

BIOSS-Seminar, 19-07-2011

Enhancing contrast and resolution in microscopy with self-reconstructing beams by confocal line detection

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Light-sheet based microscopy (LSBM) was first presented in the context of modern cell biology in 2004¹. In the meantime this technique has been widely used especially in developmental biology due to the advantages it exhibits for the observation of living organisms. In contrast to confocal microscopy, LSBM illuminates only that part of the object, that is in the plane of focus of the detection objective (DO) and therefore works light efficient, besides advantages in image acquisition speed and production costs. The principle of light-sheet microscopy is that a light sheet in the focal plane of a detection objective lens is created by a perpendicularly oriented illumination objective lens.

Recently, it has been shown that illuminating strongly scattering samples by self-reconstructing Bessel-beams is advantageous because artefacts due to scattering are reduced². Moreover, penetration depth is increased, which enables the examination of larger specimens^{3,4}. However, images obtained by sample illumination using a scanned Bessel beam suffer from reduced contrast⁴, due to the beam's large transverse extent. Rings surrounding the central lobe carry a large fraction of the total energy which depends on the beam's depth of field and NA. Since the beam's large cross-section is crucial to its self-reconstruction ability, the energy in the rings cannot be reduced. However their negative influence on image contrast can.

In the seminar, I will present a confocal line detection scheme for light-sheet microscopy with self-reconstructing beams (CL-MISERB). Experimental results will be shown that demonstrate the advantages offered by Bessel beam illumination as well as an image contrast and axial resolution superior to that of state-of-the-art light-sheet microscopy (e.g. DSLM⁵).

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