Video Rate Fluorescence Lifetime Imaging and Structured Illumination using a Blue LED

D. S. Elson, V. Poher, C. Dunsby, J. Requejo-Isidro, P. M. W. French and M. A. A. Neil

Imperial College London, London, U.K, SW7 2AZ; email: ds.elson@imperial.ac.uk

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Fluorescence lifetime imaging (FLIM) usually involves ultrafast laser technology or cw laser sources modulated extra-cavity using optical modulators. Using LEDs as light sources in microscopy is potentially a very flexible technique given the increasing average output powers, good availability, low cost and visible spectrum coverage.

We present FLIM in the frequency-domain using a Luxeon blue LED and a microchannel plate optical image intensifier (MCP). The LED is directly modulated at 40 MHz using a sinusoidal output from a signal generator, and the same signal is used to modulate the gain on the MCP. Three images were read out on a CCD camera at different detector phases and FLIM maps were calculated both from the demodulation and phase shift of the fluorescence relative to the excitation. Images were acquired at video-rate in a macroscopic multi-well plate fluorescence imager, and we have applied LED FLIM to microscopy.

![Fluorescence images](image1)

Fig. 1: (a) fluorescence intensity image and (b), (c) FLIM maps of Eosin and Coumarin in cuvettes calculated from (b) phase shift and (c) demodulation of the fluorescence relative to the excitation.

FLIM results will also be presented of samples in an inverted microscope using the LED as the light source. We have also used structured illumination [2] with LEDs to optically section fluorescence images and are currently working towards combining structured illumination and FLIM