Bi-modal microscopy for studying the dynamics at the cell membranes

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There is a large demand for microscopies allowing non-invasive near-surface imaging at spatial and temporal resolutions high enough to follow the dynamics of organelle movement, receptor diffusion, protein interaction or the formation of molecular complexes close to the basal plasma membrane. Imaging such phenomena in two-dimensional membranes is at the core of many biomedical applications and research studies ranging from cell biology to biosensing, analyte detection, surface chemistry, molecular medicine and the characterization of nanomaterials. Presently, the only surface-imaging tool compatible with extended dynamic observation and low photo-damage is Total Internal Reflection Fluorescent Microscopy (TIRFM) that probes the sample with the tail of an evanescent wave [1]. Yet, this technique is plagued by a diffraction-limited lateral resolution (~300 nm) and a non-negligible instrument- and sample-induced perturbation of the confinement of the evanescent field, which makes image interpretation difficult [2]. In this project, **we propose to increase significantly the significance of TIRFM by combining it with diffraction tomography in a Structured Illumination Microscopy (SIM) scheme**. Sophisticated numerical reconstructions of the sample taking advantage of both fluorescence and diffraction data will provide maps of fluorescence density and of refraction index with a sub-100 nm resolution. **Our approach aims at keeping the experimental implementation relatively simple, all the complexity and novelty inherent to high resolution and bi-modal imaging being concentrated in the inversion tools.** The new microscope associates the advantages of non-saturated one-photon fluorescence excitation (low photodamage of the sample), speed of up to 10 Hz full field of SIM and the nanometer-optical sectioning of TIRF. The bimodal super-resolution TIR-SIM will be tested by studying the collective dynamics of mitochondria and the endoplasmic reticulum (ER) and their interaction sites (MAMs) in a new triple-color scheme. **The simultaneous acquisition of functional and structural images of the cell membrane at the sub-100 nm level is expected to find many applications in cell biology.**

The candidate will interact with experts in optical instrumentation (Guillaume Maire), data processing (Anne Sentenac) and a specialist in cell-membrane imaging (Maia Brunstein of the Brain Physiology Lab). We are looking for talented and motivated applicants with a background in physics-engineering and/or signal processing and with a strong motivation for cell biology.

Références :

[1] H . Schenckenburger, "Total internal reflection fluorescence microscopy, technical innovations and novel applications", current opinion in biotechnology, 16, 13-18, 2005.
[2] **Brunstein** et al "near membrane refractometry using super-critical angle fluorescence ", Biophys J 112:1940, 2017.
[3] Ting Zhang, Charankumar Godavarthi, Patrick C. Chaumet, **Guillaume Maire**, Hugues Giovannini, Anne Talneau, Marc Allain, Kamal Belkebir, and Anne **Sentenac** "Far-field diffraction microscopy at λ/10 resolution", Optica, Vol. 3, 609-612, 2016.