ADVANCED CONFOCAL MICROSCOPY AND HIGH SPEED IMAGING

René Hessling
Carl Zeiss MicroImaging GmbH
Carl Zeiss Promenade 10, 07745 Jena, Germany
email: r.hessling@zeiss.de

KEY WORDS: Confocal laser scanning microscopy, multi-dimensional imaging, photomanipulation, photobleaching, sensitivity, uncaging

Today, biomedical sciences are progressing from the static analysis of structures in fixed samples towards direct observation of dynamic processes in living objects like live cells, tissues and organisms. Furthermore, recent developments of multiple variants of fluorescent proteins have opened up a large variety for new experimental setups in analysing protein-protein interactions and protein dynamics.
For studies of intracellular dynamics including diffusion processes also under steady state conditions various techniques of photomanipulation including fluorescence recovery after photobleaching (FRAP) and photoactivation are employed. Such applications demand technical solutions for the observation of fast dynamic processes under suitable environmental conditions for live samples including the need for low intensity fluorescence excitation.
Carl Zeiss has recently developed a high speed confocal laser-scanning microscope, which uses line-wise illumination and detection to achieve scanning speeds of up to 120 frames per second at full frame resolution (512 x 512 pixels). Making use of the high flexibility of the scan control system even higher frame rates e.g. more than 2000 frames/second at 512 x 25 pixel resolution are possible, while imaging up to two channels simultaneously. Even though the frame rate of this system can be more than 20 times faster than on conventional point-scanning systems, the pixel-dwell time is actually much longer resulting in better signal-to-noise ratios and lower laser dosage per time. New options in the beampath now allow imaging of up to 4 channels with up to 60 frames per second at 512 x 512 resolution. New scan options employing microscanning now enable imaging with a lateral resolution of up to 1536 x 1536 pixels.
With the LSM 5 *DUO* a new high-end confocal system has been developed combining the ultra-fast scanning properties of the line scanning module together with the usage of a second pixel-precise point scanning system. While retaining all functions of the individual line- an point scanning systems this solution enables numerous new applications in live cell imaging with parallel manipulation, e.g. uncaging approaches, photoconversion or FRAP (-related) techniques.