Absolute super-localization nanoscopy thanks to Super-critical Angle Fluorescence emission

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Key words: Super-localization, Super-critical Angle Fluorescence, isotropic, nanoscopy

Point Spread Function (PSF) engineering methods combined to Single Molecule Localization Microscopy (SMLM) allow ones to retrieve 3D position of fluorescent molecules with nanometric accuracy. Unfortunately, achieving high 3D localization precision is often at the cost of an increased experimental complexity. In addition, all these strategies only provide the relative axial positions of the fluorophores with respect to an arbitrary focal plane. Hence, 3D optical nanoscopy would strongly benefit from a method that combines high nanometric axial precision, simplicity of implementation and absolute axial positioning.

Here we propose to take advantage of the forbidden light, also called Supercritical-Angle Fluorescence (SAF) \cite{1,2}, at the single molecule level. Indeed, when a fluorophore is located in the vicinity of the coverslip interface, its near-field SAF component become propagative and can be collected by a high numerical aperture objective (Fig. 1). In the objective back focal plane, the SAF component appears as a ring beyond the critical angle $\theta_c$. For a fluorophore at the interface, the number of photons in the SAF ring, $N_{SAF}$, represents 50\% of all the photons collected $N_{tot}$. Since $N_{SAF}$ decreases exponentially with the fluorophore depth distance from the coverslip surface, the absolute axial position of each fluorescent dye is retrieved by comparing $N_{SAF}$ versus $N_{tot}$. In practice, only the detection path of our SMLM setup is modified to insert a compact home-made dual view module, which permits to simultaneously measure $N_{SAF}$ and $N_{tot}$, and compute the absolute axial position of each fluorophore in real time. This 3D absolute method, called “Direct Optical Nanoscopy with Axially Localized Detection” (DONALD), gives an isotropic 3D localization precision of 20 nm within an axial range of ~150 nm above the coverslip. DONALD localization performances allow us to observe the actin network with an axial resolution of ~35 nm, and to super-resolve the 3D hollowness of microtubules.

\cite{1} T. Ruckstuhl et al., Forbidden light detection from single molecules, Analytical chemistry, 2000

\cite{2} T. Barroca et al., Full-field Near-Field Optical Microscope for Cell Imaging, PRL, 2012