NOVEL 3D SINGLE MARKER SWITCHING MICROSCOPE WITH ISOTROPIC RESOLUTION OVER LARGE AXIAL RANGE

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KEY WORDS: isotropic resolution, 3D-imaging, single marker switching, PALM, STORM

To overcome Abbe diffraction limit in fluorescent microscopy two main methods established in the last years. The first, stimulated emission depletion (STED) microscopy, shrinks the possible area of fluorescent emission by saturated depletion of the excited state of fluorescent molecules. The size of the remaining fluorescent spot is far below Abbe limit. The other utilizes molecular switching events such that only a single fluorophore will emit at the same time within a diffraction limited volume. By localizing the center of the emerging point spread function (PSF) the position of the emitter can be determined with nanometer accuracy. Depending on the particular molecular transition used this single marker switching (SMS) technique is called photoactivated localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM), ground state depletion microscopy (GSDIM) and so on. While the expansion of STED in the third dimension (3D-STED, isoSTED [1]) is based on the same principle as in 2D, in SMS several techniques can be used to determine the labels axial position: The astigmatism and the double-helix PSF techniques unambiguously change the shape of the PSF depending on the labels axial-position, whereas in the biplane technique two axially displaced detection planes are used to enable unambiguous axial localization [2]. As none of these techniques results in isotropic localization accuracy, interferometric PALM (iPALM) utilizes detection through two opposing objective lenses in a 4Pi like geometry in order to overcome this limitation [3]. Theoretically iPALM provides a $\sqrt{2}$ fold increased lateral and about 6 fold increased axial localization accuracy [4]. Nevertheless it is restricted to layers not thicker than about $\lambda/2$. Taking into account the spherical shape of the wavefronts 4Pi-SMS extended that technique to a layer of ~1µm thickness, placed anywhere within a stained cell, providing a resolution of about 6 nm in the axial and 8-22 nm in the lateral direction [5].

In line with this development, we realized a new 3D SMS microscope with improved axial performance. Our novel microscope allows an almost isotropic resolution within a several micron thick layer inside an extended sample. This is very suitable for imaging large sample volumes and renders axial scanning obsolete.

In contrast to previous setups there are no limitations due to the overall thickness of the sample like in iPALM and there is no need for any manipulations of the PSF (astigmatism, double-helix PSF) or challenging interference procedures (iPALM, 4Pi-SMS).