Visualising Cellular Function in vivo

The BioPark Hertfordshire, Welwyn Garden City, AL7 3AX: 24th Oct 2008

This meeting has CPD accreditation

9:00 – 9:45 **Registration**

9:45 – 10:00 **Introduction by the Chair:** *Dr Rob Fowkes*,, Royal Veterinary College, UK

10:00 – 10:30 **High Resolution Fluorescence Microscopy with Structured Illumination**
Dr. Rainer Heintzmann, Kings College, London

10:30 – 10:45 **In living color: Optical imaging of multiple molecular targets *in vivo* and *ex vivo***
James R. Mansfield, Director, Multispectral Imaging Systems, Cri, UK
Insights gained in characterizing intracellular pathways and other cellular phenotypes have led to increased demands on all kinds of imaging systems, which now are being asked to report on the status of multiple targets simultaneously. One factor that has interfered with the ability to image fluorescently labeled markers *in vivo* has been unwanted autofluorescent signals. Multispectral imaging (MSI) methodologies can spectrally characterize and computationally eliminate autofluorescence, revealing otherwise invisible molecular targets. Application of MSI can increase sensitivity by orders of magnitude, allowing much less abundant (or dimly labeled) targets to be detected and measured. MSI also is a perfect complement to multiplexed analyses, with as many as five exogenous probes being imaged *in vivo* simultaneously. In addition, we will present a simple but powerful *in vivo* optical imaging approach that can create co-registered small animal anatomical maps using standard optical imaging instrumentation. The technique of Dynamic Contrast Enhancement (DyCE) takes advantage of the differing biodistribution dynamics of a small bolus of a tracer dye (such as indocyanine green). A time series of multi-view images acquired following injection can be analyzed to generate a high-resolution delineation of the major anatomical organs of nude mice, providing enhanced anatomic context for locating specifically labeled targets. Microscopy-based multi-analyte immunohistochemistry, in brightfield or fluorescence, has many potential applications in the field of drug-target evaluation. However, accurate imaging of two or more co-localized antigens, especially chromogenically labeled ones, has been hindered by difficulty in discriminating and quantifying overlaying signals. MSI can resolve overlapping labels and generate quantitative images of individual analytes. As in the *in-vivo* case, MSI is well suited to detecting and removing autofluorescence in fluorescence microscopy, allowing more sensitive and quantitative studies. Assessment of simultaneous (per-cell) expression of ER, PR and Her-2 expression in breast cancer using chromogenic labels and the imaging highly multiplexed quantum-dot-labeled immunofluorescence signals in tissue will be shown. The advantages of linking in-vivo and ex-vivo studies will be developed.

10:45 – 10:55 **Speakers photo**

10:55 – 11:30 **Mid-morning break**

11:30 – 12:00 **Live cell imaging of GnRH receptor signalling**
Professor Craig A McArdle, University of Bristol, UK
Gonadotrophin-releasing hormone (GnRH) is a decapeptide that acts via Gq/11 coupled GPCRs to control the synthesis and secretion of gonadotrophin hormones in pituitary gonadotrophs. We have been using an automated imaging platform to explore various aspects of GnRH receptor function. This will be described with emphasis on receptor trafficking and signalling via ERK and Ca^{2+}.

12:00 – 12:30 **Glucocorticoid receptor trafficking**
Professor David Ray, University of Manchester
The glucocorticoid receptor (GR) is a ligand activated transcription factor. It is found in the cytoplasm in quiescent cells, but rapidly translocates to the nucleus after ligand binding. However, there appears to be a slow, ligand-independent nuclear translocation that is linked to the cell cycle, and so immediately post-mitotic cells have exclusively cytoplasmic GR, which over hours translocates to the nucleus. Closer examination of GR distribution during mitosis revealed unexpected co-alignment of phosphorylated GR with the condensed chromosomes, while the bulk of the GR molecules locate peripherally within the cells. The phospho GR remains associated with the microtubules as chromosomes separate through anaphase and telophase. This unexpected, ligand independent trafficking of GR molecules suggests novel functions for the molecule, and these are currently under investigation

12:30 – 12:50 **Introduction to the BioPark**

12:50 – 14:00 **Lunch and Poster Viewing**

14:00 – 14:30 **Assessing Stem Cell Therapy *in vivo* using Imaging Technologies**

Dr Kishore Bhakoo, Imperial College, London

Stem cells have significant therapeutic potential to replace diseased cells since they can produce more stem cells and can generate specialised cell types. Tracking stem cell transplants by *in vivo* imaging will aid our understanding of how stem cells mediate functional recovery after transplantation. A major challenge for the development and refinement of stem cell transplantation is to map the spatial distribution and rate of migration *in situ*. We use magnetic resonance imaging (MRI) to visualise and track transplanted stem cells tagged with either paramagnetic nanoparticles, such as dextran-coated iron oxide, or other MR contrast agents. This technique could also be used to trace other cells types, such as those from a tumour and immune cells

14:30 – 14:45 **Near-Infrared Optical Imaging of Live Mice**

Dr Dan Gare, Senior Applications Scientist, Licor, UK

There are significant advantages to working in the Infrared region of the spectrum for in vivo imaging. These include less light absorption and scattering by endogenous chromophores found in living tissue. Tissue also has a lower absorption coefficient in the near infrared region, allowing for deeper penetration of light and a minimal impact upon the dyes signal by interfering tissue autofluorescence. These benefits will be discussed with examples as well as tools to enable the infrared imaging.

14:45 – 15:15 **Imaging pituitary cell function in transgenic mice.**

Dr Paul Le Tissier, National Institute for Medical Research, UK

15:15– 15:45 **Afternoon Tea/Coffee and Last Poster Viewing**

15:45– 16:00 **Technology Showcase: Novel Bioluminescent Imaging Substrates**

Dr Stephenie Richards, Product Manager, Promega.

Although luciferin has been used extensively for imaging in mice, examples of substrates that can image more specific biological processes have recently become available. Modifications of luciferins, the proluciferins, can be used to alter the tissue distribution and the pharmacokinetics in mice, and most importantly, they can be used to image specific enzyme activity in the whole animal. We have modified luciferins and coelenterazines for a variety of enzymes, some for which the utility has been demonstrated in *in vivo* imaging applications and many others for which the potential exists.

16:00 – 16:30 **Novel technologies for cell-based assays – getting information from an image**

Dr Rachel Errington, Cardiff University, UK

Robust dynamic image-based cell assays, appropriate for a high-content-screening format, demand unique solutions which enable systematic image analysis and interrogation of spatio-temporal cellular events. Our fundamental dynamic assay comprises single cell lineages tracked through each bifurcation node (eg mitosis) from which we can obtain a continuous event map to determine cell growth, cell cycle checkpoint induction and generation-to-generation inheritance patterns. The approach has been to develop bioinformatics data mining tools capable of encoding and subsequently interrogating cell cycle response profiles for use in experimental therapeutics and predictive mathematical modelling.

16:30 - 17:00 **Chairman's summing up**

18:00 **Soiree at *The Best Western Homestead Court Hotel for all the participants**

*This meeting was **organised by Euroscicon** (www.euroscicon.com), a team of dedicated professionals working for the continuous improvement of technical knowledge transfer to all scientists. Euroscicon believe that they can make a positive difference to the quality of science by providing cutting edge information on new technological advancements to the scientific community. This is provided via our exceptional services to individual scientists, research institutions and industry. The event was hosted by **'BioPark'** (www.biopark.co.uk), a research and development centre in Welwyn Garden City providing specialist facilities and support for bioscience and health technology businesses to grow, and to develop new products and technologies*

About the Speakers

Dr. Rainer Heintzmann is Head of the "Biological Nanoimaging" research group, Randall Division, King's College London

Professor David Ray, Graduated in Medicine, and later obtained a PhD from University of Manchester, before post doc post at UCLA. On return to the UK obtained a GSK fellowship to study glucocorticoid/cytokine interactions, which allowed him to establish an independent research group. Current interests are control of glucocorticoid receptor function, analysis of glucocorticoid sensitivity, and the mechanisms of action of glucocorticoids in inflammatory disease.

Dr Dan Gare, Undergrad in Biology from Imperial College and PhD in molecular parasitology from University of Aberdeen. Post docs in University of Cambridge before moving to LI-COR as application scientist. Now specialises in proteomics and optical imaging for UK, Ireland and Scandinavia.

James R. Mansfield, is responsible for the development of the award-winning Nuance and Maestro multispectral imaging systems at CRi. These systems are widely used in pathology and microscopy research as well as for optical small animal imaging.

Dr Kishore Bhakoo, received his BSc from the University of Kent at Canterbury and PhD from the Institute of Neurology, London. His Postdoc exerience was at theLudwig Institute for Cancer Research, London, Royal College of Surgeons, London and Institute of Child Health, London. He was a research Lecturer at theMRC Magnetic Resonance Unit at the University of Oxford and is now the MRC Group Head Senior and Lecturer at the MRC Clinical Sciences Centre, Imperial College London

Dr Rachel Errington's current interests focus on the development of high-content kinetic assays for screening the action of anti-cancer agents in cells and tissue. This includes the design of novel cellular systems to search for new therapeutic targets and the development of fluorescent probes for monitoring interlinking cellular events. At the core of the studies is the requirement to integrate cell biology and drug pharmacokinetics using mathematical modelling and computational biology. In addition, as part of the PK-PD modelling work it became clear we needed to develop and implement informatics tools for bringing coherence and data structures for handling microscopy and cell-assay information. To this end we have developed conversion tools for taking microscopy image data into multi-parameter databases enabling data access, mining and interrogation. Dr Errington is senior member of the UK Optical Biochips Consortium – a basic multidisciplinary research programme generating a portfolio of optical biochip technologies and prototypes, novel cell tracking dye and nanoparticle technologies and is pursuing patenting and translation activities. She is Director of Biostatus Ltd.

Professor Craig A McArdle, University of Bristol, UK has been an academic scientist at the University of Bristol since 1993. His research interests are in cell signalling and reproductive endocrinology and most of his work focuses on the hypothalamic decapeptide GnRH, that acts via G-protein coupled receptors on pituitary gonadotrophs to mediate control of reproduction by the CNS. Most of his recent work exploits image-based readouts (e.g. fluorescent reporters) using confocal microscopy and semi-automated wide-field fluorescence image acquisition and analysis (high content analysis) to monitor cellular compartmentalisation and trafficking of receptors and effectors.