SPIDER TO IMPROVE SUPER-RESOLUTION OH HIGH-DENSITY LABELING DATA

Siewert Hugelier,[1,2] P. Eilers,[3] P. Dedecker,[2] Cyril Ruckebusch[1]
[1]University of Lille, LASIR CNRS, F59000 Lille, France
[2]KU Leuven, Laboratory for Nanobiology, Heverlee, Belgium
[3]Erasmus Medical Standard, Rotterdam, The Netherlands

KEYWORDS: Fluorescence, Image, Deconvolution, Sparse, Penalized regression, Splines

Super-resolution wide-field fluorescence microscopy can provide structural information at the nanoscale and dynamic insight about biological processes in live cell samples. In general, the available information in super-resolution images is related to the density of emitters, with more emitters leading to more information. One of the strategies for obtaining a high spatial resolution is based on the sequential imaging and localization of sparse subsets of blinking fluorophores distributed over thousands of images, resulting in a high-density image of their positions and intensities. However, to obtain a high spatial resolution on short time sampling, and potentially probe dynamic processes in live cells, this principle must be extended to the analysis of high-density of emitters distributed over a few tens of movies frames only. As many emitters are simultaneously active, their emissions strongly overlap and single-emitter fitting methods collapse. Thus, analyzing high-density super-resolution data, the development of new methods remains a challenging issue for dynamic imaging and faster super-resolution.

The core of our approach is the SPIDER algorithm for SParse Image DEconvolution and Reconstruction [1]. Image deconvolution is tackled in a penalized regression framework with a combination of a sparseness and an inter-frame penalty. Sparseness of the spatial distribution of the fluorophore is obtained with an L_0-norm penalty on their estimated intensities, effectively constraining the number of fluorophores per frame. Simultaneously, continuity of the fluorophore localizations is obtained penalizing the total numbers of pixel status changes between successive frames [2]. Additionally, a high-density of the fluorophore labelling translates into the presence of significant auto-fluorescence background and strong bleaching of the fluorophores, masking the blinking. Here we propose to describe the use of signal and image smoothing procedures to handle structured fluorescence background signals at a pre-processing step [3]. The aim is to accurately separate the signal of the fluorescent emitters of interest from the heterogeneous background signals. For this purpose, smoothing approaches are flexible and powerful alternatives to data fitting methods where one would model photobleaching with multi-exponential decays, on the one hand, and filtering approaches, such as temporal median filters and spatial filtering methods, which do not rely on any statistical or phenomenological model, on the other hand.

[1] S. Hugelier, J. De Rooi, R. Bernex, S. Duwé, O. Devos, M. Sliwa, P. Dedecker, P.H.C. Eilers, C. Ruckebusch, "Sparse deconvolution of high-density super-resolution images", *Sci. Rep.* 6 (2016)
[2] S. Hugelier, P.H.C. Eilers, O. Devos, C. Ruckebusch, "Improved super-resolution microscopy imaging by sparse deconvolution with an interframe penalty", *J. Chemometr.* 31 (2017)
[3] S. Hugelier, Wim Vandenberg, P.H.C. Eilers, Peter Dedecker, C. Ruckebusch, " *Preprocessing structured background signals to improve super-resolution fluorescence images", tbp*.