3D SINGLE MOLECULE TRACKING USING FLUORESCENCE PHASE IMAGING FOR INVESTIGATIONS IN ACUTE BRAIN SLICES

Jeanne Linarès[1], Joanna Ferreira[2], Blanka Kellermayer[2], Laurent Groc[2], Pierre Bon[1*], Laurent Cognet[1]

[1]Univ. Bordeaux, CNRS, Institut d'Optique – LP2N, Talence France
[2]Univ. Bordeaux, CNRS, Interdisciplinary Institute for Neuroscience, Bordeaux, France

Email: pierre.bon@institutoptique.fr

Keywords: quantitative phase imaging, single Qdot tracking, 3D super-localization, brain

Quantitative phase microscopy with self-interferences was initially developed for white light imaging. Its extension to fluorescence imaging is possible and allows going beyond the state of the art for localizing single fluorescent molecules in 3D. More precisely, by transposing a concept previously demonstrated for absorbing particles [1], we show that the phase of the light emitted by a single emitter carries its axial position which can be determined well below the diffraction limit while analysis of the imaged intensity distribution provides the precise emitter's lateral position [2]. Importantly, this novel approach in single molecule microscopy is based on a single measurement to provide 3D localization of single emitters without the need for Point-Spread-Function shaping nor multiple plane imaging. The simplicity of the setup makes it robust and virtually transferable to any fluorescent microscope.

In this communication, we will further present our current efforts to apply this strategy toward 3D single quantum dot tracking in biomimetic samples as well as in living brain tissues at video rate. By going to 3D, our aim is to go beyond the current state of the art for studying the lateral dynamics of neurotransmitter receptors in acute brain slices [3] in order to unravel currently inaccessible knowledge about the localization and dynamics of these receptors in intact live tissue.

[1] P. Bon, N. Bourg, S. Lécart, S. Monneret, E. Fort, J. Wenger and S. Lévêque-Fort, "Three-dimensional nanometre localization of nanoparticles to enhance super-resolution microscopy", Nat. Comm., 2015
[2] P. Bon, J. Linares et al, *to be submitted*
[3] J. Varela, J. Dupuis, L. Etchepare, A. Espana, L. Cognet, and L. Groc, "Targeting neurotransmitter receptors with nanoparticles in vivo allows single-molecule tracking in acute brain slices" Nat Commun, 2016,