

Unlocking the Hidden World of Cells with Flow Cytometry

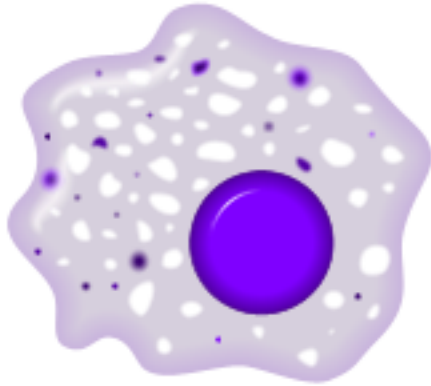
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17 June 2025



What is cytometry?

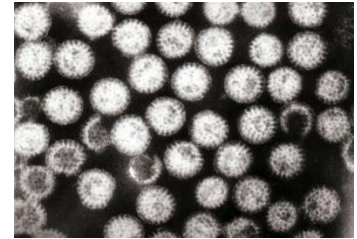
Cyto = ?



metry = ?



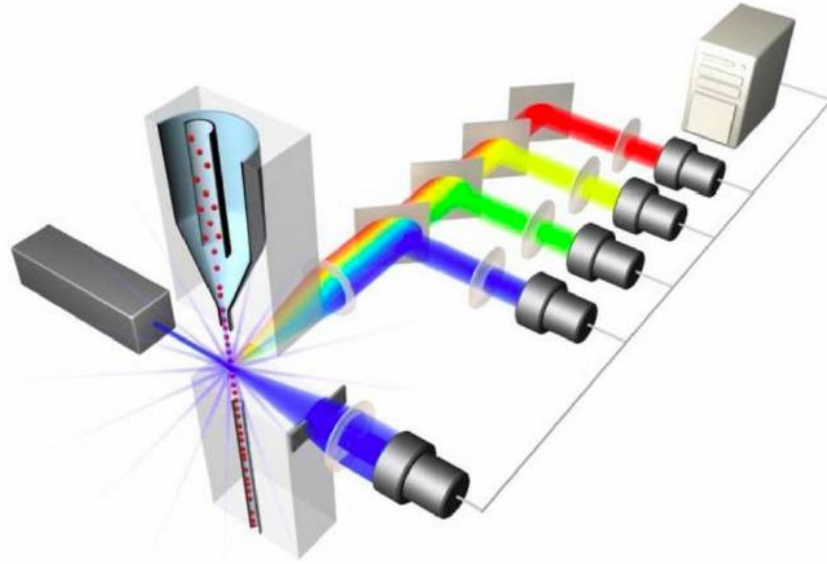
Not just cells:
also particles!



Rotavirus particles

What is flow cytometry?

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

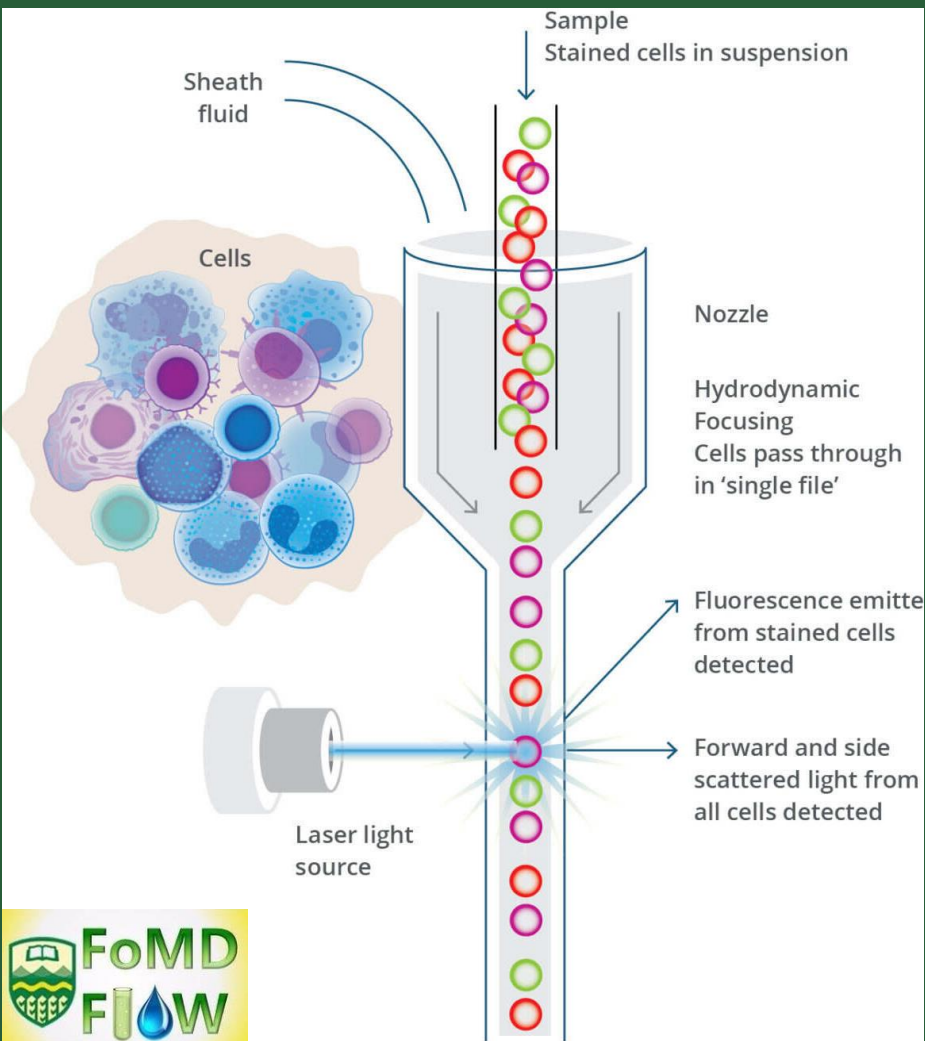


What can we learn about cells with flow cytometry?

- ✓ 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!

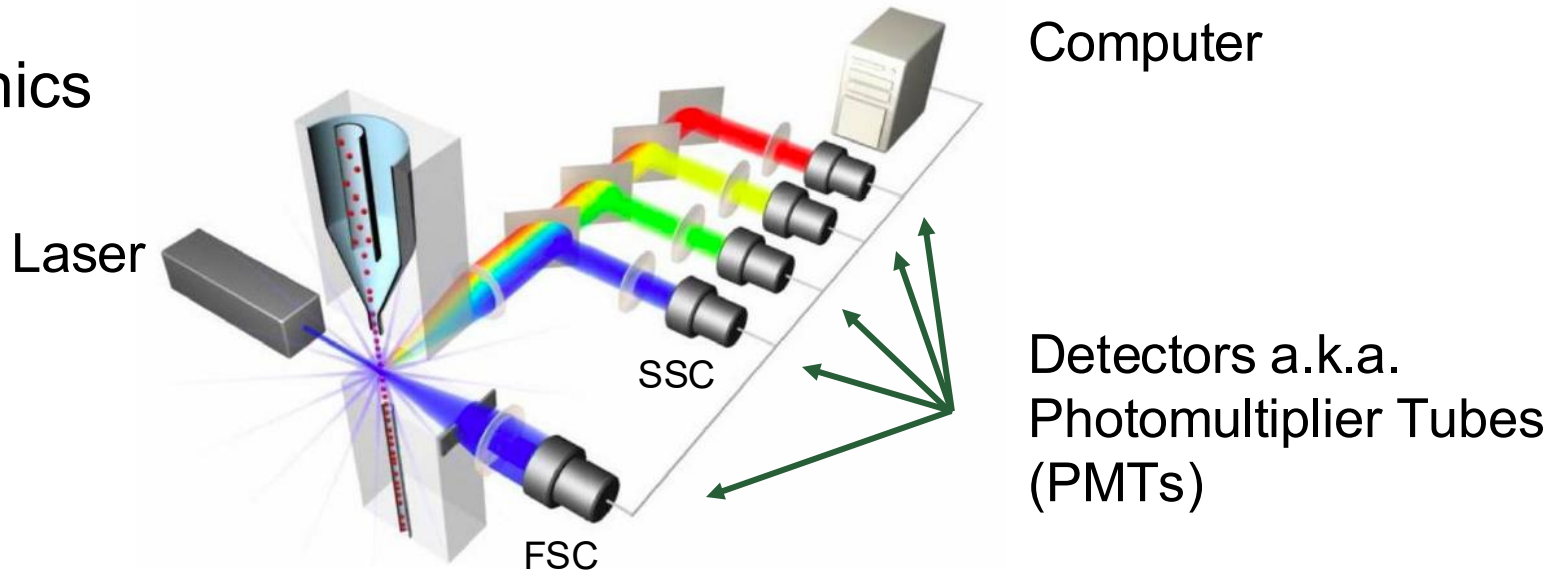




- ❑ Harvest cells or dissociate tissues, wash
- ❑ Stain with fluorescently labeled antibodies to markers (cell surface or intracellular)
- ❑ Acquire:
 - ❑ cells taken up in sheath fluid
 - ❑ directed into single cell stream
 - ❑ within flow cell encounter laser beam
 - ❑ laser excites fluorophores -> 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

Flow cytometry components

Fluidics
Optics
Electronics



FSC, forward scatter; SSC, side scatter

Types of flow cytometers



Thermo Attune NxT
Analyzer



BD FACS Aria III
Sorter – sort and
recover populations



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

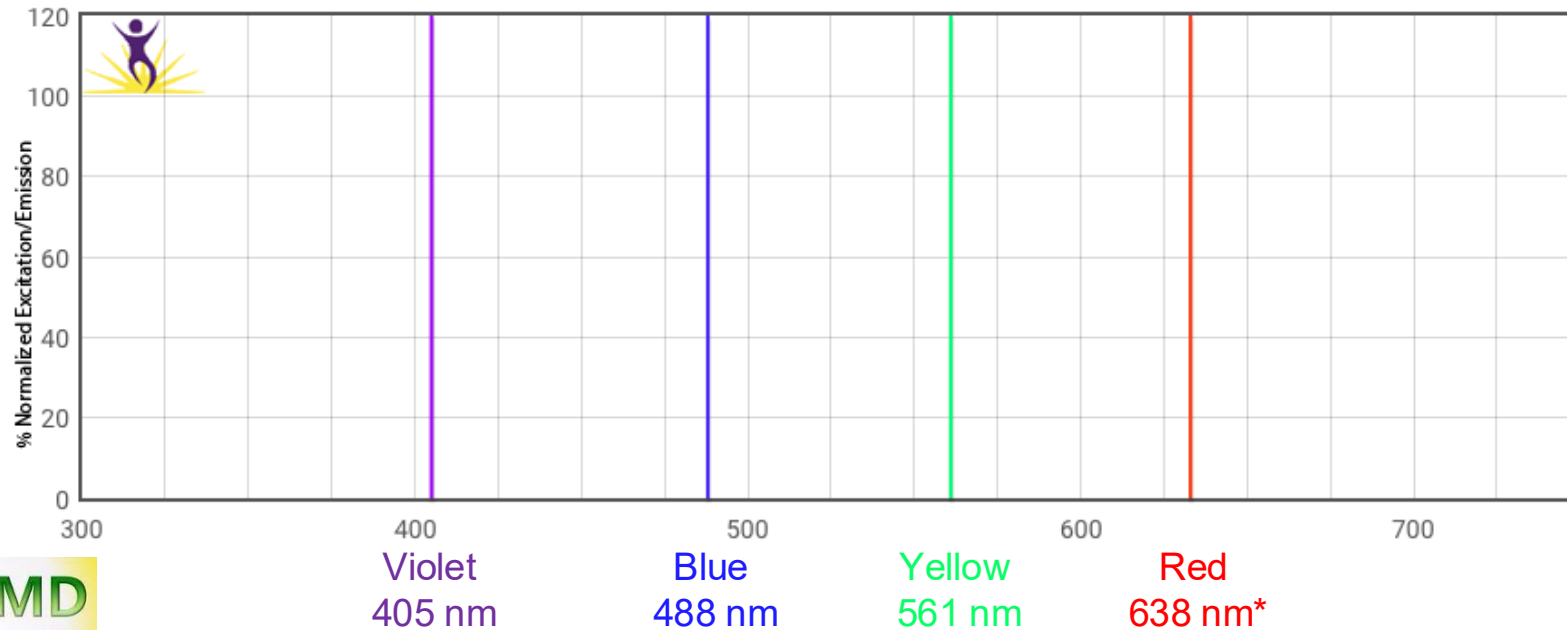
Attune NxT Flow Cytometer (analyzer)

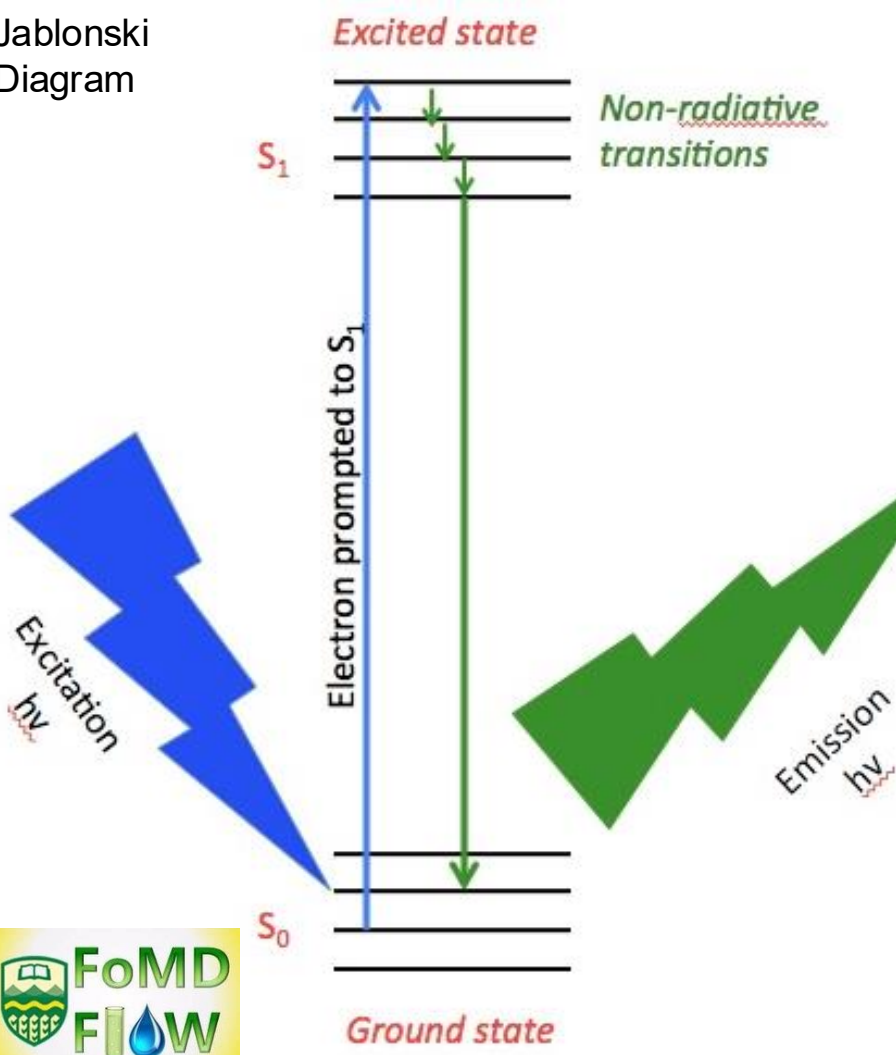


4 laser/ 16 colour

Attune NxT lasers

Spectra Analyzer





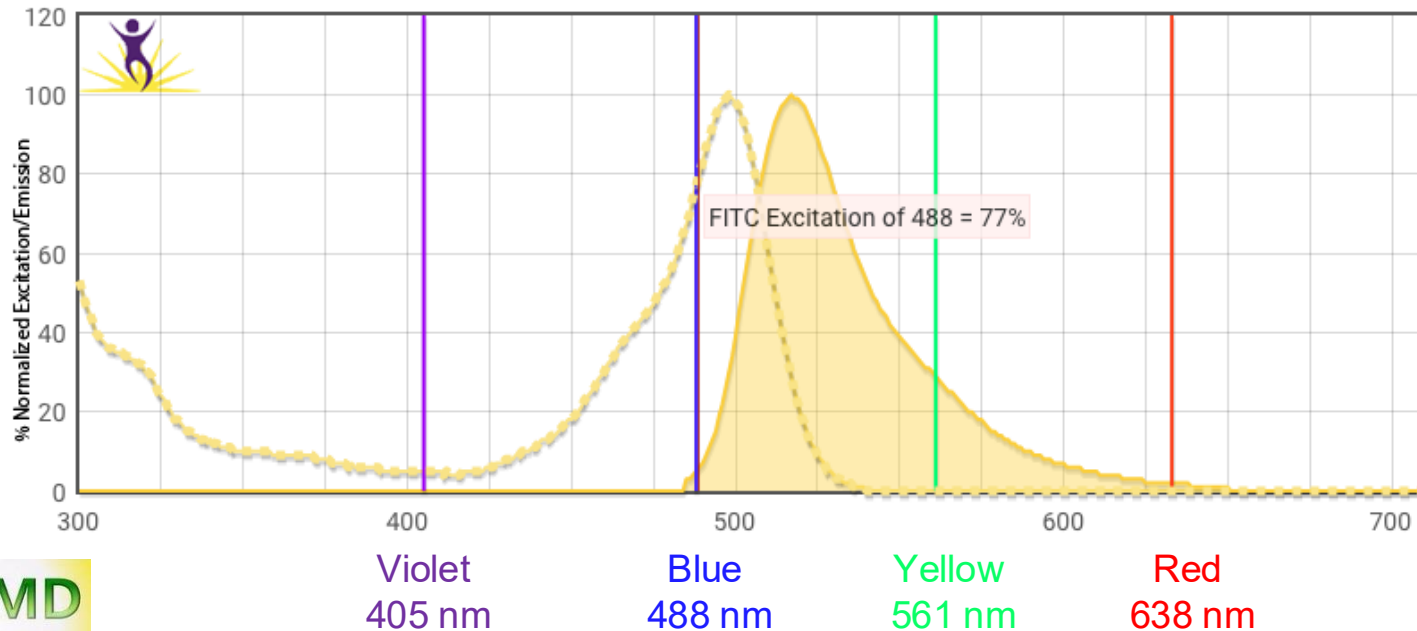
Fluorophore Excitation and Emission

- ❑ 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

FITC is excited by the blue laser (488 nm)

Spectra Analyzer

FITC ex 495/ em 519



Attune NxT Flow Cytometer (analyzer)

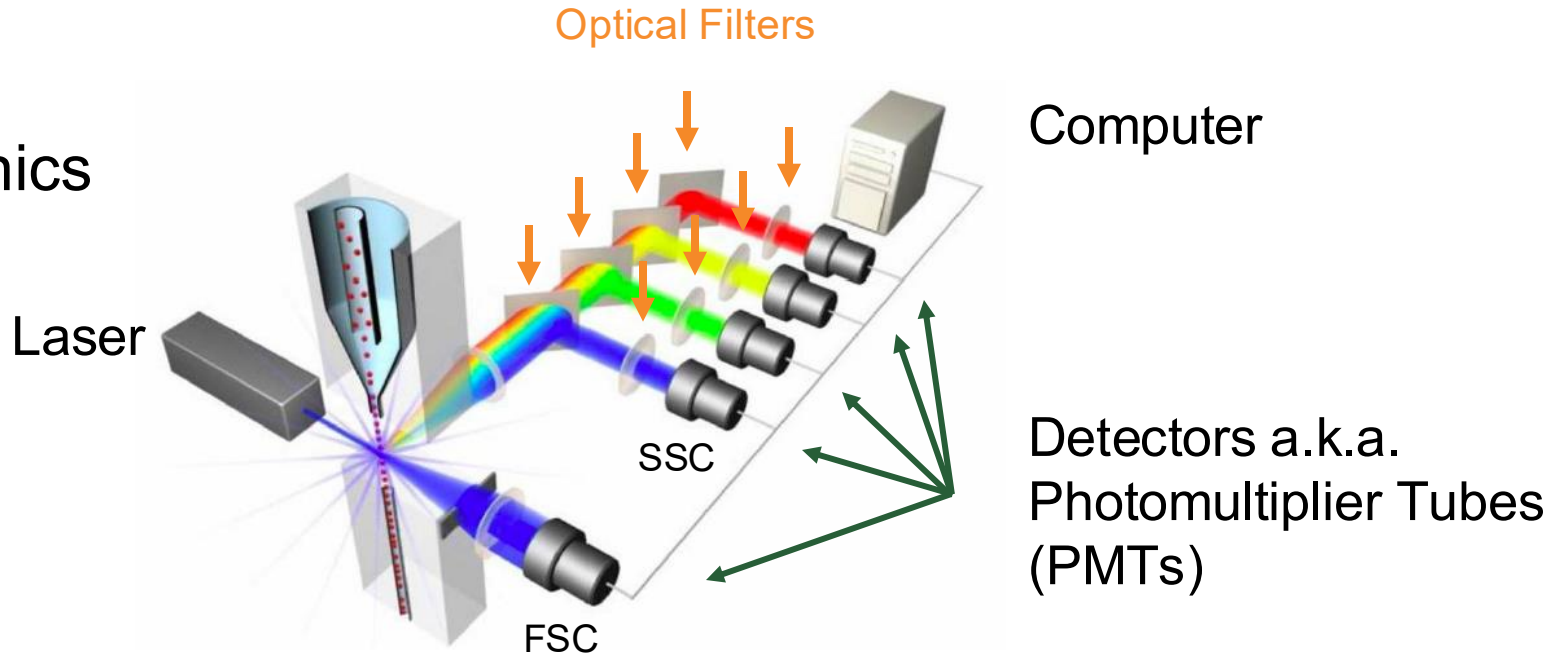


4 laser/ 16 colour

How do you get 16 colours out of 4 lasers?

Flow cytometry components

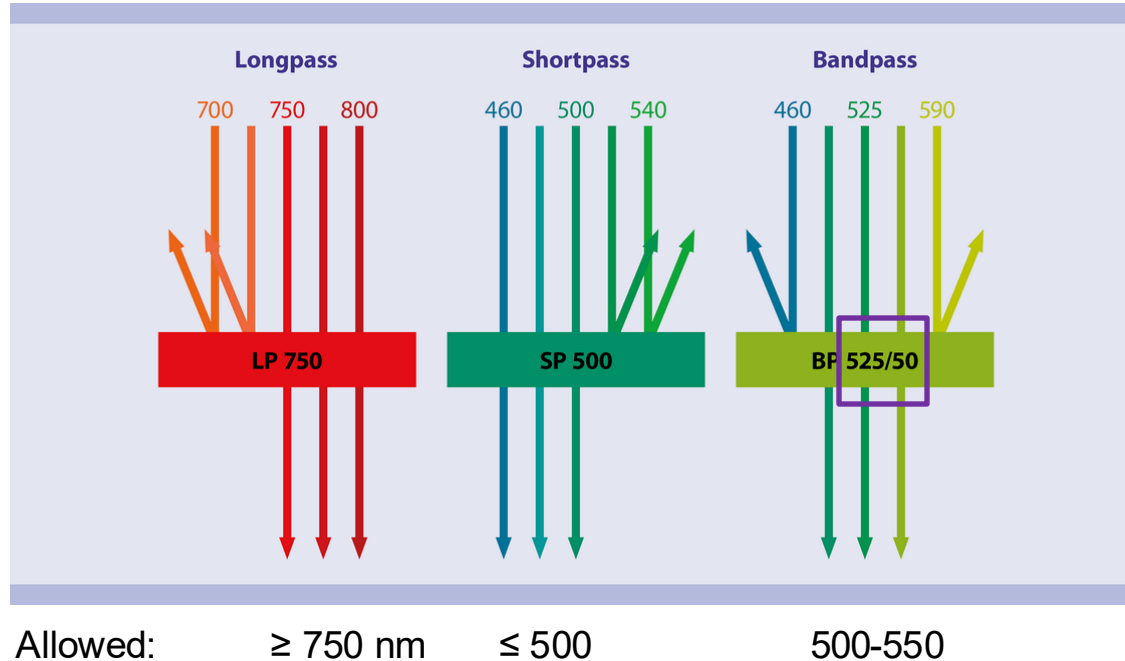
Fluidics
Optics
Electronics



FSC, forward scatter; SSC, side scatter

Optical filters distinguish wavelengths

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



$$525/50 = 525 \pm 25$$

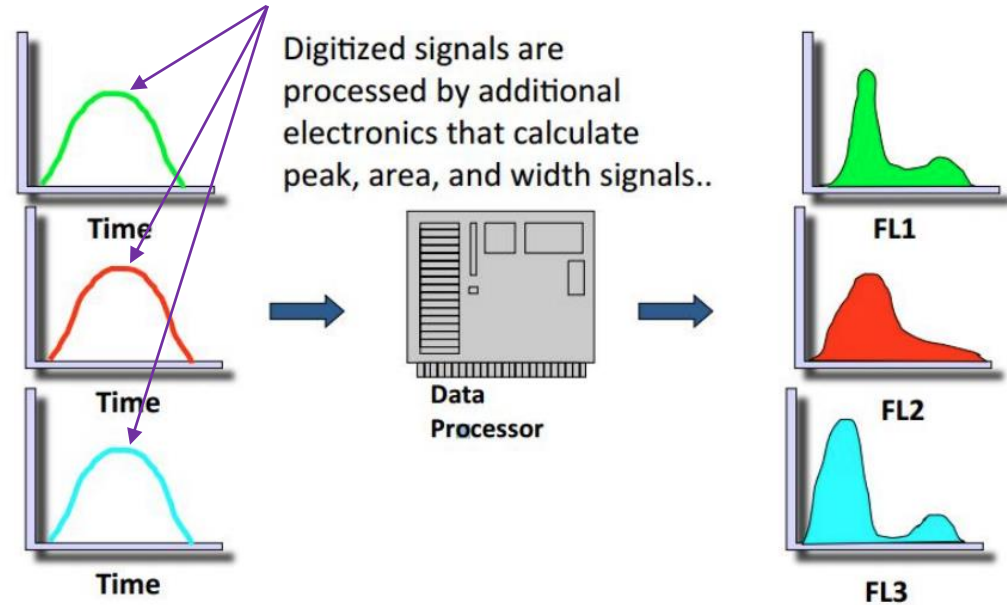
Filters are configured such that blocked light is deflected and travels to the next filter

Emitted light is detected by PMTs

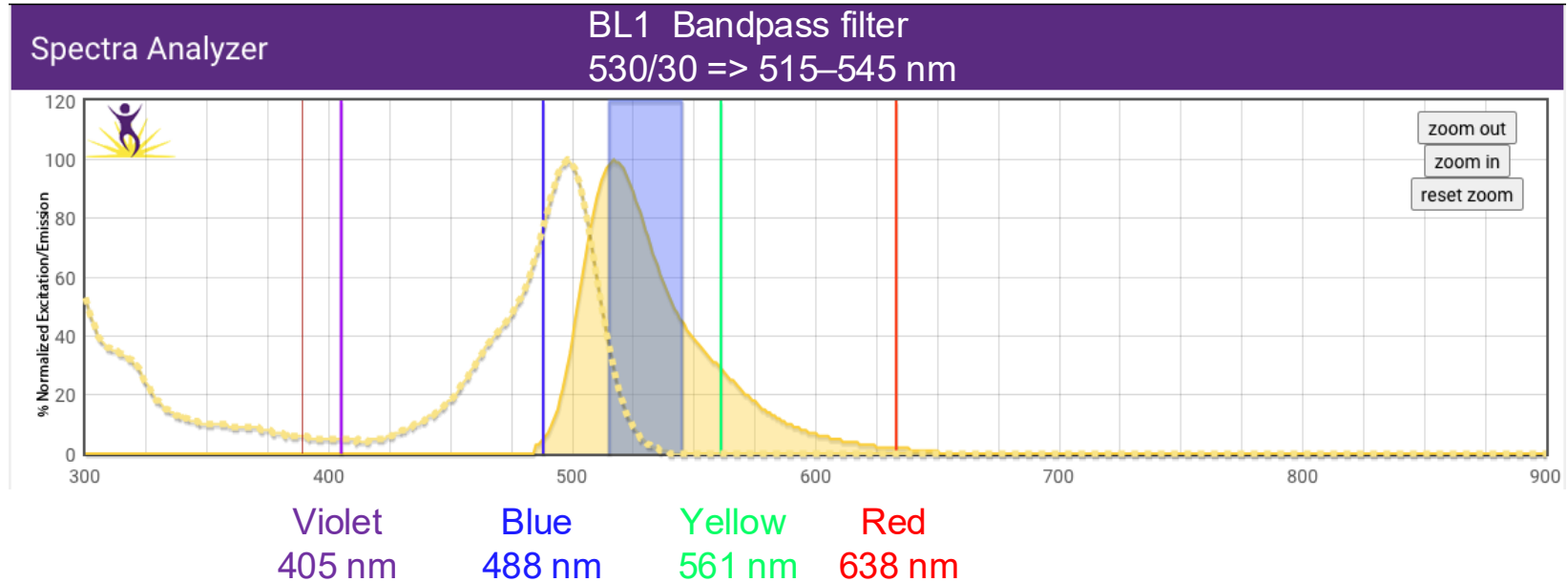
Signals are detected and amplified by PhotoMultiplier Tubes (PMTs)



Photo-electron pulses are digitized



FITC emission detected in the BL1 channel



FITC peak excitation: 495 nm
peak emission: 519 nm



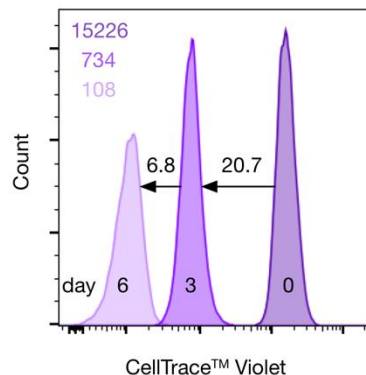
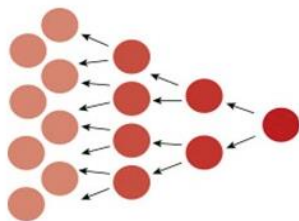
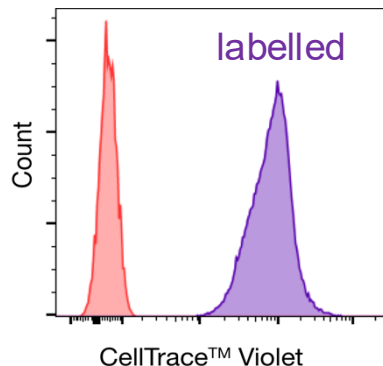
Measuring cell proliferation with CTV

one colour: histogram

Cell Trace Violet (CTV, Ex/Em = 405/450)

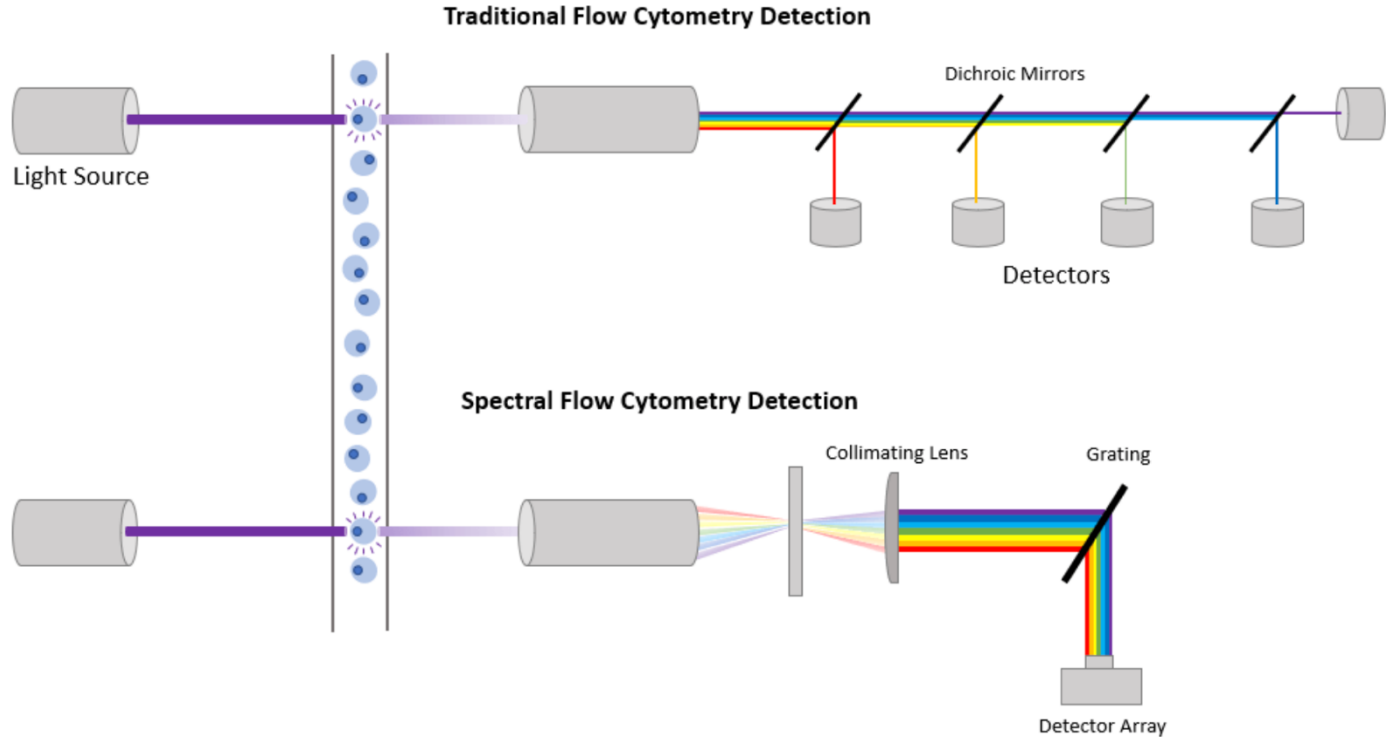
- cell labeling dye; intensity decreases as cells divide
- quantify proliferation in response to stimulus

Not labelled



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

Spectral Flow Cytometry: new and improved flow!



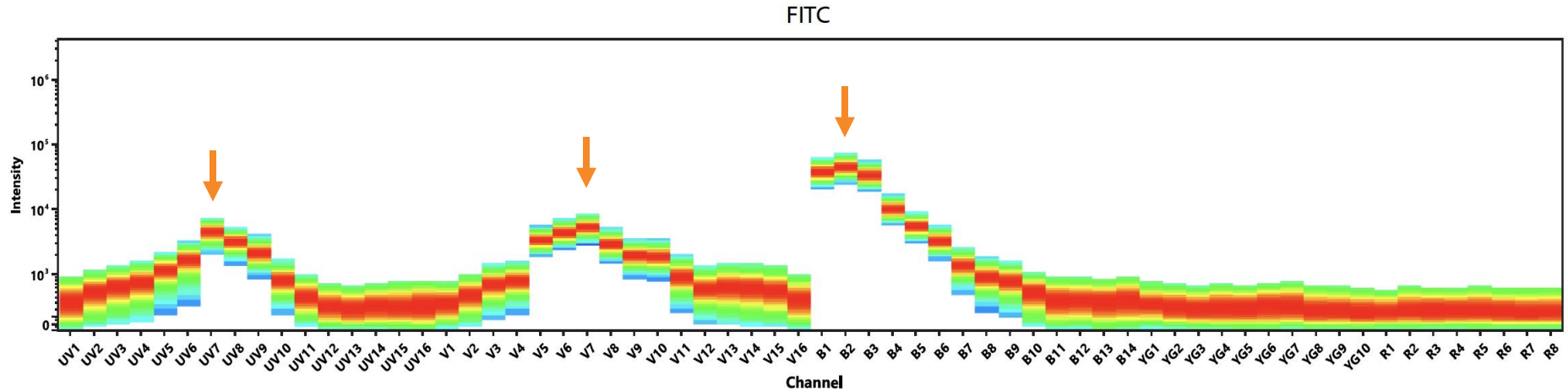
<https://fluorofinder.com/spectral-flow-cytometers/>

Cytek Aurora Spectral Flow Cytometer

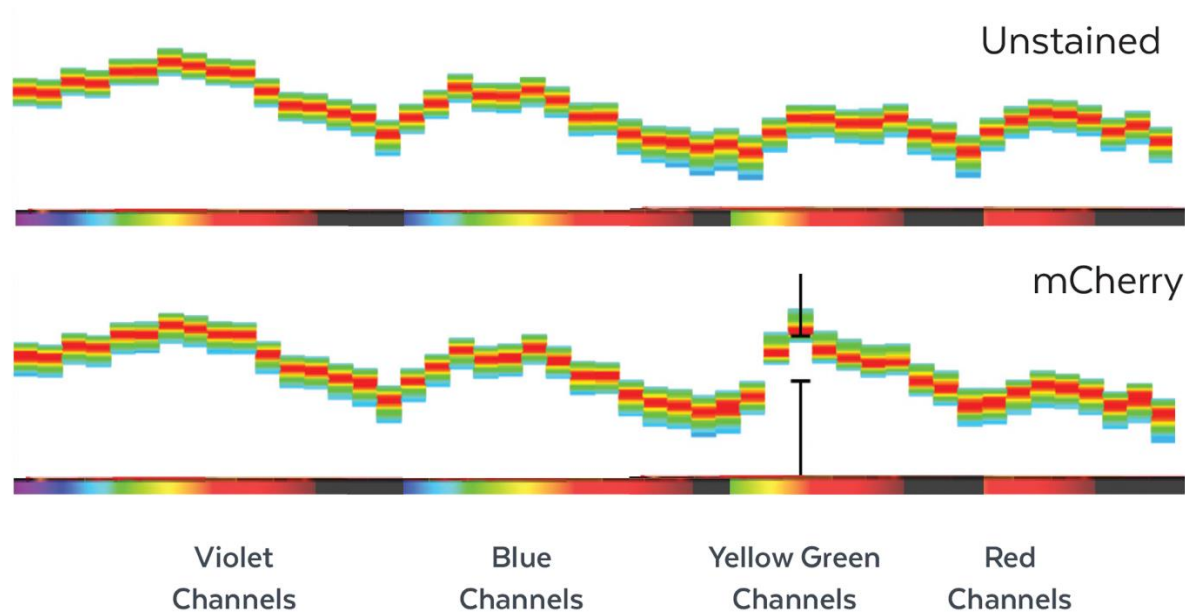


5 Lasers
64 channels

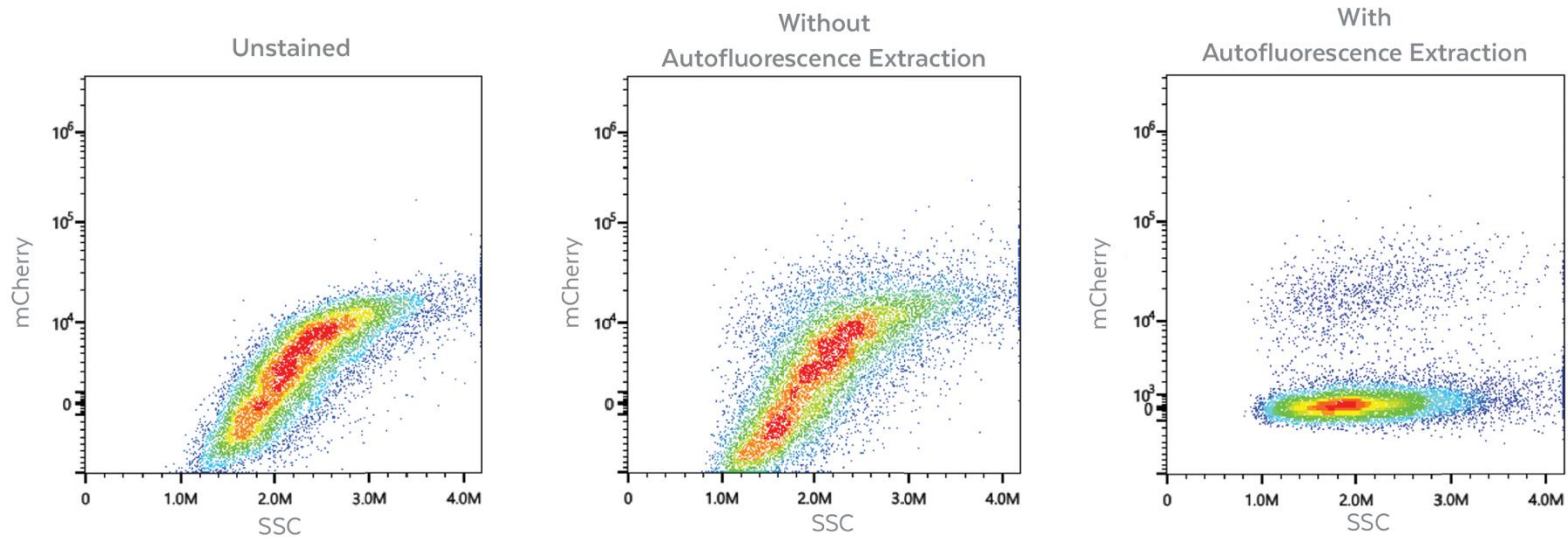
Spectral Cytometry: FITC excited by blue, violet and ultraviolet lasers



Spectral Cytometry: visualize and subtract cellular autofluorescence!

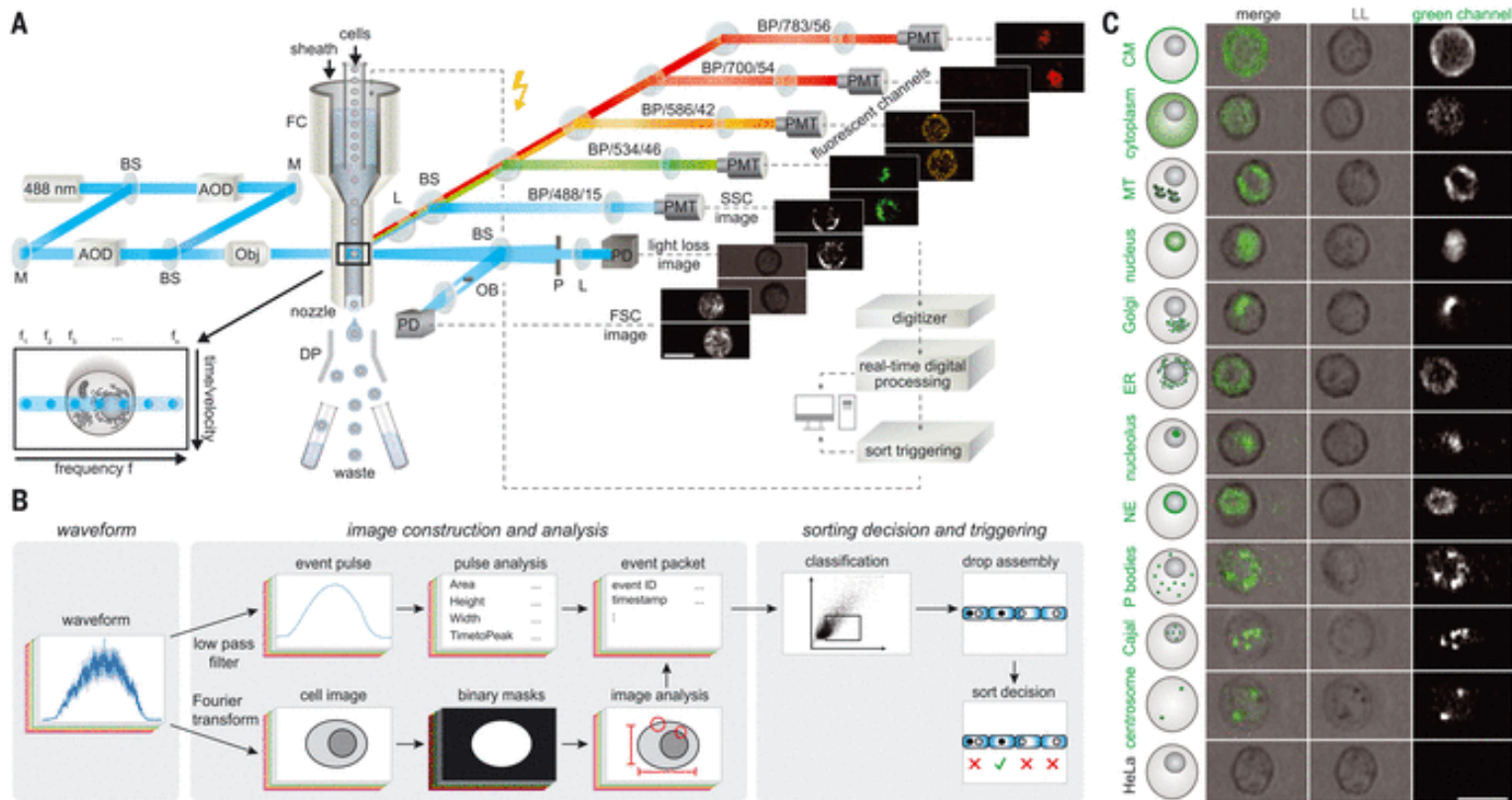


Spectral Cytometry: visualize and subtract cellular autofluorescence!



Flow cytometry is always evolving!

BD FACS
DiscoverS8
(A8 soon)
combines
spectral,
imaging flow
and sorting!!!



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.

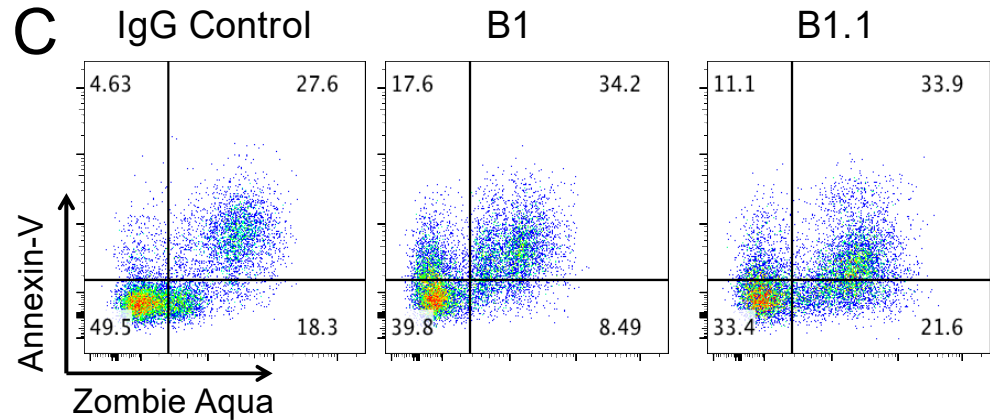
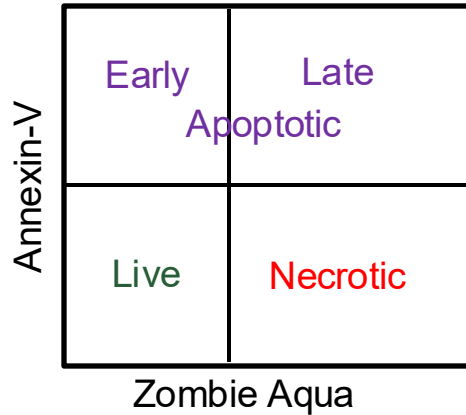


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OF ALBERTA

Cell death: apoptosis and necrosis

Anti-TCR antibodies stimulate T cells
T cells undergo activation-induced cell death (AICD)
Annexin-V is an apoptosis marker
Zombie dyes label dead cells

B1.1 antibody
induces apoptosis
in human $\gamma\delta$ T cells



Dutta I, LM Postovit and GM Siegers. $\gamma\delta$ T cell Apoptosis Induced via $\gamma\delta$ T Cell Antigen Receptor “Blocking” Antibodies: A Cautionary Tale. *Frontiers in Immunology – T Cell Biology*. 2017

Attune NxT lasers and filters

