Deciphering protein organizations and dynamics at various scales using single-objective Selective Plane Illumination Microscope (soSPIM)

Rémi Galland[1,2], Gianluca Grenci[3], Anand P. Singh[3], Corey Butler[1,2,5], Timoty E. Saunders[3], Vincent Studer[1,2], Virgile Viasnoff[3,4], Jean-Baptiste Sibarita[1,2]
[1] University of Bordeaux, IINS, Bordeaux, France.
[2] CNRS UMR 5297, F-33000 Bordeaux, France.
[3] Mechanobiology Institute, National University of Singapore.
[4] Cell Adhesion France Singapore, CNRS UMI 3639.
[5] Imagine Optics, Orsay, France.
***E-mail:* remi.galland@u-bordeaux.fr**

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ABSTRACT:
Heterogeneities of protein organization and dynamics are often the signature of proteins activities and cellular mechanisms. Developing techniques that enable to map the spatiotemporal organization of proteins at various spatial and temporal scales can therefore provides key input in our understanding of proteins functions. We recently developed an innovative single objective Selective Plane Illumination Microscope (soSPIM) relying on the combination of a beam steering unit with dedicated micro-fabricated chips featuring 45° mirrors[1]. Compatible with conventional microscopes, we demonstrated its capabilities to achieve multimodal and multiscale imaging on different biological samples ranging from drosophila embryos to single cells with single molecule resolution.

We will illustrate how soSPIM high photon collection combined with the high optical sectioning can be used to probe the spatiotemporal organization of bio-molecules in depth, at various spatial and temporal resolutions. First, we will show that the combination with imaging Fluorescence Correlation Spectroscopy approaches (im-FCS) and photo-activable proteins allows measuring proteins dynamics in depth with millisecond temporal resolution. Thanks to this combination, we were able to map for the first time the dynamics of various nuclear proteins in 3D over the whole nucleus of cells[2]. Second, we will demonstrate that the combination of the soSPIM illumination scheme with adaptive optics allows probing the organization and dynamics of proteins in depth over an entire cell with single-molecule resolution, opening new possibilities that couldn't be achieved with standard super-resolution approaches.

REFERENCES:

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