

Correlative STED and SMLM, without demons

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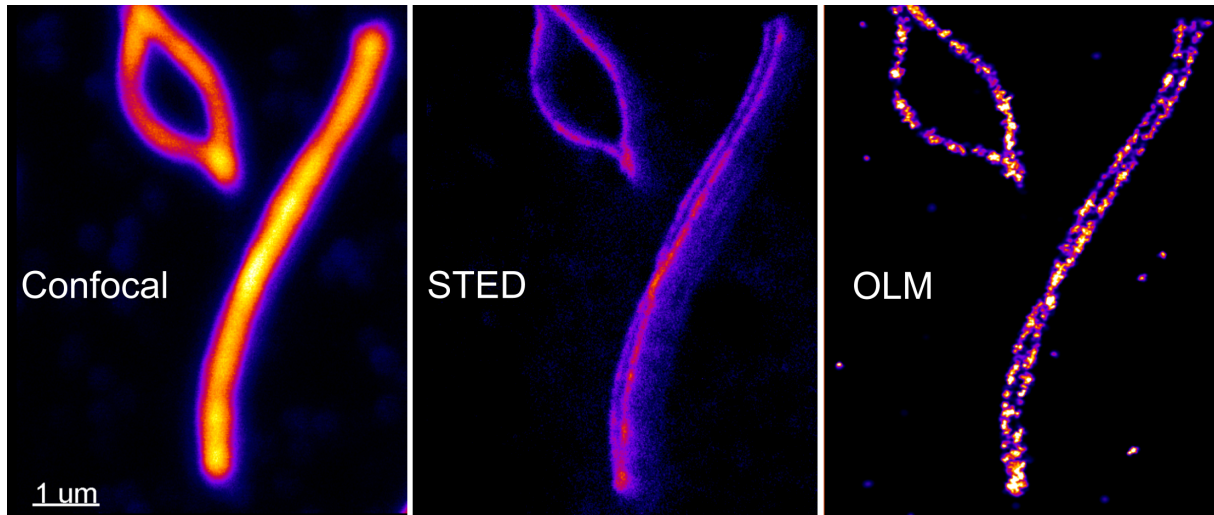


Figure 1: Synaptonemal complex (SC) proteins from mouse stained with Alexa 594. (A) The confocal image shows the entire complement of SC proteins. (B) The same chromosome imaged with STED and (C) OLM (Omnipresent Localisation Microscopy, see Prakash 2017). The two halves of the SC that unite the meiotic chromosome pair are not resolved with confocal but with STED and OLM.

The spatial resolution of light microscopy is continuously being pushed with the development of new technologies. The significant milestones have been Confocal, 4Pi, Two-photon microscopy, STED, Zero point STED, SIM, PALM, STORM. Recently, correlative light microscopy techniques have further pushed the spatial resolution down to a few nanometers. MINFLUX, a correlative STED and SMLM technique, requires very few photons and is based on adaptive updating of positions and localisation precision. The perfect localisation is described by the authors to be achievable upon the aide from "a supernatural being, a demon (1)." While the requirement of fewer photons for accurate localisation is much desired, the access to a demon might not be readily available. Moreover, the technique has only been demonstrated on DNA origami and bacterial samples (for tracking) which can be a limiting factor for biological research.

Here, I demonstrate that using deep UV reactivation (350 to 380 nm lamp excitation) instead of 405 nm laser, correlative STED and SMLM can be performed on real biological samples (mouse meiotic chromosomes) using a simple imaging medium (2). This technique, termed as inSTED, can be used by any scientist to generate high-resolution images and provides an open framework for correlative microscopy. Lastly, I hope the new photophysical observations reported here will pave the way for more in-depth investigations on the mechanisms underlying the excitation, photobleaching and photoactivation of a fluorophore.

1. Balzarotti, Francisco, et al. Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. *Science* (2016). aak9913.

2. Prakash, Kirti. High-density superresolution microscopy with an incoherent light source and a conventional epifluorescence microscope setup. *bioRxiv* (2017). 121061.