

Individual Leaflet Diffusion in Lipid Bilayers Resolved by Scanning FCS with sub 10 nm Axial Discrimination

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Understanding complex cellular processes greatly benefits from the ability to also accurately monitor slowly diffusing species. A versatile tool for studying dynamic processes that arose over the last decade is Fluorescence Correlation Spectroscopy (FCS). However, the method has a drawback when working with slower moving species, such as fluorophore labeled proteins diffusing in cell membranes: The overall measurement time has to be significantly increased to record enough independent diffusion events for good statistical accuracy, which increases the risks of photobleaching and phototoxicity.

Applying the approach called scanning FCS (sFCS) helps in circumventing these issues. In sFCS, the observation volume is scanned rapidly through the sample, which allows simultaneously measuring diffusing fluorophores at multiple locations. Thus, a significantly better statistical accuracy can be achieved within a shorter time. Furthermore, shorter residence times lower the photon dose experienced by the fluorophores, reducing also the risk of photobleaching. The latter being especially important when combining FCS with Stimulated Emission Depletion (STED) measurements. Additionally, sFCS allows determining the observation volume without calibration through spatial correlation.

The results presented here were obtained with a time-resolved fluorescence microscope (MicroTime 200 STED, PicoQuant GmbH) equipped with a galvo scanner (FLIMbee, PicoQuant GmbH). By using z-distance dependent quenching (via Metal Induced Energy Transfer (MIET) [1]) of the label fluorescence lifetime we can separate contributions from the upper and lower lipid leaflets in our FCS analysis, showing that diffusion in the upper leaflet is not affected by the supporting coverslip.

[1] Karedla *et al.* (2014). *ChemPhysChem* 15(4):705–711