Fluorescence Lifetime Imaging Endoscopy

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Abstract: We describe fluorescence lifetime imaging (FLIM) endoscopes being developed for *in vivo* applications. For depth-resolved imaging with subcellular resolution, we employ a proximally scanned imaging fibre bundle probe and time correlated single photon counting to implement confocal FLIM endomicroscopy. This has been applied to image Förster resonance energy transfer (FRET) in live cells and has been developed into a compact portable instrument for clinical applications. For tissue screening and guided biopsy applications, we have developed portable wide field imaging FLIM endoscopes for handheld operation.

OCIS codes: (170.2150) Endoscopic imaging; (170.1790) Confocal microscopy; (180.2520) Fluorescence microscopy; (170.5810) Scanning microscopy;

1. Introduction

Fluorescence lifetime imaging (FLIM) provides a means to study biomolecular interactions using Förster resonance energy transfer (FRET) and can provide label-free molecular contrast to diagnose and study disease. FLIM can provide molecular contrast and report on the local chemical environment. It is inherently ratiometric and therefore less prone to intensity artefacts associated with factors such as fluorophore concentration, non-uniform illumination and scattering. Endoscopes can provide access to interior organs in order to study disease processes *in situ* and for diagnostic applications. To study cell signalling processes and to undertake optical biopsy, it is usually desirable to image with subcellular resolution. This can be realised with confocal endomicroscopes[1,2,3,4,5,6,7,8], which can have a resolution similar to conventional histopathology and have been demonstrated using a variety of imaging methods such as reflected light[5,8], single photon[1,2,3,4,6,9] and multiphoton[10] fluorescence, multispectral[3,4] and fluorescence lifetime[7,9] imaging. These methods can be divided into coherent fibre bundle geometries[1,2,3,4,5,6] and scanning single fibres[7,8,10]. Here we describe a FLIM endomicroscope, which we have demonstrated to image FRET in live cells and have developed as a compact, portable instrument for clinical applications. The chief drawback of endomicroscopy is the relatively small field of view, which can be a disadvantage for diagnostic screening and image-guided biopsy and surgery. For such applications we are developing wide-field FLIM endoscopes based on a rigid rod lens endoscope or a flexible imaging fibre bundle instrument.

2. Confocal FLIM Endomicroscope

For endoscopic FLIM with subcellular resolution, we previously demonstrated the first laser scanning confocal FLIM endomicroscope, which we applied to tissue autofluorescence and FRET in fixed cells[11]. This instrument is represented in figure 1 and is based on the clinically approved Cellvizio® GI (Mauna Kea Technologies) incorporating time correlated single photon counting (TSCPC) to enable optically sectioned endoscopic FLIM of fields of view up to 600 µm via flexible imaging fibre-optic bundle probes. We here demonstrate the application to image FRET in live cells using this confocal endomicroscope with figure 2 showing optically sectioned FLIM images of live cos-7 cells expressing EGFP and mCherry, either separately or joined by a short peptide linker to provide a FRET sample. These FLIM images, which were analysed using SPCImage (Becker & Hickl GmbH) were acquired in just 2 s (for SPCImage binning factor = 2) and shorter acquisition times could be realised by further image pixel binning. Thus the potential for *in vivo* FRET studies of, e.g. cell signalling networks using confocal FLIM endomicroscopy is demonstrated.

For *in vivo* application, it will be important to provide a real-time FLIM preview mode to guide clinicians and experimenters. For this we have implemented the FIFO (first in, first out) mode of TCSPC acquisition, which also permits the acquisition of larger image data sets. We are also working on FLIM montaging techniques to combine

multiple fields of view in a single data set, noting that the "time-tagged" photon detection associated with FIFO TCSPC enables us to use image registration techniques if FLIM of samples moving within the total acquisition time. Clinical application will also require relatively compact and portable instrumentation. To this end we have now developed a self-contained wheeled instrument of 1.0 x 0.7m footprint, shown inset in figure 1, that incorporates a tunable ultrafast (Ti:Sapphire) excitation laser and all electronic and optical components This endoscope is being applied to FRET studies and to tissue autofluorescence to evaluate the potential for label-free biopsy.

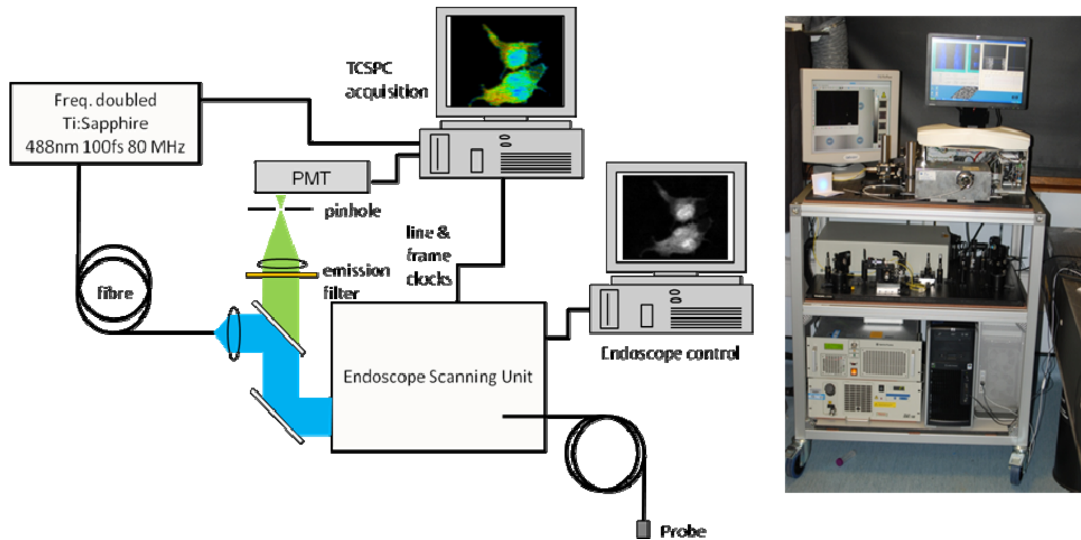

Figure 1. Experimental configuration of confocal FLIM endomicroscope with inset photograph of trolley-mounted instrument designed for in vivo experiments.

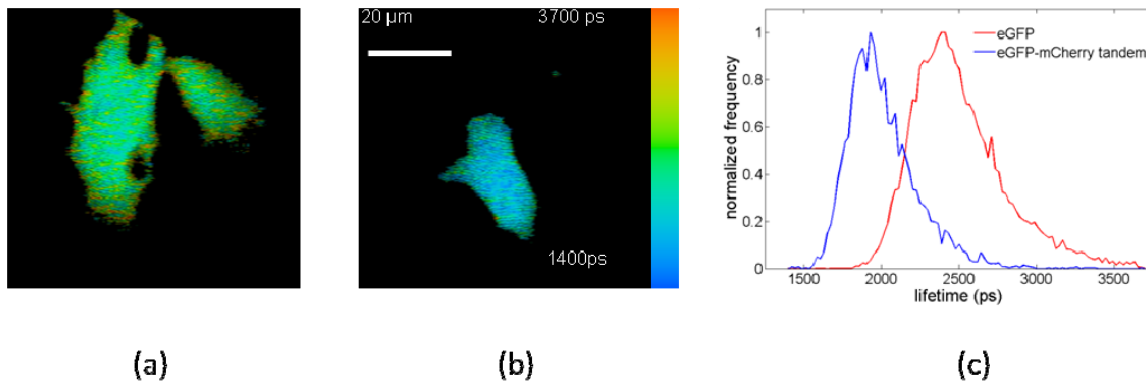

Figure 2. confocal FLIM endomicroscope images of COS-7 cells expressing (a) EGFP and (b) EGFP-mCherry tandem FRET construct, together with corresponding fluorescence lifetime histograms (c) respectively.

3. Wide-field FLIM endoscopy

For diagnostic screening and image guided biopsy "red flag" applications, a larger field of view is often desirable and to this end we have developed complementary wide-field FLIM flexible endoscopes employing either a rigid rod lens arthroscope or a coherent imaging fibre optic bundle with time-gated detection. Time-gated FLIM is implemented with 355 nm excitation from a frequency-tripled mode-locked Yb fibre laser (Fianium Ltd) and a custom-built handheld gated optical image intensifier (Modified HRI, Kentech Instruments Ltd) allowing FLIM of tissue autofluorescence with fields of view up to a few centimetres.

4. Conclusions

We have demonstrated optically sectioned FLIM FRET of live cells imaged with 2 s acquisition time using a confocal endomicroscope that has been developed as a portable instrument for *in vivo* imaging applications in diagnostic imaging and molecular cell biology. We will present our progress towards real-time clinical FLIM endomicroscopy including FIFO acquisition and image montaging. We have also developed wide field FLIM endoscopes for rapid large area FLIM of tissue, including a novel handheld unit for FLIM arthroscopy. We will present results of these instruments applied to *ex vivo* tissue.

5. References

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