# The imaging FCS diffusion law in the presence of multiple diffusive modes

Sapthaswaran Veerapathiran[1], Thorsten Wohland[1, 2]

[1]Department of Biological Sciences and NUS Centre for Bio-Imaging Sciences, National University of Singapore, 14 Science Drive 4, 117557 Singapore

[2]Department of Chemistry, National University of Singapore, 3 Science Drive 3, 117543 Singapore

Email: sapthaswaran@u.nus.edu, twohland@nus.edu.sg

**Key Words:** Fluorescence Correlation Spectroscopy, Imaging FCS, Diffusion Law, Free diffusion, Hop diffusion, Domain confined diffusion

## Abstract

The plasma membrane acts a barrier over which cells exchange materials. It also regulates key cellular functions such as cell signal transduction. Understanding plasma membrane organization and dynamics is therefore central for the understanding of cellular functions. The plasma membrane is a heterogeneous matrix, with the presence of lipid domains, protein domains and cytoskeleton meshwork. These domains and meshwork act as barriers for free diffusion of the membrane components. However, these domains and the meshwork barriers are below the optical diffraction limit and are not easily detected even in super-resolution microscopy. Therefore, dynamic measurements are often used to indirectly prove the existence of domains and barriers by analyzing the mode of diffusion of probe molecules. One of these tools is the Fluorescence Correlation Spectroscopy (FCS) diffusion law. The FCS Diffusion law, a plot of diffusion time versus observation area, helped identify at least three different diffusion modes of plasma membrane components – free diffusion, domain confined diffusion, and meshwork hindered hop diffusion. It characterizes these diffusion modes by analyzing the plot's y-intercept ($\tau_0$). However, a description of the $\tau_0$ has only been given for pure diffusive modes, and in many experimental cases, it is not evident that a protein will undergo only one kind of diffusion. Here, we investigate how the diffusion law's $\tau_0$ value changes, in the case of mixed or convoluted diffusive modes. Our experimental results supported by simulations, show that absolute $\tau_0$ values are weighted averages depending on the various diffusion modes contributing to the protein mobility with, in general, domain diffusion having a stronger contribution than hop diffusion in the biological relevant parameter range. By contrast, the relative changes of $\tau_0$ values upon disruption of a diffusion mode, typically by targeted drug treatment, are a direct indicator of the diffusion modes a protein can undergo. These findings support earlier results on epidermal growth factor receptor (EGFR) measurements (Bag et al. Biophys. J. (2015), 109(9) 1925-1936) a protein which shows domain confinement and hop diffusion due to interactions with the cytoskeleton, but which shows under all circumstances a positive $\tau_0$ value. We also show that two probes, which show different diffusion modes, can be distinguished in a single autocorrelation function, and the diffusion law can be reconstructed separately for the two components.