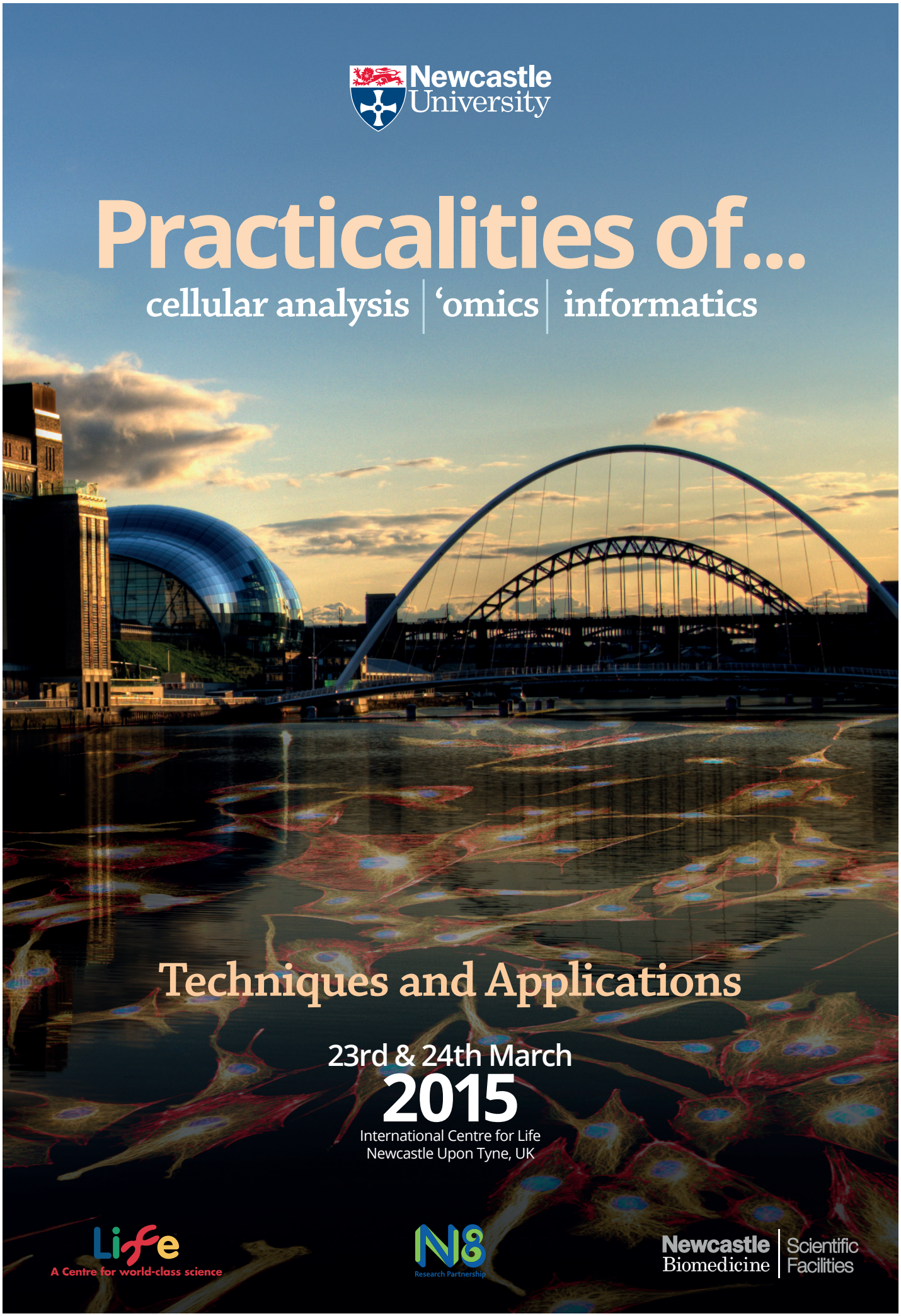
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Practicalities of...
cellular analysis | 'omics | informatics
Techniques and Applications
23rd & 24th March
2015
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Monday 23rd March 2015 — Practicalities of... **cellular analysis**

8:45 - 9:40 Refreshments and networking in sponsor exhibition space

9:40 - 9:50 Welcome & Introduction

9:50 - 10:35 Keynote

Lights will guide you: from in vivo imaging to ultrastructural detail with correlative microscopy

Matthia Winter-Karreman
EMBL

10:40 - 11:15 Parallel Sessions

Flow Cytometry
Modelling idiopathic Aplastic Anaemia using induced pluripotent stem cell technology

Dario Melguizo
Newcastle University

Imaging
Using confocal techniques and image analysis to understand the impact of mitochondrial disease on synapses

Alexia Chrysostomou
Newcastle University

Electron Microscopy
Serial blockface imaging and correlative microscopy: exploring the third dimension

Chris Peddie
Cancer Research UK

11:15 - 11:50 Parallel Sessions

Flow Cytometry
Building an encyclopaedia of the mouse immune system - challenges and opportunities of big flow datasets

Adam Laing
King's College London

Imaging
The development of CLARITY to help further understand neurodegeneration in mitochondrial disease

Jonathan Phillips
Newcastle University

Electron Microscopy
Application of serial block face electron microscopy (SBEM) to circuits found in the locust brain that operate to detect collision trajectories

Claire Rind, Newcastle University

11:50 - 13:05 Lunch and networking in sponsor exhibition space

13:05 - 13:50 Keynote

Engineering approaches to cell cycle analysis using imaging cytometry

Paul Rees
Swansea University

13:55 - 14:30 Parallel Sessions

Flow Cytometry
Practicalities of Cell cycle and Ploidy analysis

Derek Davies
Cancer Research UK

Imaging
Laser Microdissection and Optical Tweezers: Applications for the ZEISS CombiSystem

Tom Quick
Zeiss

Monday 23rd March 2015 — Practicalities of... **cellular analysis**

14:30 - 15:05 Parallel Sessions

Flow Cytometry
Practicalities of Cytof acquisition and analysis

Susanne Heck & Richard Ellis
Guy's & St Thomas' NHS Foundation Trust

Imaging
Studying homologous chromosomes segregation in mammalian oocyte using high resolution microscopy

Aicha Metchat
Newcastle University

15.05 – 15.45 Refreshments and networking in sponsor exhibition space

15.45 – 16.30 Keynote

Standardization of Flow Cytometry Assays: What for? How? Where does it lead us?

Tomáš Kalina, Charles University, 2nd Faculty of Medicine and Faculty Hospital Motol, Prague

16.30 – 17.00 Keynote

Integrative Cytometry for the Study of Immune Cell Function

Andy Filby
Newcastle University

17.00 Drinks Reception and networking in sponsor exhibition space

Tuesday 24th March 2015 — Practicalities of... **cellular 'omics & informatics**

9:00 - 10:00 Refreshments and networking in sponsor exhibition space

10:00 - 10:45 Keynote

Proteomics in a core facility setting - What can we achieve?

Sharad Mistry
University of Leicester

10:50 - 11:20 Parallel Sessions

Genomics & Informatics
Sequencing the leukaemia genome; what, where and when?

Sarra Ryan
Newcastle University

Proteomics
Exploiting the potential of Agave for bioenergy in marginal lands

Dalal Al-baijan
Newcastle University

Tuesday 24th March 2015 — Practicalities of... cellular 'omics & informatics

11:20 - 11:50 Parallel Sessions

Genomics & Informatics
Uses for genome-wide DNA methylation data in disease prediction, prevention and treatment

Caroline Relton
Newcastle University

Proteomics
A Heart for Mass Spectrometry

Achim Treumann
Newcastle University

11:50 - 13:20 Lunch and networking in sponsor exhibition space

13:20 - 13:50 Parallel Sessions

Genomics & Informatics
A year into embedding the PacBio RS II platform: Experiences and applications so far

Neil Hall
University of Liverpool

High-Throughput Screening
The Use of High Throughput siRNA Screening to Identify Kinases Involved in Prostate Cancer Progression

Scott Walker
Newcastle University

13:50 - 14:20 Parallel Sessions

Genomics & Informatics
Targeted sequencing in molecular diagnostics and clinical research

Silvia Borras
Newgene

High-Throughput Screening
Achieving a Successful High Throughput Screen

Peter Banks
Newcastle University

14.20 – 15.05 Refreshments and networking in sponsor exhibition space

15:05 - 15:50 Keynote

Towards portable, real-time genome sequencing

Nick Loman
University of Birmingham

15:50 - 16:35 Keynote

Diversity screening for chemical biology and drug discovery - the DDU experience

David Gray
University of Dundee

16.35 Closing Remarks & Prize Draw

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Practicalities of...

cellular analysis | 'omics | informatics

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9.50 - 10.35 Keynote

Lights will guide you: from in vivo imaging to ultrastructural detail with correlative microscopy

M.A. Karreman, L. Mercier, N.L. Schieber, B. Ruthensteiner, J.G. Goetz and Y. Schwab

Matthia Winter-Karreman
European Molecular Biology Laboratory, Heidelberg

Correlative microscopy combines the strengths of multiple imaging modalities in the study of a single sample. Light microscopy enables to image living samples at a large field of view, whereas electron microscopy reveals the object of interest at high resolution in its structural environment. Here we use correlative microscopy to study metastatic events *in vivo*. Fluorescent tumor cells are xenotransplanted into living mice and imaged by intravital two-photon excitation microscopy (2PEM). The region of interest (ROI) is marked on the sample surface by near-infrared branding and the sample is processed for electron microscopy (EM), involving fixation, dehydration and resin embedding steps. One of the biggest challenges in correlative microscopy is to keep track of the ROI when moving from one image modality to the next. In large complex 3D samples, like the brain and skin tissue we study, correlating 2PEM to EM is highly time-consuming and sometimes even impossible. Here we demonstrate a novel approach in which x-ray microCT is employed to image and map the EM processed sample. By correlating this to the *in vivo* obtained 2PEM dataset, the position of the ROI inside the resin block can be determined and can accurately be approached by ultramicrotomy. Next, the ROI can be imaged at high resolution in 3D with EM. Accurately targeting the event of interest and limiting data acquisition to only this area greatly speeds up the correlative workflow and prevents the formation of unnecessary large datasets.

10.40 - 11.15 Parallel Session: Flow Cytometry

Modelling idiopathic Aplastic Anaemia using induced pluripotent stem cell technology

Dario Melguizo
Newcastle University

Aplastic Anaemia is a heterogeneous disorder characterized by pancytopenia and hypocellular bone marrow. The bone marrow in Aplastic Anaemia patients is characterized by significantly reduced number or absent erythrocytes, megakaryocytes and granulocytes, with increased number of fat cells, macrophages and mast cells. Most of the cases (70-80%) of Aplastic Anaemia are acquired and the mechanisms responsible for bone marrow failure are unknown (idiopathic). Haematopoietic stem cell transplantation is the treatment of choice, although the number of histocompatible donors is very limited. Studies to model this disease are disadvantaged by the scant number of haematopoietic stem cells present in idiopathic Aplastic Anaemia patients and the difficulty establishing long-term *in vitro* cultures of these cells.

Pluripotent stem cells are capable of unlimited self-renewal and have the capacity to differentiate into all somatic cell types of the human body. By the direct reprogramming of somatic cells with a specific cocktail of factors, patient-specific pluripotent stem cells can be generated. These cells, called induced pluripotent stem cells (iPSCs), stand out as an ideal candidate for studying and modelling diseases like acquired Aplastic Anaemia. Here, by the generation of 5 different idiopathic Aplastic Anaemia iPSC lines and its subsequent haematopoietic differentiation *in vitro* we show that iPSCs are and excellent source of haematopoietic stem cells that would help to study the underlying defects that could be present in these cells.

10.40 - 11.15 Parallel Session: Imaging

Using confocal techniques and image analysis to understand the impact of mitochondrial disease to synapses

Alexia Chrysostomou[1], John Grady[1], Alex Laude[2], Robert Taylor[1], Doug Turnbull[1], Nichola Lax[1]

1. Wellcome Trust centre for Mitochondrial Research, Institute of Neuroscience, Newcastle University
2. Bio-Imaging unit, Newcastle University

Alexia Chrysostomou
Newcastle University

Mitochondrial diseases constitute a group of genetically and phenotypically heterogenic disorders. They commence due to either primary or secondary mitochondrial DNA mutations and have adverse effects on the oxidative phosphorylation system and ATP production. Hence, organs with extensive metabolic demand, like the brain, are severely affected; with neurological symptoms ranging from deafness to stroke and neurodegeneration.

Previous neuropathological investigations have documented severe neuronal loss in different brain regions and showed evidence of impaired synaptic connections in patients with mitochondrial disease. This study aims to explore neuronal communication in greater detail in an attempt to understand the impact of mitochondrial disease to synapses and gain further insights into the mechanisms of neuronal loss.

Using 5μm thick formalin-fixed paraffin-embedded (FFPE) tissue from patients with mitochondrial disease, we have developed a quadruple immunofluorescent technique that allows the precise detection of inhibitory synapses and enables the identification of key mitochondrial proteins within them.

Laser-scanning confocal microscopy and z stacking helped sample the sections throughout their depth and generated three-dimensional images of interesting areas. Analysis and processing software facilitated image deconvolution and three-dimensional reconstruction of synapses. Moreover, detection of important mitochondrial proteins within the synapses was made plausible and allowed for careful investigation of the impact of mitochondrial disease to the synapse.

This work introduces the development of a new technique for documenting mitochondrial respiratory chain deficiency in human FFPE brain tissue that represents a significant advance over existing methods. It can be modified to incorporate further markers of interest and adapted by others to study the extent of mitochondrial involvement in other neurodegenerative disorders.

10.40 - 11.15 Parallel Session: Electron Microscopy

Serial blockface imaging and correlative microscopy: exploring the third dimension

Chris Peddie
Cancer Research UK

The ultrastructural examination of cellular systems is experiencing a dramatic resurgence in interest, driven both by advances in instrumentation, and the continued development of specimen preparation techniques. As a result, we are rapidly entering an era of increasingly automated 3-dimensional visualisation, where large-scale data can be rendered with unparalleled levels of detail. At the Cancer Research UK London Research Institute, multiple volume electron microscopy methods are applied in the study cellular systems, and our SBF SEM is used almost exclusively to examine the 3-dimensional architecture of non-brain specimens. Often more tricky to work with, these types of specimen present a different set of challenges in the continual search for optimal data output. Here, I will discuss some recent projects, and strategies for SBF SEM based imaging related to the diverse range of specimens encountered at the LRI. As correlative light microscopy and volume electron microscopy become increasingly integrated, I will also highlight how we are using SBF SEM to directly correlate functional information with ultrastructural detail within a 3-dimensional reference space.

11.15 - 11.50 Parallel Session: Flow Cytometry

Building an encyclopedia of the mouse immune system - challenges and opportunities of big flow datasets

Adam Laing
Department of Immunobiology, King's College London

The Infection and Immunity Immunophenotyping (3i) consortium is conducting a comprehensive, systematic high-throughput immunological analysis of over 800 knockout mouse lines generated by the Welcome Trust Sanger Institute (WTSI). One aspect of the project is a high-dimensional flow cytometric analysis of multiple lymphoid tissues, with the aim of identifying genes involved in the regulation of the immune system in the steady state. To date, the screen has processed over 200 KO-lines and a large wild type control cohort (n=>400). Together, these have produced a high-resolution image of the normal mouse immune system and have identified multiple mutant phenotypes, ranging in severity from abnormalities in single cell subsets confined to a specific lymphoid tissue, to global dysregulation of the immune system. The screen has also identified phenotypes in both genes expressed within and outside of the immune system, many of which correlate with human disease. All data generated will be made publicly available, providing a valuable resource for the scientific community.

11.15 - 11.50 Parallel Session: Imaging

The development of CLARITY to help further understand neurodegeneration in mitochondrial disease.

Jonathan Phillips
Newcastle University

Neurological symptoms are very prominent in patients with mitochondrial disease, especially cerebellar ataxia. Cerebellar ataxia is observed in 68% of mitochondrial disease patients and is associated with a loss of co-ordination, impaired balance, speech difficulties. Neuropathological studies of the cerebellum observe cerebellar atrophy, Purkinje cell loss and dentate nucleus neuron loss with complex I deficiency in remaining cells. However the mechanisms behind the genesis of ataxia is unknown. Current methods to elucidate the mechanisms of degeneration in post-mortem tissue have been to label proteins of interest with fluorescent dyes. The major limitation with current immunofluorescent techniques is that only very thin sections (7μm) can be used, as lipids in the tissue causes the light emitted from the fluorescent dyes to scatter, reducing the resolution of the image. In order to use thicker sections (~250μm), we have been optimising a novel technique known as CLARITY (Clear, Lipid-exchanged, Acrylamide-hybridized Rigid, Imaging/immunostaining compatible, Tissue hYdrogel). CLARITY works on the principle of removing the lipids from the tissue that would otherwise scatter the light while simultaneously retaining the proteins and DNA. CLARITY will enable subtle differences in the cerebellum of mitochondrial disease patients to be observed compared to controls. Particular changes include Purkinje cell morphology abnormalities, Purkinje cell connectivity and distribution of dysfunctional mitochondria within individual Purkinje cells. In addition to human tissue, CLARITY will be applied to mouse models of mitochondrial disease, allowing us to investigate the progression of neurodegeneration, something that is not possible in humans. Applying CLARITY to tissue from mice and humans will allow us to further understand the mechanisms of Purkinje cell death in patients with mitochondrial disease.

11.15 - 11.50 Parallel Session: Electron Microscopy

Application of serial block face electron microscopy (SBEM) to circuits found in the locust brain that operate to detect collision trajectories

Claire Rind[1], Stefan Wernitznig[1,2] Peter Poelt[3], and Armin Zankel[3], Gerd Leitinger[2]

1. Centre for Behaviour and Evolution, Institute of Neuroscience, Newcastle University
2. Institute of Cell Biology, Histology and Embryology, Research Unit Electron Microscopic Techniques Medical University of Graz
3. Institute for Electron Microscopy and Nanoanalysis, NAWI Graz, Graz University of Technology

Claire Rind
Newcastle University

We have pioneered the application of serial block face electron microscopy (SBEM)[1,2], used recently for large-scale circuit reconstruction in the mouse, to reveal the pathway for the detection of approaching objects by the visual system of the locust. SBEM enables automated recording of scanning electron micrographs by repeatedly sectioning a tissue block, imaging the block surface each time a section is removed[1]. SBEM is especially well suited for elucidating brain wiring where cells are traced over long distances[1,2]

Using anatomy to reveal the precise synaptic connectivity between cells in the brain has already generated new insights, particularly into vision. By making a connectome for all cells in a single column in the visual system of *Drosophila*, for example, the elusive cells of the "elementary motion-detector" which generates a selective response to one direction of motion while ignoring all others were revealed[3,4]. We describe the use of SBEM to make detailed reconstructions of the giant collision detecting neurons in the locust visual system to finally discover how they can warn of approaching objects and trigger avoidance behaviour.

References:

1. Denk, W., and Horstmann, H. (2004). Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. PLoS Biol. *2*, e329.
2. Wernitznig, S., Rind, F. C., Pölt, P., Zankel, A., Pritz, E., Kolb, D., Bock, E. and Leitinger, G. (2015), Synaptic connections of first-stage visual neurons in the locust *Schistocerca gregaria* extend evolution of tetrad synapses back 200 million years. J. Comp. Neurol., 523: 298–312.
3. Shinomiya, K., Karuppudurai, T., Lin, T.Y., Lu, Z., Lee, C.H., and Meinertzhagen, I.A. (2014). Candidate neural substrates for off-edge motion detection in Drosophila. Curr Biol *24*, 1062-1070.
4. Takemura, S.Y., Bharioke, A., Lu, Z., Nern, A., Vitaladevuni, S., Rivlin, P.K., Katz, W.T., Olbris, D.J., Plaza, S.M., Winston, P., *et al.* (2013). A visual motion detection circuit suggested by *Drosophila* connectomics. Nature *500*, 175-181.

13.05 - 13.50 Keynote

Engineering approaches to cell cycle analysis using imaging cytometry

Paul Rees
School of Engineering, Swansea University

Multi-parameter conventional flow cytometry is routinely used to determine the position of a cell within the cell cycle. This information is particularly useful when studying the effect of anticancer agents and molecular inheritance between parent and daughter cells. Traditionally the intensity of fluorescent dyes that stoichiometrically label the nucleus in conjunction with tagged antibodies against histone modifications are used to distinguish the various phases of the cell cycle. In comparison with conventional flow cytometry, imaging flow cytometry measures not only these parameters but also the spatial position of the fluorescence within each cell in a population, also providing both brightfield and side scatter images. This rich information set makes this measurement tool an ideal candidate for the use of high content image-based screening techniques to identify cell phenotypes or the effect of anticancer agents.

In this presentation I will discuss how the brightfield cell images obtained using imaging flow cytometry can be used to detect the cell cycle position using machine learning algorithms trained on a dataset which has been annotated using tradition cell cycle markers. Also by using ergodic analysis to align the cells within their cell cycle I will outline how imaging flow cytometry can provide time lapse information on cell evolution. Finally the use of cell cycle data driven models for the analysis of cell cycle perturbations using both conventional and imaging flow cytometry will be discussed.

13.55 - 14.30 Parallel Session: Flow Cytometry

Practicalities of Cell cycle and ploidy analysis

Derek Davies
London Research Institute, Cancer Research UK

The analysis of the mammalian cell cycle was one of the first applications of flow cytometry. It has a very simple basis – that we can label DNA with a fluorescent dye which binds stoichiometrically and quantitate the amount of DNA in a cell. So we can then look at the distribution of cells across the cell cycle in different cell types or in the same cell type after different treatments or we can look for changes in total DNA content in, for example, tumour samples. Although this is a simple premise, as with many applications, some of the reasons for the practical steps have been lost, or shrouded in mystery, over the years. In this presentation we will look at the practical aspects that lead to successful DNA staining – the choice of fixative, the choice of DNA-binding dye, the way we run the samples on the cytometer and the way we analyse the data post-acquisition will be considered. In addition, there will be an overview of some of the more recent developments in the field.

13.55 - 14.30 Parallel Session: Imaging

Laser Microdissection and Optical Tweezers: Applications for the ZEISS CombiSystem

Tom Quick
Zeiss

Nowadays one is always interested in smaller and smaller scales.
It is easy to cut with a scalpel on the cm scale but on the µm scale this becomes very difficult. The same applies to tweezers: one can easily handle macroscopic things with normal tweezers but if you go down to the µm or even nm scale this is impossible. Learn how you can elegantly dissect or manipulate microscopic material with a ZEISS CombiSystem, and discover some real applications for such a technology.

14.30 - 15.05 Parallel Session: Flow Cytometry

Practicalities of CyTOF acquisition and analysis

Susanne Heck and Richard Ellis
NIHR Biomedical Research Centre at Guy's & St Thomas' NHS Foundation Trust and King's College London

Mass Cytometry has emerged as one of the new tools for single cell network analysis. Allowing simultaneous very high dimensional analysis of phenotypic and functional states it has proven to be a powerful discovery platform for developmental and disease stratification and biomarker development. Using the CyTOF platform empowers us with the measurement of around 40-50 cellular parameters at once, more than the current state-of-the-art in conventional fluorescence-based flow cytometry. Metal isotopes are used instead of fluorochromes to label antibodies or other reagents, and these metals can be resolved using time-of-flight technology - which has high specificity without any significant requirement for spill-over compensation.

The last few years have seen an emergence and evolution of workflows to use mass cytometry. The technology requires suitable reagents, many of which will be custom made and need validation. Sample preparation procedures may need to be adapted and special consideration should to be given to the number of "discovery stage" markers which will be included in assays targeted often for biomarker development. Due to the scale of typical CyTOF panels, an experiment's success will hinge upon good upfront design.

Acquisition brings new challenges, which has led to changes in both the hardware and software design since the initial release. Operators as well as users, must be trained to get the best results out of this system and to maintain it for optimum performance and to prevent inaccurate data. Data workflows should be used to ensure that only data of sufficient quality is passed to processing.

Analysis of the high-dimensional data created will often have to employ automated or machine-learning approaches. Selecting appropriate analysis packages and pipelines in conjunction with bioinformatics experts is important, and development of new algorithms will sometimes be necessary to reveal relevant biological patterns and achieve high quality results and publications.

Further information is available from the cytof.uk user group at http://cytof.uk

14.30 - 15.05 Parallel Session: Imaging

Studying homologous chromosomes segregation in mammalian oocyte using high resolution microscopy

Aicha Metchat
Newcastle University

To maintain genomic information, chromosomes must be segregated equally during cell division. However in mammalian oocytes, it is known that the frequency of chromosome missegregation is extremely high during the first meiotic division compared to other cell divisions. Fertilization of aneuploid eggs generated by such errors results in pregnancy loss and, if survived to term, severe genetic disorders such as Down's syndrome.

To understand the mechanism regulating the chromosome segregation during meiosis in mammalian oocytes, we are using 4D confocal imaging on live oocytes and super-resolution microscopy on fixed cells. In my talk, I will introduce the mouse oocytes model system and results which unravel the mechanisms regulating meiosis-I.

15.45 - 16.30 Keynote

Standardization of flow cytometry assays: What for? How? Where does it lead us?

Tomáš Kalina
Charles University, 2nd Faculty of Medicine and Faculty Hospital Motol, Prague

The presentation will focus on standardization of the flow cytometry experimental procedures so that data analysis can be performed in an interlaboratory setting, on multiple instruments and over prolonged periods of time. Various aspects of reproducibility and robustness will be discussed. Since development of standardized flow cytometry tests is essential for meaningful computational analyses of the data, the new possibilities that are just opening will be discussed. The EuroFlow Consortium has established a fully standardized "all-in-one" pipeline consisting of standardized instrument settings, reagent panels, and sample preparation protocols as well as software for data analysis and disease classification[1,2]. For its reproducible implementation parallel development of a quality assurance (QA) program was required to provide a feedback on proper execution of standardized EuroFlow flow cytometry protocols[3].

References:
1. Kalina T, Flores-Montero J, van der Velden VHJ, Martin-Ayuso M, Böttcher S, Ritgen M, et al. EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. Leukemia 2012; 26: 1986–2010.
2. Van Dongen JJM, Lhermitte L, Böttcher S, Almeida J, van der Velden VHJ, Flores-Montero J, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia 2012; 26: 1908–75.
3. Kalina T, Flores-Montero J, Lecrevisse Q, Pedreira CE, van der Velden VHJ, Novakova M, et al. Quality assessment program for EuroFlow protocols: Summary results of four-year (2010-2013) quality assurance rounds. Cytom Part A 2015; 87: 145–56.

16.30 - 17.00 Keynote

Integrative Cytometry for the Study of Immune Cell Function: Focus on Ca^{2+}

Joana Cerveira[2§], Julfa Begum[2§], Rafael Di Marco Barros[3], Annemarthe O van der Veen[4] and Andrew Filby[1,2]

1. Flow Cytometry Core Facility, Newcastle Biomedicine, Newcastle University
2. FACS Laboratory, London Research Institute, Cancer Research UK
3. Immuno Surveillance Laboratory London Research Institute, Cancer Research UK
4. Immunobiology Laboratory London Research Institute, Cancer Research UK

Andy Filby
Newcastle University

Calcium ions (Ca^{2+}) are a ubiquitous transducer of cellular signals controlling key processes such as proliferation, differentiation, secretion and metabolism. In the context of T cells, stimulation through the T cell receptor has been shown to induce the release of Ca^{2+} from intracellular stores. This sudden elevation within the cytoplasm triggers the opening of ion channels in the plasma membrane allowing an influx of extracellular Ca^{2+} that in turn activates key molecules such as calcineurin. This cascade ultimately results in gene transcription and changes in the cellular state. Traditional methods for measuring Ca^{2+} include spectrophotometry, conventional flow cytometry (CFC) and live cell imaging techniques. While each method has strengths and weaknesses, none can offer a detailed picture of Ca^{2+} mobilisation in response to various agonists. Here we report an Imaging Flow Cytometry (IFC)-based method that combines the throughput and statistical rigour of CFC with the spatial information of a microscope. By co-staining cells with Ca^{2+} indicators and organelle-specific dyes we can address the spatiotemporal patterns of Ca^{2+} flux in Jurkat cells after stimulation with anti-CD3. The multispectral, high-throughput nature of IFC means that the organelle co-staining functions to direct the measurement of Ca^{2+} indicator fluorescence to either the endoplasmic reticulum (ER) or the mitochondrial compartments without the need to treat cells with detergents such as digitonin to eliminate cytoplasmic background. We have used this system to look at the cellular localisation of Ca^{2+} after stimulating cells with CD3, thapsigargin or ionomycin in the presence or absence of extracellular Ca^{2+}. Our data suggest that there is a dynamic interplay between the ER and mitochondrial compartments and that mitochondria act as a sink for both intracellular and extracellular derived Ca^{2+}. Moreover, by generating an NFAT-GFP expressing Jurkat line, we were able to combine mitochondrial Ca^{2+} measurements with nuclear translocation. In conclusion, this method enables the high throughput study of spatiotemporal patterns of Ca^{2+} signals in T cells responding to different stimuli.

10.00 - 10.45 Keynote

Proteomics in a core facility setting - What can we achieve?

Sharad Mistry
University of Leicester

The talk will cover the basic principles of proteomic analysis by mass spectrometry. Workflows will be described with different sample types and illustrated with examples.

10.50 - 11.20 Parallel Session: Genomics & Informatics

Sequencing the leukaemia genome; what, where and when?

Sarra Ryan
Newcastle University

Chromosomal abnormalities are the hallmark of B-cell acute lymphoblastic leukaemia (BCP-ALL). The majority (~75%) of patients are stratified according to cytogenetic risk group but there are evident clinical and biological differences between individual patients. Furthermore, ~25% of patients remain unclassified, showing none of the major established chromosomal abnormalities. Next generation sequencing (NGS) technologies have provided information on the genetic profiles of individual patients and enabled the identification of recurrent genomic abnormalities; however, the method employed has been dependent on a number of factors. In this talk, I will discuss the practicalities of using whole genome sequencing (WGS) compared to whole exome sequencing (WES) in leukaemia patient samples. The range of genomic abnormalities detected, mutation profiles observed using both methods, and the limitations of using either technique to answer a specific research question will be discussed. The results of this study have demonstrated the advantages and pitfalls of using both methodologies and influenced our future NGS approaches. Through the identification of novel and recurrent genetic features, we have used these data to classify patients into genomic subtypes with distinct clinical features. Finally, the results of this study have supported the development of a targeted sequencing assay with potential clinical utility.

10.50 - 11.20 Parallel Session: Proteomics

Exploiting the potential of Agave for bioenergy in marginal lands

Dalal Al-baijan
Newcastle University

Drylands cover approximately 40% of the global land area, with minimum rainfall levels, high temperatures in the summer months, and they are prone to degradation and desertification. Drought is one of the prime abiotic stresses limiting crop production. *Agave* plants are known to be well adapted to dry, arid conditions, producing comparable amounts of biomass to the most water-use efficient C3 and C4 crops but only require 20% of water for cultivation, making them good candidates for bioenergy production from marginal lands. *Agave* plants have high sugar and fructan contents, along with high biomass yield. More importantly, *Agave* is an extremely water-use efficient (WUE) plant due to its use of Crassulacean acid metabolism. Most of the research conducted on *Agave* has centered on *A. tequilana* due to its economic importance in the tequila production industry. However, there are other species of *Agave* that display higher biomass yields compared to *A. tequilana* and could thus potentially be considered as future sources of biofuels from water-limited environments. This project set out to examine several questions and hypotheses related to the potential of *Agave* as a dedicated biofuel feedstock.

In the final experimental chapter, methods were developed to identify vacuolar sugar transporters in *Agave* which are hypothesized to play a key regulatory role in determining sucrose turnover for CAM and fructan accumulation and as such, could represent future targets for genetic engineering of increased sugar content for plants grown for bioenergy. Tonoplast isolation and integrity was determined by measuring the activity of ATPase and PPiase of leaf vesicles of *A. americana marginata*, and its sensitivity to inhibition by known ATPase inhibitors. A proteomics approach, which analysed the purified tonoplast membrane using fractionation of the proteins by SDS-PAGE and analysis by LC-MS/MS, was used to identify tonoplast transporters. Overall, the results illustrate the capacity of the vacuole as a sink for carbohydrate in *Agave* which, in turn maybe an important determinant of CAM expression with important implications for plant growth and productivity. Implications of the results presented in the project for the adoption of *Agave* as a biofuel species are discussed.

11.20 - 11.50 Parallel Session: Genomics & Informatics

Uses for genome-wide DNA methylation data in disease prediction, prevention and treatment

Caroline Relton
Newcastle University

11.20 - 11.50 Parallel Session: Proteomics

A heart for mass spectrometry

Achim Treumann, Pawel Palmowski, Rachael Watson, Julie Taggart, Nick Europe-Finner, Michael Taggart

Achim Treumann
Newcastle University

To update and upgrade the mass spectrometry capabilities of NUPPA, the University of Newcastle has awarded a new mass spectrometer to the protein and proteome analysis facility. In acquiring this equipment it is our particular intent to improve the capability of performing reliable, label-free, quantitative protein analysis of complex samples. We have therefore set about assessing the capabilities of different MS platforms to perform this task. In so doing, we have chosen to use guinea pig heart tissue. One reason for this is that in heart tissue the protein abundance is dominated by a rather small percentage of the overall proteome. This makes it particularly challenging to detect and quantify lower abundant proteins that may be physiologically very important. We thus prepared heart extracts by two different extraction methods (in an attempt to increase protein coverage) from fetal and adult guinea pigs (to assess the ability to detect biologically informative information about proteome changes in cardiac development). Each of these four experimental groups had three biological replicates and a varying number of technical replicates, to enable us to assess our ability to separate biological variability from technical variability.

I will introduce a choice of up-to-date approaches to for label-free quantitative analysis and discuss the strategies we have chosen to compare the performance of different platforms.

13.20 - 13.50 Parallel Session: Genomics & Informatics

A year into embedding the PacBio RS II platform: Experiences and applications so far

Neil Hall
University of Liverpool

13.20 - 13.50 Parallel Session: High-Throughput Screening

The Use of High Throughput siRNA Screening to Identify Kinases Involved in Prostate Cancer Progression

Scott Walker
Newcastle University

Prostate cancer (PCa) is the most commonly diagnosed cancer affecting men in the UK, with one in every eight men developing the disease within their lifetime. As an androgen dependent organ, androgen receptor (AR) activation plays a critical role in the development and progression of PCa; hence therapies targeted at antagonising AR signalling, predominantly via the depletion of circulating androgens, are initially effective in the treatment of advanced PCa. However invariably, therapeutic success is only temporary; with the majority of men relapsing to develop an incurable form of PCa, castrate-resistant PCa (CRPC), in which AR activation occurs independently of androgen stimulation. Consequently, there is an imminent need for the development of novel therapeutic strategies.

Interestingly, the AR is not solely regulated by its cognate steroid hormone, but instead by a constellation of interactions with co-regulatory proteins, some of which are responsible for modifying the AR at the post-translational level. Indeed, phosphorylation of the AR has been shown to directly modulate AR activity. However, which protein kinases are involved remains unknown.

In order to address which kinases are important in the regulation of AR activity in both androgen sensitive and independent prostate cancer, a siRNA kinome screen was performed in partnership with the High Throughput Screening Facility at Newcastle University.

13.50 - 14.20 Parallel Session: Genomics & Informatics

Targeted sequencing in molecular diagnostics and clinical research

Silvia Borras
Clinical Scientist, NewGene Ltd

NewGene is a molecular diagnostics company that has used next generation sequencing technologies since 2009 for both clinical and research purposes. This presentation will review the current applications including probe capture and amplicon PCR based target enrichment methods and their relevant laboratory pipelines. The merits of targeted specific gene panels versus sequencing of whole exomes will be discussed. Validation and verification procedures are crucial in the acceptability of clinical tests and these will be covered in the context of specific assays, the sequencing instrument and data analysis software.

13.50 - 14.20 Parallel Session: High-Throughput Screening

Achieving a Successful High Throughput Screen

Peter Banks
Newcastle University

15.05 - 15.50 Keynote

Towards portable, real-time genome sequencing

Nick Loman
Institute of Microbiology and Infection, University of Birmingham

In this talk I will describe how, using a novel handheld genome sequencer, the Oxford Nanopore MinION, we may be able move to a paradigm of rapid genome sequence-based diagnostics for infectious disease, and how genome data can be linked to national and international databases to elucidate the origins and spread of international outbreaks.

15.50 - 16.35 Keynote

Diversity screening for chemical biology and drug discovery - the DDU experience

David Gray
University of Dundee

In this presentation I will outline the capabilities underpinning diversity screening that have been developed at the University of Dundee's Drug Discovery Unit since its inception in 2006. The underpinning philosophy, selection of compound sets and investments in hardware and informatics infrastructure will be described together with a high level overview of successes in both our neglected diseases and innovative targets portfolios. Finally, I will present case studies showing in some detail how projects were moved from initial concepts through screening towards clinical assessment.