ADVANCED SINGLE-MOLECULE FLUORESCENCE MICROSCOPY FOR FAST 3D IMAGING IN LIVING CELLS

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Single-molecule (SM) imaging, localization and tracking offer a mean to investigate the nanoscale organization and dynamics of biomolecules in their cellular environment with high temporal and spatial resolution. However, the limited imaging depth of a conventional SM microscope does not reflect the inherent volumetric organization of living cells and limits the range of SM dynamics that can be observed in 3D. In addition, widefield illumination remains a method of choice for SM imaging in depth, which in turn raises many concerns. First, out of focus molecules are excited and contribute to an increase in the background noise. Second, such illumination increases photo-toxicity and photo-bleaching.

To address these challenges, we developed a new method for selective and true instantaneous volumetric imaging with SM sensitivity. We combine two cutting-edge techniques: MultiFocus Microscopy (MFM) [1], [2] and single objective Selective Plane Illumination Microscopy (soSPIM) [3]. Thanks to several diffractive elements, MFM captures an instantaneous volumetric image over up to 4 µm depth. The high sensitivity and temporal resolution dramatically extend the observable range of SM 3D dynamics. On the other hand, soSPIM architecture uses a single high numerical aperture objective to create a light sheet and collect the emitted fluorescence. soSPIM strongly reduces the out-of-focus signal, providing high signal-to-noise ratio for the detection of single-molecules at several microns inside the sample. We combined the two methods by carefully matching the thickness and shape of the excitation sheet to the observation volume of MFM. We demonstrated that our method enables to image a 3D volume of few microns with high temporal resolution and SM sensitivity (FIG 1). We believe that it could pave the way for exciting investigation of several biological functions where spatial and temporal resolutions are limiting factor.

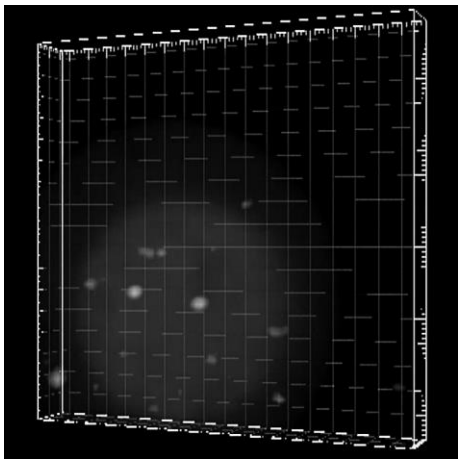

FIG. 1 – Dynamic of single CTCF proteins in the nucleus of STEM cells through instantaneous and selective volumetric imaging.

References

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