Fluorescence lifetime and polarization of environmentally-sensitive dyes for multiparameter analysis of lipid bilayers

Alix Le Marois[1], Yurema Teijeiro-Gonzalez[1], Dylan Owen[1], Rainer Heintzmann[2,3], Klaus Suhling[1]

[1]Department of Physics, Strand, King's College London, London, United Kingdom; [2]Institute of Physical Chemistry, Abbe Center of Photonics, Friedrich Schiller University Jena, Jena, Germany; [3]Leibniz Institute of Photonic Technology, Jena, Germany.

Email: alix.le_marois@kcl.ac.uk

Key Words: Membrane biophysics, Fluorescence Lifetime Imaging, Fluorescence Anisotropy, Image Analysis.

The cell membrane is an interface at which many vital processes take place. In particular, the existence of nanoscale, cholesterol-enriched lipid domains of higher order and decreased polarity remains of capital interest, though their characterisation further than the "liquid ordered/liquid disordered" phase partition concept remains difficult [1]. We seek finer understanding of the impact of the chemical nature of the lipid bilayer on membrane order, fluidity and polarity. For this, we measure these parameters independently in a single measurement, by combining time-domain fluorescence lifetime and polarization in a multi-channel confocal-FLIM microscope [2]. Artificial bilayers and live cells are stained with environmentally-sensitive membrane dyes such as laurdan and di-4-ANEPPDHQ, chosen for the sensitivity of their fluorescence lifetime to membrane polarity. Membrane order parameters are probed simultaneously by measuring time-resolved fluorescence anisotropy (TR-FAIM). The lateral diffusion of molecules is also measured using simultaneous photobleaching (FRAP) experiments [3]. We have developed novel global analysis techniques for low-intensity FLIM and TR-FAIM image data using Principal Component Analysis with a noise correction specific to photon counting experiments (NC-PCA), allowing us to resolve several distinct microenvironments with limited photon capital [4]. Our experiments show that membrane polarity and molecular rotational diffusion are correlated and sensitive to lipid composition and temperature; however, membrane rigidity, i.e. the range of freedom for molecular rotation, appears to be predominantly phase-dependent. These results show that our method offers a promising tool to obtain precise understanding of the multi-scale molecular dynamics of lipid bilayers. We hope to apply this method to identify the chemical and physical modifications of the membrane during physiological processes in cells, such as cell division, signaling, and apoptosis.

[1] D. M. Owen, D. J. Williamson, A. Magenau, and K. Gaus, "Sub-resolution lipid domains exist in the plasma membrane and regulate protein diffusion and distribution," *Nat Commun*, vol. **3**, p. 1256, (2012).

[2] A. Le Marois, D. M. Owen & K. Suhling; "Investigating cell membrane structure and dynamics with TCSPC-FLIM". *Proc. SPIE 9329, Multiphoton Microscopy in the Biomedical Sciences XV*, **932938** (2015)

[3] J. A. Levitt *et al.*, "Simultaneous FRAP, FLIM and FAIM for measurements of protein mobility and interaction in living cells," *Biomed Opt Express*, vol. **6,** no. **10**, pp. 3842–3854, (2015).

[4] A. Le Marois, S. Labouesse, K. Suhling, and R. Heintzmann, "Noise-Corrected Principal Component Analysis of fluorescence lifetime imaging data," *J. Biophoton.*, in press (2016).