Unlocking the Hidden World of Cells with Flow Cytometry

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What is cytometry?



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

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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 <u>excites</u> fluorophores -> they <u>emit</u> 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



Types of flow cytometers



Thermo Attune NxT **Analyzer**

BD FACSAria 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



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



Fluorophore Excitation and Emission

Cells encounter a laser beam as they pass through the flow cell

□ The laser <u>excites</u> fluorophores

□Emission energy < absorption energy

Fluorophores <u>emit</u> 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)

Spectra Analyzer

FITC ex 495/ em 519



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?



Flow cytometry components



Optical filters distinguish wavelengths

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





Emitted light is detected by PMTs

Signals are detected and amplified by PhotoMultiplier Tubes (PMTs)



Digitized signals are processed by additional electronics that calculate peak, area, and width signals.. Time Data Time Processor Time

Photo-electron pulses are digitized





Jan Helebrant, CC0, via Wikimedia Commons; https://commons.wikimedia.org/wiki/File:Technolo gy_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





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

Measuring cell proliferation with CTV

one colour: histogram

am 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.



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!

Traditional Flow Cytometry Detection





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

Cytek Aurora Spectral Flow Cytometer



5 Lasers 64 channels



https://biomedicalsciences.unimelb.edu.au/cytometry/our-capabilities/analysers/spectral-cytometers

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!





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Flow cytometry is always evolving!

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

FOMD



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

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.



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 γδ T cells





Zombie Aqua



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





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