HIGH-SPEED WIDE-FIELD FLIM APPLIED TO NIPKOW DISC MICROSCOPY

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Fluorescence lifetime imaging (FLIM) can contrast different molecular species and is a favoured approach for detecting Fluorescence Resonance Energy Transfer (FRET), since it avoids many of the artefacts associated with intensity based measurements. Optically sectioned fluorescence imaging is highly desirable for quantitative analysis and is usually achieved by using laser scanning confocal microscopy (LSCM) or multiphoton microscopy, for which FLIM is readily implemented using time-correlated single-photon counting (TCSPC) techniques. Unfortunately, the requirement for single photon detection and sequential pixel acquisition imposed by TCSPC typically results in acquisition times of several minutes, often making it unsuitable for live cell imaging and high-throughput applications. We report the application of high-speed (up to ~ 15 fps) wide-field time gated FLIM to a Nipkow disc-based optically sectioning microscope (Olympus IX-81, with Yokogawa Electrical Corporation CSU10) exploiting a compact electronically tuneable ultrafast excitation source based on supercontinuum generation (Fianium Ltd, SC-450) and a frequency-doubled Ti:Sapphire laser (Spectra-Physics, Tsunami).

![Diagram](image)

\textbf{Fig. 1:} (a) Nipkow microscope set-up. MF-micro-structured fibre, M-mirror, P-prism, SMF-single mode fibre, ML-micro-lenses, PH-pinhole, D-dichroic, F-filter. (b) Fluorescence lifetime variation for GFP expressing COS cells, acquired on the Nipkow and a confocal TCSPC system, for 15, 10, 5 and 1s acquisition times.

We have characterised the S/N characteristics of our time-gated detector (HRI, Kentech Instruments Ltd) and compared the optically sectioned FLIM performance to that of a LSCM with TCSPC capabilities for live cell imaging. The Nipkow disc based system is significantly faster to achieve a comparable S/N, providing useful FLIM images for acquisition times on the order of ~1s. We are currently applying this system for “rapid” time-lapse studies of protein interactions and membrane dynamics and are developing instrumentation for rapid time-domain FLIM of multiwell plate sample arrays for screening and other high-throughput applications. We will report on initial results, including observation of changes in membrane lipid order following cholesterol depletion.