Postgraduate course
Advanced applications of flow cytometry on the study of biological systems

Date: March 5-8, 2018
Place. Auditorium. CCT CONICET. La Plata
Expected: 80 students (PhD students, postdocs, Biochemists, Medical doctors, Biologists, Vets, Plant biologists)

Invited speakers

- Andrew Filby. International Society for Advancement of Cytometry (ISAC) SRL Emerging Leader. Director of Flow Cytometry Core Facility. Faculty of Medical Sciences. Newcastle University. United Kingdom
- Gustavo A. Folle. Department of Genetics. Unit of Flow Cytometry. Montevideo, Uruguay
- Fernando Unrein. Instituto de Investigaciones Biotecnológicas. Instituto Tecnológico de Chascomús (IIB-INTECH), UNSAM-CONICET. Chascomús. Argentina
- Mariela Bollati-Fogolín. Cell Biology Unit. Institut Pasteur de Montevideo. Uruguay
- Gabriel Morón. Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET). Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.
- Guillermo Blanco. Instituto de Estudios de la Inmunidad Humoral (IDEHU)(UBA-CONICET)
- Florencia Quiroga. INBIRS (UBA-CONICET)
- Balzarini, Mónica. Universidad Nacional de Córdoba, Córdoba, Argentina.
- Rodrigo Pestana. Research Platform Leader for Latin America. BD Life Sciences
- Augusto Sorrequieta (Life Technologies). Argentina

Program

8.30-9.00 Registration
9.00 Welcome message

90min. **Class 1.** Basics. In-depth principles of Flow Cytometry. Filby, Andrew
1. A general overview of Cytometry: The paradigm of single cell analysis. This talk introduces the overarching concept of what cytometry is and what it is able to achieve. (30 min max). I will introduce suspension cytometry (fluorescence, mass and also scRNA seq using Droplets aka Drop-seq). Plus make mention of image-based platforms (IFC and IMC).
2. Having introduced so-called conventional fluorescence flow cytometry I can go in to detail about this as discussed (principles, signal generation, detectors etc. analysers versus cell sorters) (45 min max)

break

60min. **Class 2.** Do´s and Dont´s in flow cytometry. Sorrequieta, Augusto
Voltage optimization or voltage walk. Spillover and compensation. Tandem dyes considerations. Fluorochrome new developments. Controls: FMOs, Isotypes, Biological relevant controls. Doublet discrimination. Live/dead discrimination. Dump channel. Autofluorescence. Backgating.
Analysis of Rare events: What is a rare event? Background and definition. Examples of rare events. Data acquisition, how many events are required?

Lunch

40min. **Class 3.** Applications in aquatic microbial ecology. Unrein, Fernando.
Identification of pigmented (algae, photosynthetic bacteria) and colorless microorganisms (heterotrophic bacteria and flagellates – protozoans) from different environments. Quantification of aquatic viruses. The concept of cytometric diversity.

40min. **Class 4.** Assessing cell nuclear DNA content by flow cytometry: principles and practice. Folle, Gustavo.

break

60min. **Class 5.** CyTOF: Mass-spectrometry detection in flow cytometry. Filby, Andrew
Mass Cytometry in suspension.

40min. **Class 6.** DNA content analysis of plant and animal cells by flow cytometry. Detection of proteins in plant cells. Folle, Gustavo

45min. **Class 7**. Analyzing Stem Cell Populations using Flow Cytometry. Sorrequieta, Augusto

break
50min. **Class 8.** Cell sorting: basis and applications. Bollati, Mariela
Basic principles. Types of sorters. Quality characteristics of a sorting: purity, recovery and efficiency. Bulk sorting vs cloning. General considerations: instruments, safety, sterility, troubleshooting. A couple of examples

40min. **Class 9.** Multiparameter analysis. Moron, Gabriel

Lunch

90min. **Class 10.** Statistics tools in flow cytometry analysis. Multivariate analysis. Part I. Balzarini, Mónica

break

60min. **Class 11.** Statistics tools in flow cytometry analysis. Multivariate analysis. Part II. Balzarini, Monica
60min. **Class 12.** Workshop: Statistics tools. Quiroga, Florencia

Wednesday 7th

60min. **Class 13.** Imaging Flow Cytometry. Filby, Andrew
Image Cytometry: Covering both imaging flow cytometry with fluorescence and label-free imagery and our new Hyperion Imaging Mass Cytometry module that allows Mass cytometry to be done in tissue sections!

40min. **Class 14.** Generation of recombinant stable cell lines using high speed cell sorting. Bollati, Mariela.
Why to select recombinant stable cell lines (surface, intracellular and secreted proteins). Different strategies. Gel Microdrops, Matrix-aided surface capture, Cold capture method, Expression of marker proteins. General considerations. A couple of examples.

Break

60min. **Class 15. Title to be confirmed**. Pestana, Rodrigo

40min. **Class 16.** Handling massive data. Filby, Andrew
Data analysis for all the above technology platforms. Essentially the way one interacts with high dimension cytometry data (non-imaging and imaging) is the same!

Lunch

60min. **Class 17.** Combining strategies to evaluate cell function by fluorescence techniques. Blanco, Guillermo
Application of fluorescent proteins and FRET in flow cytometry. Combination with cell imaging in biological studiesetria Subcelular cytometry.

30min. **Class 18. Title to be confirmed**. Pestana, Rodrigo

break

40min. **Class 19** Integration of results of mass cytometry/cell imaging/ single cell RNA seq. The Biology behind massive data. Filby, Andrew
Highlight some specific biological applications.

Thursday 8th
Data analysis (Workshop, optional). Moron, Gabriel