

Improving Immunohistochemistry

Chiltern Hall, University of Westminster, 35 Marylebone Road, London

25th April 2008

9:00 – 9:40

Registration

9:40 – 9:55

Introduction by the Chair: *Dr Will Howat, Cambridge Research Institute, Cancer Research UK*

9:55 – 10:25

In situ analysis of inflammation in man and disease models

Professor Jon D. Laman, University Medical Center Rotterdam, The Netherlands

In situ dissection of immunopathogenic mechanisms underlying inflammatory disease is an exciting challenge. Inflammation involves complex interactions of the target organs each with its respective tissue barriers, autoantigens and composition, the immune system including the draining lymph nodes and its wide array of effector cells and molecules (cytokines, chemokines, antibody), and finally microbes of both pathogens and normal flora. The talk will provide prime examples of in situ analysis of human tissues as well as animal models (mice, non-human primates). It also aims to be very practical, providing ideas and access to protocols and references suitable for your own research

10:25 – 10:55

Multimodality Imaging and the Analysis Preclinical Therapeutics Studies

Dr Kenneth Olive, Cambridge Research Institute, UK

Pancreatic Ductal Adenocarcinoma is among the most lethal of human malignancies and is lacking in effective treatments. Current approaches to drug development rely on xenografts and other ectopic models which do not accurately recapitulate the drug response of human PDA patients. We show that a recently developed genetically engineered model of PDA exhibits the innate chemoresistance characteristic of human PDA. We also demonstrate a generalized defect in small molecule delivery as the underlying mechanism of chemoresistance, using multiple imaging techniques including immunohistochemistry, confocal microscopy, high resolution ultrasound, contrast ultrasound and dynamic contrast enhance MRI.

10:55 – 11:10

Multiplexing molecular markers *in vivo* and *ex vivo* with multispectral imaging

James Mansfield, Biomedical Systems, CRI, Inc., USA

In studying gene and protein expression in solid tumors and other tissues, it can be important not just to measure overall levels, but also to monitor spatial distribution, preserving architectural clues and cell-cell arrangements. This task is difficult in the case of colocalized signals, as well as in the presence of autofluorescence commonly found in formalin-fixed specimens. Multispectral imaging (MSI) greatly alleviates these difficulties, enabling high-resolution multiplexing and increased sensitivity, especially in the presence of significant autofluorescence. It can be used on standard microscopes, with no requirement for complex confocal instrumentation. In addition, the same approach can be used for pre-clinical small animal imaging. In tissue imaging, we present a brightfield study of estrogen receptor (ER) and progesterone receptor (PR) co-expression in breast cancer, and also highly multiplexed imaging of quantum-dot immunofluorescence labels. *In vivo*, MSI is used to characterize and eliminate the significant autofluorescence that can be present, revealing otherwise undetectable labeled targets, as well as enabling high levels of multiplexing, with as many as four target analytes being imaged simultaneously. Using MSI for both *in-vivo* and *ex-vivo* imaging can be especially valuable, since the same analytes can be tracked both macro- and microscopically.

11:10– 11:15

Speakers photo

11:15 – 11:35

Mid-morning break

11:35 – 11:50

Human tissue in drugable target validation: XpressWay™ and XpressArray™

Mr Richard Bystry, Molecular Expression Profiling, Asterand

Knowing which tissues your drug target is expressed in is invaluable. Knowing which cell populations it is in is priceless. Imagine qRT-PCR data from 72 tissues, each from 3 donors with full clinical histories and validated pathology reports. Now imagine adding value to that off the shelf qRT-PCR data using validated tissue microarrays containing the same 72 tissue types from 3 donors and carrying out fully optimised immunohistochemistry to define the cell populations in which your target protein is localised. XpressWay™ and XpressArray™ allow you to do this with minimum fuss and maximum impact

11:50 – 12:20

Banking Human Tissue for Research: Vision to reality

Dr Chris Womack, AstraZeneca UK

Advances in scientific understanding of disease together with introduction of new high throughput technologies have led to increased demand for human tissue in research. In general, patients are willing to donate for research, particularly samples that are surplus to diagnostic or therapeutic requirements. New tissue-specific regulations in the UK are intended to facilitate the use of human tissue in research. Despite this positive environment there are challenges to researcher access. Coordinated, systematic collection and storage, can provide easier access. However translating a vision for a biobank into reality whether in the public or private sector, has never been simple. But it can be done

12:20 – 12:50

Quality control for In situ hybridization

Dr Howard Pringle, University of Leicester, UK

Although the technique of *in situ* hybridization (ISH) was developed over 35 years ago it has not the same prominence in the literature as immunohistochemistry. This could be due to the fact that this technique is difficult to optimize as cells and tissues samples must be suitably handled, stored and fixed to preserve RNA and achieve optimal results. This presentation will describe the factors influencing the quality of RNA ISH using non-isotopic labelled probes. Examples will be used to demonstrate the important parameters that influence this technique as well as the choice of appropriate controls. The talk will initially cover the methods for handling cells and tissue samples. Then the ISH technique will be examined including; target gene validation, probe design, labelling, hybridization conditions, detection methods and signal evaluation. Is this technique sufficiently robust with the sensitivity and specificity needed to detect and localise gene expression in human and animal tissues?

12:50 – 14:00 **Lunch**

14:00 - 14:15

An automated expression screen for mutants affected in tracheal development

Dr Stefan Mauch, INTAVIS Bioanalytical Instruments, Germany

Many organs transporting liquids or gases, such as lung, kidney and cardio-vascular system, are formed by a branched network of epithelial tubes or endothelial vessels. Key molecular mechanisms of patterning and branching are mediated by growth factors and signalling cascades conserved in humans and the fruit fly *Drosophila melanogaster*. I will present a screen setup to identify proteins involved in formation of epithelial tubes in *D. melanogaster* using a system for automated whole mount ISH and IHC. Among others, two proteins, Megatrachea and Wurst, have been identified and characterized. Both share high sequence conservation with their corresponding orthologues in mouse and human.

14:15 – 14:30

Single Gene Copy detection with Silver-ISH

Dr Uwe SCHALLES, Ventana Medical Systems, France

14:30 – 15:00

Immunogold labelling: A review

Dr Jeremy Skepper, Technical Director Multi-Imaging Centre, Physiology Development and Neuroscience, Anatomy Building, University of Cambridge.

This review will attempt to describe the major immunocytochemical strategies that are in current use for electron microscopy. Emphasis will be placed on describing what techniques are available and what are their relative merits and limitations. Expensive equipment and technically complicated methods are not always necessary to collect robust data. I hope to present a review that will enable you to select the most profitable technique (or compromise) available, to reflect both what resources you have available and their ability to address your biological questions with vigor.

15:00 -15:30

Afternoon Tea/Coffee

15:30 – 15:45

Development in Automated Research Applications

David Roche, Development in Automated Research Applications, Leica Microsystems. UK

Fully automated IHC & ISH platforms have been used in clinical laboratories for a number of years. Here we look at 2 examples of how a fully automated, open system has assisted in research applications.

15:45 – 16:00

Reproducible protein expression and colocalization measurements in tissues

Tony Bradshaw, Application Specialist, Aperio, Ltd.

To translate a protein marker into clinical practice, the expression in tissues must be measured reliably, and in a format familiar to pathologists and histologists that can be run in a preclinical or clinical test environment. This presentation will discuss algorithm techniques, controls, and things to be aware of when measuring protein expression in tissues.

- The use of color deconvolution
- Measurement of membrane, nuclear, and cytoplasm markers with IHC algorithms
- Measurement of protein colocalization
- What is “quantitation” in biological samples? What is an appropriate denominator?
- Precision results from a multisite study of semiquantitative immunohistochemistry analysis

16:00 – 16:30

Molecular Pathology of Breast Cancer

Professor Carlos Caldas, Cancer Research UK, Cambridge Research Institute, UK

16:30 – 17:00

Chairman's summing up & close.

18:00

Soiree at *The HOLIDAY INN OXFORD CIRCUS* for all the participants (further details provided on the day of the event)

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.

About the Chair

Will Howat graduated with a BSC (Hons) in Immunology & Pharmacology from the University of Strathclyde, before gaining a PhD in Pathology from the University of Southampton. After two post-doctoral positions in Southampton, he moved to the Wellcome Trust Sanger Institute in Cambridge as the leader of Research & Development for the Immunohistochemistry group of the Atlas of Protein Expression project. He is now with Cancer Research UK as the head the Histopathology/ISH facility at the Cambridge Research Institute.

About the Speakers

Dr Jeremy Skepper is a professional microscopist with some 30 years experience in biological electron microscopy. The MIC is a research centre for the School of Biological Science in Cambridge. In addition to electron microscopy we have 3 confocal and 1 2-photon microscopes for immunofluorescence and live cell imaging. Lately I have begun to be more in microscopy of nanoparticles and of the biomaterials interface. We are currently developing new methods for imaging nanoparticles in cells at high resolution.

Professor Carlos Caldas is an Honorary Consultant Medical Oncologist at Addenbrooke's Hospital, Director of the Cambridge National Translational Cancer Research Network Centre, Fellow of the American College of Physicians, Fellow of the Royal College of Physicians and a Fellow of the Academy of the Medical Sciences. He joined the Cancer Research UK Cambridge Research Institute in summer 2006. His laboratory studies the genetic alterations underlying human cancers, with a focus on epithelial malignancies. They are interested in understanding how genetic alterations accumulate and how they determine the biological behaviour of cancers. Ultimately they aim to identify patterns that are predictive of outcome and can be used to guide therapy

Dr James Pringle graduated from Bristol (1979) in Applied Biological Sciences and completed a PhD in Biotechnology at the University of Warwick (1983). He studied DNA replication as a post-doctoral scientist in the Department of Molecular Biology, University of Edinburgh. Then moved to University of Leicester in 1985 as Non-clinical Lecturer in the Department of Pathology. He is currently a Reader in Molecular Pathology in the Department of Cancer Studies and Molecular Medicine at Leicester. His research includes molecular studies of skin, breast and colorectal cancer. He has developed and applied non-isotopic *in situ* hybridisation methods on human and animal tissues since moving to Leicester in 1985.

Stefan Mauch Studied biology at Albert-Ludwigs-Universität, Freiburg, Germany, focus on biochemistry 1996-2002. PhD at Max-Planck-Institute for Plant Breeding Research, Cologne, Germany 2002-2006. Joined INTAVIS as Specialist In Situ Detection in June 2006

David Roche joined Vision Biosystems in 2003 in Australia after a number of years working in histology both in the UK and Australia. David was on the design and validation teams for both the Peloris Dual-Retort Tissue processor, and latterly, the Bondmax fully automated IHC & ISH immunostainer. After being convinced to come back to the UK in 2005, David has been involved in field-based demonstrations, technical, sales & service support for both Peloris, Bondmax, and now Bondmax Research IHC platforms. At the beginning of this year Vision Biosystems & Leica officially merged, bringing Novocastra, Bondmax and Peloris (& David!!) under the Leica banner.

Professor Jon Laman trained as a medical biologist and immunologist at the Free University Amsterdam, and obtained a PhD in 1992 from Erasmus University Rotterdam on *in situ* analysis of antibody formation against HIV-1. He worked as a postdoctoral fellow in the US with dr. Randy Noelle (Dartmouth College) on CD40-CD40L interactions in B-cell responses and autoimmunity. He joined the Dept. Immunology at Erasmus MC in 1997, where his group closely works with the clinical departments Neurology, Rheumatology and Dermatology. *In situ* analysis is a major approach we use to unravel immunopathogenesis of inflammation and contributions of microbes.

Dr Chris Womack Qualified MB BS University of London in 1978, Trained in pathology at Manchester and Nottingham, Principal Clinical Histopathologist, Cancer Discovery Medicine, AstraZeneca, Alderley Park, Cheshire since Feb 2006. HTA Designated Individual., Chairman Histologix Ltd since May 2005, Previously consultant in histopathology and cytopathology at Peterborough District Hospital, 1986 – 2006, Author and co-author of papers, abstracts and presentations relating to research tissue banking. Co-founder of the Research Tissue Bank at Peterborough. Currently lead for the cancer tissue bank at AstraZeneca and histopathologist for the Manchester Cancer Research Biobank, Past President British Association for Tissue Banking.

Dr Kenneth Olive began his training at cancer genetics at MIT in the laboratory of Tyler Jacks, describing a mouse model of Li-Fraumeni Syndrome. Following this, Ken moved his focus to more translational studies of mouse models in the laboratory of David Tuveson, first at the University of Pennsylvania and currently at the CRUK Cambridge Research Institute. In his current research, Ken is looking to assess novel therapeutics in a genetically engineered model of pancreatic cancer.

DIRECTIONS FROM THE MEETING TO THE EVENING SOIREE

- | | |
|--|--------|
| 1. Head south on Luxborough St toward Nottingham St | 269 ft |
| 2. Turn left at Nottingham St | 476 ft |
| 3. Turn right at Marylebone High St | 0.3 mi |
| 4. Turn left at Hinde St | 141 ft |
| 5. Continue on Bentinck St | 344 ft |
| 6. Turn right at Welbeck St | 164 ft |

