Parallelised Nanoscopy to study proteins in Cell Adhesion

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Until recently, coordinate targeted super resolution techniques, like STED (STimulated Emission Depletion) and RESOLFT (REversible Saturable Optical Linear Fluorescent Transitions) have been point scanning techniques, making them too slow (few seconds) to image large field of views (few tens microns). Certain critical cellular processes involved in cell shaping and migration, proceeds through cycles lasting from seconds to minutes. Parallelization of coordinate targeted super-resolution techniques is crucial to understand these biological functions. Large 2D parallelization for STED [1] and RESOLFT [2] has already been reported with optical lattices. Parallelized STED nanoscopy employs high intensities, but can resolve processes occurring in short timescales. RESOLFT imaging can image with an order of magnitude less intensities, but at the expense of speed. Thus, these two modalities have different, but complementary capabilities.

After the successful development of a parallelized 2D optical lattice STED (OL-STED), We are building a parallelized 2D Optical Lattice RESOLFT (OL-RESOLFT) designed in such a way that there will be two optical lattices: one for activation and another for switch-off. The complementary nature of these two lattices will ensure reduced photobleaching. A doughnut based RESOLFT will be used to procure the optimized intensity and timing parameters for photoactivation, switch-off and excitation by imaging cells expressing proteins fused with rsEGFP2. With OL-STED, we will measure the local forces in the nanoscale environment of focal adhesions. The OL-RESOLFT will enable us to study the nanoscale protein reorganizations in integrin based adhesion sites. These information, combined with single protein tracking experiments will provide deeper understanding about mechanotransduction and protein reorganization in focal adhesions. I will present the latest developments on OL-RESOLFT based 2D complementary lattices.

References

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