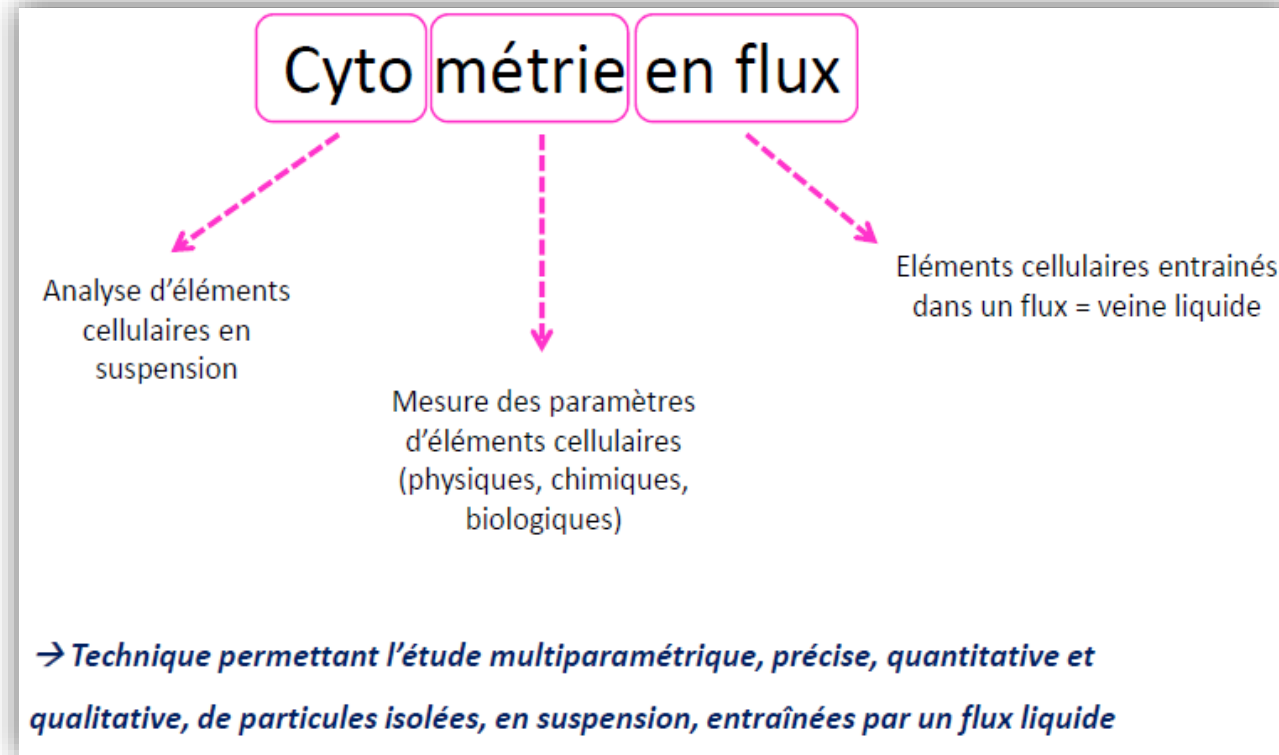# Le futur de la cytométrie en flux au laboratoire d'hématologie?
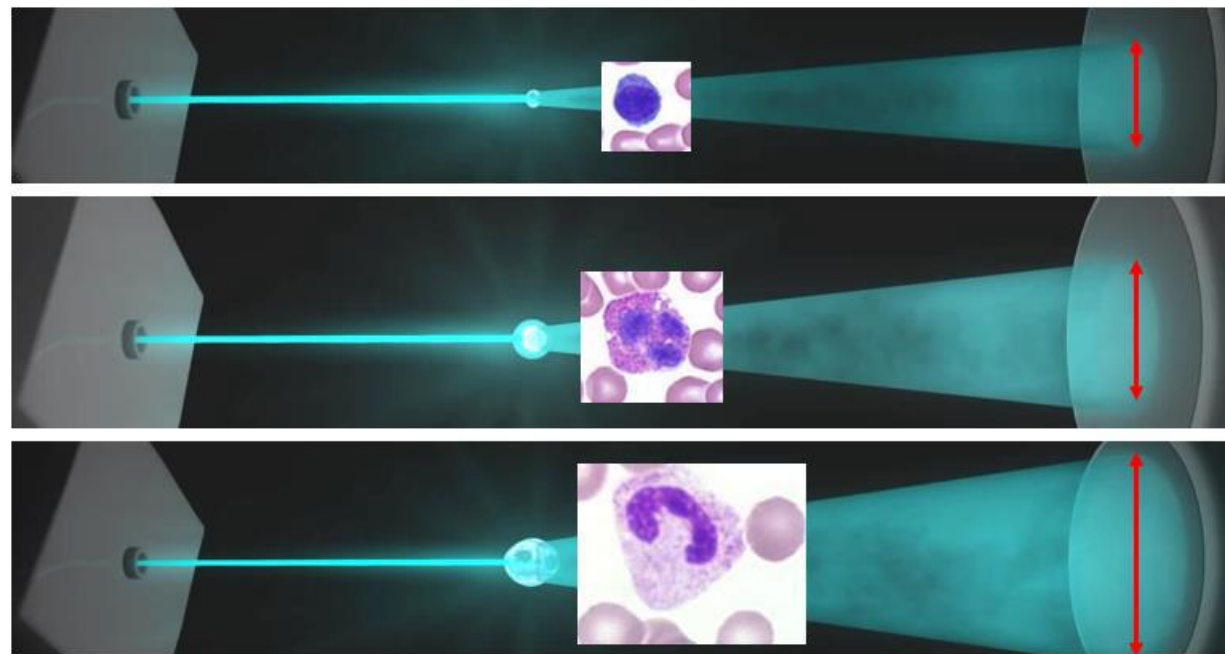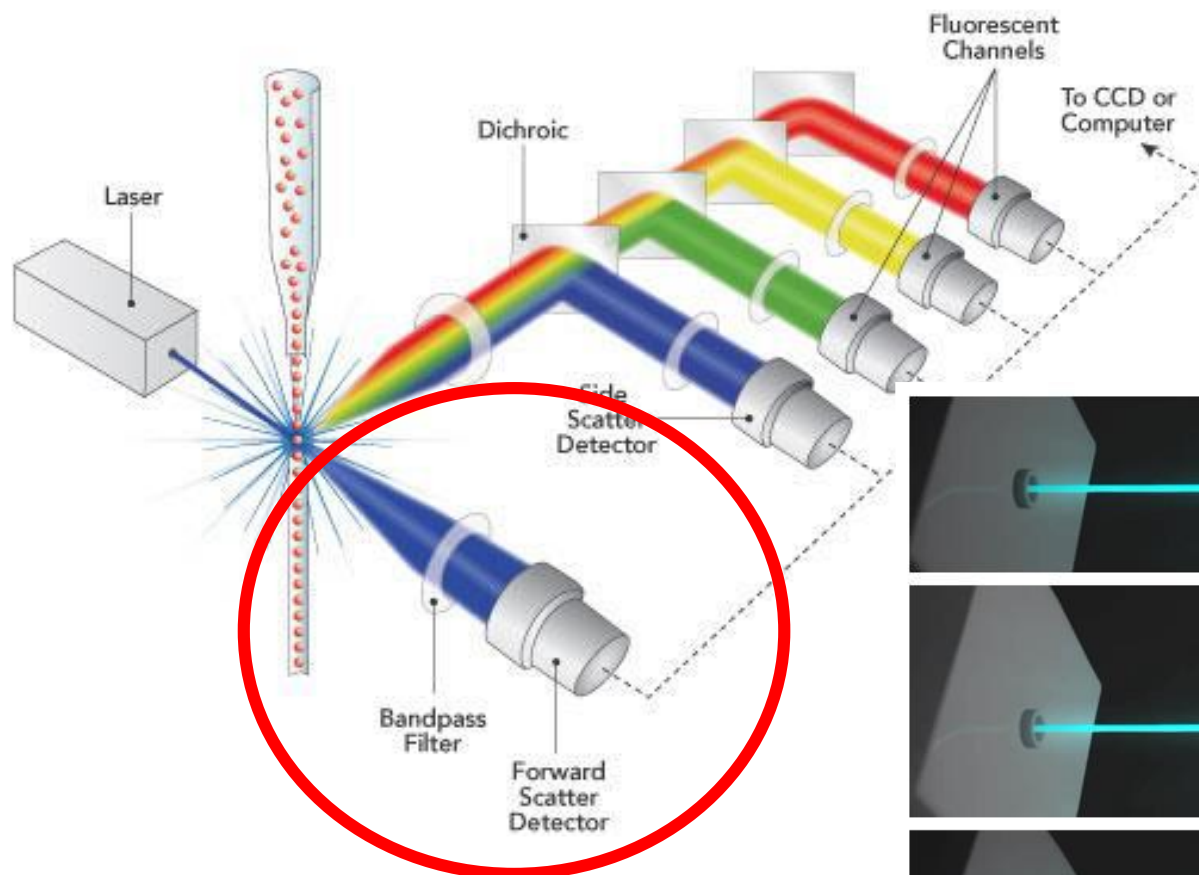
Valéry Daubie

La cytométrie

Cyto | métrie | en flux

Analyse d'éléments cellulaires en suspension

Mesure des paramètres d'éléments cellulaires (physiques, chimiques, biologiques)

Eléments cellulaires entrainés dans un flux = veine liquide

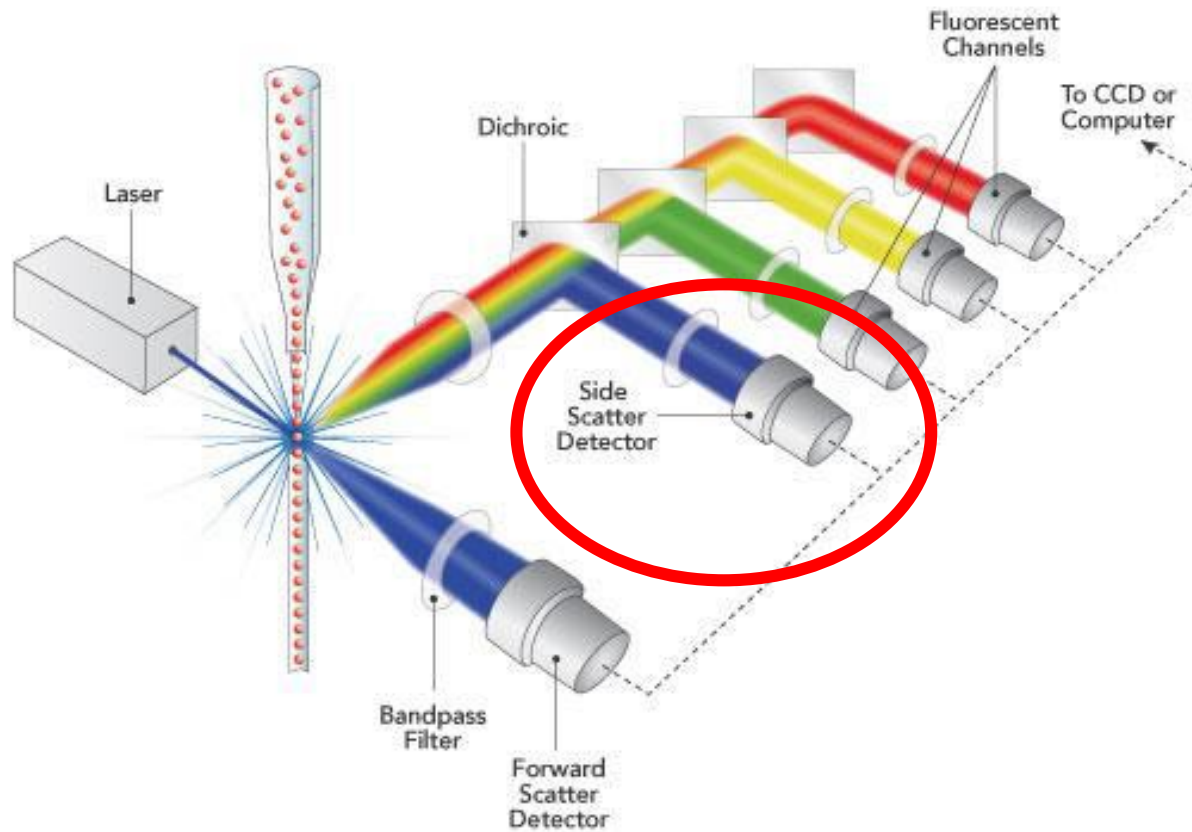→ *Technique permettant l'étude multiparamétrique, précise, quantitative et qualitative, de particules isolées, en suspension, entraînées par un flux liquide*

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

La cytométrie

La cytométrie

La cytométrie

La cytométrie

Cyto métrie en flux

Analyse d'éléments cellulaires en suspension

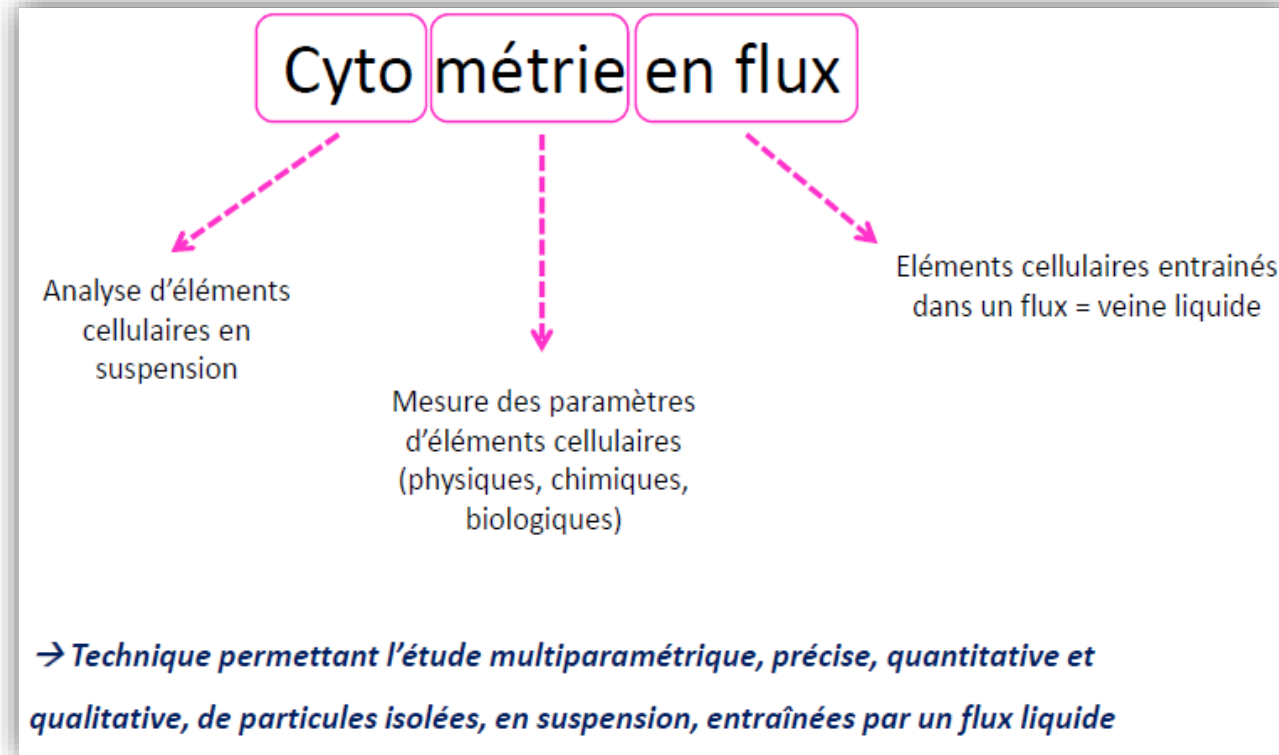Mesure des paramètres d'éléments cellulaires (physiques, chimiques, biologiques)

Eléments cellulaires entrainés dans un flux = veine liquide

→ *Technique permettant l'étude multiparamétrique, précise, quantitative et qualitative, de particules isolées, en suspension, entraînées par un flux liquide*

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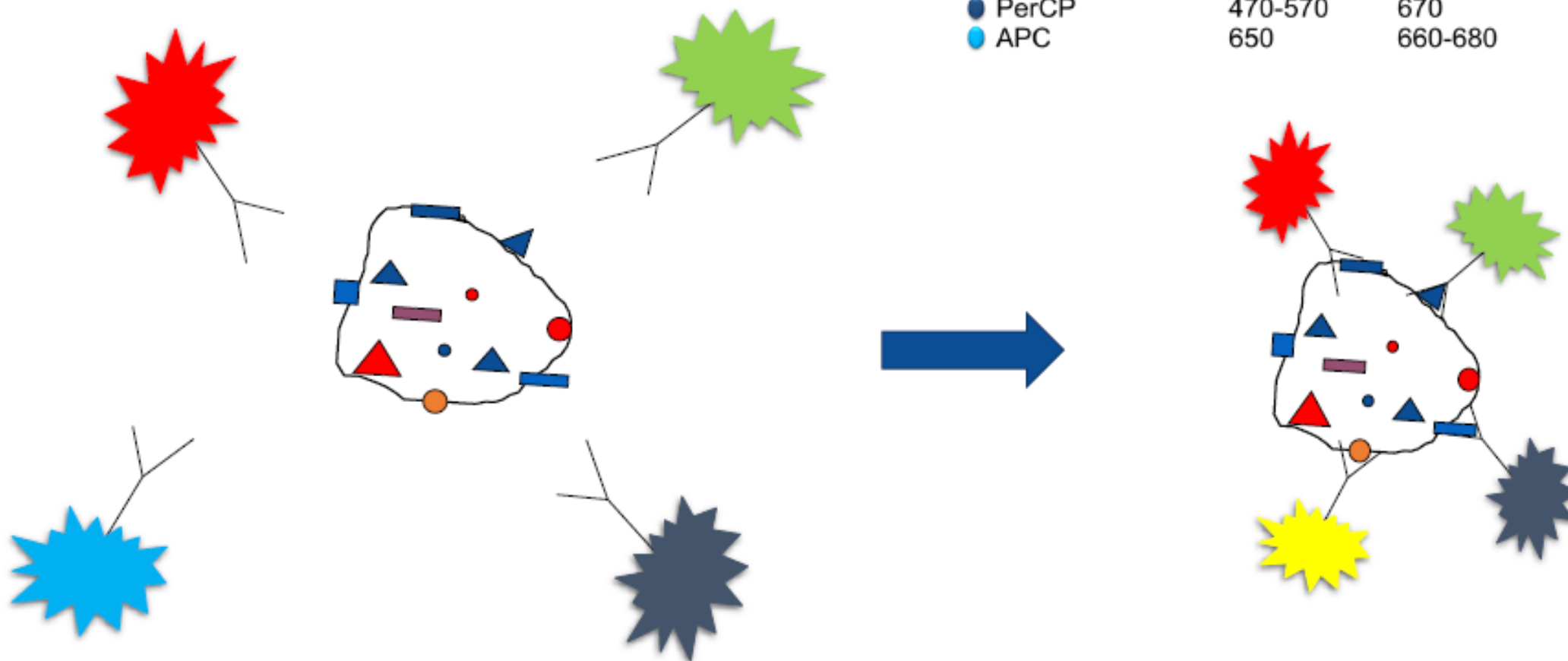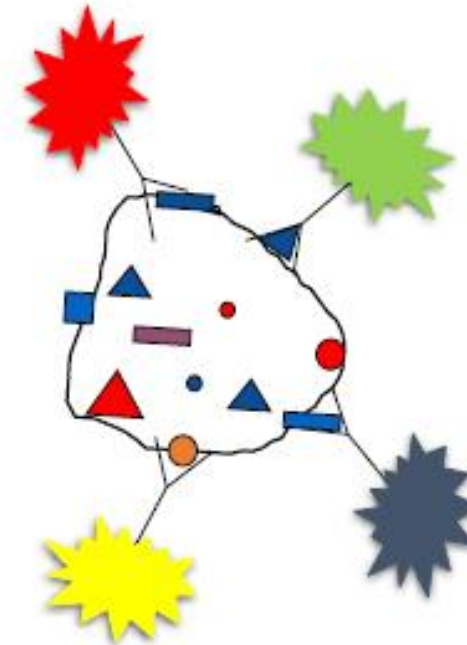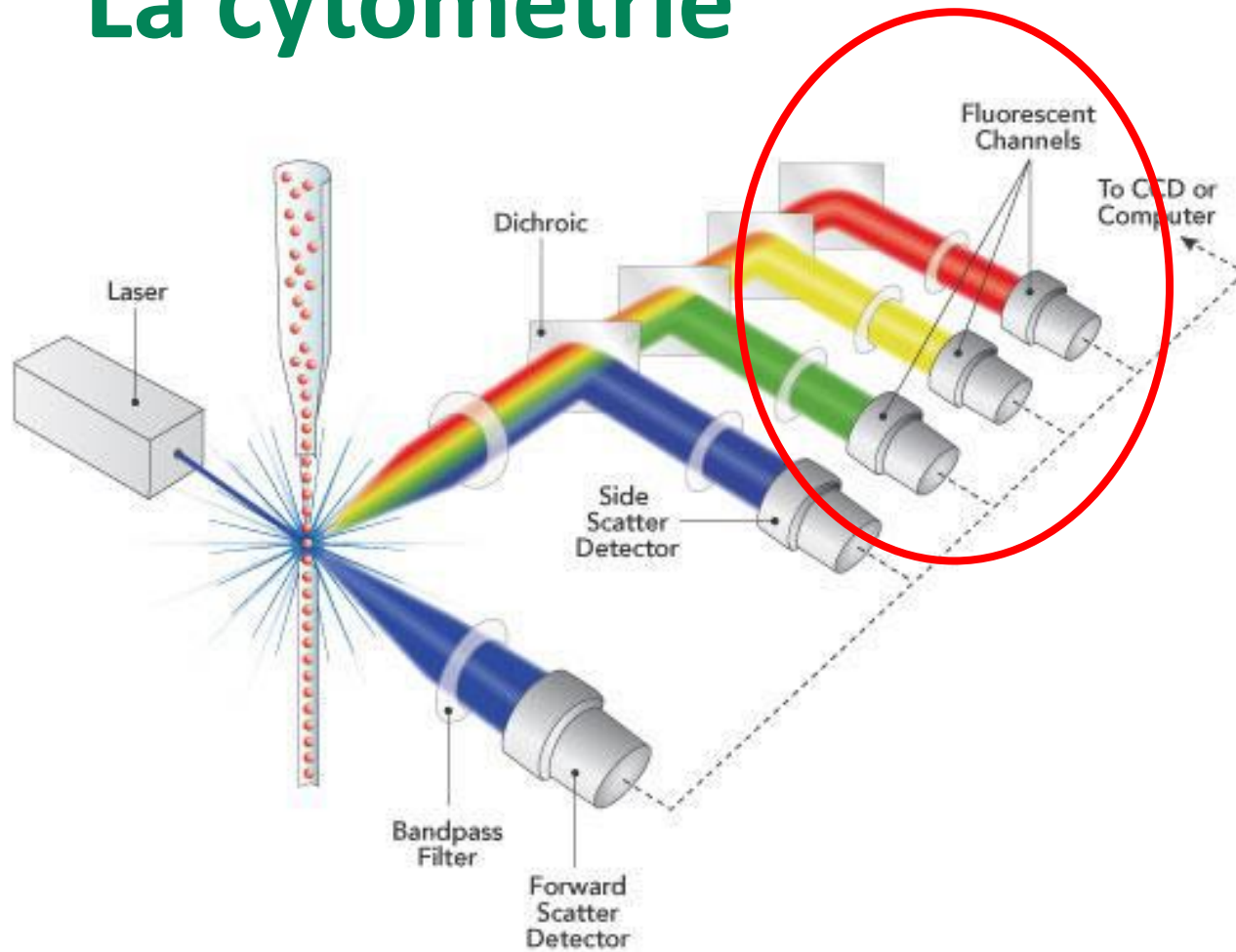- Marquage anticorps anti-CD + fluorochrome

La cytométrie

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

La cytométrie

Analyse superivsée

2 dimensions → y = f(x)

Analyse supervisée

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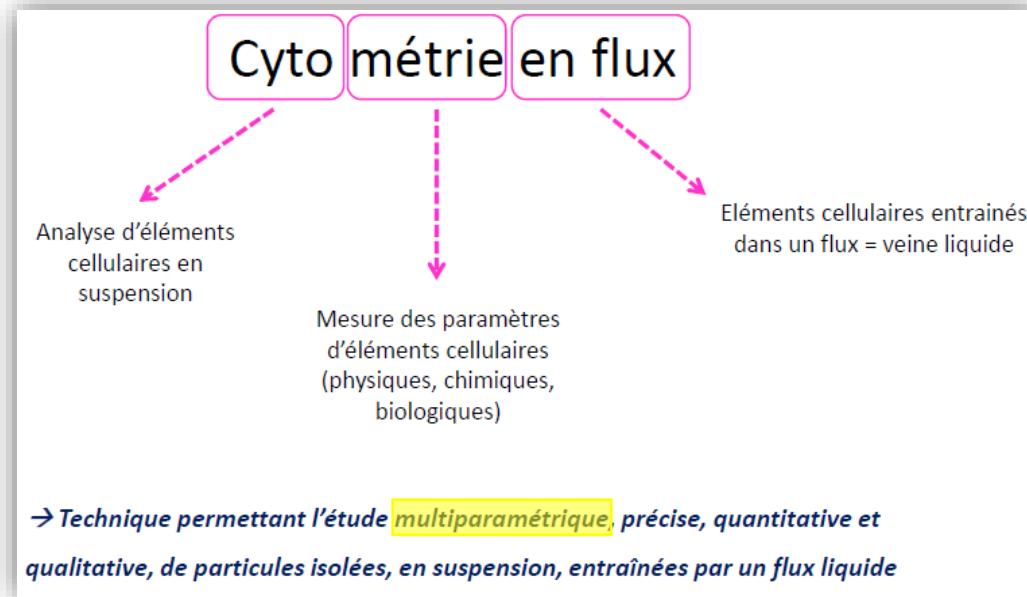
A robust and interpretable end-to-end deep learning model for cytometry data

Zicheng Hu[a,1], Alice Tang[a], Jaiveer Singh[a], Sanchita Bhattacharya[a], and Atul J. Butte[a,1]

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 → 45 graphes
n = 12 → 66 graphes
n = 15 → 105 graphes

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 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 definition…

Cyto | métrie | en flux

Analyse d'éléments cellulaires en suspension

Mesure des paramètres d'éléments cellulaires (physiques, chimiques, biologiques)

Eléments cellulaires entrainés dans un flux = veine liquide

→ *Technique permettant l'étude* multiparamétrique*, précise, quantitative et qualitative, de particules isolées, en suspension, entraînées par un flux liquide*

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.
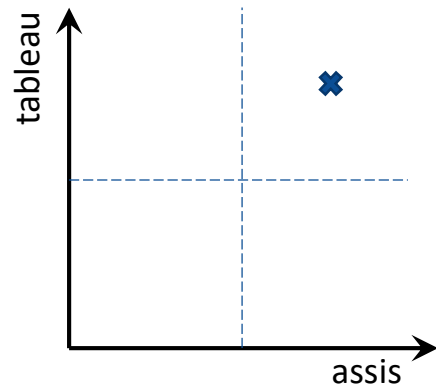
➔ Analyse multivariée!
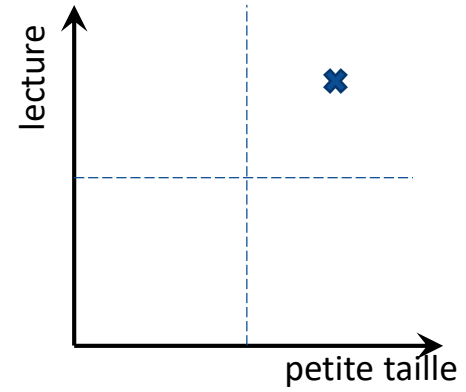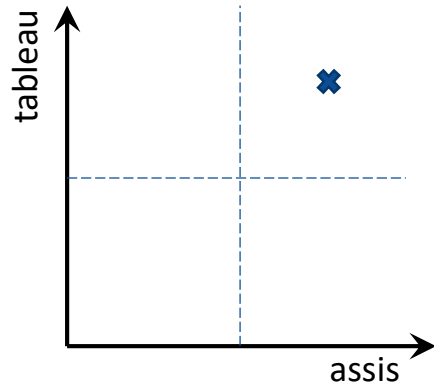
Analyse multivariée

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
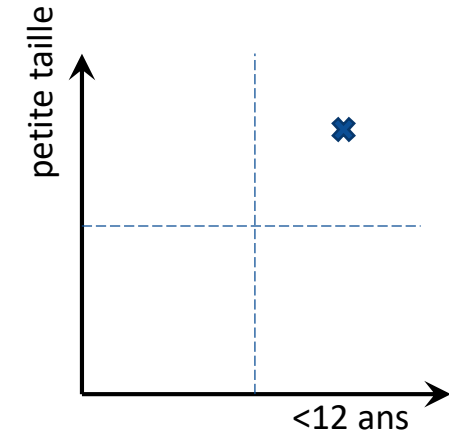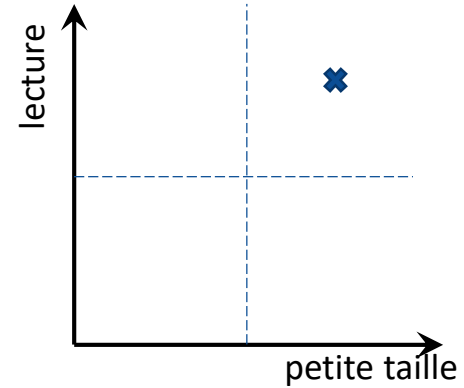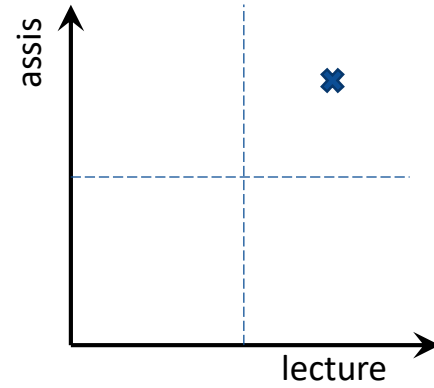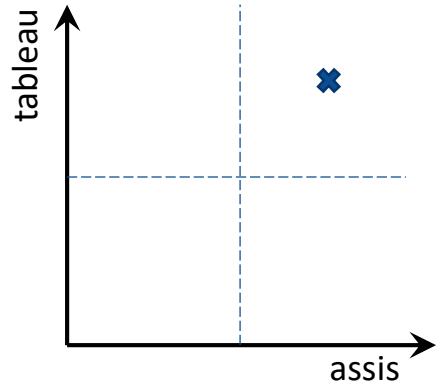
Imaginons...

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.
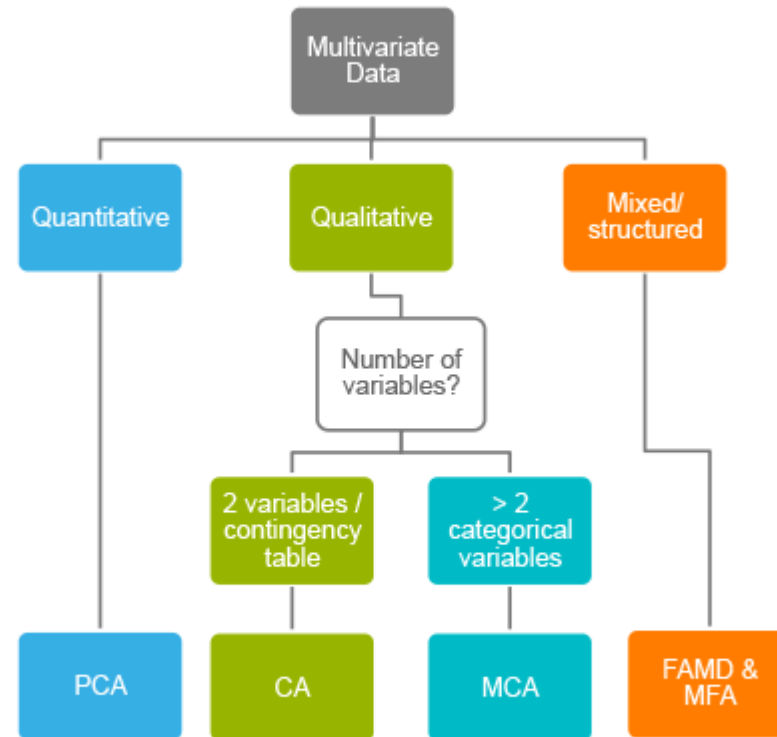
Méthodes explicatives

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.

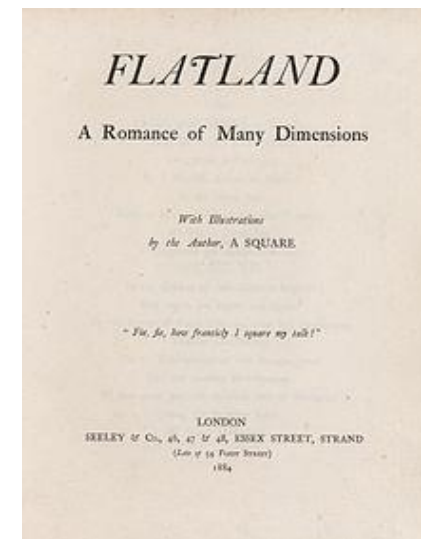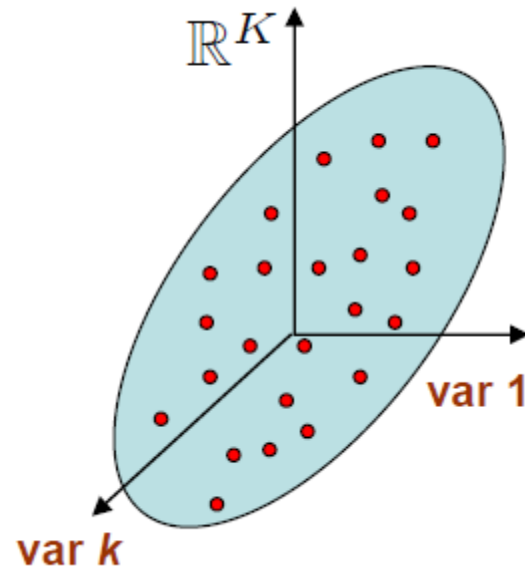# Méthodes en composantes principales

Principal Component Methods
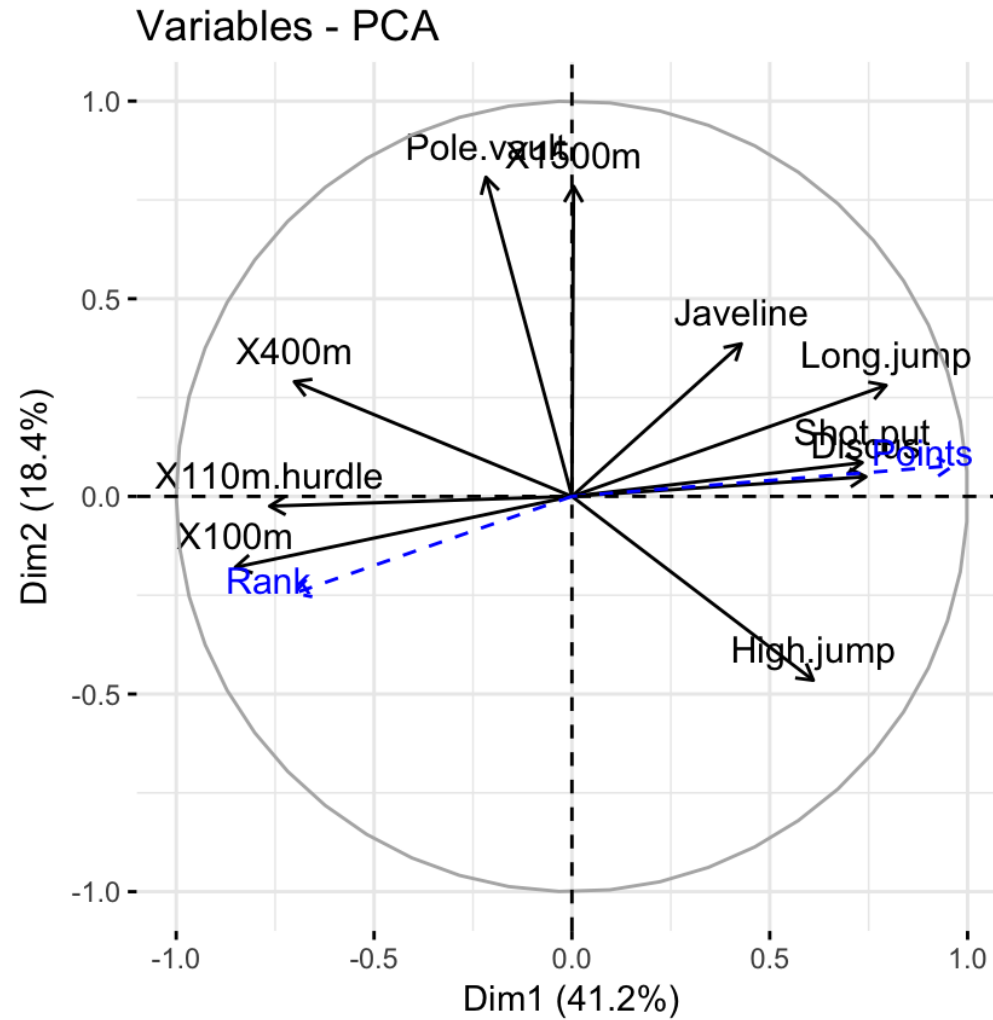*Methods* to Summarize & Visualize Multivariate Data

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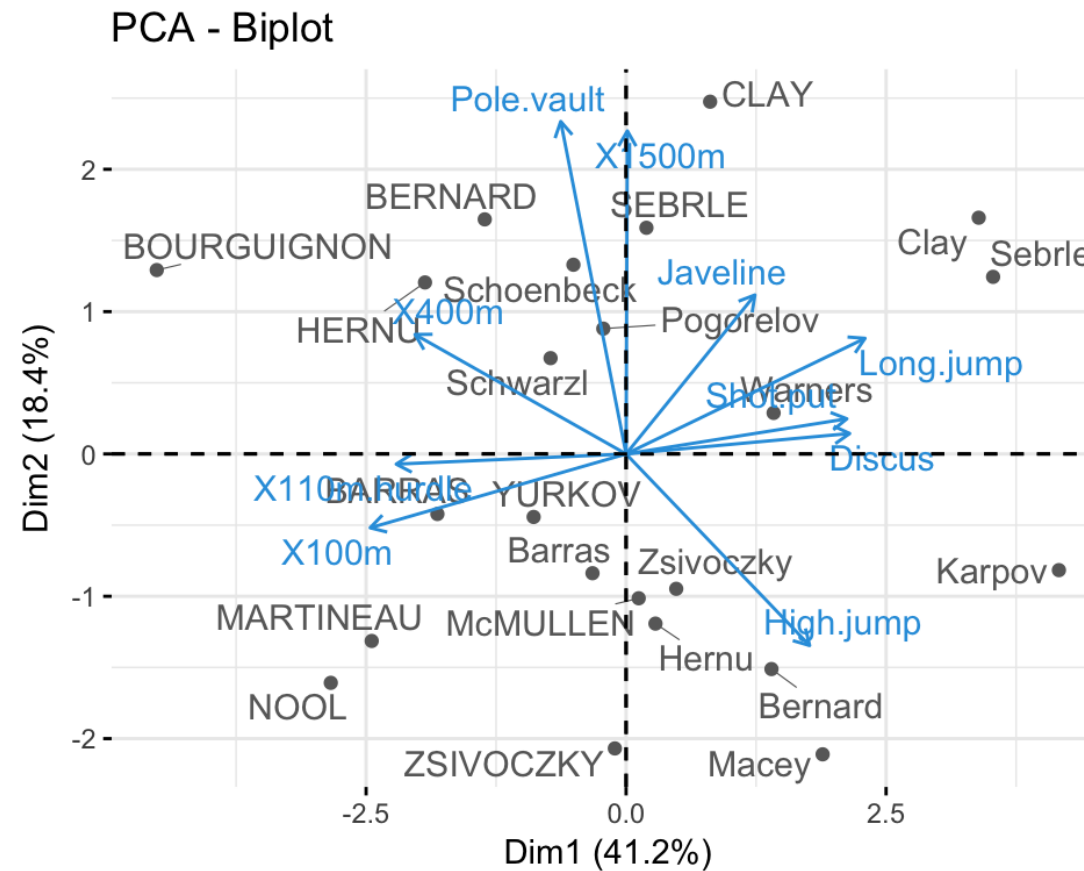- MFA: Multiple Factor Analysis

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

Patient "normal"

Marqueurs B

PCA graph of variables

PCA graph of individuals

APC et cytométrie

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

Résultats: LLC

PCA graph of individuals

PCA graph of variables

- CE: Présence d'une popultion patholoique B CD19+ CD5nég CD10nég monoclonale kappa d'expression normale, celle-ci est de phénotype: CD20+ CD22+ CD38nég CD11c neg CD103nég CD180+ CD27nég CD44+ CD25nég.

Résultats: LLC

PCA graph of variables

- CE: Persistance de l'infiltration sanguine par la population lymphocytaire représentant 74% des lymphocytes totaux (soit 17449 cellules/µl), 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)
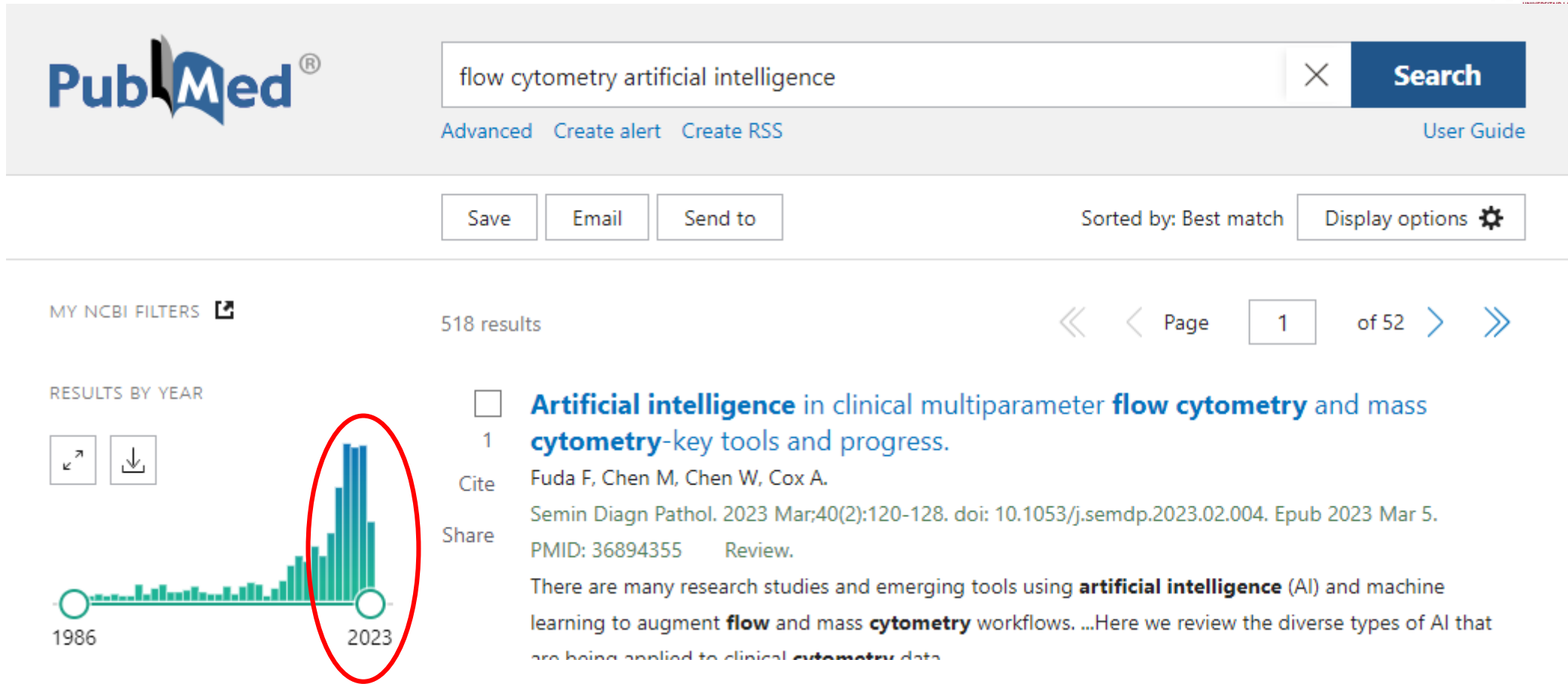- Dimensionality reduction (viSNE, t-SNE)
- ...

REVIEW ARTICLE

ISAC CYTOMETRY
Journal of Quantitative Cell Science PART A

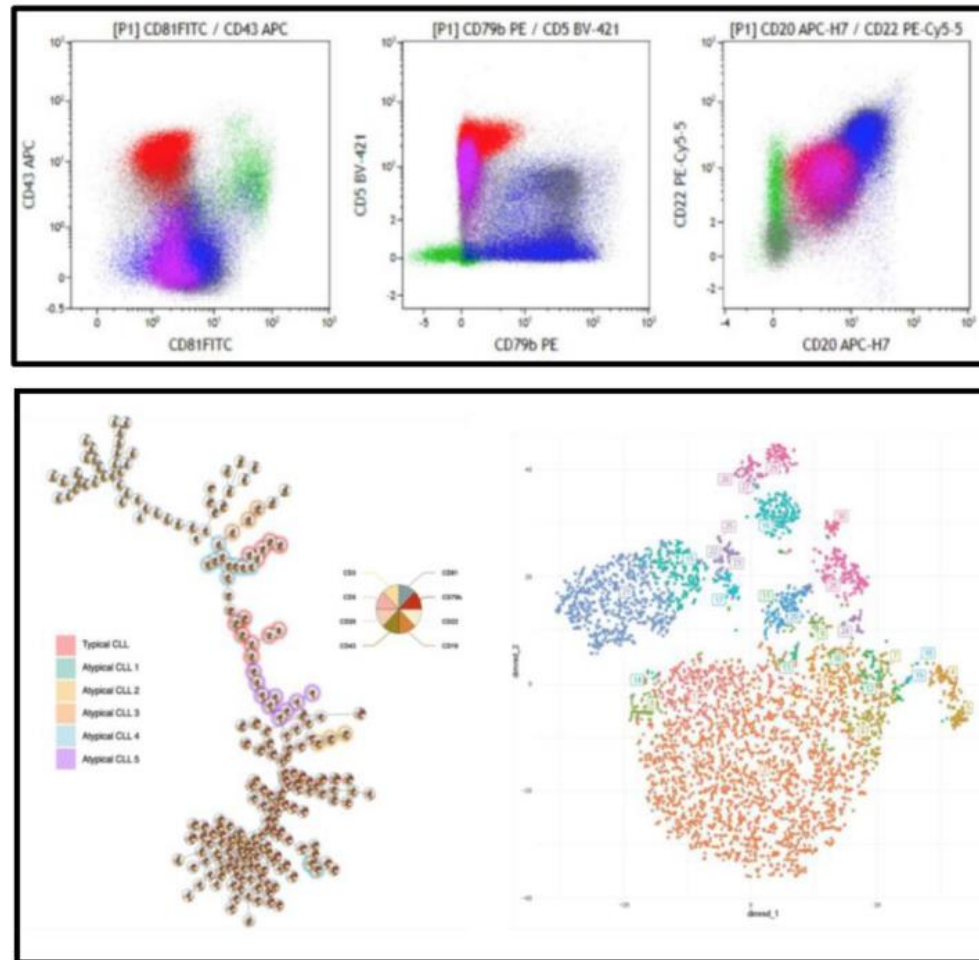## Current trends in flow cytometry automated data analysis software

Melissa Cheung[1] | Jonathan J. Campbell[2] | Liam Whitby[3] | Robert J. Thomas[1] |
Julian Braybrook[2] | Jon Petzing[1]

Flow cytometry & AI

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]).

Flow cytometry & AI

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) Software release by year

(D) Software by Programming Language

(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

Comment interpreter?

standard 2D plot gating
«classic multiparametric
Flow Cytometry»

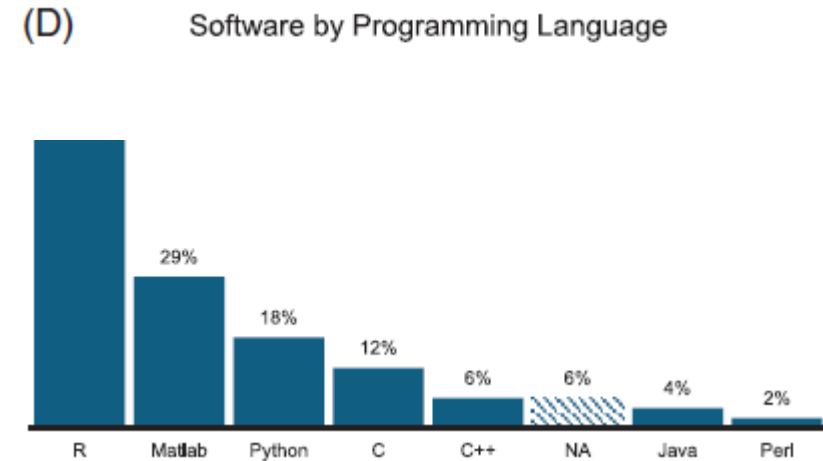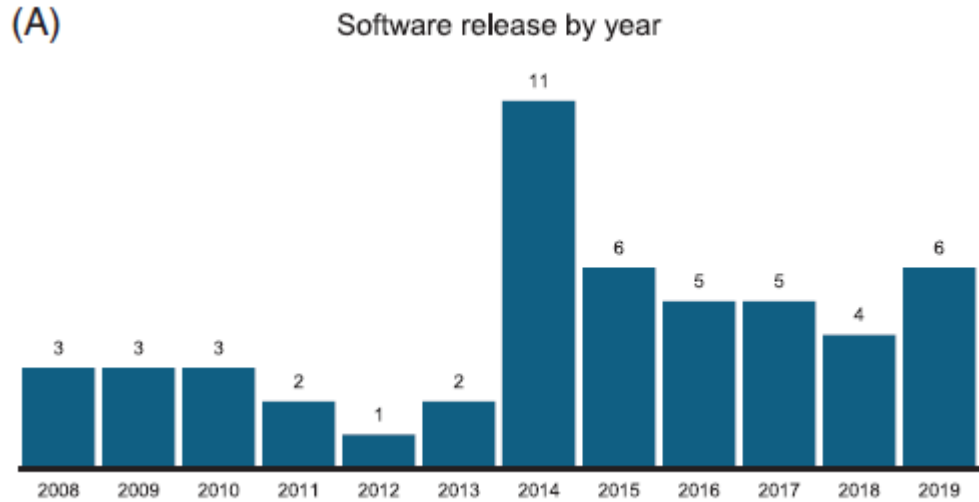Immunophenotypic
data analysis
for diagnostic
haematology

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]).
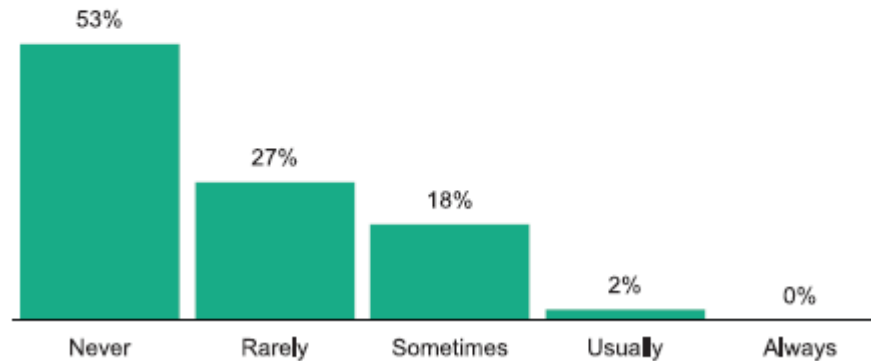
Cas clinique

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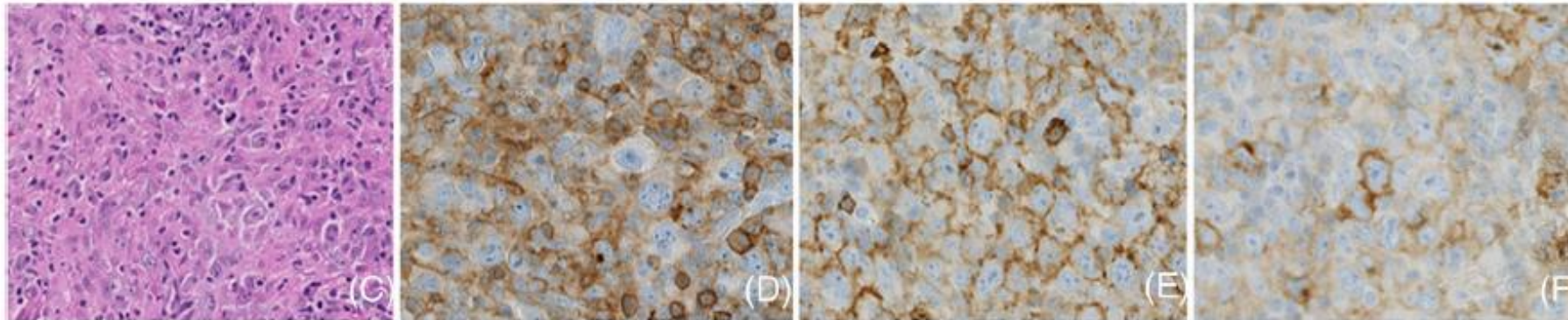
. Abdominal computed tomography scan revealed a large necrotic mass in the liver, a second necrotic mass adjacent to the superior mesenteric vessels, and numerous subdiaphragmatic lymph nodes (aorta, right kidney, retro-crural, mesenteric, iliac, and inguinal). Hypermetabolic activity was confirmed by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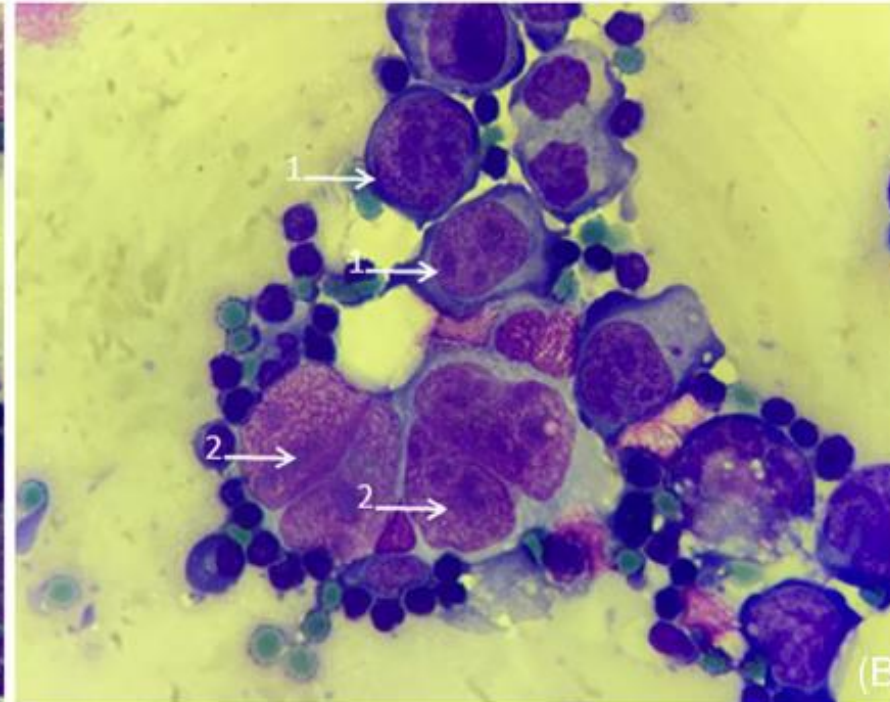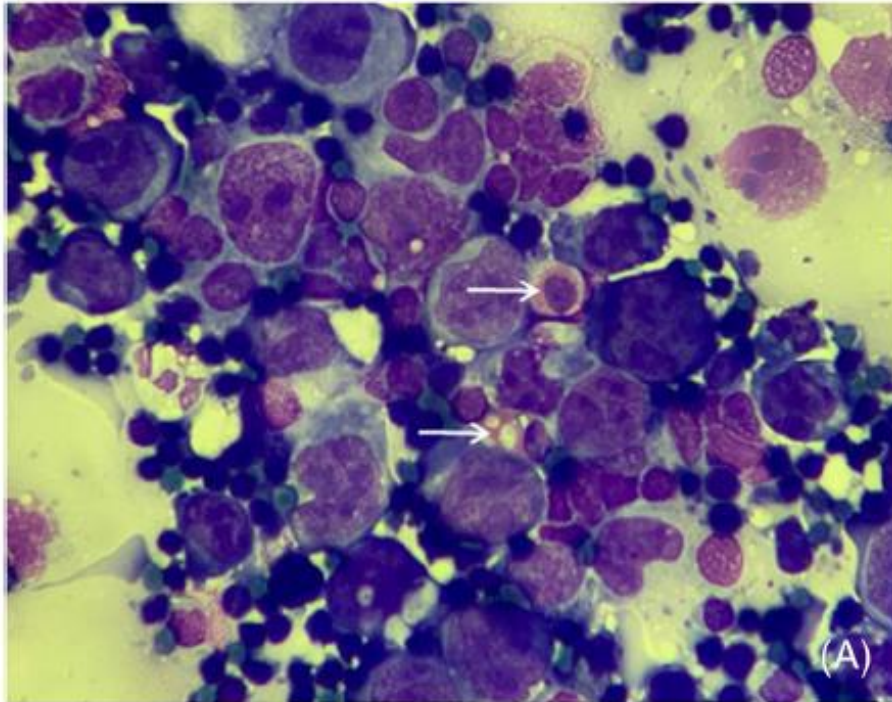
Diffuse large B-cell lymphoma (DLBCL)

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

c, and

ission

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
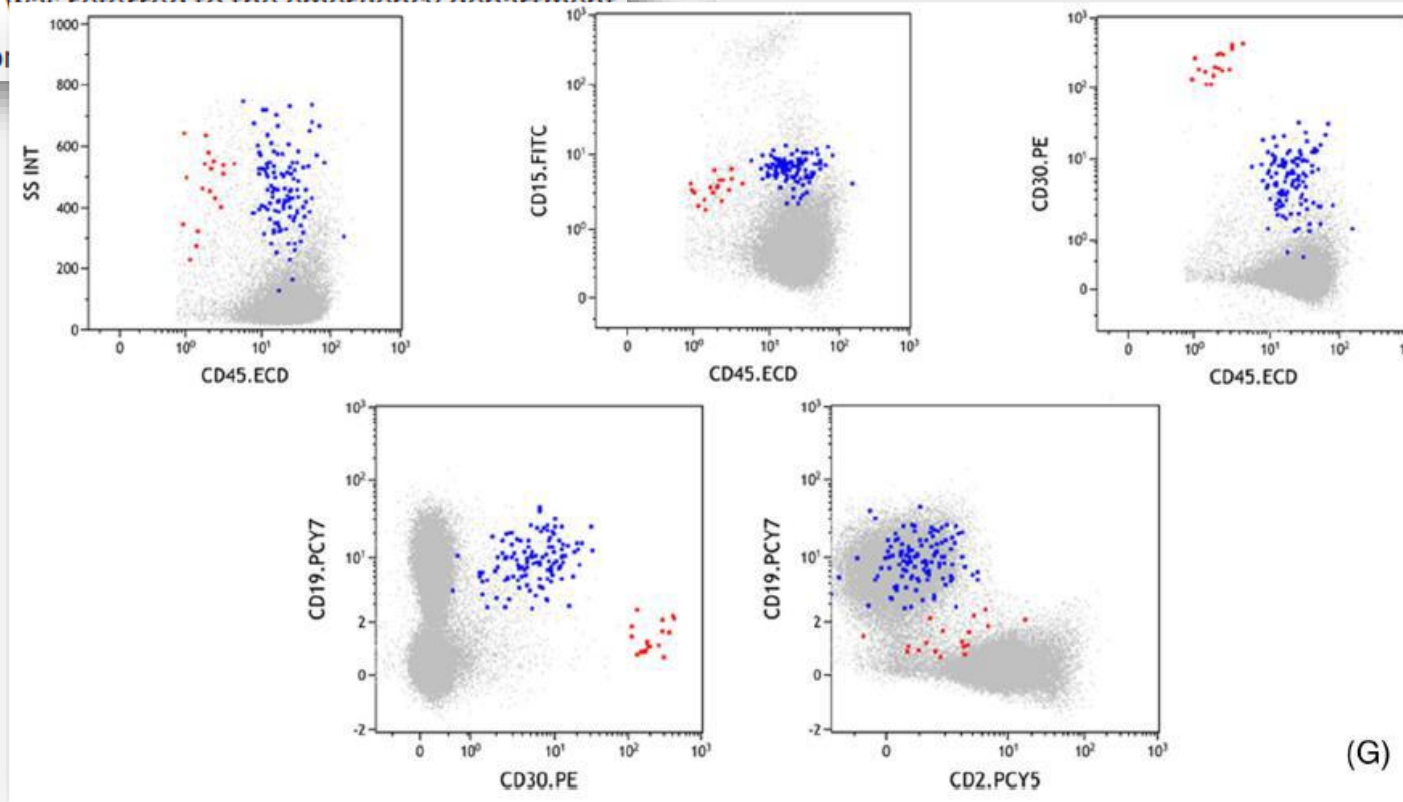
Cas clinique

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.
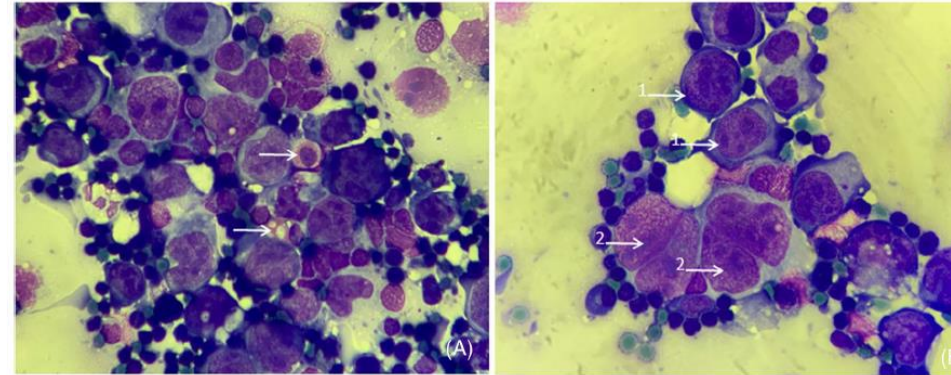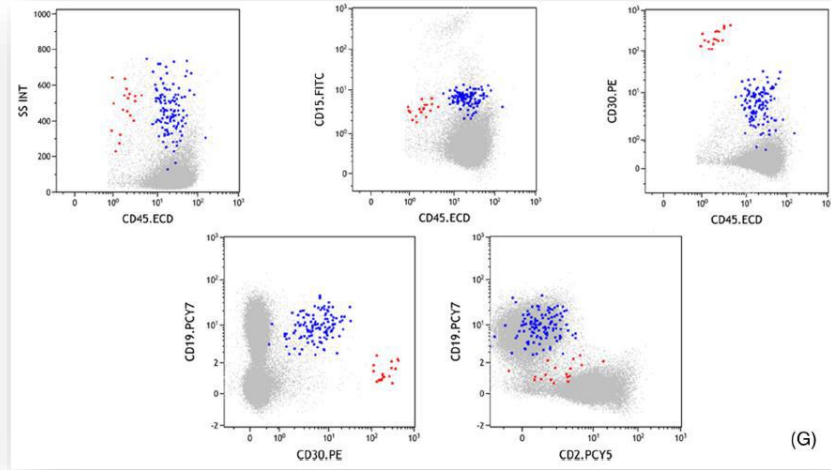
Abdominal computed tomography scan revealed ...crotic mass adjacent ...us subdiaphragmatic ...nesenteric, iliac, and ...by positron emission
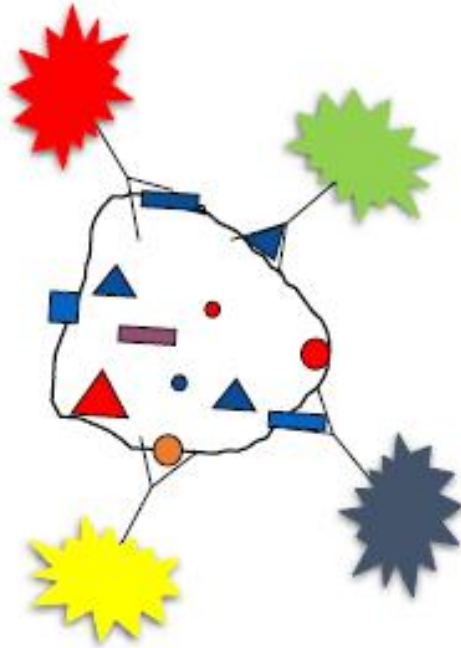
Au labo…

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

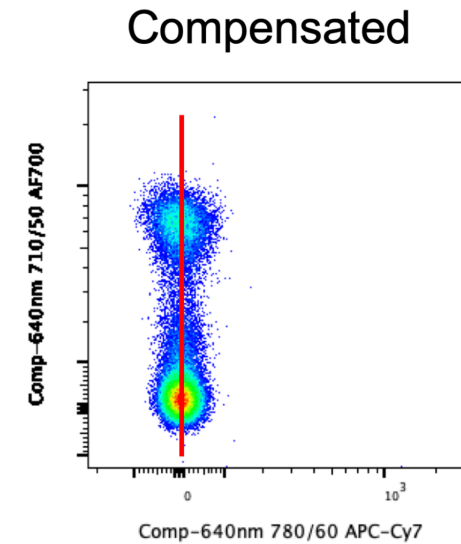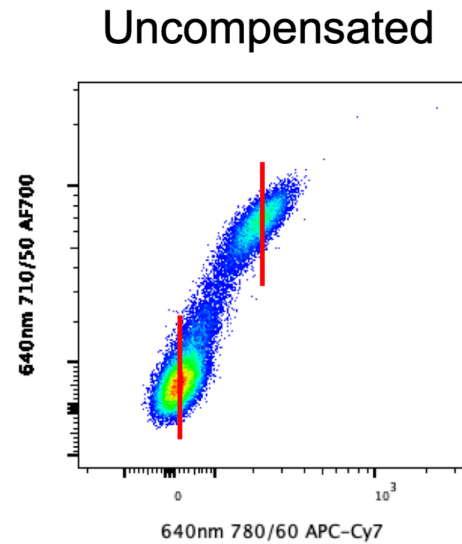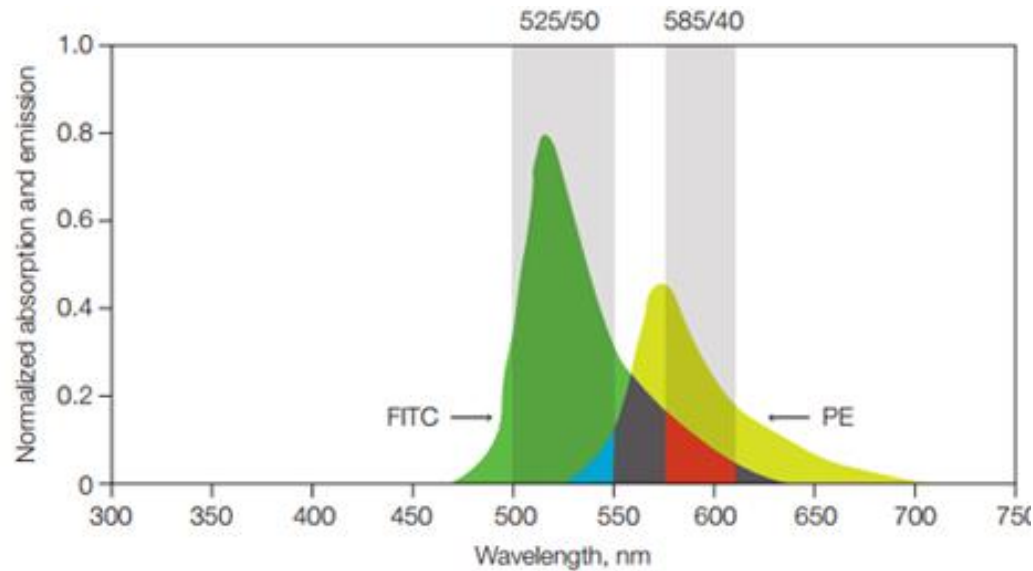**Une des faiblesses des APC → les événements rares!**

Autres methodes?

- Taille
- Granularité
- 10 couleurs

$$\begin{bmatrix} Taille \\ Granularité \\ Fluo_1 \\ Fluo_2 \\ Fluo_3 \\ Fluo_4 \\ Fluo_5 \\ Fluo_6 \\ Fluo_7 \\ Fluo_8 \\ Fluo_9 \\ Fluo_{10} \end{bmatrix}$$

- Calcul vectoriel ?
- Calcul matriciel ?

Et la technologie?

La compensation

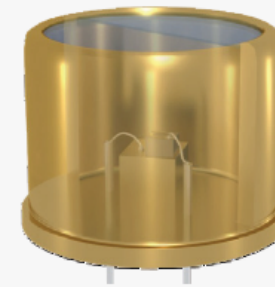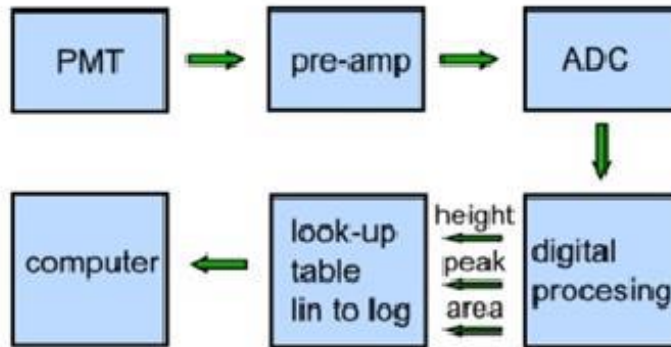Uncompensated — Compensated

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.

Miltenyi Biotec

Et la technologie?

L'effet photo-électrique

digital

PMT → pre-amp → ADC → digital procesing → height / peak / area → look-up table lin to log → computer

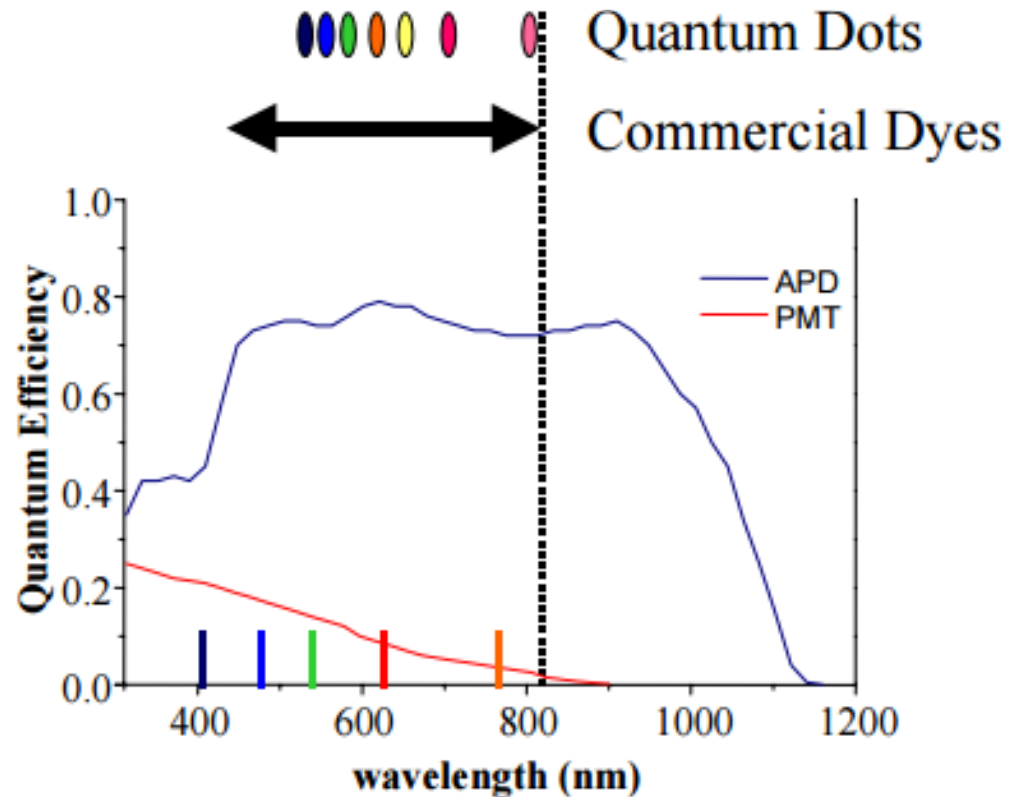**Avalanche Photodiode.**
The DxFLEX flow cytometer uses Avalanche Photodiode detectors instead of PMTs. The low electronic noise contributes to the resolution capabilities of the instrument.

Et la technologie?

Quantum Dots

Commercial Dyes

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.
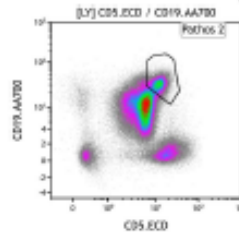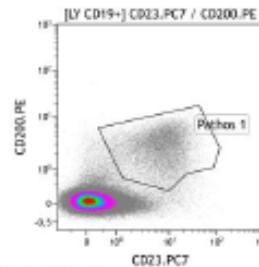
Perspectives?

- Régression linéaire
- Fonction multivariée
- ACP

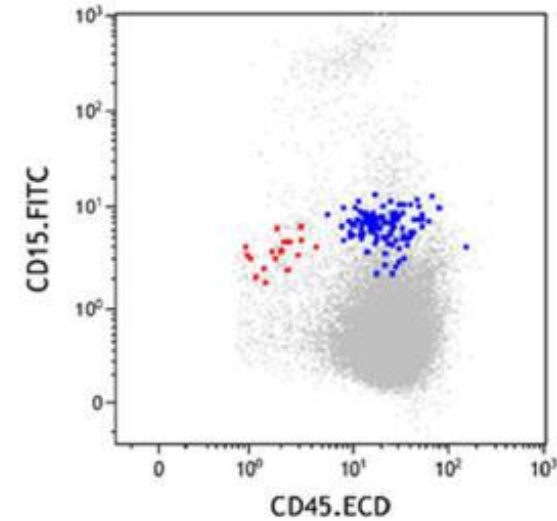Bases mathématiques de l'A.I.

Découverte de "nouveaux profils"?

Perspectives?

- Régression linéaire
- Fonction multivariée
- ACP

Bases mathématiques de l'A.I.
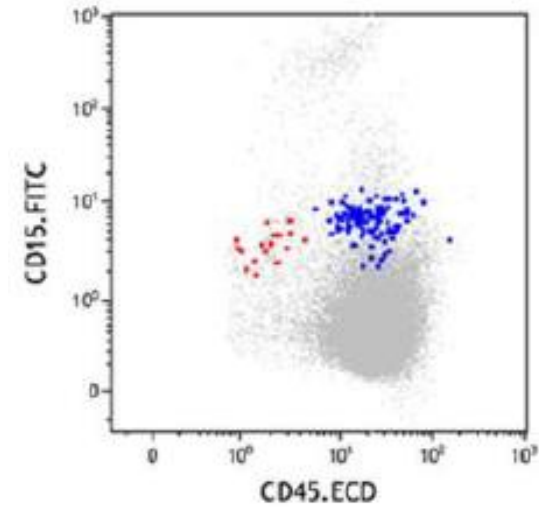
Découverte de "nouveaux profils"?

"Evénements rares"

Marqueurs d'évolution?
Marqueurs d'agressivité?

Comment comprendre?

AI
Machine learning
Big Data
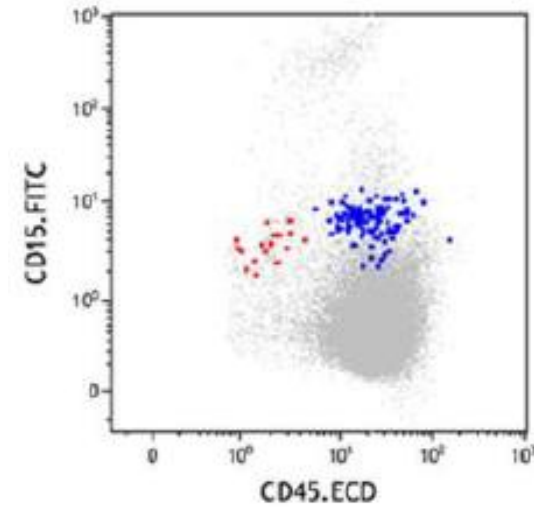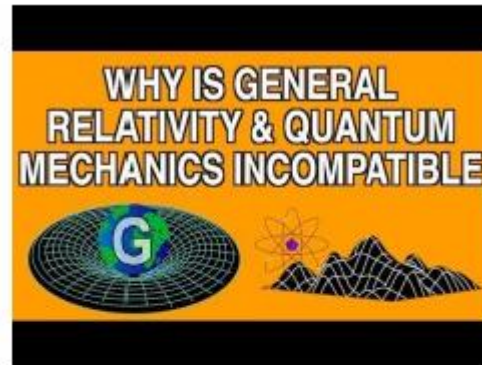
Le macroscopique

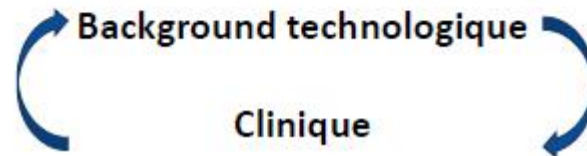Evénements "rares"

Le microscopique

Comment comprendre?

AI
Machine learning
Big Data

Le macroscopique

WHY IS GENERAL
RELATIVITY & QUANTUM
MECHANICS INCOMPATIBLE

Evénements "rares"

Le microscopique

Background technologique

Clinique

Merci…

… à toute l'équipe du LHUB-ULB