New Opportunities in Nanoscopy with Fluorescence Lifetime Tools

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Fluorescence Nanoscopy is a powerful tool for life science research to extract molecular information in the cellular context beyond the diffraction limit. The stimulated emission depletion (STED) process induces changes in the spatial lifetime distribution, therefore the combination of STED and fluorescence lifetime imaging (FLIM) can be applied to enhance applications requiring nanoscale resolution in living cells. The information contained in fluorescence lifetime is utilized in gated STED (1) and in recent approaches based on FLIM phasor analysis (2, 3). The underlying principle is the encoding/decoding of the nanoscale spatial distribution of fluorophores from the fluorescence lifetime information. The original work on this concept with FLIM phasors gave rise to the SPLIT (Separation of Photons by LIfetime Tuning) method, increasing the spatial resolution and eliminating uncorrelated background (2). Inspired by this approach, we developed a new functionality called τ-STED that enables to perform STED in gentle conditions keeping the desired resolution, eliminate the uncorrelated background contribution, and perform STED co-localization studies through lifetime-based species separation.

In this talk we will show how τ-STED works and how FALCON (FAst Lifetime CONtrast) ultra-short dead time and built-in algorithms for fast data acquisition and analysis are essential in its implementation (4). We will explain how the τ-STED approach delivers cutting-edge resolution and image quality at low light dose, key to study nanoscale dynamics of cellular processes.

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