Confocal Kilobeam Scanner with Variable Pinholes for Live Cell Imaging

Peter Lipp¹, Ken Bell², Jafer Sheblee², Lars Kaestner¹

¹Institute for Molecular Cell Biology, Saarland University, Homburg/Saar, Germany
²VisiTech International Ltd., Sunderland, UK

Our increased understanding of physiological and pathophysiological processes in living cells is driven by advancements in optical technologies in the past decades. 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 (in the ms time domain) for prolonged periods of time. A central piece of equipment enabling us to perform such studies is a real-time confocal microscope (frame rates of >30 Hz). There are various incarnations of such microscopes and we would like to discuss the different approaches to scan with several thousand beams simultaneously, the so called kilobeam scanners (Nipkow disc system and 2D-array scanner): (i) The different concepts of sample illumination by either Gaussian distribution or swept illumination and (ii) the impact of using exchangeable pinhole arrays with varying pinhole size in the range of 10-64 µm on the generation of the confocal images in terms of objective parameters, point spread function and light efficiency. The latter approach will eliminate the long-standing disadvantage of kilobeam scanners: fixed pinholes optimised only for a single objective magnification. Here, we present the above mentioned technologies for two of the major fields of applications in life cell imaging that require real time acquisition: (i) high resolution/ high speed confocal imaging of cellular signalling processes (e.g. protein translocation events and Ca²⁺ sparks) with frame rates between 1–200 Hz and (ii) 3D, 4D and 5D organelle imaging with frame rates between 0.1–10 Hz for prolonged recording periods (> 1h).

Mouse atrial myocytes were loaded with fluo4. The upper panel depicts individual time points of the calcium spark event marked with the arrow in the line-plot (lower panel). The calcium increase was coded in the height of the surface. Line-tracings show the fluorescence change at an identified calcium spark site (arrow at 30 ms). Imaging at 200 frames/second.

This work was supported by the DFG (HBFG, SFB), the Saarland University and the Medical Faculty (HOMFOR).