SUPERCRITICAL ANGLE DETECTION STED MICROSCOPY

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The wide dissemination of 3D-STED microscope has been limited by the high cost and complexity for reaching isotropic resolution. In this scenario we introduce a novel surface imaging fluorescence microscope which combines the lateral superresolution of STED microscope with the strong axial confinement of supercritical angle detection. This implementation takes advantage of the supercritical angle (SAF) emission of fluorophore closed to the interface; where their evanescent near field can become propagative and appears above the critical angle [1]. This SAF emission represents up to 50% of the emission collected by the objective for a fluorophore at the interface. To take advantage of the axial information held by SAF emission, we use a standard high NA objective (1.49) which preserves the STED beam and only requires a detection modification [2]. By filtering out the undercritical emission (UAF) in a conjugated plane of the back focal plane of the objective lens, only the SAF emission is detected. It allows nanometric axial sectioning of fluorescent emitters close to the surface; simply by adding a SAF module in the detection path of all STED microscopes.

We will show for the first time the coupling of STED and SAF microscopy. We describe the theory of image formation and highlight the benefits of this implementation by simulation. Furthermore we will demonstrate the performance by imaging fluorescent beads and sub-cellular structures as represented on fig. 1. We will also discuss the potential improvement of the raw data image quality by using deconvolution techniques.

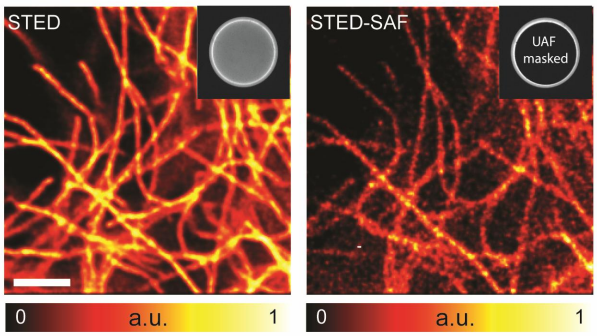

Fig. 1 STED and STED-SAF images on COS 7 cells with tubulin labelled with Alexa 488. In inset, image of the back focal plan where the undercritical emission (UAF) is filtered out in the STED-SAF configuration. Scale bar 1.5 µm.

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