VARIABLE PINHOLES IMPROVE KILOBEAM-ARRAY SCANNER FOR LIVE CELL IMAGING

Peter Lipp¹, Ken Bell², Jafer Sheblee², Lars Kaestner¹

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

E-mail: peter.lipp@uks.eu

KEY WORDS: Live cell realtime confocal imaging; kilobeam array scanner; local translocation; organelle imaging; 4D-imaging; calcium sparks

In the last 10-15 years our understanding of physiological and pathophysiological processes in living cells has been advanced by improvements in optical technologies. Today, we are in the position of visualising such processes in living cells, including those extremely shortlived (approx. ms lifetimes) in sub-femtoliter volumes as well as processes lasting for minutes or hours characterised by a high degree of spatio-temporal complexity. In all of those cases, realtime-confocal microscopy has become the prime tool of choice. Such an instrument offers acquisition rates exceeding video rate (>> 30 Hz). The most versatile realtime microscopes employ a multitude of parallel laser beams (> 2000) to allow fast scanning at reduced bleaching rates. We would like to compare two of such scanners, one of them being an established scanner based on Nipkow-disc technology, the other one represents a novel development overcoming many of the shortcomings accompanied by the former approach. We will discuss: (i) Basic concepts of sample excitation by either Gaussian light distribution or swept illumination and (ii) the impact of using exchangeable pinhole arrays with varying pinhole sizes (10-64 µm) on the generation of the confocal image with respect to objective parameters, point spread function and light efficiency. Here, we present the application of the above mentioned technologies for two of the major fields in life cell imaging requiring real time acquisition: (i) 3D, 4D and 5D organelle imaging with frame rates between 0.1–10 Hz for prolonged recording periods (> 1h) as an example for high resolution long lasting imaging (see fig. 1) and (ii) high resolution/high speed confocal imaging of cellular signalling events (e.g. local protein translocation events[1] and Ca²⁺ sparks) with frame rates between 1–200 Hz.


Work supported by DFB (SFB, HBFG), Saarland University and the Medical Faculty (HOMFOR)

Figure 1: Live cell imaging of HEK293 cell expressing mito-dsRed2. Z-stack imaging was performed every 20s with 45 slices (0.3 µm apart, exposure 250 ms) using an Andor EM-CCD camera attached to a VTinfinity. Surface rendering was achieved with Imaris. Movement of a single mitochondrion is depicted by the arrowhead.