LATTICE LIGHT-SHEET MICROSCOPY
FOR EXPANDED SPECIMEN

Anne Stockhausen, Jana Bürgers and Ulrich Kubitscheck

Institute of Physical and Theoretical Chemistry, University of Bonn, 53115 Bonn, Germany

E-Mail: stockhausen@pc.uni-bonn.de

KEY WORDS: Lattice light-sheet microscopy, light sheet fluorescence microscopy

The use of Gaussian beams for excitation in scanned Light-Sheet Fluorescence Microscopy represents the current standard [1], but results in a comparably small depth of focus in propagation direction, which limits the usable illumination field size. In comparison, the non-diffracting property of Bessel beams allows a larger field of view. Bessel beams, however, show the well-known circular sides lobes, which broaden the illumination field in an unwanted manner. Recently it was shown that an illumination of the back focal plane of the excitation objective with circular stripe patterns produces transversal optical lattice profiles with largely reduced side-lobes while retaining the self-reconstructing Bessel beam character in propagation direction [2,3]. To achieve a homogeneous planar 2D illumination the lattice is dithered in the object plane with a scanning galvanometer mirror. Therefore, the time-averaged light sheet generation is significantly faster than compared to Gaussian beam scanning.

We constructed a lattice light sheet microscope comprising micro-fabricated, fixed ring masks and optical hardware elements only to accomplish a simple and stable workhorse for imaging spatially extended samples with high resolution and high speed. Excitation with different excitation wavelengths is realized by producing the lattices separately for different colors with own ring masks, respectively. Our instrument reaches a maximum frame rate, which is limited by the camera speed only. In order to achieve a lateral illumination field of 300 µm we need a Gaussian beam diameter of 10 µm, while the lattice light sheet shows only a width of 1.4 µm.