

# COMBINING AXIAL SINGLE MOLECULE LOCALIZATION STRATEGIES TO ENHANCE 3D IMAGING OF BIOLOGICAL SAMPLES

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Retrieving fluorophore axial position in Single Molecule Localization Microscopy (SMLM) is often performed by Point Spread Function (PSF) engineering methods. We recently proposed to take advantage of the intrinsic axial information given by the supercritical emission (SAF) present in the pupil plane [1-3]. When a fluorophore is located in the vicinity of the coverslip interface, its near-field SAF component becomes propagative and can be collected with a high numerical aperture objective. Since the number of SAF photons,  $N^{SAF}$ , 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 the total number of photons collected  $N^{tot}$ . An axial localization precision down to 15 nm can be obtained within an axial range of 150 nm from the coverslip, but between 150 and 600 nm precision slightly increases. To extend the axial range of our SMLM microscope up to the first micron and maintain optimal localization precision, we couple SAF detection with a complementary astigmatism PSF engineering method, which usually only provides relative measurements. In this unique association, SAF detection brings the auto-reference for both approaches and permits extended absolute axial nano-positioning.

We will discuss the optimal axial merging position of the two techniques, implementation and performances obtained. The absolute detection depth is extended up to 1200 nm thanks to the astigmatism-based axial detection, and allows easy association of multiple labelling. After showing the interest of extending the detection range for the study of antibiotic binding site in living *Staphylococcus aureus*, we will present the improved investigation depth on images revealing 3D organization of cytoskeleton proteins in cell lines and primary neuronal cultures.

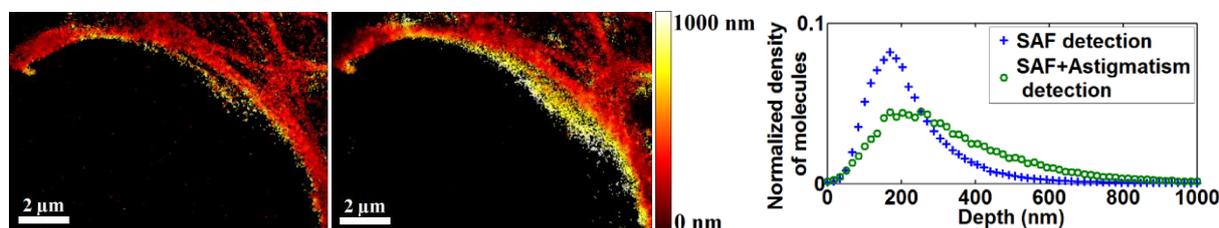


Figure 1: 3D images of actin network in CHO cells (color-coded depth). Left: SAF detection, center: coupled SAF-astigmatism detection, right: depth histogram for each type of detection.

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