Equipment Name: Flow Cytometry	**Category:** **D. In-vitro toxicity studies** **Institute:** University College Dublin **Location:** Conway Institute, University College Dublin, Belfield, Dublin4, Ireland **Contact Details of Technology Expert:** **Name:** Alfonso Blanco **Phone:** +353 1 716 6836 / 6947 **Fax:** **E-mail:** alfonso.blanco@ucd.ie

Short technology description/Overview:

Flow cytometry can be used to measure intrinsic (size, shape, granularity etc.) and extrinsic (DNA, internal and external receptors etc.) cell characteristics of cells in solution, using a range of fluorescent markers.

This has many applications in humans, animals, plants and microorganisms. The most common applications are cell cycle, apoptosis/necrosis, ploidy determination, immunophenotyping, protein expression, Ca^{2+} concentration, etc.). Cell sorting allows the physical isolation of cell populations for further procedures such as cell culture and studies of protein expression. Forward Scatter (FSC) and Side Scatter (SSC) report on the approximate cell size and the cell complexity or granularity, respectively.

UCD have developed robust protocols for quantitative determination of uptake of fluorescent nanoparticles by cells, as well as for assessment of nanomaterial impacts on cell viability (e.g. Yo/Pro; PI assays etc.) and cell cycle effects based on flow cytometry, which are available through QNano. Cell populations can also be sorted depending on fluorescence distribution, for example, based on numbers of nanoparticles taken up, or based on cellular impacts from interaction with nanoparticles.

Nanomaterial dispersion quality and nanomaterial label are key determinants of the quality of the experimental outcomes, and must be discussed at the earliest stage of experimental design.

Main Features (Equipment Capabilities):

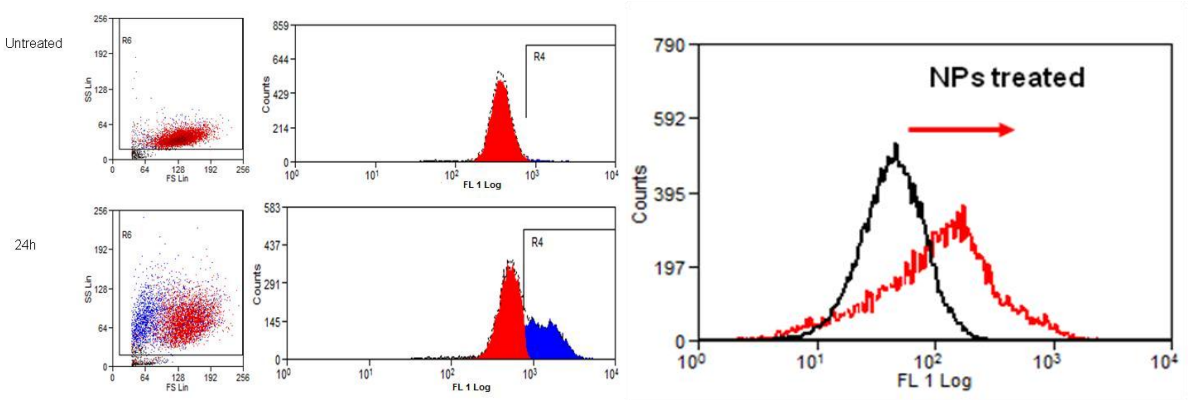
- Coulter Epics XL-MCL - simultaneous measure of FSC, SSC and up to 4 colours using a 488nm laser, with an automated sample loader, which permits a throughput of 100 samples/hour.
- Coulter FC500 - the next generation of the Epics XL-MCL, can analyse up to 5 colours using a 488nm laser. It has an automated sample loader.
- Dako Cyantm-ADP - we have two Dako Cyan ADP, each with three lasers: one with 351nm (UV), 488nm (blue) and 635nm (red) and the other one with 405 nm (UV), 488nm (blue) and 635nm (red). This allows us to detect up to 11 parameters at the same time and offers complete compensation and analysis rates of up to 50,000 events/second.

- BD Facsaria Cell Sorter - High-speed cell sorter (40,000 events/second) with 3 lasers: 488nm (blue), 633nm (red) and 407nm (violet) for detection of FSC, SSC and up to 11 parameters. It is able to sort 4 populations of cells at the same time and perform cell cloning in well plate platforms with a 100% purity level.

Issues to consider when developing TA proposal:

- Fluorophors of nanoparticle and assay should not interfere with one another or overlap
- There should be no interaction of the assay label with the test nanoparticles
- Dispersion protocol for nanomaterials should not be toxic to cells
- If comparing across cell lines, cell culture media and conditions should be identical where possible, or where not possible, appropriate control steps must be built into the experimental protocol to allow cross-comparison of data.

Typical Samples & Images:



Altered distribution of fluorescence in cells in the presence of nanoparticles.

Key publications:

- Salvati A, Aberg C, Dos Santos T, Varela J, Pinto P, Lynch I, Dawson KA. Experimental and theoretical comparison of intracellular import of polymeric nanoparticles and small molecules: toward models of uptake kinetics. Nanomedicine. 2011 Mar 29. [Epub ahead of print]

- Bexiga MG, Varela JA, Wang F, Fenaroli F, Salvati A, Lynch I, Simpson JC, Dawson KA. Cationic nanoparticles induce caspase 3-, 7- and 9-mediated cytotoxicity in a human astrocytoma cell line. Nanotoxicology. 2010 Dec 15. [Epub ahead of print]

Any further Information:

This installation can be used in conjunction with the Confocal microscopy installation or with the EM installation, if want to couple study of uptake and impacts with detailed assessment of localisation.

This installation could also be coupled with HCA installation during a single visit.