Using Bessel beam combined with optogenetics fused epidermal growth factor receptor to explore cell migration

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Epidermal growth factor receptor (EGFR) activation is an important step of cancer metastasis (cell migration). As EGFR related studies have shown that EGFR dimerization is an initial step on its signaling activation. Here we applied the function of photoreceptor CRY2olig which is homooligomerization by the blue light stimulation into the EGFR activation step. Then EGFR fusion CRY2olig (EGFR-CRY2olig) construction produced by molecular engineering. For the functional assay, we will identify cell migration by observing membrane ruffling, an essential process that occurs at the front end of migrating cells. Before the experiment initiation, we expressed CRY2olig in the U2Os cell line (human osteosarcoma) and tested the homooligomerization level of CRY2olig in different wavelength Bessel beam to determine the triggered wavelength and Bessel pattern that should be used in the experiments. We also adjusted the power of the back aperture on the excitation objective to find the better-stimulated conditions. Then following the testing condition, we used the Lattice lightsheet microscopy (LLSM) to generate the Bessel beam for stimulation EGFR-CRY2olig expressed U2Os cells. Meanwhile, the fluorescent signaling of EGFR-CRY2olig and other biomarkers (including filament actin, myosin IIB, and ARP3) collected by the original function of LLSM. In preliminary result shown that the Bessel beam triggering zone displays more area of split colors after stimulation. The figure illustrates the dynamics of EGFR-CRY2olig at six-time points, which are color-coded.

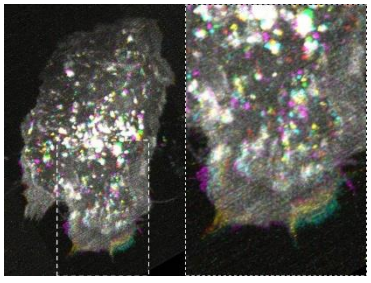

Figure 1: Maximum intensity projection images of membrane at six time points