Whole cell 3D single molecule localization microscopy using single-objective Selective Plane Illumination Microscopy (soSPIM)

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ABSTRACT:
Assessing protein organization and dynamics in their native cellular context provides invaluable insights into their activities and functions. The development of super-resolution microscopy approaches, allowing imaging proteins of interest with spatial-resolution down to the nanometre scale, has been a great step forward in this direction. Amongst these techniques, Single Molecule Localization Microscopy (SMLM) enables counting, tracking and locating biomolecules in their cellular environment with the highest spatial resolution. However, this resolution is critically dependent on strong background rejection, limiting the penetration depth of standard SMLM implementation to the first micron above the coverslips. Furthermore, conventional 3D imaging methods like confocal and Selective Plane Illumination Microscopy (SPIM) are not sensitive enough to localize efficiently single molecule events. To overcome those limitations, we developed the soSPIM technique, which uses a single high numerical aperture objective in combination with microfabricated devices. This architecture benefits from both a high collection efficiency and an optimal optical sectioning in depth, allowing to perform SMLM tens of microns above a coverslip[1,2].

Here we will discuss the capabilities and requirements of soSPIM for probing the 3D organization and dynamics of proteins in depth over an entire cell with single molecule resolution. For accurate 3D SMLM, we implemented an adaptive optics system for depth-dependent aberration correction and astigmatism control as well as a real-time mechanical drift correction system based on fiducial marker tracking to account for the long acquisition time. We will illustrate multi-colour SMLM within entire cells with 3D spatial-resolution down to few tens of nanometres.

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