DYNAMIC CELL IMAGING BASED ON 4D SOFI

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Super-resolution optical fluctuation imaging (SOFI) is a microscopy technique that achieves resolution beyond the diffraction limit by computing higher order statistics (cumulants) of time series of stochastically blinking fluorophores [1]. Instead of localizing each individual fluorophore, the resolution improvement in SOFI results from properties of spatio-temporal cross cumulants calculated from the entire image sequence. When comparing with the prominent localization microscopy techniques, SOFI is compatible with a wide range of blinking conditions and high labeling densities [2]. Recently, S. Geissbühler et al. conceived a multiplane SOFI setup allowing parallel acquisition of multiple depth planes [3]. The multiplane SOFI setup significantly reduced the overall acquisition time for 3D imaging and has been applied to visualize the mitochondrial network in fixed C2C12 cells with a three-fold resolution improvement in all spatial dimensions.

In order to reduce the acquisition time, a key requisite for live cell imaging, we developed an adapted 3D deconvolution algorithm based on Bregman iterative method [4]. Due to this algorithm, noise is suppressed and the overall signal to noise ratio is substantially improved. Therefore, smaller number of input images is required for a SOFI reconstruction and super-resolved 3D-image sequences of moving cell structures become feasible. We demonstrated the performance of our 4D-SOFI by imaging live HeLa cells expressing vimentin-Dreiklang (Fig.1). The acquisition time for one super-resolved 3D image was 0.95s.

Fig. 1: Live HeLa cells expressing vimentin-Dreiklang. 300 frames (with acquisition rate 316 f.p.s.) were used for the reconstruction. Depth color coded maximum intensity projections of the 3D stack. (a) widefield image, (b) SOFI image with adjusted dynamic range, (c) SOFI image deconvolved and linearized by our new algorithm. Thickness of the sample is 2.8 µm. Scale bar (a,b,c) 2 µm. Charts show circular averaged power spectral density and line profiles of all three images. Line profile of the images is indicated by the yellow line in (b).