EXTENDED DEPTH-OF-FIELD PARALLAX-MICROSCOPY (EDFP)
FOR 3D - REAL TIME IMAGING

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KEY WORDS: 3D imaging, real time imaging, extended depth of field microscopy, EDF, space light modulation, SLM, stereoscopy, parallax, 3D tracking

One big challenge in biological and medical sciences is the investigation of dynamic subcellular processes. Therefore a high lateral and axial resolution and a large field-of-view exceeding cell dimension (lateral and axial) are necessary simultaneously.

We present a combined technique on the basis of extended depth-of-field microscopy (EDF) and stereoscopy to visualize cell dynamic processes of an entire cell volume at once in real-time and with diffraction limited resolution in three dimensions.

In EDF-microscopy the wavefront is modulated to obtain out-of-focus information. After reconstruction EDF-images exhibit the extended field depth in a projection image of the entire depth of field volume [1, 2, 3]. Besides its benefit, this method does not give access to the corresponding depth of a signal. The intention of this work is the acquisition of axial information as enhancement to EDF-microscopy. In this context the idea of parallax is adapted [4]. A space light modulator (SLM) is positioned in a Fourier plane of a microscope. The light beam is split into two parts and the SLM modulates both parts separately. Axial resolution is evaluated by cross correlation of the center of mass of corresponding signals. Resulting local deviations of corresponding signals are correlated to an axial position within the sample.

The advantage of extended depth of field parallax (EDFP)-microscopy is that 3D-imaging is possible with a temporal resolution just limited by the camera frame rate and not by any mechanical components as in scanning methods. Furthermore, it can be used in various microscopic modes e.g. bright field, phase contrast so as for fluorescence imaging. It does not need coherence of incident and emission light (as in OCT). And markers are also not necessary for depth separation.

First results will be presented for 3D-tracking of fluorescent beads.