Enhancing 3D SMLM close to the coverslip using off-focus imaging and the supercritical angle fluorescence effect

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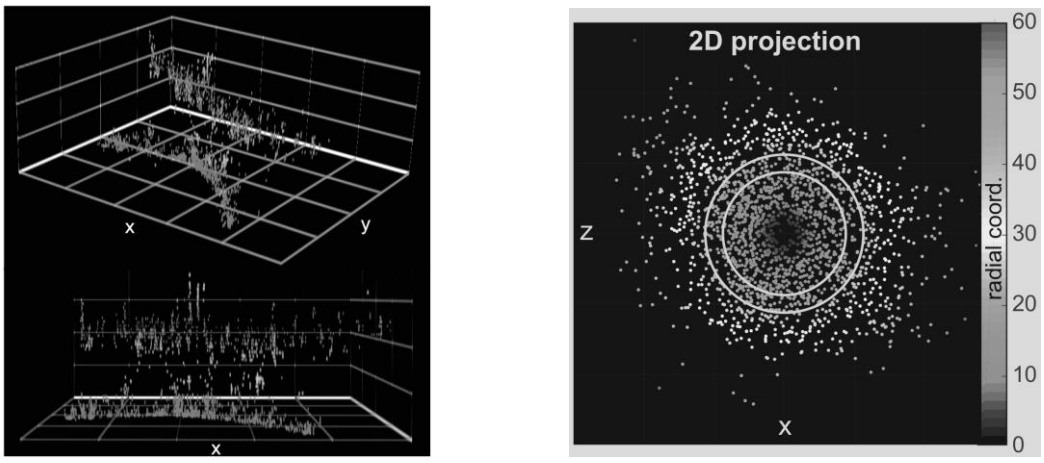
In close vicinity to a dielectric surface, for instance a microscope coverslip, fluorescent emitters couple so called “supercritical angle fluorescence” (SAF) into the glass. This light travels beyond the angle of total internal reflection and can make up almost 50% of the collected light.
Measuring the energy ratio of this SAF-light to the “undercritical angle fluorescence” (UAF) allows for improved axial localization precision. To achieve this, specific two-channel detection setups have been implemented [1, 2].
We show that off-focus imaging can obtain comparable results in x-y and even better results along z. The proposed detection scheme can be implemented in any research-grade microscope.
We support our findings by calculations of Cramér-Rao bounds and experimental results from dSTORM imaging of stained microtubule networks.

[1] J. Deschamps, M. Mund, and J. Ries, *3D superresolution microscopy by supercritical angle detection.* Optics Express, 2014. **22**(23): p. 29081-29091, 10.1364/OE.22.029081.

[2] Bourg, Nicolas, et al. "Direct optical nanoscopy with axially localized detection." *Nature Photonics* 9.9 (2015): 587.



Left: dSTORM image of Alexa647 stained microtubules in fixed COS7 cells. Right: Projection of localizations along several short microtubule sections reveals their hollow core. Two white circles bound the region of expected fluorophore positions. All units in nanometers.