

# Fluorescence Lifetime Imaging Using Low-Cost Light emitting Diodes



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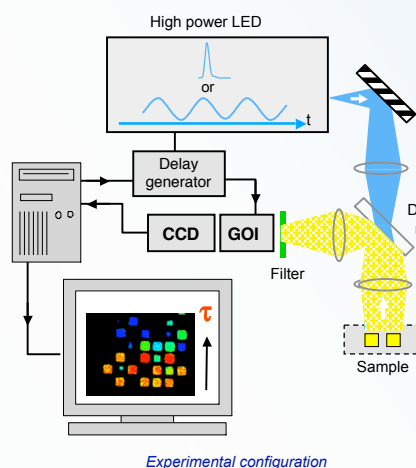
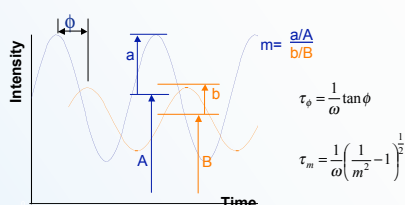


## Introduction

Fluorescence lifetime imaging (FLIM) is an important imaging modality that offers a means of contrast for different molecules and environments. One barrier to this technology is the expensive and complex laser systems that are required for the sample excitation. Recent developments in high brightness light emitting diodes (LEDs) for lighting and illumination applications have resulted in the widespread commercial availability of cheap, high-power LEDs covering a broad spectral range. We have incorporated these LEDs into wide-field FLIM systems. Both time-domain and frequency-domain FLIM have been realised using a variety of LED devices spanning the wavelength range 450 nm–640 nm.

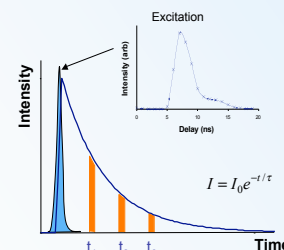
## Frequency Domain FLIM

For frequency domain FLIM the LEDs and the optical intensifier were modulated sinusoidally at a frequency of 83.3MHz and the phase between the excitation and the optical intensifier modulation was varied (homodyne detection). The fluorescence lifetime was calculated from either the phase delay or the demodulation of the fluorescence with respect to the excitation



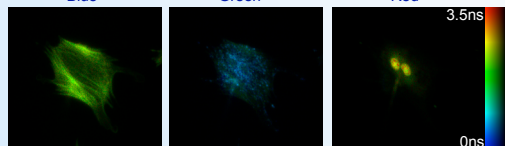
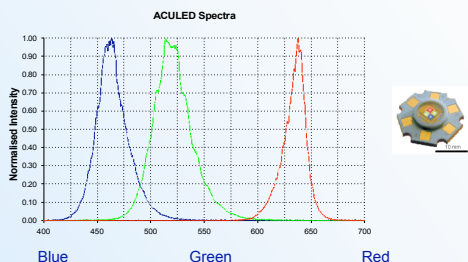
## Time-Domain FLIM

For time domain FLIM Luxeon® LEDs were excited using a pulse driver developed by Kentech Instruments Ltd. This driver has an output impedance of 12 Ohms, producing 10 amps peak current with a FWHM response of 1 ns. Optical output pulses having durations of < 4ns at a centre wavelength of 470 nm were obtained.

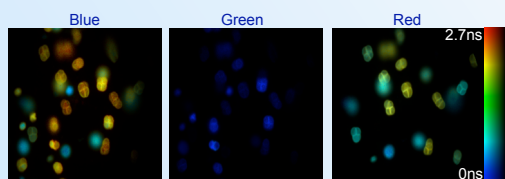


## Multi-Wavelength FLIM

We used a novel LED designed for variable colour lighting applications as a source for multi wavelength frequency domain. The ACULED™ incorporates four high brightness diodes (one blue, one red and two green) spanning the wavelength range 425nm – 670nm. This system offers the potential for fast switching multi-wavelength FLIM.

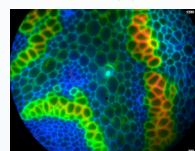
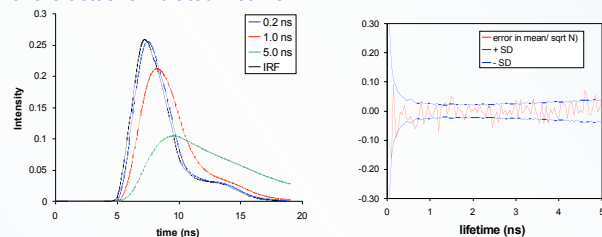


X60 merged fluorescence lifetime images of a fixed, permeabilized, and labeled muntjac skin fibroblast. F-actin was labeled with green-fluorescent Alexa Fluor 488 phalloidin. Mitochondria were labeled with anti-OxPhos Complex V inhibitor protein mouse IgG1 and visualized using orange-fluorescent Alexa Fluor 555 goat anti-mouse IgG, and the nucleus was stained with TO-PRO-3 iodide.

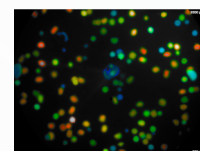


Merged FLIM image of stained pollen grains

Images of the fluorescence decay were taken using 1ns wide gates separated by 500ps. FLIM images were calculated by deconvolving the recorded instrumental response function (IRF) from the fluorescence profiles and using a non-linear least-squares fitting algorithm. Because the IRF was significantly longer using the LED than a pulsed laser source, we have characterised our fitting algorithm to assess its performance. Our simulations show that better than 5% accuracy can be obtained for lifetimes greater than 350ps and better than 10% accuracy for lifetimes greater than 150ps. Furthermore, the procedure provides an unbiased estimate of the actual simulated lifetime.



Stained convallaria  $\tau = 200\text{ps} - 1200\text{ps}$   
X20 magnification



Stained convallaria  $\tau = 600\text{ps} - 2000\text{ps}$   
X20 magnification

## Conclusions

We have demonstrated wide field fluorescence lifetime imaging using high brightness LEDs in both time and frequency domain configurations. We have also combined frequency domain FLIM with structured illumination to yield optically sectioned FLIM images. The incoherent output and flat illumination field from the LEDs enable high quality speckle free imaging and the broad range of available wavelengths makes for a cost effective alternative to supercontinuum generation for multiple-wavelength fluorescence lifetime imaging.