Towards Lens Free Super-Resolution Microscopy at Large Working Distances

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KEY WORDS: Lens Free Super-Resolution, Distributed Aperture Illumination, Single Molecule & Structured Illumination Microscopy, large working distances, nanometer resolution, nanostructure analysis

Presently, super-resolution fluorescence microscopy (SRM) methods[1] typically use objective lenses of high numerical aperture (NA), i.e. low working distances (WD) in the range of 0.2 µm. While this has allowed a substantial progress in the analysis of nanostructures at the level of individual cells in monolayers or in thin tissue sections, e.g. of nuclear genome organization[2], the low WD excluded its application to thick transparent specimens, or to objects with large topographical differences. To extend the use of SRM also in tissue diagnostics, in neuro-and developmental biology, or in the material sciences, it should be desirable to develop approaches with large WDs. A first approach towards this goal is structured illumination or other types of patterned illumination with large WD/low NA lenses. Using e.g. a typical SIM setup with a low NA objective lens and a WD of 1 mm, autofluorescent plant structures were imaged at substantially enhanced resolution. To allow still larger working distances, a novel SIM microscope system with a WD in the cm range was constructed and applied for tissue diagnostics; this system provided a substantial enhancement of contrast and resolution, compared to the same large WD using laser scanning imaging (N. Celik et al., submitted). To further enlarge the working distance and enhance the resolution, lens free microscope systems based on Distributed Aperture Illumination (DAI) may be constructed[3]. Based on numerical simulations, an illumination & detection concept is presented for a focused laser beam scanning high spatial aperture microscope for working distances up to the multicentimeter regime. In the large WD confocal mode, an optical resolution around 150 nm is estimated while in a large WD STED mode, the resolution may be enhanced several times further; the same type of lens free DAI might also be used to realize MINFLUX based super-resolution microscopy[4] and eventually make possible an optical resolution down to the 1 nm scale even at large WDs. Generally, the distributed aperture illumination principle allows great flexibility in optical design; the use of structured illumination instead of focused scanning schemes is expected to provide resolution limits like for MINFLUX, assuming similarly low photon numbers for localization[5].

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