QUANTITATIVE MICROSCOPY IN CELLS USING FLUORESCENCE FLUCTUATION SPECTROSCOPY

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One of the most intriguing challenges in life sciences is to understand how a complex mixture of molecular particles and structures can make up a living cell. To increase our understanding about the complexity of these processes in living cells, experimental data on the spatial-temporal organization is required. Fluorescence Fluctuation Spectroscopy (FFS) is a family of fluorescence techniques capable of detecting concentration, dynamics and interactions of fluorescent particles down to the single-molecule level. In this poster the basic principles of FFS will be illustrated by examples of cell signaling molecules studied in living yeast, plant and animal cells.

A key parameter for successful FFS measurements in vivo is the molecular brightness (η) of the fluorescent dye selected. Therefore optimized and Large Stokes Shift variants of Green Fluorescent Protein (FP) have been developed on basis of η, chromophore maturation and sensitivity to photobleaching & dark-state kinetics. A FCS study of YFP localized in the mitochondrial matrix showed a significant enhanced diffusion in two patient cell lines having a complex I deficiency, supporting biochemical data indicating reduced protein density inside these mitochondria. Molecular interactions are monitored for MAPK proteins involved in yeast pheromone signalling. Concentrations and apparent dissociation constants (K_D) of the complexes were calculated, which did not change upon addition of the pheromone ligand α-factor. The oligomerization of eCFP-tagged embryogenesis receptor AtSERK1 was studied by Photon Counting Histogram analysis in Cowpea plant protoplasts showing 15% homo-dimerization in absence of the brassinosteroid ligand.

Low mobility complexes suffer from photobleaching by continuous point-illumination. When scanning a line or an image instead, the diffusion coefficient of the Histamine and EGFR receptors could be determined in HeLa cells. Transport of SHortRoot (SHR-eGFP) from endodermis to cortex in Arabidopsis plants was measured by cross-correlating intensities of pixel pairs. The aggregation of Annexin A4-sGFP2, a key protein in modifying membrane morphology, is studied in HeLa cells using number and brightness analysis. Upon stimulation with ionomycin AnxA4 locates from the cytoplasm, where it’s present as monomers, to the plasmamembrane. The mobile membrane fraction consists of complexes that are three times as bright as the monomeric sGFP2 label, consistent with in vitro data in supported bilayers. In order to calculate the stoichiometry of higher-order receptor complexes cross-N&B has been applied and validated using sGFP2 and mCherry tagged proteins.