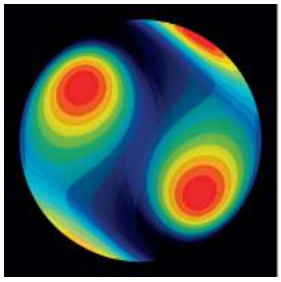


PHOTON14

Monday 1 September

Session 3: Clinical Themes in Biophotonics (16:30 – 17:00, Great Hall)

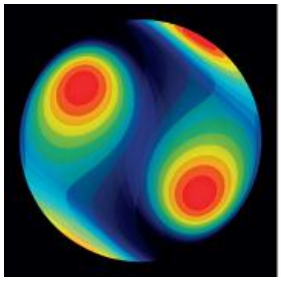
(invited) Gradient index endomicroscopes for surgery

M Hughes, P Giataganas, T Chang and G Z Yang

Imperial College London, UK

Endomicroscopy is a valuable tool for real time visualisation of tissue microstructure *in situ,* and in many cases allows determination of whether that tissue is cancerous. A potential application in oncological surgery is assisting the surgeon with identification of tumour margins. However, flexible endomicroscopes, using either fibre bundles or miniaturised scanning mechanisms, tend to suffer from either a severe resolution/field of view trade-off or low frame rates. For cases where line-of-sight access is available, such as in laparoscopic surgery, gradient index (GRIN) relay lenses could provide a convenient and low cost way of transferring images from tissue, along a rigid narrow conduit, to an external microscope.

Here, we demonstrate a handheld, fluorescence GRIN endomicroscope, with a probe that is 280 mm in length and 2.2 mm in diameter. By using a standard 0.25 pitch, 0.5 NA objective GRIN lens coupled to a customised 2.75 pitch, 0.1 NA GRIN relay lens, it achieves a resolution of better than 1.2 microns over a field-of-view of better than 300 microns, representing an order of magnitude increase in space-bandwidth product over a typical 30,000 core fibre bundle. We will show example images from a range of tissues, including *ex vivo* normal and cancerous human breast tissue, all stained using topical fluorescence contrast agents such as acriflavine. Using clinically realistic phantoms, we have simulated use of the probe during a number of surgical procedures. We will further show preliminary results suggesting that a liquid tuneable lens can be used to provide a high speed focusing mechanism in exchange for some degradation of resolution. This has possible applications in the correction of severe longitudinal chromatic aberrations which currently hinder multi-wavelength imaging.



PHOTON14

Monday 1 September

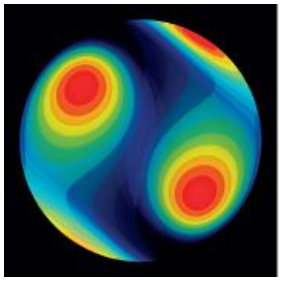
Session 3: Clinical Themes in Biophotonics (17:00 – 17:15, Great Hall)

Spatial and temporal tracking of fluorescent compounds in the skin and eye using a combined reflection and fluorescence confocal instrument

K K Buttenschoen[1,2], J M Girkin[1] and D Daly[2]

[1]Durham University, UK, [2]Reading Enterprise Centre, UK

We report on the development of a non-invasive instrument based on scanning confocal microscopy that simultaneously measures reflected and fluorescent light from tissues such as the skin and the eye. The new instrument based upon a previous concept has an axial resolution of < 100 µm and a sensitivity of < 2 nM of Fluorescein. To demonstrate the performance we have measured the diffusion of fluorescein sodium in an artificial eye in near real time as well as measuring the naturally occurring fluorescence of the crystalline lens in human volunteers of different age ranges. The performance of our technology in comparison with other commercially available ocular fluorophotometers will be discussed. We will furthermore present three-dimensional fluorescence maps of the autofluorescence of excised porcine crystalline lenses. In addition to the use in ophthalmic applications we also extended the technology for through skin measurements and initial results from this area will be presented. The instrument is now being miniaturised for better portability and cost-efficiency. We believe that it is suited to a large range of applications such as but not limited to in vitro and in vivo assessment of the diffusion of fluorescent compounds through the eye and skin, monitoring the autofluorescence from tissues and reducing the number of animals needed for pharmaceutical research. We will furthermore discuss the potential application of our technology in the clinical environment.



PHOTON14

Monday 1 September

Session 3: Clinical Themes in Biophotonics (17:15 – 17:30, Great Hall)

Compact and portable autofluorescence lifetime instrumentation and its application to the study of heart disease and osteoarthritis

J Lagarto, C B Talbot, B Dyer, D J Kelly, Nickdel, M B Sikkel, Y Itoh, N S Peters, A R Lyon, C Dunsby and P M W French

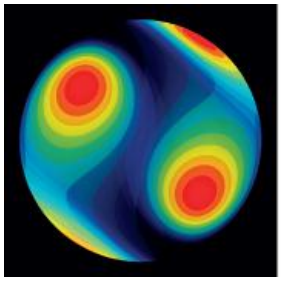
Imperial College London, UK

Autofluorescence lifetime (AFL) can be used to characterise biological activity and report structural, functional and biochemical changes occurring in biological tissue as a result of pathological transformations, thus representing a convenient non-invasive label-free additional source of contrast to fluorescence measurements. We report the development and application of a compact fibre-optic probe-based time-resolved spectrofluorometer utilizing spectrally resolved time-correlated single photon counting detection and white light reflectometry to investigate the clinical potential of AFL measurements to report structural and metabolic changes associated with heart disease and osteoarthritis.

Preliminary in vivo studies in a rat myocardial infarction heart failure model show statistically significant differences between healthy and failing myocardium, both in infarcted and distal regions. A classification algorithm based on principal component analysis was used to differentiate healthy from diseased myocardium with specificity and sensitivity of 100% in the infarcted area, and specificity >71% and sensitivity >84% in regions remote to the scar. Thus we have demonstrated the ability of our instrument to characterise the signals from scar and failing myocardium.

In osteoarthritis studies, our results suggest that AFL of cartilage tissue is sensitive to degradation of extracellular matrix components, as demonstrated through measurements of localised regions of cartilage degradation by enzymatic treatment. Thus we believe that AFL offers a potential non-invasive readout to monitor cartilage matrix integrity that may contribute to future diagnosis of early cartilage defects as well as monitoring the efficacy of therapeutic agents.

The clinical impact of AFL for label-free diagnosis would increase significantly if the cost of the instrumentation could be reduced. We present the development of a compact and portable fibre-optic spectrofluorometer utilizing low-cost FPGA-based circuitry that can be used with laser diodes and photon-counting photomultipliers. Once clinical efficiency and practicality are demonstrated, we believe that such instrument could have significant potential for commercial development and clinical deployment.



PHOTON14

Monday 1 September

Session 3: Clinical Themes in Biophotonics (17:30 – 17:45, Great Hall)

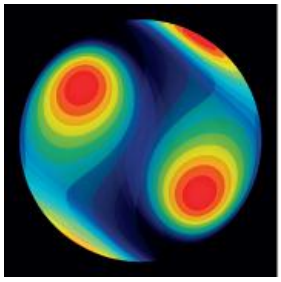
Fluorescence lifetime imaging endoscopes for biomedical applications

H Sparks[1], I Munro[1], G Kennedy[1], E Hirata[3], S Warren[1], D Kelly[1], E Sahai[3], N Guerra[1], T Tatla[2], C Dunsby[1] and P French[1]

[1]Imperial College London, UK, [2]Northwick Park Hospital, UK, [3]Cancer Research UK, UK

FLIM can provide useful molecular contrast when imaging tissue autofluorescence for label-free readouts of disease and for preclinical imaging of labelled tissues. For minimally invasive access to internal organs in vivo, it is desirable to develop FLIM endoscopes. We present a wide-field flexible FLIM endoscope using compact pulsed excitation sources with time-gated imaging for both FLIM of FRET in disease models and clinical imaging of tissue autofluorescence. This utilises a flexible coherent fiber bundle (2.3 mm diameter) that presents a spatially varying instrument response function (IRF) caused by modal dispersion in the excitation optical fiber that we have corrected in software. We will present preliminary ex vivo wide-field FLIM of human head and neck lesions and progress towards endoscopy.

We also present a scanning confocal FLIM endomicroscope utilising time-correlated single photon counting (TCSPC) that we are developing for in vivo quantitative imaging of fluorescence resonance energy transfer (FRET). This is based in the Mauna Kea Technologies Cellvizio system that provides internal access via an endoscope of less than 2.6 mm diameter. TCSPC electronics run in a first-in first-out (FIFO) mode to enable live streaming of lifetime maps. We show that it is necessary to account for background light (caused by background fluorescence from the fibre bundle) and material dispersion effects in order to achieve accurate fluorescence decay analysis. We will present our work towards *in vivo* imaging of murine xenograft models of ovarian cancer using CFP- and GFP-based FRET sensors.



PHOTON14

Monday 1 September

Session 3: Clinical Themes in Biophotonics (17:45 – 18:00, Great Hall)

Lensless measurement of chromophore optical absorption using objective speckle: application to oximetry in the retina and beyond

L R Brewer and A R Harvey

University of Glasgow, UK

The *in vivo* measurement of light absorption by chromophores, for example in blood oximetry, is often determined by optical transmissometry and application of the Lambert-Beer law. Measurement accuracy is degraded, however, by optical imaging aberrations and contrast reduction associated with imperfect lenses. This is particularly true for retinal oximetry based on imaging the optical density of retinal blood vessels. We report a novel lensless technique for measuring chromophore absorption using laser objective speckle, which eradicates the impact of imperfect imaging. The technique is based upon the inverse relation between speckle dimensions for scattered laser light and the point-spread function (PSF) for light propagation in the chromophore sample, which in turn diminishes with increasing chromophore absorption. We have demonstrated the technique for *in vitro* blood oximetry in both capillary and slab geometries, which mimic blood vessel and choroid structures in the human retina. Characterisation of speckle recorded at a range of visible-light wavelengths using tunable HeNe and argon-ion lasers demonstrated that the speckle size and PSF were strongly correlated over two orders of magnitude, facilitating calibrated oximetry. We demonstrated optical oximetry for oxygenation values ranging from 10% to 100% with high sensitivity, repeatability and accuracy. These measurements were performed as a preliminary step to developing speckle-based retinal-blood oximetry *in vivo* - information that is important for diagnosing retinal diseases involving hypoxia, such as diabetes and glaucoma. Importantly, this new mode of oximetry promises to be insensitive to aberrations and opacities of the ocular lenses, providing the unique possibility of performing oximetry of the choroid and in the capillaries, which are too small to be readily imaged. Uncertainties associated with poorly characterised light propagation through retinal tissue and vessels are also expected to be less significant using this speckle-based oximetry.