

REAL TIME CONFOCAL MICROSCOPY IN MULTIPLE DIMENSIONS: DISK OR ARRAY - THAT IS THE QUESTION.

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Our understanding of physiological and pathophysiological processes in living cells has gone a long way in the past decades. Optical technologies have driven that development in the last 10 years to a great degree and we are nowadays able to visualise processes in living cells that occur in extremely small volumes (< 1 fl) that are of a very short lifetime (ms time domain) for extended periods of time. A central piece of equipment enabling us to perform such studies is a real-time confocal microscope. There are various incarnations of such microscopes and we would like to discuss the different setups but also introduce an exciting new technology with a great potential of driving this development even further.

Despite years of research and development, high imaging frame rates are still a remaining request in confocal microscopy. A new kilo-beam confocal scanner has been developed based upon a novel optical geometry where the pinhole and microlens arrays are stationary. The light path contains a single moving part – a galvano-driven mirror serving a triple function: (i) it scans a few thousand parallel laser beams across the specimen, (ii) descans the returning fluorescence on a stationary, exchangeable pinhole array and (iii) rescans the same emission onto CCD-camera detectors. Rapidly and automatically interchangeable novel dichroic mirrors and emission filters enable a great flexibility during the experimental process. The novel design of the dichroic mirrors is such that slight variations of its angle relative to the light path do not effect the position of the image.

Compared to today's Nipkow-disk based scanners, the new kilo-beam 2D-array scanner conserves all positive features like real time capability, high resolution and low bleaching rates, but introduces more flexibility and an optimal synchronisation between frame generation and image acquisition with CCD-cameras. With the recent introduction of high speed electron multiplication CCD-cameras such a frame-to-frame precise synchronisation has become an essential issue for signal quality.

Nevertheless, for ultra-high speed confocal imaging, the acousto-optic deflector based technology is still the most flexible approach with the best speed/resolution ratio.

Here, we compare the above mentioned technologies for two of the major fields of applications in life cell imaging that require real time acquisition: (i) high resolution ultra-high speed confocal imaging of elementary Ca²⁺ signals (e.g. Ca²⁺ sparks) with frame rates between 100–500Hz and (ii) 3D, 4D and 5D imaging of protein translocation and organelle imaging with frame rates between 0.5–100Hz.