

Recent advances in Flow cytometric techniques and instrumentation

The BioPark Hertfordshire, Welwyn Garden City, AL7 3AX - 14th Nov 2008

"Instrumentation has to keep up with the rapidly moving field of science served by flow Cytometry, this meeting will bring together the most recent advances in clinical and research flow Cytometric techniques and the hardware that has been developed to keep pace with the scientific requirements". Meeting Chair - Dr Ian Dimmick

This meeting has CPD accreditation

- 9:00 – 9:30 **Registration**
- 9:30 – 9:45 **Introduction by the Chair:** *Dr Ian Dimmick, Newcastle Upon Tyne University.*
- 9:45 – 10:15 **Current trends in flow cytometry-New tools for old problems-**
Dr Ian Dimmick, Newcastle Upon Tyne University. A resume of what are the most useful new tools that I am using for flow Cytometry within the core facility.
- 10:15 – 10:45 **Lies, damned lies, statistics, and truth. Imaging flow cytometry's role in cell analysis**
Dr. David Basji, Amnis Corporation, US
Imaging flow cytometry combines the information richness of microscopy with the statistical power of flow cytometry. Imaging flow cytometry broadens the applications of traditional flow cytometry to include signal localization and morphologic analysis while effectively eliminating the false positive and false negative results that result from traditional flow cytometry's inability to visualize cells. By capturing over 100,000 images per minute, imaging flow cytometry broadens the applications of traditional imaging techniques to include the analysis of rare cells in primary tissue samples. The very large image data sets produced by the technique allow the statistical characterization of any image parameter, including the degree of NFkB translocation, localization of internalized antibodies to specific cellular compartments, the number of FISH spots in a cell, changes in cell or nuclear shape, and any intensity measurement that can be done by traditional flow cytometry. Numerous example applications will be reviewed.
- 10:45 – 11:15 **The MACSQuant Analyser – a new milestone in flow cytometry**
Mark Twigden, Miltenyi Biotec Ltd, Germany
The MACSQuant analyser is a completely new 7 colour flow cytometer, designed with unique features unavailable on any comparable machine. These include non-pressurized sample uptake; defined volume uptake; absolute cell counting without beads; automated sample labelling and analysis; magnetic pre-enrichment unit for ultra-sensitive detection of rare cells; automated compensation using beads and or cells; PC-based software with automated analysis. I will highlight the application of these various features in the modern flow cytometry lab.
- 11:15 – 11:25 **Speakers photo**
- 11:25 – 12:00 **Mid-morning break**
- 12:00 – 12:30 **The applications of FRET in flow cytometry**
Dr Gary Warnes, Institute of Cell & Molecular Science, Barts & Royal London, UK
Flow Cytometric use of Fluorescence Resonance Energy Transfer (FRET) has become more prevalent due to increased use of Fluorescent Protein (FP) technology to investigate not only molecule proximity but protein functionality when single molecules are dual labelled with two types of FP *e.g.* CFP and YFP. FRET can be also used to show changes in protein proximity using antibody conjugates and fluorescent dyes. Other uses of FRET include the use of the gene reporting substrates CCF2 and CCF4 as well as BrdU detection by the use of PI and ToPro3. Cell sorting can be an aid to the imaging of FRET by maintaining cell cultures that have a high proportion of cells displaying FRET. Examples of all these approaches will be presented.
- 12:30 – 13:00 **Risk stratification in chronic lymphocytic leukaemia - applications of flow cytometry**
Dr Chris Pepper, Department of Haematology, Cardiff University, UK
The diagnosis of CLL is now a routine affair but defining the prognosis of individual patients is much more challenging. Over the last 10 years numerous novel prognostic markers have emerged but questions still remain about the reliability and accuracy of many of these parameters. In this talk I will review the current state of play, highlight some of the problems that are commonly encountered and suggest ways in which we can improve the risk stratification of CLL patients in order to aid clinicians and benefit patients.
- 13:00 - 13:10 **Introduction to the Biopark**
- 13:10 – 14:15 **Lunch and Poster Viewing**

- 14:15 – 14:45 **The use of 24-bit analog-to-digital converters (ADC) in flow cytometry simplifies data collection and enhances data analysis**
Dr. Leo Ostruszka, Accuri Cytometers (Europe) Ltd
 Digital signal processing (DSP) has revolutionized the field of flow cytometry through more accurate representation and processing of fluorescence and scatter signals. DSP also provides more flexibility in data analysis, since functions like gating and fluorescence compensation can be performed after data collection. Present technology remains limited, however, by the use of (at most) 18-bit analog-to-digital converters (ADCs) to digitize detector signals. Although 18-bit conversion provides an expanded range of data channels (262,144 vs. 1024 on older machines) and with it, increased data resolution, the cytometrist must still adjust detector voltage and/or gain settings, depending on the application (e.g., small or large beads/cells, dim or bright fluorescence). This scenario has several drawbacks. First, information is often lost from the data; it is almost impossible, for example, to find a signal amplification compromise for forward and side scatter signals that allows simultaneous resolution of platelets and eosinophils in the same data file. An amplification setting sufficient to allow analysis of platelets will most likely result in signals from larger cells, such as granulocytes and eosinophils, falling off scale. Once amplification is set and data are collected, there is no way to accurately retrieve off-scale signals due to over- or under-amplification. Gain and voltage setting is also highly subjective. While an individual lab or flow core may suggest appropriate amplification settings for a particular application, staining protocol and instrument used, it is virtually impossible to maintain any level of consistency between different labs and core facilities. Furthermore, significant amounts of sample are consumed during the setup phase of data collection. The Accuri C6 cytometer, by contrast, uses 24-bit ADCs to digitize the analog signals from each of its six detectors (two scatter and four fluorescence). This increases resolution to approximately 16.7 million channels for each signal, essentially eliminating the need for users to separately control signal amplification. Consequently, the data collection and analysis processes are nearly independent of each other. This report demonstrates some of the advantages of 24-bit ADCs and DSP for flow cytometric data collection and analysis, using the Accuri C6 in concert with certain features unique to its companion CFlow collection/analysis software.
- 14:45 – 15:15 **Dielectrophoresis cytometry**
Professor Paul J Smith, Department of Pathology, School of Medicine, Cardiff University, Wales
 Microelectrode structures are increasingly used in biochip formats for sensing and in particular actuation. Electrokinetic actuation employs a range of typically AC electric fields to induce a force, and hence motion, on particles suspended in a sample medium. The simplest AC electrokinetic principle is dielectrophoresis where particles exposed to a non-uniform AC electric field experience a force either towards or away from the regions of highest field intensity. The speed and direction of motion is a function of the dielectric properties of the cell and suspending medium, the electric field geometry and the magnitude and frequency of the electric field. Dielectrophoresis (DEP)-based devices have found applications in cancer research and areas of drug discovery - one attraction being the 'problemless' nature of the approach. The presentation will overview DEP cytometry, its integration with conventional approaches and nanoparticle-based methods, discuss innovations in data analysis with a focus on applications in the area of cell therapeutics
- 15:15 – 15:45 **Afternoon Tea/Coffee and Last Poster Viewing**
- 15:45 – 16:15 **Problem solving difficult samples with red/far-red nuclear dyes**
Roy Edward, Biostatus, UK
 There are many intractable samples for flow cytometry. Often this is due to problems with contaminating debris, RBCs, inability to segment populations based on scatter properties or phenotype, etc. Far-red nuclear dyes can assist by providing additional information without impacting on the other diagnostic parameters. Recent examples will be presented.
- 16:15 – 16:45 **A New era in cytometry set up : automated process controls**
Dr John Lawry, BD Biosciences, UK
 Flow cytometry has evolved significantly in the last ten years in terms of robust QC schemes, automated acquisition processes and most recently Cytometer Setup and Tracking systems. These procedures will be reviewed in terms of their use, relative significance, and most importantly, as an aid to the cytometrist in getting the best from their experiments and cytometer.
- 16:45 – 17:15 **Chairman's summing up**
- 18:00 **Soiree at *The Best Western Homestead Court Hotel for all the participants**

About the Meeting Chair

Mr Ian Dimmick, Institute of Genetics International centre for life, UK Previously a European Flow Cytometry applications specialist, now in charge of a busy analysis and sorting facility in Newcastle upon Tyne, UK

About the Speakers

Professor Smith has been active in the fields of DNA repair, drug development, cytometry, nanomedicine and imaging technologies for more than 25 years. His current research focuses on the cell cycle and anticancer drugs, imaging technologies and mathematical modeling of complex biological systems. He was a senior scientist with the UK Medical Research Council in Cambridge before being appointed to the Chair of Cancer Biology at Cardiff University in 1995. He leads the UK Optical Biochips Consortium exploring chip-based cytometry systems

Roy Edward - Biochemist with 25+ years in bioscience commerce, product development and tech support. Experience in molec and cell biology – specific knowledge in sample preparation, mass spectrometry, immunology and high content screening. Has written several book chapters and most recent publication in Analytical Chemistry in 2005.

Dr David Basiji received his Ph.D. from the University of Washington department of Bioengineering, where he holds an affiliate faculty position. He is one of the founders of Amnis, a co-inventor of the ImageStream technology, and has co-authored 27 issued patents.

Dr Gary Warnes interest in flow cytometry started at St. Mary's in 1986, analysing T-cell subsets. Completed a PhD investigating the immunosuppression of HIV-ve haemophiliacs at St.Thomas' Hospital. Post-doctoral position in the US investigated the regulation of Tissue Factor expression by immune costimulatory molecules in sepsis. Core Facility Manager at the Flow & Imaging Core Facilities at the MRC Clinical Science Centre at Hammersmith Hospital. Worked with Derek Davies at Cancer Research UK and now running the new Flow Cytometry & Imaging facilities at the Institute of Cell & Molecular Science, Queens Mary's London University.

Dr John Lawry was formally an academic flow cytometerist running a research group plus core flow cytometry facility in the Medical School. University of Sheffield (18 yrs). He is now an application Specialist with BD (since 2001).

Dr. Leo Ostruszka received his Ph.D. in Pharmacology from the University of Michigan in 2001. He subsequently completed his Post-Doc at the University of Michigan and was a key member of the University of Michigan Flow Cytometry Core Facility. His research interests have focused on cell cycle, anticancer drugs, and radiation therapy. Since 2006, he has been integrally involved in the development of the Accuri C6 Flow Cytometer and is currently an Applications Scientist for Accuri Cytometers, in Ann Arbor, Michigan.

Dr Chris Pepper obtained his PhD in Medicine Chemistry (1993) from Cardiff University. He is currently a Senior Lecturer in Haematology in the School of Medicine in Cardiff where he runs a successful research team and provides a University-wide high speed cell sorting facility. For the past 14 years his research career has been focussed on chronic lymphocytic leukaemia. During this time, he has published over 50 Peer reviewed papers and has been an invited speaker at numerous national and international meetings. He is also the Scientific Secretary of the UKCLL Forum and sits on the UKCLL/NCRI CLL Clinical Trials Sub-Group.

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 Hertfordshire' (www.biopark.co.uk), a new 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

EFFECTS OF THALIDOMIDE & RITUXIMAB IN LOW GRADE NON-HODGKINS LYMPHOMA

THACL & RASSAM S

Maidstone & Tunbridge Wells NHS Trust, Haematology & Blood Transfusion, Maidstone Hospital, Hermitage Lane, Maidstone, Kent, ME16 9QQ

Low Grade Non-Hodgkin's Lymphomas (LG-NHL) have been found to be formed from B cells with clonal proliferations arising as B cells differentiate. There are 8 main groups: Follicular Lymphoma (FL), Lymphoplasmacytic Lymphoma (LPL), Hairy Cell Lymphoma (HCL), Hairy Cell Leukaemia variant (vHCL), Splenic Lymphoma with Villous Lymphocytes (SLVL), Marginal Zone Lymphoma (MZL), Mantle Cell Lymphoma (MCL) and Chronic Lymphocytic Leukaemia (CLL) (WHO, 2005). LPL, HCL, SLVL (a subset of MZL) and MCL are the rarer forms of LG-NHLs and are currently not well studied. Patients with these types of LG-NHL may go on to develop secondary malignancies, such as Basal Cell Carcinoma of the skin, which suggests an impaired immunity to control the emergence of these tumours. Quantification analysis of these 4 types of LG-NHLs together with CLL will provide information about the changes in the immune profile, such as CD4+:CD8+ T cell ratio. Use of Fox-p3 (an intracellular staining T regulatory cell specific marker) with CD4 and CD25, as well as CD3 and CD56 intensity, could provide data about these cells which are important in the transformation of lymphomas to secondary malignancies. Possible analysis by ELISA and Affimetrix cards to analyse TNF α , IFN γ and perforin may provide some insight on whether these cells are able to undergo apoptosis effectively, based on the phenotypes for naïve, memory and effector cells (with CD45RA, CD45RO and CCR7 surface markers). The investigation will involve analysis of pre-/during/post-treatment EDTA blood samples from LG-NHL patients who attend the Kent Oncology Centre at Maidstone Hospital between 2008 and 2011. Comparison between these patients (administered Thalidomide and Rituximab) and others taking alternative chemotherapeutic agents may provide information to immune function when coupled with quantitative analysis of naïve, effector and memory cells.

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