NANOSCALE DIFFUSION IN 3D IN LIVE CELLS BY STED-FCS

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The intracellular environment is a complex, heterogeneous and dynamic assembly of macromolecules forming transient structures that can range from several microns down to few tens of nanometers. The observation of molecular diffusion at different spatial scales, and in particular below the optical diffraction limit (< 200 nm), can reveal details of the subcellular topology and, most importantly, its functional organization.
In this respect, super-resolution STED microscopy has been combined efficiently with Fluorescence Correlation Spectroscopy (FCS) for investigating molecular diffusion in 2D systems, such as the cellular plasma membrane [1,2], whereas, unfortunately, its potential has not been fully exploited for investigations in 3D environments.
We have recently introduced a STED-based method for super-resolution imaging, based on the separation of fluorophore lifetime components rather than fluorescence inhibition [3]. Here we combine this method, named separation of photons by lifetime tuning (SPLIT), with fluorescence lifetime correlation spectroscopy (FLCS) [4], to measure the 3D diffusion of fluorescent proteins at spatial scales tunable from the diffraction size down to 100 nm range in live cells. We discuss the obtained results and the limitations of the method imposed, in live cells, by the simultaneous requirements of minimal photodamage and high signal-to-noise ratio.

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