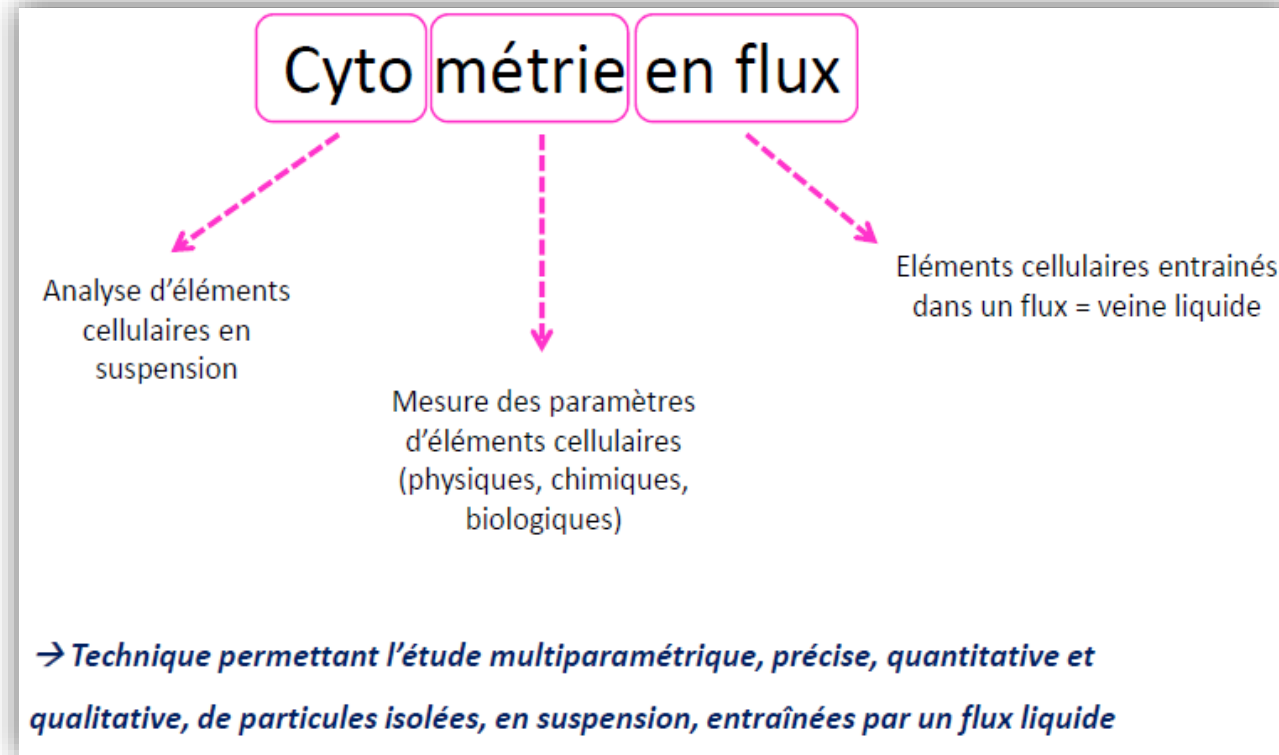


Le futur de la cytométrie en flux au laboratoire d'hématologie?

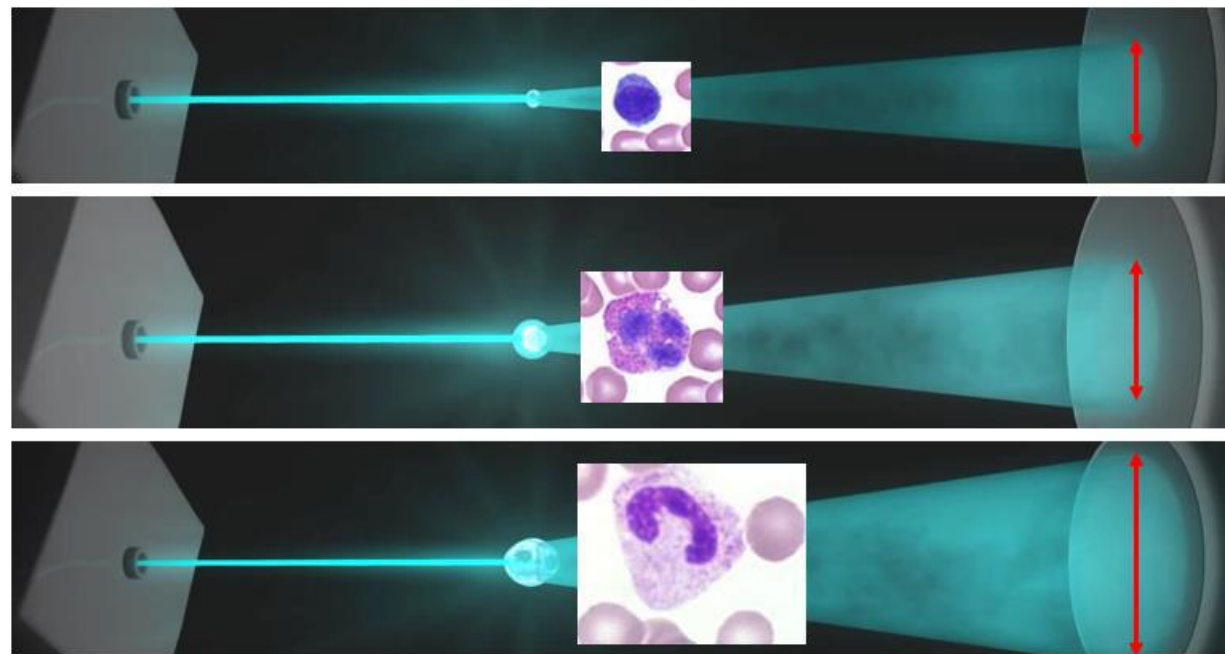
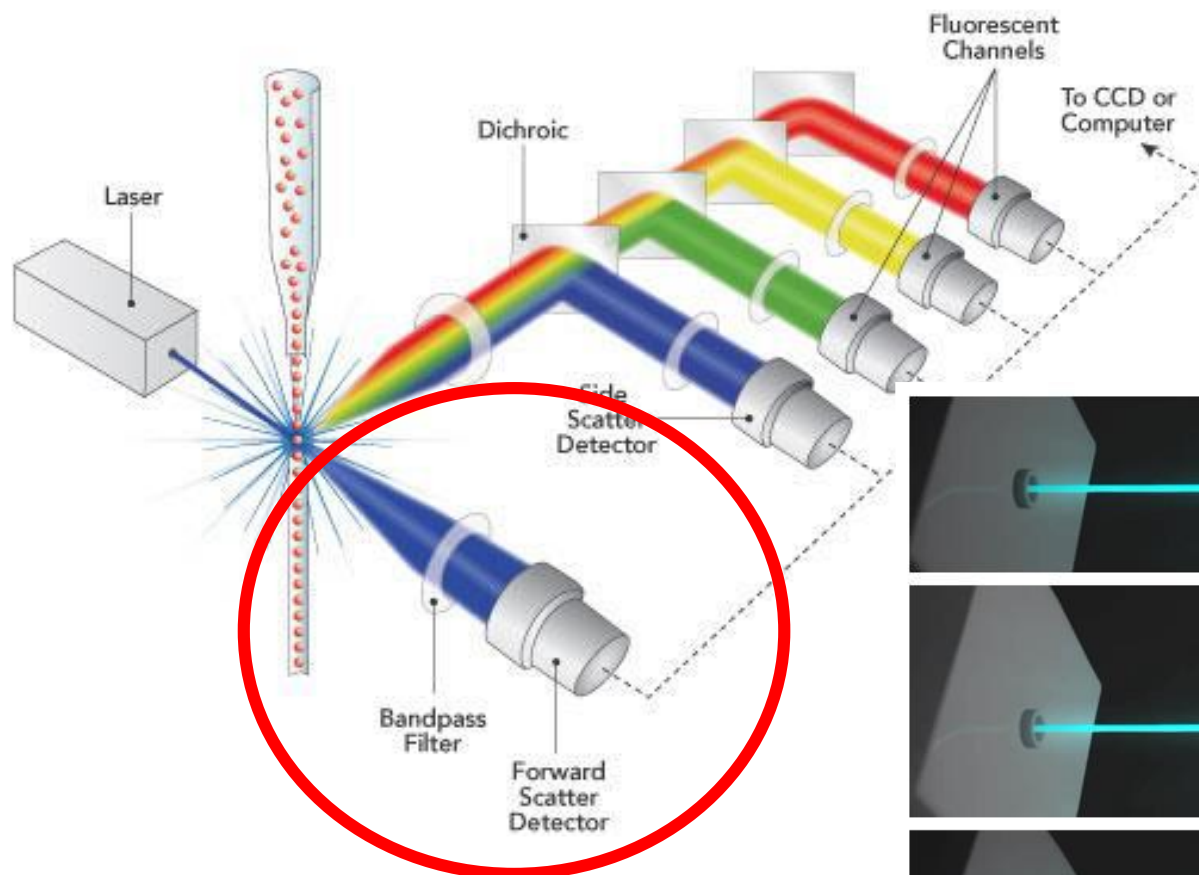
Valéry Daubie

La cytométrie

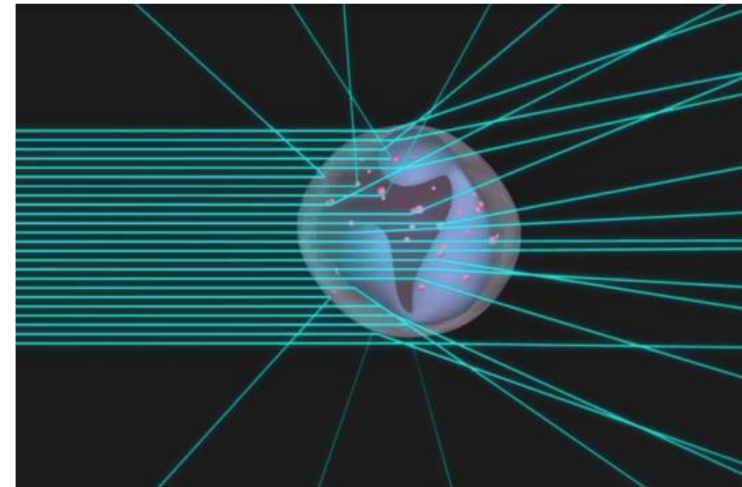
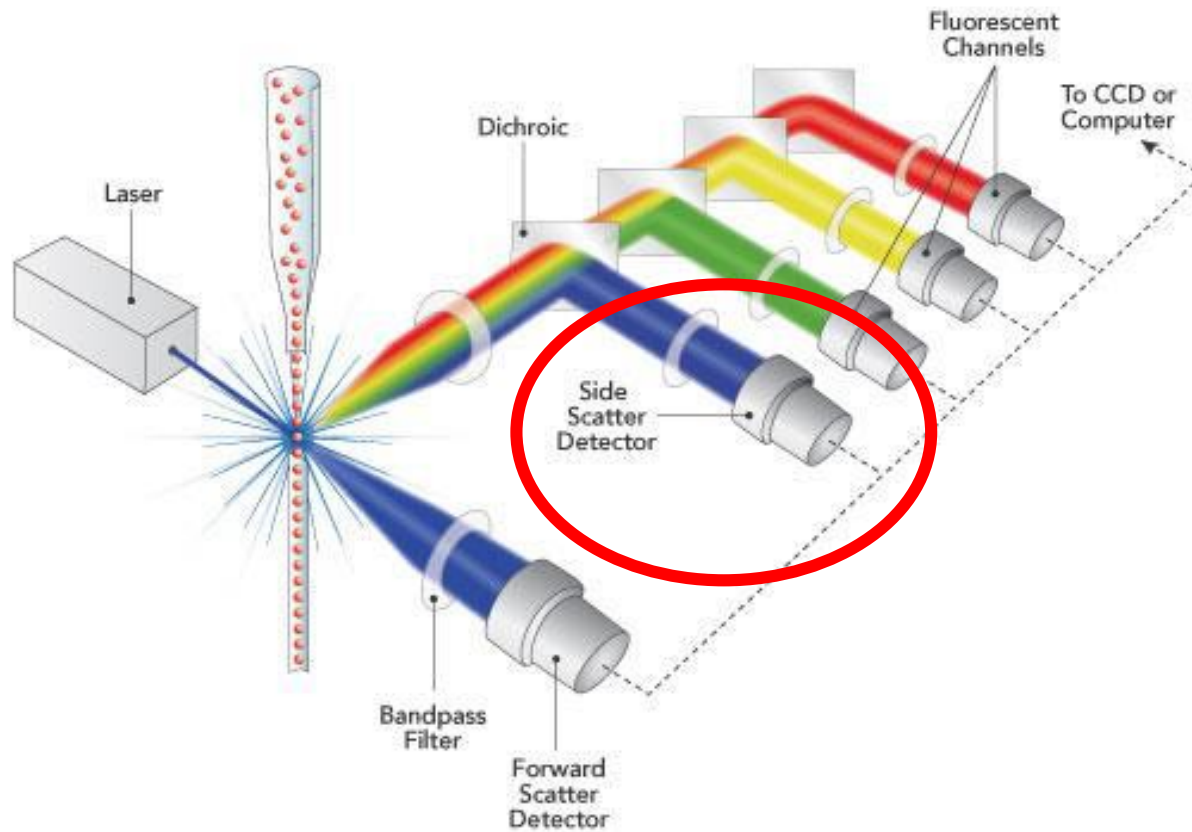


- Cellules en suspension < sang, moelle osseuse, liquides (LCR, LBA, ...), ganglion

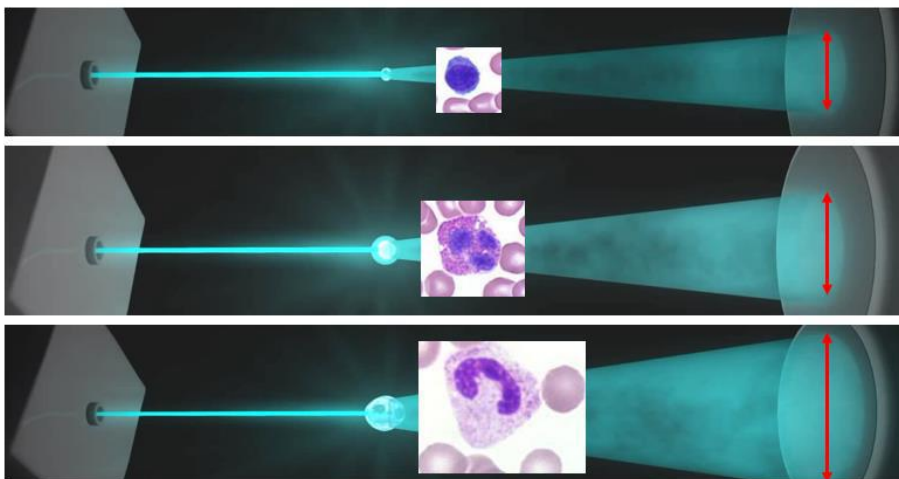
La cytométrie



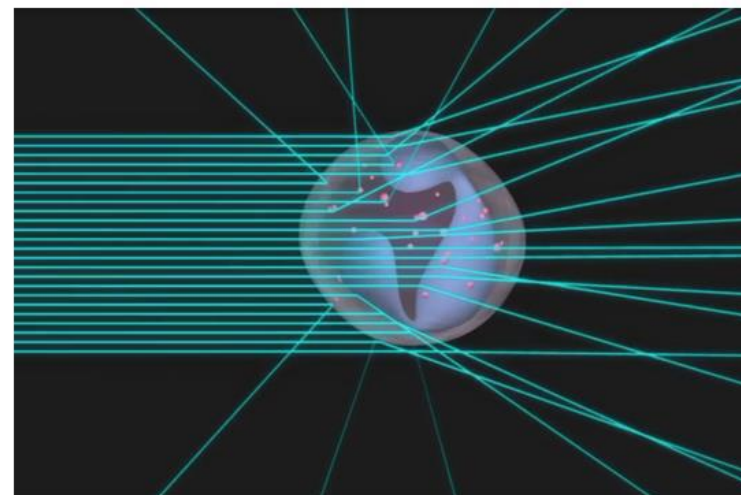
La cytométrie



La cytométrie

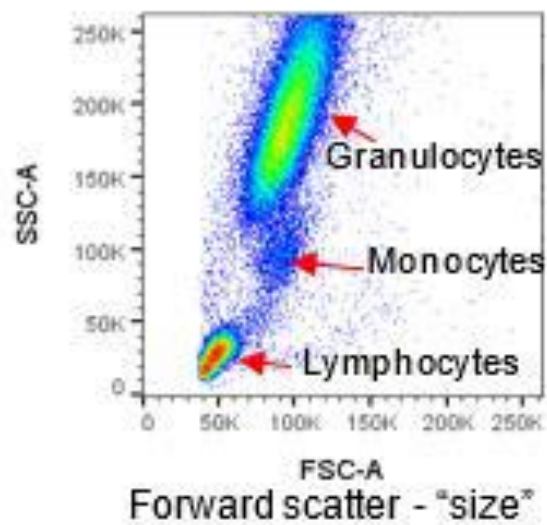


+

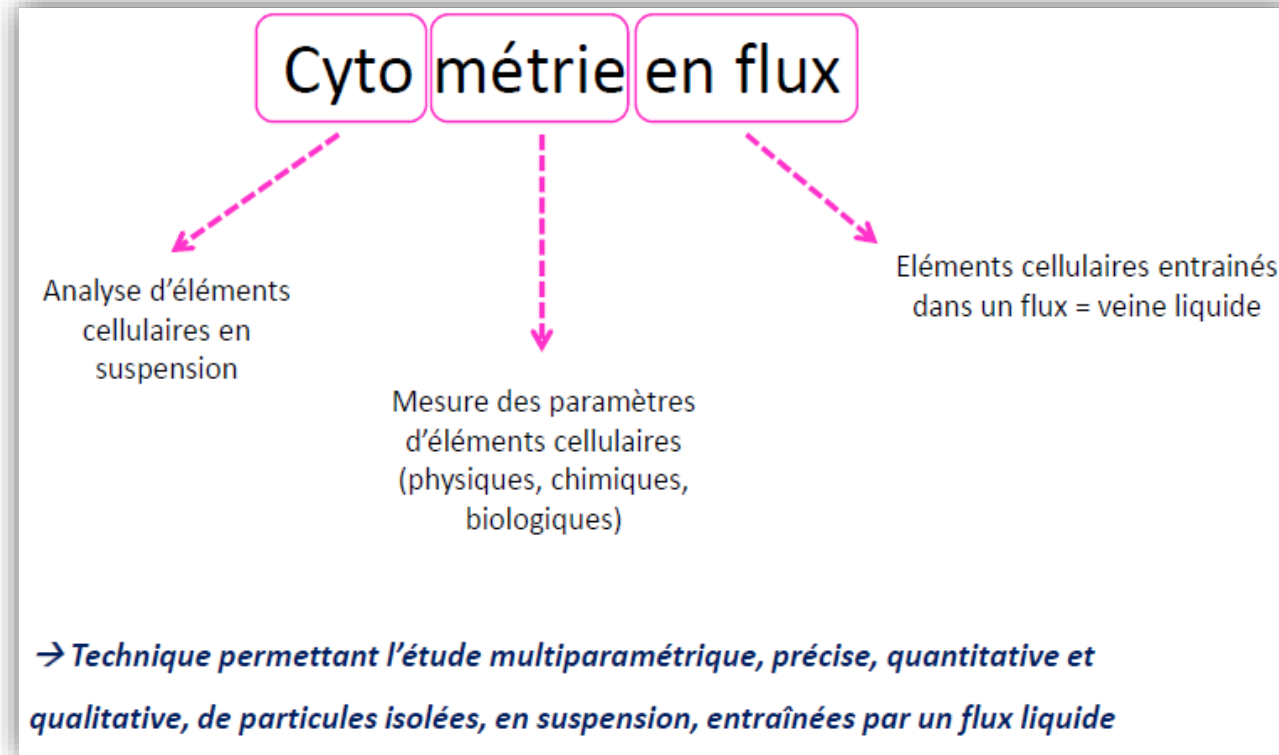


||

Side scatter
"complexity"







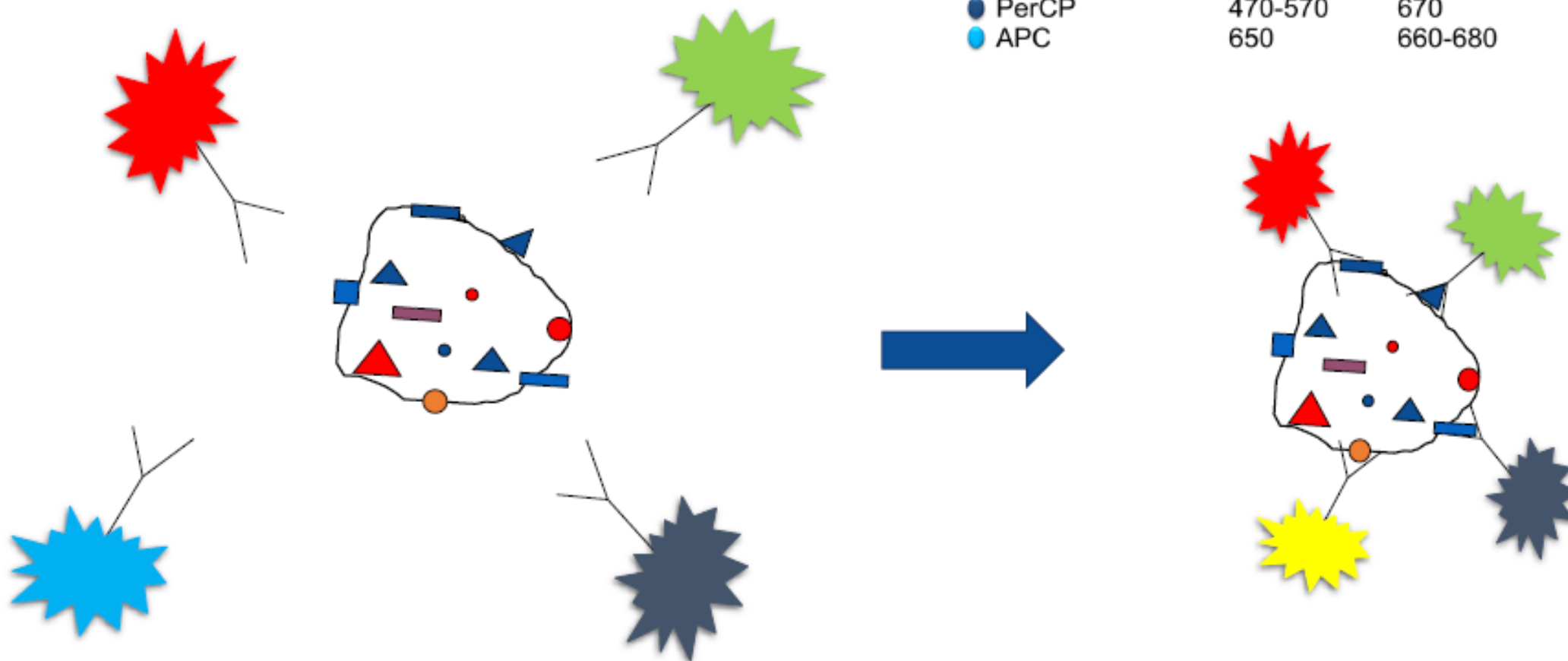
La cytométrie



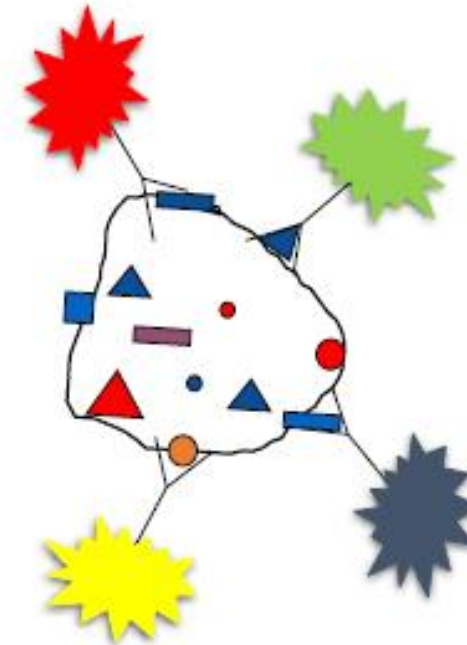
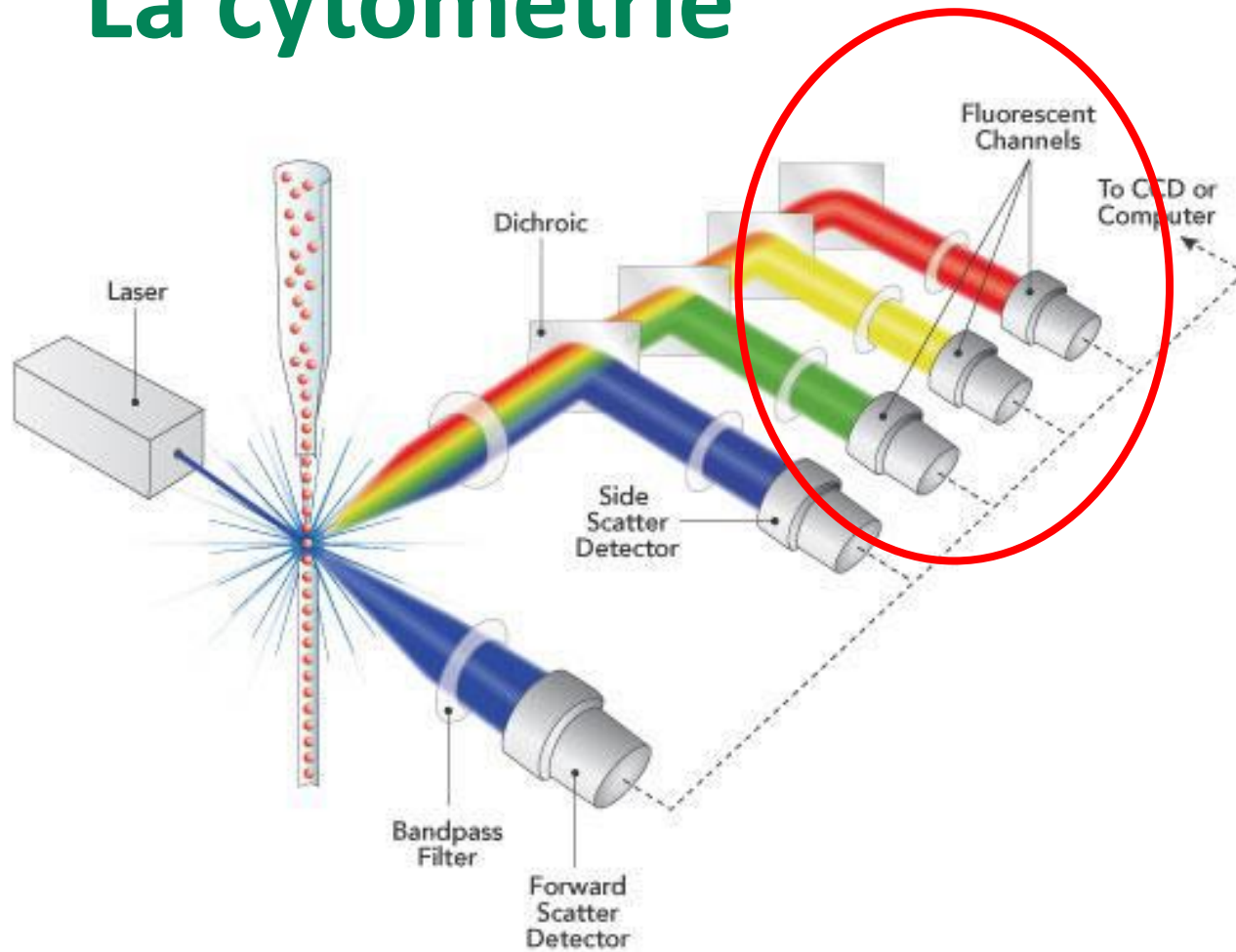
- Cellules en suspension < sang, moelle osseuse, liquides (LCR, LBA, ...), ganglion
- Marquage anticorps anti-CD + fluorochrome

La cytométrie

Fluorochrome	Excitation	Emission
 FITC	494-517	518
 PE	470-570	576
 PerCP	470-570	670
 APC	650	660-680

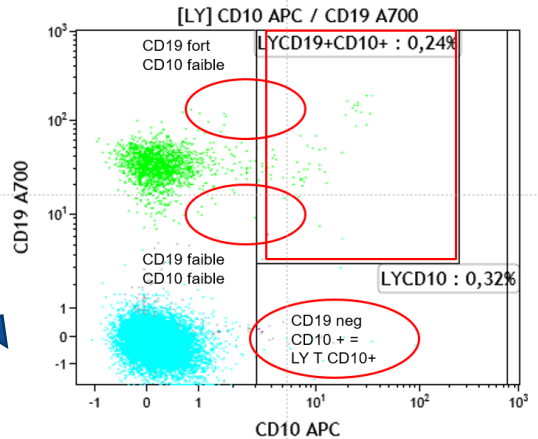
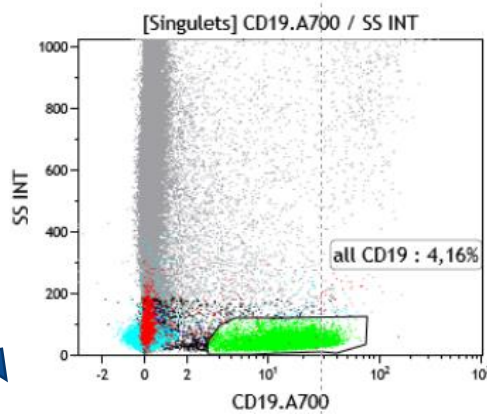
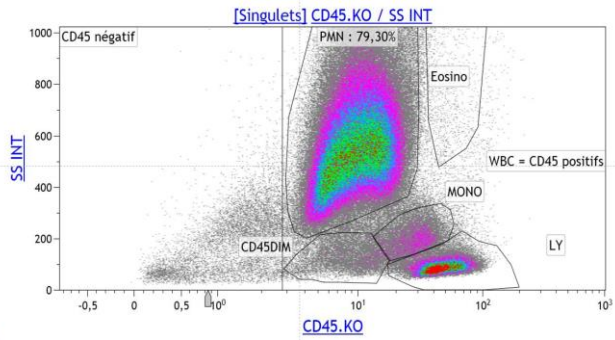


La cytométrie



Analyse supervisée

2 dimensions → $y = f(x)$



...

Item	Value
Date in format YYYYMMDD	20180109
TESTFILE	Tube B 10c new combi 20 BV
TESTNAME	Tube B 10c new combi 20 BV
CYTOMETERID	A550034
all CD5 % Gated	69,29
LYCD5 B % Gated	71,51
LYCD19 B % GATED	13,17
LYCD20 % Gated	35,71
LYCD22 % Gated	87,66
LYCD19_CD20 % Gated	14,00
LYCD19_CD22 % Gated	13,43
LYCD10_CD19 % Gated	1,30
LYCD5_CD19 % Gated	1,30
S_LAM % Gated	34,95
S_LAM % Gated	45,45
LYCD38 B % Gated	48,05
CD45DIM B % Gated	0,75
SAMPLEID1	P19
SAMPLEID3	
SAMPLEID1	C7747C13

Analyse supervisée

BIOINFORMATICS ORIGINAL PAPER Vol. 28 no. 15 2012, pages 2052–2058
doi:10.1093/bioinformatics/bts300

Data and text mining Advance Access publication May 17, 2012

flowPeaks: a fast unsupervised clustering for flow cytometry data via K-means and density peak finding

Yongchao Ge* and Stuart C. Sealfon
Department of Neurology and Center of Translational System Biology, Mount Sinai School of Medicine, New York, NY 10029, USA

it is (i) **highly subjective**, depending on the users' expertise and the sequences of the markers to draw the gates and where to draw the gates

(ii) tedious, for data consisting of n channels, the user needs to check and draw the gates on possibly $\binom{n}{2}$ pairs of 2D contour plots.

$n = 10 \rightarrow 45$ graphes
 $n = 12 \rightarrow 66$ graphes
 $n = 15 \rightarrow 105$ graphes

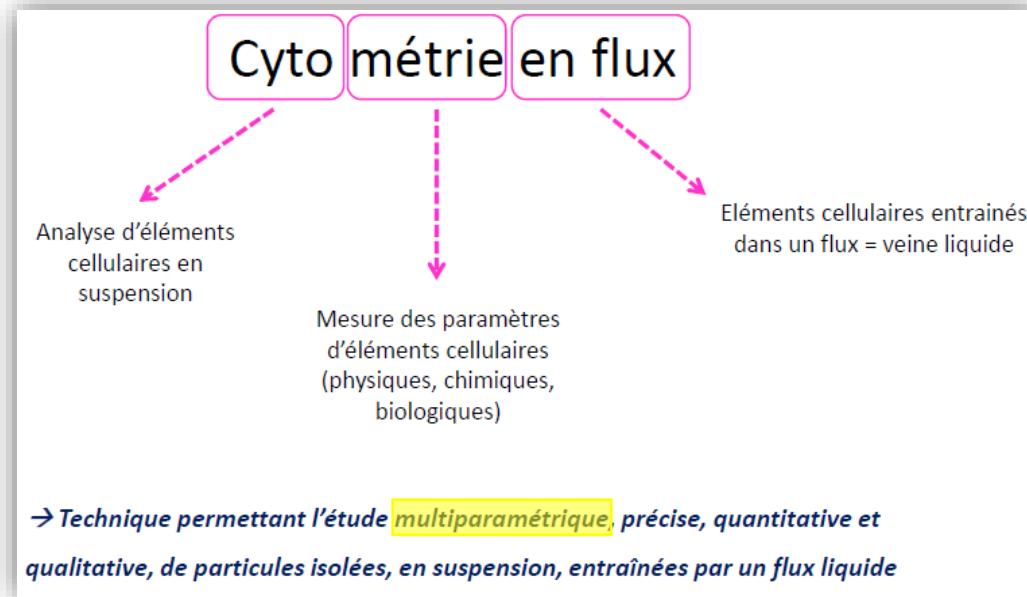
A robust and interpretable end-to-end deep learning model for cytometry data

Zicheng Hu^{a,1}, Alice Tang^a, Jaiveer Singh^a, Sanchita Bhattacharya^a, and Atul J. Butte^{a,1}

First, in the cell gating step, the original cytometry **data are reduced to summary statistics** of cell subsets, potentially leading to the loss of important information such as **the correlation** between cell markers and the distribution of marker expression within each cell subset. Second, the com-

Second, the commonly used approach requires all samples to **be clustered in the same way**, making it sensitive to batch effects and the choice of clustering methods.

Revenons à la définition...



Qu'est ce que la Cytométrie en flux?

C'est une technologie qui permet la mesure **simultanée de plusieurs caractéristiques** physiques d'une particule (cellule) en suspension.

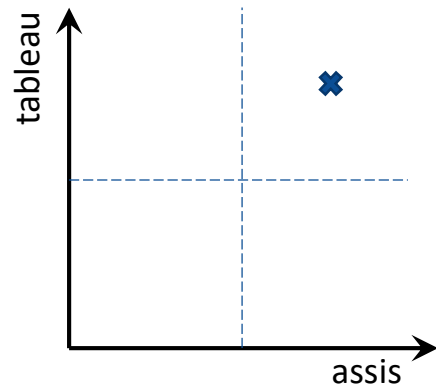
→ Analyse multivariée!

Analyse multivariée

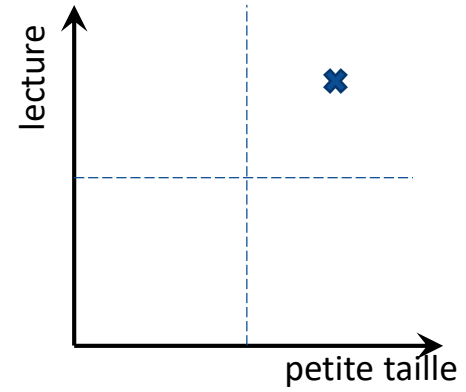
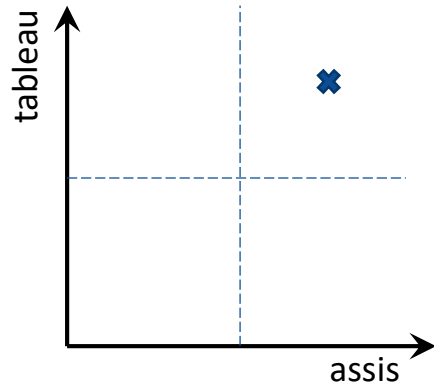
Qu'est-ce que l'Analyse multivariée ?

L'analyse multivariée regroupe les méthodes statistiques qui s'attachent à l'observation et au traitement simultané de plusieurs variables statistiques en vue d'en dégager une information synthétique pertinente. Les deux grandes catégories de méthodes d'analyse statistique multivariées sont, d'une part, les méthodes dites descriptives et, d'autre part, les méthodes dites explicatives. Les méthodes descriptives ont pour objectif

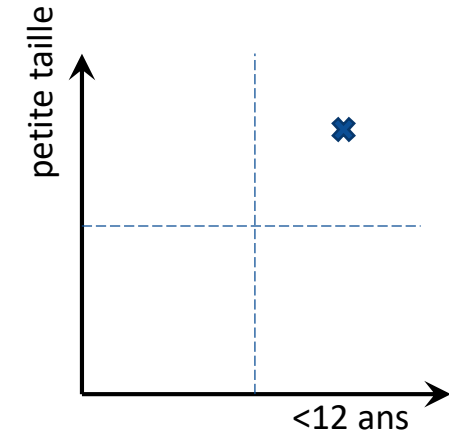
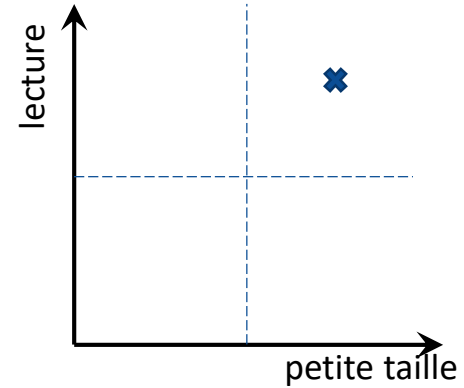
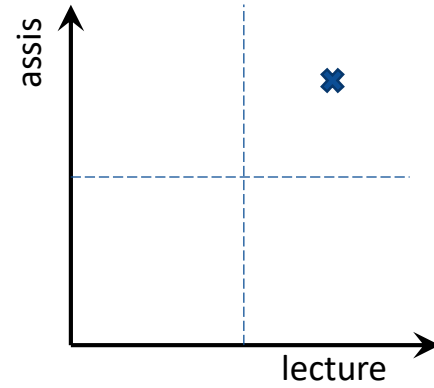
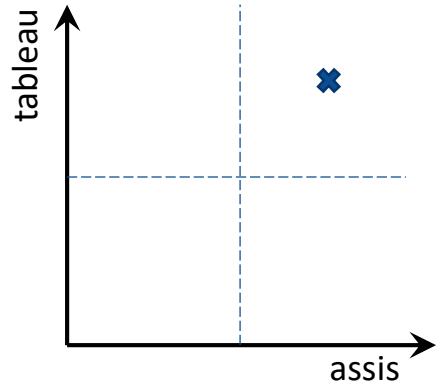
Imaginons...



Imaginons...



Imaginons...



Personne de <12 ans en train de lire assis devant un tableau...



Analyse multivariée

Méthodes descriptives

structurer et résumer un ensemble de données issues de plusieurs variables sans privilégier particulièrement l'une de ces variables

Les traitements et représentations graphiques visent à apporter une vision globale la plus exacte possible de l'ensemble des données analysées, en minimisant la déperdition d'information

- l'ACP ou analyse en composantes principales,
- l'AFC ou analyse factorielle des correspondances,
- l'ACM ou analyse des correspondances multiples,
- la typologie,
- les méthodes de classification.

Méthodes explicatives

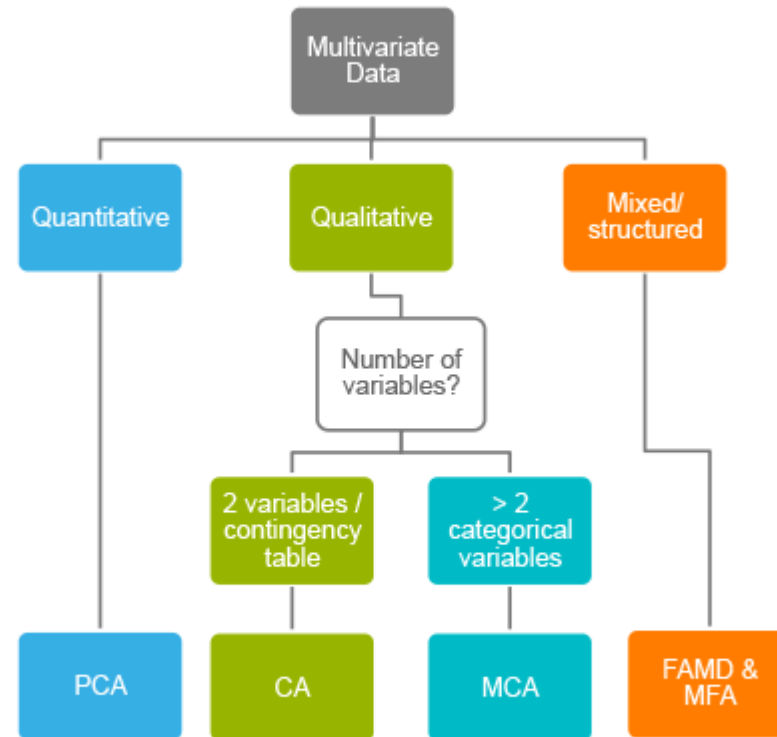
expliquer l'une des variables (dite dépendante) à l'aide de deux ou plusieurs variables explicatives (dites indépendantes)

- la régression multiple,
- la segmentation,
- l'analyse de la variance ou ANOVA,
- la régression logistique,
- l'analyse discriminante,
- l'analyse conjointe,
- les arbres de décision.

Méthodes en composantes principales

Principal Component Methods

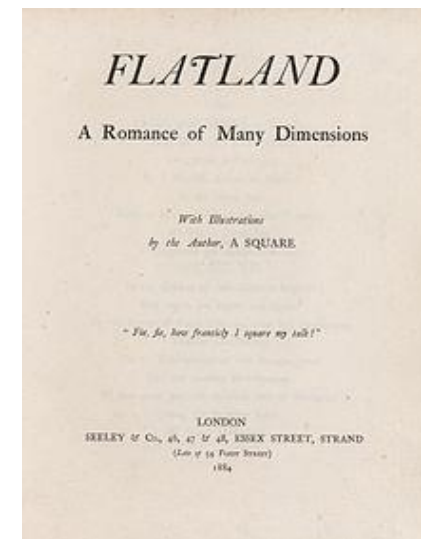
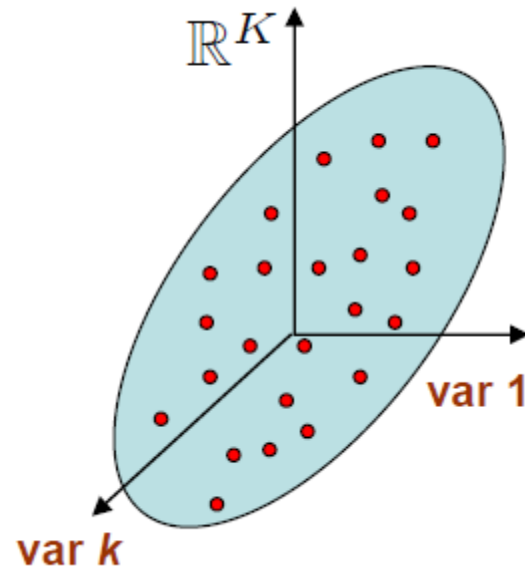
Methods to Summarize & Visualize Multivariate Data



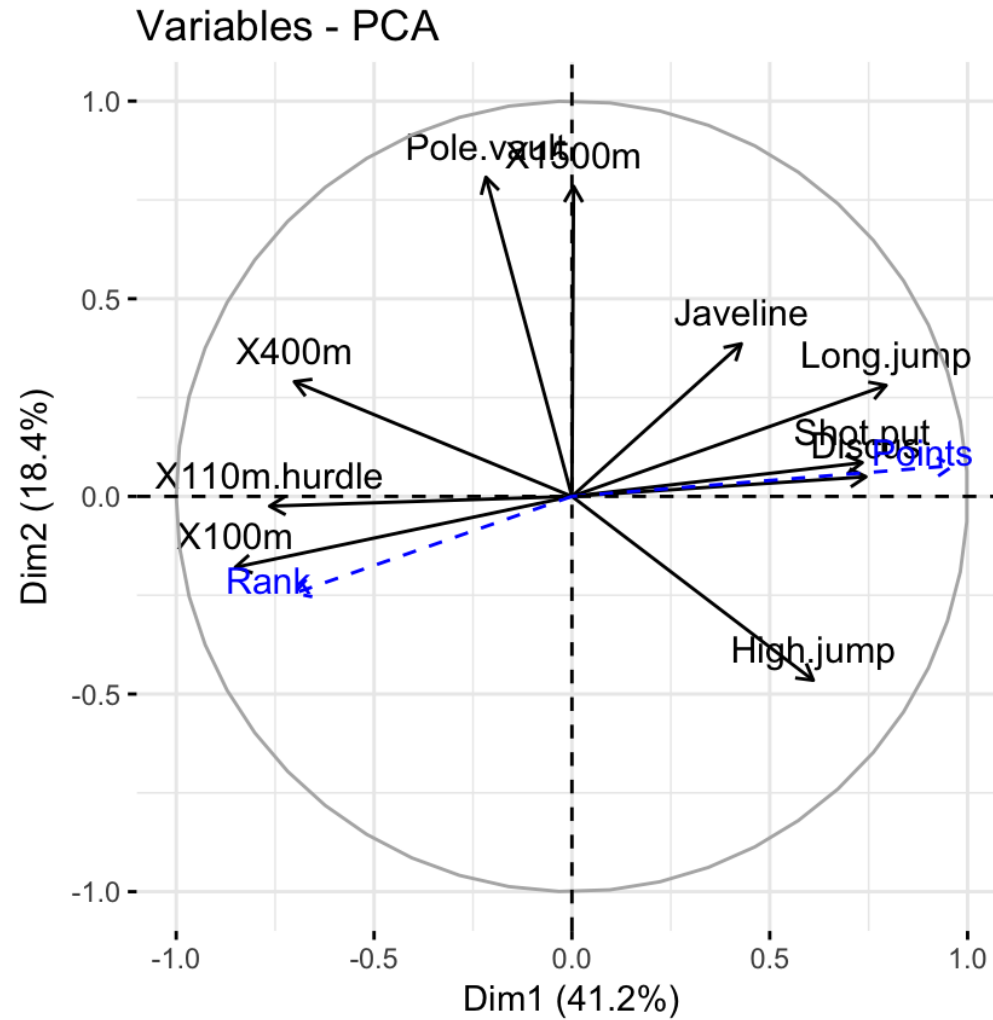
- PCA: Principal Component Analysis
- (M) CA: (Multiple) Correspondence Analysis
- FAMD: Factor Analysis of Mixed Data
- MFA: Multiple Factor Analysis

La philosophie

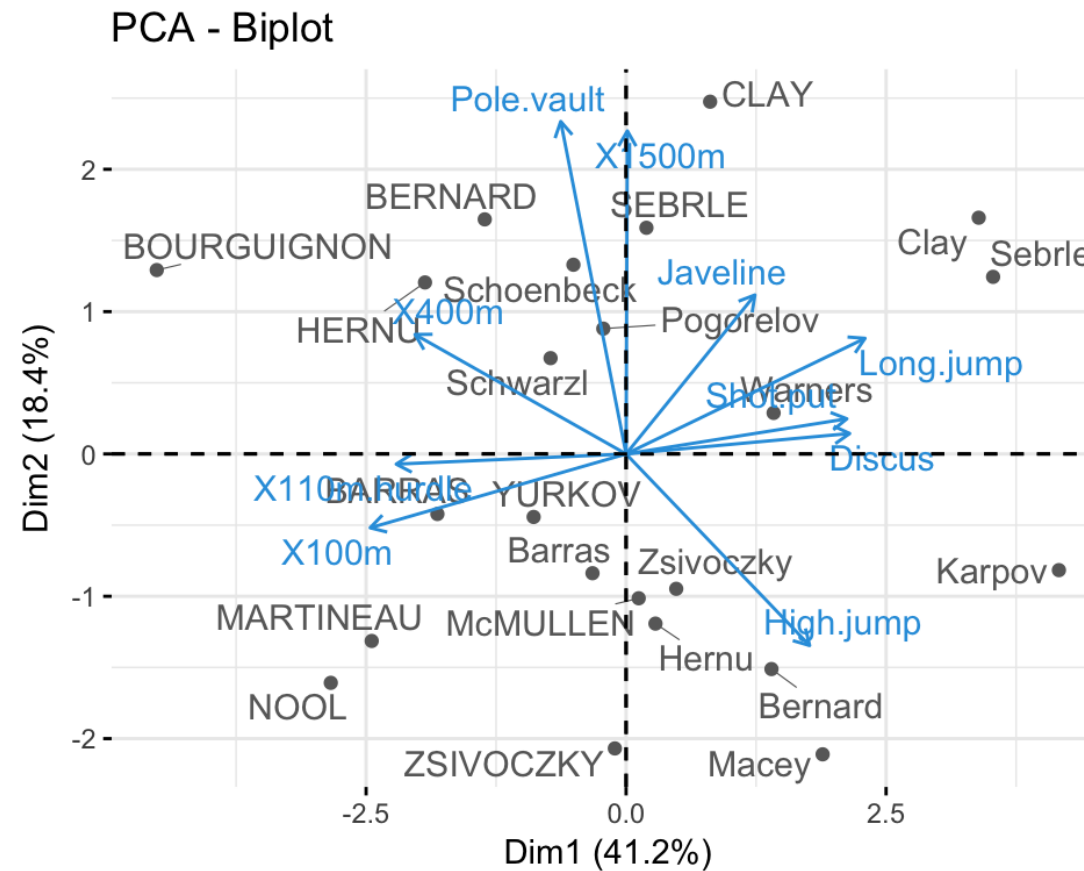
Placer les points dans un espace à n dimensions...
...et les projeter dans un espace à 2 dimensions!



Le cercle des corrélations



Le graphe des individus



APC et cytométrie

Programmation



1. Sauver les valeurs dans un fichier .csv
2. Lancer le code R

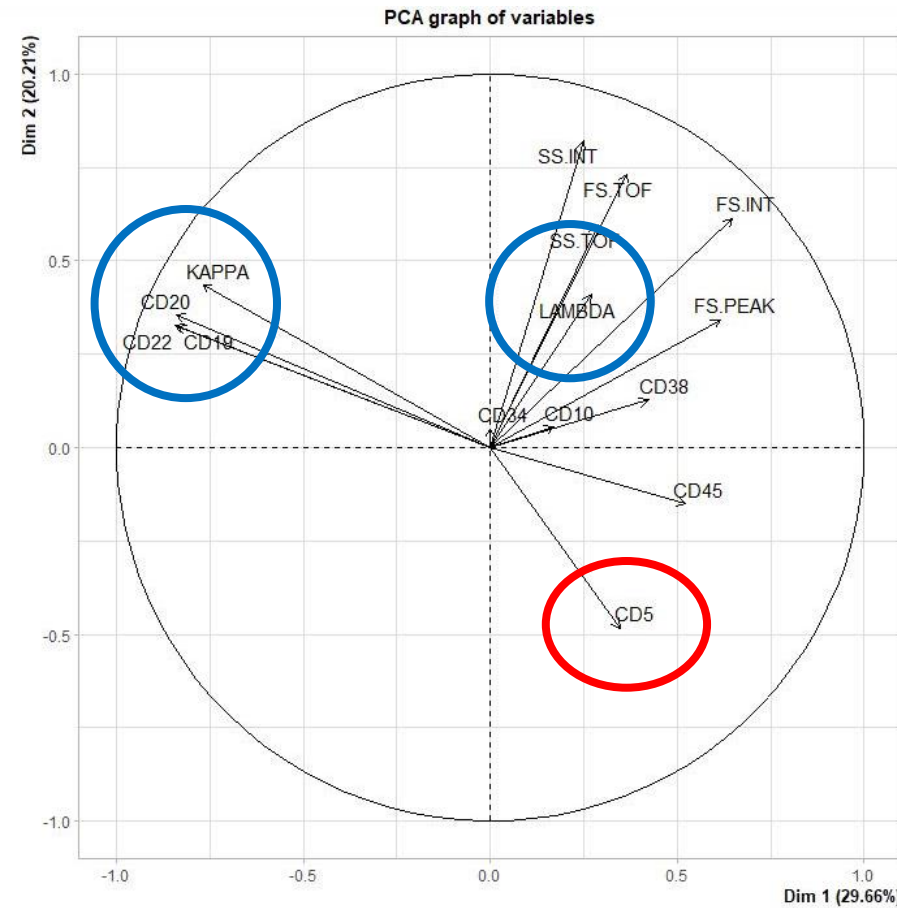
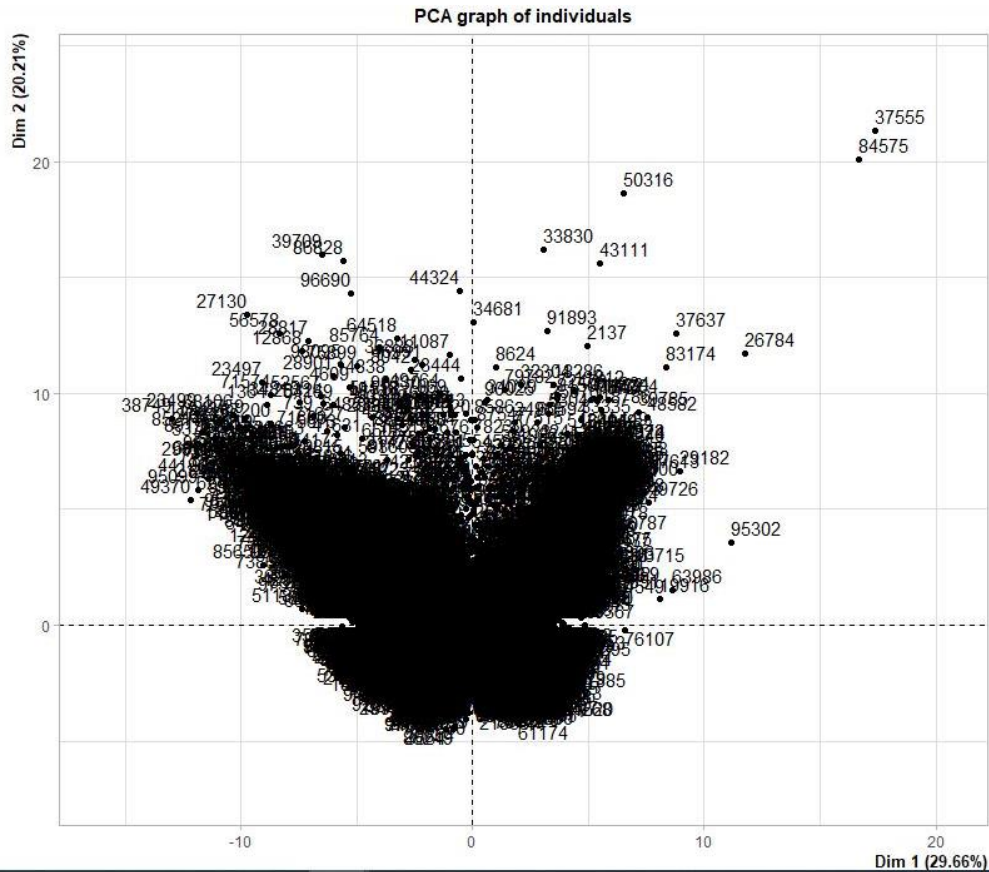
```
Cyto_ACPR.R
1 library(FactoMiner)
2 library(Factoshiny)
3 library("corrplot")
4 library(Factoextra)
5 data_cyto <- read.csv2("C:/Users/User/Documents/03_Boulot/LHUB-ULB/20200218_Sem1
6 summary(data_cyto)
7 res_cyto <- PCA(data_cyto)
8 summary(res_cyto)
9 fviz_eig(res_cyto, addlabels = TRUE, ylim = c(0, 50))
10 #summary(res_cyto, nbelements = Inf, file = "C:/Users/User/Documents/03_Boulot/LI
11 #res_cyto_2 <- PCASHiny(data_cyto)
12
13
8:1 (Top Level) ↓ R Script ↕

Environment History Connections
Global Environment
Environment is empty

Files Plots Packages Help Viewer

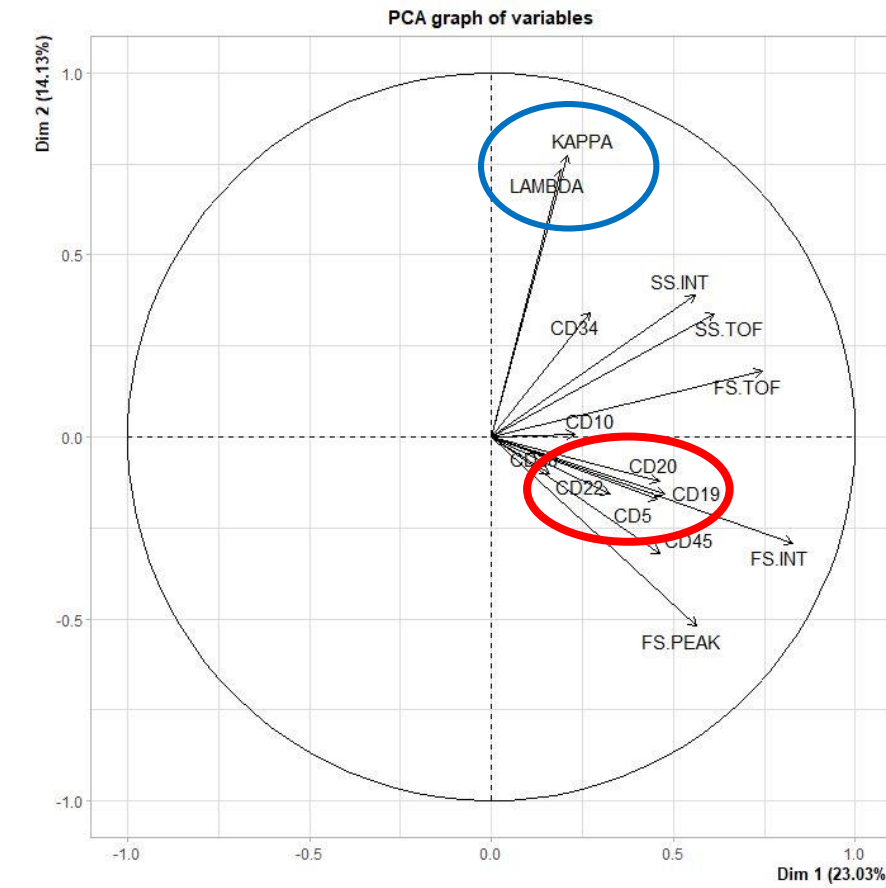
1st Qu.: 68.00 1st Qu.: -118.7 1st Qu.: 1343 1st Qu.: 43.6
Median : 73.00 Median : -7.0 Median : 2197 Median : 142.4
Mean : 74.03 Mean : 932.5 Mean : 3051 Mean : 186.4
3rd Qu.: 77.00 3rd Qu.: 99.1 3rd Qu.: 3614 3rd Qu.: 237.4
Max. :398.00 Max. :982921.6 Max. :109172 Max. :76488.4
CD22 CD38 CD10 CD19
Min. : 289.2 Min. : -692.2 Min. : -1439.67 Min. : 3616
1st Qu.: 2170.3 1st Qu.: 593.1 1st Qu.: 46.11 1st Qu.:13864
Median : 3851.7 Median : 1682.1 Median : 279.59 Median :20367
Mean : 5213.1 Mean : 3430.2 Mean : 463.65 Mean :22543
3rd Qu.: 6607.8 3rd Qu.: 4023.3 3rd Qu.: 540.37 3rd Qu.:29193
Max. :48649.3 Max. :169252.7 Max. :101520.40 Max. :84574
CD5 CD20 CD45
Min. : -1402 Min. : 74 Min. : -38857
1st Qu.: 5134 1st Qu.: 9609 1st Qu.: 15884
Median : 8008 Median : 27323 Median : 20609
Mean : 9676 Mean : 44099 Mean : 22275
3rd Qu.: 11965 3rd Qu.: 59962 3rd Qu.: 26427
Max. :283569 Max. :680341 Max. :468909
> res_cyto <- PCA(data_cyto)
> |
```

Résultats: LLC



- CE: Présence d'une population pathologique B CD19+ CD5^{neg} CD10^{neg} monoclonale kappa d'expression normale, celle-ci est de phénotype: CD20+ CD22+ CD38^{neg} CD11c^{neg} CD103^{neg} CD180+ CD27^{neg} CD44+ CD25^{neg}.

Résultats: LLC



- CE: Persistance de l'infiltration sanguine par la population lymphocytaire représentant 74% des lymphocytes totaux (soit 17449 cellules/ μ l), de phénotype LLC-B : CD19+CD5+CD10-CD20+dim et CD22+ dim CD38+ partiel et dont le rapport kappa lamda est ininterprétable en surface.

Analyse non supervisée

- Hierarchical clustering (SPADE algorithm)
- K-means clustering (flowMeans, flowPeaks)
- K-medoids clustering
- Density-based clustering (DBSCAN)
- Model-based clustering (FLAME, flowClust, flowMerge, SWIFT)
- Spectral clustering (SamSPECTRAL)
- Self-organizing (flowSOM)
- Dimensionality reduction (viSNE, t-SNE)
- ...

Received: 26 November 2020 | Revised: 21 January 2021 | Accepted: 28 January 2021
DOI: 10.1002/cyto.a.24320

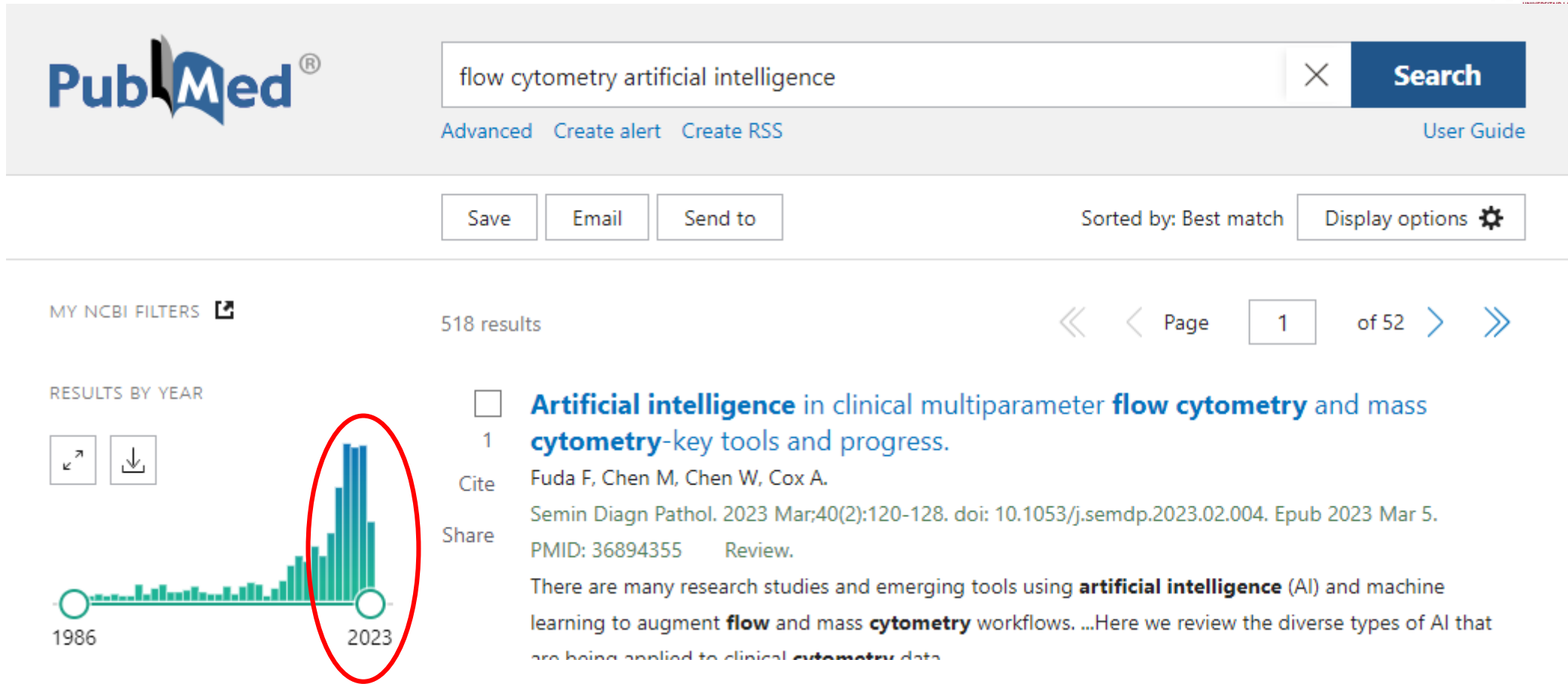
REVIEW ARTICLE

 **CYTOMETRY**
Journal of Quantitative Cell Science PART A

Current trends in flow cytometry automated data analysis software

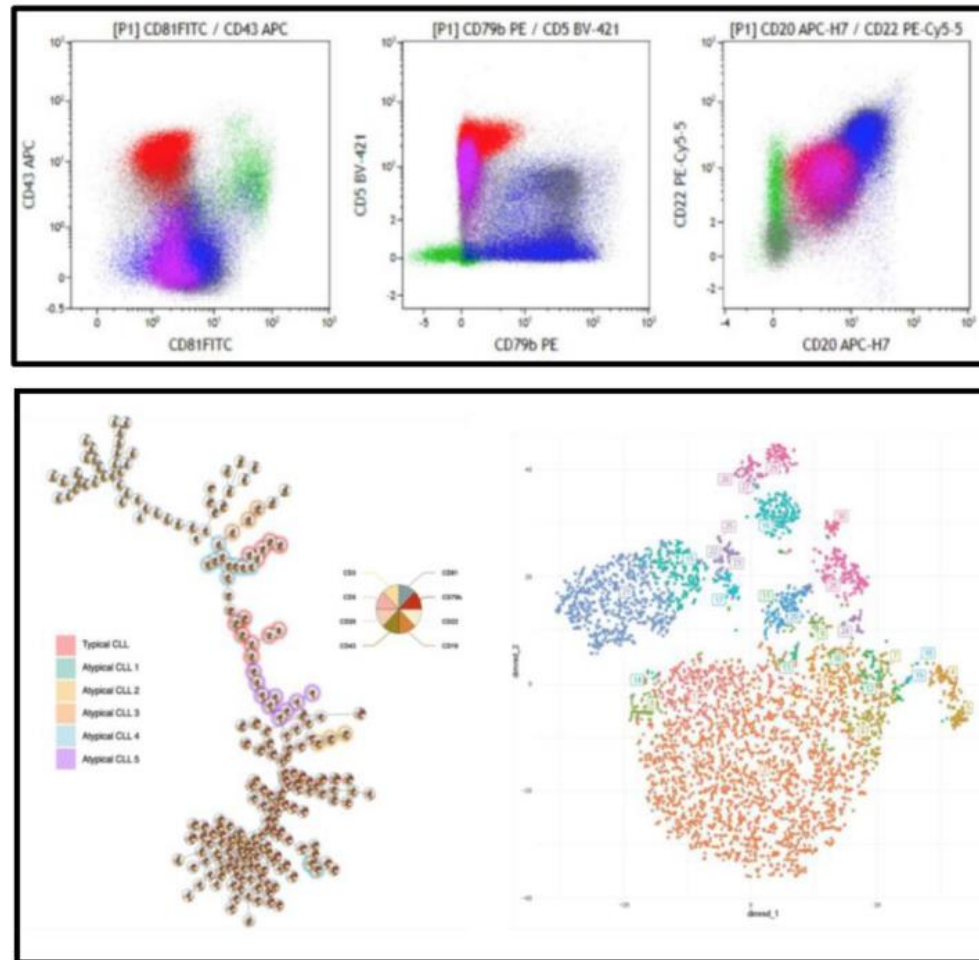
Melissa Cheung¹ | Jonathan J. Campbell² | Liam Whitby³  | Robert J. Thomas¹ | Julian Braybrook² | Jon Petzing¹

Flow cytometry & AI



The screenshot shows a PubMed search interface. At the top left is the PubMed logo. A search bar contains the text "flow cytometry artificial intelligence" with a search button labeled "Search". Below the search bar are links for "Advanced", "Create alert", "Create RSS", and "User Guide". Below the search bar are buttons for "Save", "Email", and "Send to". To the right of these buttons, it says "Sorted by: Best match" and "Display options" with a gear icon. Below the search bar, there is a section for "MY NCBI FILTERS" and "518 results". To the left of the results is a "RESULTS BY YEAR" section with a bar chart showing the number of results per year from 1986 to 2023. The chart shows a significant increase in results starting around 2015, peaking in 2023. A red circle highlights the 2023 data point. Below the chart are two icons: a left arrow and a download icon. The first search result is displayed, starting with a checkbox, the number "1", and the title "Artificial intelligence in clinical multiparameter flow cytometry and mass cytometry-key tools and progress." The authors are listed as Fuda F, Chen M, Chen W, Cox A. The journal is Semin Diagn Pathol. 2023 Mar;40(2):120-128. The PMID is 36894355 and it is a Review. The abstract text is partially visible: "There are many research studies and emerging tools using artificial intelligence (AI) and machine learning to augment flow and mass cytometry workflows. ...Here we review the diverse types of AI that are being applied to clinical cytometry data."

Flow cytometry & AI



standard 2D plot gating
«classic multiparametric
Flow Cytometry»

*Immunophenotypic
data analysis
for diagnostic
haematology*

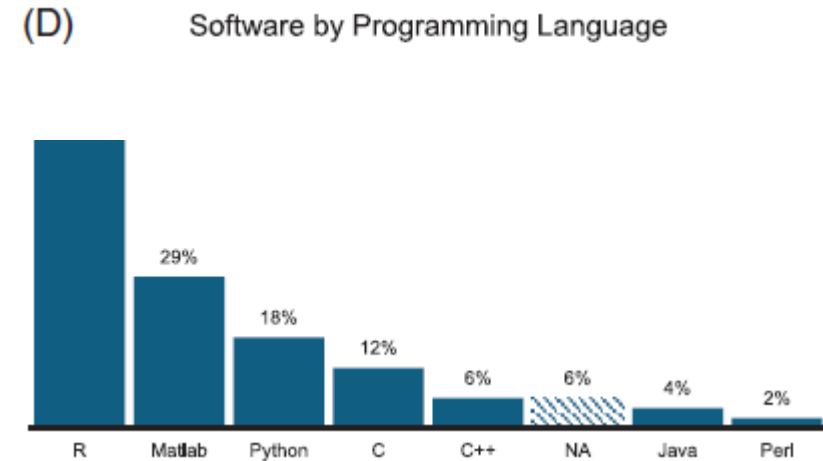
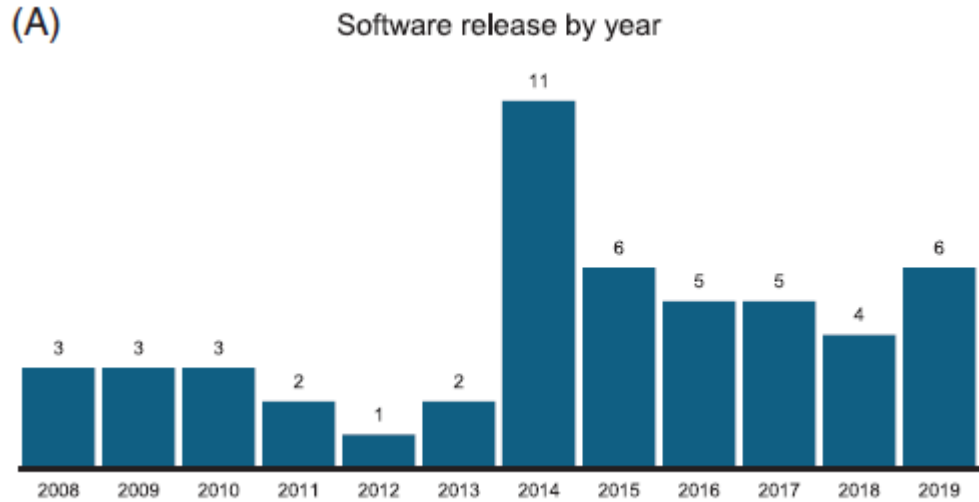
**AI-based
clustering algorithms**
«Computational Flow
Cytometry» (CFC)

FIGURE 1 Evolution of immunophenotypic data analysis (2D plots, top; MST, bottom left; t-SNE, bottom right; all graphs from Nguyen et al.¹).

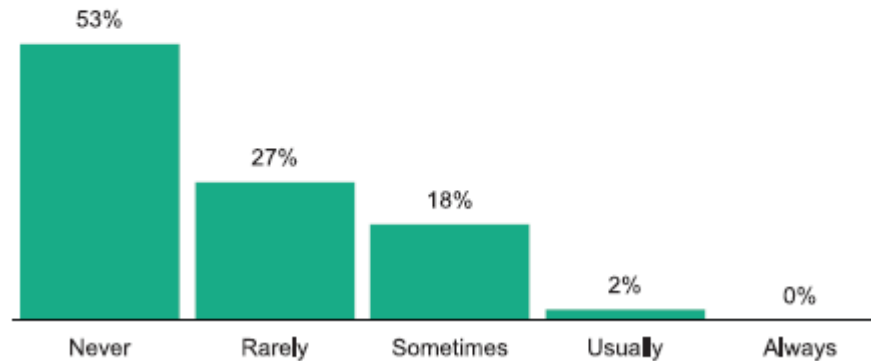
L'intelligence artificielle peut-elle remplacer l'humain ?

LE CERCLE - Digitalisation, intelligence artificielle (IA), robots performants, on pourrait croire à la fin de l'emploi humain. Pour Charles Cuvelliez, professeur à l'école polytechnique de Bruxelles, les compétences sociales propres à l'homme sont un avantage majeur face l'IA et la robotisation, encore faut-il renforcer ces acquis par des formations adéquates.

Flow cytometry & AI



(A) How often do you use automated flow cytometry data analysis software to identify cell populations?
Responses: 49



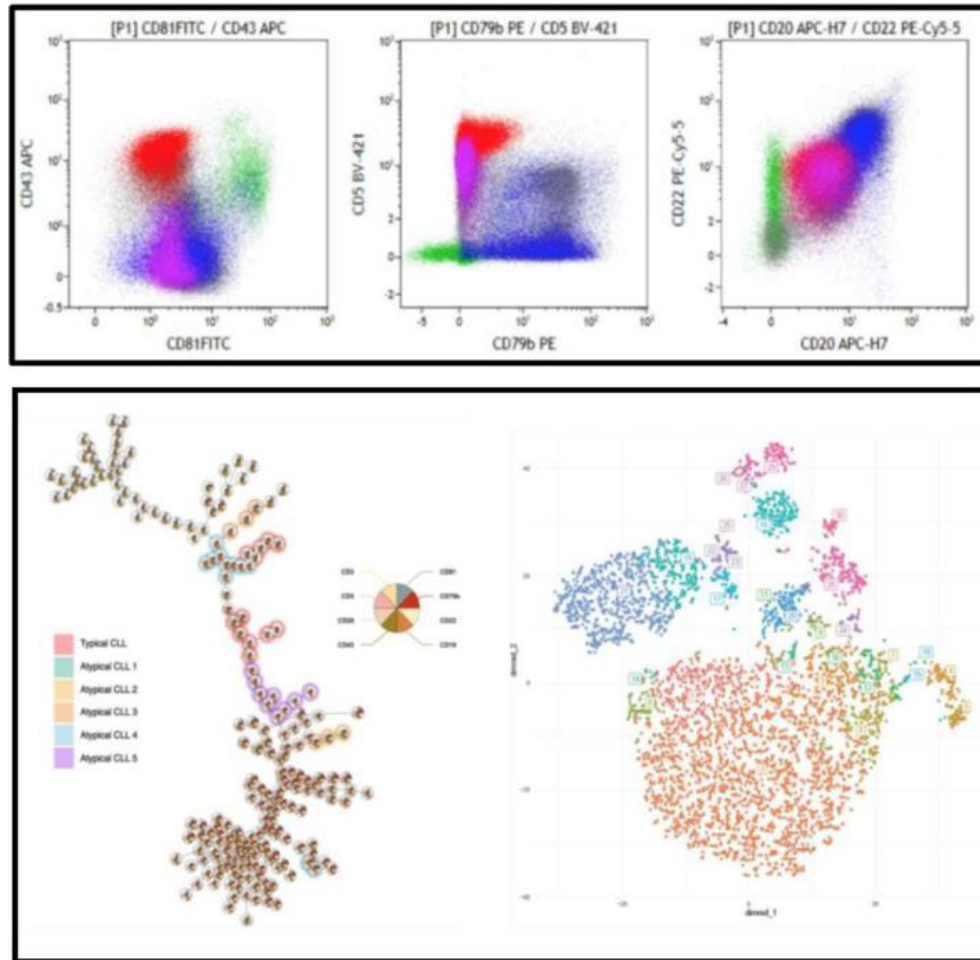
Results of a survey of clinical laboratories on the use of automated flow cytometry software

REVIEW ARTICLE

Current trends in flow cytometry automated data analysis software

Melissa Cheung¹ | Jonathan J. Campbell² | Liam Whitby³ | Robert J. Thomas¹ | Julian Braybrook² | Jon Petzing¹

Comment interpreter?



standard 2D plot gating
«classic multiparametric
Flow Cytometry»

*Immunophenotypic
data analysis
for diagnostic
haematology*

**AI-based
clustering algorithms**
«Computational Flow
Cytometry» (CFC)

FIGURE 1 Evolution of immunophenotypic data analysis (2D plots, top; MST, bottom left; t-SNE, bottom right; all graphs from Nguyen et al.¹).

Cas clinique

A 55-year-old man with a 1-month history of asthenia, right hypochondrial pain, and weight loss was referred to the emergency department for hepatic mass evaluation.

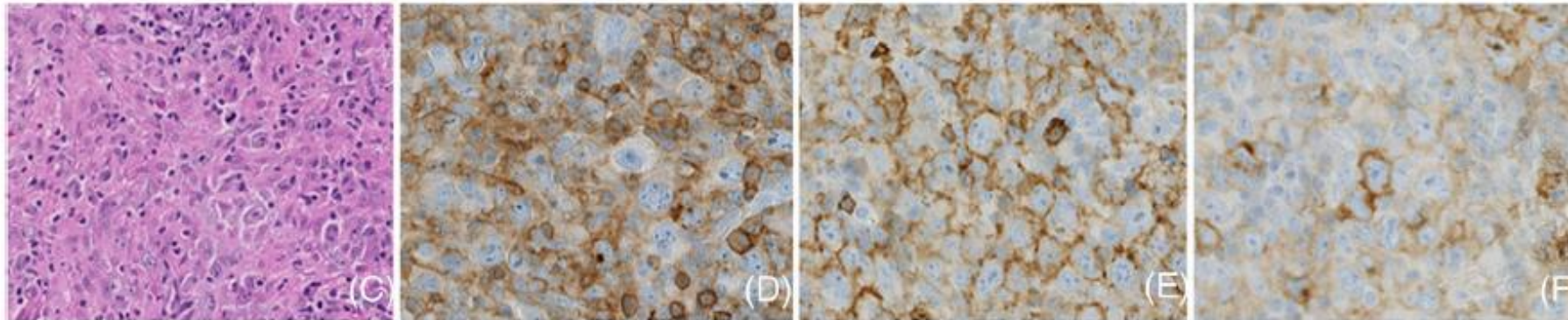
Abdominal computed tomography scan revealed a large necrotic mass in the liver, a second necrotic mass adjacent to the superior mesenteric vessels, and numerous subdiaphragmatic lymph nodes (aorta, right kidney, retro-crural, mesenteric, iliac, and inguinal). Hypermetabolic activity was confirmed by positron emission

➔ **Biopsie inguinale**

Cas clinique

A 55-year-old man with a 1-month history of asthenia, right hypochondrial pain, and weight loss was referred to the emergency department for hepatic mass evaluation.

Abdominal computed tomography scan revealed a large necrotic mass in the liver, a second necrotic mass adjacent to the superior mesenteric vessels, and numerous subdiaphragmatic lymph nodes (aorta, right kidney, retro-crural, mesenteric, iliac, and inguinal). Hypermetabolic activity was confirmed by positron emission



Histological examination revealed infiltration by sheets of large lymphoid cells displaying irregularly shaped nuclei with macronucleoli and moderately abundant, finely granular cytoplasm.

cells were positive for common leukocyte antigen (CLA), CD20, and CD30 and negative for anaplastic lymphoma kinase (Figure 1D-F)

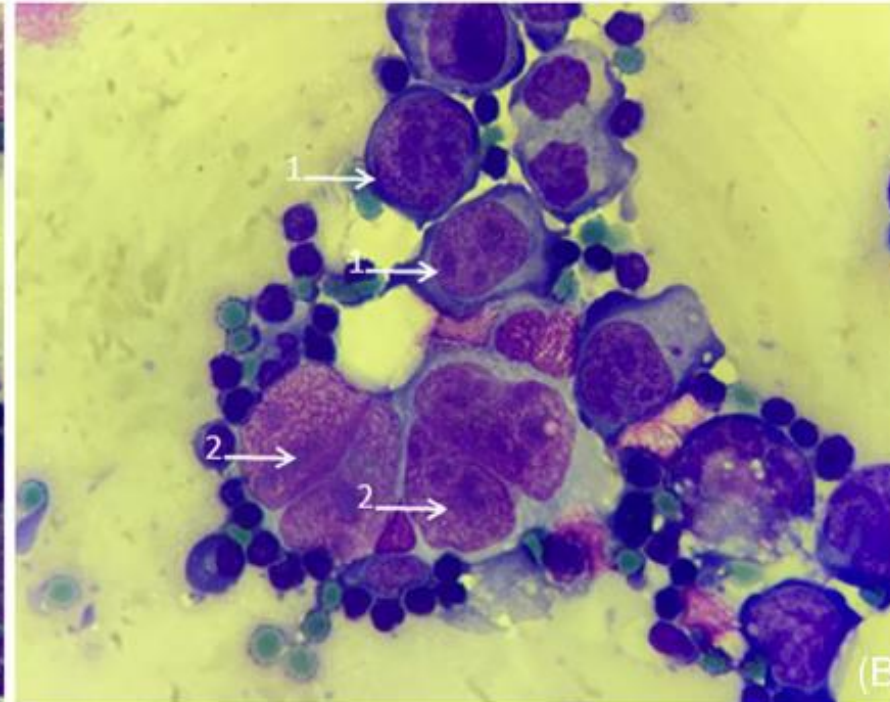
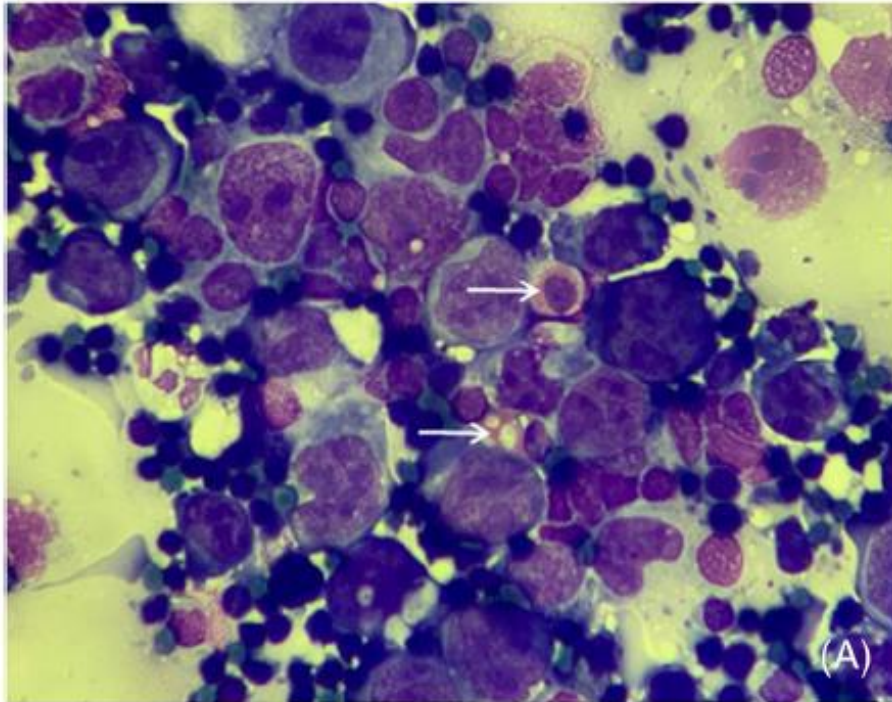
➔ **Diffuse large B-cell lymphoma (DLBCL)**

Cas clinique

A 55-year-old man with a 1-month history of asthenia, right hypochondrial pain, and weight loss was referred to the emergency department for hepatic mass evaluation.

Abdominal computed tomography scan revealed a large necrotic mass in the liver, a second necrotic mass adjacent to the superior mesenteric vessels, and numerous subdiaphragmatic

Au labo...



Inguinal lymph node biopsy imprint showing a heterogeneous background containing (A) eosinophils and (B, 1) large cells with various nucleocytoplasmic ratios, occasionally irregular nuclear contour, dispersed and delicately condensed mixed chromatin, multiple prominent nucleoli and deeply basophilic cytoplasm and (B, 2) scattered cells with sternbergoid features and decreased cytoplasmic basophilia

Cas clinique

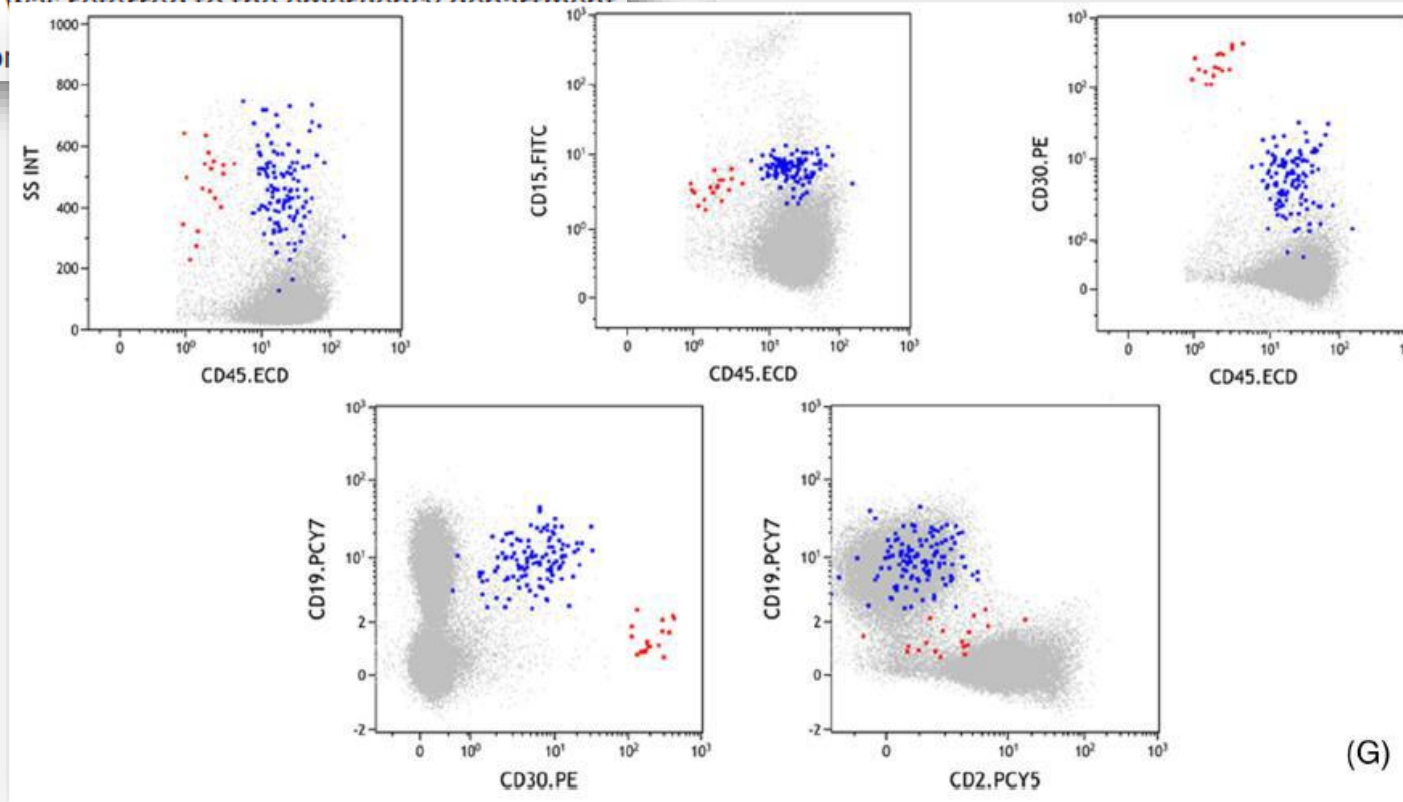


LABORATOIRE HOSPITALIER UNIVERSITAIRE DE BRUXELLES
HUB-ULB
UNIVERSITAIR LABORATORIUM BRUSSEL

A 55-year-old man with a 1-month history of asthenia, right hypochondrial pain, and weight loss was referred to the emergency department for hepatic mass evaluation.

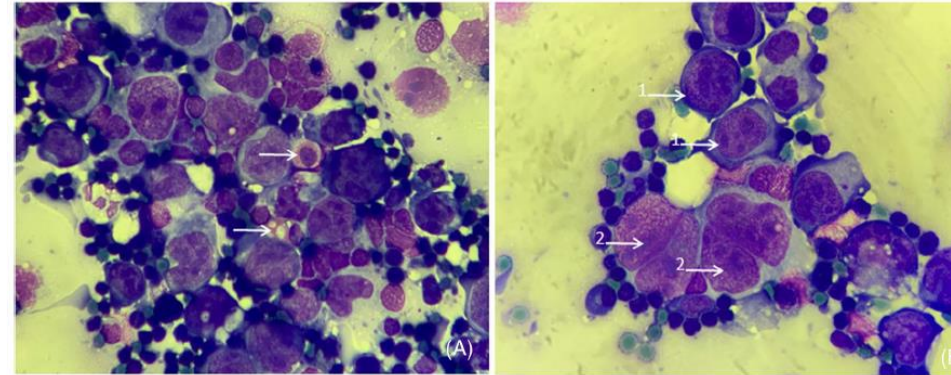
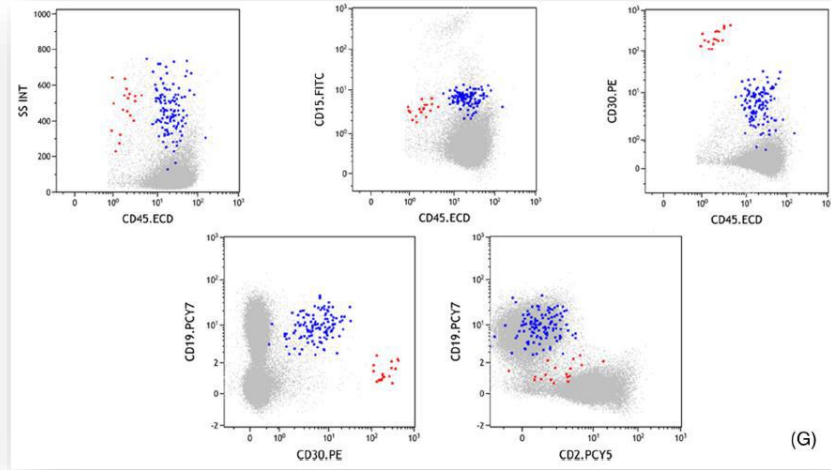
Abdominal computed tomography scan revealed a hypodense, heterogenous, necrotic mass adjacent to the liver, with subdiaphragmatic mesenteric, iliac, and retroperitoneal lymphadenopathy by positron emission tomography.

Au labo...



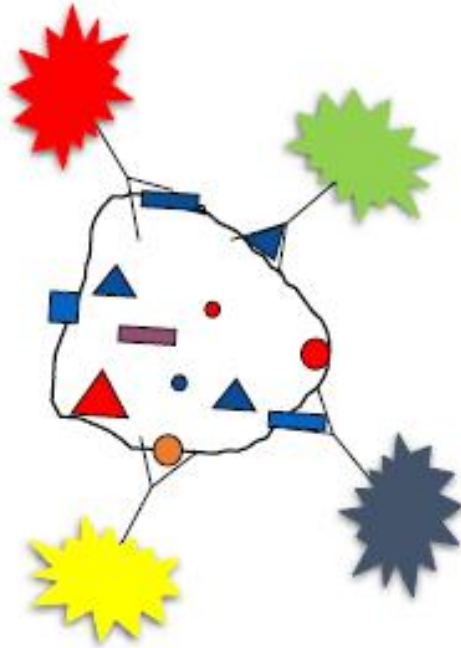
Flow cytometry charts showing the presence of two cell populations with different immunophenotypes: a population with high side scatter (SSC), CD19+, CD20+, CD22+, CD15+dim, CD30+dim, CD45+ cells (blue population) and a few CD19-, CD20-, CD15+dim, CD30+bright, CD45- cells (red population).

Cas clinique



Une des faiblesses des APC → les événements rares!

Autres methodes?



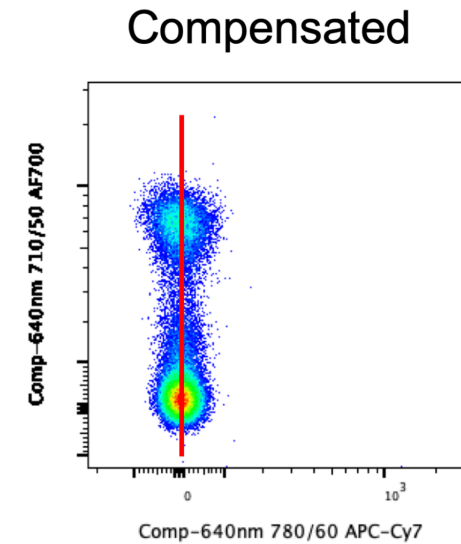
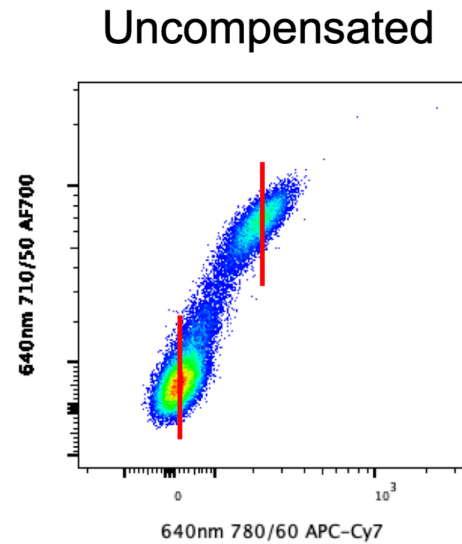
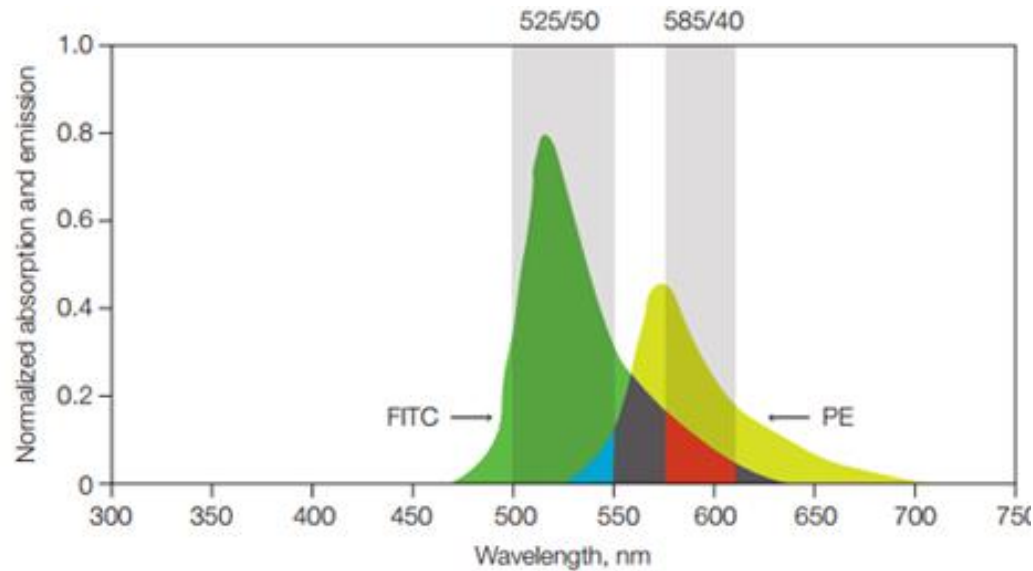
- Taille
- Granularité
- 10 couleurs

$$\left[\begin{array}{l} \textit{Taille} \\ \textit{Granularité} \\ \textit{Fluo}_1 \\ \textit{Fluo}_2 \\ \textit{Fluo}_3 \\ \textit{Fluo}_4 \\ \textit{Fluo}_5 \\ \textit{Fluo}_6 \\ \textit{Fluo}_7 \\ \textit{Fluo}_8 \\ \textit{Fluo}_9 \\ \textit{Fluo}_{10} \end{array} \right]$$

- Calcul vectoriel ?
- Calcul matriciel ?

Et la technologie?

La compensation



Video

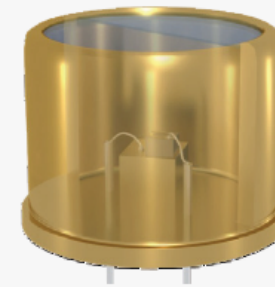
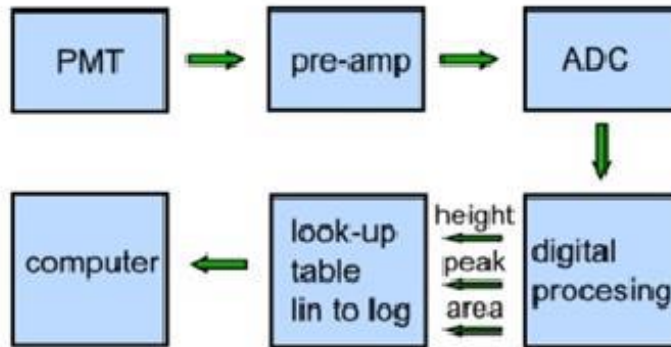
Automated flow cytometric compensation with the MACSQuant Analyzer Family

Free your lab staff from unnecessary tasks so they can get on with their research: the MACSQuant Analyzer Family boasts automated compensation (and more) to save time, money, and to reduce opportunities for human error. It's as simple as press play and walk away.

Et la technologie?

L'effet photo-électrique

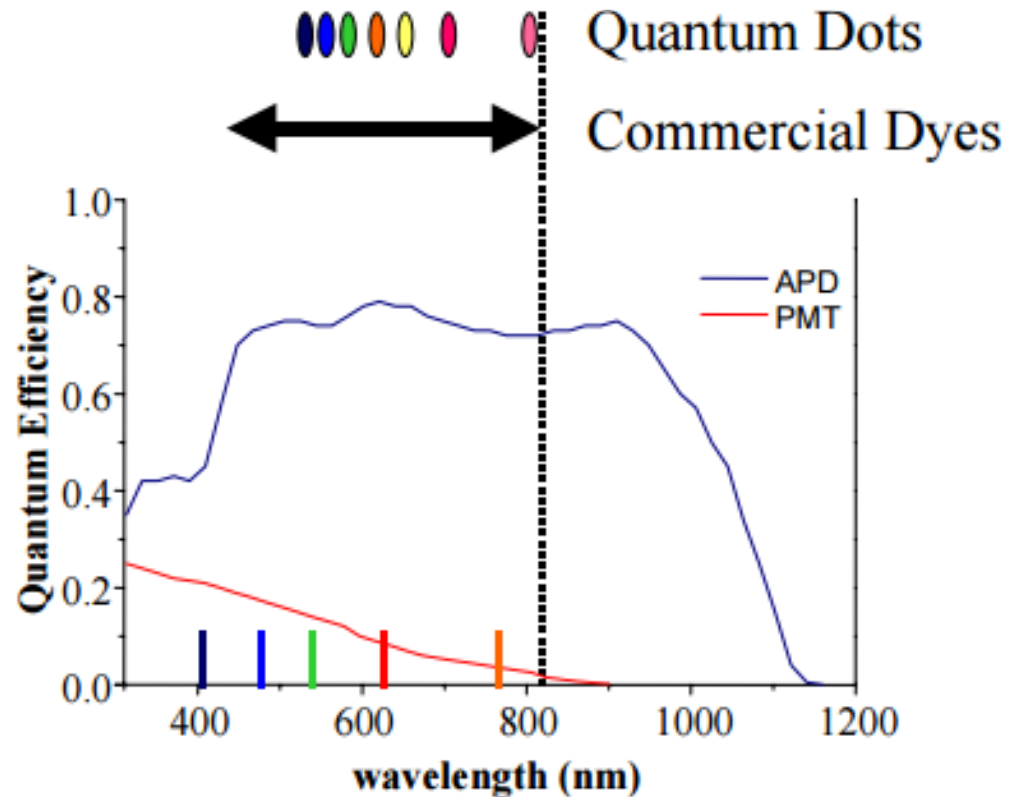
digital



Avalanche Photodiode.

The DxFLEx flow cytometer uses Avalanche Photodiode detectors instead of PMTs. The low electronic noise contributes to the resolution capabilities of the instrument.

Et la technologie?

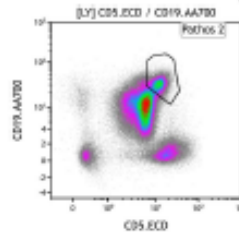
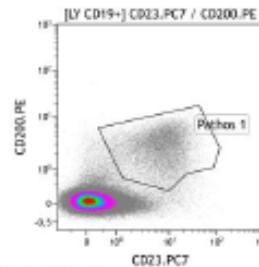


Perspectives?

- Régression linéaire
- Fonction multivariée
- ACP

Bases mathématiques de l'A.I.

Découverte de "nouveaux profils"?

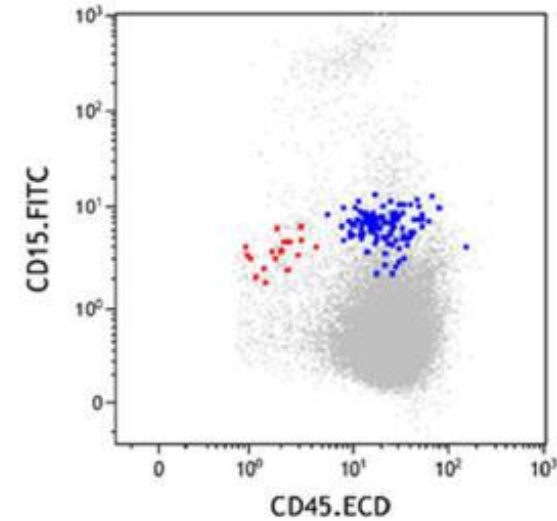


Perspectives?

- Régression linéaire
 - Fonction multivariée
 - ACP
- Bases mathématiques de l'A.I.



Découverte de "nouveaux profils"?



"Evénements rares"

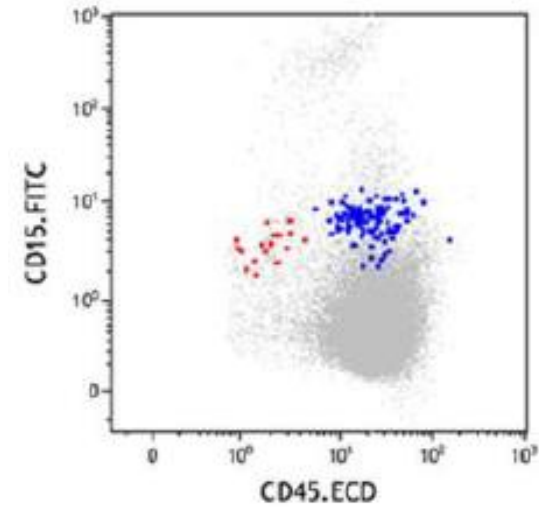
**Marqueurs d'évolution?
Marqueurs d'agressivité?**

Comment comprendre?



AI
Machine learning
Big Data

Le macroscopique



Evénements "rares"

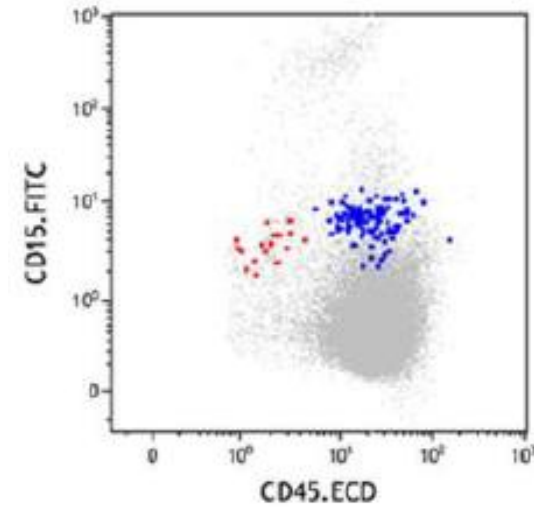
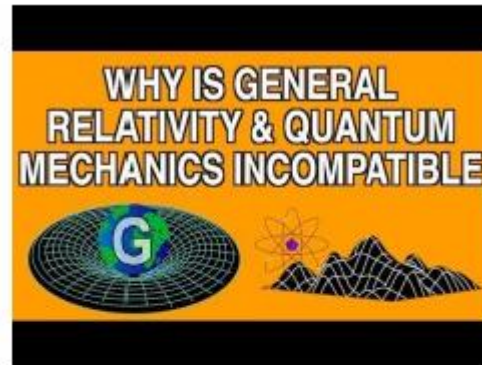
Le microscopique

Comment comprendre?



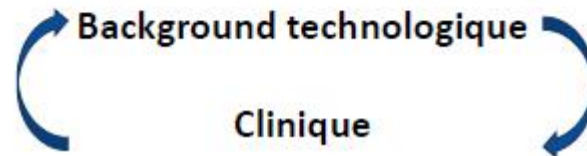
AI
Machine learning
Big Data

Le macroscopique



Evénements "rares"

Le microscopique



Merci...



... à toute l'équipe du LHUB-ULB