

# Where to look for valuable Flow Cytometry information? Tips & Tricks

Swiss Lunch Cytometry Program 2025/26

By Sarah Cattin and Florian Mair

08 December 2025









# Online resources for Flow Cytometry

#### Why do we need reliable Flow related information?

Flow Cytometry is a complex technology, with numerous applications, and the need to understand technology and biology.

It's also an evolving field and technology that is changing with time.

- Efficient training (faster, more effective, will give you confidence in experimental results)
- Avoid technical errors (using validated protocols)
- Stay updated on new techniques (foster innovation, cutting-edge insights, keep you curious)

In short: knowing where to find trusted, peer-reviewed, and authoritative information about flow cytometry is essential for accuracy, reproducibility, ethics, innovation, training, and collaboration.







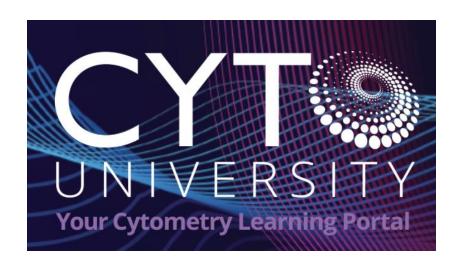
#### **International Society for Advancement of Cytometry (ISAC)**

The leading global flow cytometry organization, offering conferences, training, and standards for cytometry research.



#### https://isac-net.org

- Need to be a member (min. 45 \$ per year) to access the content.
- Many webinars + e-learning modules (not all free): https://learning.isac-net.org
- Organize CYTO annual international conference (you can find some of the recordings)
- Provide links to the OMIPs and FlowRepository.





# **ISAC**

#### **Optimized Multicolor Immunofluorescence Panel (OMIP)**

Special peer-reviewed Cytometry Part A publication type that reports on newly designed and optimized multicolor panels for flow cytometry, fluorescence microscopy, image cytometry, and other polychromatic fluorescence-based methods

#### OMIPs are aimed:

- to alleviate the development time for researchers in need of the same or highly similar panels,.
- to provide a starting point for the creation of novel OMIPs.
- to give the developers of the panels credit via citation or the publication.









https://onlinelibrary.wiley.com/doi/toc/10.1002/(ISSN)1552-4930.OMIPscollection

https://public.tableau.com/app/profile/fanny2212/viz/OMIP\_ISAC/Menu



# **MIFlowCyt**

#### **International Society for Advancement of Cytometry (ISAC)**

FlowRepository & MIFlowCyt

### http://flowrepository.org

FlowRepository is a database of flow cytometry experiments where you can query and download data collected and annotated according to the MIFlowCyt standard.



# MIFlowCyt: The Minimum Information About a Flow Cytometry Experiment

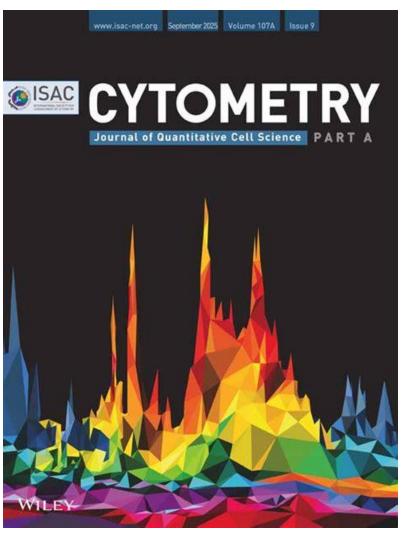
Jamie A. Lee, <sup>1†</sup> Josef Spidlen, <sup>2†</sup> Keith Boyce, <sup>3</sup> Jennifer Cai, <sup>1</sup> Nicholas Crosbie, <sup>4</sup> Mark Dalphin, <sup>5</sup> Jeff Furlong, <sup>6</sup> Maura Gasparetto, <sup>2</sup> Michael Goldberg, <sup>7</sup> Elizabeth M. Goralczyk, <sup>8</sup> Bill Hyun, <sup>9</sup> Kirstin Jansen, <sup>6</sup> Tobias Kollmann, <sup>10</sup> Megan Kong, <sup>1</sup> Robert Leif, <sup>11</sup> Shannon McWeeney, <sup>12,13,14</sup> Thomas D. Moloshok, <sup>8</sup> Wayne Moore, <sup>15</sup> Garry Nolan, <sup>16</sup> John Nolan, <sup>17</sup> Janko Nikolich-Zugich, <sup>18</sup> David Parrish, <sup>3</sup> Barclay Purcell, <sup>19</sup> Yu Qian, <sup>1</sup> Biruntha Selvaraj, <sup>19</sup> Clayton Smith, <sup>2</sup> Olga Tchuvatkina, <sup>7</sup> Anne Wertheimer, <sup>20</sup> Peter Wilkinson, <sup>21</sup> Christopher Wilson, <sup>6</sup> James Wood, <sup>22</sup> Robert Zigon, <sup>23</sup> The International Society for Advancement of Cytometry Data Standards Task Force, Richard H. Scheuermann, <sup>1,24</sup> Ryan R. Brinkman<sup>2\*</sup>

List of the minimum information about a flow cytometry experiment that should be included in all publication of flow data.

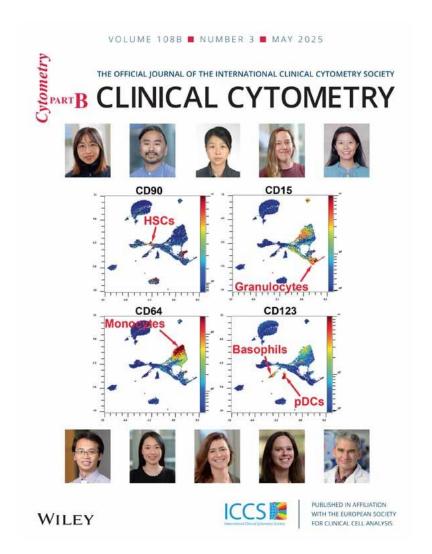
DOI: <u>10.1002/cyto.a.20623</u>



Peer-Reviewed Journals.



Covers fundamental and applied aspects of flow and image cytometry.



Focuses on diagnostic and clinical applications





# Commercial resources

#### Flow Instrument vendors

Vendors of instrumentation are a great source of valuable knowledge about Flow Cytometry, in the form of e-learning and webinars.



https://cytekbio.com/blogs/webinars-videos

https://cytekbio.com/blogs/spectrolearn



https://www.thermofisher.com/ch/en/home/c/a/lets-talk-flow-webinar-series.html



https://www.sonybiotechnology.com/webinars





BD Bioscience & Beckman Coulter offer also e-learning, but with a fee, except for some webinars...



# Protocols - vendors

# Thermo Fisher SCIENTIFIC

https://www.thermofisher.com/ ch/en/home/references/proto cols/cell-and-tissueanalysis/flow-cytometryprotocol.html

#### Preparing for your flow cytometry experiment

- Human CD & Other Cellular Antigens
- Mouse CD & Other Cellular Antigens
- Antibody Fixation Considerations
- \mathbb{T} Flow Cytometry Staining Index
- Phospho Flow Cytometry Antibody Clone Buffer Selection Guide
- Intracellular Staining Quick Guides
- Intracellular Staining Buffer Selection Guide

#### General protocols for flow cytometry

- X Super Bright Staining Buffer protocol
- Cell Preparation for Flow Cytometry Protocols (Invitrogen eBioscience reagents)
- Red Blood Cell Lysis Protocols Using eBioscience Lysis Buffers (Invitrogen eBioscience reagents)
- Staining Cell Surface Targets for Flow Cytometry (Invitrogen eBioscience reagents)
- Staining Intracellular Antigens for Flow Cytometry (Invitrogen eBioscience reagents)
- OneComp and UltraComp Compensation Beads Protocols for Flow Cytometry (Invitrogen eBioscience reagents)

#### Cell preparation protocols for single cell suspensions

- Isolation of human peripheral blood mononuclear cells from whole blood
- Monocyte isolation from peripheral blood mononuclear cells
- NK cell isolation from peripheral blood mononuclear cells
- T cell isolation from peripheral blood mononuclear cells
- CD4<sup>+</sup> T cell isolation from peripheral blood mononuclear cells
- CD8<sup>+</sup> T cell isolation from peripheral blood mononuclear cells
- Regulatory T cell isolation from peripheral blood mononuclear cells
- · T cell isolation from secondary lymphoid organs



# Protocols - vendors



https://www.bdbiosciences.co m/endk/resources/protocols/flowcytometry

#### **Choosing the Right Fix Perm Kits**

#### Choosing the Right Fix Perm Kits Cell Fixation/Permeabilization Kits for Intracellular Cytokine Analysis

Explore three cell fixation/permeabilization kits to simplify the preparation of cells for intracellular staining of cytokines.

#### Reducing non-specific staining with Fc Block

#### **Uses of Fc Block**

Learn how antibody binding to Fc receptor can cause background staining and how to use the BD Fc Block™ Reagents in immunophenotyping of mouse or rat leucocytes..

#### Protocol for Direct Immunofluorescence Staining of Mononuclear Cells

Find the procedure for specimen collection, preparation and direct immunofluorescence staining of mononuclear cells...

#### Cell Surface Staining of Human PBMCs and Suspension Cell Lines

Find a step-by-step procedure for cell surface staining of human peripheral blood mononuclear cells and cell lines.

#### **Phosflow Protocol for Adherent Cells**

Find the optimal protocol for detecting intracellular signaling molecules from adherent cells using BD Phosflow™ Reagents.

#### Intracellular Flow and Phosflow FAQ

Find answers to most commonly asked questions on intracellular flow cytometry and use of BD Phosflow™ Reagents...

#### **Indirect Staining Mononuclear Cells**

#### **Indirect Immunofluorescence Staining of Mononuclear Cells**

The indirect method is used to enhance the fluorescence signal and also to facilitate multicolor staining of human cells when direct conjugated reagents are not available. Find the procedure for specimen collection, preparation and staining of mononuclear cells.

#### BD™ Phosflow Protocols for Human PBMCs

Explore four protocols using different methods (detergent method, mild or harsh alcohol method or harsh detergent method) for the activation of cell signaling molecules in human peripheral blood mononuclear cells.

#### BD™ Phosflow Protocols for Human Whole Blood Samples

Explore four protocols using different methods (detergent method, mild or harsh alcohol method or harsh detergent method) for the activation of cell signaling molecules in human whole blood samples.



# Protocols - vendors



#### Fluorophore Families

Diverse families of fluorophores allow us to continue to expand the limits of flow cytometry. Learn more about our fluors and the unique advantages offered by each, including Spark Dyes, Fire Dyes, KIRAVIA multimers, and Brilliant Violet™ polymers.

Learn about fluorophores >

#### Fluorophore Equivalents

With companies each having their own naming system for their fluorophores, it can be difficult to keep track of them all. This tool will help you find BioLegend's equivalent fluorophores compared to other commercially available fluorophores for flow cytometry.

View fluorophore equivalents >

#### Spectral Cytometry

Spectral cytometry is quickly allowing researchers to create larger multicolor panels with its unique technology. Learn the basics of spectral flow cytometry and how BioLegend is providing unique fluorophores to fill spectral gaps previously unused in conventional flow.

Learn about spectral cytometry >

#### **Brightness Index**

This convenient Fluorophore Brightness Index lists the relative brightness of dyes offered by BioLegend. This is particularly helpful for the balancing of brightness with antigen expression levels in a panel.

View the Brightness Index >

#### Tandem Dyes

Our tandem dye page provides useful information on the background of tandem dyes, important notes, and how best to use them in flow cytometry applications. We also provide data on lot-to-lot consistency and links to products and resources.

Learn about tandem dyes >

# https://www.biolegend.com/fr-lu/flow-cytometry-tools

#### **Cell Surface Protocols**

Veri-Cells™ Protocol

Anti-Neu5Gc Antibody Kit Protocol: Flow Cytometry

Precision Count Beads™ Protocol and Applications

Cell Surface Flow Cytometry Staining Protocol

Cell Surface Flow Cytometry Staining of Whole Blood

 $\textbf{Flex-}\mathbf{T}^{\scriptscriptstyle\mathsf{TM}}\,\textbf{Tetramer}\,\,\textbf{Preparation}\,\,\textbf{and}\,\,\textbf{Flow}\,\,\textbf{Cytometry}\,\,\textbf{Staining}\,\,\textbf{Protocol}$ 

 $Flex-T^{\scriptscriptstyle\mathsf{TM}}\ Fixed\ Peptide\ Tetramer\ Preparation\ and\ Flow\ Cytometry\ Staining\ Protocol$ 

#### Intracellular Protocols

Veri-Cells™ Phospho PBMC (MAPK/ERK Pathway) and Activated (Cytokine) PBMC Kit Protocols

Stimulation Guide for Intracellular Staining of Cytokines/Chemokines

True-Nuclear  $^{\tiny \mathsf{TM}}$  Transcription Factor Staining Protocol for 96-Well U Bottom Plate

True-Nuclear™ Transcription Factor Staining Protocol for 5mL Tubes

Propidium Iodide Cell Cycle Staining Protocol



# Protocols - journals



# in Cytometry

reviews related to the quantitative analysis of cells and cell systems. The section covers the detection and analysis of various cellular populations, including instrumentation, data processing and analysis, cell and molecular imaging, and more.

The Cytometry section of Current Protocols is a comprehensive source for step-by-step protocols and

https://currentprotocols.on linelibrary.wiley.com/journ al/19349300

#### **Articles**

Most Recent Most Cited

Isolation and Flow Cytometric Analysis of Natural Killer Cells from Human Glioblastoma Multiforme (GBM) Tissues

Soumyajit Das, Kyle B. Lupo, Veronika Slivova, Aaron A. Cohen-Gadol, Sandro Matosevic

Current Protocols | First Published: 25 November 2025

Abstract | Full text | PDF | References | Request permissions

#### Open Access

Optimization of the Blocking and Signal Preservation Protocol in High-Parameter Flow Cytometry

Oliver T. Burton, James Dooley, Adrian Liston

Current Protocols | First Published: 25 September 2025

Abstract | Full text | PDF | References | Request permissions

#### **Open Access**

Introducing the Dish Soap Protocol: A Unified Approach for Multi-Modal



**Get Access** 



Submit a Proposal

#### **Editor's Choice**

**Flow Cytometry Method Validation Protocols** 

Preparing a Minimum Information about a Flow Cytometry Experiment (MIFlowCyt) Compliant Manuscript Using the International Society for Advancement of Cytometry (ISAC) FCS File Repository (FlowRepository.org)

Flow Cytometry Instrumentation – An Overview

Cell Cycle Analysis of Hematopoietic Stem and Progenitor Cells by Multicolor Flow Cytometry

**Modern Laser Scanning Confocal Microscopy** 





#### A learning program:

https://expertcytometry.com/technical-programs/expert-cytometry/

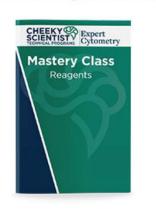
By Tim Bushnell, University of Rochester (NY)



#### 40+ Advanced Training Videos

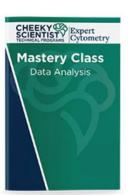


#### **Mastery Class Workbooks & Presentations**









#### 10 Training Quizzes





#### Valuable blogs:

https://expertcytometry.com/blog/



**Combining Flow Cytometry** With Plant Science, Microorganisms, And The **Environment** 



The Power Of Spectral **Viewers And Their Use In Full Spectrum Flow Cytometry** 



**4 Critical Rules For Spectral** Unmixing

## https://bitesizebio.com/technical-channels/flow-cytometry/



**How Fluorescent Molecules** 

By Kristen Haberthur

Work



FLOW CYTOMETRY Nail Your Unmixing for **Full Spectrum Flow** Cytometry: 7 Top Tips

By Jess Rowley



Flow Cytometry Gone Viral: Introducing Flow Virometry

By Alex Chen

FLOW CYTOMETRY



The History and Future of Fluorescent Labels: We've Come a Long Way, Baby!



How to Destroy your Flow Cytometry Data in 3 Easy Steps: Snap, Crackle, and Pop

By Kristen Haberthur

By Kristen Haberthur



#### Valuable blogs:

https://voices.uchicago.edu/ucflow



By the facility of the University of Chicago Plenty of diverse information, webinars, list of resources



#### Experiment Design

Attune NxT, Basics of Flow,
Bigfoot, CytoFLEX LX,
Experimental Design, FACSAria,
Fortessa/LSRII, Penteon,
Quanteon



#### Spectral Experiment Design

Aurora, Bigfoot, Experimental

Design



#### Flow Basics 2.0

Basics of Flow, Training Courses







#### Setting Voltages

Attune NxT, Basics of Flow,
CytoFLEX LX, Fortessa/LSRII



#### Compensation

Attune NxT, Basics of Flow, CytoFLEX LX, Fortessa/LSRII, Penteon, Quanteon



#### Sample Preparation

Attune NxT, Aurora, Basics of Flow, CytoFLEX LX, FACSAria, Fortessa/LSRII, Penteon, Quanteon



#### Valuable blogs:

https://www.colibri-cytometry.com

By Dr. Oliver Burton from the University of Cambridge



Cytometry Blog

Tips, tricks and technical advice for flow cytometry.



**Useful Papers** 

References and literature for anything flow related. Suggestions welcome.



**Protocols** 

Flow techniques. Links to relevant technical papers plus tips and improvements.



Links

A collection of sites that may be useful for flow cytometry. This does not constitute an endorsement of these services



Let your data take flight!





Data Analysis

Code and instructions for performing high dimensional flow analysis.



Antibody info

Information on how to use various antibodies with overnight staining



#### Other sources:

http://www.cyto.purdue.edu/hmarchiv

It's an electronic list maintained by Dr. Paul Robinson at Purdue University and has a 20+ year archive that can be searched

https://www.reddit.com/r/flowcytometry// https://discord.gg/ZmyPYUQr9Q

Discord is an instant chat platform like Slack, Microsoft Teams, WhatsApp, or AOL Instant Messenger. Discord allows you to throw a question out to the community and get an instant response.

https://learncytometry.com

R course, free, for flow cytometrist + mass analysis using Al.

https://www.youtube.com/@RAIFCA3284/videos

Small videos and podcasts on Flow Cytometry.

https://flowstars.bitesizebio.com/

Podcasts with personalities from the Flow Cytometry world.



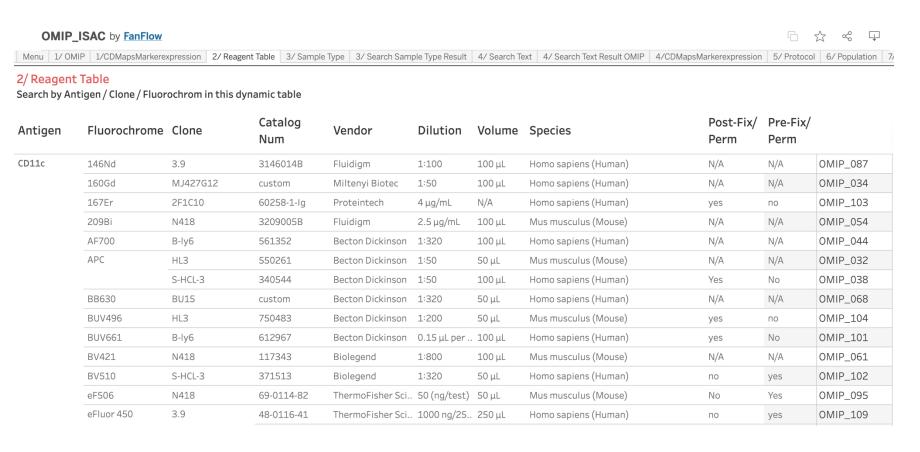


# Developing a new panel – how to leverage OMIPs (1)

#### **Optimized Multicolor Immunofluorescence Panel (OMIP)**

Need to include titration data in their online material section

- → Can get an idea about expresion patterns of targets of interest
- → Performance of different clones



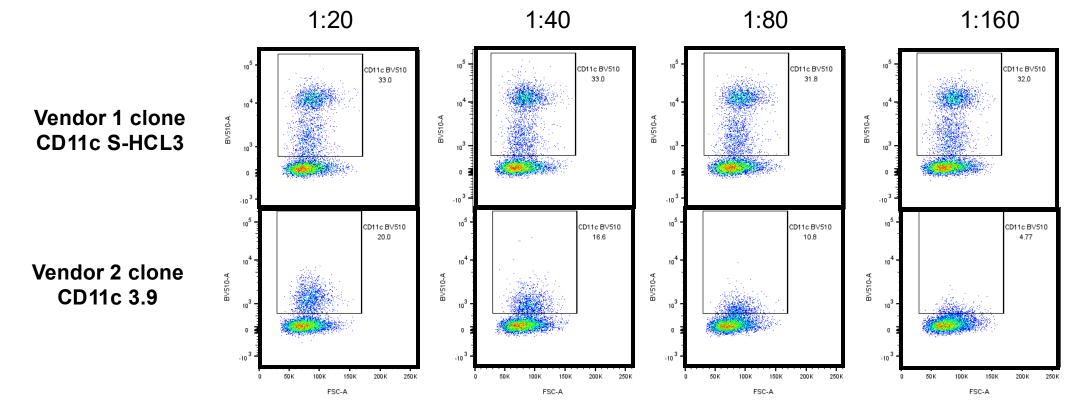


# Developing a new panel – how to leverage OMIPs (2)

#### **Optimized Multicolor Immunofluorescence Panel (OMIP)**

Need to include titration data in their online material section

→ Example different CD11c clones (ETH Zurich, unpublished, but similar to public OMIP data)



Christoph Capelle and FCCF ETH Zurich

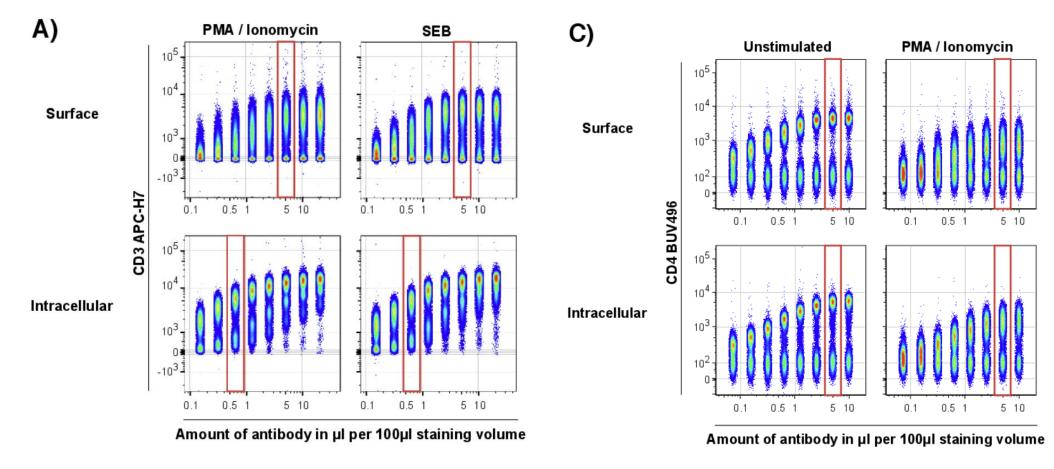


# Developing a new panel – how to leverage OMIPs (3)

#### **Optimized Multicolor Immunofluorescence Panel (OMIP)**

Need to include titration data in their online material section

→ Example for different staining conditions after T cell restimulation with PMA/lono: OMIP-060 (Liechti and Roederer, CytoA 2019)





# Working with a new technique – leveraging blogs

#### Colibry-cytometry.com

olivertburton · Aug 11 · 7 min read

Very broad and up-to-date discussions of current problems in the field of spectral flow

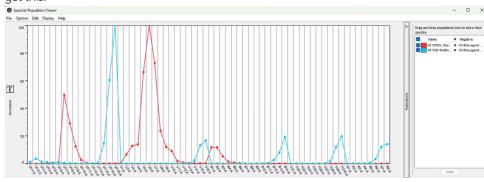
→ Let's say you want to understand more thoroughly what a spectral unmixing matrix really is: the blog features descriptions, references, and step-by-step screenshots

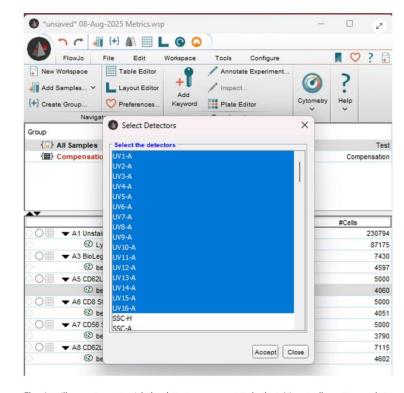
# What's in a spectral matrix?

Updated: Aug 14

In this series of posts, I'm going to discuss a bit about the mathematics and metrics we use in spectral flow cytometry. Now, since I am not a mathematician by training, I'm probably going to get some of this wrong, so please chime in when you spot errors or misconceptions. My hope is that this lack of a mathematical background is going to force me to explain things in a relatively simple way.

In contrast, by gating on the positive beads and subtracting the negative bead signal, we get this:





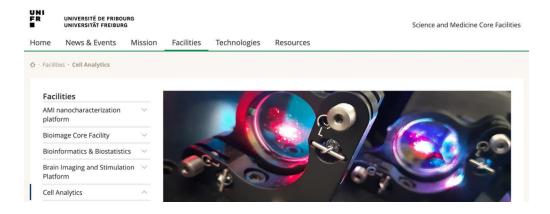
FlowJo will prompt you to pick the detectors you want to look at. It's actually pretty good at detecting these, so hit "Accept".



# Saving time – ask your local technology experts

### Leverage the knowledge of the experts in your Flow Cytometry Core!

- → They have been exposed to lots and lots of different problems
- $\rightarrow$  Even if they do not know the solution, they might be able to point you to another person



Fribourg, ETH Zurich, and many more...



Welcome to the Flow Cytometry Core Facility at the ETH Zurich



# See you next year Enjoy the end of the year



### **Swiss Lunch Cytometry Program**

Seminar list for 2025 26

Online at 12h00 via Microsoft Teams

Take the opportunity to learn more about flow cytometry and related technologies. A good way to refresh or gain knowledge and perform your experiment in a more efficient way!

Oct.

2025 Nov.

Dec.

Cytolution: a new tool to help with your flow analysis Can Pinar from Cytolytics



S8 Technology - Sorting beyond the dots Jean-François Mayol (UNIL), Claudia Dumrese (UNIZH) and Stefan Müller (UNIBE)





Where to look for good Flow Cytometry information-



Sarah Cattin (UNIFR) and Florian Mair (ETHZ)

Feb. 9

2026 March

May

Using Machine Learning with ImageStream for bacterial quantification - a practical case Mara Esposito, University of Basel



How to get the best from your compensation and spectral unmixing Gabriele De Simone, EPFL



Considerations for cell sorting for genomics, proteomics and metabolomics



Cécile Gameiro (UNIGE) and Malgorzata Kisielow (ETHZ)



No need to register, just click on the title of the seminar and you will be redirected to the Teams meeting directly. Don't hesitate to advertise these dates around you! Everybody is welcome.

For any question: Sarah Cattin (sarah.cattin@unifr.ch)















