FLOW CYTOMETRY CORE UNIT

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**Titulado Superior* (Advanced Degree)
***Plan de Empleo Joven* (Youth Employment Plan)

OVERVIEW

Flow Cytometry is a fast and multiparametric technology, and a very valuable tool in the oncology field. It is an important workhorse for the identification, quantification and isolation of defined subpopulations of cells, based on the expression levels of fluorescent markers and their relationship to each other at the single cell level.

Our aim is to provide CNIO Groups with technical and scientific advice regarding the use of cytometric technologies, collaborating with them in the design, acquisition, data analysis, and interpretation of flow cytometry data.

We currently have 5 analysers and 3 high-speed cell sorters with different optical configurations to cater to our users' needs. We also have an automated magnetic bead separation system (AutoMACS), 2 automated cell counters (Countess) and a tissue homogenizer (GentleMACS). Analysers are user-operated upon appropriate training, and cell sorters are operated by the Unit staff. Our sorters can separate up to 4- or 6- defined populations simultaneously, as well as perform single cell cloning and index sorting. We can accept human samples to sort under BSL2 regulations.

"We incorporated a full spectrum cytometer to expand the number of parameters we could study per sample, increasing our knowledge on the role of different immune subsets in cancer progression, and elucidating new biomarkers with potential therapeutic value."

RESEARCH HIGHLIGHTS

We provide state-of-the-art equipment and software packages in flow cytometry and collaborate with CNIO investigators in setting up and optimising flow cytometry techniques relevant to their research projects. Some applications developed and validated at our Unit include:

- → Cell proliferation studies (CFSE, Cell Trace Violet, BrdU or EdU, DNA content, etc.).
- → Apoptosis studies (Annexin V, Mitochondrial Membrane Potencial, Caspase 3, etc.).
- → Multicolour immunophenotyping panels (B and T cell development, Tregs, inflammation, etc.).
- → Functional assays (side population detection, Ca^{2+} flux, intracellular pH, etc.).
- → Cytometric bead arrays to measure several cytokines from cell extracts and plasma.
- → Platelets studies.
- → Extracellular vesicles detection (microvesicles and exosomes).
- → CTC detection and isolation.
- → Single cell sorting for OMICs analysis.

We further optimised our multicolour flow cytometry panels to characterise immune response in various samples from haematopoietic tissues, pancreas, skin, liver, lung, brain, as well as different tumour types. Single cell deposition using index sorting into 96 or 384 PCR plates to perform single OMICs techniques is now part of our routine portfolio. We also improved the performance characteristics of our instrumentation by creating voltration templates in all our instruments to assess optimal voltage for each detector and expand our training capacities with many more workshops and small practical analysis sessions. This provides our users with more tools to successfully perform their flow cytometry experiments. ■

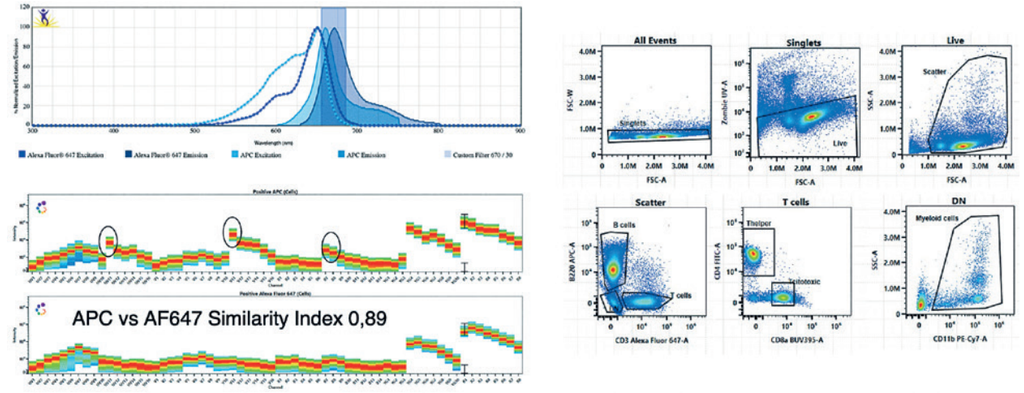

FIGURE Immunophenotyping panel of mouse spleen combining antibodies coupled to AF647 and APC, a combination not possible with conventional cytometry. Full spectral cytometry opens the door to increase panel complexity by allowing the use of fluorophore combinations that were not possible before.

PUBLICATIONS

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