IMPROVED LIGHT SHEET FLUORESCENCE CORRELATION SPECTROSCOPY MODELS FOR IN VIVO MEASUREMENTS OF MOLECULAR DYNAMICS

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Imaging based Fluorescence Correlation Spectroscopy through Total Internal Reflection Fluorescence Microscopy (TIRF) and Selective Plane Illumination Microscopy (SPIM) have been successfully used to measure diffusion coefficients of molecules over space in 2D and 3D environments, respectively. However, the parallel detection of contiguous pixels on a camera and the constant illumination intensity in planes parallel to the focal plane, lead to cross-talk due to single molecules being detected by multiple pixels. This cross-talk further increases if the depth of field of the detection objective is smaller than the illumination profile thickness. Therefore, in TIRF-FCS it represents a minor problem. But in SPIM-FCS the cross-talk can lead to a diffusion coefficient bias of up to 30% in autocorrelation analysis and leads to improper fits in spatial cross-correlation functions.

However, the inclusion of cross-talk into the model derivations for imaging FCS does not lead to an analytic solution for FCS models and so we introduce here a GPU-supported numerical integration approach that can be used for data fitting with different point spread function models.

As a proof of principle, we first demonstrate the amount of cross-talk expected and the errors introduced in the determination of diffusion coefficients in correlation analysis of simulated 2D and 3D diffusion in TIRF and SPIM systems. We then demonstrate that we can obtain unbiased results using the new fitting approach. The model is then tested with free diffusing fluorescent dyes and beads in solution and compared to the diffusion coefficient values obtained by a confocal FCS system. Finally, this model is used to quantify the diffusion of fluorescently tagged signaling molecules in the extracellular space of live zebrafish embryos in a distance dependent manner.