Whole cell 3D SMLM using single-objective Selective Plane Illumination Microscopy

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Fluorescence microscopy is an unevaluable tool to observe targeted molecules in their cellular environment, but the diffraction of light limits its capability to resolve structures at the molecule scales and thus to decipher proteins organization of dense and complex structures. Over the past 20 years, several methods have been proposed to overcome this spatial limitation. Amongst them, Single Molecules Localization Microscopy (SMLM) enables counting, tracking and locating biomolecules in their cellular environment with the highest spatial resolution, down to 20 nm. However, this resolution strongly depends on out of focus light rejection and efficient photons collections limiting the penetration depth of standard SMLM implementations which use either Total Internal Reflection Fluorescence (TIRF) or highly inclined (HILO) illumination scheme. On another hand, if Selective Plane Illumination Microscopy (SPIM) allows efficient optical sectioning in depth, the need of a side illumination objective induces important mechanical constraints, preventing the possibility to use high NA objective and making the implementation of SPIM for SMLM not straightforward.

To overcome this limitation, our team developed a single-objective Selective Plane Illumination Microscope, named soSPIM[1]. This new SPIM architecture combines micro-fabricated devices displaying 45° mirrors, with a single high NA objective, allowing both high photon collection and in depth of out-of-focus light rejection. It therefore enables to perform SMLM in depth up to 40 µm above the coverslip.

Here we will discuss the capabilities and requirements of the soSPIM system to probe the 3D organization of proteins in depth over an entire cell with single molecule resolution. To do so, we implemented an adaptive optics system to reduce the impact of optical aberrations in depth and precisely localize molecules in 3D, as well as a real time drift correction system to account for drift in all directions, allowing automatization of long time acquisitions processes for multicolor 3D SMLM of whole cells.

[1] R. Galland, G. Grenci, A. Aravind, V. Viasnoff, V. Studer, and J.-B. Sibarita, “3D high- and super-resolution imaging using single-objective SPIM,” *Nat. Methods*, vol. 12, no. 7, pp. 641–644, Jul. 2015.