Flow Cytometry Core Facility

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Blizard Institute

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Website: http://www.icms.qmul.ac.uk/flowcytometry/

Blizard Flow Cytometry Core Facility

Flow cytometry is a powerful tool for the multiparameter analysis of cells of all types. Three high powered high speed flow cytometers are available including a BD Aria I cell sorter, as well as two simpler cytometers and a five colour LED upright Fluorescence microscope in the Blizard Institute. The analyser instrumentation is open 24/7 with a trouble shooting guide/contact number for instrument problems outside working hours.

The Core Facility also trains new beginners and gets all Users up to speed by offering a compulsory Training Course which includes use of all analyzers, software, the practicalities of colour compensation and helps the user set up experimental templates so that all Users get what they want in terms of results from their experiment were possible. The Core Facility Staff have over 25 years experience and gives, advice and support in experimental design. The Core Facility also offers the possibility of designing new assays or improving known techniques as well as free FACS tubes, range of viability dyes, Comp Beads and other reagents for free, see Reagent Shop for full list

http://www.icms.qmul.ac.uk/flowcytometry/shop/index.html

Website

The Flow Cytometry Core Facility is currently rated by Google as the best academic website on the planet. There are multiple examples of methods with full protocols with expert Guides on instrument use, How to set Compensations Correctly (highlighting some of the mis-information about this dark art), Cell Cycle Guide and lots more about what Flow Cytometry is and how it works.

http://www.icms.qmul.ac.uk/flowcytometry/index.html

Range of New Assays Developed at the Blizard

FRET Applications **(J Cell Sci 2012),** 6 Autophagy assays **(J Clin Exp Imm 2012, Intech Publication 2013, Cytometry 2013),** a real-time differential necrosis-apoptosis assay **(Cytometry 2011),** receptor quantification **(BJH 1998),** Flow FISH for telomere length semi-quantification, soluble analyte bead assays, protein translocation assay **(Apoptosis 2007),** NADH analysis, neuron analysis, antibody binding real-time analysis, multiplexing of apoptosis assays **(Cell Res 2009, Macromolecular Bioscience 2011),** cell cycle phase sorting **(Nature Cell Biology 2006, Cell Cycle 2007),** single cell cloning **(EMBO 2001),** bacterial viability assay **(J Med Microbiol 1993).**

Instruments & Charges all instruments are bookable on the Google Calendar - Grant Codes are required.

Cell Sorting

The Blizard Aria III (upgraded to model II in 2014) has 4 lasers (argon, violet, Yellow-green & red HeNe). The instrument can analyze 2 colours from the argon laser (FITC, PerCP), analyse 5 colours from the Yellow-Green laser (PE, PE-Texas Red, PE-Cy5, PE-Cy5.5, PE-Cy7) 5 colours from the violet laser (Pacific Blue, AmCyan or BV510, BV 570, BV605, BV655 or BV785) and 3 colours from the red HeNe (APC, Alexa700 or APC-Cy5.5, APC-Cy7). The instrument can sort 4 populations at 20,000 cells/sec (lymphocytes) or 10,000 cells/sec for cells larger than 7um. The instrument can single clone into any TC plate in a sterile manner under cool conditions. The charge rate is £50/h without setup or cancellation charges.

BD LSRII

The BD LSRII can analyze 6 colours from the argon laser (FITC, PE, CF590, PerCP, PE-Cy5.5, PE-Cy7), 3 colours from the violet laser (Pacific Blue, AmCyan or BV 570, BV605), 2 colours from the UV laser (Hoechst 33342 or DAPI, Indo-1 Green) and 2 colour from the red HeNe (APC, Alexa700 or APC-Cy5.5 or APC-Cy7). The charge rate is £30/h without cancellation or time change charges – what you use is what you pay.

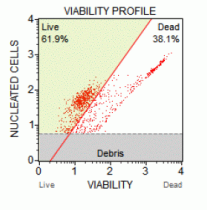
BD FACSCanto II

The Blizard BD FACSCANTO II can analyze 4 colours from the argon laser (FITC, PE, PerCP or PE-Cy5.5, PE-Cy7), 2 colours from the violet laser (Pacific Blue, AmCyan or BV 570) and 2 colour from the red HeNe (APC, APC-Cy7). The charge rate is £30/h without cancellation or time change charges – what you use is what you pay.

BD FACScan

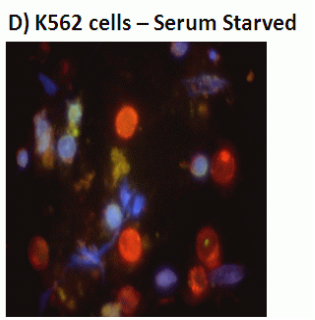
The Blizard BD FACScan can analyze 3 colours from the argon laser (FITC, PE, PerCP or PE-Cy5.5 orPE-Cy7). The charge rate is £5/h without cancellation or time change charges – what you use is what you pay.

Millipore Muse



The Blizard Millipore Muse can analyze 2 colours from the 532nm Green HeNe (PE, PI, 7–AAD, LDS751, RFP, Alexa546 or 555). This instrument is of a new simple design without the need to set voltage or compensation by the User. The instrument can perform simple FACS assays, such as cell viability and absolute counts, annexin V assay, PI cell cycle analysis, mitochondrial & caspase function by the purchase of reasonable priced kits from Millipore. The charge rate is £10/h without cancellation or time change charges – what you use is what you pay. This instrument is easy to use for beginners and occasional Users.

LED Epi-Fluorescent Upright Microscope



The Flow Cytometry Core Facility has a five colour LED epi-fluorescent upright microscope with UV, violet, Green, Blue and Bright Field illumination. This instrument can take jpg of DAPI, FITC, TRITC and violet excitation probes, which can be overlaid in other supporting software. This allows FACS Users to have a quick look at their cells for free.

Software & Data

The Core Facility has a large screen Apple computers for data analysis with FlowJo software all bookable on the Quartzy booking syastem. All data is backed up every 3 months by the Core Facility onto Removable Hard Drives, at which time some data is removed from the instruments Database, but users usually have easy access to 3-6 months of data. Older data can be re-installed onto instruments computers as required if the User has the date of the original experiment. Finally it is the User that is responsible for their data.

Assistance

The Manager & Research Assistant are bookable on the Quartzy booking system for expert help and advice with setting up experiments and run samples if required. The Core Facility tries to promote Users to ask questions and ask for help with experimental design, instrument setup, sample collection and data analysis when needed by Users; most of this is given free of charge but for complex use of the instrument this is charged at £50/h.

Flow Cytometry Standards & Publications

NEW MIFlowCyte criteria for good practice in data publication, see Cytometry the scientific journal for the International Society for the Advancement of Cytometry, **ISAC**, see details and **download form** **in** **Flow Cytometry Guides.** All such data for publication in Cytometry has to be uploaded to **FlowRepository.org**

These standards include the quoting of voltage & compensation values as well as controls used (FMO, isotype as appropriate), as well as gating strategies. These standards have been accepted in numerous journals such as Nature, Science & Cell.

Projects & Collaborations

Currently the Flow Cytometry Core Facility offers under and post-graduate (MSc) student place for projects studying autophagy by flow cytometry. The Core Facility also collaborates with PIs requiring the study of autophagy. Currently there are 7 projects with the Centre for Cutaneous Research, Diabetes, Neuroscience & Trauma, Paediatrics, & the Dental Institute.

Flow Cytometry Methods

Immunophenotyping

The use of directly fluorescently conjugated tagged monoclonal antibodies has historically been carried out on lymphocytes and the Core Facility can detect commonly used fluorophores *e.g.* FITC, PE, PerCP, PerCP-Cy5.5, PE-Cy7, APC, APC-Cy7 & Pacific Blue. The instruments at the Blizard can also detect new fluorophores such as Horizon V450 & 500, Pacific Orange & Green, CF590, BV421, BV570, BV605, BV655, BV710, BV785, as well as BUV dyes. The only Alexa Fluor dyes that can be used are Alexa405, 488 and 647. The Muse instrument can analyse Alexa546 and 555 but these can only be run in single colour mode. There are numerous other techniques associated with immunophenotyping including intracellular cytokine analysis as well as PhosFlow protein analysis http://www.icms.qmul.ac.uk/flowcytometry/uses/multicolouranalysis/index.html

Viability Dyes

The Core Facility has a free supply of commonly used viability dyes such as DAPI, PI, 7-AAD and DRAQ7. A useful tip when using the LSRII, is that DAPI can still be used with the Pacific Blue fluorophores as this dye is only excited by the violet laser whilst DAPI is excited by the UV & Violet and hence dead cells can be excluded on the UV parameter and Pacific Blue events gated on the Violet parameter. The Muse instrument is supplied with a cell viability dye for absolute counts for rapid analysis http://www.icms.qmul.ac.uk/flowcytometry/uses/viability/index.html

Cell Cycle Analysis

Cell cycle analysis of DNA content can be made with DAPI, PI & 7-AAD as well as with live cells using Hoechst 33342 (only on the LSRII). More complex analysis can also be used to show DNA content versus antigens using the Click-IT kits from Invitrogen; as well as standard Brdu analysis for investigating cell proliferation rates; DNA breaks can also be investigated using anti-H2AX antibody, as well as PARP, histone H2 to determine m phase. Rapid cell DNA content can be performed using a rapid Triton X-100 method allowing the user to rapidly analyse DNA content with DAPI even with Violet laser excitation rather than UV. Polyploid cells can also be analysed flow cytometrically, as well as cell quiescence using RNA probe, Pyronin Y http://www.icms.qmul.ac.uk/flowcytometry/uses/cellcycleanalysis/index.html

Fluorescent Proteins

The Core Facilities analysers can detect CFP, GFP, YFP & DSRed. The new Muse instrument with its Green HeNe can detect RFP & Cherry Fluorescent proteins. Routinely, GFP, YFP & CFP can be sorted by the Aria I http://www.icms.qmul.ac.uk/flowcytometry/uses/fluorescentproteins/index.html

Flow FISH

Telomere length can also be rapidly analysed flow cytometrically using the DAKO commercial kit for estimations of relative telomere lengths
http://www.icms.qmul.ac.uk/flowcytometry/uses/flowfish/index.html

Cell Death - Apoptosis, Autophagy, Oncosis & Necrosis

Cell death includes various recognized processes including classic apoptosis, which is studied by a wide variety of techniques *e.g.* SubG_1 DNA analysis, annexin V assay, mitochondrial function and active caspase detection. Autophagy or Type II apoptosis is a cell survival mechanism when the cell is under environmental pressure due to lack of nutrients. The archetypal double membrane structure, the autophagosome is formed to sequester cytoplasm, misfolded protein and cell organelles; this then fuses with lysosomes to form a single membrane structure, the autolysosome in which the contents is digested by hydrolases, releasing energy in the form of ATP thus helping the cell to survive. If nutrient starvation persists then the cell dies. Autophagy has only recently been studied by flow cytometry, and this Flow Cytometry Core Facility has been at the fore-front in assay development in this area of science. Quantification of autophagy marker, LC3II or B and lysosome mass has been demonstrated to measure autophagy in live and fixed cells. Flow cytometric organelle phagy assays have also been published.

The 15 year old problem of not being able to differentiate cells dying via apoptosis or necrosis (oncosis) has been solved by the use a real-time mitochondrial function assay as well as a method that distinguishes early and late necrotic cells.

http://www.icms.qmul.ac.uk/flowcytometry/uses/apoptosis/index.html
http://www.icms.qmul.ac.uk/flowcytometry/uses/autophagy/index.html
http://www.icms.qmul.ac.uk/flowcytometry/uses/oncosis/index.html
http://www.icms.qmul.ac.uk/flowcytometry/uses/necrosis/index.html

Calcium Flux

Calcium flux into cells or release from internal stores is indicative of cell activation. The Core Facility provides Indo-1 for Ratiometric analysis of calcium flux using the UV laser on the BD LSRII. The use of Indo-1 allows the use of most available antibody fluorophores including Pacific Blue. Organelle (ER & mitochondria) calcium flux can also be analysed using calcium indicator dye, Rhod-2
http://www.icms.qmul.ac.uk/flowcytometry/uses/functionalanalysis/calciumflux/index.html

Cell Proliferation

CFSE is supplied free by the Core Facility for proliferation generation analysis after cell stimulation. Other colours of CFSE, such as violet, orange and red are available from Invitrogen and more economically from eBioscience. This data can be analysed using the special Platform within FlowJo Software available for Users of the Core Facility http://www.icms.qmul.ac.uk/flowcytometry/uses/functionalanalysis/cellproliferation/index.html

Bead Assays

BD and eBioscience are manufacturers of soluble protein analyte capture beads for the detection of a large range of cytokines and other soluble proteins. This method is setup to run on the BD FACSCanto II and the Core Facility provides expert help in the use of these expensive kits http://www.icms.qmul.ac.uk/flowcytometry/uses/cba/index.html http://www.icms.qmul.ac.uk/flowcytometry/uses/flowcytomix/index.html#

FRET

Fluorescence Resonance Energy Transfer (FRET) allows the analysis of protein interactions (<10nm) rapidly by flow cytometry and provides an easy mechanism of providing a second method of FRET analysis to combine with microscopic analysis of FRET. Donor and Acceptor fluorophores have to be within 10nm and also lie in parallel for energy to transfer to occur. This typically from the Donor fluorophore with a lower wavelength than the Acceptor, typically <50nm lower in order for the Acceptor to be excited by the Donor fluorophore and hence show fluorescence or increase in the intensity of this fluorophore. Flow cytometry can also use FRET to detect the presence of BRDU (without antibody); gene transfer reporting by CCF2/4 reagents (Invitrogen); cell hybridoma formation by use of green & red carbocyanine dyes; protein interactions in live cells using Fluorescent Proteins CFP-YFP. Antibodies can also be used to detect homo-hetro dimer receptor levels as well as kinetic measurement of second messenger interactions http://www.icms.qmul.ac.uk/flowcytometry/uses/fret/index.html