Multi-color 3D super-resolution imaging by combining strong astigmatism and supercritical angle fluorescence

C. Cabriel[1], N. Bourg[1], P. Jouchet[1], G. Dupuis[2], C. Guillaume[3], A. Baron[4], M.-A. Badet-Denisot[4], B. Vauzeilles[4,5], C. Leterrier[6], E. Fort[7], S. 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] Abbelight, 6 rue Jean Calvin, 75005 Paris, France
[4]Centre de Recherche de Gif, Institut de Chimie des Substances Naturelles CNRS, Gif-sur-Yvette, France
[5]Laboratoire de Synthèse de Biomolécules, ICMO, Université Paris-Sud, CNRS UMR 8182, Orsay, France
[6]Aix-Marseille Université, CNRS, INP UMR7051, Marseille, France
[7]Institut Langevin, ESPCI ParisTech, CNRS, PSL Research University, Paris France
E-mail: sandrine.leveque-fort@u-psud.fr

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The use of 3D Single Molecule Localization Microscopy (SMLM) for multicolor imaging currently remains limited because several issues may considerably limit the relevance and reliability of obtained data. In particular, the anisotropy between the lateral and axial precisions as well as the strong dependence of these precisions on the axial position may restrict 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 to combine astigmatic imaging with Supercritical Angle Fluorescence (SAF) detection [2] in order to provide an absolute axial reference to the PSF shaping approach. Furthermore, the dual view optical setup which allows us to decouple lateral and axial information, permits to introduce a strong astigmatism PSF shaping, thus extending the axial performances without degrading the lateral precision. This technique, called Dual-view Astigmatic Imaging with SAF Yield (DAISY), provides 3D absolute information over a 1-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 [3]. DAISY is ideally suited for multicolor imaging as it only provides drift-free but also chromatism-insensitive data over the whole imaging range. Sequential dual-color (AF555/AF647) will be presented on whole living *E. coli* bacteria in the framework of the study of new click chemistry labelling techniques and to decipher the 3D organization of adducin and β2-spectrin scaffold within the axons of cultured neurons.
We will also present simultaneous 3-color acquisitions with red dyes only, which are conveniently excited with the same laser at 637 nm and present optimal photophysics for dSTORM. Spectral demixing [4] is obtained by inserting a dichroic mirror to create two spectral imaging channels for both the SAF path and the epifluorescence path, leading to four images acquired by two cameras in a quad-view system. The intensity ratio between the two spectral channels is used to identify the fluorophore associated to each PSF. Evaluation of cross-talk coefficients will be provided to evidence the benefit of spectral demixing in 3D. We will also present preliminary 3D-3-color images of cytoskeletal components in COS7 cells and neurons.

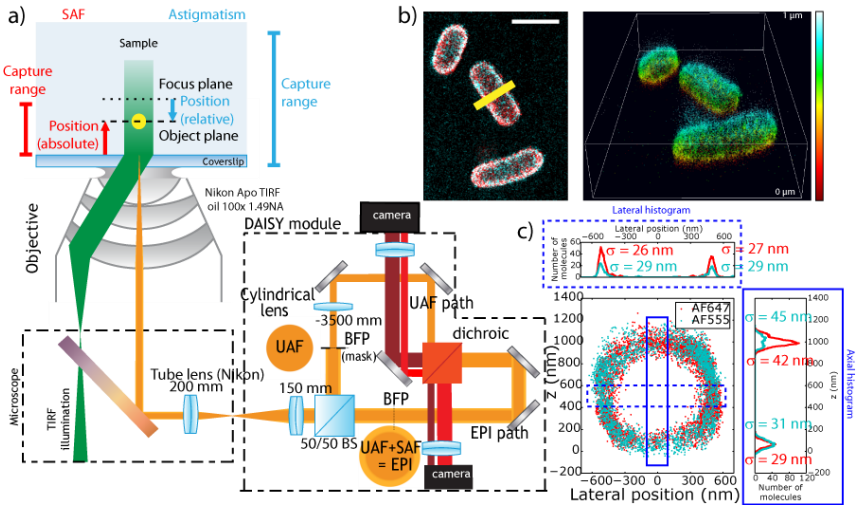

Figure 1: a) Quad view for spectral demixing with DAISY , b) 2D and 3D image of E. coli bacteria labelled with AF647. c) 3D profile of a bacterium along the displayed blue line.

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