**Balanced Super-resolution Optical Fluctuation Imaging**

Super-resolution optical fluctuation imaging (SOFI) achieves 3D super-resolution by computing higher-order cumulants of stochastically blinking fluorophores [1]. In contrast to localization microscopy, SOFI is compatible with weakly emitting fluorophores and a wide 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.

Balanced super-resolution optical fluctuation imaging (bSOFI) [3], extends SOFI by the combination of several cumulant orders to map fluorescence-related molecular statistics, such as molecular state lifetimes, concentrations and brightness distributions with super-resolution. Since these parameters are often linked to the chemical microenvironment of the fluorophores, they report on static differences and/or dynamic changes within cells and thus add a “functional” dimension to super-resolution microscopy based on stochastic switching. Furthermore, the information obtained can be used to correct for the nonlinear brightness and blinking response of cumulants.

We show experimental results of Alexa647-labeled microtubules in fixed HeLa cells with an up to five-fold resolution improvement compared to diffraction-limited widefield microscopy. Using a total-internal-reflection illumination scheme, we obtain depth information through the estimation of the spatial distributions of the molecular brightness as well as the blinking on-ratio.

[1] Dertinger, T., Colyera, R., Iyer, G., Weiss, S., and Enderlein, J., *Proc. Natl. Acad. Sci. U.S.A.,* **106**, 22287–22292 (2009).   
[2] Geissbuehler, S., Dellagiacoma, C., and Lasser, T., *Biomed. Opt. Express,* **2**, 408-420 (2011).  
[3] Geissbuehlers, S., Bocchio, N., Dellagiacoma, C., Berclaz, C., Leutenegger, M., and Lasser, T., *Opt. Nanoscopy*, **1**:4 (2012)