ABSOLUTE DIFFUSION COEFFICIENTS IN MEMBRANES MEASURED BY A COMBINATION OF FLUORESCENCE CORRELATION SPECTROSCOPY MODALITIES

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Imperfect focusing resulting in the broadening of the effective PSF in the plane of the membrane is a frequent source of artefacts in molecular diffusion measurements in membranes (live cell membranes as well as artificial model systems such as SLBs). Increasing the robustness of measurements in membranes has motivated the development of several advanced FCS modalities. We have compared 3 of them (line-scan FCS, RICS and imaging FCS) with standard confocal point FCS on diffusion measurements of fluorescent lipid analogues in SLBs and of fluorescent protein analogues in plasma membranes of live plant cells.

When the measurements are performed with near ideal focusing and analysed with correct PSF calibration (where needed), all 4 modalities provide consistent results. Point FCS is the most prone to artefacts due to incorrect focusing. It has, however, the highest temporal resolution (unlimited if using multiple detectors) and can provide the most complete information on fast photo-physical processes or rotational diffusion. The temporal resolution of line-scan FCS is limited by the scanning speed (time per line; 278 μs in our setup). The key advantage of line-scan FCS lies in the robust intrinsic PSF calibration obtained from spatial correlations between different points along the line. RICS is also in principle calibration-free, although it is considerably less robust in this aspect than line-scan FCS. On the other hand, RICS data are collected from a relatively large area, which is a better representation of an inhomogeneous membrane. In conclusion, combination of all the 3 above mentioned confocal FCS modalities provides the most robust and complete picture of the membrane system under investigation, with the ultimate time resolution (point FCS), large acquisition area (RICS) and robust intrinsic PSF calibration (line-scan FCS).

We have compared the confocal modalities with imaging FCS, a camera-based wide-field FCS modality. Imaging FCS offers truly simultaneous FCS mapping in hundreds to thousands points at the cost of time resolution limited by the frame rate of the camera (ms order). Despite the low temporal resolution, the mapping capability and robust FCS calibration make it an interesting alternative for membrane measurements.