

# Improving Immunohistochemistry - 2010

UCL Institute of Child Health, Friday, 30 April 2010

This popular annual event, now in its 7th year, is dedicated to the technique of immunohistochemistry and in situ hybridisation. This exciting meeting has been created to merge the need for technical-based updates in the areas of immunohistochemistry, clinical histopathology and *in situ* hybridisation. With a mixed array of speakers, this meeting should appeal to clinical, academic and pharmaceutical organisations. This event has CPD accreditation and will have a troubleshooting panel session. On registration you will be able to submit your questions to the panel that will be asked by the chair on the day of the event.

Meeting Chair: *Dr Will Howat*, Cambridge Research Institute, Cancer Research UK

- 9:00 – 9:45      **Registration**
- 9:45 – 10:00    **Introduction by the Chair:** *Dr Will Howat*, Cambridge Research Institute, Cancer Research UK
- 10:00 – 10:30    **Towards the molecular characterization of Circulating Tumor Cells**  
*Dr George V. Thomas*, Institute of Cancer Research and Royal Marsden Hospital, Surrey, UK  
There is increasing evidence that the presence of Circulating Tumor Cells (CTCs) in the blood is associated with the potential for metastases and subsequently poor prognosis. The recent developments have focused on the identification and enumeration of CTCs. I will present our efforts to molecularly characterize CTCs and personalize cancer therapy in the process.
- 10:30 - 10:45    **Leica Novocastra antibodies past, present and future: advances in IHC**  
*Dr Nigel H Piggott*, Principal Development Scientist - Leica Biosystems Newcastle.  
Novocastra TM has been in the fore front of the development of clinically diagnostic antibodies over many years. It is through rigorous academic studies that the clinical utility of these reagents are assessed. The academic studies of a number of antibodies, including some of the latest antibody releases will be discussed.
- 10:45 – 11:15    **Immunohistochemistry of Breast Biomarkers: Are we any closer to standardisation?**  
*Dr. Merdol Ibrahim*, Manager UK NEQAS-ICC, University College London (UCL), London  
Breast cancer diagnosis routinely employs immunocytochemistry for the classification and subsequent selection of patients for specific therapies. However, correct interpretation is dependent on the quality of the immunohistochemical staining, which can vary enormously between laboratories, and even show day-to-day variation within the same laboratory. External quality assessments (EOA) of breast biomarkers, including; HER2, oestrogen and progesterone receptors, will be used to illustrate acceptable and unacceptable levels of staining as affected by the choice of antibody, retrieval methods, and inhouse tissue controls. Furthermore, 'real-world' clinical data will be illustrated, as collected by a web based breast biomarker auditing system.
- 11:15 - 11:45    **Speakers photo and then Mid-morning break**
- 11:45 – 12:15    **Tissue Crossreactivity**  
*Dr Andy Postoyalko*, Covance Laboratories Ltd, Europe
- 12:15 – 12:30    **Tissue cross-reactivity studies for Regulatory Submission**  
*Dr Julia Stevens*, Asterand UK Ltd.  
It is now a requirement by the FDA (and strongly recommended by other regulatory authorities) that pharmaceutical companies submit tissue cross-reactivity data on therapeutic antibodies prior to their use in clinical trials. In the UK, by law, such regulatory studies may only be carried out by laboratories with current membership of the UK GLP Compliance Program (or its equivalent in other countries). Asterand UK Ltd. has recently achieved membership of this compliance program after an audit by the UK GLP Monitoring Authority (UK GLPMA), and this presentation will detail the IHC services we are now able to offer clients for regulatory submission, and how GLP has been implemented in our IHC laboratories.

- 12:30 – 12:45 **Quantitating multiple proteins in tissue sections: imaging and analysis**  
*Dr James R. Mansfield, Cambridge Research & Instrumentation, Inc, USA*  
Treatment for breast cancer has benefited significantly from advances in molecular biology. IHC tests for protein receptors ER, PR, and Her2 have lead to a new patient classification system. Traditional approaches to assessing multiple proteins use serial sections, staining for one protein per serial section. Multiple proteins can be assessed in the same tissue section, determining phenotype on a per-cell basis, possibly revealing significant subtypes and leading to more targeted and more effective treatments and therapies. Multispectral imaging (MSI) was performed on two sets of a 712- core TMA (356 patients, in duplicate). One set was stained for ER and ki67, the second for ER, PR and Her2 (both plus counterstain). IHC signals were spectrally unmixed from each other and counterstain. Machine-learning-based automated image analysis was performed to locate cancer cells, segment subcellular compartments, and extract IHC signals on a per-cell basis. Per-cell co-expression subtypes were detected using flow-cytometry data analysis software. Percent double and triple positivity were determined, revealing subtypes. Correlation between subtypes and clinical outcomes will be the topic of future publications. MSI and analysis software, coupled with flow-cytometry analysis tools, can be used to reveal molecular subtypes, which may lead to new targeted strategies for breast cancer research and clinical care.
- 12:45 - 13:45 **Lunch**
- 13:45 – 14: 45 **Question and Answer Session**  
Delegates will be asked to submit questions to a panel of experts. Questions can be submitted before the event or on the day
- 14: 45 – 15:15 **Laser capture microdissection and analysis of gene expression**  
*Professor Stephen Bustin, Professor of Molecular Science, Barts and the London School of Medicine and Dentistry, London*  
Accurate description of gene expression in complex tissues requires accurate delineation of the starting material. Laser capture microdissection (LCM) is a powerful technique that permits the isolation and subsequent analysis of single cells, or groups of related cells. Both fresh and archival material can be analysed, with both types of sample characterised by certain advantages and drawbacks. LCM in combination with real-time RT-PCR (RT-qPCR) is an effective replacement for in-situ hybridisation. As the importance of positional effects of cells within tissue, and of mRNA within cells becomes increasingly understood, it is clear that a combination of LCM, RT-qPCR and immunohistochemistry is essential for a complete description of gene expression.
- 15:15– 15:45 **Afternoon Tea/Coffee**
- 15:45 – 16:00 **HistoFAXS: providing “FACS-functionality” for immunohistochemistry**  
*Dr Rupert Ecker, TissueGnostics, Austria*  
HistoFAXS is the microscopic equivalent to flow cytometry – applicable to tissue sections. It provides automated recognition and cytometric measurement of individual cells within histological samples. Recognition of nucleus and cytoplasm for each cell allows to measure comparative marker expression. For each cell or cellular compartment up to 14 parameters are measured. Cellular parameters are displayed in a FACS-like manner.  
The functionality of HistoFAXS will be demonstrated by studies in cancer research, immunology and signal transduction research. Using this technology observer-biased visual estimation in immunohistological analysis of tissue samples is replaced by observer independent measurements on the single-cell level.
- 16:00 – 16:30 **Use of automated image analysis for large-scale tissue microarray datasets: Experiences from BCAC**  
*Dr Will Howat, Cambridge Research Institute, Cancer Research UK*  
The Breast Cancer Association Consortium (BCAC) is a multi-centre collaboration of investigators interested in the inherited risk of breast cancer. Comprising over 50 individual groups, it is an invaluable resource tool. We have examined 16,000 cores from about 9,700 breast cancer tumours from participants in 10 separate studies in BCAC for 5 IHC markers, ER, PR, HER2, EGFR and CK5/6. Due to the volume of the dataset, automated image analysis with the Ariol System was used as a first pass tool for the IHC scoring followed by individual validation by pathologist. This presentation will detail the advantages and disadvantages of using this methodology.

16:30 – 16:45 **Using histology pattern recognition to automate whole slide analysis of immunohistochemistry**  
*Dr Kate Lillard, Sr. Manager of Applications Science, Aperio UK*

16:45 – 17:15 **Creation of a human protein atlas and the search for interesting proteins**

*Dr Caroline Kampf, Rudbeck Laboratory, Sweden*

Background. Completion of the human genome sequence has opened up a possibility for global expression profiling of human tissues and cells, allowing for comparative studies between normal and disease tissues.

Methods. Recombinant protein fragments selected from unique regions called Protein Epitope Signatures Tags (PrESTs) were used as immunogens to generate antibodies. Analysis of protein expression patterns was performed on tissue and cell microarrays containing >700 spots of normal and cancer tissues as well as in vitro cultured cells.

Results. We have used this strategy to construct a comprehensive, antibody-based protein atlas for expression and localization profiles in 48 normal human tissues and 20 different cancers ([www.proteinatlas.org](http://www.proteinatlas.org)). The results are presented in a publicly available database containing images and data from protein profiling using over 6,000 antibodies. Each image has been manually annotated and curated by a certified pathologist to provide a knowledge base for functional studies and to allow searches and queries about protein profiles in normal and disease tissue.

Conclusions. Our results suggest that it should be possible to extend this analysis to a majority of all human proteins thus providing a valuable tool for medical and biological research. We believe that the presented approach combining immunohistochemistry and tissue microarray technology can be used as an effective strategy to identify and evaluate novel markers, with potential clinical importance, of cell lineages and tumors.

17:15 **Chairman's summing up.**

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About the Chair

**Dr 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

*This meeting was **organised by Euroscicon** ([www.euroscicon.com](http://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 Speakers

**Dr Kampf** obtained her Ph.D. in Cell Biology 2001. Dr Kampf is the site-director at the Uppsala site in the Human proteome resource project (HPR) responsible for overall organisation and personnel. The HPR project is set to generate antibodies towards the entire human proteome, and to use the antibodies for expression analysis in situ in a multitude of human tissues and cells. Dr Kampf has been responsible for setting up most of the techniques and modules at the Uppsala-HPR site including the tissuemicro array facility, the digitalisation unit and the validation and annotation of the immunohistochemically stained tissues.

**Dr Nigel H Piggott**, Principal Development Scientist has over 25 years experience of developing antibodies, both polyclonal and latterly mouse monoclonal, in both universities and the business environment. Since joining Novocastra over 15 years ago he has been responsible for the development of a number of Novocastra TM best known clones.

**Dr Julia Stevens** graduated from the University of Newcastle upon Tyne in 1994 with a BSC Hons in microbiology. She was awarded a PhD in cellular microbiology by the University College London in 2001 and went on to work at Imperial College London as a PostDoctoral Research Assistant, on the development of Group B meningococcal vaccines. Julia joined Asterand in 2004 as a Scientist in the department of Exploratory Drug Profiling, before joining the Molecular Pathology group in 2006. She is currently a Senior Scientist in the Group and a trained Study Director for Regulatory Studies in IHC.

**Dr James Mansfield** is one of the primary developers of CRi's Nuance and Maestro multispectral imaging systems and has been working in the field of spectral imaging for over 15 years. He began his career at the National Research Council of Canada and for the last 6 years has been at CRi working towards commercializing multispectral imaging in the fields of microscopy and in-vivo imaging. He has authored 45 publications and holds numerous patents in this field.

**Dr. Rupert Ecker** studied Biology with focus on cell biology at the Universities of Graz and Vienna in Austria (1990-2000). He did his thesis "Strategies for standardisation and automation of immunohistology" at the General Hospital of Vienna, Medical University of Vienna, . After a postdoctoral fellowship (2001-2003) at the Competence Centre for Bio Molecular Therapeutics, a joint venture between the Medical University of Vienna and the Novartis Research Centre Vienna, he founded TissueGnostics, a company that focuses on development of microscopy based tools for tissue cytometry and automated single cell analysis, where he is now in charge of product development.

**Dr George Thomas** joined The Institute of Cancer Research in 2008 as Reader in Molecular Pathology and Team Leader. George obtained his medical degree from the Royal College of Surgeons in Ireland and then went to do his residency in pathology at the Beth Israel Deaconess Medical Center/Harvard Medical School in Boston. He subsequently moved to the University of California, Los Angeles where he did his basic science postdoctoral training in the laboratory of Dr Charles Sawyers. Prior to joining The Institute, George held the position of Assistant Professor at the Department of Pathology and Laboratory Medicine, UCLA.

**Professor Stephen Bustin** obtained his PhD in molecular genetics from Trinity College Dublin. He is Professor of Molecular Science at Barts and the London School of Medicine and Dentistry and visiting Professor of Molecular Biology at the University of Middlesex. His area of research is focused on the large bowel, with particular emphasis on colorectal cancer. He also has a special interest in real-time PCR and has written and edited two books on this subject. He coordinated the recent Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) initiative and is regularly invited as speaker at international meetings and courses.

**Dr Merdol Ibrahim** was appointed the manager of the UK NEQAS ICC & ISH in 2004, where he oversees the quality of clinical immunocytochemistry produced in 600 clinical laboratories, from 54 countries. Obtained his PhD from the University of London and concentrated on immunohistochemical and morphological analysis of CNS myelination. 1997-2001 worked in Switzerland, initially within the department of Histology in Fribourg then with Novartis Pharma (Basel), within the department of Toxicopathology. 2001-2004. Research scientist at the institute of Psychiatry (London), studying mechanisms of brain plasticity