MULTICOLOR 3D SINGLE PARTICLE TRACKING USING SPECTRALLY DISPLACED LOCALIZATION

Corey BUTLER[1,2,3], Rémi GALLAND[1,2], Vincent STUDER[1,2], Jean-Baptiste SIBARITA[1,2]
[1]University of Bordeaux, Interdisciplinary Institute for Neuroscience, UMR 5297, 146 rue Léo-Saignat, 33077 Bordeaux, France
[2]CNRS, UMR 5297, F-33000 Bordeaux, France
[3]Imagine Optic, 18 rue Charles de Gaulle, 91400 Orsay, France
[1]Email: corey.butler@etu.u-bordeaux.fr

Keywords: single particle tracking, multicolor, dual-objective, super-resolution, optics

Single particle tracking (SPT) techniques such as sptPALM, uPAINT, and quantum dot tracking have given unprecedented insight into molecular dynamics in living cells. They allow monitoring the behavior of individual proteins in the plasma membrane as well as their molecular interaction with scaffold proteins with millisecond temporal resolution and high spatial resolution (<30 nm) by fitting the point spread function (PSF) of individual emitters and tracking their position over time.

While these SPT methods have been extended to study the temporal dynamics and co-organization of multiple proteins, conventional experimental setups used to perform multicolor imaging are typically limited to two simultaneous wavelengths. Increasing the number of colors requires additional filters for specific fluorescent tags and is usually performed at the expense of a loss of spatial or temporal resolution. This limits the minimum diffusion coefficient that can be measured, thereby degrading the ability to differentiate between molecular diffusion regimes like immobilization and confined diffusion.

By employing a dual-objective imaging configuration compatible with live cell imaging, we will present a single molecule tracking technique that allows for simultaneous 3D single molecule localization and tracking of multiple distinct species, without compromising the spatio-temporal resolution. A dispersive element introduced into the second optical path (Fig. 1a) induces a spectrally-dependent displacement, which is used to separate numerous fluorescent species of single emitters based on their emission spectra. We will characterize the system's spectral separation capabilities using multicolor fluorescent beads (Fig 1b,c) and demonstrate the technique's capability to simultaneously track several fluorescing species in live cells.

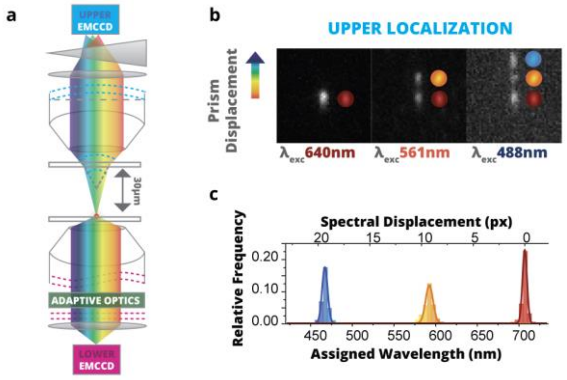

Figure 1: (a) A dispersive element placed in the upper collection path converts wavelength into a spatial displacement. (b) This wavelength to displacement conversion can be easily visualized by exciting a Tetraspeck bead at different wavelengths. (c) The spectrally-induced displacement can be measured relative to the lower camera localization to separate multiple fluorescent species and retrieve the corresponding wavelength.