
Advances in Gated CW STED Microscopy: Toward a Versatile Implementation

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Résumé

Stimulated emission depletion (STED) microscopy based on time-gated detection is a straightforward implementation, which substantially reduces the STED beam (peak) intensity to obtain effective sub-diffraction imaging. Furthermore, when combined with STED beam operating in continuous-wave (CW) lowers the complexity and the cost with respect to the early STED implementations, the so called gated CW-STED implementation (gCW-STED). However, the time-gated detection reduces the fluorescence signal that forms the images. Hence, in a situation of high background and/or weak signal, the images degrade in terms of signal-to-noise/background ratio (SNR and SBR) and the benefits of time-gated detection could vanish.

In this work we reported about synergetic combinations of hardware and software methods able to compensate for the SNR and SBR reduction. In particular we demonstrate the benefits of (i) time-gated detection to provide the sharpest images with the lowest instantaneous intensity ; (ii) subtractive methods to remove the anti-Stokes emission background caused by the direct excitation from the STED beam ; (iii) Separation of Photons by Lifetime Tuning (SPLIT) based on the encoding/decoding of spatial information through manipulation of molecular dynamics; (iv) tailored image deconvolution algorithms to improve the contrast. We exemplify the benefits of these methods by imaging sub-cellular structures with Two-Photon Excitation STED Microscopy. Finally, we discuss about the use of these a posteriori proposed methods for improving the quality of the image and the performance of actual STED microscopes.

Mots-Clés: STED microscopy, gated detection, superresolution

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