A SIMPLE LATTICE LIGHT-SHEET MICROSCOPE

FOR MULTI-COLOR IMAGING OF EXPANDED SAMPLES

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In current research Light-Sheet Fluorescence Microscopy (LSFM) proved as a useful method for the investigation of small structures in cells and tissue [1], but the mainly employed Gaussian beams for excitation of scanned light-sheets exhibit divergence and thus a limited depth of focus. The usable illumination field size can be improved by the use of low-diverging Bessel beams. The beam profile of a Bessel beam, however, shows circular side-lobes broadening the illuminated region.

A suppression of the side-lobes is possible by confining the illumination in the back focal plane of the excitation objective to an annular stripe pattern. The resulting intensity distribution in the sample plane is a thin optical lattice, whose individual beams maintain their Bessel character [2, 3]. To achieve a homogeneous illumination, the lattice is dithered with a fast scanning galvanometer mirror.

We constructed a low-cost microscope using a micro-fabricated fixed ring mask for lattice light sheet generation. The use of optical hardware elements only enables a stable and simple illumination path. In combination with long-working distance objectives and the possibility for simultaneous dual-color imaging, the constructed lattice light sheet microscope features ideal conditions for the imaging of expanded mouse brain slices with a frame rate of 14 Hz. With a 3.7 μm thick lattice light sheet we reached a homogeneous axial resolution of 1.2 μm over a (330x330) μm^2 sized field of view, whereas a Gaussian beam light-sheet with the same waist-diameter shows a strong divergence and thus a decrease of axial resolution at the image borders.

We present experimental data from expanded mouse brain hippocampus to demonstrate the high homogeneous resolution throughout the full imaged volume.

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[2] Chen BC et al., 2015. Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. *Science* 347, 543-8
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