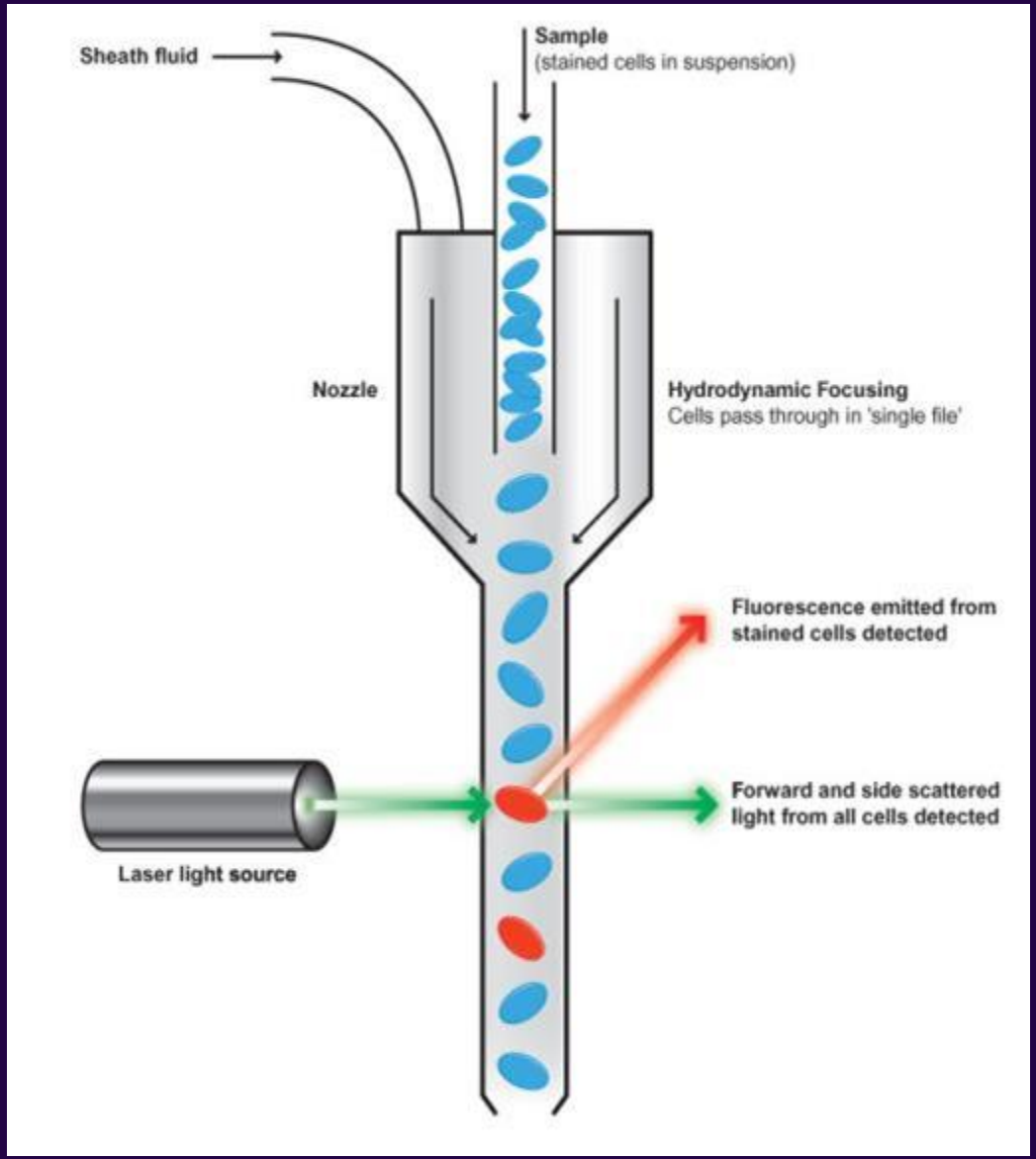
Gulbenkian Institute *for* Molecular Medicine

Flow Cytometry Platform at GIMM

Mariana Fernandes

Flow Cytometry

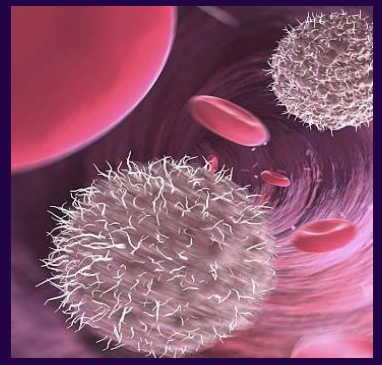
- used to detect and measure physical and chemical characteristics of a population of cells or particles
- a sample containing cells or particles is suspended in a fluid and injected into the **flow cytometer** instrument



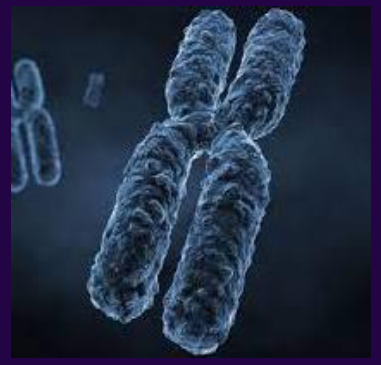
- Cell count
- Cell analysis

Flow Cytometry *Samples*

Primary cells

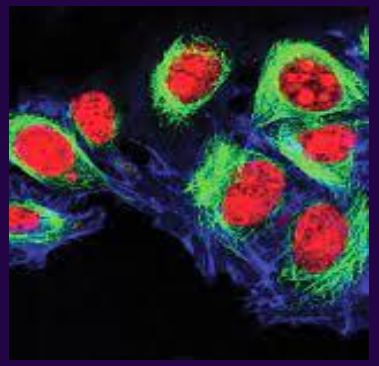


Chromosomes



Protozoa

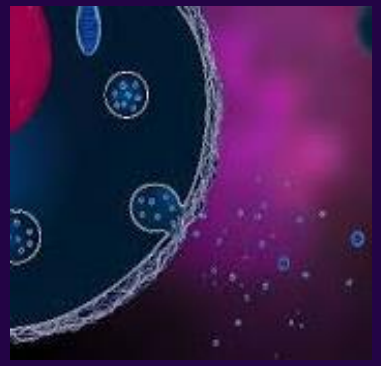
Bacteria



Cell Lines

Algae

Yeast



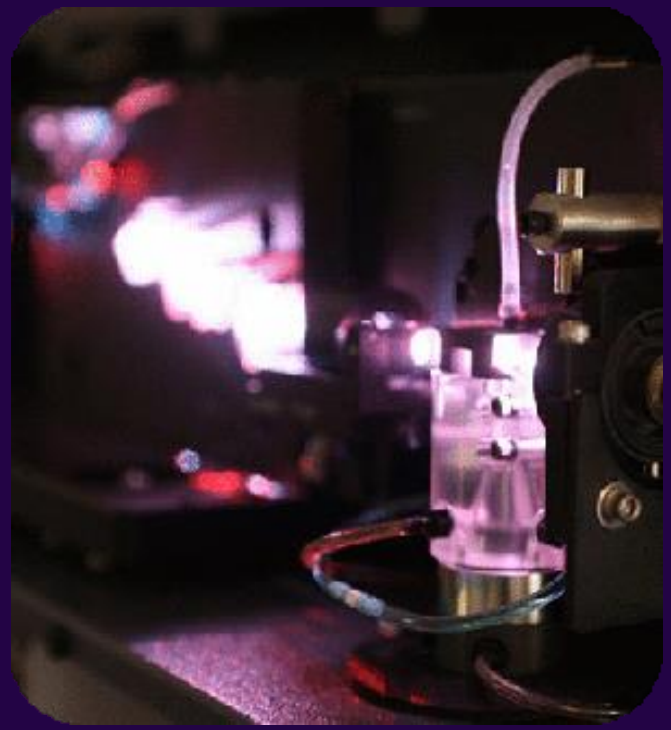
Small Particle (e.g. exossomes)

Flow Cytometry *Advantages*

Population data
- Measure thousands of cells/particles per second
- Measure multiple parameters simultaneously

Detection of extremely rare populations

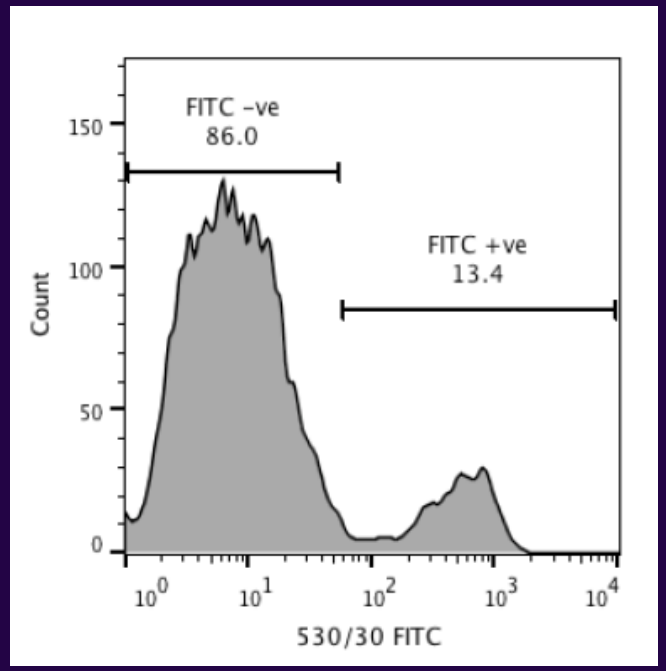
Cell sorting with high purity, speed and yield



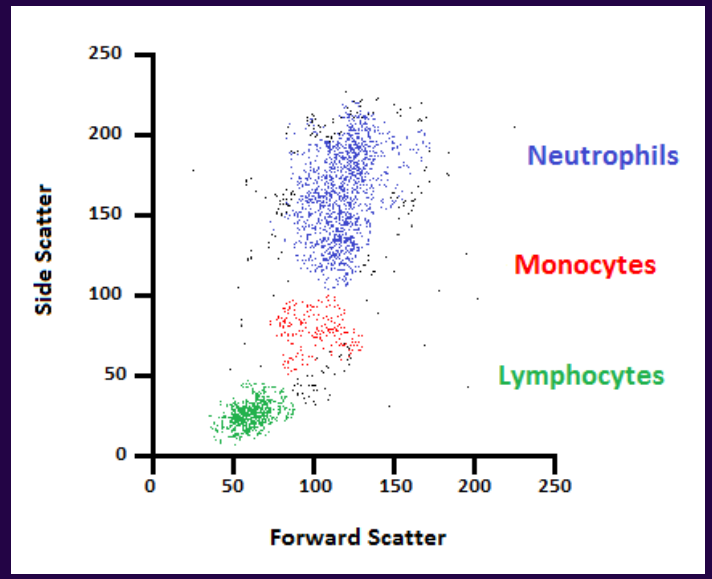
Flow cytometry offers:
- High-throughput
- Automated quantification of specified optical parameters on a cell-by-cell basis.

Flow Cytometry *Advantages*

How many cells express a given protein?



How many populations exist in my sample?



Flow Cytometry does not…

- provide information on where a given protein is within a cell
- measure distribution of cellular components
- measure cell morphology

Flow Cytometry *Instruments*

Analyzers
quantitative information
on cell populations properties

BD FACSCalibur

BD FACSCelesta

BD LSR Fortessa

Miltenyi MACSQuant

BC Cyan ADP

BD Accuri C6 Plus

Bio-Rad ZE5

BC Cytoflex

Cytek Aurora

BD Symphony
A3/A5/A3 SE

Sony ID7000

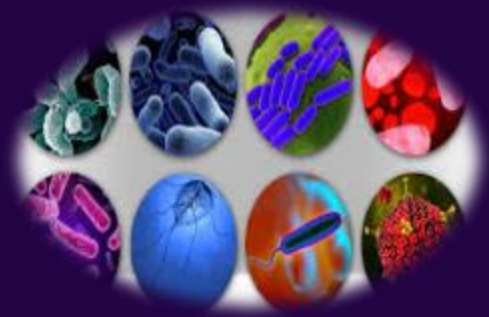
…

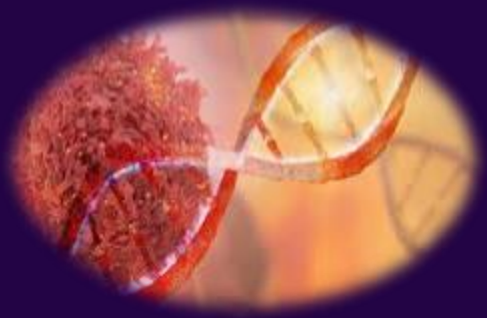
Sorters
separation of
populations of cells

BD FACSAria
Beckman Coulter MoFlo
BD FACSVantage
Bio-Rad S3E
BD Influx
Sony SH800S
Propel-Labs Big Foot
BD FACSAria Fusion
BD FACS Symphony S6
BD FACS Discover S8
Cytek Aurora CS
BC CytoFLEX SRT
…

Flow Cytometry *Research Applications*

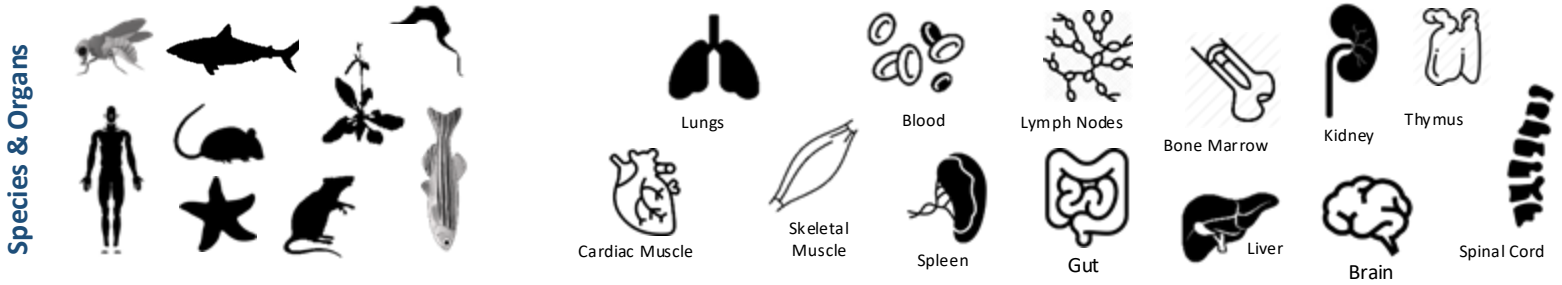


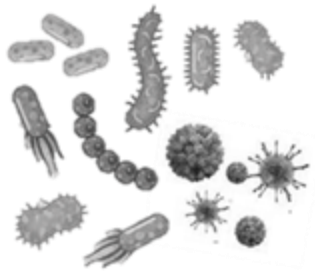


A full world of options to support research in Biosciences

Flow Cytometry *Research Applications*





CELL STATE

- Immunophenotyping
- Cell cycle
- Apoptosis
- Cell proliferation
- Cell ploidy
- Cell viability

CELL FUNCTION

- Cell activation
- Calcium flux
- Cytokine Secretion
- Activation of signalling pathways
- Levels of intracellular reactive oxygen species

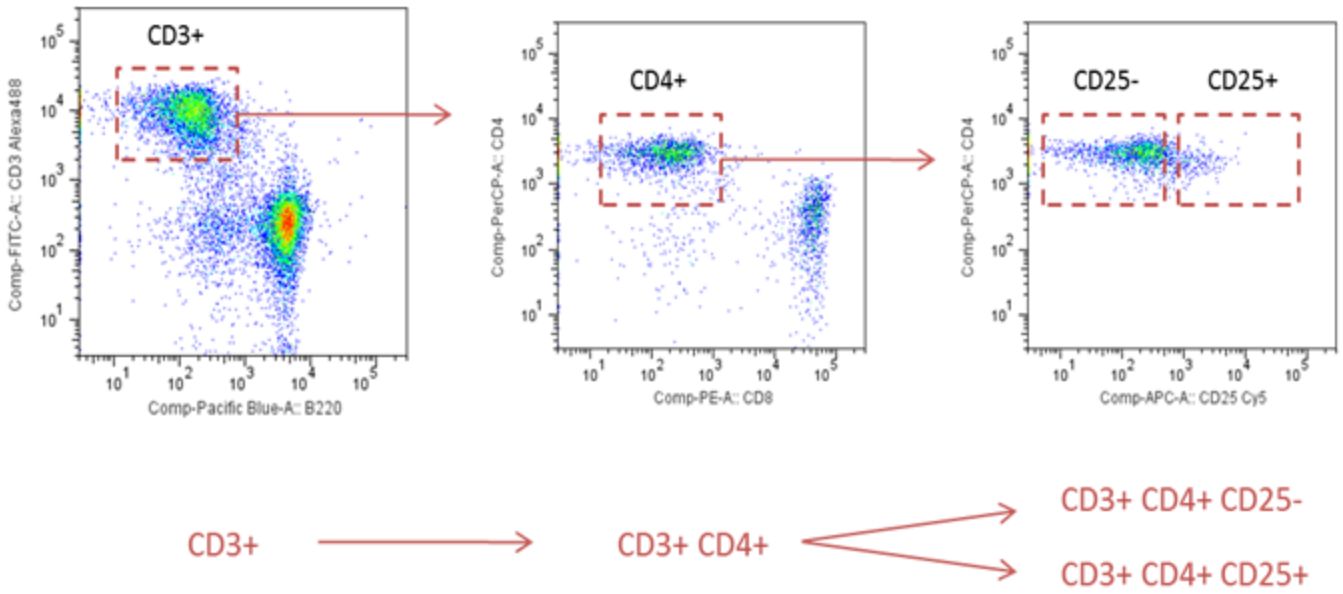
CELL SORTING

- Isolate subpopulations
- Establishing cell clones
- Stem cell sorting
- Establishing fluorescent cell lines
- Chromosome sorting

MICROBIOLOGY

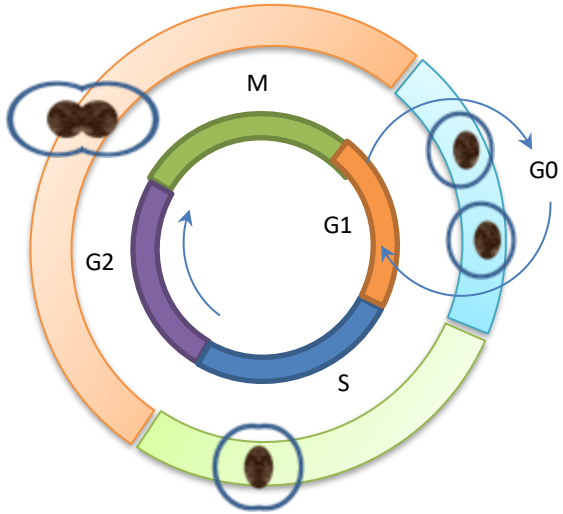
- Detect bacteria, virus or parasites
- Dead/Live Discrimination
- Absolute counting
- Aquatic Microbiology

Research Applications *Immunophenotyping*

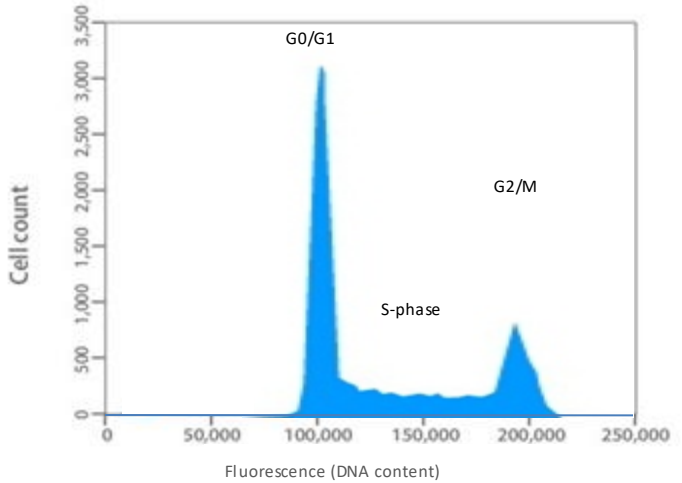


- Uses labeled antibodies (Abs) to identify cells of interest
- Determination of cell surface antigens
- Allows for detailed identification of cellular subsets (simultaneously measure multiple parameters cell by cell)
- Targets on both surface and intracellularly

Research Applications *Cell Cycle*

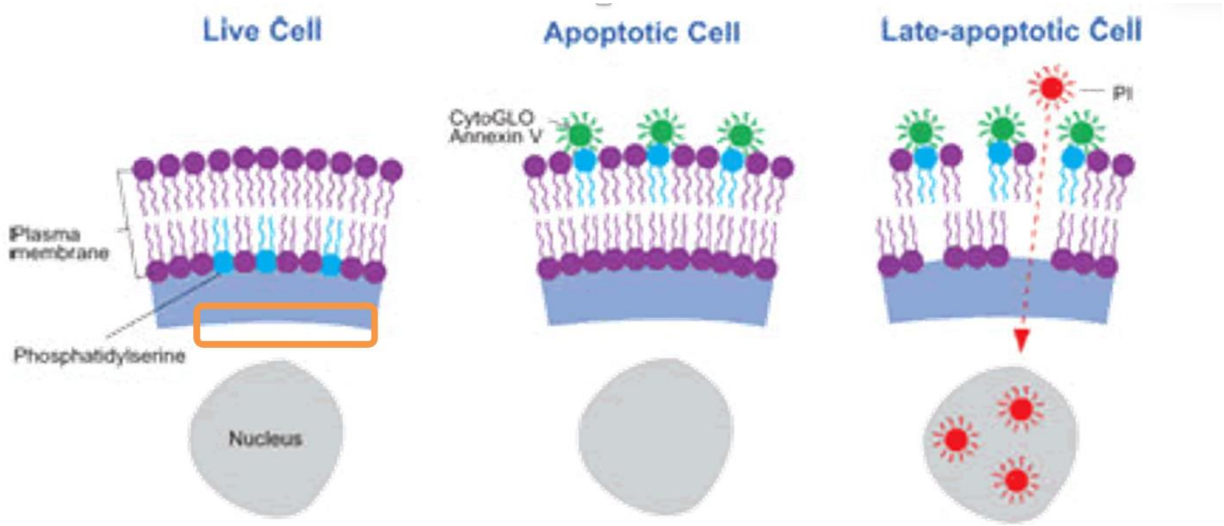


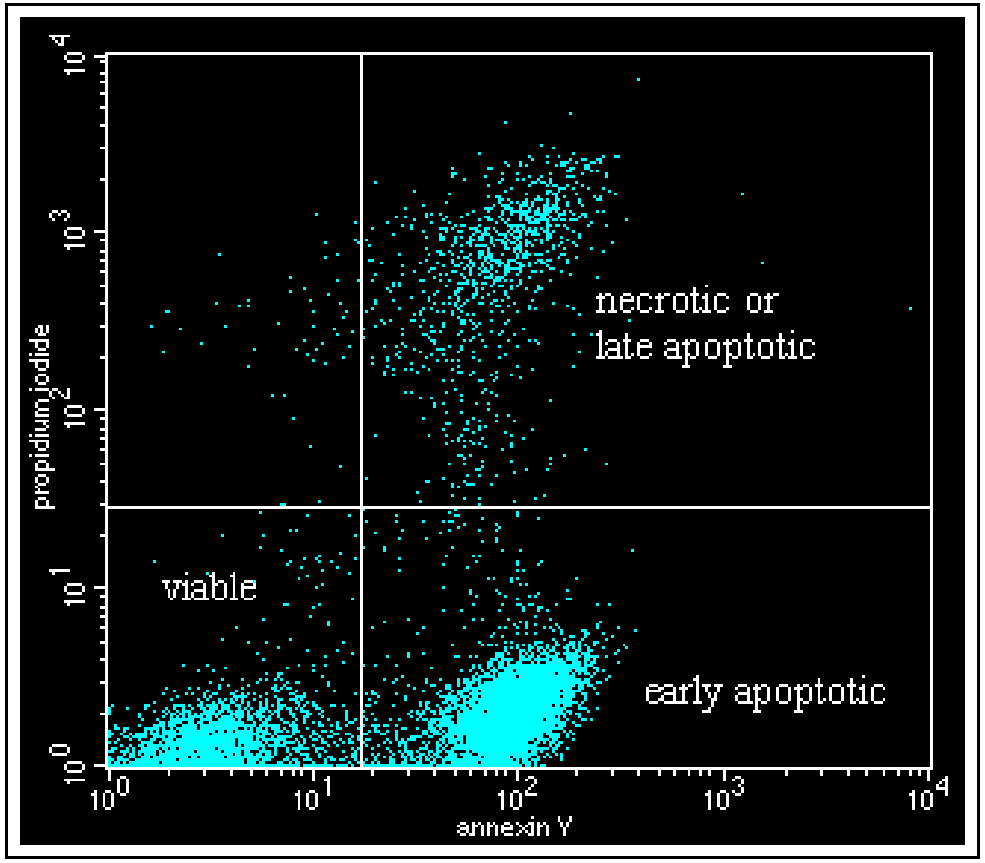
DNA content analysis - Propidium Iodide (PI)



Research Applications *Apoptosis*

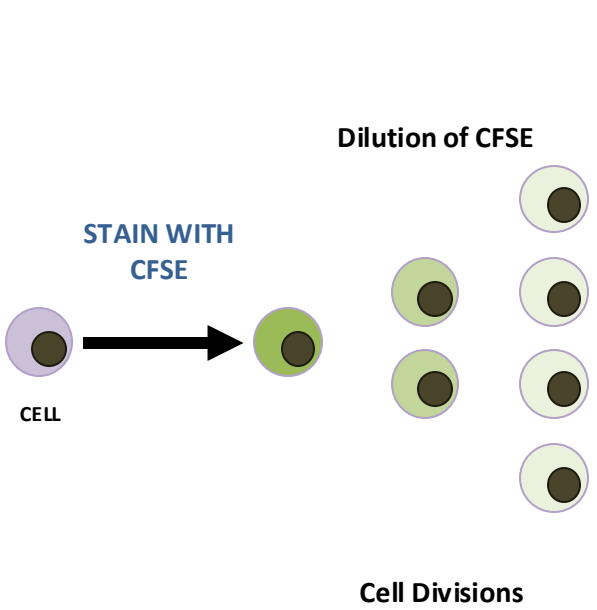
Annexin V-fluorochrome *plus* **Propidium Iodide** (non-fixed cells)

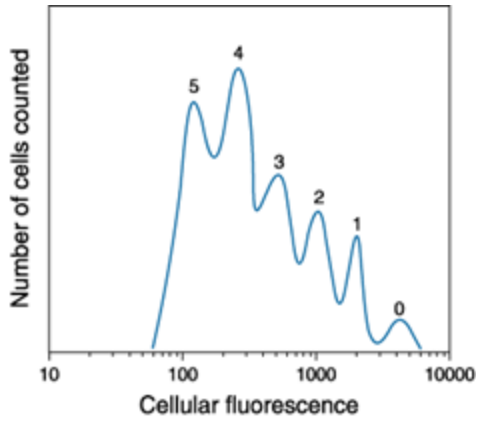


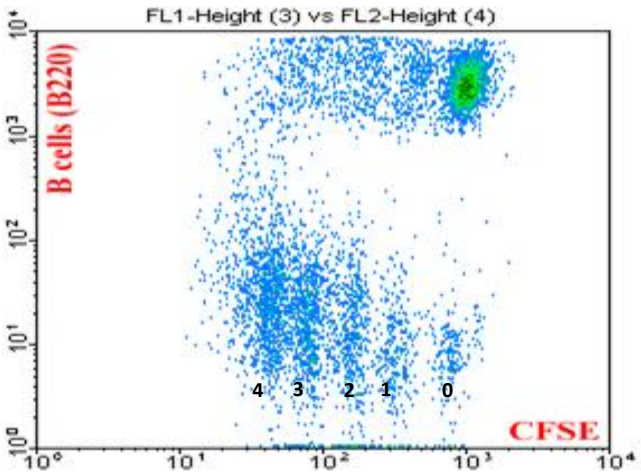


Research Applications *Cell Proliferation*

Tracking Cell Proliferation with CFSE (Carboxyfluorescein Succinimidyl ester)







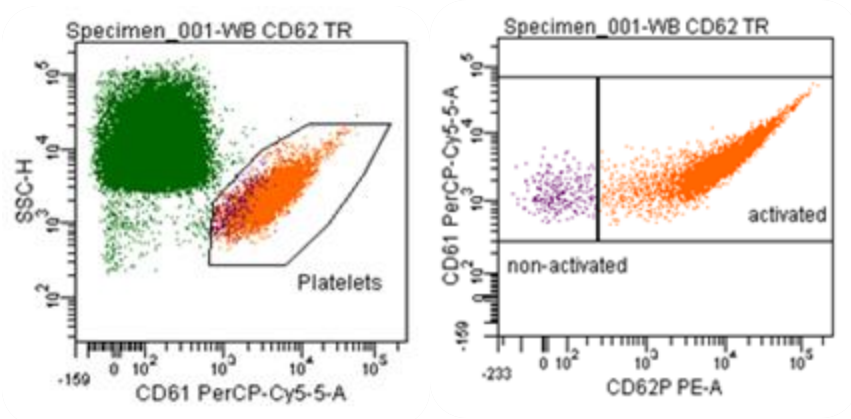
Research Applications *Cell Activation*

Platelets

Biochemical Parameters of Platelet Activation:

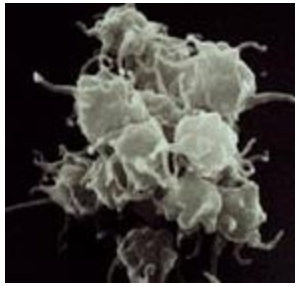
- **Activated GPIIb/IIIa receptor expression (PAC1)**
- **Granules GP expression (CD62)**
- **Protein phosphorylation**
- **Others**

GATING STRATEGY example

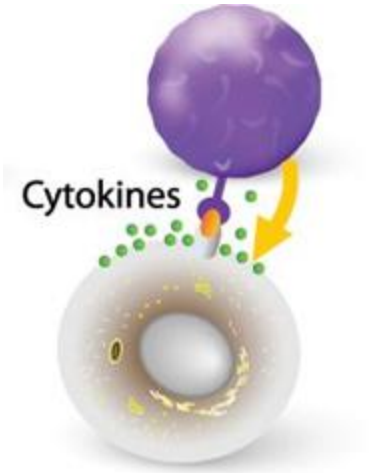


Resting Platelets

Platelets Activated



Research Applications *Cytokine Secretion*



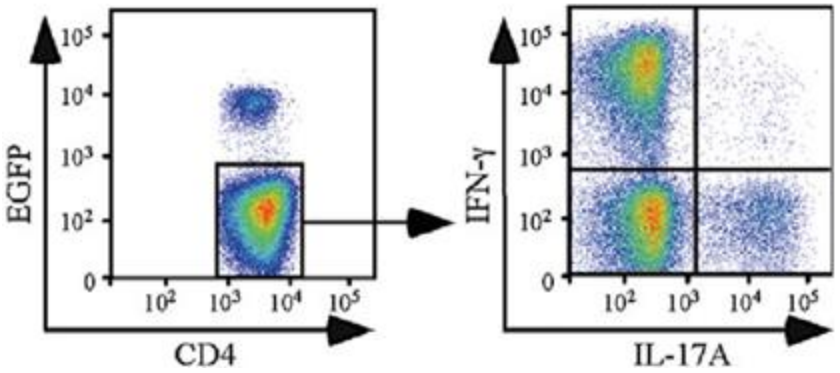
IMPORTANT IN HEALTH AND DISEASE SPECIALLY IN HOST RESPONSE TO:

- Infection
- Immune responses
- Inflammation
- Trauma
- Sepsis
- Cancer
- Reproduction

Their release has an effect on the behavior of cells around them.

Examples:

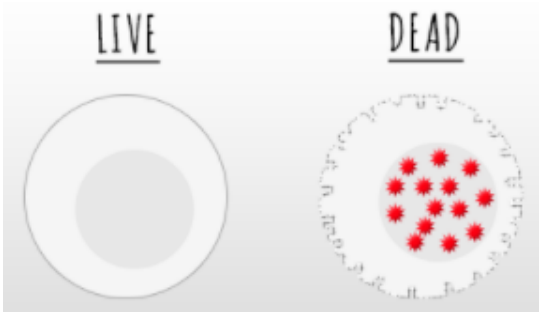
Interleukin 4 (IL-4), IL-2, IL-6
Tumor necrosis factor alpha (TNF-α)
Gamma interferon

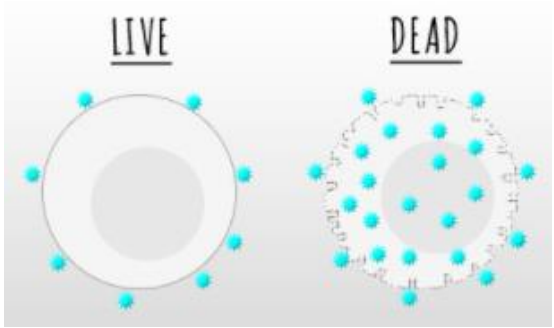


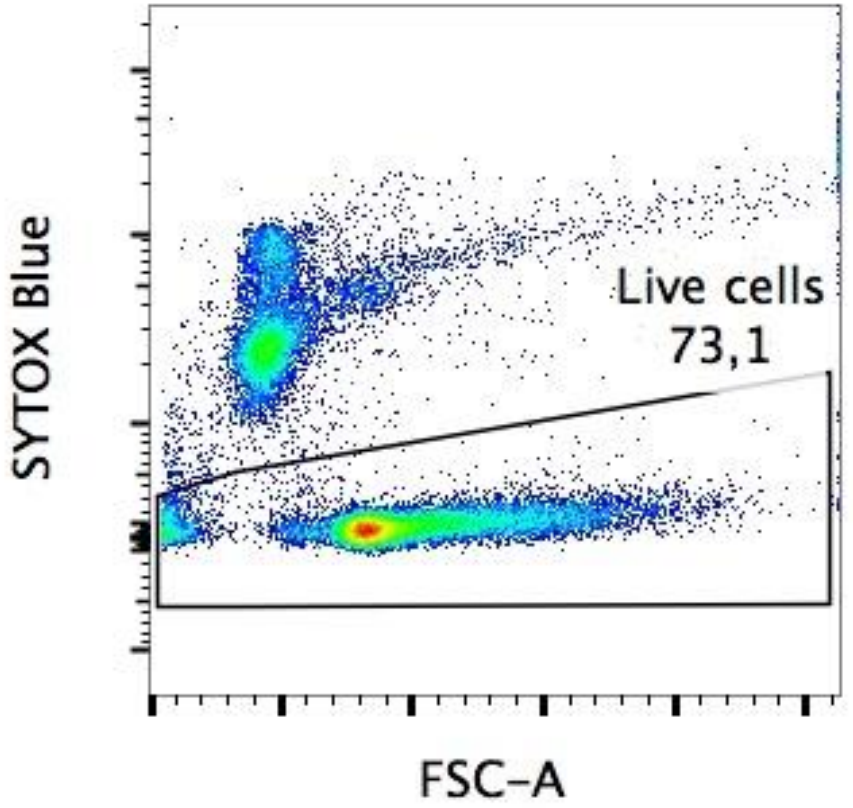
Analysis of T-cell cytokine production. Flow cytometry analysis of ex-vivo stimulated MLN lymphocytes by intracellular staining for IFN-γ and IL-17A.

Research Applications *Live/Dead Staining*

- Non-fixable dyes
  - DAPI, SYTOX Blue
  - SYTOX Green, Propidium Iodide, 7-Aminoactinomycin D
  - TO-PRO-3, DRAQ7
- Fixable dyes
  - Fixable Viability Stains
  - LIVE/DEAD
  - Vivafix





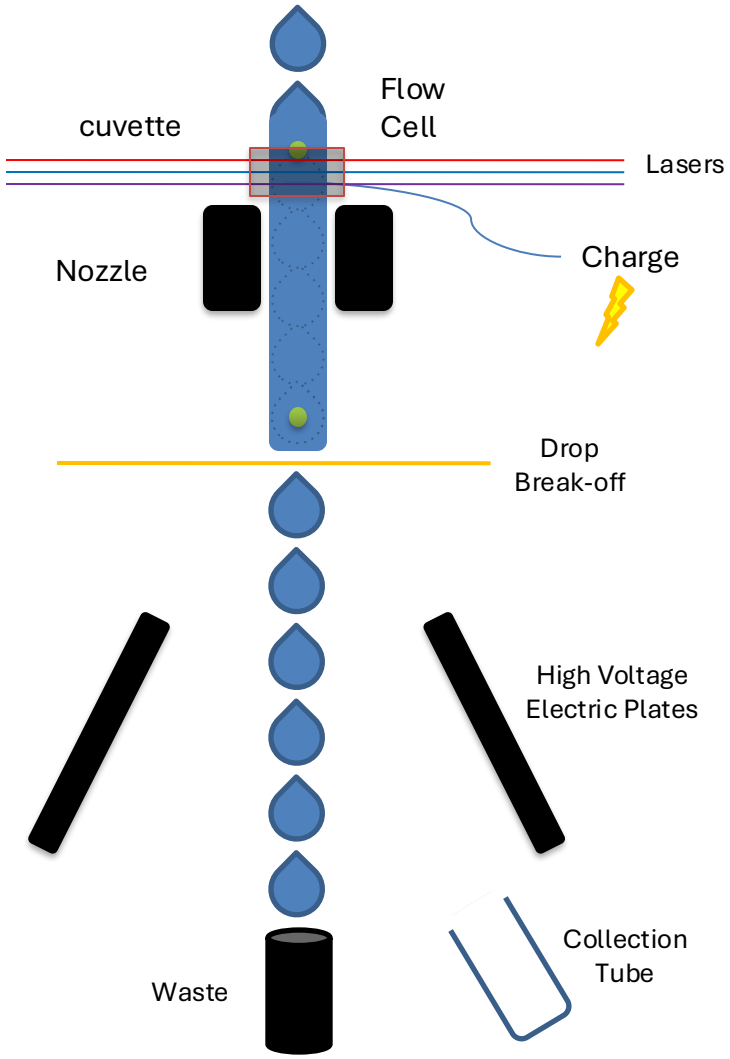


Research Applications *Fluorescent Activated Cell Sorting*

Physical separation of a cell or particle of interest from a **heterogeneous** suspension of cells or particles.

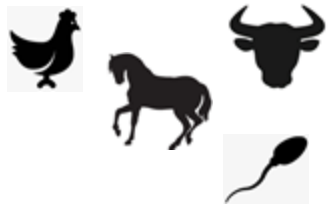
Drop Generation
Break Stream into Droplets (30-100,000 drops/sec)
Charge Drop
Vibrating Particle of interest
Deflect Charged Drop

*A cell sorter sorts **drops**, not cells!*



Flow Cytometry *Industry Applications*

Species & Organs



PHARMACEUTICAL INDUSTRY

- Early drug discovery
- Membrane potential
- Intracellular pH
- Cellular components levels (DNA, Protein, Surface receptors, Calcium..)

MARINE BIOTECHNOLOGY

- New species isolation
- Microalgae culture conditions monitoring
- Fish aquaculture food studies
- Oyster aquaculture parasites control

FOOD INDUSTRY

- Fermentation studies
- Food spoilage
- Quality control (water)

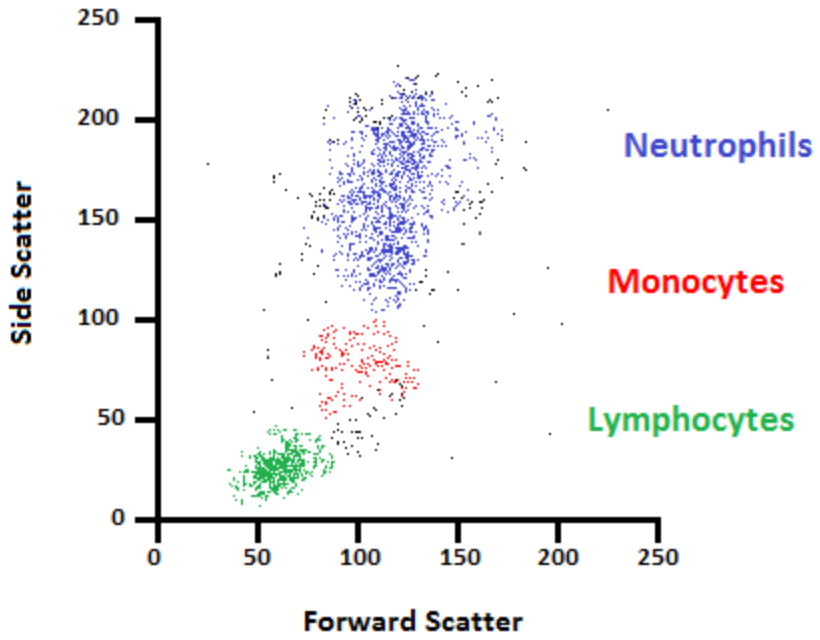
ANIMAL HUSBANDRY

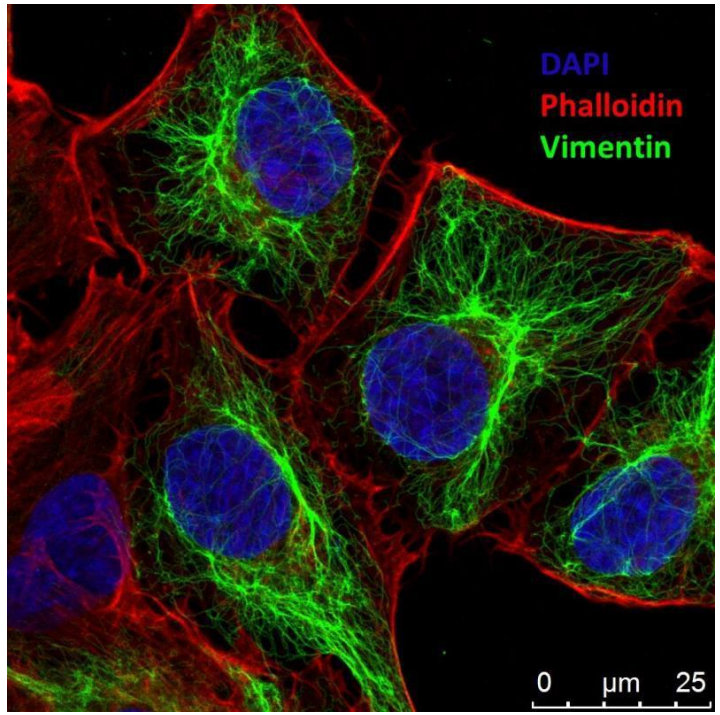
- Spermatology
- Welfare on poultry production farms (immune system check)

OTHER

- Pulp and Paper industry (biofilm growth control)

Last Developments *Imaging Flow Cytometry*





www.genetex.com

Flow Cytometry does not…

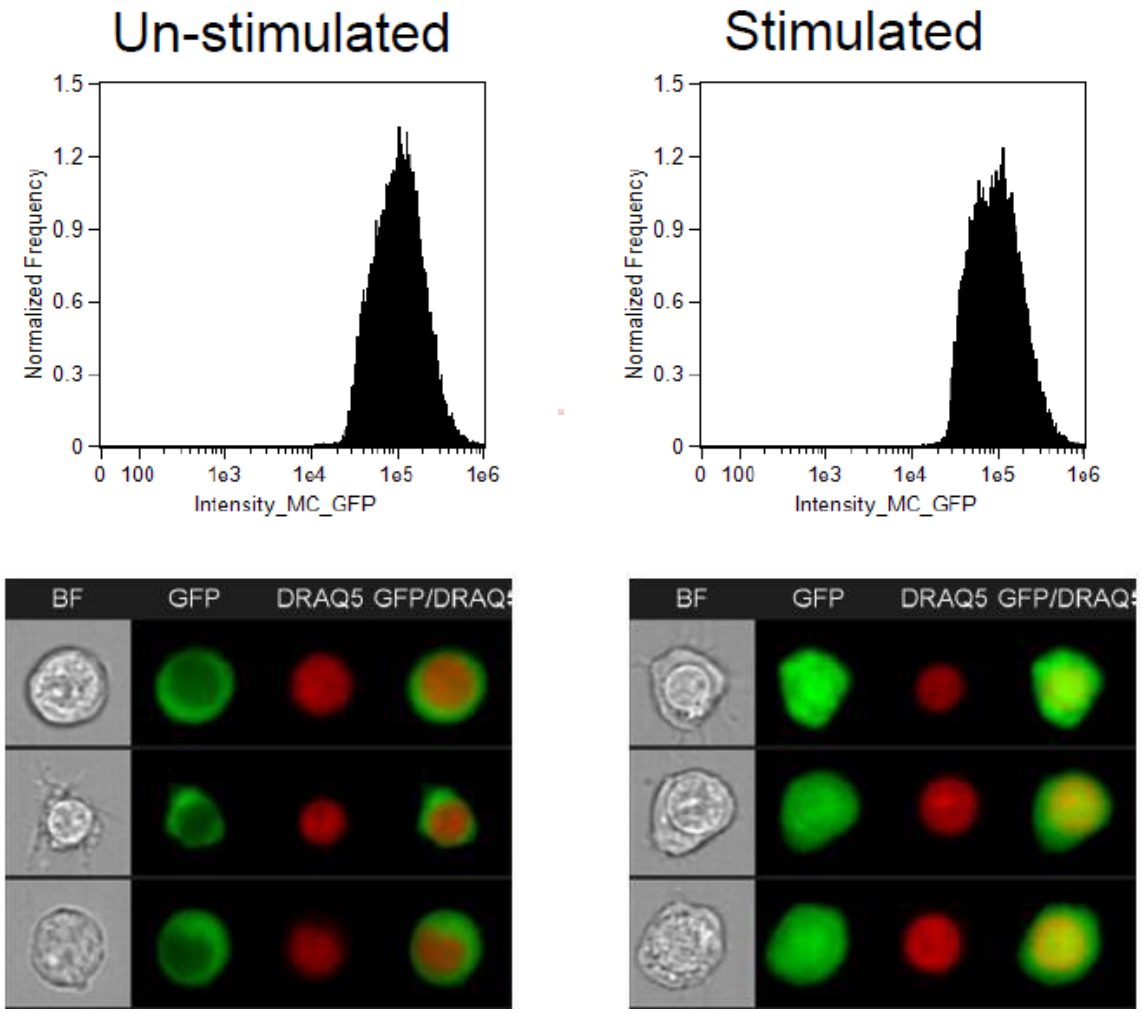
- provide information on where a given protein is within a cell
- measure distribution of cellular components
- measure cell morphology

https://www.labome.com/method/Flow-Cytometry-A-Survey-and-the-Basics.html

Last Developments *Imaging Flow Cytometry*

	Flow Cytometry	Microscopy
Speed	✓	✗
Statistical Power	✓	✗
Imaging	✗	✓
Information Content	✗	✓

Last Developments *Imaging Flow Cytometry*

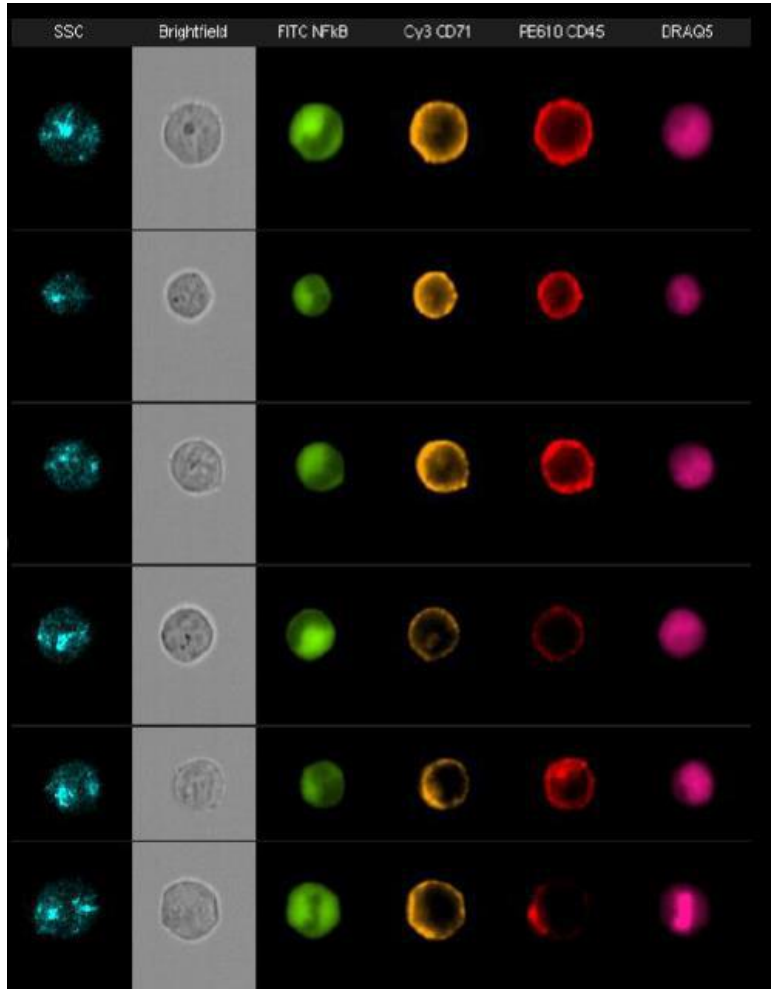


The fluorescence intensity may be the same but the location very different.

Andrew Filby, PhD
FCCF, Newcastle University

Last Developments *Imaging Flow Cytometry*

CYTEK Amnis ImageStream MK II



Predefined wizards for:

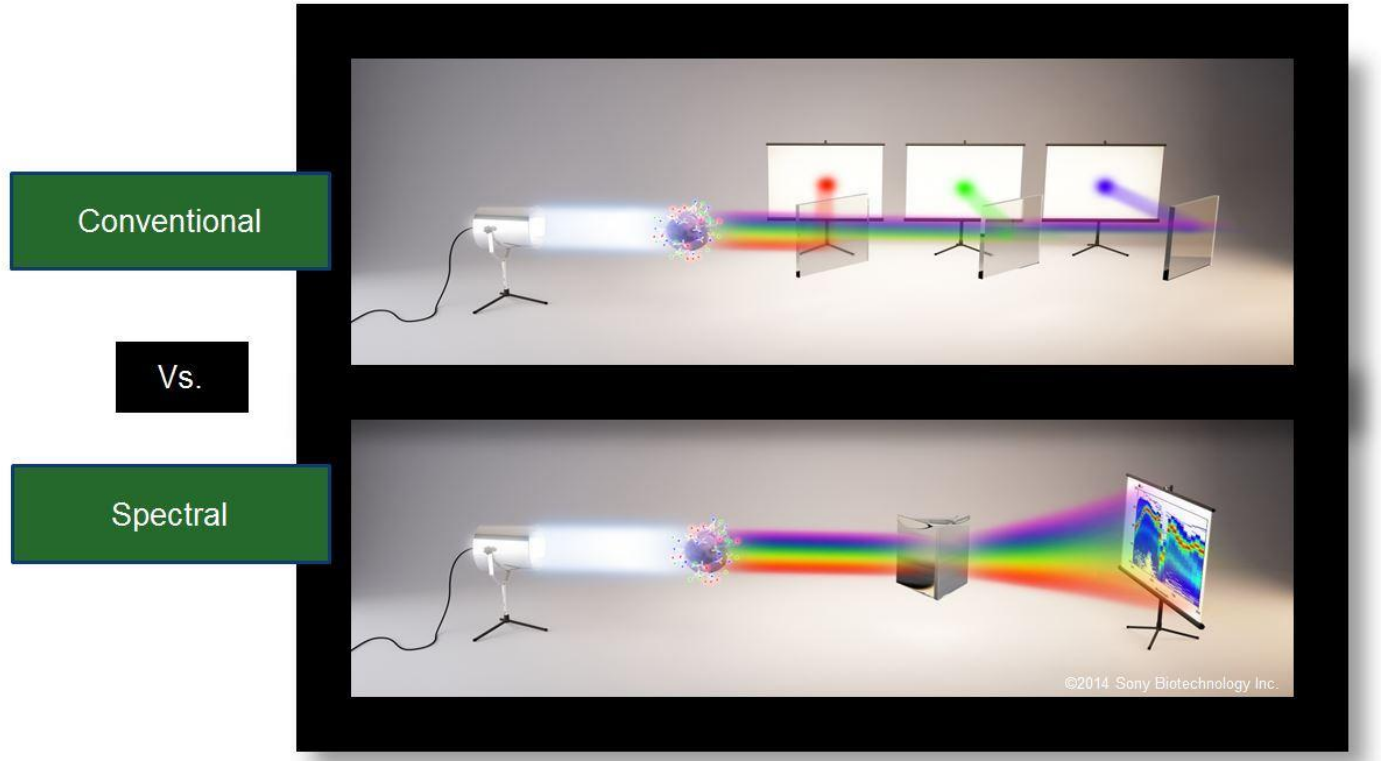
- apoptosis
- cell cycle
- co-localization
- internalization
- shape change
- spot counting
- others

amnis
part of Merck Millipore

Last Developments *Spectral Flow Cytometry*

Conventional Flow Cytometry uses mirrors and filters to select specific wavelength ranges for detection of signal from different fluorophores on individual PMTs.

Spectral Flow Cytometry uses dispersive optics, such as prisms or gratings, to disperse the collected light across a detector array, allowing the full spectra from each particle to be measured.

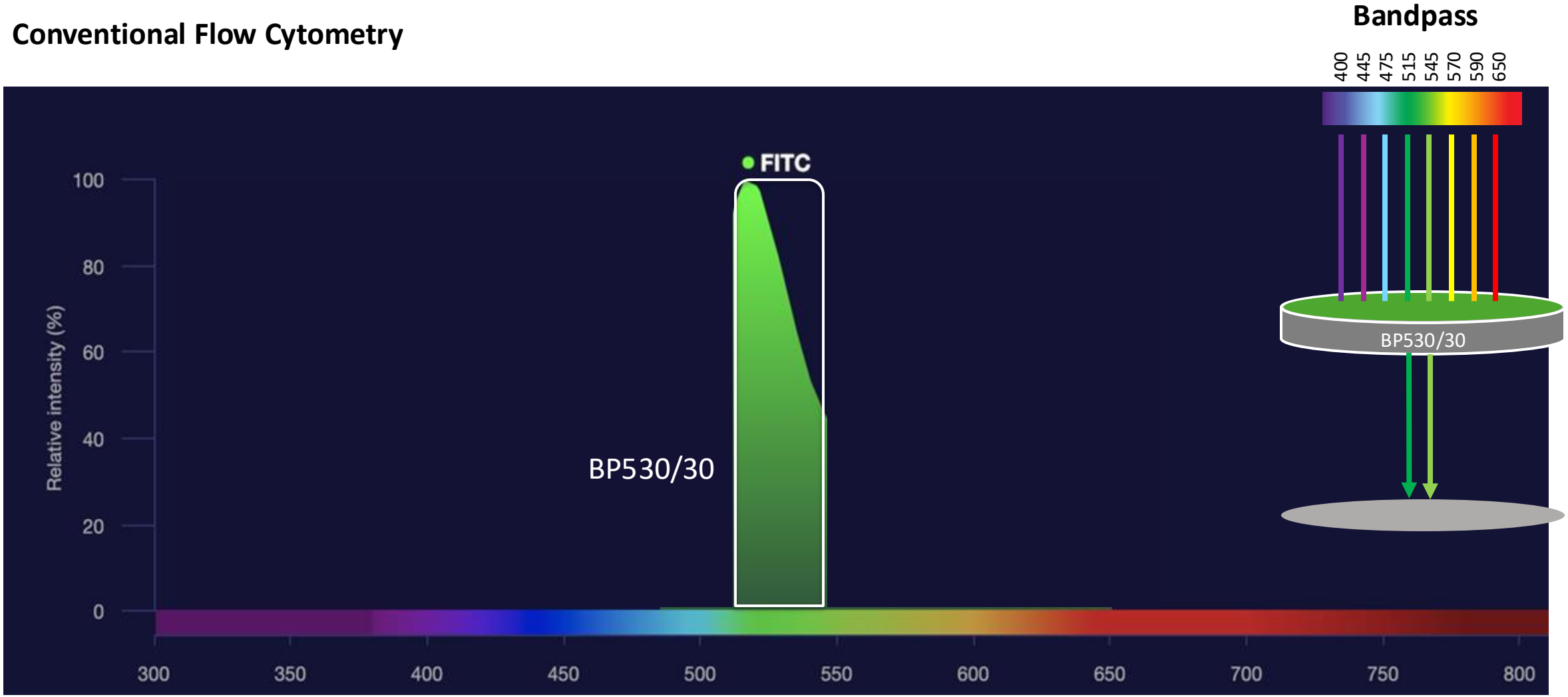


Nolan and Condello (2013) Current Protocols in Cytometry

Marsha Griffin and Mike Zordan, Sony Biotechnology

Last Developments *Spectral Flow Cytometry*

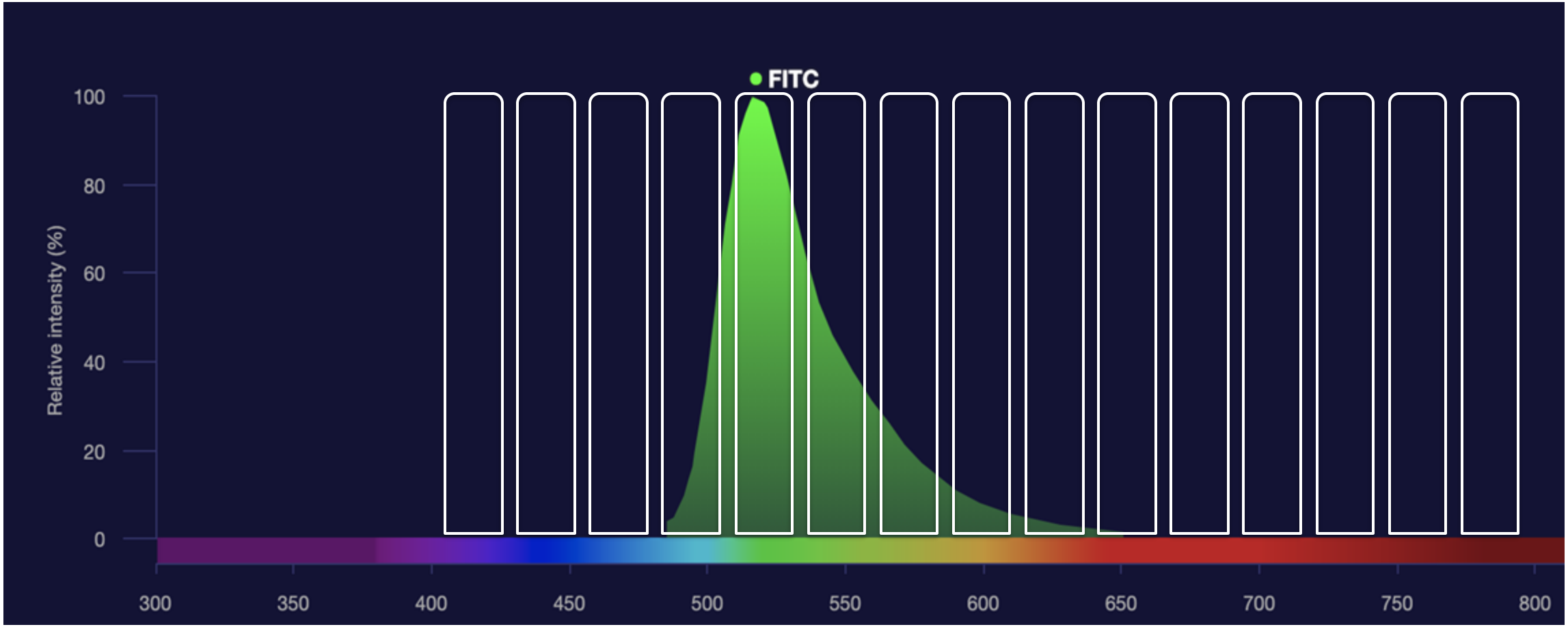
Conventional Flow Cytometry



FITC – peak emission from 515 to 545

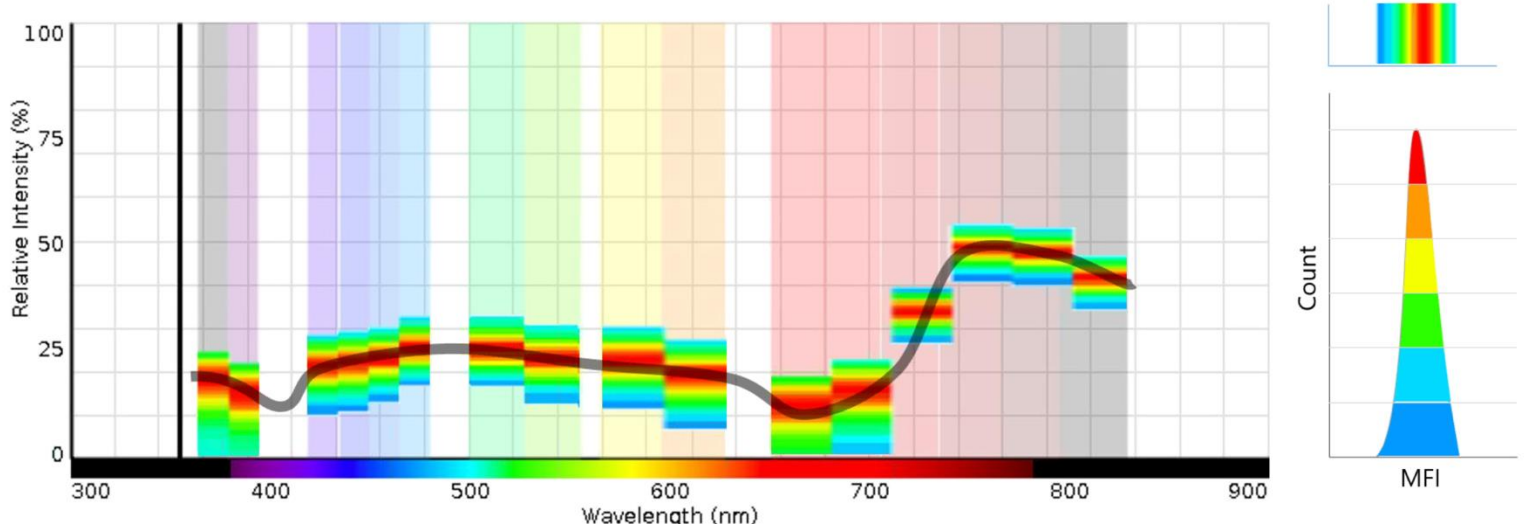
Last Developments *Spectral Flow Cytometry*

Spctral Flow Cytometry

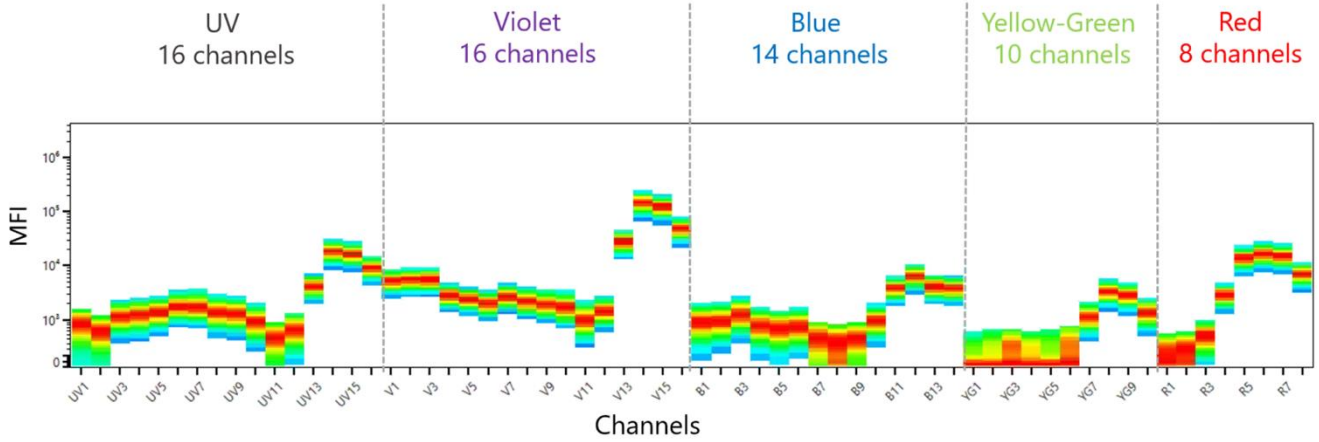


FITC – full emission from 490 to 650

Last Developments *Spectral Flow Cytometry*



Building a spectral signature



Full a spectrum signature

CYTEK
TRANSCEND THE CONVENTIONAL

Last Developments *Spectral Flow Cytometry (Cell Analysis & Cell Sorting)*

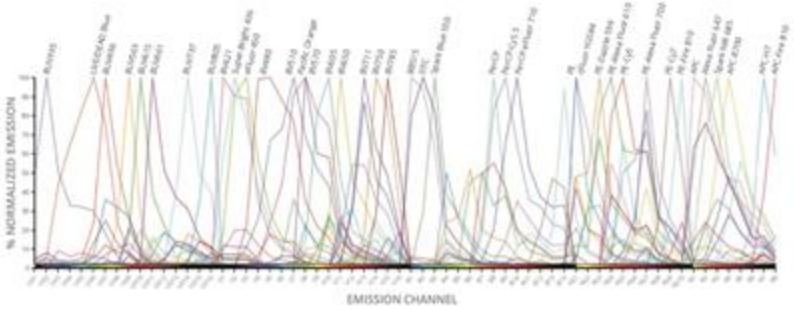
Main Advantages:

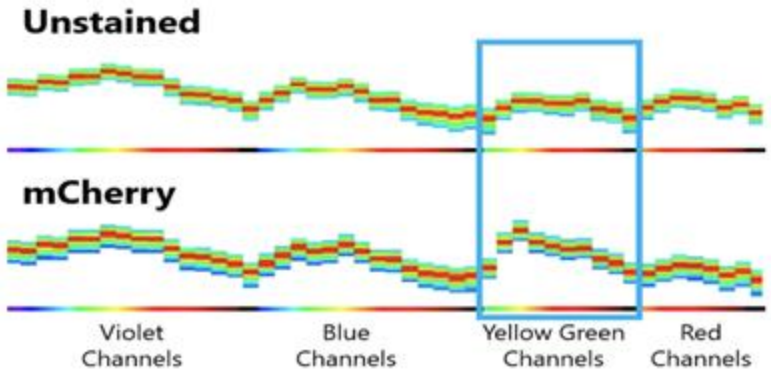
✓Easier to increase of possible number of fluorochromes

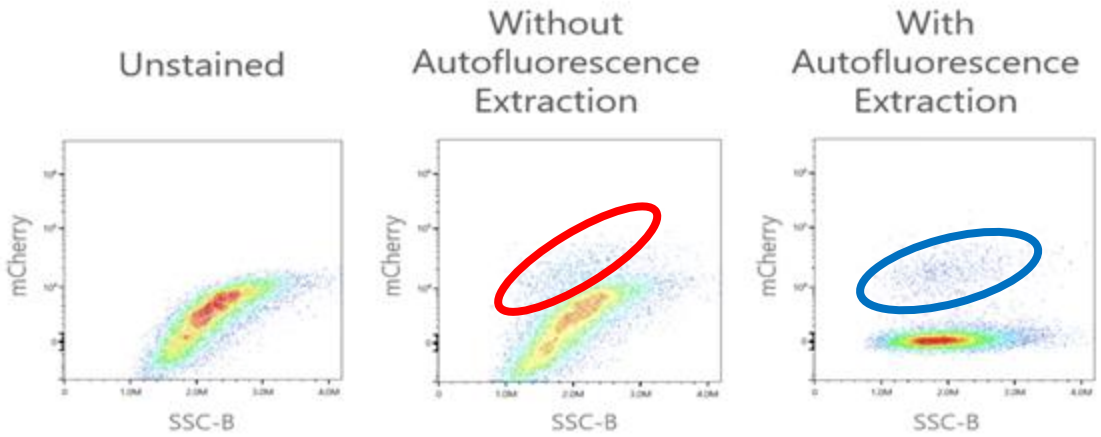
✓Better discrimination between dim and rare populations (high sensitivity)

✓A new level of flexibility (can distinguish close emitting fluorophores)

✓Allows autofluorescence Extraction



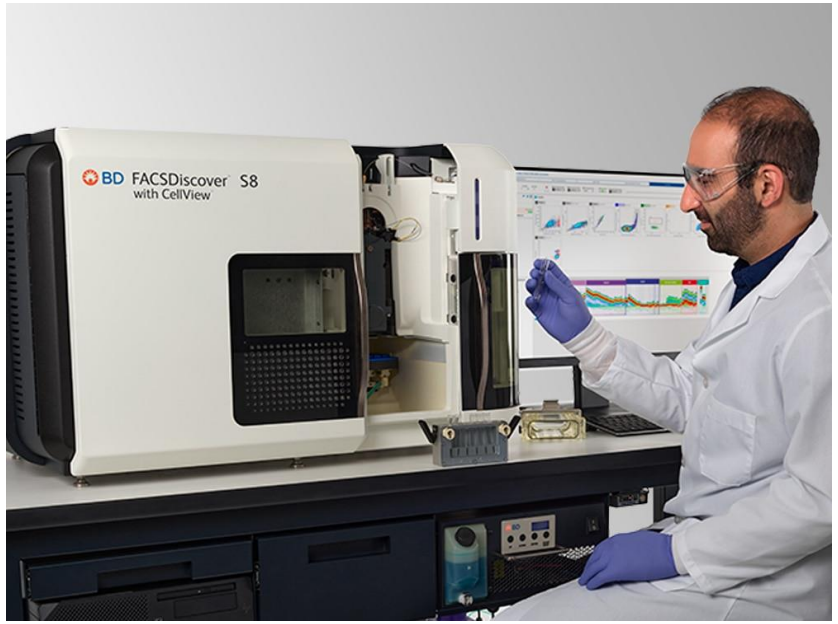




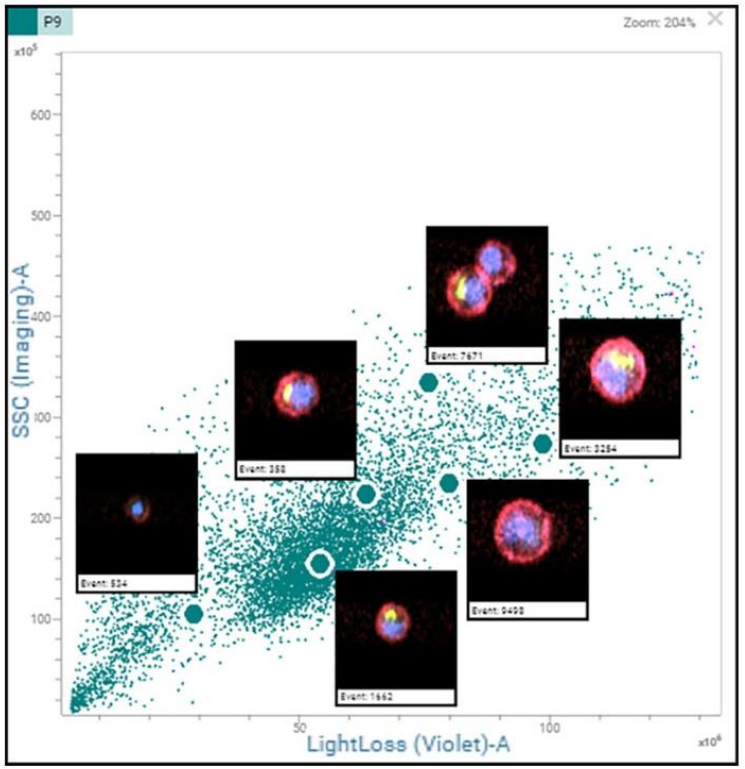
Just because you don't see it doesn't mean it isn't there

Last Developments

Spectral & Image- Enabled Cell Sorting



BD FACSDiscover™ S8 Cell Sorter



High-dimensional and image –enabled Cell Sorting

Flow Cytometry

Downstream Applications

sample processing
fluorescent staining

Flow Cytometry
Cell Sorting

Downstream Applications

Flow Cytometry

Downstream Applications

***In vitro* studies:**

- Cloning (single-cell sorting)
- Single-cell sorting for transcriptomics
- Cell differentiation and expansion
- Cell function
- Co-culture studies
- Drug therapy studies

***In vivo* studies:**

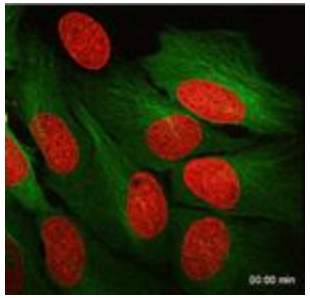
- Injection of sorted cells to evaluate immune response and cell/disease development
- Injection of model organisms with sorted cells
- Transplantation studies

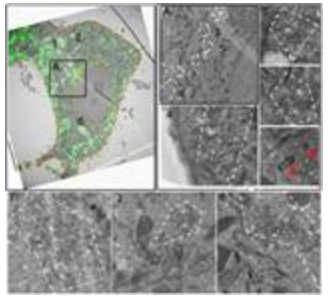
Genomics:

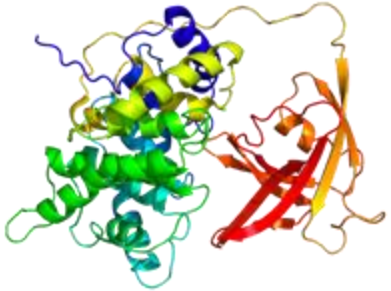
- DNA and RNA sequencing
- 10X Genomics Chromium system (single cell RNAseq)
- miRNA sequencing
- RT-PCR for specific genes

Flow Cytometry

Downstream Applications







Microscopy studies:

- Light microscopy
- Electron microscopy
- Expansion microscopy

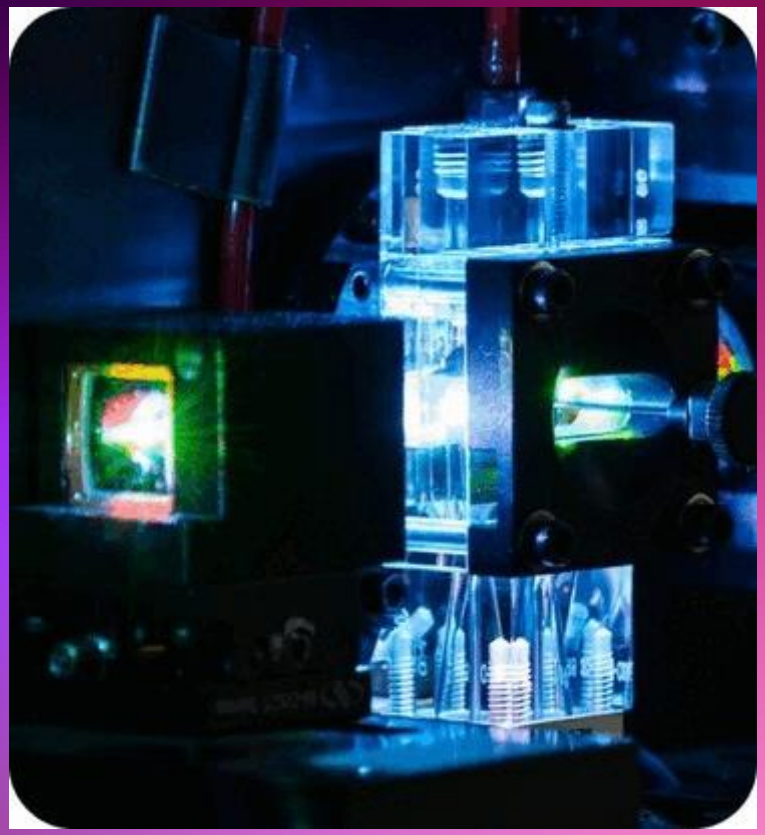
Protein analysis studies:

- Western blot of specific cell subsets
- Proteomics of specific cell types
- Quantitative single-cell proteomics

Clinical Trials & Personalized Medicine:

- Immunotherapy and clinical research: patient follow-up (e.g. optimal dosing approach and evaluation of predictive biomarkers)

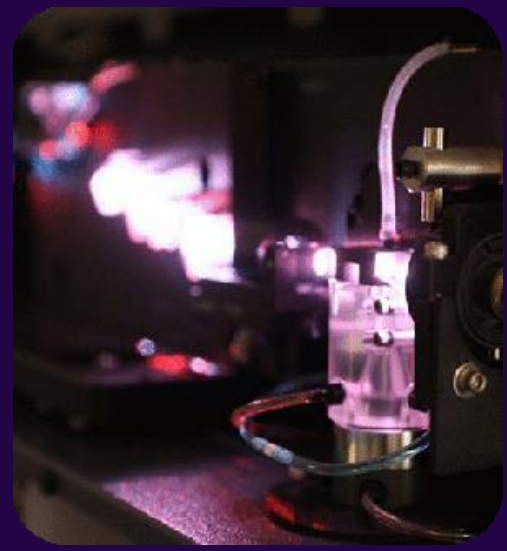
Flow Cytometry @GIMM



Ready to the future.

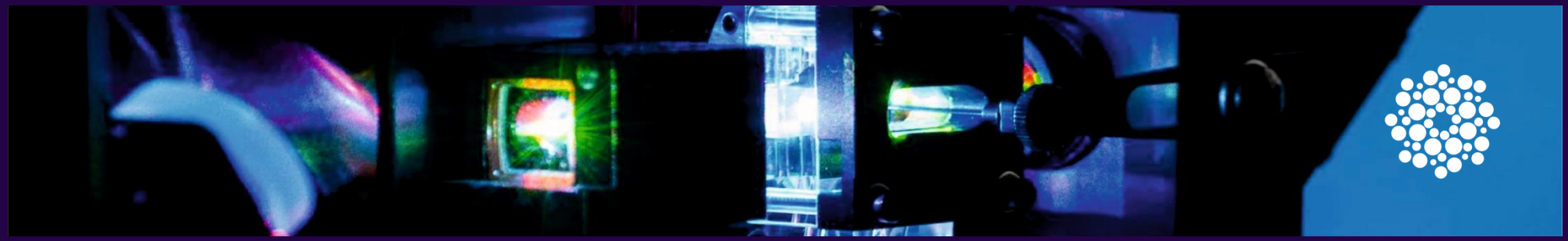
About the Flow Cytometry Platform

The Flow Cytometry Platform provides a state of the art flow cytometry service to GIMM researchers as well as external groups from other research institutes.



GIMM Flow Cytometry Plaform

Mission: Support users in their research projects



Consulting in Experiment Planning

Advice on Panel Design and Sample Preparation

Training and Support

Cell Analysis
Cell Sorting
Imaging Cytometry

Data Analysis and Reporting

GIMM Flow Cytometry *Team*

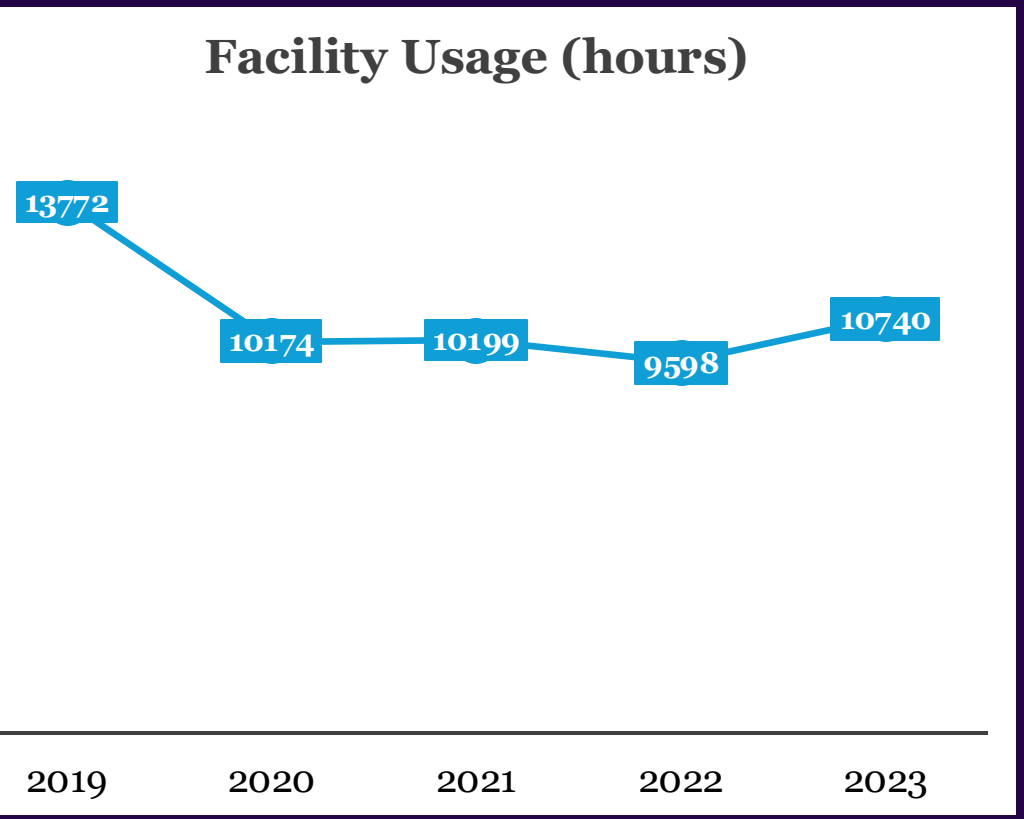
Head of Platform: Mariana Fernandes

Technical Support:

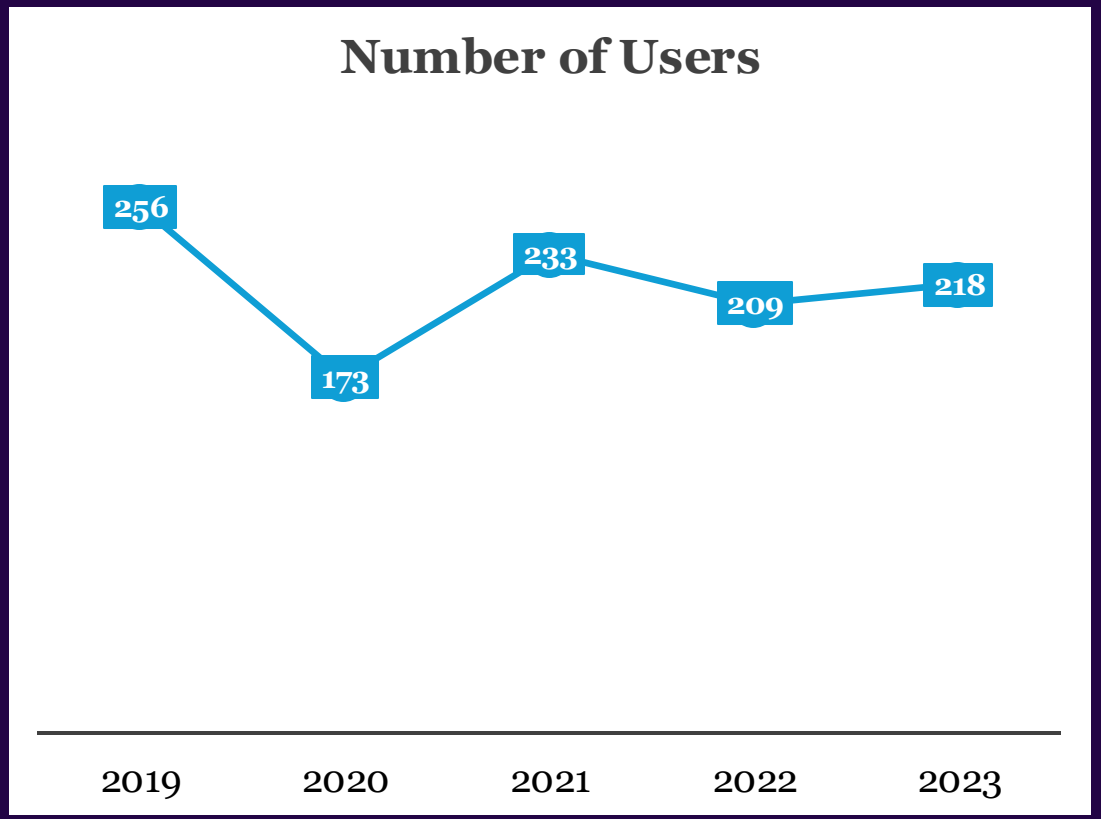
Marta Monteiro
André Branco
Andreia Santos
Maria Neves
Rute Gonçalves
Sónia Pereira
Telma Costa

Facility Usage

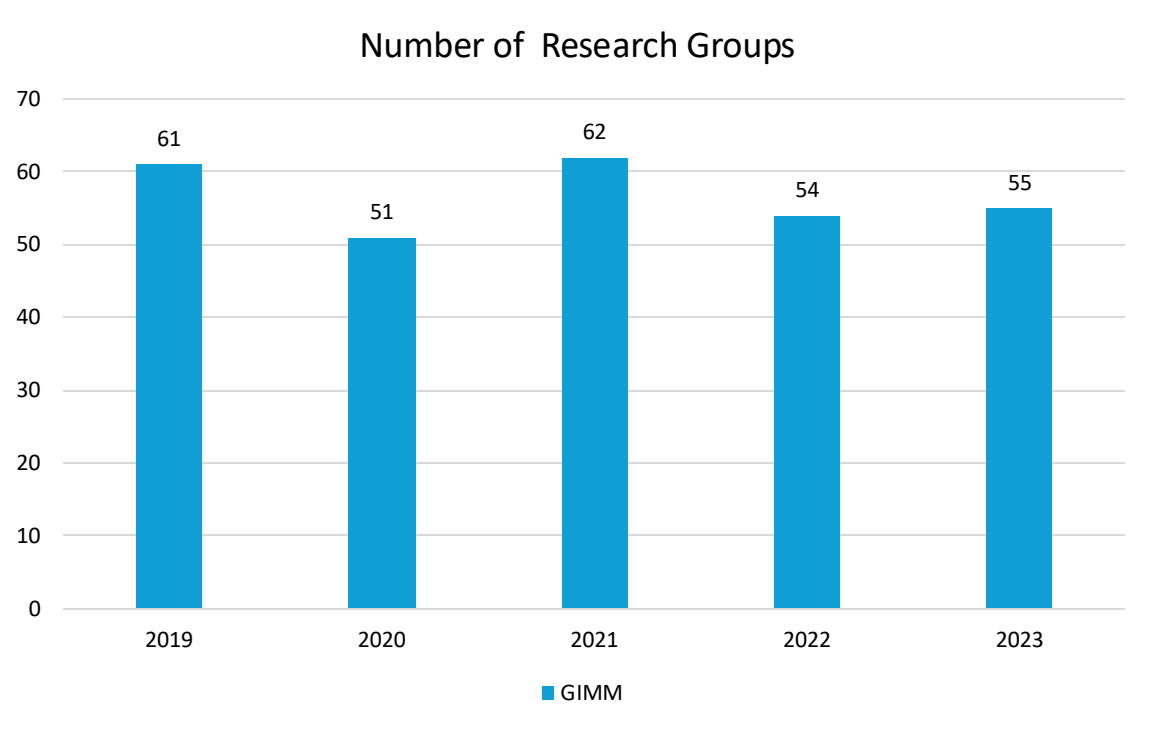
Usage

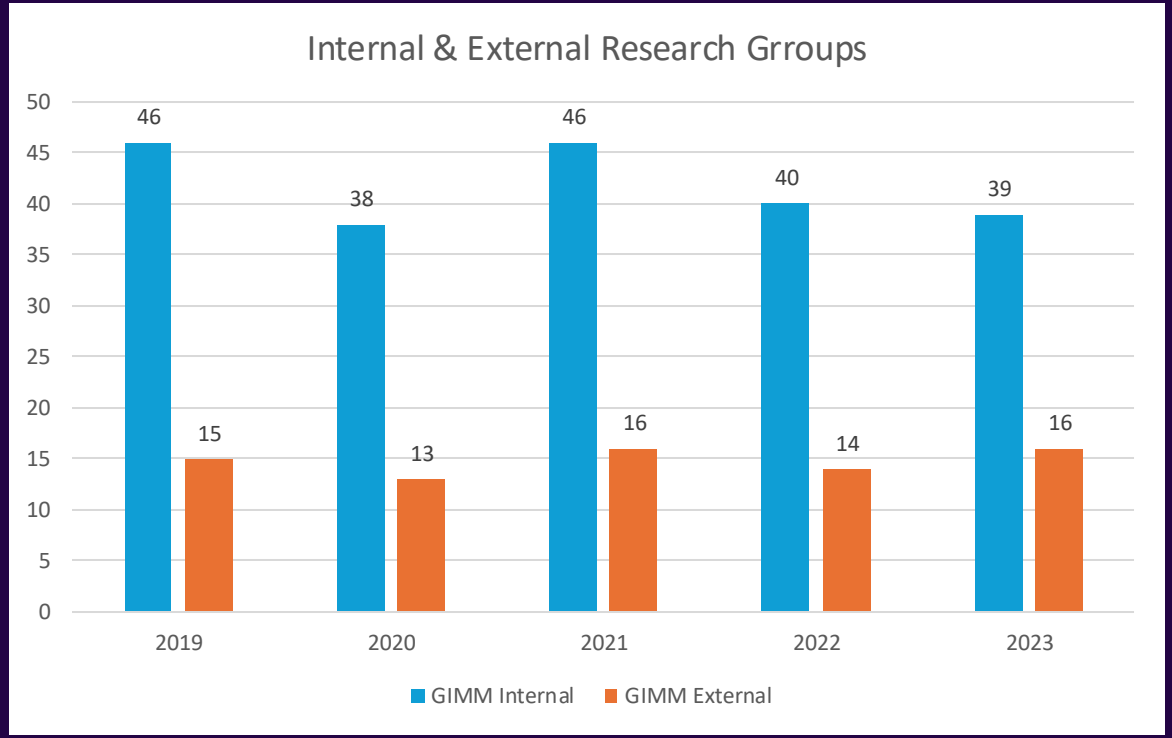


Users



Facility Usage





Flow Cytometry *Users*

Cell and Molecular Biology

Immunology & Infection

Evolution

Regeneration & Aging

Neurosciences

Metabolism and Nutrition

Biochemistry and Nanoparticles

Translational Research

Flow Cytometry *Instruments – Oeiras Site*

Cell Analyzers

Conventional

BD LSR Fortessa SORP + HTS (optimize to bacteria)

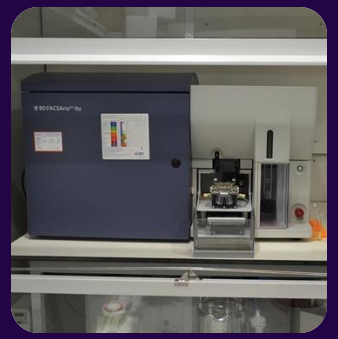
BD LSR Fortessa X-20

Spectral

Cytek Aurora +MAS

Cell Sorters

Conventional



BD FACSAria IIu

Spectral

Cytek Aurora CS

Flow Cytometry *Instruments – Lisbon Site*

Cell Analyzers

Conventional

BD Accuri C6 Plus

BD LSR Fortessa + HTS

BD LSR Fortessa X-20

Spectral

BD FACSymphony A5 SE

Cytek Aurora + ASL

IFC

Cytek Amnis ImageStream^{X} MK II

Cell Sorters

Conventional

BD FACSAria Fusion

BD FACSAria III

Spectral

BD FACSymphony S6 SE

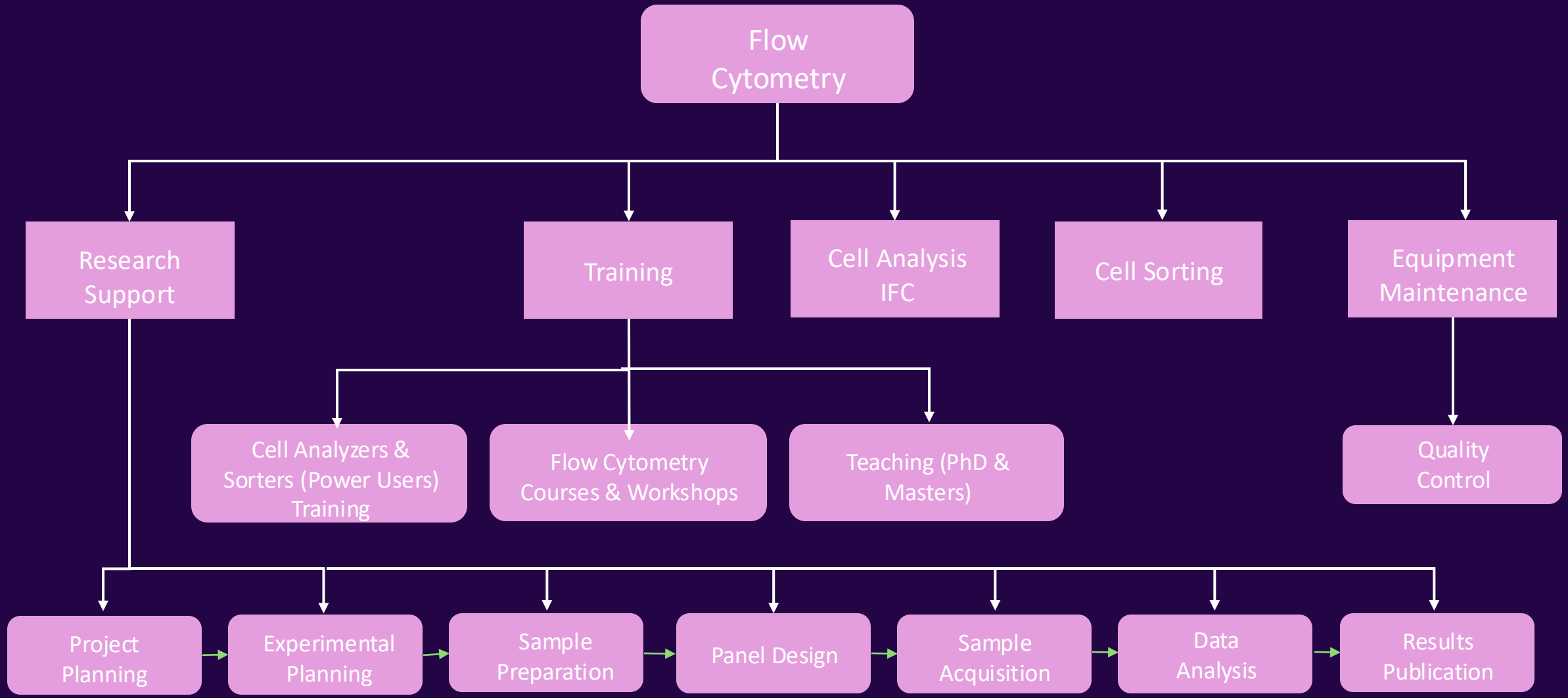
Flow Cytometry *Workstations*

Multiple Workstations with:

- FlowJo
- FCS Express
- SpectroFlo
- IDEAS
- FACSDiva
- ModFitFL

High-performance data analysis workstations (on-site and remote)

Flow Cytometry *Technical and Research Support Services*



Flow Cytometry *Training*

Advanced Training (Internal & External Users)

Facility Users

- Basic Introduction
- Theoretical Introduction
- Practical Session in a specific system

Workshops

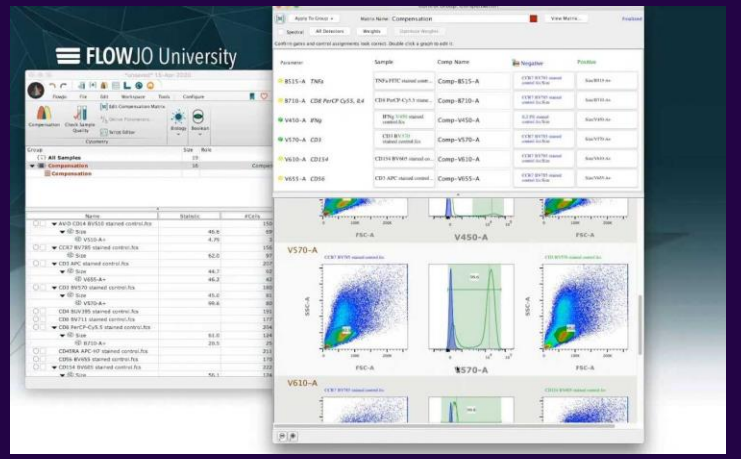
- Sample Preparation
- Experimental Planning and Troubleshooting
- Panel Design
- Data Analysis

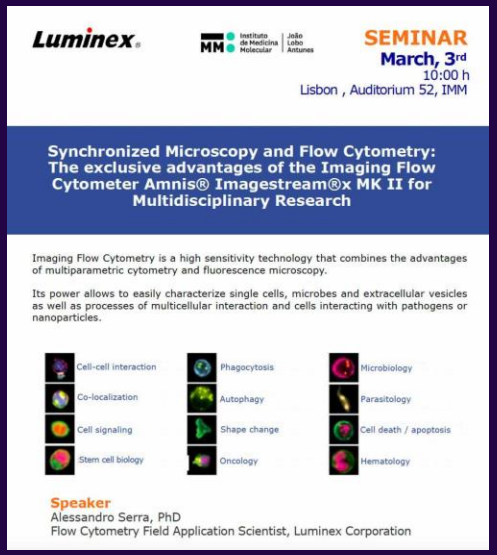
Courses

- Flow Cytometry
- Cell Sorting
- Spectral
- Imaging Flow Cytometry (IFC)

Flow Cytometry *Training*

Exploring New Technologies





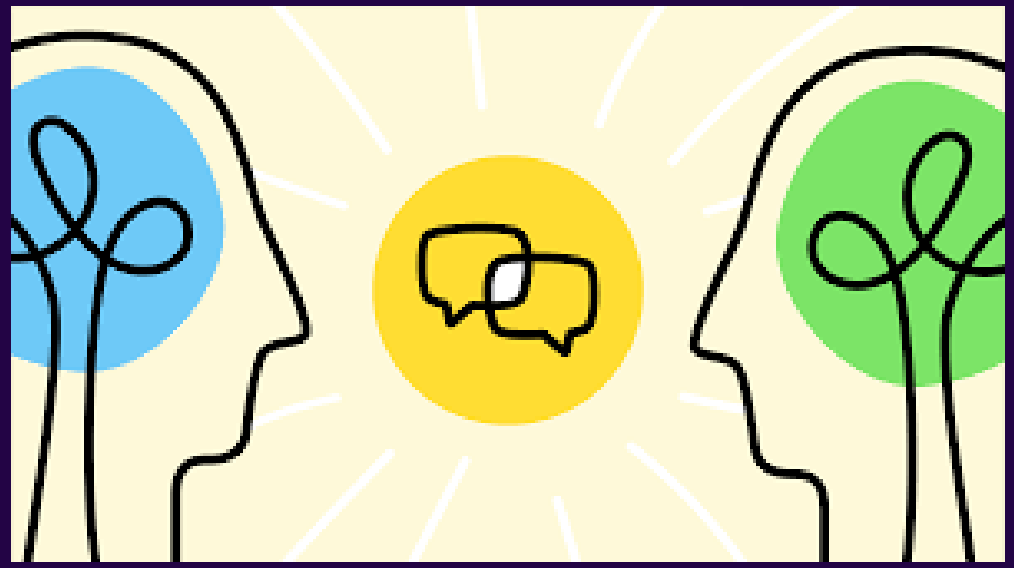
International FC Course

Technical Webinars & Workshops

Flow Cytometry *Advice & Support*

Research Consulting

- Advise in projects & experimental set-up
- Help in scientific writing/communication



Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

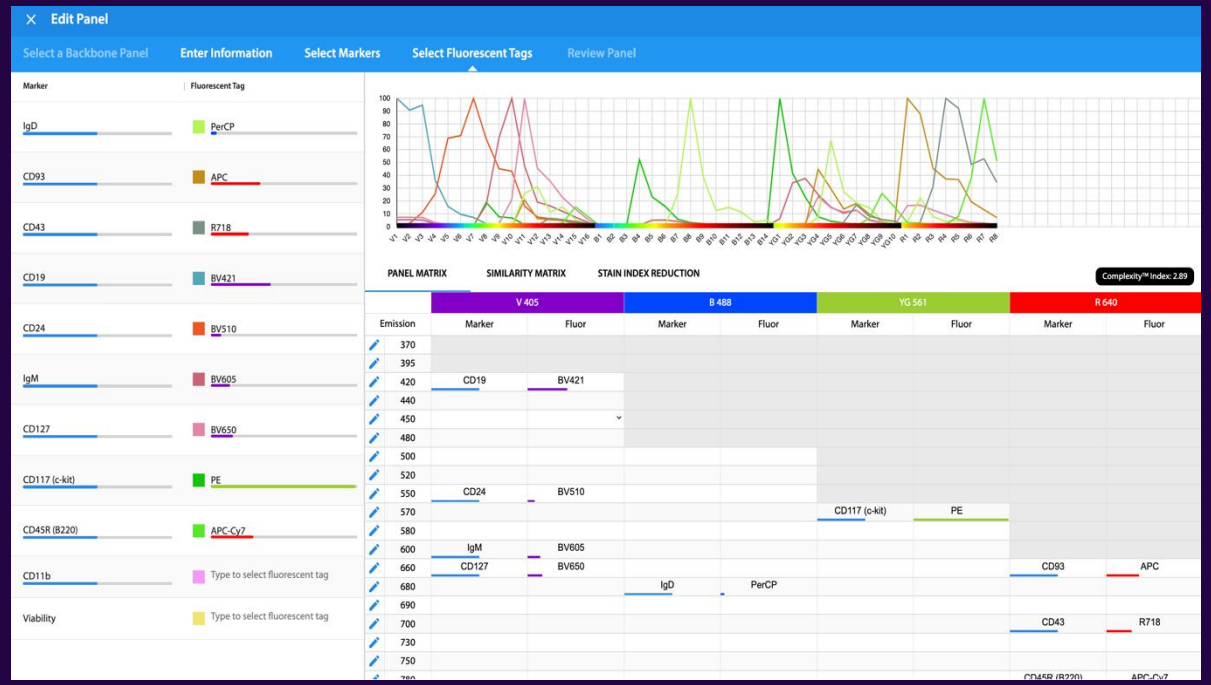
Methodology

Sample preparation	AnTat1.1 GFP::PAD1utr reporter parasites isolated from blood and adipose tissue were fixed prior to flow cytometer analyses. Lister 427 parasites from culture or isolated from blood and adipose tissue were fixed, permeabilized and stained with Click-iT™ HPG/DAPI/PI prior to flow cytometer analyses. Lister 427 parasites from culture were stained with PKH26 or CellTrace™ Violet prior to flow cytometer analyses. Also, PKH26 or CellTrace™ Violet stained parasites isolated from blood and/or adipose tissue were analyzed by flow cytometry.
Instrument	BD LSRFortessa™, BD LSRFortessa™ X-20, BD FACSAria™ IIu, BD FACSAria™ III
Software	Data analyses were performed in FlowJo.
Cell population abundance	Sorted cell populations were within 100s to 100000s events. Samples were pure, with purity defined based on a gating strategy where a pure culture sample was used to gate for parasites followed (when applicable) by gating of DAPI/PI positive cells to exclude debris.
Gating strategy	SSC-A/FSC-A to determine parasites gate, FSC-W/FSC-A to exclude doublets, Count/FITC-A- to determine the % of stumpy cells (threshold determined with culture GFP- and GFP+ cells). SSC-A/FSC-A to determine parasites gate, FSC-W/FSC-A to exclude doublets, Count/Comp-Pacific Blue-A-to determine DAPI+, Count/Comp- Texas Red-A- to determine HPG MFI (compensation performed with pure samples of double negative and DAPI + and HPG+ cells). SSC-A/FSC-A to determine parasites gate, FSC-W/FSC-A to exclude doublets, Count/Texas Red-A- to determine PKH26 positive cells (threshold determined with culture PKH26- and PKH26+ cells). SSC-A/FSC-A to determine parasites gate, FSC-W/FSC-A followed by SSC-W/SSC-A to exclude doublets, FSCA/Pacific Blue-A- to sort CTV positive/CTV negative cells (threshold determined with culture CTV- cells).

Flow Cytometry *Advice & Support*

Panel Design

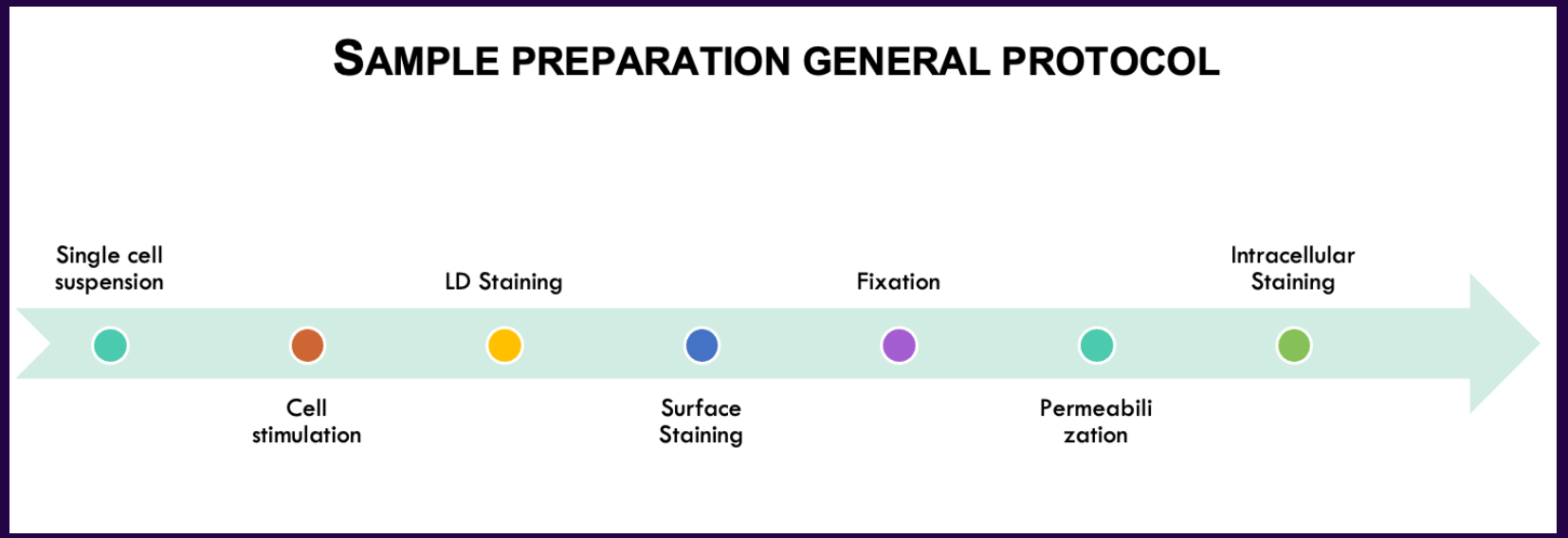
- Support to develop new panels for Flow Cytometry
- Panel Optimization & Validation
- Troubleshooting



Flow Cytometry *Advice & Support*

Sample Preparation

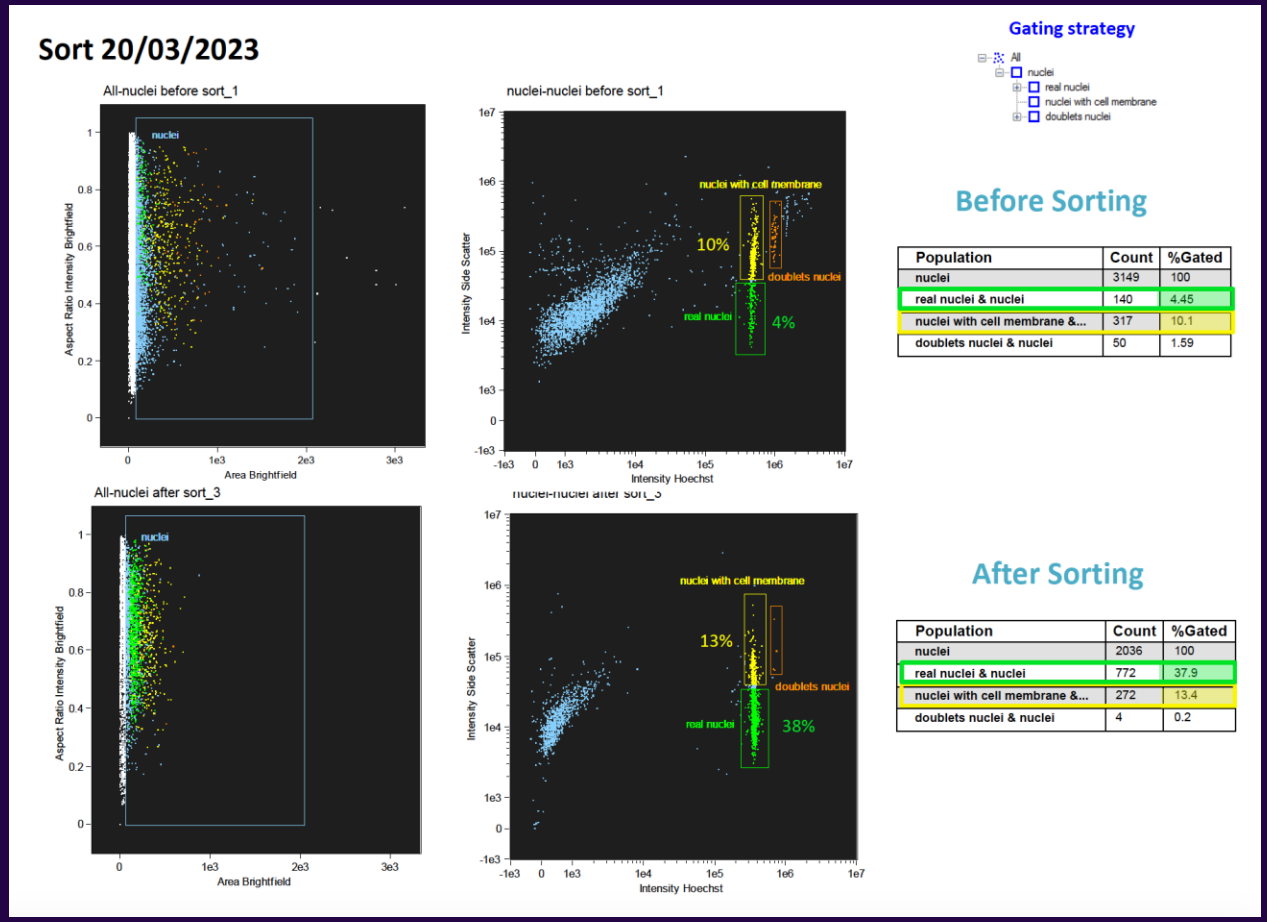
- Database with sample preparation protocols for Flow Cytometry
- Advice and troubleshooting



Flow Cytometry *Advice & Support*

Imaging Flow Cytometry

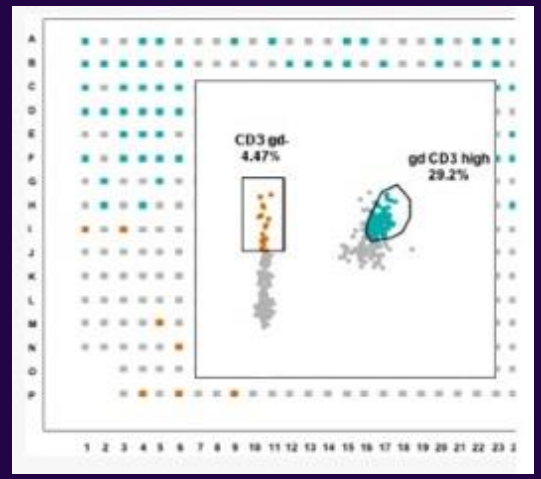
- Support and Advice on IFC

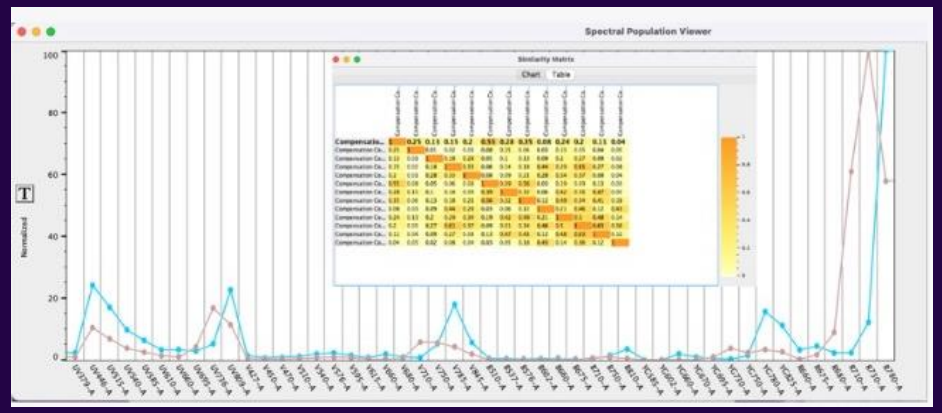


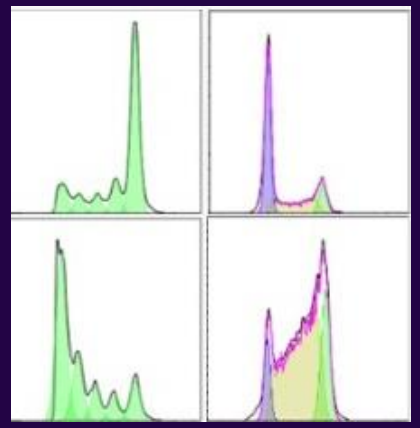
Flow Cytometry *Advice & Support*

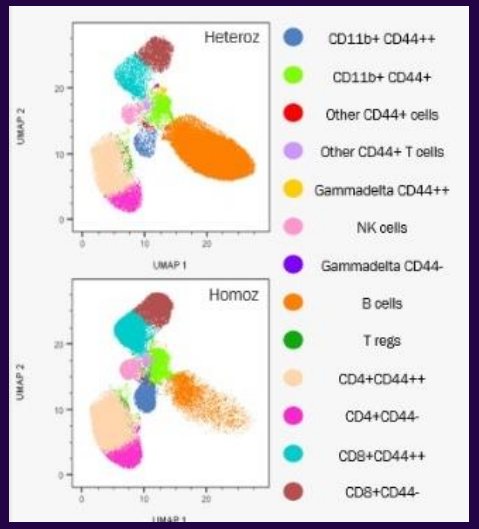
Data Analysis

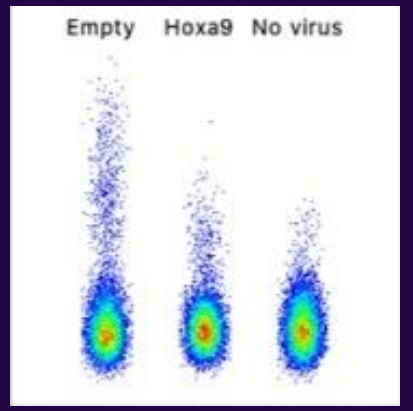
- Training
  - FlowJo
- Consultation
- Advice on Data Analysis







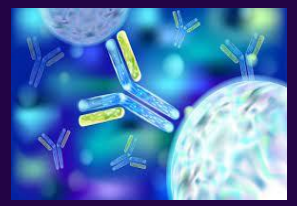
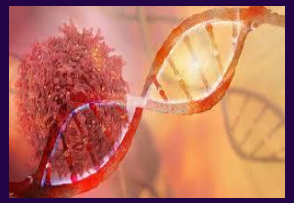




Optimized Workflows for

Downstream Applications

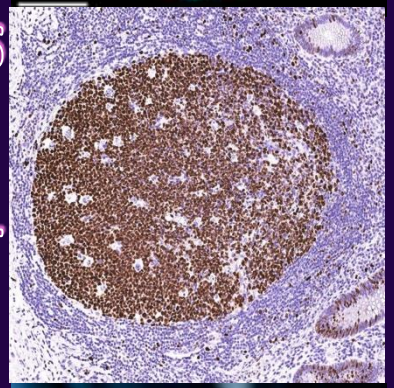
Research Groups


Scientific Question

Bioimaging

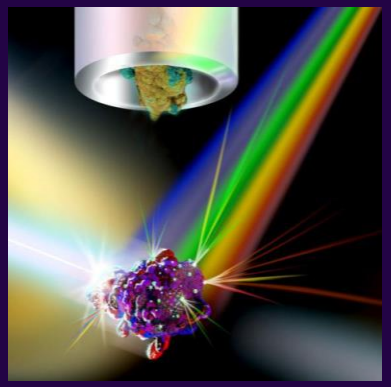
Histopathology

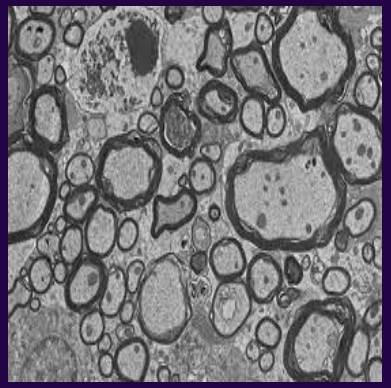


Data Science

Facilities

Flow Cytometry

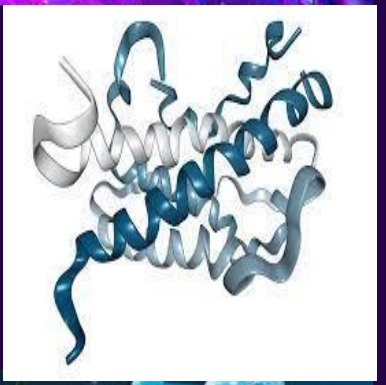




E. Microscopy

Genomics

Proteomics

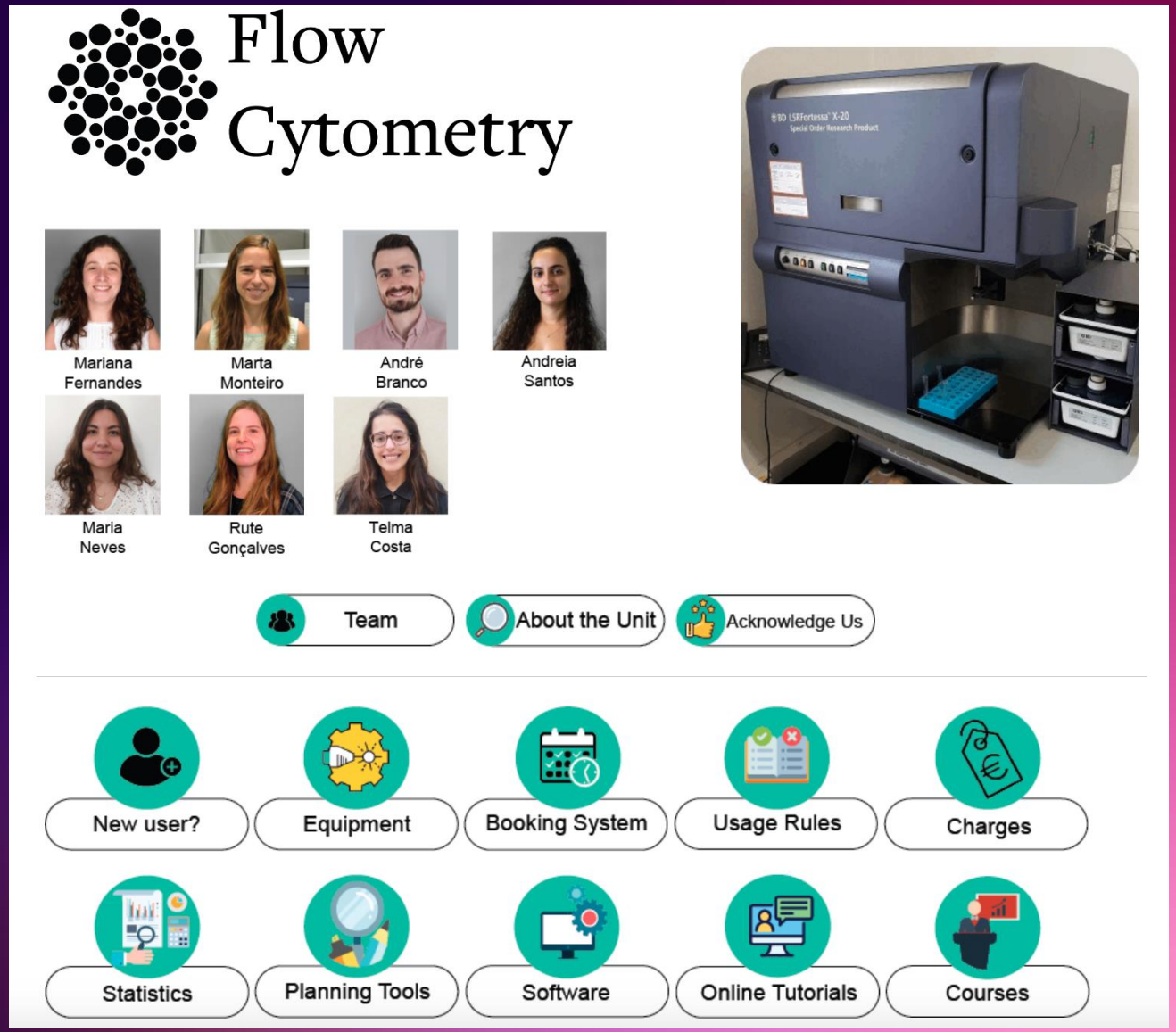


Metabolomics

Mariana Fernandes

mariana.fernandes@gimm.pt

Thank you.



https://imm.medicina.ulisboa.pt/facility/flowcytometry/doku.php?id=start