THREE-DIMENSIONAL SPECTRAL PRECISION DISTANCE MICROSCOPY (SPDM): PRINCIPLE AND METHODS

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Novel developments in optical technology and photophysics made it possible to radically overcome the diffraction limit of conventional far-field fluorescence microscopy1. Here, the focus will be on a special variant of localization microscopy, called ‘Spectral Precision Distance Microscopy’ (SPDM). As other far-field localization microscopy methods using fluorescent emitters, like GSDIM, PALM/FPALM, STORM, dSTORM etc., it is based on the high precision localization of point sources possible even in the case that the distance between them is smaller than the conventional resolution limit of about 200 nm. In the original SPDM method described this was achieved by using photostable fluorochromes with differences in their absorption/emission spectra, or their fluorescence life times2,3. Applying the Rayleigh criterion of optical (two-point) resolution, in this way the positions of individual adjacent point sources were determined with a localization precision down to the few tens of nanometer range, both in the object plane and in 3D. Although in this way, an enhancement of structural resolution (as defined by the Nyquist theorem) is still difficult to achieve, localization microscopy using photostable emitters may contribute substantially to the spatial analysis of biological nanostructures, especially if combined with photoswitching based localization microscopy approaches and other superresolution techniques.


