LIVE CELL IMAGING STED-FCS FOR MEASURING FAST DYNAMIC PROCESSES APPROACHING THE NANOMOLECULAR SCALE RESOLUTION

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Nowadays, the application of super-resolution spatial and temporal imaging techniques to living samples is still challenging. STED-FCS (stimulated emission depletion fluorescence correlation spectroscopy)[1] is a cutting-edge method that combines super-resolved spatial and temporal information. The application of STED-FCS in biology allows retrieving a plethora of information, e.g. protein diffusion dynamics, stoichiometry or particle numbers, with high precision. STED-FCS applications in living cells, however, are still limited. The purpose of this work was to apply STED-FCS to a range of living samples to demonstrate the feasibility of this technique. Investigating autophagosomal transport in neurons, our diffusion data revealed the switch of a key component during amphisome transport in pre-synaptic boutons. Secondly, we were able to use STED-FCS as a tool for determining shortened carbohydrate chain lengths in enzyme-deprived melanoma cells. Finally, we quantified clear changes in the membrane fluidity of Alzheimer's disease peptide Abeta-treated neuronal cells. STED-FCS allowed to detect dynamics in the milliseconds regime in extremely small volumes (50x50x50nm³). Our results are a step forward in the measurement of fast dynamic processes in living samples with a spatial resolution approaching the molecular level.

[1] C. Eggeling, C. Ringemann, R. Medda, G. Schwarzmann, K. Sandhoff, S. Polyakova, V.N. Belov, B. Hein, C. von Middendorff, A. Schönle, S.W. Hell. "Direct observation of the nanoscale dynamics of membrane lipids in a living cell." Nature, **457**(7233), 1159-62.