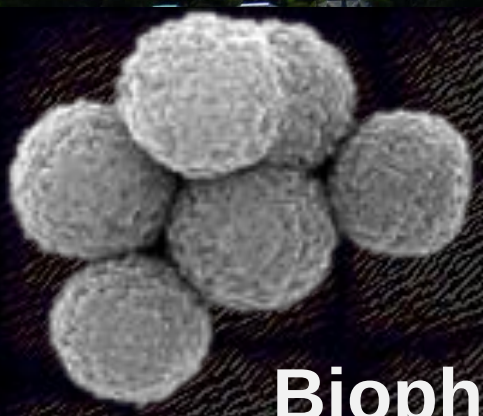
PHOQUS conference 2016

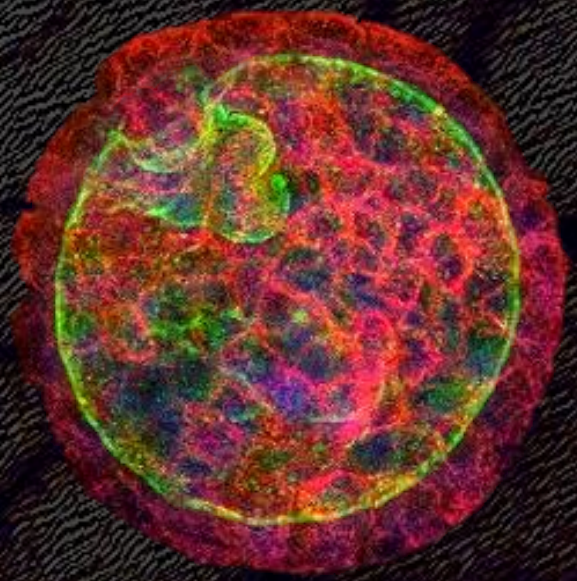


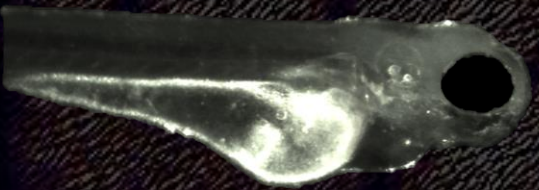
Biophotonic approaches: From molecules to living systems

(2nd PHOQUS Conference)

22 – 23 August 2016

West Park Conference Centre, Dundee, Scotland





This event has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under REA grant agreement n° 608133.

PHOQUS

UNIVERSITY OF DUNDEE

Biophotonic approaches: From molecules to living systems

Venue: General Information

Fire Exits

- Auditorium: Doors are at either side of the auditorium to the left and right hand side of the stage
- Foyer: exits are located at either end of the corridor

Fire Alarm

- The Fire Alarm is tested on a Monday at 1pm. Outwith these times you should make your way to the nearest exit and muster at the grass in front of the Sidlaw Suite

Smoking

- Smoking is permitted at the designated area outside of the Sidlaw suite and at the designated area in front of the Mansion House

Toilets

- Toilets are located in the foyer area of the Sidlaw Suite, additional toilets are available in the Mansion House

West Park are proud to be part sponsors of 'Glow' one of the many Oor Wullie's to be found on the Bucket Trail.

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Biophotonic approaches: From molecules to living systems

PROGRAMME - Monday 22nd August 2016

08:00 **Registration and Coffee**

08:30 **Introduction and Welcome**
Kees Weijer, PHOQUS Coordinator

Session 1 (Chair: Alessandra Cecchini)

08:40 **Intravital imaging of drug target engagement and therapy failure**
Erik Sahai, Francis Crick Institute, UK

09:10 **STED nanoscopy combined with optical tweezers reveals protein dynamics on densely covered DNA**
Gijs Wuite, VU Amsterdam, Netherlands

09:40 **OME's OMERO & Bio-Formats: Open Tools for Image Data and Metadata Access @ Scale**
Jason Swedlow, University of Dundee, UK

10:10 **In situ bacterial detection in human alveolar lung tissue using Smart Probes and fluorescence lifetime imaging microscopy**
Tushar R. Choudhary, Heriot-Watt University, UK

10:25 **Using Raman spectroscopy for the detection of drug concentrations inside of cells**
Thomas Rabl, University of Dundee, UK

10:40 **Coffee Break**

Session 2 (Chair: Ricardo Correia)

11:00 **Scalable quantitative characterization of three-dimensional multicellular aggregates at single cell resolution with light sheet microscopy**
Ernst H.K. Stelzer, Goethe Universität Frankfurt am Main, Germany

11:30 **Optically pumped semiconductor lasers; a flexible low cost laser source for bio-medical imaging applications**
Keith Wilcox, University of Dundee, UK

12:00 **An in vitro engineered epithelia that bears the hallmarks of living tissue**
Marija Plodinec, Universität Basel, Switzerland

12:30 **Tagging and tracking multiple generations of cells with intracellular lasers**
Marcel Schubert, University of St Andrews, UK

12:45 **Polarised Light Sheet Tomography**
Sascha Reidt, University of Dundee, UK

13:00 **Lunch**

Session 3 (Chair: Salvatore Smirni)

14:00 **Wavefront shaping techniques for advanced optical imaging**
Monika Ritsch-Marte, Medinische Universität Innsbruck, Austria

14:30 **Investigation of cell mechanics using Nanodevices and Nano-instruments: some examples**
Christophe Vieu, LAAS-CNRS, France

15:00 **Imaging strategies for scattering tissues**
Martin Leahy, National University of Ireland, Galway, Ireland

15:30 **Novel single-fibre probes for advanced biophotonics applications**
Ivo Leite, University of Dundee, UK

15:45 **Coffee Break**

Session 4 (Chair: Valerie Bentivegna)

16:05 **Looking Deeply in Living Biology: Finding Nemo's Heart**
John Girkin, Durham University, UK

16:35 **Multidimensional Proteomic Analysis of Disease Mechanisms**
Angus Lamond, University of Dundee, UK

17:05 **Learning from examples in optics**
Demetri Psaltis, EPFL, Switzerland

17:35 **Airy beams for light-sheet microscopy**
Jonathan Nylk , University of St Andrews, UK

17:50 **Close – Day 1**

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Biophotonic approaches: From molecules to living systems

PROGRAMME - Tuesday 23rd August 2016

08:00 **Arrival and coffee**

Session 5 (Chair: Daniel O'Brien)

08:30 **Multidimensional Fluorescence Imaging across the scales**
Paul French, Imperial College, UK

09:00 **Advances in adaptive optics for nanoscopy**
Martin Booth, University of Oxford, UK

09:30 **Imaging cortical activity in vivo: from neuronal networks to single spines**
Nathalie Rochefort, University of Edinburgh, UK

10:00 **Experimental study of optimum repetition rate and pulse duration for multiphoton microscopy at 1030 nm**
Shamil Mirkhanov, University of Dundee, UK

10:15 **Coffee Break**

Session 6 (Chair: Valentina Ferro)

10:35 **Feeling for cell function: mechanical phenotyping at 1,000 cells/sec**
Jochen Guck, TU Dresden, Germany

11:05 **Increased variability in precancerous $Apc^{Min/+}$ intestinal tissue can be measured with microultrasound**
Inke Näthke, University of Dundee, UK

11:35 **Elastic Resonator Interference Stress Microscopy (ERISM) – A new and versatile tool to gauge biophysical cell-substrate interactions**
Nils M. Kronenberg, University of St Andrews, UK

11:50 **High-speed wavefront modulation in complex media**
Sergey Turtaev, University of Dundee, UK

12:05 **Poster Session Introduction**

12:30 **Lunch**

13:30 **Poster Session**

15:00 **Coffee Break**

Session 7 (Chair: Kees Weijer)

15:20 **Imaging protein function and cancer phenotypes**
Alan Serrels, University of Edinburgh, UK

15:50 **Snapshot 3D-Ranging Microscopy with Extended Depth-of-Field**
Andrew R. Harvey, University of Glasgow, UK

16:10 **Challenges in building a fast scanning Super-Resolution STED microscope**
Piotr Zdankowski, University of Dundee, UK

16:25 **Encapsulated SERS Nanosensors – Fibre-based pH Sensing**
Holly Fleming, University of Edinburgh, UK

16:40 **Catching the perfect wavelet: multi-resolution signal analysis for biophysics**
Quintin Davis, University of Dundee, UK

16:55 **Coffee Break**

17:15 **Lightwedge and Lightsheet-Raman Microscopy**
Rainer Heintzmann, FSU Jena, Germany

17:45 **Holographic micro-endoscopy based on multimode waveguides**
Tomas Cizmar, University of Dundee, UK

18:15 **Closing Remarks**

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SPEAKER ABSTRACTS

Session 1: Monday 22nd August 08:40 – 10:40

Intravital imaging of drug target engagement and therapy failure
Erik Sahai, Francis Crick Institute, UK

The lack of durable efficiency of cancer drugs is a major clinical problem. This results from heterogeneity in drug distribution, heterogeneity in biological response, and ongoing tumour evolution. We use a combination of *in vivo* and 3D co-culture imaging methodologies to study all these problems. To accurately measure chromatin binding by the topoisomerase inhibitor Doxorubicin, we have developed a FLIM assay that measures changes in GFP-histone lifetime when Doxorubucin is bound to DNA. In collaboration with Imperial College, we have used a newly developed confocal fluorescence lifetime laparoscope to monitor Doxorubicin binding to chromatin in a model of ovarian cancer peritoneal metastasis. We will present data on both intra- and inter-tumour heterogeneity in drug – target engagement.

The Ras-MAPK pathway is mutated in the majority of melanoma, with BRAF mutations being most common. Tumours with BRAF mutations show an initial response to BRAF inhibitors before genetic resistance emerges. However little is known about the spatio-temporal response to BRAF inhibitors in vivo and how this relates to the failure of targeted therapy. We have used both intravial ratiometic FRET and FLIM of an ERK/MAP kinase biosensor to investigate heterogeneity in signalling in melanoma models. We have longitudinally monitored responses to targeted therapy and identified areas that become refractory to drug action. BRAF mutant melanoma cells can rapidly become tolerant to PLX4720 in areas of high stroma. The rapid kinetics of this process indicate that it is not caused by genetic events. We demonstrate that PLX4720 has an unexpected effect on the tumour stroma leading to enhanced matrix remodelling. The remodelled matrix then provides signals that enable melanoma cells to tolerate PLX4720. We propose that this safe haven enhances the population of cancer cells from which genetically resistance emerges.

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STED nanoscopy combined with optical tweezers reveals protein dynamics on densely covered DNA

Gijs Wuite, VU Amsterdam, Netherlands

Dense coverage of DNA by proteins is a ubiquitous feature of cellular processes such as DNA organization, replication, and repair. We present a single-molecule approach capable of visualizing individual DNA-binding proteins on densely covered DNA and in presence of high protein concentrations. Our approach combines optical tweezers with multicolor confocal and STED fluorescence microscopy. Proteins on DNA are visualized at a resolution of 50 nm, a 6-fold resolution improvement over confocal microscopy. High temporal resolution (<50 ms) is ensured by fast one-dimensional beam scanning. Individual trajectories of proteins translocating on DNA can thus be distinguished and tracked with enhanced precision. We demonstrate our multimodal approach by visualizing the assembly of dense nucleoprotein filaments with unprecedented spatial resolution in real time. Experimental access to the force-dependent kinetics and motility of DNA-associating proteins at biologically relevant protein densities is essential for linking idealized *in vitro* experiments with the *in vivo* situation.

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OME's OMERO & Bio-Formats: Open Tools for Image Data and Metadata Access @Scale

Jason R. Swedlow, University of Dundee, UK

Despite significant advances in cell and tissue imaging instrumentation and analysis algorithms, major informatics challenges remain unsolved: file formats are proprietary, facilities to store, analyze and query numerical data or analysis results are not routinely available, integration of new algorithms into proprietary packages is difficult at best, and standards for sharing image data and results are lacking. We have developed an open-source software framework to address these limitations called the Open Microscopy Environment. OME has three components—an open data model for biological imaging, standardised file formats and software libraries for data file conversion and software tools for image data management and analysis. The OME Data Model provides a common specification for scientific image data and has recently been updated to more fully support fluorescence filter sets, the requirement for unique identifiers, screening experiments using multi-well plates. The OME-TIFF file format and the BioFormats file format library are easy-to-use tools for converting data from proprietary file formats. These resources enable access to data by different processing and visualization applications, sharing of data between scientific collaborators and interoperability with third-party tools like Fiji/ImageJ. The Java-based OMERO platform includes server and client applications that combine an image metadata database, a binary image data repository and visualization and analysis by remote access. The current stable release of OMERO (OMERO 5.2) includes a single mechanism for accessing image data of all types-- regardless of original file format-- via Java, C/C++ and Python and a variety of applications and environments (e.g., ImageJ, Matlab and CellProfiler). OMERO includes SSL-based secure access, distributed compute facility, filesystem access for OMERO clients, and a scripting facility for image processing. An open script repository allows users to share scripts with one another. A permissions system controls access to data within OMERO and enables sharing of data with users in a specific group or even publishing of image data to the worldwide community. OMERO 5.0 includes updates and resources that specifically support large datasets that appear in digital pathology and high content screening. Importing these large datasets is fast, and data are stored in their original file format, so they can be accessed by 3rd party software. Several applications that use OMERO are now released by the OME Consortium, including FLIMfit, a fluorescence lifetime analysis module; u-track, an object tracking module; image-based search applications, OMERO.searcher and WND-CHARM; an automatic image tagging application, a biobanking application. OMERO and Bio-Formats run the JCB DataViewer, The Image Data Repository and are used to publish 3D EM tomograms in the EMDataBank. They also power several large project image data. All OME software is available at http://openmicroscopy.org.

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In situ bacterial detection in human alveolar lung tissue using Smart Probes and fluorescence lifetime imaging microscopy (FLIM)

Tushar R. Choudhary, Heriot-Watt University, UK

Antibiotic resistance is a serious global concern. One way to tackle this problem is to develop new and sensitive approaches to diagnose bacterial infections and prevent unnecessary antibiotic use. With recent developments in optical molecular imaging, we are one step closer to *in situ* rapid detection of bacterial infections. We present nitrobenzoxadiazole (NBD) based Smart Probes for bacterial detection in *ex vivo* human lung tissue using fluorescence lifetime imaging microscopy (FLIM). NBD is an environmentally sensitive fluorophore and has different fluorescence intensity and lifetime depending on the environment. Two NBD based Smart Probes (Bac One and Bac Two) were used in this study to detect and differentiate between Gram positive and Gram negative bacterial strain using their fluorescence lifetime in the *ex vivo* human lung tissue. The average fluorescence lifetime of live bacteria labelled with Bac One (n=12) was 2.40 ± 0.25 ns and Bac Two (n=12) was 6.73 ± 0.49 ns. The human lung tissue (n=12) average fluorescence lifetime value was found to be 3.43 ± 0.19 ns. We demonstrated here use of novel Smart Probes for detection and discrimination of Gram positive and Gram negative bacteria using FLIM. Both BAC One and BAC Two have now been administered to humans (NCT02491164) and we have developed Fibre-FLIM methods to enable clinical translation within the PROTEUS Project (www.proteus.ac.uk).

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Using Raman spectroscopy for the detection of drug concentrations inside of cells

Thomas Rabl, University of Dundee, UK

Uptake of novel drugs into target cells is a major challenge in early drug discovery. Current measurement methods such as Ultra Performance Liquid Chromatography-tandem Mass Spectrometry (UPLC-MS/MS) and the measurement of Fraction Unbound (FU), only detect and quantify drug concentrations in the whole cell. Therefore, novel techniques for drug localization are sought after. Raman spectroscopy detects vibrational states of molecules allowing for label-free chemical finger printing. This work reports the use of spontaneous Raman spectroscopy to collect spectra of single cells grown on top of a quartz coverslip. Different cell types can be distinguished by measuring the ratio of intensities of peaks corresponding to general building blocks of cells. Furthermore, different approaches for detection of drugs in single cells are investigated. Raman spectra of mammalian cells in general show a region where no intrinsic signal can be observed (cell silent region) allowing for the easier detection of drugs showing peaks in this specific region. However, only few drugs show intrinsic peaks in the cell silent region (e.g. Alkyne groups, Erlotinib, EdU). Drug molecules are generally small and do not allow to attach of Raman tags without altering the physico-chemical properties. Here we report preliminary results by using a different approach: drug deuteration. We will show the shifting of a Raman peak into the cell silent region by deuterating of the base substance. This could help to lower the detection limit for substances inside of cells without altering their physico-chemical properties.

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Session 2: Monday 22nd August 11:00 – 13:00

Scalable quantitative characterization of three-dimensional multicellular aggregates at single cell resolution with light sheet microscopy

Ernst H.K. Stelzer, Goethe Universität Frankfurt am Main, Germany

Three-dimensional multicellular aggregates closely resemble real *tissue* and provide an excellent model for cell-based assays in modern cell biology. They offer a suitable framework to investigate gene expression patterns, cell differentiation and therapeutic effects of drugs in a tissue-like context. High resolution light sheet-based fluorescence microscopy (LSFM) of cleared three-dimensional cell aggregates (*e.g.* spheroids and organoids) provides an *in toto* view. The organization and morphology of cells in large aggregates with diameters of several hundred micrometers comprising several ten thousand cells are systematically observed to discover properties of individual cells in their native microenvironment. Because of the large cell numbers and the high cell density, a robust and precise quantitative characterization of acquired images is a challenge. We developed an image analysis pipeline that performs a robust, accurate three-dimensional cell nuclei segmentation and returns >30 features to quantitatively characterize three-dimensional cell aggregates. We applied our pipeline to a set of breast carcinoma spheroids of different sizes and identified a transition in structural morphology for spheroids of more than 30,000 cells. Our approach provides new perspectives for the characterization of three-dimensional multicellular aggregates and the investigation of therapeutic toxicity effects of drugs on tumors with sub- und super-cellular detail.

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Optically pumped semiconductor lasers; a flexible low cost laser source for bio-medical imaging applications

Keith Wilcox, University of Dundee, UK

Semiconductor lasers are the most common type of laser in the world – ranging from lasers in DVD players and blu-rays to telecoms. However, for many applications in bio-medical imaging semiconductor lasers have struggled. Edge emitting semiconductor lasers struggle with the combination of high power and excellent beam quality.

An alternate approach is the optically pumped semiconductor laser (OPSL). The OPSL maintains the low cost, designer active medium – the semiconductor, but uses a surface emitting geometry and optical pumping to enable uniform carrier injection over large areas. The combination of this, along with a low loss external cavity makes the laser low noise and excellent beam quality. In CW operation OPSLs have achieved significant success in confocal microscopy, marketed by Coherent in the Obis range.

Our research focusses on femtosecond pulse generation in OPSLs which are of potential interest as low cost lasers for multi-photon imaging (MPI) systems. Here I will describe the underlying physics of our mode-locked OPSL devices and discuss some of the engineering challenges that this presents in the context of developing OPSLs ideally suited for MPI.

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An in vitro engineered epithelia that bears the hallmarks of living tissue

Marija Plodinec, Universität Basel, Switzerland

Regulation of cell functions by physical properties of the extracellular matrix (ECM) is a critical contributor to development and disease. ECM composition, dimensionality and stiffness, each influence cell signaling and function. It is appreciated that epithelial cells adapt to their microenvironment via vast signaling networks that have a reciprocal effect on their cellular functions. However, it remains elusive how mechanical signals that arise from anchoring cells to the specialized basement membrane (BM) ECM, influence properties of the epithelia such as mechanical stability, basal-apical polarity and cell-cell contacts that result in tissue barrier formation, directed ion transport and sensory perception. We show that In contrast to current interconnected meshwork model of BMs, native BMs from human retinas, distal tubules of the kidney and mammary glands exhibit multi-layered structures with side specific morphology, architecture and stiffness contributions from either laminin or collagen IV rich sides that are distinct from mice. By using internal limiting membrane (ILM) isolated from human retina for culturing MDCK epithelial cells, we demonstrate that BM composition, architecture and stiffness are required to act jointly in order to achieve apico-basal polarity, tissue barrier formation and stiffness within the same epithelial layer similarly to tissues in vivo. We reveal that activating ß1 and ß4 integrins has a key role, not only as previously thought for maintaining cell polarity but in establishing physiological mechanophenotype. These integrins are specifically activated by the BM laminin microarchitecture and stiffness on native BMs that is not present in the artificial substrates. Consequently, since epithelial functions that are linked to human development and disease, are tightly regulated by the composition, architecture and stiffness of human tissue BMs, our data indicate that reconstituted and animal models of BMs need to be used with caution.

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Tagging and tracking multiple generations of cells with intracellular lasers

Marcel Schubert, University of St Andrews, UK

Tracking individual cells among large cell populations is critical to a number of important biomedical questions e.g. for studying the progression of cancerous tissue, the composition of circulating tumour cell clusters or the migration of cells during differentiation. Surprisingly, generating laser light directly within live cells bears huge potential to realize a new generation of fast, robust and reliable cell tags.

Polymer microspheres doped with a fluorescent dye trap light by total internal reflection and the resulting whispering gallery modes (WGM) facilitate lasing upon optical pumping. Recently, we and others showed that these WGM microresonators can be internalized by live cells and that intracellular lasing can be achieved without harming the cells. Importantly, due to minute differences in size, each resonator features a characteristic lasing spectrum providing each cell with a unique barcode-type tag.

Here, we demonstrate that treating the surface of WGM resonators strongly improves the efficiency of resonator delivery to a broad range of cell types, including primary cells from the nervous system as well as cancer and neuronal cell lines. We show that tracking experiments can be performed over several days and in particular that resonators remain inside cells during cell division. The ability to robustly tag and track mitotic and non-phago-cytic cells over an extended time-frame may help to reveal distinct properties of single cells that are so far hidden by the dynamics and characteristics of large cell populations.

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Polarised Light Sheet Tomography

Sascha L. Reidt, University of Dundee, UK

Three-dimensional imaging is key to the discovery and understanding of larger systems in developmental biology or plant science. Light-sheet imaging has made a large impact in this field, such that it is becoming a widely used modality for monitoring developing samples. While light-sheet microscopy is mostly used for fluorescence imaging, Light-Sheet Tomography, a related technique that solely relies on scattered light, shows great promise. The method was successfully applied to imaging of plant roots in transparent soil, but is limited when it comes to more turbid samples.

Imaging at depth is a well sought after addition to 3D imaging. This is especially a challenge when imaging in highly scattering samples. For successful imaging at depth the highly scattered photons must either be filtered or used to benefit the data acquired. One such way to achieve this is to utilise the polarisation aspect of light, which has been used to great effect in skin pathology, but has never been applied to orthogonal collection of polarised light, in a light-sheet regime, before.

We present a Polarised Light-Sheet Tomography system and its advantages when imaging in highly scattering turbid media. The experimental configuration is guided by Monte Carlo Radiation Transfer methods, which model the propagation of a polarised light sheet in the sample. The method shows this new form of light sheet tomography for phantoms in a complex collagenous matrix, and allows for a large increase in contrast through the reduction of Rayleigh scattered light when compared to Mie scattered light.

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Session 3: Monday 22nd August 14:00 – 15:45

Wavefront shaping techniques for advanced optical imaging
Monika Ritsch-Marte, Medinische Universität Innsbruck, Austria

Wavefront shaping with spatial light modulators (SLMs) has become an indispensable tool in optical imaging. With this option microscopy becomes programmable and customizable with respect to a given sample. Another strength of this synthetic holography approach with programmable phase masks is the possibility to “pack several things into one hologram”, for instance to multiplex different imaging modalities, focus depths, or other parameters. Opportunities as well as limitations of the approach will be discussed in terms of a general information-content argument applicable to optical imaging.

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Investigation of cell mechanics using Nanodevices and Nano-instruments: some examples

Christophe Vieu, LAAS-CNRS, France

It is now well established that to perform their various functions, cells undergo a large range of intra and extracellular events, which involve mechanical phenomena at both the micro and nanoscale. Cells are able to sense forces and stiffness (mechanosensing) and to transduce them into a cascade of biochemical signals leading to a context specific cell response (mechanotransduction). At the core of the mechanical activity of cells are the components of their cytoskeleton acting as contractile cables actuated by proteic nanomotors. The nanoscale is thus the appropriate one for investigating the organisation of the active mechanical components and also for the measurement of the exerted forces at a subcellular level. On the other hand the microscale is adapted for upscaling these investigations to cell aggregates and tissues. The nanomechanics of cells is today a flourishing domain of activity in which new methods derived from micro/nanotechnologies have been developed for shedding some light and quantitative values in the mechanosensing properties of cells. This fundamental activity in cell biology meets some medical perspectives as mechanical properties of cancer cells and tumours turned out to differ significantly from normal cells or tissues.

After a short presentation of the biological knowledge related to cell mechanics, I will present some elegant methods coming from the micro/nano community that starts to become standard methods. In particular at the nanoscale, the use of Atomic Force Microscopy (AFM) to sense the rigidity of cells or to measure the force exerted by living cells will be exemplified through the investigation of human macrophages. At the microscale, I will show how the forces generated by adherent cells can be investigated using flexible micrometric pillars of polydimethylsiloxane (PDMS) and how this method can be upscaled to measure the forces generated by growing aggregates of cells in the context of tumor growth and metastasis nucleation.

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Imaging strategies for scattering tissues

Martin J. Leahy, National University of Ireland, Galway, Ireland

This paper will report several modalities developed for imaging of human skin. Imaging the skin helps to reveal it structure and function which are still not well understood. Better knowledge is required to understand the mechanism of diseases such as various forms dermatitis, eczema, psoriasis, progeria and cancers, especially melanoma. Laser Doppler has been available commercially since the late 1980s and in imaging form since the 1990s. It is a versatile imaging instrument which has been applied to many research questions, but has struggled to find mainstream acceptance as a diagnostic imaging instrument, with the possible exception of burns. We became interested in optical coherence tomography (OCT) when it became possible to use 1300 nm light sources and image sufficiently fast to avoid motion artefact on living subjects. We developed the correlation mapping optical coherence tomography (cmOCT) algorithm to more elegantly render the microcirculation. cmOCT operates by comparing two adjacent or better repeated B-Scans in the same location and rendering the areas with low correlation in a bright colour to indicate flow. Examples of skin imaging will be shown. While the images of structure and function with OCT are impressive, it is generally limited in depth penetration to about 1 mm in practice. Photoacoustics combines the advantages of power and light to get greater penetration depth along with spectral sensitivity to view chromophores and measure oxygen saturation. These measurements are currently limited due to the lack of knowledge of the local fluence, but several schemes have been proposed to solve this. Depths of many millimetres can be imaged using photoacoustics and the limit is the diffuse penetration depth of the light used. This can be up to 5-7 cm in theory. Imaging scattering tissues has advanced significantly, but there is much more to do to obtain the resolution and sensitivity at clinically useful depths. Indeed, as will be shown, regardless of modality, the depth resolution is never better than the depth divided by 200.

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Novel single-fibre probes for advanced biophotonics applications

Ivo T. Leite, University of Dundee, UK

Optical imaging within turbid media such as biological tissue is typically limited to penetration depths of up to hundreds of micrometres due to multiple scattering events occurring at inhomogeneities in the volume of the sample, which limit the attainable imaging resolution. Applications demanding imaging with optical resolution deep within the turbid sample thus require the use of an endoscopic probe, which naturally can pose constraints by damaging the surrounding environment.

Recent advances in holographic beam-shaping in multimode fibres have shown a great potential for devising minimally invasive probes comprised of a single optical fibre. Until now, most work in this field has been grounded on the well-established technology of optical fibre manufacturing, which was developed mainly for applications in optical communications. However, qualities such as low-cost and low-attenuation which are greatly appreciated for kilometre-scale networks are in general not beneficial for imaging applications - where the scales involved are in the order of centimetres - as they typically come with a cost in unnecessarily large cladding dimensions and low NA.

In this contribution, we will introduce a new concept of optical fibres with optimised design for applications as single-fibre imaging probes, and high-performance imaging and optical manipulation which will pave the way for the further development of advanced biophotonics applications.

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Session 4: Monday 22nd August 16:05-17:50

Looking Deeply in Living Biology: Finding Nemo's Heart
John Girkin, Durham University, UK

Since its invention around 400 years ago optical microscopy has played a crucial role in biology, as observation is the key component of all science. In many cases the ultimate test is to observe sub-cellular events *in vivo* and this clearly creates challenges; namely sample movement and the distortion of the images as one observes ever more deeply within the sample. This presentation will explore the recent advances in imaging methods to overcome these issues. After a brief discussion on the approach to multidisciplinary research, work will be presented in which live beating Zebrafish hearts have been imaged in real time with micron resolution through novel methods of optical gating. However, as one looks more deeply into the sample the tissue through which you are imaging distorts the view. This is similar to the challenge faced by ground-based telescopes. By borrowing adaptive optics technology, originally developed by astronomers, using a single plane illumination microscope we have been able to image at depth within a range of live samples with both high spatial and temporal resolution.

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Multidimensional Proteomic Analysis of Disease Mechanisms

Angus Lamond, University of Dundee, UK.

Cell regulation and disease mechanisms can now be studied in unprecedented detail, combining high throughput '*omics*' techniques, including mass spectrometry (MS) based proteomics and RNA seq with image-based phenotypic assays using microscopy. If properly designed, these methods provide the opportunity for making unbiased, system-wide, quantitative measurements of gene expression and phenotypes that underpin mechanisms carrying disease. The opportunity here is clear: modern quantitative proteomics in particular can reveal detailed insights into proteome dynamics, providing a flexible suite of quantitative assays that we can use to characterize, system-wide, a multi-dimensional array of '*Protein Properties*'. Thus, time-dependent measurements, with isoform resolution, of protein abundance, subcellular protein localization, turnover rates, post-translational modifications, cell cycle variation and specific protein complexes and protein-protein interactions etc. allow a detailed characterization of cell phenotypes in both healthy and diseased cells. One of the major challenges in using proteomics and other '*omics*' methods to study molecular mechanisms affecting biological regulation is how to manage, analyse and integrate the huge volumes of complex, quantitative data that are generated. I will describe some of the current projects in which we are doing quantitative proteomics to study gene expression mechanisms relevant to cancer, immune cells and nutritional responses in humans and in model organisms. I will also review our progress in building user-friendly, computational tools for the effective management and sharing of large, multidimensional data sets with the life sciences and biomedical research community.

This event is funded from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under REA grant agreement n° 608133

Learning from examples in optics

Demetri Psaltis, Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland

We consider optical imaging systems in which the detected output is not directly a reproduction of the object yet it is possible to derive an image of the object by post-detection digital processing. We will focus on learning methods to assist in the image formation process. Specifically the optical system is illuminated with various input patterns and the corresponding optical response is recorded. The set of input-output pairs can be thought of as examples with which we can train the system. We consider two cases: Imaging through multimode fibers and optical tomography. For multi-mode fibers the learning procedure can be thought of as a calibration step that converts the fiber into a device with imaging capabilities similar to a lens. This can lead to interesting applications in endoscopy. In the case of tomography, we construct a multi-layer neural network that simulates light propagation. The adaptable parameters of the network correspond to the voxel values of the 3D object. Training the network to reproduce the experimentally recorded input-output pairs yields the 3D image of the object. Our results suggest that the learning method yields an image quality better than other tomographic reconstruction methods.

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Airy beams for light-sheet microscopy

Jonathan Nylk, University of St. Andrews, UK

Propagation-invariant light modes have played a key role in developing high-resolution light-sheet microscopy (LSM) techniques as they can overcome the divergence associated with a traditional Gaussian beam, enabling uniform light-sheet thickness over large distances. Of these special beam types, Bessel beams and Airy beams have been of particular interest. The propagation-invariance of these beam types is achieved at the cost of an extended transverse beam profile, leading to a broad light-sheet profile. Bessel-LSM techniques achieve high-resolution imaging through pre-processing methods (e.g. confocal line scanning or structured-illumination), adding complexity to the optical system or acquisition protocols. The properties of the Airy beam make Airy-LSM very suitable for computational deconvolution, and thus high-resolution imaging can be achieved through post-processing only. Airy-LSM can yield isotropic high-resolution imaging over a ten-fold larger field-of-view (FOV) compared to a Gaussian light-sheet, and without additional, unnecessary irradiation of the specimen.

I will introduce the concept of Airy-LSM, explaining the benefits of the Airy beam for LSM; high spatiotemporal resolution, large FOV, low photo-bleaching, and photo-toxicity. The use of controlled aberrations in simple optical components will be discussed, enabling ultra-compact implementations of Airy-LSM.

I will describe recent experiments to quantify the ability of Airy-LSM to image at depth in turbid specimens. Preliminary results show a three-fold improvement in the signal-to-noise ratio (SNR) with Airy-LSM compared to traditional Gaussian-LSM at a depth of 50µm within mouse brain tissue, and enables imaging at 50% greater depth into tissue.

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Session 5: Tuesday 23rd August 08:30 – 10:15

Multidimensional Fluorescence Imaging across the scales
Paul French, Imperial College, UK

We are developing a multidimensional fluorescence imaging platform to provide 2-D and 3-D functional imaging across the scales with a particular emphasis on fluorescence lifetime imaging (FLIM) to provide contrast between different molecular species, including fluorophore labels or endogenous fluorophores, and variations in the local fluorophore molecular environment. The inherently ratiometric nature of FLIM, which makes it relatively insensitive to fluorophore concentration or signal attenuation by the instrument or sample, enables quantitative FLIM-based readouts to be translated from in vitro assays to in vivo preclinical and clinical imaging. The potential for enhanced optical molecular contrast has led to FLIM being increasingly applied to tissue autofluorescence to read out changes in cellular metabolism or tissue matrix components, with our laboratory applying FLIM to cancer, heart disease and osteoarthritis. The capability to sense the local fluorophore environment may be exploited using probes sensitive to specific analytes, such as signalling molecules, or it may be used to probe interactions by sensing the close proximity of other fluorophores via Forster resonant energy transfer (FRET), which can be exploited to assay cell signalling processes through protein interactions and genetically expressed biosensors.

We apply FLIM technology across the scales from 3-D STED FLIM microscopy, incorporating adaptive optics to correct for aberrations, through high-speed FLIM of fixed and live cells, e.g. for HCA including FRET assays of protein interactions or cellular metabolism in automated multiwell plate readers, to in vivo preclinical and clinical imaging. For in vivo preclinical studies, we are developing optical projection tomography (OPT) with FLIM, particularly applied to zebrafish, and FLIM endoscopy. For clinical studies we are developing multiphoton multispectral FLIM to diagnose skin cancer and single point fibre-optic probes for time-resolved and spectrally resolved fluorometry of autofluorescence, including an ex vivo and in vivo studies of cancer in skin and colon.

Our FLIM analysis utilises our open source software, *FLIMfit*, which provides rapid global fitting capabilities and is available as an OMERO client. We are working to develop a comprehensive low-cost, modular, open source fluorescence microscopy platform, utilising µManager and have implemented this for FLIM HCA and OPT.

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Advances in adaptive optics for nanoscopy

Martin J Booth, University of Oxford, UK

Adaptive optics (AO) is used to correct the effects of specimen induced aberrations. These distortions of the optical wave front are compensated usually with a deformable mirror or a liquid crystal spatial light modulator. AO methods have been applied to different microscopes with various applications in biomedical imaging and other areas. Adaptive microscopy is now being developed for super-resolution microscopes. We report on specific recent developments in adaptive aberration correction for stochastic optical reconstruction microscopy (STORM) and stimulated emission depletion (STED) microscopy. STORM – and equivalent methods – usually rely upon fitting to an aberration-free point-spread-function model, which may not represent the actual imaging properties in aberrating specimens. We have introduced adaptive optics to compensate specimen-induced aberrations showing improved 2D and 3D STORM imaging with cells at depths up to 10um. STED microscopy provides enhanced resolution by restricting the region of fluorescence emission to a size far smaller than the diffraction limited focus of the excitation laser beam. This is achieved by superimposing the excitation focus with a second structured depletion focus that has a region of zero intensity at the centre of a bright ring. The depletion focus is however susceptible to the effects of aberrations, leading to a filling in of the zero intensity or a change in intensity in the bright ring focus. This in turn leads to compromised resolution and a drop in fluorescence signal. We have developed AO methods to overcome these problems by correcting aberrations in tissue specimens. We demonstrate a range of implementations of AO including in a 4pi STORM microscope that uses two opposing objective lenses to increase the signal and resolution of 3D super-resolution images.

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Imaging cortical activity in vivo: from neuronal networks to single spines

Nathalie Rochefort, University of Edinburgh, UK

Brain functions such as sensations and thoughts depend on the coordinated activity of neuronal networks in precise spatial and temporal sequences. In vivo calcium imaging fills a unique niche in correlating the activity of neuronal populations to behaviour. This approach allows monitoring the activity of specific types of neurons at the scale of neuronal populations, as well as at the level of individual dendrites and spines, in awake behaving animals.

I will present applications of this method in the mouse primary visual cortex, showing how behavioural context modulates neuronal activity in this cortical area.

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Experimental study of optimum repetition rate and pulse duration for multiphoton microscopy at 1030 nm

Shamil Mirkhanov, University of Dundee, UK

Multi-photon imaging (MPI) allows high resolution deep tissue imaging with less phototoxicity and photodamage than confocal microscopy. Traditionally, MPI systems use a relatively high cost titanium sapphire laser, but recently the significant increase in both available florescent molecules and compact laser systems such as fiber lasers has provided the potential for lower cost MPI systems. These new laser systems can have different repetition rates and pulse durations to titanium sapphire lasers, therefore a figure of merit (FOM = $P_{peak}P_{ave}$) was introduced, suggesting that provided the FOM remain constant, the repetition rate, average power and pulse duration could be varied with no effect on MPI signal within an optimum operating range [1]. Here we experimentally test the applicability of the FOM using a commercial fiber laser and microscope. We measure the signal produced from both fluorescein labeled beads and fixed SYTOX Green labeled mouse intestine samples as a function of average power and repetition rate between 90MHz and 2.85MHz with 350fs pulses, and as a function of average power and pulse duration from 120fs to 3ps at 45MHz repetition rate. We find that for both fluorescent markers there are operating ranges where the FOM describes our observations well, but observe operating ranges where the signal does not scale as predicted due to photodamage, microscope dispersion and photobleaching. These studies provide insights for optimizing laser systems for low cost, compact MPI microscopes.

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Session 6: Tuesday 23rd August 10:35 – 12:05

Feeling for cell function: mechanical phenotyping at 1,000 cells/sec
Jochen Guck, TU Dresden, Germany

The mechanical properties of cells have long been heralded as a label-free, inherent marker of biological function in health and disease. Wide-spread utilization has so far been impeded by the lack of a simple and convenient measurement technique with sufficient throughput. To address this need, we have introduced real-time deformability cytometry (RT-DC) for the continuous mechanical single-cell characterization of large populations (> 100,000 cells) with analysis rates up to 1,000 cells/s, approaching that of conventional fluorescence-based flow cytometers. Using RT-DC we can sensitively detect cytoskeletal alterations, distinguish cell cycle phases, track hematopoietic stem cell differentiation into distinct lineages and characterize cell-populations in whole blood by their mechanical fingerprint. Our results indicate that cell mechanics can define cell function, can be used as an inherent cell marker and could serve as target for novel therapies. Mechanical phenotyping adds a new functional, marker-free dimension to flow cytometry with diverse applications in biology, biotechnology and medicine.

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Increased variability in precancerous *Apc*$^{Min/+}$ intestinal tissue can be measured with microultrasound

Inke S. Näthke, University of Dundee, UK

Altered tissue structure is a feature of many disease states. Usually it is measured by microscopic methods, limiting analysis to small areas. The ability to rapidly and quantitatively measure the structure and organisation of large tissue areas would represent a major advance not just in the clinic but also for research. To establish whether ultrasound could provide such a tool, we compared measurements using microultrasound and three-dimensional, high-resolution microscopy. We used a well-established mouse model system for intestinal cancers. Specifically, we aimed to measure changes in tissue organisation that result from heterozygosity in *Apc*, a precancerous situation in the intestine. Optical microscopy and microultasound both revealed a significant increase in the variability of tissue organisation in *Apc* heterozygous tissue. This was despite its normal appearance in conventionally examined cross-sections suggesting that the changes we detected preceded the formation of aberrant crypt foci or adenoma. Measuring premalignant changes using microultrasound provides a potential means to detect microscopically abnormal regions in large tissue samples, independent of visual examination or biopsies. This provides a powerful tool for studying tissue structure in experimental settings. It also establishes microultrasound as a tool for detecting and monitoring tissue changes that could be developed into a powerful adjunct to screening endoscopy in the clinic. Based on these results, we are exploring means for incorporating ultrasound into capsule endoscopy to permit access to the small intestine, which is difficult to reach and thus screen by conventional endoscopy.

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Elastic Resonator Interference Stress Microscopy (ERISM) – A new and versatile tool to gauge biophysical cell-substrate interactions

Nils M. Kronenberg, University of St Andrews, UK

Monitoring mechanical forces applied by cells is considered a key factor to the understanding of a range of fundamental biological processes. Today, various biophysical techniques to investigate different types of interactions between cells and the substratum they adhere to exist (e.g. Traction Force Microscopy) each having a specific set of advantages and limitations.

With ERISM we present a completely new and highly versatile approach to measure a wide range of cell-substrate interactions by interferometrically detecting deformations of an elastic probe with which the cells are in contact. The centrepiece of our innovation is an elastic, optical micro-cavity that enables fast displacement mapping with a large dynamic range and with nm-accuracy (sub-Pa stress resolution) by analysing local changes in resonance wavelengths. The micro-cavities used for ERISM have excellent long-term stability and the optical readout avoids any phototoxic effects. This allows us to continuously monitor cellular forces at high frame rate (up to 0.5 Hz), even for prolonged biological processes lasting hours or days. Cellular stress maps can be recorded without the need for zero-stress images, which eliminates the need to detach non-migrating cells after investigation and allows measurements of multiple cells on one substrate.

In this presentation we will explain the working principles of ERISM and discuss stress and lateral resolution by means of AFM indentation analysis. We will demonstrate the high accuracy and long-term, time-lapse capability of ERISM by showing mechanical measurements for different cell types, e.g. during amoeboid migration through spatial confinement and podosome-assisted migration of leukocytes.

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High-speed wavefront modulation in complex media

Sergey Turtaev, University of Dundee, UK

Since liquid-crystal-based spatial light modulators (LC-SLMs) became commercially available in the last decade, holographic algorithms have given birth to numerous exciting applications, such as aberration-free deep-tissue imaging, holographic optical manipulation as well as fibre-based holographic endoscopy.

Even though controlling of the wavefront allowed to correct for aberrations in tissues and enables deep-tissue imaging, samples with short speckle decorrelation time as well as raster-scan imaging techniques still pose a significant challenge for the current technology based on LC-SLMs, owing to limitations in their switching rate (typically 10s-100s Hz).

In recent years, following the trend on the market of multimedia projectors, where LC-SLM are being ousted by a new technology – the digital micromirror device (DMD), the interest in these modulators is rapidly growing in the community of complex photonics, mainly due to their comparatively higher speed and lower price. Off-axis holographic modulation algorithms allow converting DMDs into powerful instruments for complex field modulation with three order of magnitude higher switching rates.

In this work, we perform experiments on aberration correction in the ballistic as well as diffusive regimes, namely focusing through turbid media, using both DMD and LC-SLM. We highlight pros and cons of each type of SLM not only in terms of speed but also efficiency, scattering properties, and pixel cross-talk. We also show high-speed imaging through a single multimode fibre, which promises to open new avenues for real-time deep-brain imaging, allowing to study dynamic processes while minimising photobleaching and photodamage of targeted cells in raster-scanning fluorescent imaging.

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Session 7: Tuesday 23rd August 15:20 – 18:15

Imaging protein function and cancer phenotypes
Alan Serrels, University of Edinburgh, UK

Confocal and multiphoton imaging in the mouse has become a powerful tool in pre-clinical cancer research, and has yielded new insights into cancer cell behaviour, the tumour microenvironment, and the molecular mechanisms driving the pathogenesis of cancer. We have taken advantage of emerging technologies to custom build a multi-modal microscope combining two-photon fluorescence with CARS and SRS, and show that this enables detailed in niche imaging of cancer and the complex tumour environment without the need for extensive labelling. Furthermore, we show that SRS enables imaging of anti-cancer drugs in cells, highlighting the potential of this technology for cancer imaging and drug studies.

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Snapshot 3D-Ranging Microscopy with Extended Depth-of-Field

Andrew R. Harvey, University of Glasgow, UK

We describe a novel computational-imaging microscopy, Complementary-Kernel Matching (CKM), which provides, for the first time, snapshot three-dimensional imaging and ranging while also yielding an order-of-magnitude increase in depth of field. We demonstrate its application in bright-field and fluorescence microscopy and also for particle-tracking velocimetry of micro-fluid flow.

CKM is based upon wavefront coding in which a cubic-phase placed in the exit pupil of the microscope encodes the point-spread function in such a way that an in-focus image can be recovered for an extended depth of field. In our CKM technique two complementary images are recorded with differential defocus on a single detector array and the differential translation of the CKM point-spread functions with defocus enables z-displacement to be determined which in turn enables accurate image recovery devoid of the image artefacts that plague conventional wavefront-coded images. Recovered images have comparable image quality to those reconstructed using conventional z stacks of several tens of images, but with the advantage of snapshot operation. It thus uniquely offers extended depth-of-field, 3D imaging of transient phenomena and for particle-tracking velocimetry.

We report the implementation of CKM with a commercial microscope for reflection and transmission bright-field imaging and fluorescence imaging. Using a 40X, 0.75NA objective with a conventional DOF of < 1µm, the measured RMS error in the inferred depth was 0.15µm for an image SNR of 60dB and 0.25µm for an SNR of 30dB over a depth range of ~14µm. Additionally, we were able to localise 0.39µm-diameter fluorescent beads with a mean precision of ~14nm in the lateral dimensions and ~100nm axially using 4,000 photons per localisation over a depth range of ~12µm. The equivalent axial precision for a 100Å~, 1.4NA system would be 22nm. This is comparable to what is reported for 3D STORM using astigmatism from 6,000 photons but over a depth range more than twice as large and this can potentially be increased further.

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Challenges in building a fast scanning Super-Resolution STED microscope

Piotr Zdankowski, University of Dundee, UK

Fluorescence microscope has been a well-established and extremely useful technique to image biological structures. However, its resolution has always been restricted by the diffraction limit. Introduction of the super-resolution microscopy techniques that emerged at the turn of the last century allowed us to see into the nanostructures and step into the world of nanoscopy. Among the super-resolution techniques, Stimulated Emission Depletion (STED) Microscopy has been especially interesting as it relies solely on optical phenomena and does not require any additional processing. STED has proven able to resolve structures as small as 20nm. To date, there are a few companies that offer commercial STED microscopes, but their price tag is extremely high and they do not offer the flexibility of a home-built system. What is more, imaging through the thick tissues is a very challenging task as every biological sample introduces aberrations and scatters propagating light. This problem becomes even more significant in the STED microscopy as even small amount of aberrations can degrade the depletion beam shape substantially. Lastly, STED microscopy relies on very high depletion beam powers which can cause photodamage very quickly and it is important to minimise the exposition of the sample to the STED beam. It can be achieved with the fast resonant scanning of the sample. For that reasons we decided to build our own STED microscope with the AO correction of the depletion beam. Here we show progress towards building the fast scanning super-resolution AO STED microscope and the challenges this nontrivial task holds.

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Encapsulated SERS Nanosensors – Fibre-based pH Sensing

Holly Fleming, University of Edinburgh, UK

The identification of respiratory illness can be a complex process resulting in a multitude of drugs being prescribed, which may not always remedy the ailment. The implementation of a bedside based fibre-optic system, capable of sensing within the alveolar regions is highly desirable. Determining physiological parameters such as pH, O2, and CO2 could help facilitate an earlier diagnosis.

Here, we have developed a method of measuring pH in these difficult to reach regions, incorporating Surface Enhanced Raman Spectroscopy (SERS) sensors with an optical fibre based approach. Gold nanoparticles functionalised with the pH responsive reporter molecule were encapsulated within a microsphere and deposited onto the end of an optical fibre providing a robust method of measuring pH.

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Catching the perfect wavelet: multi-resolution signal analysis for biophysics

Q. Tyrell Davis, University of Dundee, UK

As physical scale decreases, mechanical systems are subject to an increasing amount of thermal perturbation relative to any deterministic activity of the system. By definition, studying a system where the activities of interest and thermal motion occur at the same order of magnitude means operating in a low signal-to-noise regime. This presents both a signal processing challenge and insight into the system under study: how does a given mechanical system with principal activities below the thermal noise floor decide what to do next? This question and accompanying signal processing challenges are fundamental to the molecular machines of biology, which often carry out their respective functions in single molecules and small cohorts. Multiresolution wavelet analysis of biophysical data (in this case optical tweezers data) can be optimized for relevant frequency/displacement scales of even weak molecular motor activity. Furthermore, wavelet analysis provides a framework for identifying signal changepoints associated with the activity of molecular machines which don't exhibit active transport.

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Lightwedge and Lightsheet-Raman Microscopy

Rainer Heintzmann, FSU Jena, Germany

Two recently developed modes of lightsheet imaging will be presented. **Lightwedge microscopy** aims at mesoscopic imaging of fixed and optically cleared samples at 1 µm isotropic resolution without the need for sample rotation. The key-idea is to focus a lightsheet at an unusually high NA (thus the name "lightwedge") and still obtain a large field of view due to refocusing of the lightwedge and stitching the multiple small regions of thin illumination back together. This has been simplified by electrical tunable lens technology which has become available recently.

The second mode is **hyperspectral Raman imaging** in a lightsheet illumination configuration. To recover the spectral information a full-field Fourier-spectroscopic approach has been chosen. The difficulty here is that in a Michelson approach, it would be technically very hard to maintain the angular stability and common path approaches usually tolerate a relatively low product of étendue and maximal optical path difference. We thus developed an optically stable Mach-Zehnder like scheme based on the use of retro-reflecting corner cubes, which is inherently stable. This enabled us to obtain full spectrally-resolved Raman images consisting of over four million spectra in about 10 minutes. Advantages over the conventional Raman imaging are the reduced maximum power on the sample and out of focus heating, the lightsheet-inherent good suppression of crosstalk from the illumination side and the avoidance of glass close to the sample mounting.

Light sheet illumination for Raman imaging at few specific wavelengths was previously reported. With a total laser power of 2 W at an illumination wavelength of 577 nm, we obtained images (2048 × 2048 pixels) of polystyrene beads, zebrafish and a root cap of a snowdrop at a spectral resolution of 4.4 cm^{-1} with only few minutes of exposure. The olefinic and aliphatic C-H stretching modes, as well as the fingerprint region are clearly visible along with the broad water peak of the embedding medium.

Spectrally resolved spontaneous Raman microscopy therefore promises high-throughput imaging for biomedical research and on-the-fly clinical diagnostics.

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Holographic micro-endoscopy based on multimode waveguides

Tomas Cizmar, University of Dundee, UK

The turbid nature of refractive index distribution within living tissues introduces severe aberrations to light propagation thereby severely compromising image reconstruction using currently available non-invasive techniques. Numerous approaches of endoscopy, based mainly on fibre bundles or GRINlenses, allow imaging within extended depths of turbid ĕssues, however their footprint causes profound mechanical damage to all overlying regions and their imaging performance is very limited.

Progress in the domain of complex photonics enabled a new generaĕon of minimally invasive, high-resoluĕon endoscopes by subsĕtuĕon of the Fourier-based image relays with a holographic control of light propagating through apparently randomizing multimode optical waveguides. This form of endo-microscopy became recently a very attractive way to provide minimally invasive insight into hard-to-access locations within living objects.

Here, we review our fundamental and technological progression in this domain and introduce several applications of this concept in bio-medically relevant environments. We present isotropic volumetric imaging based on advanced modes of light-sheet microscopy: by taking advantage of the cylindrical symmetry of the fibre, we facilitate the wavefront engineering methods for generation of both Bessel and structured Bessel beam plane illumination. Further, we demonstrate the first utilization of multimode fibers for imaging in living organism. We present a new fibre-based geometry for deep tissue imaging in brain tissue of a living animal model.

Lastly we show the development and exploitation of highly specialised fiber probes for numerous advanced bio-photonics applications including highresolution imaging and optical manipulation.

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Biophotonic approaches: From molecules to living systems

Poster Session: Tuesday 23rd August 13:30 – 15:00

1. **Single-lens Quantitative Phase Microscopy**
   Henry Axt, University of Dundee, UK

2. **A tumour associated APC fragment changes the response of MDCK cells to compression**
   Valerie Bentivegna, University of Dundee, UK

3. **Imaging to Assess the Risk of Cardiovascular Disease Using a Targeted Nanoparticle DNA Reporter System**
   Kirsty Callan, University of Strathclyde, UK

4. **MIPs coupled to quantum dots as tools for the detection of tumour angiogenesis in zebrafish**
   Alessandra Cecchini, University of Dundee, UK

5. **Characterisation of Bessel and Gaussian beam illumination modes on LSM imaging quality of early chick embryo development**
   Ricardo Bango Da Cunha Correia, University of Dundee, UK

6. **Improved antireflection coated microspheres for biological applications of optical tweezers**
   Valentina Ferro, University of Dundee, UK

7. **Towards SESORS Imaging of Breast Cancer Using Responsive Enhanced Raman Reporters**
   Anastasia Kapara, University of Strathclyde, UK

8. **Water-Based Optofluidic Distributed Feedback Lasers with Evanescent Pumping**
   Markus Karl, University of St Andrews, UK

9. **Characterisation of heterogeneity in prostate biopsies using optical coherence elastography and second harmonic generation microscopy**
   Yuting Ling, University of Dundee, UK

10. **Organic light-emitting diodes optimised for optogenetics**
    Andrew Morton, University of St Andrews, UK

11. **Photonic Quantum Metrology With Blue Photons: Towards Application of Quantum Metrology in Bioimaging**
    Jason Mueller, University of Bristol, UK

12. **Lectin-Functionalised Silver Nanoparticles for Bacterial Detection by Surface Enhanced Raman Spectroscopy (SERS)**
    Evita Ning, University of Strathclyde, UK

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13. **Diagnosis of kidney disease by structured illumination microscopy**
    Jonathan Nylk, University of St Andrews, UK

14. **Light-sheet fluorescence lifetime imaging**
    Daniel O'Brien, University of Dundee, UK

15. **Imaging plant- pest interactions in vivo in transparent soil**
    Felicity O'Callaghan, James Hutton Institute, UK

16. **Development of Fluorescent, Targeted, Ultrasonic Contrast Microbubbles for Optical and Ultrasonic imaging**
    Adeel Shafi, University of Edinburgh, UK

17. **Combined Optical Flowmetry, Oximetry and Fluorescence Measurement of Novel Markers for Vascular Dysfunction**
    Salvatore Smirni, University of Dundee, UK

18. **Applications with compact Light Sheet Fluorescent Microscope**
    Zhengyi Yang, University of St Andrews, UK

19. **Probes for oxygen sensing in biological systems**
    Patricia Zhu, University of Edinburgh, UK

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PHOQUS conference 2016

Some information on PHOQUS

PHOQUS (PHOtonic tools for Quantitative imaging in Cells and tissUeS) is funded by the EU's FP7 Marie Curie Innovative Doctoral Programme to train 13 early stage researchers as interdisciplinary scientists at the interface between Physics/Photonics, Medicine and Life Sciences. The fellows are primarily based at the University of Dundee in Scotland however they also have access to academic and commercial expertise and training provided by academic and industrial and partners from across Europe.

Specific aims of PHOQUS are to

- Train a new generation of exceptional scientists in the life and physical sciences, without the historic barriers that have existed between these disciplines
- Develop new photonics tools that will feed into the design and development of smaller, more cost effective instruments
- Use these new tools to investigate the cellular and molecular dynamics which drive the process of cell division
- Use newly developed imaging techniques to investigate the role of cell behaviours during embryonic development and disease.

For more information see www.phoqus.eu

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