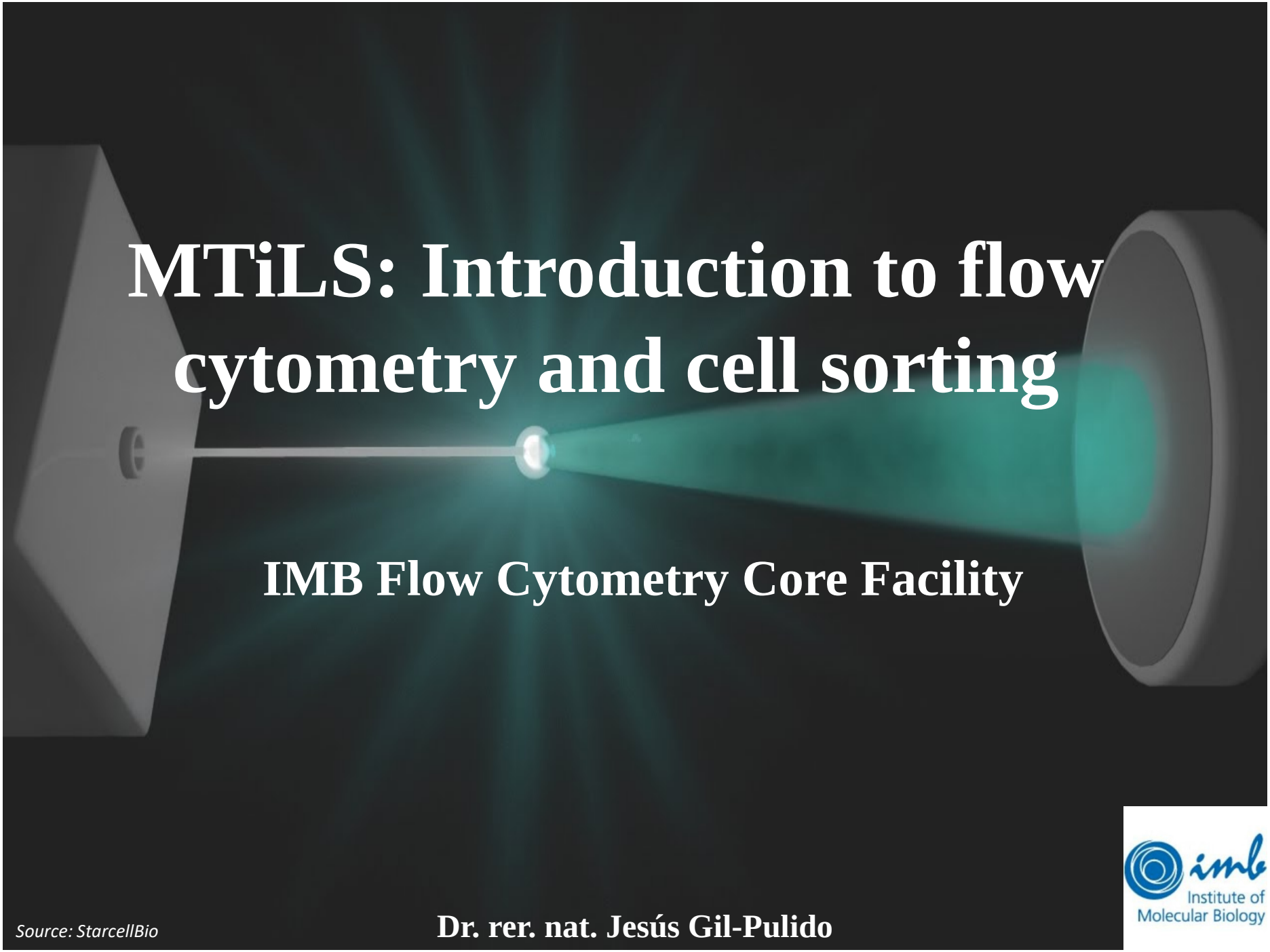
MTiLS: Introduction to flow cytometry and cell sorting

IMB Flow Cytometry Core Facility



Source: StarcellBio

Dr. rer. nat. Jesús Gil-Pulido

Institute of Molecular Biology

Not only basic research, but also used in clinical settings

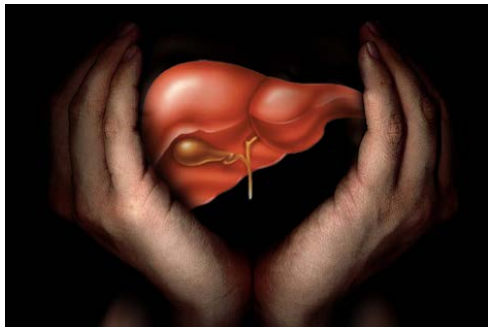
Diagnosis and staging of lymphomas and leucemias

SciencePhotoLibrary

Minimal residual disease

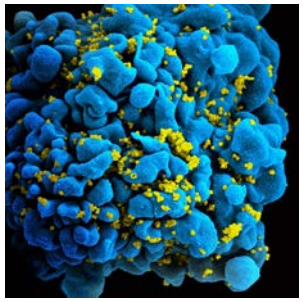
Depositphotos

Solid organ transplantation (monitoring)



Georgetown University

HIV infection



NIAID

Immunodeficiencies
Fetomaternal hemorrhage…

Overview

- What is flow cytometry?
  - FSC – SSC (relative size, granularity / complexity)
  - Fluorescence
- Looking inside a flow cytometer
  - Fluidics
  - Optics
  - Electronics
- Data presentation and gating
- Introduction to electrostatic cell sorting
- Applications of flow cytometry & cell sorting
- Examples of current flow cytometers
- Advantages of flow cytometry over other techniques

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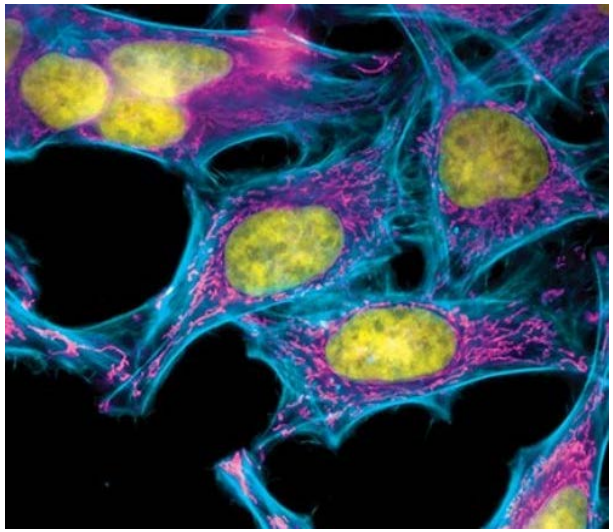
What is flow cytometry?

Flow - Fluid

Source: pbase.com

Cyto - Cell



Source: smithsonianmag.com

Metry - Measurement

Source: clipartkid.com

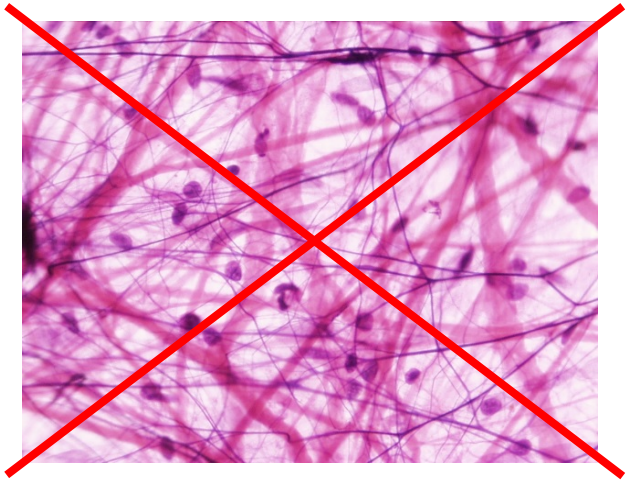
Meaning that a feature from a "cell" in suspension will be measured (at single cell level).

How do flow cytometers do that?

Thanks to lasers and detectors

„…in suspension…“

No solid tissue



…only cell in suspension

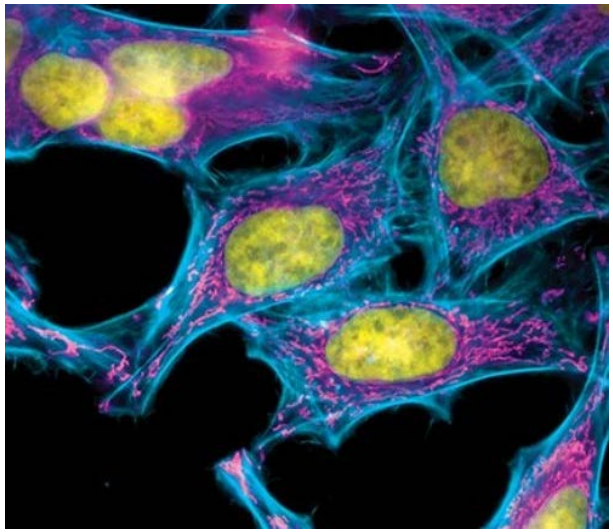
Mechanical disruption
Enzymatic dissociation

What is flow cytometry?

Flow - Fluid

Source: pbase.com

Cyto - Cell



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Metry - Measurement

Source: clipartkid.com

Meaning that a feature from a "cell" in suspension will be measured (at single cell level).

But how does a flow cytometer do that?

Thanks to lasers and detectors

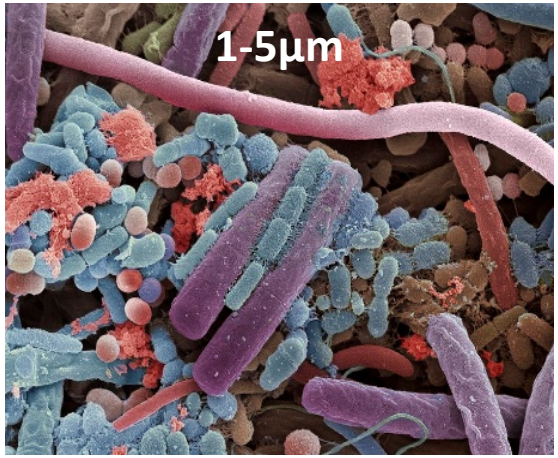
Which features can be measured?

1. Characteristic of the „cell“ (relative size, complexity/granularity)

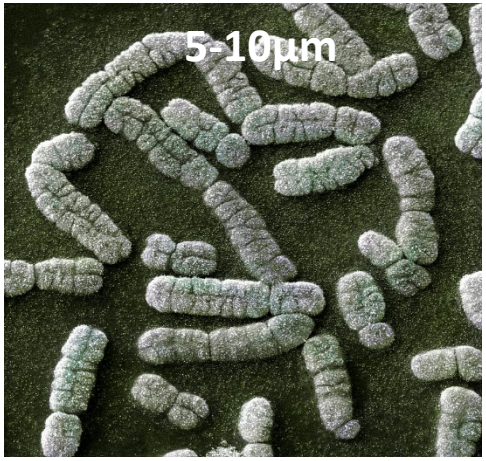
2. Fluorescence

1. Characteristic of the „cell“

Human microbiota (tongue)



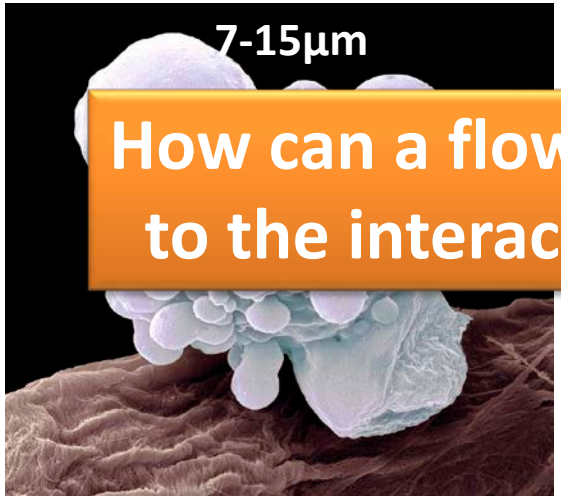
Human chromosomes



Relative size

Granularity/ Complexity

Human lymphocyte



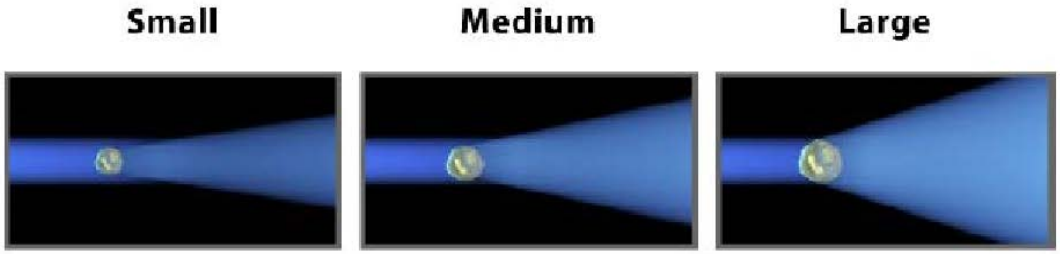
Stem cell

How can a flow cytometer do that? Thank to the interaction of light with the cells.

Source: FineArtAmerica (all)

1. Characteristic of the „cell“: FSC and SSC

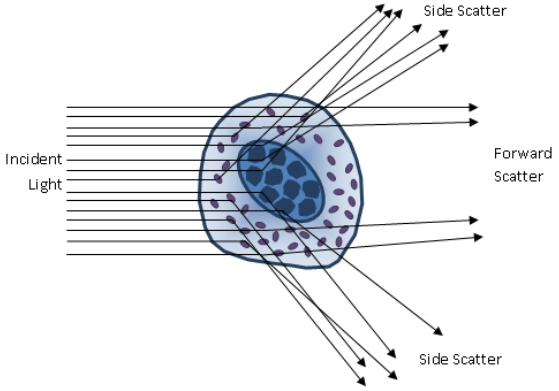
FSC = **F**orward **Sc**atter (diffraction) → Relative size of a particle (signal collected at ~10°)



Source: Adapted from *ThermoFisherScientific*

The bigger the cell, the more diffracted light will be collected.

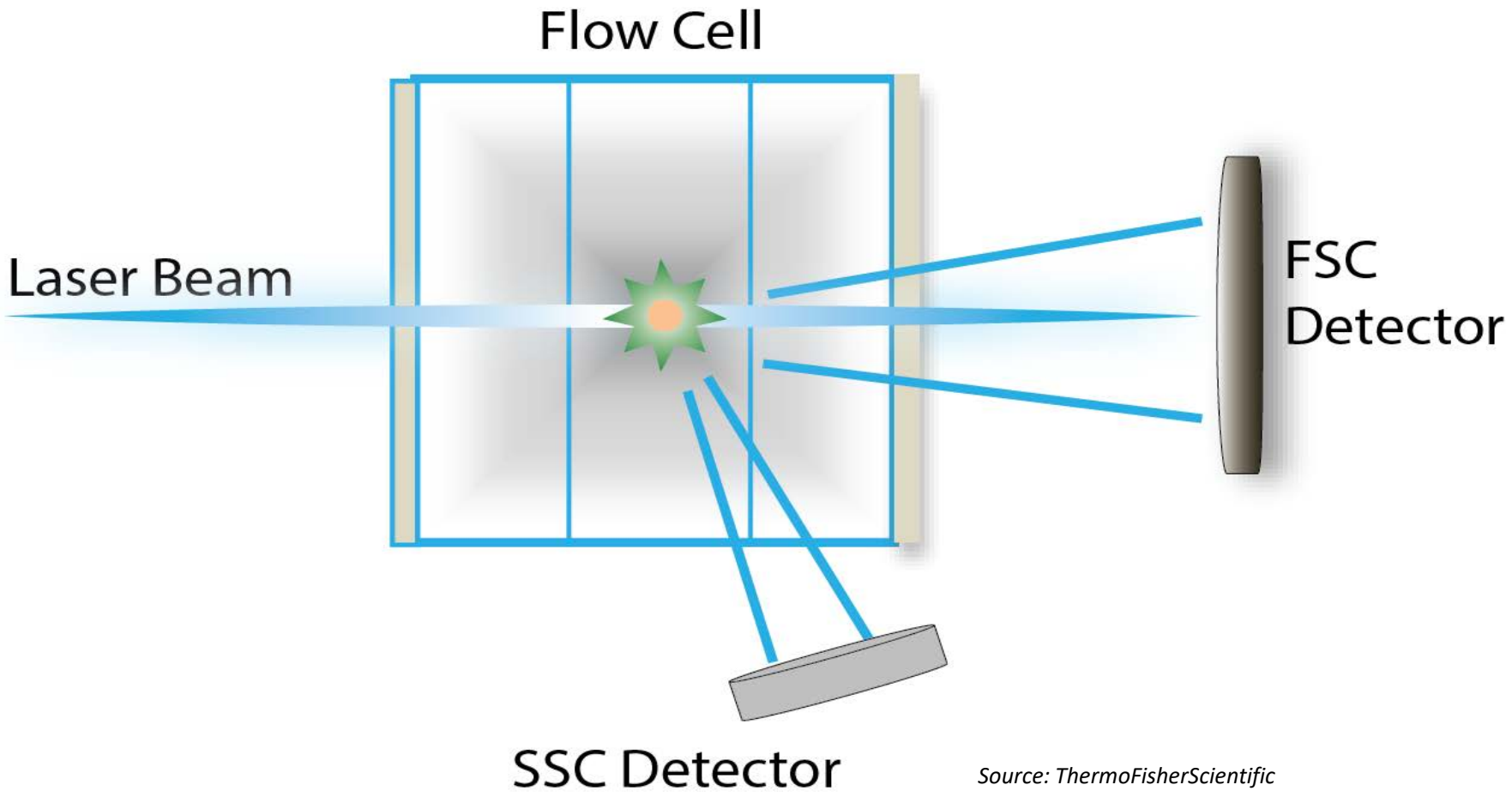
SSC = **S**ide **Sc**atter (reflection/refraction) → Granularity/complexity (signal collected at ~90°)



Source: The University of Queenslad

The more complex a cell is, the more light will be reflected/refracted and collected by the detector!

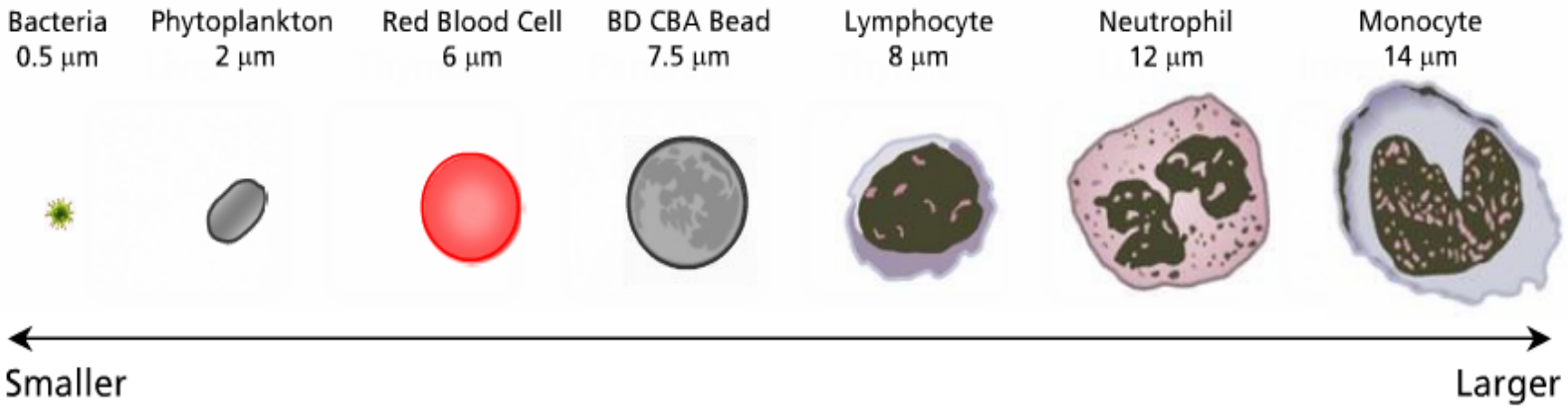
1. Characteristic of the „cell“: FSC and SSC



Source: ThermoFisherScientific

1. Characteristic of the „cell“: FSC and SSC

Conventional flow cytometers



Up to 50 µm

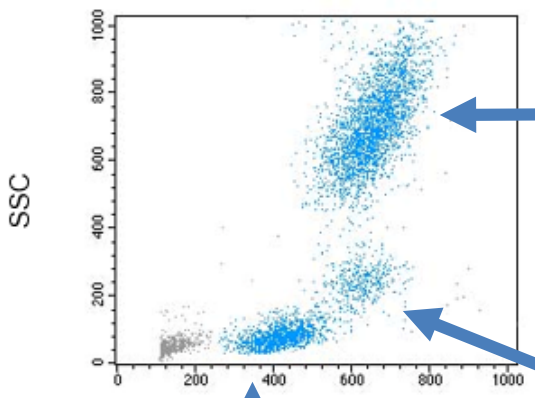
Source: BDBiosciences (modified)

Special instruments

Smaller particles (<0.5µm): Extracellular vesicles (0.03µm – 1µm)
Larger particles (>50µm): Whole organisms or cell clusters: *C. elegans, Drosophila* larvae, pancreatic islets (100µm – 1800 µm)

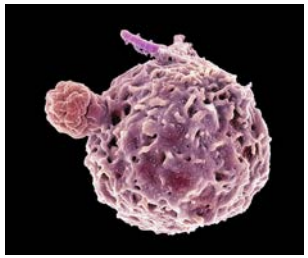
1. Characteristic of the „cell“: FSC and SSC

Lysed human blood



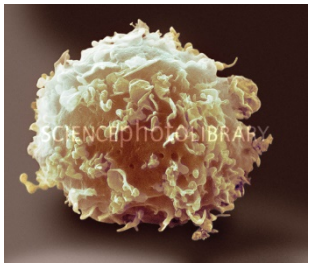
Source: Nordic Mubio

Neutrophils



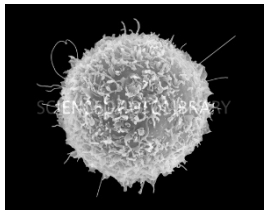
Source: FineArtAmerica

Monocytes



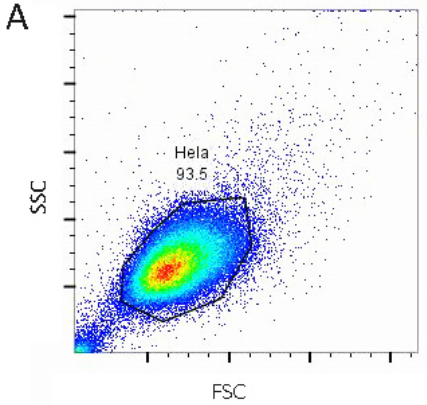
Source: SciencePhoto Library

Lymphocytes



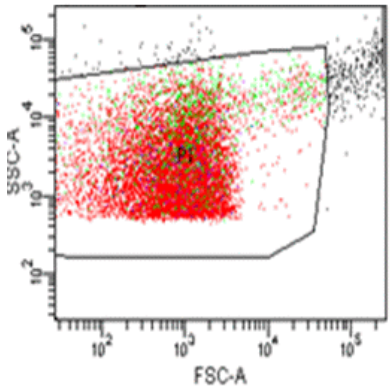
Source: SciencePhoto Library

HeLa cells in culture



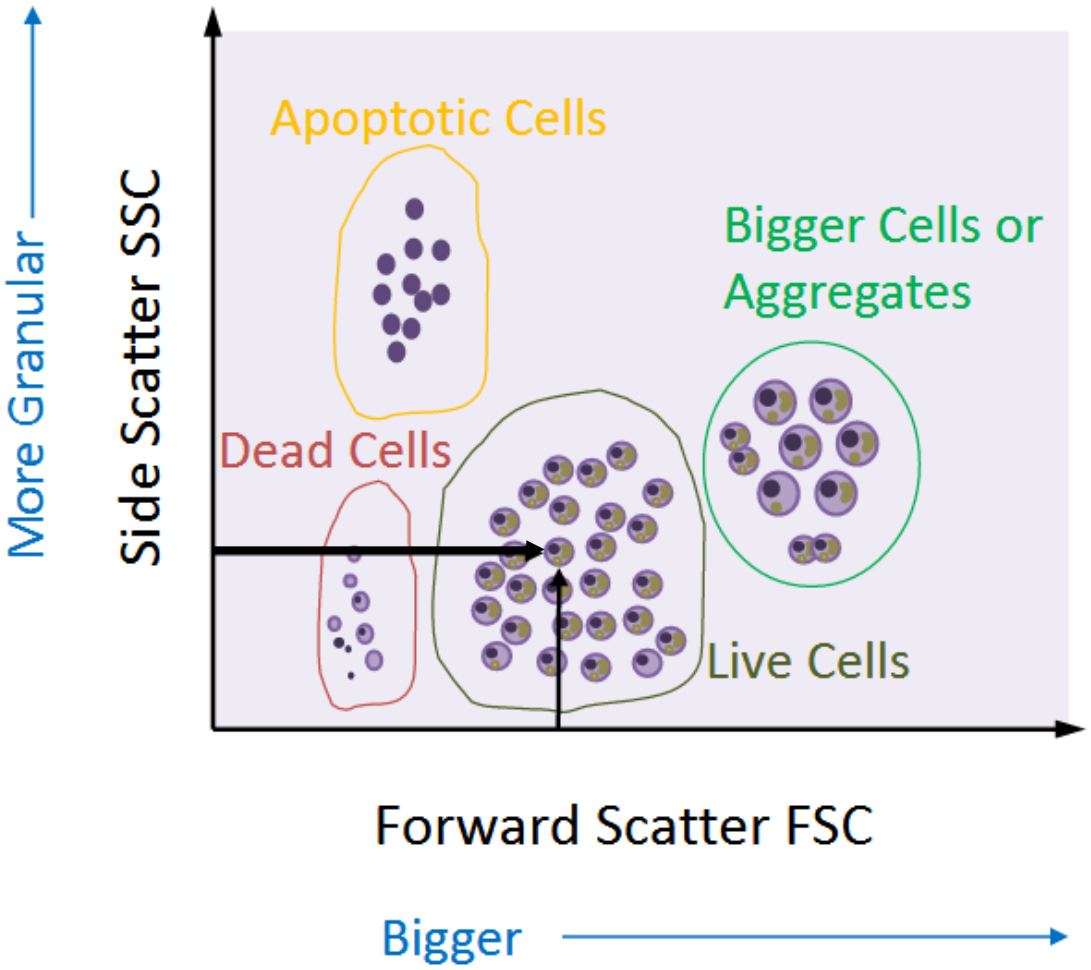
Source: Bioprotocols

Exososomes



Source: Barts and The London

1. Characteristic of the „cell“: FSC and SSC



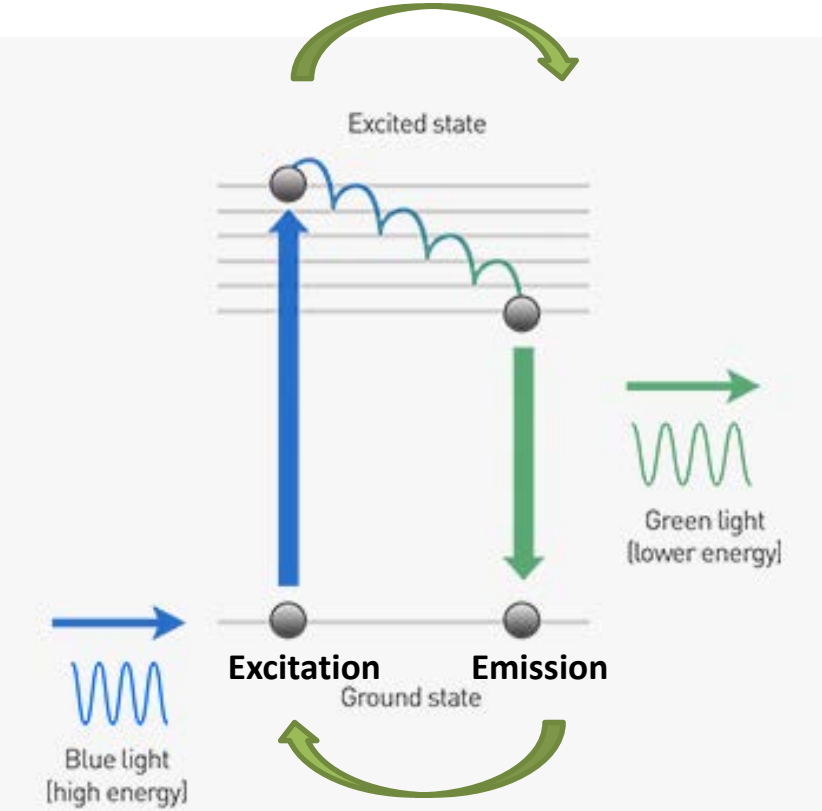
Flow cytometry properties: FSC, SSC and fluorescence

1. Characteristic of the „cell“ (relative size, complexity/granularity)

2. Fluorescence

2. Fluorescence

- Molecules with the ability to fluorescence are fluorophores



Excitation: absorbance of photons

Emission: release of photons

Source: ThermoFisherScientific

2. Fluorescence in flow cytometry

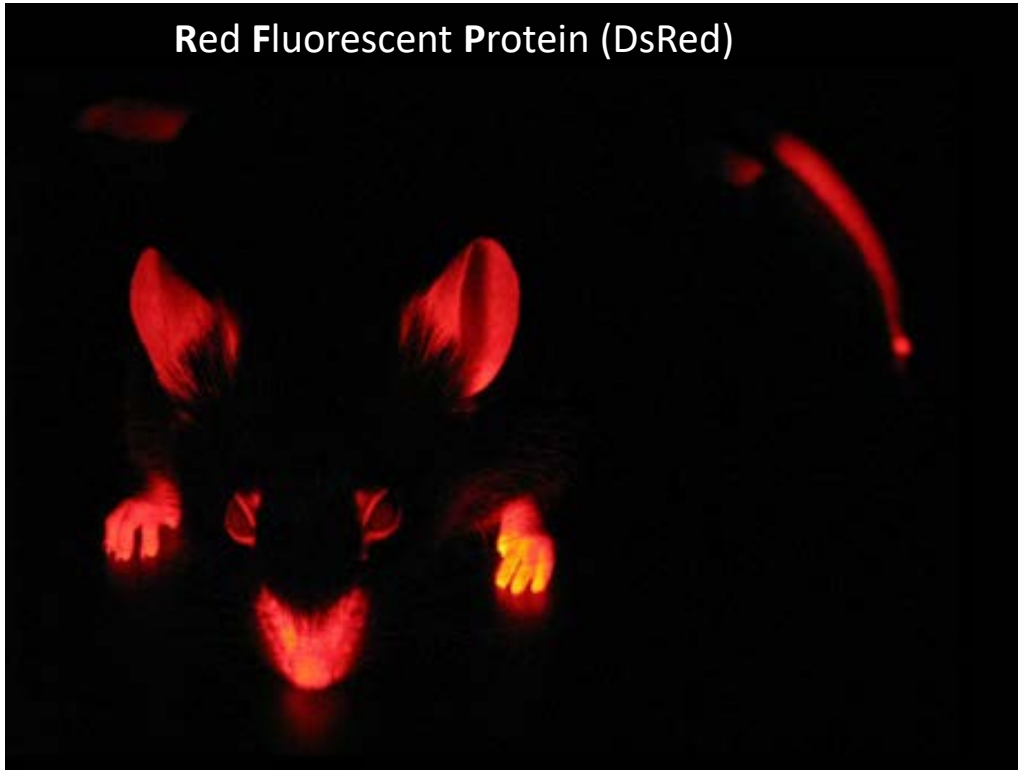
- **How many fluorophores are?**

APC

PE

Fluorescent proteins („natural“)

Source: Microbiological Blogs



Source: NightSea.com

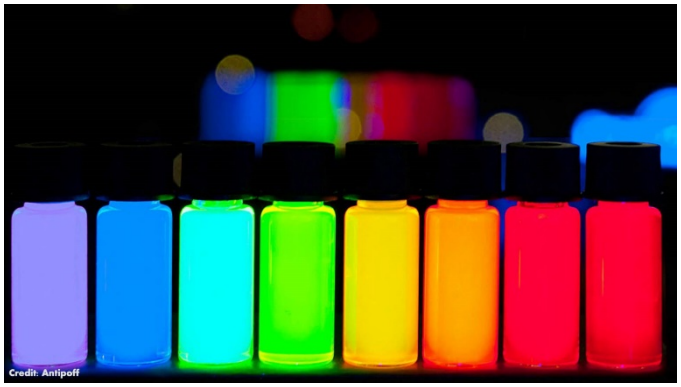
2. Fluorescence in flow cytometry

- **How many fluorophores are?**

Synthetic compounds

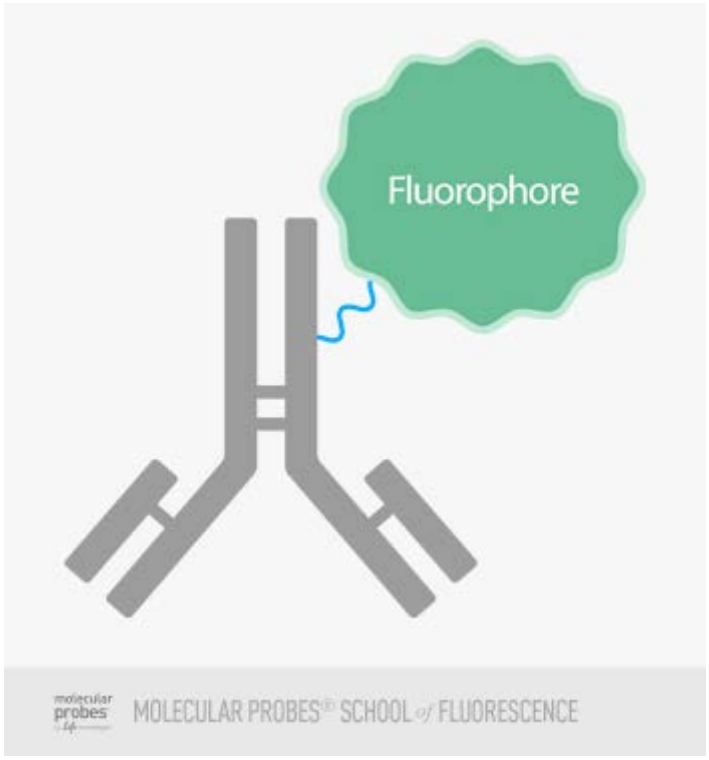
- Cyclic ring compounds: FITC, Texas Red, Alexa 488
- Tandem dyes: BV575, PE-Cy5, APC-Cy7
- Polymer and nanocrystal dyes: BV421, Quantum Dots

Quantum Dots



Source: Medium.com

2. Fluorescence: antibodies conjugated to fluorophores



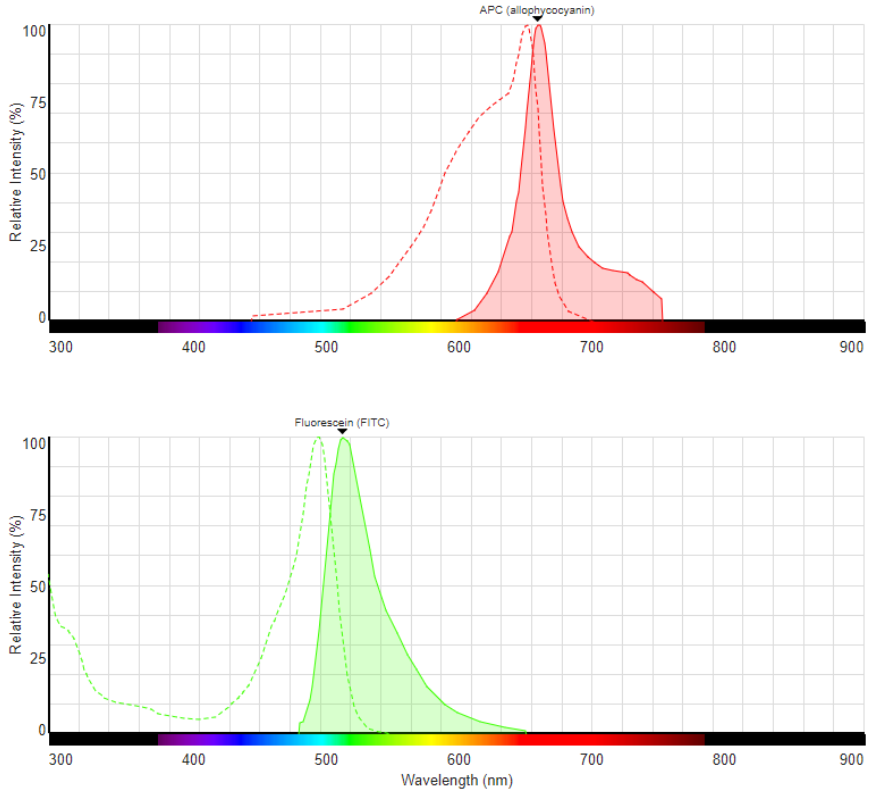
- They specifically bind your antigen (i.e. FITC-antibody recognizing CD3 in T cells) → plenty of options (multiparametric flow cytometry)

- Conjugated directly with fluorophores or biotin (=secondary stainings)

2. Fluorescence

- Every fluorophore has an excitation and an emission range, which are **unique** (important for *spectral cytometry*)

- In flow cytometry, knowing excitation and emission ranges are **critical** (panel design)



Emitted light will always have a longer wavelength = less energy („Stokes shift“)

Source: ThermoFisherScientific Spectral Viewer

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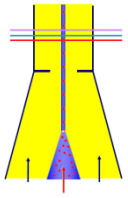
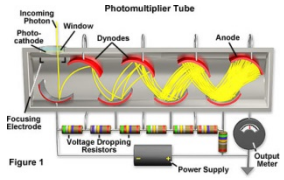
Digital flow cytometer

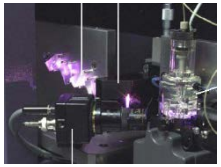


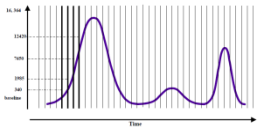
Fluidics

LSRFortessa

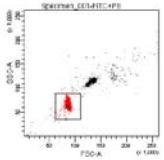
Flow Cytometer

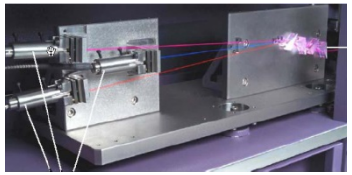






Electronics

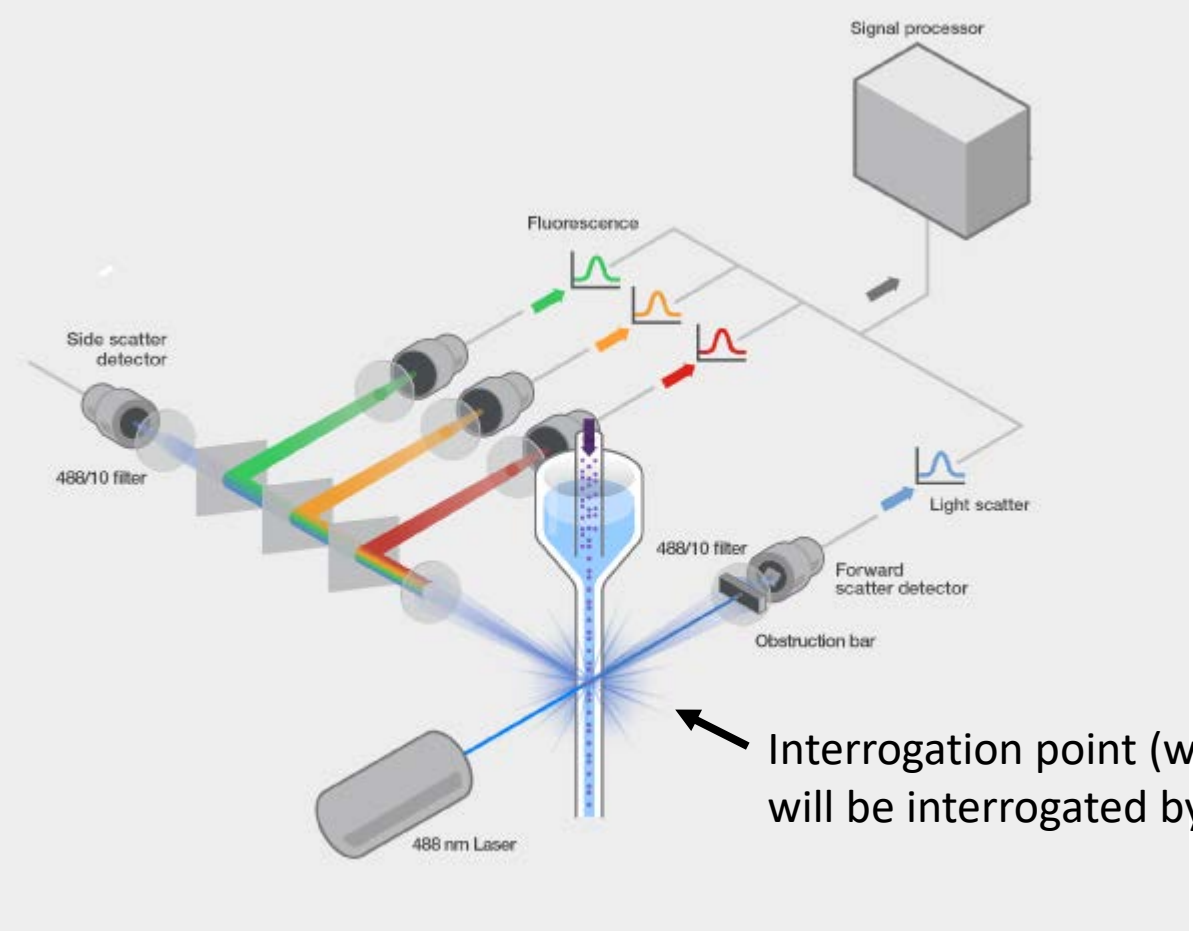




Optics

Fluidics

- Responsible for moving your precious samples to the interrogation point (and to the waste)



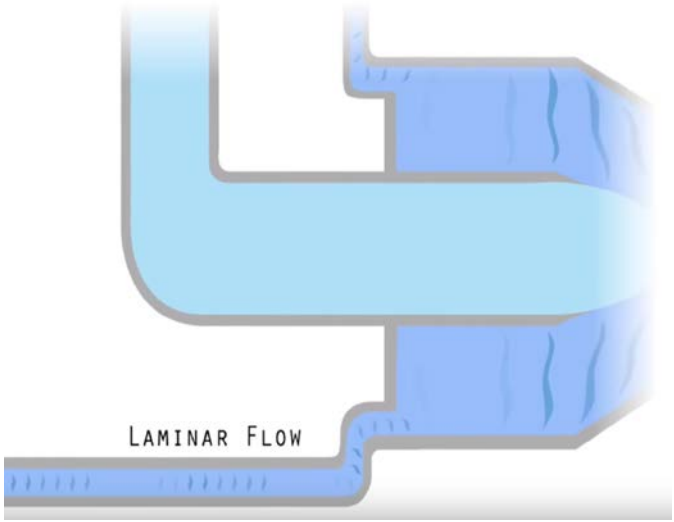
Source: ThermoFisherScientific (Modified)

Fluidics: the sheath fluidic

Flow - Fluid

- Flow cytometers are filled with a fluid, the **sheath fluid**, which runs in a laminar way.

Adapted from SimScale.com



Adapted from iBiology Techniques

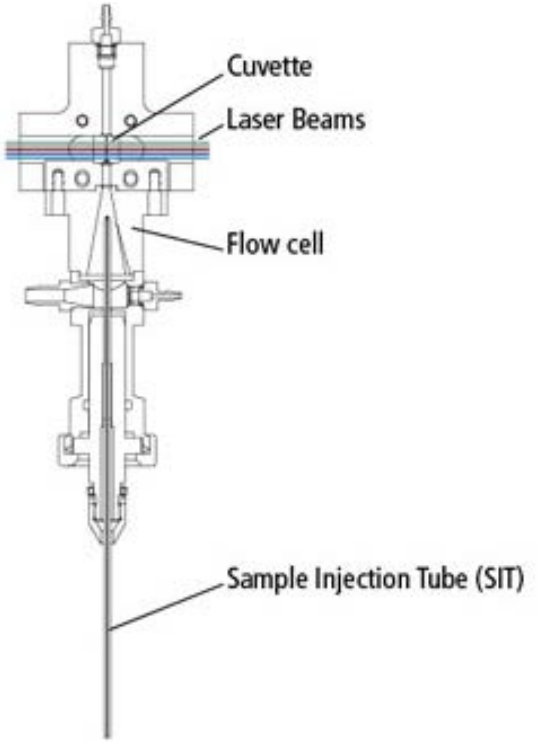
- The sample (in <u>suspension</u>) is introduced into the flow cytometer

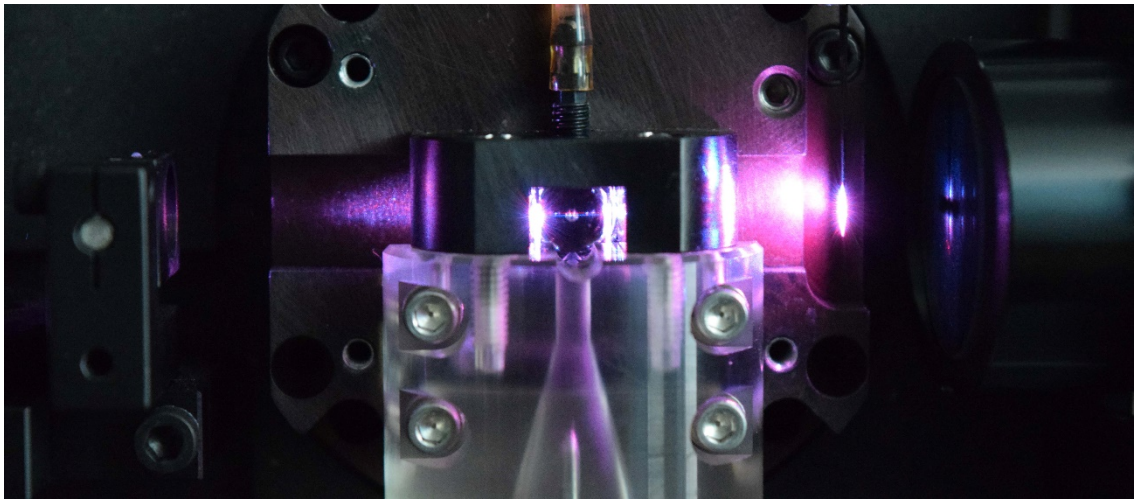
Sample Injection Port (SIP)

High Throughput Sampler (HTS)

Fluidics: the flow cell

- Cells are going to be interrogated in the flow cell. This part of a flow cytometer is also where **hydrodynamic focusing** takes place.



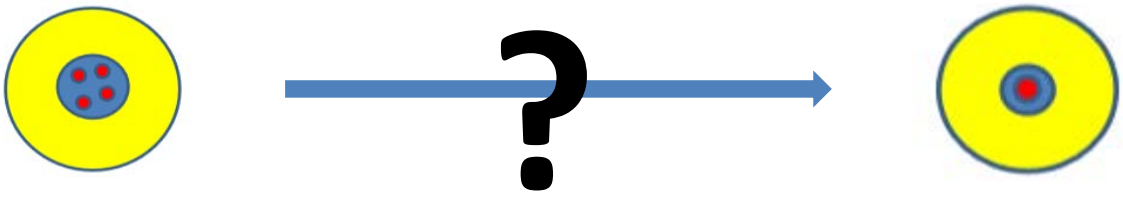


Source: Innovation.ca

Fluidics: hydrodynamic focusing

Core diameter and cells at time of sample injection

Core diameter and cells at time of measurement



Hydrodynamic focusing

Fluidics: hydrodynamic focusing

Gulf of Alaska

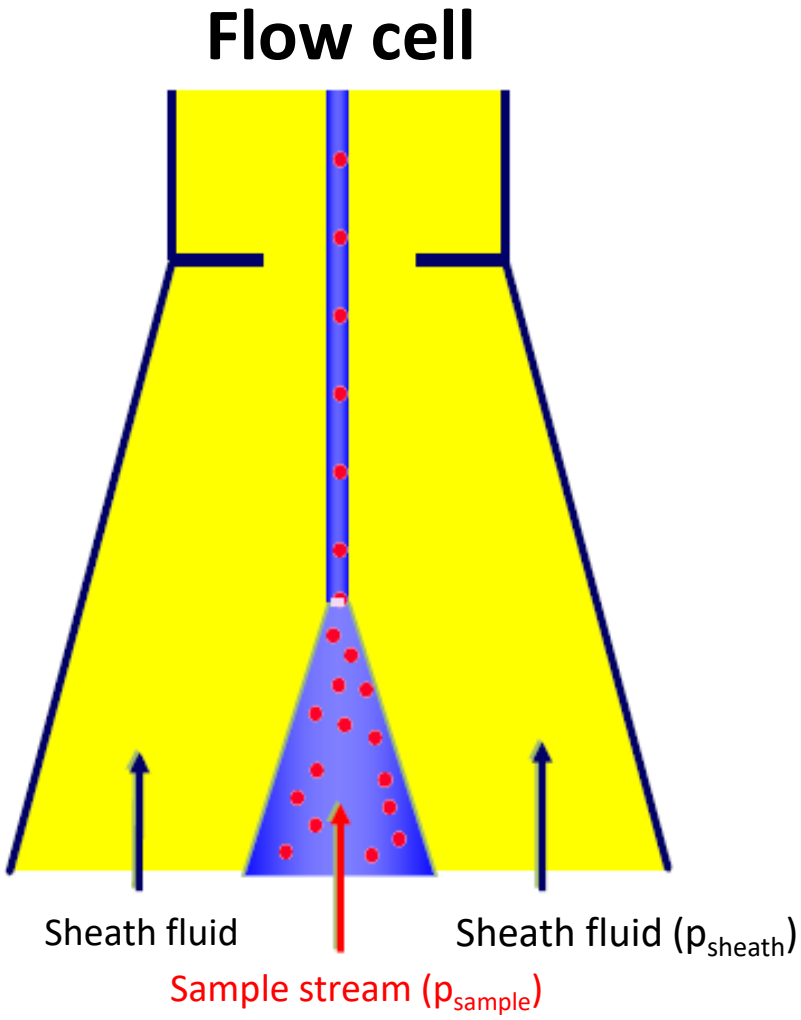
Source: Hipertextual.com

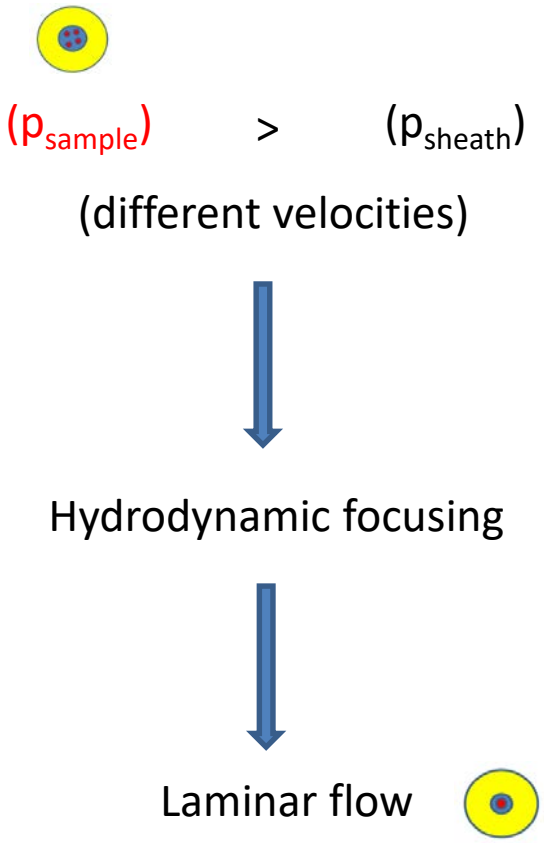
Rio Negro Vs Rio Solimoes

Source: Nuestroclima.com

- If two fluids differ enough in density and/or velocity, they behave as two independent fluids, without mixing > They form a two layer stable flow (laminar)

Fluidics: hydrodynamic focusing





Source: BD Biosciences (modified)

Fluidics: the importance of the flow rate

Low: ≈ 12 µl/ min

Medium: ≈ 35 µl/ min

High: ≈ 60 µl/ min

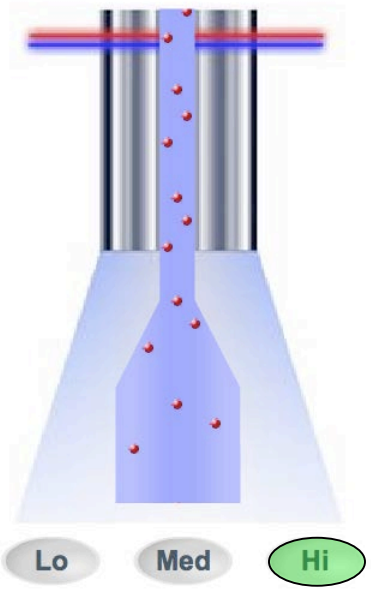
Source: BD Biosciences (modified)

Fluidics: the importance of the flow rate

Low: ≈ 12 µl/ min

High: ≈ 60 µl/ min

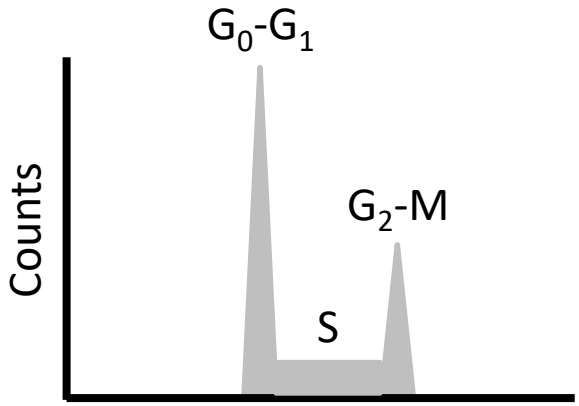
LOW	HIGH
Decreased coincident events	**Increased** coincident events
Better signal resolution	**Lower** signal resolution



Source: BD Biosciences (modified)

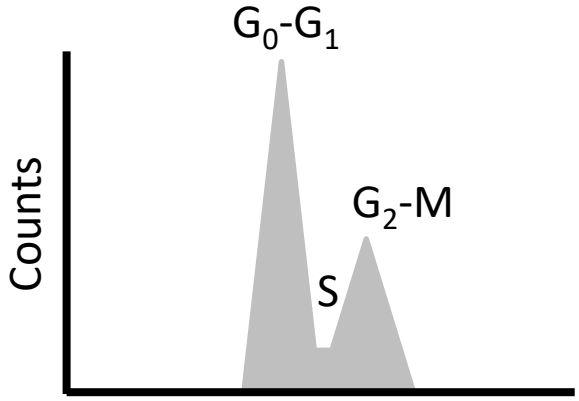
Fluidics: the importance of the flow rate. Example

- **LOW** (**Decreased** coincident events, **Better** signal resolution)



✓ Quantifiable

- **HIGH** (**Increased** coincident events, **Lower** signal resolution)



X Quantifiable

Source: BD Biosciences (modified)

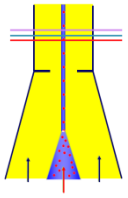
Fluidics: summary

1. It allows your sample to travel from the injection point to the interrogation point to the waste.

2. Hydrodynamic focusing makes your cells to pass in a single line through the interrogation point.

3. Flow rate impacts hydrodynamic focusing and therefore also your results. Higher flow rates > higher coincident events, less signal resolution; lower flow rates > lower coincident events, better signal resolution.

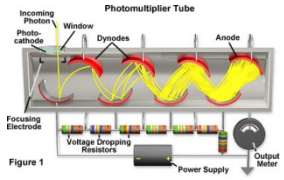
Digital flow cytometer

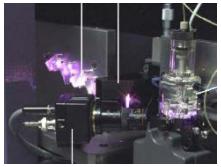


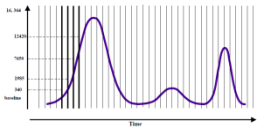
Fluidics

LSRFortessa

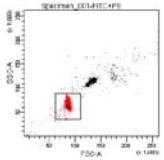
Flow Cytometer

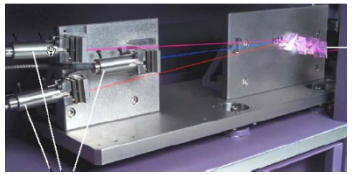






Electronics





Optics

What is flow cytometry?

Flow - Fluid

Source: pbase.com

Cyto - Cell

Source: smithsonianmag.com

Metry - Measurement

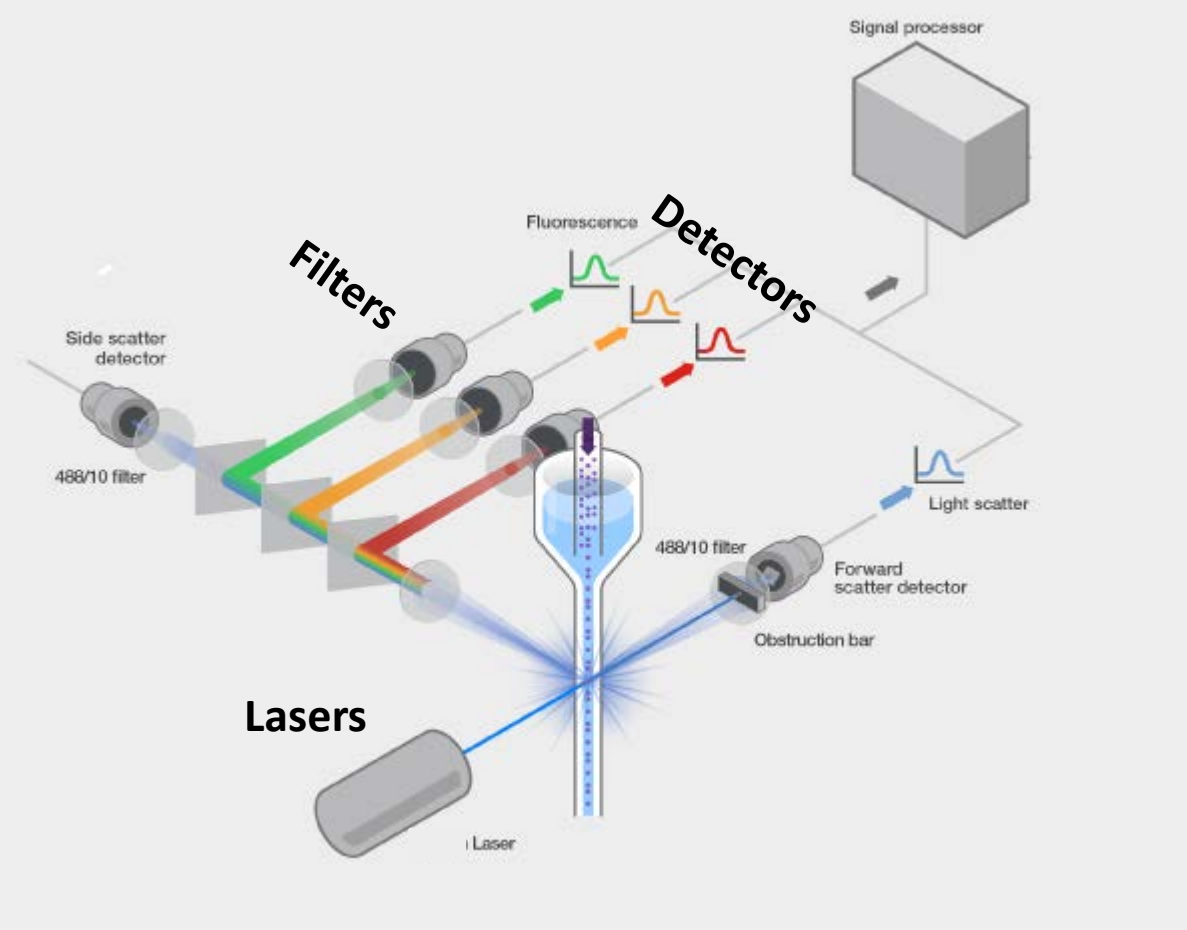
Source: clipartkid.com

Meaning that a feature from a "cell" in suspension will be measured (at single cell level).

How do flow cytometers do that?

Thanks to lasers and detectors

Optics: lasers, filters and detectors

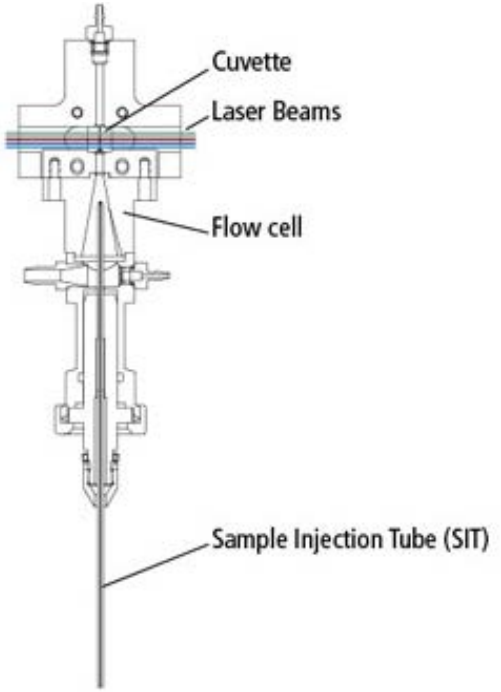


Source: ThermoFisherScientific (Modified)

Optics: lasers

LASERS are the core of your flow cytometer!
The number of laser a flow cytometer is equipped with will vary among different machines.
The more lasers, the more parameters to analyse!

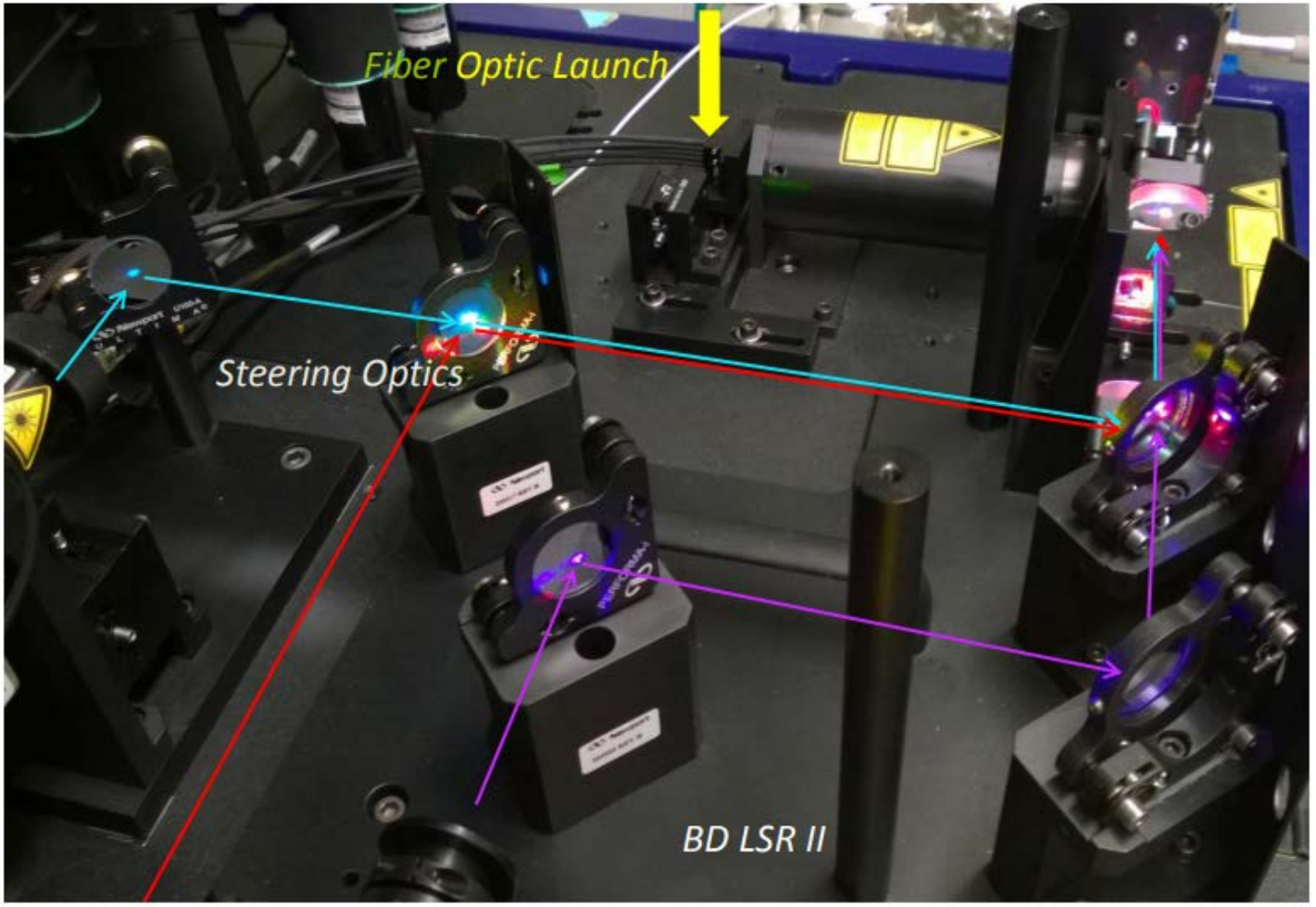
How can they be so close?



Regardless of the number of laser your machine is equipped with, laser needs to be directed to the flow cell…

Optics: lasers

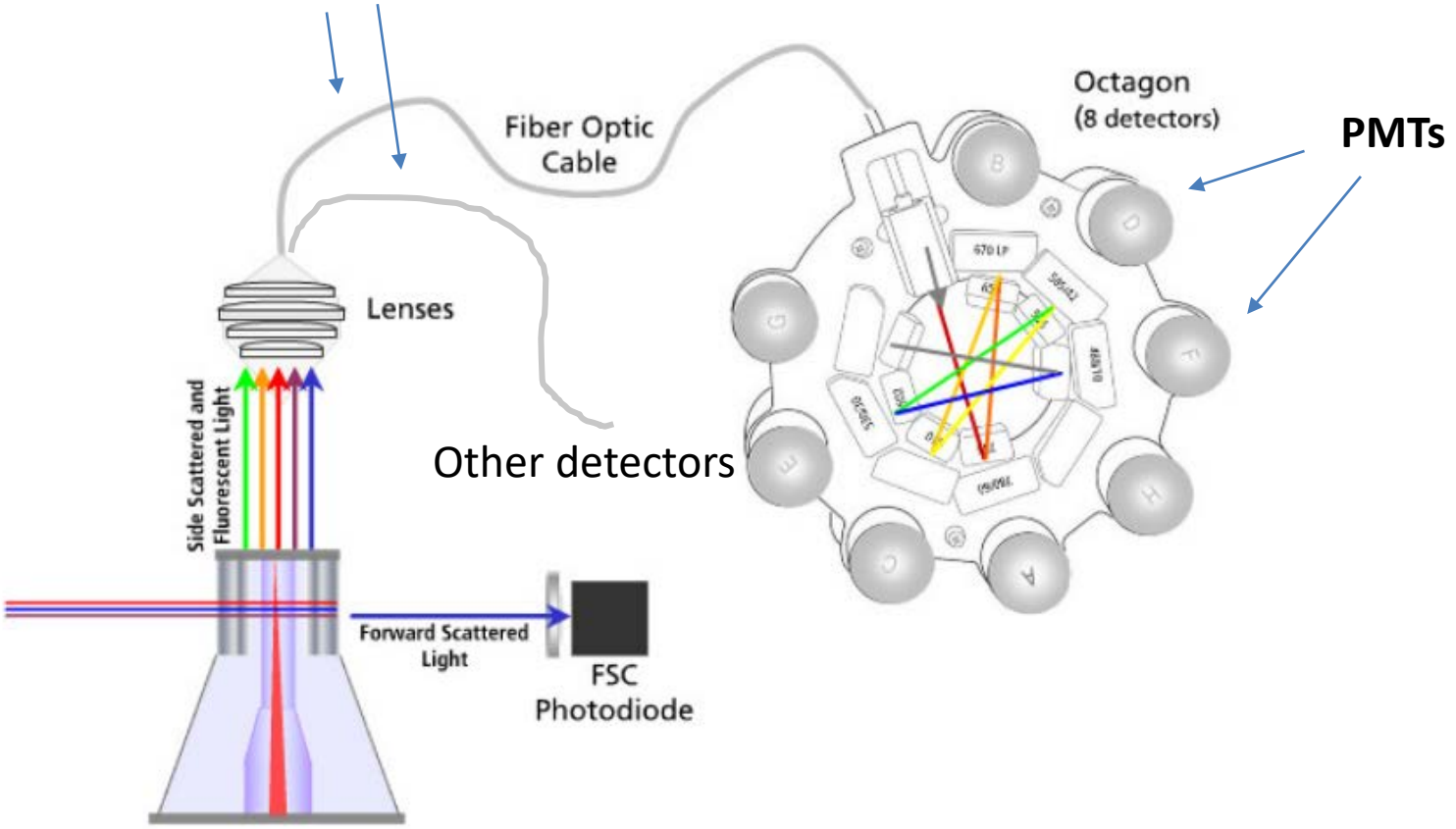
BD LSRII



Source: Washington University

Optics: lasers

Collecting light from specific laser (i.e.: blue laser)

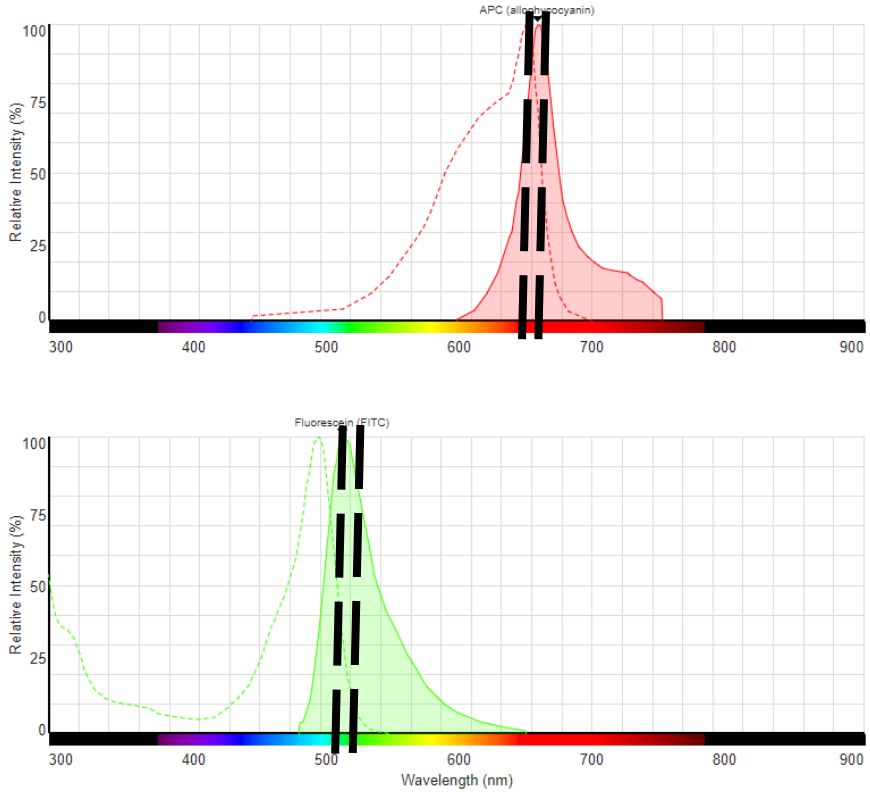


Note: emitted light from different lasers will be collected in different fiber optic cables

2. Fluorescence

- Every fluorophore has an excitation and an emission range, which are **unique** (important for *spectral cytometry*)

- In conventional flow cytometry, knowing excitation and emission ranges are **critical**



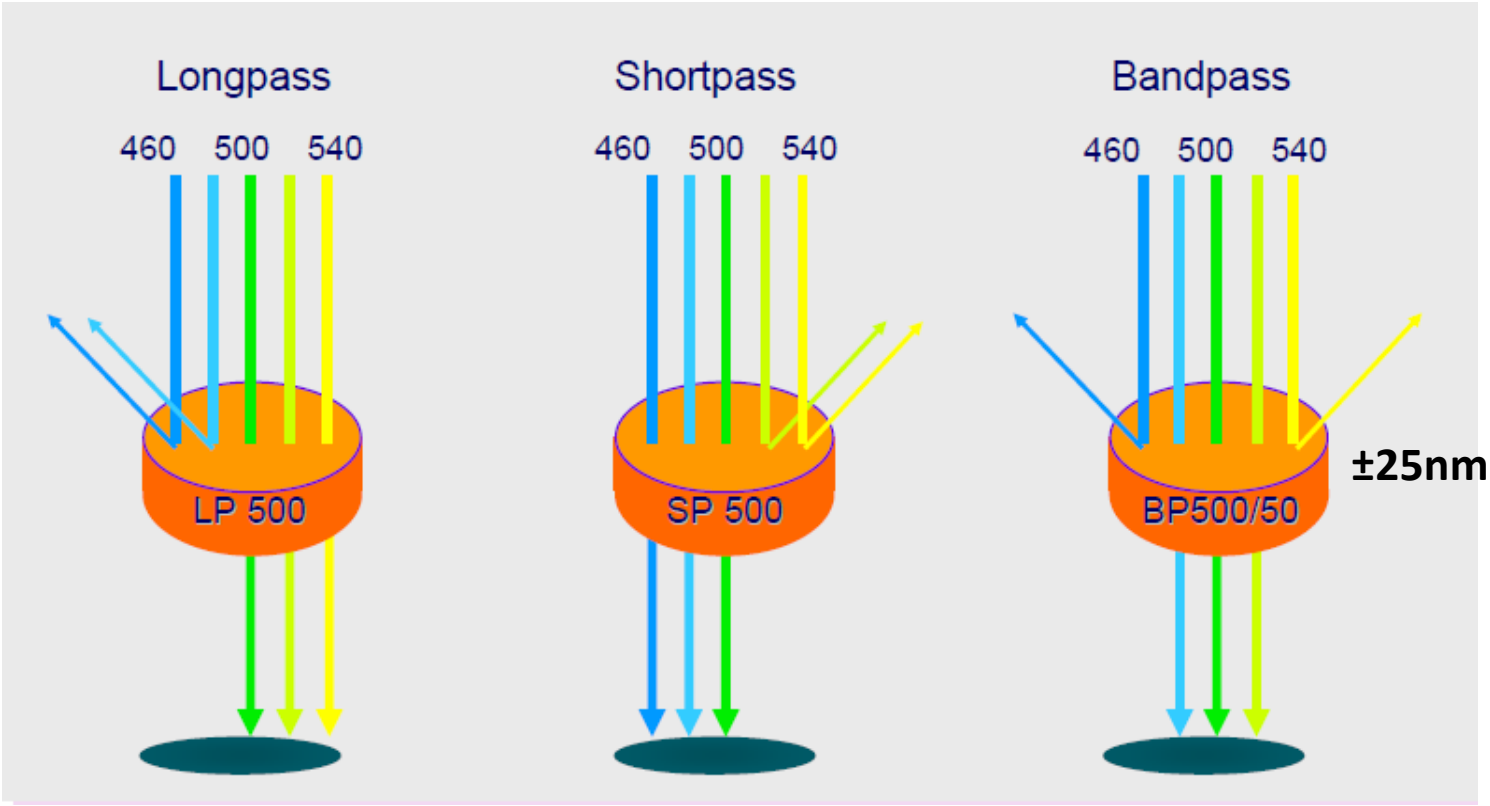
A conventional flow cytometer is not going to measure the whole emission spectrum > we need filters!

Emitted light will always have a longer wavelength = less energy („Stokes shift“)

Source: ThermoFisherScientific Spectral Viewer

Optics: filters

- With wavelength discrimination: dichroic mirrors

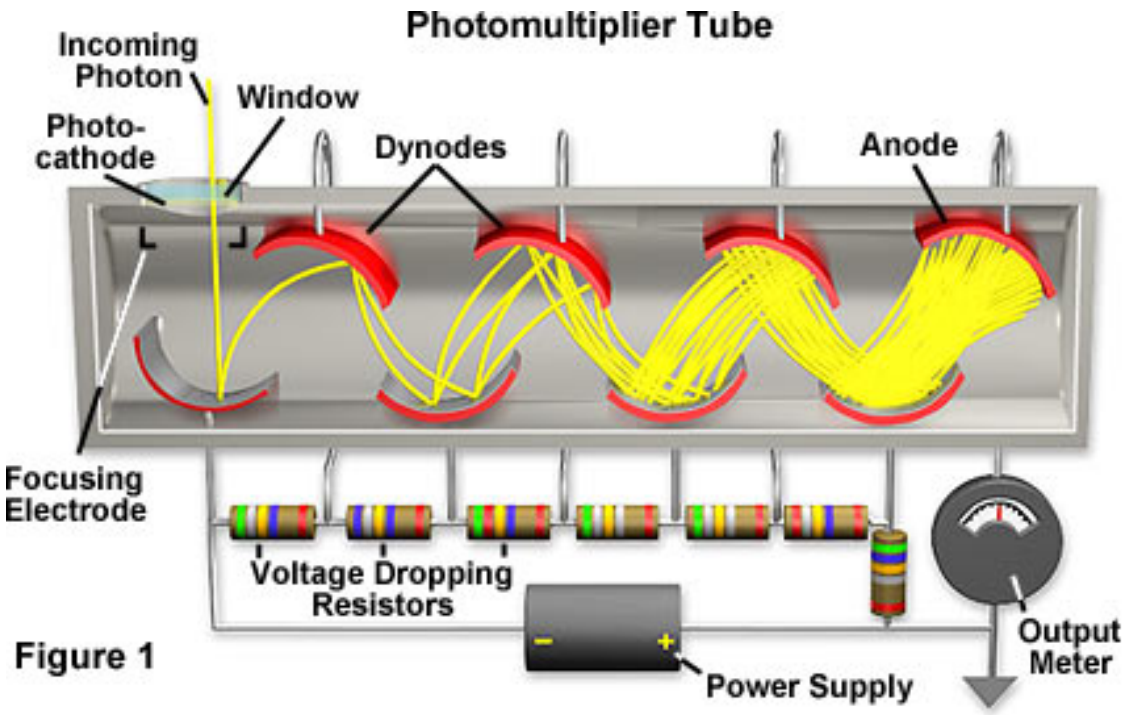


Source: BD Biosciences

Optics: detectors

- Detectors are devices that sense the light, then convert it to an electronic signal

Optics: detectors. The PMT

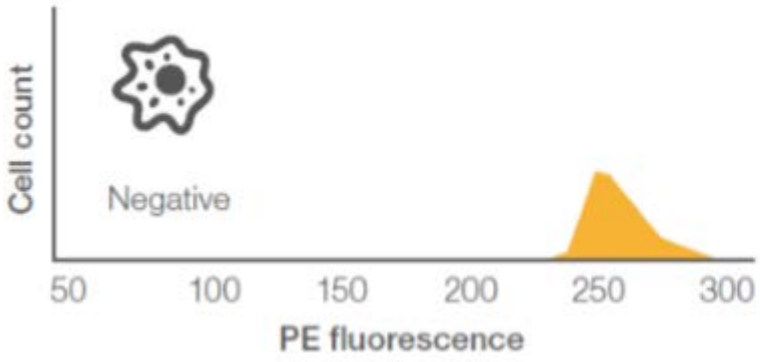


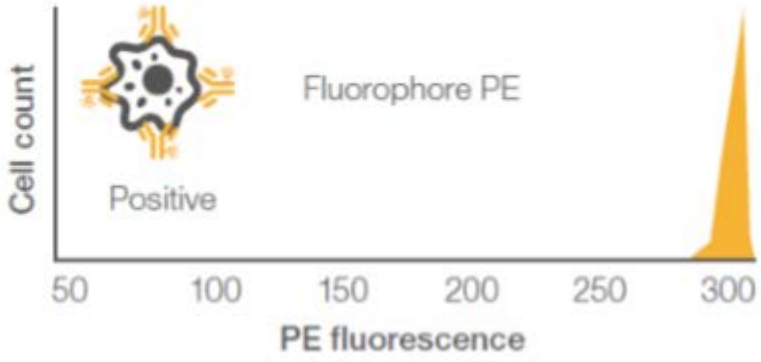
Source: Florida University

- Amplification: up to 10^8 (voltage dependent)
- Supply voltage: up to 1000 V >> You can change using Flow´s Software
- PMT provides current output proportional to light intensity

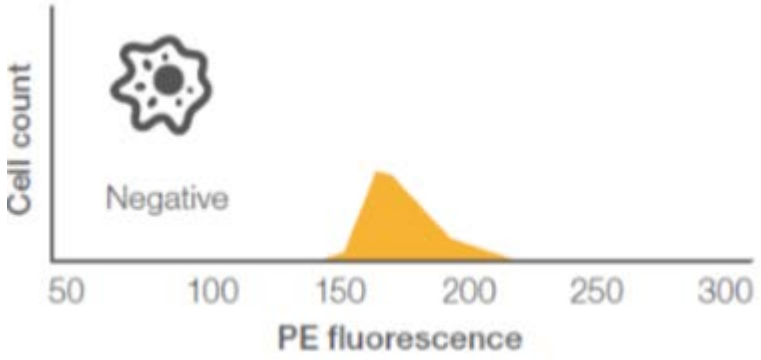
Optics: detectors. The PMT. Voltages

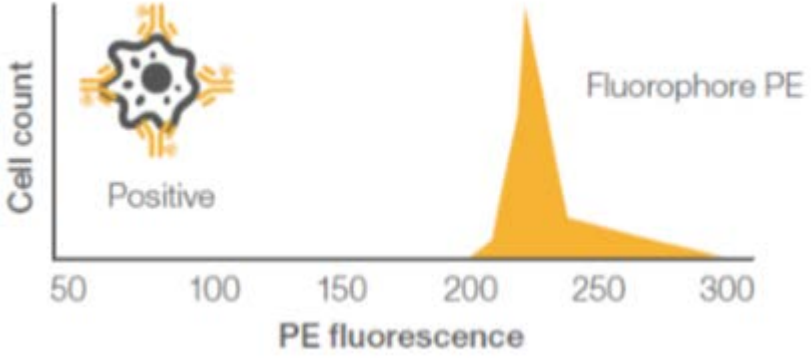
PMT voltage too hight: positive signal off scale





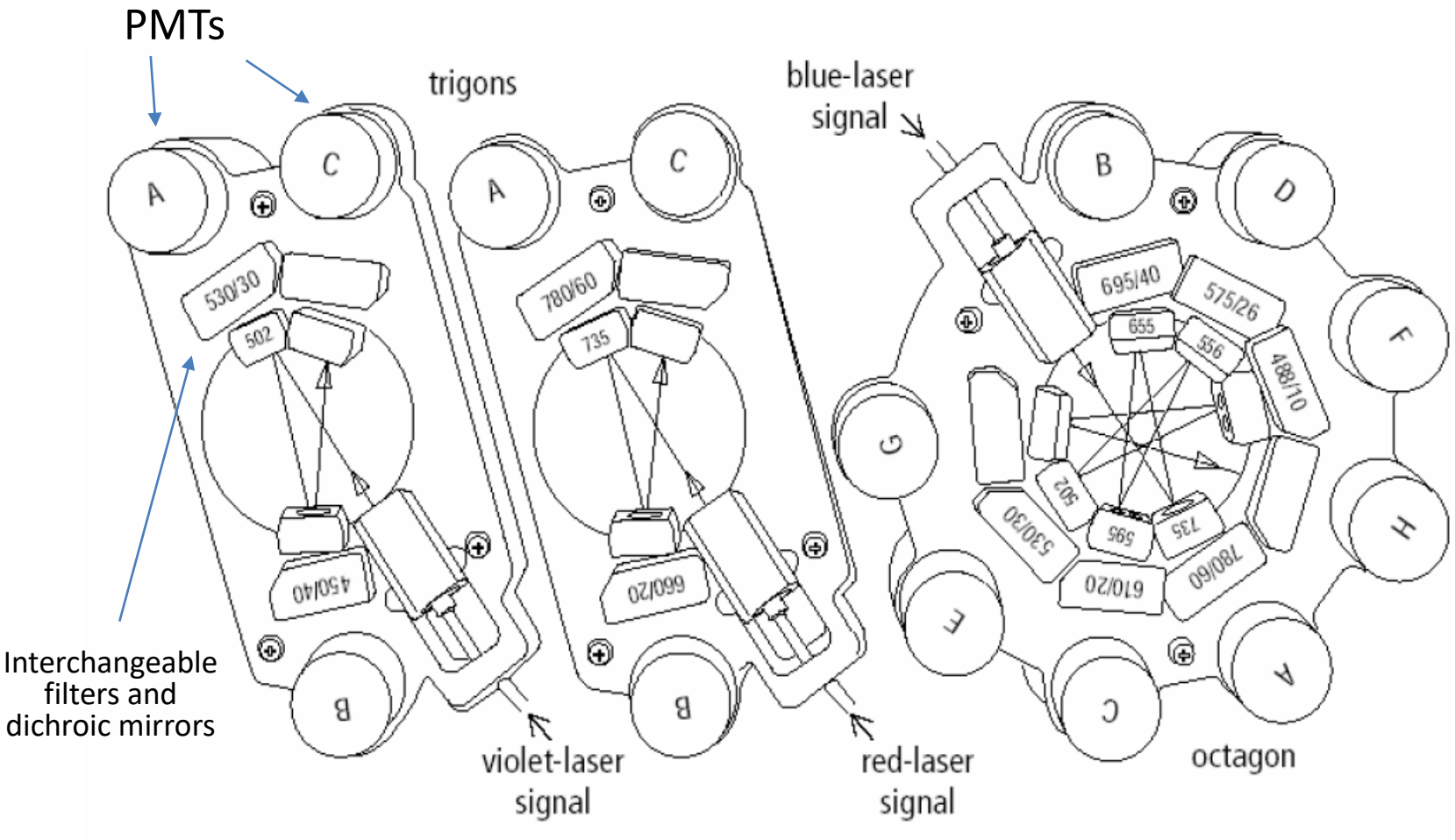
Reduced PMT voltage: positive signal fully resolved





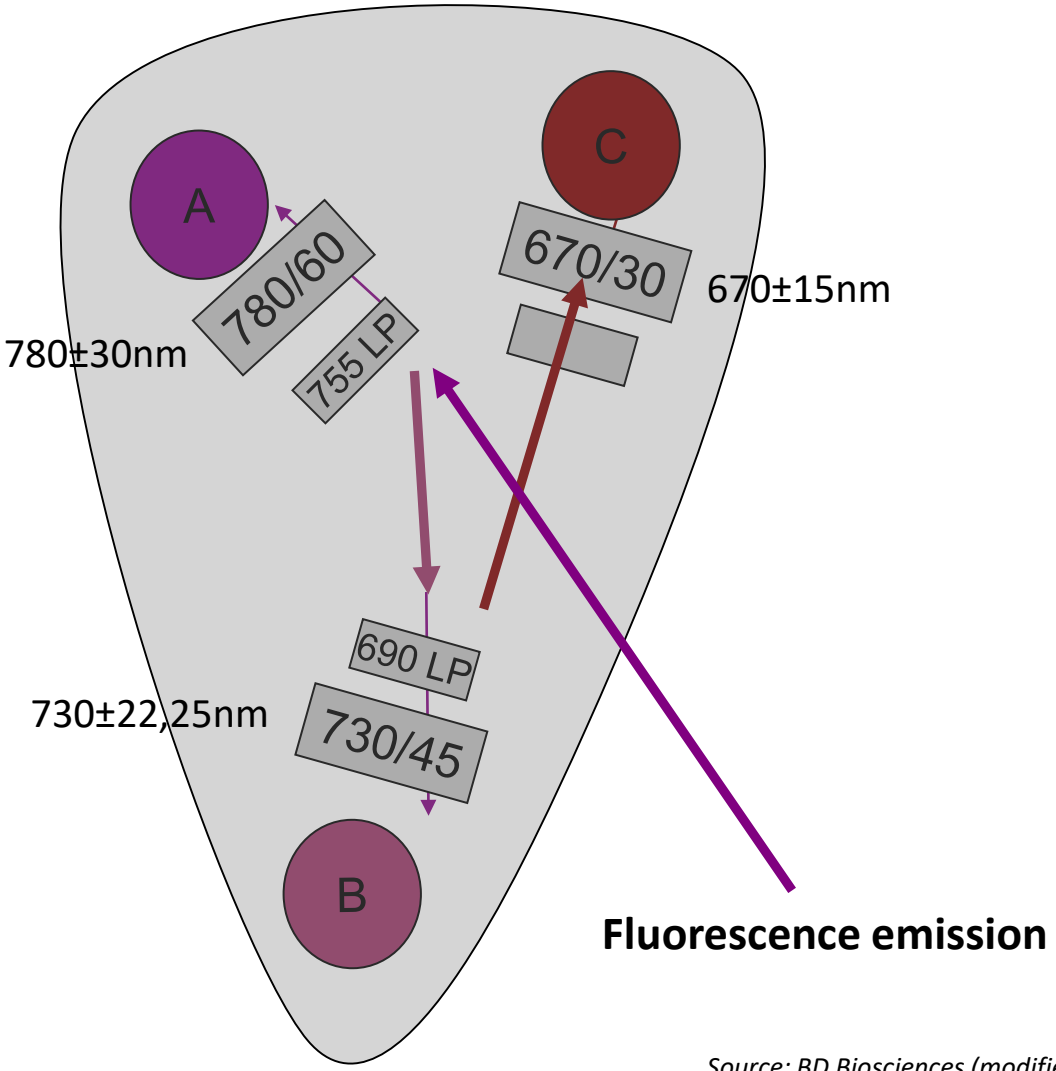
Source: Thermo Fisher Scientific

Optics: detectors. The PMT. Trigons and octagons



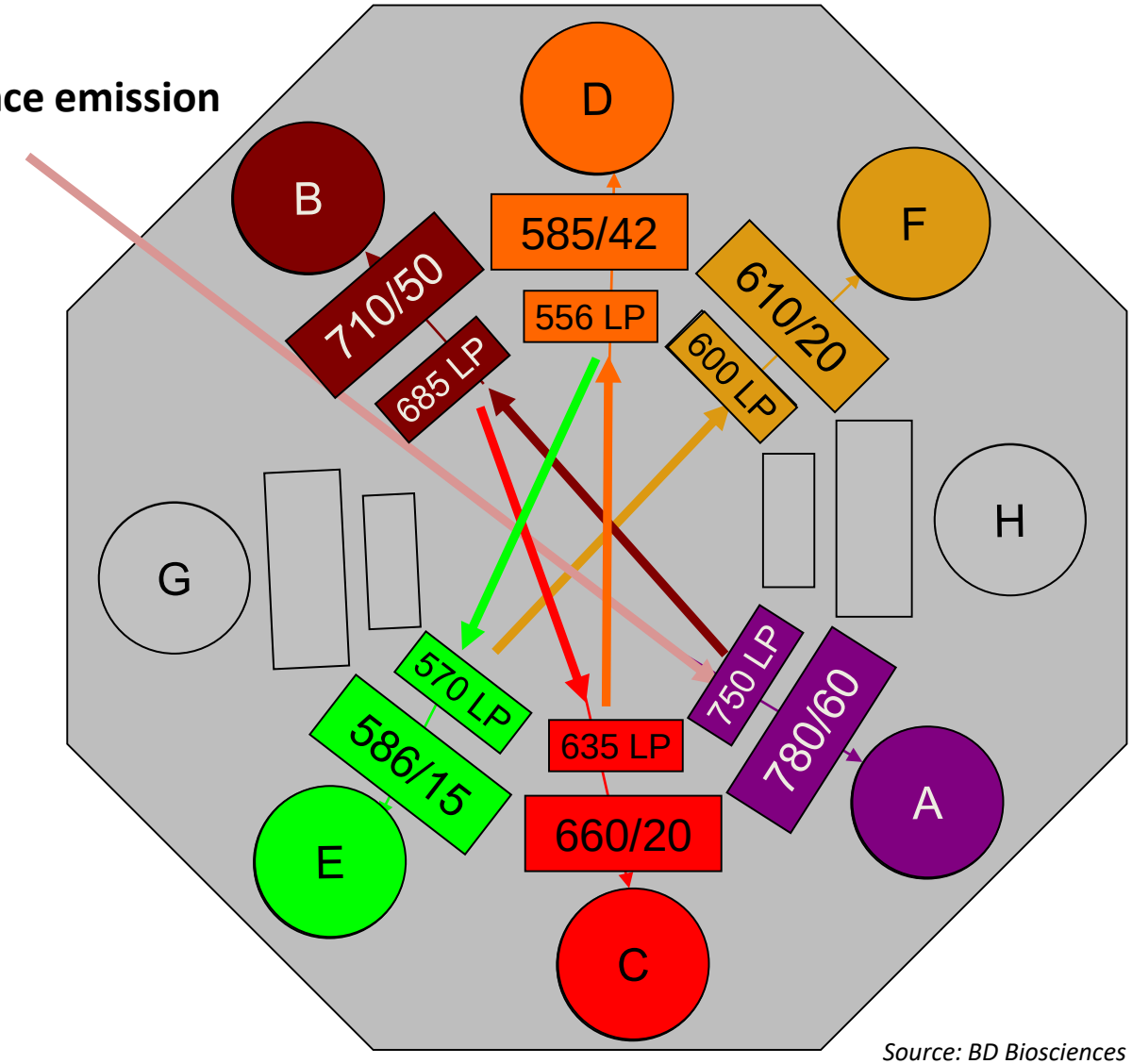
Source: BD Biosciences

Optics: detectors. The Trigon



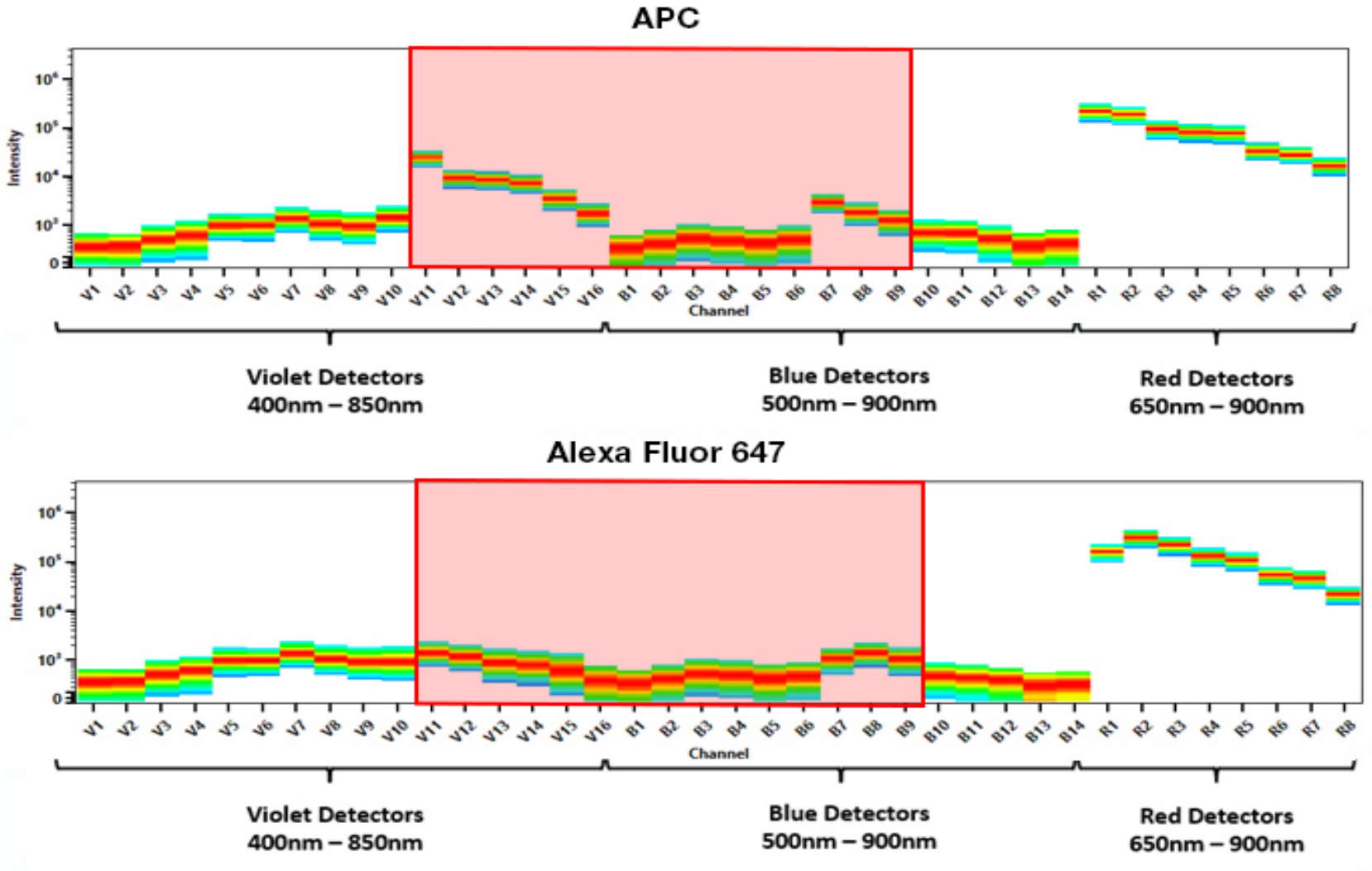
Source: BD Biosciences (modified)

Optics: detectors. The Octagon



Source: BD Biosciences (modified)

Optics: spectral flow cytometry, a new era in flow



Source: ThermoFisher Scientific

Optics: summary

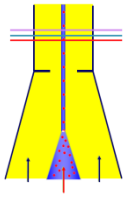
1. Lasers with specific emission wavelengths excite different fluorophores.

2. Emitted light (fluorescence and scatter) is organized into wavelength ranges using filters.

3. The specific wavelength ranges are sent into the detectors (PMTs).

4. Spectral flow cytometers do not only detect a small portion of the emission range of a fluorophore, but it records the whole „emission fingerprint“, allowing the detection of fluorophores that conventional flow cytometry cannot resolve.

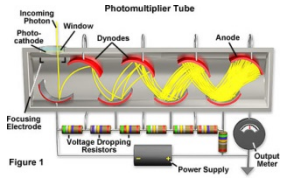
Digital flow cytometer

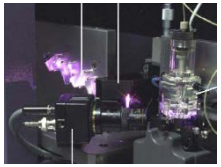


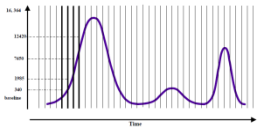
Fluidics

LSRFortessa

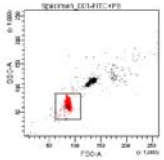
Flow Cytometer

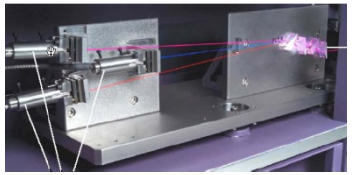






Electronics

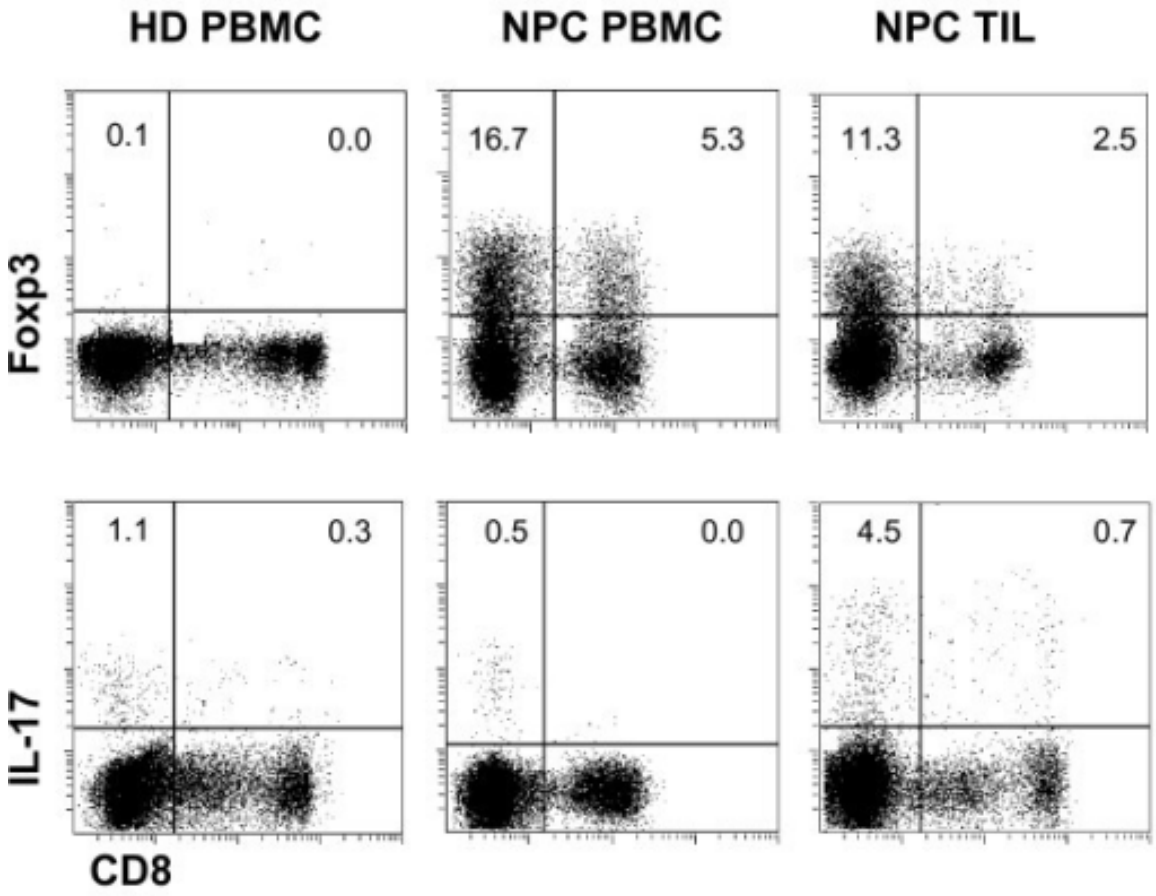




Optics

Electronics: voltage pulse by single cells

- From photoelectric current to dots on a plot (via **A**nalog to **D**igital **C**onverter)



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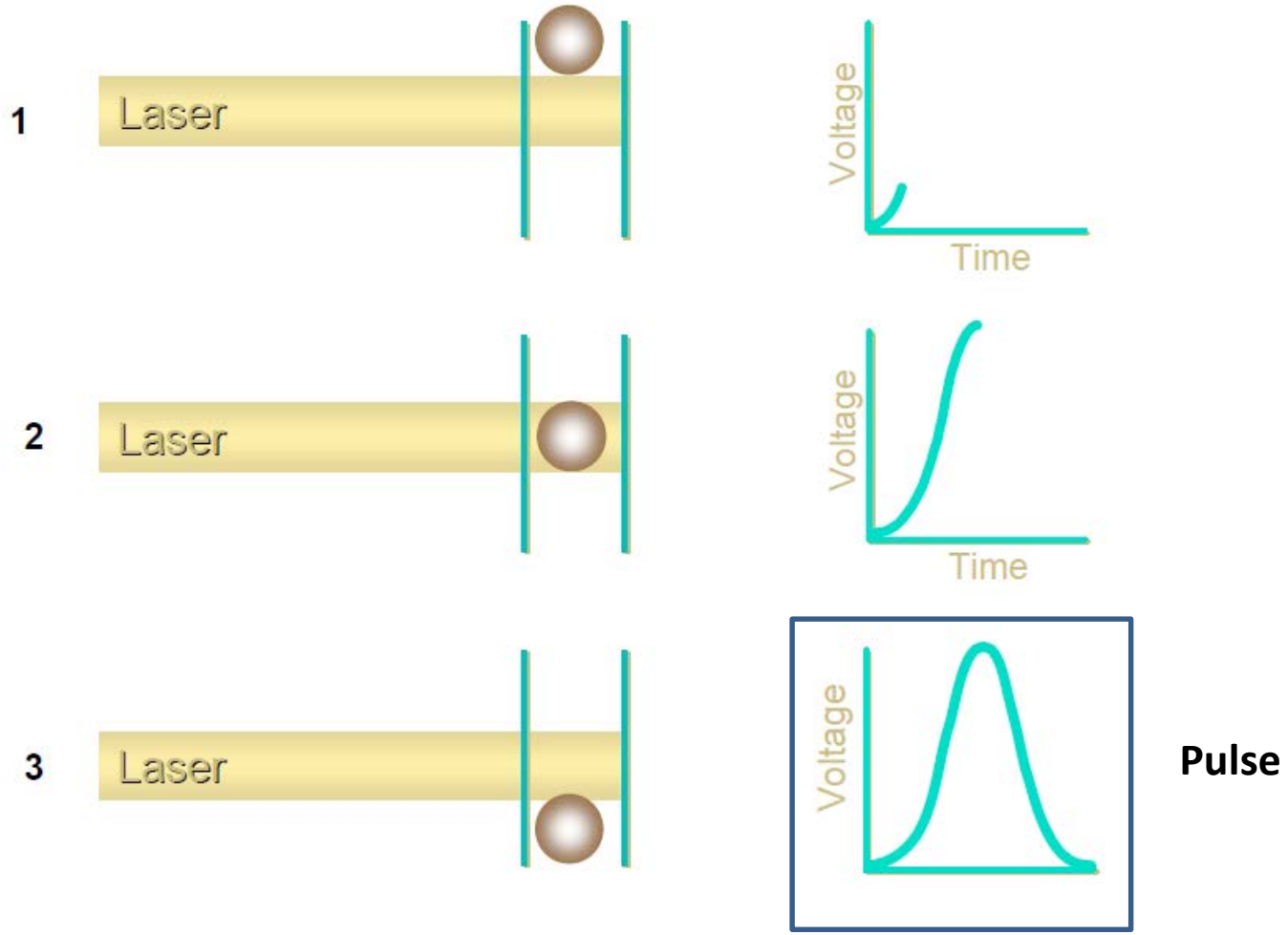

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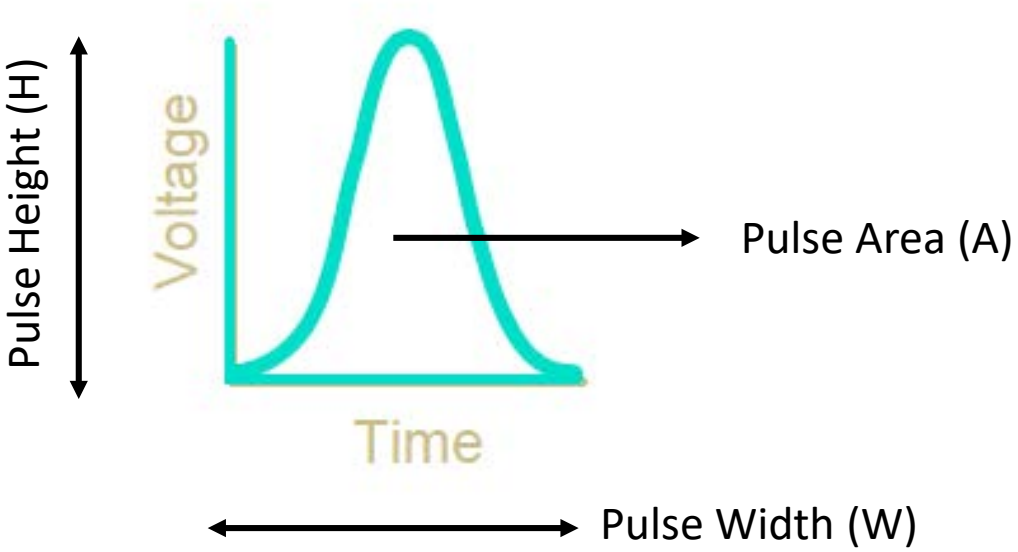
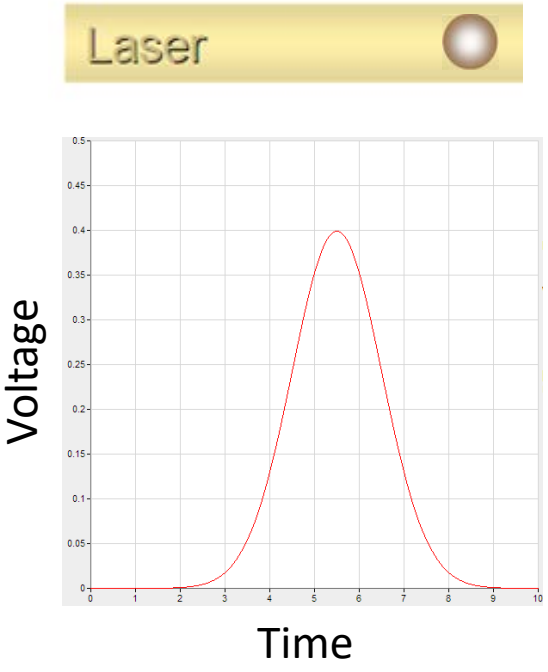


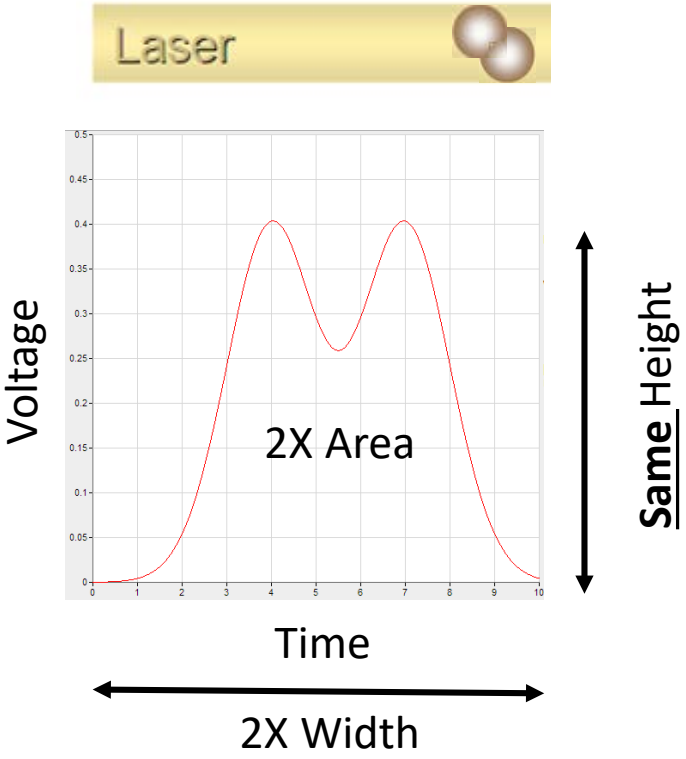
Electronics: voltage pulse by aggregates

- Aggregates negatively impact your precious data: identify them!

Single cell



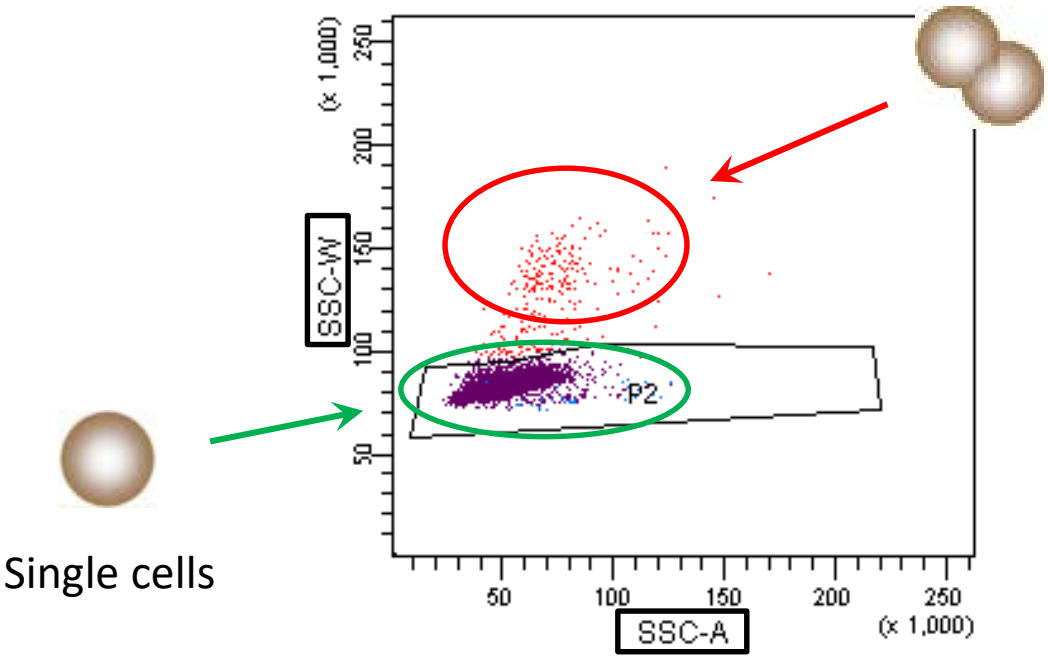
Doublets/Aggregates/Cell clusters



Electronics – Doublet discrimination

- Remove your doublets before start analyzing/sorting any parameter

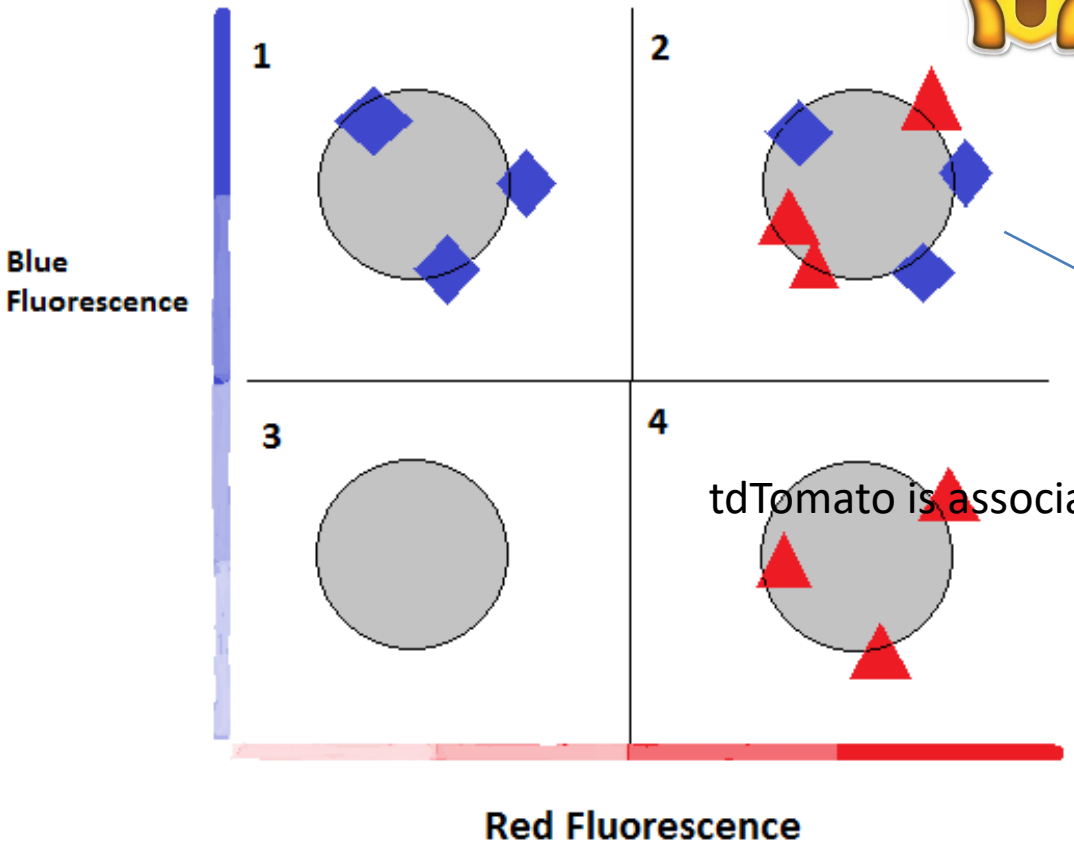
Doublets/ Aggregates/cell clusters



Single cells

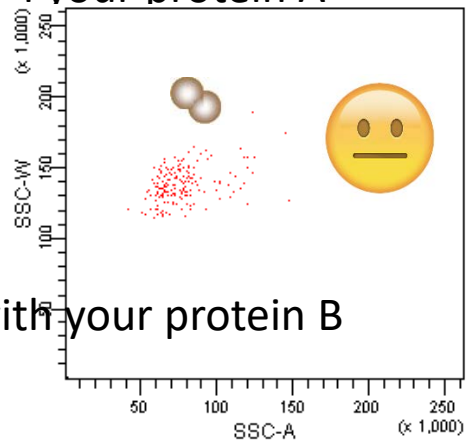
- Doublet exclusion possibilities: FSC or SSC.

Electronics – How do aggregates impact your results?



My sample has the two proteins, great!

ı your protein A



tdTomato is associated with your protein B

- **Blue and red signal are coming from two different cells, not from only one!**

Electronics – Summary

1. The current is converted into a pulse, characterized by an area, width and height.

2. Characteristics of the pulse can be used for removing doublets, which can impact. your results

3. Each pulse is converted and represented as a dot in a plot.

Overview

- What is flow cytometry?
  - FSC – SSC (relative size, granularity / complexity)
  - Fluorescence
- Looking inside a flow cytometer
  - Fluidics
  - Optics
  - Electronics
- Data presentation and gating
- Introduction to electrostatic cell sorting
- Applications of flow cytometry & cell sorting
- Examples of current flow cytometers
- Advantages of flow cytometry over other techniques

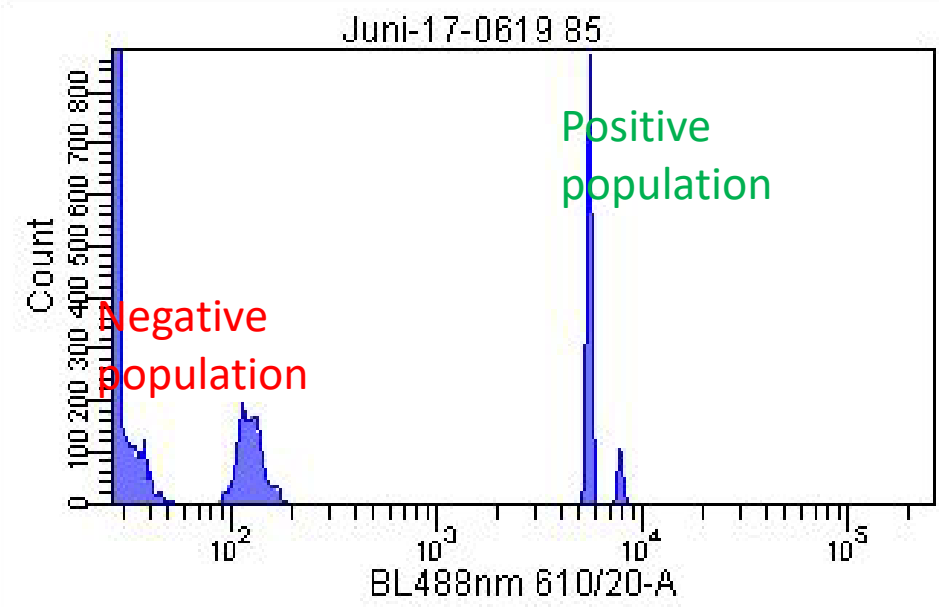
Data presentation

- Each pulse will be processed and presented as a graphic in the software.
- You can choose between different data presentation styles according to your experiment (or you PI, reviewer…etc.).

Data presentation

Plot types

- Histograms
- Dot plots
- Contour plots
- Density plots



Data display

- Linear scale
- Logarithmic scale
- Biexponential scale

Data presentation

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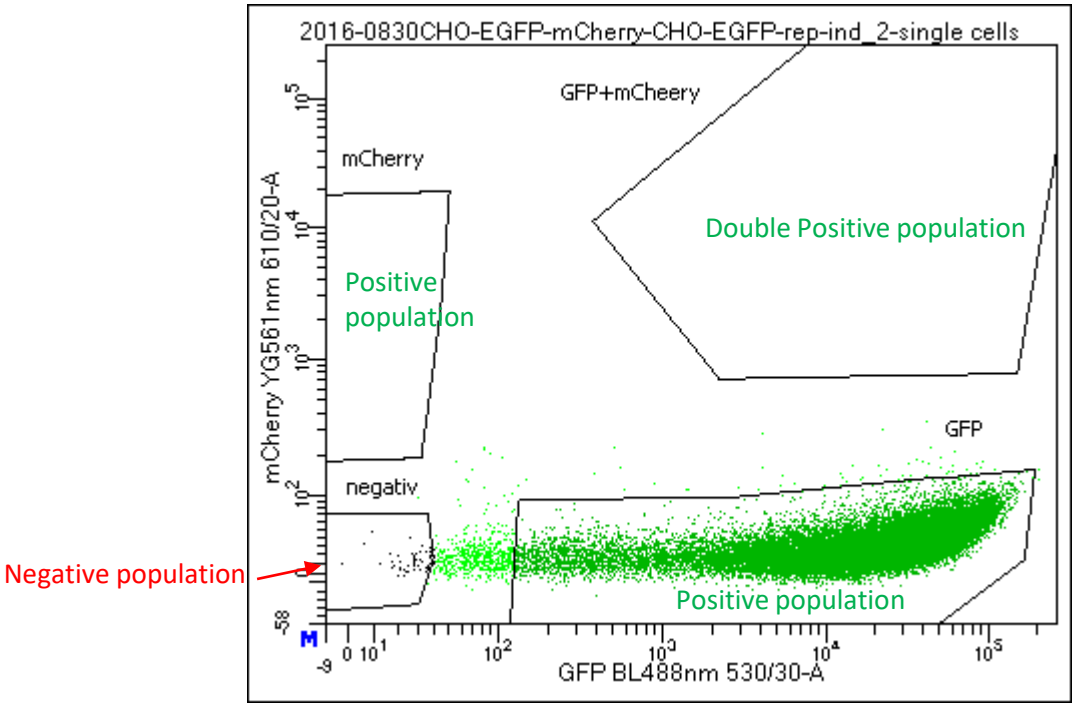

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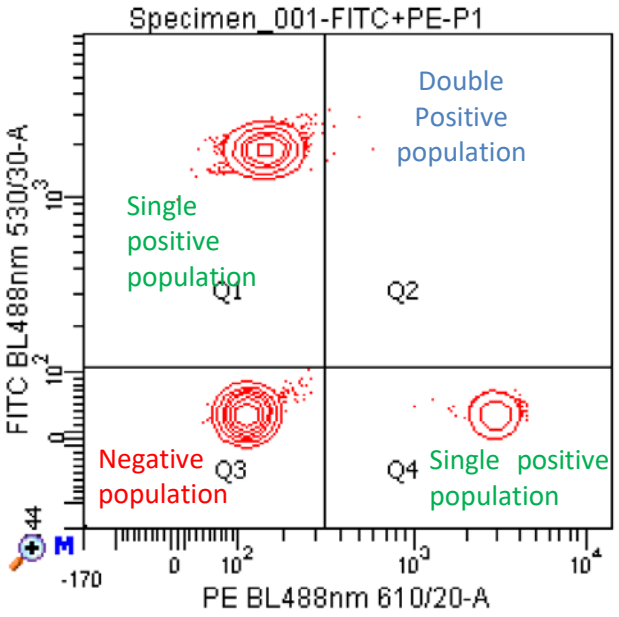

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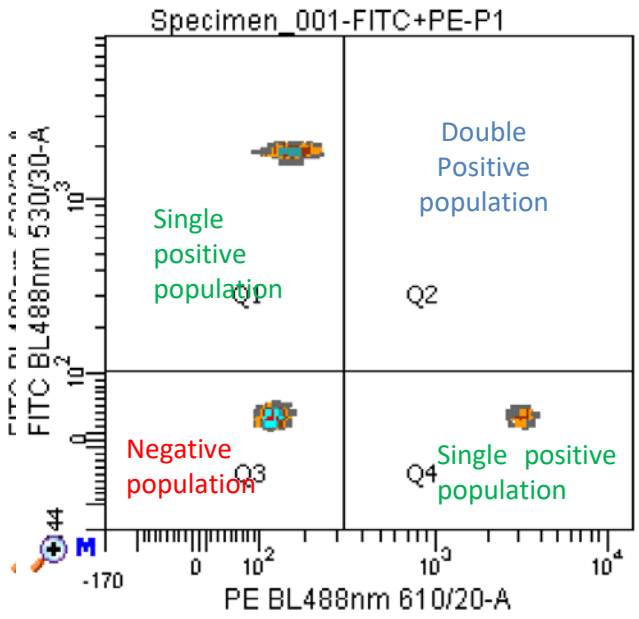


Data display

- Linear scale
- Logarithmic scale
- Biexponential scale

Data presentation: gating

- At the end of your experiment, you will generate a FCS (**F**low **C**ytromery **S**tandard) file.
- FCS files can be analyzed by different softwares (FlowJo, DIVASoftware…etc.)

- Gating is the process of narrowing your population of interest.
- The selected gating strategy impacts your results

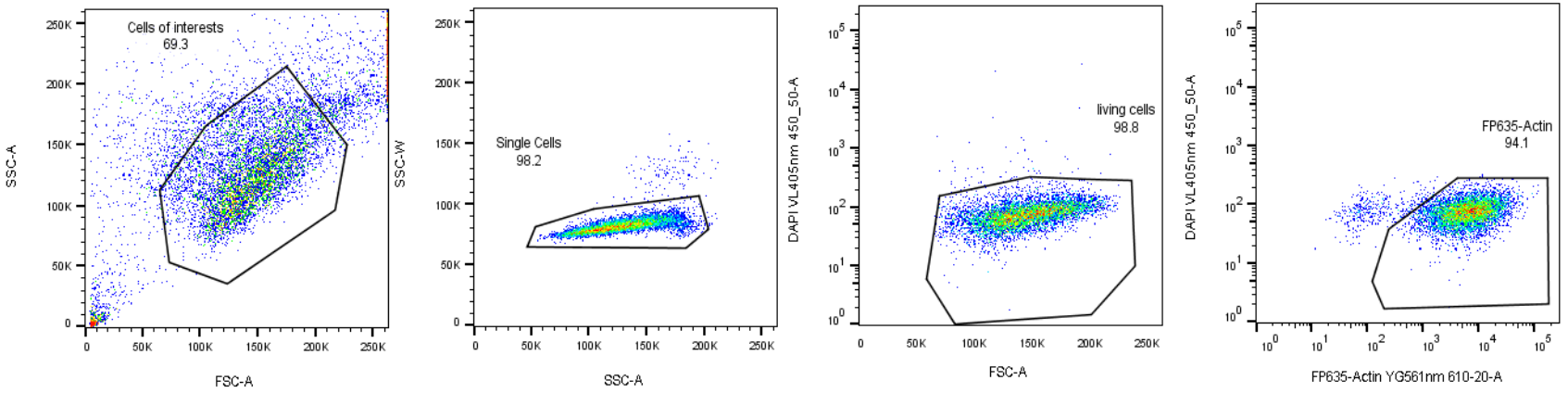
What is important to know when gating your cells of interest?

DOUBLET DISCRIMINATION

DEAD CELLS EXCLUSION

Data presentation: gating

Example: you want to see how many of your cells are RFP⁺



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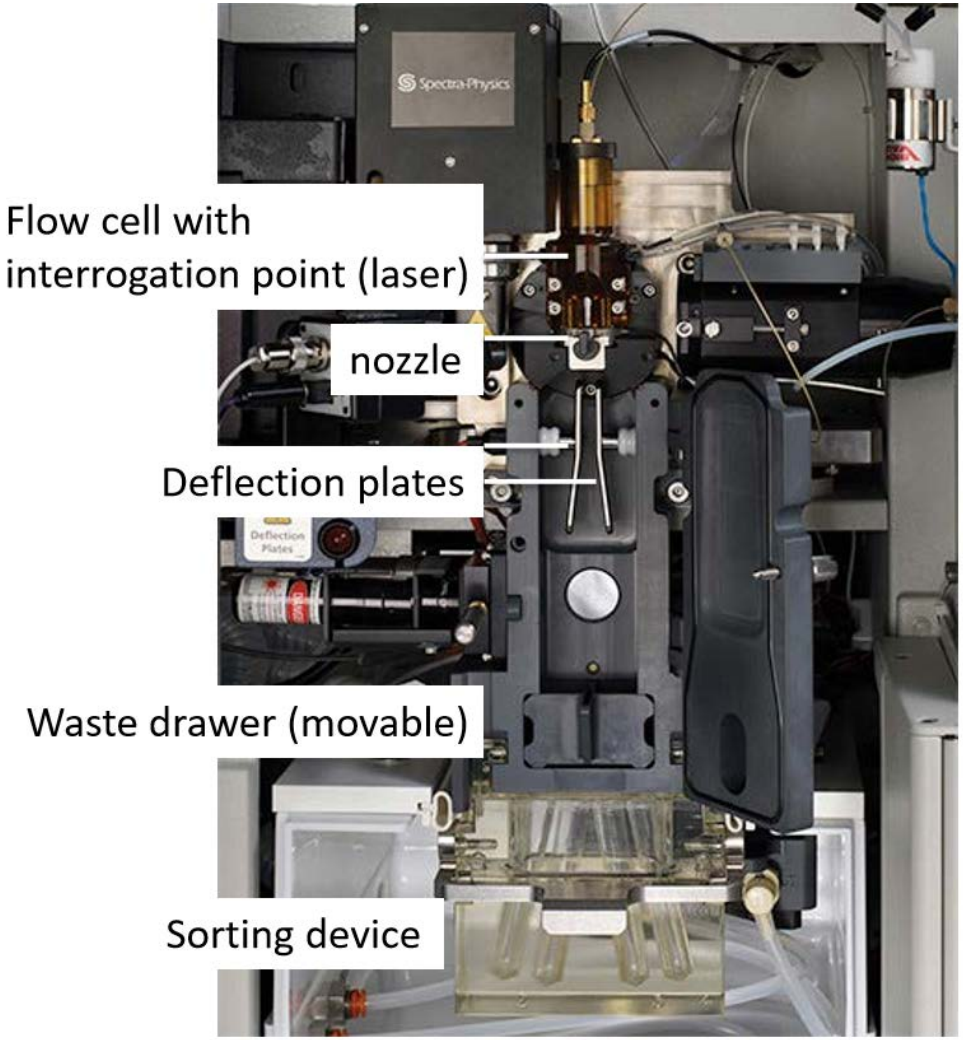
Cell sorting – a briew overview

- There are different ways to sort (*separate*) cells (magnetic, mechanical, microfluidic, electrostatic).
- We will focus on electrostatic sorting (most extended)
- In any case, cell sorting allows you to enrich a population of interest.

Electrostatic cell sorting – the principle

- A vibrating mechanisms („transducer“) causes a liquid stream to break into single droplets.
- One drop contains one cell (in theory).
- Cells are interrogated before the droplet breaks off.
- At the stream break-off point, the droplet is charged, if it contains cell of interest.
- Charged droplets are deflected in an electric field and collected in the respective sorting device



Possible collection devices for cell sorting

- Tubes

Source: Eppendorf

Source: StemCell Technologies

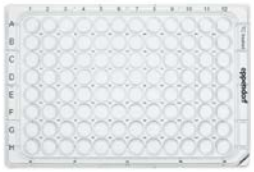
Source: Amazon

- Microscopy slides

Source: Parco Scientific

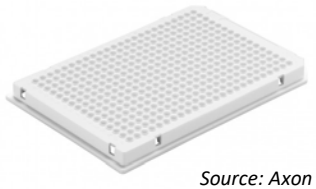
- Microtiter plates (it allows you single cell sorting)

96-well plates



Source: Cole Parmer

384-well plates



Source: Axon Lab

- Ibidi chamber

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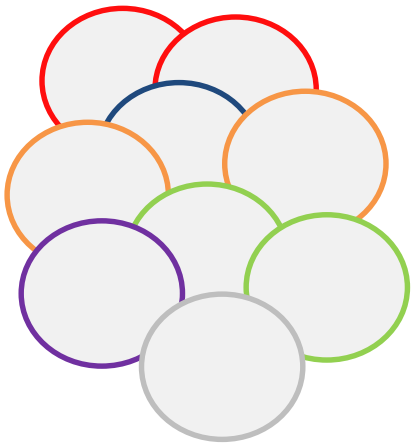
Applications: phenotyping (Multiparametric FC)



- If you know **surface** or **intracellular** antigens being expressed in a specific cell population, you can use multiparametric FC for the identification of populations (very popular application)

- For example, peripheral blood can be analyzed for the detection of B cells, T cells, monocytes…and even its activation status. **Up to 50 parameters at the same time.**

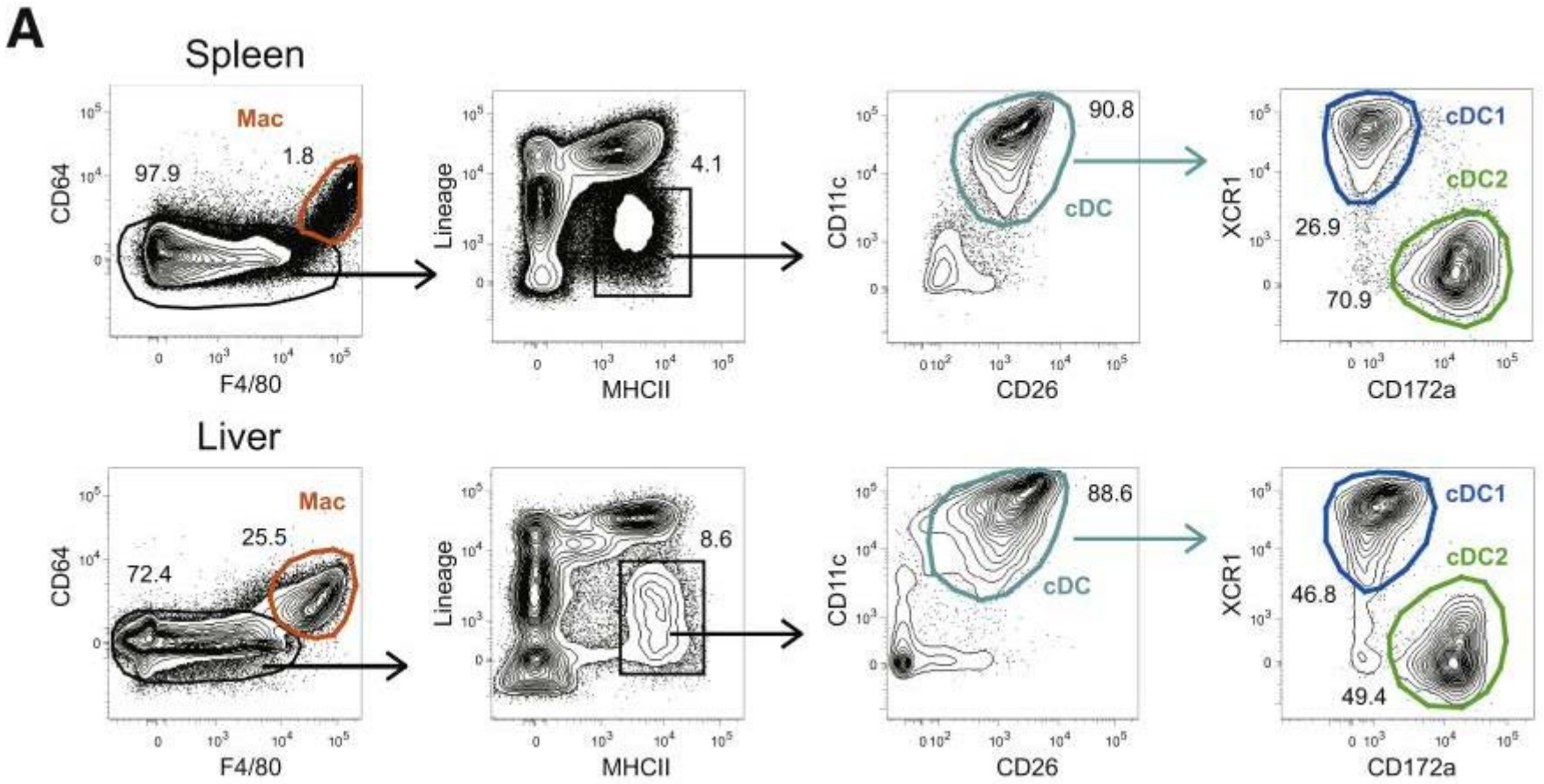
- Immunophenotyping, for example, is a really important tool nowadays to monitor patient responses after immunotherapy drug administration.

- In theory, your imagination is the limit!*

*and, of course, the availability of reagents…

Applications: phenotyping

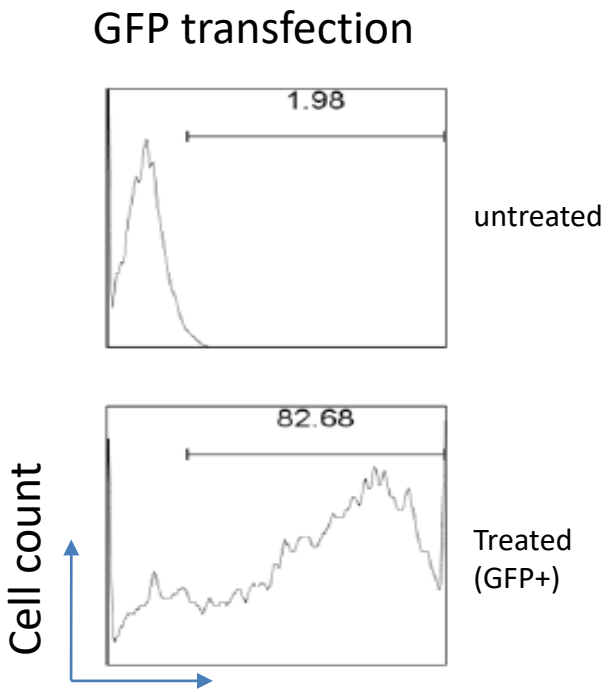
Characterization of dendritic cells (a complex subset to look at…) in different mice organs



10.1016/j.immuni.2016.08.015

Applications: transfection/transduction/transformation efficiency

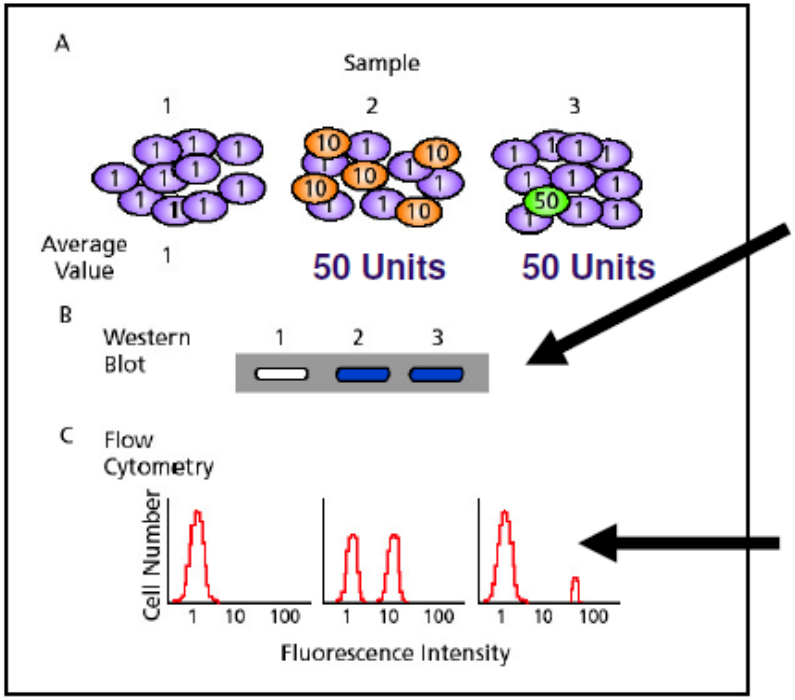
- You could look under the microscope, but quantification might be tedious >> Flow cytometry is really fast and gives you a fast % of + cells.
- AND if you only want + cells…→ **cell sorting!**



Applications: phopho-protein profiling

→ Typically: phospho-protein detection is done by Western Blot

→ Currently there are several antibodies recognizing non-phosphorilated and phosphorilated version.



Western Blot

- No opportunity to view variability
- Requires sorting of subsets to gain access to intracellular antigens
- Requires larger numbers of cells 10^6

Flow Cytometry

- Differentiation possible
- Subset Typing via surface markers possible
- Possibility to observe heterogeneity in the population
- Requires fewer cells 10^3-10^4

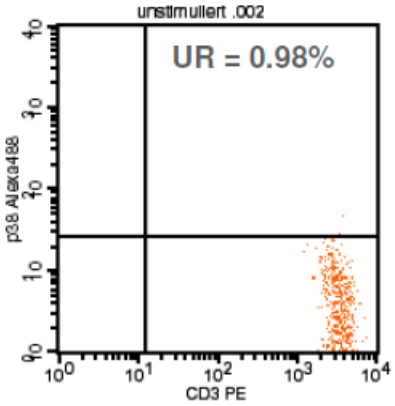
http://www.bdbiosciences.com/research/phosflow/

Applications: phopho-protein profiling

→ You can even easily analyze the status of phosphorilation over time

- Kinetics of MAPK p38 on CD4+ T cells out of a mixture of cells

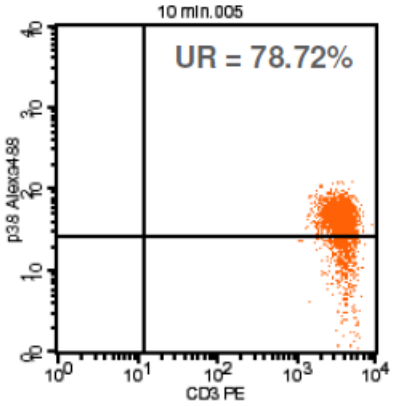
unstimulated



File: unstimulert .002
Tube: 20µl p38
Gate: CD4 pos T-Lymphozyten

Quad	% Gated	Y Geo Mean
UL	0.00	***
UR	0.98	29.12
LL	0.00	***
LR	99.02	2.54

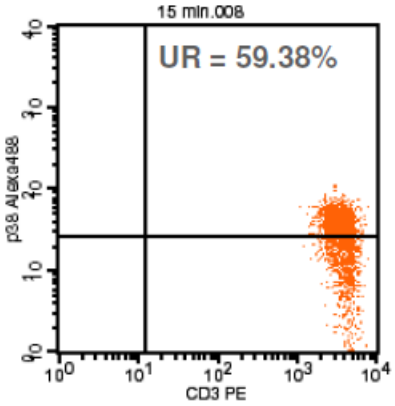
10 min. PMA



File: 10 min.005
Tube: 20µl p38
Gate: CD4 pos T-Lymphozyten

Quad	% Gated	Y Geo Mean
UL	0.00	***
UR	78.72	46.45
LL	0.00	***
LR	21.28	12.00

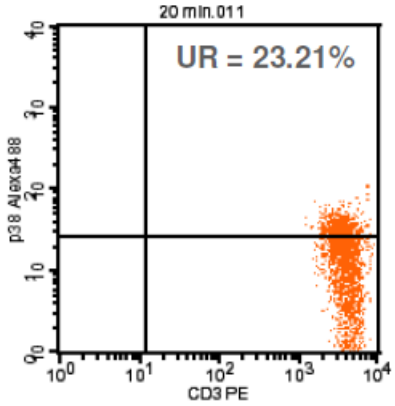
15 min. PMA



File: 15 min.008
Tube: 20µl p38
Gate: CD4 pos T-Lymphozyten

Quad	% Gated	Y Geo Mean
UL	0.00	***
UR	59.38	39.25
LL	0.00	***
LR	40.62	10.10

20 min. PMA



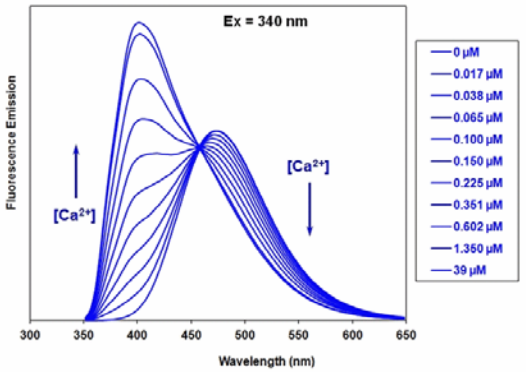
File: 20 min.011
Tube: 20µl p38
Gate: CD4 pos T-Lymphozyten

Quad	% Gated	Y Geo Mean
UL	0.00	***
UR	23.21	33.83
LL	0.00	***
LR	76.79	6.66

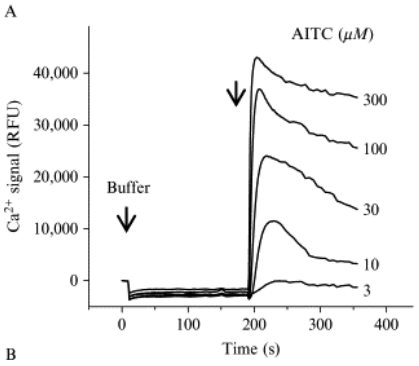
BD Biosciences PhosFlow Tutorial

Applications: calcium flux

- Why any interest in study Ca2+ flux?
  - Ca2+ is related with cell activation. You may want to check Ca2+ levels after several treatments, to see whether Ca2+ is affected and thus, also activation status of a cell (by multiparametric flow cytometry together with surface markers)
  - Remember: Ca2+ is one of most important second-messengers in a cell.



AAT Bioquest



https://doi.org/10.1016/B978-0-12-381296-4.00017-8

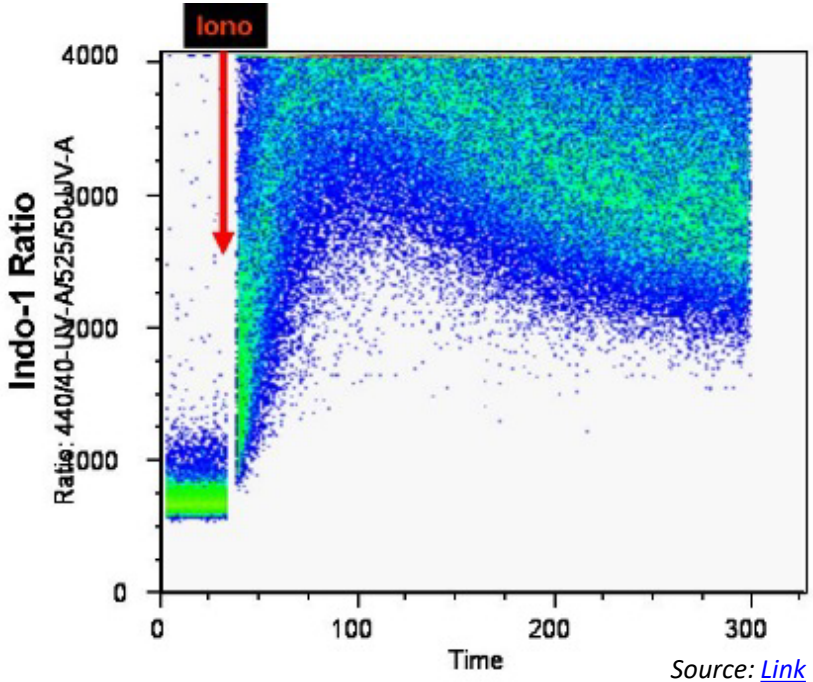
- How do you measure Ca2+ flux? With dyes whose emission spectra changes after binding Ca2+:
  - Indo-1: 475nm to 400nm after Ca2+ binding (excitation: UV) > We measure the ratio
  - Fluo-4: 100x more emission at 506nm after Ca2+ binding (excitation: blue laser

Applications: calcium flux with Indo-1

$$\frac{\text{Indo-Ca2+bound (400nm)}}{\text{Indo-Ca2+free (475nm)}}$$

If Ca2+bound > Ca2+free then higher Ratio = Ca2+flux inside the cell

- Stimulation of Jurkat T cells, monitoring of cell activation over time

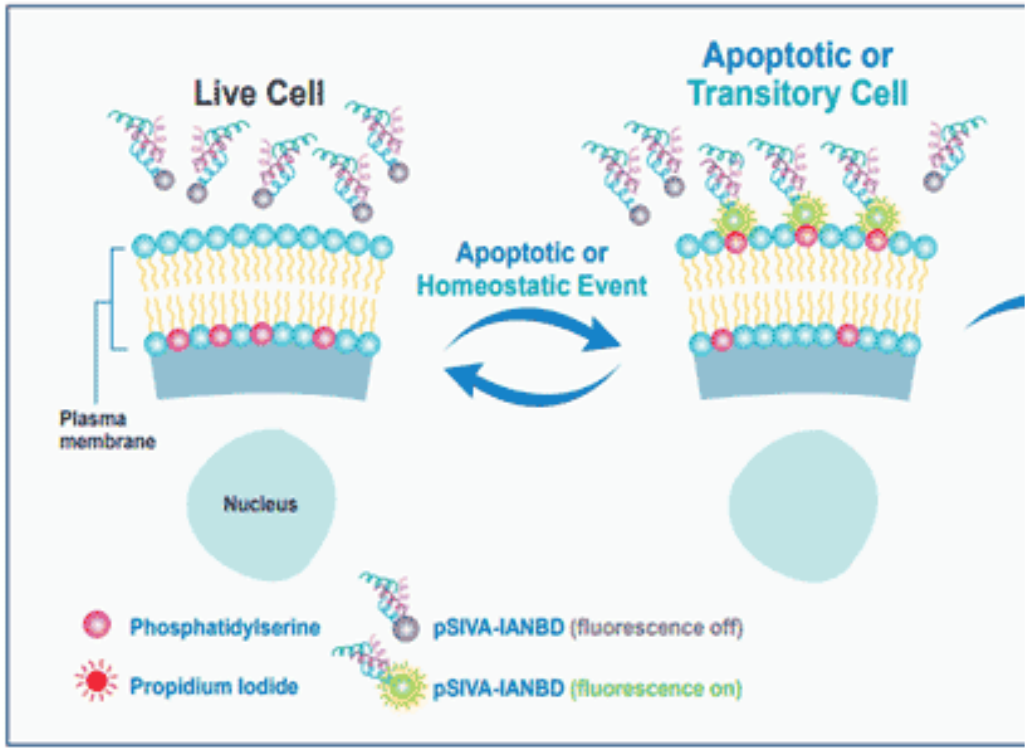


Source: Link

Applications: Apoptosis

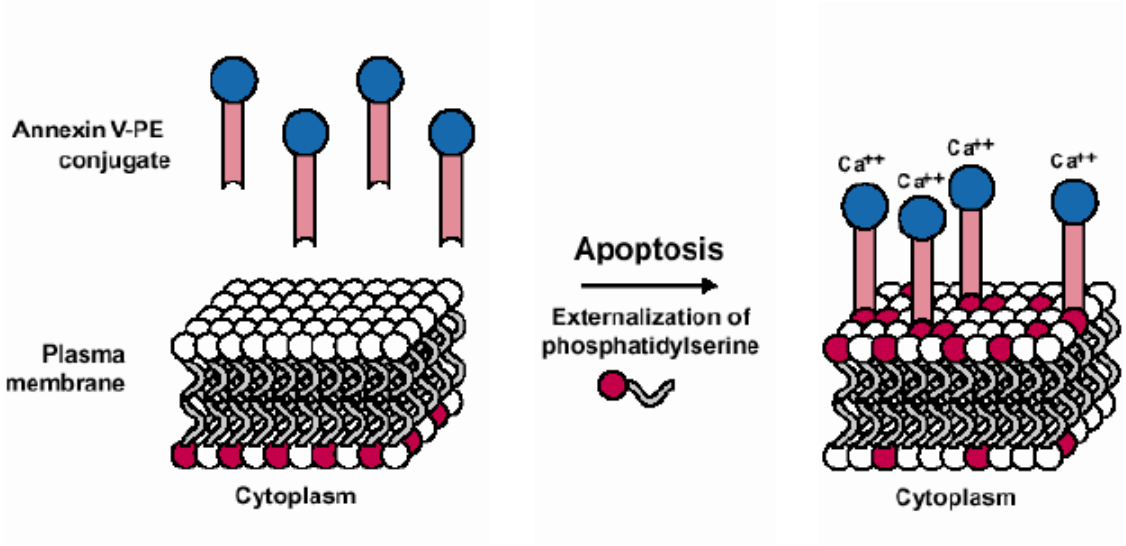
Apoptosis = programmed cell death. During the process several molecules can be analyzed by flow cytometry to follow and characterize apoptosis.

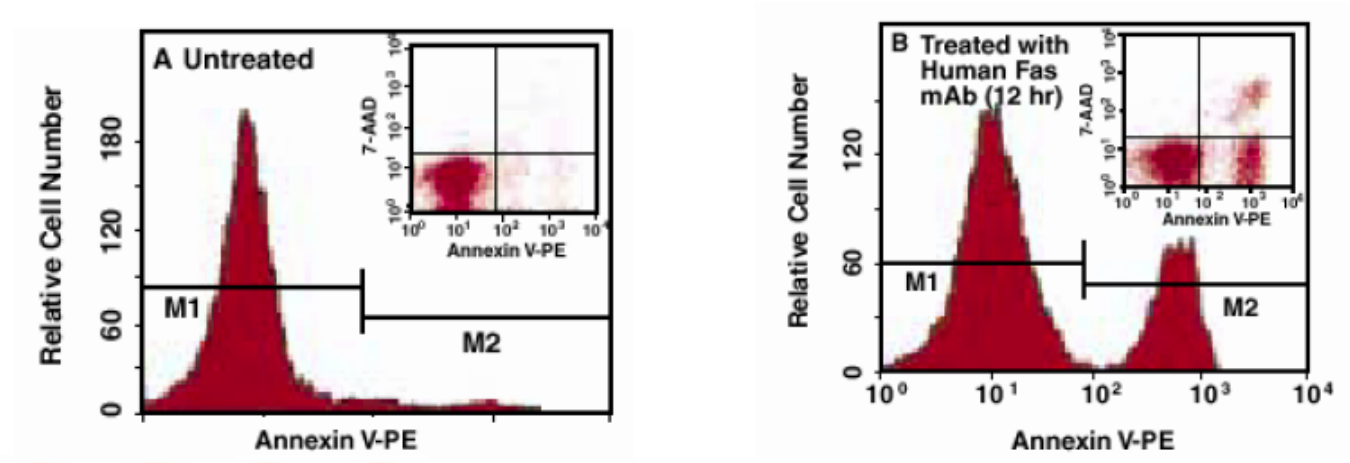
Example 1: phosphatidylserine translocation



Source: Novus Biological

Applications: apoptosis (PS by Annexin V)



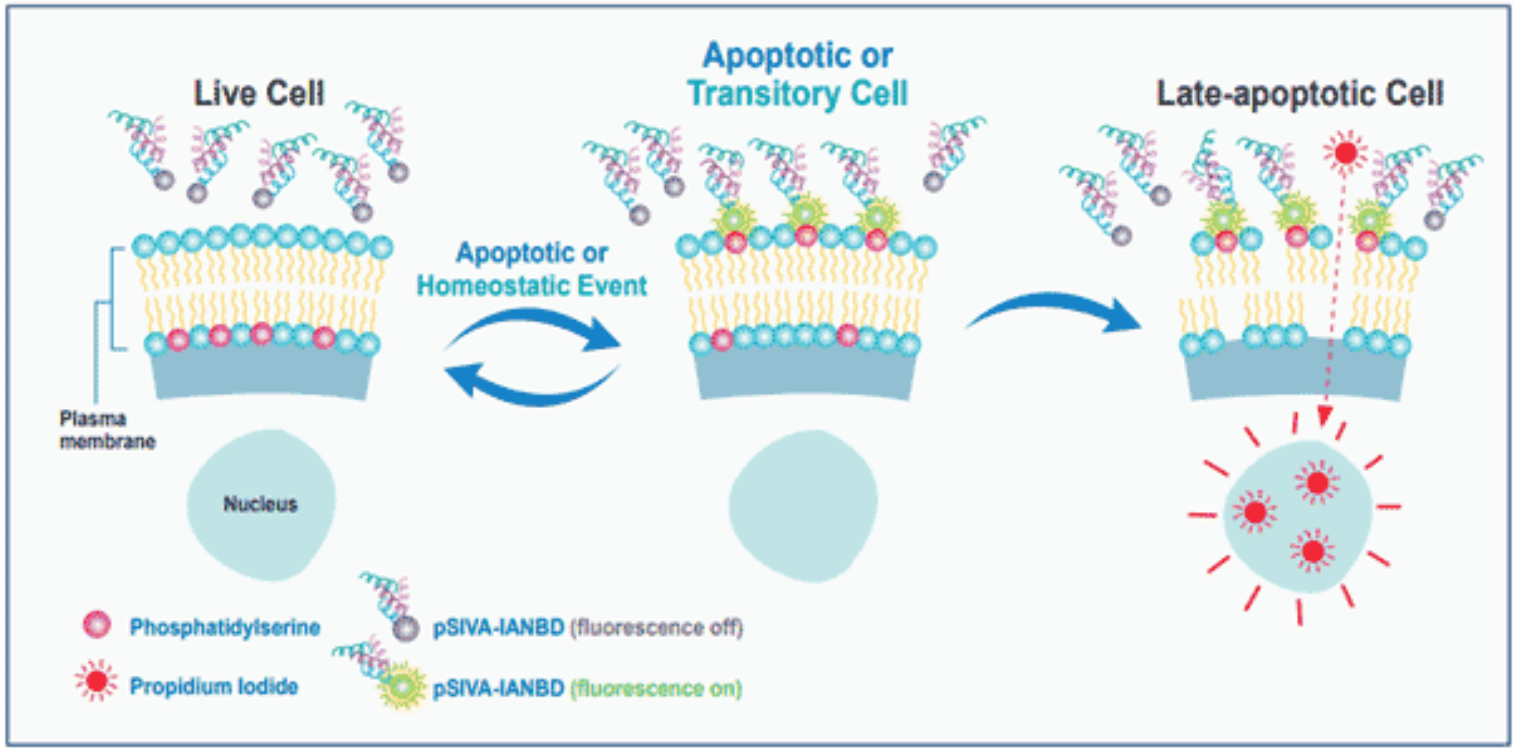


Source: Applications in flow cytomety: Tim Schenkel BD Biosciences Training Center Heidelberg

Applications: Apoptosis

Apoptosis = programmed cell death. During the process several molecules can be analyzed by flow cytometry to follow and characterize it

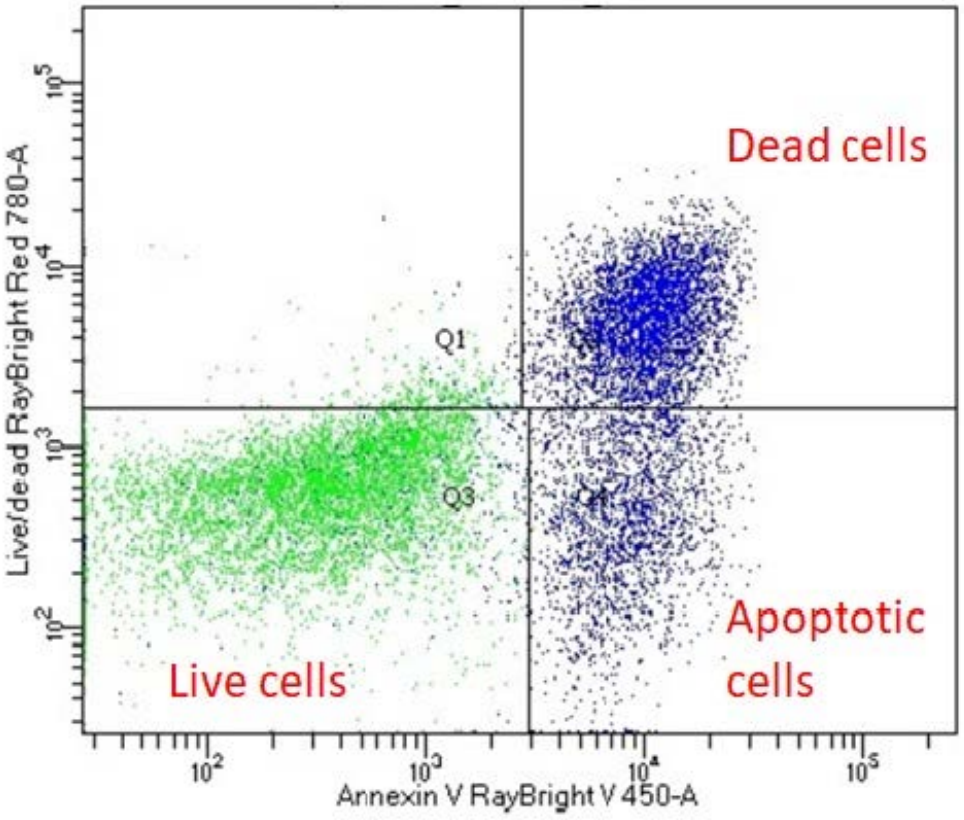
Example 2: phosphatidylserine translocation



Source: Novus Biological

Applications: Apoptosis

- You need dyes binding DNA: PI, Sytox, DAPI, 7-AAD…



Source: RayBiotech

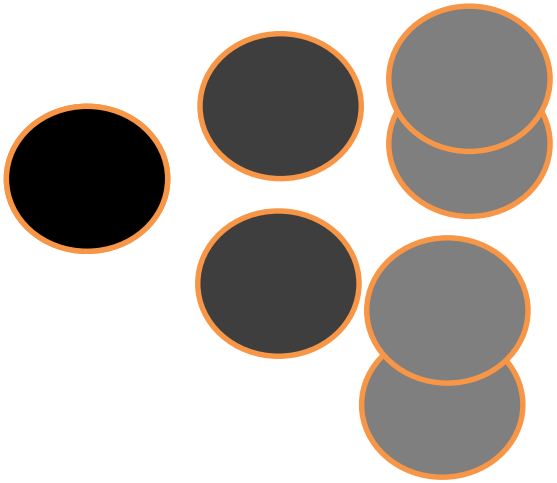
Applications: apoptosis

Change	Assays
Translocation of phophatidylserine from the inner to the outer leaflet of the plasma membrane	Annexin V
Changes of mitochondria membrane potential	BD MitoScreen test
Activation of caspases	e.g. activation of caspase-3
DNA fragmentation	APO BrdU Kit (TUNEL)

Source: Applications in flow cytomety: Tim Schenkel BD Biosciences Training Center Heidelberg

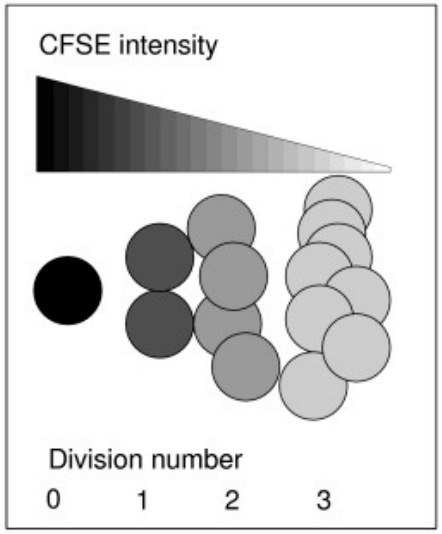
Applications: cell proliferation

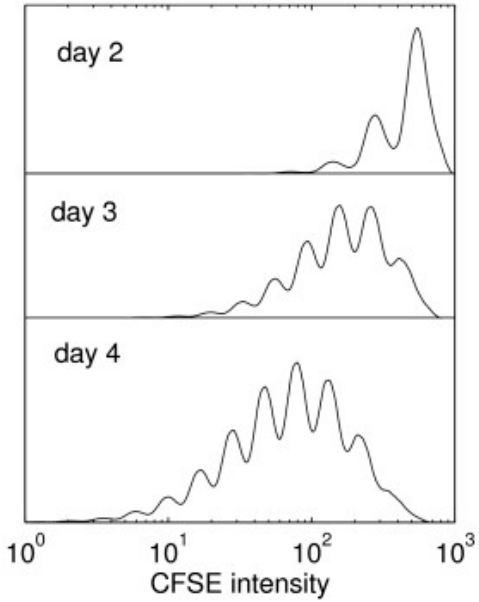
The principle:



In each generation, the amount of dye is ½ (theoretically)

Most common dyes: CFSE, CellTrace Violet…

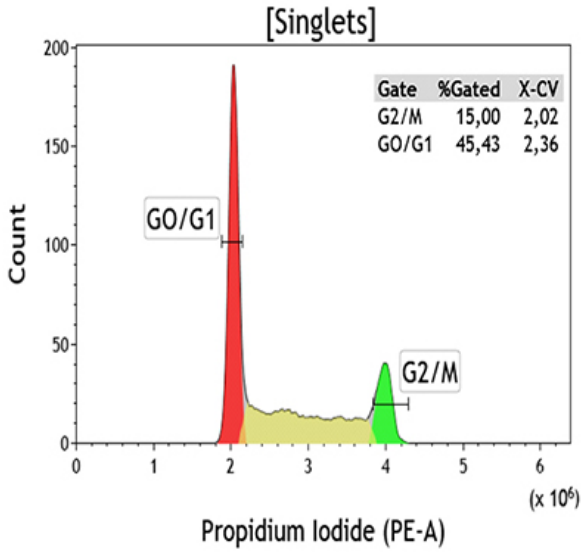




Source: Luzyanina et al. Theoretical Biology and Medical Modelling 2007 ***4****:26*

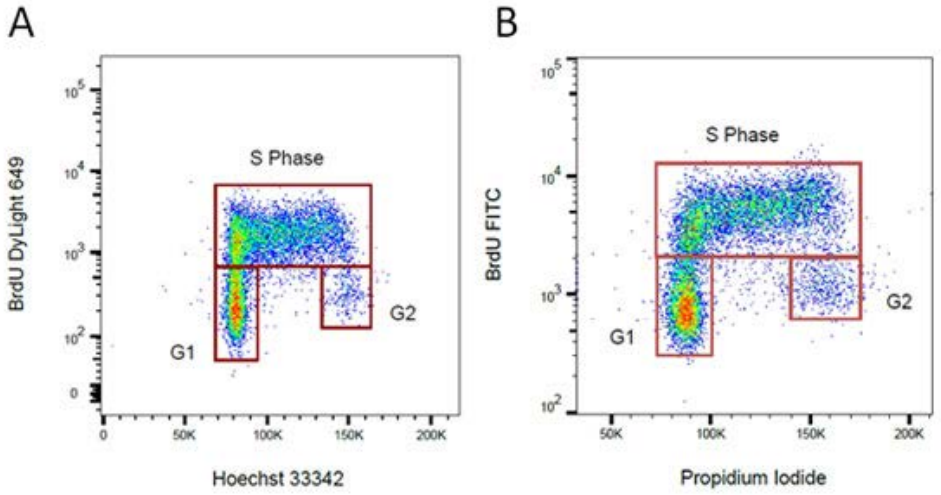
Applications: cell cycle 1D and 2D

Cell cycle 1D = one parameter using DNA-binding dyes (as in apoptosis): PI, 7-AAD, DAPI…etc.



Source: Beckman Coulter

Cell cycle 2D = 1D + another parameter (normally an antibody), to fully reveal S-phase



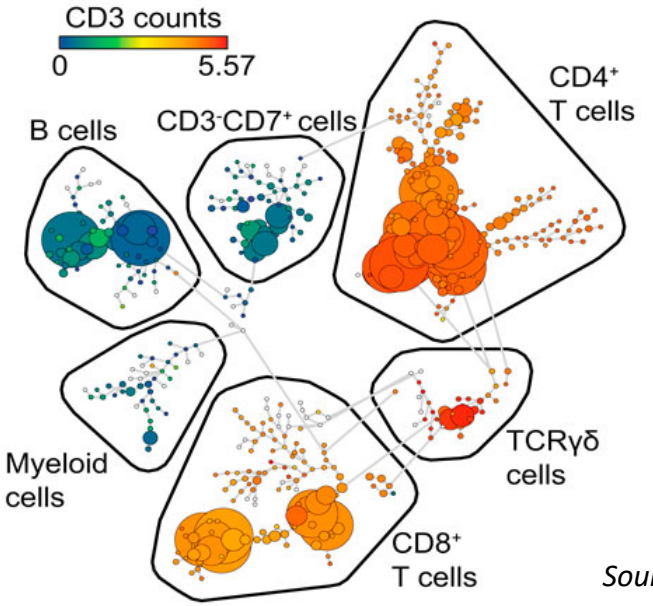
Source: BioRad

Applications of cell sorting

- Enrichment of transfected cells (i.e. GFP+ only, mCherry+ only, double positive…etc.) or any cell of interest (you can sort up to 6 populations **at the same time**, at the IMB only 4)

- Single-cell sorting for single cell clone expansion (available only for some cells, as others might not like to be alone!)

- Single-cell sorting for single-cell sequencing (collaboration with Genomics)



Source: Cytosplore

Overview

- What is flow cytometry?
  - FSC – SSC (relative size, granularity / complexity)
  - Fluorescence
- Looking inside a flow cytometer
  - Fluidics
  - Optics
  - Electronics
- Aquisition software, data presentation and gating
- Introduction to electrostatic cell sorting
- Applications of flow cytometry & cell sorting
- Examples of current flow cytometers
- Advantages of flow cytometry over other techniques

Examples of conventional flow cytometers

BD Flow cytometers

FACSFortessa (18p.)

BDFACSSymphony (50p.)

Beckman coulter cytometer

Gallios (12p.)

Miltenyi Biotech cytometer

MACSQuant (10p.)

ThermoFisher Cytometer

Attune NxT (16p.)

Examples of spectral flow cytometers

Cytek

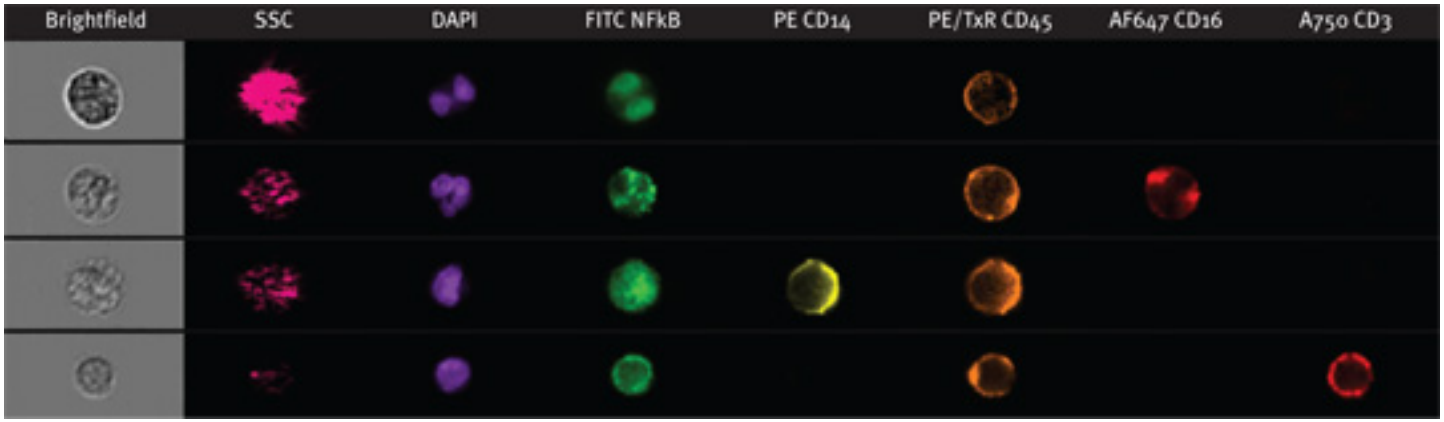
Aurora (?)

Sony

SP6800

Imaging Flow Cytometry: ImageStream

- Combination of flow cytometry + imaging
  - For each cell, one picture



Imaging Flow Cytometry: ImageStream

Advantage

- Information about signal distribution within the cell (spatial distribution) and about cell morphology of a high number of cells

Disadvantages

- Low data acquisition rate (5000 evt/sec)
- Only up to 10 colours
- Huge data amount per measurement
- Image quality

Overview

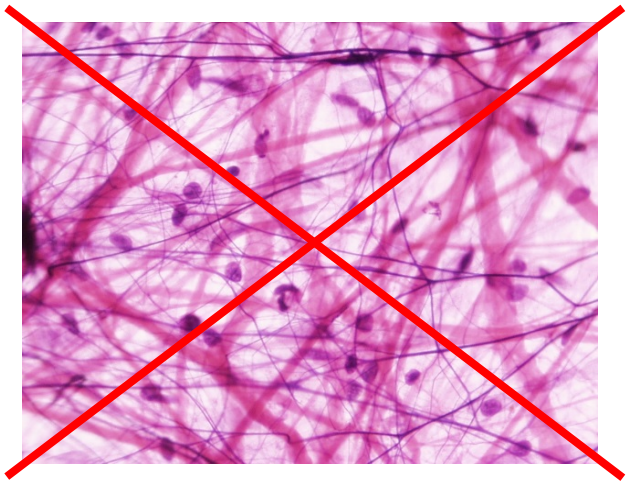
- What is flow cytometry?
  - Which features can be measured? (FSC/SSC, fluorescence)
- Looking into a flow cytometer
  - Fluidics
  - Optics
  - Electronics
- Fluorescence in flow cytometry
- Aquisition software, data presentation and gating
- Introduction to electrostatic cell sorting
- Applications of flow cytometry & cell sorting
- Examples of current flow cytometers
- Advantages of flow cytometry over other techniques

Advantages of flow cytometry

- Quick sample processing (up to 10,000 events/s). Generation of high amounts of data.
- Possibility of automatization (e.g. using 96/384-well plates)
- „Easy“. Sample preparation can be easily done.
- Analysis of multiple cell types in one simple tube.
- Detection of rare population, e.g.: circulating tumour cells.
- Possibility of cell sorting > cell purification for downstream analysis (PCR, qPCR, culture, single cell sequencing…etc.)

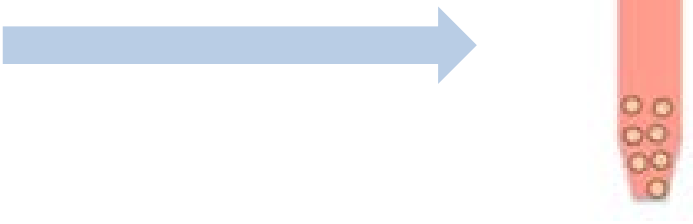
BONUS I: **Do not forget!**

No solid tissue



…only cell in suspension

Mechanical disruption
Enzymatic dissociation

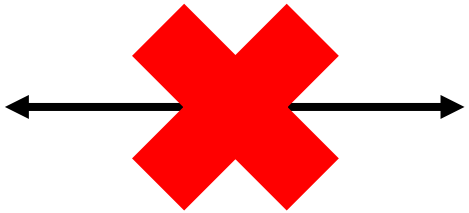


X It cannot locate a component within the cell

X It cannot see how the fluorescent compound is distributed in the cell

X No detailed intracellular morphology

BONUS II: Is Flow Cytometry and FACS the same?

Flow cytometry  **FACS**

Fluorescent **A**ctivated **C**ell **S**orting
(BD Trademark)

If you are only analyzing samples > Flow Cytometry

If you are going to sort your cells of interest > Cell Sorting

Everything will be clearer!

TAKE HOME „PHRASES“

Cells/Cells-like-particles in suspension

FSC – Relative Size
SSC – Cell complexity
Fluorescence

Sheath Fluidic

Hydrodynamic focusing

Lasers - Detectors

Many applications available! Enjoy!

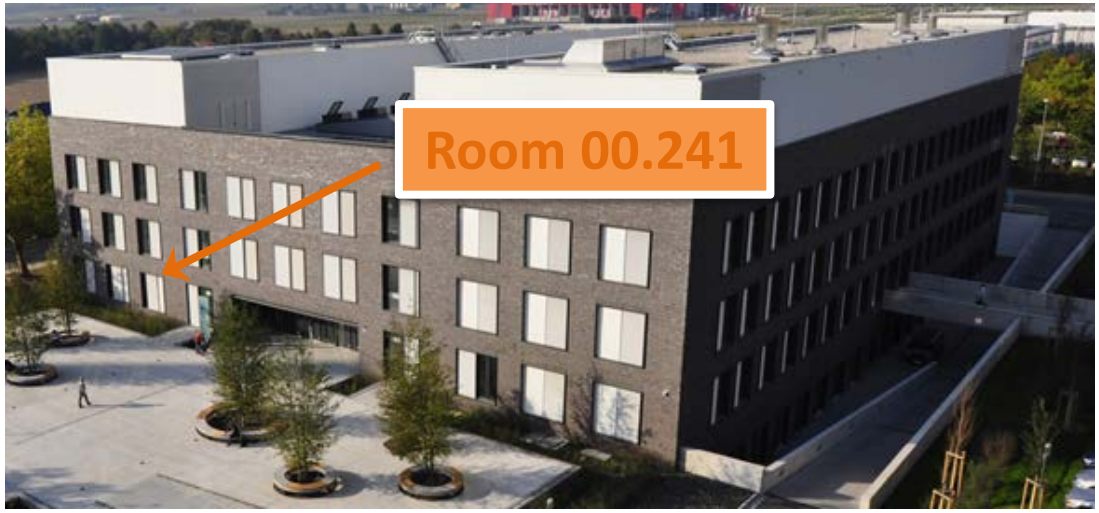
Contact us: **cytometry@imb-mainz.de**

Who are we?

Dr. Stefanie Möckel

Dr. Jesús Gil-Pulido

Where can you find us?



THANKS FOR YOUR ATTENTION!

Do not hesitate to contact us under:

cytometry@imb-mainz.de

Or come by to our Facility ☺

What am I seeing? Melilla, a spanish city in the North of Africa

Dr. rer. nat. Jesús Gil-Pulido