Simultaneous STED-FLIM imaging of actin and EGFR

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In this study a home-built Stimulated Emission Depletion (STED) microscope was combined with Fluorescence lifetime imaging (FLIM). The setup uses both a pulsed excitation and a pulsed STED beam. FLIM requires much higher signal levels than intensity imaging which hampers its use in super resolution microscopy. Here, we optimized imaging conditions to minimize photobleaching and obtain high enough signal levels for FLIM. FLIM images were recorded using custom-built electronics equipped with 62, 200ps wide time channels. An optimized binning scheme for the time channels was used in combination with custom-written software; this approach allows analyzing lifetimes of pixels with as few as 70 counts.
The FRET-FLIM microscope was used for simultaneous imaging of actin and Epidermal Growth Factor Receptors (EGFR) in A431 cells. Phalloidin-Atto647N was used to label actin and an anti-EGFR nanobody followed by an anti-VHH and Atto647N conjugated secondary antibody was used to label EGFR. The large lifetime difference between the phalloidin-Atto647N (~3.5ns) and the labelled EGFR (~2.0ns) allows for lifetime based segmentation. The reduction of the lifetime of the antibody-Atto647N is believed to be due to self-quenching of the Atto647N dye on the secondary antibody. EGFR is present as monomers and small clusters and after stimulation with EGF, the number and size of EGFR clusters increases. This results in reduction of the fluorescence lifetime that can be observed in the lifetime images. In these experiments the optical resolution amounts to about 60nm.

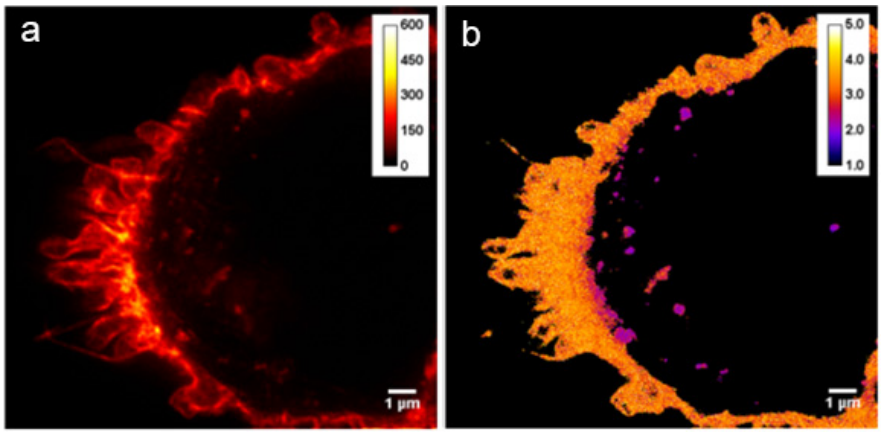

Figure 1: Intensity image a) and lifetime image b) of an A431 cell showing actin (phaloidin-Atto647N) and EGFR (antibody-Atto647N).