FREQUENCY DOMAIN LIFETIME IMAGING AT HIGH SPEED AND ACCURACY

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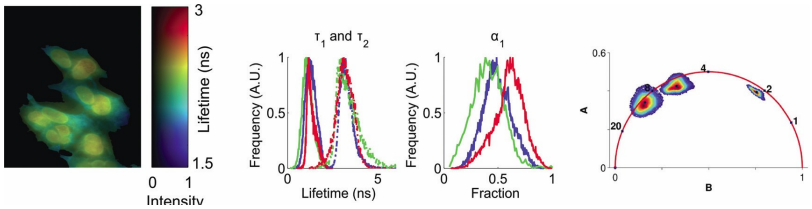
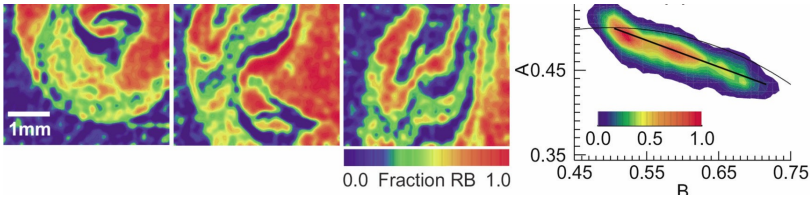
ABSTRACT: Frequency domain Fluorescence Lifetime Imaging Microscopy (FD-FLIM) is a popular technique to quantify the molecular environment of a fluorophore in biological applications. There are drawbacks, however. The photon efficiency of FD FLIM has generally been considered worse than that of time correlated single photon counting (TCSPC) FLIM. Furthermore, most implementations of FD FLIM are restricted to the measurement of single exponential fluorescence decays (because they are sampled at a single frequency). Further problems arise from detector imperfections and sub-optimal excitation waveforms, leading to the problem of frequency aliasing. In practice, one is therefore required to perform significant oversampling of the signal. Typically one samples over 8 or more phase steps although the Nyquist criterion prescribes that >=2 phase steps should suffice for sinusoidal signals. The prevention of frequency aliasing thus comes at a cost of measurement speed, and possible overexposure of the specimen to light. We present here a new type of FD FLIM system that addresses these shortcomings. In particular the system permits operation in two distinct modes that are optimised 1) for measurement precision so that multiple lifetime components can be reliably recovered from the sample and 2) for measurement speed, so that high fidelity lifetime data can be obtained at frame rates exceeding several Hz. The instrument relies on two novel techniques developed in our group: multi-harmonic FLIM (**mhFLIM**) [1] and **φ²FLIM** [2]. Several applications will be presented, including heterogenous lifetime decay measurements from biological samples (Fig. A) and rapid imaging fluid mixing phenomena (Fig. B). Novel data analysis and representation schemes will be described.

(A)



(B)



References:

1. A. D. Elder, et al. "A new technique for alias-free frequency-domain fluorescence lifetime imaging measurements," submitted.
2. S. Schlachter, et al. "mhFLIM: Resolution of heterogeneous fluorescence decays in widefield lifetime microscopy," Optics Express. submitted.