STED nanoscopy without background using polarization switching

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Stimulated emission depletion (STED) super-resolution microscopy (or nanoscopy) offers significant enhancement of optical resolution compared to conventional microscopy [1]. To achieve resolution beyond the diffraction-limit, STED nanoscopy uses orders of magnitude (roughly ~10^5) more photons than the conventional confocal microscopy. Those additional 'STED' photons, which are designed to deplete the fluorescence at the periphery of focus, can induce unintended background noise. Increased low spatial frequency background noise decreases the signal-to-background ratio (SBR) and deteriorates the image quality by masking the high spatial frequency, super-resolved signal.

Here, we report a simple and easy-to-implement method, which we call polarization switching STED (psSTED), that can efficiently suppress the low spatial frequency background appearing in STED images. In psSTED, we switch the STED beam polarization between two different circularly polarized states to record a regular STED image and a background noise image. A simple, unambiguous subtraction process between these two images accomplishes a background-free super-resolved image. With both simulation and experimentation, we demonstrate psSTED works universally for different STED conditions. Finally, we compare the performance of psSTED with other state-of-the-art background subtraction methods and highlight its capability of efficient background suppression with a much simpler hardware implementation.

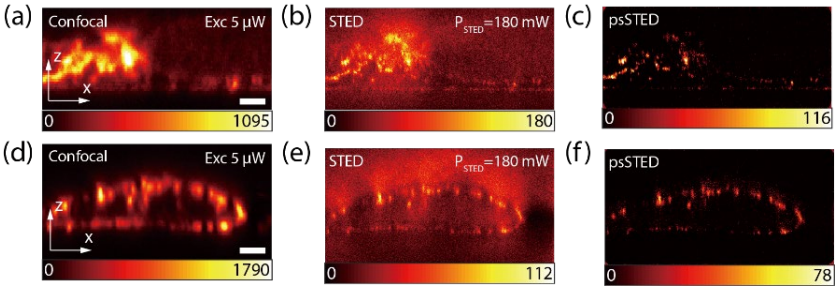

Figure 1. Background noise problem in STED and significant background reduction by psSTED. Scale bar: 2 µm. (a) Confocal, (b) conventional STED, (c) psSTED images of microtubules in a 3T3 cell labelled with SiR-tubulin. (d) Confocal, (e) conventional STED, (f) psSTED images of actin filaments in a LN229 cell labelled with SiR-actin

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