

COMBINING LIFETIME AND SPECTRAL INFORMATION TO QUANTIFY FRET

Simon Schlachter¹, Alessandro Esposito¹, Gabriele S. Kaminski Schierle¹, and Clemens F. Kaminski¹

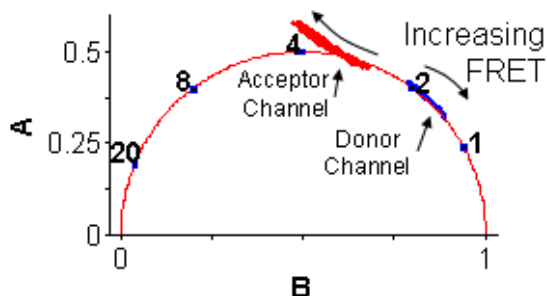
1) Laser Analytics Group, Department of Chemical Engineering and Biotechnology, University of Cambridge, New Museums Site, Pembroke Street, CB2 3RA, Cambridge, (UK)

E-mail: ss678@cam.ac.uk

KEY WORDS: multi-dimensional imaging, supercontinuum laser, confocal microscopy, Fluorescence Lifetime Imaging Microscopy, Förster Resonance Energy Transfer.

ABSTRACT: Förster Resonance Energy Transfer (FRET) is a widely used technique in the biological sciences. It allows inter-molecular distances, protein-protein interactions, concentrations and many other biochemical quantities to be measured.

Among the simplest and most accurate methods to quantify FRET is Fluorescence Lifetime Imaging Microscopy (FLIM), particularly by Time Correlated Single Photon Counting (TCSPC). Through careful selection of the emission filter and excitation wavelength one aims to ensure that only fluorescence from donor molecules is measured. The FRET efficiency is then estimated from a measurement of the donor fluorescence lifetime reduction caused by energy transfer to the acceptor chromophore.



Often, the fraction of donor and acceptor molecules engaging in FRET is the important quantity from a biological point of view; however, typical FLIM methods provide limited information in this respect. Sensitized emission

FRET (seFRET), on the other hand, can quantify the interacting fractions for both the donor and the acceptor populations. However, seFRET requires use of several calibration samples to provide accurate estimates and is much more complex to perform in practice.

Here we propose a new method that combines the advantages of TCSPC and seFRET through measurement of both the donor and the acceptor fluorescence lifetimes. By collecting additional acceptor-channel information and applying global analysis algorithms, we show how both FRET efficiency and information on the interacting fractions of donors and acceptors can be retrieved with a minimal number of calibration samples.

References:

A. Esposito et al., "FRET Imaging of Hemoglobin Concentration in Plasmodium falciparum-Infected Red Blood Cells," PLoS ONE 3(11), e3780 (2008).

A. D. Elder et al., "A quantitative protocol for dynamic measurements of protein interactions by Förster resonance energy transfer-sensitized fluorescence emission," J. Roy. Soc. Interface 6(Suppl 1), S59–S81 (2009).