

cnio AD HOC SEMINAR

“MULTICOLOR FLOW CYTOMETRY WORKSHOP”

Venue: CNIO 2nd Floor Seminar Room & Online

Speakers: *Oliver Burton, Senior Scientist, Liston-Dooley Lab. University of Cambridge. UK*
Lola Martínez, Head of the Flow Cytometry Unit. CNIO. Madrid. Spain
Ana Vieira, European Technical Application Specialists. Cytek Biosciences
Jamie McCarthy, European Application Specialist. DeNovo Software.
Ioannis Panetas, European Application Specialist, FlowJo, BD Biosciences.

PROGRAMME

Multicolor Flow: panel design fundamentals and rules.

Monday 20th May 2024 (CNIO 2nd floor seminar room)

*** Multicolor Flow – rules and considerations! (L. Martínez)**

09:30-11:00

We will introduce concepts such spectral overlapping, compensation/unmixing, and go through what you need to take into consideration when planning your Multicolor Flow Experiments. Rules you must follow and the available tools for you to systematically design your multicolor flow panels following good practices incl. sample prep tips, strategies on how best acquire your data and standardize them, and controls need it.

*** Panel Design Exercise (L. Martínez)**

11:00-12:00

From a practical perspective we will put in practise all the learned rules to design together a Multicolor Flow panel. In small groups you will go through the different steps of the experimental design (assigning the markers, choosing the best antibody-fluorochrome combinations, defining controls and using different tools to succeed on panel design (spreading error matrices).

*** Tips & Tricks: Getting the most out of your high parameter flow cytometry (O. Burton) 12:00-13:00**

Oliver will be talking on best practices in umixing, comparing between dimensionality reduction approaches, the different types of data analysis (autogating, clustering or FlowComb), standardization of cell numbers and impact of data scaling on dimensionality reduction analysis and panel design affects data analysis.

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*** Practical exercise setting a panel in the cytometer (CNIO FCCU)**

14:00-16:00

We will connect to the CNIO core instruments on the facility to run live some multicolor flow samples both in polychromatic and spectral cytometers. How to set up the experiment, do compensation or unmixing and troubleshoot your experiment.

*** Cytex Tools to deal with autofluorescence Tools (A. Vieira)**

16:30-17:30

Ana will be sharing with us the different tools Cytek have available to deal with autofluorescence removal from your data experiment and how that could improve your panel resolution. Ana will sharing with us examples on how they could be best put to work.

Multicolor Flow Data Analysis & Troubleshooting.

Tuesday 21st May 2024 (CNIO 2nd floor seminar room & Online)

*** Analysing a multicolor flow experiment using FCS Express (J. McCarthy)**

09:30-11:30

Jamie will be showing you the tools to analyse a multicolor flow experiment using FCS Express v7. We will cover the tools for titration analysis, compensation, unmixing, getting SSM, stats and reporting data plus a brief introduction to the high dimensional analysis tools available within the software.

*** Analysing a multicolor flow experiment using FlowJo v.10 (I. Panetas).**

11:45-13:45

Serena will cover using FlowJo v10. how to clean your data files, compensation, unmixing and how to critically investigate your data, getting stats and reporting data plus a brief introduction to the high dimensional analysis tools available within the software.

*** Data Troubleshooting guide: case study (A. Vieira)**

15:00-17:00

Using practical examples Ana will cover how to identify and assess “errors” in your data – assessing your controls, comparing spectral fingerprint on sample vs control, unmixing errors. She will also cover what tools SpectroFlo have available to deal with AF removal, and when and how to use them.

* The Multicolor course is highly recommended if you are doing or planning to do soon immunophenotyping panels. Some knowledge of how cytometers work is required.

Registration is need it through the link below, and the cost of the course is 50 Euros (CNIO Members), 75 Euros (Non CNIO Members) and 125 Euros (Industry):

<https://www.cnio.es/eventos/advance-multicolor-course-20-21-may-2024/>

It includes theoretical and practical sessions in panel design, data acquisition and data analysis using some of the common analysis software in the market. The Friday before the workshop starts, participants will receive further information on the different sessions.

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