Three-dimensional Super-resolution Optical Fluctuation Imaging

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Super-resolution optical fluctuation imaging (SOFI) achieves three-dimensional super-resolution by computing higher-order spatio-temporal cross-cumulants of stochastically blinking fluorophores [1]. In contrast to localization microscopy, SOFI is compatible with weakly emitting fluorophores and a wider range of blinking conditions [2]. The main drawback of SOFI is the nonlinear response to brightness and blinking heterogeneities in the sample, which limits the use of higher cumulant orders.
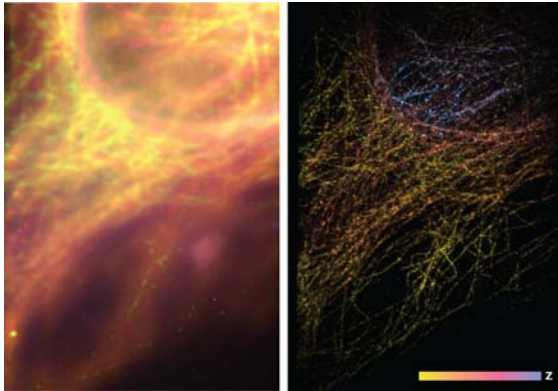

Figure 1: Maximum intensity projections of a three-dimensional fluorescence image of microtubules in a HeLa cell. Left: average intensity image. Right: third-order balanced SOFI image. Image size: $20 \times 29 \times 2.5$ μm^3.

We present a balanced SOFI algorithm for mapping molecular parameters and for linearizing the brightness response [3] and we outline a MATLAB toolbox for two- and three-dimensional SOFI analysis. We show super-resolved three-dimensional cell structures imaged with a multi-plane wide-field microscope. The simultaneous acquisition of several focal planes significantly reduces the acquisition time and helps limiting the photo-bleaching of the marker fluorophores.

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[3] S. Geissbuehler, N. L. Bocchio, C. Dellagiacoma, C. Berclaz, M. Leutenegger and T. Lasser, "Mapping molecular statistics with balanced super-resolution optical fluctuation imaging (bSOFI)," *Opt. Nanoscopy,* **1:**4 (2012).