CHROMATIC ABERRATION IN 3D MULTICOLOR STED NANOSCOPY

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The super-resolution nanoscope based on stimulated emission depletion (STED) technique allows to characterize subcellular details like molecular structures in real time, not visible with a conventional confocal microscope [1].

We realized a custom made STED nanoscope, based on a supercontinuum pulsed laser (ALP-710-745-SC, Fianium, Southampton, UK) that has 20MHz repetition frequency and three synchronized outputs: a supercontinuum laser beam in the spectral window 450 - 2000nm, used for excitation, and two laser beams at 710 and 745nm respectively, which have a bandwidth of about 10nm, used for depletion [2].

We obtained three-dimensional super-resolved images, [3] by dividing the STED beams in two parts and shaping them in donut and bottle profiles. Such beam shapes are achieved by the use of two phase plates: a vortex and a homemade 0-π one respectively. The possibility of adjusting the power ratio in the two depletion pathways allows a better flexibility in the choice of the final resolution.

The laser source is the core component for multicolor STED imaging. Although we are using achromatic lenses and the STED beams are coupled together through the same fiber an almost negligible chromatic aberration could have a dramatic impact in 2 colors colocalization nanoscopy. In this work we focused our attention on the sources of chromatics aberrations and how to overcome them consequently.

Therefore by optimizing fluorophore choice, we are able to do up to 3 colors super-resolved imaging.