Wednesday, February 24, 2021
1:30 PM-2:00 PM
Leica Microsystems

High Photon-Count Rate FCS and STED-FCS to Study Diffusion Dynamics in Live Cells

Investigating diffusion dynamics of proteins and small molecules has become a routine measurement all across the life sciences, chemistry and physics. It provides valuable insights into reaction dynamics, oligomerization, molecular interactions or cellular (membrane) heterogeneities [1]. Fluorescence correlation spectroscopy (FCS) is a versatile tool to determine diffusion dynamics in membranes (2D diffusion) and solution or the cytoplasm (3D diffusion). Measuring the intensity fluctuations over time due to the diffusion of molecules through the observation volume is the basis for FCS. Temporal autocorrelation of the signal allows for the calculation of the autocorrelation curve which provides insights into the underlying dynamics as well as the concentration of the observed species [2]. Until now, the concentration regime for reliable measurements has been limited by the detection electronics which could not efficiently and accurately time-tag photons at high photon-count rates. This restricted the range of measurable fluorophore concentrations and data quality of the FCS recordings, especially in combination with super-resolution stimulated emission depletion (STED)-FCS.

In this talk, we will show the applicability and reliability of FCS at high photon-count rates (average intensities of more than 1 MHz and concentrations higher than 1 µM) using novel detection equipment based on hybrid detectors, namely HyD SMDs, and real-time gigahertz sampling of the photon stream using the Leica SP8 STED FALCON FCS implementation [3]. By measuring the diffusion of fluorophores in solution and cytoplasm of live cells, as well as in model and cellular membranes, we show that accurate diffusion and concentration measurements are possible in these previously inaccessible high photon count regimes on a turn-key instrument. This may reduce the bias when performing live cell measurements where varying expression levels occur routinely and increases the experimental flexibility. In STED-FCS data quality suffers from the fluorescence depletion and can be greatly improved by using higher confocal count rates. The presented data show a path towards robust FCS and STED-FCS measurements in living cells.

Speakers

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[1] E. Sezgin et al., "Measuring nanoscale diffusion dynamics in cellular membranes with super-resolution STED–FCS," Nat. Protoc., vol. 14, no. 4, pp. 1054–1083, Apr. 2019.
[2] J. Lackowicz, Principles of Fluorescence Spectroscopy, Third. Boston, MA: Springer US, 2006.
[3] F.Schneider et al., "High photon count rates improve the quality of super-resolution fluorescence fluctuation spectroscopy," J. Phys D: Appl. Phys., vol. 53, no. 16, 2020