LIVE 5-D SMLM FOR SIMULTANEOUS MULTI-RECEPTOR TRACKING IN NEURONS

Corey Butler[1,2], G Ezequiel Saraceno[1,2], Adel Kechkar[3], Vincent Studer[1,2], Laurent Groc[1,2], Rémi Galland[1,2], Jean-Baptiste Sibarita[1,2]
[1] University of Bordeaux, IINS, Bordeaux, France.
[2] CNRS UMR 5297, F-33000 Bordeaux, France.
[3]Ecole Nationale Supérieure de Biotechnologie, Constantine, Algeria
Email: corey.butler@u-bordeaux.fr

KEYWORDS: *Single Molecule, Tracking, Spectral Imaging, Living Cells*

ABSTRACT

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 and molecular interaction of individual proteins at 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 spatial or temporal resolution and/or field of view. This limits the minimum diffusion coefficient that can be measured and reduces the statistics that can be gathered from a single experiment, thereby degrading the ability to differentiate between molecular diffusion regimes like immobilization and confined diffusion. Moreover, simultaneous multi-receptor tracking could also reveal specific interactions between different protein populations, which could previously only be inferred from the behavior of a single population without any knowledge of the presumed partner behavior.

By employing a dual-objective imaging configuration compatible with routine live cell imaging, we will present a single molecule tracking technique that allows for simultaneous 3D single particle tracking of multiple distinct species without compromising spatio-temporal resolution. A dispersive element introduced into the second optical path induces a spectrally-dependent displacement, which is used to separate numerous fluorescent species of single emitters based on their emission spectra, similar to *Zhang et al*[1]. A proof of concept of the spectral separation abilities of the system will be shown via simultaneous 3D DNA-PAINT of fixed samples, where the acquisition time is significantly reduced compared to conventional sequential multicolor imaging. Lastly, we will demonstrate how the technique can be applied to track multiple receptors in live neuron cultures, and we will discuss possibilities of how advanced data analysis techniques can fully exploit the 5-dimensional data (x,y,z,t,λ) to extend the capabilities of conventional single particle tracking, such as the investigation of protein-protein interactions.

REFERENCES

1. Zhang, Z. *et al.* Ultrahigh-throughput single-molecule spectroscopy and spectrally resolved super-resolution microscopy. *Nature Methods* **12,** 935–938 (2015).