WHOLE CELL 3D SINGLE MOLECULES LOCALISATION MICROSCOPY USING SINGLE-OBJECTIVE SELECTIVE PLANE ILLUMINATION MICROSCOPY (soSPIM)

H. Forrière, M. Cabillic, C. Butler, M. Sambrano, J. Roissbroich, F. Levet, J-B Sibarita and R. Galland
Institut Interdisciplinaire de NeuroScience (IINS)
Centre Broca Nouvelle-Aquitaine, 146 rue Léo-Saignat, 33000 Bordeaux, France
Email: Hisham.Forriere@u-bordeaux.fr

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Fluorescence microscopy is an unevaluable tool to observe proteins of interest within their native cellular environment. However, diffraction of light limits its capability to resolve structures below 200 nm and thus to decipher proteins organization at the molecules scale. Over the past 20 years, several methods have been proposed to circumvent this limitation. Amongst them, Single Molecules Localization Microscopy (SMLM) allows localizing, tracking and counting biomolecules with the highest spatial resolution, down to 20 nm. However, this resolution strongly depends on out of focus light rejection and efficient photon collection. Hence, standard SMLM implementation uses high numerical aperture (NA) objective in combination with Total Internal Reflection Fluorescence (TIRF) or Highly Inclined Laminated Optical (HILO) microscopy to provide high photon collection and efficient optical sectioning respectively. However, it limits the illumination to the first 0.1 to 1 µm above the coverslip, intrinsically restricting the achievable imaging depth. On the other hand, Single Plane Illumination Microscopy (SPIM) allows efficient optical sectioning in depth, but their standard multi-objectives architectures are subject to important mechanical constraints, preventing the use of high NA objectives.

To make in depth SMLM possible, we developed a single-objective SPIM (soSPIM) [1] system, that integrates micro-fabricated devices presenting 45° mirror to produce light sheet illumination and signal collection with a single objective. This architecture benefits from the optical sectioning of light-sheet-based illumination systems, and is compatible with high NA objectives for high photon collection. It thus allows SMLM imaging in depth up to tens of microns above the coverslip.

Here we will discuss the capabilities and requirements of the soSPIM technique for probing the 3D organization of proteins over an entire cell with single molecule resolution. Especially, we implemented an adaptive optics system in order to maximize the localization precision by limiting optical aberrations and allow for their precise 3D localization. We developed a real-time drift correction and automatic acquisition workflow to allow daylong 3D DNA-PAINT acquisition of entire cells over volume of 20x20x20 µm^3 with nanometric resolution ($\sigma_{x,y} = 10\ nm$ and $\sigma_z = 40\ nm$). As an illustration, we will investigate the 3D nanoscale organization of CD3 receptors over a whole T-Cell membrane surface using tessellation techniques [2][3].

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