

Flow Cytometry Core Unit



Lola Martínez

Unit Head

Lola Martínez, born in Madrid in 1973, studied Chemistry at the *Universidad Autónoma de Madrid*, obtaining her BSc in Biochemistry and Molecular Biology in 1997.

Shortly after receiving her degree she moved to London, UK, and joined the Wolfson Institute for Biomedical Research Core Support Facilities where she first used flow cytometry. In 2001 she moved to a more research-driven environment and joined D. Beach's lab at University College London (UCL), where she focused on molecular pathways that regulate the proliferation and fate determination of mammalian cells, performing genetic screening for cDNAs bypassing replicative senescence using a MoFlo High Speed Cell Sorter.

In 2004 she joined the Stem Cell Consortium Initiative (funded by the Wellcome Trust) and worked closely with C. Boshoff's lab developing new flow cytometric methods to identify and isolate endothelial precursors from bone marrow aspirates.

At the end of 2005 she joined the Cancer Research UK London Research Institute as Deputy Head of the Flow Cytometry Laboratory, where she worked with D. Davies at the largest flow cytometry facility in Europe.

She returned to Spain in June 2009 and joined the CNIO as Head of the Flow Cytometry Unit. Her main interest is to promote flow cytometry methodology and technology at the Centre.

Lola has been a Member of the International Society for Advancement of Cytometry since 2003 and she is also part of the European Cytometry Network. Her contributions to the flow cytometry field are reflected by her numerous publications that she has co-authored in high-impact factor journals.

Summary

A great deal of early oncology research is based on cell biology studies. Flow cytometry has proven to be an indispensable tool in cancer research for its use in the identification and isolation of defined subpopulations of cells, helping us to further understand cell function.

The Unit provides CNIO Groups with all the necessary technical and scientific advice in flow cytometry technology, offering an array of instrumentation for multicolour analysis as well as cell sorting in defined cell populations. We are committed to evaluating and implementing new technologies as well as actively developing new techniques for CNIO research groups. Regarding the implementation and improvement of protocols, the Unit has recently acquired two new instruments that incorporate yellow-green and UV lines to cater for all users' needs, allowing us to do DNA profiles in live cells using Hoescht as well as employ a wide array of new generation red fluorescent proteins.

Main Objectives

- Provide state-of-the-art equipment and software packages in flow cytometry, including technical and scientific advice and support to CNIO scientists
- To set-up and optimise flow cytometry techniques for research at the CNIO
- Develop new technologies and tools involving flow cytometry applications
- Evaluate new equipment of potential interest to CNIO researchers
- Organise training courses in flow cytometry technology and data analysis

Highlights

The Flow Cytometry Core Unit at the CNIO is equipped with four benchtop analytical systems available for multiparameter cellular analysis: the FACScalibur (BD) dual laser system, equipped with 488 and 633nm lines and the capacity to detect four fluorescence parameters; the FACSArray (BD), a 96-well platform dual laser system with capability to detect four fluorescence parameters; a LSRII Fortessa (BD) equipped with 355nm, 488nm, 561nm and 633nm lines capable of detecting up to sixteen fluorescence parameters in the actual configuration; and the FACSCanto II (BD) equipped with lines of 405nm, 488nm and 633nm, capable of detecting up to eight fluorescence parameters. This instrument is equipped with a high-throughput system (HTS module) that runs 96-well and 384-well microtiter plates.

The Unit also counts with two high-speed cell sorters capable of sorting up to 4-defined cell populations simultaneously: the FACS ARIA (BD), equipped with lines of 407nm, 488nm and 635nm, and its current optical configuration allows for the detection of up to 12 parameters; and the InFlux (Cytospeia, BD) equipped with 355nm, 488nm, 561nm and a 640nm lines, its current optical configuration allows for the detection of up to 16 parameters. Both cell sorters are equipped with sample temperature control systems at sample acquisition and collection points and can sort into various receptacles, including 96-well and 384-well microtiter plates. InFlux is contained into a biological safety cabinet that allows sorting human samples in accordance to safety regulations.

Analytical and cell sorting flow cytometric applications developed and validated at the Flow Cytometry Core Unit over the last few years include:

- Determination of DNA content by cell cycle analysis



Technicians: Ultan P. Cronin and Aranzazu García.

- Quantification of cell proliferation by BrdU and EdU incorporation
- Apoptosis studies
- Immunological characterisation of surface, cytoplasmic and nuclear markers in cell subpopulations from tissue samples as well as cell lines, by multicolour antibody staining
- Protocols to determine the concentrations of intracellular proteins, including the phosphorylation status of proteins involved in critical intra-cellular signalling cascades (p38, JNK, AKT, ERK...), and the monitoring of transfection / infection efficiency of fluorescent proteins to monitor gene expression
- Cytometric bead arrays to measure cytokine production, reducing sample and time requirements compared to traditional ELISA or Western blot methods

The Unit has been designing and setting up multicolour panels to assess inflammation in different tissues including skin by combining up to 7 fluorescence parameters in the same tube, which has proven useful for extracting lots of information from one due sample.

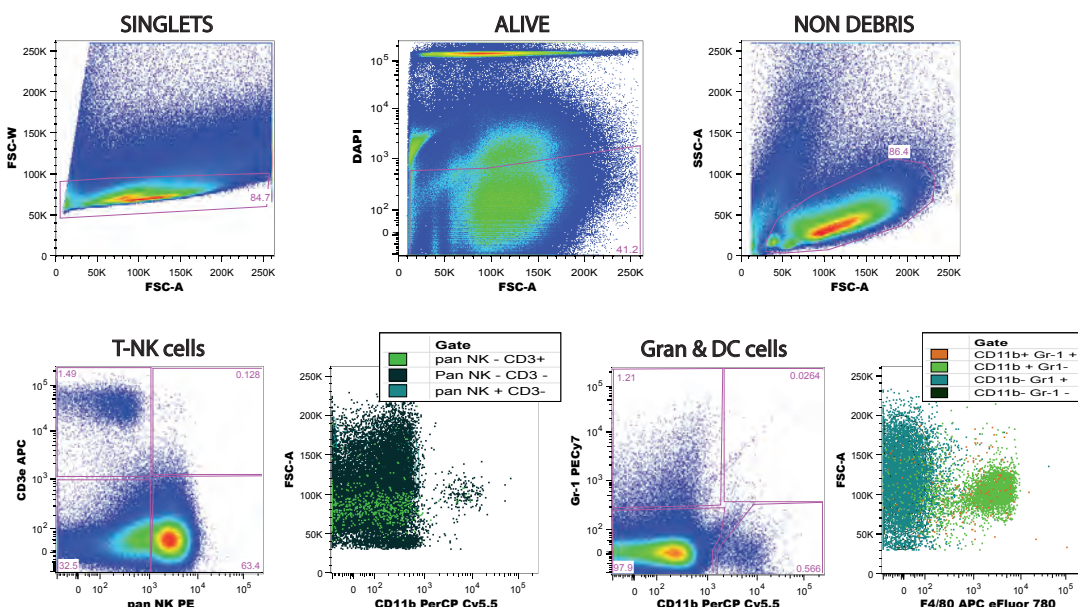


Figure: Multicolour Flow Cytometric Analysis. Example of a 6-colour inflammation panel in skin where we combined DAPI to get rid of dead cells and then we looked for the expression of CD3e, pan NK, CD11b (Mac-1), Gr-1 and F4/80 on a freshly isolated mouse skin sample.