Two-Dimensional Fluorescence Lifetime Imaging using a 5 khz/110 ps Gated Image Intensifier

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ABSTRACT

We report the demonstration of a high temporal resolution fluorescence lifetime imaging (FLIM) system using a time-gated image intensifier to provide whole field FLIM images. The gate width has been optimised to 110 ps, and changes in the environment of a fluorescent phantom, causing lifetime differences of 20 ps, have been detected. Environmental changes of the fluorescent indicator, Lucifer Yellow, have been sensed by measuring changes in its fluorescence lifetime when unbound and when bound to the protein albumin.

Keywords: fluorescence lifetime imaging, medical imaging, functional imaging, all solid state lasers, gated image intensifier

1. FLUORESCENCE LIFETIME IMAGING

In recent years optical imaging in the visible/near infrared spectral region has been investigated as an alternative to traditional medical diagnostic techniques. However, optical imaging is limited by the high scattering cross section of biological tissue, which impacts the ability to detect variations in the optical properties of the tissue under investigation. A method of increasing the contrast between tissue types is to use fluorescent marker dyes (fluorophores). Fluorophores can be designed such that they are selectively absorbed in the specific area of tissue under investigation. In medical diagnostic techniques the presence of a particular tissue type (e.g. cancerous tissue) can be established by detecting the emission-wavelength signature, 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 and hence a map of the tissue under investigation. However, traditional fluorescence imaging techniques, which rely on quantitative intensity measurements, become increasingly difficult when imaging into greater tissue depths, due to scattering. An alternative method is fluorescence lifetime imaging (FLIM) where the lifetime of the fluorescence signal, rather than its intensity, is measured. Fluorescence lifetime is a signature of a fluorophore which is only weakly affected by the increase in photon propagation times due to scattering in tissue, making it possible for measurements to be made through greater tissue depths.

In biochemical applications of fluorescence imaging the effect of environment on the process of fluorescence is used to map chemical or physical changes within a sample. The quantum efficiency of fluorescence is a function of the radiative and non-radiative decay rates. The radiative decay rate is considered constant for a given fluorophore, while the non-radiative decay rate can vary with environment. Unfortunately the quantum efficiency is not easy to determine as it is difficult to measure the exact quantity of fluorophore in a particular region, and to quantify how much pump-light is absorbed. However, fluorescence lifetime is also a function of fluorophore environment.[1] Since determination of fluorescence lifetime requires only relative intensity measurements, knowledge of the fluorophore concentration or excitation flux in the sample is no longer required. Thus imaging using fluorescence lifetimes may provide functional data about a tissue, sample under investigation. Fluorescence Lifetime probes already exist for the measurement of e.g. Ca^{2+} concentration, oxygen concentration and pH. Non-biomedical applications also exist for FLIM, such as determination of impurities in metal samples for nuclear process control,[2] and in combustion related studies.[3]

2. MEASUREMENT OF FLUORESCENCE LIFETIME

Fluorescence lifetime may be measured in the frequency domain or the time domain. The most common technique is in the frequency domain where the sample is illuminated with a sinusoidally modulated continuous-wave laser and the fluorescence lifetime determined from the phase change between the excitation and measured fluorescence modulation.[4] The work presented in this paper is in the time domain, where a short pulse of light is used to excite the fluorophore and the intensity of the fluorescence is then measured as a function of time. Previously this technique has used detectors which have only been demonstrated with temporal resolutions of a few nanoseconds.[5,6,7] We report a fluorescence lifetime imaging system based on a time-gated intensifier which allows simultaneous measurement of the fluorescence lifetime at all pixels in the field of view with an accuracy significantly better than alternative time domain FLIM techniques.[8,9]

Using the apparatus shown in figure 1, fluorescent samples were illuminated by ~ 10 ps pulses of up to 1 μJ energy at 415 nm, at a repetition rate of 5 kHz. These were derived from a commercial ultrafast Ti:sapphire laser (Spectra-Physics Tsunami) and amplified in a Cr:LiSAF regenerative amplifier whose output was tuneable from 800 - 880 nm (400-440 nm in the second harmonic signal). This could be replaced by the diode pumped system reported by Hyde et al,[10] allowing this FLIM technology to become compact, portable and relatively inexpensive. An image of the fluorescent sample was relayed onto the cathode of the time-gated image intensifier (Kentech Instruments Ltd. Gated Optical Imager), for which the gate width was measured to be 110 ps (this measurement includes triggering jitter). The intensifier was triggered by the switching of the regenerative amplifier Pockels cell via an electronic delay (Stanford Research Systems DG535) to set its position relative to the arrival time of the excitation light.

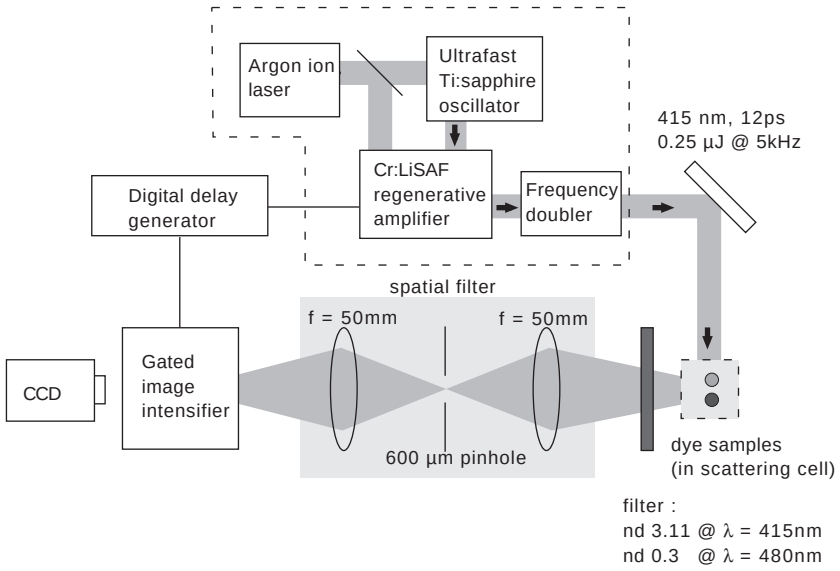


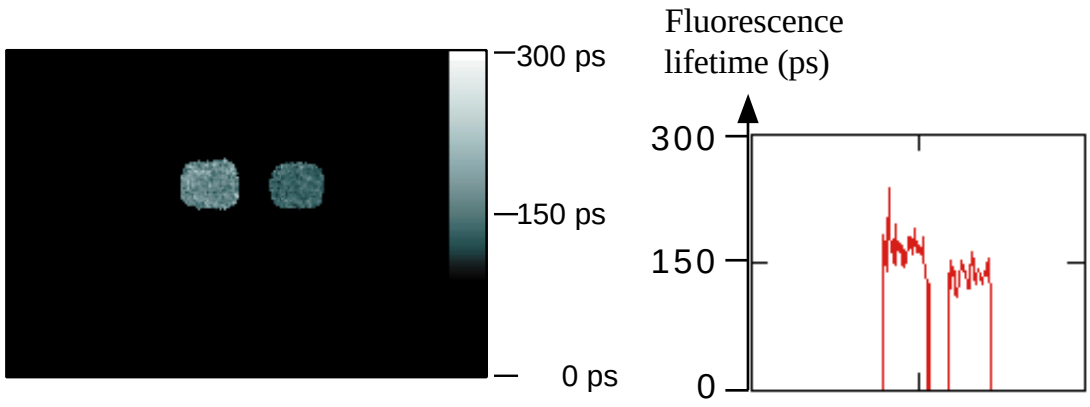
Figure 1. Experimental set up for fluorescence lifetime imaging.

By recording images of the fluorescence at different delays after excitation, a temporal profile of the fluorescence could be obtained simultaneously for each point in the field of view. By performing a least-squares fit to these profiles for each pixel the distribution of fluorescence lifetime can be displayed - a FLIM map. By knowing how the environment of a chosen fluorophore perturbs its lifetime, a FLIM map can be translated to a map of the distribution of environment.

3. TEMPORAL RESOLUTION

To demonstrate the temporal resolution of the FLIM imaging system presented here, a short lifetime laser dye was used. The dye selected was 3,3'-diethylthiacyanine iodide (DTI), because its lifetime is a sensitive function of the viscosity of its solvent,[11] and so a fluorescent phantom with a controllable fluorescence lifetime can be realised. The solvent used was ethanol which was mixed with different amounts of glycol to provide variable viscosity. The solvent was adjusted such that two samples were prepared with lifetimes differing by 20 ps. As can be seen in the lifetime maps in figure 3, the measured lifetimes were 160 ps (left hand side) for the phantom with a higher viscosity and 140 ps (right hand side) for the less viscous solution. While these lifetimes are comparable with the detector gate width. It is possible to measure lifetimes to a greater accuracy than the width of the intensifier gate provided that the data is

recorded over a sufficient number of decades.[12] The noise on the FLIM lifetime map is largely due to the limited bit depth (measured to be 5) of the CCD camera used. Further improvements in resolution could be made by reducing the system response time and increasing the bit depth of the image acquisition. Work is underway to reduce the system response to ~ 80 ps by eliminating most of the jitter.



(a) (b)

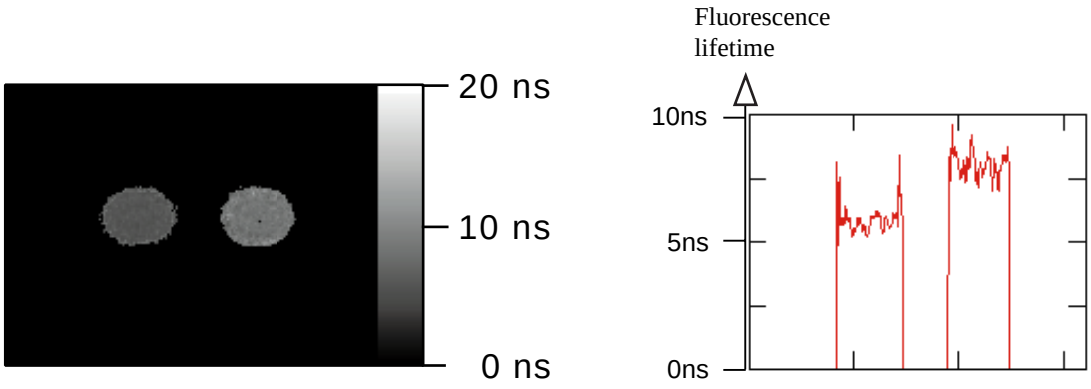
Figure 3 (a) FLIM environment map showing two samples of DTI, with differing solvent viscosity. The separation of their lifetimes is than 20 ps. (b) shows a cross-section of the FLIM map.

4. FLUOROPHORE ENVIRONMENT IMAGING

To demonstrate the ability of the fluorescence lifetime imaging system to image the environment of a fluorophore, we used the indicator lucifer yellow, a fluorophore used as a marker in fluorescence microscopy,[13] which can also be used in vivo. To provide a change in environment two samples were prepared One was an aqueous solution of lucifer yellow and the other a solution of lucifer bound to the protein albumin. The resulting lifetime map, shown in figure 2, demonstrates that a fluorescence lifetime difference is detectable between the two samples. The measured lifetimes were 5.7 ns for the unbound and 8.3 ns for the protein bound lucifer yellow samples. While there is clearly uncertainty in the in the absolute values of the lifetimes measured, which was again due to the limited bit depth of the CCD camera, it is clear that the FLIM map shows the difference in lifetime caused by the environment perturbation.

5. CONCLUSIONS

In conclusion, we have demonstrated the operation of a fluorescence lifetime imaging system based on a time-gated image intensifier and a solid state regenerative oscillator/amplifier system. We have shown 110 ps temporal resolution for this system, with the ability to detect fluorescence lifetimes separated by 20 ps. The laser source for this system has also been demonstrated diode-pumped, potentially yielding a commercially viable instrument. The application of FLIM imaging to environment mapping has also been demonstrated with the marker lucifer yellow.



(a) (b)

Figure 2 (a) FLIM environment map showing the change in fluorescence lifetime of Lucifer Yellow when unbound and bound to protein, (the unbound sample is on the left of the field of view), and (b) a cross-section of the FLIM map.

6. ACKNOWLEDGEMENTS

The authors gratefully acknowledge invaluable discussions with Roy Taylor. Funding for this research was provided by the UK Engineering and Physical Sciences Research Council (EPSRC). K. Dowling acknowledges an EPSRC CASE studentship supported by Imperial Cancer Research at The Royal Marsden NHS Trust. S.C.W. Hyde acknowledges an EPSRC CASE studentship supported by Kodak Ltd.

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