ISM-ASSISTED TOMOGRAPHIC STED MICROSCOPY ALLOWS FOR SAMPLE-GENTLE SUPER-RESOLUTION IMAGING

Julia Kratz, Claudia Geisler, and Alexander Egner

Department of Optical Nanoscopy, Institute for Nanophotonics Göttingen e.V., Hans-Adolf-Krebs-Weg 1, 37077 Göttingen, Germany
E-mail: julia.kratz@ifnano.de

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Fluorescence nanoscopy allows to non-invasively resolve three-dimensional cell structures beyond the diffraction limit [1]. One of these high resolution imaging techniques is stimulated emission depletion (STED) microscopy [2]. However, the practically achievable resolution of a STED microscope is often limited by photobleaching [3]. One method to overcome this limitation is tomographic STED (tomoSTED) microscopy [4]. In tomoSTED microscopy, excited fluorophores in the sample plane are depleted by a 0-π phase step modified and subsequently focused STED beam, which leads to an effective narrowing of the fluorescence-allowed area in a single direction. A full highly resolved spatial information of the object in two dimensions is then obtained by rotating the depletion pattern. The number of required pattern rotations depends on the ratio of the resolutions in the depleted and non-depleted directions. Since the resolution per pattern orientation is only increased along a single direction, imaging along the other direction is still diffraction-limited. Therefore, the resolution along this direction can be increased by utilizing the concept of image scanning microscopy (ISM). ISM allows to improve the resolution of a scanning microscope by factor of $\sqrt{2}$ [5]. By combining tomoSTED with ISM, the number of required pattern orientations can therefore be reduced leading to a lower overall acquisition time which translates directly into a lower light dose. On simulated as well as on experimental data, we show that approximately a factor of 1.4 lower number of orientations are required to achieve the same resolution and image quality as in tomoSTED microscopy without ISM assistance. Under typical recording conditions, this corresponds to a six-fold reduction of the required light dose as compared to a classical STED microscope.

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