Beyond the diffraction limit by Light-Sheet Microscopy

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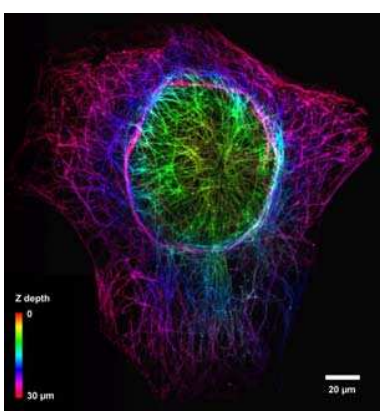
By combination with the advantage of localization microscopy and light-sheet microscopy, one could get super-resolved cellular imaging in 3D across large field of view. The localization process is determined by on-off switching of spontaneously blinking fluorophore based on intramolecular spirocyclization reaction without intense laser irradiation or additives8. With high-density labeled spontaneous blinking fluorophore and wide-field detection of light-sheet microscopy, these allow us to construct 3D super-resolution multicellular imaging at high speed (~minutes) by light-sheet single-molecule localization microscopy. Extended from cultured cells, an intact tissue imaging with high spatial resolution is technically challenging and required for the understanding of whole brain connectome. Due to the sample-induced aberration, the diffraction-limit resolution and beyond is hard to achieve at the tissue level. One could reduce the refractive index discontinuity within the sample by optical clearing. The transparent whole thick tissue could be imaged by light-sheet single-molecule localization microscopy without the preparation of sliced brain sample. It is feasible to have 3D super-resolution imaging with synaptic-scale connectomics. For live tissue imaging, we could use adaptive optics to correct the aberration so that the aberration-free imaging at the diffraction-limit regime could be obtained9. Together with live functional imaging of Drosophila brain; these enable us to connect the gap between neuronal structure and complex behavior of Drosophila.

Instead of sweating on the super-resolution techniques to pursuit high spatial resolution, expansion microscopy (ExM) is invented to detour the optical diffraction limit by physically expanding the samples to ~4 times larger than original with swellable polymer10. However, the main concept in ExM is that all the components inside cell or tissue under physical expansion in all directions isotropically. Firstly, we would like to confirm this and subsequently, by the combination of ExM and light-sheet to visualize the nanoscale structure inside subcellular component with large volume imaging in a second.



A 4x expanded HeLa cell with microtubule (Alex488) imaged with lattice light-sheet microscopy