Flow cytometry is a fast and multiparametric technology, indispensable in the field of cancer. It allows for the identification, quantification and isolation of defined subpopulations, based on the expression levels of fluorescent markers at the single cell level, as well as the quantitative measurement of cell proliferation and cell death rates upon genetic modifications or drug treatments.

Our aim is to provide CNIO Groups with technical and scientific advice on the use of flow cytometry needed to answer their biological questions, developing new methodologies or pipelines when needed.

We currently have 4 analysers and 3 high-speed cell sorters with different optical configurations to cater to user needs, plus other small instruments to help standardise sample preparation. We offer extensive training so users can independently operate the analysers, while the Unit staff operate the cell sorters, which 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.

“So keep an eye out for new technologies and developments potentially useful to the CNIO community. This year we tested several cell sorters and analysers with spectral as well as imaging capabilities to push CNIO capabilities further.”

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 the following:

- Cell proliferation studies (CFSE, Cell Trace Violet, BrdU or EdU, DNA content, etc.).
- Apoptosis studies (Annexin V, Mitochondrial Membrane Potential, Caspase 3, etc.).
- Multicolour immunophenotyping panels (B and T cell development, Tregs, inflammation, etc.).
- Functional assays (side population detection, Ca²⁺ flux, intracellular pH, etc.).
- Cytometric bead arrays to measure several cytokines from cell extracts and plasma.

We have 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 do single OMICS techniques is now part of our routine portfolio. We keep expanding our training capacities with many more workshops and small practical analysis sessions, to provide our users with more tools to successfully perform their flow cytometry experiments.

**RESEARCH HIGHLIGHTS**

- Platelet studies.
- Extracellular vesicles detection (microvesicles and exosomes).
- CTC detection and isolation.
- Single cell sorting for OMICS analysis.

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**FIGURE 1** Example of applying imaging-derived parameters available at the CytoPix (ThermoFisher) cytometer to further elucidate the CAR-T–Jurkat synapse. CAR-T cells (CellTraceTM Violet) co-cultured with Jurkat cells expressing GFP. Samples provided by the IdiPaz-CNIO Pediatric Onco-Hematology Clinical Research Unit.

**PUBLICATION**


**AWARDS AND RECOGNITION**

- Lola Martínez: Visiting Scholar, invited to teach the 1st Flow Cytometry Course in Latin America, under the auspices of the International Society for the Advancement of Cytometry (ISAC).