CONTRAST ENHANCEMENT IN MICROSCOPY WITH SELF-RECONSTRUCTING BEAMS BY STIMULATED EMISSION DEPLETION

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Imaging of large organisms often suffers from a limited image quality deep inside the sample due to the scattering of illumination light at refractive index inhomogeneities within the sample. Microscopy with Self-Reconstructing Beams (MISERB) is an imaging technique designed to address exactly this problem. It is derived from the concept of light sheet microscopy [1]. In contrast to regular light sheet microscopy however, where the light sheet is either created by a cylindrical lens or a laterally scanned Gaussian beam, the light sheet in MISERB is created by a laterally scanned self-reconstructing beam [2]. The most prominent member within the class of self-reconstructing beams is the Bessel beam. It recuperates its beam profile a short distance behind a scattering object. In addition it also features a very thin, highly uniform main maximum compared to the commonly used Gaussian beam. These properties make the Bessel beam highly suitable for the illumination of both, large and small samples, in a light sheet based imaging scheme. They come however to the prize of a relatively broad ring system surrounding the main maximum of the beam. This reduces the optical sectioning capability of a light sheet microscope and leads among others to a decrease in image contrast. The main challenge in employing Bessel beams for light sheet based microscopy is thus to reduce the influence of the ring system on image formation. One way of achieving this goal is the selective detection of the central lobe of the Bessel beam by the application of an appropriate mask in the detection pathway [4]. Others are the use of multi-photon excitation and structured illumination. Here we present an alternative approach that is based on the non-linear suppression of the background by exploiting the principles underlying stimulated emission depletion (STED) microscopy [4]. We will discuss the right choice of the illumination beams and the potential of this new illumination scheme in dependence of the scattering induced wavefront deformation.