IMAGING FLUORESCENCE CORRELATION AND CROSS-CORRELATION SPECTROSCOPY ANALYSE LIPID AND PROTEIN ORGANIZATION IN LIVE CELLS

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Imaging Fluorescence Correlation and Cross-Correlation Spectroscopy (Imaging FCS and FCCS) are powerful quantitative tools that provide diffusion, concentration and interaction maps on live samples over observation areas of 100 µm² and larger (1). Imaging FCS and FCCS are applicable in total internal reflection (TIR) setups for the investigation of lipid membranes with high signal to noise ratio (2, 3) or can be applied in light-sheet based microscopes to investigate molecular dynamics in 3D samples (4, 5). Here we use imaging TIR-FCS and FCCS (ITIR-FCS and FCCS) to measure the lipid and protein organization of several cell types over a temperature range between 25-37 °C. We demonstrate that different proteins reside in different lipid environments and experience specific activation energies for diffusion. In addition, we show that epidermal growth factor receptor (EGFR) proteins can be found as oligomers in cell membranes even before activation and we provide maps detailing the EGFR interaction. ITIR-FCS and FCCS are powerful tools that can now measure large areas of a cell membrane in a single measurement providing information on lipid and protein localization, their environment and their interactions.

Fig.1: Imaging Total Internal Reflection Fluorescence Cross-Correlation Spectroscopy (ITIR-FCCS) measurements of co-expressed epidermal growth factor receptors (EGFR), which were labelled either with mRFP or GFP, in CHO cells. (A) Histogram for positive control, negative control, and the co-expression experiments. (B) Interaction map showing the degree of cross-correlation for every pixel. Green lines show the outline of the cell. Pixel size: 270 nm.