

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. Amongst others, we will present measurements of glycoproteins in melanoma cells, autophagosomal components in neurons and injectisome complexes in pathogenic bacteria. We combined z-STED-FCS with DyMIN-STED to detect dynamics in the milliseconds regime in extremely small volumes ($50 \times 50 \times 50 \text{ nm}^3$). 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.