Investigation of Membrane Dynamics by Lattice Light-sheet Microscopy

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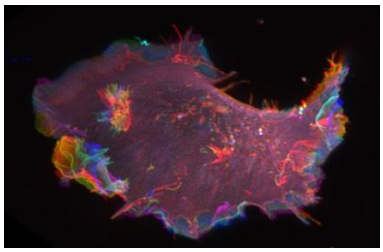
Membrane ruffling is an essential process at the leading edge of the migrating cells, which contains protrusion and retraction of plasma membrane. The extension membrane determines the direction of migration. The dynamic of membrane ruffling depends on the interaction between filament actin and motor proteins. Upon the activation of motor proteins by calcium ions, the migration process starts. Therefore, it is important to study the correlation between local calcium concentration and the membrane dynamics. To study the dynamics of the membrane ruffling, we established several stable cell lines, which contain chemical and optogenetic inducible dimerization. In addition, the proteins of interest such as actin, myosin, membrane anchors and calcium indicators were labeled with fluorescence proteins. The dynamics of membrane ruffling was investigated by lattice light sheet microscope (LLSM), which is capable of recording the three-dimensional images at a speed of three seconds per volume [1]. Our system can be used to trigger a specific area of the optogenetic inducible cell. We have recorded the spatiotemporal behavior of calcium flickering at cellular edge upon chemical or optogenetic stimulation.



The membrane ruffling can be triggered by rapamycin and/or blue light. After stimulation, the three-dimensional dynamics of membrane ruffling has been recorded by LLSM. The figure illustrates the dynamics of membrane at six time points, which are color coded.

Figure 1: Maximum intensity projection images of membrane at six time points

Reference;

[1] B.C. Chen, W.R. Legant, K. Wang, L. Shao, and E. Betzig, Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution, Science, 346(6208):1257998 (2014).