3D STED imaging with unprecedented resolution at minimal light dosages (DyMIN)

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Super resolution light microscopy has gained significant momentum in the past few years, thanks to new dyes, methods, and increased user-friendliness of commercially available instruments[1]. Still, essentially every nanoscope is in one way or the other more invasive than its diffraction limited counterpart, be it due to higher light intensities and/or longer observation times, which cause fluorophore bleaching and phototoxicity. These are major obstacles towards super resolution (live cell) imaging. Recently, a number of imaging schemes were introduced for STED nanoscopy that share the common goal of reducing light dosages in the sample, namely RESCue[2], DyMIN[3] and MINFIELD[4]. They are commonly summarized under the term *Adaptive Illumination*, because by adapting the illumination intensity to the underlying structure they seek to put light only where it is needed in order to induce a major gain in resolution, instead of unconditionally shining high laser intensities everywhere in the sample. Here, we report on the latest improvements, in particular concerning DyMIN STED microscopy. We give an overview of the technique and demonstrate that the application of DyMIN STED and the concomitant manifold reduction of the light dosages and therefore bleaching in many cases enables imaging in the first place, which would not be possible at such high resolutions with conventional STED. Notably, the recording of three-dimensional volumes and time lapse data, which both entail scanning the same region in the sample many times, especially with 3D-STED, highly benefits from reducing the illumination intensities. Additionally, these novel concepts expand the scope of applications to samples which are perturbed by high light intensities like malaria parasites and pigmented cells.

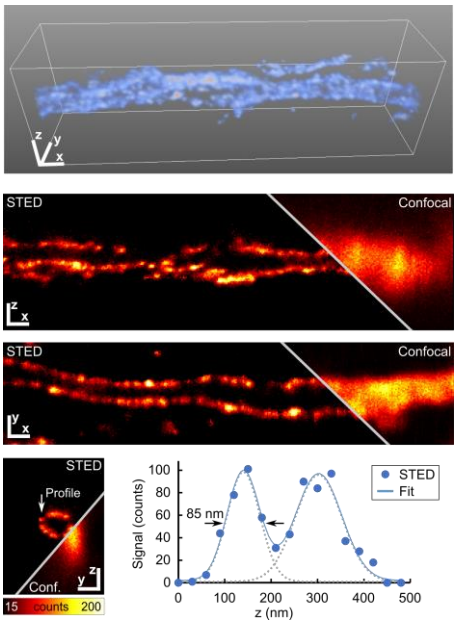

Figure 1: 3D DyMIN STED in Primary hippocampal neurons

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