Type of presentation: Invited

ID-3-IN-1939 Measuring 2D and 3D diffusion coefficient maps on lipid bilayers, vesicles and cells with light sheet based fluorescence correlation spectroscopy

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Fluorescence Correlation Spectroscopy (FCS) is a widely used quantitative spectroscopy technique which allows measurements of molecular parameters and interactions with single molecule sensitivity even in live samples. However, up to now FCS was used mainly in a confocal mode at single points in a sample. With the recent development of fast array detectors and light sheet based illumination, we extended this approach to Imaging FCS, i.e. the recording of FCS measurements at all pixels in an image. Despite the fact that the time resolution of Imaging FCS is lower than for single spot measurements, Imaging FCS has several advantages. The most important being that i) the illumination modes (total internal reflection (TIR) or singe plane illumination microscopy (SPIM)) expose the sample to much lower light doses and allow more measurements per sample, and ii) that it contains much more information since spatial and temporal correlations can be evaluated between any pixels or group of pixels in the image. The combination of spatial and temporal information in the same measurement allows the application of the so-called FCS diffusion law. The FCS diffusion law relates the mobility of molecules on different spatial scales to the underlying structure of the medium in which the diffusion takes place, providing information even below the diffraction limit.

Here we demonstrate the measurement of 2D diffusion coefficient maps on supported lipid bilayers (SLBs), giant unilamellar vesicles (GUVs) and live cells. We investigate the lipid organization of SLBs of different composition and live cells of different lines at different temperatures to extract diffusion coefficients and diffusion activation energies. We demonstrate that SLB organization and phase behaviour can be elucidated even blow the diffraction limit and that the corresponding live cell measurements correlate with their lipid and protein content. Finally we extend these studies to 3D diffusion maps on GUVs and live cells. Our experiments demonstrate that Imaging FCS is a quantitative imaging technique which provides a wide range of information form concentrations, diffusion and transport coefficients, to structural and organizational parameters in an imaging format.

Acknowledgement: This project was supported by a Life Science Institute – Baden Wuerttemberg grant (R-143-000-422-646) and a grant by the Singaporean Ministry of Education (R-154-000-543-112).