

Developing Instrumentation for Endoscopic Fluorescence Lifetime Imaging

Introduction

The aim of the research that I am involved in is to develop endoscopic instrumentation for use in Fluorescence Lifetime Imaging (FLIM). General advantages of FLIM over conventional fluorescence intensity imaging include the fact that the fluorescence lifetime is sensitive to the micro-environment of the fluorophore¹ (e.g. pH² and solvent viscosity³) and that it is relatively robust against variations in fluorophore concentration and the intensity of the excitation source⁴. FLIM can also be applied to imaging intrinsic tissue autofluorescence from biomolecules such as NADH, elastin and collagen. A number of studies have now shown that FLIM has the ability to provide intrinsic contrast between different tissue states⁵, including between healthy and diseased tissues^{6, 7}. Despite this, there have been relatively few studies into endoscopic FLIM, aimed at *in vivo* diagnostic applications.

Endoscopy, in itself, is a fairly wide field of research and a number of groups have proposed methods employing optical fibres to permit *in vivo* imaging. One approach to this is confocal endomicroscopy, which can be achieved using several techniques. The first of these is to use a single optical fibre to deliver the illumination light – and carry back the fluorescent or reflected light – with a scanning mechanism and objective lens situated at the distal end of the fibre⁸. Alternatively a fibre bundle can be used, consisting of 10,000 or so fibre cores, with a scanning system at the proximal end, which sequentially addresses each fibre core. The objective lens is situated at the distal end as before. In this setup the scanning mechanism is usually very similar to that used in a confocal microscope (i.e. scanning mirrors)⁹ but it is also possible to use a digital micromirror device (DMD) to translate a single spot across the fibre bundle¹⁰. A very different approach is used in wide-field endoscopes where the light is delivered and collected by two different fibres with a fibre bundle typically being used to relay the image from the sample to the detector.

As mentioned above, relatively few studies have been published on endoscopic FLIM. Systems demonstrated to-date include the work by Mizeret et al using a frequency domain detection technique¹¹ and a system developed at Imperial College London which uses a wide-field time-gated detection technique to achieve endoscopic FLIM in the time domain at acquisition rates of up to 29 frames/s⁴. Part of our research is now aimed at improving this system and packaging it such that it is suitable for *in vivo* studies in humans (e.g. so that it can be easily sterilised, etc.).

Research in the Photonics group at Imperial is also underway using a second endoscope. This system is a microconfocal endoscope designed for fluorescence intensity imaging, which consists of a fibre bundle and two scanning mirrors at the proximal end. Currently the endoscope operates at a wavelength of 488nm.

Part of my work is to investigate the practical issues associated with *in vivo* FLIM. One such problem is that the fibre bundle used to carry the excitation light can exhibit unwanted background fluorescence – this produces noise that can adversely affect experiments. Data regarding the background emission from some similar fibres has recently been published¹² and we are currently undertaking an investigation into the spectral and lifetime characteristics of our fibre.

Current Research

Various studies have been carried out here at Imperial based on evaluating the potential of FLIM as a clinical tool. One such example is reference 6, where biopsies were taken from 25 patients identified to have Basal Cell Carcinomas (BCCs). The unstained samples were then excited using a pulsed 355nm laser and imaged using FLIM. Although no consistent relationship was observed between the autofluorescence intensities of the areas of BCC and the areas of surrounding normal skin, a statistically significant difference was seen between the two regions in fluorescence lifetime. The regions of BCC were found to have shorter mean fluorescence lifetimes than the surrounding normal tissue and this trend was consistently observed using different combinations of two emission filters and two fitting methods⁶ (single exponential and stretched exponential¹³).

The fluorescence lifetime measurements taken were then used to produce FLIM maps of all of the samples. In each case 2D false-colour maps of lifetime alone were generated as were intensity-weighted lifetime images. In the majority of situations clear visual contrast was observed between the areas of BCC and the surrounding normal skin. An example of one of the images produced is shown below in figure 1 where clear contrast can be seen between the central region of BCC (short lifetime) and the outer regions of normal skin (long lifetime).

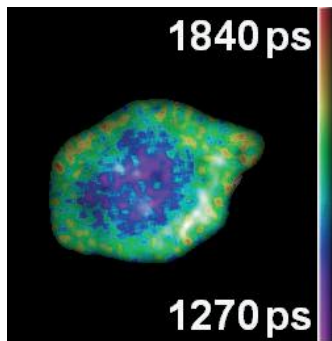


Figure 1: Intensity-weighted lifetime map of a skin biopsy with basal cell carcinoma (BCC)⁶.

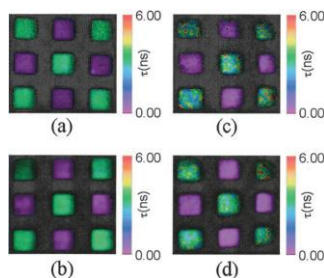


Figure 2: Intensity weighted FLIM maps of a multi-well plate array of alternating solutions of Stilbene 1 and Coumarin 314 obtained using (a), (b) the macroimager and (c), (d) the endoscope⁴.

In the study discussed, as well as in others³, FLIM has been shown to provide intrinsic contrast between different states of tissue. For this reason, it is desirable to use FLIM *in vivo* to see if this useful contrast remains. In order to do this, it is necessary to have instrumentation to apply FLIM endoscopically.

For this reason, the group has produced a wide-field FLIM endoscope which retains the intrinsic contrast observed in lifetime images obtained using a wide-field macroimager (i.e. a system where the endoscope is replaced by a standard camera lens), although the quality of the images is degraded slightly. The system has been tested by imaging a multi-well plate array of two solutions with differing lifetimes as well as by imaging autofluorescence of a sample of bisected lamb kidney. In this study, it was found that the signal-to-noise ratio was lower in the endoscopic images than in those acquired with the macroimager but, importantly, that the fluorescence lifetime contrast was not adversely affected⁴. Figure 2 shows images comparing the performance of the macroimager and the endoscope.

The important result obtained from the study described in reference 4 is not just that the system discussed can reproduce the previously obtained fluorescence lifetime contrast, but also that it can do so at update rates that are high enough to suggest that the endoscope can be used in a clinical setting. In figure 2, it can be seen that there is clear lifetime contrast in each of the four images. Of the two images acquired using the endoscope, figure 2(c) was obtained at an update rate of 29Hz while 2(d) was obtained at 7.2Hz⁴. These are both very rapid acquisitions and the former is certainly fast enough to be implemented in clinical diagnostics.

Future Direction

As discussed above, it has been demonstrated that FLIM provides intrinsic contrast between healthy and diseased states of tissue and that applying FLIM endoscopically is feasible. The aim of our work in the near future is, therefore, to develop instrumentation for endoscopic FLIM that is suitable for use in a clinical environment. We currently have two endoscopes set up in the laboratory – one wide-field endoscope built “in-house” and a second microconfocal endoscope. The wide-field system is already equipped with FLIM and needs simply to be housed in such a way that is appropriate for clinical use (i.e. so that it can be easily sterilised, etc.). Work on the microconfocal system will also be a significant part of my PhD.

It is intended that all of these steps be carried out soon such that we can use both systems for endoscopic FLIM. Once this is the case, the systems will be tested and characterised by comparing their performance to that of a hyperspectral FLIM microscope. They will then be used to study various tissue samples, *ex vivo*, in an investigation aimed at improving understanding of the molecular origins of the fluorescence lifetime contrast observed between healthy and diseased tissue. Finally, we hope that the endoscopic FLIM systems will be applied to *in vivo* clinical imaging – for example, to look at cancer and pre-cancer in the gastrointestinal tract.

In conclusion, our research is based around the use of FLIM for biomedical and clinical applications, such as the diagnosis, monitoring and study of various diseases. Previous investigations have shown that FLIM can provide useful contrast between healthy and diseased tissue and that endoscopic FLIM is a feasible and potentially useful tool for clinical diagnostics. Thus, current work is aimed at designing and improving two FLIM endoscopes, characterising their performance and using them for *in vivo* clinical applications.

References

1. Lakowicz J., *Principles of Fluorescence Spectroscopy*, KA/PP, 1999.
2. Sanders R., Draaijer A., Gerritsen H.C., Houpt P.M. and Levine Y.K., *Quantitative pH imaging in cells using confocal fluorescence lifetime imaging microscopy*, *Anal Biochem*, 1995, **227**(2):302-8.
3. Elson D., Requejo-Isidro J., Munro I., Reavell F., Siegel J., Suhling K., Tadrous P., Benninger R., Lanigan P., McGinty J., Talbot C., Treanor B., Webb S., Sandison A., Wallace A., Davis D., Lever J., Neil M., Phillips D., Stamp G. and French P., *Time-domain fluorescence lifetime imaging applied to biological tissue*, *Photochem Photobiol Sci*, 2004, **3**(8):795-801.
4. Requejo-Isidro J., McGinty J., Munro I., Elson D.S., Galletly N.P., Lever M.J., Neil M.A., Stamp G.W., French P.M., Kellett P.A., Hares J.D. and Dymoke-Bradshaw A.K., *High-speed wide-field time-gated endoscopic fluorescence-lifetime imaging*, *Opt Lett*, 2004, **29**(19):2249-51.
5. Cubeddu R., Comelli D., D'Andrea C., Taroni P. and Valentini G., *Time-resolved fluorescence imaging in biology and medicine*, *Journal of Physics D-Applied Physics*, 2002, **35**(9):R61-R76.
6. Galletly N.P., McGinty J., Dunsby C., Teixeira F., Requejo-Isidro J., Munro I., Elson D.S., Neil M.A.A., Chu A.C., French P.M.W. and Stamp G.W., *Fluorescence lifetime imaging distinguishes basal cell carcinoma from surrounding uninvolved skin*, *British Journal of Dermatology*, 2008, **159**(1):152-161.
7. Tadrous P.J., Siegel J., French P.M., Shousha S., Lalani el N. and Stamp G.W., *Fluorescence lifetime imaging of unstained tissues: early results in human breast cancer*, *J Pathol*, 2003, **199**(3):309-17.
8. Dickensheets D.L. and Kino G.S., *Micromachined scanning confocal optical microscope*, *Optics Letters*, 1996, **21**(10):764-766.
9. Gmitro A.F. and Aziz D., *Confocal Microscopy through a Fiberoptic Imaging Bundle*, *Optics Letters*, 1993, **18**(8):565-567.
10. Lane P.M., Dlugan A.L.P., Richards-Kortum R. and MacAulay C.E., *Fiber-optic confocal microscopy using a spatial light modulator*, *OPTICS LETTERS*, 2000, **25**(24):1780-1782.
11. Mizeret J., Stepinac T., Hansroul M., Studzinski A., van den Bergh H. and Wagnieres G., *Instrumentation for real-time fluorescence lifetime imaging in endoscopy*, *Review of Scientific Instruments*, 1999, **70**(12):4689-4701.
12. Udovich J.A., Kirkpatrick N.D., Kano A., Tanbakuchi A., Utzinger U. and Gmitro A.F., *Spectral background and transmission characteristics of fiber optic imaging bundles*, *Appl Opt*, 2008, **47**(25):4560-8.
13. Lee K.C.B., Siegel J., Webb S.E.D., Leveque-Fort S., Cole M.J., Jones R., Dowling K., Lever M.J. and French P.M.W., *Application of the stretched exponential function to fluorescence lifetime imaging*, *Biophysical Journal*, 2001, **81**(3):1265-1274.