FLOW CYTOMETRY
CORE UNIT

Overview

Flow Cytometry is an indispensable tool in the oncology field. It allows multiparametric analysis for the identification, quantification and isolation of defined subpopulations of cells, based on the levels of expression of fluorescent markers and their relation to each other.

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.

We have 4 analysers and 3 high-speed cell sorters, with different configurations of lasers and detectors, to cater to all our users’ needs. We also have an automated magnetic bead separation system (AutoMACS), 2 automated cell counters and a tissue homogeniser (GentleMACS). Analysts 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. We can accept human samples to sort according to Biosafety regulations.

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 of their interest. Some of the applications developed and validated at our Unit are:

- 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, Ca⁺⁺ flux, intracellular pH, etc.)
- Cytometric bead arrays to measure several cytokines from cell extracts and plasma
- Platelets studies
- Extracellular vesicles detection (microvesicles and exosomes)
- 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 into 96 or 384 PCR plates to perform single omics techniques is now part of our routine portfolio. We perform 4-way sorting based on DNA content on live stained samples and are advancing to separate even further to isolate 6 different fractions of DNA content. Additionally, we are also pushing the power of our analytical tools by moving towards high dimensional analysis, performing ‘unsupervised’ clustering analysis on our multiparametric panel assays.

Figure

Upgrade of CNIO’s LSR Fortessa. We have optimised an 18-marker immunophenotyping panel in human blood samples and we are characterising different T and Myeloid subsets and developing a 20-marker one in murine tissues.

AWARDS AND RECOGNITION

- Vice-Treasurer of the European Association ‘Core Technologies for Life Sciences (CTLS)’. 
- Re-elected Member of the Board of Directors of the Iberian Cytometry Society (SIC).