

In Situ Hybridisation Symposium 2013

Thursday 10th October 2013
Cineworld: The O2, London, SE10 0DX, UK

This technical workshop is dedicated to the discussion of in situ hybridisation (ISH), its capabilities, difficulties and troubleshooting. This event will be an ideal place to network, establishing lines of communication for the future if you ever need help with setting up the technique and giving you short cuts to improving your results. This event has CPD accreditation.

Why you should come: This is a specialist meeting, with no other forum comparable internationally, as it is rare to have so many experts in the field, together in one room, giving you unrivalled access, all in one place.

Even experienced people who are interested in keeping up with the technique should be at this unique event.

Meeting Chair: Dr Julia Jones, Cancer Research UK, Cambridge

Who Should Attend: PhD students, postdocs, technicians and any scientists trying to set up ISH or already running it.

The Deadline for abstract and poster presentations has now passed.

Talk times include 5 – 10 minutes for questions

9:00 – 9:45 **Registration**

9:45 – 10:00 **Introduction by the Chair:** Dr Julia Jones, Cancer Research UK, Cambridge

10:00 – 10:30 **Probe technology for in situ hybridisation**

Dr James Howard Pringle, Reader, University of Leicester, UK

In situ hybridization (ISH) is a powerful technique developed over 40 years ago for localizing specific nucleic acid targets within fixed tissues and cells and can provide temporal and spatial information about gene expression and genetic loci. Although the technique of ISH has not the same prominence in the literature as immunohistochemistry, probe technology is currently improving and new clinically important applications are being developed. In this talk I will compare different probe technologies for visualising RNA and DNA targets by in situ - fluorescence (FISH) and chromogenic (CISH) detection. I will discuss target gene validation, probe design, sensitivity and the use of suitable control probes. I will also highlight the differences and the advantages of FISH and CISH probes in different applications. .

10:30 – 11:00 **In situ hybridisation and immunohistochemistry: better together or apart?**

Dr Richard Poulson, Head, Molecular Pathology Facility, Queen Mary, University of London, UK

Localising the expression of an mRNA and a protein can offer different views of what is happening within tissues, especially when the protein is secreted, or there is rapid movement and maturation of cells. Colocalisation is sometimes possible but at the expense of greatly reduced sensitivity. I will describe some of the strategies available to tackle this problem and the related problem of localising DNA in situ together with specific epitopes defining the phenotype of cells.

11:00 – 11:30 **Speakers' photo then mid-morning break and poster exhibition and trade show**

Please try to visit all the exhibition stands during your day at this event. Not only do our sponsors enable Euroscicon to keep the registration fees competitive, but they are also here specifically to talk to you.

11:30 – 12:00 **4-colour FISH probes in Prostate Cancer**

Dr Jeremy Clark, SRA, University of East Anglia, UK

Androgen Receptor (AR) amplification in Prostate Cancer is associated with advanced treatment-resistant disease and death. Our work using 4-colour FISH probes has revealed the presence of AR gain and amplification in small clonal growths within a tumour field, highlighting the complexity of clonal variation in prostate cancer. Rehybridising the same tissue section with other FISH probes has enabled additional genomic changes in the same cells to be evaluated. This has revealed further divergent poor-prognosis clones in single prostates.

- 12:00 – 13:00 **Lunch, poster exhibition and trade show**
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- 13:00 – 14:00 **Question and Answer Session**
- 14:00 – 14:30 **Talk title to be confirmed**
Dr Kai Wilkens, Advanced Cell Diagnostics, Inc
- 14:30 – 15:00 **Afternoon Tea, last poster session and trade show**
- 15:00 – 15:30 **High speed whole mount labeling technology**
Mr Jacques Thélu, Flogentec Ltd, Scientist CNRS, University of Grenoble, France
 Flogentec's innovative new device uses a patented process to subject multiple samples to a computer controlled flow of reactants. ISH or immunochemistry tests are carried out in situ on whole biological samples. The device ensures that solvents wash continuously through 3D samples at optimum conditions, maximizing the transfer of solvents to the specimens' core and by the way, reducing analytic lead time to 22 hours down from 3 days. Similarly, because it is a "stand alone" device, results are improved significantly. I will discuss some results, and techniques that can potentially be applied. FLO 400, the compact robot for the lab bench, is presented on the Flogentec's stand.
- 15:30 – 16:00 **Physical Approaches to Studying the Chromosomal Evolution of *Anopheles darlingi* and *Aedes aegypti***
Dr Miriam Silva Rafael, Researcher and Teacher, INPA-Instituto Nacional de Pesquisas da Amazônia, Petrópolis, Brazil
Anopheles darlingi is the main vector of malaria in the South America. The GST, actin, myosin, HSP70 and rDNA genes were physical mapped in the chromosomes of *An. darlingi*, which provide data for chromosomal homologies arm 3L in *Anopheles gambiae*, 2L in *Anopheles stephensi*, 3L in *Anopheles funestus* and 3R in *Anopheles albimanus*. We also studied the effect of semi-synthetic 1KL39-B and 1KL43-C dillapiol on *Aedes aegypti* as a potential control. Due to their high toxic and genotoxic effects, the two semi-synthetic can be considered a alternative for *Ae. aegypti* control. Funding: universal projects nos. 480926/2011-5 (FAPEAM) and 1036/2011 (CNPq).
- 16:00 – 16:30 **Multiplex in situ hybridisation to detect circulating tumour cells in breast cancer patients**
Professor Raoul Charles Coombes, Professor of Medical Oncology, Imperial College, London, UK
 The objectives of this study were to develop and characterise an ultrasensitive multiplex fluorescent RNA in situ hybridisation (ISH)-based CTC detection system called CTCscope. This method detects a multitude of tumour-specific markers at single-cell level in blood. CTCscope detected CTC transcripts of eight epithelial markers and three epithelial-mesenchymal-transition (EMT) markers for increased sensitivity. CTCscope was used to detect CTCs with minimal enrichment, and did not detect apoptotic or dead cells. In patient blood samples, CTCs detected by CellSearch, but not CTCscope, were positively correlated with CA15-3 levels. Circulating tumour cells detected by either CTCscope or CellSearch predicted PFS (CTCscope, HR (hazard ratio) 2.26, 95% CI 1.18–4.35, P.0.014; CellSearch, HR 2.50, 95% CI 1.27–4.90, P.0.008).
- 16:30 - 17:00 **Chairman's summing up and Close of Meeting**

Keywords: RNA, in situ hybridization, nucleic acid probes, non-isotopic labels, ISH, FISH, miRNA, 35S riboprobes, tyramide enhancement, miRNA detection, CISH, FISH, DNA, breast cancer, IHC validation, RNAscope, digital pathology, gene expression, Hybridisation, Probe, automation, mRNA detection, antigen retrieval, DNA FISH, sequential detection, 4-colour FISH, prostate, GST, malaria, dillapiol extract, genotoxicity, dengue, in situ hybridisation, breast cancer, CTC's, whole mount, ISH, immunochemistry, embryo, robot

Registration Web Site: www.regonline.co.uk/insitu2013

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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:

Julia Jones graduated with a BSc (Hons) in Biomedical Science from the University of Southampton before gaining a PhD in Neuroscience from Cambridge University. She is now with Cancer Research UK as a Senior Scientific Officer (ISH) where she has run the ISH service in the Histopathology/ISH facility at the Cambridge Research Institute for 6 years.

About the Speakers:

James Howard 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 lung, 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.

Richard Poulosom, together with a team of expert colleagues, operated a core facility for localisation of mRNAs for many years; looking at the expression of hundreds of mRNAs in around 89,000 sections of routinely fixed and processed human and experimental tissues, mostly using 35S and 3H riboprobes. Recently he has been exploring the newer signal amplifying non-isotopic ISH methods with the intention to compare their results to isotopic ISH and to combine them with immunohistochemistry.

Jeremy Clark has researched molecular changes in cancer for over 25 years, and has discovered a number of novel oncogenes including the SYT/SSX fusion genes in synovial sarcoma, 3 fusion partners of TFE3 in papillary renal cell carcinoma and the EWS/CHN fusion in chondrosarcoma. His current research concentrates on prostate cancer detection and prognosis.

Jacques Thélu, the Flogentec patent inventor, is a research manager for the French national research agency: CNRS. He is an expert in several wide-ranging domains including: chick embryo development and in situ hybridisation applied to cancer research. Dr Thélu has also acquired a wide range of industrial experience in the fields of automation and biotechnology with several blue chip organizations. He has filed and obtained patents for several innovative technologies including: the procedure for a vaccine production, the automated production of *P. falciparum* cell culture, an innovative immunological test, the automated device for ISH.

Miriam Silva Rafael researches on chromosomal evolution, genomics of the malaria mosquito, and mutagenesis in vectors of dengue at INPA, Coordenação de Sociedade, Ambiente e Saúde, Laboratório de Malária e Dengue. She teaches and guides students of MSc and PhD at Programa de Pós-Graduação em Genética, Conservação e Biologia Evolutiva and Programa de Pós-Graduação em Biotecnologia e Recursos Naturais, Universidade do Estado do Amazonas. Dr. Miriam Rafael is member of the Câmara de Pesquisa Científica of FAPEAM, and coordinates scientific research projects funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico and FAPEAM.

Charles Coombes is Professor of Medical Oncology (Breast Cancer). He is theme leader for Cancer at Imperial College London and he leads the Imperial CRUK Centre. He is engaged in developing novel methods for prediction of response to endocrine therapy in breast cancer and also carrying out research aimed at understanding the mechanisms of resistance to endocrine therapy and development of novel anti-cancer drugs. His laboratory is focusing on elucidating molecular signals controlling aberrant growth of breast cancer cells with specific focus on the oestrogen receptor and allied cell signalling pathways. He works with scientists engaged in molecular target identification and chemists whose remit is to target specific signalling abnormalities to develop novel therapies. He also runs a translational laboratory which focuses on detection of micrometastatic disease and application to the treatment of breast cancer.

NOTES ABOUT THIS EUROSCICON EVENT

For your convenience we would like to bring your attention to the following

- You will be issued with a FULL delegate list within 14 days of the event, which will include the email addresses of the delegates (we are sorry that there is this delay in emailing the list, but we need to make sure that it takes into account any late arrivals). You will not be included in this list if you have opted out and you can do this by logging into your registration details. This list will not be sold or ever give out to third parties. Only people attending or sponsoring the event have access to the list
- There may be an independent meeting report published within a few months of this event. If this is published we will send you an email to let you know the reference details
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