3D SINGLE MOLECULE LOCALIZATION MICROSCOPY CLOSE TO THE COVERSLIP: A COMPARISON OF METHODS

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At distances up to a few hundred nanometers from the coverslip, evanescent wave coupling into the glass (known as *Supercritical Angle Fluorescence* or SAF) can strongly improve the axial precision in single molecule localization microscopy (SMLM). Specific detection systems such as *virtual* [1] or *direct* [2] *Supercritical Angle Localization Microscopy* (SALM) or *Direct Optical Nanoscopy with Axially Localized Detection* (DONALD) [3], have been developed to exploit SAF in modified two-channel imaging configurations.

Recently, we have shown that off-focus microscopy, that is imaging at an intentional slight defocus, can effectively exploit this effect as well [4, 5], which raises the question if and how much other 3D SMLM techniques can profit from SAF.

Here we compare the performances of popular 3D SMLM techniques in the near field regime. Our comparison includes off-focus, cylindrical lens and biplane imaging as well as SALM. We find that all methods gain from SAF and identify a high numerical aperture as the only major key requirement to unlock the SAF benefit. We identify optical parameter settings for off-focus, cylindrical lens and biplane imaging and compare the methods in view of robustness to aberrations, fixed dipole emission and double-emitter events.

We find that biplane imaging provides the best overall performance and support our findings by DNA-PAINT experiments on DNA-nanoruler samples.

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