The latest tech innovations in flow cytometry

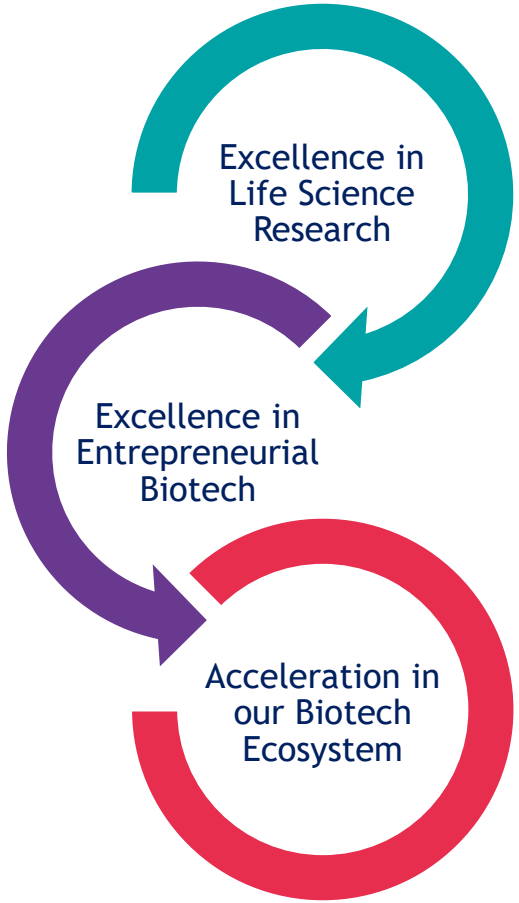
Gert Van Isterdael

Katrien Van der Borght

10 February 2026

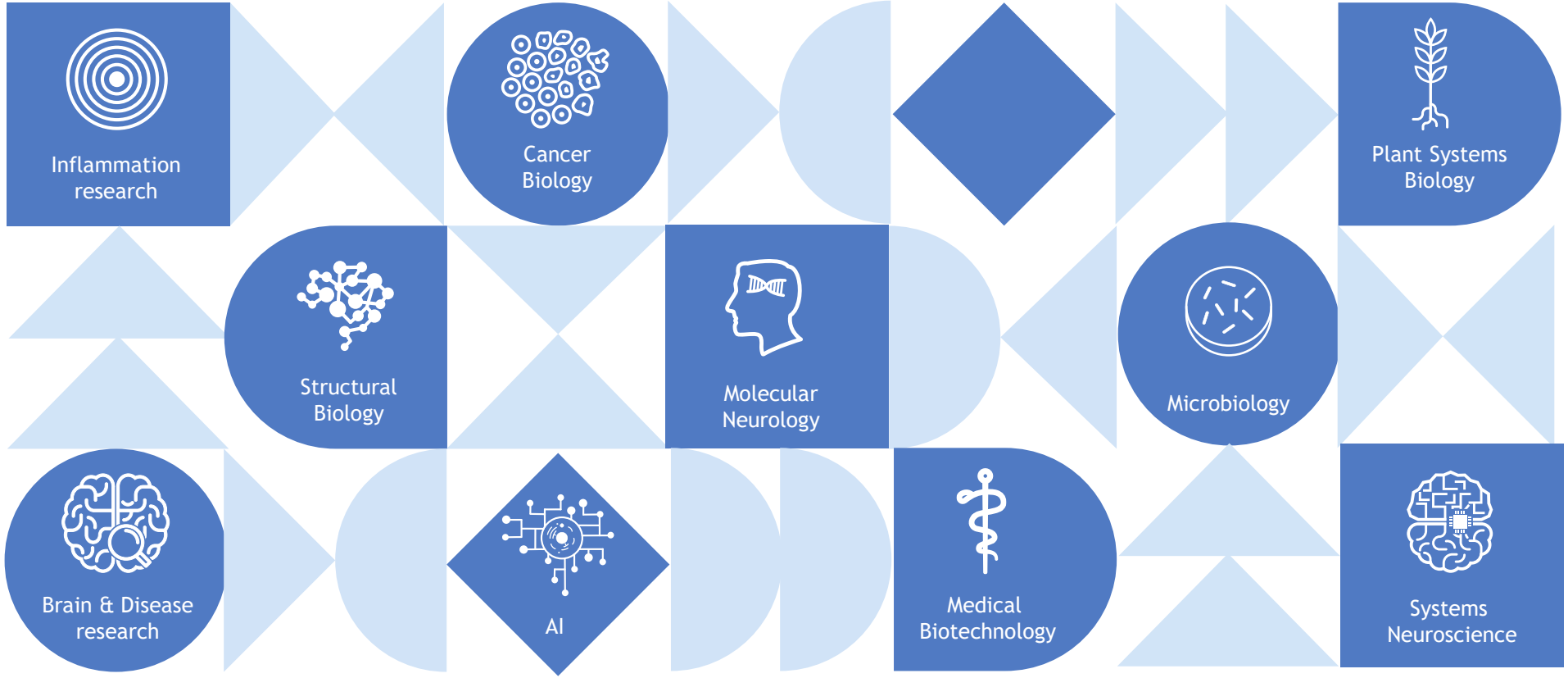
VIB

VIB is a **leading life sciences research institute in Belgium with an entrepreneurial spirit.** We are a vibrant, multidisciplinary, and inclusive community of highly skilled scientists.

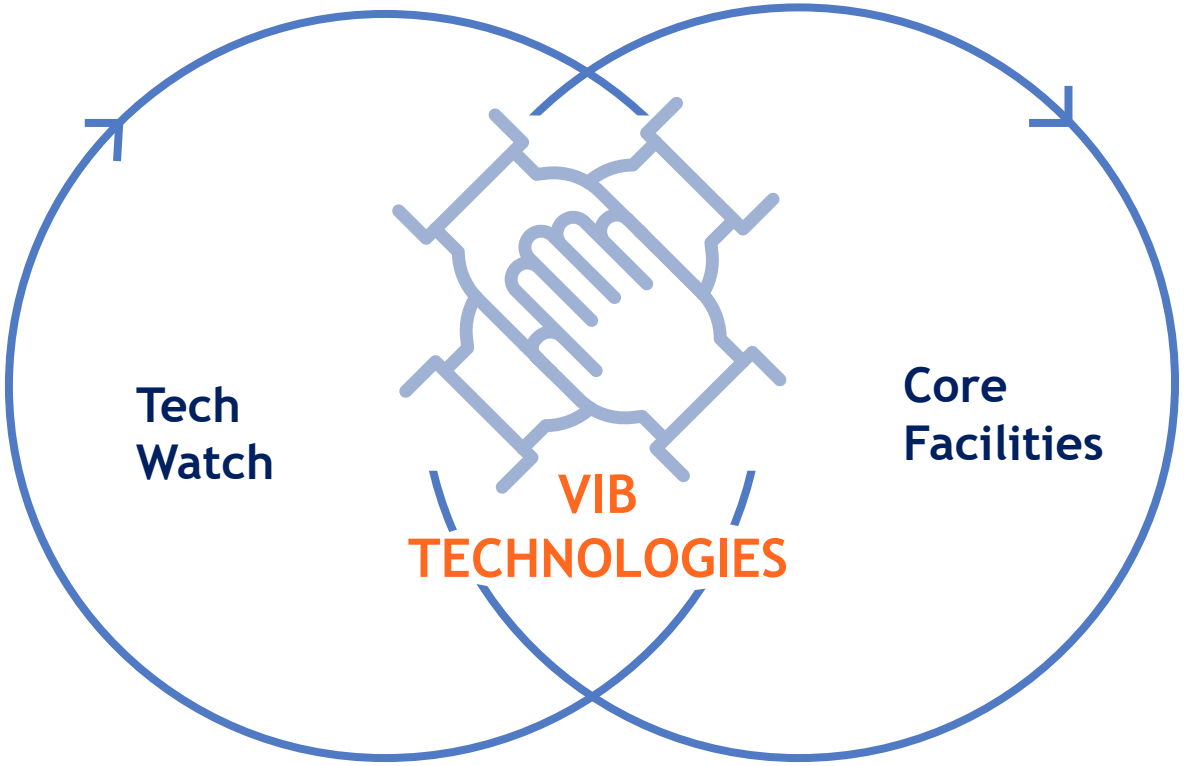


VIB Pioneering life sciences research

10 focused research areas



VIB Technologies



110 FTEs

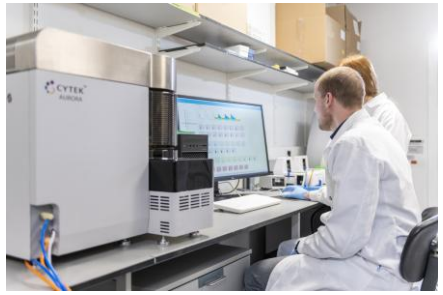
17 nationalities

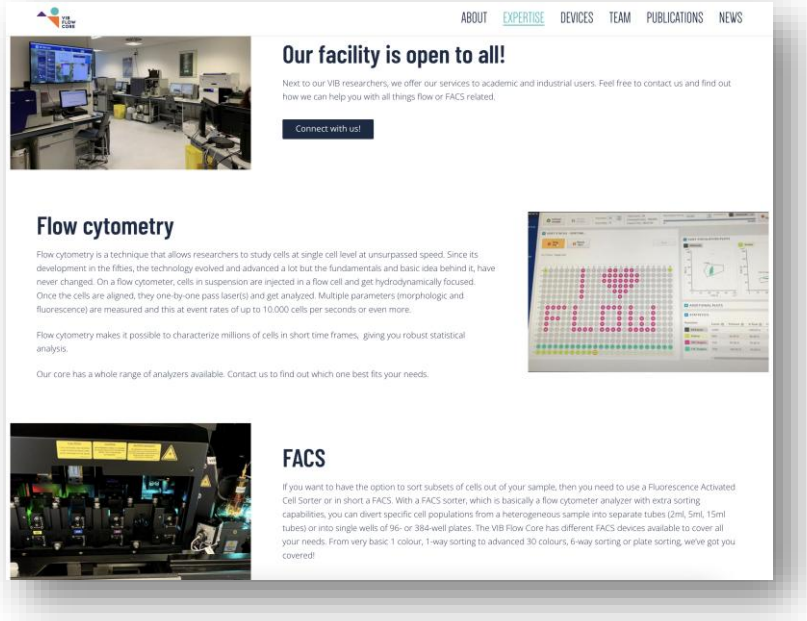
MISSION:

To provide a competitive advantage to VIB researchers and the local life science landscape by enabling access to technology and expertise.

VIB Flow Core Ghent

• Katrien Van der Borght	FC Expert
• Julie Van Duyse	FC Expert
• Elien Ruyssinck	Lab Manager
• Margot Van Zandweghe	Lab Technician
• Janto Malfliet	Jr. Lab Technician
• Gert Van Isterdael	Head of Core





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• Sofie Van Gassen	FC Expert, data analysis
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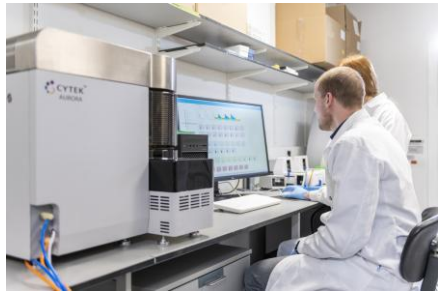
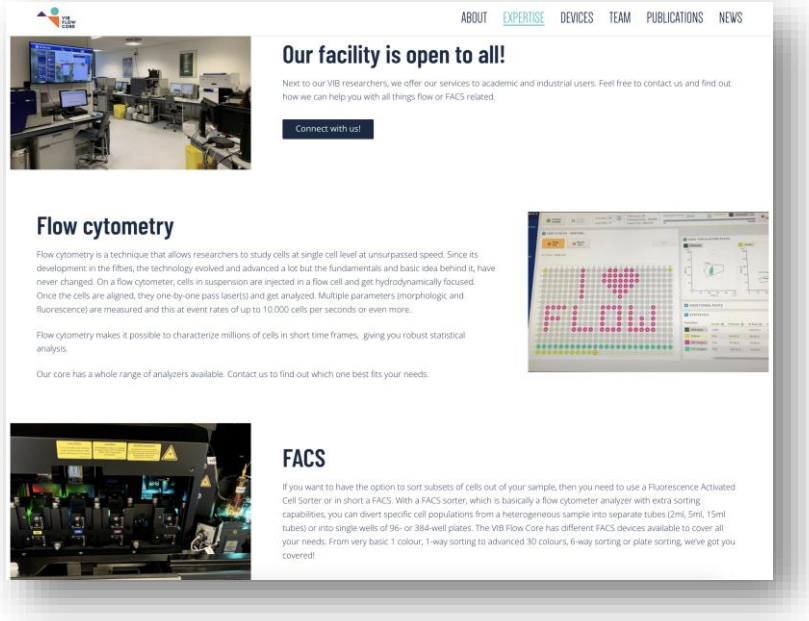




VIB Flow Core's Mission

- Provide quality **service** and access to **state-of-the-art equipment**
  - Specialized staff
  - Quality controlled flow cytometers, cell sorters and multiplex machines
- **Train** current and next generation of scientists
  - Full theoretical training program, hands-on courses
- Help with **design, execution, and analysis** of experiments
- New assay **development**
- Implementation of **new technologies/applications**

All-in service

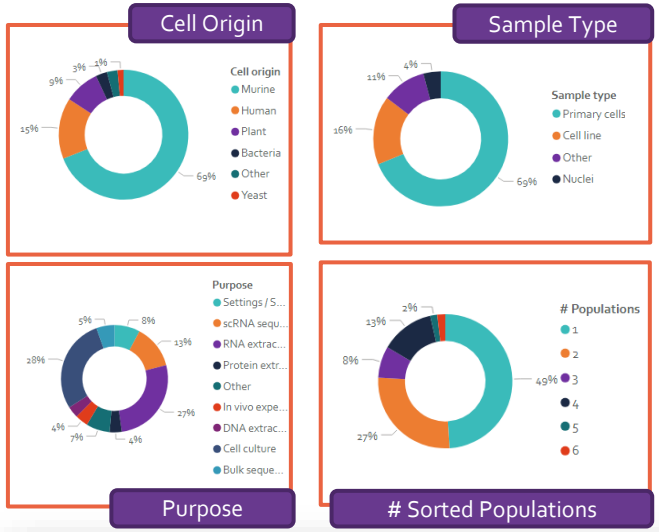
Training

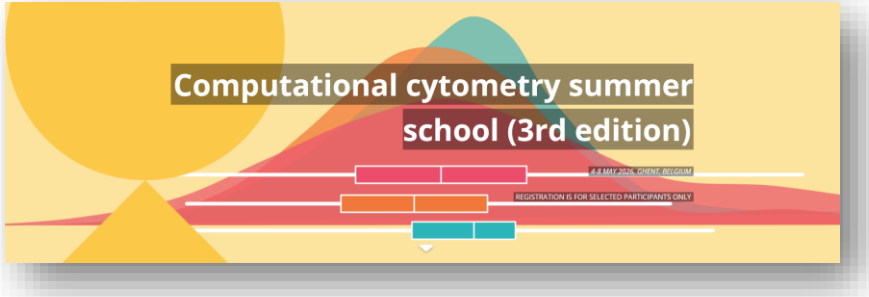
Collaboration

Technology scouting,
adaptation, implementation

VIB Flow Core Ghent in numbers

- Flow Cytometer analyzers: 13
- FACS cell sorters: 8
- Users: >270 active users
- Booked device time: >9.000h usage/year
- Number of sorting experiments: >1100 exp./year
- Training:
  - Flow demos >300 students/year
  - Theoretical introduction sessions >80 participants/year
  - Hands-on trainings >90 participants/year
  - FlowJo trainings >45 participants/year
  - Computational Summer School >40 participants/year





4-8 May 2026, Ghent, Belgium

1. The history and basics of Flow Cytometry

History of Flow Cytometry

Birth of ‘real’ FC is debated, because it combines several technical concepts
→ fluidics, optics, electronics, fluorescence chemistry and computation

1950s-1960s

Coulter

Coulter principle

Electronic counting and sizing of cells in suspension

Fundamental concepts emerge

Foundations

1965-1970s

Fulwyler · Göhde · Sweet

1st cell sorter (1965)

Coulter-based detection coupled to electrostatic droplet sorting

Pulse cytophotometry

Fluorescence-based FC

First commercial FACS by BD (1974)

Birth of FACS

1980s-1990s

FACS popularized

Becomes widely available

Immunophenotyping

Monoclonal antibodies and multi-color fluorescence

Clinical adoption

Hematology and HIV

Commercialization & Immunology Revolution

2000s

8+ colors becomes standard

Functional assays mature

Standardization and advanced software

High-Parameter & Functional Cytometry

2005-2010s

Imaging FC

ImageStream

Mass cytometry

CyTOF

Diversification

2020s-Today

Spectral FC

40-50+ colors possible

Machine learning-assisted analysis

Integration with single-cell omics

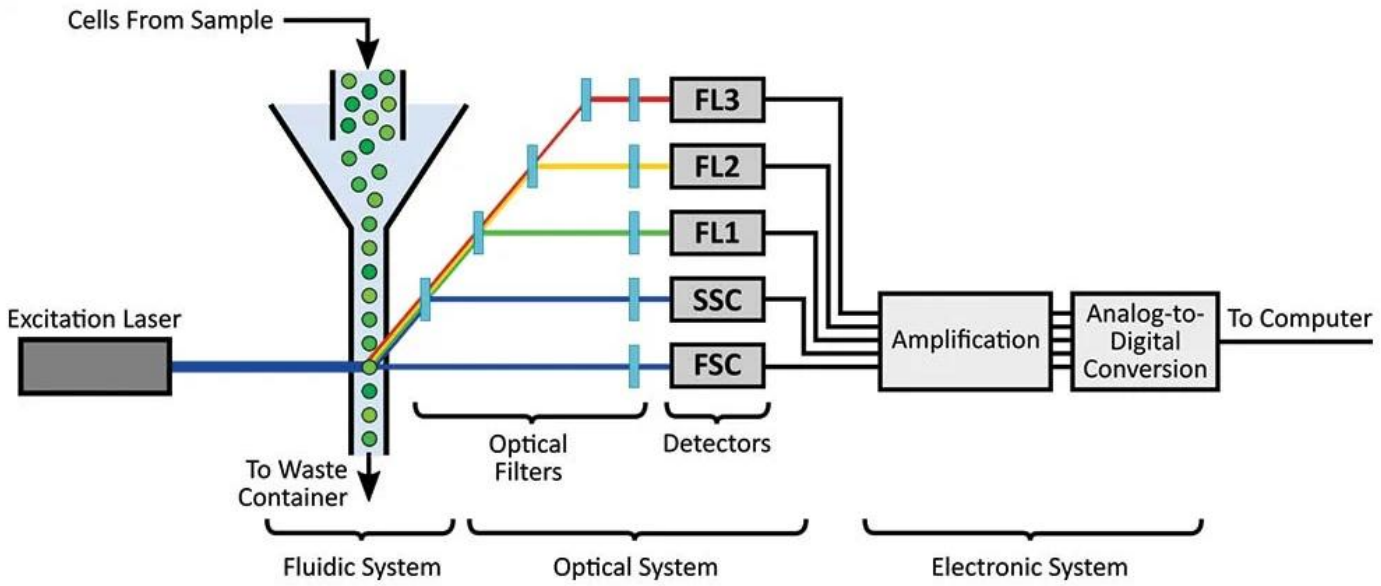
The “Diamond Age”

As a cornerstone technology, flow cytometry has driven major advances in biology, medicine and healthcare.

Basics of Flow Cytometry

Principle of FC did not fundamentally change over the past ~ 65 years

- Enabling rapid, multiparameter single-cell analysis and sorting
- Build your own FC – Open-source - Low Cost - Kit Form
- **CYKIT-K-1:** Kit containing fluidic components, optical components, solid state detectors, electronics hardware



Cytkit

https://microbenotes.com/flow-cytometry/

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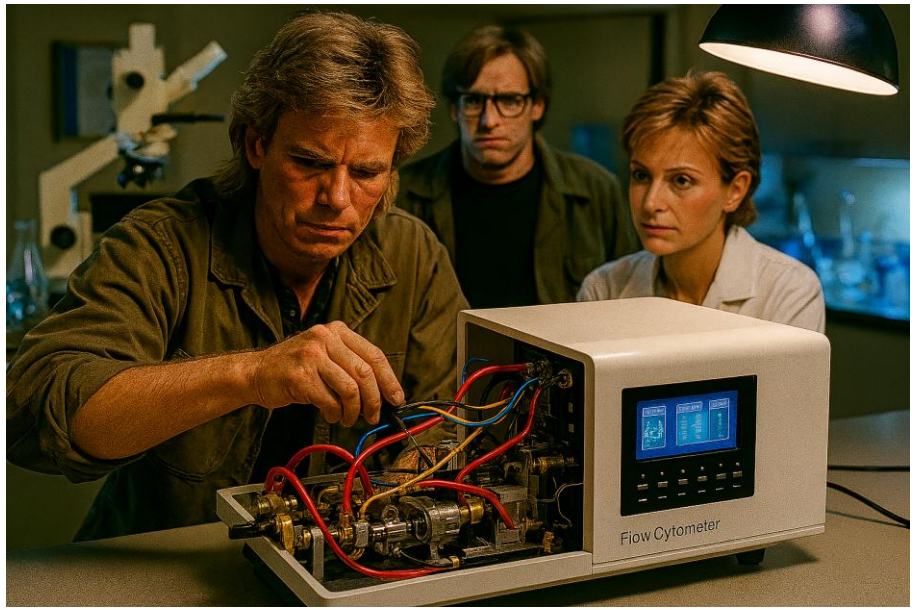

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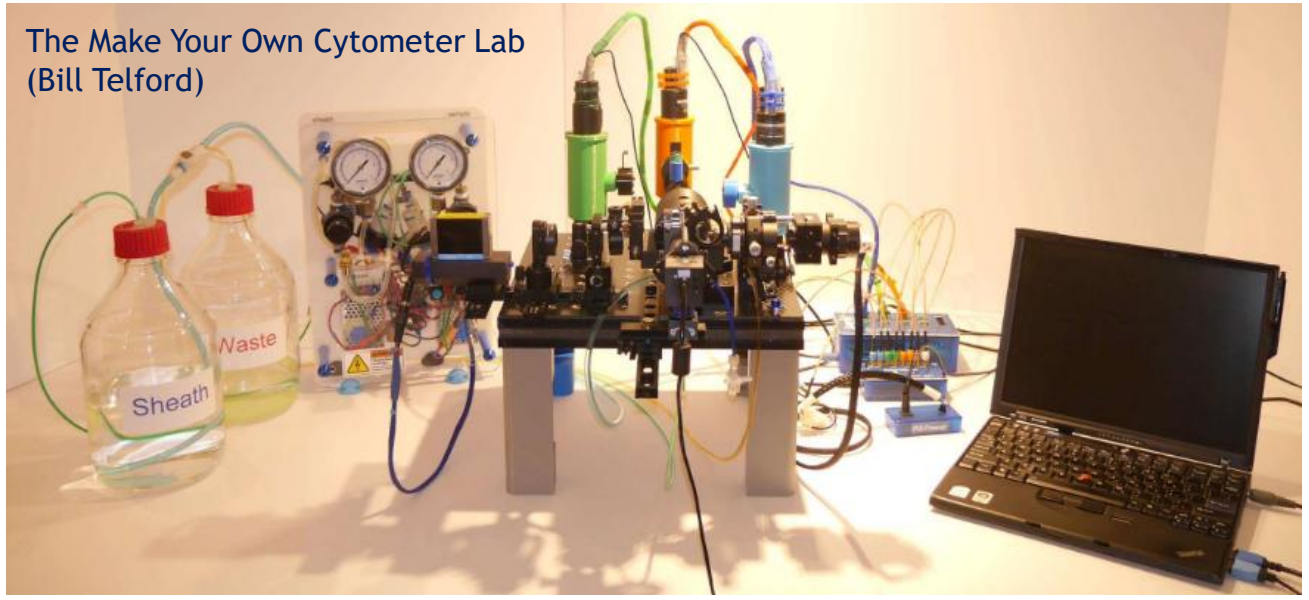


The Make Your Own Cytometer Lab
(Bill Telford)

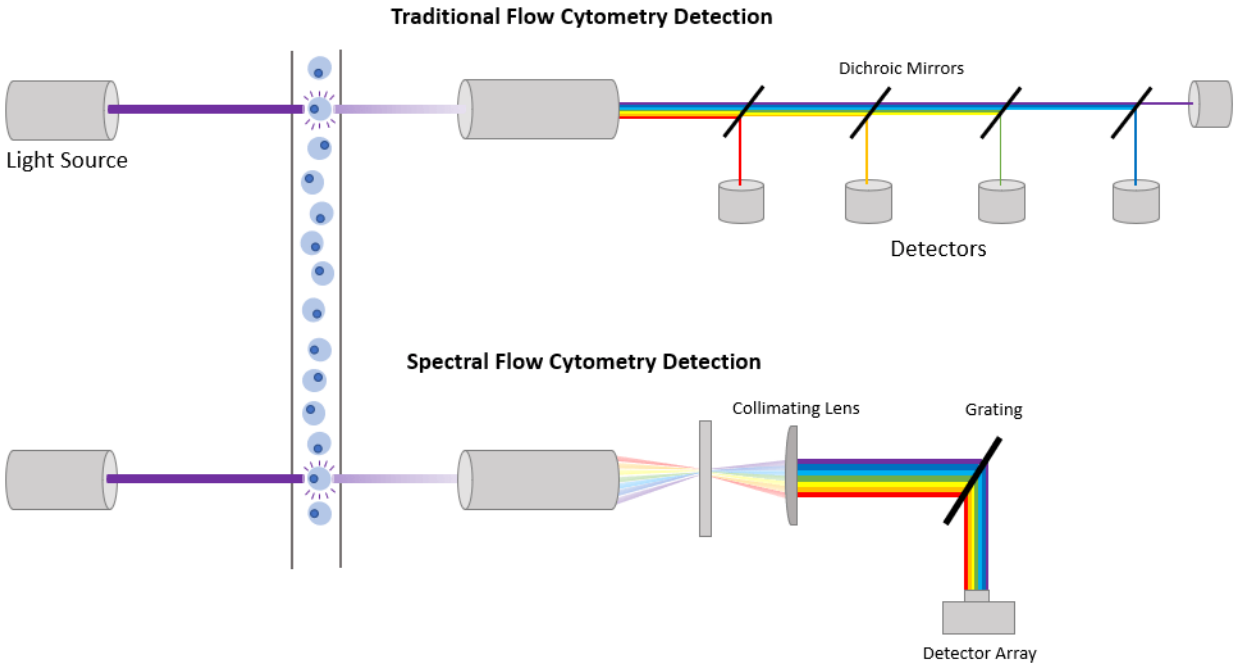
- Teaching & training programs
- Resource-limited labs
- Citizen science / maker communities / Tech nerds / Lab hackers

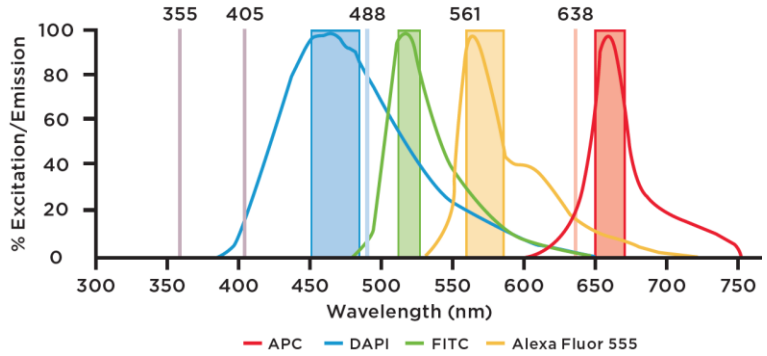
*https://cytometryunderground.com/**Telford**_MYO_Cytometer_UNAM_111120.pdf*

Basics of Flow Cytometry

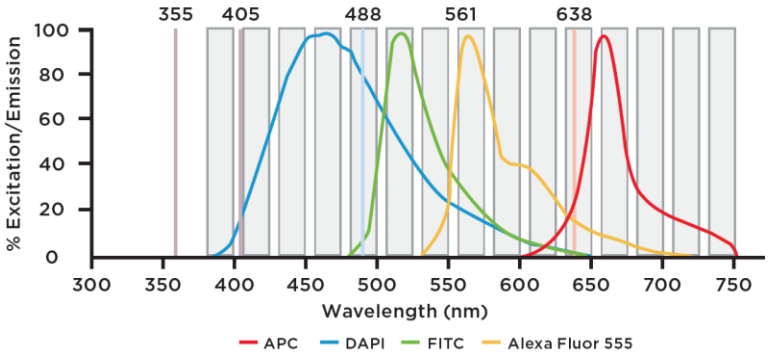
Conventional versus Spectral Flow Cytometry

- DIY Flow Cytometer has:
  488-nm laser, forward scatter, side scatter, **one spectral fluorescence array** (15 channels total)





Part of emitted spectrum detected



Full emitted spectrum detected

https://fluorofinder.com/spectral-flow-cytometers/

https://www.beckman.com/resources/reading-material/application-notes/spectral-flow-cytometry-detailed-scientific-overview

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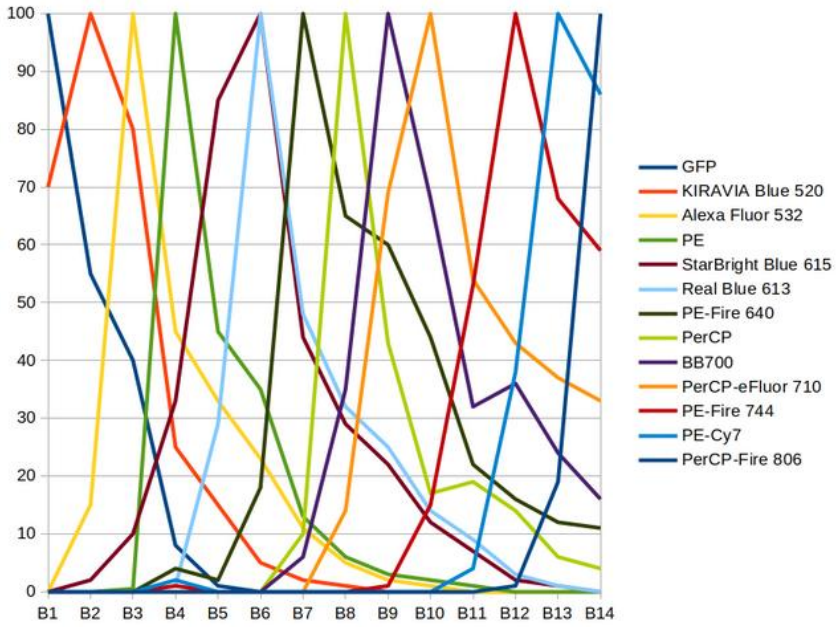


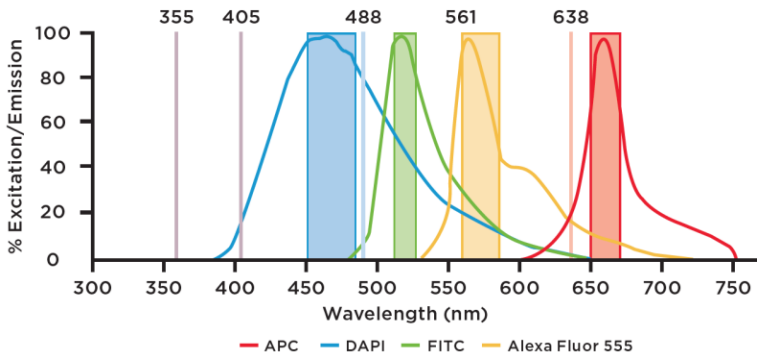
Example of 13 color panel in one laser DIY FC:
GFP, KIRAVIA Blue 520, Alexa Fluor 532, PE, StarBright Blue 615, Real Blue 613, PE-Fire 640, PerCP, BB700, PerCP-eFluor710, PE-Fire 744, PE-Cy7, PerCP-Fire 806

2. High-Parameter Flow Cytometry

High-parameter Cytometry

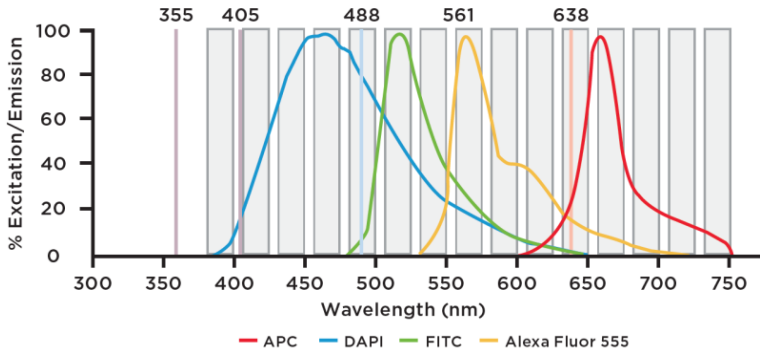
Conventional

- Part of emitted spectrum detected
- # colors = # detectors
- **Max. 28 color panel**



Spectral

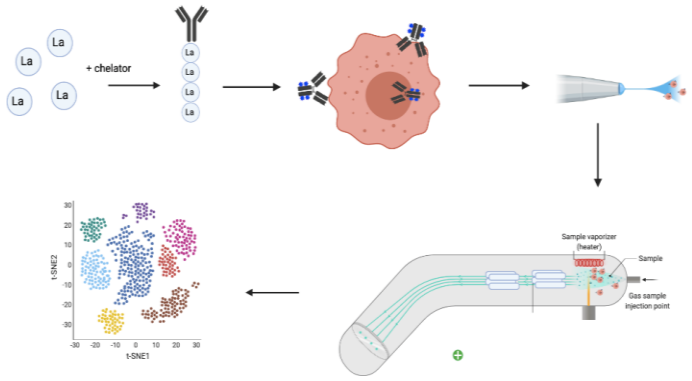
- Full emitted spectrum detected
- # colors < # detectors
- **+50 color panel**



Mass

- Heavy-metal labeled antibody
- Time-of-flight mass spectrometry

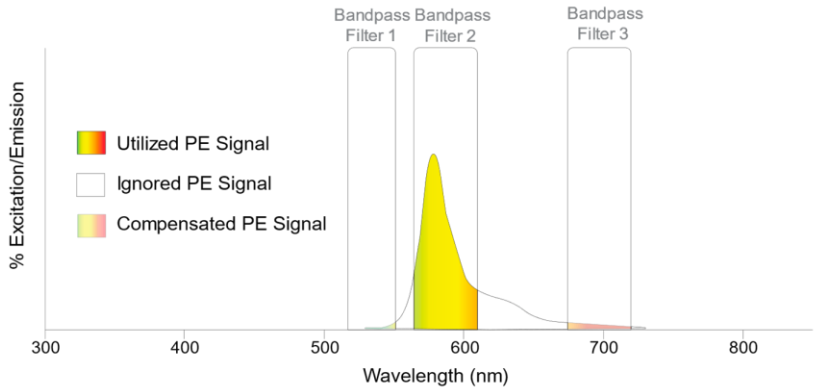
- 48 parameter panel



High-parameter Cytometry

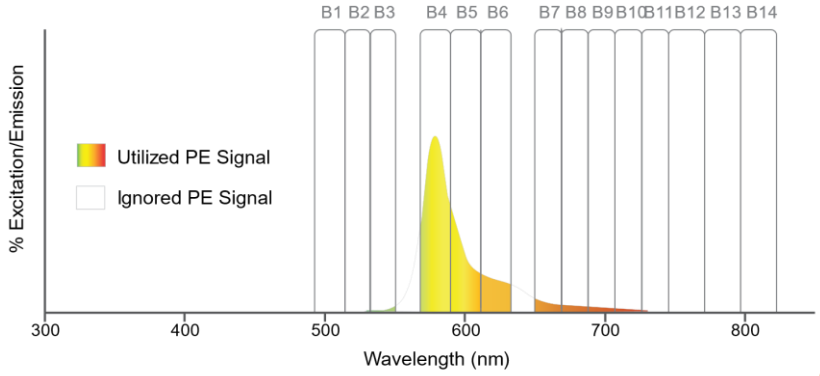
Conventional

- Part of emitted spectrum detected
- # colors = # detectors
- Max. 28 color panel
- Signal in other detectors **= compensated**



Spectral

- Full emitted spectrum detected
- # colors < # detectors
- +50 color panel
- Specific signature of each fluorochrome known **= spectral unmixing**



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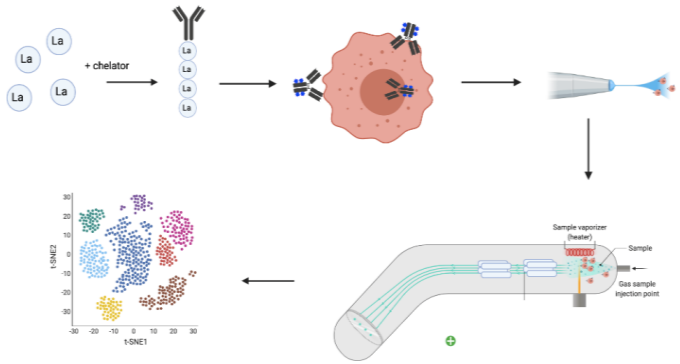
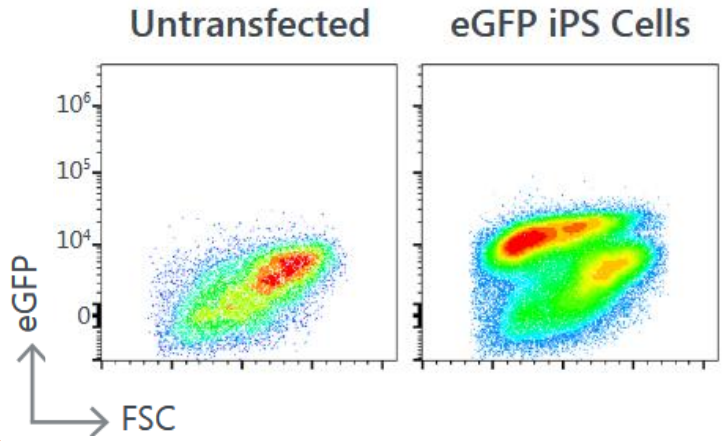


High-parameter Cytometry

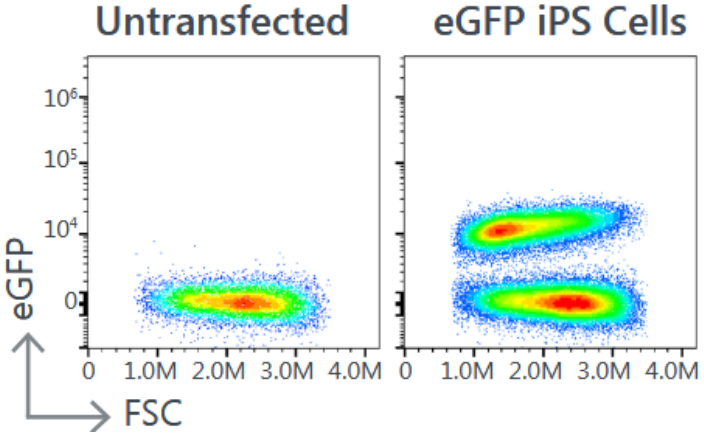
Conventional

- Part of emitted spectrum detected
- # colors = # detectors
- Max. 28 color panel
- Signal in other detectors = compensated
- Autofluorescence problem



Spectral

- Full emitted spectrum detected
- # colors < # detectors
- +50 color panel
- Specific signature of each fluorochrome known = spectral unmixing
- Autofluorescence subtraction



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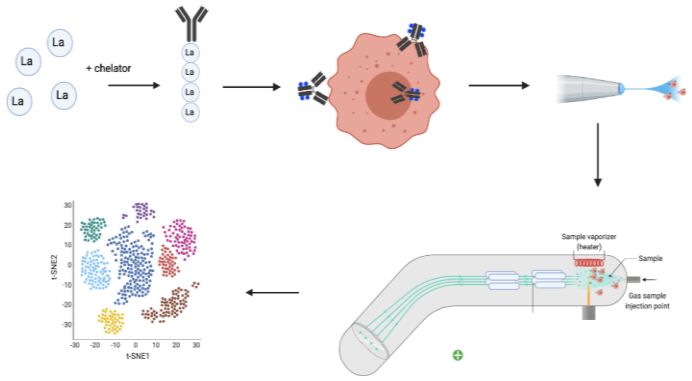
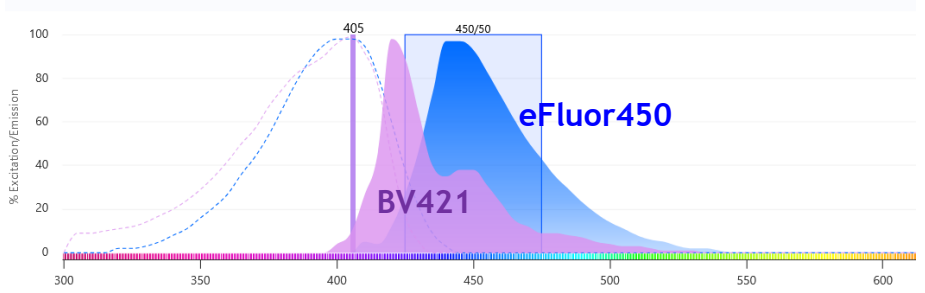


https://welcome.cytekbio.com/hubfs/WebsiteDownloadableContent/DataSheets/N9-20076PowerofAFExtraction.pdf

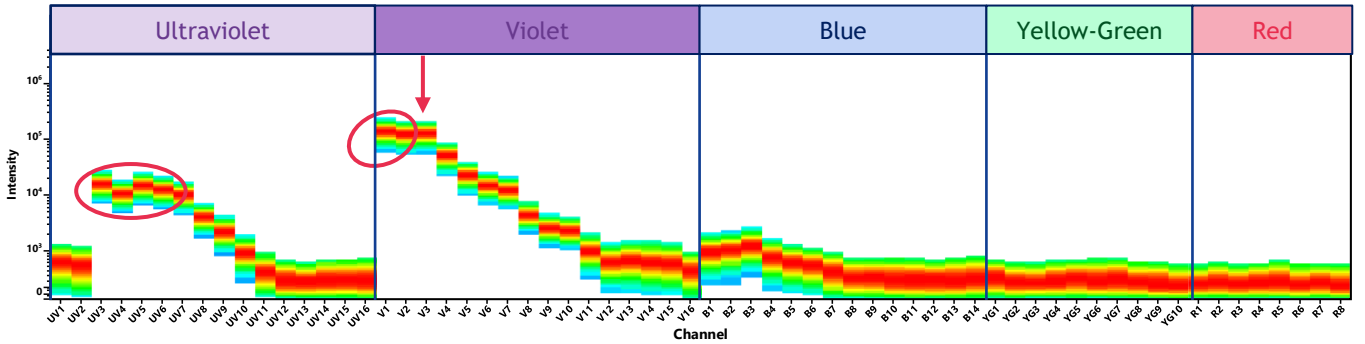
Spectral Flow Cytometry

Spectral fingerprint or signature

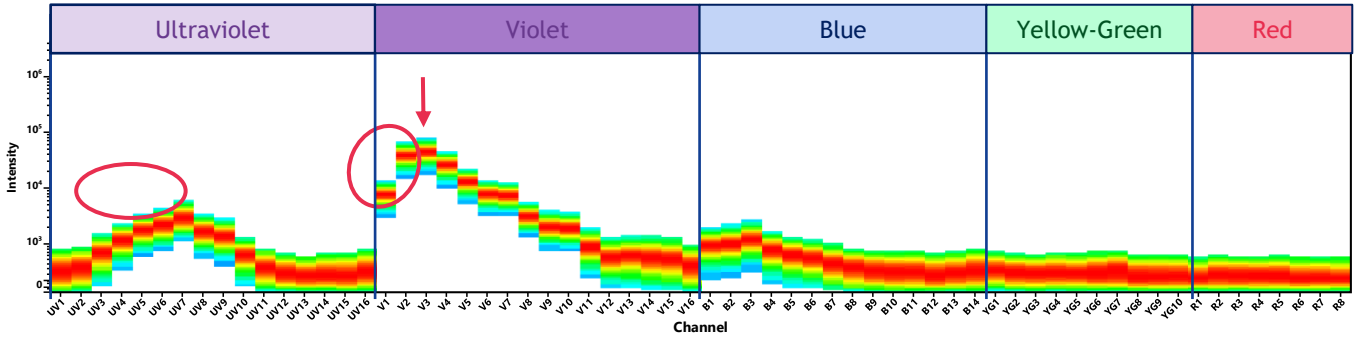
- Unique to fluorochrome
- Overlapping emission
  - Conventional = problem
  - Spectral = same emission max. but different spectrum
- Spectral unmixing of similar fluorochromes is possible



BV421



eFluor450



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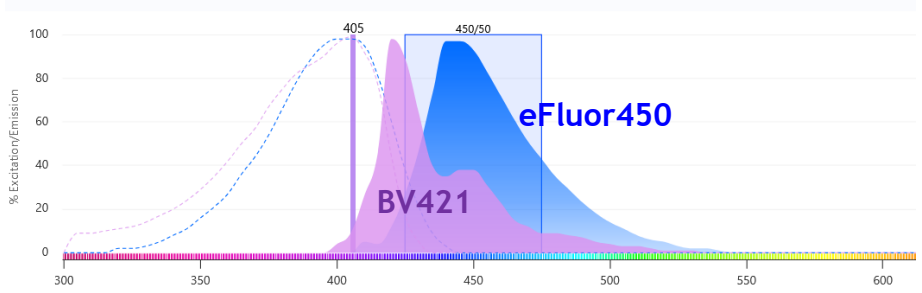
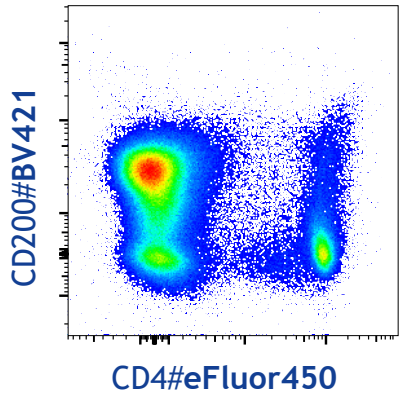


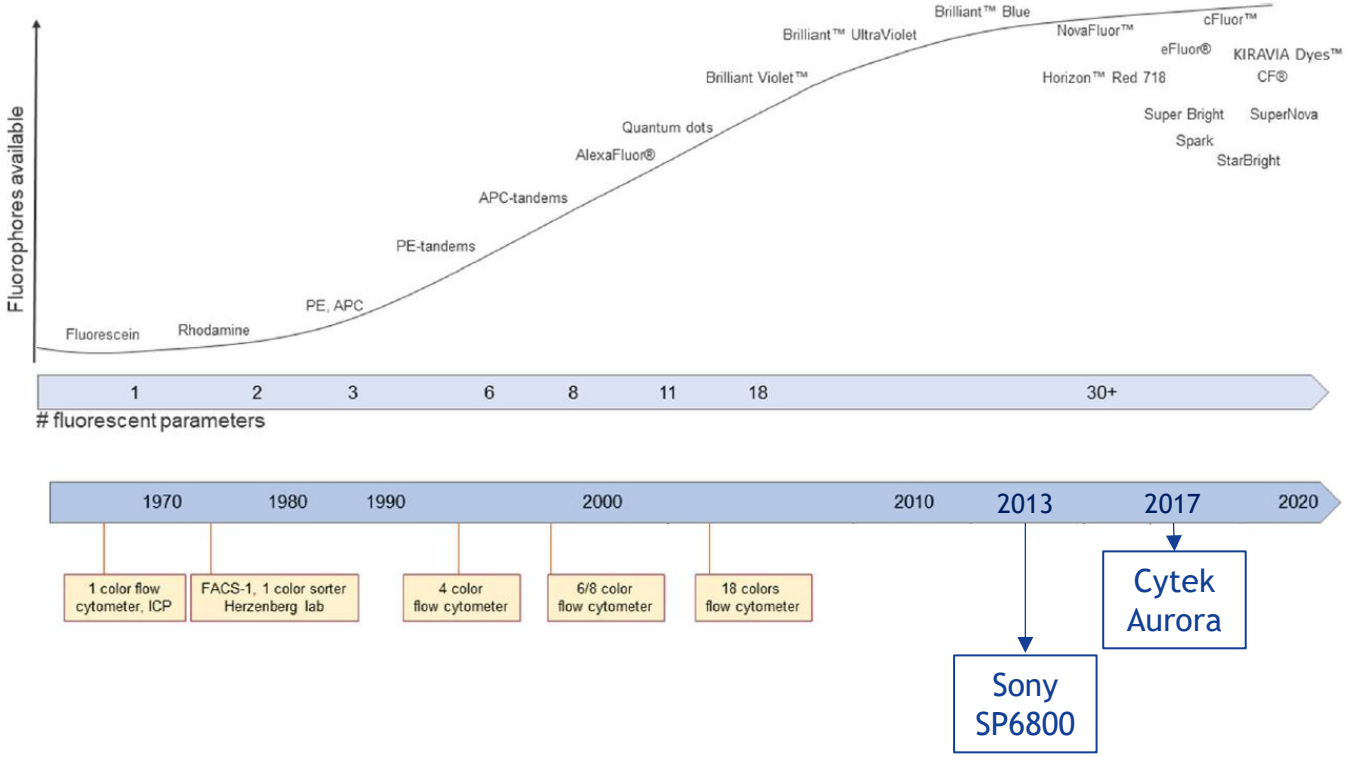


⇨ More colors and thus more markers possible

⇨ multiplexing capabilities increase → Fewer panels needed
→ less sample needed → interesting for rare clinical samples with low cell number

Spectral Flow Cytometry

The “dye revolution”

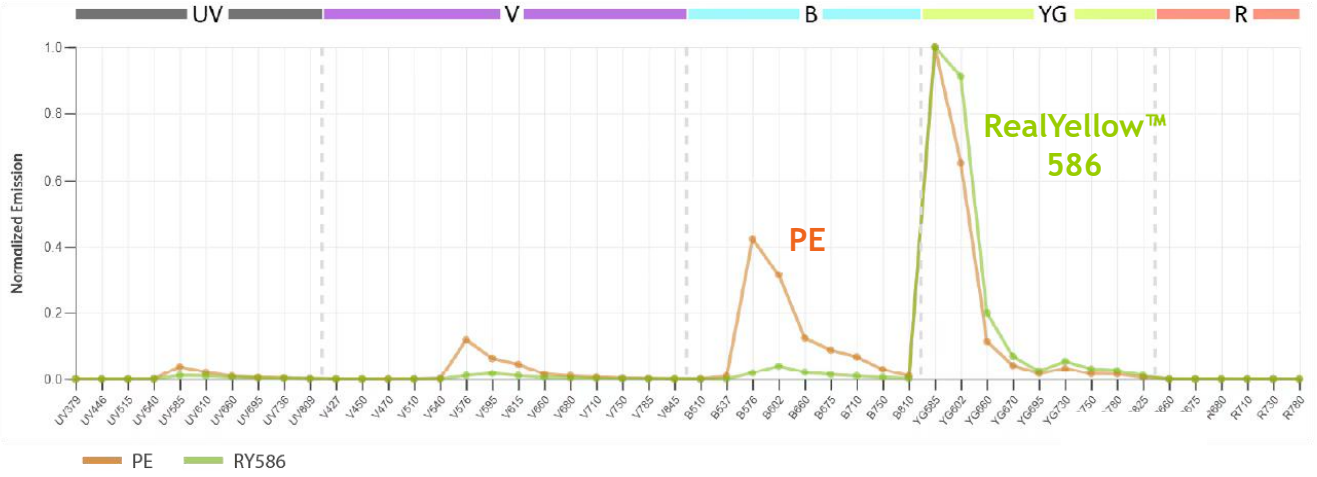


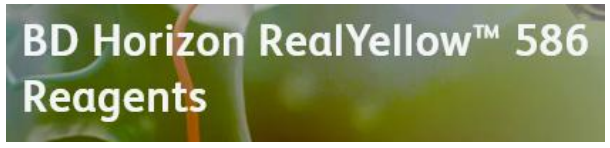
- Many new dyes on the market thanks to spectral FC
- Goal
  - Reduce spillover
  - Minimal cross laser excitation
  - Narrow emission
  - Fill ‘spectral’ gaps, expand panels to >60 dyes
  - Brighter
  - More stable (less issues with fixatives)
  - Extending into near-IR, beyond 820nm
- Conventional FC also benefits from better dyes

https://link.springer.com/article/10.1208/s12248-021-00633-6

Spectral Flow Cytometry

The “dye revolution”





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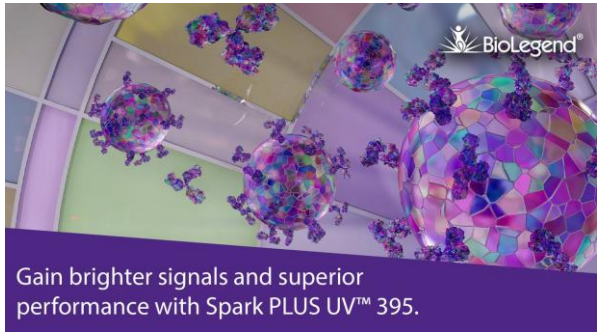
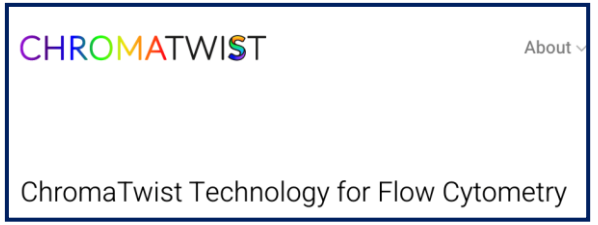
The “dye revolution”

- BD Biosciences
  - Real dyes: RealYellow™, RealBlue™, RealRed™,…
- Biolegend
  - Spark PLUS™
  - Fire™ tandem dyes
- Thermo Fisher
  - NovaFluor™ dyes
- Beckman Coulter
  - SuperNova Fluorescent Polymer Dyes
  - Infrared fluorescent IR820 and IR870 dye
- BioRad
  - StarBright™ dyes
- Cytek Biosciences
  - cFluor® portfolio
- *New, not yet commercialized:*
  - *Chromatwist*





Ultra-high dimensional Flow Cytometry

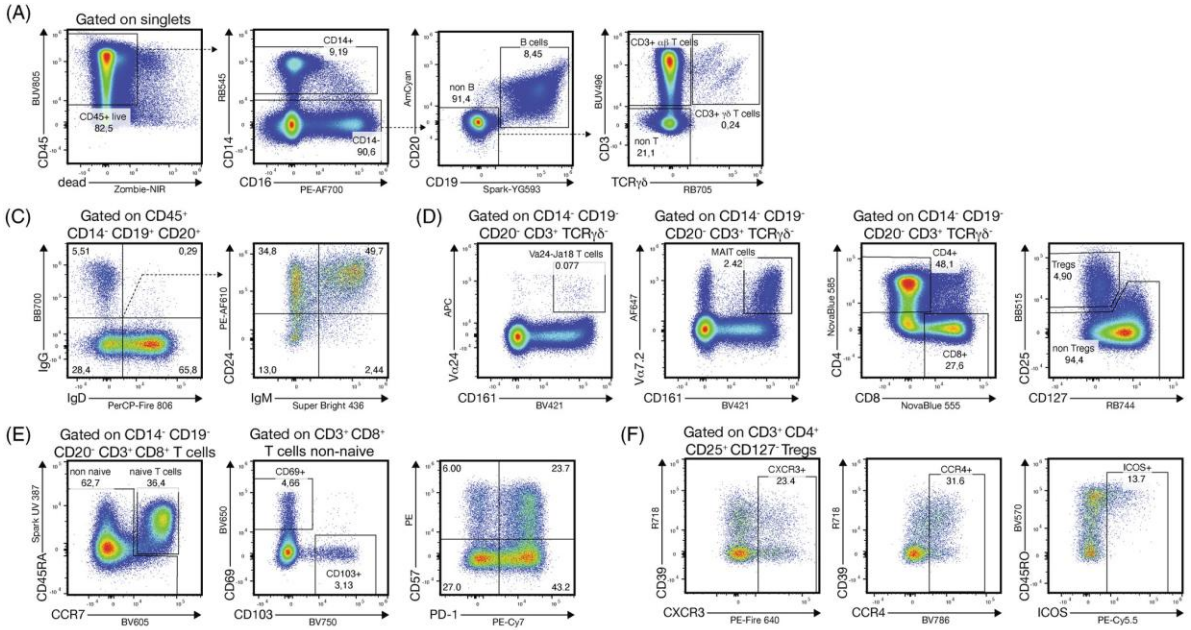
Cytometry PART A | Journal of Quantitative Cell Science | ISAC

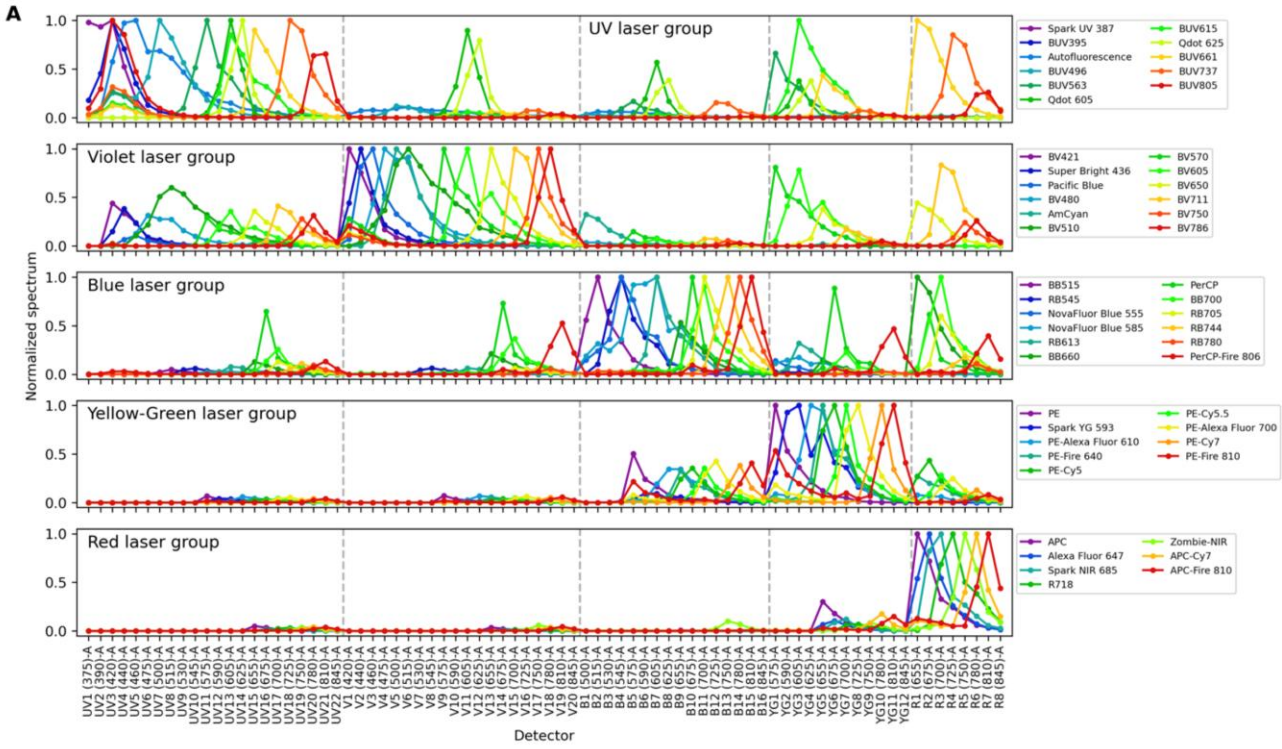
OMIP | Open Access

OMIP-102: 50-color phenotyping of the human immune system with in-depth assessment of T cells and dendritic cells

Andrew J. Konecny, Peter L. Mage, Aaron J. Tyznik, Martin Prlic, Florian Mair

First published: 18 April 2024 | https://doi.org/10.1002/cyto.a.24841 | VIEW METRICS





(B)

List of markers in panel (**total of 50** with Live/Dead):

Lineage markers (19): CD45, CD14, CD16, CD20, CD19, CD2, CD3, TCRγδ, Vα24 TCR, Vα7.2 TCR, CD4, CD8, CD11c, CD141, HLA-DR, CD123, CD303, CD56, Nkp46

B cell phenotyping (4): IgG, IgD, IgM, CD24

T cell phenotyping (20): CD161, CD25, CD127, CD57, PD-1, CD69, CD103, CD45RA, CD45RO, CCR7, CXCR3, CCR4, ICOS, CD39, BTLA, CD27, CD28, CD38, TIGIT, KLRG1

APC phenotyping (6): CD86, CD40, CD11b, CD163, FcER1, CD1c

High-parameter Spectral Cytometry

In 2026, most vendors have a spectral FC on the market

	Cytek Aurora	Sony ID7000	BD FACS Discover A8	Thermo Xenith	Agilent Opteon	Beckman Coulter Mosaic
Laser Config.	≤5	≤7	≤5	≤6	≤5	≤6
Fluorescent Channels	≤64	≤186	≤72	≤51	≤73	≤88
Fluorescent Detectors	APD	PMT	APD	PMT	APD	APD
Unmixing Model	Ordinary Least Squares (OLS)	Weighted Least Squares-based (WLSM)	SpectralFX	Ordinary Least Squares (OLS)	Least Squares Regression	LSM Unmixing or Poisson Unmixing

Present at VIB Flow Core Ghent

Demo at VIB Flow Core Ghent

Differences in:

- Hardware
- Software
- Unmixing algorithms
- Nomenclature
- Single stain controls
- Autofluorescence MGMT
- Post-unmixing actions
- Data output

Need for standardization!

High-parameter Cytometry

Conventional

- Part of emitted spectrum detected
- # colors = # detectors
- **Max. 28 color panel** (per tube)
- Signal in other detectors
  = compensated
- Autofluorescence problem

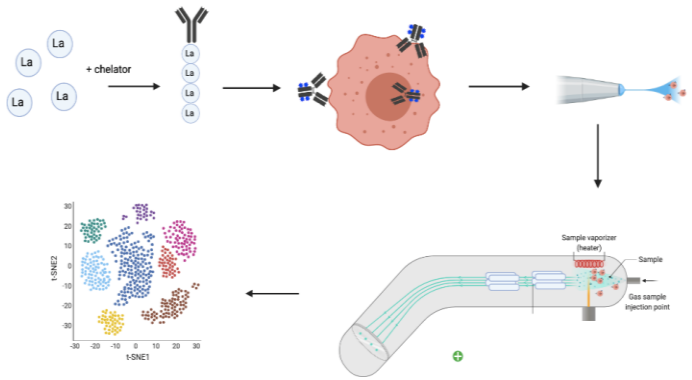
Spectral

- Full emitted spectrum detected
- # colors < # detectors
- **+50 color panel**
- Specific signature of each fluorochrome known
  = spectral unmixing
- Autofluorescence subtraction

Mass

- Heavy-metal labeled antibody
- Time-of-flight mass spectrometry

- **48 parameter panel**



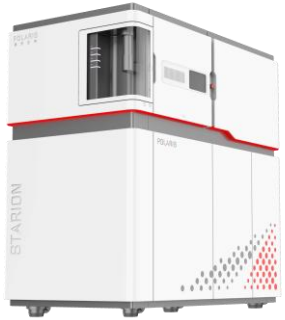
High-parameter Mass Cytometry

VIB FLOW CORE

Two main players on the market

- CyTOF™ single-cell proteomics

- Polaris Starion™ mass cytometry system



Example of a Biological Application

CyTOF reveals predictive biomarkers for response and survival in lung cancer immunotherapy

Conventional panel in spectral fluorescence	25 surface markers*
Additional simultaneous coverage on on CyTOF system	+25 intracellular/functional markers*

MMRF Biomarker for identification of drug targets in multiple myeloma trial

MERCK[1] | pembrolizumab
Predictive biomarker of survival in pembro Phase 1 trial

BMS[1] | nivolumab
Predictive biomarker of response in combination nivo Phase 2 trial

NOVARTIS[1] | CAR T
Predictive biomarker of response in CAR T

→ Unique predictive biomarker

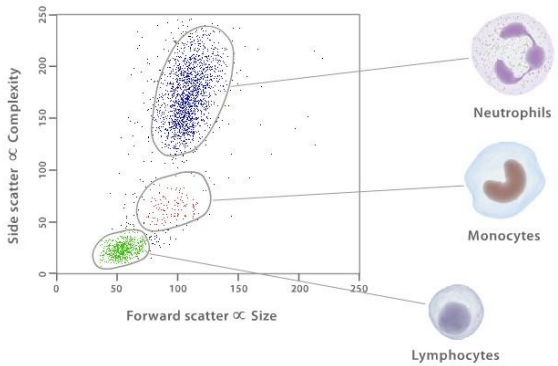
Borders between technologies are fading > Mass Spectrometry + Flow Cytometry = Mass Cytometry

3. Imaging Flow Cytometry

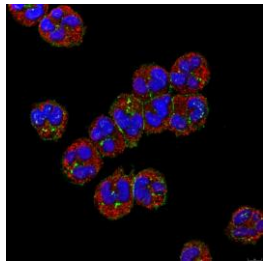
Imaging Flow Cytometry

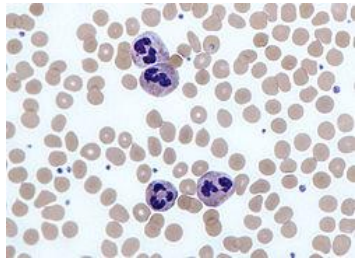
Merges flow cytometry speed with microscopy-level single-cell imaging

Flow Cytometry

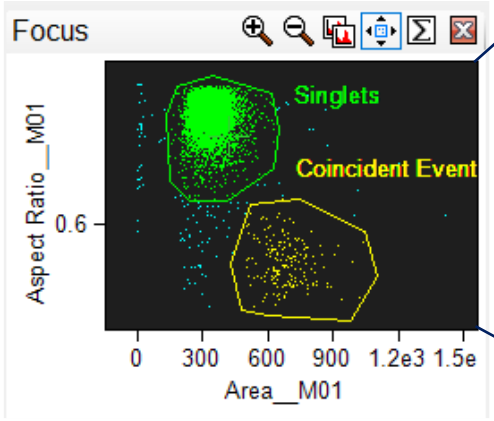


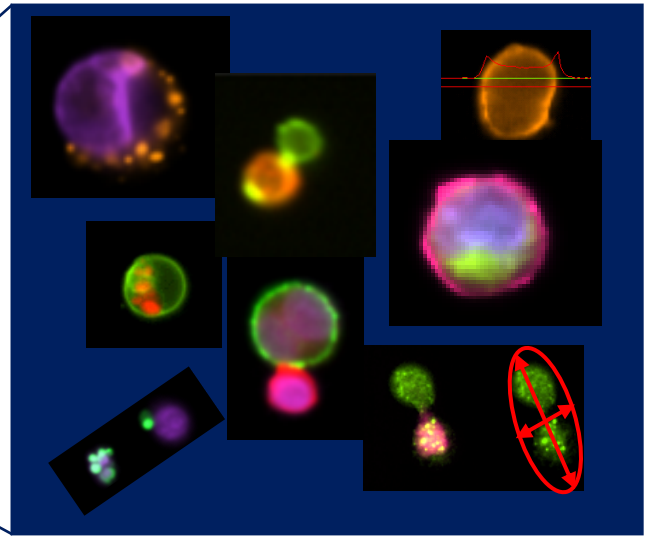
Microscopy





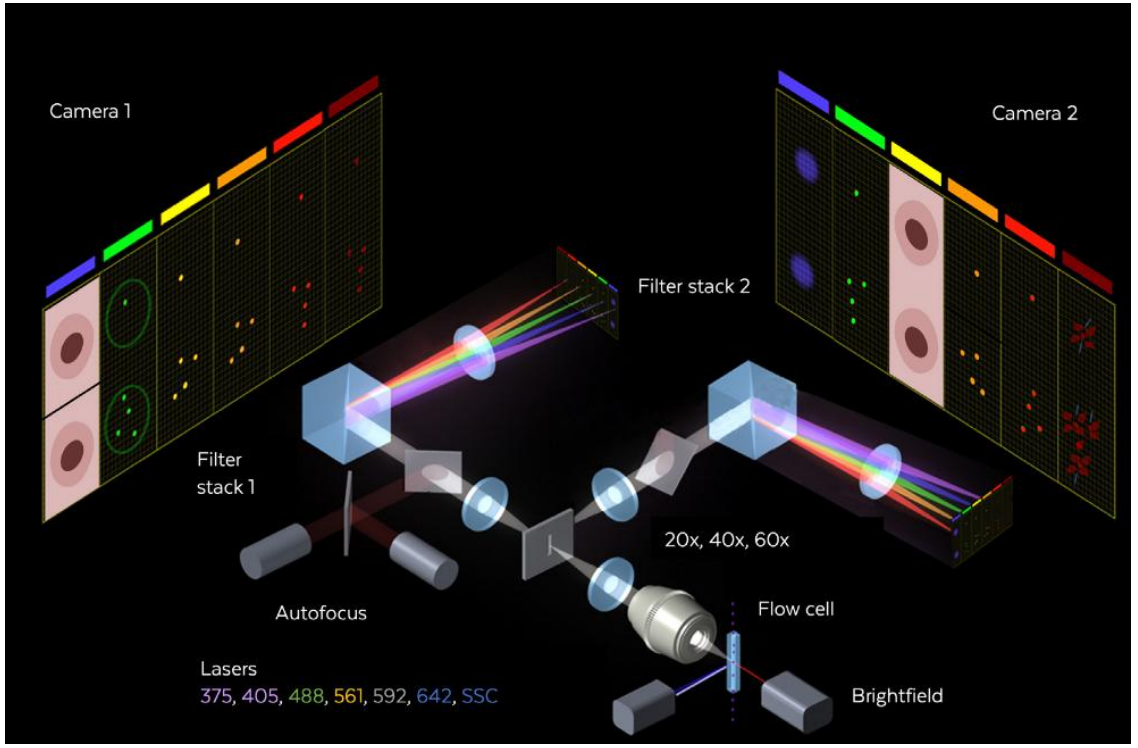
Imaging Flow Cytometry





Imaging Flow Cytometry

Merges flow cytometry ‘speed’ with microscopy-level single-cell imaging



Cytek® Amnis® **ImageStream**®X Mk II
imaging flow cytometer

First commercial instrument launched in **2005**

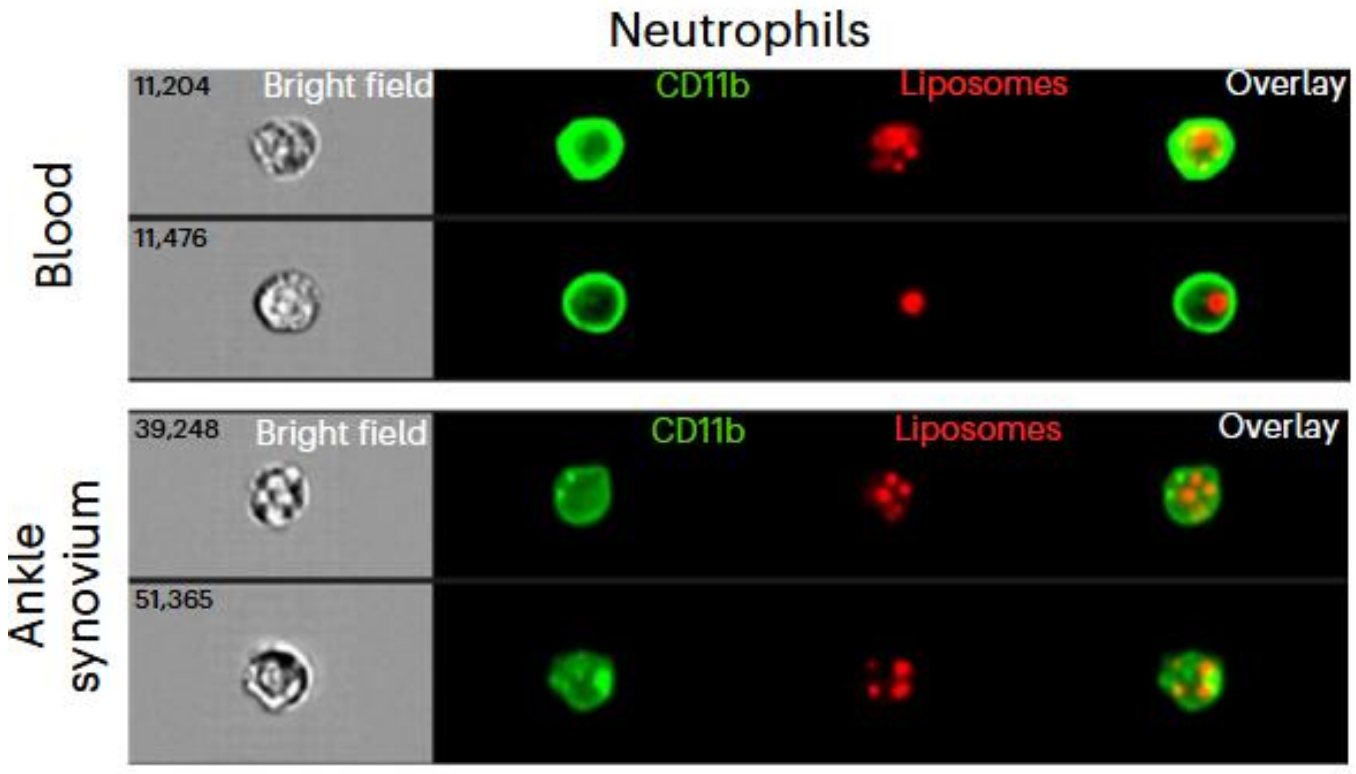
Run at ~1,000/s for routine experiments
to balance throughput and image quality

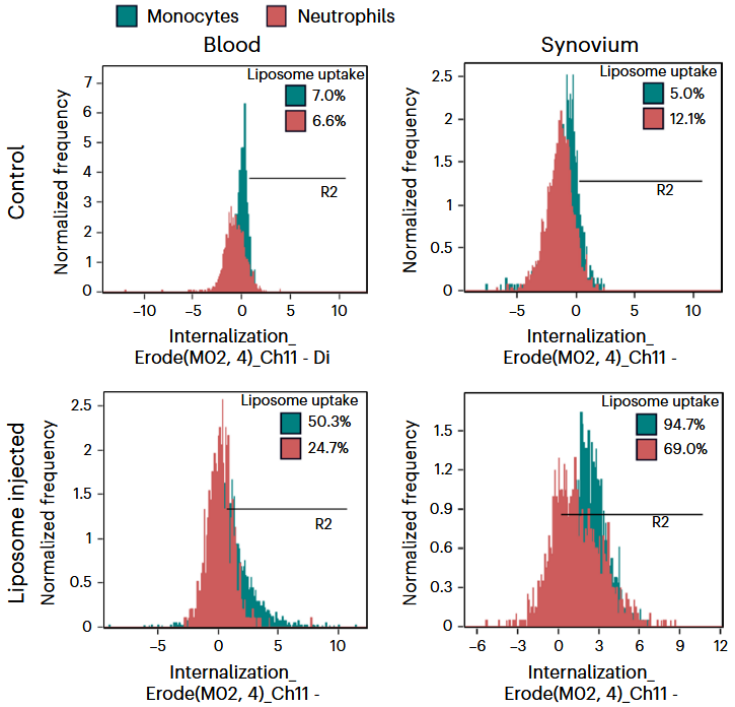
Imaging Flow Cytometry

Example of a Biomedical Application

Do myeloid cells actively transport liposomes to arthritic joints thereby fueling inflammation?

Yes → **Visual confirmation and quantification by imaging FC** that these cells internalize liposomes in blood and inflamed synovium





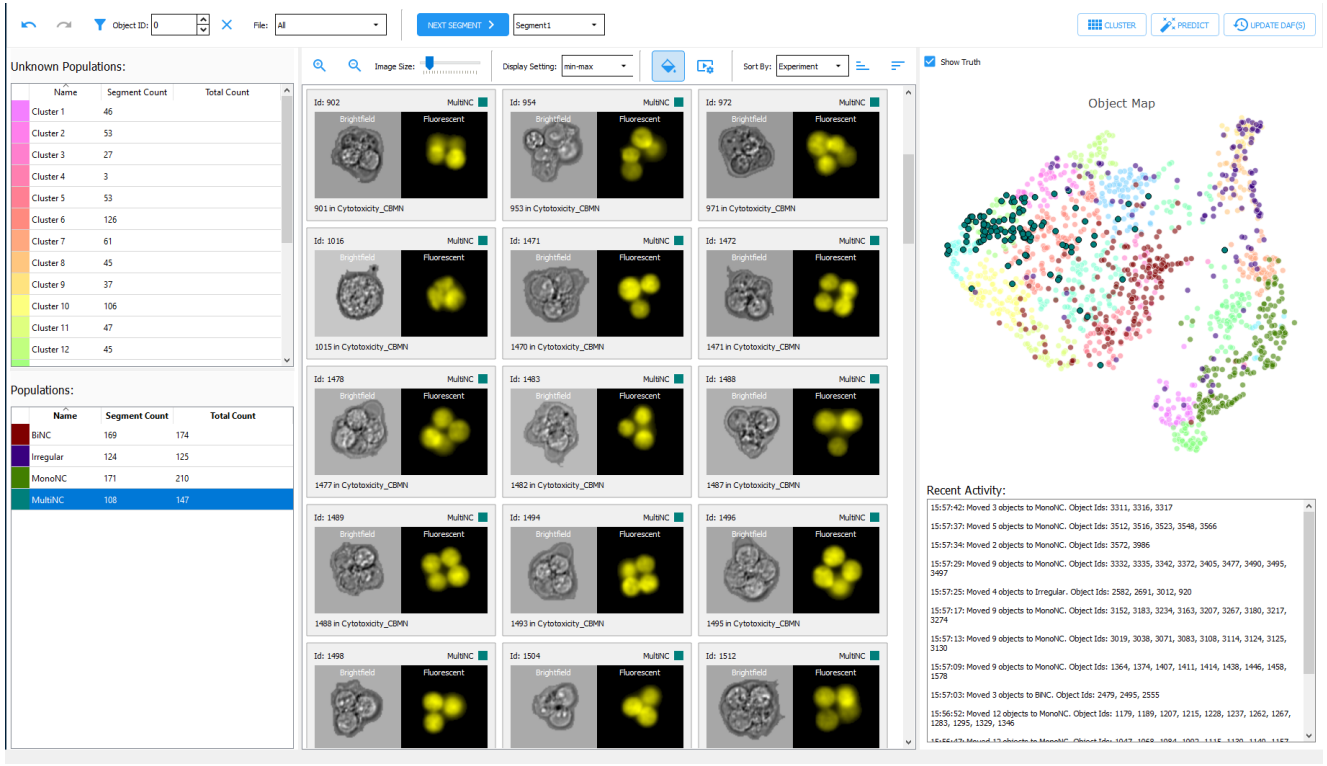
Julie Van Duyse

Deprez et al. Nat Nanotechnol, 2023

Imaging Flow Cytometry meets AI

Amnis® AI Image Analysis Software

- Next generation of image analysis software
- Visually hand-tag multiple cell types → train the software to identify those populations in unknown samples



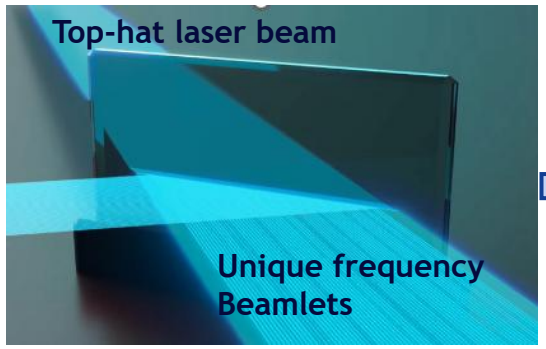
- Population clustering
- Deep Learning
- Accuracy analytics to verify that results match the expectation

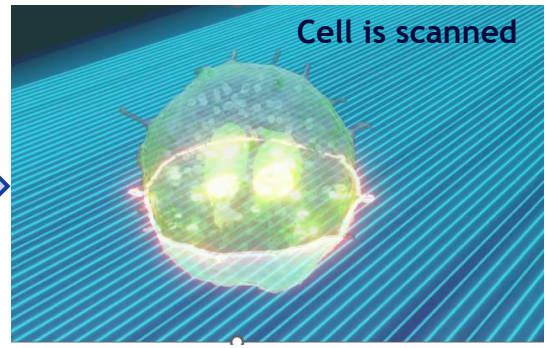
Low throughput + No sorting possible

Imaging FC transitions to high-throughput analysis and cell sorting

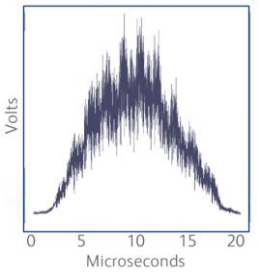
BD FACSDiscover™ A8 (Analyzer) and S8 (FACS) use BD CellView™ Image Technology

FIRE Microscopy: Fluorescence Imaging using Radiofrequency-tagged Emission (*FIRE*)





Generates a highly complex pulse



Mathematically derived image

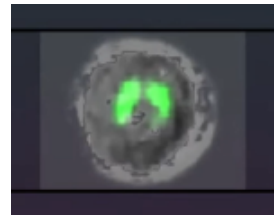


Image laser beam (blue)

Waveform

No cameras!

6 imaging PMT detectors

Lower resolution images compared to camera-based FCs like ImagestreamX

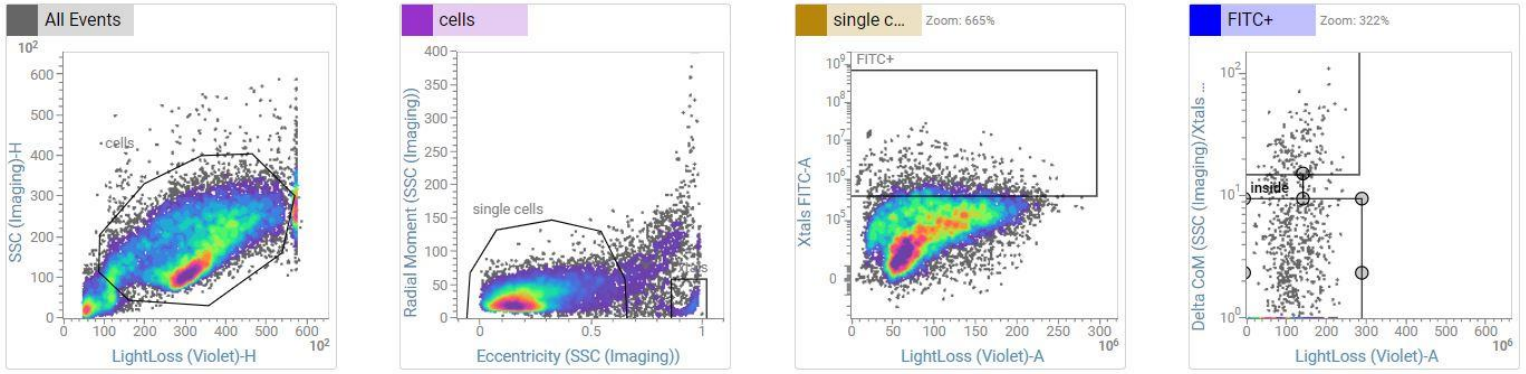
High throughput + Sorting possible → 12.500 images per second and instantly sort on image-derived parameters

New biological insights using BD FACSDiscover™ S8

Example of a Biomedical Application

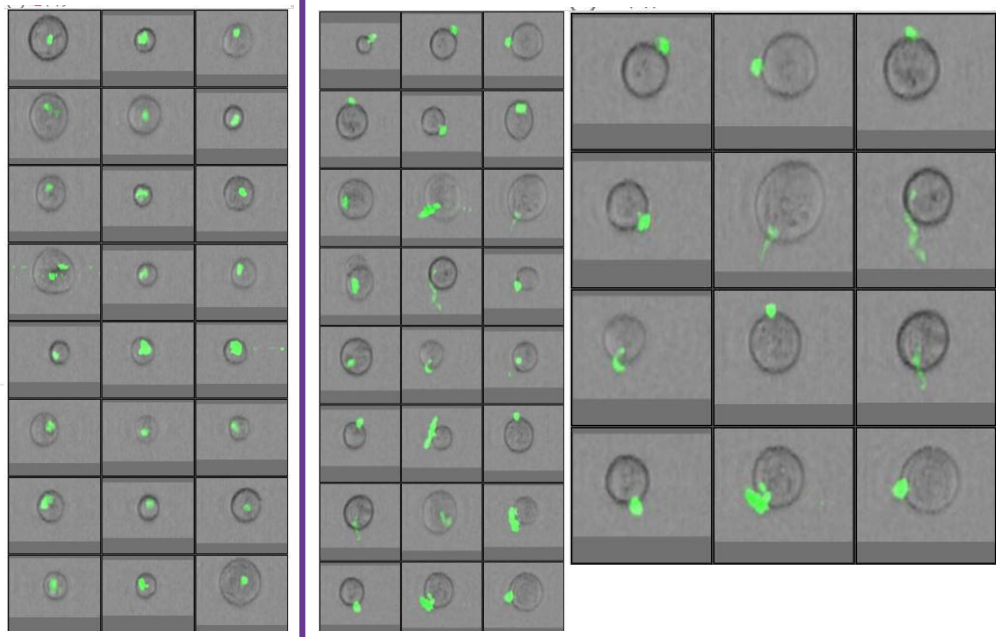
Assess uptake of immunogenic protein crystals

Only sort cells that phagocytosed crystals



Unpublished data

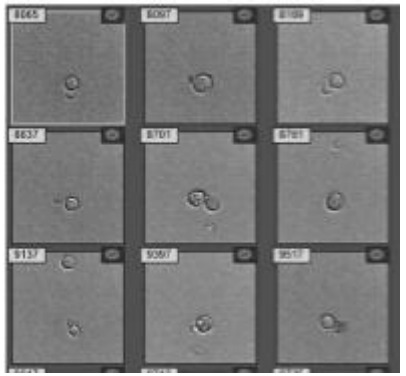
inside | outside



Imaging Flow Cytometry: The Future Is Now

VIB FLOW CORE

- Attune™ **CytPix**™ from Thermo Fisher



High-throughput FC with a **high-speed brightfield camera** that captures images at 6,000 images/sec of a configurable subset of events

Label-free imaging to analyze e.g. cell morphology, sample quality, …

- **VisionSort** from Thinkcyte- 'Ghost Cytometry'

THINKCYTE

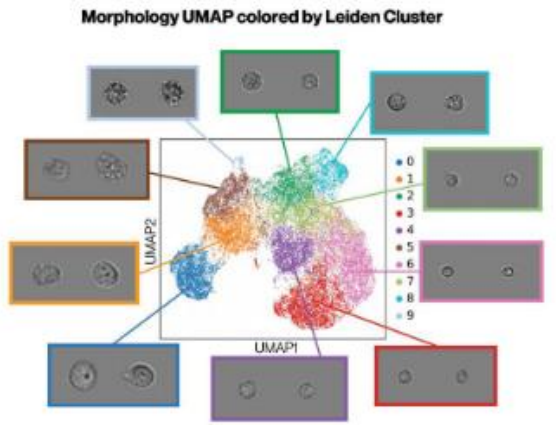
Merges fluorescence FC with high-dimensional **AI-driven morphological profiling** to create a cellular fingerprint

Label-free, image-free cell sorting based on cell morphology

- **REM-I** from Deepcell

Brings deep-learning-powered microscopy into live, label-free single-cell sorting

Deepcell®



Imaging Flow Cytometry: The Future Is Now

VIB FLOW CORE

- VERLO™ cell sorter from NanoCellect

NanoCellect

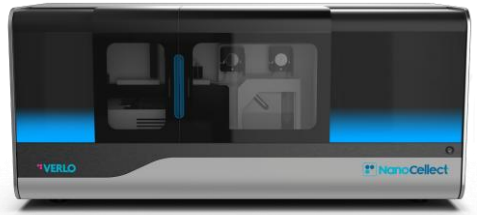
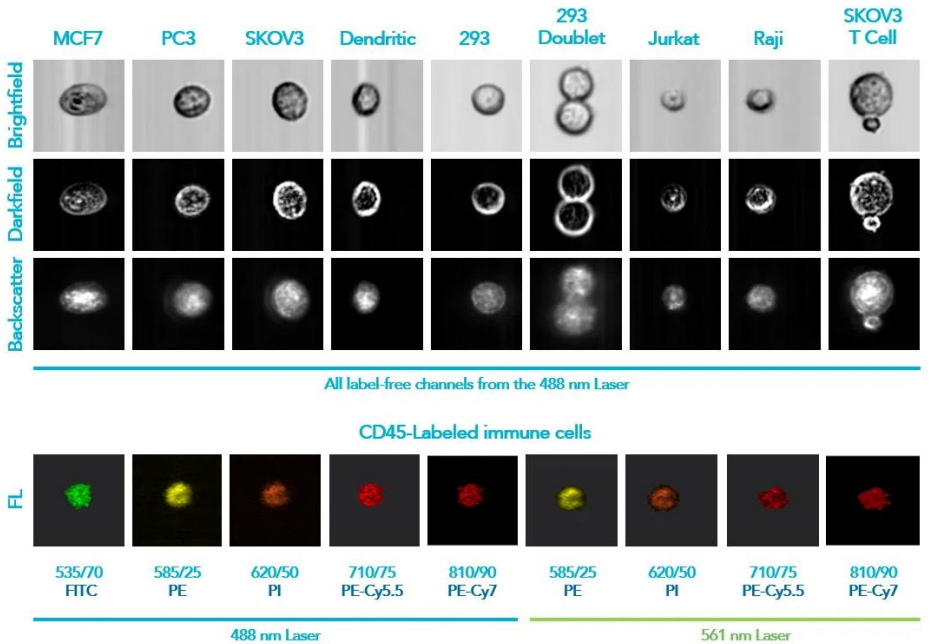


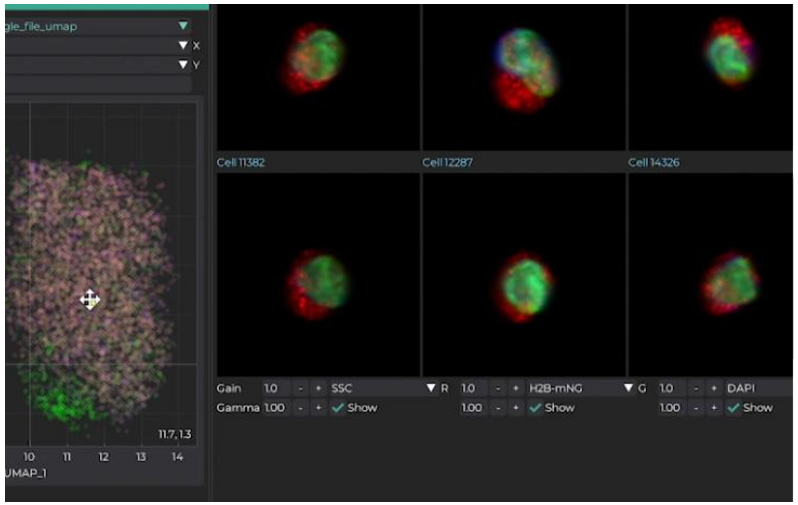
Image-guided sorter combined with gentle microfluidic-based cell sorting to visualize and sort cells based off image features and cell morphology

3 label-free channels and 9 fluorescent channels



- ZOMP™ imaging flow cytometer

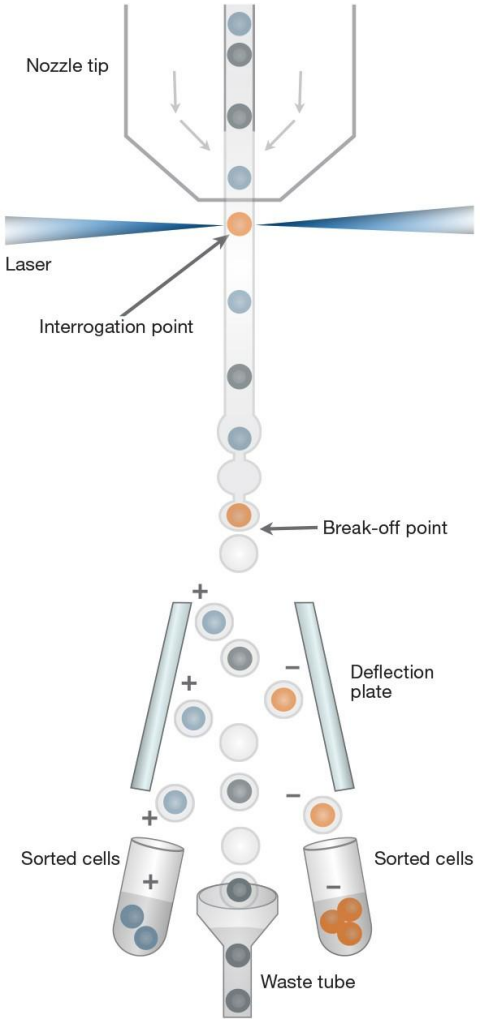
  Multicolour **3D** imaging cytometry to quantify complex cell structures



4. Cell Sorting Innovations

Conventional Cell Sorting

- Key vendors
  - BD Biosciences
  - Beckman Coulter
  - Sony Biotechnology
  - Cytek Biosciences (Aurora CS can be used as conventional)
  - Thermo Fisher (BigFoot is hybrid device)
- Established technology
  - Droplet cell sorters
  - 3-5 laser
  - Up to 30 fluorescent parameters
  - 2- to 6- way sorting
  - Multi-well plate sorting option
  - Cuvette based or jet-in-air systems



https://www.bioradiations.com/expanding-flow-core-capabilities-by-shifting-routine-sorts-to-self-sort-on-the-s3e-cell-sorter/

Conventional Cell Sorting

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  - BD Biosciences
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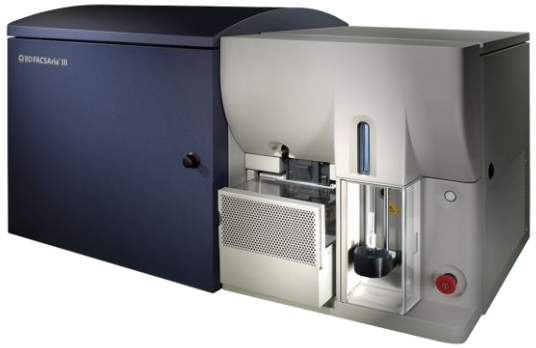
  - 3-5 laser
  - Up to 30 fluorescent parameters
  - 2- to 6- way sorting
  - Multi-well plate sorting option
  - Cuvette based or jet-in-air systems
- End of an era?



Cell Sorting Innovations

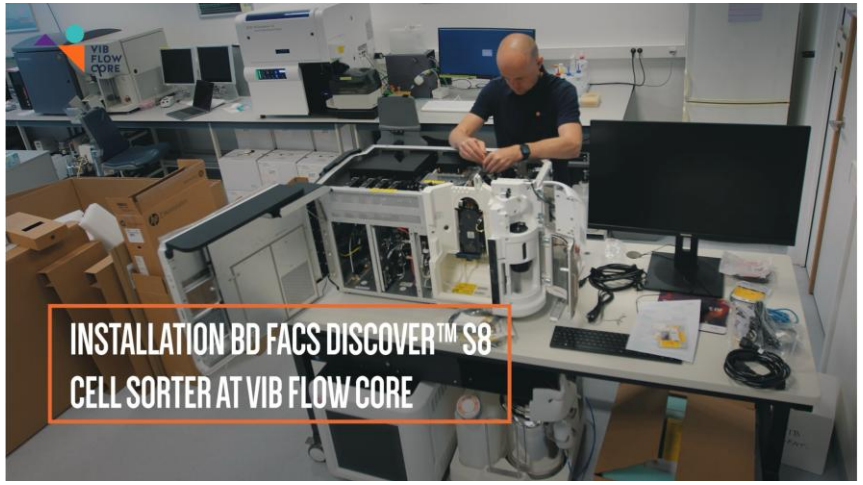
- Spectral cell sorters
- Image based spectral sorting
- Cartridge based / microfluidic cell sorters

Spectral Cell Sorting

- Key vendors
  - BD Biosciences
  - Sony Biotechnology
  - Cytek Biosciences
  - Thermo Fisher
- Based on established technology, enhanced with Spectral capabilities:
  - Droplet cell sorters
  - 3-5 laser
  - Up to 182 fluorescent parameters
  - 2- to 6- way sorting
  - Multi-well plate sorting option
  - Cuvette based or jet-in-air systems

Image based spectral cell sorting

- BD FACSDiscover™ S8 Cell Sorter
  - 5 laser image based spectral cell sorter
  - BD CellView™ technology with 15 imaging derived parameters
  - 6 imaging detectors
  - BD SpectralFX™ with 78 fluorescent detectors
  - 6-way sorting
  - Fast index plate sorting
  - 85, 100, 130 µm nozzle



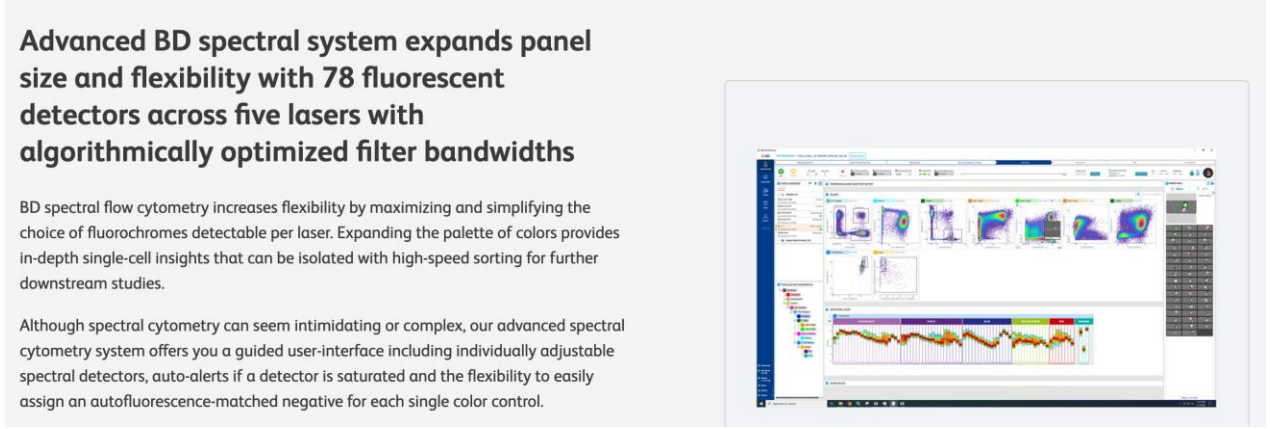
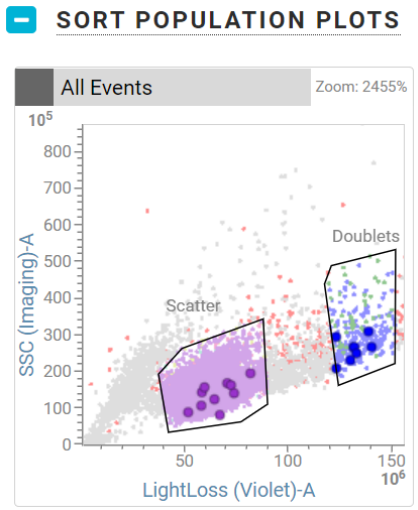
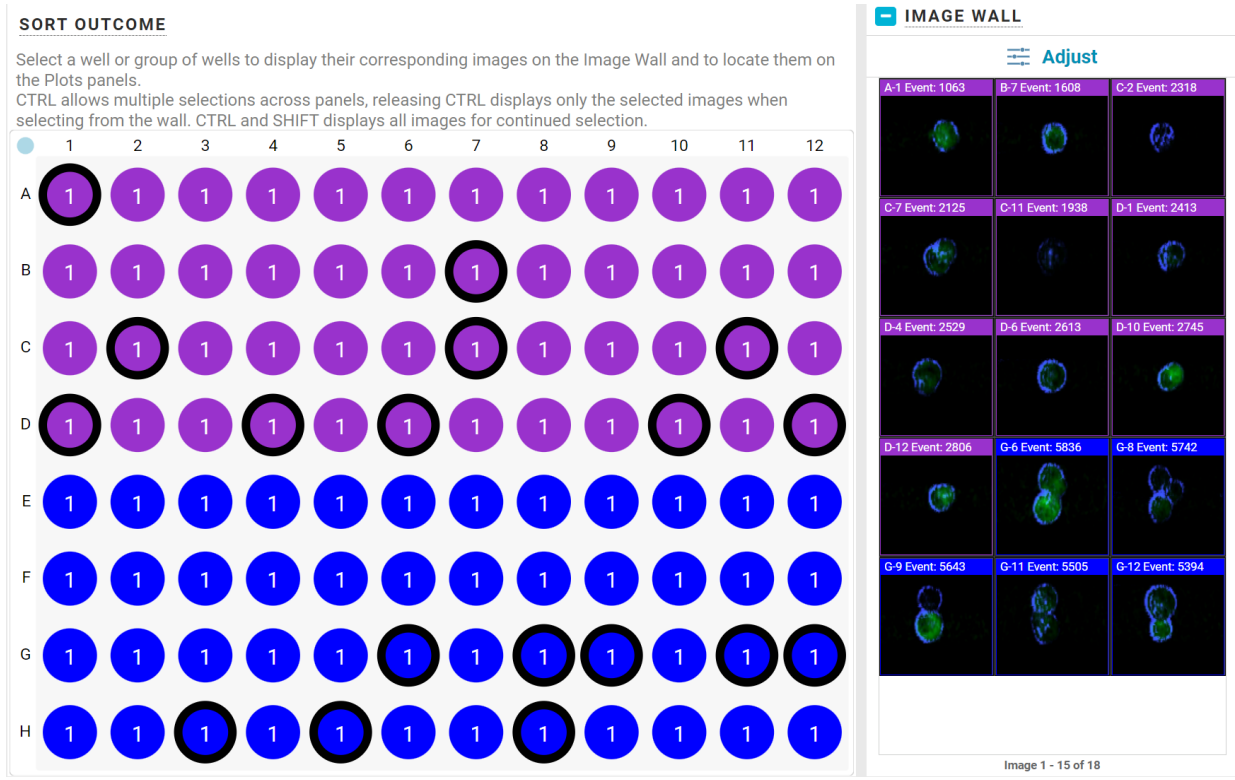


Image based spectral cell sorting

Fast image-based index sorting on BD FACSDiscover S8™

- Development of Smart-Seq3 pipeline for single cell sequencing in collab with VIB single cell core

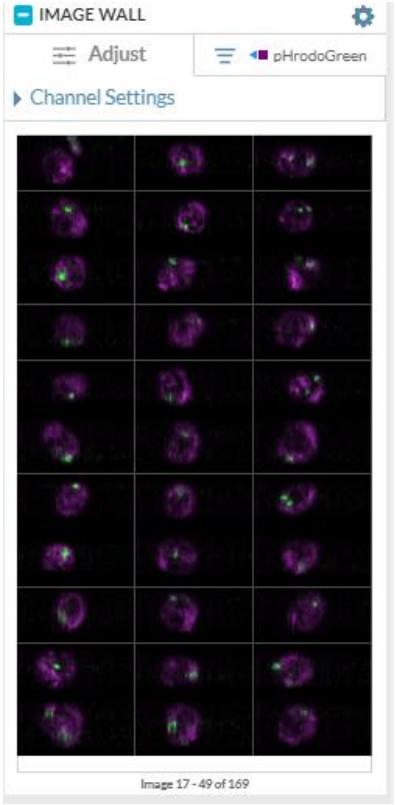




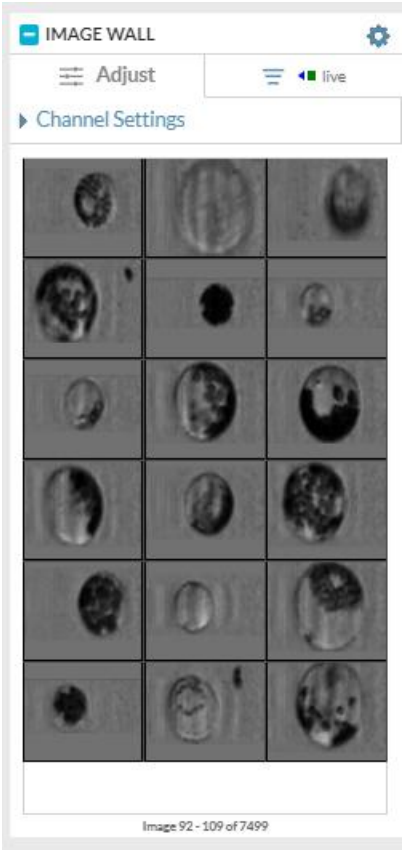
- 85, 100, 130 µm nozzle

Image based spectral cell sorting

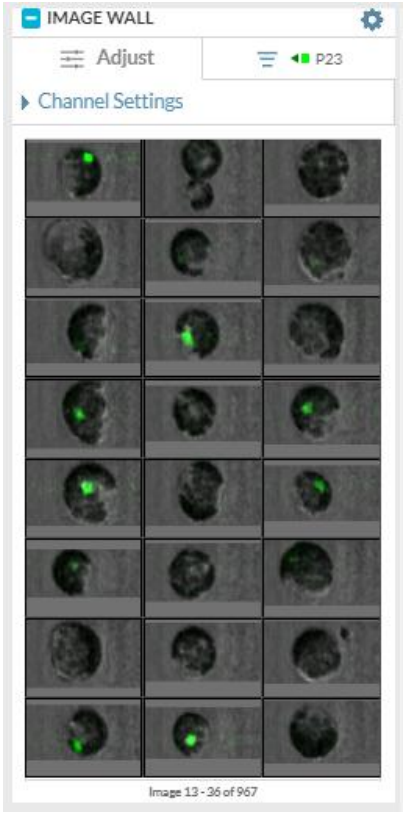
Diverse species, diverse applications



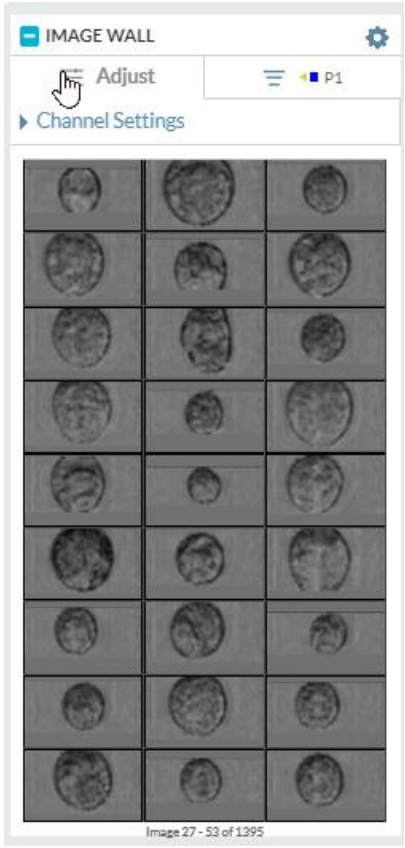
Macrophages
(Phagocytosis bacteria)



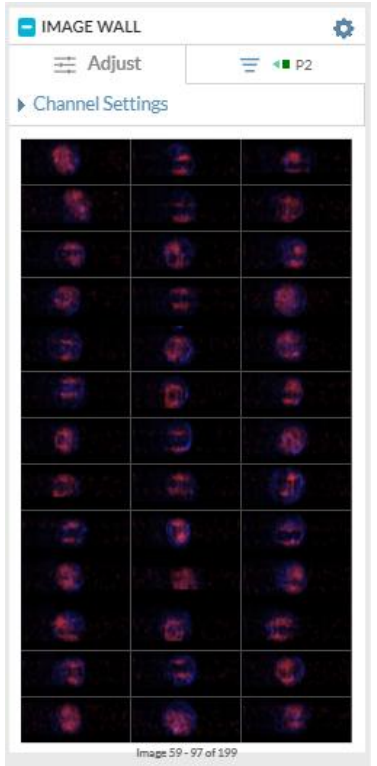
Canola -leaf



Arabidopsis - leaf



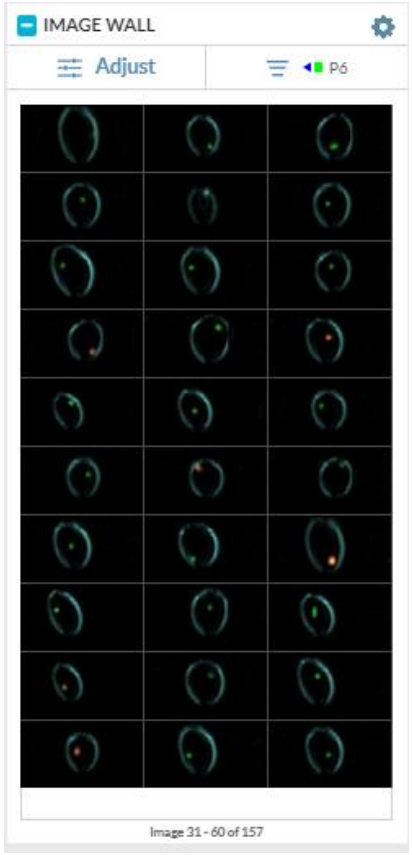
PSB-D cell line



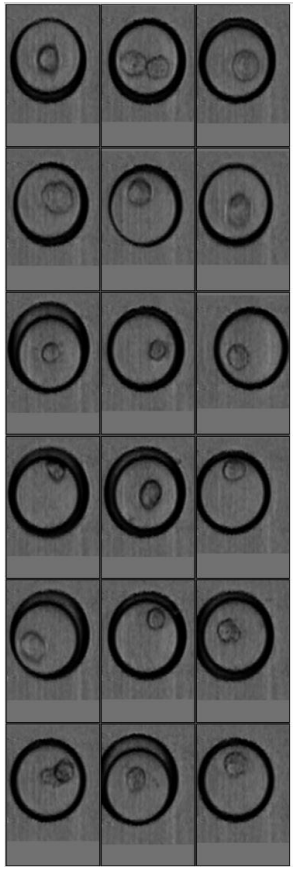
HACAT (cell cycle)

Image based spectral cell sorting

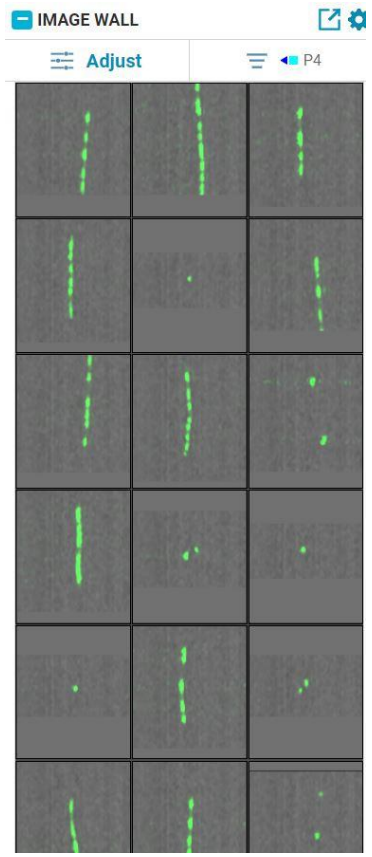
Diverse species, diverse applications



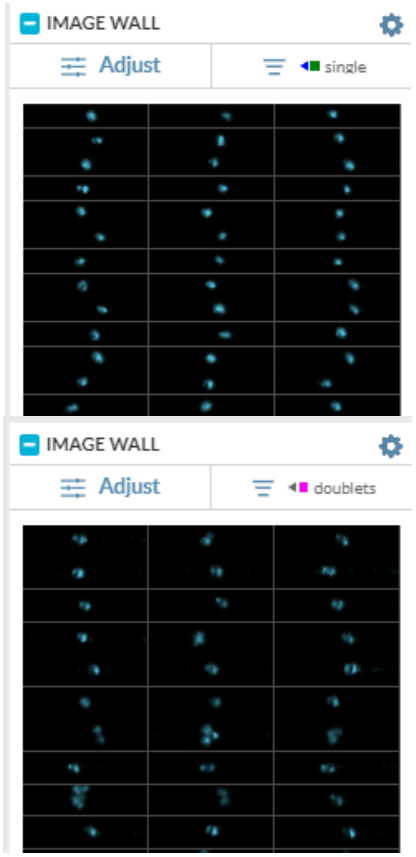
DE-droplets beads



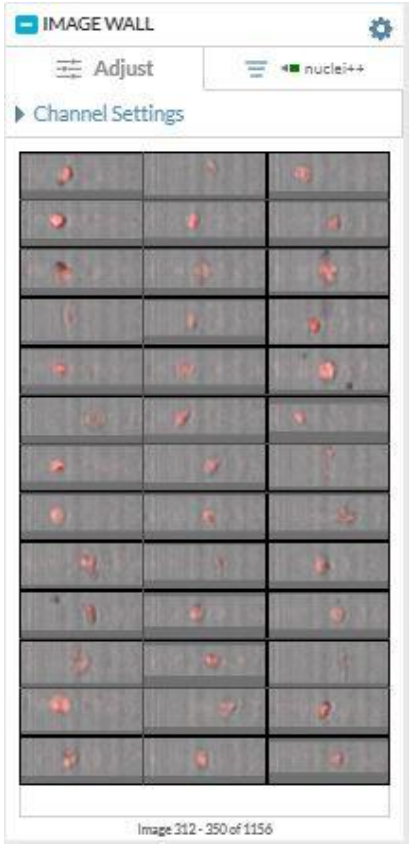
DE-droplets cells



Archaea



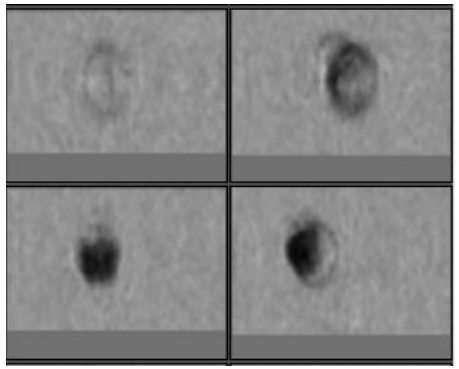
Yeast

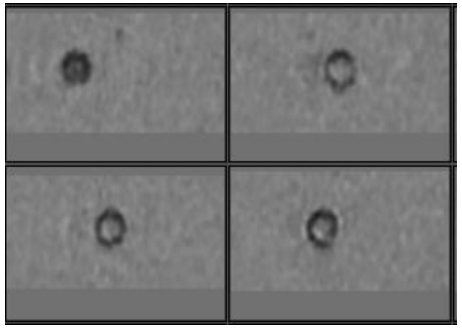


Nuclei (tomato)

Image based cell sorting

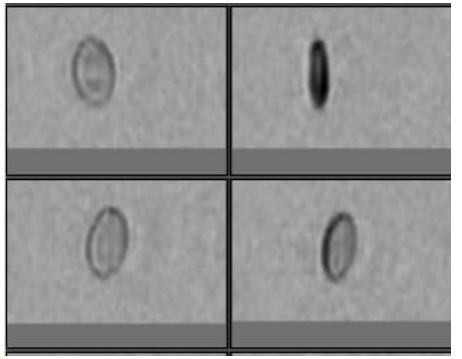
Diverse species, diverse applications



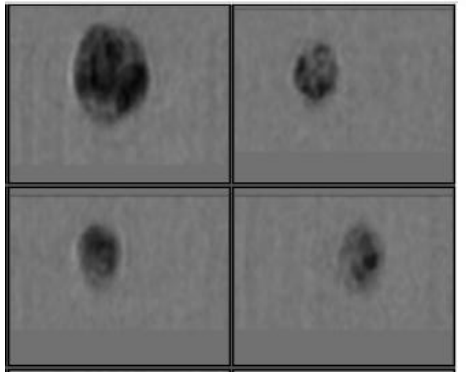


Liver neutrophils

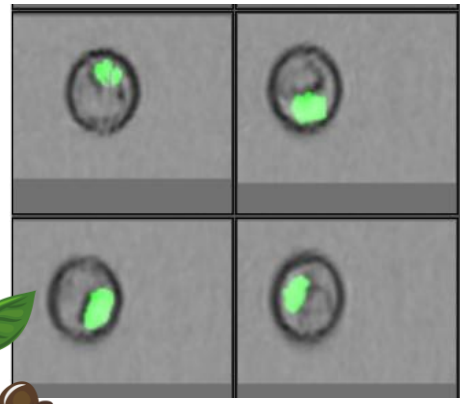
Red blood cells
of *Xenopus trop.*



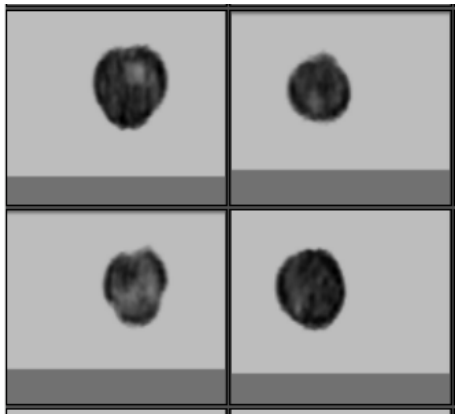
Cells
of honeybee



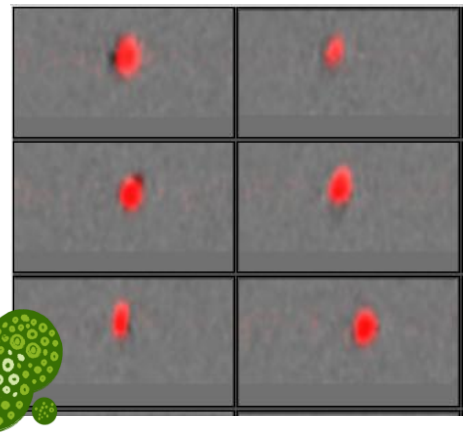
Arabidopsis th.
root protoplasts



Brassica napus
(rapeseed) microspores

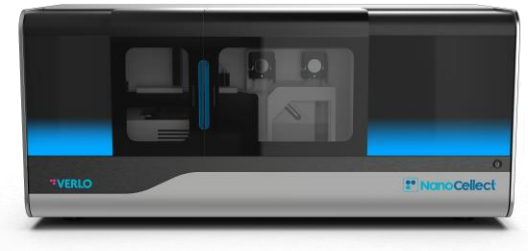


Autofluorescent gametes
of Ulva (microalgae)



Cartridge based cell sorting

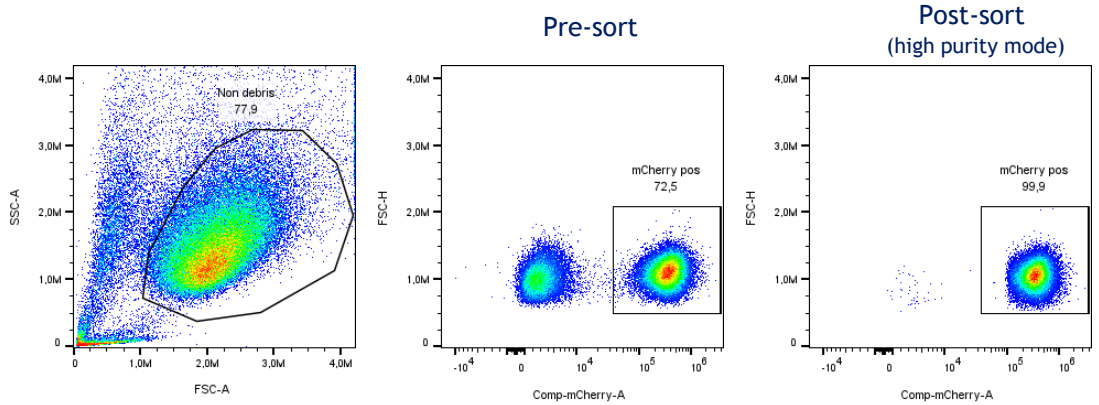
- Key vendors
  - Miltenyi
    - *MACSQuant Tyto*
    - *MACSQuant Tyto LUX*
  - NanoCellect
    - *Verlo*
    - *Wolf G2*
  - STEMCELL technologies
    - *Highway1 Cell Sorter*
- Microfluidic based systems
  - Closed, sterile workflows
  - Sample buffer as sheath fluid
  - Gentle, low-pressure
  - Small sorting volumes
  - Compact in design
  - Low parameter, max 2-way sorting
  - Consumable cost of cartridges/chips



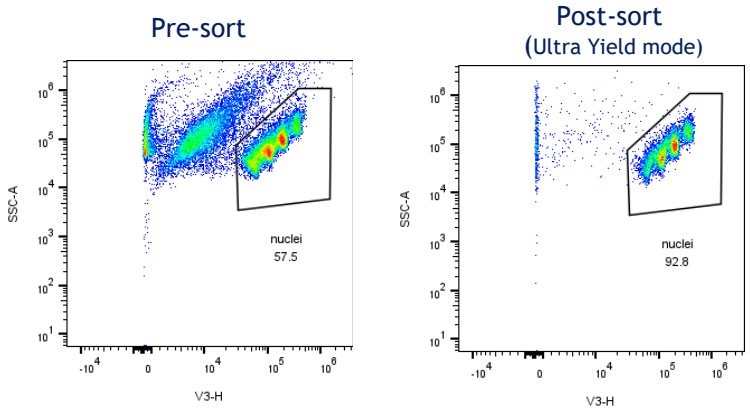
MACSQuant Tyto LUX

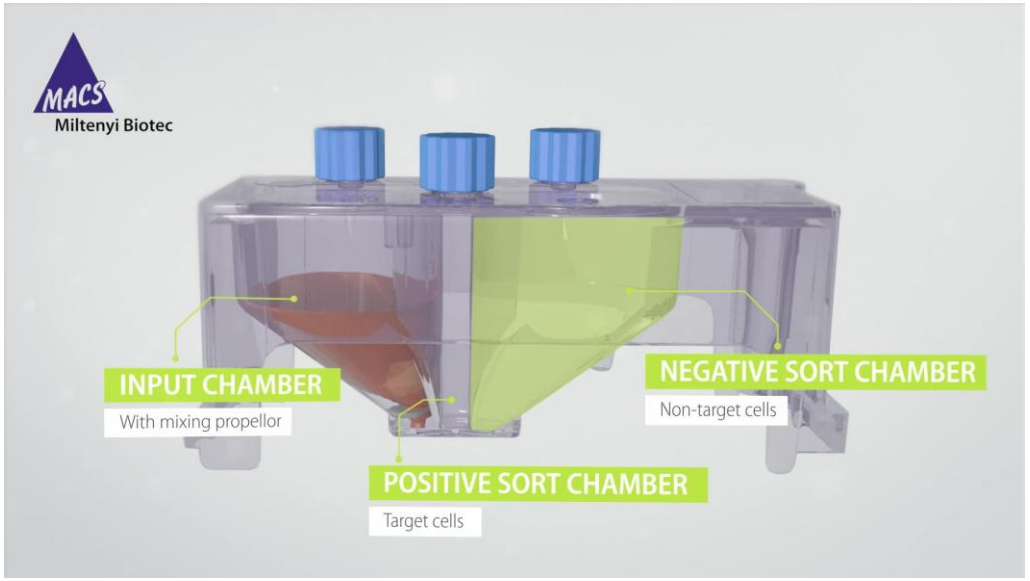
- Samples tested:
  - Eosinophils
  - Hepatocytes
  - Neutrophils
  - Macrophages
  - Nuclei (plants and murine)
  - Human and mouse cell lines
    - tdTomato, mCherry, mRFP

B16-mCherry (mouse melanoma cell line)



Arabidopsis th. (plant nuclei)





5. Automation

Automation

- Automation is transforming every step of the experimental workflow

Online panel design tools

AI guided

Tissue Dissociation/ Homogenization

Staining

Washing

Advanced plate loaders

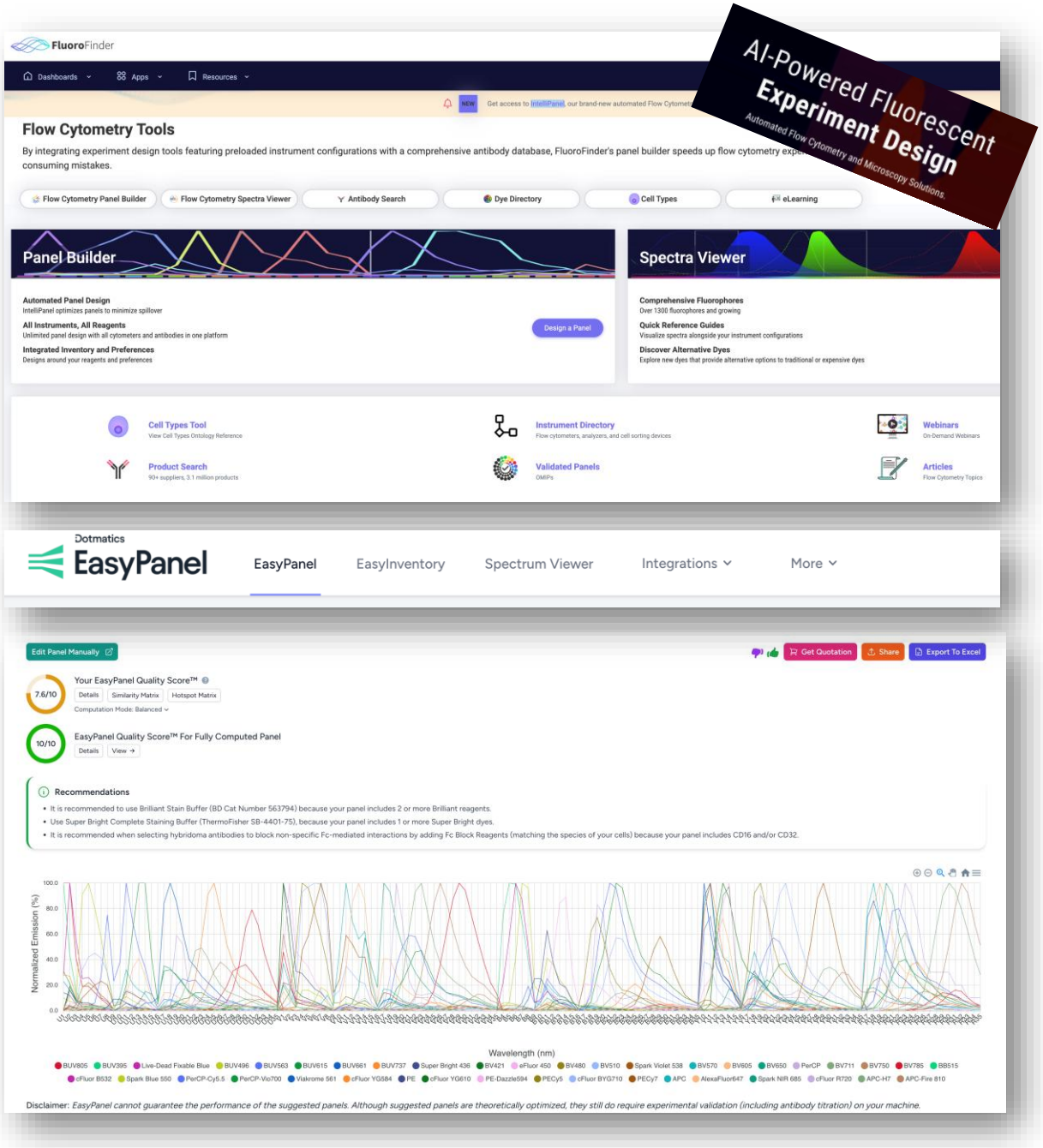
Startup / Shutdown

Advanced data analysis tools

Advanced algorithms

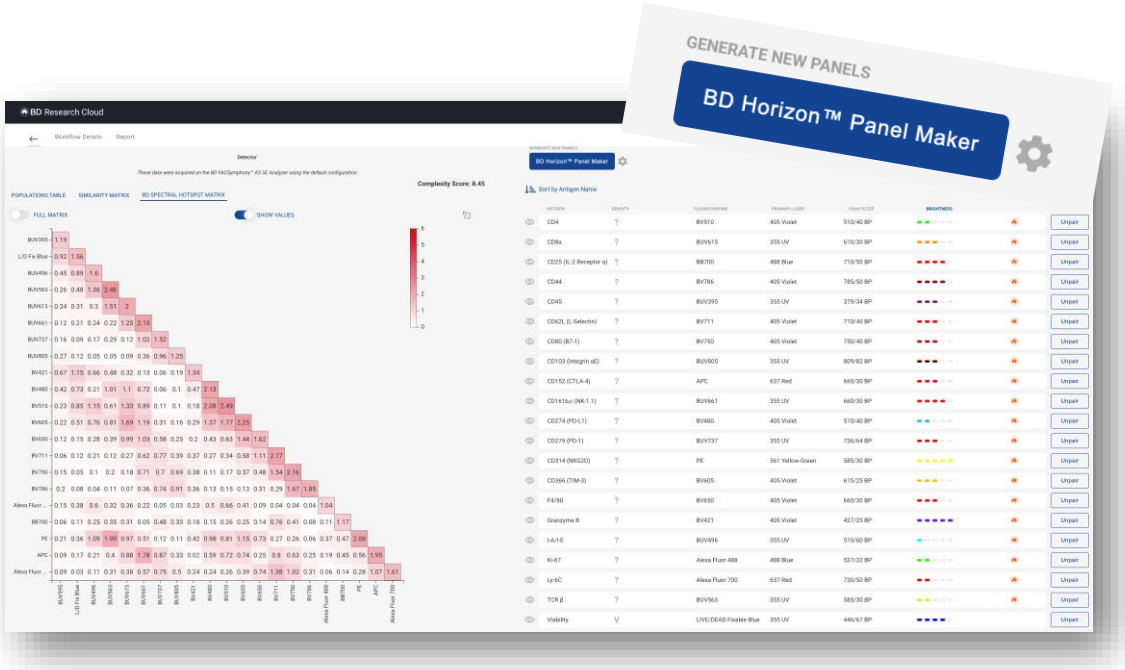
Panel Design

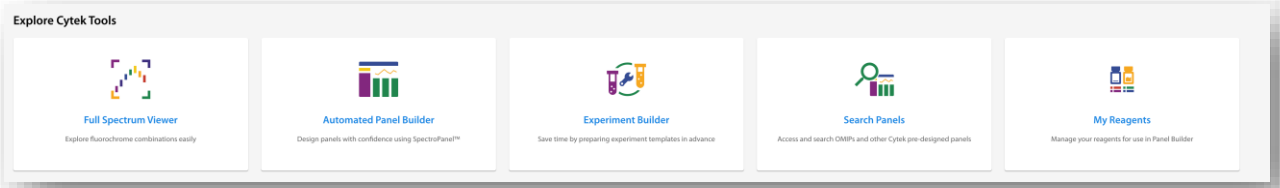
- Online panel design tools with “AI” option
  - Fluorofinder - *IntelliPanel*
  - Easypanel

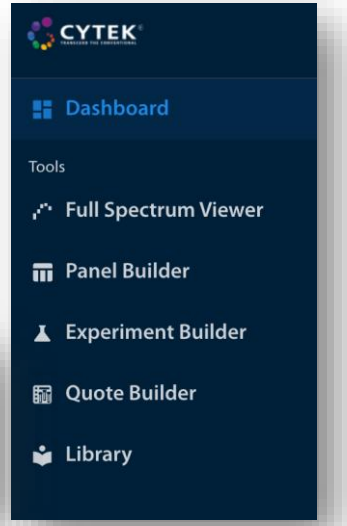


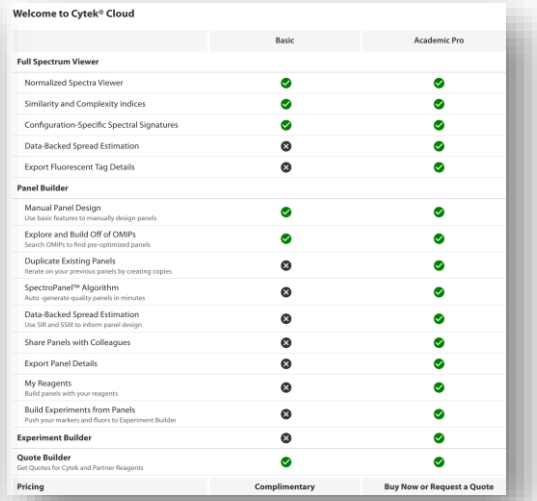
Panel Design

- Online panel design tools with “AI” option
  - Fluorofinder - *IntelliPanel*
  - Easypanel
  - BD Research Cloud - *BD Horizon™ Panel Maker*
  - Cytek cloud - *SpectroPanel™*









Panel Design → **Sample preparation**

- Tissue dissociation / homogenization
  - Semi automated Miltenyi GentleMACS

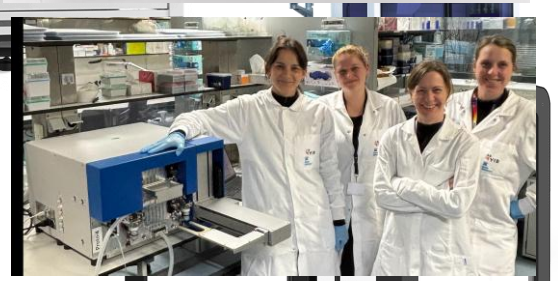
Goal: Standardization, reproducibility, contamination free

- Sample staining
  - Automated staining robots
  - Commercialized for flow cytometry stainings:
    - Cytek Orion, BD FACSDuet™, Curiox C-FreePluto, Sysmex PS10
  - Liquid handlers
    - Dispendix I-DOT
    - Beckman Coulter Echo Acoustic dispenser
    - Traditional pipetting: Tecan, Hamilton, OpenTrons, Biomek, ….

Goal: Standardization / Reduce reagents / Walk-away / Minimize human error / Speed

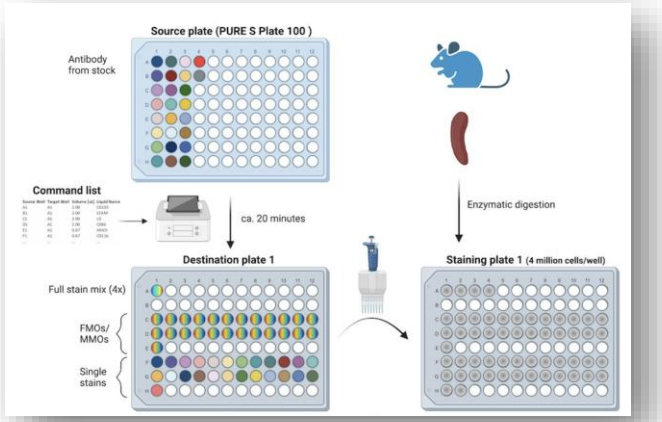
- Sample washing
  - BlueCatBio, Curiox,…

Goal: Replace centrifugation, less cell loss,….



Automated antibody dispensing

- 25 color test panel
  - All FMO's
  - Single stains on beads
  - Single stains on cells
  - Full stains
  - <20min



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TECHNICAL NOTE

MIFlowCyt

ISAC CYTOMETRY Journal of Quantitative Cell Science PART A

Automated antibody dispensing to improve high-parameter flow cytometry throughput and analysis

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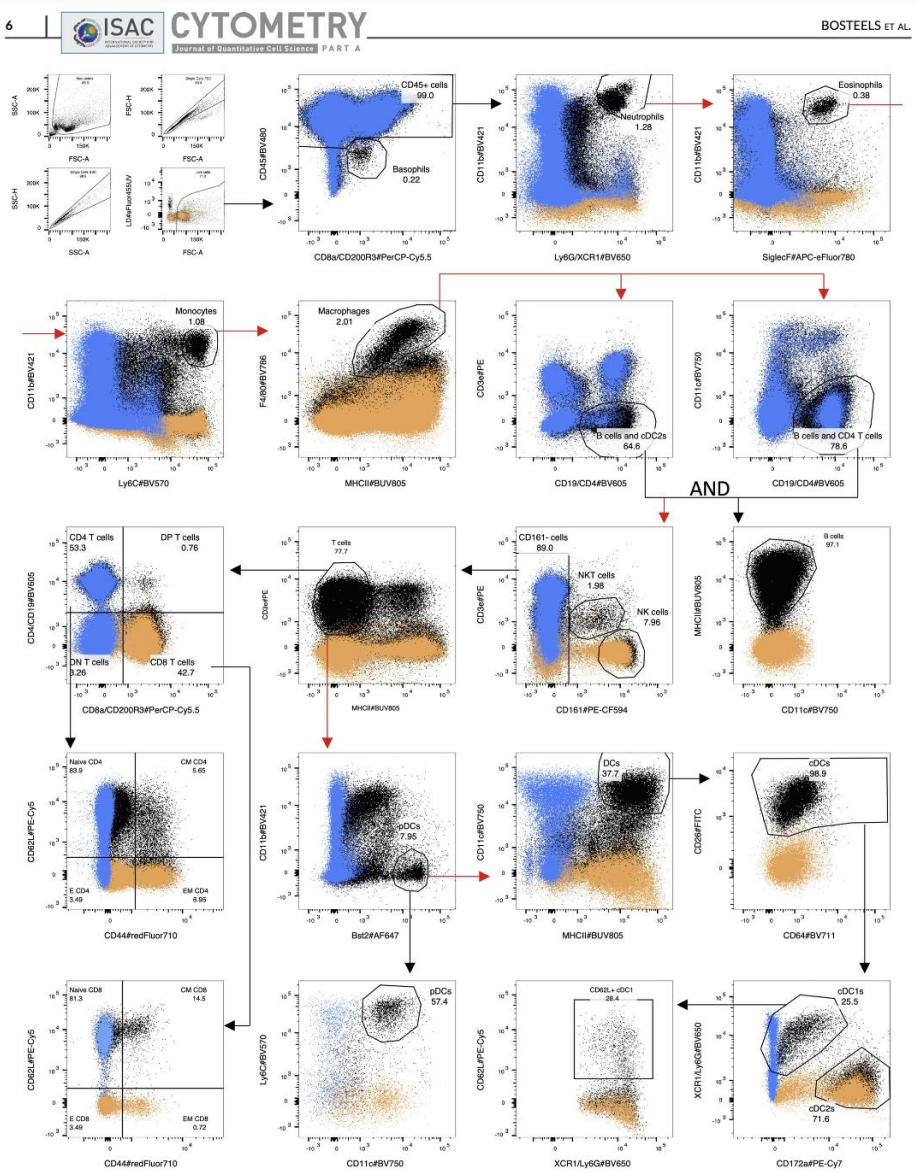


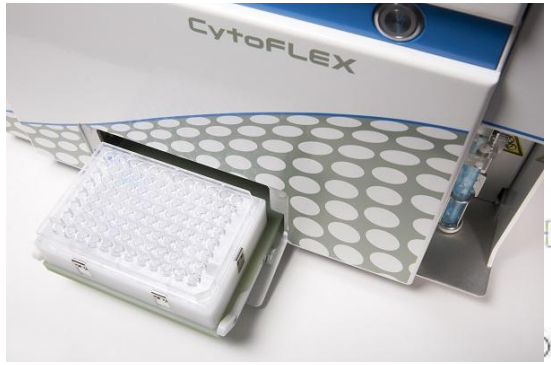
Panel Design → Sample preparation → Sample Acquisit

- Advanced plate loaders on FC Analyzers
  - Sony ID7000
  - BD FACSDiscover A8
  - Agilent Novocyte Opteon / Penteon / Quanteon
  - Cytek Aurora
  - BC Cytoflex
  - Thermo Fisher Xenith / Attune

  Key features:
    - 40 tube racks, 96-well, 384-well, deepwell plates
    - Flow sensor, clog detection
    - Automated washing
    - Minimized dead volume, low carryover
    - Temperature control, sample mixing
    - Volumetric measurements
- Programmed start up and automated shutdown
  - Some vendors have it (Sony, Agilent, Cytek,…)
  - Start measurement at end of the day.

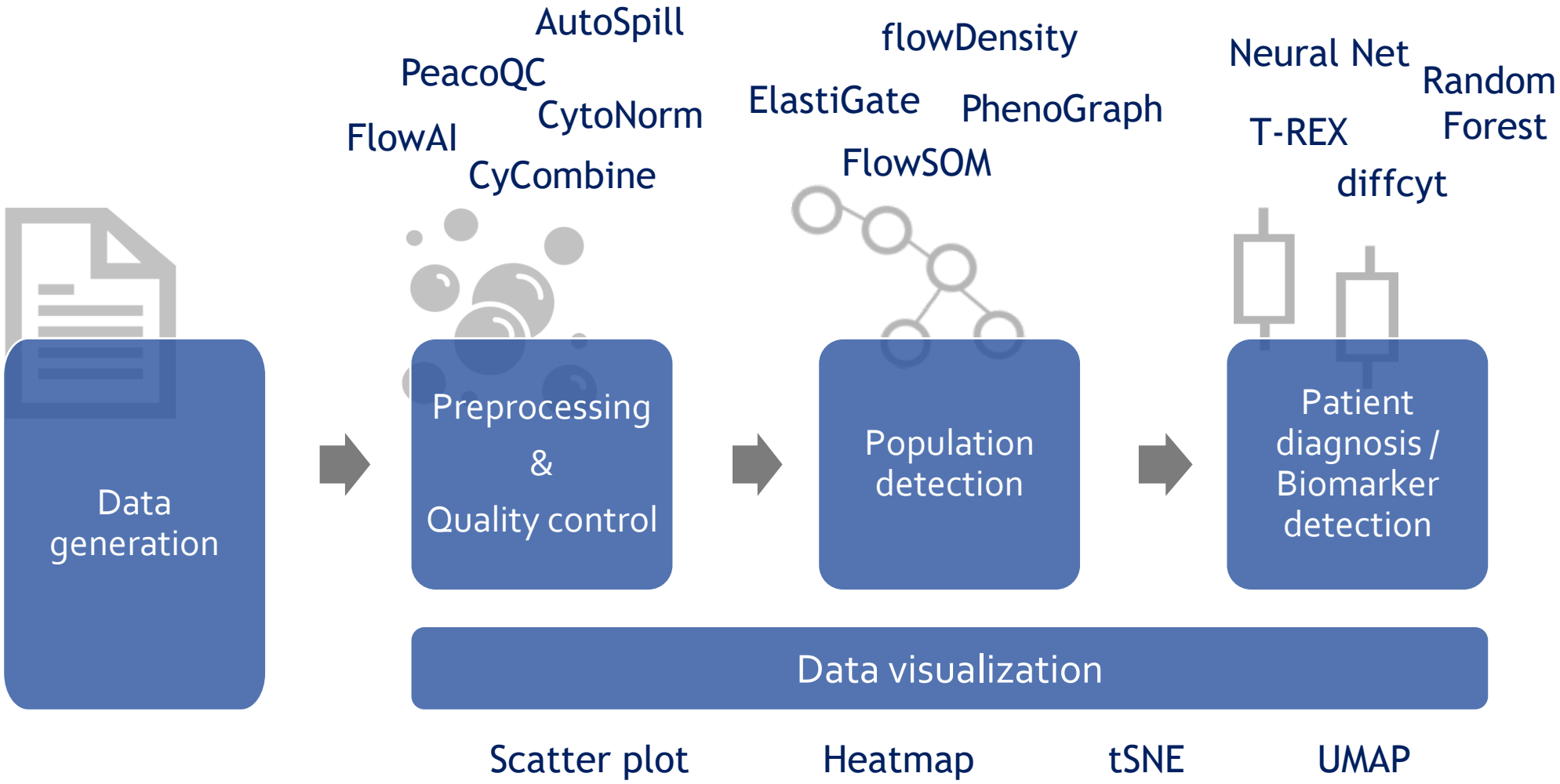
Sony ID7000

Agilent NovoCyte



High-Throughput Data Analysis

Panel Design | Sample preparation | Sample Acquisition | Data Analysis



High-Throughput Data Analysis

Manual

Gate on a specific population of interest

Intuitive interpretation

Less practical for high-dimensional data
(e.g. Time vs all parameters)

Inconsistency between scientists
(e.g. small shifts in gates)

Manual adjustment of gates

Computational

Look at all cells/parameters simultaneously

Less straightforward interpretation of results

Automated steps possible

Reproducible
(given same input and hyperparameters)

More sensitive to batch effects/changes

6. Take-Home messages

Take-Home messages

- Flow cytometry field is changing at laser light speed
- Automation and AI will dramatically change our way of working
- More advanced data analysis is needed —lots of opportunities!
- Flow Core facilities are evolving into technology & training hubs

VIB Flow Core is ready for the future!

Spectral Flow Cytometers

Sony ID7000

2x BD FACSDiscover A8

Cytek Aurora

Imaging Flow Cytometer

Cytek ImageStream X

Technical and data analysis expertise

Spectral + Imaging Sorter

BD FACSDiscover S8

Cartridge based Sorter

Miltenyi Tyto Lux

VIB Flow Core: An Open and Future-Ready Facility

Interested in one of our services or want to try one of our state-of-the-art devices?

Flow Cytometry Consultancy

FACS Consultancy

Connect website – request training

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