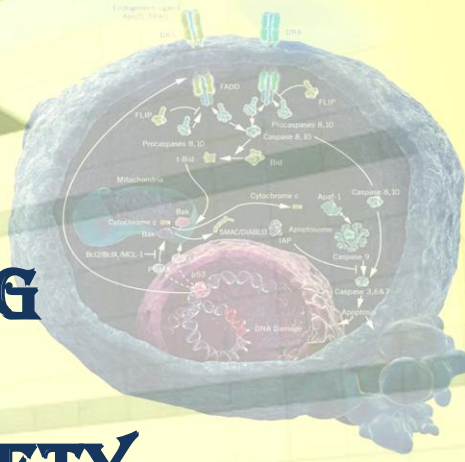

5TH ANNUAL MEETING
OF THE
IRISH CYTOMETRY SOCIETY

17TH – 18TH NOVEMBER 2009

SCIENCE GALLERY (TCD)

ABSTRACTS

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TUESDAY, NOVEMBER 17TH

9:50 – 10:35 *Stem Cell Therapy for Tissue Repair: The Stem Cell-Host Interaction*

F. Barry
Regenerative Medicine Institute, National Centre for Biomedical Engineering Science, National University of Ireland Galway, Galway, Ireland

Abstract:
Introduction: Mesenchymal stromal cells (MSCs) reside within the stromal compartment of bone marrow and other tissues. These cells have generated a great deal of interest because of their potential use in regenerative medicine and tissue engineering. While the therapeutic testing of these cells has progressed well, there are still many questions to be addressed concerning the role of endogenous populations of stem cells in the adult and the function of various stem cell niches.
The purpose of this study was to evaluate the nature of the transplanted stem cell-host interaction that underlies the therapeutic mechanism of action. Three animal models of human disease were used, each of which allows an assessment of aspects of the host response. The disease models were (1) osteoarthritis (OA) of the knee, (2) myocardial infarction (MI) and (3) human breast cancer xenografts. Each of these models allows an assessment of the mode of action of the transplanted cells. The results of these studies lead to the conclusion that neither extensive engraftment nor differentiation of the transplanted cells are prerequisites for a useful therapeutic response.
Methods: MSCs were isolated from bone marrow aspirates from multiple animal species, and characterised by measurement of cell surface antigens. OA was induced by complete medial meniscectomy in goats and MSCs expressing GFP were delivered by intraarticular injection. MI was induced by irreversible ligation of the LAD coronary *artery* in Fischer rats and PKH26-lebelled MSCs were delivered by myocardial injection. Female athymic nude mice received a subcutaneous injection of $2x10^7$ T47D cells. When tumors had reached a volume of ≥100 mm^3 the mice received a subcutaneous injection of $1x10^6$ PKH26-labelled MSCs.
Animals were sacrificed at several time points post delivery of cells and the target tissue was harvested, processed and sectioned for histological evaluation. In the case of the infarcted rats the hearts were harvested, digested with a mixture of collagenase and trypsin and the resulting cell suspension was separated by high speed cell sorting. The retrieved labelled MSCs were analysed for expression of tissue-specific and cell-specific markers and for differentiation potential.
Results: In each disease model labelled transplanted cells were observed at the site of injury. Levels of engraftment appeared low in the OA joints and in the infarcted hearts and higher in the xenograft tumours, even when they cells were delivered by IV infusion. Cells retrieved from the infarcted hearts up to 7 days after delivery showed no evidence of cardiomyocytic differentiation but appeared to retain the stem cell phenotype.
Discussion & conclusions: MSCs delivered to the injured host have the capacity to migrate to the site of injury and engraft, although with low efficiency. Engrafted MSCs apparently do not differentiate in a tissue-specific manner, but certainly remain viable. It

appears unlikely that the engrafted cells proliferate but this cannot be ruled out. These observations suggest that the therapeutic effect associated with MSC delivery is unrelated to their capacity to differentiate and more likely associated with their capacity to deliver soluble factors to the injured host.

Acknowledgements: This work was supported by Science Foundation Ireland

10:35 – 10:55 *In Vivo Imaging (Optical and Radionucleotide Imaging)*

A. Byrne
Royal College of Surgeons of Ireland, Dublin, Ireland

10:55 – 11:15 *External quality assurance of circulating tumor cell enumeration by automated immunomagnetic enrichment and image cytometry: A pilot study*

J. Kraan, M. Strijbos, S. Sleijfer, J.A. Foekens, J.W. Gratama
Erasmus MC, Rotterdam, The Netherlands

Abstract:
Circulating tumor cells (CTC) are cancer cells that have detached from solid tumors and entered the blood stream. CTC can be detected, among other methods, by automated immunomagnetic enrichment and image cytometry (CellSearch system; Veridex, Raritan, NJ). Using the latter technique, studies of patients with metastatic breast, colon and prostate cancer have shown that CTC enumeration has prognostic value and can serve as an early marker to assess response to systemic therapy. Periodic quality assurance of image interpretation is provided by the manufacturer. Here, we studied the feasibility of external quality assurance of the entire procedure from blood draw to interpretation of test result. Six fresh blood samples from patients with various tumor types were distributed to 13-15 laboratories with the request to enumerate CTC using the CellSearch system. These samples contained a median of 9.5 CTC per 7.5 ml (range 2 to 34). Using previously published cut-off values and on the basis of consensus scores of all laboratories, 5 of the 6 samples were scored "positive". Three samples were scored correctly by all labs, while misclassifications were made by 1-3 individual labs on the remaining samples, respectively.
In addition, a website was established showing 509 blinded images of the 6 samples; all participants were requested to classify each image as "positive" or "negative". Although the interrater agreement varied from "substantial" on 4 samples (Fleiss' κ between 0.61 and 0.80) to "almost perfect" on 2 samples (κ between 0.81 and 1.00), image interpretation did contribute to between-laboratory variation; specifically, poor sample quality and a high number of dead or apoptotic cells contributed to inconsistency in scoring. We conclude that in spite of the extensive guidance provided by the manufacturer to the users, inconsistency in image scoring largely contributes to inconsistency in results between laboratories and therefore remains a point of attention.

11:15 – 11:35 *Tissue-Based Imaging: Automated Image Analysis in Histopathology*

W. Gallagher
University College Dublin, Dublin, Ireland
OncoMark Ltd., Ireland

Abstract:
Virtual pathology, the process of assessing digital images of histological slides, is gaining momentum in the laboratory environment of today. Indeed, digital image acquisition systems are becoming commonplace and associated image analysis solutions are viewed by most as the next critical step in automated histological analysis. In this presentation, an overview of the recent advances in this technology area will be provided. Oncology has been a primary target area for these approaches, with example studies in this therapeutic area being covered here. Toxicology-based image analysis solutions are also profiled as these are steadily increasing in popularity, especially within the pharmaceutical industry. With there being several image analysis software programs focusing on the quantification of immunohistochemical staining, particular attention will be paid to this aspect, including showcasing of a novel in-house image analysis solution, IHC-MARK, that can quantify biomarker expression within nuclear, cytoplasmic and membranous compartments. In addition, the role of high-throughput pathology mediated via the use of tissue microarray technology will also be covered.

11:50 – 12:10 *Developing an effective scientific program within a core flow cytometry facility*

J.D. Gagnon, S.B. Lazo-Kallanian, J.F. Daley II
Dana-Farber Cancer Institute, Harvard Medical School, Harvard University, Boston, USA

Abstract:
Flow Cytometry technology has permeated many areas of science, is well established on a global scale and is used on a daily basis in many diverse fields, most notably basic research and clinical medicine. Although the technology has been constantly evolving to satisfy the current research and clinical application demands, two factors have predominately restricted the ubiquitous use of the methodology (with few exceptions): cost and instrument complexity. To overcome these limitations, centralization of cytometry instrumentation and specialized expertise has been concentrated in localized "core" facilities that interact with a wide user base and depending on demographics, experience various levels of demand.
One of the potential shortcomings of such an arrangement is the unbalanced emphasis on instrumentation automation and the eventual diminution of the thought process in improving and adding valuable insight to current research projects. To alleviate that condition from spreading and having a detrimental effect on the overall health of the core, we have taken a number of steps to revitalize the facility to operate in a science priority format mode while still maintaining an effective service component. In fact the implementation of constant experimentation in the facility related to current and future user applications has improved the homeostasis of the facility and makes it thrive in an otherwise very stressful environment.

Examples and certain case studies will be presented that illustrate how we have attempted to implement an aggressive scientific program into the core and what effect it has had in improving the dynamics of the interaction between the operators ,users, instrumentation, and most importantly the experiments that are processed through the facility.

12:10 – 12:30 *Hypoxia Mirrored in the Epigenome*

A. McCann
University College Dublin, Dublin, ireland

Abstract:
Increasing levels of tissue hypoxia have been reported as a natural feature of the aging prostate gland and may be a risk factor for the development of prostate cancer. In this study we have used PwR-1E benign prostate epithelial cells and an equivalently aged hypoxia-adapted PwR-1E sub-line to identify phenotypic and epigenetic consequences of chronic hypoxia in prostate cells. We have identified a significantly altered cellular phenotype in response to chronic hypoxia as characterised by increased receptor-mediated apoptotic resistance, the induction of cellular senescence, increased invasion and the increased secretion of IL1beta, IL6, IL8 and TNF alpha cytokines. In association with these phenotypic changes and the absence of HIF-1α protein expression we have demonstrated significant increases in global levels of DNA methylation and H3K9 histone acetylation in these cells, concomitant with the increased expression of DNA methyltransferase DMNT3b and gene-specific changes in DNA methylation at key imprinting loci. In conclusion, we have demonstrated a genome-wide adjustment of DNA methylation and histone acetylation under chronic hypoxic conditions in the prostate. These epigenetic signatures may represent an additional mechanism to promote and maintain a hypoxic-adapted cellular phenotype with a potential role in tumour development.

This work was supported by Health Research Board (HRB) in Ireland [HRB NS/2004/2], The Irish Cancer Society, and the UCD Horizon Scanning Seed funding scheme.

12:30 – 12:50 *A Solution to Sorting Unscreened, Live, Human Stem Cells*

R. Grenfell
Cancer Research UK – Cambridge, Cambridge, UK

Abstract:
Biosafety has always been an issue in flow cytometry, particularly with aerosol generation of droplet forming cell sorters. The safety of instrument operators can sometimes take second place behind a researcher's requirements.
With translational research moving from animal models into humans, the change in Hazard Group needs to be considered for cell sorting. In Europe, Human samples obtained for use in this research is very rarely screened for pathogens such as HIV, Hepatitis or others, that may be passed on through aerosol generation in a cell sorter.

We will show a solution to sorting at Containment Level 2 that complies with, and is validated to, the European Standard BS:EN 12469:2000, by incorporating a cell sorter completely within a biosafety cabinet, yet still allowing for cabinet fumigation and instrument service.

13:40 – 14:25 *Apoptosis: Controlled Demolition at the Cellular Level*

S.J. Martin
Molecular Cell Biology Laboratory, Dept. of Genetics, The Smurfit Institute, Trinity College Dublin, Dublin, Ireland

Abstract:
Apoptosis is characterized by a series of dramatic perturbations to the cellular architecture that contribute not only to cell death, but also prepare cells for removal by phagocytes. Much of what happens during apoptosis is orchestrated by members of the caspase family of cysteine proteases. These proteases target several hundred proteins for restricted proteolysis during this complex process of cell demolition. Here, we discuss how caspases provoke many of the signature events of apoptosis and also highlight unresolved issues relating to their role in this process.

14:25 – 14:45 *The human omentum as an immunological tool*

L. Lynch[1,2], M. Nowak[1], D. O'Shea[2], M. Exley[1], C. O'Farrelly[3]
1 Beth Israel Deaconess Medical Centre, Harvard Medical School, Boston, USA
2 Obesity and Immunology, St. Vincent's University Hospital, Dublin, Ireland
3 School of Biochemistry and Immunology, Trinity College Dublin, Ireland

Abstract:
Prediction Invariant natural killer T (iNKT) cells recognize lipid antigens and respond rapidly by killing tumor cells and releasing cytokines that activate and regulate adaptive immunity. We have previously described an accumulation of functional iNKT cells in human omentum, a fat storing organ. Here we show that murine fat also harbours iNKT cells, in larger numbers than in liver, and that adipose-derived iNKT cells are phenotypically different from their splenic and hepatic counterparts. In vivo and in vitro stimulation with aGC, followed by flow cytometric analysis for intercellular cytokines shows that fat derived iNKT cells in fat produce less IFNg and more IL10 than other NKT cells. To investigate the origin of fat derived iNKT cells, we used adoptive transfer of iNKT cells from human Va24 transgenic mice into wt mice and examined each organ by flow cytometry after 2 and 24 hours post transfer. iNKT cells home to fat within 2 hours but not other organs. In vitro transwell homing assays using murine fat as a target found that chemokine receptors CCR5 and CCR6 are important for iNKT cell migration to fat. Concentration of anti-tumor iNKT cells in adipose tissue, both in humans and mice suggests a unique anti-metastatic or immunoregulatory role for the adipose tissue. Omental adipose tissue is easily accessible and is therefore a potential source of iNKT cells for therapeutic use.

14:45 – 15:05 *Using Imaging Flow Cytometry to Examine Intracellular Delivery of Polymeric Nanosensors*

R. Pineda
University of Nottingham, Nottingham, UK
R.G Pineda1[*+], M. Herod[2*], V. Mautner[2], J. Aylott[1]
[1] School of Pharmacy University Nottingham, UK
[2] CRUK Institute of Cancer Studies Birmingham, UK
*equal contributors
[+] Correspondence to paxrp1@nottingham.ac.uk

Abstract:
Optical polymeric nanosensors know as PEBBLEs (Probes Encapsulated by Biologically Localised Embedding) have been developed for a range of analytes based around analyte sensitive fluorescent dyes. These nanoscale devices allow quantitative measurements to be made in the intracellular and extracellular environment and have significant advantages over the use of free fluorescent sensitive dyes, electrochemical measurement or fibre optic probes for measurement of analytes. Advantages include protection of the analyte sensitive dye sensing component from interference from intracellular or extra cellular proteins, protection of the intracellular environment from toxicity of the analyte sensitive dyes and high spatial resolution. Oxygen sensitive PEBBLEs have previously described characterised and delivered to cells in culture. Here we build on previous studies and take this work further and describe the use of Imaging Flow Cytometry in the first stage of optimisation of delivery of nanosensors to the intracellular environment. This is part of a larger project to allow the mapping of oxygen levels in 3D tissue culture models initially multicellular spheroids and then multicellular layers (MCL).

15:05 – 15:20 *Multiplex Analysis of Cytokine and Immunoglobulin Production in Invariant Natural Killer T Cell – B Cell Interactions using Cytometric Bead Arrays*

S. Grace Zeng, A.E. Hogan, D.G. Doherty
Dept of Immunology, School of Medicine, Trinity College Dublin, Ireland

Abstract:
Invariant natural killer T (iNKT) cells are an innate subset of T cells that express receptors that can also be found on natural killer cells. They have been described as mediators that bridge innate and adaptive immunity as they have the ability to interact with and activate a myriad of cells such as natural killer cells, dendritic cells and B cells. iNKT cells have been reported to interact with B cells by providing direct help to the latter. However, not much is known about the dynamics and mechanisms behind the interaction. Here we attempt to gain greater understanding of what happens during the interaction by generating a temporal profile of the cytokines and immunoglobulins produced in the interaction. iNKT cells and B cells were co-cultured in vitro for 3 days and 10 days, and supernatants were analysed using cytometric bead arrays, which allowed us to perform multiplex analysis of the cytokines/immunoglobulins present in the supernatant. We observed production of IL-4, IL-5, IL-6, IL-12p70, IL-13, IgG, IgA and

IgM in the co-cultures. Overall, the results suggest that interaction between iNKT cells and B cells results in a bias towards Th2 cytokine production and that iNKT cell help to B cells is sufficient to cause antibody production by the latter *in vitro*.

15:20 – 15:35 *Marrow Stromal Cells (MSCs) potently inhibit the primary induction of T-helper 17 Cells through Cell-Cell Contact*

M. Duffy, J. Pindjakova, S. Hanley, G. Weidhofer, K. English, B. Mahon, R. Ceredig, M. Griffin.
Regenerative Medicine Institute, NUI Galway, Galway, Ireland
Institute of Immunology, NUI Maynooth. Dublin, Ireland

Abstract:
Background: MSCs are immunosuppressive with potential to treat autoimmune/inflammatory disease. Pro-inflammatory T-helper 17 cells (Th17) are pathogenic in some autoimmune diseases. In this study the interactions between mouse MSCs and CD4+ T-cells undergoing primary Th17 induction in vitro were examined. Th17 induction consisted of stimulation with anti-CD3 and autologous APCs in the presence of IL-6, TGFβ1, anti-IFNγ and anti-IL-4. Analyses included ELISA, surface/intracellular flow cytometry, and cell division analysis by CFSE dilution. In some experiments, MSC effects on flow-sorted naïve (CD4+/CD62Lhi/CD25-) and memory (CD4+/CD62Llo/CD25-) T-cells were separately examined.
Results: MSCs potently inhibited primary Th17 induction in co-culture at T-cell:MSC ratio as low as 400:1 ratio. This effect was characterised by preserved T-cell viability, moderately reduced proliferation, suppression of CD25 up-regulation and decreased production of IL-17 upon re-stimulation in the absence of MSCs. Th17 inhibition occurred when either naïve or memory T-cells were induced in the presence of MSCs. Skewing to other T-helper phenotypes was not observed although an IFNγ-secreting component was preserved among memory T-cells. Th17 suppression was mediated by either autologous or allogeneic MSCs but not by fibroblasts. The effect was maintained when APCs were replaced by antiCD3/CD28-coated beads and was unaffected by pre-treatment of MSCs with IFNγ or irradiation. MSC-conditioned medium failed to suppress primary Th17 induction.
Conclusion: In the mouse, the capacity of MSCs to modulate T-cell activation extends to primary Th17 induction through a contact- or proximity-dependent mechanism. Of interest, the effect is quite potent in vitro and applies to both naïve and memory-phenotype responders.

15:35 – 15:50 *The Role of Cytochemistry and Flow Cytometry in the Modern Diagnosis of Acute Myeloid Leukaemia*

N. Chanzu[2], K. Whitting[2], R. Saso[3], R. Morilla[1]
[1] Institute of Cancer Research, Sutton, UK
[2] Kingston University, London, UK
[3] Royal Marsden Hospital, Sutton, UK

Abstract:
Cytochemical and morphological staining has been used in the diagnosis of Acute Leukaemias for many years. Immunophenotyping by Flow Cytometry has become an essential component of Acute Leukaemia diagnosis. This study was designed to evaluate whether cytochemical staining adds any potential useful information to the diagnosis of Acute Leukaemias by Flow Cytometry. To assess if one technique was more accurate and to analyse if the results and diagnostic values were comparable. A total of 64 Acute Leukaemia patients were studied. They were subclassified as follows: 54 Acute Myeloid Leukaemias (AMLs), 1 Juvenile Myelomonocytic Leukaemia (JMML), 3 Biphenotypic Acute Leukaemias (BALs), 1 Dendritic Cell Leukaemia (DCL), 4 Relapsed Leukaemias and 1 Residual Leukaemia. Flow Cytometry and Cytochemistry results were selected for retrospective review if the initial diagnosis was of Acute Leukaemia. Based on the light microscopy morphology, cytochemical reactions and surface antigen expression, the majority of the leukaemias were accurately diagnosed, characterised and classified. Multiparametric Flow Cytometry, i.e. MPO, CD13 and CD33 (pan-myeloid markers) diagnosed 100% of Leukaemias in the study. Morphology and Cytochemistry only diagnosed 88.71% of the Acute Leukaemias but failed to identify the more rare types of Biphenotypic Acute Leukaemias (BAL) and Dendritic Cell Leukaemia (DCL). The new WHO classification (2008) established that MPO positivity was the gold standard to diagnose BAL. In these series of 3 BAL, 1 was both SBB and MPO (By FC) negative and 1 SBB negative, MPO (By FC) negative and 1 SBB negative and MPO (By FC) positive. Flow Cytometry had a major role in defining major subgroups of AMLs, in particular Minimally differentiated Acute Leukaemia (AML-M0), Biphenotypic Acute Leukaemia and Dendritic Cell Leukaemia. Results for this study indicate that with a few exceptions, Flow Cytometry alone provides sufficient information to diagnose and classify Acute Leukaemias. Nevertheless cytochemical staining should be available for those cases in which Flow Cytometry results fail to allow a definitive diagnosis.

16:05 – 16:35 *Leukaemic Stem cells in Acute Myeloid Leukaemia*

N. Goardon
University of Oxford, Oxford, UK

16:35 – 16:50 *Nucleoids Remodeling and Impaired Mitochondrial Dynamics in Human Malanoma Cells in Response to UVR*

L. Zanchetta[1,3], A. Garcia[2], J. Walsh[2], F. Lyng[3], J.E.J Murphy[1]
[1] Mitochondrial Biology and Radiation Research Group, IT Sligo, Sligo, Ireland
[2] Radiation & Env. Science Centre, Dublin, Institute of Technology, Dublin, Ireland
[3] School of Physics, Dublin, Institute of Technology, Dublin, Ireland

Abstract:
Introduction: Ultraviolet-radiation (UVR) causes cell damage or death by disrupting cellular energy metabolism at multiple levels directly altering DNA and protein structures and by production of reactive oxygen species (ROS). Mitochondrial dynamics changes, more precisely mitochondrial fusion disruption, are associated with mtDNA nucleoids loss and decreased mitochondrial respiratory function. Damaged mitochondria are possibly recycled thought mitophagy. In order to study whether UVR causes mitochondrial nucleoid remodelling and if such reorganizations are dependent on or associated with specific metabolic energy sources, melanoma cell line (A375) were metabolic challenged with/without subsequent exposure to a Q-Sun solar simulator and assessed by confocal microscopy.
Methods: In the present study the mitochondrial nucleoid size and distribution patterns, mitochondrial membrane potential, morphology and distribution was accessed by DNA, mitochondria and lysossome specific-fluorescent staining in human malignant melanoma (A375) using a LMS 500 confocal microscope. Cells were glutamine and glucose bio-energetically challenged, mitochondrial fusion inhibited (FCCP) or stimulated (creatinine), with and without subsequent UVR. Mitochondrial nucleoids quantification and cytoplasmic distribution was reported through in vivo fluorescent mtDNA stainning. Mitochondria and lysosomes morphology and distribution were studied thought organelle-specific fluorescent dyes.
Results
Decrease in MMP, mitochondrial mass and changes in the mitochondrial nucleoid number and size were observed as an early response to UVR, with or without energetic sources challenging, on human melanotic melanoma cells. Confocal image analyses revealed that a fraction of the mitochondria were devoid of mtDNA post UV irradiation and that the nucleoid size and distribution changed on a dose-specific and energetic substrate-dependent manner. UV irradiation associated to bioenergetics challenging (low glutamine and low glucose availability) was associated with lysosomes accumulation.
Conclusions: We found mitochondrial and nucleoid number and distribution to be altered in human melanoma cells subjected to simulated sunlight irradiation, suggesting that mitochondrial dynamics may play an important role in the cell response to UVR light damage. Simulated sunlight irradiation lead to mitochondria and mitochondrial nucleoid changes possible due to effects on the regulation of mitochondrial dynamics as well as lysosomes accumulation, suggesting possible changes in the patterns of the mitochondrial recycling dynamics thought the mitophagic process. Moreover, we have found that mitochondrial nucleoid undergoes remodelling thought UVR and metabolic challenging alone and, on a greater extent, when both cell-stressing agents were used in association. Impaired mitochondrial bioenergetics, dynamics and recycling may play a role in the

melanoma tumour initiation and progression in humans post systematic sunlight over-exposure.

16:50 – 17:05 *Translation of Novel Anti-Cancer Cytotoxicity Biomarkers Detected with High Content Analysis from an In Vitro Predictive Model to an In Vivo Cell Model*

N.T. Lieggi[1], L.A. Edvardsson[1], S. Hancock[2], P.J. O'Brien[1]
[1] Veterinary Science Centre and Advanced Diagnostics Laboratory, University College Dublin, Ireland
[2] GE HealthCare, Cardiff, UK.

Abstract:
Using high content analysis (HCA), we demonstrated an in vitro cytotoxicity model for predicting human toxicity, and furthermore, that cytotoxicity could be assessed in peripheral blood lymphocytes. Here we assessed whether cytotoxicity biomarkers translate from in vitro to in vivo models using anticancer drugs (arabinoside C [AC], arsenic trioxide [AT], doxorubicin [DOX], and mitoxanthrone [MX]) and staining live cells with Hoechst for DNA (NA nuclear area, NI nuclear intensity), Fluo-4 for calcium (IC), TMRM for mitochondrial membrane potential (MMP) and TOTO3 for plasma membrane permeability (PMP). Ionomycin, FCCP and Triton were used as positive controls for IC, MMP and PMP, respectively.

	AC		AT		DOX		MX	
	HepG2	Jurkat	HepG2	Jurkat	HepG2	Jurkat	HepG2	Jurkat
NA	20	-	-	-	2	2	2	-
NI	-	-	-	-	2	2	2	2
MMP	200	20	-	-	2	2	2	0.02
IC	20	-	-	-	0.02	2	-	0.2
PMP	-	20	-	20	-	-	2	2
CN	20	20	20	20	0.02	0.02	0.002	0.002
μM Concentration for half observed effect at 72 h								

Human HepG2 hepatocytes and Jurkat T-lymphocytes were treated with 10-fold increasing concentrations of drug from 0.002μM or 0.02μM for either 24hr or 72hr. In both cell types, cytotoxicity was typically detected at one concentration point lower at 72hr than at 24hr. Hormesis (biphasic response) was observed for NA, NI, MMP, PMP, and cell number (CN), especially at 72 hr. We conclude cytotoxicity biomarkers translate from the in vitro, predictive, hepatocyte model to the in vivo lymphocyte model. The in vivo model was at least as sensitive as the in vitro model. Thus, HCA cytotoxicity biomarkers may not only be predictive of human toxicity potential but may be translatable for detection and monitoring of subclinical toxicity.

WEDNESDAY, NOVEMBER 18TH

9:30 – 10:00 *Multiparametric Analysis of Mesenchymal Stromal Cells from Different Sources*

F. Lanza
Hospital de Cremona, Italy

10:00 – 10:30 *New Technological Advances and their Effects on Polychromatic Flow Cytometry*

V. Toxavidis, J. Tigges, S. Lazo-Kallanian
Harvard University, Boston, USA

Abstract:
As the scientific process becomes more complex and diverse, the advancement of technology must follow suit. At the least, from a flow cytometric standpoint, antibodies, instrumentation, and software must be one step ahead. Experiments have made the transition from only a few colors to many colors in just a short time period. As the need to look at more targets simultaneously increases, so do the need for greater antibody combinations and the ability to analyze those combinations effectively. Even simple scatter property analysis is requiring enhanced sensitivity for separation of populations, especially when dealing with submicron particles. Recent developments, from both leading companies and start-ups, have made the analysis possible and much easier to do. With the development of more efficient optics, greater resolution, and laser line diversity, both compensation and rare event analysis have become much simpler. In addition, greater instrument stability, available temperature control, and advanced sorting options have made for greater cell purity and better overall cellular health. Finally, with more efficient software, experimental design to final analysis has become seamless.
Generally speaking, the advancement of flow cytometry technologies has immensely benefited the research process. With software that can handle large amounts of data at tremendous speeds, and researchers having the ability to label cells with more and more antibody combinations, the process of discovery has become more efficient and faster. At this point, we are not sure what the next technological breakthroughs will be, but we have a wish list. However, we are sure they will be, not only exciting, but extremely beneficial in helping carry research and discovery into the future.

10:30 – 11:00 *Cytomics for Asessment of Drug Safety and Prediction of Human Acute and Chronic Toxicity*

G. Herrera[1], A. Martínez-Romero[1], L. Díaz[1], A. Gomes[1], S. Pinto[1], T. Donato[2], M.J. Gómez-Lechón[2], J.V. Castell[2], J.E. O'Connor[1]

[1] Laboratory of Cytomics, Mix Research Unit, Principe Felipe Research Centre and University of Valencia, Valencia, Spain
[2] Laboratorio de Patología Experimental, Centro de Inestigación, Hospital Universitario La Fe, Valencia, Spain

Abstract:
Prediction of toxicity is highly relevant for drug safety assessment and regulatory Toxicology for consumer protection. To both purposes, validated assays and test strategies which replace, reduce or refine use of animals (3R principle) are required. On the one hand, pharmaceutical companies may benefit from early detection of acute or chronic toxicity of a drug candidate. On the other hand, the recent EU policy REACH for Registration, Evaluation and Authorisation of Chemicals implies toxicity assesment for more than 30,000 chemicals. While the acute toxicity of a substance or a drug candidate can be easily detected in vitro, anticipating long-term toxicity proves more complicated. In order to predict acute human toxicity we have set up novel in vitro cytomic methods based upon flow cytometry (FCM) and High-Content Analysis (HCA). 96-well format assays are grouped as Cytomic Panels for Cytotoxicity (CPCS) and Oxidative Stress Screening (CPOSS) with three human cell lines and include FCM assays of viability, intracellular calcium, mitochondrial and plasma membrane potentials, superoxide anion and peroxides, and HCA assay of oxidative damage to genomic and mitochondrial DNA. To assess the predictive value of cytomic assays, IC50 or EC50 in vitro values for 58 chemicals of reference have been correlated by cluster analysis and hierarchization with reported in vivo LC50 or LD50 in humans, rat and mouse. Most assays have excellent correlation with in vivo human toxicity data, and classify compounds better than the United Nations Global Harmonization System (GHS) based on rat toxicity parameters. In addition, our assays identify compounds labelled as non-toxic by the GHS classification and provide alerts for specific toxicity. For prediction of chronic hepatotoxicity we have designed and validated wo novel, complementary short-term in vitro cytomic assays of steatosis and cholestasis, major causes of drug withdrawal from the discovery pipeline or the market. Risk of steatosis is approached by an assay consisting of 24-hour exposure of HepG2 cells to free fatty acids in the presence of test compounds. Cholestasis is predicted from the interference of test compounds on bile acid uptake by isolated rat hepatocytes, with a kinetic assay of novel bile-acid fluorescent derivatives. Our data show that cytomic assays and data mining by array analysis may be very useful in assessment of drug safety and chemical hazard to humans. Sponsored by European Comission (grants LSHB-CT-2004-512051, grants LSHB-CT-2004-504761 and LSSBCT-2005-037499) and Spanish Ministry of Education and Science (BIO2007-65662).

11:20 – 11:35 *Regulation of the NLRP Inflammasome by phosphoinositide-3-kinases and Autophagy*

J. Harris, M. Hartmann, A. O'Shea, E. Creagh, O. Hanrahan, E. Lavelle
School of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland

Abstract:
The assembly and activation of inflammasomes are essential processes in the immune response to many inflammatory stimuli. Inflammasomes are typically formed by at least one member of the cytosolic innate immune sensor family, the NOD-like receptors (NLR). The NLR family members NLRP3, NAIP5 or Ipaf and the adaptor ASC are involved in caspase-1 activation in response to bacterial infection, triggering the processing and secretion of IL-1β and IL-18. Recent studies have demonstrated that TLR-dependent inflammasome activation in macrophages is modulated by autophagy, a homeostatic mechanism for the catabolism of cytosolic constituents. Autophagosome biogenesis and maturation requires activation of the class III PI3K, Vps34 and can be inhibited with the PI3K inhibitors wortmannin and 3 methyladenine (3MA). In contrast, activation of Akt, via class I PI3K, results in inhibition of autophagy. We have examined the effects of inhibiting autophagy on inflammasome activation in response to TLR ligation. Secretion of active IL-1β is enhanced in dendritic cells and macrophages treated with 3MA or wortmannin in combination with LPS or poly(I:C). Similarly, inhibition of autophagy by siRNA knockdown of beclin 1 (Atg6), a tumour suppressor that is critical for autophagy, enhances IL-1β secretion by macrophages in response to LPS treatment. This effect is dependent on NLRP3 and the production of reactive oxygen species (ROS). Moreover, there appears to be a specific role for TIR-domain-containing adapter-inducing interferon-β (TRIF) in this response. These data further demonstrate that autophagy plays an important role in inflammasome activation in macrophages and dendritic cells and highlight some of the mechanisms behind this regulation.

11:35 – 11:50 *Use of Hermetic Cell Culture Containers (PetakaG2T) for Cell Biological Research*

J.E. O'Connor[1], G. Herrera[1], L. Díaz[1], E. Barberá-Guillem[2]
[1]Laboratory of Cytomics, Mix Research Unit, Principe Felipe Research Centre and University of Valencia, Valencia, Spain.
[2]Celartia Europe, S.L., Valencia, Spain

Abstract:
The development of testing strategies for the study of long-term in vitro toxicity is an essential issue in toxicology, as previous works which attempt model systems mimicking chronic exposure attest. Complex flow-cell and static cell bioreactor systems have been compared satisfactorily with conventional cell culture flasks in sustained toxicity testing, but simple and cheap systems allowing long-term exposure of unperturbed cell cultures are still lacking. Recently, a novel concept of environment-independent, hermetic container (Petaka™, Celartia Ltd., www.celartia.com) has evolved to maintain long-term cell cultures without need of CO2 incubator. Petaka™ is a hermetic flat flask with the

footprint of a 96-well plate (150 cm2 culture surface) designed to exchange very low amounts of CO2, due to an integrated gas transfer quenching system. As a result, the level of CO2 is maintained within the container, allowing a pH compatible with the growth of 16-22 million cells. In addition, no added humidity is required, as the hermetic culture is not influenced by environmental dehydration. Petaka™ provides the cells with a gradually decreasing oxygen concentration within the physiological limits of living tissues. Cell culture may begin with a concentration high enough to promote exponential cell growth. The concentration is then progressively reduced avoiding cell damage and facilitating cell differentiation. Cell monolayers grown in Petaka™ at room temperature and low pH, reduce DNA replication practically to zero, enter a dormant-like state of the cell cycle and remain viable for long periods of time. Dormancy is dependent on the cell type. At least, 7 days survival is common for most cells, while others may live for more than 200 days. MultiCytox-LT® is an assay consisting of a long-term exposure of relevant cell cultures grown in Petaka™ to test compounds and controls. Toxic effects are monitorized in situ and quantified by a cytomic panel of miniaturized assays which determine cell death and quantify Ca2+, mitochondrial- and plasma membrane potential, polar and non-polar lipids, peroxides, mitochondrial generation of superoxide, and oxidative damage to genomic and mitochondrial DNA. MultiCytox-LT® is designed for sustained in vitro toxicology studies but can be applied to general, mechanistic or predictive approaches in Toxicology and Pharmacology.

11:50 – 12:10 *Combining Atomic Force Microscopy with Confocal Microscopy: Illuminating the Role of Forces in the Life Cycle of Cells*

M. Van Es, G. Kelly, P. Weafer, S.P. Jarvis
University College Dublin, Dublin, Ireland

Abstract:
Most cell types are subjected to forces from their surroundings and it has recently become clear that cells are capable of sensing these mechanical stimuli through specific proteins that have force-sensing elements with catalytic activity. This process of converting a mechanical stimulus into chemical activity is known as mechanotransduction and it modulates a broad range of cellular functions including migration, proliferation, differentiation and apoptosis. Thus any defects in this process, for example by misregulation of proteins, can have implications for the development of a number of diseases.
Atomic Force Microscopy (AFM) has traditionally been used to characterize surfaces at the nanoscale. However, it can also be used to apply nanoscale forces at specific locations. We use a combined AFM / Confocal / Total Internal Reflection Fluorescence microscope to apply forces to specific locations on a cell surface and simultaneously check the response of cells using fluorescent markers. In this way we have studied cell adhesion to various substrates, which is an important factor in tissue engineering and the development of new materials for implants. We have also investigated differences in cellular signaling under mechanical loading between healthy and pathological cells and at different stages in the life cycle of cells, indicating the value of these combined techniques in understanding the role of forces in cell function

12:10 – 12:30 *Flow Cytometric Response and Comparative Analysis of Immunofunctionalized Silica coated Magnetic Nanowire and Nanoparticle*

A. Prina-Mello
Trinity College Dublin, Dublin, Ireland

Abstract:
Flow cytometry is the one of the gold standard technique used in clinical medicine for quantitative immuno-assaying. Recently, quantitative multiplexed immunoassay has challenged the use on nanoparticles as probes. This work investigate the comparative response of functionalized fluorescent silica based magnetic nanowires and nanoparticles under flow cytometry as a novel probe category. Preparation and full characterization of these multimodal nanowires is reported and compared to silica based magnetic nanoparticles by flow cytometry. This work shows that loaded silica nanowires have intrinsic geometrical advantages when compared to similar particles due to their unique "flow cytometry fingerprint" when utilized as magnetic carriers for immunodetection applications.

12:30 – 12:45 *Instantaneous Imaging of Protein Structure and Function for High Throughput Fingerprinting*

I.P. Mercer
University College Dublin, Dublin, Ireland

Abstract:
We have been limited on our ability to generate detailed fingerprints of molecular structure in combination with high throughput. Since its introduction, NMR has seen rapid and widespread take up and within a generation has become a common tool for undergraduate chemistry and industry laboratories. NMR generates fingerprints that relate to molecular structure by mapping the couplings between the nuclei of molecules. Although indispensible in its fields of applicability, NMR has drawbacks in terms of the required sample exposure time and preparation.
We present a novel laser method, Angle-Resolved Coherent (ARC) mapping, that for the first time directly separates out the mechanisms of protein electronic couplings (analogous to those for nuclear couplings in NMR) to give two-dimensional mappings that represent molecular function and structure. This method has recently been demonstrated to instantaneously map new information on electronic couplings in a protein at ambient temperature in solution.
We are interested in exploring avenues for high content screening and generally for high throughput fingerprinting of molecular function. Complete maps, rich in detail, can be taken from solutions, surface films or solids of between 1 and 1000μL. Each ARC mapping is generated instantaneously, with high throughput (currently up to 1kHz frame rate) and is noninvasive.

13:45 – 14:30 *Looking Cell Death in the Eye*

T. Cotter
Department of Biochemistry, University College Cork, Cork, Ireland

Abstract:
The eye is the organ though which we see and image the world and any loss of light detecting cells in the retina will have a detrimental effect on one of our most important senses. In this presentation I will describe how we have used a variety of biological methods, including several imaging techniques, to investigate how cells struggle for survival in the face of molecular stresses that are behind so many degenerative conditions in the eye.

Oxidative stress is a key stress component that drives cell death in the eye (one of the most metabolically active organs in the body), but paradoxically it also has a role in cell survival. This Janus like role is dependent on concentration, with low concentrations of reactive oxygen stimulating cell survival, but higher concentrations pushing the cell towards death. The Nox family of enzymes has the ability to produce reactive oxygen species and we describe how these enzymes contribute to, for example to the activation of the PI3k/Akt cell survival pathway as cells struggle to stay alive under molecular stress. I will also describe how recombinant fibroblast growth factor (FGF) promotes cell survival, both in cell lines and in animal models of retinal disease.

14:30 – 14:50 *The Relevance Of Ultrastructural Analysis in the Diagnosis Of Human Disease*

D. Cottell
The Electron Microscopy Laboratory, Core Technology, Conway Institute of Biomolecular and Biomedical Research, UCD Dublin, Ireland

Abstract:
Image resolution in light microscopy is essentially limited by the wavelength of light, λ = 500nm. Even using optimum conditions and the highest quality equipment light microscopy is incapable of revealing structures of less than 0.25 μm (limit of resolution). This places serious limitations to our understanding of biological structures smaller than 0.25 μm and hence to our comprehension of cell biology and disease. However, electron beams can be generated having a wavelength of less than 0.004nm and when such radiation is used in transmission electron microscopy (TEM) image resolution is increased to less than 0.2 nm which allows scientific exploration of molecular and even atomic structures.
The foregoing underscores the importance of ultrastructural analysis in biological science and in the diagnosis of certain human diseases. To illustrate this I will concentrate on three areas of interest where TEM is not only useful but in fact where it is necessary if the diagnosis is to be secured. (1) In renal pathology, TEM distinguishes membranous glomerulonephritis from membranoproliferative glomerulonephritis. (2) In Cardiac disease, amyloidosis involvement may simulate cardiomyopathy, congestive heart failure or coronary heart disease. However, in cardiac biopsies the identification of the

characteristic fibers of amyloid by TEM secures the diagnosis as cardiac amyloidosis. (3) The hereditary absence of dynein arms in the cilia of epithelia causes a lengthy sequence of pathology in all histological systems which are ciliated. This, ciliary dyskinesia syndrome is elucidated only by ultrastructural examination of bronchial or nasal biopsy
This presentation will draw on ultrastructural observations from biopsies which have been studied by the author over the past 30 years. In addition, all relevant background histological data will be discussed for the audience to better comprehend the significance of the diagnosis.

14:50 – 15:10 *Investigating Autophagy in Human Antigen Presenting Cells and Mouse Knockout Models using Imagestream*

K. Phadwal
University of Oxford, Oxford, UK

Abstract:
Autophagy is a catabolic process whereby cytosolic components like long lived proteins and damaged organelles are taken into autophagosomes, which later fuse with Lysosomes, where they are degraded by acid hydrolases. It has also emerged as a major immune mechanism against pathogens in macrophages. Although mitochondrial Autophagy (mitophagy) has been found as a mean of mitochondrial removal from developing erythroid cells in vivo, the role of the core autophagy gene in erythroid development has not yet been studied. We have created mice with conditional autophagy knockout in the hematopoietic system to study the role of core autophagy genes in erythroid development.
We have observed that mice lacking the essential Autophagy gene Atg7 in the hematopoietic system develop a lethal anemia. The Autophagy deficient erythrocytes have a decreased life span in vivo and they show an overall increased susceptibility to cell death. The removal of mitochondria is impaired in these erythrocytes and damaged mitochondria accumulate in them.
In parallel we have developed a high throughput Autophagy assay using Imagestream. This assay is developed on PBMCs and Human antigen presenting cells. This will ultimately allow us to do further studies to understand the genetic basis of certain idiopathic human anemia like Myelodysplastic Syndrome and also to investigate the role of autophagy in various viral diseases.

15:10 – 15:25 *Characterisation of the Phenotype, Developmental Potential and Immunosuppressive Properties of Mouse Mesenchymal Progenitor Cell Lines and Clones.*

A. Stocca[1], M. Duffy[1], S. Hanley[1], S. Gaughan[1], M. Griffin[1], S. Elliman[2], R. Ceredig[1]
[1] NCBES, National University of Ireland, Galway, Galway, Ireland
[2] Regenerative Medicine Institute, Pro-Cure Laboratories, Ireland

Abstract:
Controversy exists as to whether the mesenchymal stromal cells of bone, cartilage and fat derive from a single stromal "stem" cell or rather from a group of differentially committed stromal progenitors. In the mouse, mesenchymal stromal cells are difficult to isolate, propagate and manipulate. Single cell experiments have not yet unambiguously demonstrated the existence of tri-lineage (osteoblast, chondrocyte, and adipocyte) progenitor cells. In order to address this issue, we have begun characterising pre-existing lines and clones of mouse stromal cells. Phenotypic analysis confirmed that all lines were homogeneously CD44+ and CD45- but differed in their expression of CD73, CD90 and CD105, markers typically used to identify mouse stromal cells. One line (B16-14) derived from an Interleukin-7 transgenic mouse line and containing a temperature-sensitive form of SV40T antigen was cloned by limiting dilution following sorting on an ARIAI flow cytometers using automatic cell deposition into 96 well microtiter plates. The plating efficiency of sorted cells was about 1/19 and from these experiments, nine independent B16-14 clones have been maintained for further study. Analysis of the differentiation potential along the osteogenic lineage showed heterogeneity with B16-14 being one of two with potent osteogenic potential. Interestingly, one of the nine B16-14 clones did not differentiate along the osteogenic lineage. Likewise, the lines showed heterogeneity in their ability to differentiate along the adipocyte lineage. However, as measured by flow cytometry of in vitro-activated CFSE-labelled CD4+ T cells, all stromal cell and clones could potently inhibit T lymphocyte proliferation. Further experiments are planned to characterise these cell lines.

15:25 – 15:40 *Study of cell death induced by amine-modified polystyrene in 1321N1 cells*

F. Wang[1], M. G. Bexiga[1,2], J. Varela[1], A. Salvati[1], I. Lynch[1], K. Dawson[1]
[1] Centre for BioNano Interactions, University College Dublin, Belfield, Dublin 4, Ireland
[2] PhD Programme in Experimental Biology and Biomedicine, Center for Neuroscience and Cell Biology, University of Coimbra, 3004-517 Coimbra, Portugal

Abstract:
Cationic nanoparticles (NPs) have been shown to induce apoptosis in various cell types. Using cationic NPs to target diseased cells and selectively induce apoptosis could be a promising tool for nanomedicine. For this purpose, a full understanding of the apoptotic mechanism triggered by these NPs is required, in order to elucidate which pathway is activated and determine the timing of the events along the apoptotic cascades induced by NPs.
Functional assays and imaging techniques were used to study apoptosis induced by 50nm amine-modified polystyrene in an astrocytoma cell line - 1321N1. YoPro 1/ Propidium

iodide staining, western blot of PARP cleavage and activation of different CASPASEs have previously confirmed that the cell death occurs mainly by apoptosis. Electron microscopy images showed damage in lysosomes and mitochondria. Both flow cytometry and live cell imaging were used to follow lysosomal rupture, ROS generation, mitochondria membrane potential collapse and intracellular and mitochondria calcium flux change. Moreover, fluorescently labelled cationic NPs were used to visualise the uptake and sub-cellular distribution at different times by confocal microscopy.
All the evidence shows that stimulating 1321N1 cells with 50 nm amine-modified polystyrene NPs results in disruption of both lysosomes and mitochondria. Further studies will be focused on the stress in the endoplasmic reticulum to further the study of the apoptotic signalling pathway activated by cationic NPs.

15:55 – 16:15 *A Flow-Cytometric Method for a Continuous Measurement of Intracellular Ca^{2+} Concentration*

A. Vines[1], G.J. McBean[1], A. Blanco Fernández[2]
[1] UCD School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland.
[2] Flow Cytometry Core Facilities, UCD - Conway Institute of Biomolecular & Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland.

Abstract:
Alterations in intracellular Ca2+ concentration is one of the most rapid and significant responses to a variety of stimuli in majority of mammalian cells. In the nervous system in particular, responses occur within nanoseconds. A major challenge in intracellular Ca2+ recording is to achieve measurements within this very fast time frame. To date dynamic intracellular Ca2+ concentration has been monitored using a variety of techniques such as confocal microscopy and flow cytometry. An advantage of confocal microscopy is the examination of the response of a small number of cells throughout the recording, however the amount of cells examined may be as little as 10 per field. While flow cytometry provides analysis of thousands of cells, it has never been possible to add compounds to be examined without a gap in recording with significant lost of detail of a rapid Ca2+ response. New technologies, such as high content screening are trying to address this issue.
A new generation of flow cytometers (such as Accuri C6), which operates an open as oppose to the standard pressurised system resolves this and allows for the addition of test compounds with continuous recording, therefore providing a method for highly accurate dynamic calcium measurements.
This system was tested using a combination of commonly used Ca2+ modulating agents. Thapsigargin (TG), a blocker of Ca2+ uptake into the ER, causes a significant increase in the intracellular calcium concentration via ER emptying followed by Ca2+ entry via store operated Ca2+ channels (SOCC). This is well established pathway can be partially inhibited by 2-APB, a blocker of SOCC. Both the increase with TG alone and the partial increase when co-incubated with 2-APB were observed with continuous recording along with calibration curves.

With these new cytometers, dynamic Ca2+ concentration measurement becomes extremely accessible and accurate, while also providing extensive and valuable data regarding population health and responsiveness.

16:15 – 16:30 *Identification of Allo-Reactive Lymphocytes in a B Cell-Based MLR*

E. Piscianz, E. Valencic, M. Rabusin, A. Tommasini
Institute for Maternal and Child Health IRCCS Burlo Garofolo, Italy

Abstract:
Background. Graft-versus-Host Disease (GvHD) is one of the major complications of aploidentical HSCT, due to donor allo-reactive T lymphocytes injected during the transplantation. Current strategies to minimize the risk of GvHD include immunosuppressive drugs and T cell depletion with selection of CD34+ cells or with anti-lymphocyte antibodies. Unfortunately, these strategies can increase the risk of relapses or infections.
Aim. To set up a method for identifying and removing allo-reactive T lymphocytes from donor cell preparations.
Methods. B cells were expanded from peripheral blood obtained from normal donors and used as stimulator cells in standard mixed lymphocyte reactions (MLR). Enhanced MLR (with IL-4 and anti CD28 antibodies) was also performed. Allo-reactive cells were identified using flow cytometry as IFN-gamma producing cells or as cells expressing activation markers (CD25, CD69, HLA-DR).
Results. A well-defined population of IFN-gamma positive cells could be identified after 10 hours of co-culture: 0.5% in standard MLR and 1.5% in enhanced MLR (positive control PHA=2.5%; negative controls=0.01-0.1%).
After 24 hours of co-culture 15-17% and 4-6% of cells expressed respectively CD69 and CD25 (negative controls 4-6% and 0.5-1%). In enhanced MLR with the addition of anti-CD28 and/or IL-4, the sensitivity of the test resulted increased without loss of specificity.
Conclusions. Allo-reactive cells could be identified using an enhanced MLR as soon as after 10h hours of stimulation. Production of IFN-gamma seems to be highly specific, as only few cells expressed this cytokine in negative controls, even in enhanced MLR. Secondary MLR will be performed in order to measure the residual allo-reactivity using different methods for allo-depletion.

16:30 – 16:45 *A Novel NLS sequence in PEDF Mediates its Interaction with Transportin-SR2 and Nuclear Import*

S. Anguissola, W. McCormack, M. Morrin, M. Worrall
University College Dublin, Dublin, Ireland

Abstract:
PEDF (Pigment epithelium-derived factor) is a non-inhibitory member of the serpin gene family (Serpin F1) that displays anti-angiogenic and anti-cancer properties. PEDF

contains a secretion signal, but although originally regarded as a secreted extracellular protein, endogenous PEDF is found in the cytoplasm and nucleus of several mammalian cell types. In this study we use GFP-tagged PEDF transfected cells as well as exogenously administered PEDF to show that nuclear import of PEDF is an active process. In a yeast two-hybrid screen we identified transportin-SR2, a member of the importin-β family of nuclear transport proteins, as a possible binding partner. The interaction was supported in vitro by GST-pulldown and co-immunoprecipitation. A motif (YxxYRVRS) shared by PEDF and the unrelated transportin-SR2 substrate, RNA binding motif protein 4b, was identified and we investigated its potential as a novel NLS sequence. Site-directed mutagenesis of this helix A motif in PEDF resulted in a GFP-tagged mutant protein excluded from the nucleus; mutation of two arginine residues (R67, R,69) was sufficient to abolish nuclear localization assessed both by confocal microscopy and flow cytometry as well as interaction with Transportin-SR2.

16:45 – 17:00 *Development of Fluorescence Activated Cell Sorting Methodology to Isolate Sub-populations of Lactococcus lactis Subsp. Cremoris AM2 with Enhanced Intracellular Enzyme Release*

I.A. Doolan, M.G. Wilkinson
University of Limerick, Limerick, Ireland

Abstract:
Lactic Acid Bacteria (LAB) play an important role in the manufacture of Cheddar cheese. During the manufacture and ripening process, the LAB starter bacteria contribute to the flavour profile by releasing important intracellular enzymes during permeabilisation and autolysis. Lactococcus lactis subsp cremoris AM2 contains many intracellular enzymes which are released by permeabilisation or autolysis depending on the growth conditions. However, traditional microbiological methods preclude the selection of a permeabilised sub-population of interest within a liquid culture. The current study was undertaken to develop methodology based on cytometry and fluorescence activated cell sorting (FACS) to isolate specifically labelled sub-populations of intact, permeable or autolysed cells of the AM2 strain within a single growth medium. Strain AM2 was grown under various conditions including a simulated Cheddar cheese manufacturing process (Pearce Test) and under nutritional deprivation. At various stages of the above treatments samples were removed, stained using Syto9 and PI combinations and analysed by flow cytometry for the presence of specific live, dead or permeabilised populations. These populations were sorted using a MoFlo cell sorter and analysed for viability on solid agar, and intracellular enzyme activity by sorting into 96 well microtitre plates containing substrates for PepX and Pep N. As well as identifying sub-populations, BD TruCountTM beads were included in the cytometric assay to enumerate sub-populations cells and results compared with traditional plate counts. Overall, laboratory scale methodology for the cytometric profiling of AM2 was developed which allowed FACS sorting of sub-populations with maximal release of intracellular Pep X and Pep N. This approach will be further progressed to generate highly permeabilised LAB cell sub-populations or autolysed cell free extracts for use in the production of cheese flavour concentrates.

PARALLEL SESSION
WEDNESDAY, NOVEMBER 18TH

COMPANY TUTORIALS

15:00 – 15:50 *Follow-Up: VenturiOne® Offline Data Analysis Software - Making flow faster*

Gillian Byrne
Applied Cytometry

14:35 – 15.05 *In Situ Cytometry for Vital Cells*

Gareth Jones
Cyntellect

15.05 – 15:35 *Reducing Variability in Flow Cytometric Analysis with the Accuri C6*

Kate Easten
Accuri Cytometers

15:35 - 16:05 *ImageStreamX® Cytometry: High Speed High Content Image Analysis of Cells in Flow – See what you've been missing!!*

D. Coulson
Alpha Technologies

Abstract:
Alpha Technologies proudly presents the new ImageStreamX imaging flow cytometer from Amnis. This tremendous breakthrough sets the standard for cell analysis and maintains the leadership role in imaging flow cytometry. Amnis has dedicated 10 years to combining the most advanced technologies in optics, sensors, and image processing to achieve what has never been possible before – a fusion of the analytical power of flow cytometry with the visual detail of imaging.
The ImageStreamX quantifies both the intensity and the location of fluorescent probes and can image at incredibly high rates of speed, allowing analysis of rare sub-populations and highly heterogeneous samples with statistically robust and objective results. By combining the speed, sensitivity, and phenotyping abilities of flow cytometry with the detailed imagery and functional insights of microscopy, the ImageStreamX will rapidly advance your research.

15:50 – 16:40 *Automated Imaging and High-Content Analysis of Cell-Based Assays - A complementary method to flow cytometry*

Yannick Marrari
Becton Dickinson

16:05 - 16:35 *Multicolour Flow Cytometry: Configuration, Design and Practicalities*

K. Lundsten
Cambridge Bioscience Ltd

Abstract:
With advancements in digital flow cytometry instrumentation, continued development of a more diverse array of fluorophores useful on this platform and importantly a wider availability of direct conjugates, multicolor analysis becomes ever more useful in flow cytometry. Here we will discuss the steps to scaling up to 8+ color flow cytometry, including instrumentation, fluorophore combinations and optimization. We will cover examples of 10 color T-cell immunophenotyping.

16:35 – 17:05 *Partec CyScope: High performance, portable, fluorescence microscopy*

Jane Wood
Partec

The Partec CyScope® is a revolutionary portable fluorescence and light microscope which is powered by a battery or electrical power supply. The CyScope® features innovative LED light cubes with different excitation wavelengths: 365nm (ultraviolet UV), 455nm (royal blue), 470nm (blue), green and red are available. A white LED is used for transmitted light. The light level of all LED light sources is adjustable and all LEDs are set for Köhler illumination and are electronically and thermally stabilized.
The Partec CyScope® is ideal for Clinical routine and research applications: Immunofluorescence, cell biology and pathology as well as educational programs. For routine microbiology and parasitology tests Partec offers dedicated fixed set-ups with Partec CyStain TB and Partec Rapid Malaria Tests. The Partec CyScope® can also be used for contrasting techniques and polarization microscopy.

POSTER PRESENTATIONS

1. *The nanoparticle-cell dialogue: a cell cycle study*

J.A. Kim, A. S., I. Lynch, K. A. Dawson

Centre for BioNano Interactions, School of Chemistry and Chemical Biology and Conway Institute, University College Dublin, Dublin, Ireland

Nanoparticles hold great promise as drug delivery vehicles for cancer treatment, where the lack of drug specificity for malignant cells limits the efficacy of current treatments and often leads to undesired side effects. Different parameters affecting nanoparticle uptake by cells are being studied in order to assess their potential role in preferential targeting of cancer cells. Some of these parameters include physicochemical characteristics of nanoparticles such as size, material and surface modifications (which can influence epitope conjugation of adsorbed proteins). It has been reported that rates of endocytosis change during cell cycle progression, indicating that certain phases favour particle uptake relative to others. In this study, flow cytometry is used to investigate the interaction of A549 cells with fluorescent polystyrene and silica nanoparticles. Different assays such as DNA staining and nucleoside analogue incorporation are used to study which phases are optimal for particle uptake and also to analyse any possible disruptions of the cell cycle progression caused by the presence of the nanoparticles studied.

2. *pDMAEMA causes apoptosis in human intestinal cells but necrosis in human monocytes*

L.A.B. Rawlinson, D. J. Brayden

Food Science and Veterinary Medicine, UCD School of Agriculture, University College Dublin, Ireland

Poly(2-(dimethylamino ethyl)methacrylate)) (pDMAEMA) is a mucoadhesive cationic polymer. pDMAEMA has use in drug delivery as a polymeric carrier for drugs and genetic material. It also has antimicrobial activity and has potential as an antimicrobial coating for implanted medical devices. In order to use pDMAEMA in a clinical setting, its cytotoxicity must be investigated. Therefore the aim of this research was to investigate the cytotoxicity of pDMAEMA in human cell lines. To do this, a multiparameter cytotoxicity assay was carried out using high content analysis (HCA). Four fluors were used: Hoechst 33342, Fluo 4-AM, TOTO-3 and TMRM. Six parameters of cytotoxicity were tested: 1) nuclear area (NA), 2) nuclear intensity (NI), 3) intracellular calcium (IC), 4) mitochondrial membrane potential (MMP), 5) cell membrane permeability (CMP) and 6) cell number (CN). The capacity for pDMAEMA to induce phospholipidosis was also investigated using HCA with the dyes Hoechst 33342 and nile red. The results found that against the human monocyte-like cell line

U937, pDMAEMA increased all parameters except IC compared to untreated controls. The decrease in IC was correlated with an increase in CMP. The results suggest a necrotic mechanism of cell death in these cells. Against the human intestinal epithelial cell line Caco-2, pDMAEMA was found to increase NI, IC and CMP with an increase in CN at low concentrations compared to untreated controls. A decrease was observed in NA with a decrease in MMP from low concentrations and above and in CN at high concentrations. The results suggest an apoptotic mechanism of cell death in these cells. The lowest IC50 values against U937 cells were found at 25- 50 μg/ml and at 140-250 μg/ml against Caco-2 cells. pDMAEMA was not found to induce phospholipidosis in either cell line at incubation times up to 72 hours. In conclusion, pDMAEMA induces apoptosis in Caco-2 intestinal epithelial cells. In U937 cells, the method of cytotoxicity induced by the polymer is necrosis. The minimum IC50 values of the tested parameters against U937 cells were approximately 5-fold less than against Caco-2 cells. pDMAEMA did not induced phospholipidosis. The next stage will be to test it against whole tissue. With these results and the lack of phospholipidosis found, pDMAEMA could eventually prove to be safe as a mucosal or topical formulation for drug delivery or antimicrobial treatment.

3. *Understanding the role of small RNAs in hybrids between Arabidopsis accessions.*

A.Giakountis, D. Baulcombe

Department of Plant Sciences, University of Cambridge, UK

Small RNAs in plants are known to be involved in a variety of processes such genome stability and transposon regulation, gene regulation and antiviral defence. Such functional diversification arise from the specialization of the small RNA biogenesis machinery, which results into two major classes of small RNAs the 21 nt long miRNAs and the 24 nt long siRNAs. Plant miRNAs are responsible for post-transcriptional gene silencing (PTGS) mainly through transcriptional cleavage of the target mRNA while siRNAs are involved into transcriptional gene silencing (TGS) and they are associated with transposon silencing via histone modifications and DNA methylation. Intraspecific natural variation in *Arabidopsis* results in a large number of genetically distinct ecotypes. In addition extensive epigenetic variability at the level of DNA methylation has also been reported in the species although our knowledge for the full extent of natural epigenetic variation in *Arabidopsis* has been limited. Furthermore extensive plasticity and heterosis have both been reported in F1 hybrids between *Arabidopsis* accessions. We are interested in characterizing the role of small RNAs in hybrids between Arabidopsis ecotypes. For that reason we will be sequencing small RNA libraries constructed from F1 hybrids from two reciprocal crosses between Col-0 and other accessions. The small RNA pattern of the hybrids will be compared with the corresponding parental small RNA pattern and with the pattern from crosses between mutants that affect small RNA production. In order to test the role of small RNAs in transgenerational inheritance we will use FACS in order to

check for epigenetic changes specifically at the meristem of the hybridized plants against the parents.

4. *Reducing Variability in Flow Cytometric Analysis by Pre-optimizing Instrument Optics and Electronics*

K. Easten, C. Rogers, B. Eckert, M. Dinkelman, C. Rich

Institution: Accuri Cytometers, Inc. Ann Arbor, Michigan, USA and St. Ives, Cambs, UK.

Flow cytometric analysis relies on the interaction of a number of systems, each having inherent variability. These include the functional systems of the instrument itself (fluidics, optics, electronics and software) as well as factors such as sample preparation, reagent quality and stability, and the skill and experience of the human operator. This variability at every step in the process often frustrates efforts to standardize analysis and to compare data collected on different types of instruments at various locations. The Accuri C6 Flow Cytometer is manufactured to optimize overall instrument performance before leaving the factory. The optical alignment of lasers, flow cell, light filters and detectors is "locked down" during the manufacturing process, and the voltage and gain on photomultipliers and diode detectors is factory-set, using industry standard beads. The result is a cytometer with highly predictable and reproducible performance. Data is presented showing that instruments retain their predictable performance for up to 12 months after installation. In addition, the benefit to the user of pre-optimization of detector performance is illustrated in the highly predictable nature of the fluorescence spillover of FITC and PE on the C6 system. Data collected on a wide range of cell and bead types, and on at least 10 different C6 systems, was combined and used to mathematically model fluorescence spillover of these fluorochromes. Analysis regions drawn on 2D FITC vs. PE plots based on this modeling allowed accurate population percentage data to be obtained without applying fluorescence compensation to the data sets. However, if compensation is desired to improve data visualization, the predictability of spillover on the C6 has allowed average compensation values to be determined for specific fluorochrome combinations. These values can be applied by the C6 user and then "tweaked" based on the autofluorescence signals for any given experiment.

5. *Using flow cytometry to derive a novel pTα KO x FoxP3EGFP KI mouse strain.*

R. Ceredig[1,2], A.G. Rolink[1]

[1]Department of Biomedicine, University of Basel, Switzerland

[2]Regenerative Medicine Institute, NCBES, National University of Galway, Ireland.

Mice in which the gene encoding the pre-T cell receptor alpha (pTα protein have been deleted have a major block in thymocyte development. Their thymus is small because in the absence of pTα proliferation of progenitor thymocytes is severely impaired at the DN3-to-DN4 transition. As a result of diminished thymus size and decreased thymic output, pTα mice are severely lymphopenic. Two additional defects were reported to be present in pTα mice, namely that the cell size of DN2 thymocytes was reduced and that they contained increased numbers of CD25+, FoxP3+ regulatory T cells, some of which expressed the TCR□□ receptor. In order to address these issues, we decided to derive homozygous pTα KO mice expressing EGFP knocked into the FoxP3 locus. The FoxP3 gene is located on the X chromosome; consequently, genotyping mice had to be carried out by flow cytometry on peripheral blood leukocytes stained for CD4 and CD25. Details of this screening strategy will be presented as well as phenotypic analysis of the pTα KO x FoxP3EGFP KI mouse strain.

6. *IHG-1 Regulates Mitochondrial Biogenesis and Mitochondrial Network Integrity*

F.B. Hickey, J. Corcoran, B. Griffin, D. Cottell, F. Martin, C. Godson, M. Murphy

University College Dublin, Dublin, Ireland

Induced in high glucose-1 (IHG-1) is a novel gene that we have described to be upregulated in human diabetic kidney disease. To date a mitochondrial localisation sequence is the only predicted functional domain identified in IHG-1. We have confirmed the mitochondrial location of IHG-1 by immunocytochemistry and confocal microscopy following overexpression of V5-tagged IHG-1. Immunogold cryosection electron microscopy has indicated that IHG-1 is located predominantly in the mitochondrial matrix and is associated with the inner mitochondrial membrane.

We have further investigated the role of IHG-1 in mitochondrial function. These analyses have been carried out in both cell lines that over-express IHG-1 and using shRNAi-mediated knockdown of IHG-1. Overexpression of IHG-1 has been found to lead to increased mitochondrial mass as measured by mitotracker uptake and flow cytometry. In addition, we have studied the effect of IHG-1 on fusion of mitochondria by measuring fluorescence recovery after photobleaching (FRAP) and other live cell imaging fluorescent techniques. Increased mitochondrial fusion is seen in cells overexpressing IHG-1, indicating that, in DN, IHG-1 may lead to altered mitochondrial function.

7. *$CD39^+Foxp3^+$ regulatory T cells suppress INFLAMMATORY Th17 cells and are impaired in multiple sclerosis*

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Despite the fact that CD4+CD25+Foxp3+ regulatory T cells (Treg cells) play a central role in maintaining self-tolerance and that IL-17-producing CD4+ T cells (Th17 cells) are pathogenic in many autoimmune diseases, evidence to date has indicated that Th17 cells are resistant to suppression by human Foxp3+ Treg cells. It was recently demonstrated that CD39, an ectonucleotidase which hydrolyses ATP, is expressed on a subset of human natural Treg cells. We found that although both Foxp3+CD39+ and Foxp3+CD39- T cells suppressed proliferation and IFN-γ production by responder T cells, only the Foxp3+CD39+ Treg cells suppressed IL-17 production, whereas Foxp3+CD39- cells produced IL-17. An examination of T cells from multiple sclerosis (MS) patients revealed a normal frequency of CD4+CD25+Foxp3+, but interestingly a deficit in the relative frequency and the suppressive function of Foxp3+CD39+ Treg cells. The mechanism of suppression by Foxp3+CD39+ Treg appears to require cell contact and can be duplicated by adenosine, which is produced from ATP by the ectonucleotidases CD39 and CD73. Our findings suggest that Foxp3+CD39+ Treg cells play an important role in constraining pathogenic Th17 cells and their reduction in MS patients might lead to an inability to control IL-17 mediated autoimmune inflammation.

8. *Visual Tracking of Cell Movement and Deformation over Image Sequences*

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This project involves the motion analysis of multiple cells over image sequences. The automated tracking of a large number of cells faces many challenges, including identification of cells in high density data set, segmentation of close contact cells, and cell morphology changes under a variety of experimental conditions. At present, the research work focuses on the movement and deformation of multiple cells within in vitro microscopy images. There are two general approaches to tackling cell tracking problems. One is referred to as the model free segmentation method. For this type of method, cells are first segmented in each image independently, and then the detected cells from consecutive frames are paired by using certain probabilistic objective functions. The second one can be classified as the model evolution method: parametric or non-parametric active contour based representation of cell appearance or shapes are evolved throughout the image sequences. The first method provides no, or only poor descriptions of the cellular shape and can be problematic with higher density of cells due to the

inability to discriminate between multiple neighboring cells and cells in close contact. Therefore, the active contour model based method is chosen as the main model in the proposed project. First, for image preprocessing purposes, techniques such as noise removal, thresholding by combining both global and local properties of images, and edge enhancement approaches can be applied. Then for correctly segmenting nearby cells in clustering, repulsive forces are integrated into the active contour models. This can be achieved either by modification of the edge map of the target images or adding volcano forces between neighbor snakes. In future, the measurements of cell dynamics can include the quantification of different cell behaviors, such as mitosis (division), apoptosis (dying), cell migration, and so on.

9. *Cell death responses in bystander cells exposed to signals from irradiated cells*

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Considerable evidence now exists relating to radiation-induced bystander effects but the mechanisms are still unclear. The aim of this study was to investigate cell death responses in bystander cells.

Human keratinocyte cells (HaCaT cells) were irradiated using a Cobalt-60 teletherapy source at doses of 0.005Gy, 0.05Gy and 0.5Gy. One hour after irradiation the medium was harvested and filtered. This irradiated cell conditioned medium (ICCM) was then added to parallel HaCaT cells. Mitotic cell death was measured using immunofluorescence staining of MPM2 and confocal microscopy. The cells were treated with 0 Gy, 0.5 Gy, 0.05 Gy and 0.005 Gy irradiated cell conditioned medium (ICCM) and observed after 24, 48 and 72 hours of incubation. Mitotic cell death was observed in cells treated with ICCM for 72 hours. To confirm mitotic cell death, the distribution of cell cycle phases was investigated using flow cytometry. Future work will involve other cell death responses and inhibitors of different pathways to modulate cell death.

The research into radiation induced bystander effects may have important consequences for health risk assessment and, consequently, for radiation protection.

10. *Evaluation of commercial antibodies combined with FACS for the detection of food pathogenic bacteria*

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Flow cytometry combined with labelled monoclonal antibodies has made possible the routine identification of specialized cell lines in a range of clinical applications. Fluorescence-activated cell sorting (FACS) allows the process to be taken one step further where cells with specific characteristics can be separated from the sample for further analysis or growth. In contrast to the advances made in clinical applications, the use of cytometry for routine microbial analysis lags behind considerably. This gap has arisen because of a lack of specific cytometric methodology for microbial detection and the unavailability of good quality monoclonal antibodies against target microorganisms.
In this study a number of commercially available monoclonal antibodies against *Staphylococcus aureus* and *Listeria monocytogenes* were sourced and evaluated for their ability to quantitatively label cultures in broth systems. R-PE conjugated secondary antibodies were used to reveal labelled cells and were analyzed using a MoFlohigh speed cell sorter. Both of the commercially available primary antibodies against *Staphylococcus aureus* appeared capable of distinguishing this strain within mixed culture systems. None of the four commercially available antibodies against *Listeria monocytogenes* enabled detection using flow cytometry. Cell sorting of labelled *Staphylococcus aureus* enabled successful identification using a combination of selective agars and microscopic analysis.
In comparison to traditional plate counting, FACS analysis of labelled cells was possible within six hours and has highlighted the potential for FACS as a rapid detection method within the food industry

11. *Evaluation of commercial culture media for use in CHO cell culture by flow cytometry*

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Chinese hamster ovary cells (CHO cells) are the most commonly used mammalian cells for biopharmaceutical production. CHO cell media selection and optimisation is a critical part of the bioprocess affecting cell proliferation and productivity. Current culture media evaluation employ several methods including cell growth monitoring, HPLC for nutrient and by-product detection, titration experiments, viability tests and cell counting apparatus. All of these protocols involve various pieces of equipment and is time consuming. The use of flow cytometry in the area could lead to a more efficient process. In this study CHO-S cells were grown in several commercially available media and cell density, viability and the onset of apoptosis were all evaluated on the flow cytometer.

12. *Using Chicken Immune Phage Display Technology to Generate Stem Cell-Specific Monoclonal Antibodies for flow cytometry*

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Generating stem-cell specific antibodies has proven problematic as membrane proteins involved in stem cell growth and differentiation are likely to be highly conserved across closely related species, they are therefore unlikely to provoke a high affinity IgG response in rodents. This makes monoclonal antibody (MAb) generation particularly challenging, especially when the membrane target is unknown, cannot be purified and therefore no attempt can be made to break immune tolerance. Several attempts to generate high affinity, truly stem cell-specific reagents using various techniques including hybridoma, screening of a naive human antibody phage display library, and aptamer technology have been essentially unsuccessful. To overcome immune tolerance and generate a panel of highly specific MAb's, we have generated chicken immune phage display libraries targeted to human mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs). The phylogenetic distance between humans and chickens is much greater than between humans and rodents greatly increasing the chance of generating high affinity recombinant antibodies to a human protein with conserved sequence in mammals. We have identified an MSC-specific antibody 'TMSC3' that binds to Limbin/EVC2 on the primary cilium of quiescent CD45low lineage-ve MSCs in human bone marrow, and to RANKhigh pre-osteoclasts in both bone marrow and peripheral blood. We have also identified an EPC-targeted antibody that binds to circulating CD3+ve and CD14+ve subsets of cells, that are enriched in EPC cultures and may play a role in neovasculogenesis. Our results underline how robust and broadly applicable this method of stem cell marker discovery may become in the future.

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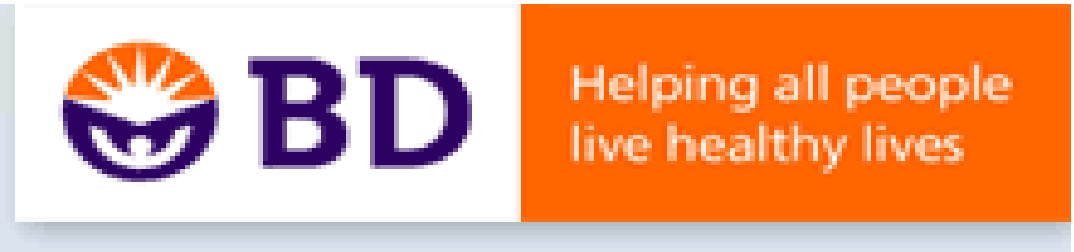

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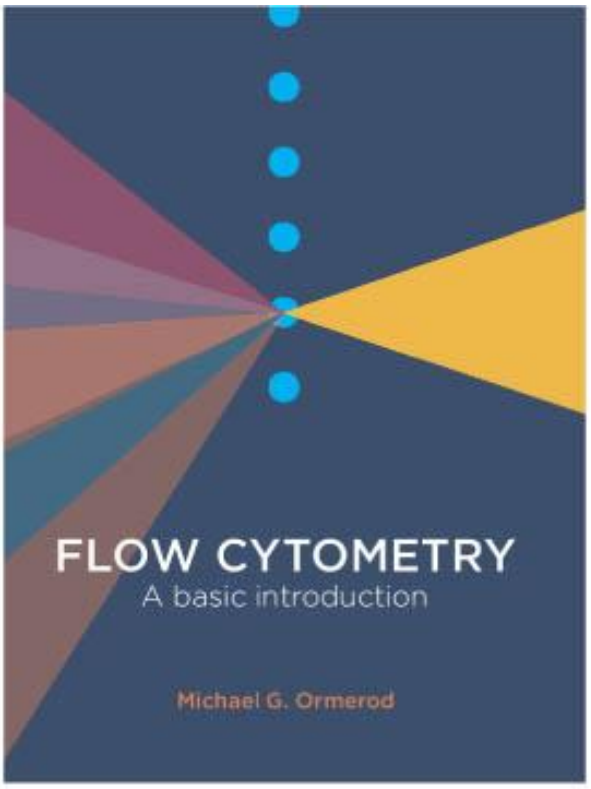

This new introduction to flow cytometry has two unique features.

Most of the figures have links to the data file from which they were derived. If you have downloaded FCS Express Reader, the layout used for each figure can be accessed. In some cases, extra information is supplied, including other data files. The reader can experiment with the data, changing the position of the gates, etc.

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The book is free for you to read on the Web site above. A conventional paperback version is available for purchase at www.denovosoftware.com/site/introtoflowormerod.shtml..

We hope that you enjoy this new venture and look forward to hearing any comments.

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