Highly reproducible multi-color 3D super-resolution imaging

Clément Cabriel[1], Nicolas Bourg[1], Guillaume Dupuis[2], Aurélie Baron[3], Boris Vauzeilles[3,4], Emmanuel Fort[5], Sandrine Lévêque-Fort[1]

[1]Institut des Sciences Moléculaires d'Orsay, Université Paris-Sud, CNRS UMR 8214, Orsay France
[2]Université Paris-Sud, Centre de Photonique BioMédicale, Féd. LUMAT, CNRS FR 2764, Orsay France
[3]Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles CNRS, Gif-sur-Yvette, France
[4]Laboratoire de Synthèse de Biomolécules, ICMO, Université Paris-Sud, CNRS UMR 8182, Orsay, France
[5]Institut Langevin, ESPCI ParisTech, CNRS, PSL Research University, Paris France

sandrine.leveque-fort@u-psud.fr

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The use of 3D Single Molecule Localization Microscopy (SMLM) in structural and dynamic biological studies currently remains limited because of its lack of versatility, but a great deal of efforts on labelling, fluorophores brightness and software are being made to render it both more precise, quantitative and reproducible. Still, to actually improve the performances and the repeatability of acquisitions, it is necessary to address several problems that may considerably limit the relevance and reliability of obtained data. In particular, the anisotropy of the localization between the lateral and axial precisions as well as the strong dependence of these precisions on the axial position may restrict the potential applications. Besides, axial drifts, chromatic aberrations and field aberrations (especially spherical aberrations) often hamper Point Spread Function (PSF) based experiments, inducing dramatic losses of resolution and axial biases [1].

We propose a detection scheme that addresses these issues to provide more reliable results for biological applications. To minimize the loss of lateral information when improving the axial resolution and detection range, we developed a dual-view optical setup that decouples lateral and axial detections. Moreover, we combined strong astigmatism PSF shaping with supercritical angle fluorescence (SAF) detection [2] in order to give an absolute axial reference to the astigmatism approach. This technique, called depth astigmatic imaging with SAF yield (DAISY), provides 3D absolute information over a 1.2 µm capture range above the glass coverslip and an axial localization precision down to 15 nm with minimal loss of lateral resolution and little sensitivity to field aberrations.

We will discuss the implementation and the calibration [3] as well as the optimal merging of the axial information sources and the performances in terms of localization precisions and intrinsic biases corrections. In particular, we will show how the SAF absolute information helps retrieving drift-free and chromatism-insensitive data over the whole imaging range. After presenting dual-color images on cytoskeletal networks, we will show that DAISY enables the imaging of whole living *E. coli* bacteria in the framework of the study of new click chemistry labelling techniques.

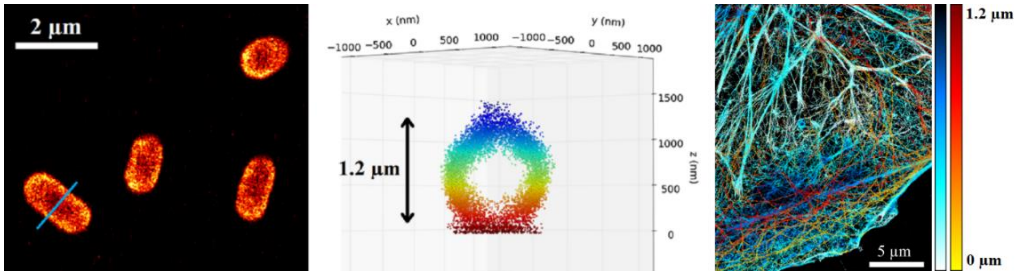

Figure 1. (left) 2D image of *E. coli* bacteria labelled with AF647. (center) 3D profile of a bacterium along the displayed blue line. (right) Dual color 3D image of the cytoskeleton of a COS-7 cell (cyan-blue: actin, yellow-red: tubulin).

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