Currently, two general approaches allow for three-dimensional resolution in localization microscopy. On one hand, the z position of single emitters can be determined from the shape of the point spread function (PSF) either by imaging two focal planes (bi-plane) or after PSF engineering (e.g. astigmatic PSF, double helix PSF or self-bending PSF). Nevertheless, the axial resolution is usually worse than the lateral one, which itself is compromised by the modification of the PSF. On the other hand, the z position can be extracted from relative intensities of single-molecule images when employing an interferometric detection scheme (iPALM, 4Pi-SMS). Unfortunately, such interferometric microscopes are very difficult to built and cumbersome to use.

Here we present a fundamentally different way of achieving 3D resolution in localization microscopy, based on the principle of surface-generated fluorescence. This near-field fluorescence occurs when a fluorophore is in the vicinity of a water-glass interface. It is emitted into large angles above the critical angle and its intensity strongly depends on the distance of a fluorophore from the interface. By splitting high and low emission angles and imaging them simultaneously we can determine the precise axial position of single molecules from their relative intensities in the two channels. A theoretical analysis shows that isotropic resolution on the nanometer scale can be expected in a range of a few hundred nanometers above the coverslip, without compromising the lateral resolution.

We developed a simplified setup that we calibrated with fluorescent beads and provided proof-of-principle by imaging DNA-origami tetraedra. We present first biological data on three-dimensional imaging of clathrin-coated pits and microtubules and discuss ideas for approaching the theoretical resolution limit.