STIMULATED EMISSION DEPLETION IMAGE SCANNING MICROSCOPY WITH A SPAD ARRAY

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ABSTRACT:

Stimulated Emission Depletion (STED) is one of the most influential nanoscopy (or diffraction-unlimited microscopy) techniques[1,2]; theoretically, it allows achieving any desired spatial resolution by increasing the STED laser intensity, but at the non-negligible cost of photo-damaging the sample. In particular, the higher is the dose of stimulating photons, the better is the expected resolution, but the higher is photo-bleaching and photo-toxicity.

Albeit bounded by diffraction, Image Scanning Microscopy (ISM) technique[3,4] over-performs traditional confocal microscopy for what concerns resolution and signal-to-noise ratio (SNR), while the usage of low excitation intensities averts possible photo damaging effects.

Here we explore the synergistic combination of these two techniques to mitigate their drawbacks and enhance their strengths. We show that the pixel reassignment approach of ISM is compatible with STED imaging, and grants improved SNR of the final result compared to the raw STED counterpart. The resolution is increased as well if the STED power is lower than a given threshold. In other words, STED-ISM allows substantially reducing the STED power to achieve a target resolution.

The recent ISM implementation based on a single-photon avalanche diode (SPAD) array detector[5] allows reconstructing the ISM result *a posteriori*, as opposed to previous all-optical ISM realizations[6]. We demonstrate this feature to be fundamental for STED-ISM, and beneficial in case of large field-of-view imaging - condition for which the effective point spread function (ePSF) may be no longer a *spatio*-invariant characteristic of the imaging system - and, in general, in case of optical aberrations that alter the ePSF.

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