Flow cytometry: recent advances in techniques and instrumentation

Cutting-edge advances in the field of flow cytometry were the focus of a EuroSciCon meeting held in Hertfordshire last November. Ruth Challis reports.

Modern flow cytometry instruments with multiple lasers and fluorescence detectors mean that this technology has a wide and ever-expanding range of applications in fields including molecular biology, pathology, immunology, plant biology and marine biology. This wide applicability is due to the more precise phenotypic identification of target populations permitted by multiparametric analyses.

A meeting held last November, organised by EuroSciCon (www.euroscicon.com) and chaired by Dr Ian Dimmick (Institute of Genetics, International Centre for Life, Newcastle upon Tyne), aimed to bring together the most recent advances in clinical and research flow cytometric techniques, together with information on the hardware that has made these advances possible.

POLYCHROMATIC FLOW CYTOMETRY

Dr Ian Dimmick was the first speaker of the day and he set the scene with a clear introduction to the optimisation and compensation issues associated with multilaser, multiparameter flow cytometers used for polychromatic flow cytometry. Increasing the number of lasers and detectors permits multiple antibody labelling and can identify more precisely a target population by the phenotypic expression of surface (or intracellular) markers. However, one of the hardest challenges in these complex characterisations is the ability to ensure the continued output of meaningful data, without influence from fluctuations in instrument performance or the obscuring of biological effects due to poorly compensated data. Dr Dimmick concluded that this can only be achieved by a good choice of fluorochrome combination and the continual tracking of instrument performance.

FLOW MEETS MICROSCOPY

The second speaker, Dr David Basiji (Amnis Corporation, Seattle, USA), presented applications of the new flow technology made possible by the ImageStream flow cytometer (Amnis Corporation). Typically, normal and abnormal blood (and other) cells are analysed by either histological or flow cytometric approaches. Histology facilitates the examination of complex visual traits, but with a relatively limited numbers of cells. Flow cytometry allows characterisation of millions of cells by multiple fluorescence parameters, but it lacks morphological or subcellular spatial detail.

The remarkable ImageStream instrument blends morphology and flow cytometry by its ability to acquire more than 15,000 cells per minute while capturing brightfield, darkfield and multiple fluorescence images of individual cells in flow (Fig 1). The capture rate broadens the application of traditional imaging techniques to allow discrimination of rare populations of cells, while the applications of traditional flow cytometry are broadened to include signal localisation and morphological analysis. Additionally, the morphological information effectively eliminates the false-positive and false-negative results that can occur due to traditional flow cytometry's inability to visualise cells.

MILESTONE IN FLOW CYTOMETRY

The first session was concluded by Mark Twigden (Miltenyi Biotec, Germany), who introduced the MACSQuant analyser, a new seven-colour flow cytometer. This instrument offers the widest range of optical fluorescence parameters (three lasers, seven fluorescence channels) from the relatively new range of small instruments now available from several companies. These instruments use non-pressurised sample uptakes, based on capillary action, and are extremely compact – most are small enough to fit inside a standard laminar flow hood.

Analysis rates are comparable with conventional flow cytometers, with up to 10,000 events per second, but this new breed of instrument offers other advantages such as accurate volumetric sampling allowing absolute cell counting without the need for beads, and minimal sampling volumes (25 µL). Sheath fluid consumption is also much lower (2–10 mL/minute) than with conventional flow cytometers.

The MACSQuant analyser can also be used with an integrated pre-analysis enrichment column for detection of rare cell populations. The result is a compact

'Flow cytometry has expanding range of applications in molecular biology, pathology, immunology, plant biology and marine biology'

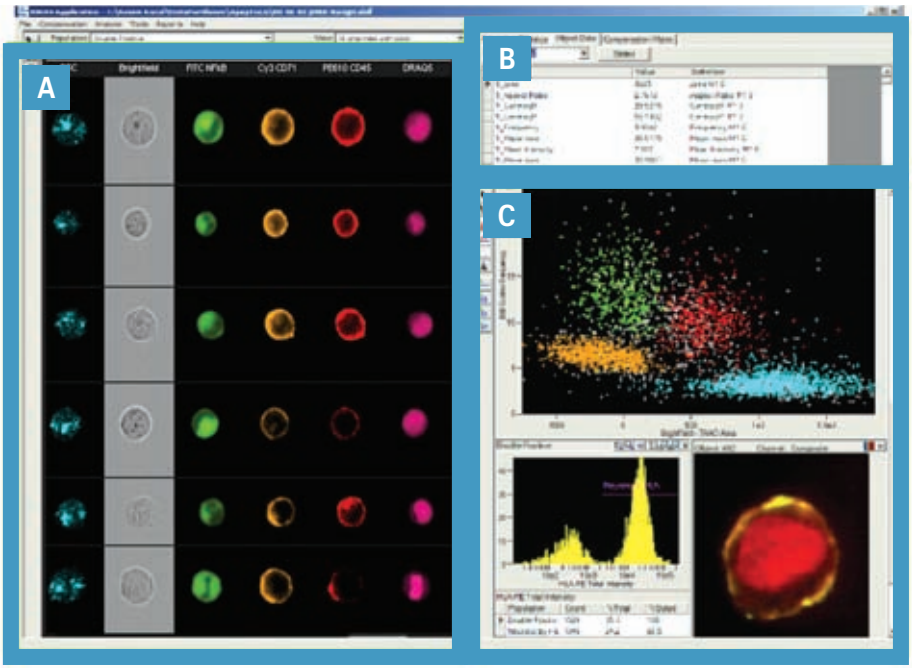

Fig 1. Data acquired by the ImageStream system: A) image gallery, B) tabular data, C) analysis area. Reproduced by kind permission of Amnis users USA and Dr Andrew Filby, Cronus Technologies, UK.

yet versatile alternative that should offer more affordable alternatives to the previous market leaders.

APPLICATIONS OF FRET IN FLOW CYTOMETRY

Dr Gary Warnes (Institute of Cell and Molecular Science, Barts and the Royal London, UK) then introduced some applications of fluorescence resonance energy transfer (FRET) in flow cytometry. It can be used to show that molecules are within 10 nm of each other, and this technique has become more prevalent in the investigation of both molecule proximity and protein functionality.

Applications of FRET discussed included a gene reporter system developed by Invitrogen, which uses the FRET-based fluorescent substrates CCF2-AM and CCF4-AM, detection of protein interactions or biological processes (eg phosphorylation) using fluorescent proteins (most commonly CFP-YFP) and analysis of the 'peptide editing' function of tapasin in its role in peptide loading of MHC class I molecules.

Some of these applications can even be followed in real time where conformational changes that take minutes to occur can be followed by measuring how FRET varies over this time.

RISK STRATIFICATION IN CHRONIC LYMPHOCYTIC LEUKAEMIA

The next speaker, Dr Chris Pepper (Department of Haematology, University of Cardiff, Wales), described a specific application of flow cytometry whereby the diagnosis of chronic lymphocytic leukaemia (CLL) is made by complex flow cytometric analysis. He went on to discuss the variation in disease prognosis, what makes a good prognostic marker, the difficulties in characterising such markers, and various candidate markers in CLL.

His conclusion of the challenges involved in finding such prognostic markers underlined the difficulty in harmonising data between multicentre and multinational trials. The lack of standardisation of protocols between laboratories leads to hugely confounding differences in prognostic value. In order to address this problem, the European Research Initiative on CLL (ERIC) harmonisation study has been working towards standardised protocols for flow cytometric analysis of CLL samples.

Dr Pepper's view was that such standardisation, particularly of instrumentation rather than samples, is crucial in order to enable large multicentre trials to be meaningful and effective.

ANALOGUE-TO-DIGITAL AGE

Digital signal processing (DSP) has revolutionised the field of flow cytometry through more accurate representation and processing of scatter and fluorescence signals. It also has added advantages in its increased flexibility in data analysis, as functions such as fluorescence compensation can be performed after data collection.

Much of the increase in accuracy of digital data is due to the use of 18–20-bit analogue-to-digital converters to digitise detector signals. An 18-bit conversion provides an expanded range of data channels (262,144 compared to 1024 on older analogue machines) and therefore increased data resolution. However, the cytometrist must still adjust detector voltage and/or gain settings depending on the application.

Dr Leo Ostruska (Accuri Cytometers, Europe) presented the advantages of 24-bit analogue-to-digital convertors (as used in the Accuri C6 cytometer), which have increased resolution to approximately 16.7 million channels for each parameter. The company claims that this increased resolution virtually eliminates set-up time as voltages and gain settings remain fixed, as signals now are never 'off scale'.

Improved data resolution accompanied by the companion CFlow collection/analysis software also allows virtually infinite plot resolution, enabling distinct populations to be apparent and accurately gated among 'clouds' of data.

DIELECTROPHORESIS CYTOMETRY

Professor Paul Smith (Department of Pathology, School of Medicine, Cardiff University, Wales) then introduced microflow cytometry fluidics driven by micro-electro-mechanical devices, and various applications of this interesting technique.

Cell behaviour can be engineered through micropatterned surfaces so that cellular properties may be investigated at a single-cell level, or by trapping cells for large-scale single-cell analysis. Micro-engineering and manipulation of the cells using their dielectrophoretic properties can allow analysis of cell division along microtrenches, where the division can only occur in one direction due to the physical constraints, thus allowing determination of exact cell heritage by their position in relation to surrounding cells.

Cell electromanipulation can also allow cells to be 'trapped' or levitated in an electrical field, and, combined with micro-manufacture of slides, produce dielectrophoresis (DEP) 'cages' where cells are organised for rapid analysis as the cells are positioned in a 'check board' position, effectively lining up for analysis. Combining DEP manipulation with flow cytometry allows accurate and high-throughput cell sorting. One application of these phenomenal technologies was discussed in relation to the study of small cell lung cancer.

RED/FAR-RED NUCLEAR DYES

The next speaker, Roy Edward (Biostatus, UK), presented many examples of the use of the red/far-red nuclear dyes DRAQ5 and CyTRAK Orange, and their application to the analysis of difficult samples in flow cytometry. Often, this allows staining in whole unlysed blood or bone marrow samples where agents used to lyse red blood cells may cause loss of some antigenic labels. Examples included simple and reliable gating of intact cells from pancreatic islets, nucleated cell gating of whole unlysed blood for the detection of rare circulating endothelial cells, and diagnostic information based on non-lysed bone marrow aspirations. These examples were used to demonstrate how the far-red nuclear dyes can be utilised to provide additional information without impacting on other diagnostic parameters.

INSTRUMENT TRACKING PROCEDURES

The day concluded with a return to one of the major challenges in flow cytometry, that of tracking instrument performance. Dr John Lawry (BD Biosciences, UK) gave a review of the uses of manufactured beads in flow cytometry, from their first uses as tools to aid laser alignment, through to more recent applications such as replacing cells for compensation set-ups, in quality control procedures, and in cytometer set-up and tracking (CST). The CST beads, combined with the latest BD software, provide a convenient quality control for the instrument, as well as providing 'best setting' adjustments for each parameter, thus eliminating day-to-day variations in instrument performance. In addition, photomultiplier tube voltage and also laser and flow cell performance are tracked, thereby helping to troubleshoot potential component failure.

TECHNOLOGY LEADS THE WAY

Flow cytometry applications are ever increasing and cover a huge range of biological processes. The technological advances demonstrated by new instrumentation, software analysis and experimental components (microstructured or in fluorochrome development) mean that the technological advances often far outstrip the published literature in the public domain.

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Therefore, the recent EuroSciCon meeting was of great value in bringing together technological hardware developments with advances in clinical and research flow cytometry. ■

- *IBMS members can attend EuroSciCon meetings at reduced rates.*

FURTHER READING

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