On the importance of simulated datasets in localization microscopy

Daniel Sage[1], Hagai Kirshner[1], Thomas Pengo[2], Suliana Manley[2] and Michael Unser[1]

[1]*Biomedical Imaging Group, EPFL, Lausanne, Switzerland*
[2]*Laboratory of Experimental Biophysics, EPFL, Lausanne, Switzerland*

In single-molecule localization microscopy, there exist numerous software tools that have been developed for localizing photoactived fluorophores. Theses tools introduce a trade-off between relevant estimation of the fluorophore positions, accuracy, and computational complexity, and we aim to provide data to validate and benchmark the available software.

For this purpose, we have created realistic sets of sequences that simulate molecule-based data. Each image sequence consists of millions blinking fluorophores that are randomly activated and excited over thousands of images. The 3-D location of each fluorophore is determined by a pre-defined continuous-domain structure such as tubes of various radii values. The density of fluorophores on the surface of these tubes and the aggregation factor resemble the experimental values. The lifetime property of the fluorophores has been incorporated and autofluorescence has been also added into our simulated data. The optical setup is the Gibson and Lanni 3-D point-spread function. In terms of noise, datasets include Gaussian readout noise, scattering Poisson noise, EMCCD noise (Electron Multiplying CCD) and Poisson distribution of the emission rate. The datasets also incorporate spatial drift by applying a slowly varying 3D transformation.

Synthesized images are extremely useful for comparing algorithms and we believe that these simulated datasets will play a similar role in single particle localization microscopy. Knowing the ground truth of the data, one can examine the following algorithmic properties: local maxima detection, localization accuracy, geometrical measures such as lengths and radii, and running time. Our datasets aim to further contribute to the standardization of the process of publishing and reviewing localization microscopy data.