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2-D fluorescence lifetime imaging using a time-gated image intensifier

K. Dowling [a], S.C.W. Hyde [a], J.C. Dainty [a], P.M.W. French [a,1], J.D. Hares [b]

[a] *Femtosecond Optics Group, Physics Department, Imperial College, London SW7 2BZ, UK*
[b] *Kentech Instruments Ltd., Unit 9, Hall Farm Workshops, South Moreton, Didcot, Oxon, OX11 9AG, UK*

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Abstract

We report a 2-D fluorescence lifetime imaging system based on a time-gated image intensifier and a Cr:LiSAF regenerative amplifier. We have demonstrated 185 ps temporal resolution. The deleterious effects of optical scattering are demonstrated.

In recent years optical imaging in the visible/near infrared spectral region has been investigated as an alternative to traditional medical diagnostic techniques. Optical imaging, however, is limited by the fact that this light is highly scattered by biological tissue, which impacts the ability to detect variations in the absorption and scattering properties of the tissue under investigation. A method of increasing the contrast between tissue types is to use fluorescent marker dyes (fluorophores). Fluorophores, which have a "signature" emission wavelength, can be designed such that they are selectively absorbed in a specific area of tissue that is under investigation. This property is widely used in medical diagnostic techniques as a method of detecting the presence of a particular tissue type (e.g. cancerous tissue) by detecting the emission wavelength of the fluorophore (which will not be present if the tissue of interest is not present). This spectroscopic technique can be combined with optical imaging techniques to produce a "map" of the localisation of the fluorophore. However, traditional fluorescence imaging techniques, which rely on quantitative intensity measurements, become increasingly difficult when imaging into greater tissue depths, due to optical scattering. An alternative method is fluorescence lifetime imaging (FLIM) where the lifetime of the fluorescence signal, rather than its intensity, is measured. The fluorescence lifetime is another signature of the fluorophore which is only weakly affected by the increased average photon propagation times due to optical scattering in tissue, making it possible to image into greater tissue depths. Imaging using fluorescence lifetimes may also provide functional data about the tissue being probed since the lifetime of a fluorophore can be a function of its chemical environment, (e.g. Ca^{2+} concentration, pH) [1].

One technique for the measurement of fluorescence lifetime is to illuminate a sample with a modulated continuous wave laser and determine the fluorescence lifetime from the phase change between the excitation and measured modulation [2]. An alternative technique, which is used in the work presented in this paper, uses a short pulse of light to excite the fluorophore. The intensity of the fluorescence is then measured as a function of time. The influence of scattering on the resultant intensity-time profile is relatively weak since only the relative intensities at different time delays are required. Previously this technique has only been demonstrated with sufficient temporal resolution to measure lifetimes of a few nanoseconds [3–5]. It has been suggested, however, that the use of fluorophores with such long fluorescence decay lifetimes

[1] E-mail: paul.french@ic.ac.uk.

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will degrade FLIM images in thick tissue due to the diffusion of fluorophore away from the region of interest [6]. Short lifetime fluorophores allow improved FLIM measurements through temporal separation of fluorescence from different regions of fluorophore. We report a fluorescence lifetime imaging system based on a time-gated intensifier which offers a much faster temporal response than previously demonstrated time domain systems. The apparatus described below simultaneously measures the fluorescence lifetimes at all pixels in the field of view and is capable of collecting the data for a FLIM image in under one second. The experimental configuration is shown in Fig. 1.

The fluorophores used to test the fluorescence imaging system were solutions of the laser dyes Coumarin 314 and DASPI, with fluorescence lifetimes of 1.2 ns and 150 ps respectively (measured with a Hamamatsu OOS-01 sampling optical oscilloscope). Each dye solution was contained in a 1 mm diameter glass capillary. The samples were illuminated with ~ 10 ps pulses of 0.25 μJ energy, at 415 nm, at a repetition rate of 5 kHz. These pulses were generated by amplifying 100 fs pulses from a commercial femtosecond Ti:sapphire laser (Spectra-Physics Tsunami) in a Cr:LiSAF regenerative amplifier and then frequency doubling to the blue. It was necessary to frequency double the amplified pulses to excite appropriate, visible emitting, fluorophores in order to achieve maximum sensitivity at the intensifier photocathode. However, for clinical applications, where it would be appropriate to use near infra-red (NIR) light because of its lower absorption in tissue, an alternative photocathode could be used. This laser/amplifier system has the potential to be diode-pumped, as reported in Ref. [7], and therefore compact and portable.

The gated image intensifier (Kentech Gated Optical Imager) has a gate width of 120 ps, and was operated at 5 kHz. The jitter of the triggering and the delay units degraded the system response to ~ 185 ps, but it should be possible to reduce this to less than 100 ps by optical triggering and decreasing the intensifier gate width to its minimum of 80 ps (with reduced sensitivity).

The trigger signal was swept through a range of delays using an electronic delay generator (Stanford Research Systems DG535) and a snapshot of the output of the time-gated image intensifier was taken at each time delay using a standard CCD camera (Pulnix PE 500). Combining these frames produced an intensity-time profile of the fluorescence decay for each pixel in the field of view. For each of these intensity-time profiles, a nonlinear least squares fit to a mono-exponential decay was made, yielding a fluorescence lifetime value for each pixel in the field of view. The spatial distribution of these calculated average lifetimes forms the FLIM lifetime map.

To demonstrate the fluorescence lifetime imaging apparatus we prepared the phantom shown in Fig. 2(a). This phantom consisted of two dye filled capillaries, separated by 1 mm, which were placed in a larger dye cell (10 mm by 10 mm) which could contain a scattering solution. The excitation light passed through the DASPI sample and any that was not absorbed illuminated the Coumarin 314 sample. Fig. 2(b) shows a three-dimensional plot of the resultant FLIM lifetime map.

To investigate the effects of scattering on the FLIM lifetime maps, the same phantom was used but the large dye cell was filled with a scattering solution. This consisted of 0.46 μm diameter polystyrene spheres suspended in water. The concentration of the polystyrene spheres was

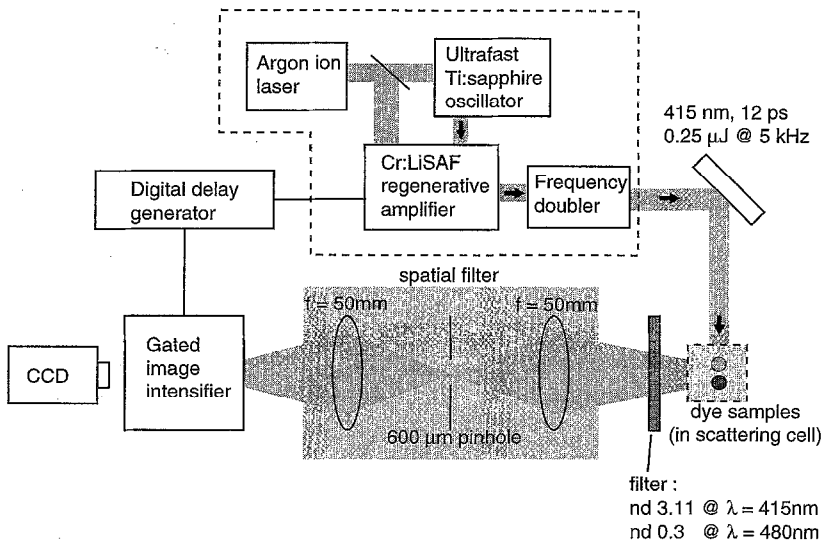

Fig. 1. Experimental set-up for recording of fluorescence lifetime maps.

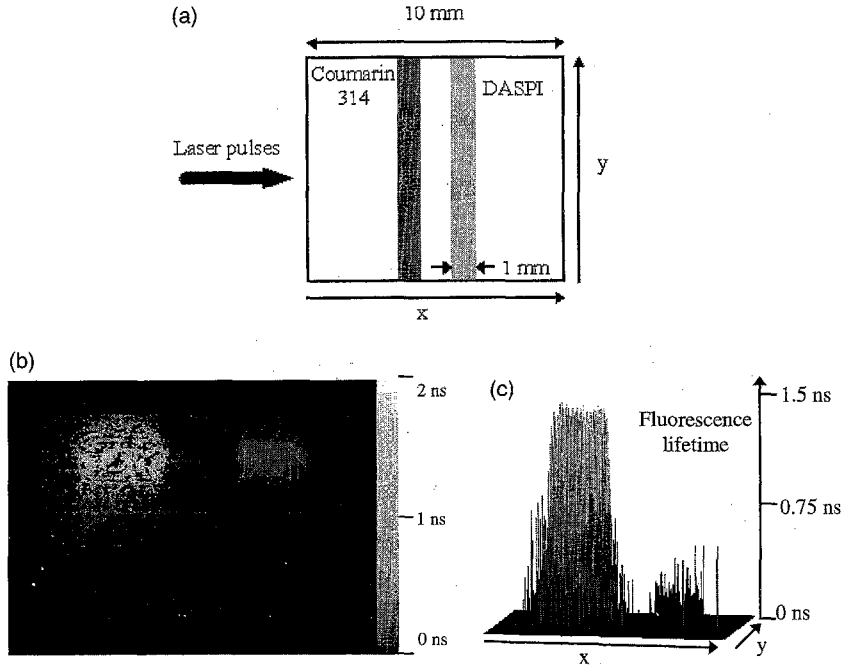

Fig. 2. (a) Schematic diagram of the phantom used to test the fluorescence lifetime imaging apparatus, (b) FLIM lifetime map of the phantom, (c) three-dimensional rendering of the FLIM lifetime map.

varied to achieve scattering thicknesses of 4.9, 7.2 and 9.7 mean free paths. The resultant FLIM lifetime maps are shown in Figs. 3(a)–(d). The FLIM lifetime maps obtained show how the increase in the depth of scattering medium degrades localisation of the fluorophore. For all of these scattering solutions, the measured lifetime remained con-

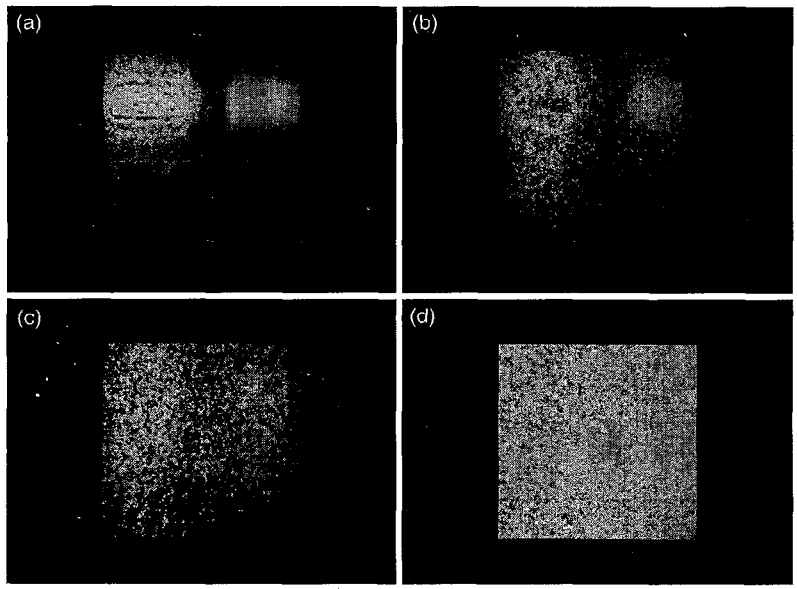

Fig. 3. FLIM lifetime maps of Coumarin 314 and DASPI dyes, viewed (a) through no scattering medium, (b), (c), (d) through 4.9 mfps, 7.2 mfps and 9.7 mfps of scattering medium respectively.

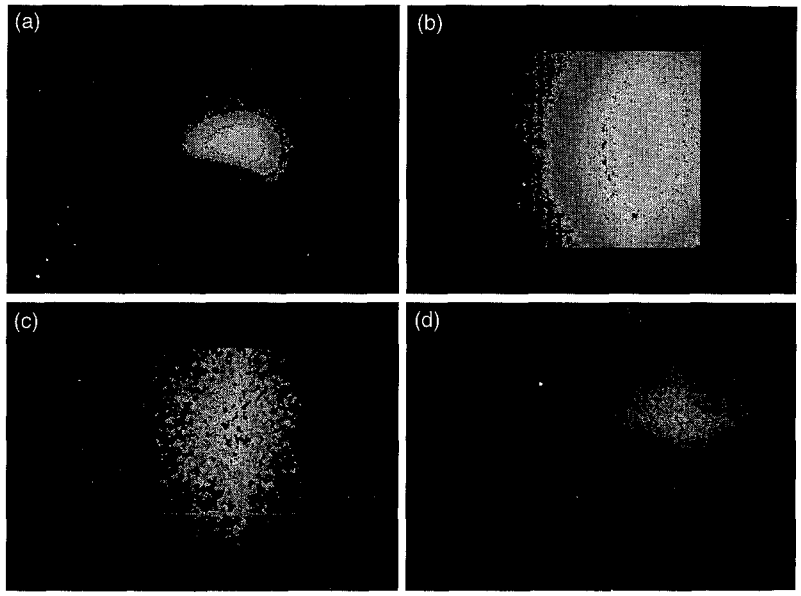

Fig. 4. FLIM lifetime maps of the edge of the Coumarin 314 dye capillary. Viewed through (a) no spatial filter with no scattering medium, (b) no spatial filter and 7.2 mfps of scattering medium, (c) a spatial filter with a 600 μm pinhole and 7.2 mfps of scattering medium and (d) a spatial filter with a 100 μm pinhole and 7.2 mfps of scattering medium.

stant within our experimental resolution. In a clinical environment this would mean that, although information about the position of the fluorophore would have had been lost, functional information could still be derived.

The degradation of images with increasing scattering depth is similar to that encountered when using optical imaging techniques based on absorption. A simple step to reduce the image degradation due to scattered light, used in some absorption imaging techniques, is to introduce a spatial filter. FLIM images of the edge of a single capillary tube containing Coumarin 314 were recorded to investigate how the effect of scattering on the degradation of localisation could be reduced using a spatial filter. 100 and 600 μm diameter pinholes were used in the experimental set-up shown in Fig. 1. The results of these experiments are shown in Figs. 4(a)–(d). These results show that the spatial resolution of the FLIM lifetime maps improved as the size of the pinhole in the spatial filter is reduced. This is only a first step to improving the image quality. A more sophisticated approach would be to incorporate an inverse scattering algorithm, such as described in Ref. [8].

In this paper we have demonstrated a FLIM instrument with a temporal resolution of 185 ps, with the potential, using optical triggering, to reduce this figure to below 100 ps. The instrument requires no pixel by pixel scanning and can run at up to 10 kHz frame rate. The required microjoule pulse energy is within the range of the diode pumped oscillator-amplifier system described in Ref. [7], suggesting that the apparatus described here could be adapted to a relatively low-cost compact, portable system. The current data acquisition time can be as short as 1 s, thus providing near real-time FLIM imaging. We have demonstrated that the localisation of the fluorescence image is greatly degraded by even modest scattering, although lifetimes can still be determined, allowing the determination of functional information. This degradation is significantly reduced, however, by spatial filtering. In future work we hope to improve upon the system described here by using fluorophores that emit in the NIR and by optimising the detection system for this wavelength region, thereby increasing the tissue penetration depth. We aim to apply inverse scattering techniques to the FLIM lifetime maps to further improve the localisation of the fluorophores.

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