Combining advanced microscopy techniques for fast 3D imaging at the single molecule level in living cells

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Single molecule fluorescence microscopy allows investigating the nanoscale organization and dynamics of biomolecules in their cellular environment with high temporal and spatial resolution. However, conventional single molecule imaging is limited in the imaging depth and thus does not reflect the inherent 3D organization of cells. Also, the volumetric extension of the sample is the cause of high background noise, originated by out-of-focus fluorescent sources when the cell is excited through unspecific wide-field illumination. Consequently, molecules are excited with high laser powers to improve the signal to noise ratio which in turn increases photo-toxicity and photo-bleaching. Finally, efficient 3D single particle tracking requires high temporal resolution and sensitivity over an extended volume. To address these challenges, we developed a new method for instantaneous 3D volumetric imaging with single molecule sensitivity, combining two cutting-edge techniques: MultiFocus Microscopy (MFM) [1] and single objective Selective Plane Illumination Microscopy (soSPIM) [2].

MFM permits the simultaneous acquisition of several planes without sample scan and using a single camera. It allows the fluorescence detection over an axially extended volume with a high temporal resolution only limited by the acquisition speed of the camera. The high temporal resolution of MFM dramatically extends the observable dynamics range of single molecules.

To confine the excitation beam, we use the soSPIM architecture. It uses a single high numerical aperture objective to create a light sheet and collect the fluorescence thanks to 45°mirrors located on the glass coverslip beside the sample. soSPIM strongly reduces the out-of-focus signal, allowing to detect single molecules at several microns inside the sample.

We demonstrated that our method enables to image a 3D volume of few microns with a time resolution of 30 ms with single molecule sensitivity (FIG. 1). We believe that it could pave the way for exciting investigation of biological functions where spatial and temporal resolutions are limiting factors.

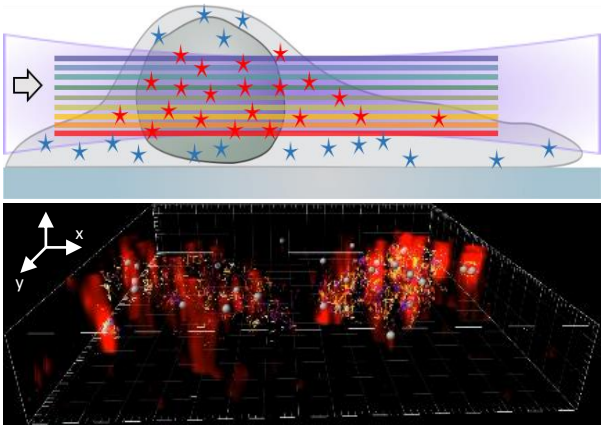

FIG. 1 – Matching the acquisition and excitation volume allows instantaneous imaging of single H2B histones dynamics over a 4 µm volume. Time resolution is 30 ms.

References

[1] Abrahamsson *et al.*, Fast multicolor 3D imaging using aberration-corrected multifocus microscopy, **Nat. Meth.**, 2013.

[2] Galland *et al.*, 3D high and super-resolution imaging using single-objective SPIM, **Nat. Meth.**, 2015.