flowcytometry UK

15th-17th July 2009

Keble College, Oxford

Final Programme

(2nd July 2009)

For updated information email: admin@flowcytometryuk.org

Registration : http://www.rms.org.uk/event_flowuk09.shtml

www.flowcytometryuk.org

This meeting is run in conjunction with the Royal Microscopical Society

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Wednesday 15th July

12:00-14:00	Registration, lunch and poster set-up
14:00-14:10	**Derek Davies (London)** - Welcome to *flowcytometry***UK** 2009
	Session 1: Cancer and stem cells
14:10-14:55	**Paul Smith (Cardiff)** - *Cancer Stem Cells and Drug resistance*
14:55-15:20	John Stingl (Cambridge) - *Analysis of the mammary epithelial cell hierarchy by the use of flow cytometry and functional assays*
15:20-15:45	Bill Godfrey (Oregon, USA) - *Proliferative and phenotypic characterization of human mesenchymal stem cells by flow cytometry*
15:45-16:30	Coffee and Exhibition
	Session 2: Clinical cytometry
16:30-17:15	**Peter Openshaw (London)** - *Lung inflammation caused by viral infections*
17:15-17:35	Qing Chang (Ontario, Canada) - *Effects of hypoxia on tumour progression in human primary pancreatic cancer xenografts studied using multiparametric flow cytometry*
17:35-17:55	Steve Garner (Cambridge) - *Identifying inherent variation in blood donors' platelet function: Characterising influences on therapeutic products and a strategy for measuring the effect of sequence variation in genes regulating platelet responses.*
1800-20:00	Exhibition and Poster session
20:00	Dinner in Keble Dining Hall

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Thursday 16th July

Session 3: Practical Flow Cytometry

09:00-09:20	Raif Yucel (Aberdeen) - *Measuring intracellular phospho-signalling events using flow cytometry*
09:20-09:40	Gary Warnes (London) - *Applications of FRET in flow cytometry*
09:40-10:00	Anna Petrunkina (Cambridge) - *Cytometric evaluation of experimental systems for studies on male gamete maturation*
10:00-10:20	Alfonso Blanco (Dublin) - *A flow cytometric method for continuous measurement of intracellular calcium concentration*
10:20-10:40	Sonja Tattersmuch (London) - *Multiparameter Flow Cytometry: Screening for Cell Populations Involved in Anti-HTLV-1 Immune Responses*
10:40-11:20	Coffee and exhibition

Session 4: Imaging cytometry

11:20-12:05	**Bill Telford (Bethesda, USA)** - *Laser scanning cytometry: where does it fit in with modern biomedical imaging?*
12:05-12:25	Andy Filby (London) - *The use of imaging flow cytometry to quantifiably determine cell phenotype and function*
12:25-12:45	Emma Gudgin (Cambridge) - *Potential clinical applications of imaging flow cytometry*
12:45-13:30	Lunch in Keble College Dining Hall
13:30-14:00	Coffee and exhibition
14:00-16:00	Commercial tutorials Beckman Coulter UK Ltd Cambridge Bioscience Ltd CompuCyte Corporation Flowjo Miltenyi Biotec Partec UK Ltd
16:00-16:30	Coffee and exhibition

Session 5: Stem cell biology

16:30-117:15	**Austin Smith (Cambridge)** - *Capturing pluripotency*
17:15-17:30	Rachael Walker (Cambridge) - *Stem cells - the single cell*
17:30-17:45	Richard Grenfell (Cambridge) - *Biosafety in stem cell research*
17:45-18:00	Simon Monard (Edinburgh) - *Colour compensation for stem cell research*

18:00-18:45 Bob Spencer Memorial Lecture

David Hedley (Ontario, Canada) - *Integrating flow cytometry into molecular cancer therapeutics*

1900 - 20:00	Drinks reception (Exhibition area)
20:00-00:00	Conference Dinner in the Great Hall, Keble College

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Friday 17th July

Session 6: Micro-cytometry

09:30-10:15	**Ger van den Engh (Seattle, USA)** - *Use of flow cytometry in marine biology*
10:15-10:30	Rebecca Dragovic (Oxford) - *New dimensions in the quantification and characterisation of plasma microparticles*
10:30-10:45	Gerhard Nebe von Caron (Bedford) - *Membrane integrity staining as a measurement of bacterial viability: pitfalls and limitations*
10:45-11:00	Nigel Rust (Oxford) - *Isolating coccoliths from sediment for geochemical analysis*
11:00-11:30	Coffee and Exhibition
11:30:12:00	**Alexis Perez Gonzalez (Germany)** *- Cytometry networking*
12:00-13:00	Lunch and depart

We are grateful to the following companies for their support of this meeting:

Exhibitors

AbD Serotec
Accuri Cytometers (Europe) Ltd
Applied Cytometry
BD Biosciences
Beckman Coulter UK Ltd
Cambridge Bioscience Ltd
CompuCyte Corporation
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Other Sponsors

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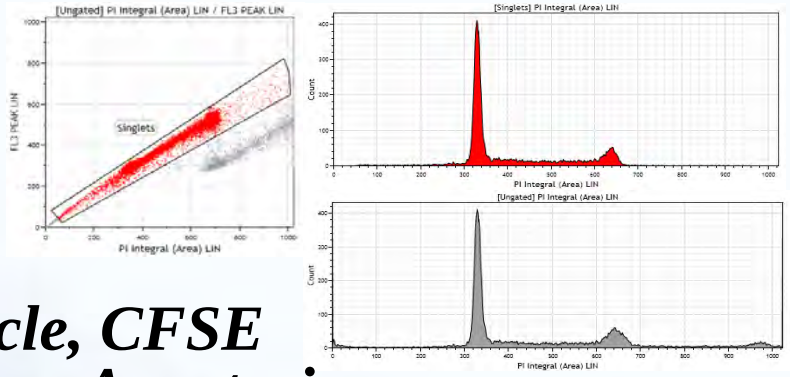
Thursday 16th 2-4pm

BECKMAN COULTER
We're better together

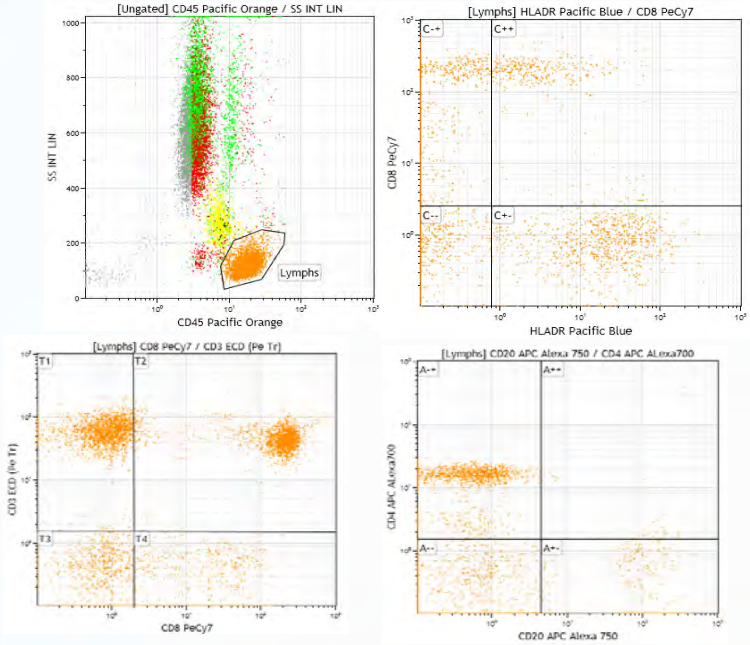
Beckman Coulter Applications Workshop

(14:00-15:00 then repeated 15:00-16:00, Thurs 16th July 2009)

Data Analysed using NEW Beckman Coulter Kaluza Software



Cell Cycle, CFSE Proliferation, Apoptosis, CD Marker Staining etc



System setup and use of Blue, Red and Violet excited Fluorochromes

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Cambridge Bioscience - Vijay Mhaiskar (Biolegend Business Manager)

Accelerating towards Multi-Colour Flow Cytometry

As development of digital flow cytometry instrumentation continues, the range of fluorescence-based labeling chemistries grows, making ever more complex polychromatic analysis possible. Examples of research areas benefiting from these developments are T-cell differentiation and Th subset immunophenotyping. Information gained from using multi-parametric flow cytometry and other high content analytical tools such as Luminex cytokine assays is helping to extend our knowledge of immunologic pathways. In this talk we will discuss multi-colour flow cytometry from many different perspectives.

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Compucyte Corporation

Quantitative Imaging Cytometry in Biomedical Research, Drug Discovery and Biomarker Development

Introduction to Laser Scanning Cytometry Technology and Virtual Demonstration of Its Application to Multiplexed Cell Cycle Analysis – Mel Henriksen, CompuCyte Corporation

The iGeneration LSC technology is a novel platform combining many of the advantages of flow cytometry and laser scanning microscopy, and significantly extending the boundaries of quantitative single-cell analysis. Unlike conventional flow cytometry, the technology analyzes samples attached to a horizontal surface (e.g., cultured cells on slides or in a variety of carriers), tissue sections, and tissue microarray specimens. Unlike confocal microscopy, LSC provides high-resolution imaging and quantitative analysis on large numbers of cells. iGeneration instruments use an automated nanostep X-Y stage to pass the samples by the objective lens, applying up to three different lasers to excite fluorescent emissions or detect laser light absorbance and scatter. LSC technology is uniquely able to simultaneously quantify both fluorescent excitation, using an array of four photomultiplier tube (PMT) detectors, and laser light loss and scatter, employing two photodiode detectors. Images are developed from these signal arrays and quantification via various segmentation routines is accomplished on a cellular or sub-cellular level.
The platform provides many researchers with a powerful new way to analyze cells and tissues, enabling in-depth understanding of a broad spectrum of areas of biomedical science, including cancer biology, stem cell research, immunology, autoimmune diseases, and infectious diseases.
We will present an overview of the technology and its unique features and capabilities. We will then demonstrate the use of LSC technology in one of its hallmark applications: direct and multiplexed cell cycle measurement. This demonstration will show direct assessment of cellular DNA content for phase separation of interphase cells similar to flow cytometric analysis, coupled with the LSC's ability to distinguish G2 from M phase cells based on the amount of chromatin condensation. Use of additional cell cycle-related markers allows a detailed look at the stages of mitosis. Visualization of the cell images provides validation of the quantitative data.

Focal Adhesion Kinase (FAK) Regulates Progenitor B Cell Growth And Localization in Bone Marrow Niches - Shin-Young Park, Children's Hospital Boston and Immune Disease Institute/Harvard Medical School, Boston, MA USA

In the bone marrow (BM), progenitor B cell growth and survival depend on cues from distinct microenvironments, e.g., niches that are defined by cellular components, soluble regulators, and the extracellular matrix (ECM). Niche-specific signals, involving cytokines, chemokines and adhesion molecules, also influence the localization and migration of normally developing B cells in BM. However, the intracellular pathways downstream of these niche-specific signals are not fully understood. Collectively, our previous studies suggest that the CXCR4-FAK-VLA4 pathway is important for sustained adhesive interactions between progenitor B cells and the BM microenvironment. Based on these findings, we hypothesize that FAK may play an important role in niche-induced signaling regulating progenitor B cell fate decisions, e.g., proliferation, differentiation, apoptosis, and migration.

To test the role of FAK function in B lymphopoiesis, we generated CD19-Cre FAK floxed mice in which FAK was conditionally deleted in B lineage cells. FAK deletion led to a selective decrease (30-40%) in the number of BM pro-, pre- and immature B cells. FAK-deleted pro-B cells showed increased cell division followed by an increase in apoptosis (70%) in vitro cultured in semisolid methylcellulose medium with IL-7 alone or with CXCL12. We also found that FAK deletion leads to increased mobilization of pro-B cells to the peripheral blood. Using laser scanning cytometry, we objectively quantified the morphological localization of progenitor and immature/mature B cells in the longitudinal cryo-preserved 5 micron sections of wild type and FAK KO mouse femurs. LSC analysis of B cell subpopulations is validated by identification of the known follicular organization of B220+ cells within the spleen and also by identification of negligible B220+ IgM+ population in the femoral BM cavity of Rag2-deficient mice, which have a maturation defect in V(D)J recombination at pro-B cell stage and therefore have no IgM expression.

We found that niche environments in the metaphyses as well as the diaphysis serve as major sites for B lymphopoiesis in wild-type mice. In the diaphysis, early progenitor B cells (B220 + c-Kit +) localize in the endosteal region while mature (B220+ IgM+) B cells localize in the central medullary region. To examine whether FAK deletion affects localization of progenitor B cells in BM, we examined the distribution of B220 + c-Kit + cells in the femoral BM of FAKFAK-deleted mice. In comparison to wild type (C57BL/6) B220 + c-Kit + cells, lodging of B220 + c-Kit + cells in the metaphyses is significantly impaired in FAKFAK-deleted mice. This impaired lodging of B220 + c-Kit + cells is also observed in the endosteal region of the diaphysis, albeit to a lesser degree. Taken together, we propose that FAK functions as a key intermediary of BM niche-induced signals controlling early B lineage development.

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FlowJo - Claudio Vallan (Application Specialist, Treestar)

14:00 – 14:30
Basics of Flowjo for PC (Version 7.5.3)

FLowJo is a cytometry data analysis package that works on different concepts than any other cytometry analysis software. Experiments with differently stained samples are not just conceived as samples that have to be analyzed on a sample by sample basis, but as an entity belonging together. Once you become acquired with the concept, FlowJo becomes the most powerful tool, giving you the means to automate your approaches and saving you hours and hours of analysis time. And it's not only a tool for complex analyses. You will have much more time to spend drinking coffee with your colleagues and discussing about ideas for your next publications.

14:30 – 15:00
New and advanced features in FlowJo for PC

The new version of FlowJo (7.5) has been completely recoded from scratch and has now become may times faster. Proliferation analysis, cell cycle analysis, calibration, compensation, kinetic analysis and transformations have become much more manageable. Furthermore there are many new tools as 2D cell cycle analysis, 3D plots, automatic compensation, heat maps on tables, derived parameters... Come and see what's new.

15:00 – 15:30
Basics of FlowJo for Mac (Version 8.8.6)

The Mac version of FlowJo works with the same concepts as the PC version, but includes some features now yet available there. A walk trough the basics showing magnetic gates, gate molding, spider gates and many more.

15:30 – 16:00
New and advanced features in FlowJo for Mac

Ever heard about FJML gates? See how gates can calculate themselves their position. You will be fascinated!

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MACS

Miltenyi Biotec

Workshop

MACSQuant® Analyzer

A new milestone in cell analysis

- **Compact benchtop flow cytometer**
- **Multiparameter cell analysis**
- **Absolute cell counting (volumetric)**
- **Highly sensitive detection of rare cells**

You are cordially invited to our workshop, so you can see the new MACSQuant Analyzer—ideal for both inexperienced and experienced flow users.

Date: Thursday 16th July
Time: 2–4 pm
Workshop leader: Dr. John Campbell

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Partec- Jane Wood (Chief Operating Officer, Partec UK)

HIV remains a global health problem of unprecedented dimensions.

Unknown 27 years ago, HIV has already caused an estimated 25 million deaths worldwide and has generated profound demographic change. Due to the close link between HIV/AIDS, TB and Malaria it is important to cover patients with a complete set of diagnostics tests.

Partec Essential Healthcare provides diagnostic solutions specifically dedicated to these 3 diseases: the CyFlow Counter, CyScope Malaria and CyScope TB.

The CyFlow® technique has provided the world with the first mobile/portable flow cytometry instrument, the CyFlow Counter. This instrumentation provides a robust, easy-to-operate, accurate and affordable method for HIV monitoring and AIDS patient follow-up. As such it is targeted at low and middle income countries.

The Partec CyScope® is a new unique, battery operated fluorescence microscope designed for Malaria and TB diagnostics. It is the first truly portable fluorescence microscope and can be used with a CCD camera in order to provide slide images.

By combining these three techniques Partec significantly contributes to the specific requirements of patients by providing mobile solutions to where they are needed most.

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