

Time-Correlated Single Photon Counting-based FLIM, FRAP and FAIM to investigate intracellular dynamics and their environments

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It is well known that fluorescence microscopy techniques are of great value in biological research due to its applicability on the extraction of numerous features of cells and their structure. It is of special interest obtaining as much information as possible in a single experiment in order to decrease possible damage of the biological sample and increase the efficiency of the limited photon budget before the fluorophore is irreversibly bleached, which means using different techniques simultaneously [1].

Different Time-Correlated Single Photon Counting (TCSPC) techniques are used and being set up for the moment in our laboratory in order to fulfil this purpose. These techniques are: Time-Resolved Fluorescence Lifetime Imaging (FLIM), Time-Resolved Fluorescence Anisotropy Imaging (tr-FAIM), and Fluorescence Recovery After Photobleaching (FRAP), which are all carried out simultaneously. The last two techniques give information about polarization, monitoring the rotation and translation of the fluorophore and the molecule it attaches to, respectively [2]. We will report on the latest progress with this approach.

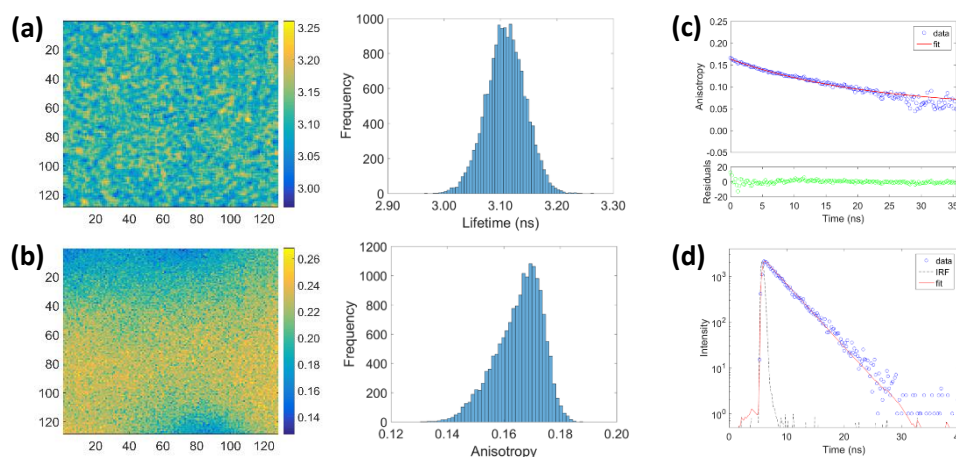


Fig. 1 Representative data set measuring R6G in a glycerol-methanol mixture. (a) FLIM image and histogram. (b) Steady-state fluorescence anisotropy image and histogram. (c) Time-resolved fluorescence anisotropy decay from the entire image. (d) Representative fluorescence decay and fitting from a 3x3 pixel region.

[1] J.A. Levitt, P.E. Morton G.O. Fruhwirth, G. Santis, P.-H. Chung, M. Parsons and **K. Suhling**. Simultaneous FRAP, FLIM and FAIM for measurements of protein mobility and interaction in living cells. *Biomed Opt Express* 6(10) 3842-3854, 2015.

[2] Becker, W. (2015). *Advanced Time-Correlated Single Photon Counting Applications*, Springer Series in Chemical Physics 111, Springer (Berlin, Heidelberg).