

FLUORESCENCE LIFETIME IMAGING WITH A WHITE-LIGHT SUPERCONTINUUM SOURCE

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Fluorescence lifetime imaging microscopy (FLIM) is a burgeoning technique for exploiting intensity independent contrast. In particular these techniques enable the quantitative determination of protein-protein interactions via measurement of the fluorescence resonance energy transfer (FRET) between donor and acceptor fluorescently tagged proteins.

To perform time correlated single photon counting (TCSPC) FLIM, a pulsed excitation source is required whose excitation wavelength should ideally be well-matched to the absorption wavelength(s) of the sample. Currently, the preferred options are either pulsed laser diodes or via multi-photon excitation. In the former case, only a very limited number of discrete, fixed wavelengths are available. Although multi-photon excitation inherently provides optical sectioning capability, the lower excitation efficiency, limited tuning range (particularly the 1000-1200 nm range) and simultaneous excitation of multiple fluorescent molecules are limitations. Optical parametric oscillators have been used to perform both single and multi-photon excited TCSPC FLIM, however, these sources are complex and difficult to operate.

We report a novel approach to overcome these limitations using a white-light supercontinuum source based upon photonic crystal fibre (PCF) technology. This simple and stable broadband light source comprised of a short section of PCF pumped by a fs-pulsed Ti:Sapphire laser. The resulting output was wavelength-selectively filtered for optimal excitation of the sample. Using a laser scanning microscope equipped with a TCSPC module (Becker-Hickl SPC830), fluorescence lifetime data was recorded. This method enables both single- and multi-photon TCSPC FLIM using the same Ti:Sapphire platform source. The wide range of accessible wavelengths broadens the choice of fluorescent molecules that can be investigated using this powerful technique.

We will present lifetime measurements and FLIM images from a range of samples. Furthermore, we will discuss the potential benefit of this wavelength flexible source for the single-photon, separate excitation of certain acceptor and donor molecule pairs for quantified FRET, currently not possible using existing multi-photon methodologies.

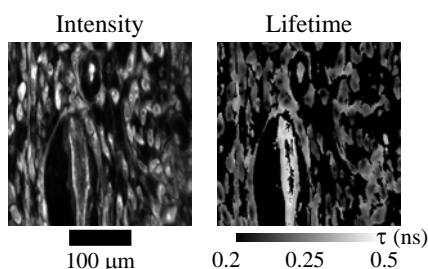


Figure 1: Intensity and TPSPC FLIM images of a mouse fibrosarcoma stained with haematoxylin and eosin obtained using $\lambda=540 \pm 10$ nm radiation spectrally filtered from a white-light supercontinuum.