SPIDER – AN ALGORITHM FOR FAST SUPER-RESOLUTION BIOIMAGING

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Super-resolution wide-field fluorescence microscopy can provide nanoscale structural information about live cells. In general, the available information in these images is related to the density of emitters, with more emitters leading to more information. Traditionally, thousands of sequential image frames are acquired to obtain a sufficient spatial resolution. However, increasing efforts are now focused on obtaining a high spatial resolution on shorter time scale, as dynamic imaging is required when investigating the nanoscale structure of biological systems such as live cells.

This dynamic imaging also requires the principle of sequential imaging (localization of sparse subsets of blinking fluorophores distributed over thousands of frames) to be extended to the analysis of high-density frames of which the emissions are strongly overlapping. The analysis involves several step ranging from pre-processing (e.g. background handling) to post-processing (e.g. image rendering). The core of our approach is the SParse Image DEconvolution and Reconstruction algorithm (SPIDER) [1,2], which tackles the deconvolution problem in a penalized regression framework by implementing multiple penalties. In simulations, we show that SPIDER provides more quantitative images, with reduced bias, better recall rate and higher localization precision than other available high-density algorithms (CSSTORM [3] and FALCON [4]). Moreover, we performed live-cell super-resolution microscopy by applying our approach to an image series of HEK293-T cells expressing Dronpa targeted to the mitochondria. Despite the fact that the data were obtained with protein labels expressed at very high levels, which may cause imaging artifacts, we show that hollow morphologies of the structures could be resolved (spatial resolution of 50 nm). We also show that the study of dynamical processes at the sub-second time scale is not only possible but allows recovering images that are less prone to artifacts for samples that change or move over time (time sampling as short as 0.5 s).

[1] Hugelier et al. *Sci. Reports*, **6**, 21413–21423 (2016).
[2] Hugelier et al. *J. Chemom.* **31**, e2847 (2017).
[3] Zhu et al. *Nat. Methods*, 9, 721–723 (2012).
[4] Ming et. al. *Sci. Reports*, 4, 4577 (2014).