision mechanical carriage and thus can be exchanged into the exact optical position – all motorized and computer controlled. The arrangement is designed as self adjusting and therefore does not require technical personnel. It is sufficient to decide during switch-on which mode is required. The rest is done fully automatically by the system. Changing the mode is simply done by the re-initialization of the system.

Two worlds in one

The full range of scan speeds in one single system

The advantages for a user of such a configuration are obvious: two completely different experimental requirements can be satisfied by one single system. On one hand classical morphology, e.g. research on structures of cytosceleton, organelles or tissues, where highest spatial resolution is required and the images should not show residual noise. On the other hand, physiology and biophysics, where naturally temporal resolution becomes very important and the images may, but must not necessarily look nice as long as data are recorded fast and safely. Of course, one can also create nice images with a resonant scanner by line- or frame averaging.

The Leica TCS SP5 merges these contradictory fields of application in a single system. This is economical on one hand, and allows to do completely different experiments with one system without having to move the sample (which would be impossible e.g. for brain sections with micropipettes implanted).

Conventional Scanner

- True confocal point-scanning – real optical sectioning
- Extra large field of view (23 mm)
- Superior resolution: 64 Megapixel images (8k x 8k)
- Beam Park data acquisition
- Spot-bleaching FRAP
- Zoom- and pan function
- Slow scan mode
- Up to 5 confocal channels simultaneously
- 2-channel Spectral Fluorescence Lifetime Imaging (SP FLIM)

Resonant Scanner

- True confocal point-scanning – real optical sectioning
- Large field of view (16 mm)
- Zoom function
- Panning function
- 16.000 lines per second
- 250 frames per second
- 25 frames per second 512 x 512
- Up to 5 confocal channels simultaneously
- Low photobleaching during image acquisition

www.confocal-microscopy.com

Leica

MICROSYSTEMS

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