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3D SINGLE MOLECULE LOCALIZATION MICROSCOPY: KEY OUTCOMES OF THE SOFTWARE BENCHMARKING

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ABSTRACT

With the large diffusion of the super-resolution localization microscopy technique, a large number of software packages have been developed to accurately localize blinking fluorescent emitters in frames. To guide developers of software and practitioners on their critical choice of software, we have designed a large-scale software benchmarks, first in 2D and very recently in 3D. The 3D setting is challenging computational task because the axial information is encoded by the shape of PSF in 2D images. Our framework is based on 1) the simulation of 3D highly-realistic datasets in the most used 3D modalities, astigmatism (AS), double helix (DH), biplane (BP), 2) the definition of common metrics, and 3) thanks to the involvement of many groups of researchers we have the localization results of more than 30 software on the same datasets. This provides a unique board assessment of the 3D SMLM software and a holistic view of the performances of the 3D software. The complete method and material is available at http://bigwww.epfl.ch/smlm/.

The analysis of the performances of the software allows us to draw trends of the recent developments. The large size of the engineered PSF degrades drastically the performances of the software in high-density, both the rate of detection (estimated with the Jaccard) and the localization precision (estimated with the RMSE). This is the case for the DH-PSF which have a large support (~1um). In this case, the software packages are not able to reconstruct a decent 3D super-resolution images in high-density. In low density, the axial precision of software on the DH dataset is better than AS and BP over a large range of depth. On the average of the top performers, the isotropic ratio is 1.0 for DH, 0.60 for AS, and 0.53 for BP. It shows that the specific signature of the shape of DH PSF can guarantee an isotropic localization precision even far from the focal plane. This is confirmed by the theoretical Cramer-Rao lower bounds.

To address the 3D problem, we used experimental 3D PSF to well imitate the aberrations (e.g, axial wobble) that practitioners are facing with real experiments. Our study confirms the importance of learning the shape of PSF (e.g., by spline functions) instead of using the classical Gaussian function. This was the choice of two software, CSpline and SMAP, which achieve good performances in all 3D modalities.

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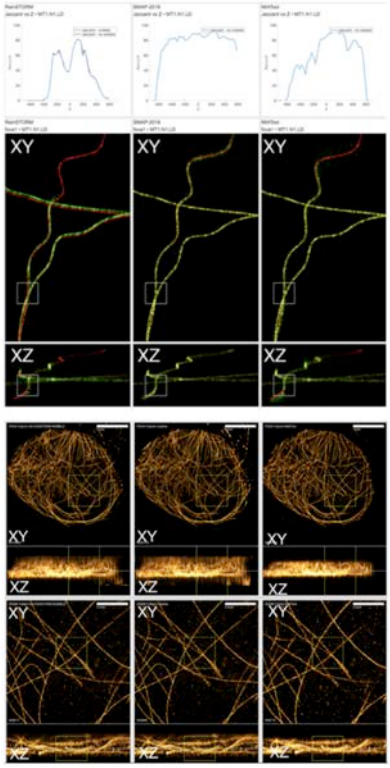

Figure 1. Side-by side rendering of super-resolution localization. Top: illustration of the results of 3 software, Jaccard, and rendering, ground-truth in red, tested software in green. Bottom: rendering of real datasets. More illustrations are available on http://bigwww.epfl.ch/smlm/

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