Introduction to Flow Cytometry

Flow cytometry

- History & definition
- Principles
- Applications
- Other

Flow cytometry

- History & definition
- Principles
- Applications
- Other

Flow cytometry

History & definition

Relatively old origins

- 1934 : First « baby cytometer » designed by Moldavan :
  Capillary cell counting/Photoelectric sensor (Science , 1934)

  Moldavan, A. Photo-Electric Technique For The Counting Of Microscopical Cells. Science 1934: 188-189.

- 1949 : Wallace Coulter files patent: Apparatus for counting and measuring cell size

- 1965 : Kamentsky : addition of cell constituents analysis

Flow cytometry

History & definition

→ 1969 one of the first article describing the sorting of mammalian cells (HERZENBERG , Stanford), then in 1973 its applications:

FACS ou Fluorescence Activated cell Sorter

Hulett, H.R., Bonner, W.A., Barrett, J. and Herzenberg, L.A. (1969) Cell sorting: Automated separation of Mammalian cells as a function of intracellular fluorescence. *Science* **166**; 747–9.

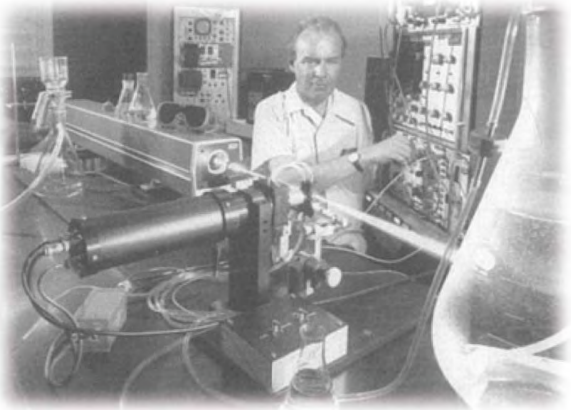
Hulett, W.R., Bonner, W.A., Sweet, R.G. and Herzenberg, L.A. (1973) Development and application of a rapid cell sorter. *Clin. Chem.* **19**, 813–16.

→ 1969 Marvin Van Dilla : use of laser as a light source

B. Shoor et L. Herzengerg before

The first flow cytometer

Becton Dickinson



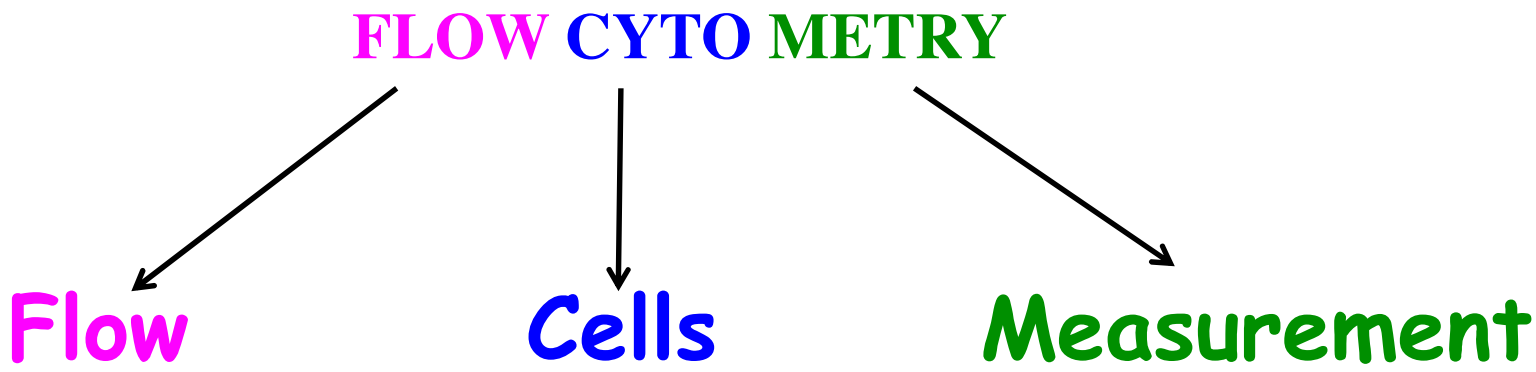
Flow cytometry

History & definition

- 1974 : Commercialization by Becton-Dickinson (FACSII)
- 1980 : Analysis of 3 Fluorescence parameters per cell
- 1990 : Analysis of 7 fluo parameters per cell
- 2001: Analysis of 11 fluo / cell parameters
- 2006 : Analysis of 18 fluo / cell parameters
- 2016 : Analysis of 20 parameters per cell .....

Flow cytometry

History & definition



Flow cytometry

History & definition

Precise study of isolated cells driven by a fluid flow.

Caracterization :

- Individual
- Quantitative
- Qualitative
- Functional

of particules suspended in a liquid.

Flow cytometry

History & definition

Consists of:

- analyse the ***optical or physical signals***
- Emitted by a particule
- Cutting the ***light beam of a laser*** or an arc lamp.

Flow cytometry

- History & definition
- Principles
- Applications
- Other

Flow cytometry

Principle

Flow cytometry?

Simultaneous measurements

- Of different characterisitics of a cell or a particule

Measurement made while the cells are scrolling one by one

- in an analysis chamber,
- at more or less high speed.

Flow cytometry

Principle : which informations?

Which informations about the cells?

- Its size(Forward scatter : FSC)
- Its granularity or internal complexity (Side Scatter = SSC)

Diffraction

- Its fluorescence intensity (depending on fluorochromes)

Fluo

Flow cytometry

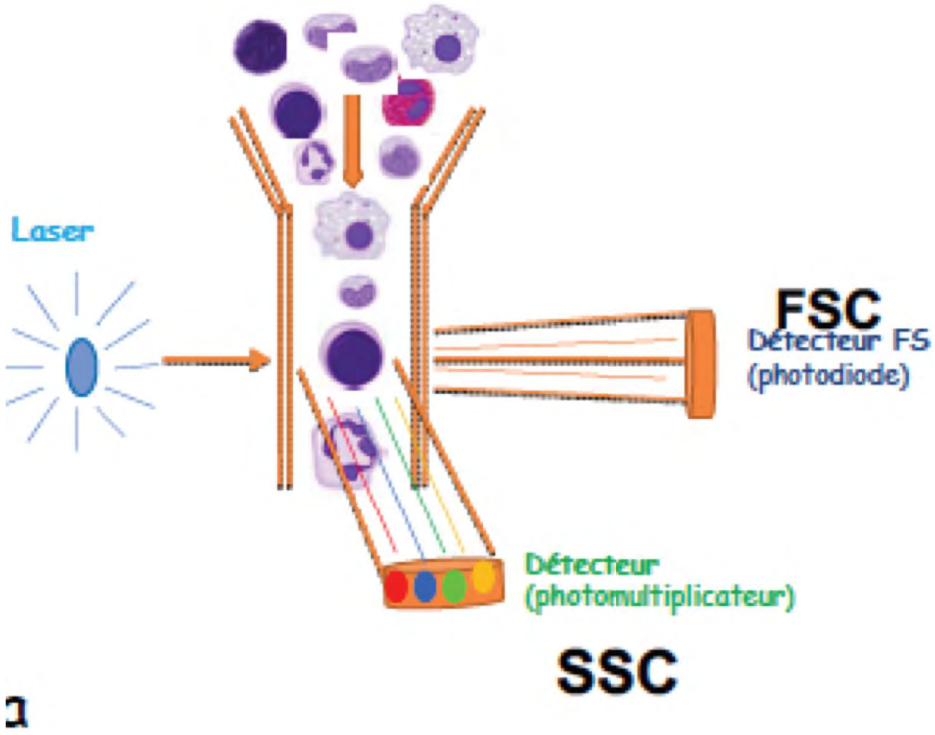
Principle : which informations?

Diffraction

Each event passing in front of the laser will diffracted
We measure:

- Size of the cells (FSC)
  Diffraction proportional to cell size

- Structure of the cells (SSC)
  Diffraction proportional to the granularity or complexity of the cell



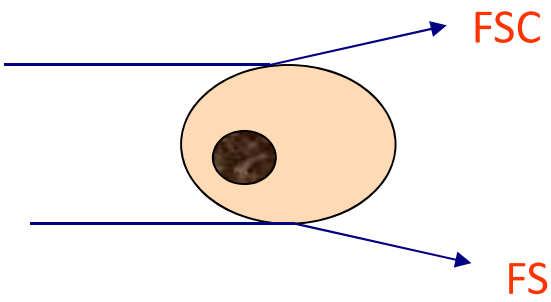
Flow cytometry

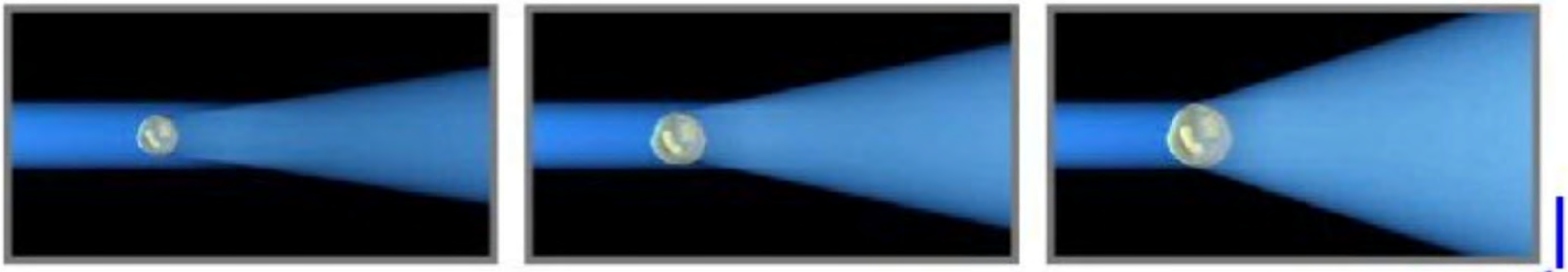
Principle : which informations?

FORWARD SCATTER = Light diffracted at small angles

➟ The light diffracted is collected at a small angle (between 1 and 10 degrees)

➟ The signal is proportional to **the size of the cell**





FSC : Forward SCatter

Evaluate cell size.

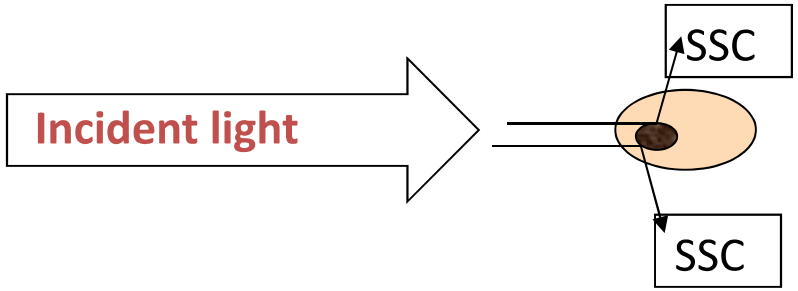
The detector is almost aligned with the laser, it is in the cone of diffracted light

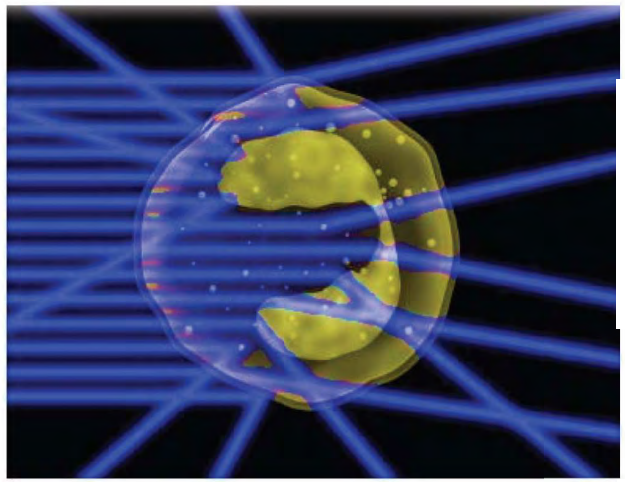
Flow cytometry

Principle : which informations?

SIDE SCATTER = Diffracted light at large angles

- The diffracted light is collected at 90° from the laser axis
- The signal is proportional to the **granularity and cellular complexity**.



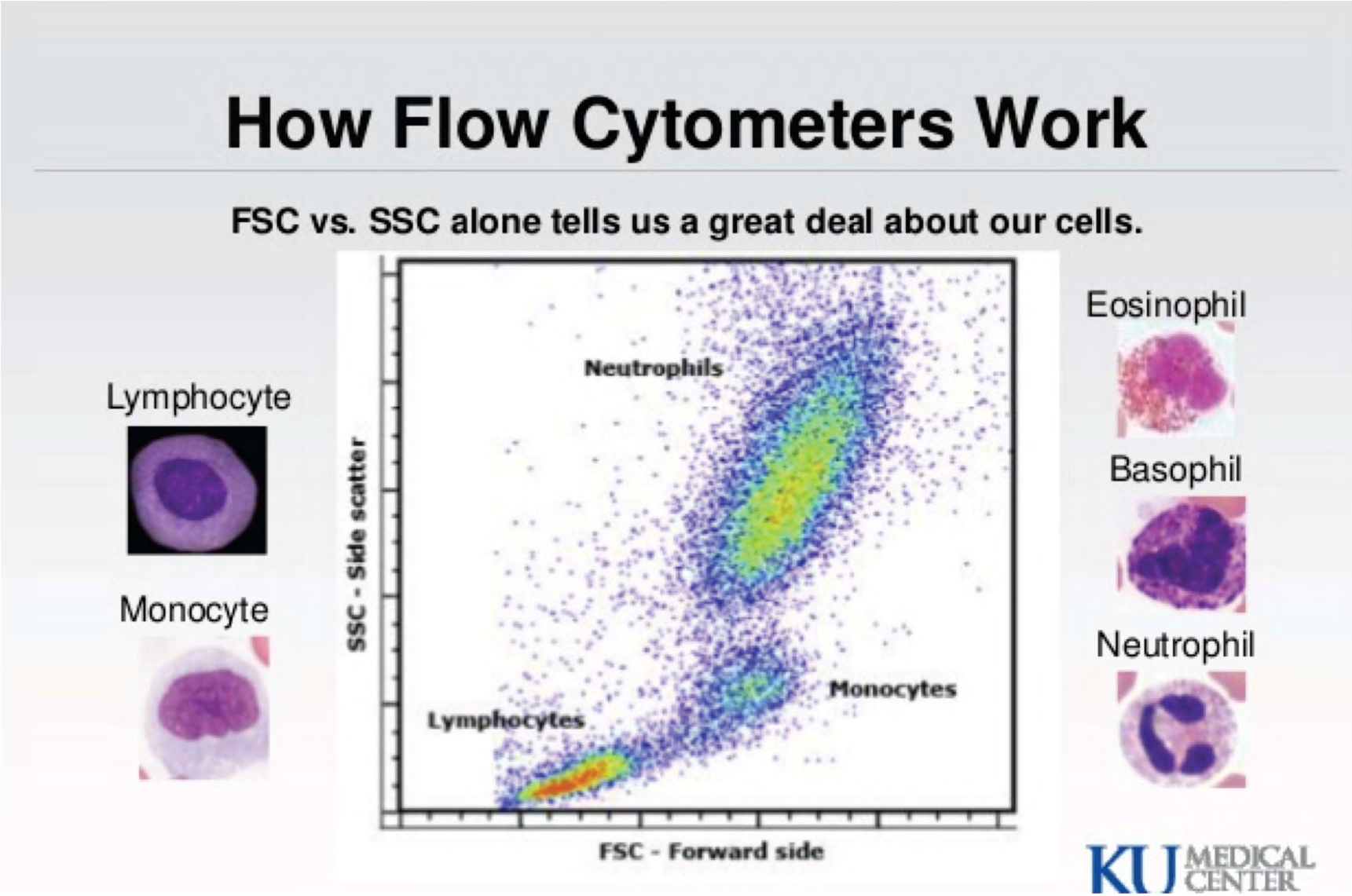


the detector is more or less perpendicular to the laser beam. it is in the area of widely diffracted light

Approximate position of the detector

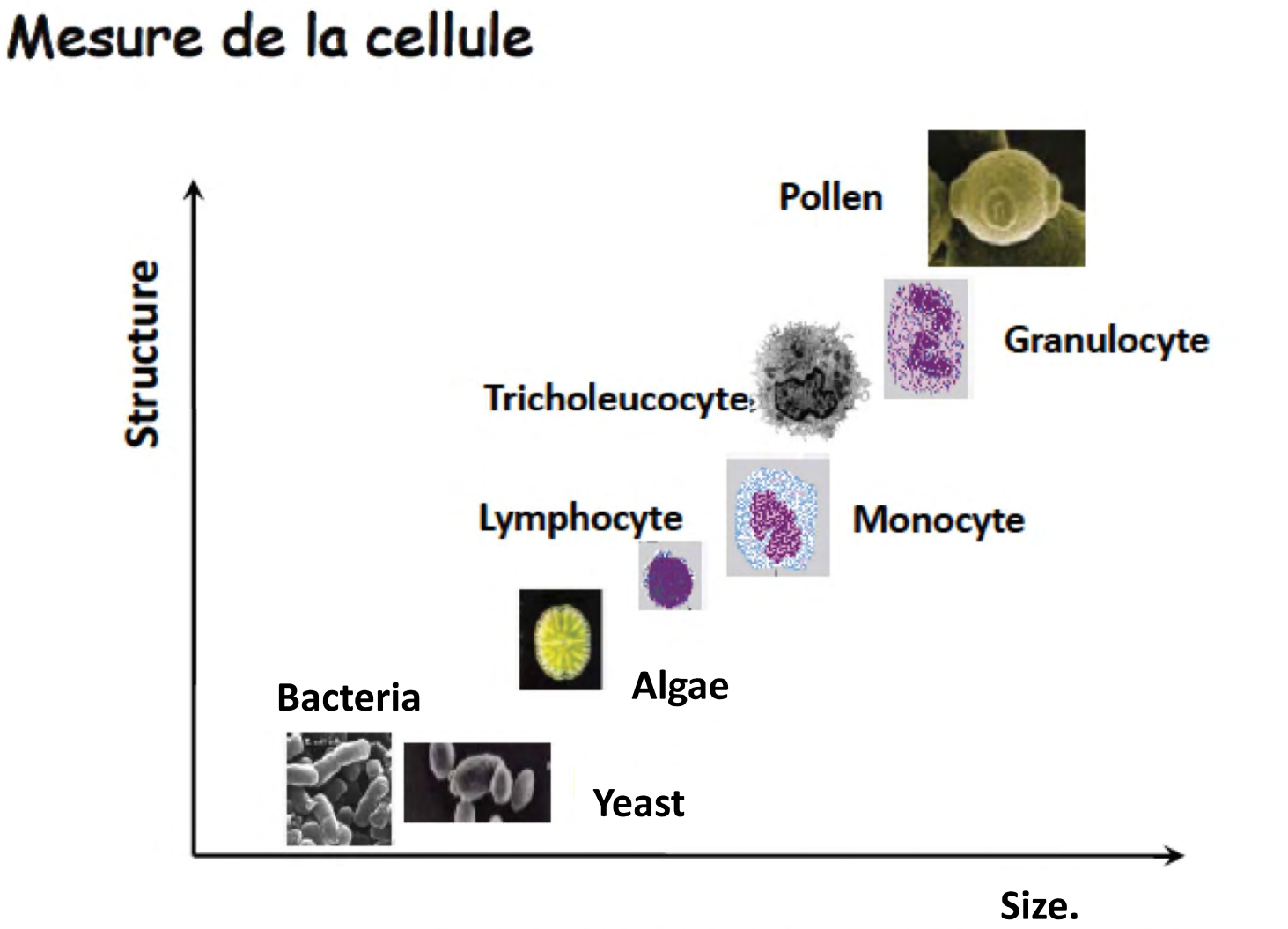
Allows a first sort by characterizing size and structure

Exemple: Whole blood lysed



Flow cytometry

Principle : which informations?

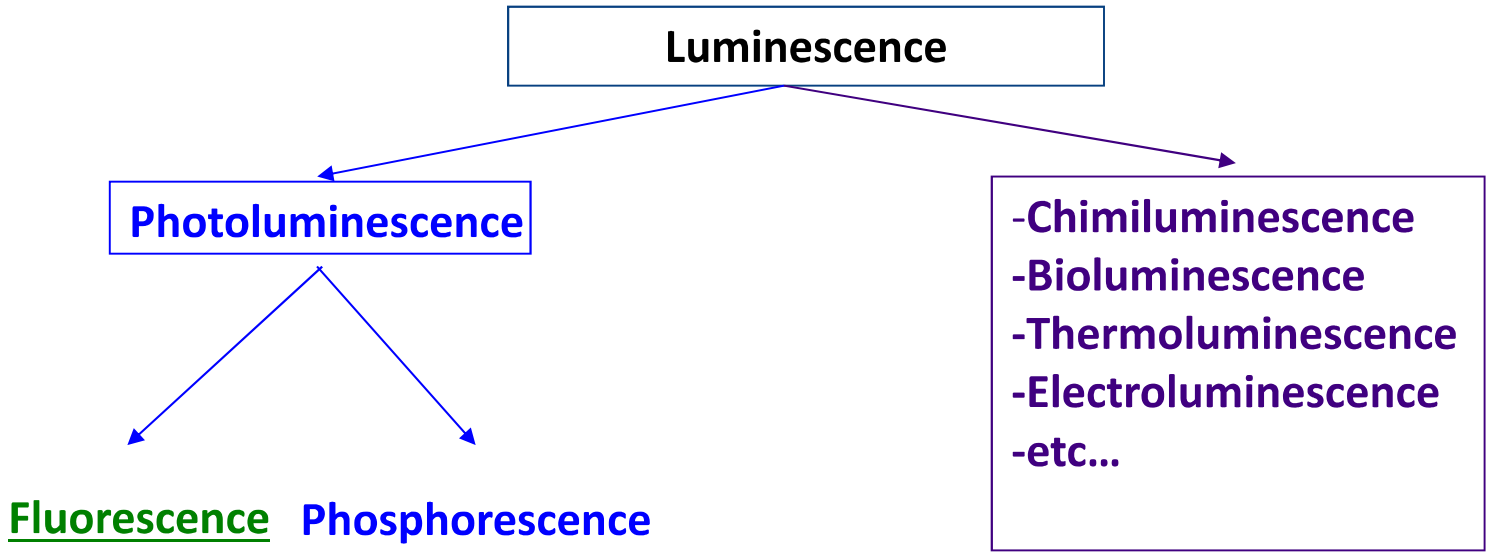


Measurement of particles ranging from 100nm to 100um

Flow cytometry

Principle : which informations?

Fluorescence : each event passing in front of a laser can also emit fluorescence which will be measured at 90 ° C.



- **The photoluminescence** is a radiative phenomenon following a *light excitation* (photons of visible light or UV)

- **La chimiluminescence** is a radiative phenomenon consecutive to a *chemical reaction* (true chemiluminescence) ....

Flow cytometry

Principle : which informations?

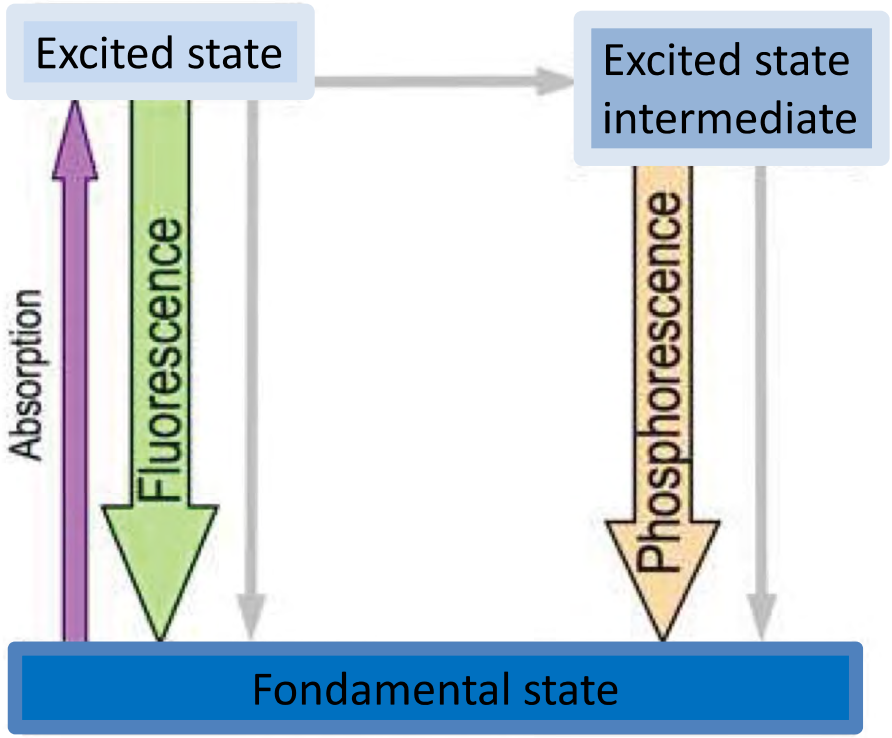
Fluorescence is a light emission that follows

1-the excitation of a molecule (absorption of a photon)

2- rapid spontaneous emission

Different fromPhosphorescence :much slower

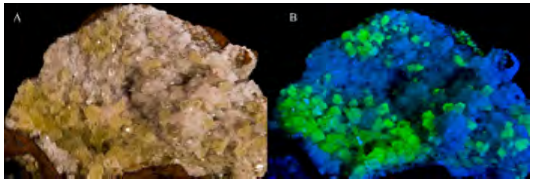
because goes though a transition state S0 to S1



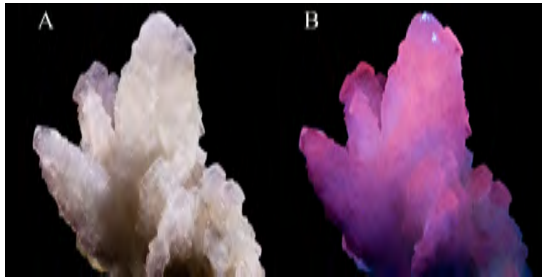
Flow cytometry

Principle : which informations?

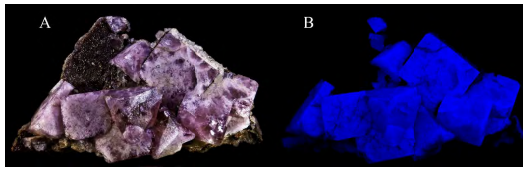
Natural fluorescence :



Adamite: green
Hémimorphite : light blue



Calcite



Fluorite

Under UVA

Quinine under uv

Flow cytometry

Principle : which informations?

Imported fluorescence :

Mouse or other GFP under ubiquitous promoter

Flow cytometry

Principle : which informations?

Natural phosphorescence :

Flow cytometry

Principle : which informations?

Artificial Phosphorescence :

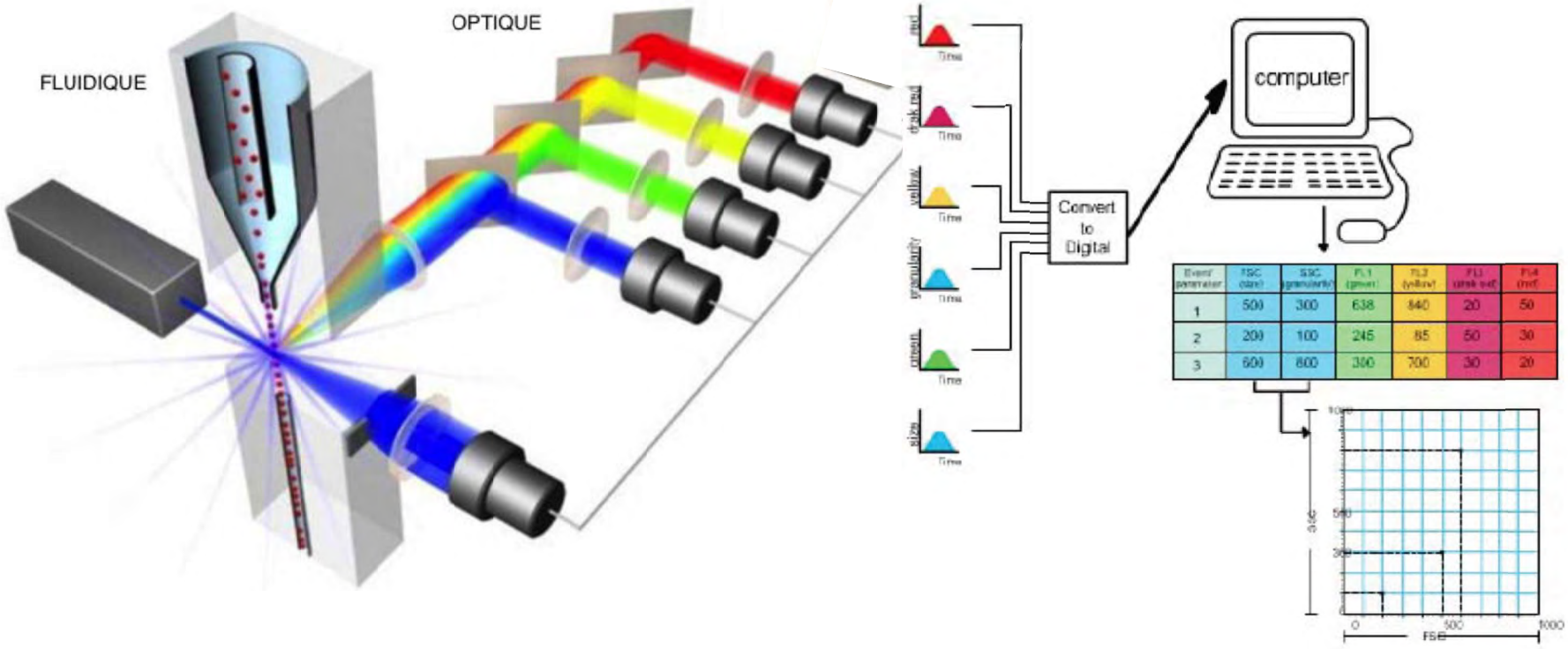
Flow cytometry

working principle?

FLUIDIC
Introduction and positioning of cells

OPTICAL
Production et collection of light signals

ELECTRONIC
Conversion of optical signals into electronic signals and digitization of theses signals for computer analysis



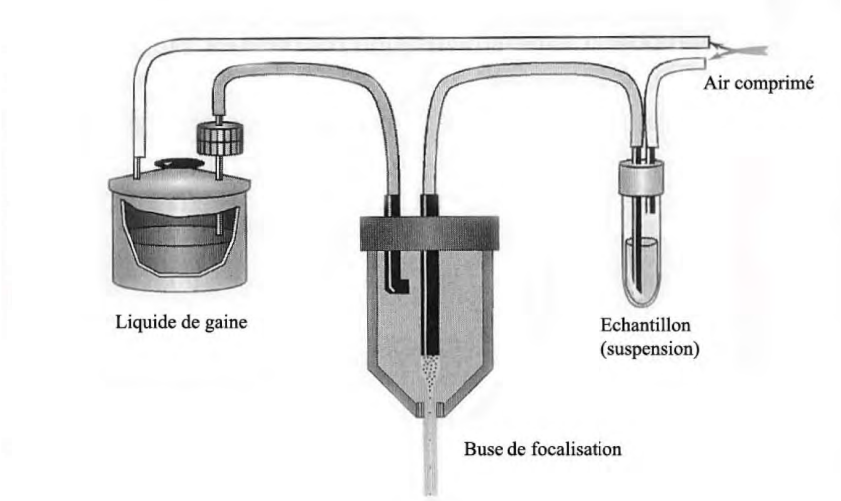
Flow cytometry

Principle : Fluidic

Special feature of cytometers is to **analyse one cell at a time**, for that:

→ Cells are suspended

→ They are driven by a pump and sent one by one (principle of hydrofocusing) in front of one or more lasers



- **Centering** cells in a laminar **flow** (hydrodynamic focus)

- Progressive acceleration (**cell**) **Separation)**

- Passage through a nozzle (**channeling**, centering)

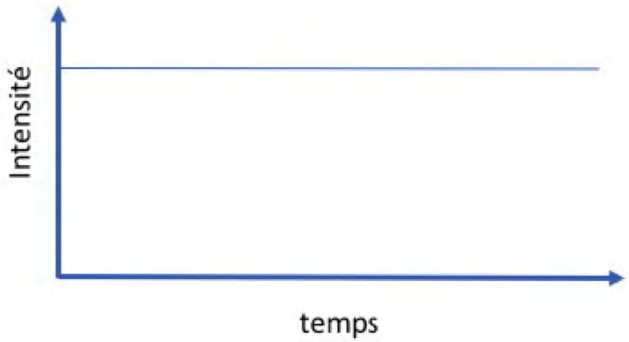
Flow cytometry

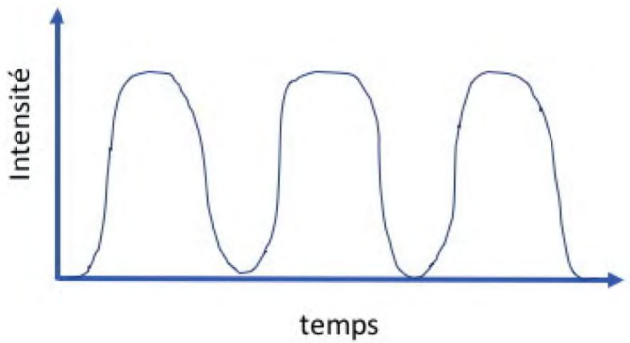
Principle : Fluidic

Need for **centering** cells in a **laminar flow** : **hydrodynamic focus**

For it:

- Progressive acceleration (**cell separation**)
- Passage through a nozzle (**channeling**, centering)



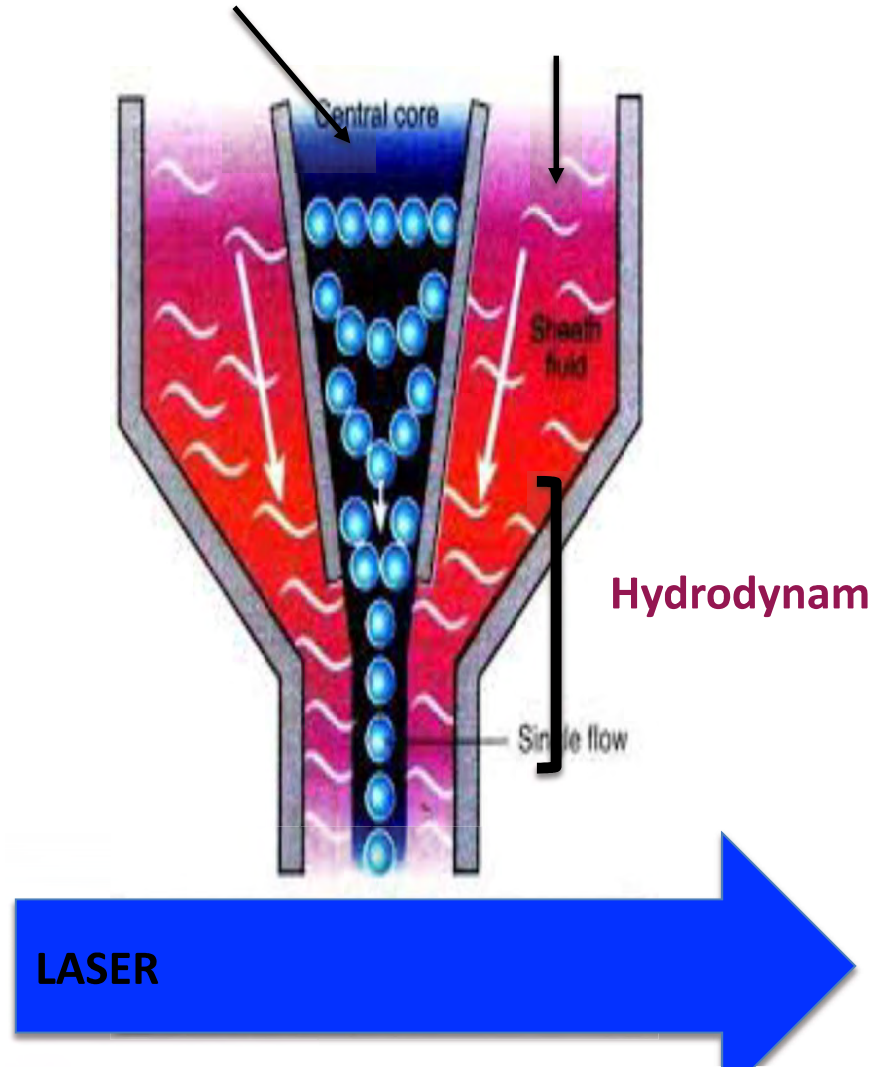


Flow cytometry

Principle : Fluidic

Sample under pressure

Sheath fluid under pressure



Hydrodynamic focus area

- Centering et alignement of cell **in a laminar flow** (hydrodynamic focus)

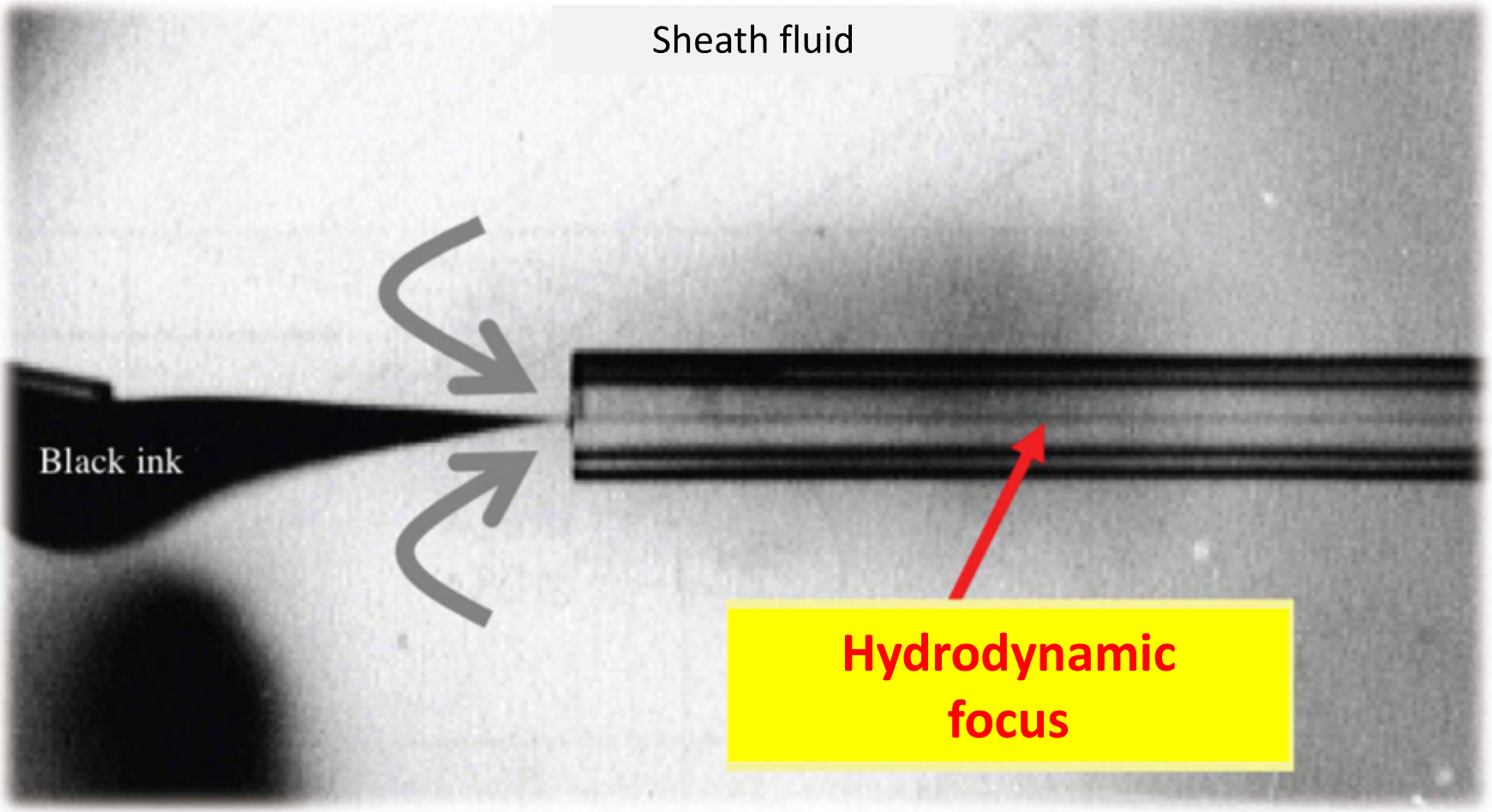
- Progressive acceleration of the sheath = stretching of the sheath fluid (**separation of the cells**

- Passage in a nozzle(**channeling**, centering)

No mixing between the sheath fluid and the sample because pressure difference

Flow cytometry

Principle : Fluidic



Flow cytometry

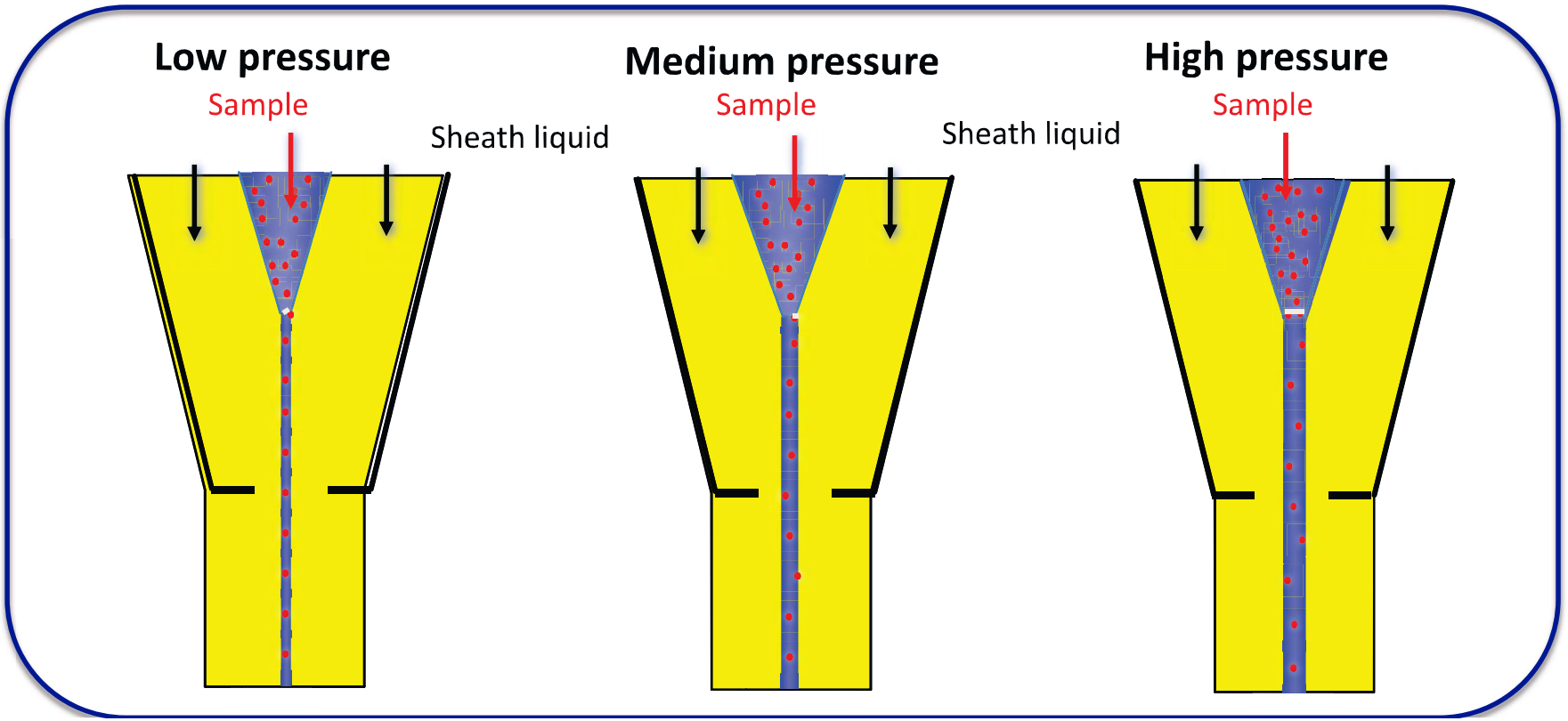
Principle : Fluidic

Différences de pressions

The sheath fluid pressure remains the same, unlike the sample

An increase in pressure causes an increase in flow

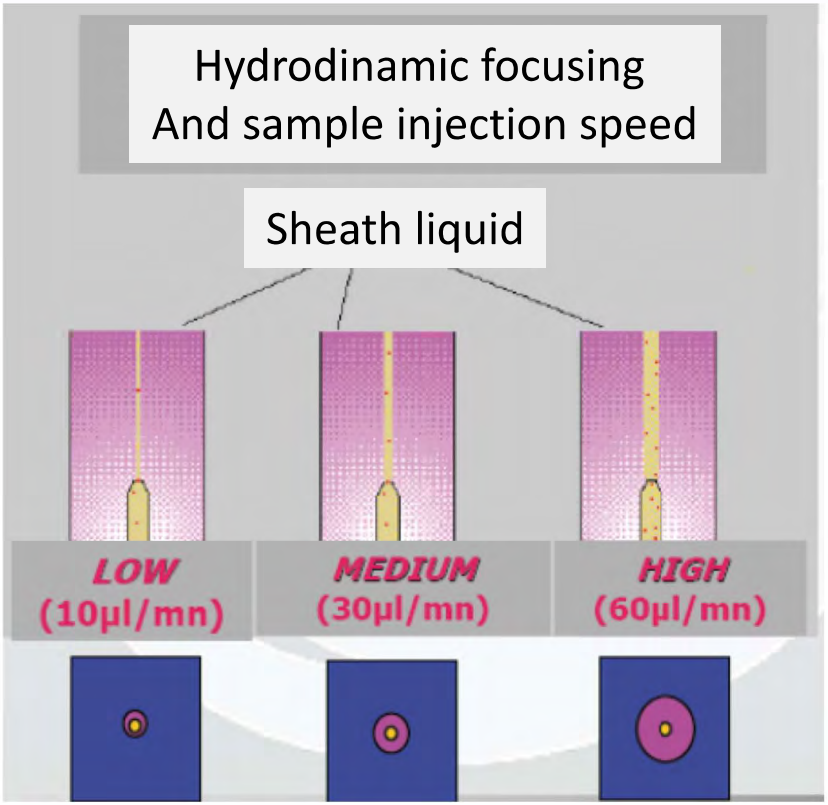
The higher the pressure, the more cells have freedom of movement

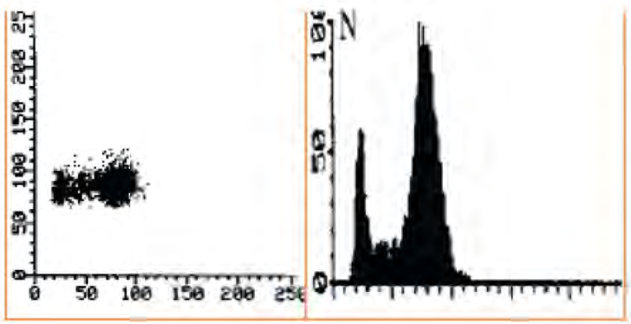


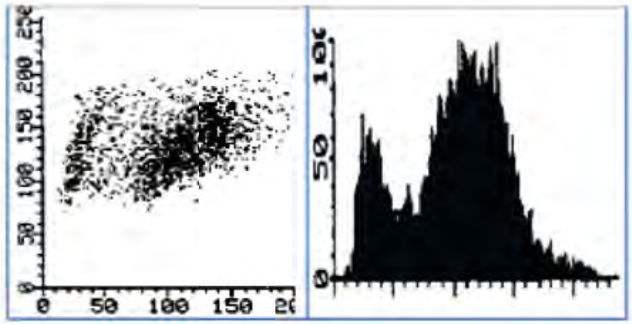
?

Flow cytometry

Principle : Fluidic

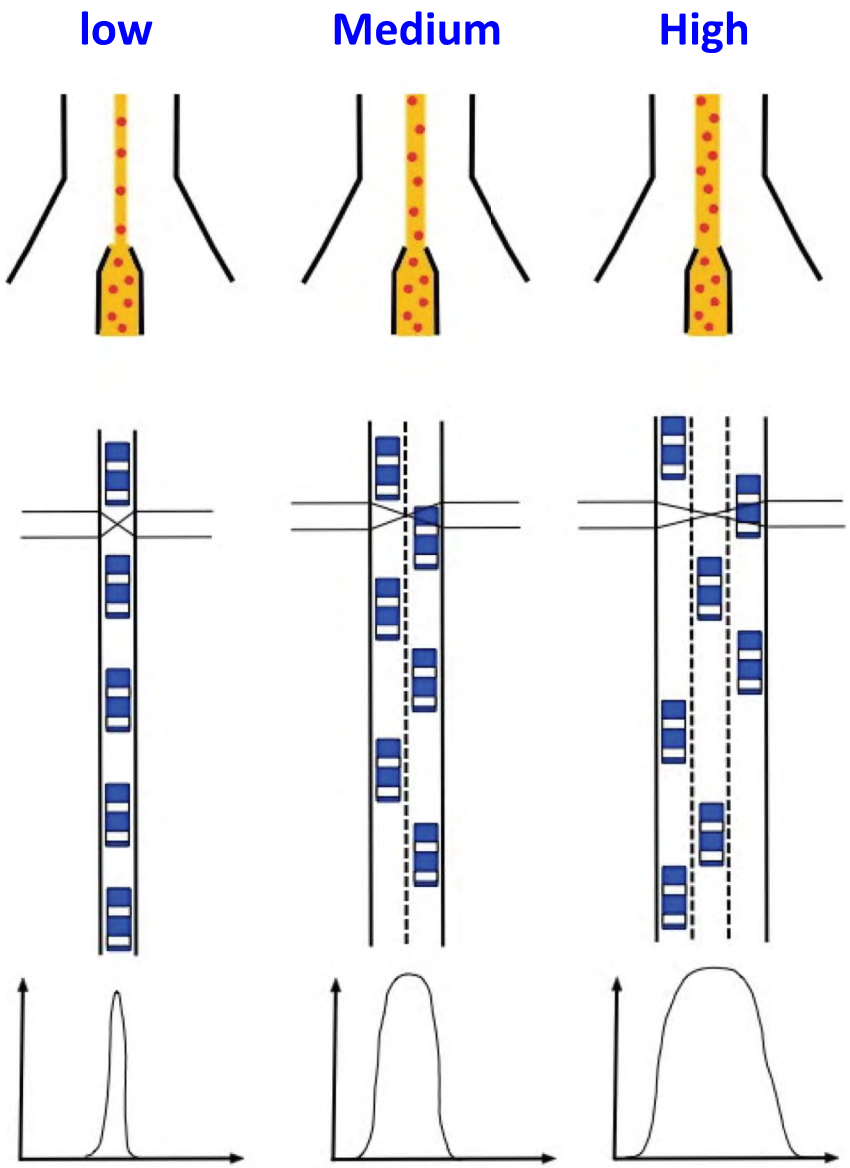






Flow cytometry

Principle : Fluidic



Flow cytometry

Principle : optical

- It is necessary to **focus the light source** on the cells
- Must allow illumination of dyes at a wavelenght close to their excitation max
- For it:
  - Lasers are the most frequently used : power, stability
  - Steam lamps ; (mercury, xenon) are less expensive but less accurate

Flow cytometry

Principle : optical

1-The excitation field is composed of :

→ One or more sources of excitation (laser(s)…)

→ lenses and prisms to focus the laser beam

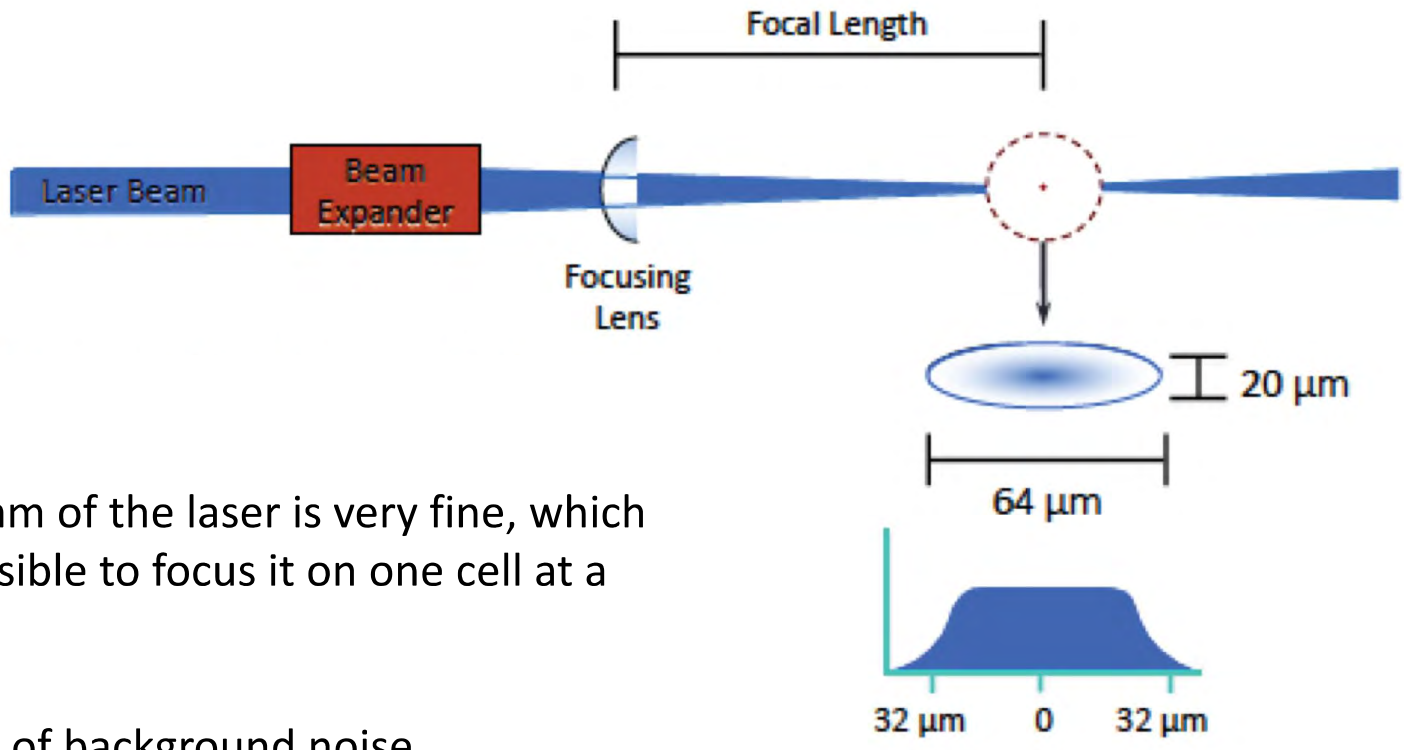
2-The reception field consist of :

→ a lens to collecte the emitted light

→ a system of mirrors and optical filters to direct specific wavelenghts on the corresponding detector

Flow cytometry

Principle : emission optical



The light beam of the laser is very fine, which makes it possible to focus it on one cell at a time.

-> Limitation of background noise
-> Energy concentrated on a small surface
-> Very powerful monochromatic light excitation

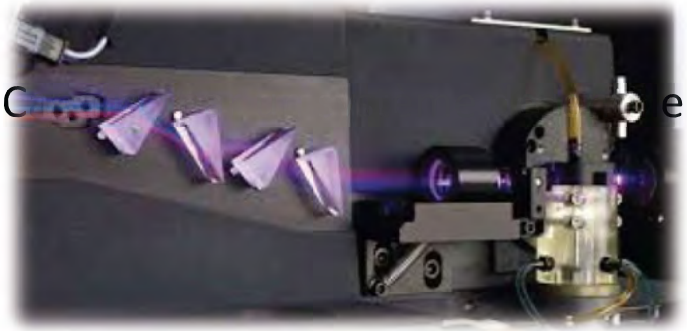
Flow cytometry

Principle : emission optical

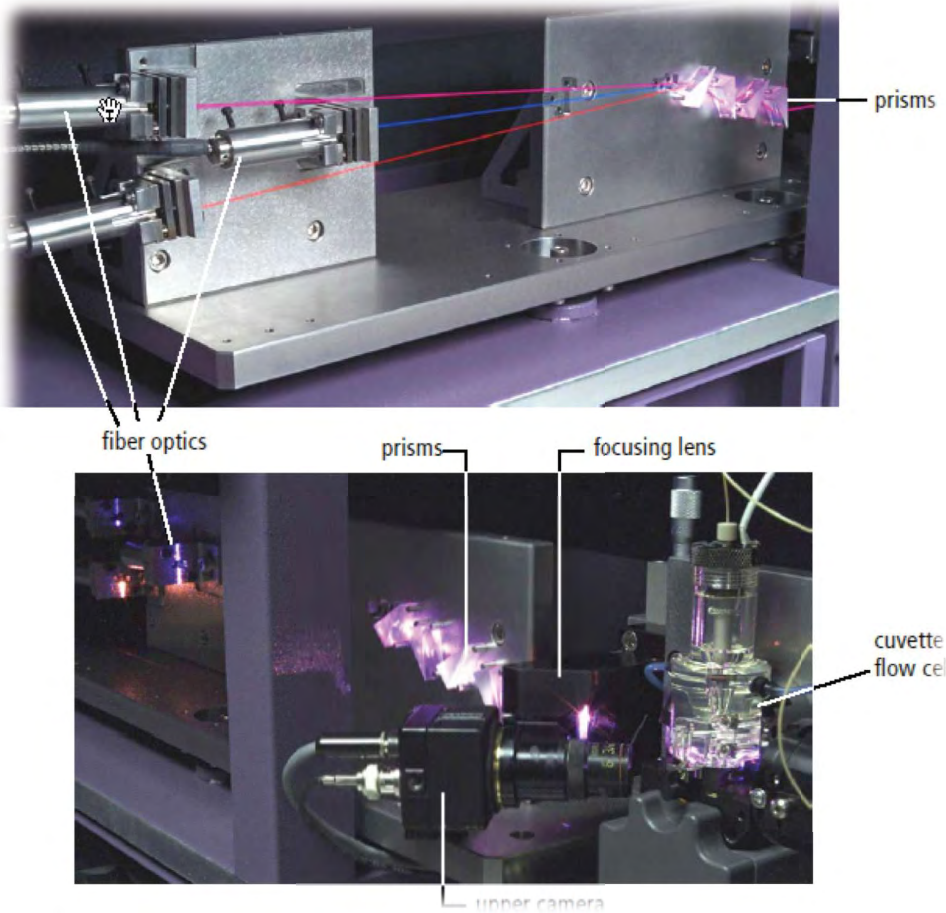
- **The light coming from a laser is**:
  - monochromatic
  - Unidirectional

- **The most used lasers:**
  - Laser UV 325 nm : UV
  - **Laser 405 nm : violet**
  - **Laser 488 nm : blue**
  - **Laser 532nm : green**
  - **Laser 561 yellow**
  - **Laser 592 nm : orange**
  - **Laser 635nm : red**

- C e



A prism and lens system channels the laser to the analysis chamber



Flow cytometry

Principle : reception optical

The different optical signals emitted by the cell must be

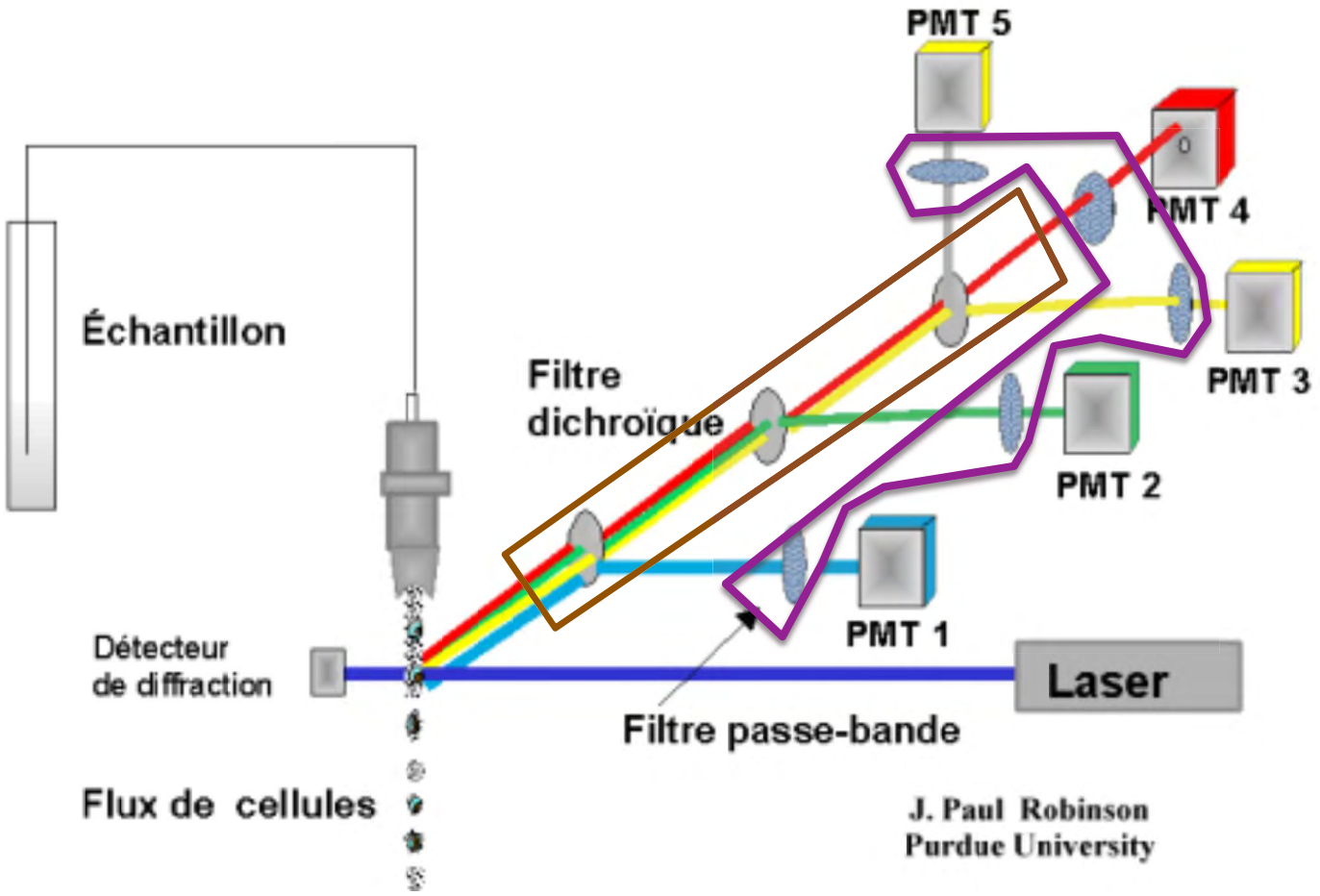
- focused
- separated
- routed to detection systems:
  photomultipliers or
  photodiodeses

For this: selection by different **optical circuits**
composed of **mirrors and filters**

Flow cytometry

Principle : reception optical

Composed of **dichroic mirrors** and **optical filters**

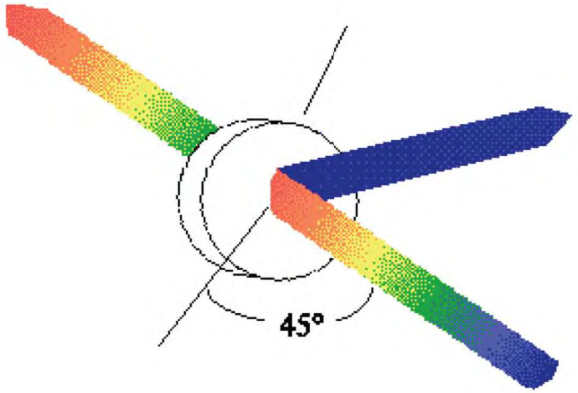


Flow cytometry

Principle : reception optical

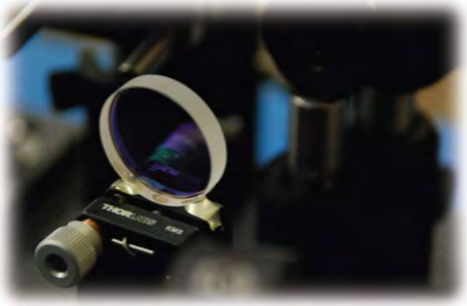
The dichroic mirror makes it possible to **direct each signal towards the appropriate detector.**

Transmitted light
In the axis of the source
(**Transmission**)



Reflected light at 90°
(**Reflexion**)

LASER



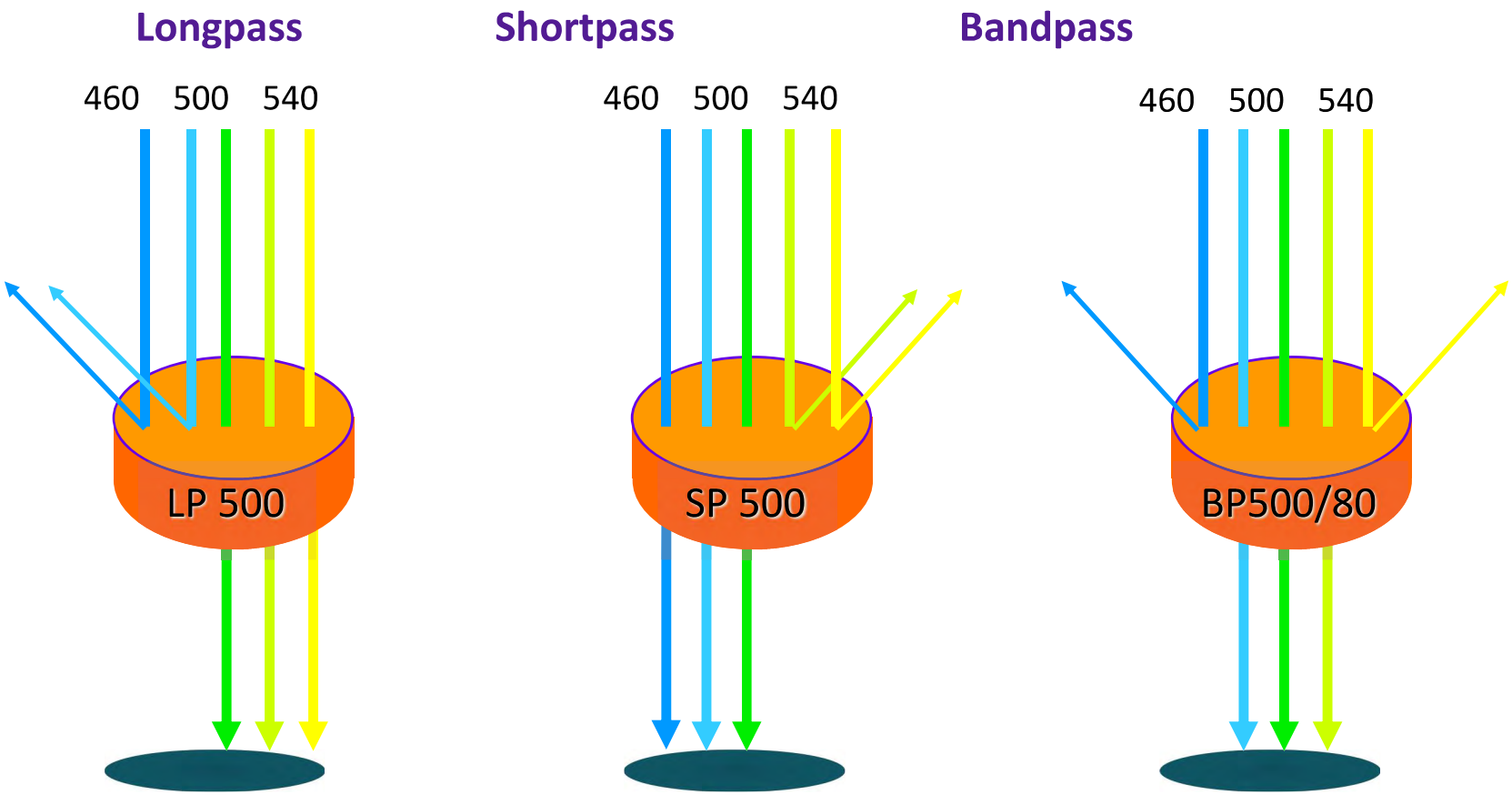
Allows the **passage** of the signal in one direction : **Transmission**
Allows **sending** the signal in oter direction : **Reflexion**

Flow cytometry

Principle : reception optical

Optical filters: compounds absorbing certain wavelengths and pass on the others

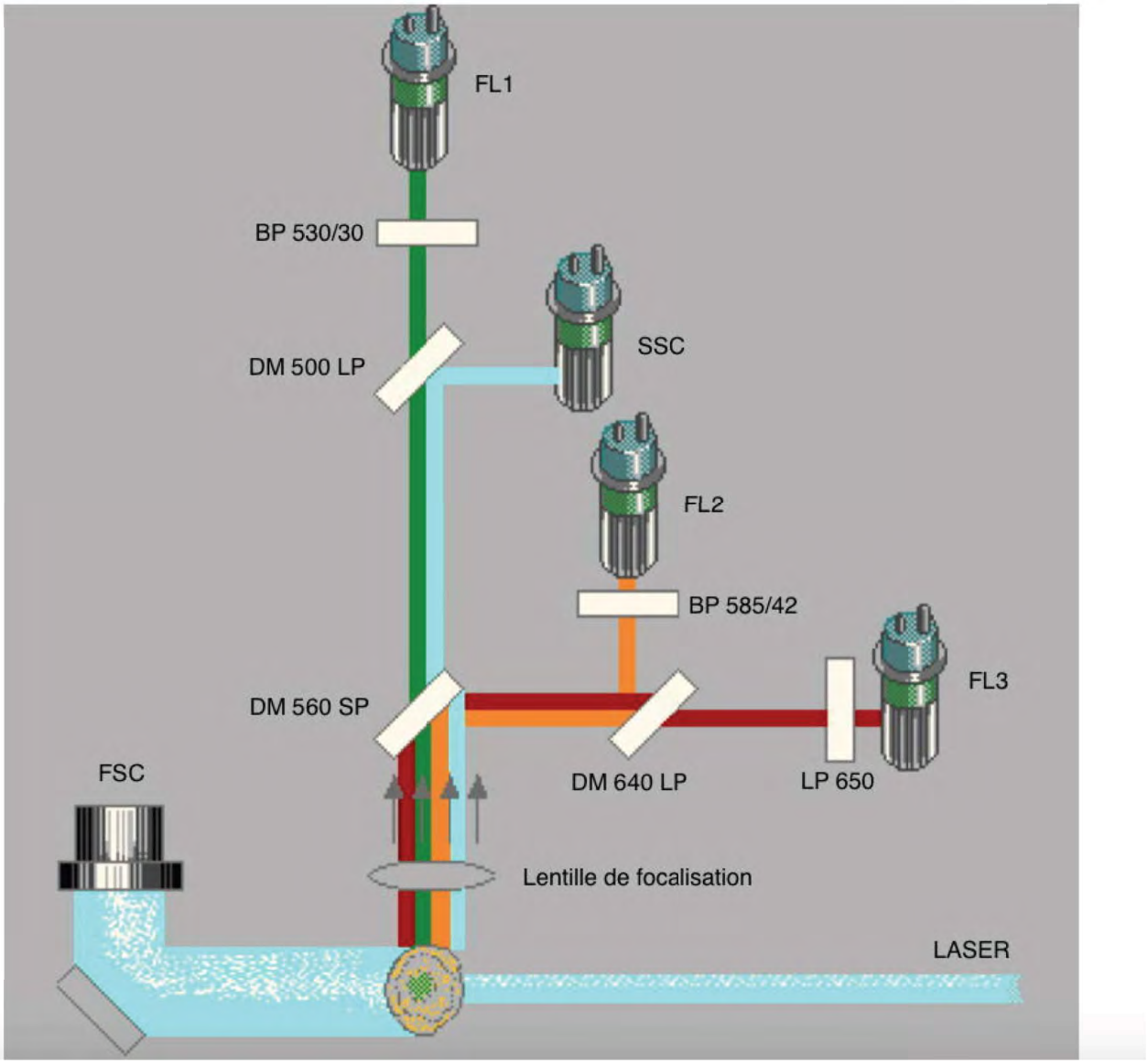
Passage according to three modalities:



Flow cytometry

Principle : reception optical

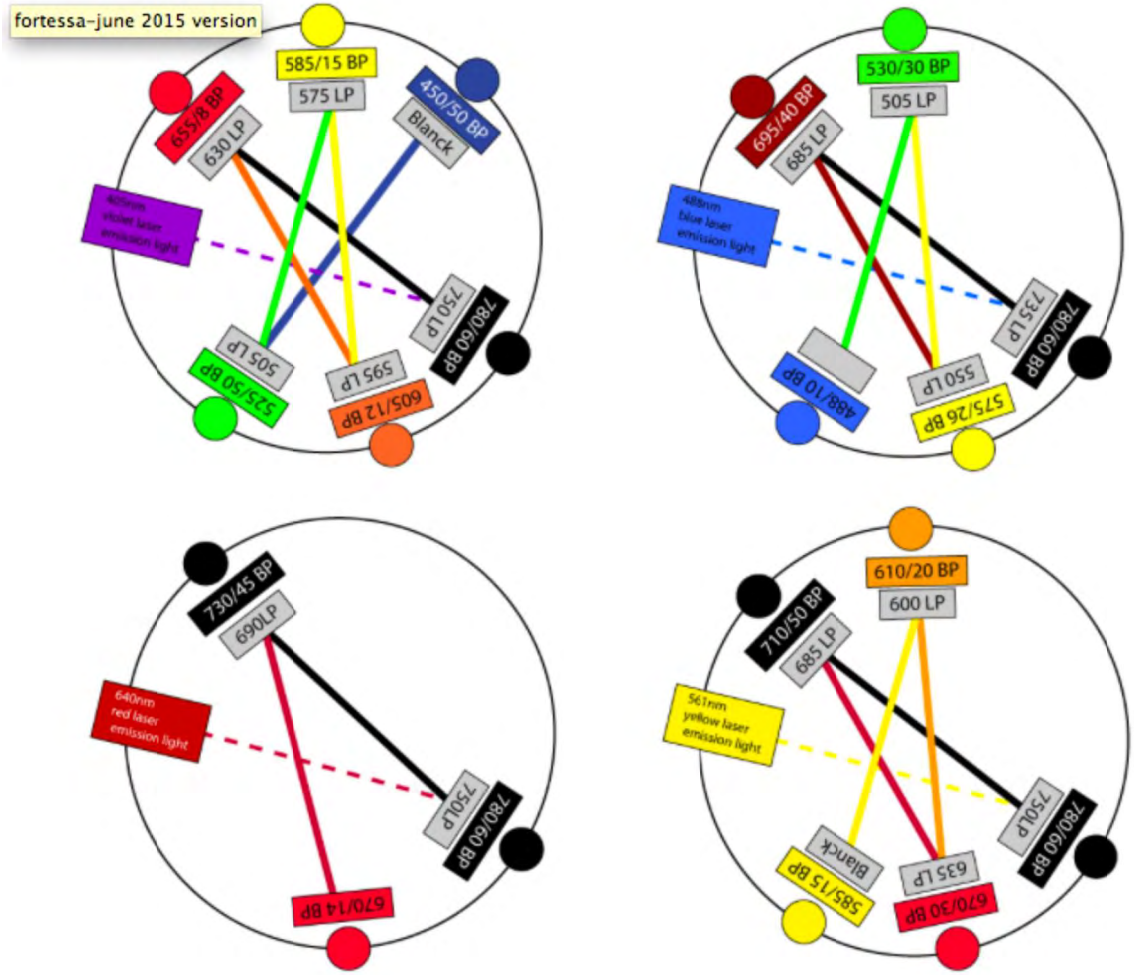
Example:
an optical path
simple



Flow cytometry

Principle : reception optical

Example:
an optical path
more complexe



Flow cytometry

Principle : reception optical

- After crossing the sucession of mirrors and filters

- The light is collected and transformated into an electricla signal by:

Photomultiplier (PMT) :

very sensitive
used for weak signals
significant gain
-> fluorescence/structure

Photodiode (PD)

lower sensitivity
For strong signals, very intense
When the saturation of the detector is a potential problem (no gain)
-> The size

Flow cytometry

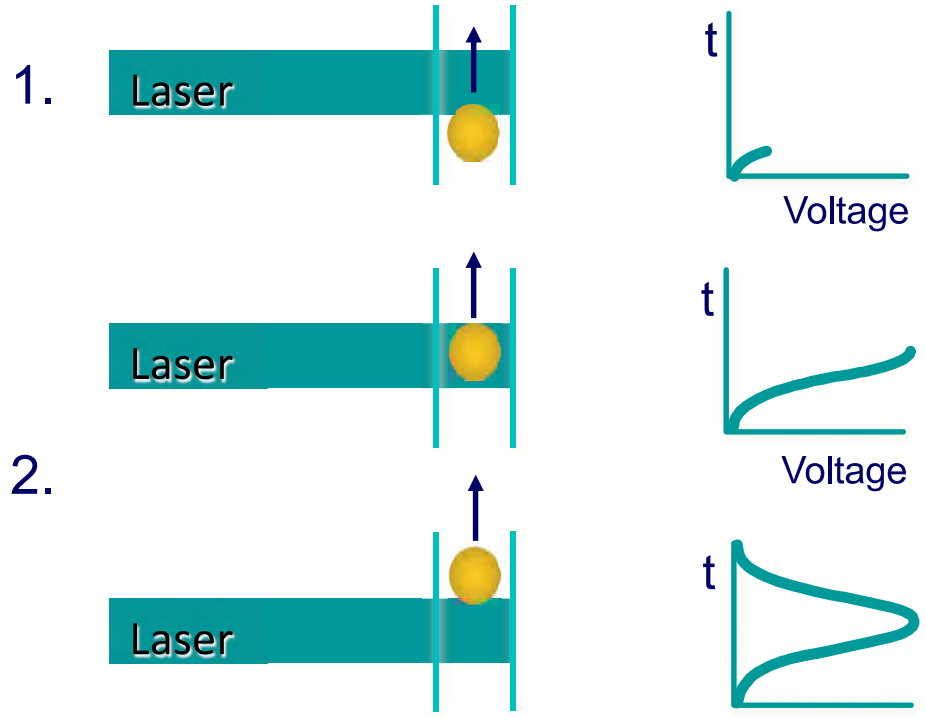
Principle : electronic

GOAL:

Transform the optical light signals (photons) into electrical signals to be digitized.

It is the **PMT (photomultiplier**) or photodiodes **that transform a photon into an electric current**

Creating an impulse



Flow cytometry

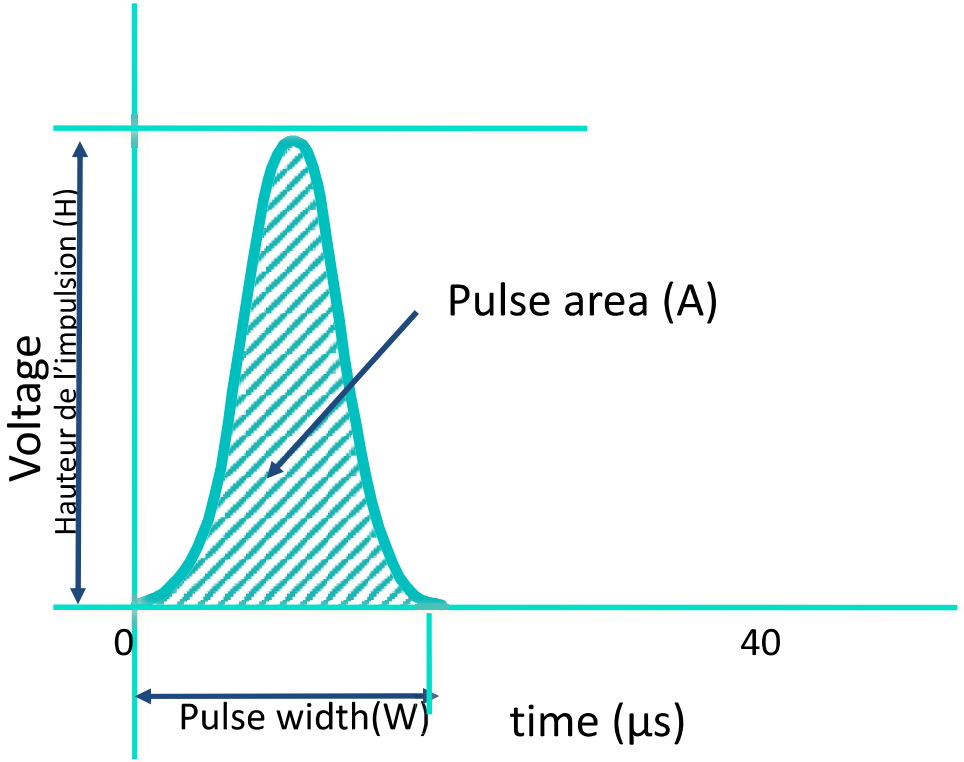
Principle : electronic

The impulse is characterized by:

1 / height (pulse height)
measures the intensity of the light scattered by the particle

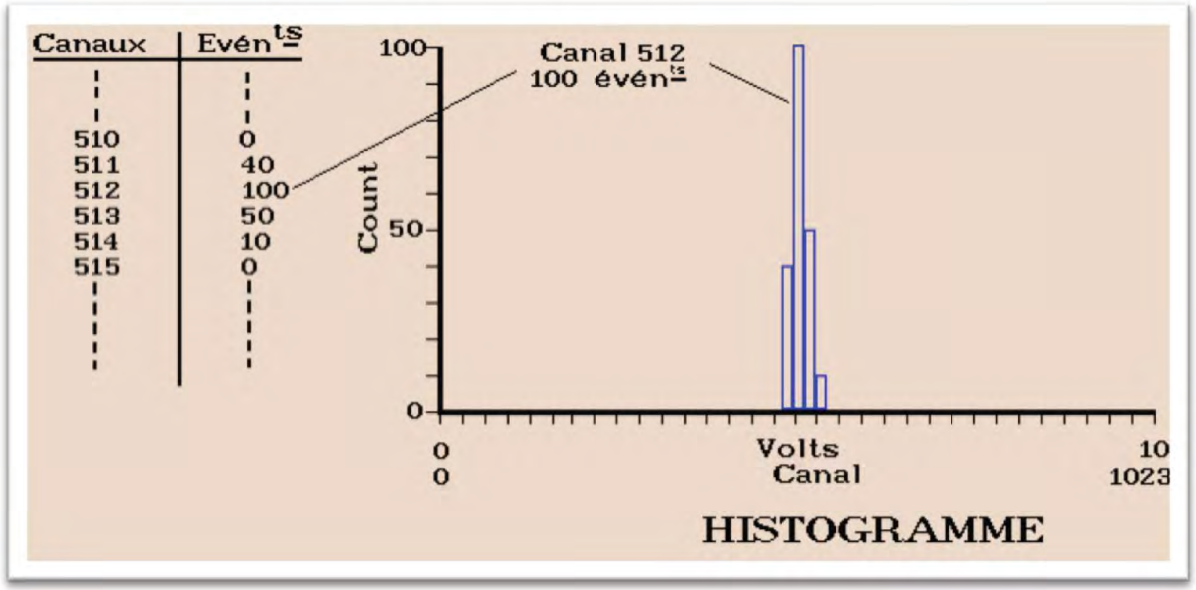
2 / The area (pulse area)
provides information on the overall fluorescence of the particle

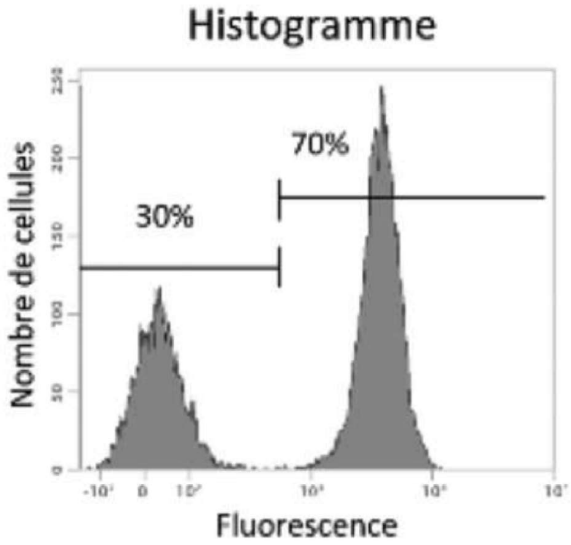
3 / The duration of the pulse (Pulse width)



Flow cytometry

Principle : electronic





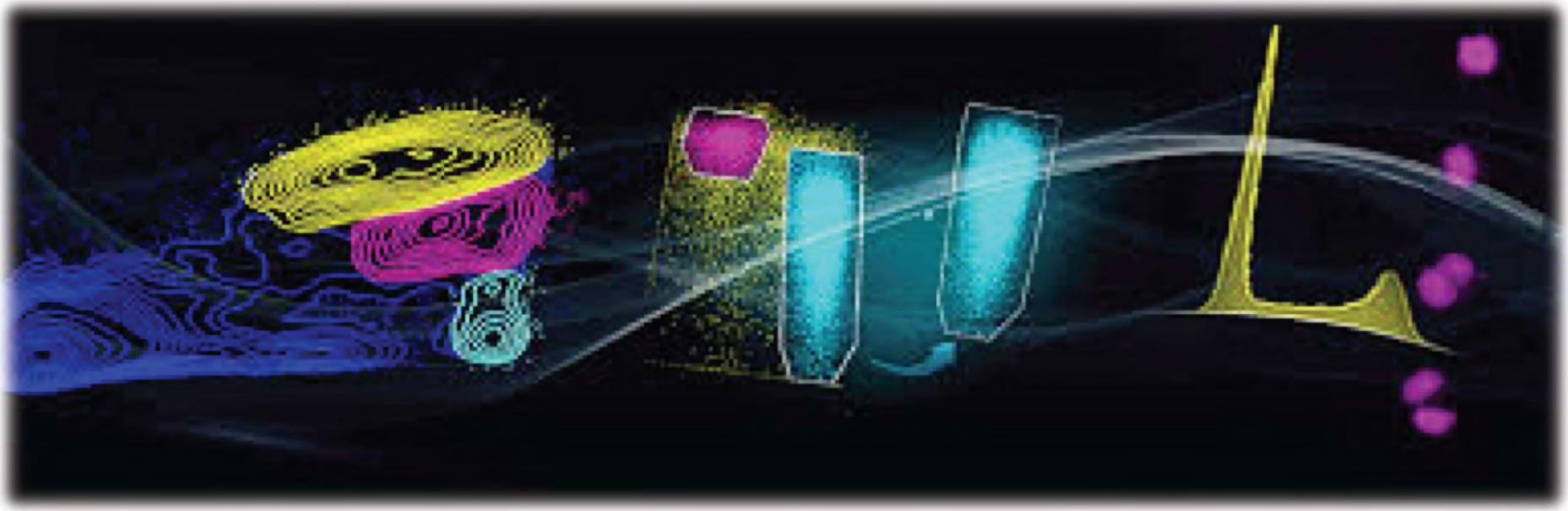
Each numerical value corresponds to a channel whose counter is incremented by the number of events

The plot of the number of events per channel is called **Monoparametric Histogram**

Flow cytometry

Principle : electronic

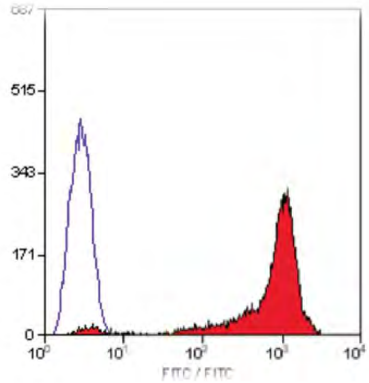
Présentation of results

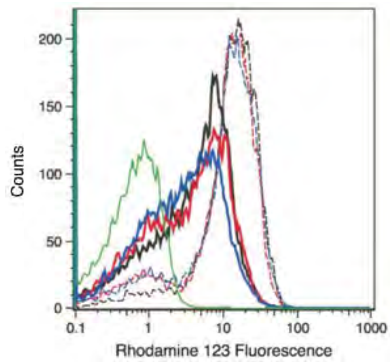


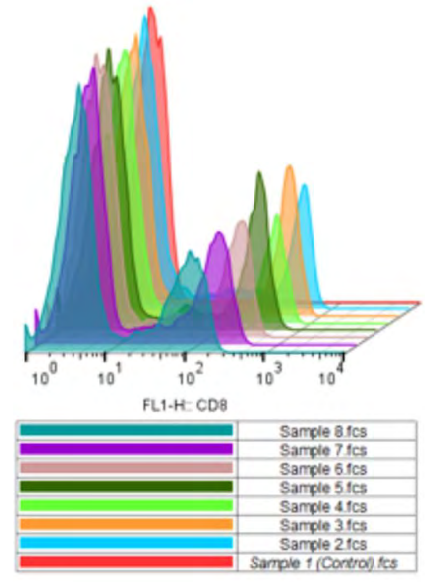
Flow cytometry

Principle : electronic

The simplest: **histogram** :



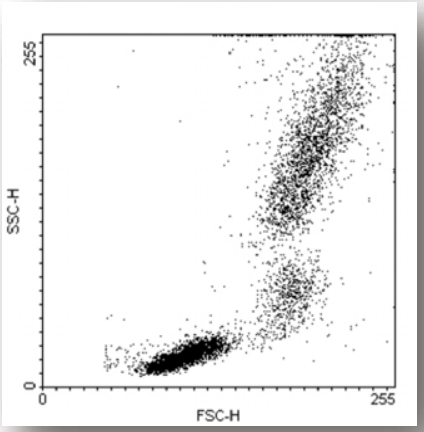


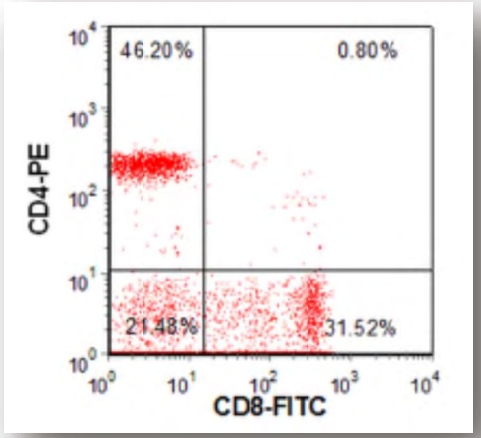


The relative fluorescence with respect to the number of events: on one parameter

But to appreciate the characteristics relative to the other parameters: dot plot

the **dot plot** :





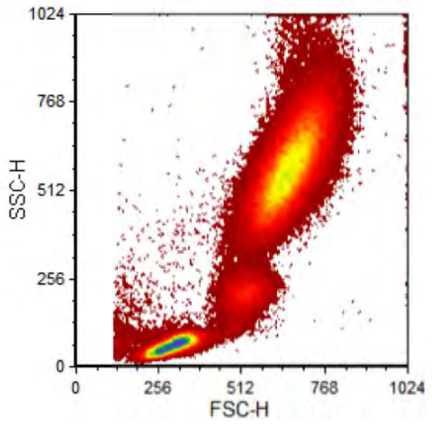
Good way to detect a small number of events whose pop are well separated

No information on the relative density of events
Density plot

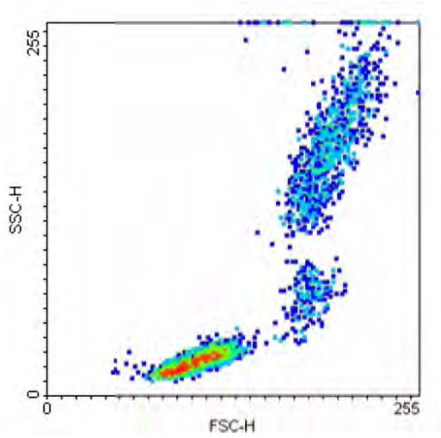
Flow cytometry

Principle : electronic

Density plot

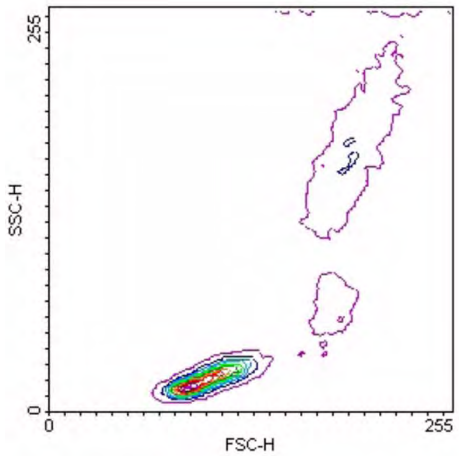


Pseudo colors plot



Simulates a 3D representation where the 3rd parameter is the number of evts
Lets highlight a discrete pop

Contour plot

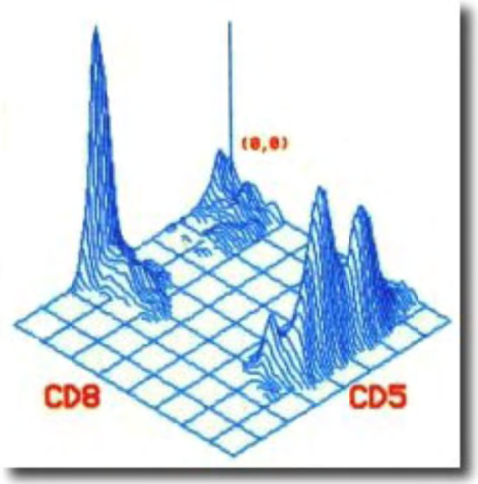


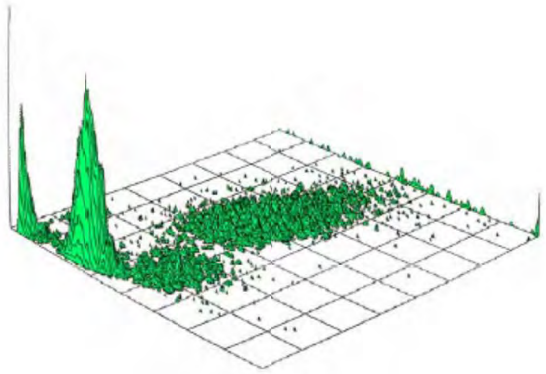
Another 2D representation of pop whose number of evts is similar.
Populations present in low percentage may not appear

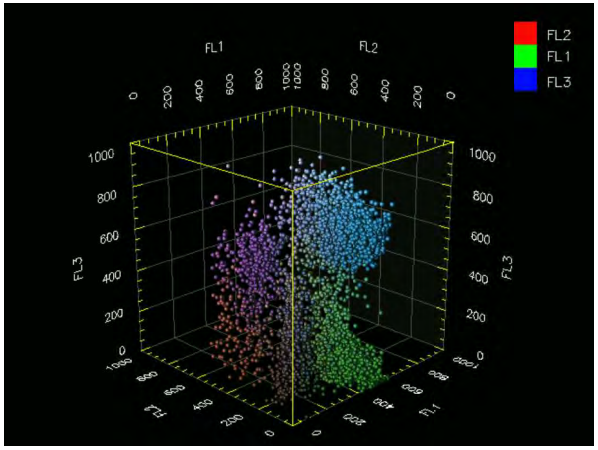
Flow cytometry

Principle : electronic

Three-dimensional histogram :

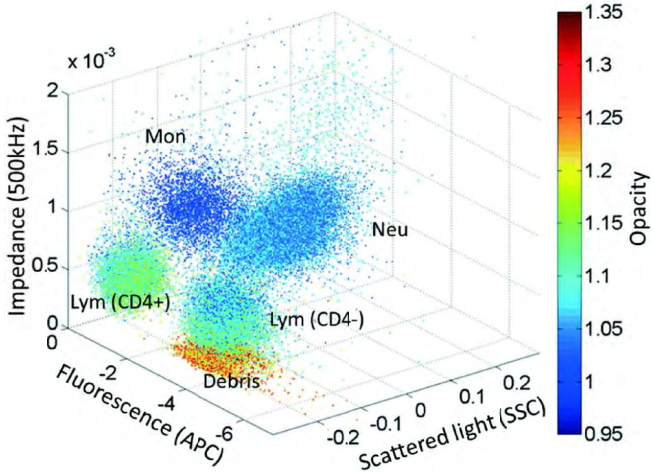






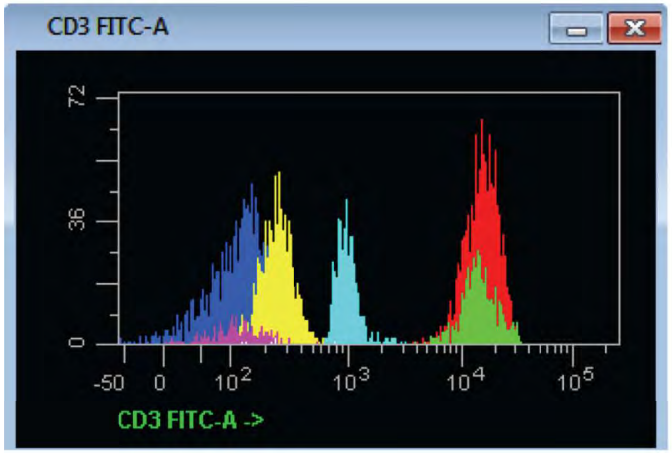
-This triparametric histo is obtained from a biparametric with a third dimension = number of cells

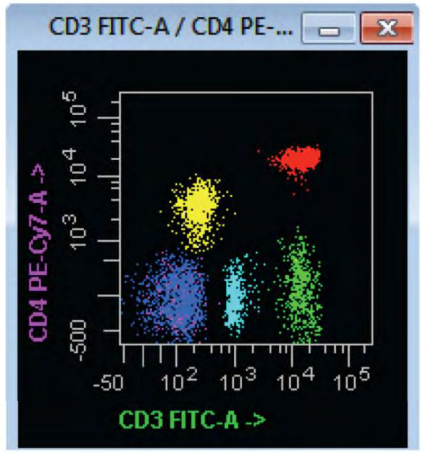
-- Allows to get an idea of the proportion of different categories of cells from one to the other

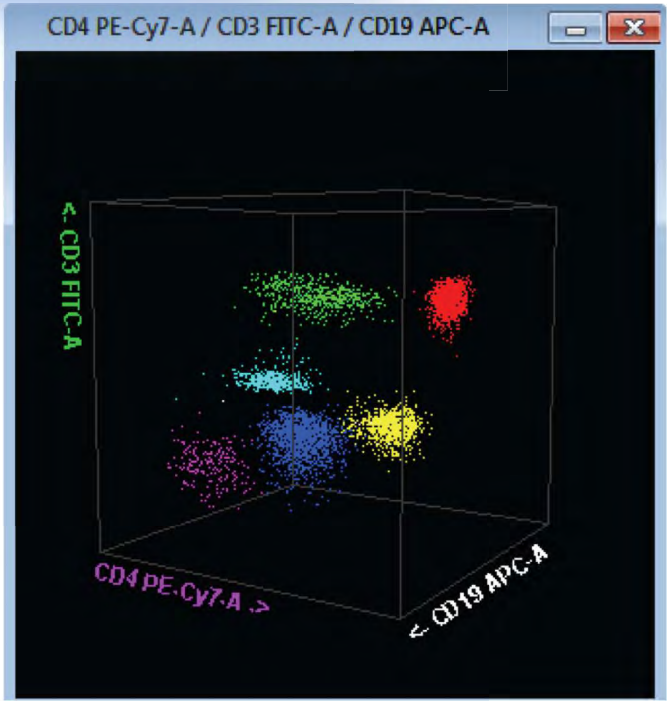


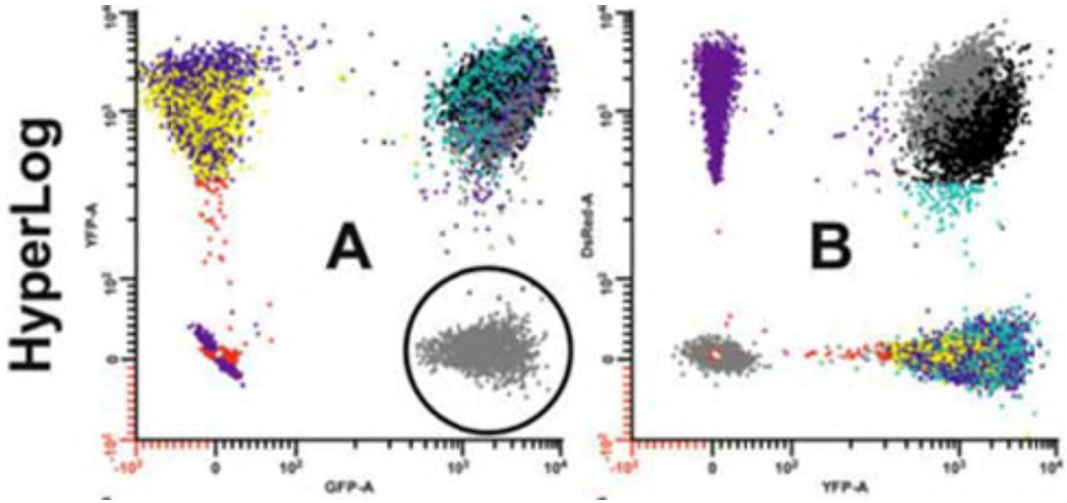
Flow cytometry

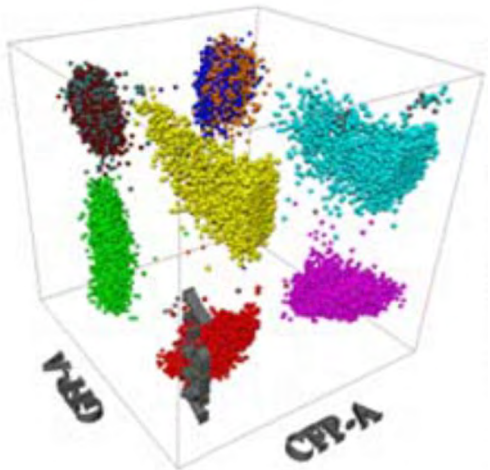
Principle : electronic





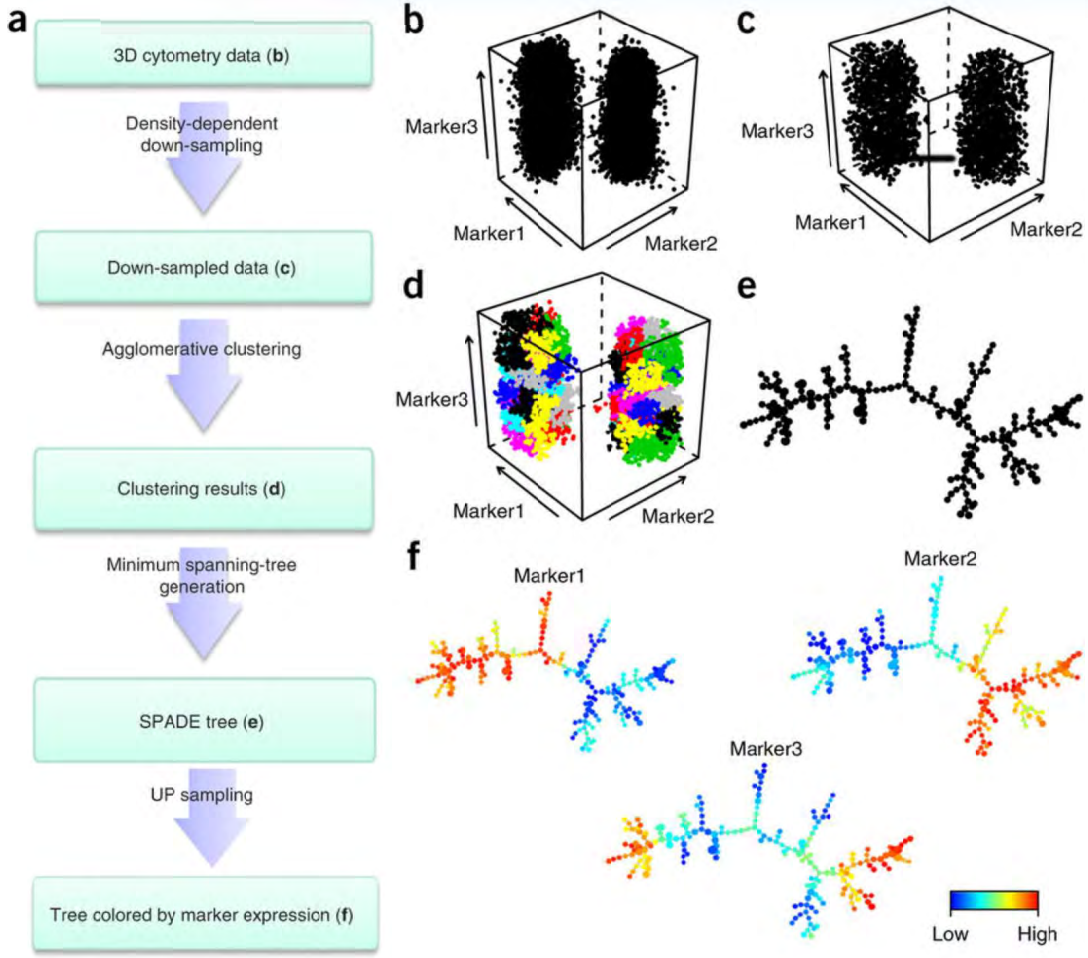


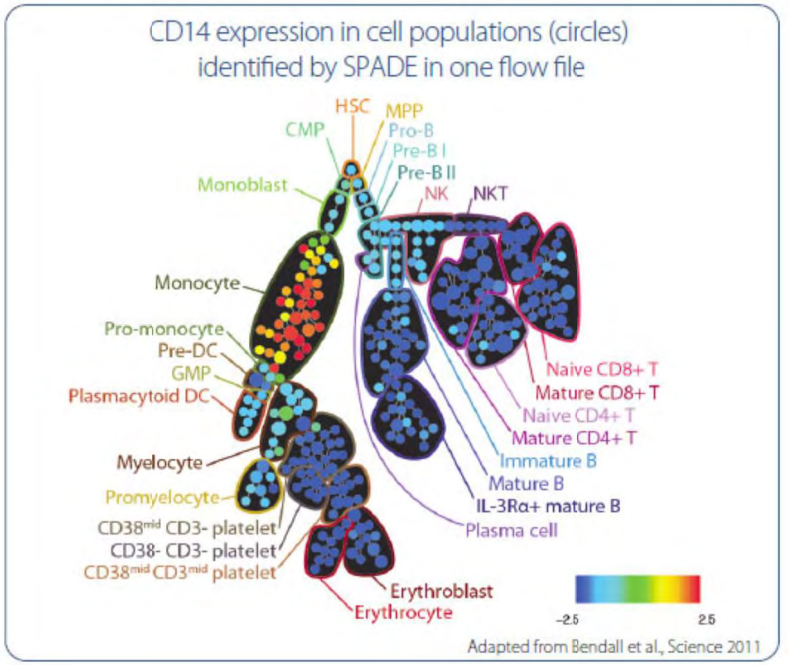




Flow cytometry

Principle : electronic





Flow cytometry

- History & definition
- Principles
- Applications
- Other

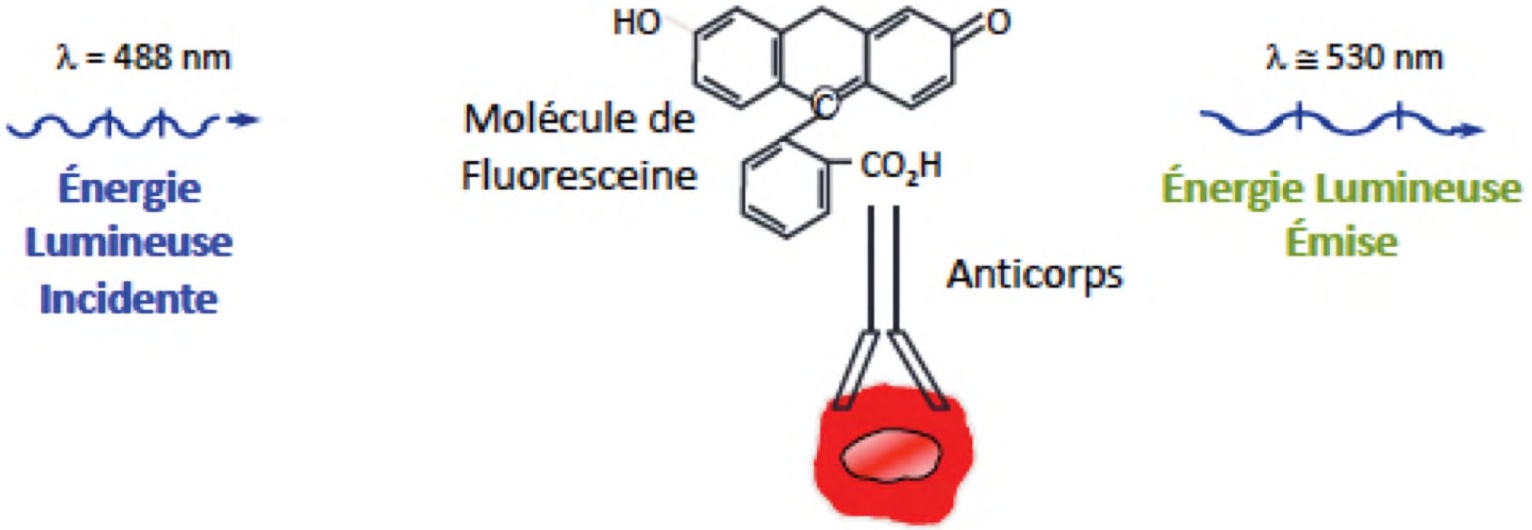
Flow cytometry

Applications : Fluorochromes

Definition of fluorochrome:

- Molecule capable of emitting fluorescence light after excitation

- The emission wavelength being greater than the emission wavelength



Flow cytometry

Applications : Fluorochromes

Un peu d'histoire…

Fin du 19ème siècle: methyl violet, malachite green, safranin O, methylene blue…
Base pour le développement des futures sondes fluorescentes comme la fluorescéine, la rhodamine ou l'acridine orange.

Début des années 1920: Développement de la microscopie de fluorescence: premiers marquages vitaux pour bactérie, protozoaires

Début des années 1940: Développement (par Albert Coons) d'une technique pour marquer les anticorps avec des sondes fluorescentes: développement des techniques d'immunofluorescence.
Développement d'un large spectre d'anticorps secondaires couplés à une large variété de fluorochromes permettant des marquages multiples.

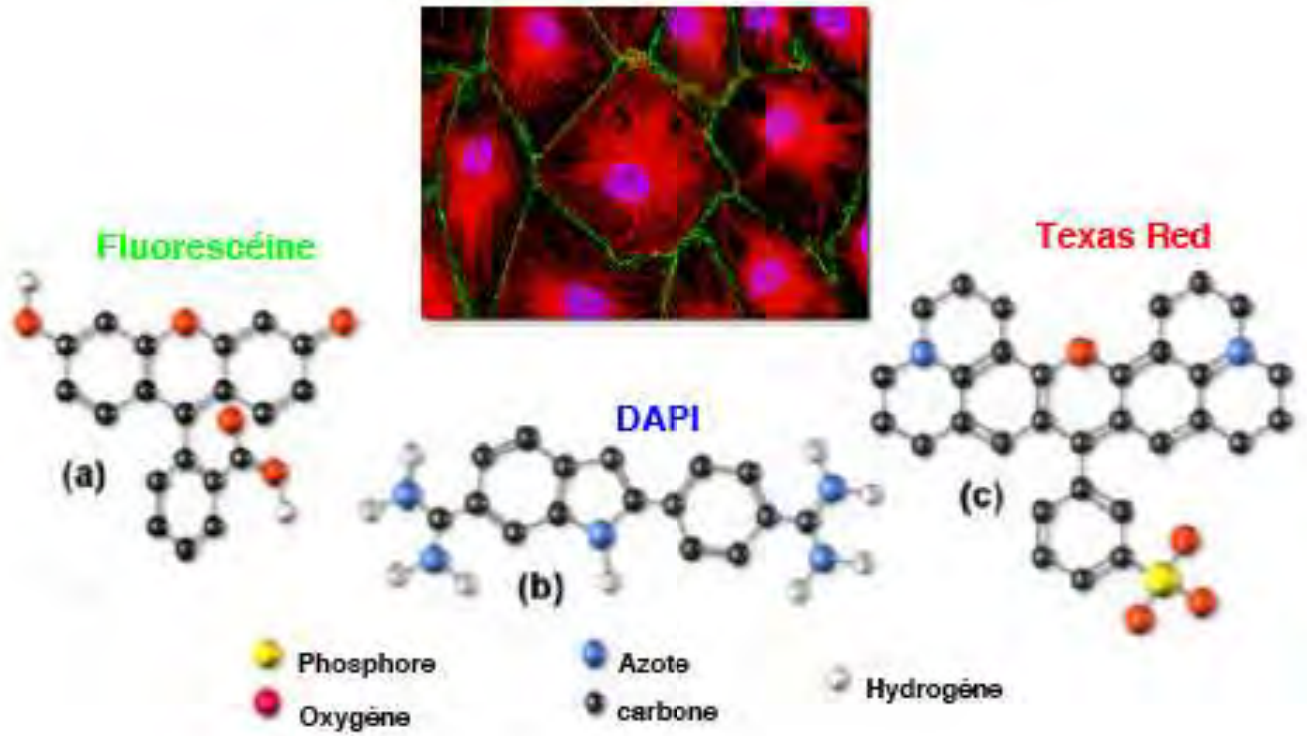
1992: Clonage du gène codant pour la GFP à partir de la méduse du pacifique Aequorea victoria: développement des techniques de production de protéines de fusion.
Développement de nombreux variants spectraux de la GFP et découverte d'autres protéines fluorescentes

Plus récemment: Développement des nano-particules fluorescentes semi-conductrices: quantum dots permettant le suivi d'objet individuel.

Flow cytometry

Applications : Fluorochromes

Chemicals composed of several conjugated aromatic rings
or even planar and cyclic molecules having one or more bonds
Are exited by a specific wavelength and emit at a specific wavelength.
Are exited by a specific wavelength and emit at a specific wavelength.

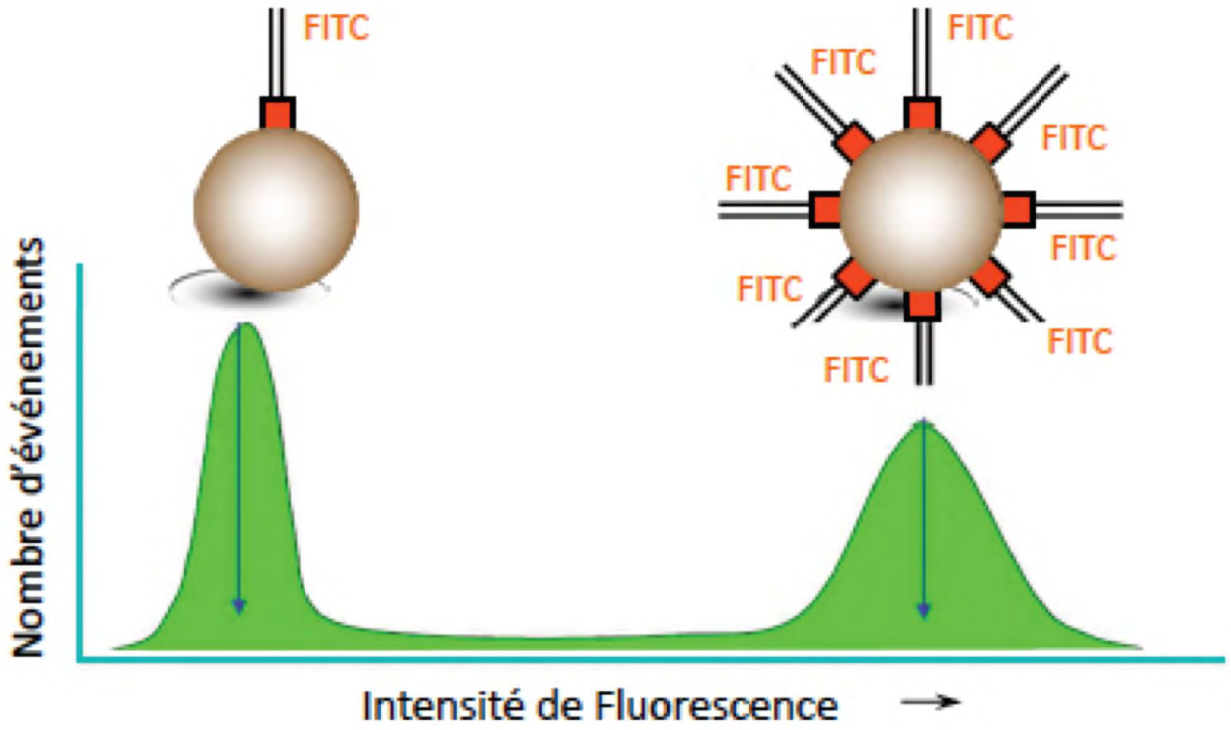


Flow cytometry

Applications : Fluorochromes

Intensité de fluorescence émise $\propto$ Nombre de sites de fixation

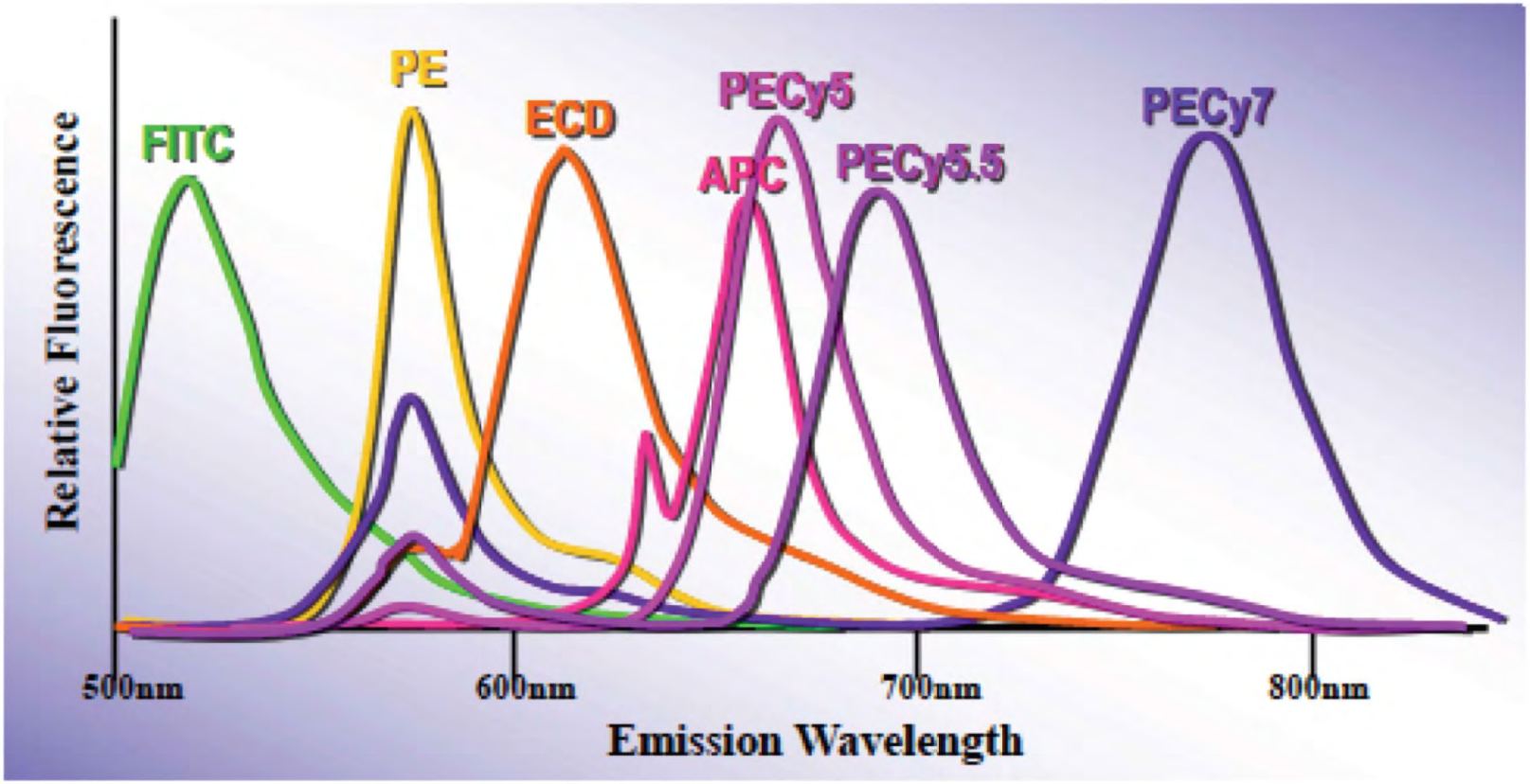
(QB)



Flow cytometry

Applications : Fluorochromes

Each fluorochrome is characterized by an excitation spectrum and a fluorescence emission spectrum



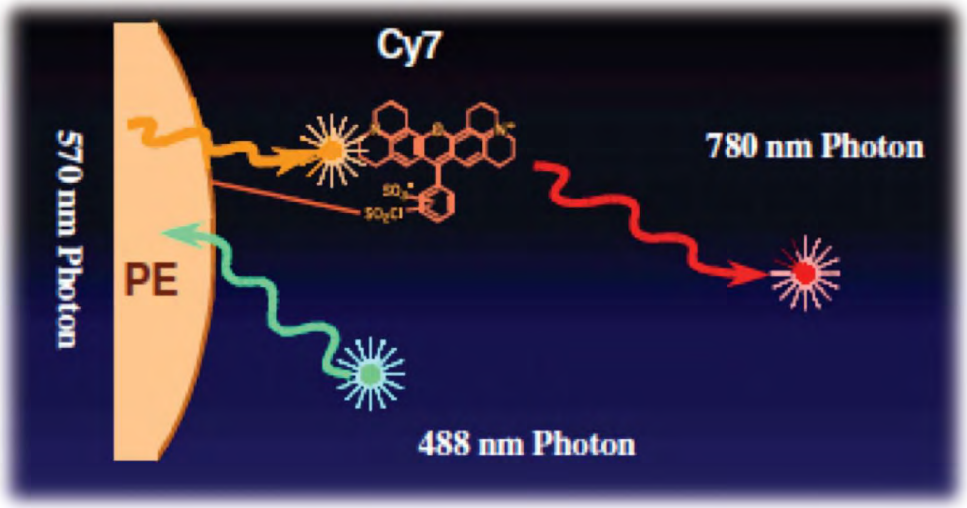
Flow cytometry

Applications : Fluorochromes : tandem

PerCP-Cy5,5, PE-Cy5, APC-H7, PE-Cy7……..

What is it?

- 2 fluorescent molecules covalently attached
- One serves as a donor and the other as an acceptor (FRET Fluorescence Resonance Energy Transfer process)
- Property of excitement of the donor and emission of the receiver
- High Stokes Displacement: facilitates multi-color marking



Flow cytometry

Applications : Fluorochromes ; tandem

historically

- End of the year 1980 with PE as the donor: PE-cy5 or PE-cy7 .....
- In the 1990s use of APC or PerCP: APC-CY7 or PerCP-CY5.5
- Much more recently, new tandem class like those using Brilliant Violet as a donor (advantage they are less fragile)

Flow cytometry

Applications : Fluorochromes ; tandem

Limits and preservation of performance :

- There is always a residual fluorescence of the donor which will depend on the quality of th tandem

- The effectiveness of FRET can be different from one batch to another of the same tandem

- Degradation or decoupling
  light
  Cell-specific metabolic activity (APC-X)
  PFA (PE-X, APC-X ....)

Flow cytometry

Applications : Fluorochromes ; tandem

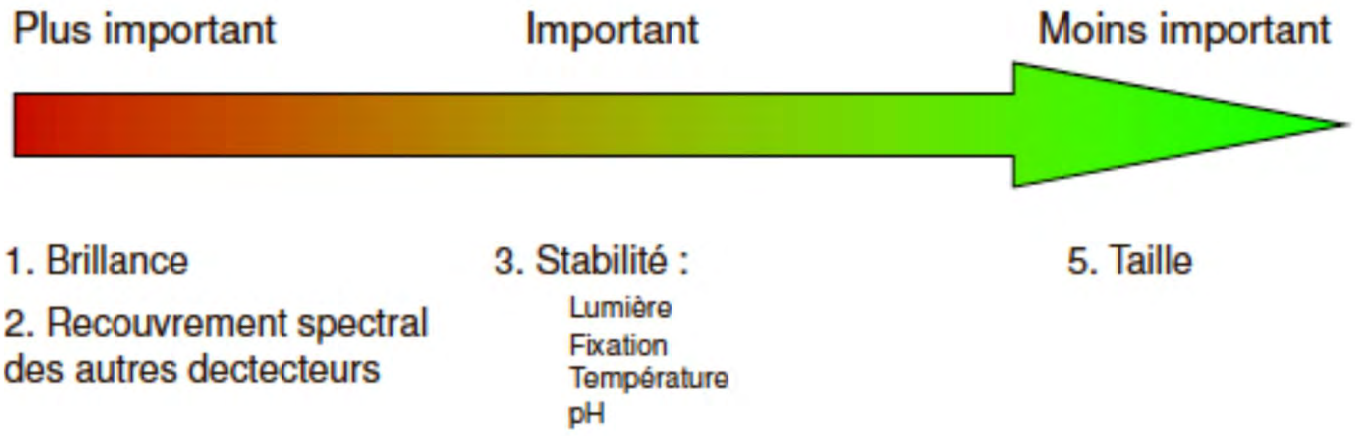
Good practices for using tandems :

- Keep the vials of antibodies and markings at 4 ° C
- Keep flasks and markings away from light
- Fixation max 30 minutes followed by a wash
- Calculate compensations with the same Ac as used in the final marking
- Check compensation after batch change

Flow cytometry

Applications: Fluorochromes choice

To take into account for a label :



Flow cytometry

Applications: Fluorochromes choice

Know the question or questions that arise

Know your material well:

Facs: laser available, possibility
Cells, tissues
Fluorochromes available

They do not all have the same performance

Antigen is not detected in the same way
with the same Antibody if it is coupled to different fluorochromes

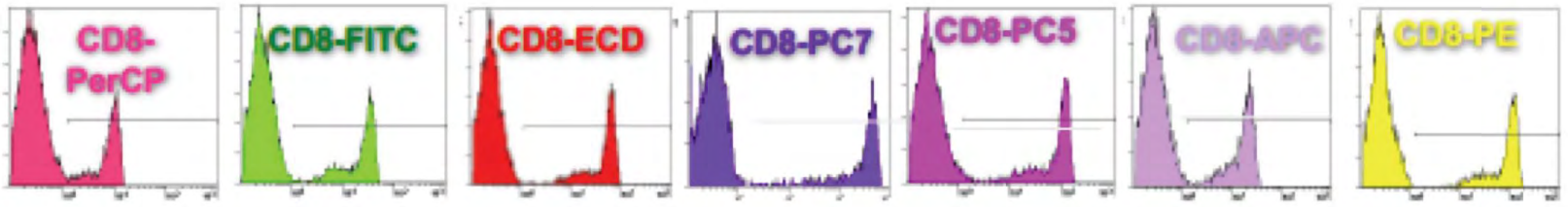
Flow cytometry

Applications: Fluorochromes choice

Rendement quantique (ψ)

$$\frac{\text{Nombre de photons émis}}{\text{Nombre de photons absorbés}} \text{ par molécule}$$

PerCP < FITC < ECD < PC7 < PC5 < APC < PE



Proportionnel à la brillance

Flow cytometry

Applications: Fluorochromes choice

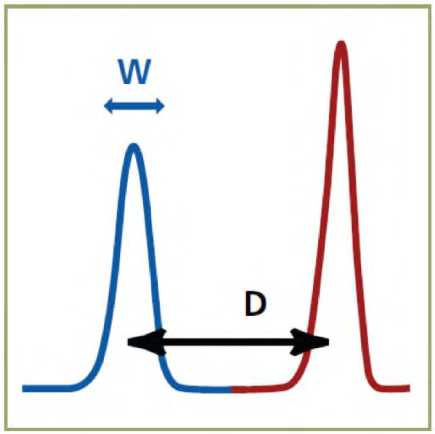
high

Which is the most Fluorescent?

low

Brightness of various fluorochrome conjugates

Relative Brightness	Reagent	Filter
BRIGHTEST	Brilliant Violet™ 421	450/50
	PE	575/26
	Brilliant Violet 605	610/20
	BD Horizon PE-CF594	610/20
	PE-Cy5	670/14
	APC	660/20
BRIGHT	PE-Cy7	780/60
	Alexa Fluor® 647	660/20
	PerCP-Cy5.5	695/40
MODERATE	Alexa Fluor® 488	530/30
	FITC	530/30
	BD Horizon V450	450/50
	Pacific Blue™	450/50
DIM	Alexa Fluor® 700	730/45
	PerCP	695/40
	APC-Cy7	780/60
	AmCyan	525/20
	BD Horizon V500	525/20
	BD APC-H7	780/60



Flow cytometry

Applications: Fluorochromes choice

Choice depending on what you want to label

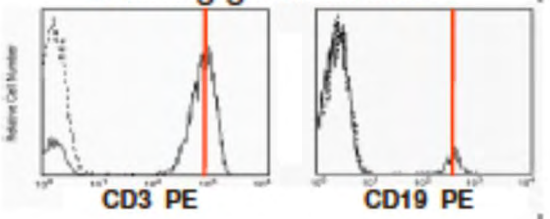
Niveau d'expression ?

Use literature-data about # of antigen-molecules per cell

Antigen	Antigen-Density	Expression Level
CD3	90.000	++
CD4	100.000	++
CD8	124.000	+++
CD14	110.000	++
CD19	18.000	+
CD45	200.000	+++
CD56	10.000	+
CD80	2.000	+

Antigen-expression High / Intermediate / Low:
+++ / ++ / +

Compare examples of PE-staining given in TDS

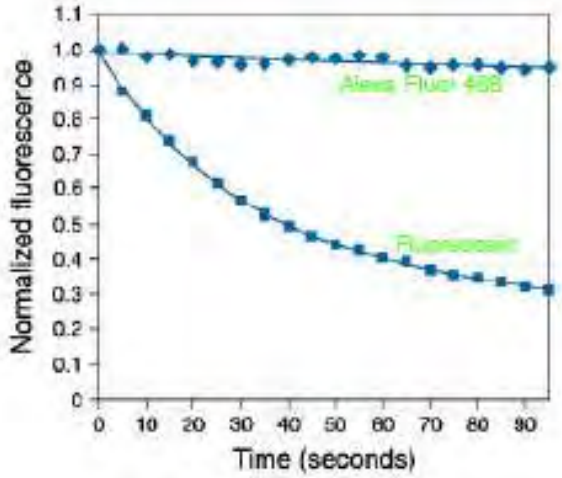


Flow cytometry

Applications: Fluorochromes choice

Watch out for the exposure of your marked samples, the fluo is not permanent

Comparaison du photoblanchiment de deux fluorochromes



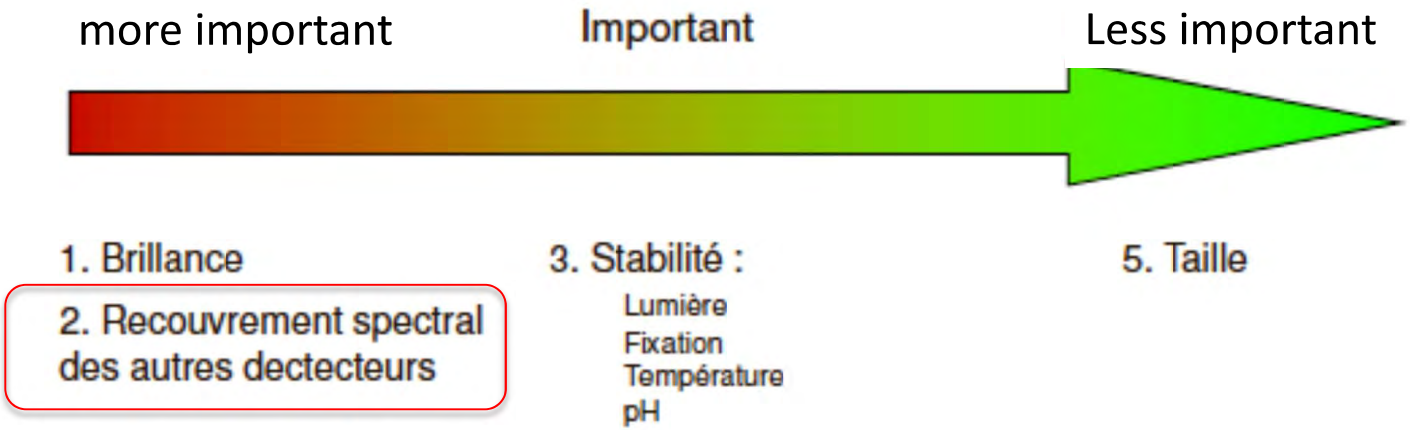
Les échantillons ont été illuminés continuellement et une image a été enregistrée toutes les 5 secondes avec une caméra CCD.

(D'après C. Poujol)

Flow cytometry

Applications: Fluorochromes choice

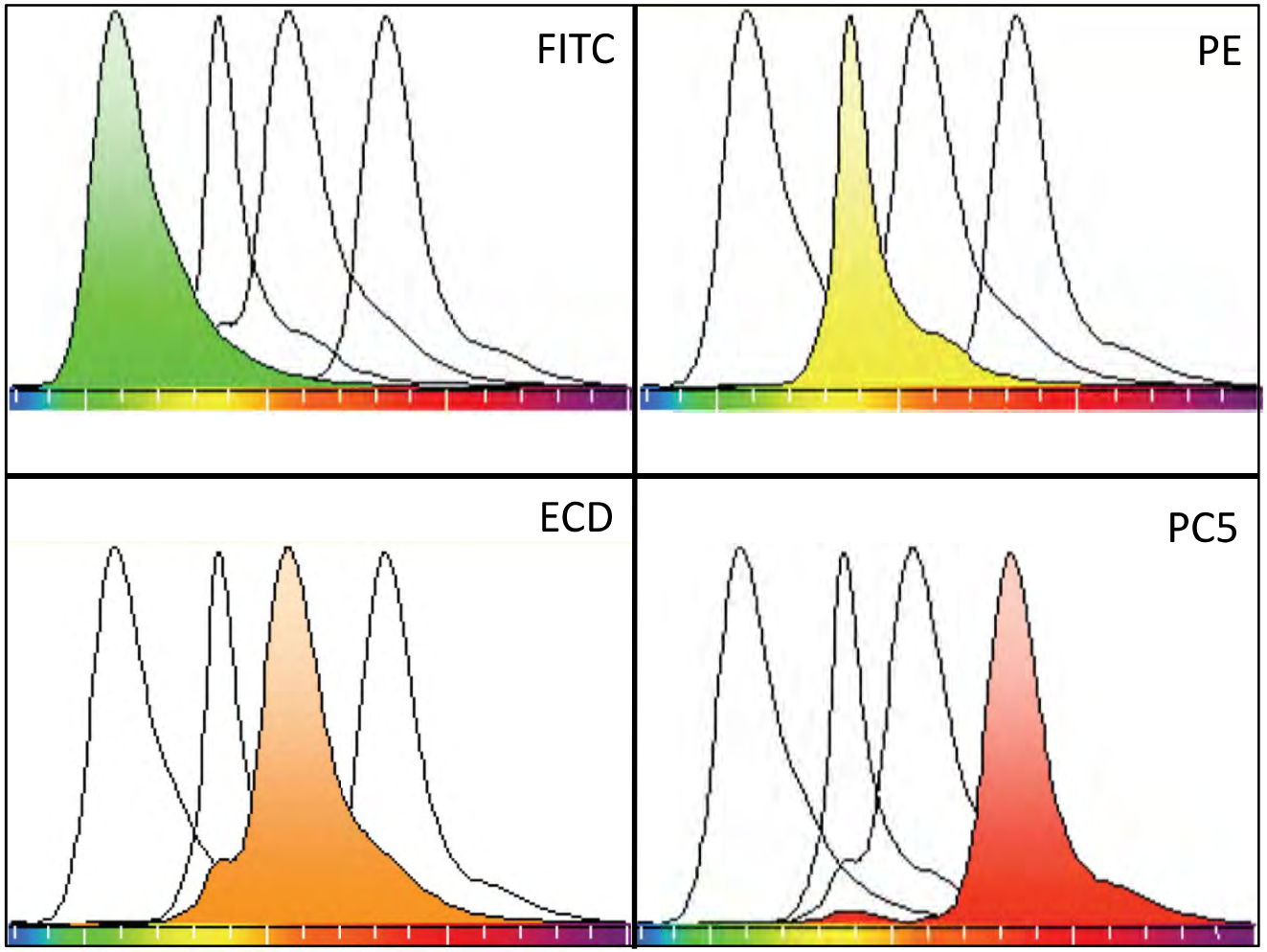
To take into account for a label:



Flow cytometry

Applications: Fluorochromes choice

Each fluorochrome has a spectrum of its own

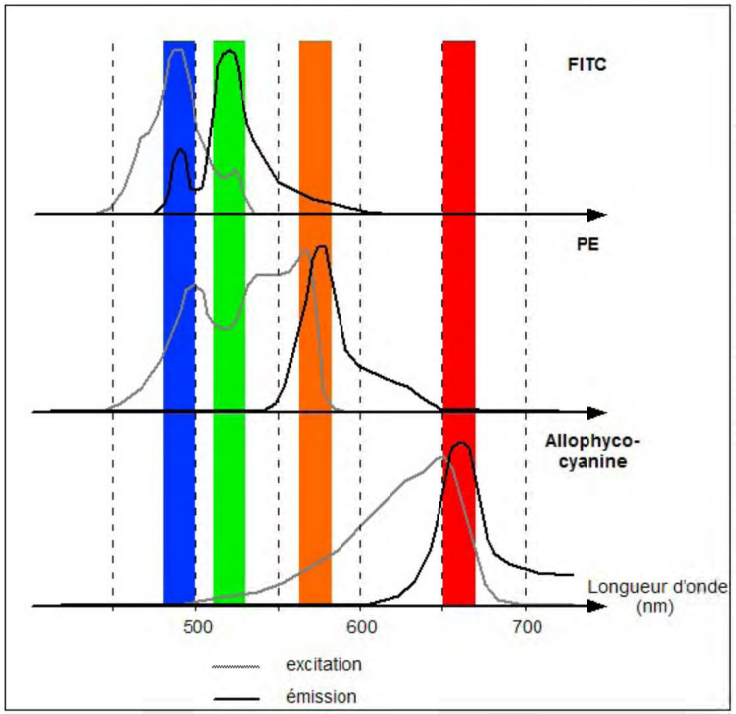


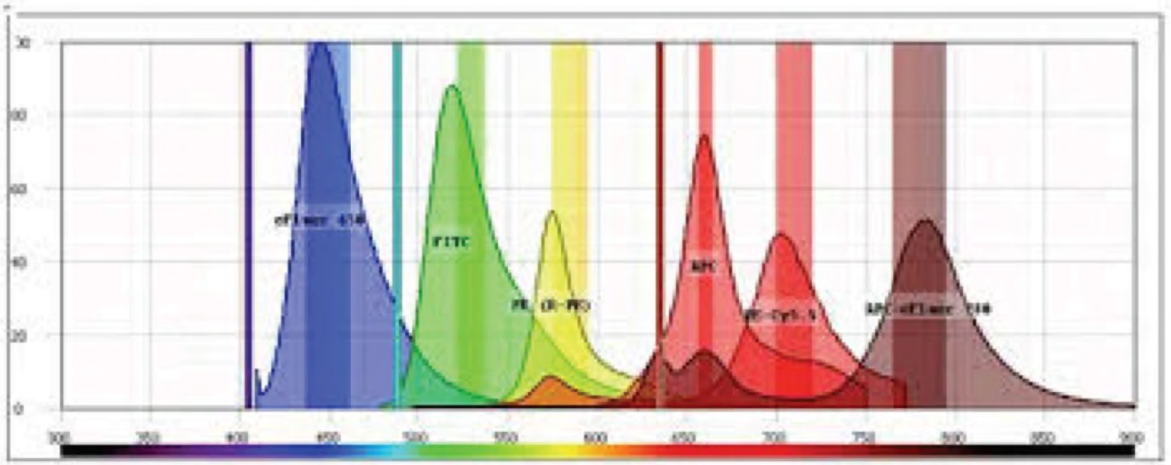
-The emission spectra are not fine lines but more or less wide spectra

Some emission spectra overlap

Flow cytometry

Applications: Fluorochromes choice



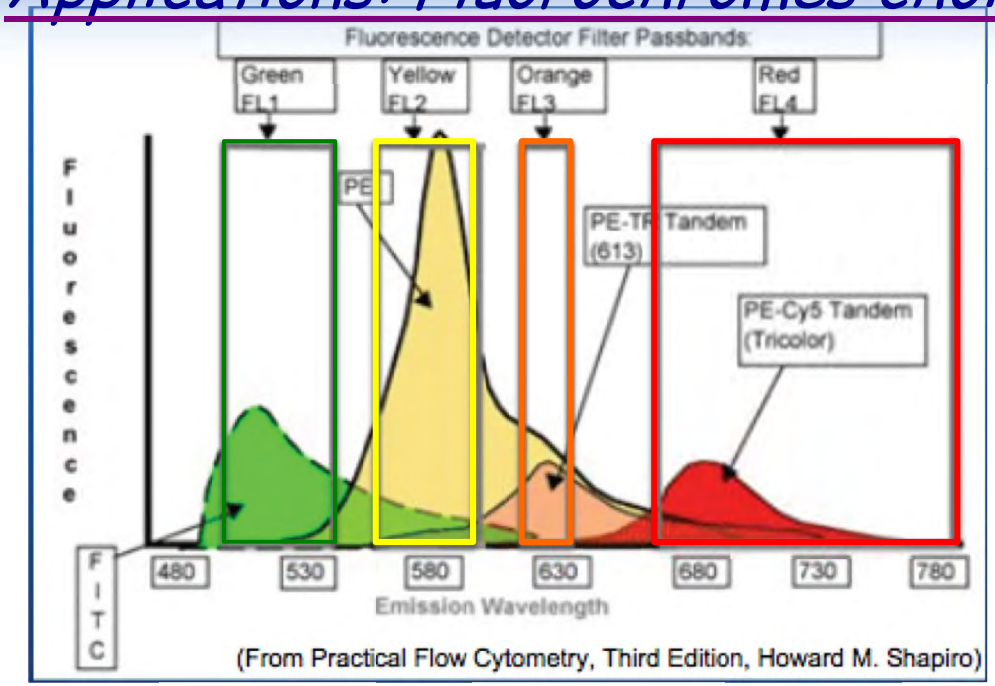


Detection of signal from 1 fluorochrome by 2 (or more) detector :

=➔ COMPENSATIONS

Flow cytometry

Applications: Fluorochromes choice



It is almost impossible to avoid a partial overlap of the emission spectra of fluorochromes

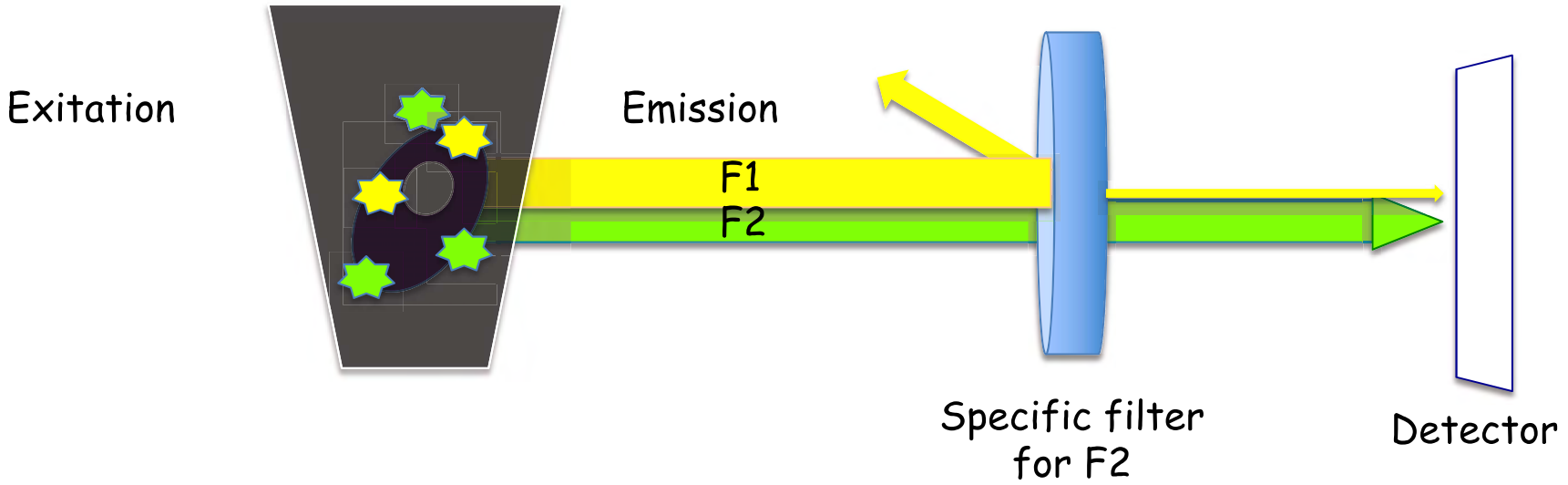
Artifactual fluorescences are then observed because of these fluorescence leaks read on the other PMTs.
Interference creation (false positive)

It is therefore essential to remove electronically a certain percentage of the parasite fluorescence: it is the compensation

Flow cytometry

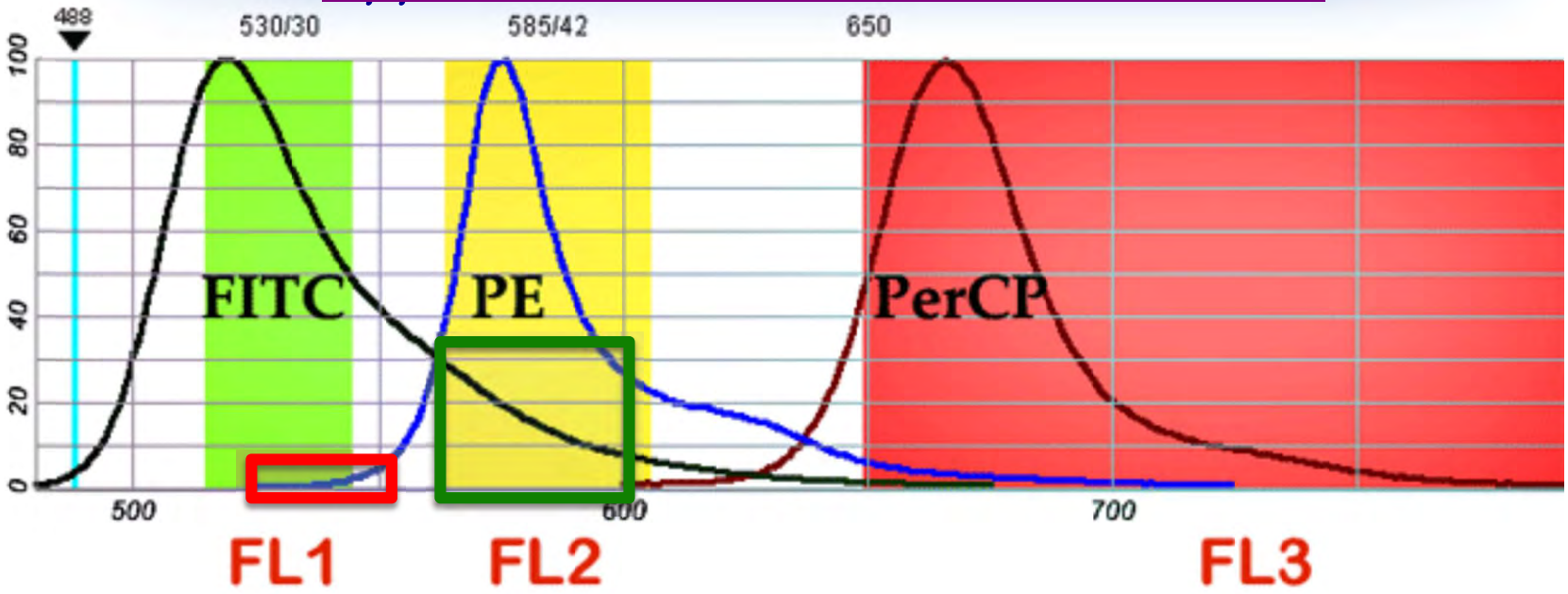
Applications: Fluorochromes choice

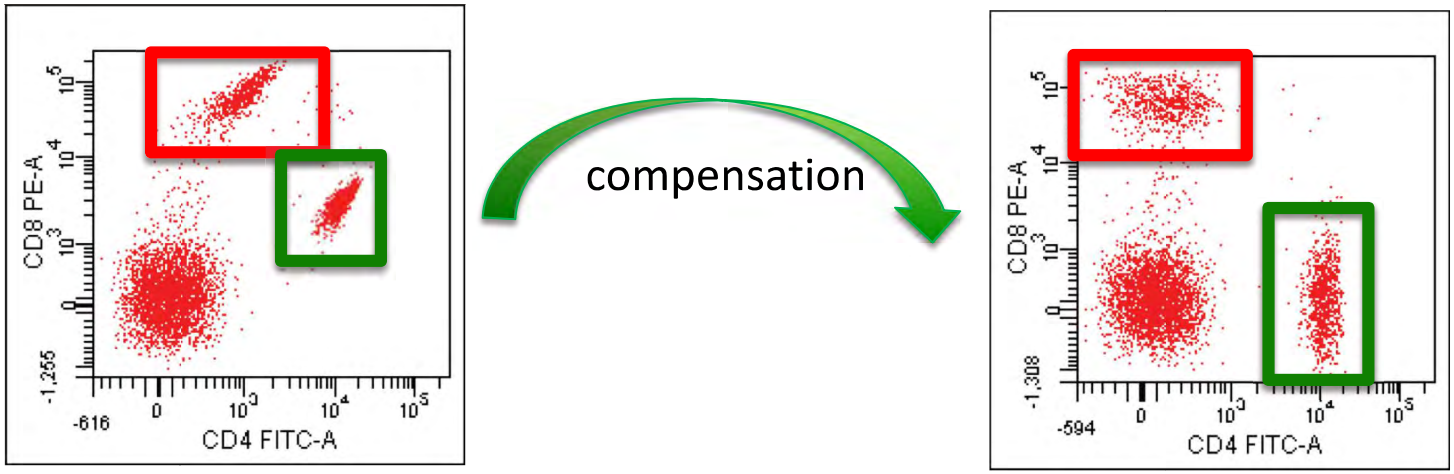
In practice:



Flow cytometry

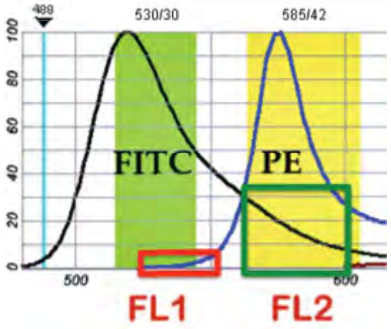
Applications: Fluorochromes choice

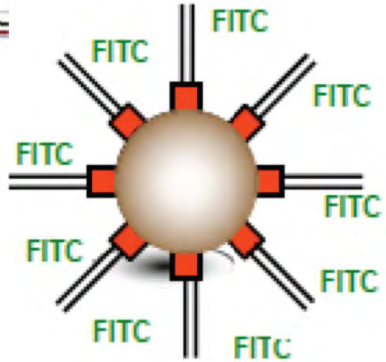


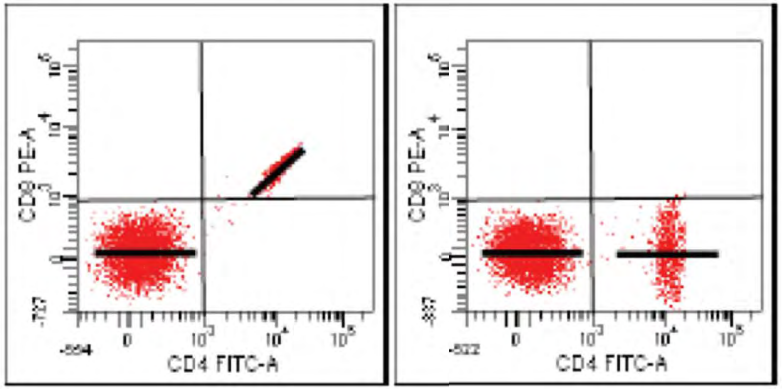


Flow cytometry

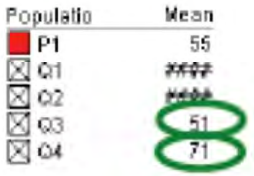
Applications: Fluorochromes choice







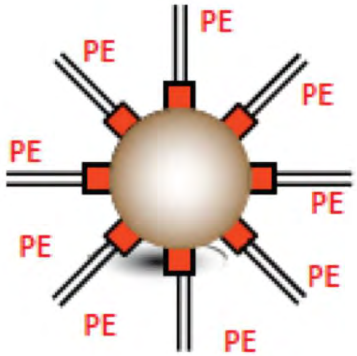
$$FL2' = FL2 - n\% \times FL1$$

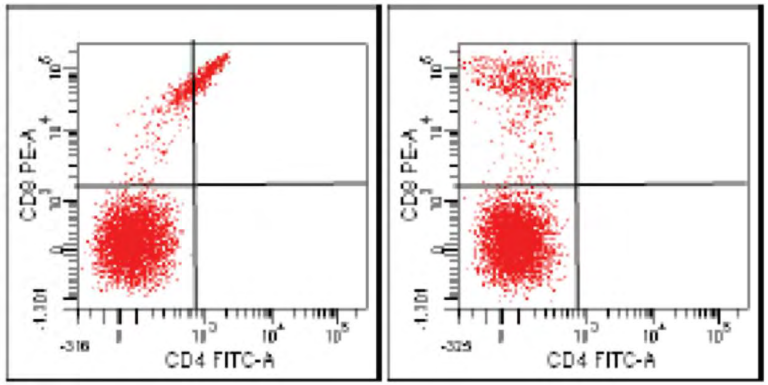


the correct compensation is obtained when the median of the positive population to be compensated becomes the same as that of the negative population

Flow cytometry

Applications: Fluorochromes choice



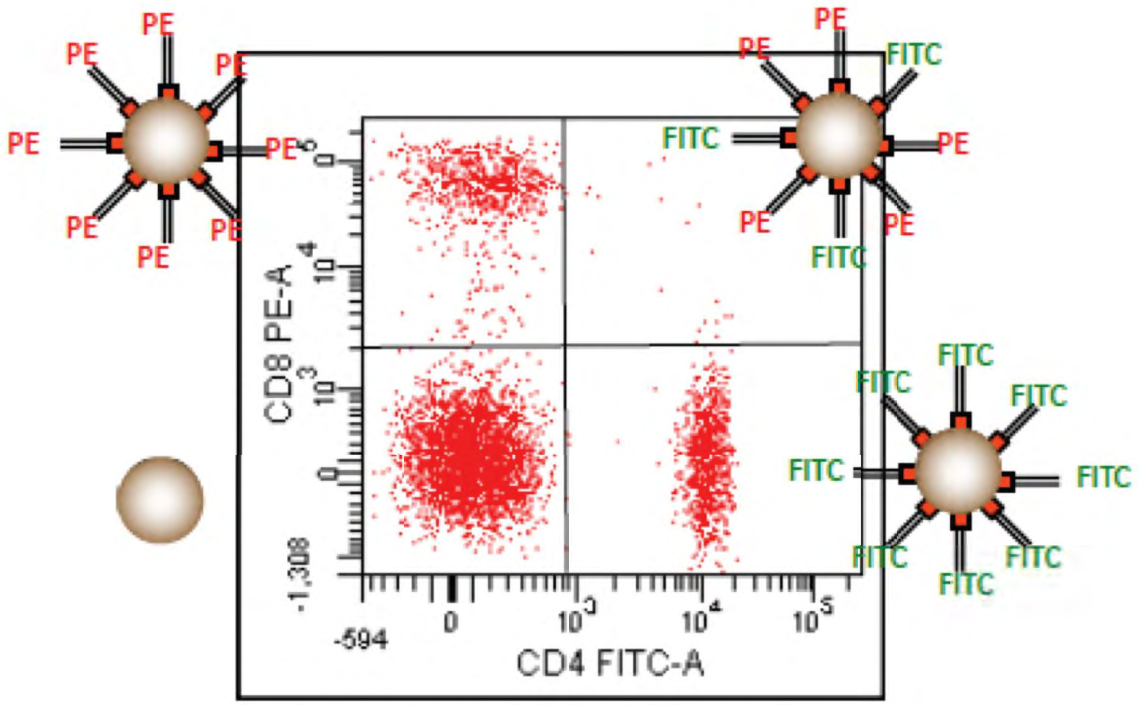


FL1 ' = FL1 - n'% x FL2

Population	Mean
P1	77
Q1	118
Q2	****
Q3	69
Q4	****

Flow cytometry

Applications: Fluorochromes choice



FL1 ' = FL1 - n% x FL2

FL2 ' = FL2 - n% x FL1

Flow cytometry

Applications: Fluorochromes choice

In practice:

Tube with containing:

unlabeled cells (positivity threshold and negativity)

and / or cells marked by isotypic control

PMT adjustment

FSC

SSC

Definition of a "gate" on cells of interest

Condition the plots on this gate

Passage of single-celled cells (or beads) with fluorochromes of interest

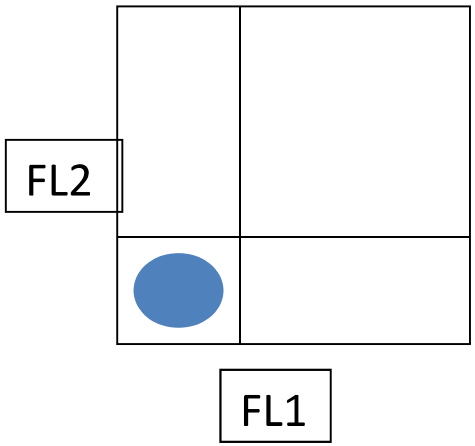
Flow cytometry

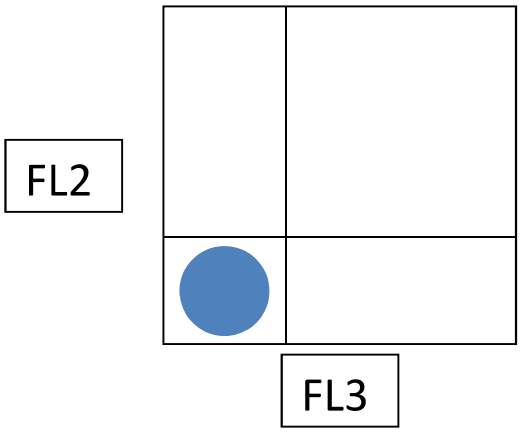
Applications: Fluorochromes choice

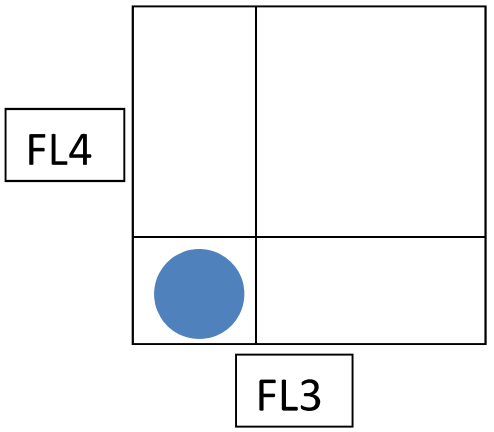
Tube containing:

unlabeled cells (positivity threshold and negativity)

and / or cells marked by isotypic control



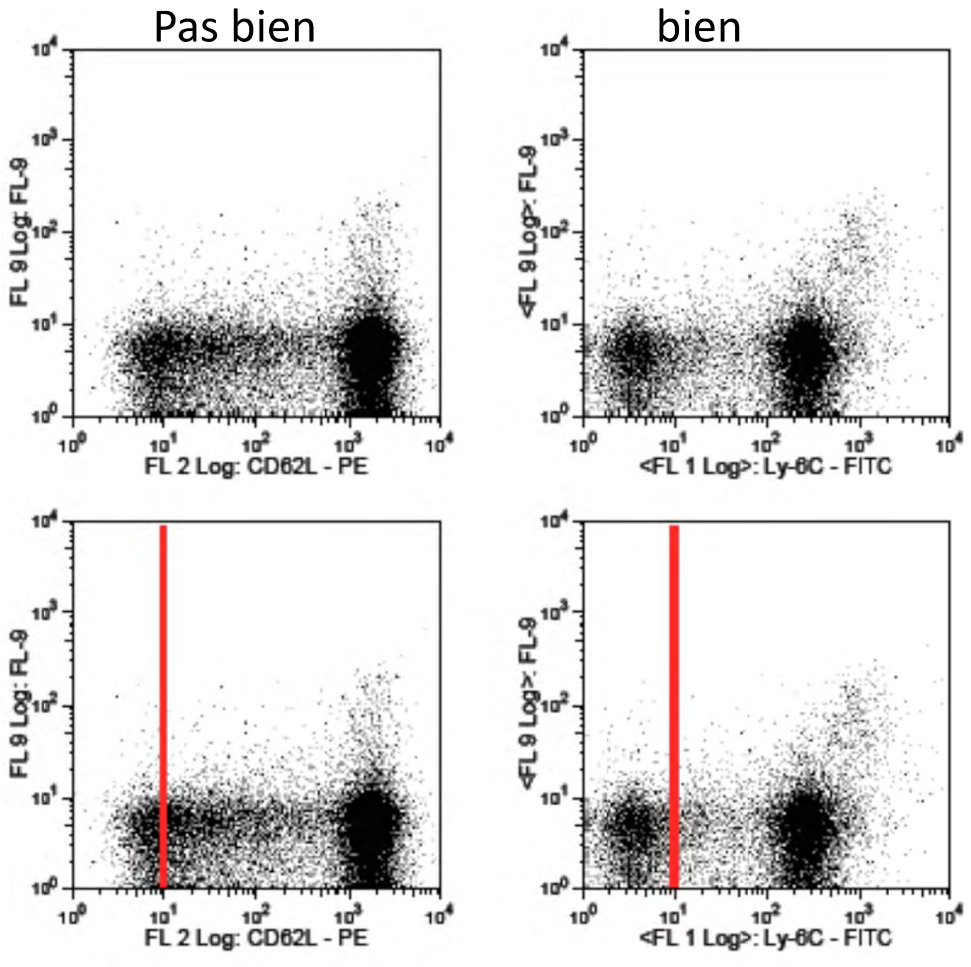




Flow cytometry

Applications: Fluorochromes choice

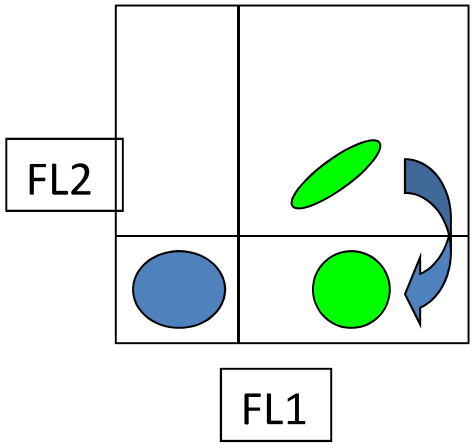
Positivity and negativity threshold definition for offsets

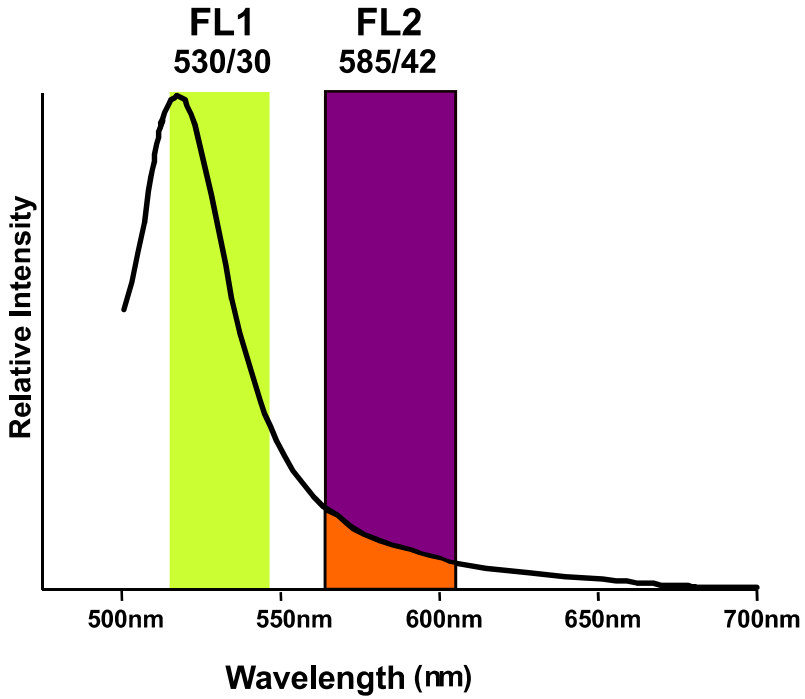


Flow cytometry

Applications: Fluorochromes choice

Tube with only one label: FITC





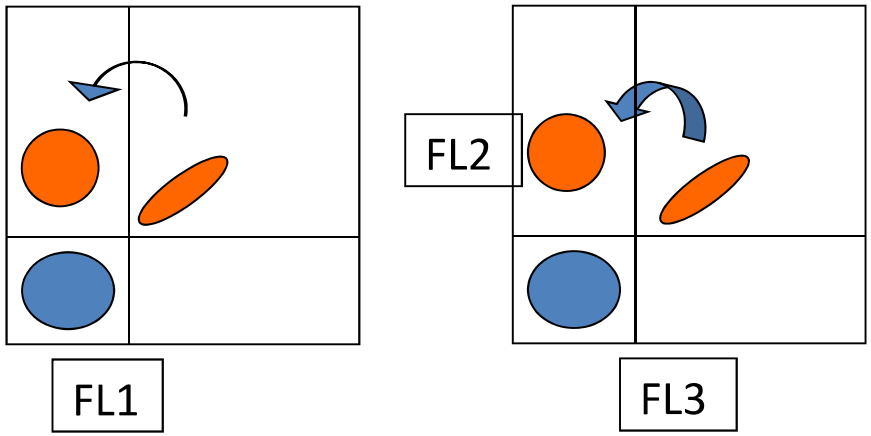
The negative population for FITC (FL1) must be align with the negative population of FL2

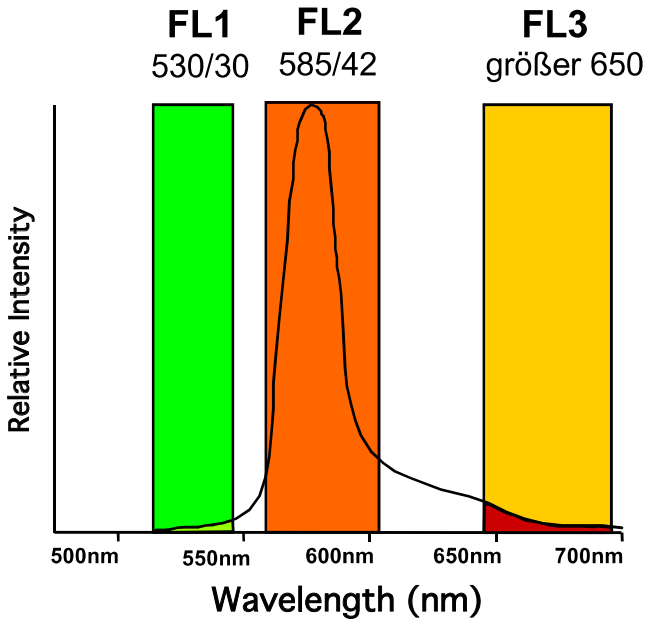
➔ We subtract fluo FL1 to FL2
Corresponding to the superposition
FL2 -x% FL1

Flow cytometry

Applications: Fluorochromes choice

Tube labelled with PE (FL2)





The PE population must align with
The negative pop FL1 and FL3
- > We subtract fluo FL2 to FL1 and FL3
Corresponding to the superposition of emission spectra
FL1-% FL2 and Fl3-% FL2

Flow cytometry

Applications: Fluorochromes choice

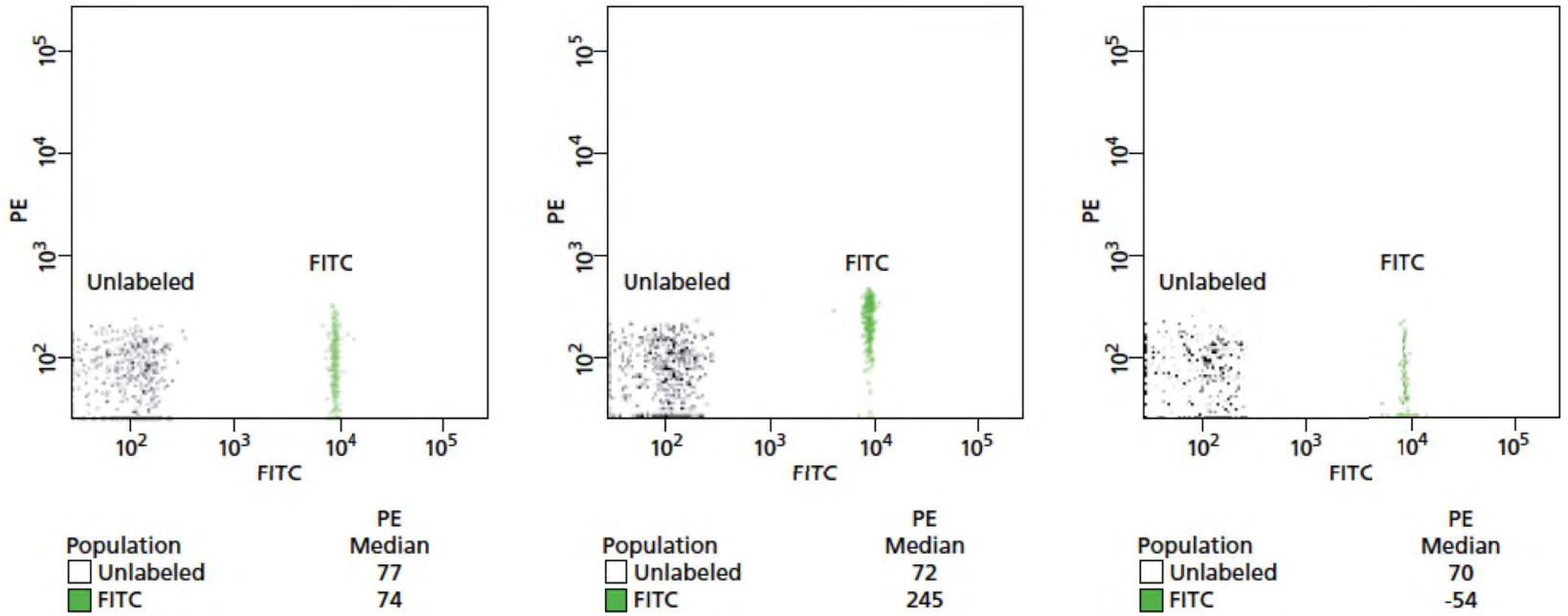


Figure 3.

Compensated properly
The medians of the positive and negative FITC populations are equal in the PE channel.

Undercompensated
Not enough fluorescence subtraction. The PE MFI of the positive FITC population is greater than that of the negative FITC population.

Overcompensated
Too much fluorescence subtraction. The PE MFI of the negative FITC population is greater than that of the positive FITC population.

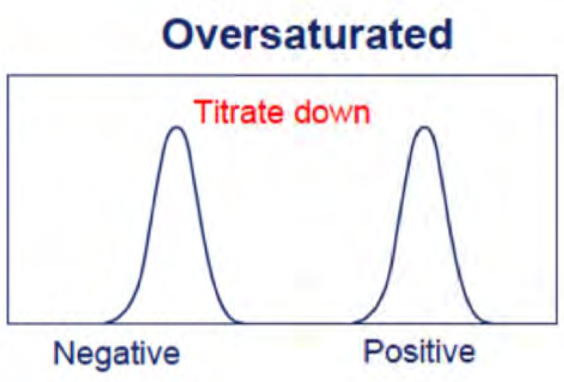
Flow cytometry

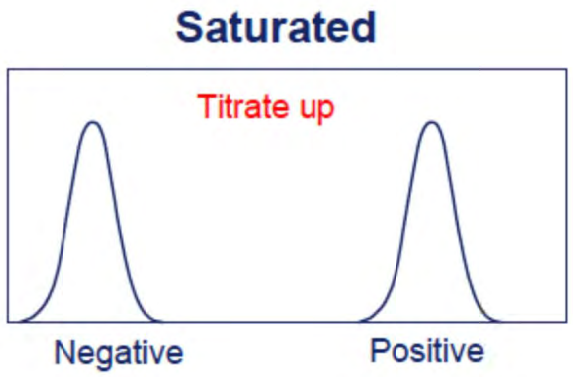
Applications: Fluorochromes choice

Same principle for markings> 4 colors

-> more complex
-> adapted compensation software
-> sometimes need to reward

⚠ your labeled Ac should be well calibrated and therefore used at optimal dilution otherwise you have a risk of signal loss (not diluted enough) or poor positive / negative discrimination. (too diluted)





Flow cytometry

Applications: Fluorochromes choice

Warning : tandem cases:

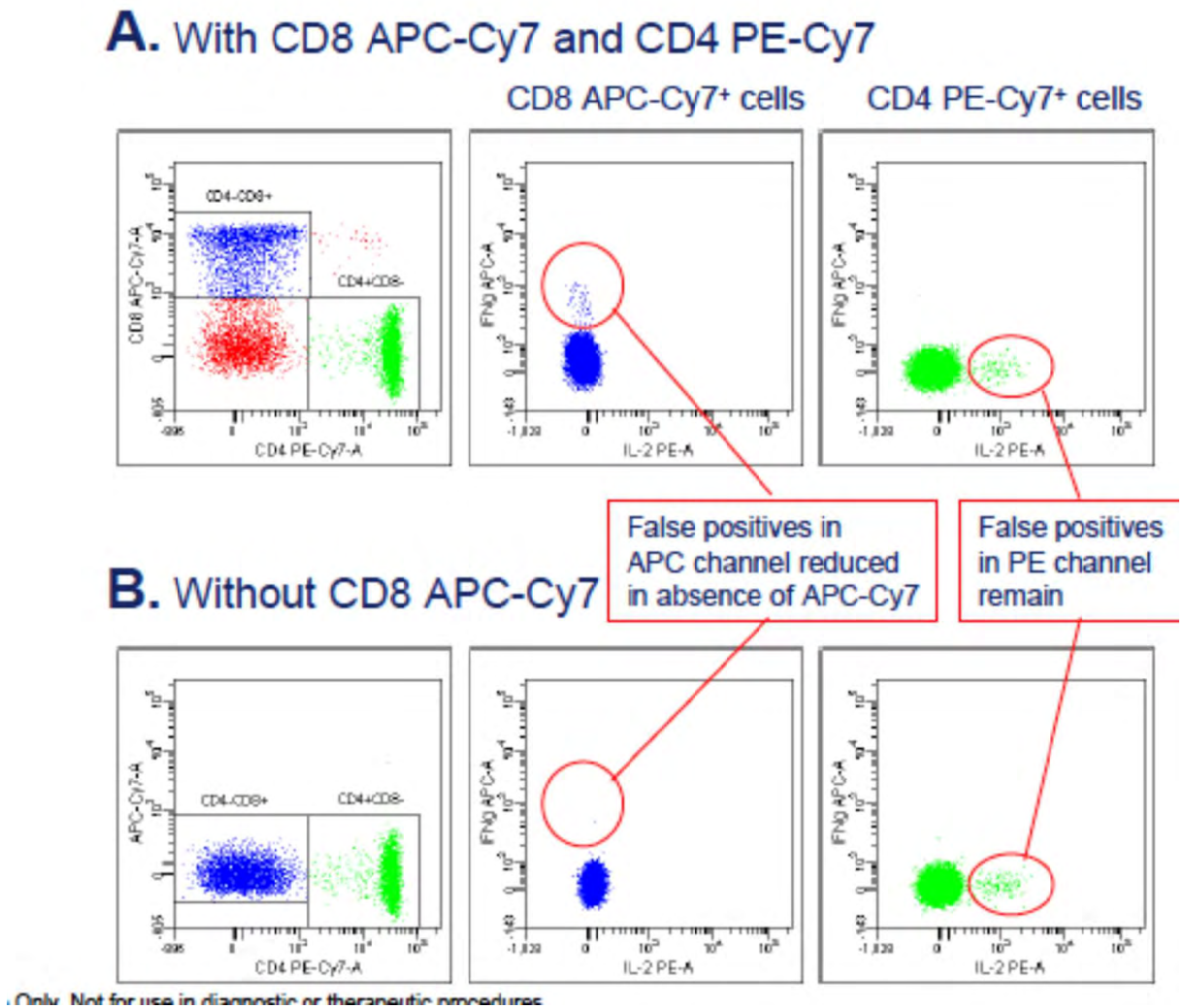
Compensation for tandems may vary with the same Ac from one experience to another (same or different lot)

- decoupling phenomenon
- systematically compensate

Some tandems (APC-Cy7, PE-CY7)
degradation: expo light
temperature
fixation

Flow cytometry

Applications: Fluorochromes choice

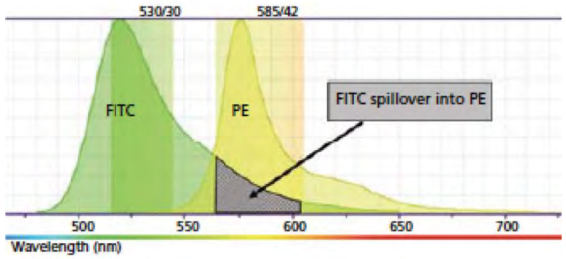


Only. Not for use in diagnostic or therapeutic procedures

Flow cytometry

Applications : multi colors panel

1-Associate the brilliant fluorochrome according to the density of Ag
so little Ag -> stain important index
if a lot of Ag -> stain index lower



2- Minimize spill over

3- Attention to tandem and their specificities (coupling, cold, permeabilization, fixation)

4- Know your device (filters)

5- Use the right controls

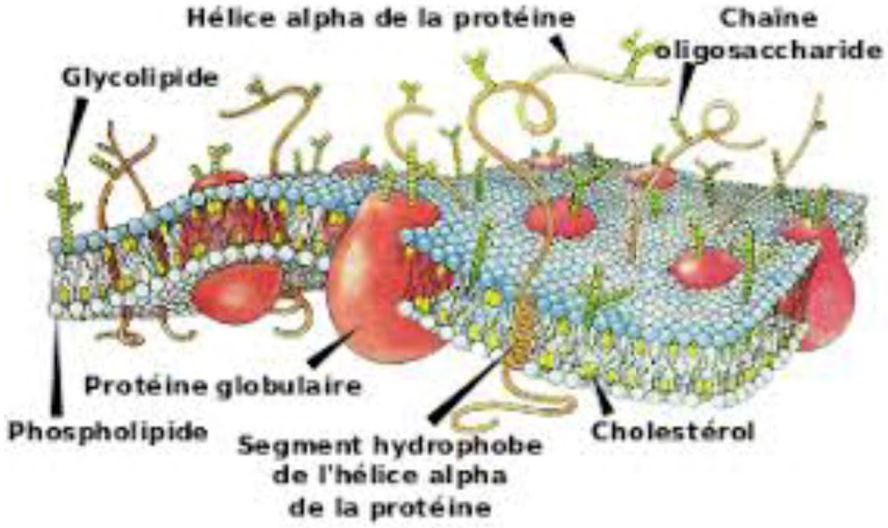
Flow cytometry

Applications : multi colors panel

Cell	Antigen	Molecules per Cell
T cell	TCR	100,000
	CD2	55,000
	CD3	124,000
	CD5	90,000
	CD7	20,000
	CD45	>200,000
CD4+ T cell	CD4	100,000
	CD28	20,000
	CCR5	4,000-24,000
CD8+ T cell	CD8	90,000
	CD28	15,000
B cell	CD19	18,000
	CD20	109,000
	CD21	210,000
	CD22	14,000
	HLA-DR	85,000
	CD11a	10,000
	CD40	2,000
	CD86	16,000
	CD80	2,000
Dendritic cell	CD11a	27,000
	CD40	17,000
	CD80	132,000
	CD86	208,000
Monocyte	CD14	110,000
	CD32	21,000
	CD64	13,000
Neutrophil	CD14	3,500
	CD16	225,000
NK cell	CD56	10,000
Red Blood Cell	Glycophorin A	340,000
Basophil	CD23	15,000

Achieve a good association
Ag density/ brightness

Steric hindrance phenomenom



Flow cytometry

Applications : multi colors panel

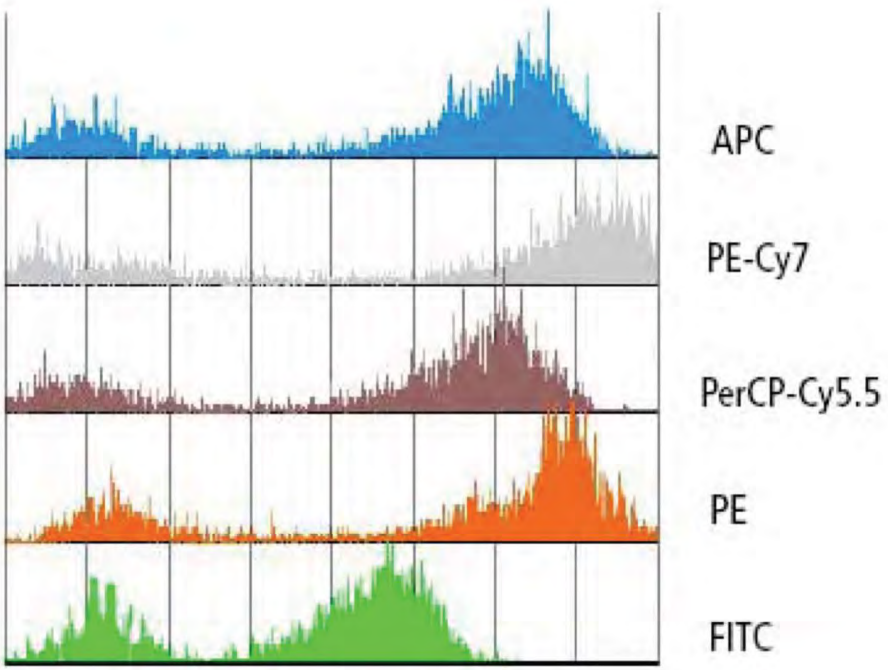
Fluorochrome	Stain Index
PE-Cy™5	353
PE	302
APC	278
Alexa Fluor® 647	214
PE-Cy™7	139
PerCP-Cy™5.5	107
BD Horizon™ V450	**85**
Pacific Blue™	80
Alexa Fluor® 488	73
Alexa Fluor® 700	61
FITC	56
APC-Cy7	37
PerCP	37
AmCyan	25
APC-H7	24

See web site

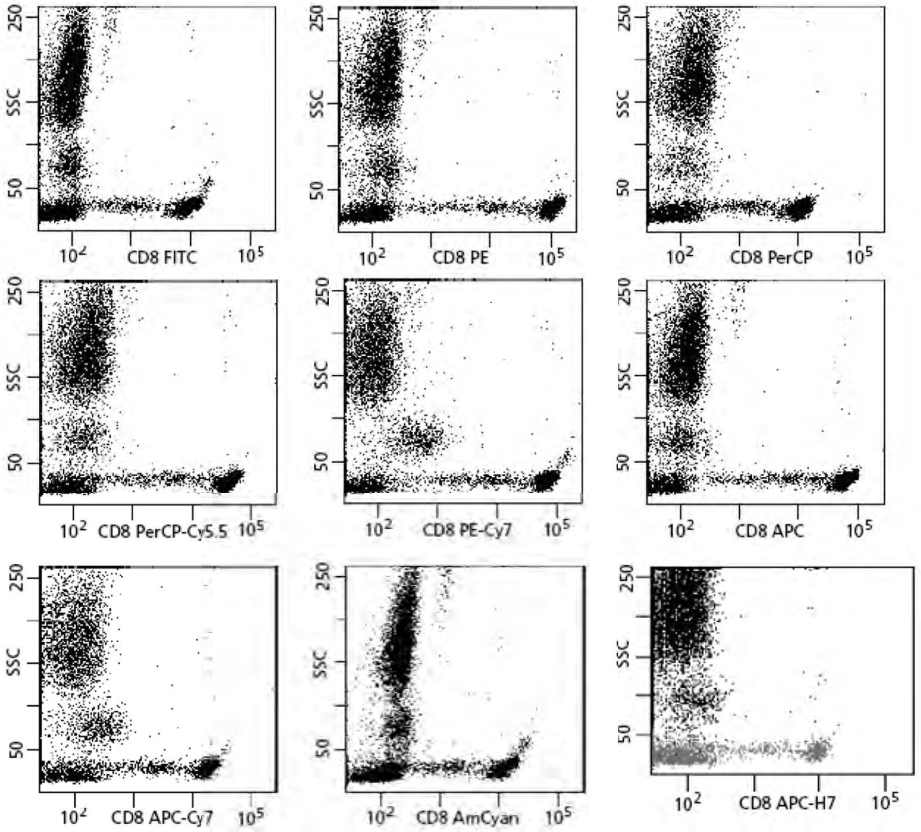
Flow cytometry

Applications : multi colors panel

CD5



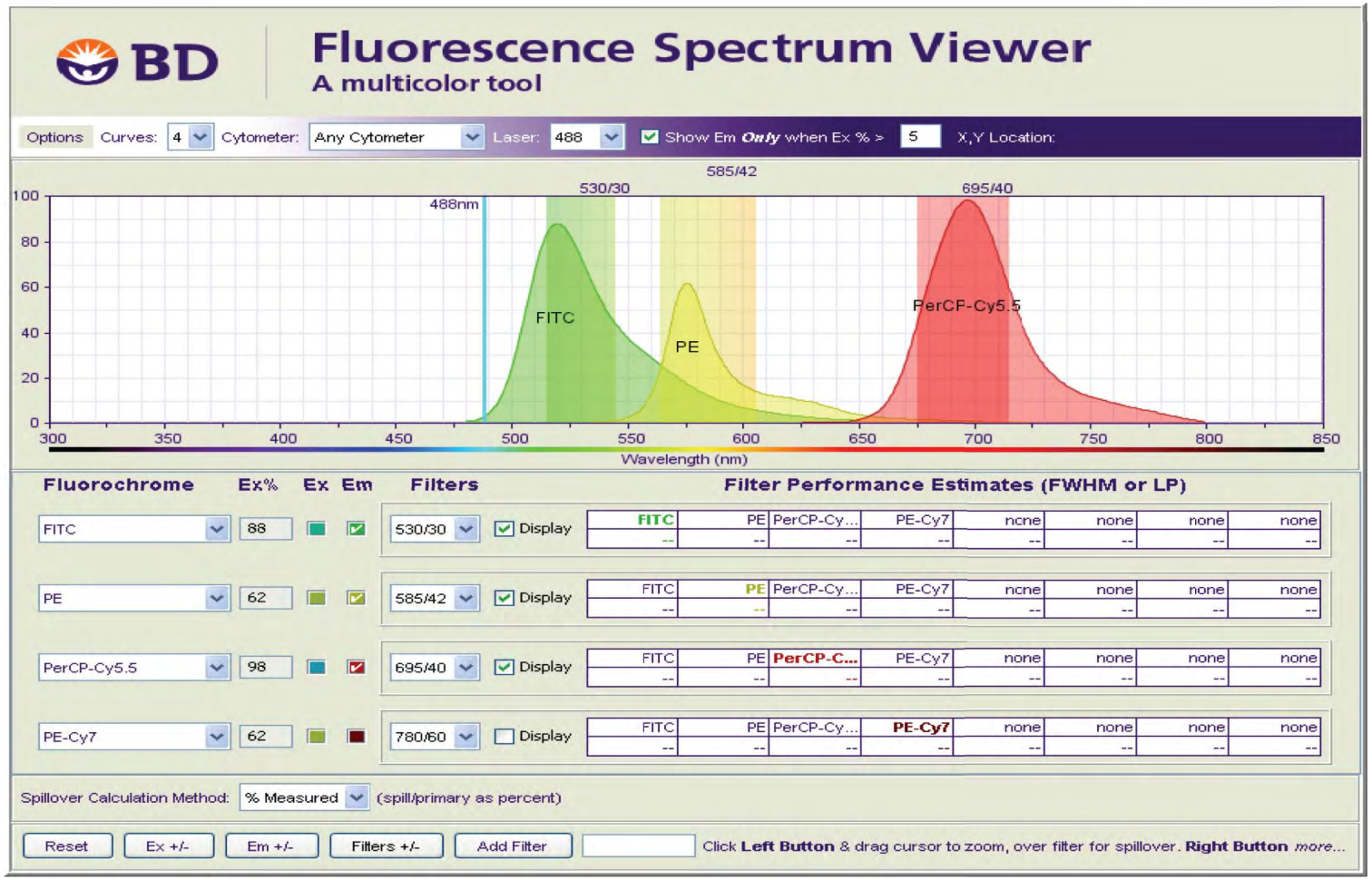
CD8



Warning : choice of fluorochromes

Flow cytometry

Applications : multi colors panel



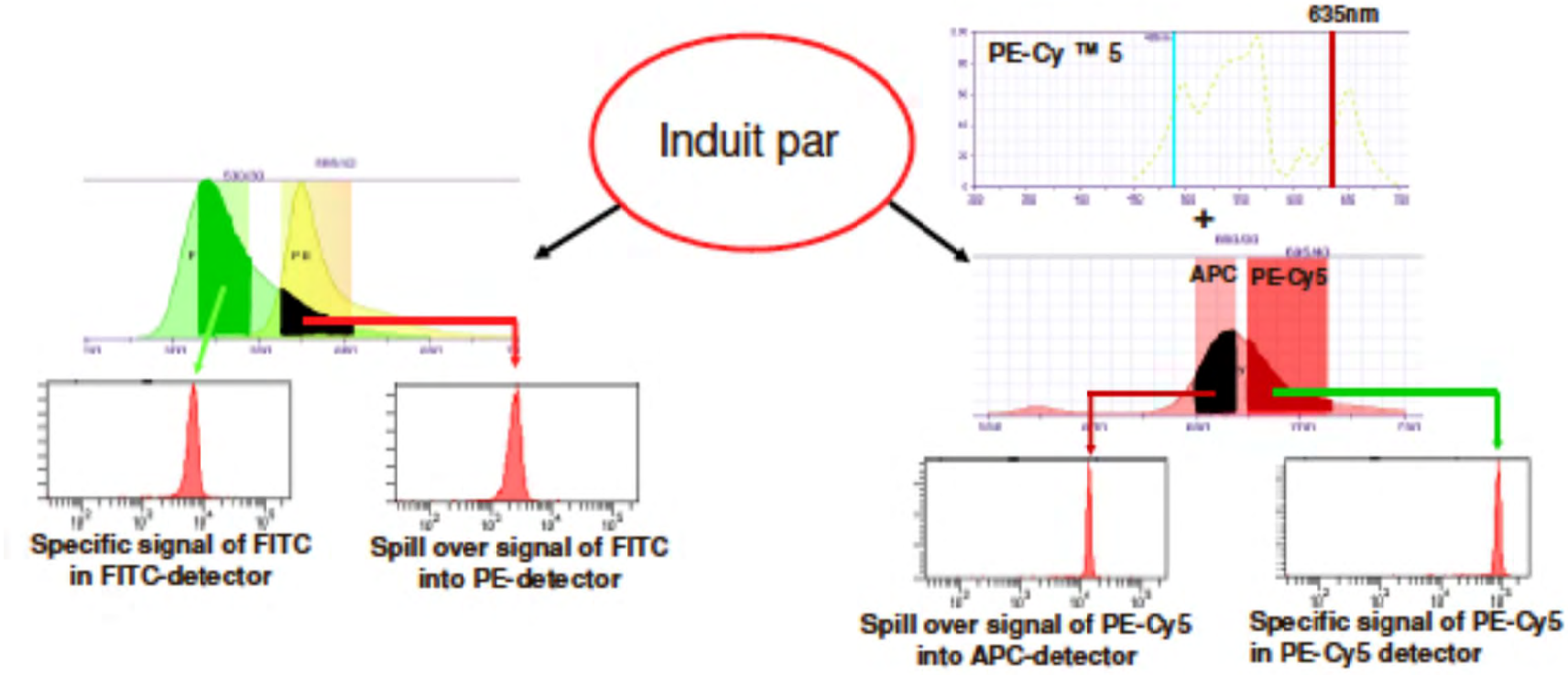
Choice of fluorochrome and spill over, on site supplier

Flow cytometry

Applications : multi colors panel

Le spill over

Détection du signal d'un fluorochrome par 2 détecteurs (ou plus)



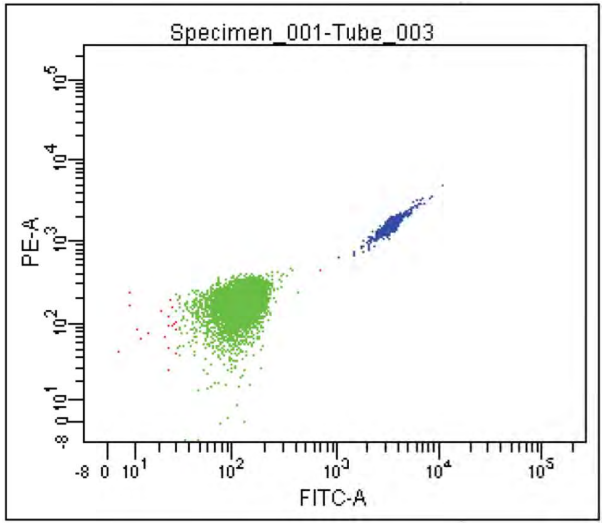
False positive

Flow cytometry

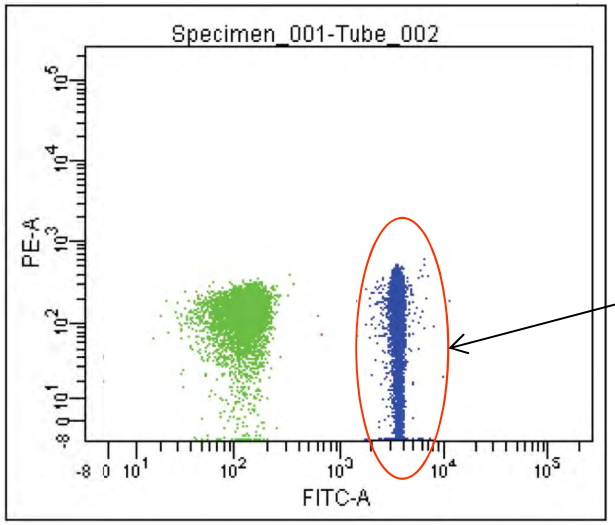
Applications : multi colors panel

Le spill over

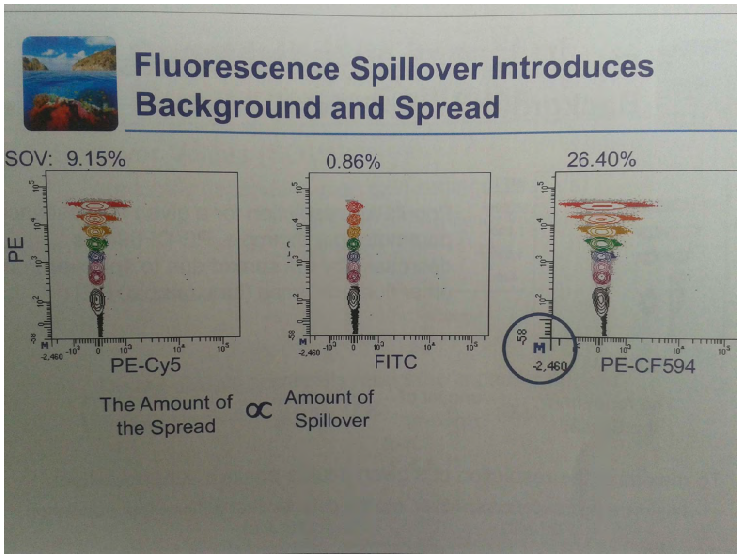
No

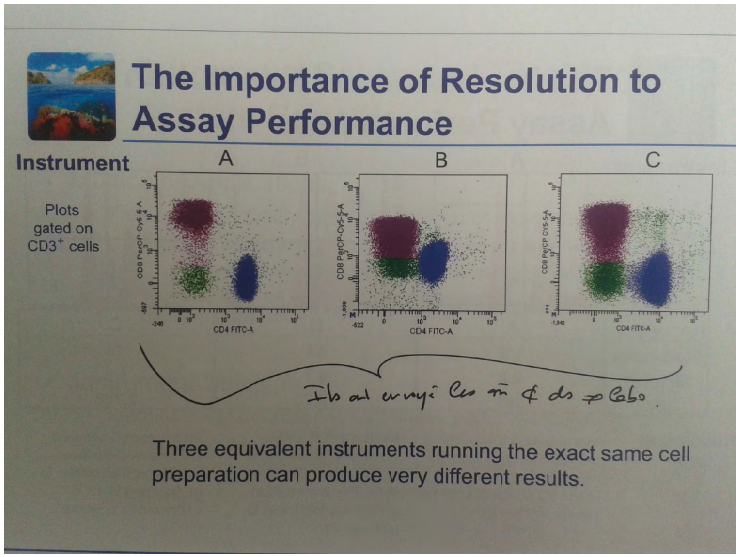


Yes



Spread data
Du
Au spill over





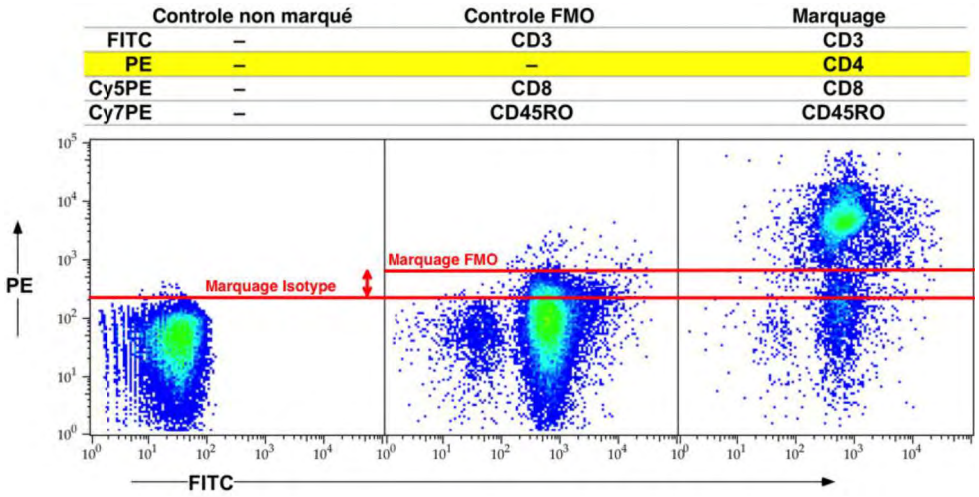
Flow cytometry

Applications : the threshold of positivity

How defin it?

- Check of l'autofluorescence (cells unlabeled only)
- Make isotype control
  (non-reactive antibody of the same isotype as the directly coupled test antibody)
- Make biological controls (eg stimulated / unstimulated cells)
- Make FMO type controls (Fluorescence Minus One)

  All the fluoresces less one, to achieve during a new combination of marking and / or whenever the threshold of positivity is difficult to determine



Flow cytometry

Applications : exemples

In a clinical laboratory:

HIV immunophenotype
Absolute accounts CD4
Immunophenotyping leukemias and lymphomas
cell cycle
Progenitor Cell Count (CD34)…

In a research laboratory:

Immune system studies
hematopoietic cells
Kinetic studies
Detection
Cell sorting

…

Flow cytometry

Applications : exemples

More precisely:

- Viability / cell mortality
- Surface membrane markings
- Intracellular markings
- Cell Division
- Cell cycle
- Membrane potential
- Intracellular calcium fluxes
- Intracellular pH
- Cytochrome P450 activity
- Enzyme markings
-Apoptosis.

everything depends on the fluorochrome, the cell preparation and the lase

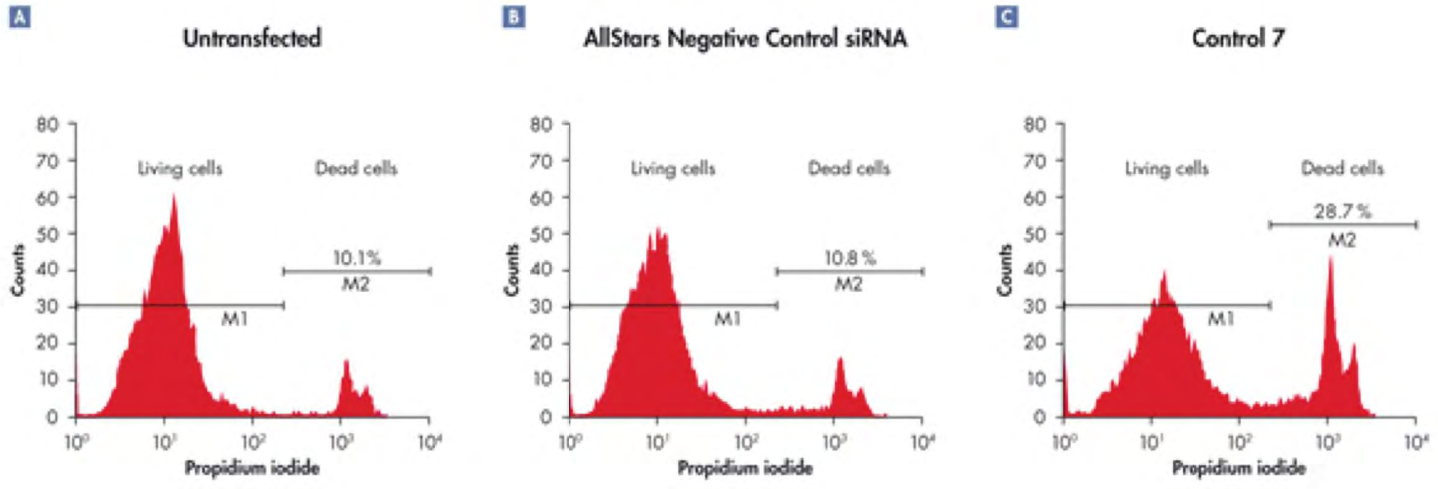
Some examples:

Flow cytometry

Applications : exemples

Viability / cell mortality :

Iodure de propidium
7 AAD (7 amino-actinomycin D)
live dead
....
-> DNA interlayer (BET, IP), Bases A-T (DAPI, Hoechts), Base C-G (7AAD)
-> Penetrate into dead cells

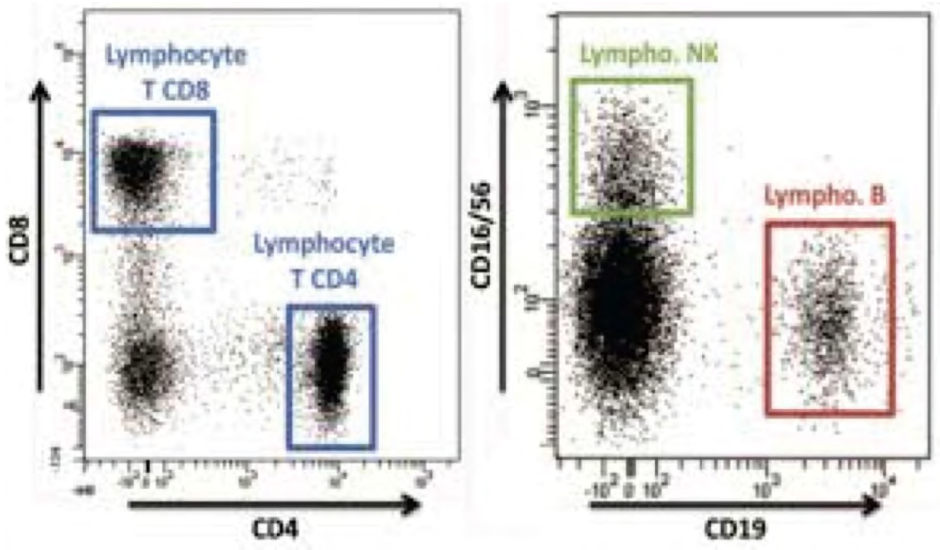


Flow cytometry

Applications : exemples

Surface membrane labelling :

Antibody coupled to a fluorochrome directly or to biotin
Direct incubation marking
Example: CD4, CD8, CD19TCR….

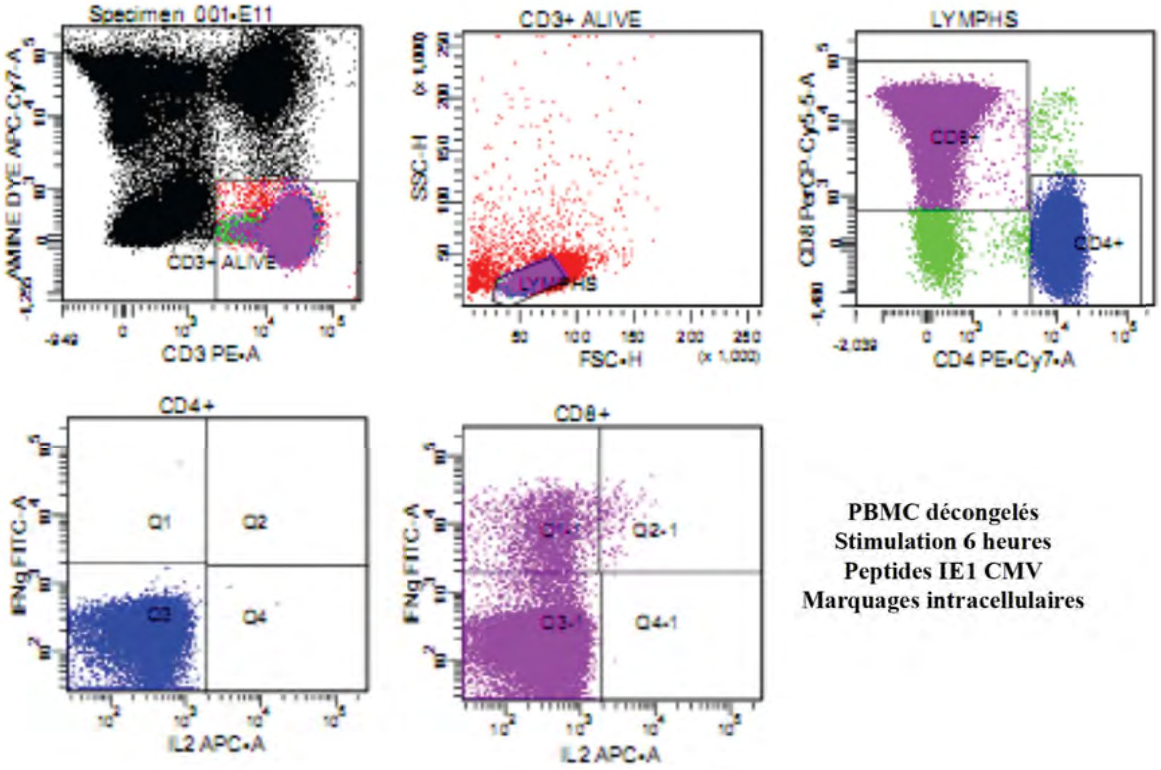


Flow cytometry

Applications : exemples

Intracellular labelling :

Antibodies coupled directly to a fluorochrome (better)
Permeabilization phase of the membrane before marking
Example: FoxP3, IL2, IFNg…

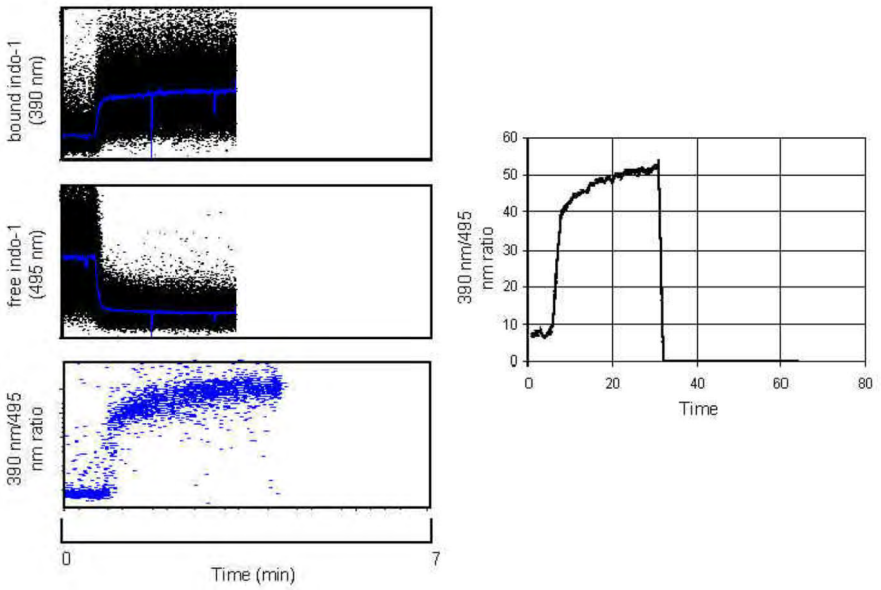


Flow cytometry

Applications : exemples

Potentiel de membrane :

DIOC5(3) Dipentylaxocarbocyanine iodide
carbocyanine Familly
hyperpolarized cells captures the DIOC
Example:
used in neurology for studies on neuron activation



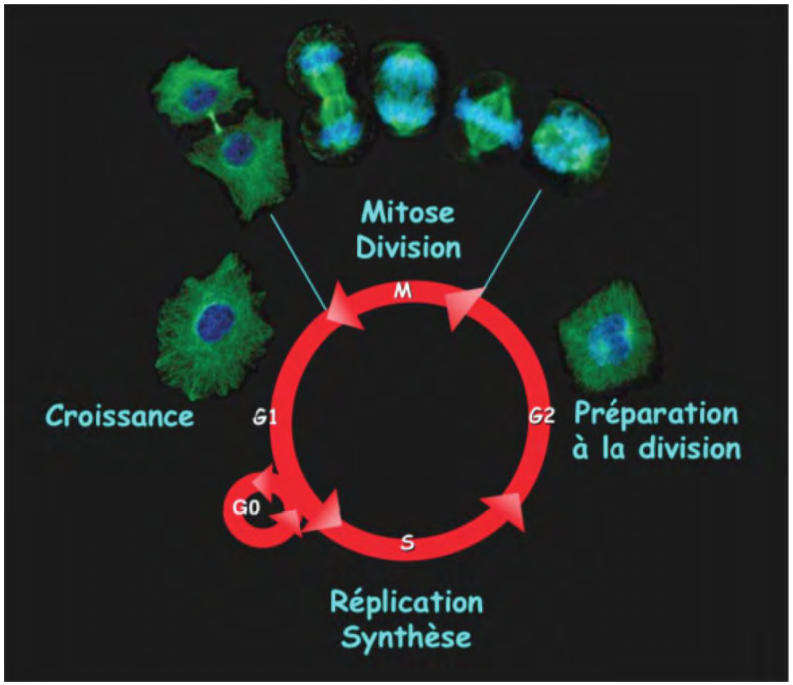
Flow cytometry

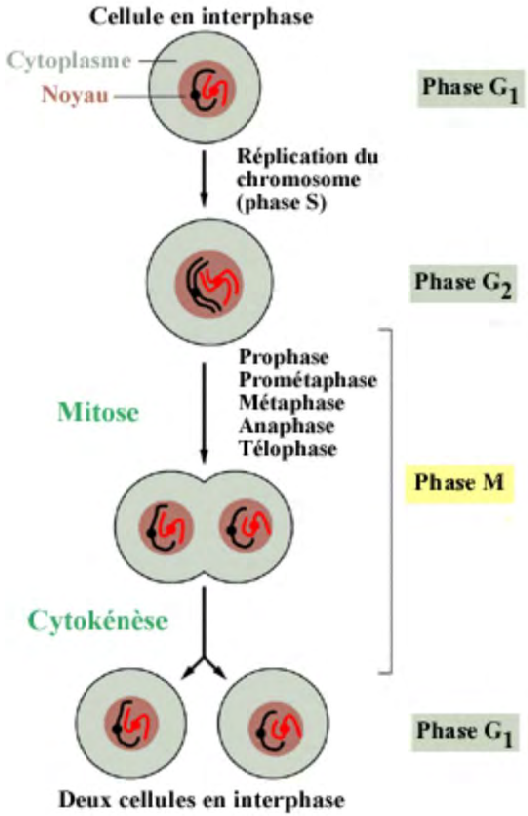
Applications : exemples

Cell Cycle :

Mesurement of DNA ploidy: the more DNA there is, the stronger the signal

- Iodure de propidium
- Hoechst (The most lipophilic)
- DAPI (di amino phenyl Indol)
- BrDU





Flow cytometry

Applications : exemples

Cell proliferation:

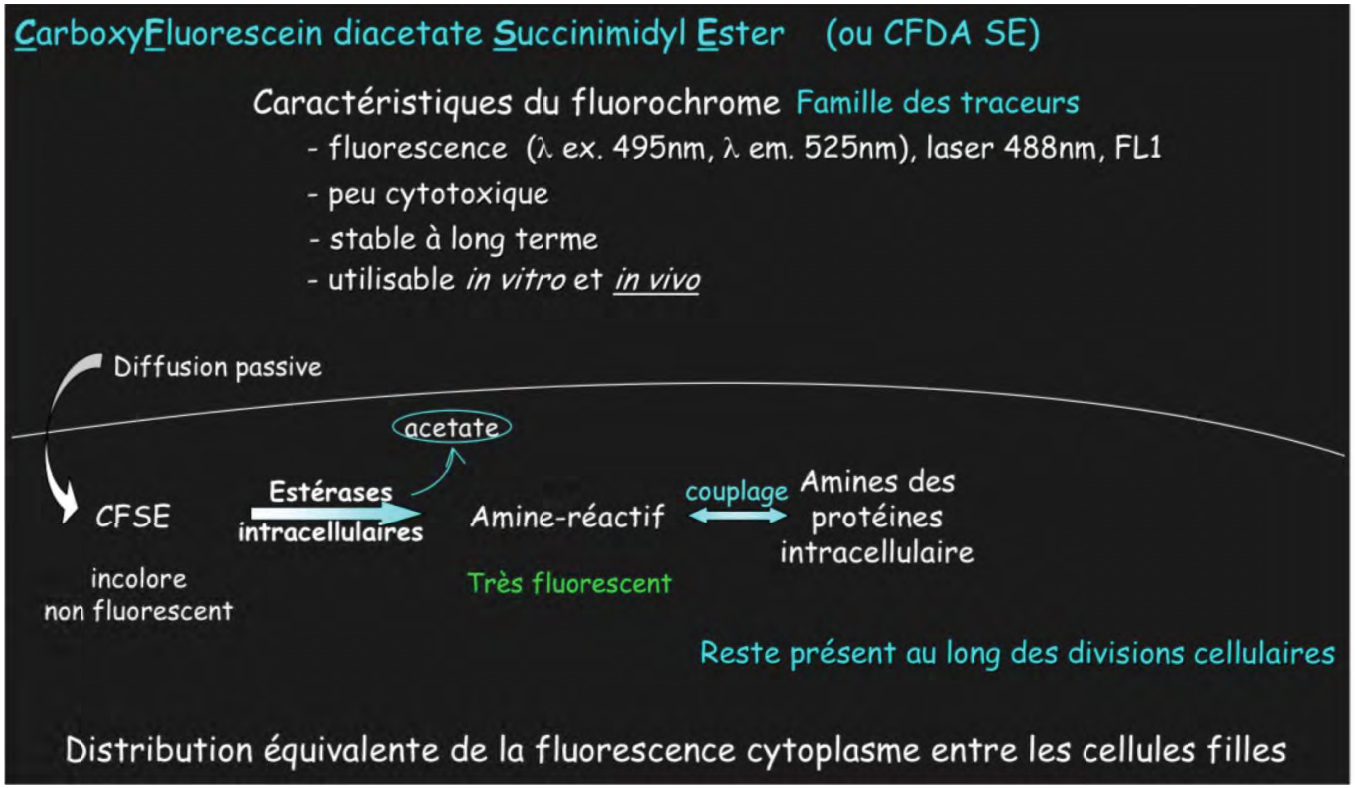
CFSE: 5 (6) carboxyfluorescein diacetate N succinidyl ester

Lipophilic: penetrates easily into the living cell then esterification:

cleavage leading to fluo and trapping in the cytoplasm (protein-permeabilization possible)

-> the more the cell divides the less it fluoresces.

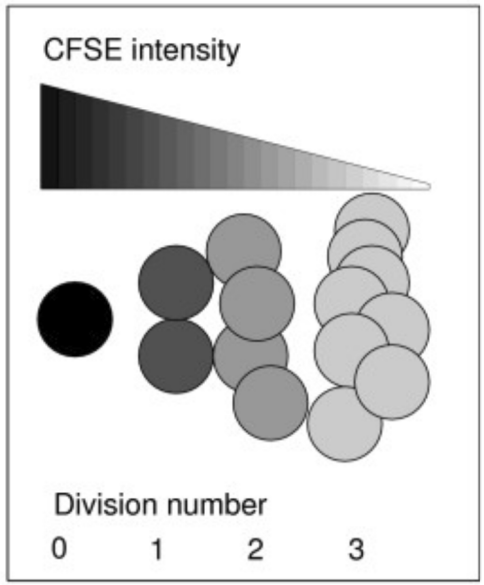
-> Typical image

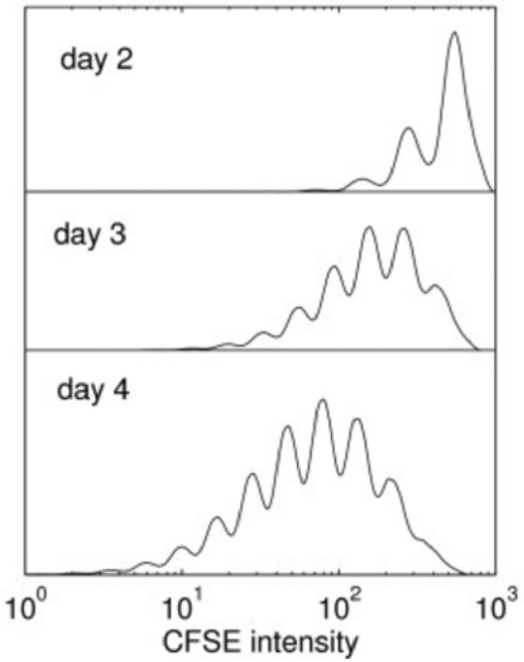


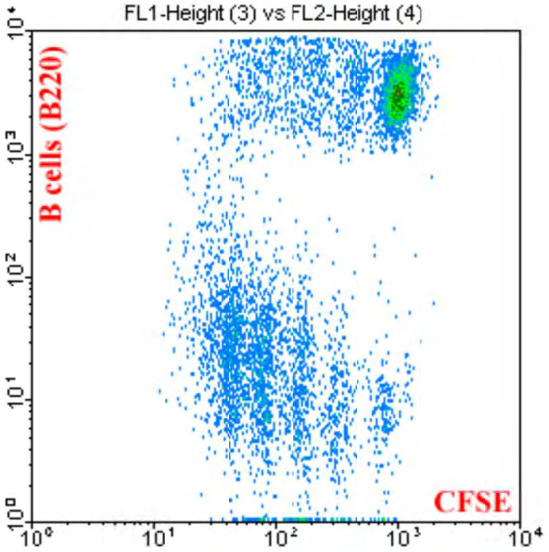
Flow cytometry

Applications : exemples

Cell proliferation:







Flow cytometry

Applications : exemples

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Absolute accounts CD4
Immunophenotyping leukemias and lymphomas
cell cycle
Progenitor Cell Count (CD34)…

In a research laboratory:

Immune system studies
hematopoietic cells
Kinetic studies
Detection
Cell sorting

…

Flow cytometry

Applications : cell sorting

- Enrichment of a sub-population.
- Sorting of rare events (HIV-dendritic cells - stem cells).
- Cloning in 96 well microplates (production of hybridomas, receptor-transfected cell lines).
- Remove dead cells from a crop

Flow cytometry

Applications : cell sorting

- Base: the cells are separated according to the information given by the phenotype
- The fluidic column is fractionated and each droplet is analyzed
  a cell / droplet
- According to the values: we decide the separation

Flow cytometry

Applications : cell sorting

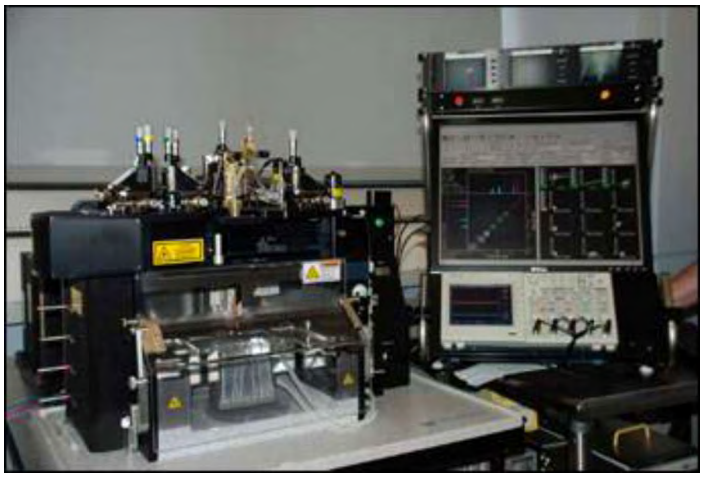
- This separation is obtained by electrifying the drop
- Then deflection by electric or magnetic fields
- Depending on the devices you can sort 1, 2 to 4 different populations

Flow cytometry

Applications : cell sorting

ARIA

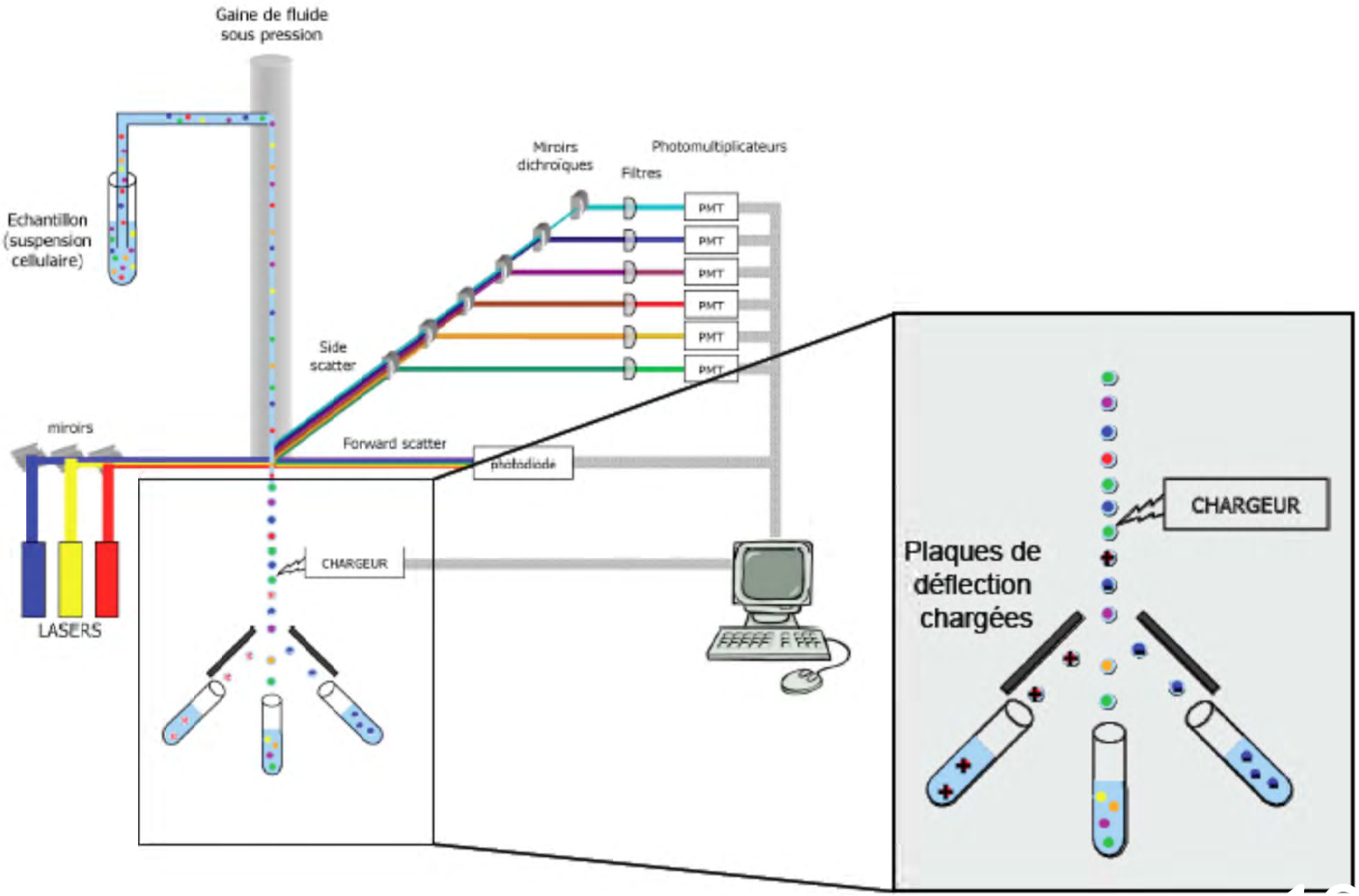
INFLUX



ASTRIO-MOFLO

Flow cytometry

Applications : cell sorting



Flow cytometry

- History & definition
- Principles
- Applications
- Other

Flow cytometry

Technical evolution

Mass cytometry

- From 18 parameters to 135

- Ultrafine characterization of even very rare cells

- Biological markers are detected using antibodies with metal tags
120kD <PM metals> 215 kD

- The cells are vaporized in a plasma chamber

- the cell generates an ion cloud

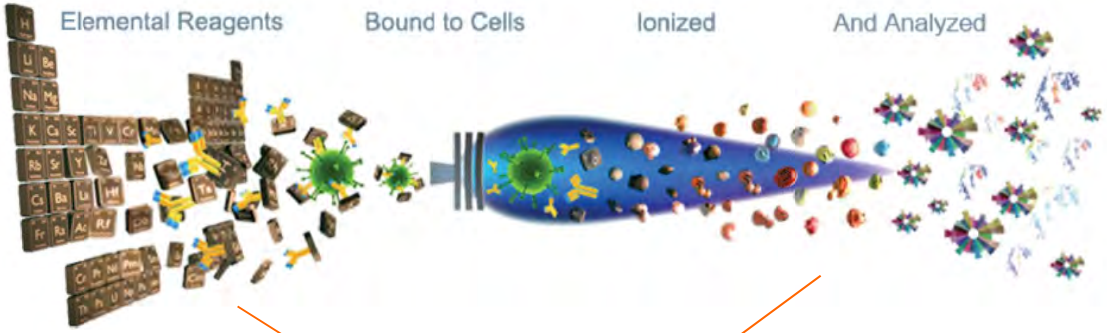
- Mass spectrometry analysis

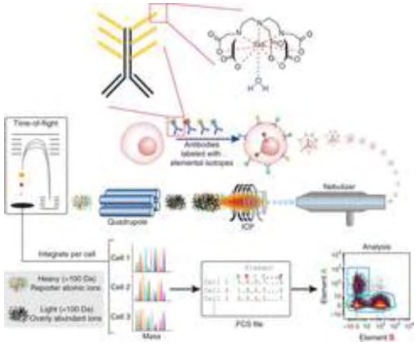
- The mass spectrometry output signals are devoid of spectral overlap

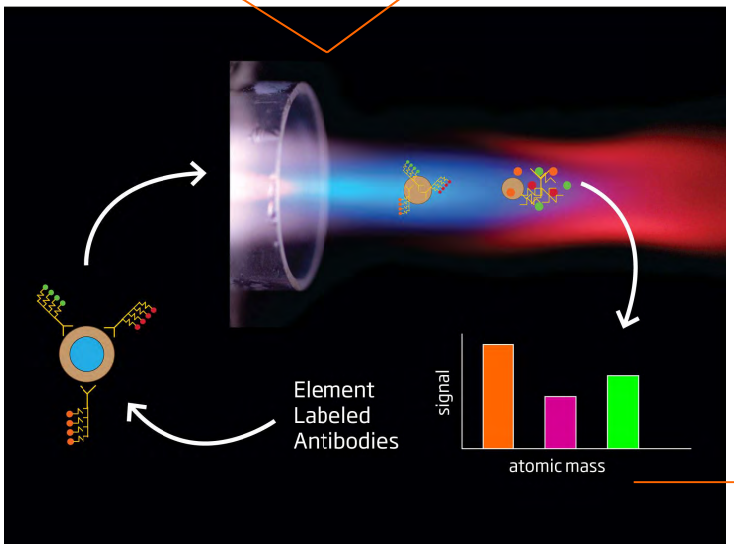
Flow cytometry

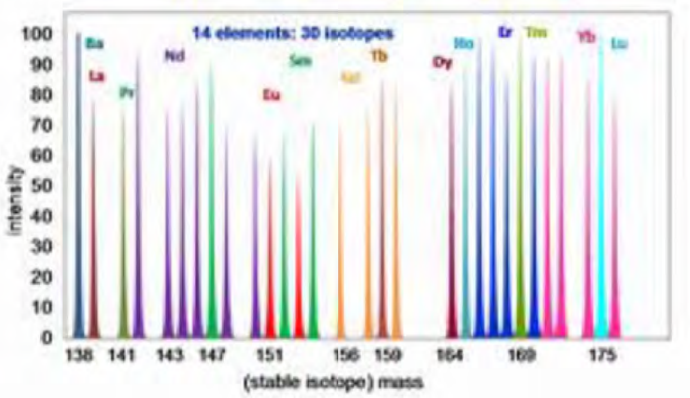
Technical evolution

Mass cytometry





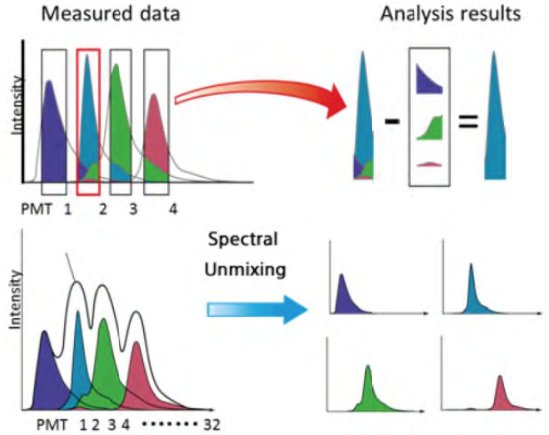


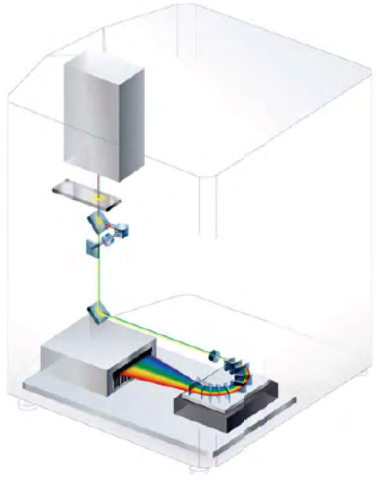


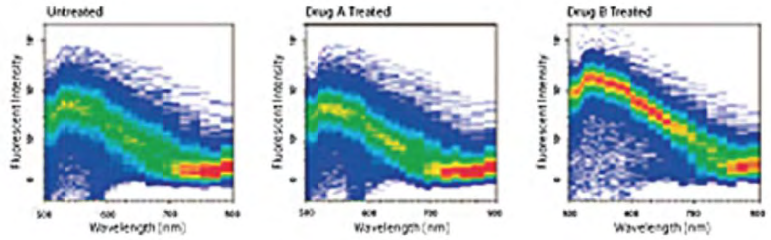
Flow cytometry

Technical evolution

Spectral cell analyser





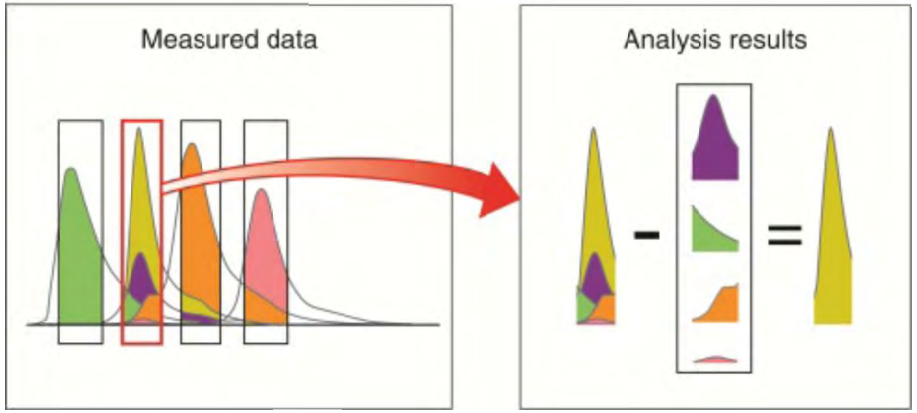


Flow cytometry

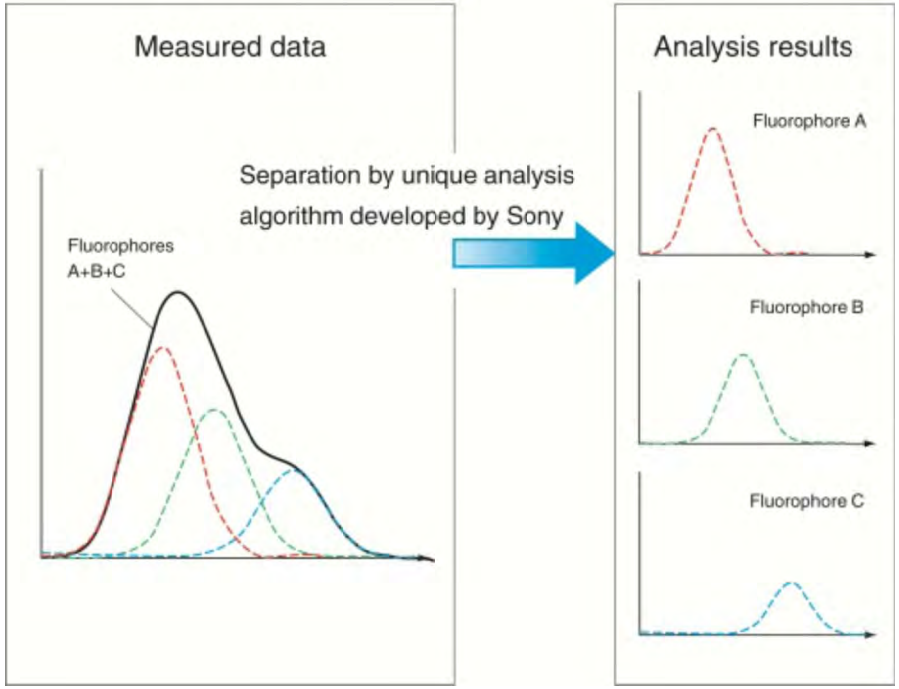
Technical evolution

Spectral cell analyser

Analysis by Conventional Cell Analyzers



Analysis by the Spectral Cell Analyzer



division of fluorescence emissions of all fluorophores through a network of Sony's unique design prisms

in combination with a newly developed '32 - channel photomultiplier

Allows analysis of spectral emissions of virtually all overlapping fluorophores by dividing them into individual fluorophore emissions with the independently developed analysis algorithm of Sony.

Expected results:
No more need to make compensation
Increase in the number of determiners

Flow cytometry

Security

1/ Electrical risks

2 / Class 3 laser hazards

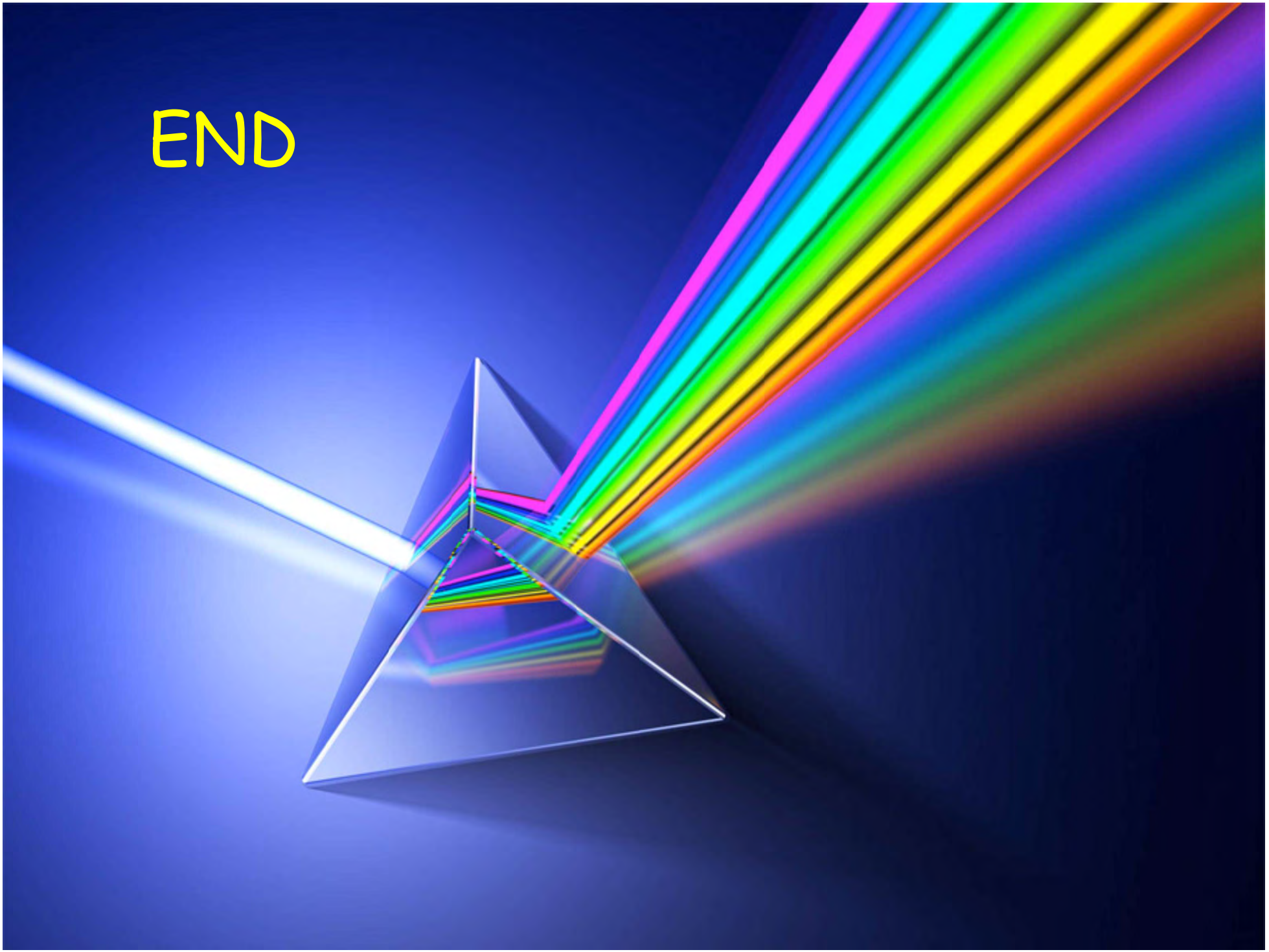
3 / Chemical risks

- fluorochromes
- Formaldehyde
- Parformaldéhyde

pay attention to waste disposal

4 / Biological risk

- aerosols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