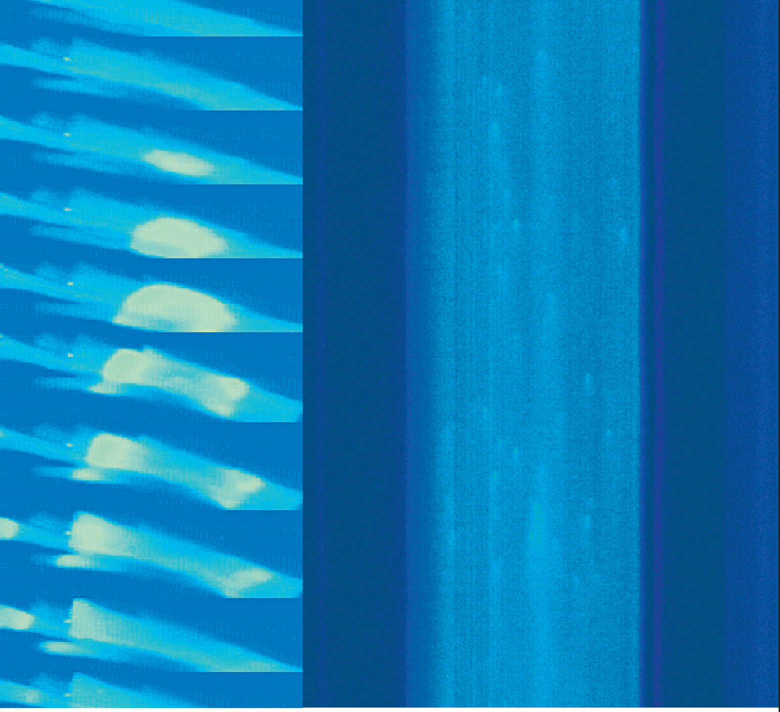
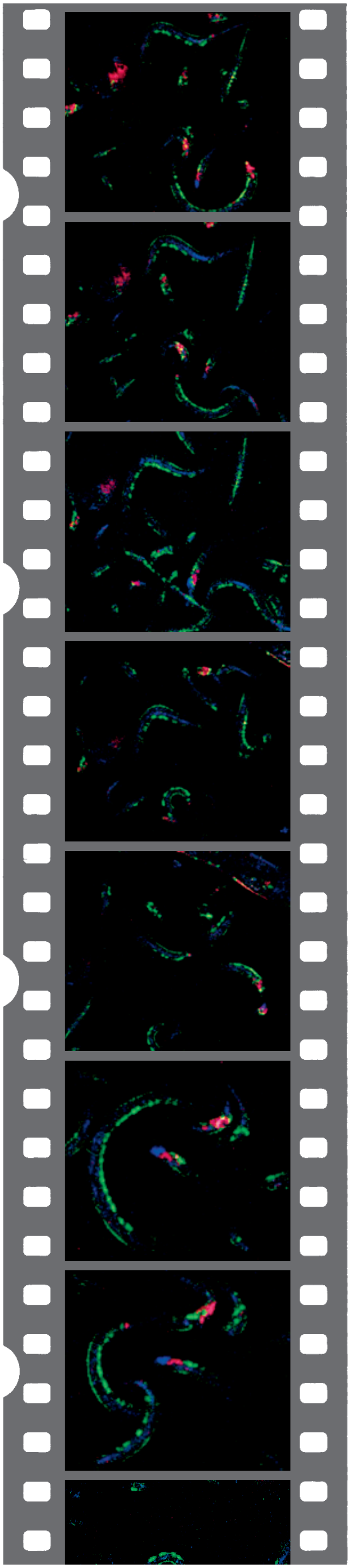


Resonant Scanner

Leica TCS SP5: The Broadband Confocal

Really Confocal Fast Optical Sectioning

MICROSYSTEMS



Caenorrhabditis elegans
(neurons)
Time Sequence
Green:
GABAergic neurons, GFP
Red:
glutamagergic, DsRed
Blue:
sensory neurons, DiD
Courtesy of Dr. Harald Hutter, Max Planck Institute for Medical Research, Heidelberg, Germany

Fast acquisition . . .

Modern microscopy is not any longer only a matter of excellent still images, multiple colors or high optical resolution. The trend is clearly quantitative data recording of living samples. This is why scientists in this research field require the best possible equipment for high speed image acquisition.

Many solutions are now on the market, and some of them claim to be even confocal. But on a closer look, true confocal systems for high speed are missing. Typically, the currently available "confocal" instruments illuminate and observe in parallel. The resulting image, therefore, is not really confocal, but still shows a significant background (out-of-focus haze) which is not, what you expect from a true confocal microscope. Your goal is clear optical sectioning, producing very thin slices for best contrast and structural localization capabilities – at high speed, of course.

. . . and a close look?

High optical resolution and high speed imaging. Can we have both? Yes we can. Leica has developed a solution to have both by one system: with the implementation the new resonant scanning system into the Leica TCS SP5 the system combines a true confocal scanner for excellent imaging and a resonant scanner for high speed imaging. The Leica TCS SP5 provides the two – today very much required – technologies in one single system – a broadband confocal for all applications.

The resonant scanner provides the benefits of true confocal imaging and fast frame recording; with some additional edges. It is indeed quite tricky and challenging to implement a resonant scanner into a quantitative imaging instrument, but our engineers took this need as a challenge – and succeeded.

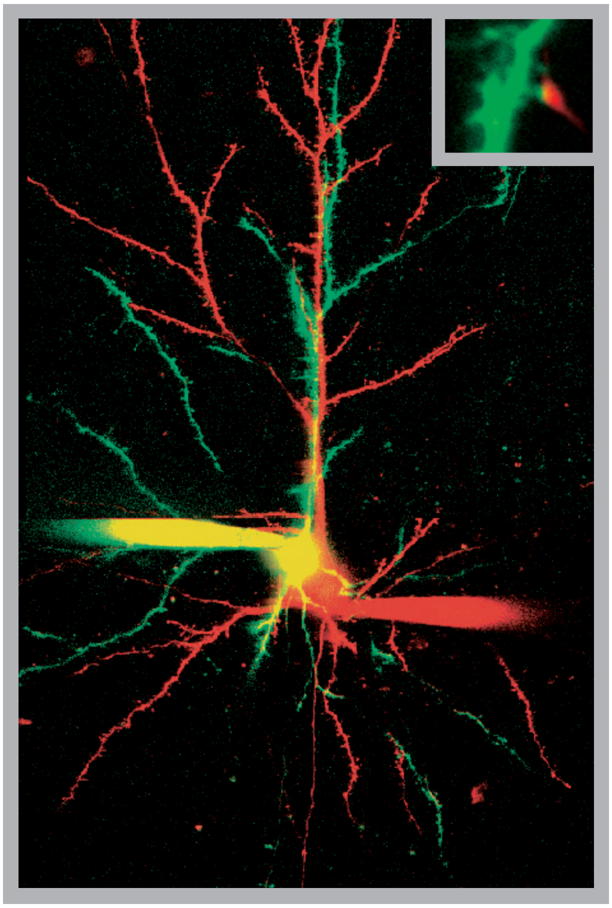
Speedy Life

The technology behind

Most scanning systems employ galvanometric driven mirrors. This galvanometer devices work like electric motors – but with the rotator axle fixed to the anchor at one side. Conventional scanners have a feedback loop, which allows for halting the position at any angle, for fine tuning the movement during scanning and for tuning the scanning frequency over a wide range – but all this at the expense of speed. A resonant scanner works ten times faster, but without control of speed, as it operates at its resonant frequency. Very much like a pendulum, you can charge the resonant scanner with energy – which results in variable amplitudes, but at a single constant frequency. This frequency can be much higher as compared to conventional scanners. The Leica TCS SP5 uses the current top model, working at 8 kHz.

Besides for confocal imaging, this device is also a platform for multiphoton excitation by pico-second laser sources; dentically to current multiphoton systems – just at high speed.

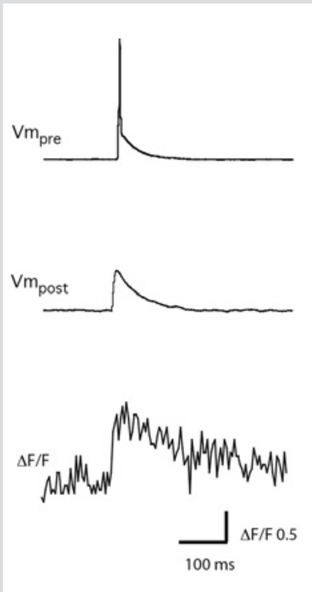
What does this mean in terms of speed?
Current research in neurobiology, cell biology and other disciplines asks for constant improvement of time resolution to investigate the dynamics of signaling, transport and development in Biology. This is the case for both structural imaging and analytical methods like FRAP-experiments. To have an idea of the performance of the resonant scanner: Let us assume a standard image size of 512 x 512 pixels. A conventional scanner allows not more then about two to three frames per second in this format. The resonant scanner can acquire the data 10 times faster: up to 25 frames per second. This is sufficient to display flicker-free real data. But also much faster processes can be recorded and analyzed, if restricted to fewer lines. At a frame format of 512 x 32 pixels, the resonant scanner acquires up to 250 frames per second. This is sufficient for even speedy Ca^{2+} changes, fast liquid streaming or particle movement.



Reliable Numbers

Variable frame sizes
Variable frame sizes allow also for the restriction to a single line (xt-scan) in bidirectional mode. Here, the acquisition rate arrives 16 kHz, corresponding to a time resolution of about 60 microseconds! This is fast enough to even show details of membrane-potential changes and Ca^{2+} sparks.

On the other side, also very slow time lapse recordings are required for embryonic development, cell migration and related areas of research. With the resonant scanner, time resolution can be reduced by averaging, which improves the signal-to-noise: a additional free-of-charge benefit. Slower frame rates can also be achieved by recording images at tunable delay times, keeping the amount of energy on the sample as low as possible – and the cell more comfortable. There is virtually no limit on the slow side.



High speed Ca^{2+} recording: Synaptically connected neurons from rat brain. Overview of two cells with injected stain and pipettes for electrical recording. The inset shows the connecting point of the two neurons at high magnification. The charts show electrical signal of an Action Potential (upper), the postsynaptic electrical response (mid) and the Ca^{2+} response in the postsynaptic spine recorded in xt-mode by fluorescent Ca^{2+} indicator.

Courtesy of Dr. Helmut J. Koester, Baylor College of Medicine, Div. of Neuroscience, Houston, TX, USA

Optical Slicing . . .

The resonant scanner is based on the true confocal concept of point-illumination and point-observation. Only here, the thinnest optical sections can be achieved. Other concepts, like spinning discs, array scanners or line scanner devices, suffer from spatial leakage. This instantly impairs the depth-discrimination. Only the Leica TCS SP5 with resonant scanner combines high speed and high resolution!

. . . at any z-position . . .

An inherent advantage of true confocal optical sectioning is the possibility to record images at any z-position inside the sample – within the free working distance of the objective lens. This is not possible with evanescent field optical sectioning (TIRF), where only a single section at the surface of the sample is imaged. This allows for following movements of particles and metabolites deep inside the sample, as well as 3D stacking and reconstruction; including time lapse 4D series.

Really Confocal

. . . and at real time!

Real time is a precondition for work in live brain slices, where imaging is intimately connected to experimental feedback. Offline deconvolution would not solve this problem. Moreover, deconvolution is still time consuming, and high speed acquisition typically produces very huge data files – quite unpleasant objects for deconvolution.

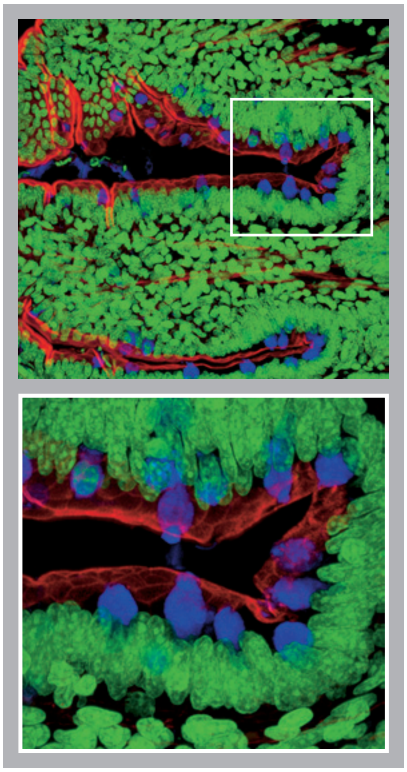
From a broad horizon . . .

Even at 250 frames per second, the resonant scanner offers a large field of view. 15 mm fov, corresponding to 400 µm object diameter with a 10x lens. Conventional scanners have to restrict to fields down to 3 mm fov, when operated at frequencies above 1,5 kHz. Here the resonant approach is much more beneficial, especially for whole mounts of live embryos, e.g. drosophila or zebra fish samples.

. . . zoom in . . .

As described above, the amplitude of a resonant scanner is tunable. This allows for zooming in, just by applying smaller amplitudes. This feature, which one expects anyway, is not directly available from camera-based systems. By applying zoom factors, it is possible to restrict photon loading on the sample and to reduce the amount of data, when only those areas being of interest are acquired. Zooming allows to fine tune the voxel size as well, a crucial parameter to transfer optical resolution to the final image.

Effect of zoom and pan function: a smaller area anywhere in the field of view can be imaged at high resolution-transfer-efficiency.



. . . and panning.

The last challenge about resonant scanners, was the pan-function – a very helpful device to move quickly into areas of interest, which are not necessarily in the center of the microscopic field. As the position of the resonant scanner cannot be controlled, the pan-function has been introduced into the TCS SP5, compensating this restriction. As a consequence, you can take an overview image, mark a position of interest anywhere in that image, and the system will automatically move to the desired location. No gearing by hand and constant exposure of the sample to laser light. In this point, too, resonant scanning outperforms any system based on camera images.

The play of colours

Multispectral imaging with the AOBS® (Acousto Optical Beam Splitter) applies as well to the resonant scanning system. Your research does not suffer of any restriction in excitation wavelength or in spectral band selection for emission. There is no chromatic aberration from micro lens arrays. Profit from the full spectral performance and work with five confocal channels simultaneously!

MotionSpy software

MotionSpy is one out of the long list of software options. MotionSpy is dedicated to the analysis of movement, tracking of objects and rearranging regions of interest for illumination and quantification. It meets perfectly the requirements of the current trend in fluorescence microscopy: dynamic data acquisition and analysis – at high and slow speed.

More dimensions

The new galvanometric driven z-stage SuperZ moves in parallel to the field plane over up to 1,5 mm travel distance. In combination with resonant scanning, the SuperZ-stage opens the world to really fast 4D sequences with subsequent time-space visualization and analysis. This is where the future is – at high speed and high resolution and with some additional benefits along the way.

Resonant Scanner

- True confocal point-scanning – real optical sectioning
- Large field of view (16 mm)
- Zoom function
- Panning function
- Variable frame formats (up to 1024 x 1024)
- 16.000 lines per second
- 250 frames per second
- 25 frames per second at 512 x 512 pixels
- Up to 5 confocal channels simultaneously

www.confocal-microscopy.com

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