Ultrafast switchable depletion patterns for tomoSTED microscopy generated by conical diffraction

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In STED microscopy, super-resolution is achieved by employing a depletion intensity distribution to confine the fluorescence to a sub-diffraction sized area [1]. In the canonical implementation, a doughnut-shaped depletion pattern is chosen to confine the sub-resolved area in two dimensions, yielding a resolution of up to 20nm [2].

Recently, a novel approach for a low-intensity STED microscope with uncompromised resolution and image quality, named tomoSTED, has been presented. This STED variant was shown to yield super-resolved 2D images with only ¼ of the light dose compared to conventional STED microscopy [3] and therefore has the potential to reduce photobleaching and sample damage significantly. TomoSTED is based on confining the fluorescent area not in two dimensions at a time, but only in one, by using an intensity distribution with two lobes and a line of zero intensity, rather than a spot of zero intensity, in the middle. It was shown that this pattern, further referred to as 1D depletion pattern, is preferable in terms of STED efficiency due to its higher curvature in the vicinity of the intensity minimum [3].

In tomoSTED, a 2D super-resolved image is reconstructed from a series of single 1D super-resolved images, each acquired with a different orientation of the 1D depletion pattern [3]. Here, different sequences of rotating the pattern and scanning over the sample are possible, from rotating the pattern only after a full image scan, over line by line rotation up to rotation on every pixel. The latter is advantageous in terms of robustness against drift and bleaching issues and is therefore the favorable implementation. The pattern creation method realized so far allows for an image-wise and line-wise switching, but a pixel-wise switching was inaccessible.

We addressed this problem and present a new method to create 1D depletion patterns based on conical diffraction. The pattern orientation is completely controlled by polarization and can therefore be ultrafast switched with a rate of up to several 100 kHz by means of electro-optical devices. This feature greatly improves the performance of tomoSTED. Ultrafast switching by conical diffraction allows changing the pattern orientation on the single pixel level, enhancing the microscope's robustness against sample drift and making it a promising candidate for live-cell imaging.

References

[1] S.W. Hell, Science, 316, 1153-1158 (2007)

[2] B. Harke, J. Keller, C.K. Ullal, V. Westphal, A. Schönle and S.W. Hell, Opt. Express 16(6), 4154 – 4162 (2008).

[3] J. Krüger, Tomographic STED Microscopy, PhD Thesis, Göttingen University (2017)