Unlocking the Hidden World of Cells with Flow Cytometry

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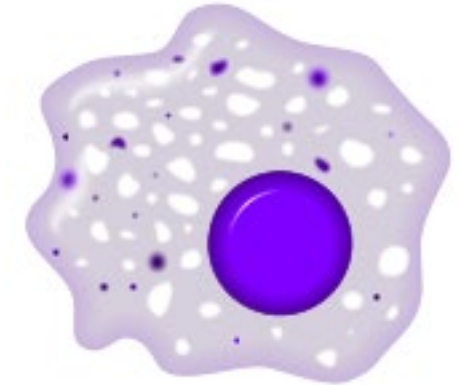
UNIVERSITY OF ALBERTA

FoMD FLOW

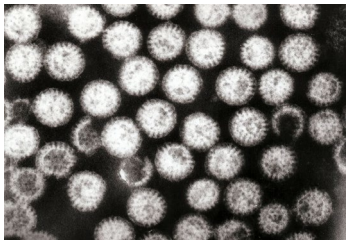
What is cytometry?

Cyto = ?



metry = ?

Not just cells:
also particles!



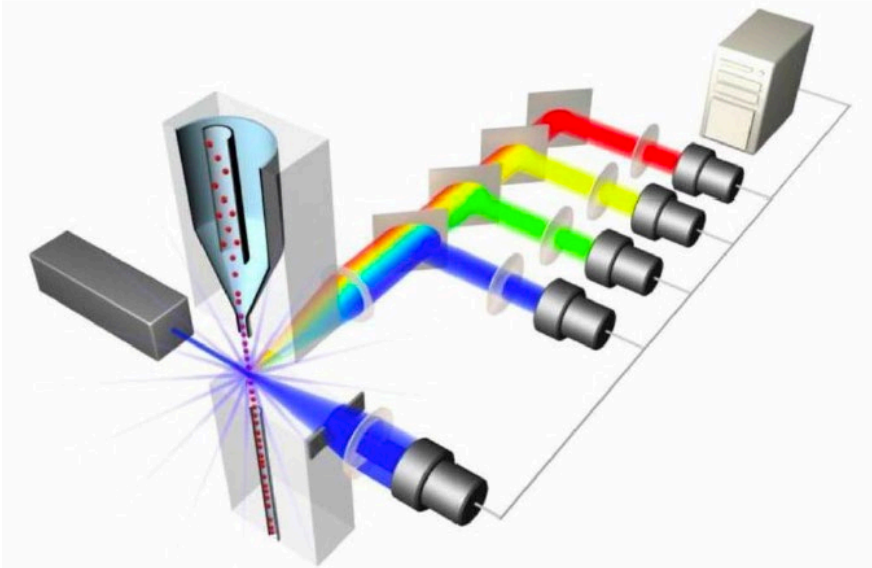
Rotavirus particles

https://upload.wikimedia.org/wikipedia/commons/0/0e/Macrophage.png Simon A. Eugster, CC BY-SA 3.0 <https://creativecommons.org/licenses/by-sa/3.0>, via Wikimedia Commons; https://upload.wikimedia.org/wikipedia/commons/5/5e/Tape_measure_colored.jpeg

Dr Graham Beards, CC BY 3.0 <https://creativecommons.org/licenses/by/3.0>, via Wikimedia Commons https://upload.wikimedia.org/wikipedia/commons/7/7c/Multiple_rotavirus_particles.jpg

What is flow cytometry?

measurement of cells or particles as they pass by a laser within a stream or “flow” of fluid

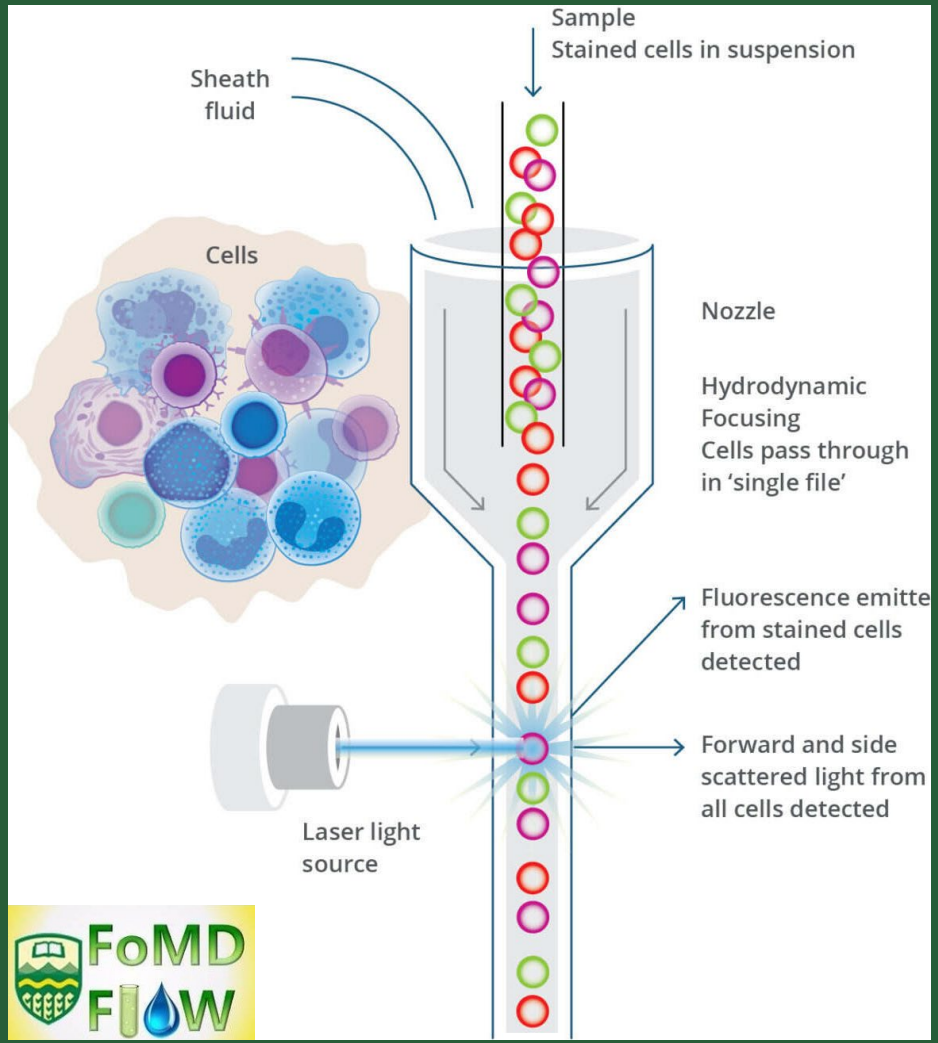


https://wwwndmc.ndmctsgh.edu.tw/files/web/192/file_up/100043/7314/20200820%20Attune%20Training%20deck%20BRYV6_NDMC.pdf

What can we learn about cells with flow cytometry?

- Who are they?
- How big are they?
- How many are there?
- What stage of life are they in?
- How healthy are they? (living or dying?)
- How active are they? (resting or activated?)
- What are they doing? (proliferating, killing?)
- What are they making?

- AND MORE!

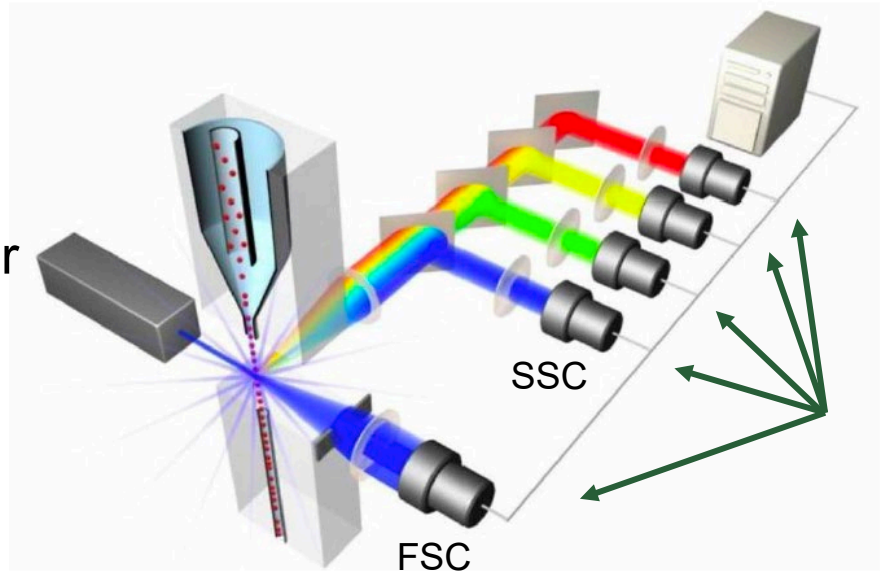


- Harvest cells or dissociate tissues, wash
- Stain with fluorescently labeled antibodies to markers (cell surface or intracellular)
- Acquire:
  - cells taken up in sheath fluid
  - directed into single cell stream
  - within flow cell encounter laser beam
  - laser excites fluorophores -> they emit light at a different wavelength
- Optical detectors collect
  - cell's refracted light, indicating
    - size (Forward Scatter, FSC)
    - granularity (Side Scatter, SSC)
  - emitted fluorescence
- translated into digital signals that can be analyzed

https://www.streck.com/blog/flow-cytometry-a-powerful-tool-for-clinicians/

Flow cytometry components

Fluidics
Optics
Electronics



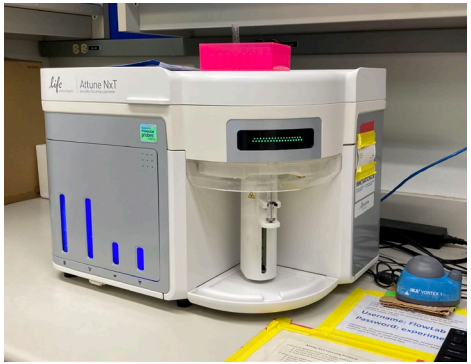
Computer

Detectors a.k.a. Photomultiplier Tubes (PMTs)

FSC, forward scatter; SSC, side scatter

https://wwwndmc.ndmctsgh.edu.tw/files/web/192/file_up/100043/7314/20200820%20Attune%20Training%20deck%20BRYV6_NDMC.pdf

Types of flow cytometers



Thermo Attune NxT
Analyzer

Sony MA900
Sorter – sort and recover populations

Cytek Amnis ImageStream mkII
Imaging Flow Cytometer – combines imaging and flow cytometry

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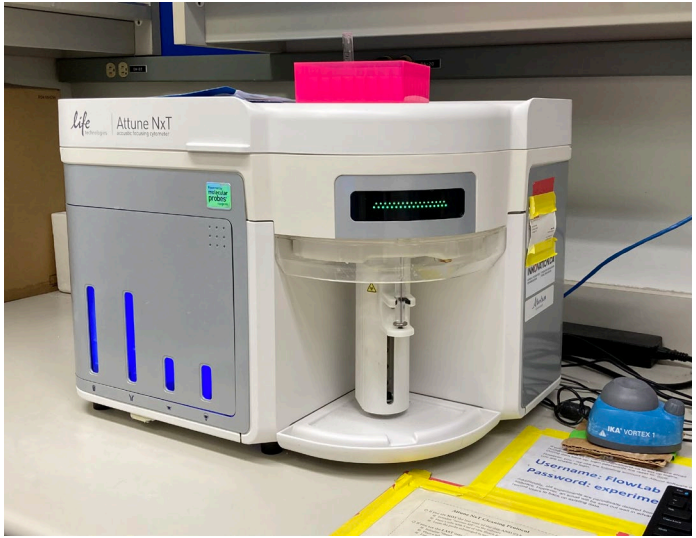
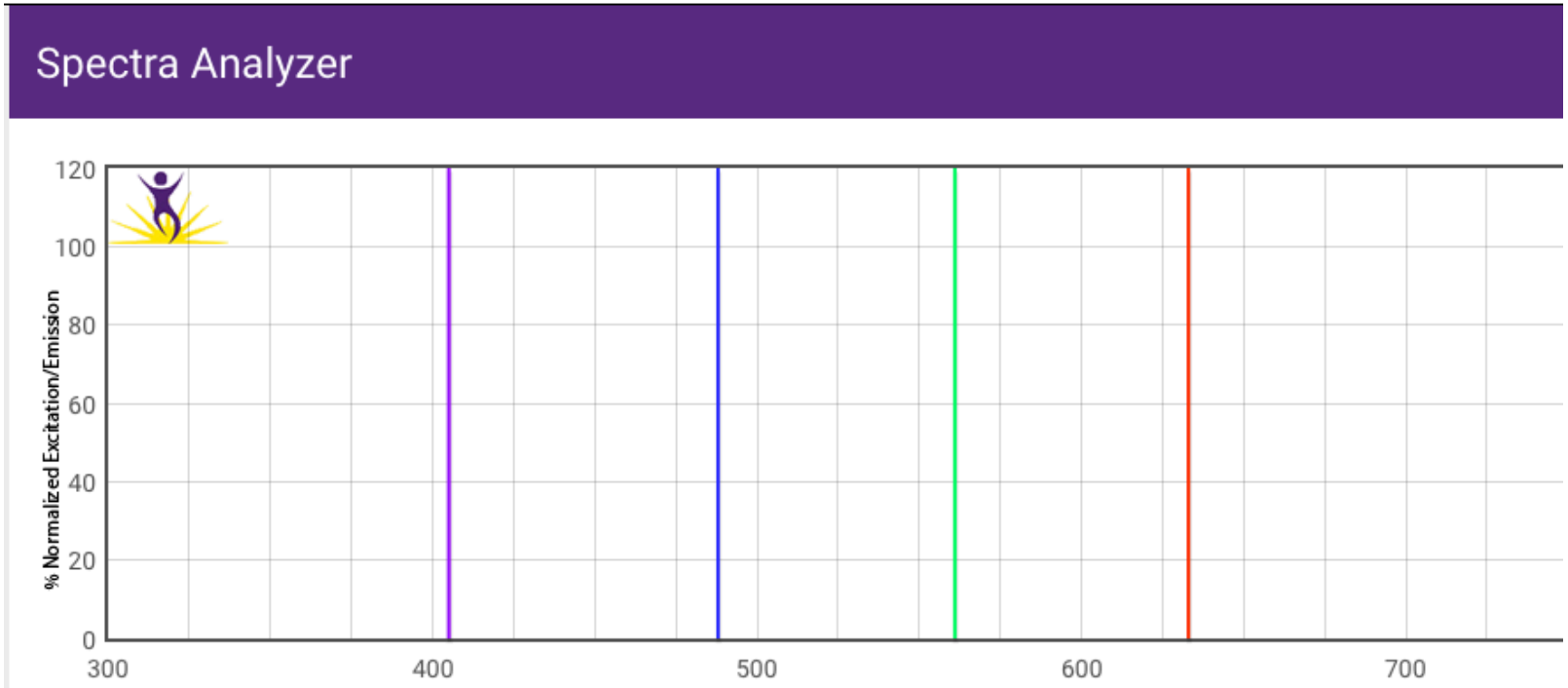
Attune NxT Flow Cytometer (analyzer)



4 laser/ 16 colour

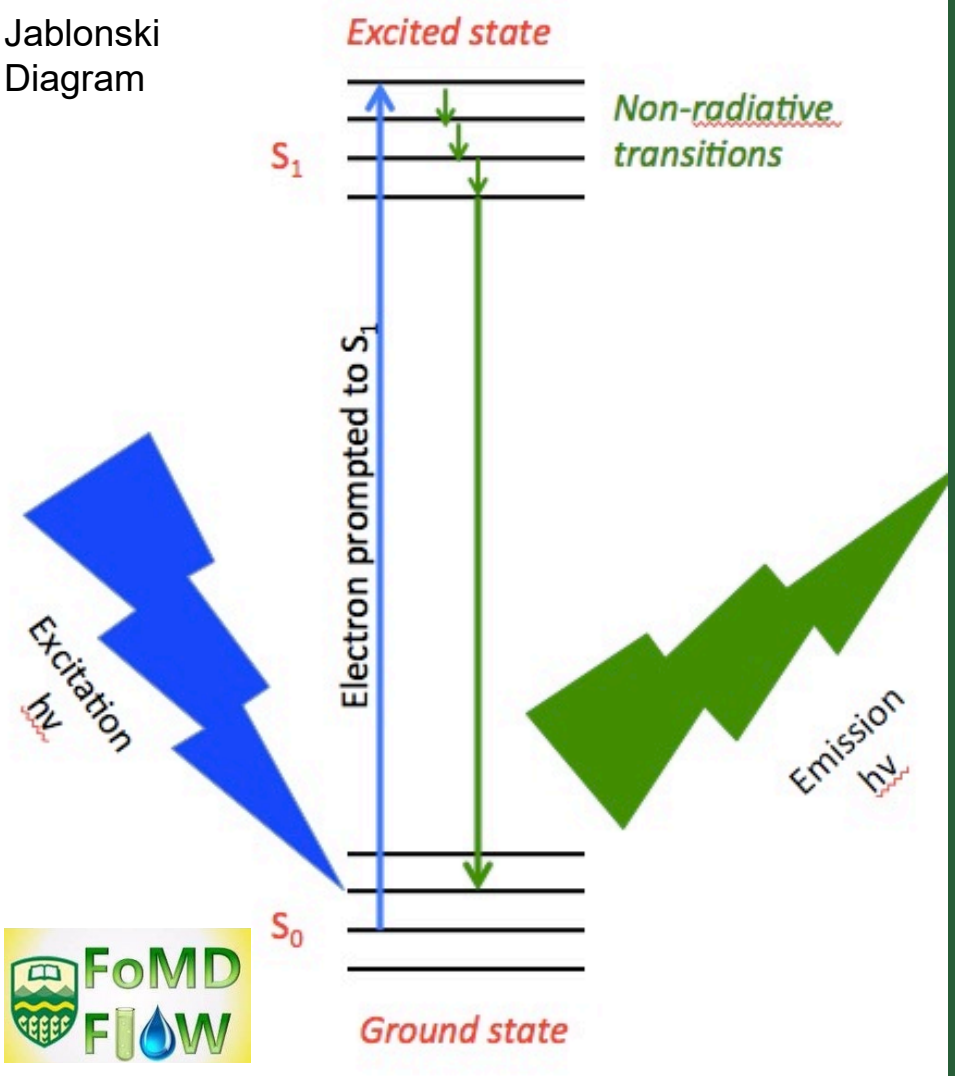
Attune NxT lasers



Violet	Blue	Yellow	Red
405 nm	488 nm	561 nm	638 nm*

https://www.biolegend.com/en-us/spectra-analyzer *633 on Spectra Analyzer

Jablonski Diagram

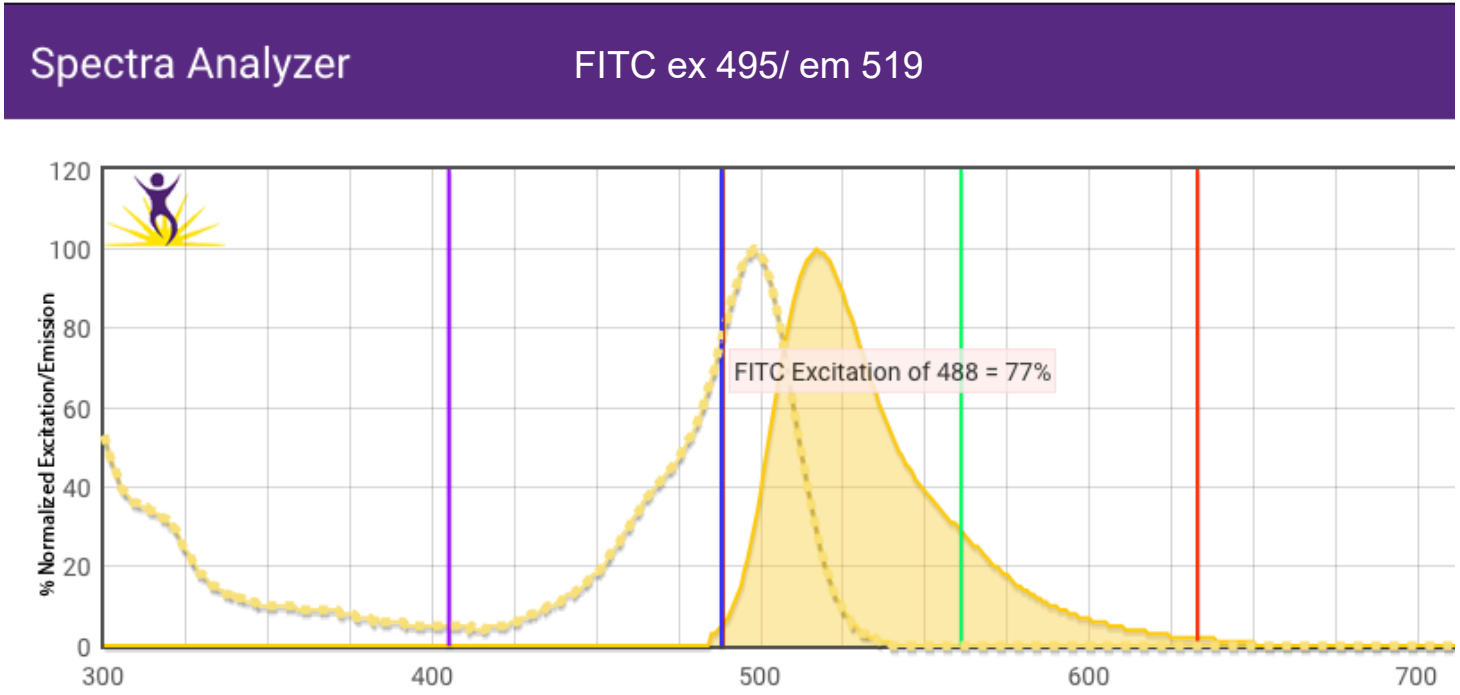


Fluorophore Excitation and Emission

- Cells encounter a laser beam as they pass through the flow cell
- The laser excites fluorophores
- Emission energy < absorption energy
- Fluorophores emit light at a longer wavelength

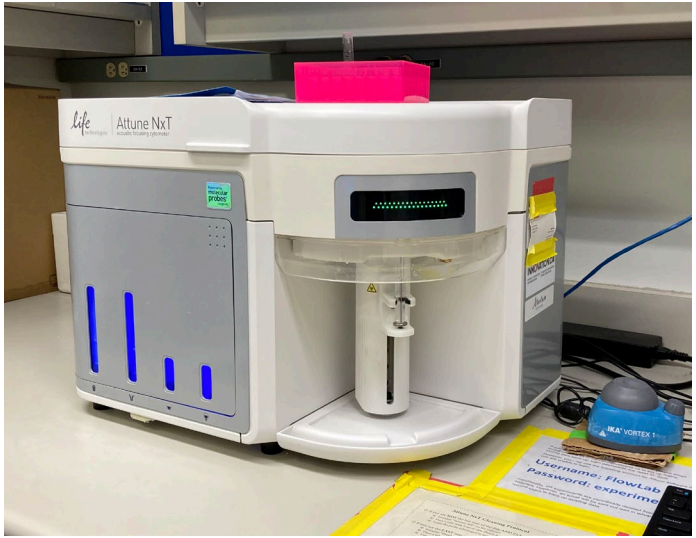
https://expertcytometry.com/the-jablonski-diagram-freshly-presented-in-new-excyte-courses/

FITC is excited by the blue laser (488 nm)



https://www.biolegend.com/en-us/spectra-analyzer

Attune NxT Flow Cytometer (analyzer)



4 laser/ 16 colour

How do you get 16 colours out of 4 lasers?

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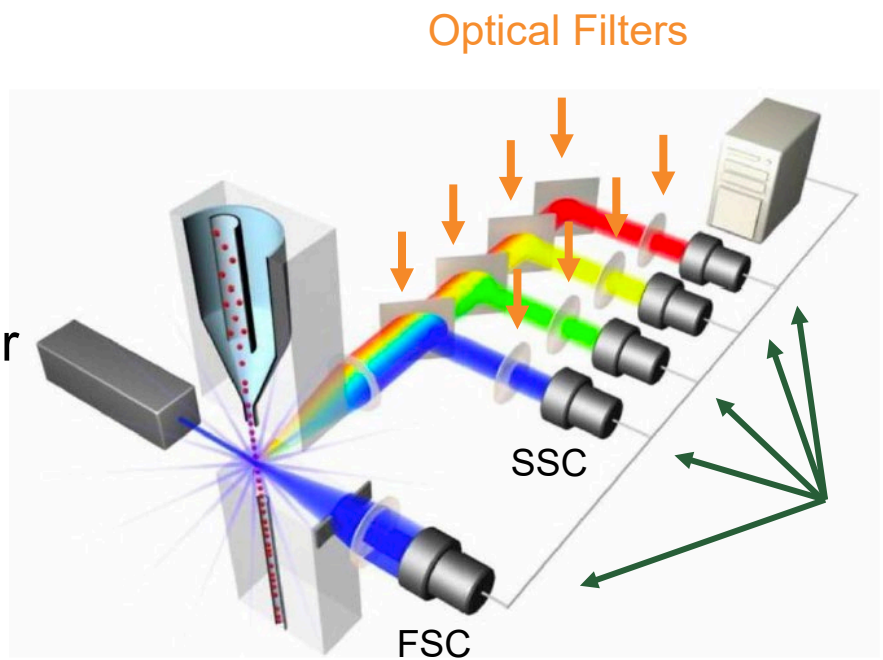
Flow cytometry components

Fluidics
Optics
Electronics



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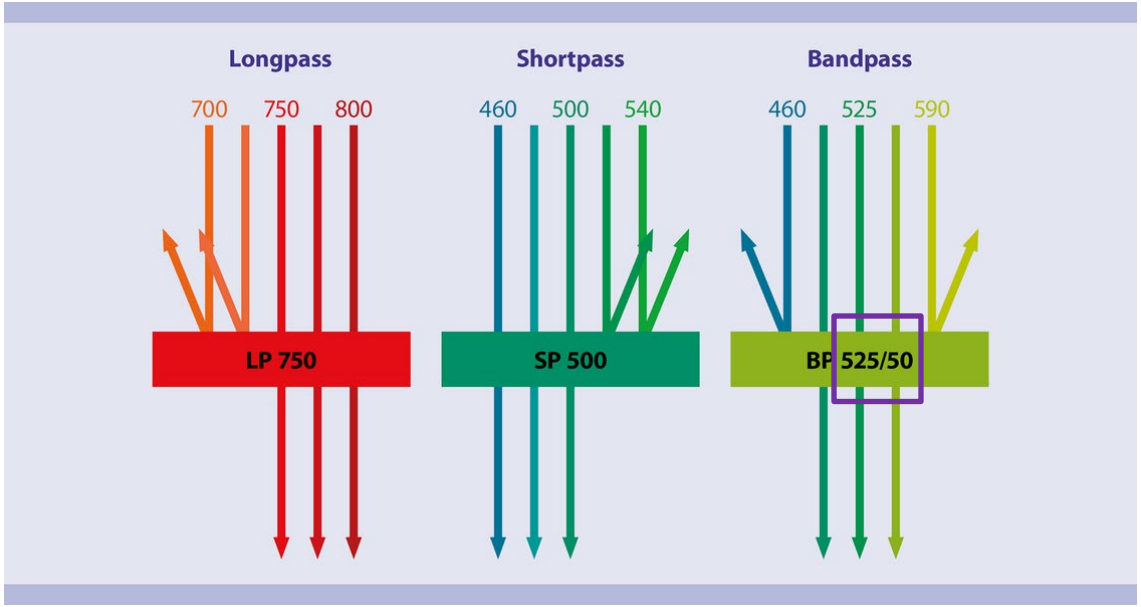
Optical filters distinguish wavelengths

Emitted light encounters optical filters that control which wavelengths of light can be detected



525/50 = 525 ± 25

Allowed: ≥ 750 nm ≤ 500 500-550

Filters are configured such that blocked light is deflected and travels to the next filter

https://www.miltenyibiotec.com/NO-en/support/macs-handbook/macs-technologies/flow-cytometry/flow-cytometry-basics.html

Emitted light is detected by PMTs

Signals are detected and amplified by PhotoMultiplier Tubes (PMTs)

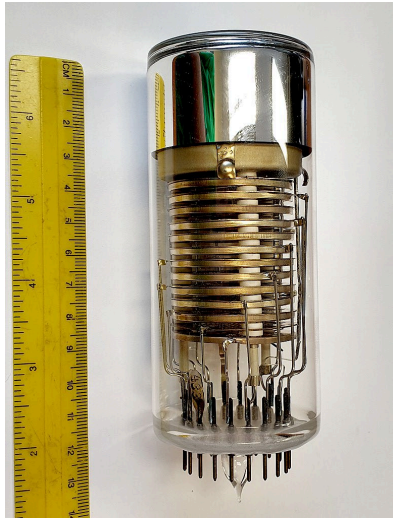
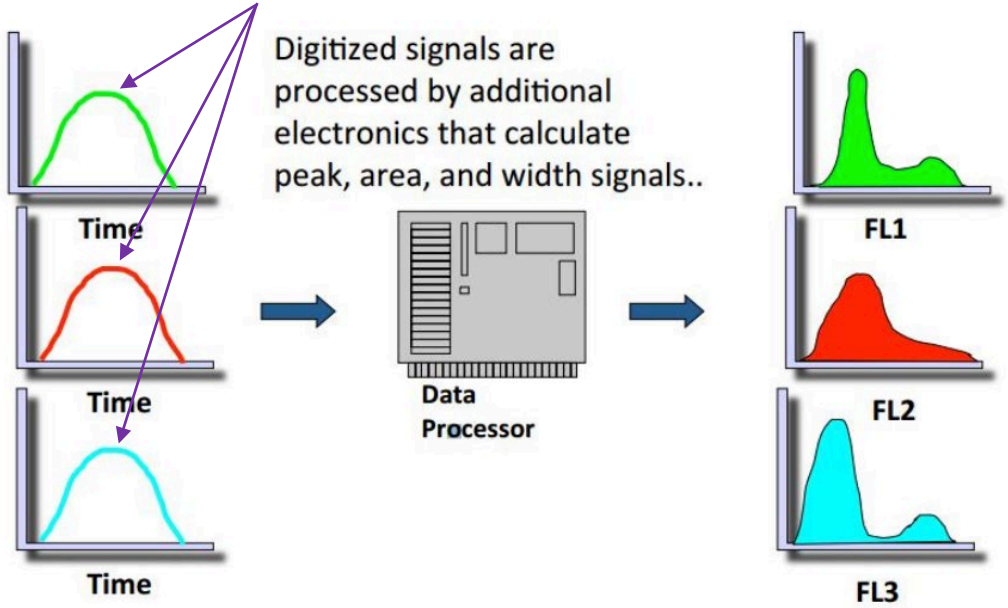


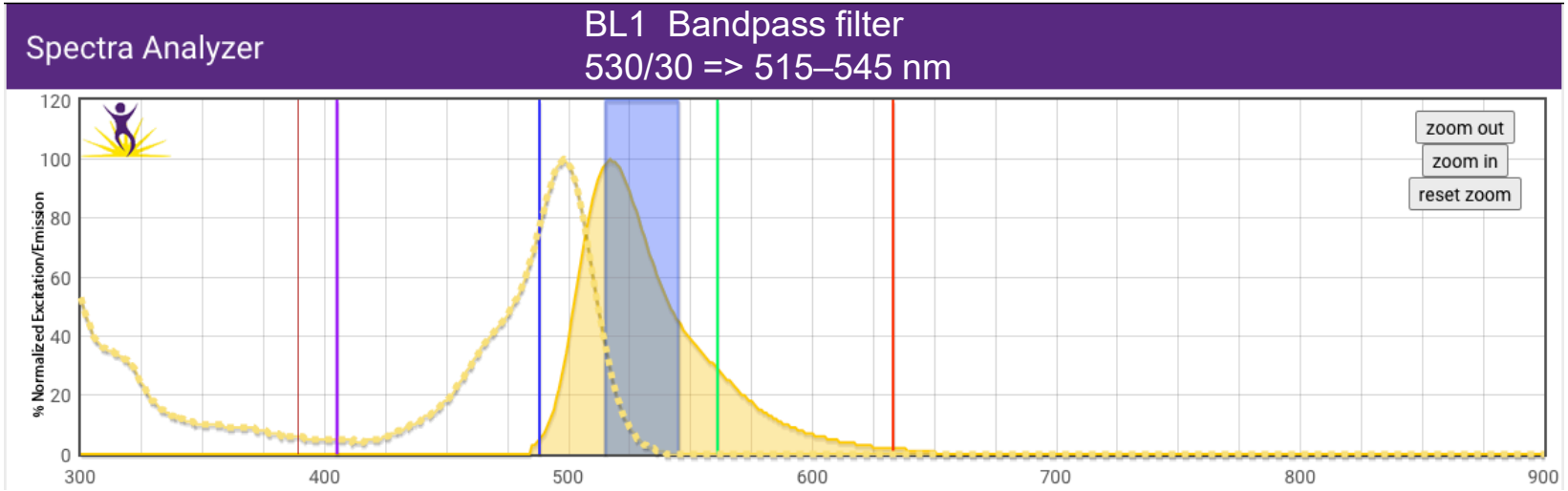
Photo-electron pulses are digitized



Jan Helebrant, CC0, via Wikimedia Commons; https://commons.wikimedia.org/wiki/File:Technology_photomultiplier_tube_202_(52280871801).jpg

https://www.bidmc.org/-/media/files/beth-israel-org/research/core-facilities/flow-cytometry-core/flow-cytometry-basics.pdf

FITC emission detected in the BL1 channel



Violet 405 nm | Blue 488 nm | Yellow 561 nm | Red 638 nm

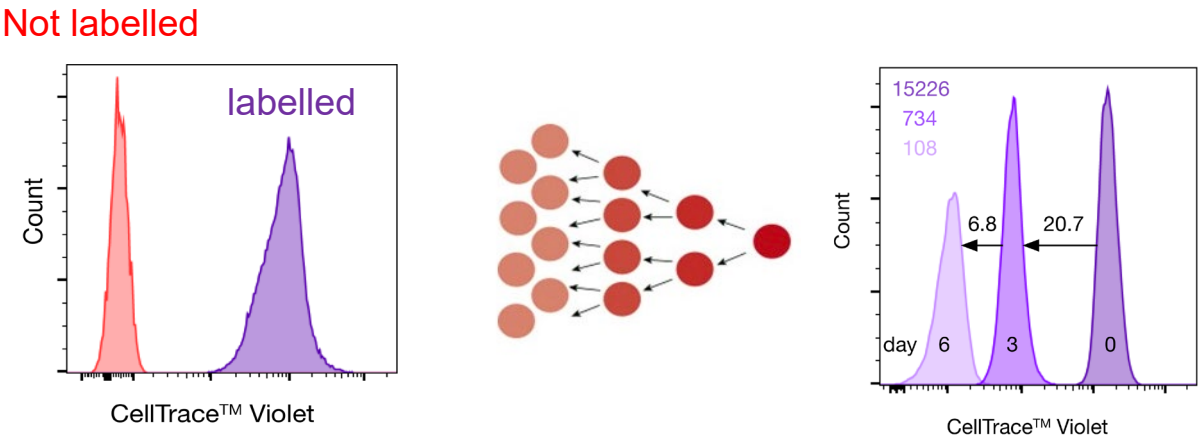
FITC peak excitation: 495 nm
peak emission: 519 nm

https://www.biolegend.com/en-us/spectra-analyzer

Measuring cell proliferation with CTV

one colour: histogram

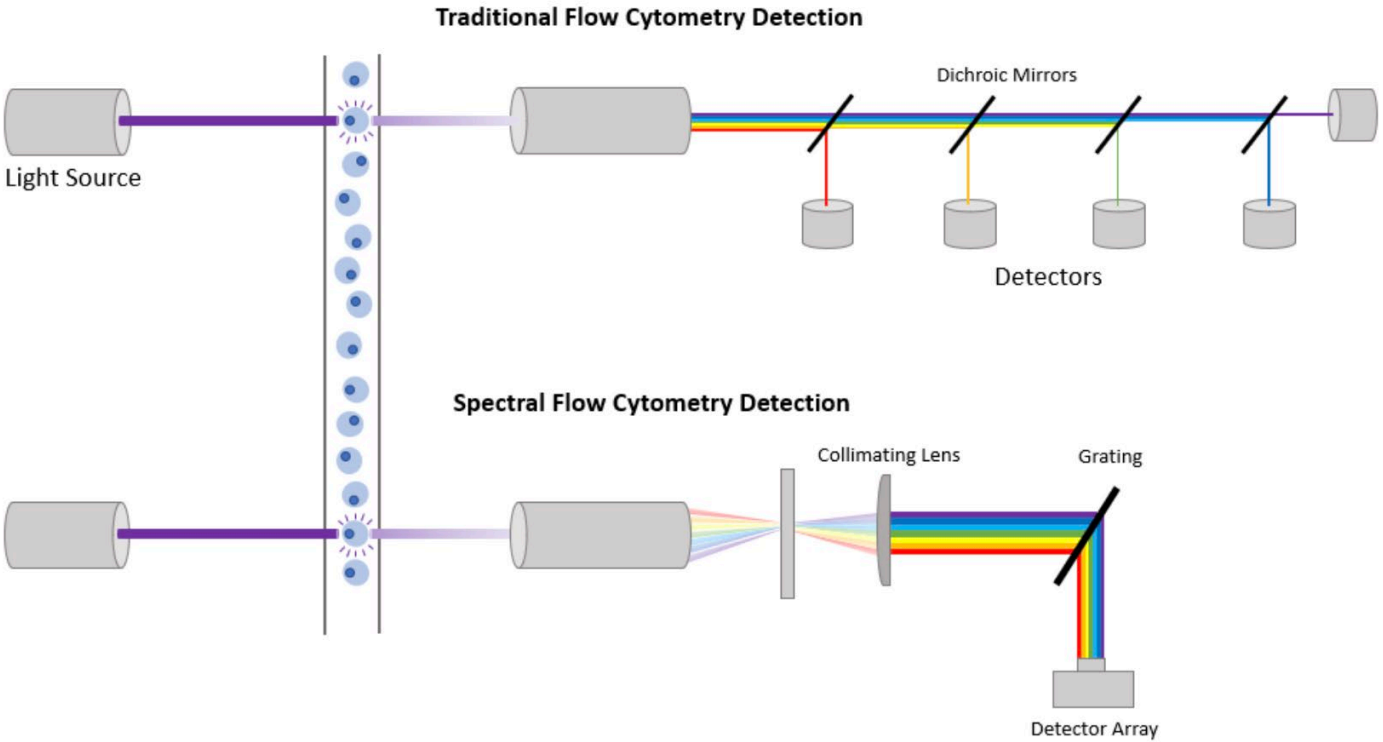
Cell Trace Violet (CTV, Ex/Em = 405/450)
- cell labeling dye; intensity decreases as cells divide
- quantify proliferation in response to stimulus



Median fluorescence intensity (MFI) and fold change in MFI indicated. Log scale on x-axis.

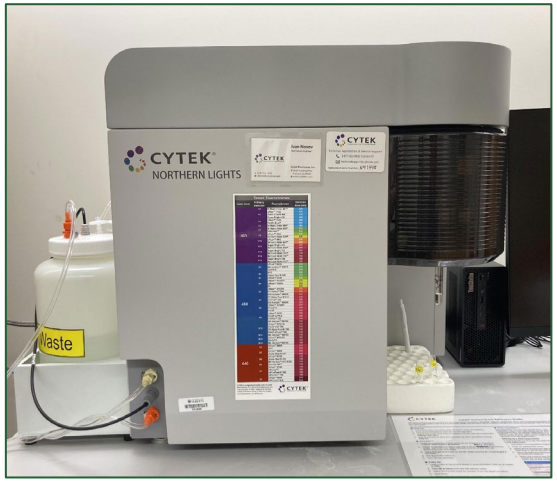
Lemieszek, Findlay and Siegers: CellTrace Violet™ Flow Cytometric Assay to Assess Cell Proliferation. *Methods in Molecular Biology*. 2022; 2508:101-114. doi: 10.1007/978-1-0716-2376-3_9; image from ThermoFisher website describing CellTrace reagents.

Spectral Flow Cytometry: new and improved flow!



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

Spectral Flow Cytometers in our core



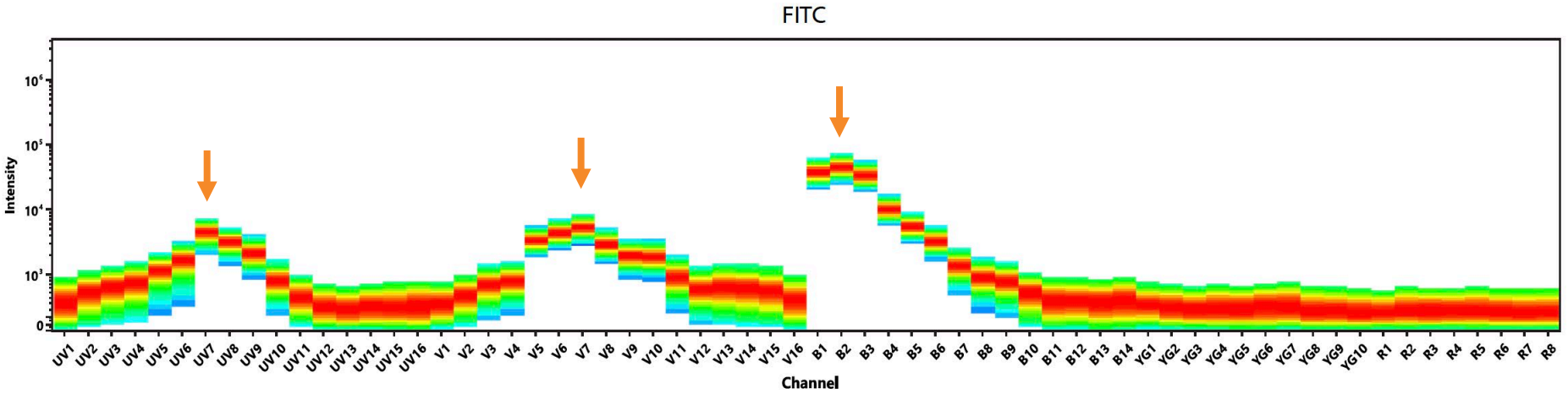
Cytek®Northern Lights™
3 laser/38 channels
BSL1

Cytek®Aurora™
5 laser/64 channels
BSL1

FoMD FLOW

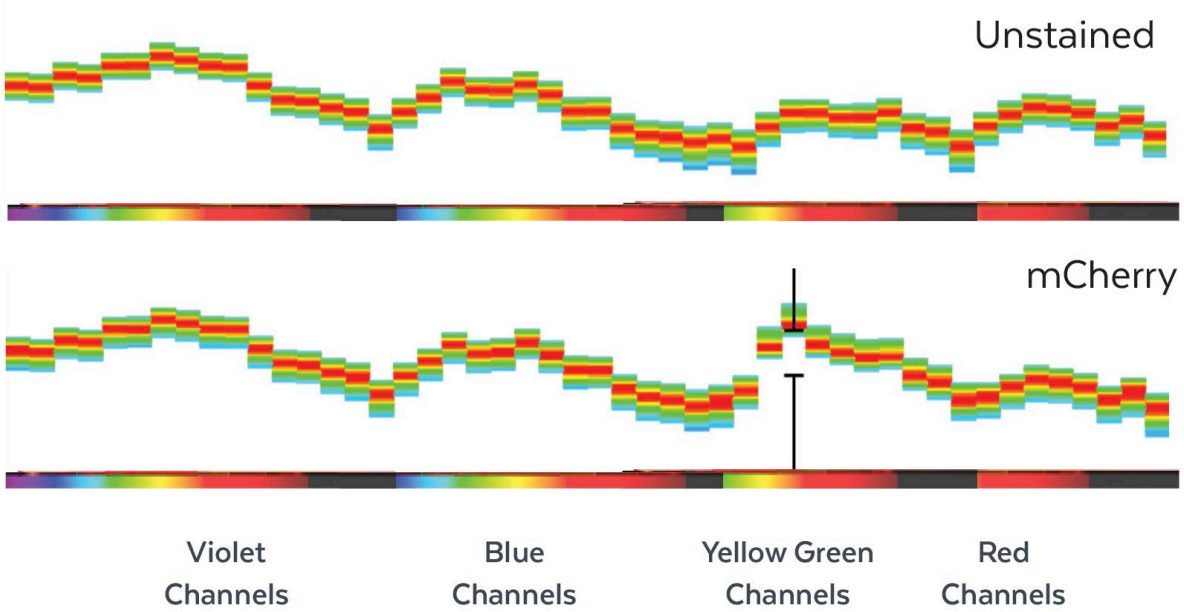
BSL, Biosafety Level

Spectral Cytometry: FITC excited by blue, violet and ultraviolet lasers



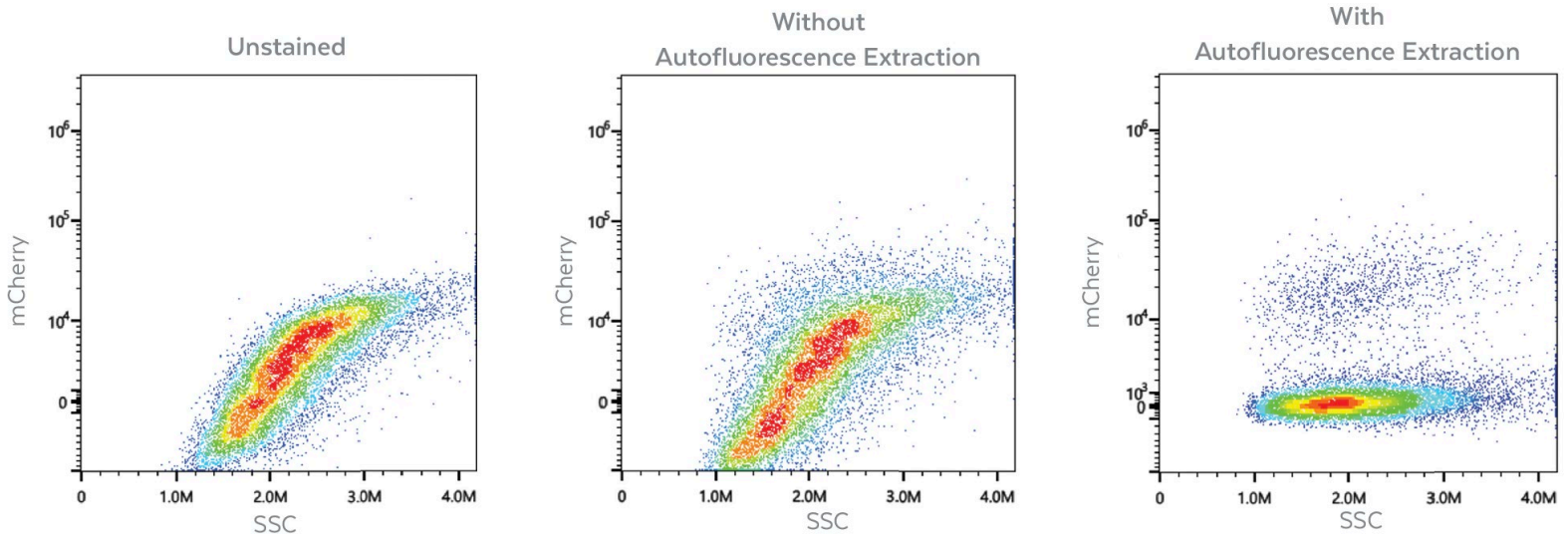
https://cloud.cytekbio.com/spectrum/cloudspectrumviewer

Spectral Cytometry: visualize and subtract cellular autofluorescence!



https://welcome.cytekbio.com/hubfs/Posters/Autofluorescence%20Extraction%20Poster.pdf

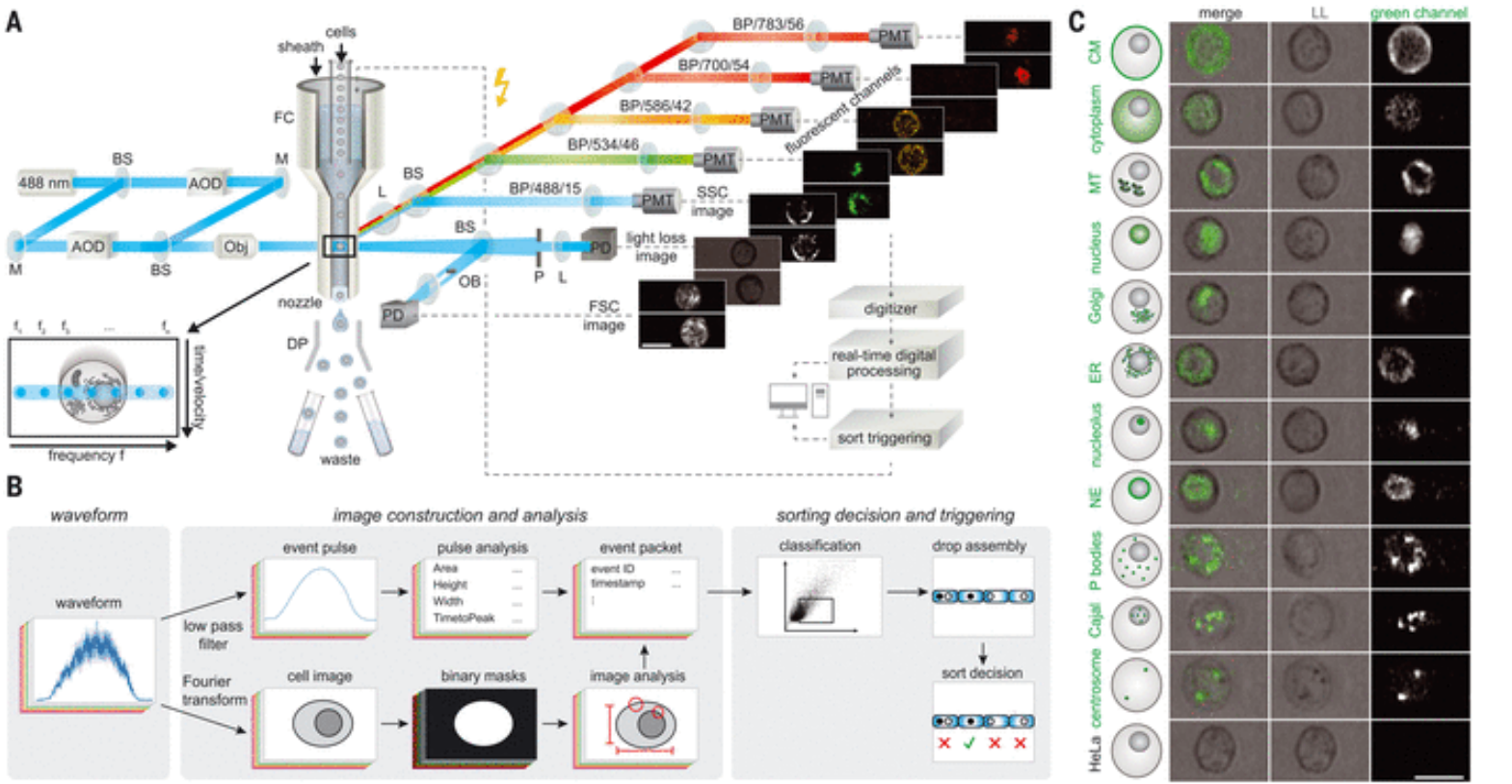
Spectral Cytometry: visualize and subtract cellular autofluorescence!



https://welcome.cytekbio.com/hubfs/Posters/Autofluorescence%20Extraction%20Poster.pdf

Flow cytometry is always evolving!

BD FACS Discover S8 combines spectral, imaging flow and sorting!!!



High-speed fluorescence image–enabled cell sorting, Volume: 375, Issue: 6578, Pages: 315-320, DOI: (10.1126/science.abj3013)

Who and where are we?

The Faculty of Medicine and Dentistry Flow Cytometry Facility is a multi-user facility at the University of Alberta

HMRC 6-67 main facility
HMRC 6-50 analysis room
Katz 6-125 BSL2 analyzer
Katz 6-127 BSL1 spectral analyzer

Hannah McGillis
Flow Core Technologist

Photo credit: Olga Ivanova

Lai Xu, MSc
Lead
Flow Core Technologist

https://www.ualberta.ca/en/medicine/research/corefacilities/flow-cytometry-facility/

How to Access the Flow Core

Go to Flow Core Home Page: https://www.ualberta.ca/en/medicine/research/corefacilities/flow-cytometry-facility/ for detailed instructions

- Create a Stratocore PPMS Account*
- Access virtual training program
    - 9 videos (Intro to Flow Cytometry playlist, each <20 min)
    - contact me at gmsiegers@ualberta.ca for a link to the quiz
    - 24h to submit, 80% to pass, multiple versions in case of fail
    - after pass: request hands-on training** -> for conventional flow cytometers and Sony MA900 sorter
- For access to spectral cytometers:
    - review additional information package
    - contact me for spectral cytometry quiz
    - 24h to submit, 70% to pass
    - after pass: request hands-on training*
- **Submit EHS form (in PPMS under “Request”) -> must be validated prior to hands-on training

Ideally: connect with us before requesting hands-on training to ensure training on most suitable instrument to achieve experimental goals

*you can request assisted analysis or sorting here

Flow Cytometry Summary

- ✓ powerful technology to measure properties of cells or particles
- ✓ always evolving: conventional to spectral, imaging flow cytometry
  - ✓ increasing number of features – 50-colour panels!
- ✓ Combine with other techniques to deepen understanding
  - ✓ single cell genomics
- ✓ FoMD flow cytometry core has conventional and spectral analyzers, core-assisted and non-assisted sorters, and imaging flow cytometry

Leading with Purpose.

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