CYTOMETRIE EN FLUX
PRINCIPES ET APPLICATIONS
MYELOME MULTIPLE

BUT Montpellier

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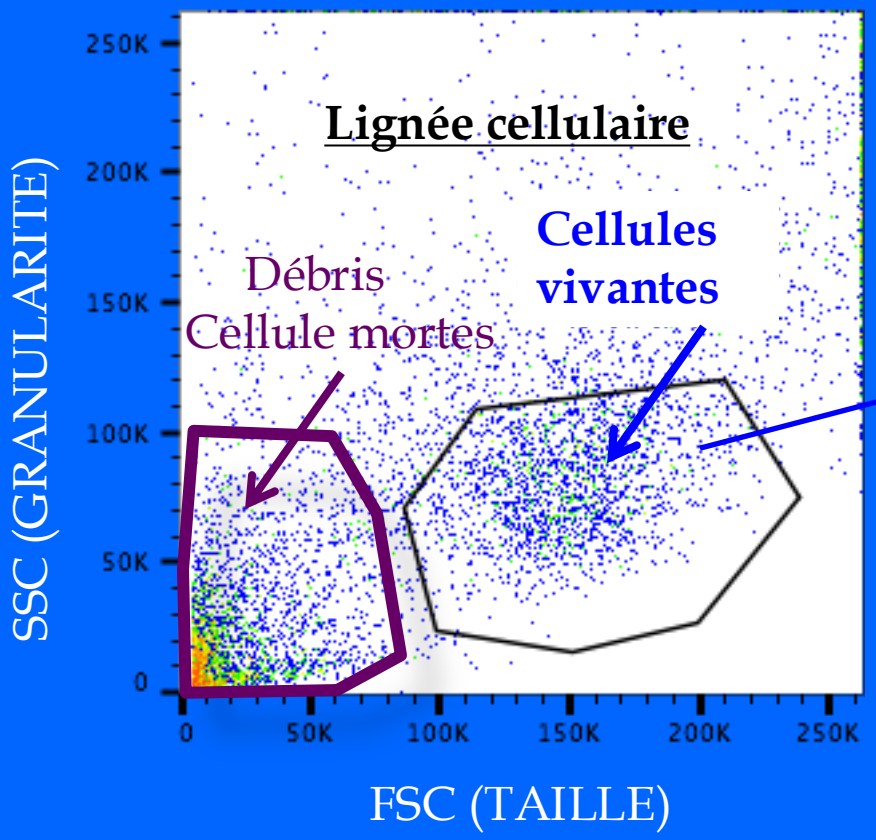
Laboratoire Suivi des Thérapies Innovantes

CYTOMETRIE EN FLUX

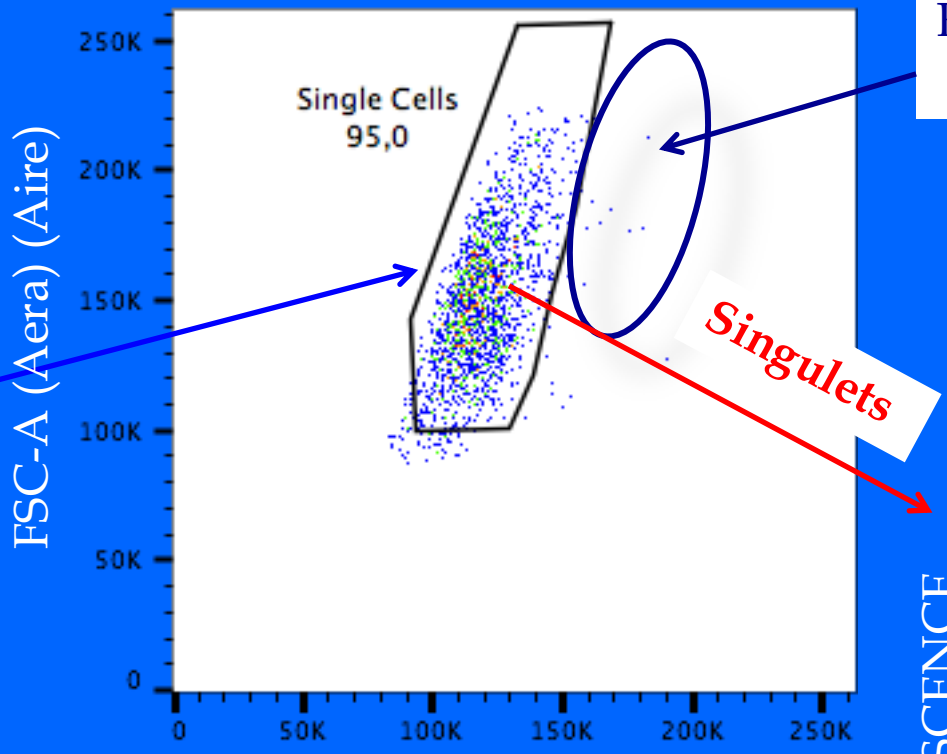
3. Strategy of Gating :
   Analyse et interprétation en cytométrie
   Notion de « Gate » (fenêtre)

ANALYSE EN CYTOMETRIE EN FLUX « STRATEGY OF GATING »

TOUTES LES CELLULES



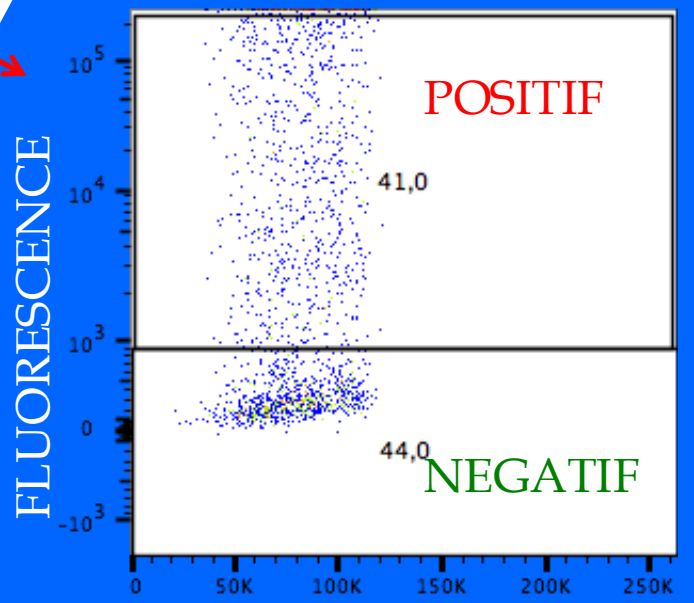
CELLULES VIVANTES



Elimination des doublets

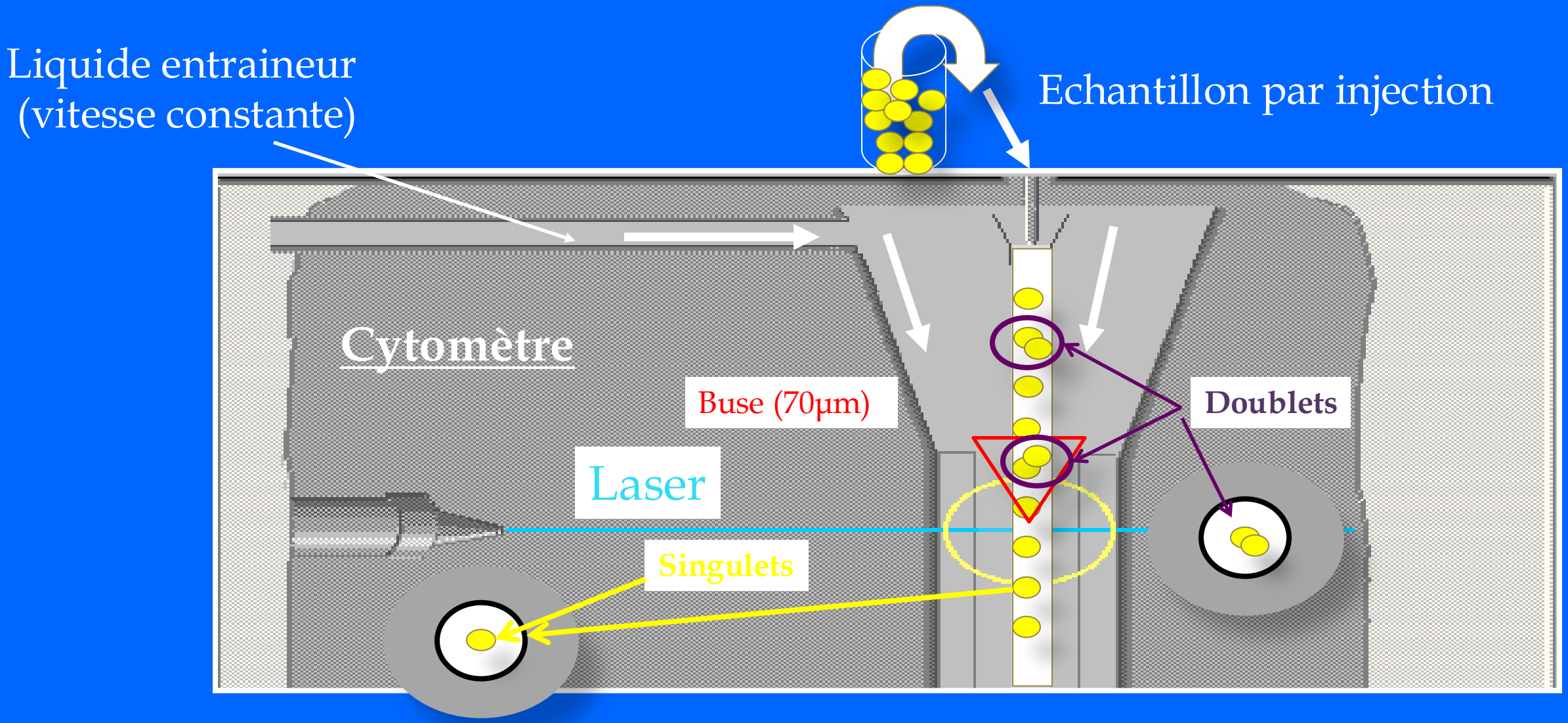
Singulets

SINGULETS



FSC / SSC / FSC-W / FSC-A = échelle Linéaire (Lin)
FLUORESCENCE = échelle Logarithmique (LOG)

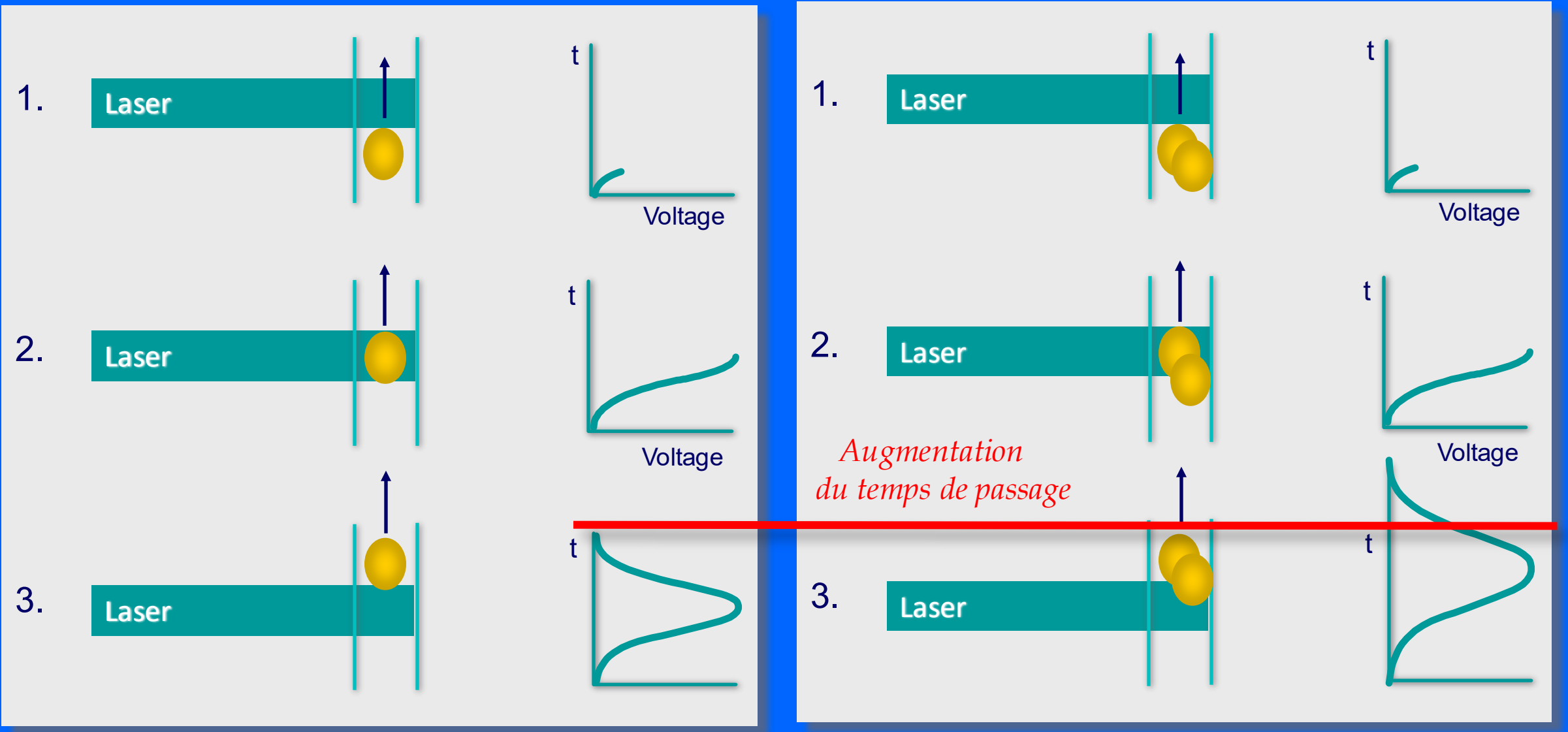
FORMATION DES DOUBLETS



VOLTAGE INDUIT PAR LES PMT

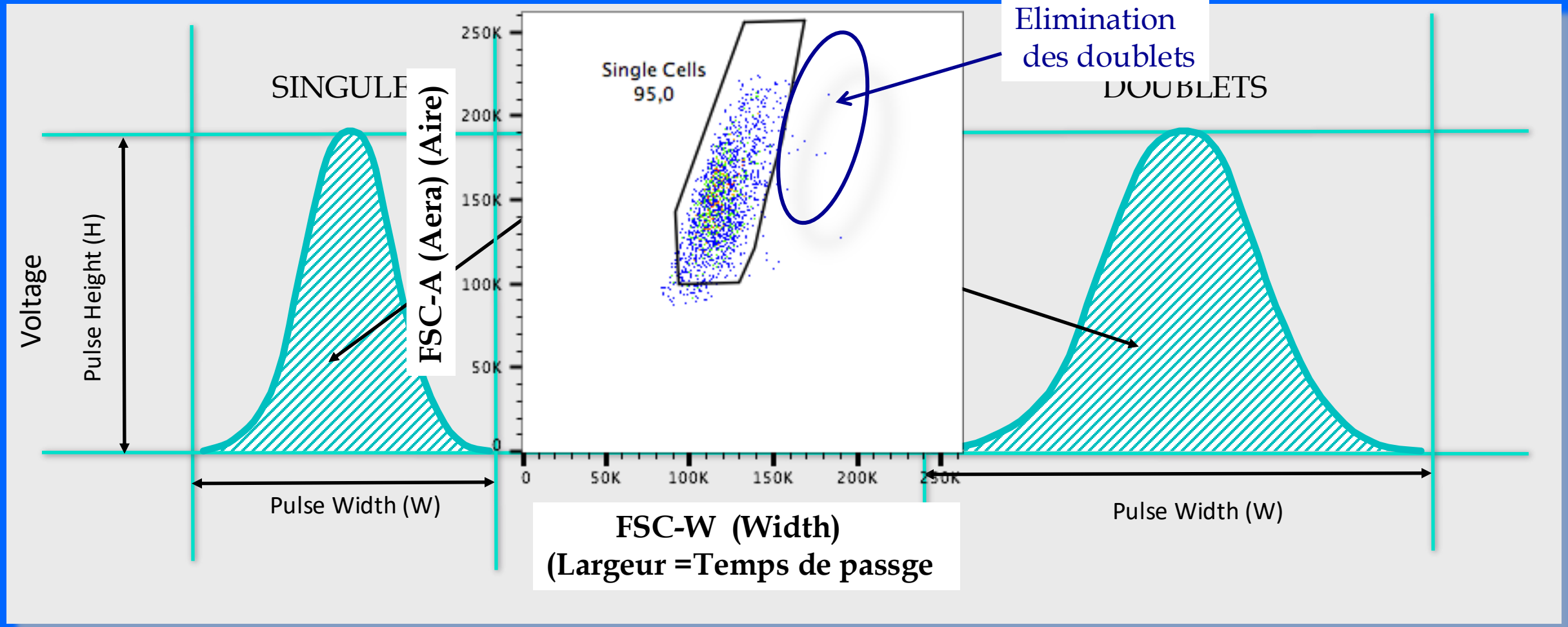
SINGULETS

DOUBLETS

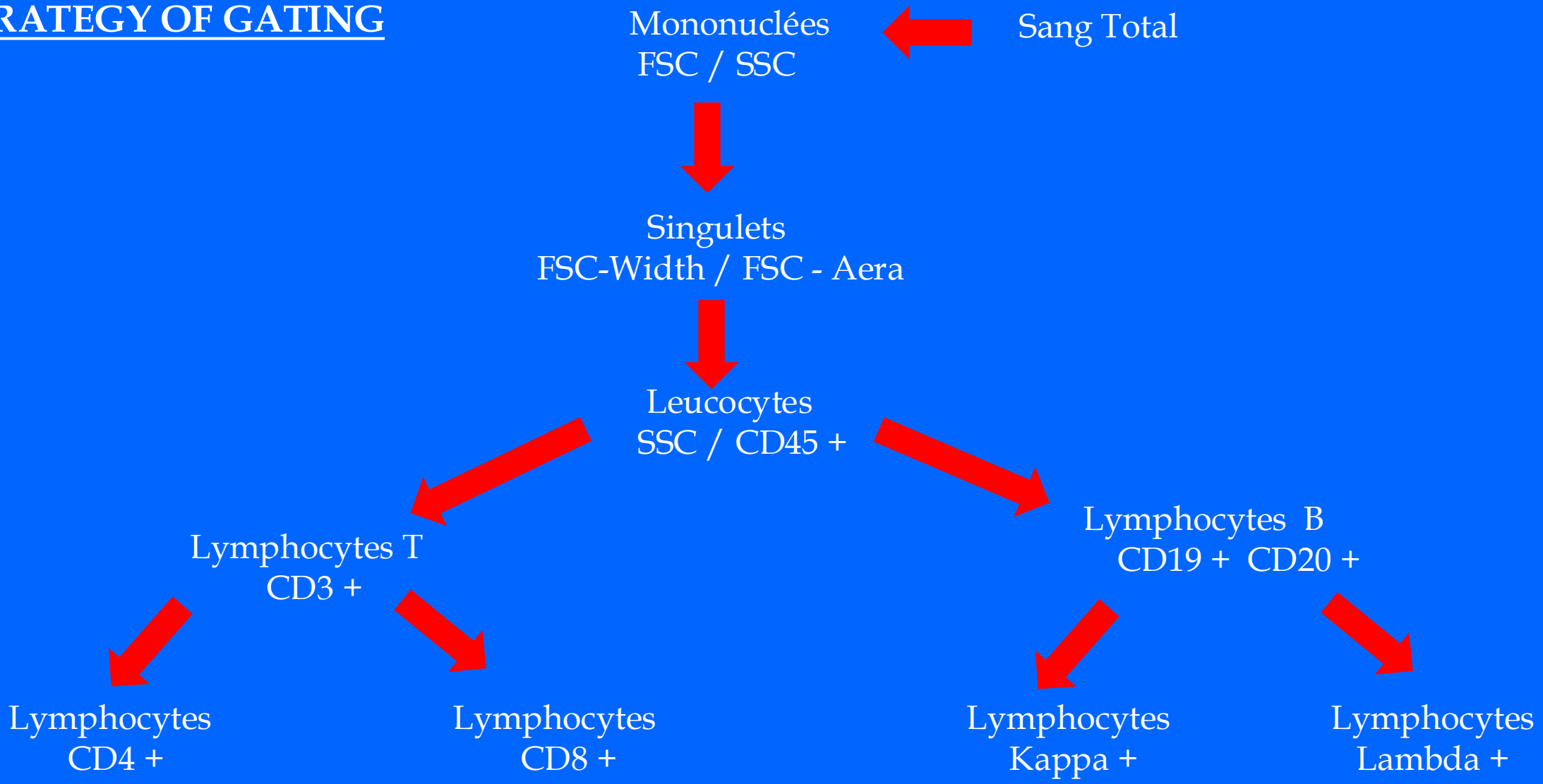


PRINCIPE D'ELIMINATION DES DOUBLETS

(Height, Area, and Width)

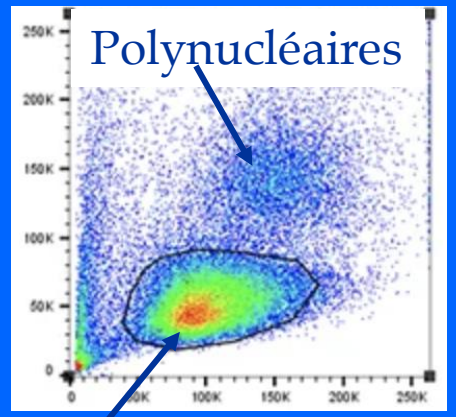


STRATEGY OF GATING

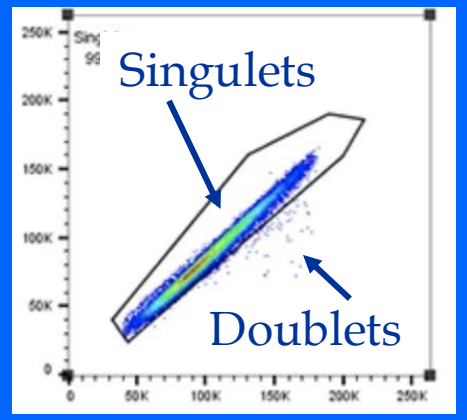


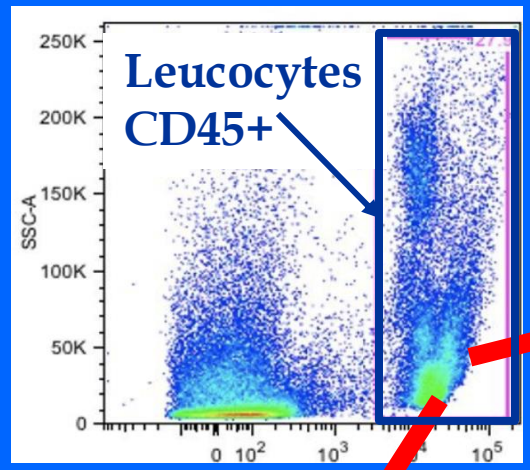
STRATEGY OF GATING

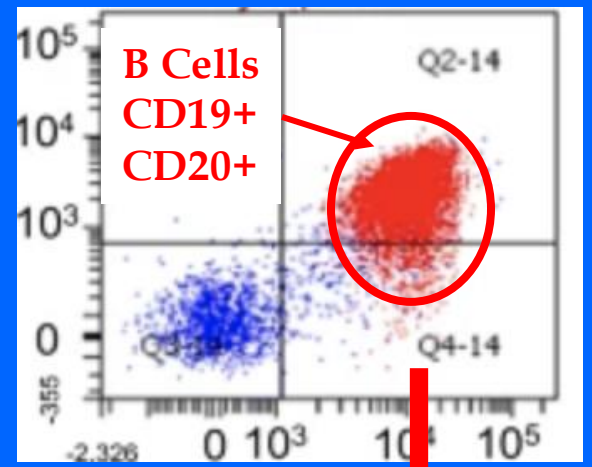
Sang Total → Mononucléées → Singulets → Lymphocytes B



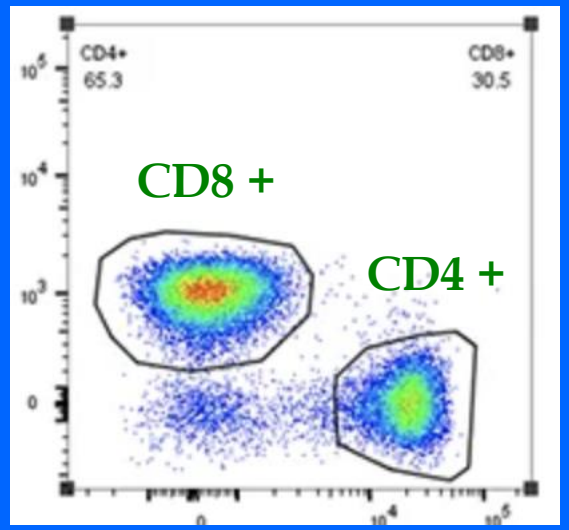
Mononucléées

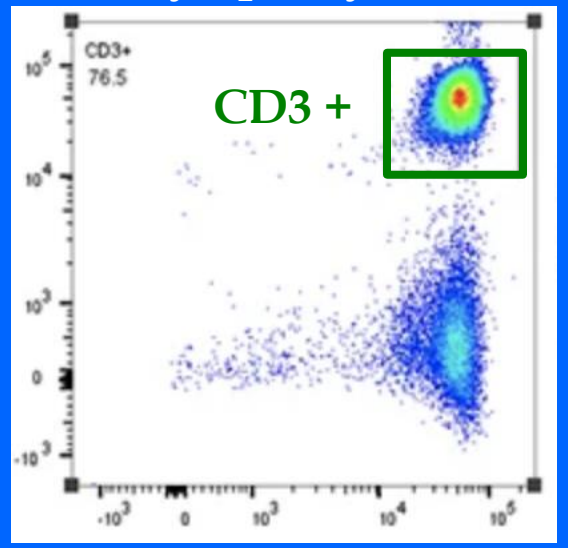


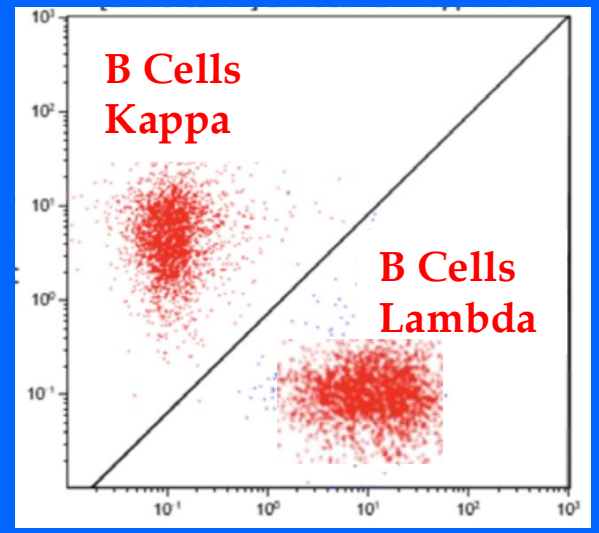




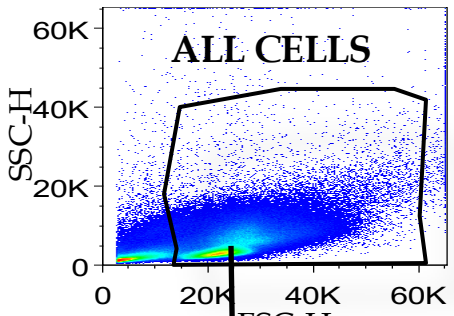
Lymphocytes T4 / T8 ← Lymphocytes T → Lymphocytes B

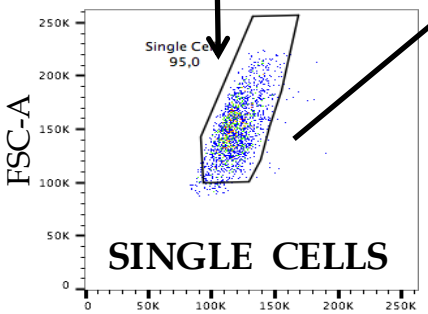


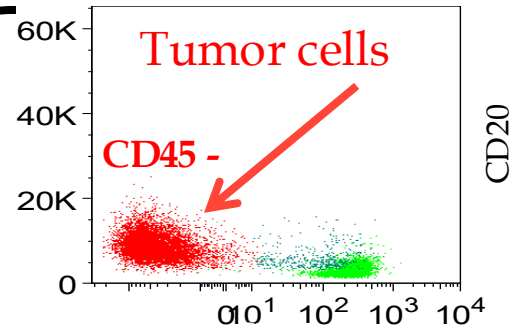


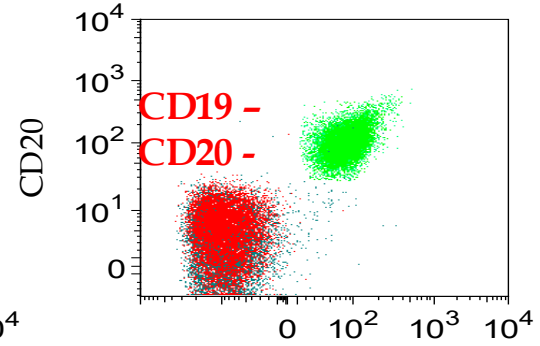


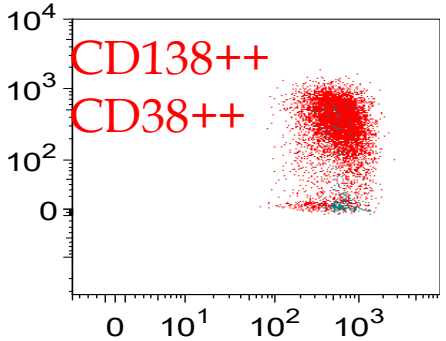
« Strategy of gating » Multicolor Flow Cytometry

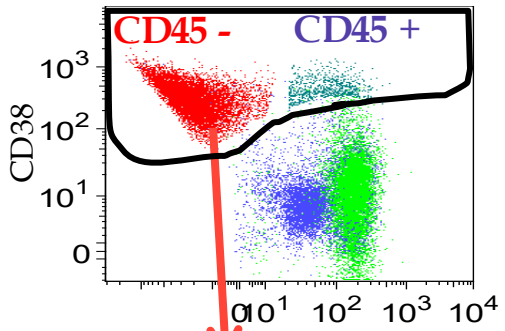


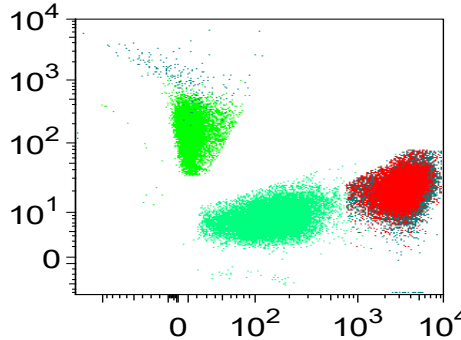




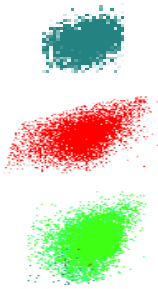








Kappa Monoclonal

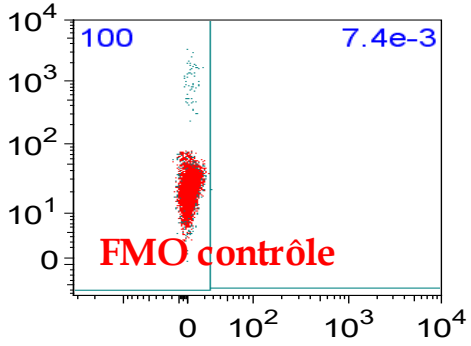


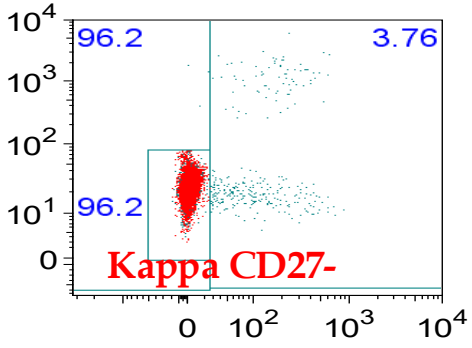
Normal Plasma cells

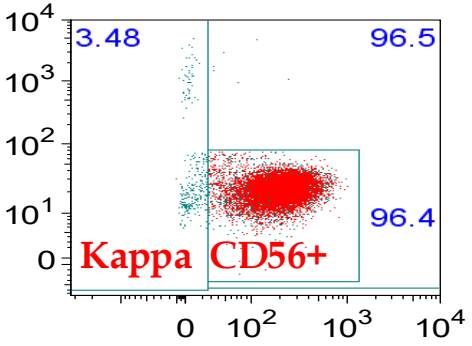
MM cells

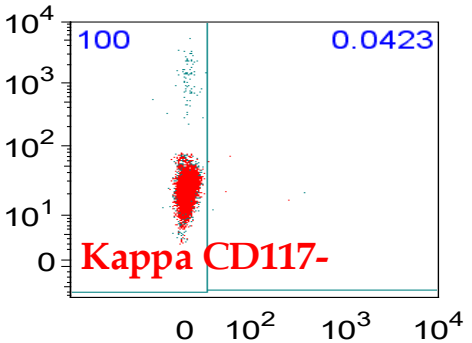
Normal B cells

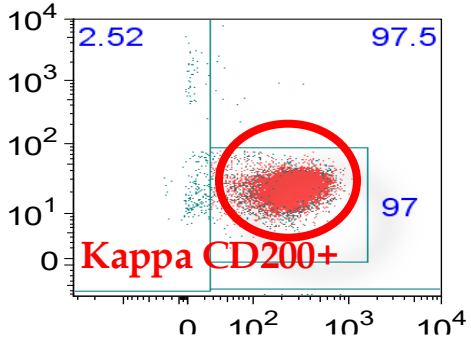
Tumor cells (Kappa, CD138+, CD38+, CD45-, CD19-, CD20-,CD27-, CD117-,CD200+)







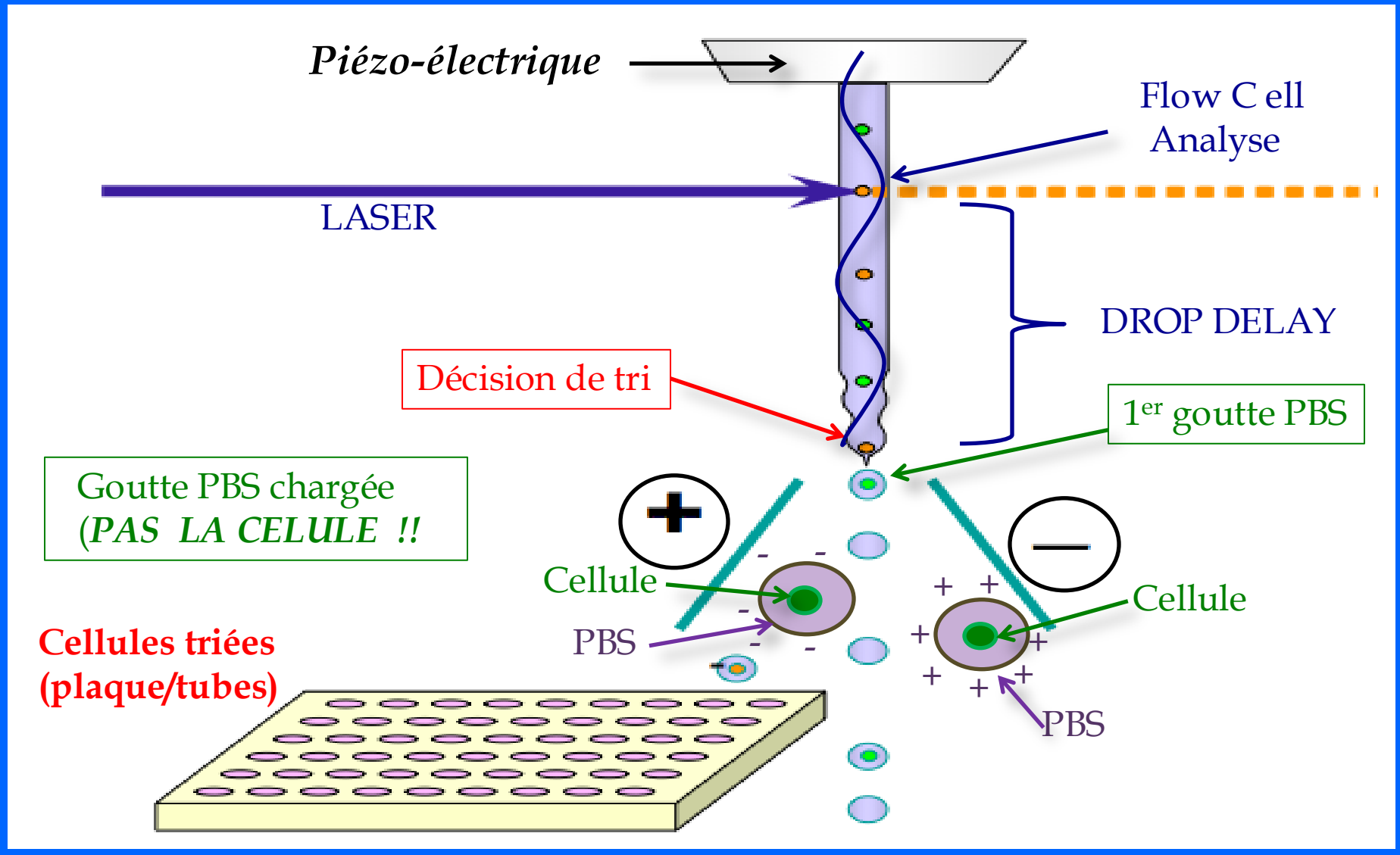


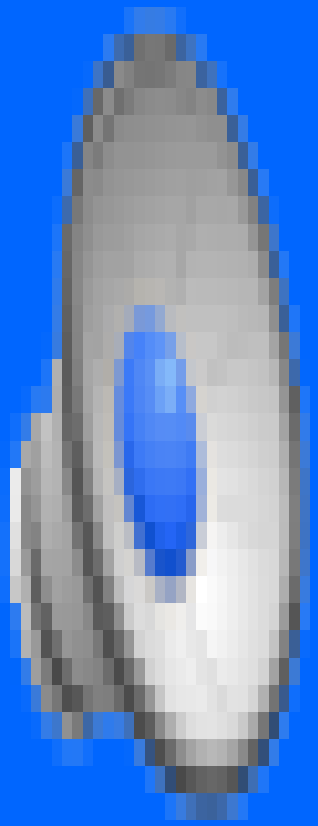


CYTOMETRIE EN FLUX

Cytométre : Trieur de cellules à Haute vitesse

PRINCIPE DU TRIEUR à HAUTE VITESSE





FACSARIA III

CONCLUSIONS (Partie 3)

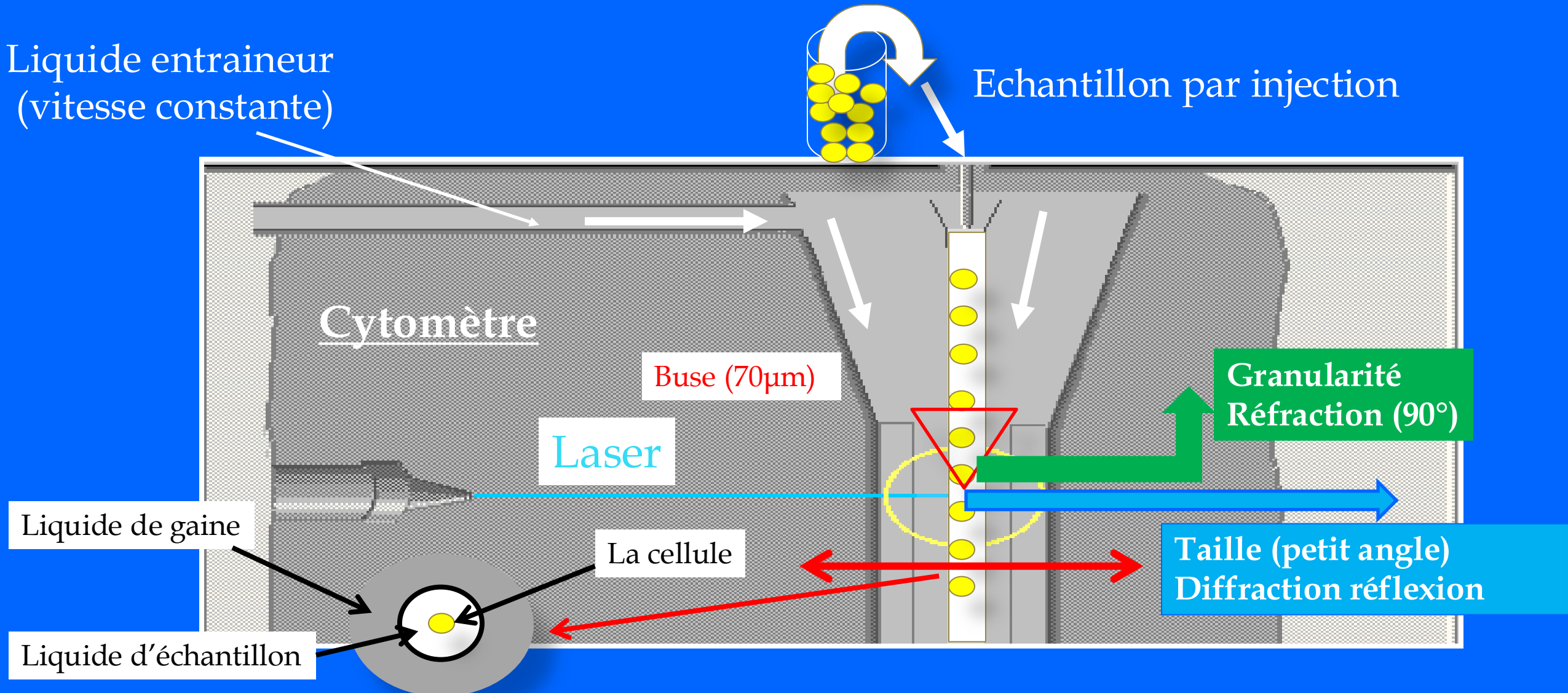
- « Strategy of Gating »:
  - ↳ Logique d'analyse en cytométrie
- *Graphe 1*: Toutes les cellules (FSC taille/ SSC morphologie)
- *Graphe 2*: Sélectionner les cellules vivantes
- *Graphe 3*: Eliminer les doublets (FSC-W width / FSC-A Aera)
- *Graphes suivants:*
  - identifier toutes les populations par représentations bi-paramétriques (comparaison 2 fluorochromes deux à deux)
  - Identifier la population recherchée à partir des graphes précédents
  - Caractériser le phénotype complet et quantifier la population d'intérêt recherchée en comparant avec le tube négatif (FMO)

CYTOMETRIE EN FLUX

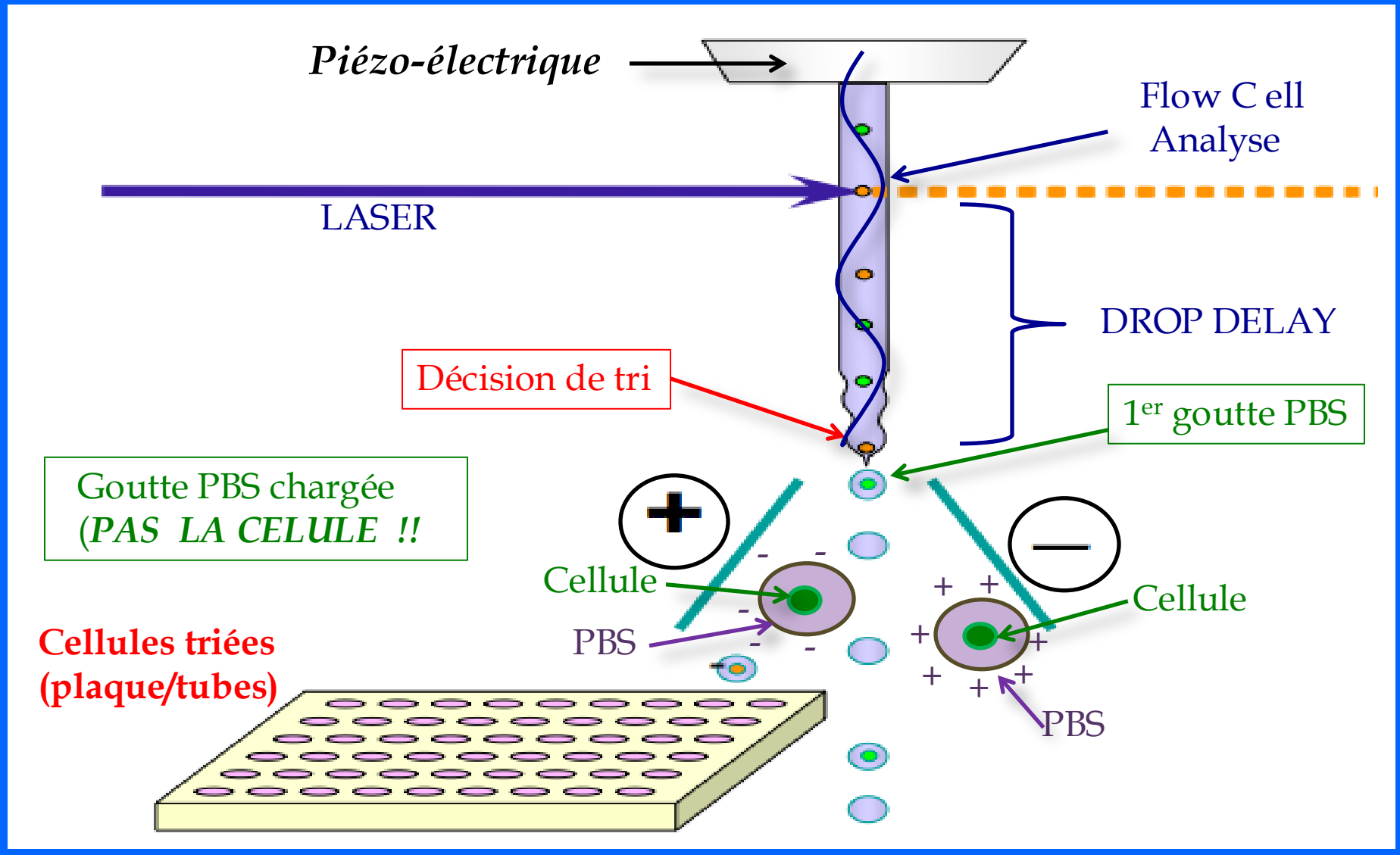
Cytométre : Trieur de cellules à Haute vitesse

TRIEUR DE CELLULES à HAUTE VITESSE

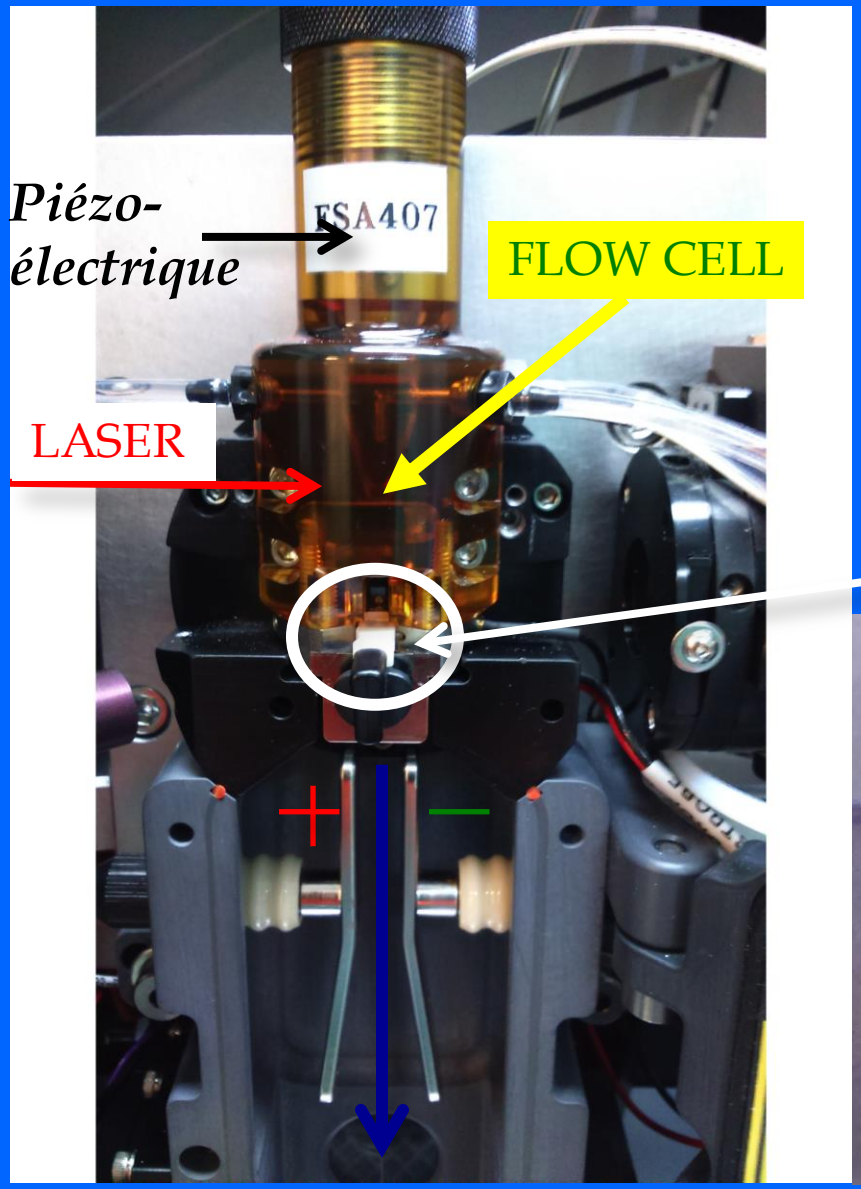
Même Principe du centrage hydrodynamique



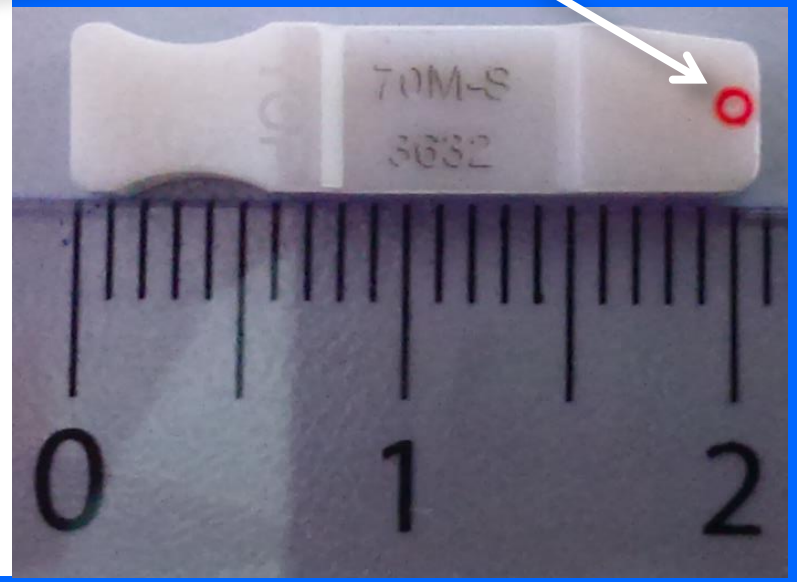
PRINCIPE DU TRIEUR à HAUTE VITESSE



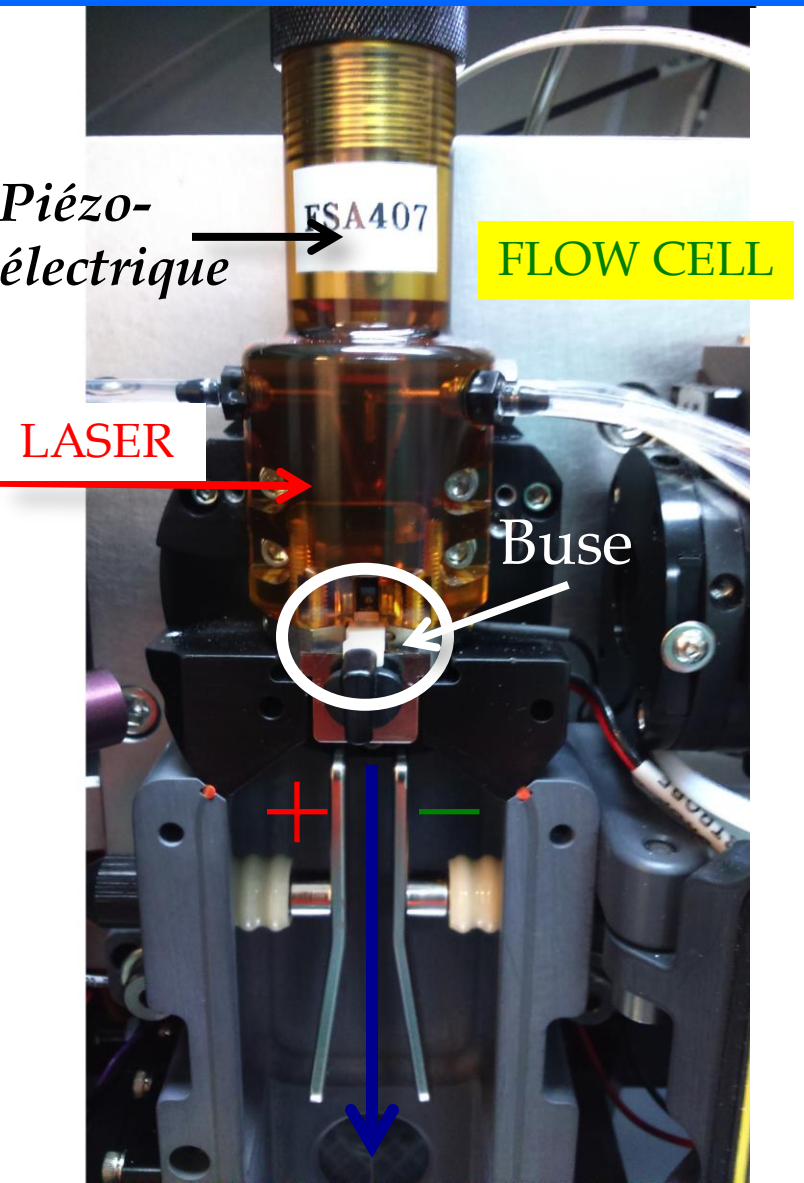
PRINCIPE DU TRIEUR à HAUTE VITESSE

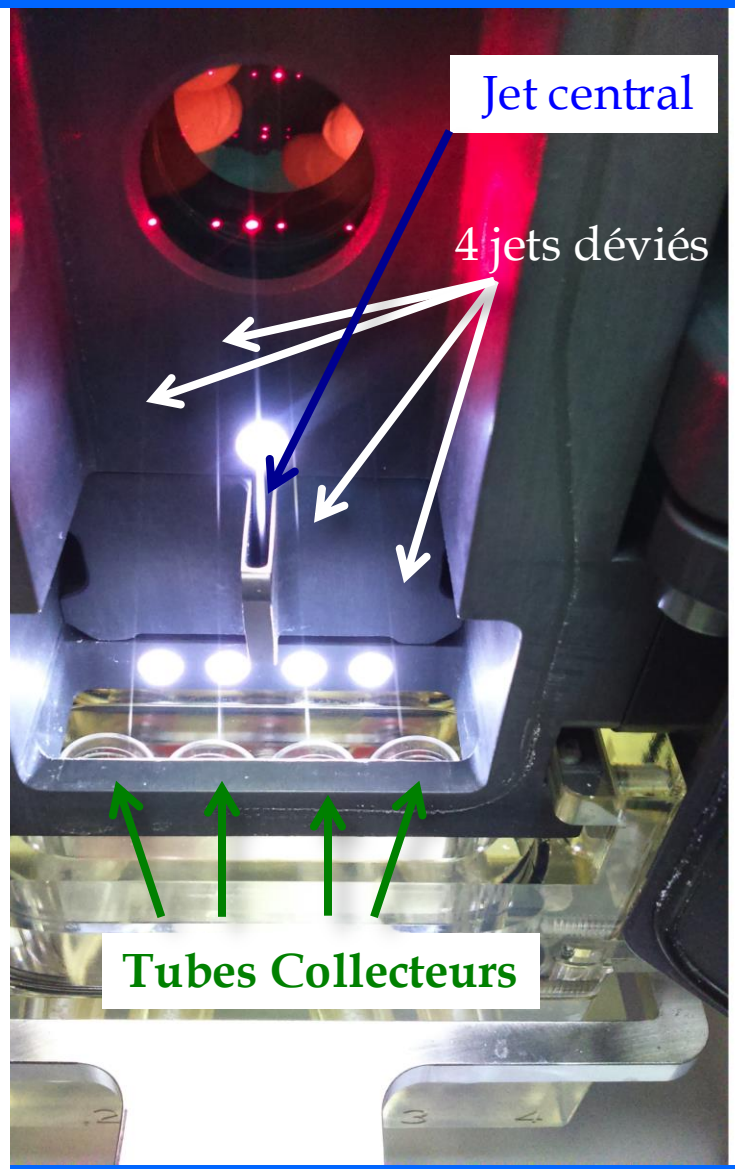


Buse (70 µm)

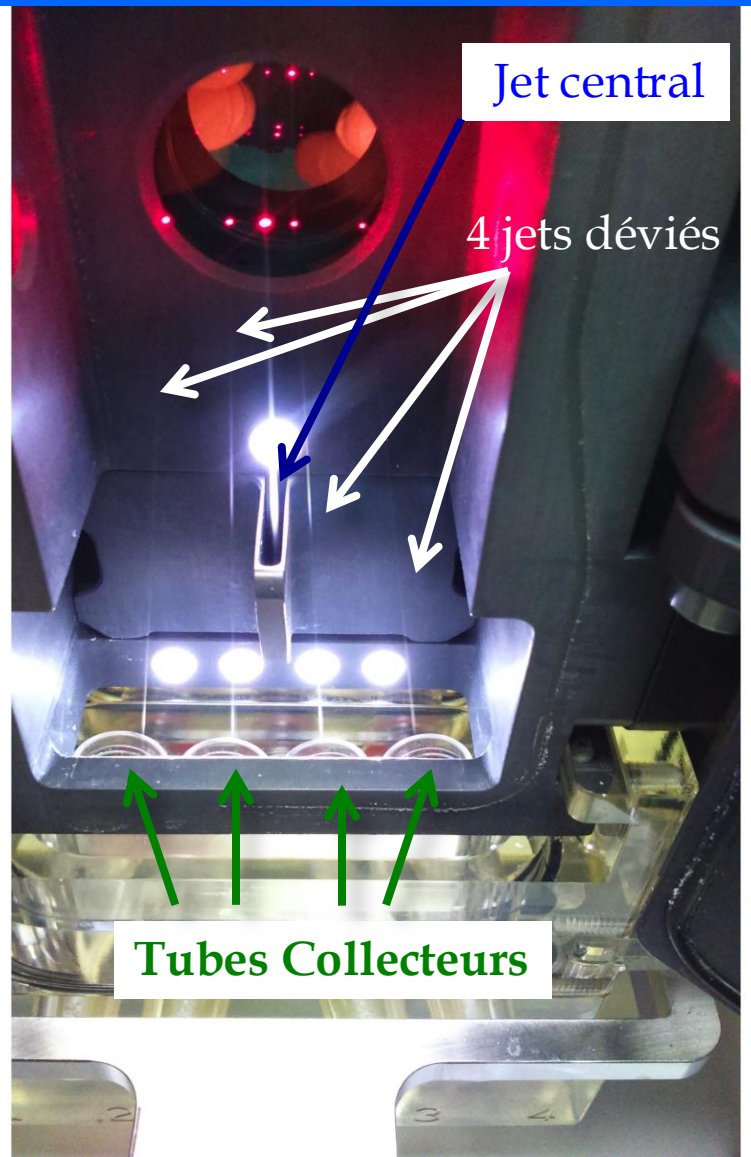


PRINCIPE DU TRIEUR à HAUTE VITESSE

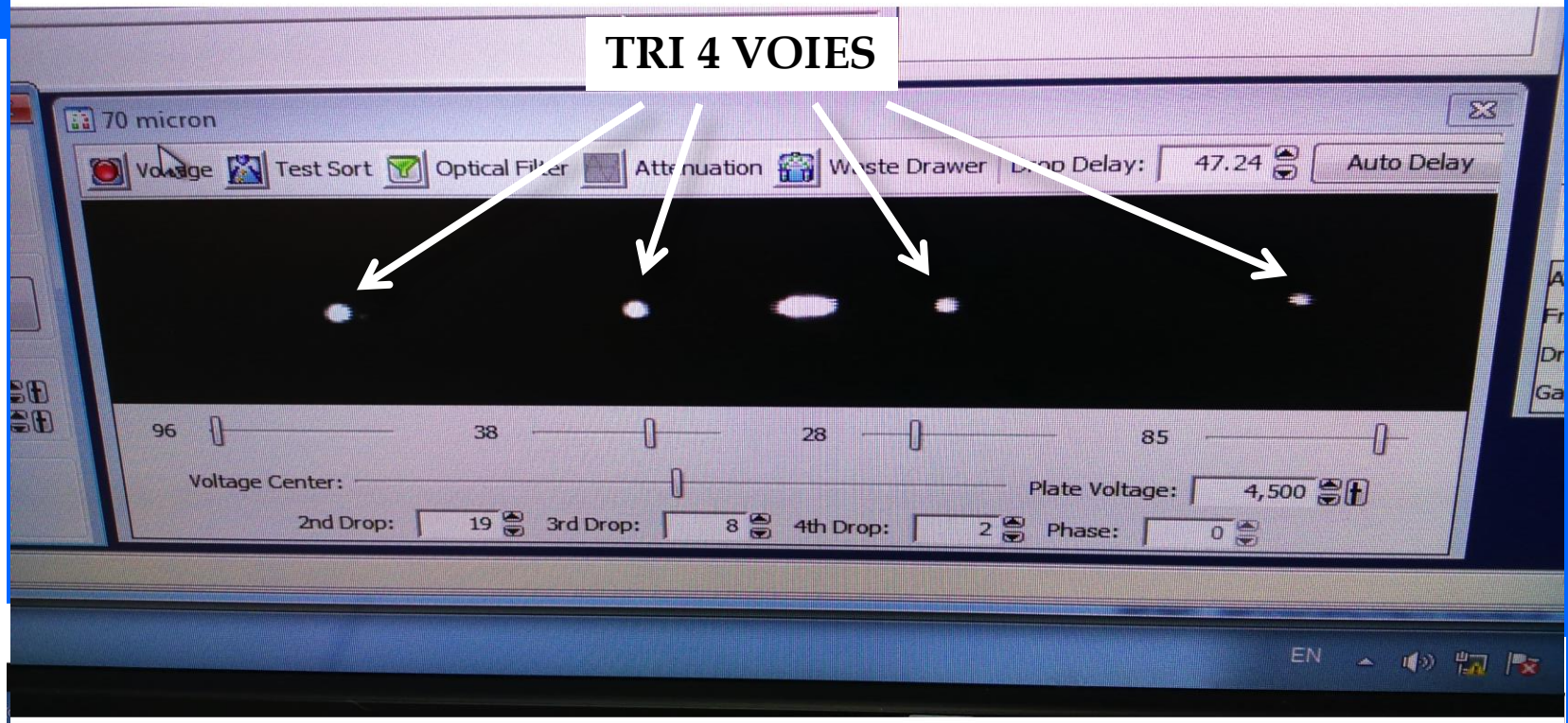




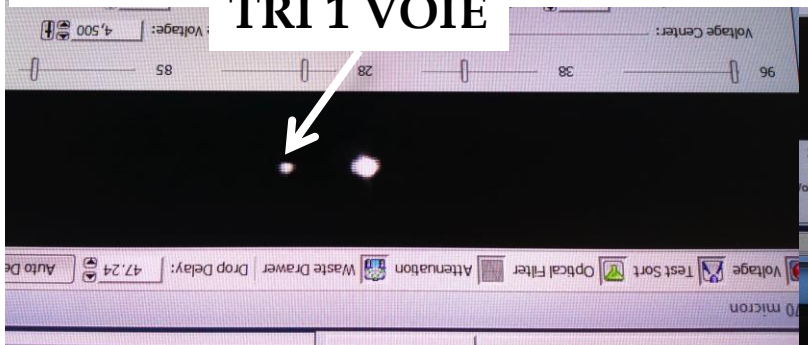
PRINCIPE DU TRIEUR à HAUTE VITESSE



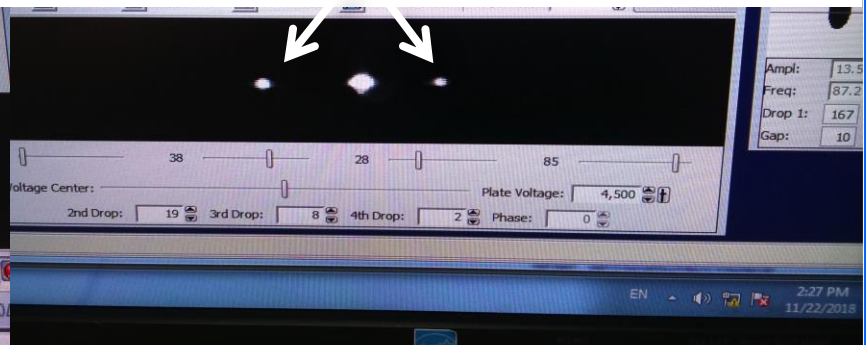
TRI 4 VOIES

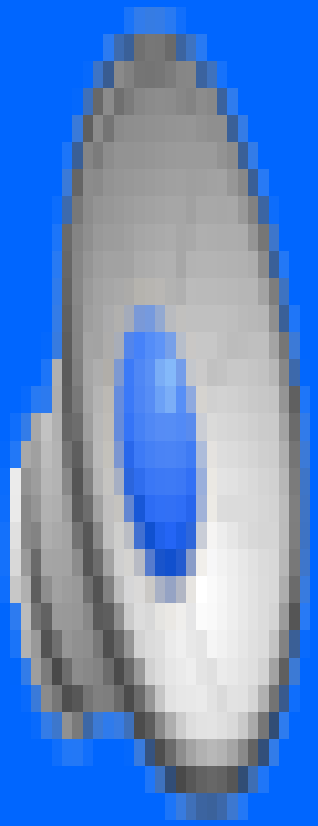


TRI 1 VOIE



TRI 2 VOIES





FACSARIA III

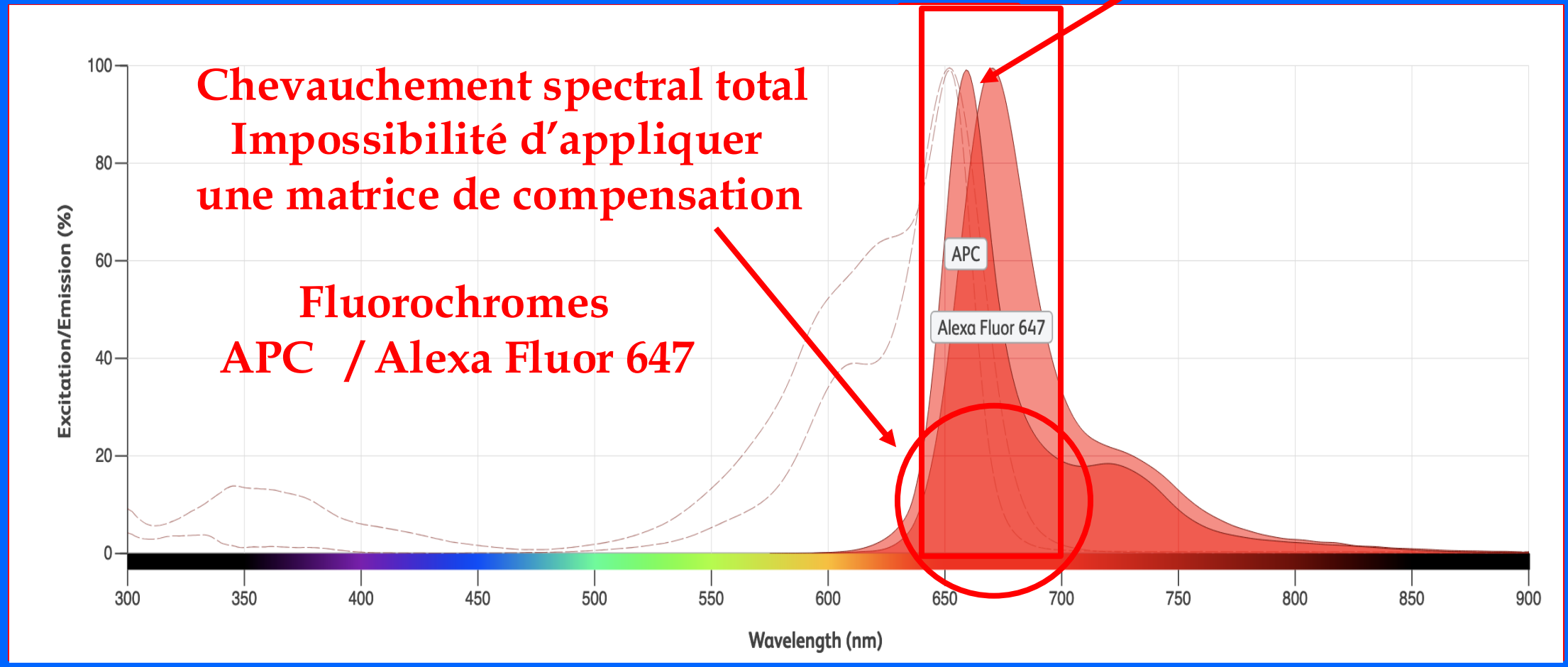
CYTOMETRIE EN FLUX

4. Nouvelles technologies spectrales

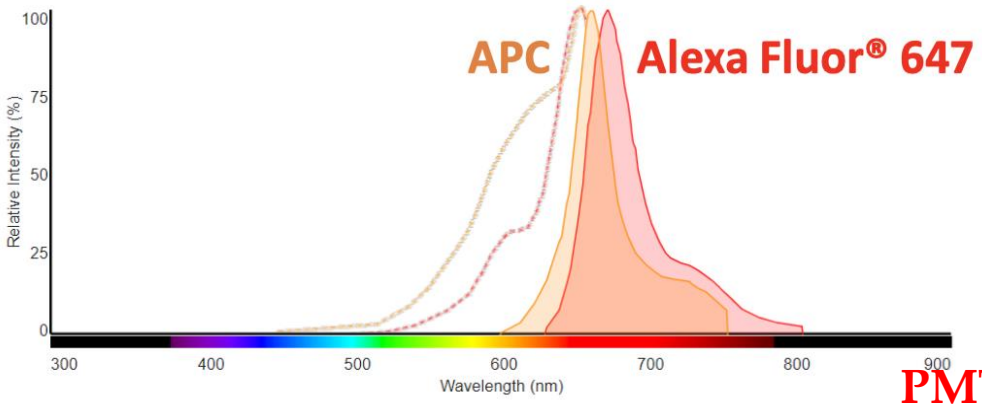
Principe de la Cytomérie classique

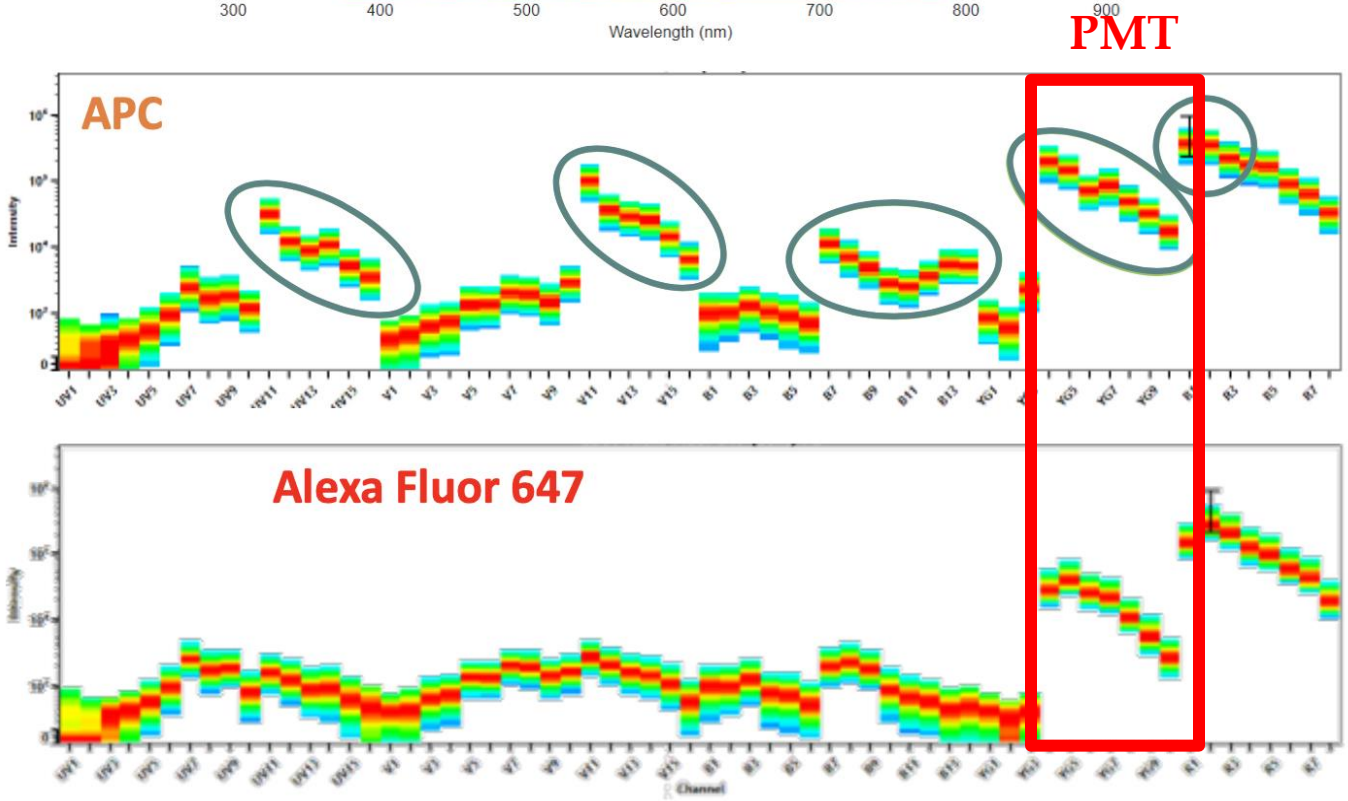
Détection Emission uniquement PMT

PMT

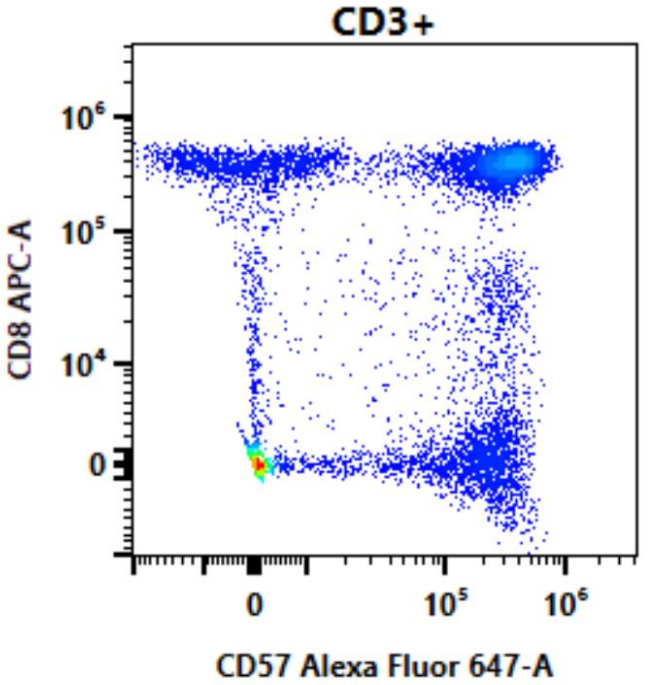


Use of Highly Overlapping Dyes





Plot gated on singlet lymphocytes



Fluorochromes with highly overlapping emission spectra can be used effectively on co-expressed markers

CONCLUSIONS (Partie 4)

- Cytométrie:
  - ↳ Possibilité de trier plusieurs populations cellulaires

- *Cytométrie Spectrale:* (nouvelle technologie)
  - ↳ Analyse de la fluorescence sur l'ensemble du spectre du fluorochrome et non pas sur la bande de détection du PMT

  - ↳ Augmentation du nombre de paramètres (nombre d'antigènes détectables)pour un même nombre de fluorochrome

CYTOMETRIE EN FLUX

5. Assurance Qualité en cytométrie:

* Alignement des lasers sur la FlowCell
* Centrage des fibres optiques
* Sensibilité et la linéralité de détection des PMT
* Stabilité du cytométre (fluidique)
* Reproductibilité des signaux

A FAIRE TOUS LES JOURS AVANT TOUTES ACQUISITIONS

ASSURANCE QUALITE EN CYTOMETRIE

Maintena

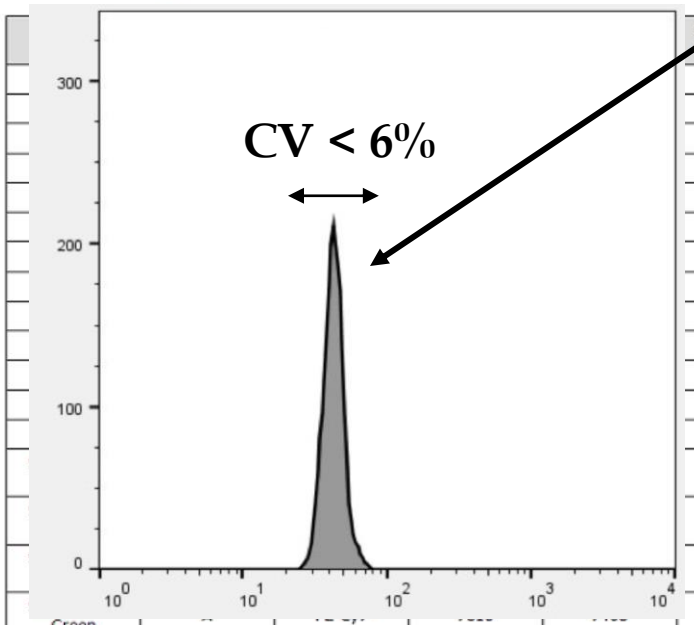
Cytometer Performance Report

Cytometer:	FACSAriaIII	User:	Administrator
Cytometer Name:	FACSAriaIII	Institution:	N/A
Serial Number:	P658919000001	Software:	BD FACSDiva 8.0
Input Device:	Manual	Date:	02/21/2020 09:06 AM
Cytometer Configuration:	70-70 488, 561, 633, 405- (3-4-3-6)	Cytometer Baseline:	02/13/2020 11:18 AM
		P/F:	Pass

Setup Beads

Bead Product:	C ST	Part #:	910858
Lot ID:	9 30	Expiration Date:	11/30/2021
Bead Lot Information:	A vai		

Detector Settings



CV < 6%

% Difference Target Value	Bright Bead % Robust CV	Mid Bead Median Channel	Mid Bead % Robust CV
-1	3.38	124378	3.44
0	6.25	125987	6.46
4	3.86	128	13.37
-2	4.91	407	14.9
-3	3.3	462	7.44
-4	4.33	317	7.56
-5	4.66	300	7.64
-1	4.46	187	10.33
-1	4.05	276	8.21
0	5.19	407	23.97
-1	4.38	975	12.69
-4	5.36	409	12.22
-4	6.92	332	16.06
-4	4.89	828	7.66
-5	5.24	231	13.9
-2	6.06	271	16.77
-6	6.6	236	11.32

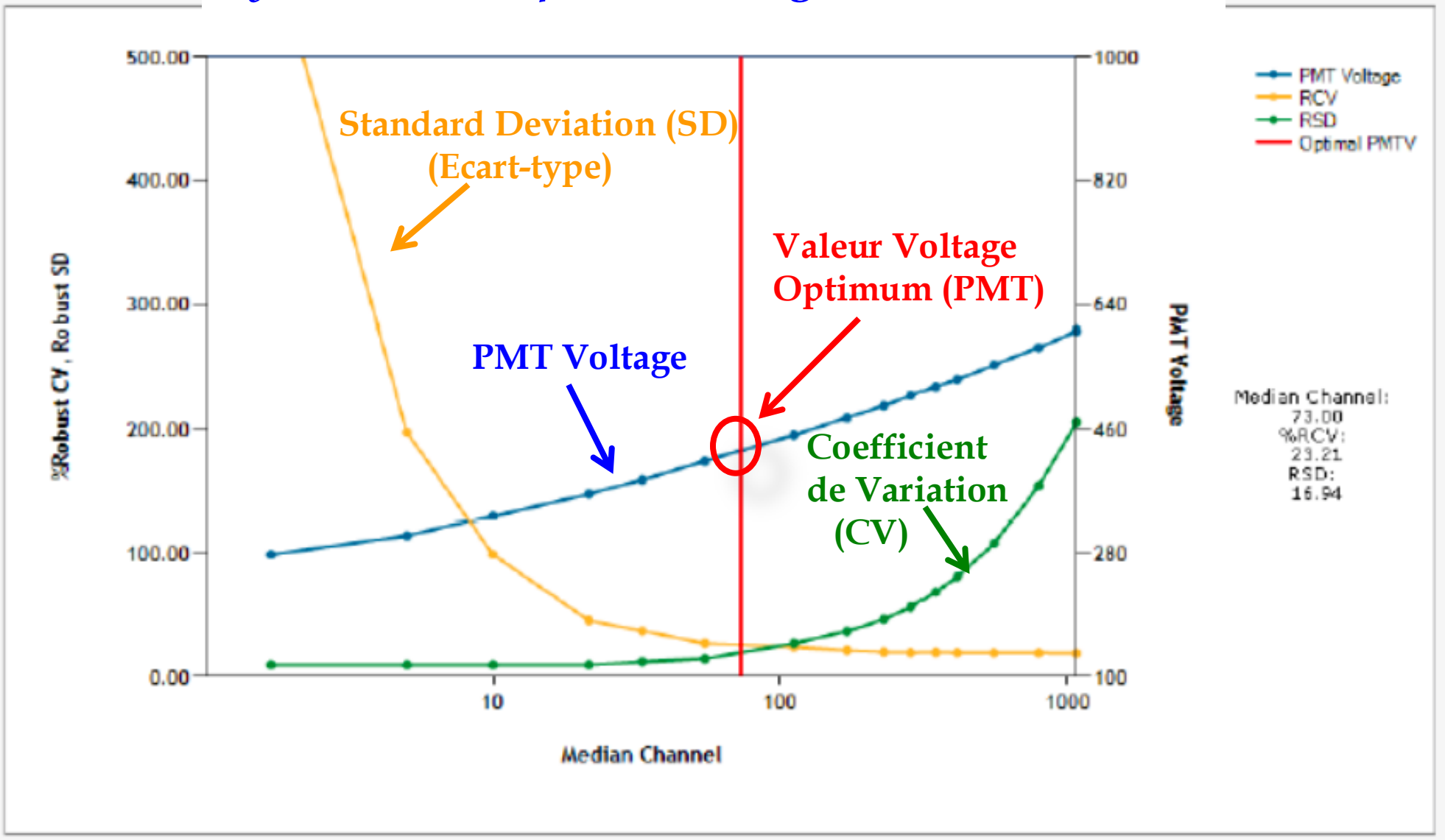
n Laser

We perform daily C
detection efficiency
If there is a major a

formance metrics such as

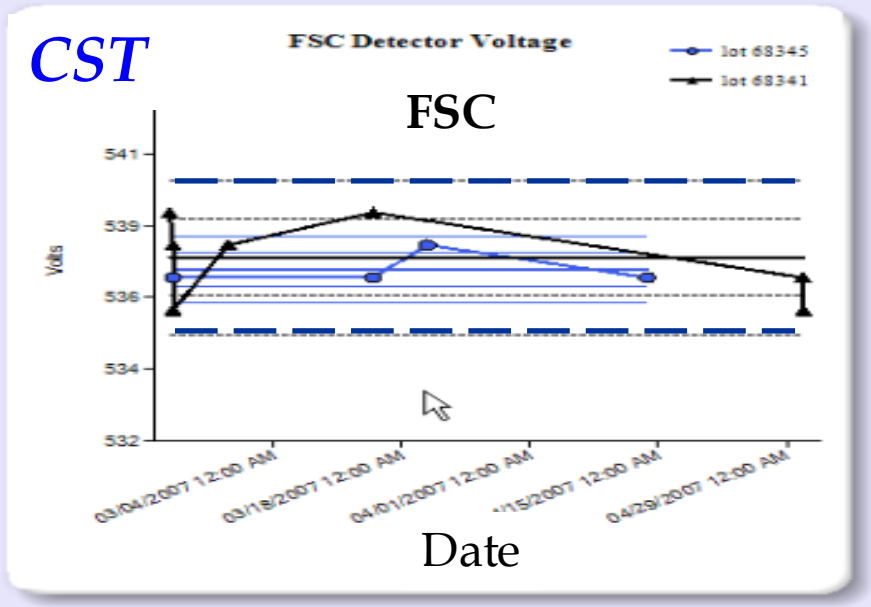
ASSURANCE QUALITE EN CYTOMETRIE

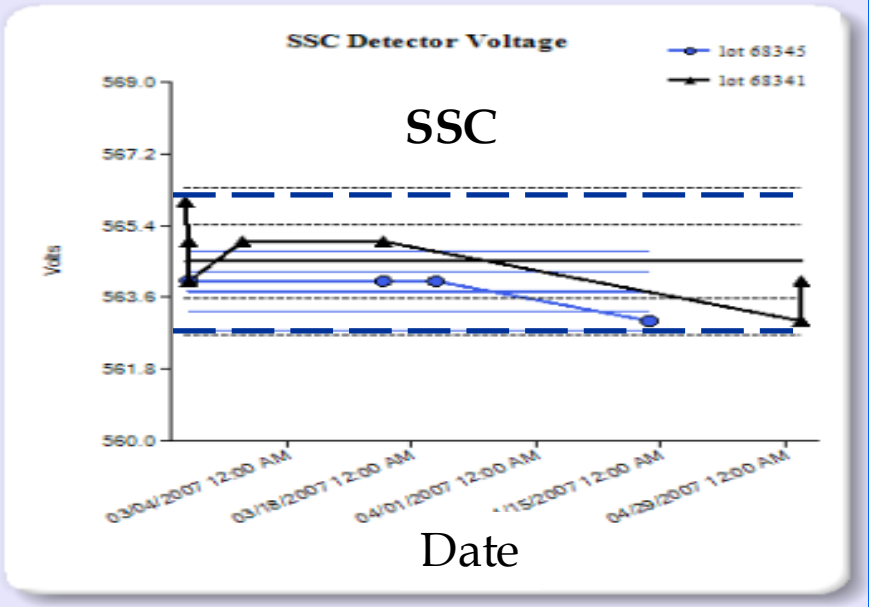
Cytometer Setup & Tracking (CST) Beads

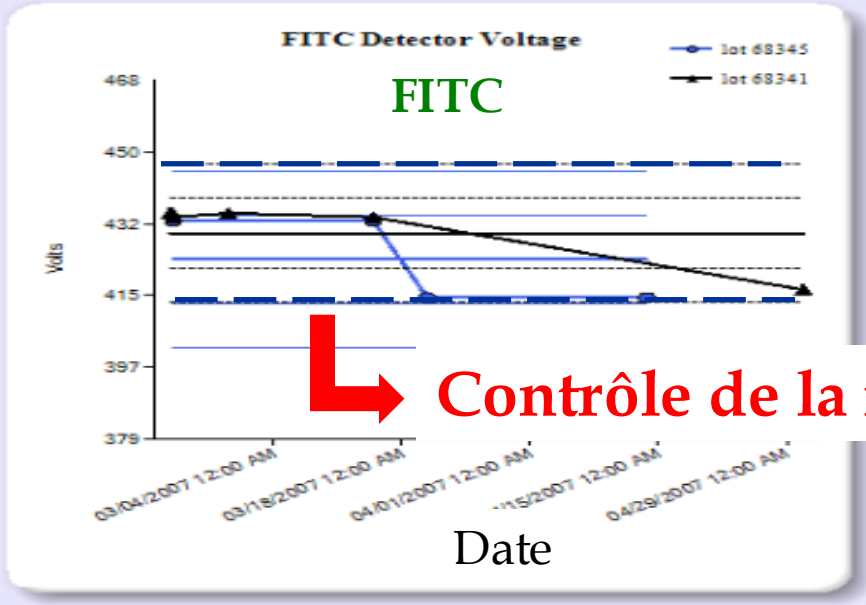


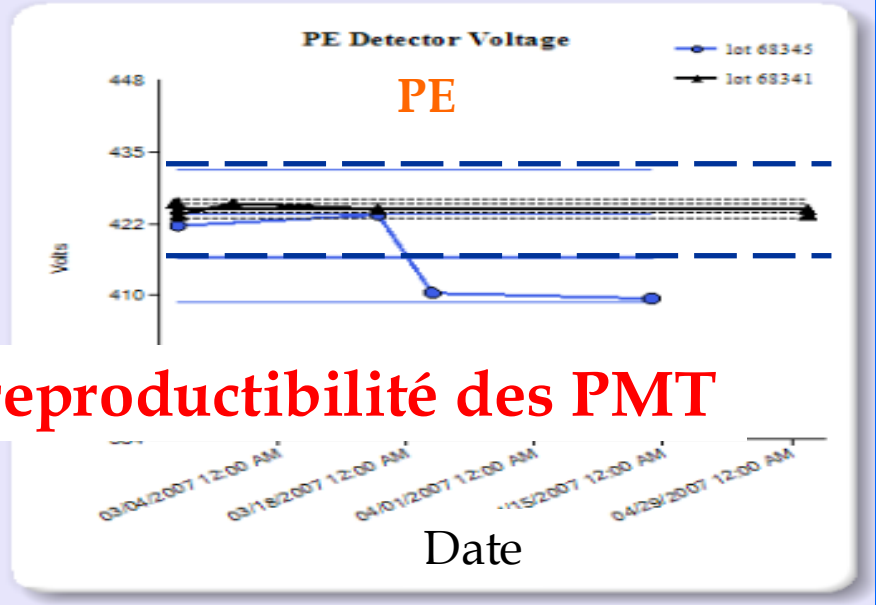
ASSURANCE QUALITE DU CYTOMETRE

CST





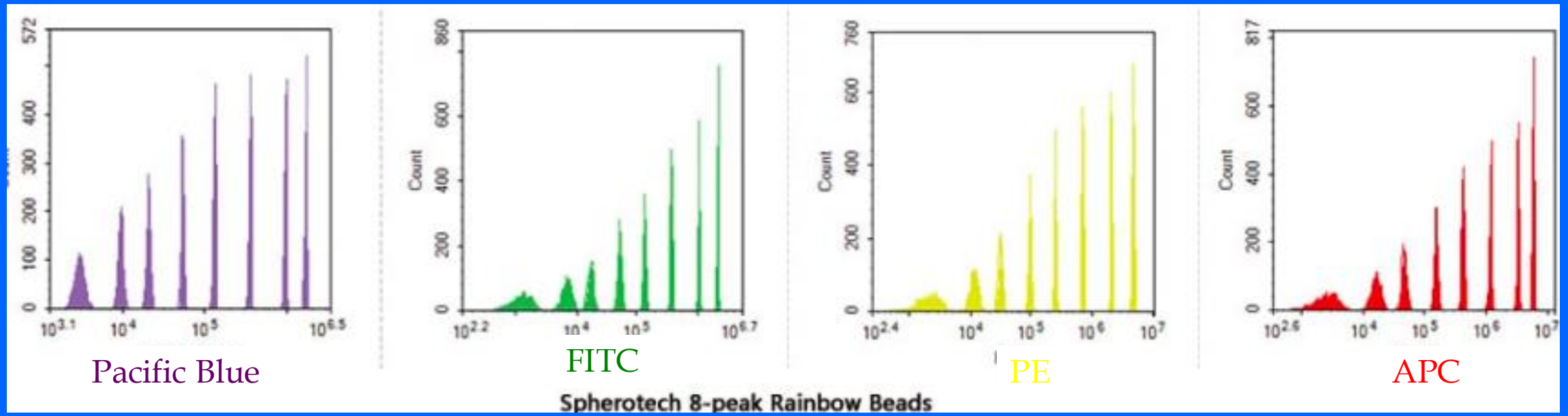




Contrôle de la reproductibilité des PMT

ASSURANCE QUALITE DU CYTOMETRE

SPHEROTECH 8 PEAKS RAINBOW BEADS



Pacific Blue FITC PE APC

VERIFICATION DE LA DETECTION DES PMT (Linéarité)

CYTOMETRIE EN FLUX

6. Applications :

Cycle cellulaire
Apoptose
Expression
Activité cellulaire

Les Phases du cycle

Mitose

G_2

Check-point

Augmentation du volume cellulaire

Préparation à la mitose

Contenu en ADN 4N

G_0

Quiescence

G0 = en dehors du cycle cellulaire

Contenu en ADN 2N

G_1

Préparation à la synthèse

Augmentation de la synthèse protéique et d'ARNm

Augmentation du volume cellulaire

Contenu en ADN 2N

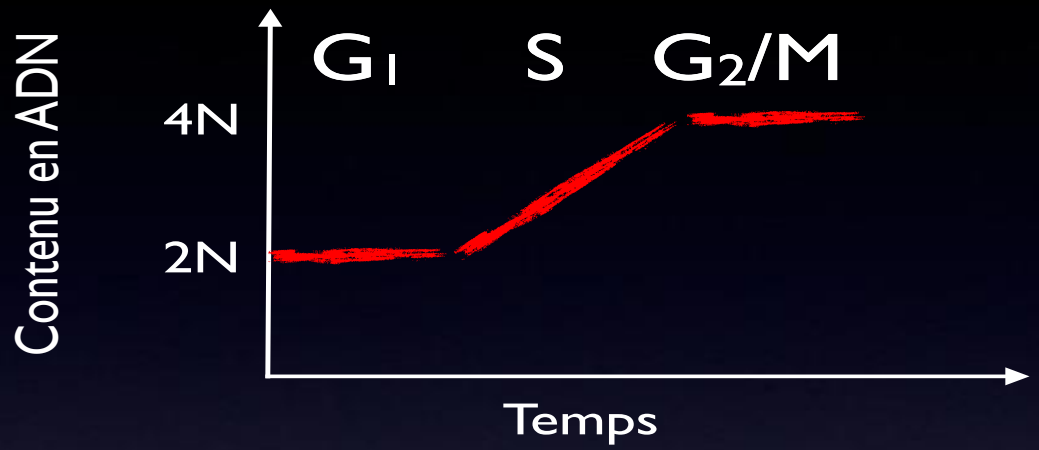
S

Réplication du génome

Contenu en ADN variable (2N 4N)

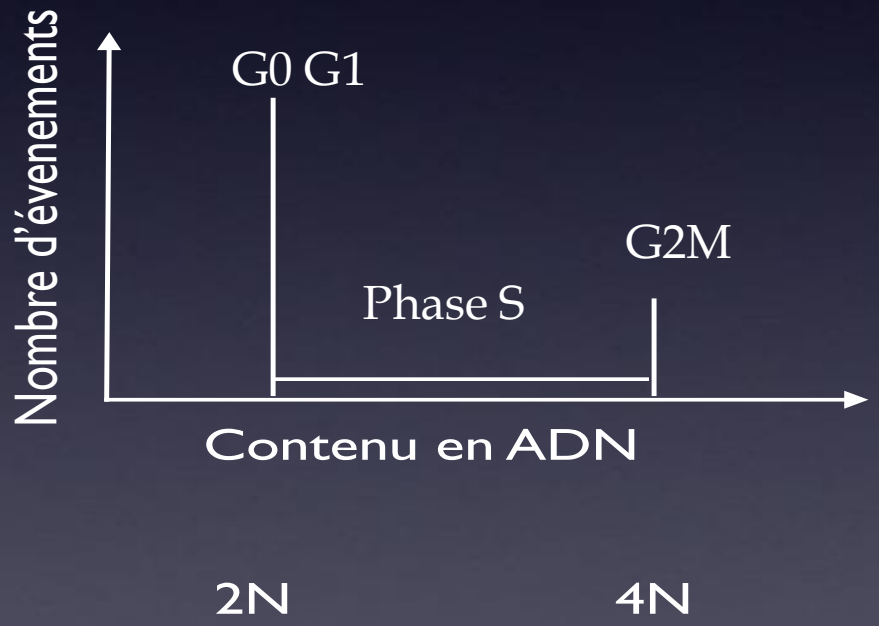
Cellules non quiescentes = phases du cycle cellulaire (G1/S/G2/M)

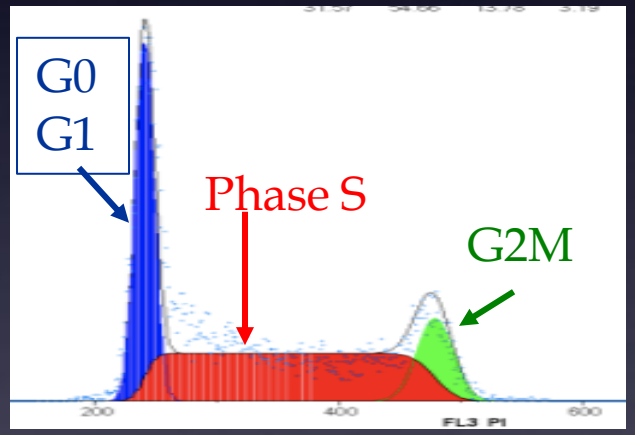
Le cycle celullaire en cytométrie en flux



Analyse Cycle Cellulaire:

- CV < 5%
- Singulet
- Fluorochrome spécifique ADN
- Pas de fixation ARN
- Perméabilisation





$$\frac{SD}{Mean} \times 100\% = CV$$

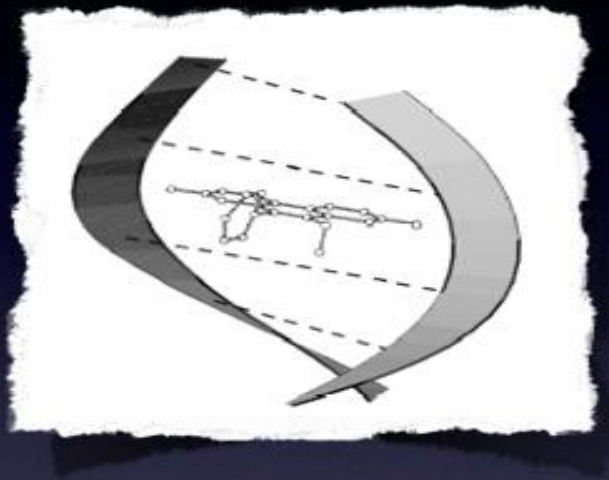
Cellule normale = diploïdie

Utilisation de fluorochrome spécifique de l'ADN

Les fluorochromes

Modes de liaison aux acides nucléiques

Les intercalants



Dans une double hélice

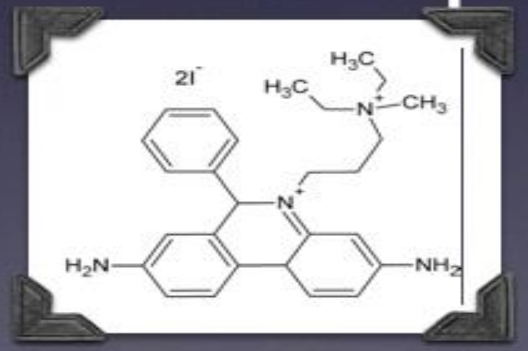
ADNADN

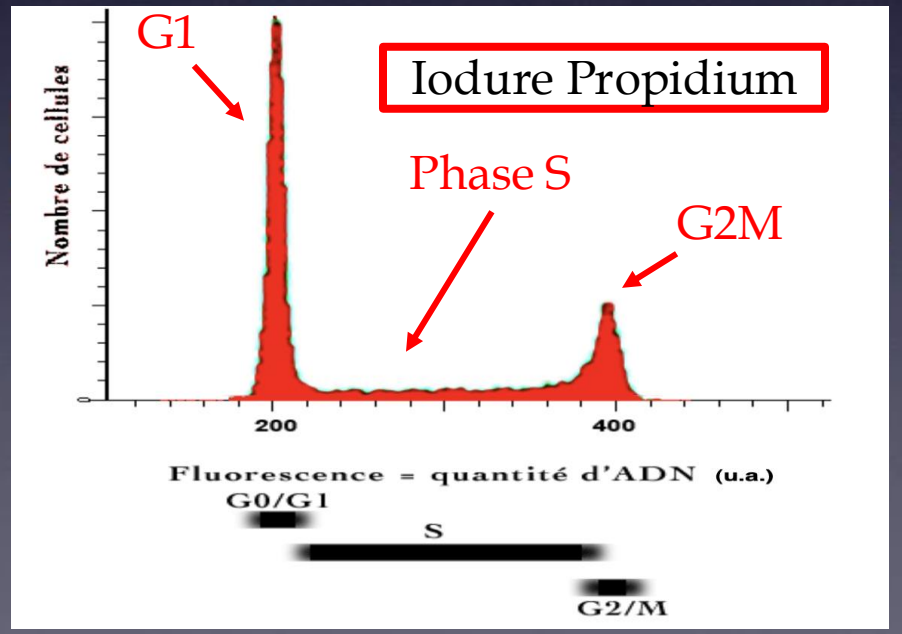
ADNARN

ARN ARN

→ Rnase
Perméabilisation

Iodure de Propidium



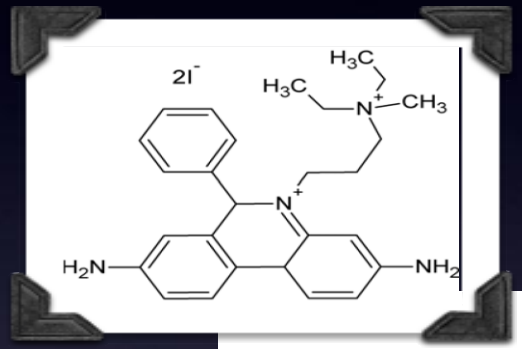


Les fluorochromes

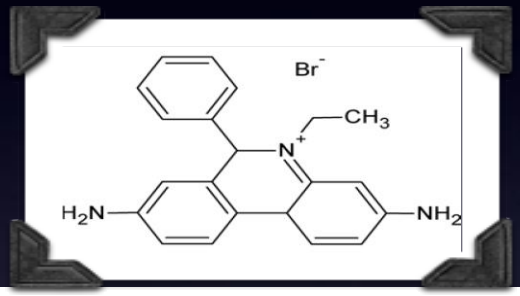
Modes de liaison aux acides nucléiques

Les intercalants

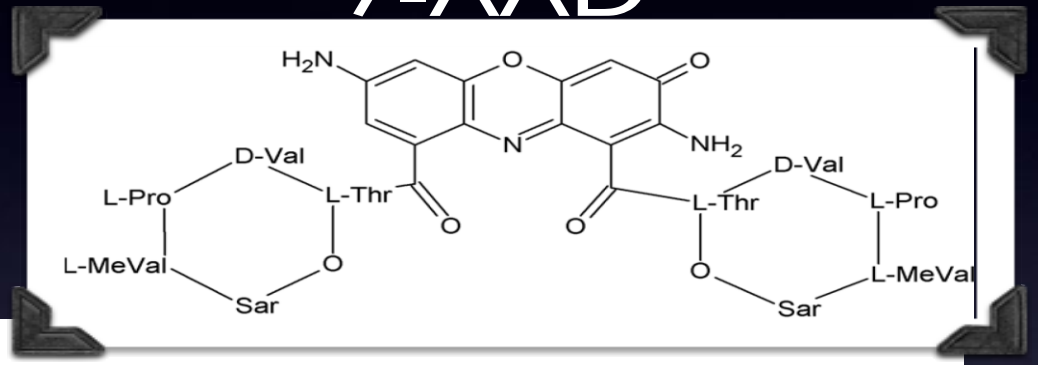
IP

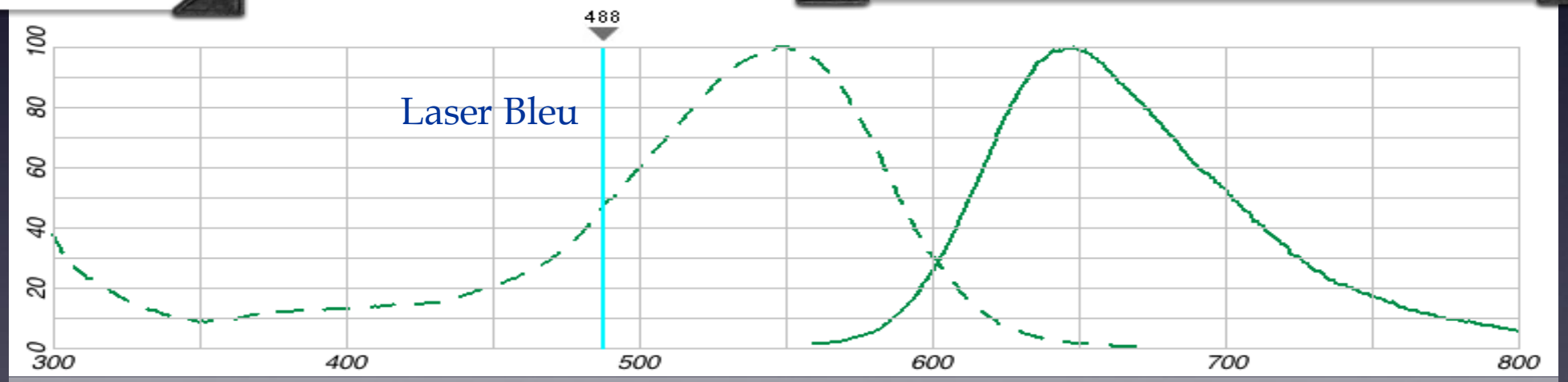


Ethidium



7-AAD





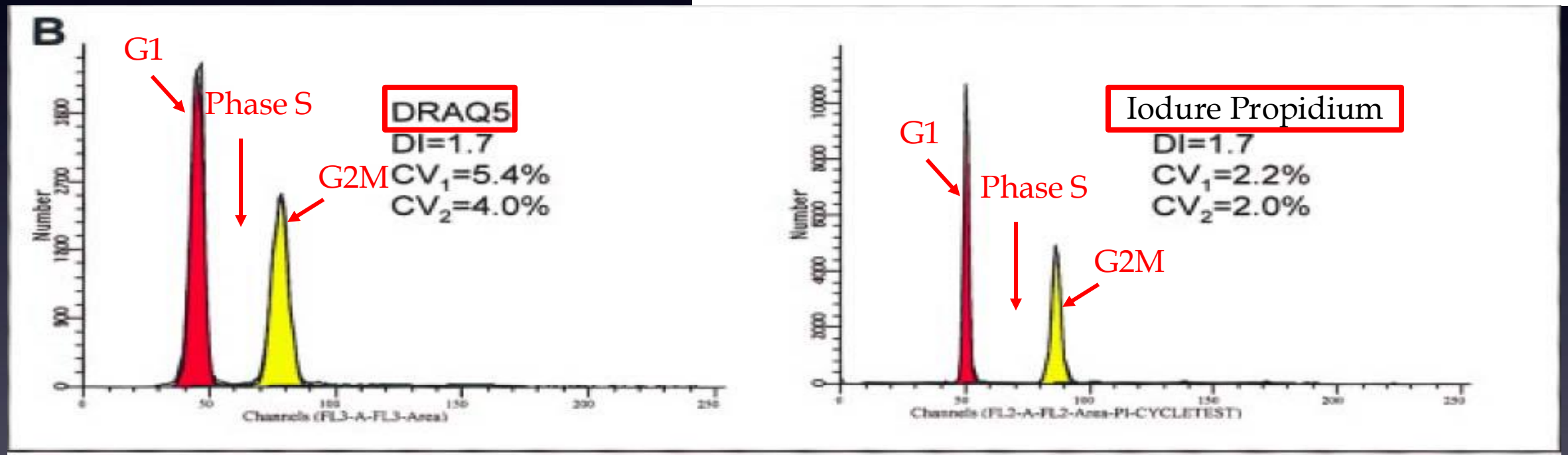
Les fluorochromes

Modes de liaison aux acides nucléiques

Les intercalants

DRAQ5 Diffuse librement à travers les membranes

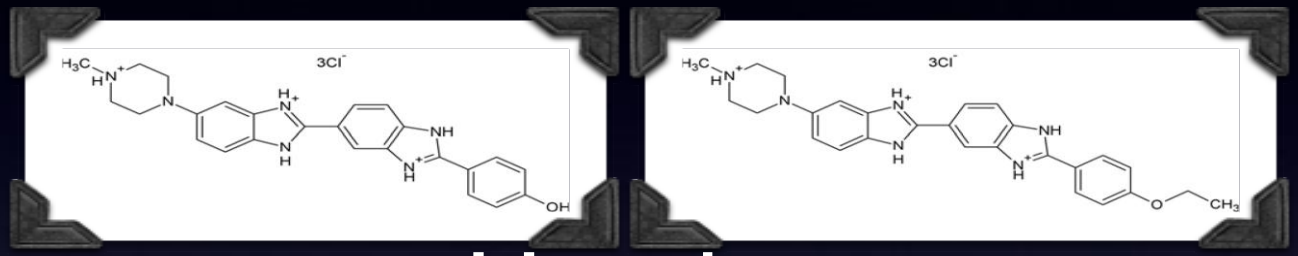
Cytometry Part B (Clinical Cytometry) 58B:47–52 (2004)



Les fluorochromes

Modes de liaison aux acides nucléiques

Liaison électrostatiques



Hoechst

33 258

Cellules fixées

33 342

Cellules fixées + vivantes

Régions riches en bases AT

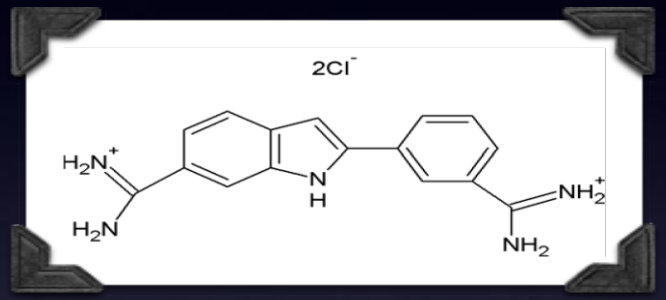
Pas de traitement RNAse

Les fluorochromes

Modes de liaison aux acides nucléiques

Liaison électrostatiques

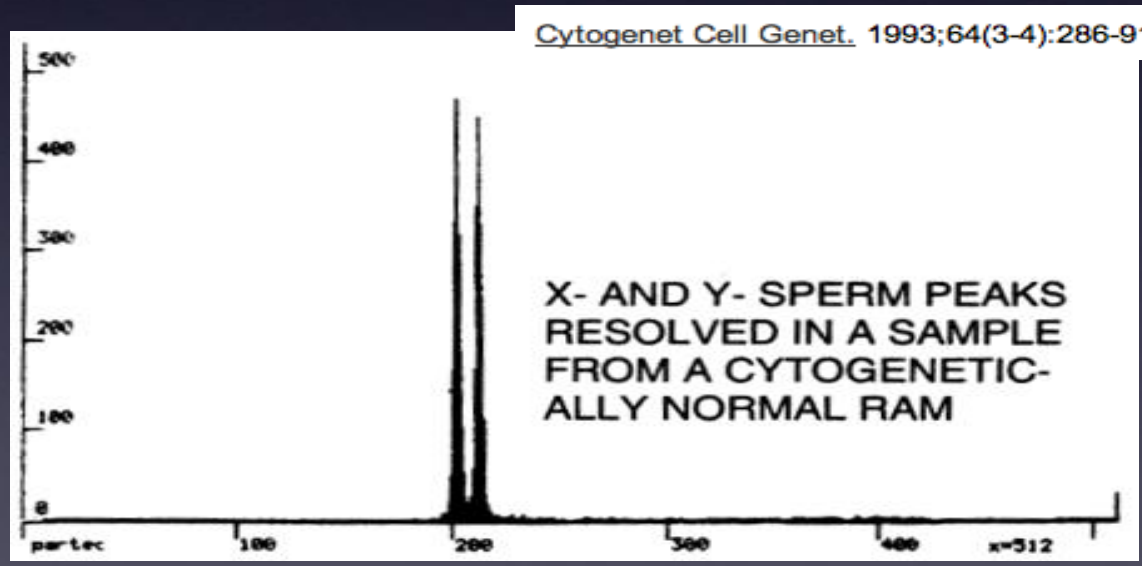
DAPI



Petit sillon de la double hélice
région riche en bases A-T
«Semi-perméant»

Très bons CV

Différence d'intensité de fluorescence : 4,8 %
CV : 0,6 - 0,8%



Méthode de marquages ADN

Propriétés nécessaires:

- **Fluorescence:**
  La fixation doit être stœchiométrique (saturation fluorochrome de l'ADN)

- **Marquage ADN seul**:
  Les cellules doivent être perméabiliser pour faire pénétrer le fluorochrome pour le marquage de l'ADN, soit avec l'éthanol (fixe et perméabilise) ou Saponine (Tween 20)

- **Multimarquage Antigène et ADN simultanément**:
  Les cellules doivent être fixer (PFA4%) pour garder les marquages membranaires et intra-cytoplasmiques, mais également perméabiliser pour faire pénétrer le fluorochrome pour le marquage de l'ADN.

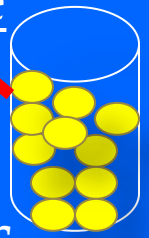
- **Iodure Propidium**:
  Traitement à la RNAse pour dégrader l'ARN (qui fixe l'IP)

Protocole d'un Marquage ADN SEUL

Sang périphérique

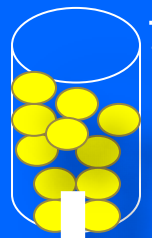
Comptage de la concentration cellulaire

~~Tube contrôle~~
~~1. 10°6 cellules~~



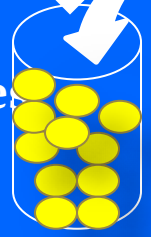
Pas de Saturation Récepteur Fc

Tube test
1. 10°6 cellules



Perméabilisation des membranes cytoplasmique et nucléaire

Tube test



Ethanol ou Saponine

Pas d'anticorps couplé à un fluorochrome
Marquage de l'ADN SEUL

Tube test

Marquage ADN par un intercalant fluorescent

Iodure Propidium

DAPI

Protocole d'un MultiMarquge ET d'un marquage ADN

ETAPE 1

Sang périphérique → **Comptage de la concentration cellulaire**

Tube FMO
(Tube Contrôle)

Tube Multicouleurs
(Tube Test)

Saturation Récepteur Fc

Tube FMO

Tube Multicouleurs

Immunomarquage Multicouleurs

Tube FMO

Isotype contrôle IgG1 FITC
CD45 BV421, CD38 PeCy5.5
CD19 PECF594, CD20 APC,
CD3 PE, CD8 PeCy7

IgG1 CD4 FITC
CD45 BV421, CD38 PeCy5.5
CD19 PECF594, CD20 APC,
CD3 PE, CD8 PeCy7

Tube Multicouleurs

Protocole d'un MultiMarquge ET d'un marquage ADN

ETAPE 2

Après Saturation Récepteur Fc
Après Marquages Membranaires

Tube FMO
(Tube Contrôle)

Tube Multicouleurs
(Tube Test)

PFA

Fixation membranaire

Tube FMO

Tube Multicouleurs

Saponine

Perméabilisation membranaire et nucléaire

Tube FMO

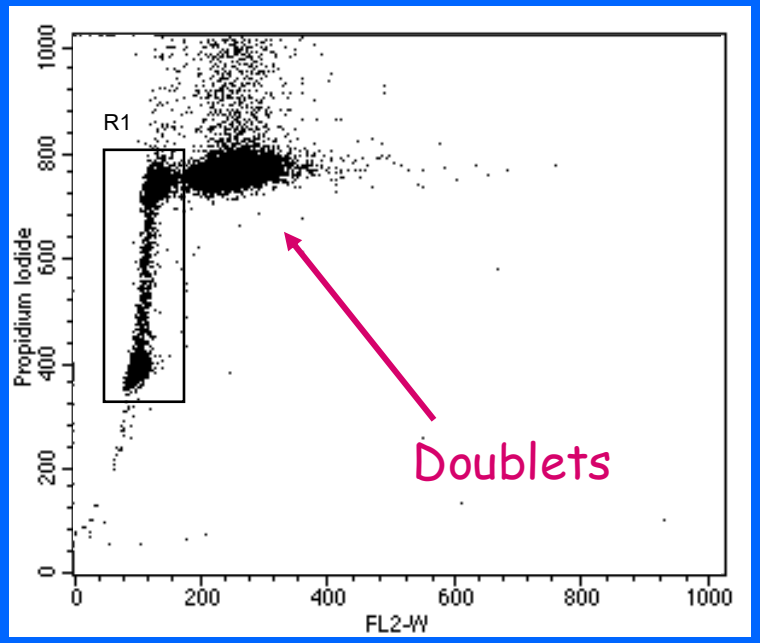
Tube Multicouleurs

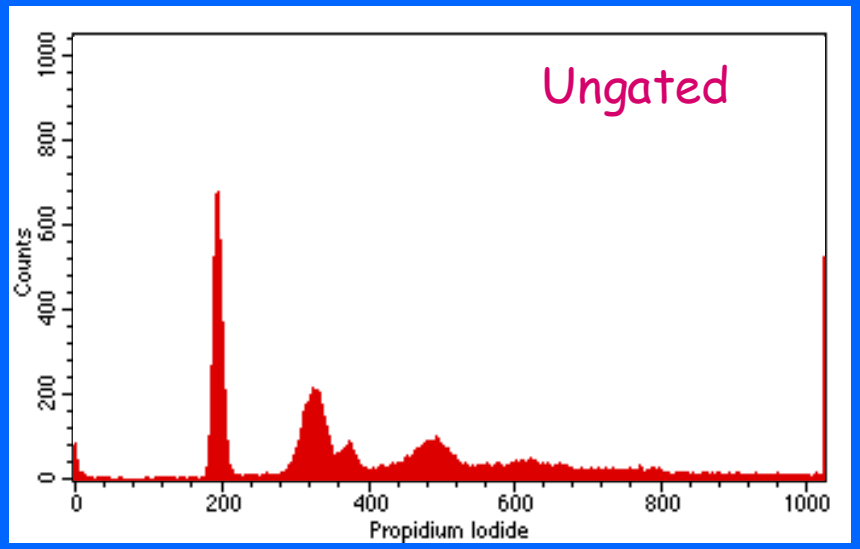
DAPI

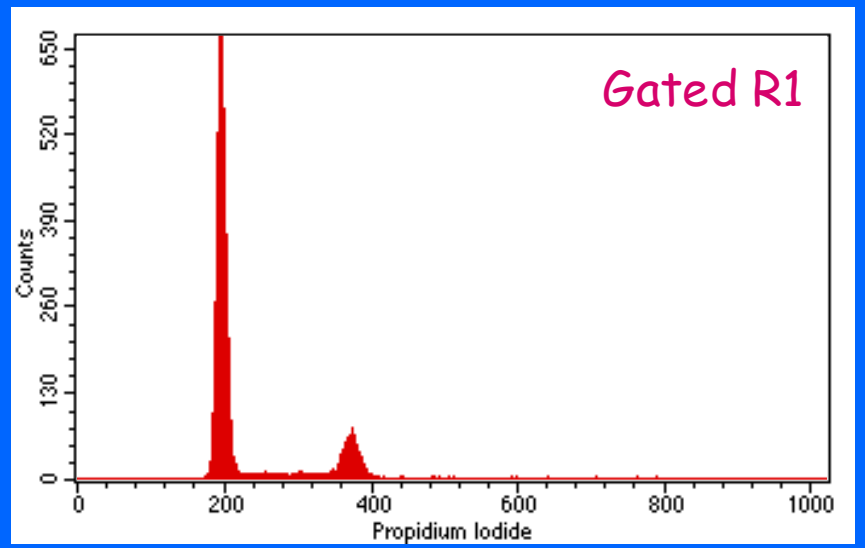
Iodure Propidium

Marquage ADN par un intercalant fluorescent

Eliminer les doublets de cellules



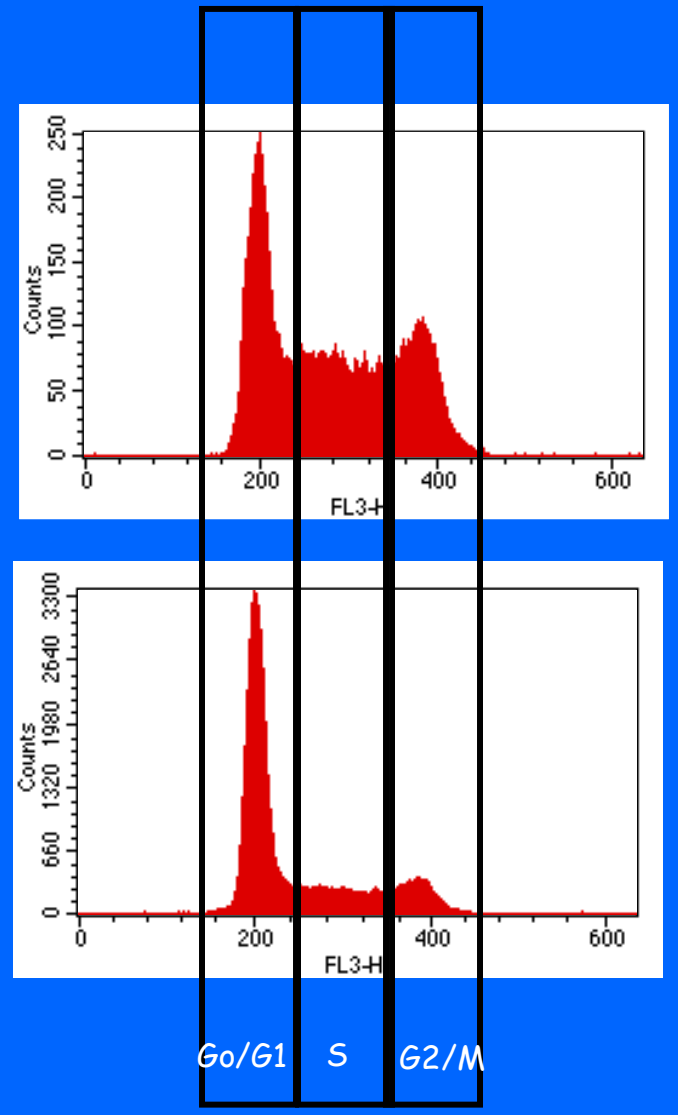




Comparaison des Cycles Cellulaires

Jour 1

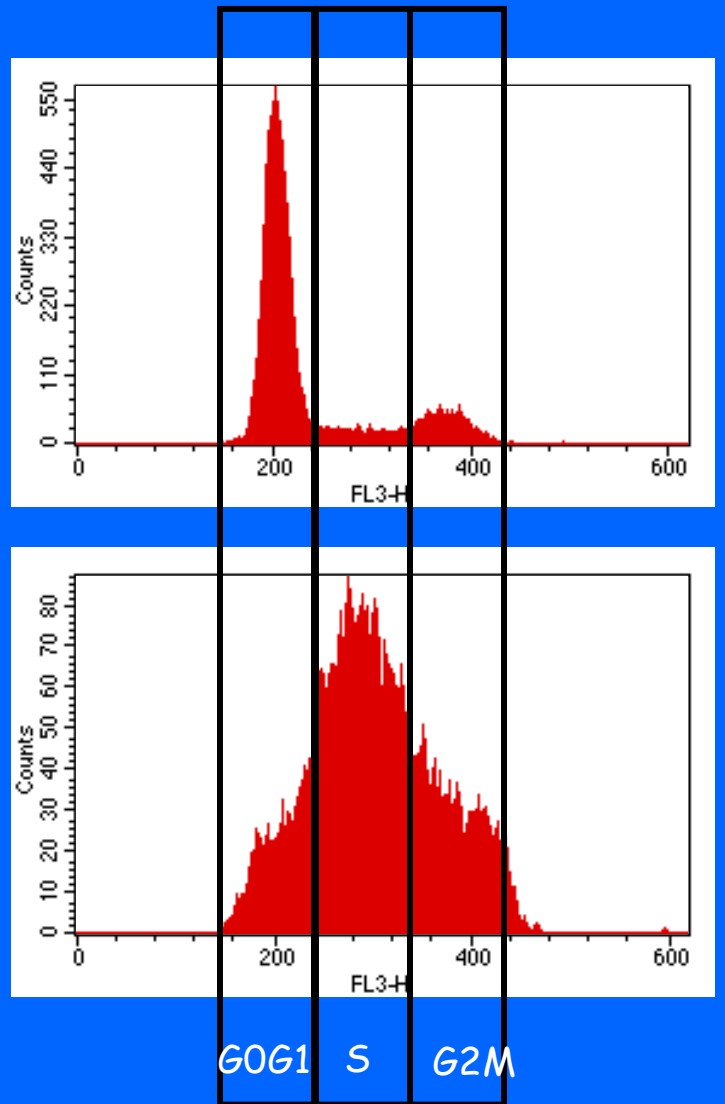
Jour 3



Blocage de la phase S

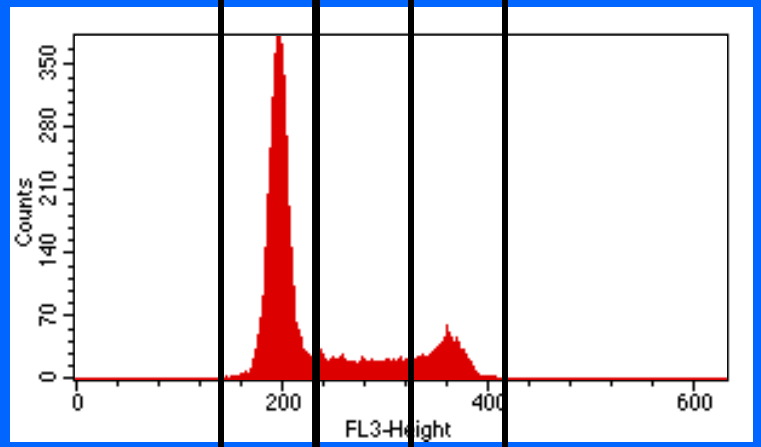
T0

T24

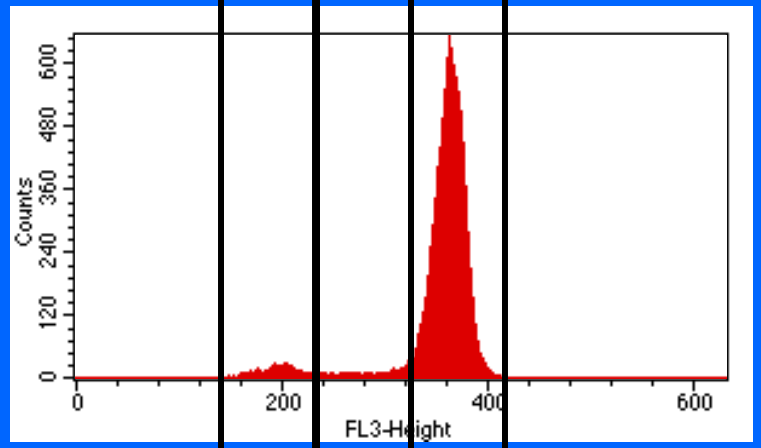


Blocage de la phase G2M

T0

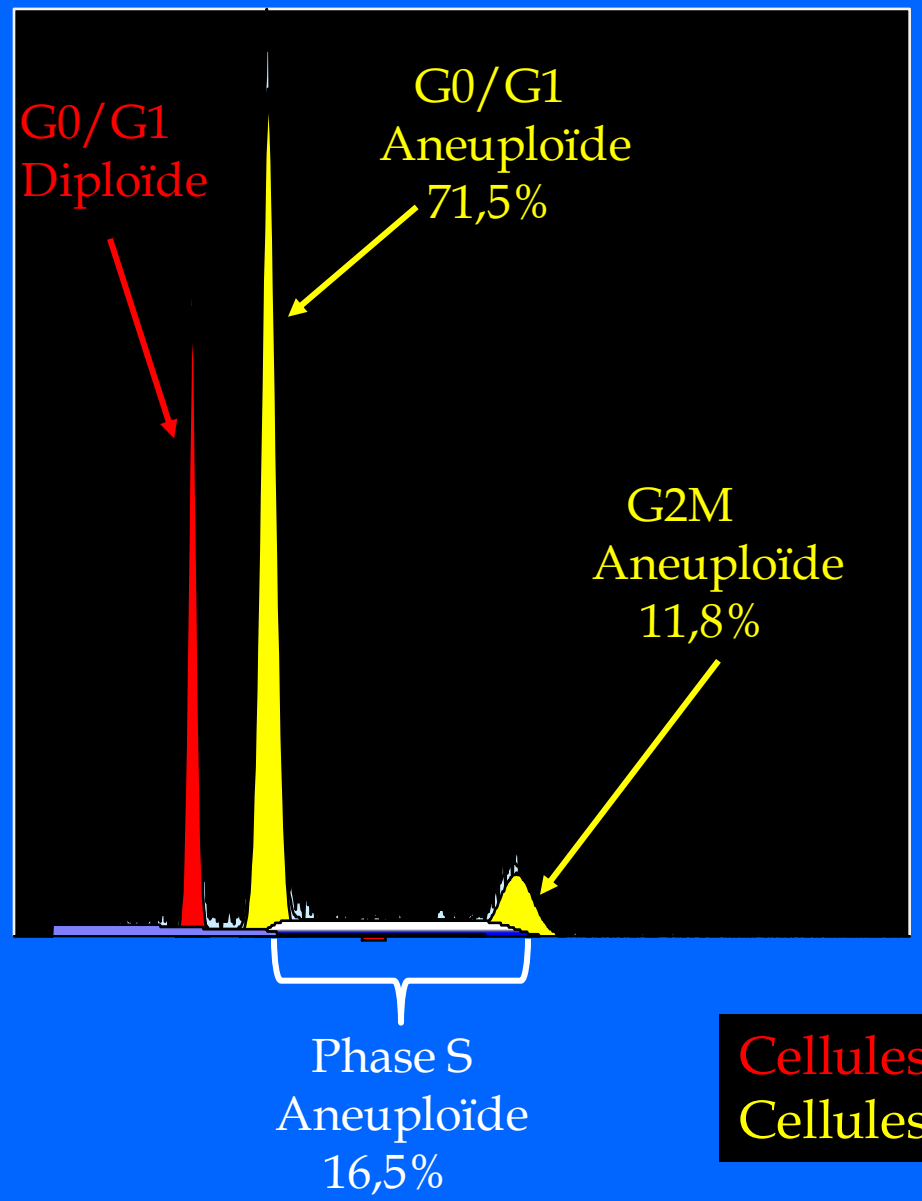


T24



G1 S G2M

Tumeur ADN – Aneuploïdie



- **Patiente de 35 ans**
- **CCI (carcinome) , Grade III**

- **Cellules normales = Diploïde**
- **Cellules tumorales = Aneuploïdie**

- **Index d'ADN = 1.41**

- **Proportion : 77.2% cellules tumorales**
- **%An G0-G1: 71.5**
- **%An S: 16.7**
- **%An G2-M: 11.8**

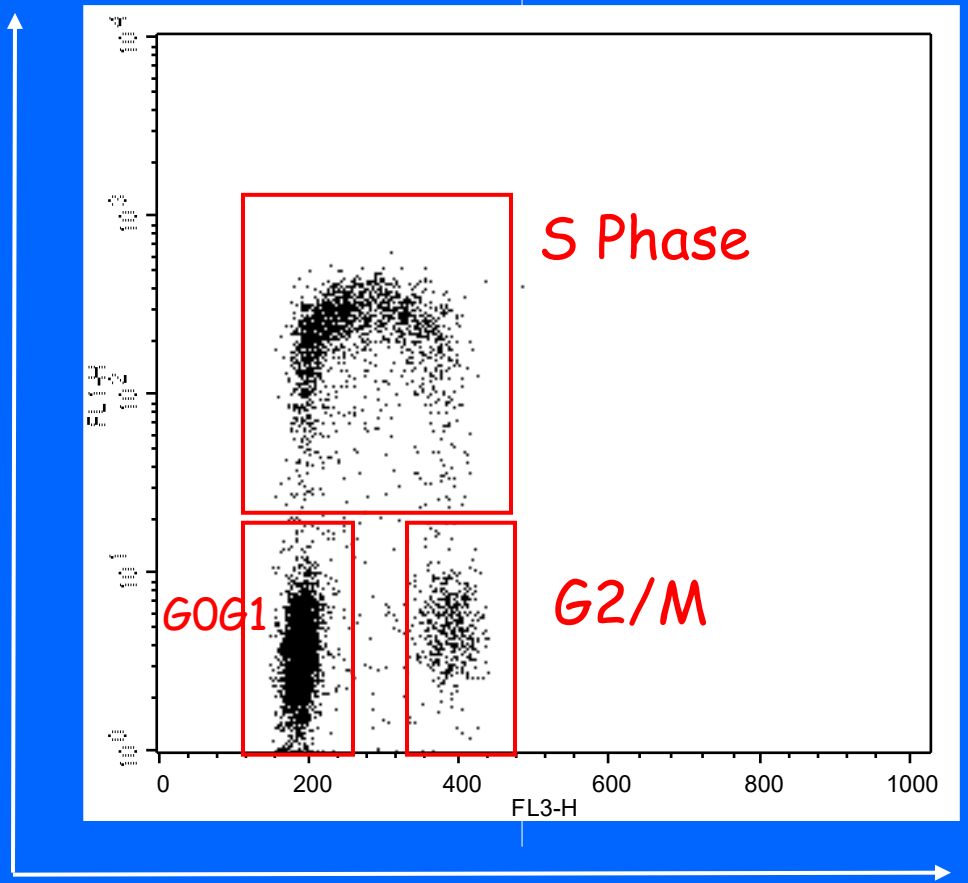
Cellules normales en rouge (Diploïde)
Cellules tumorales en jaune (Aneuploïde)

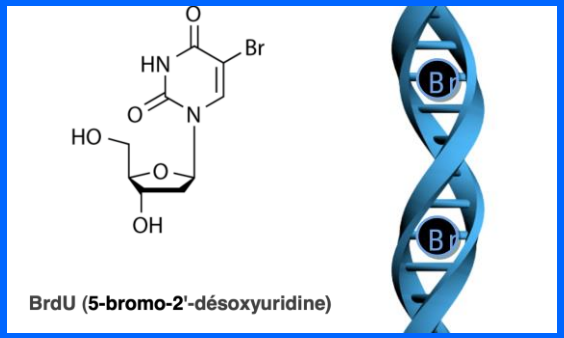
Cycle cellulaire avec BrdU

BrdU= 5-Bromo-2-déoxyUridine: Analogue artificiel de la Thymidine

Incorporation dans l'ADN

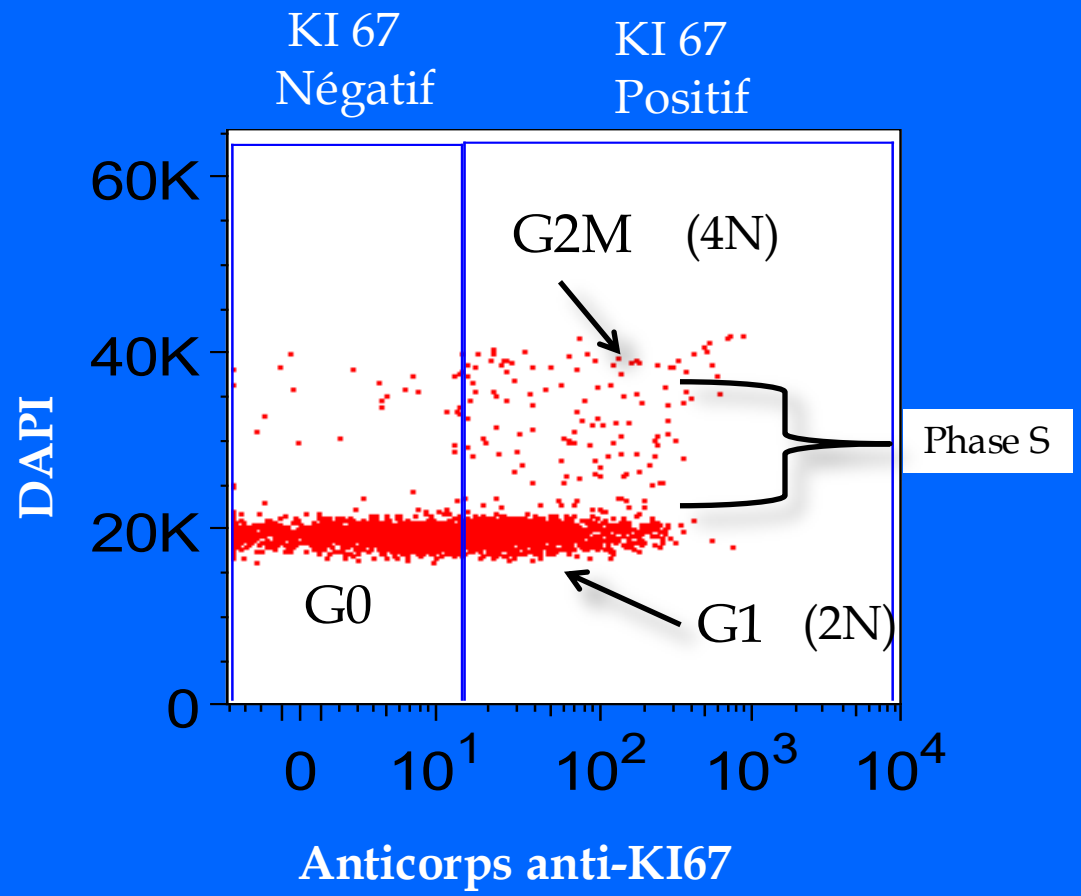
Révélation par un anticorps anti-BrDU





Protéine KI 67

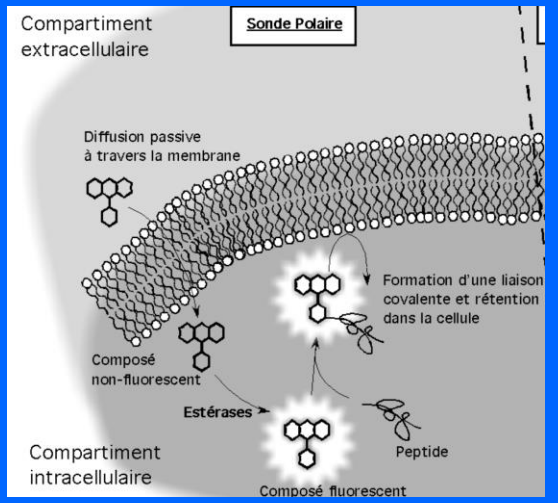
KI 67: Protéine nucléaire exprimée durant les phases du cycle cellulaire (G1, Phase S , G2M)
Mais pas durant la phase de quiescence (G0)

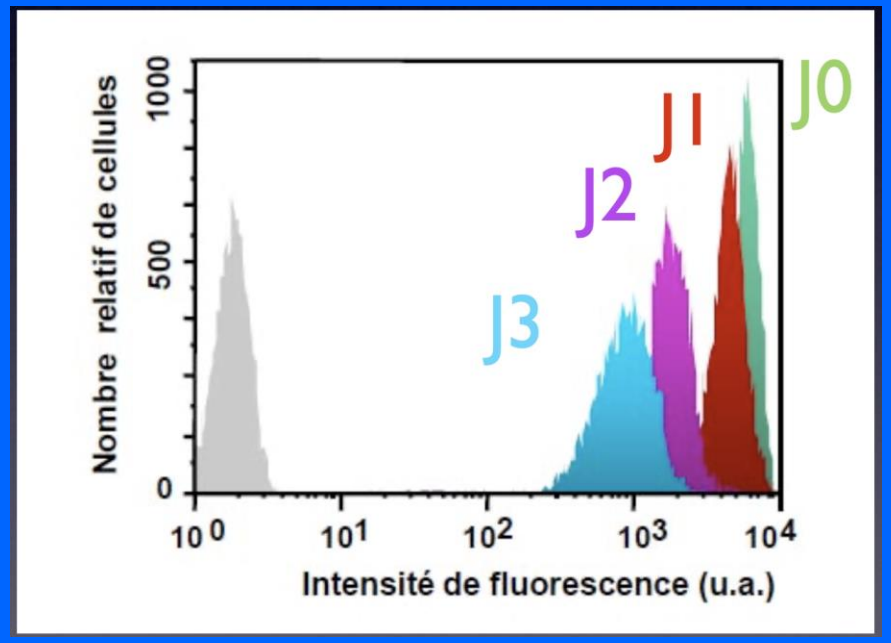


Fluorochrome CFSE

CFSE = Fluorochrome vert (sonde polaire) qui se lie aux amines libres de la cellule

Principe = La fluorescence décroît à chaque nouvelle génération cellulaire



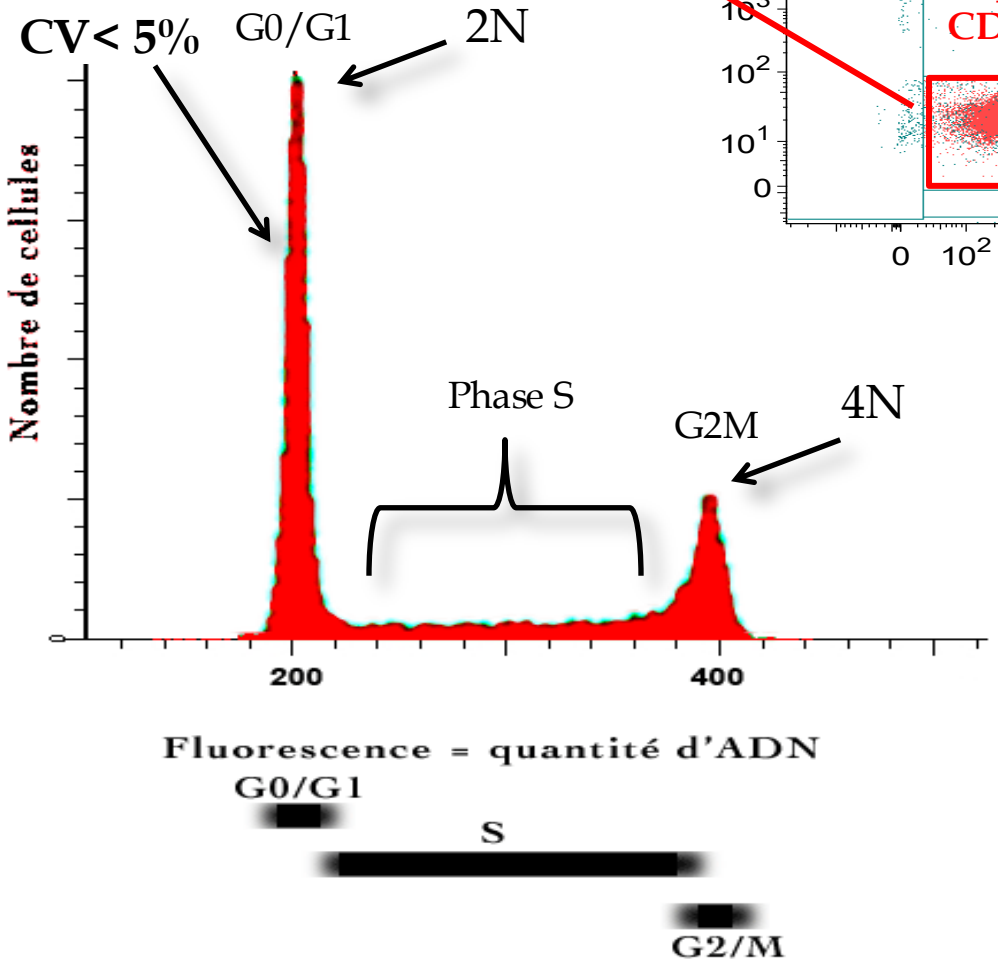


$$\text{Indice de prolifération} = \frac{\text{Mean Fluo J0}}{\text{Mean Fluo Jn}}$$

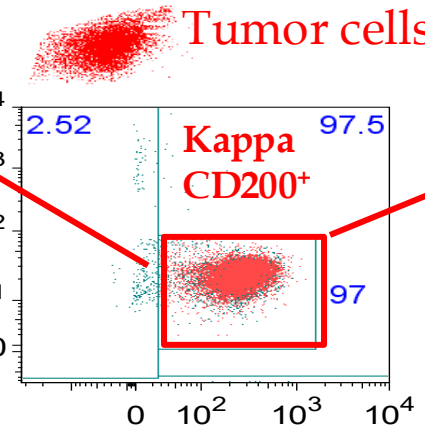
Représentation du Cycle Cellulaire

$\frac{SD}{Mean} \times 100\% = CV$

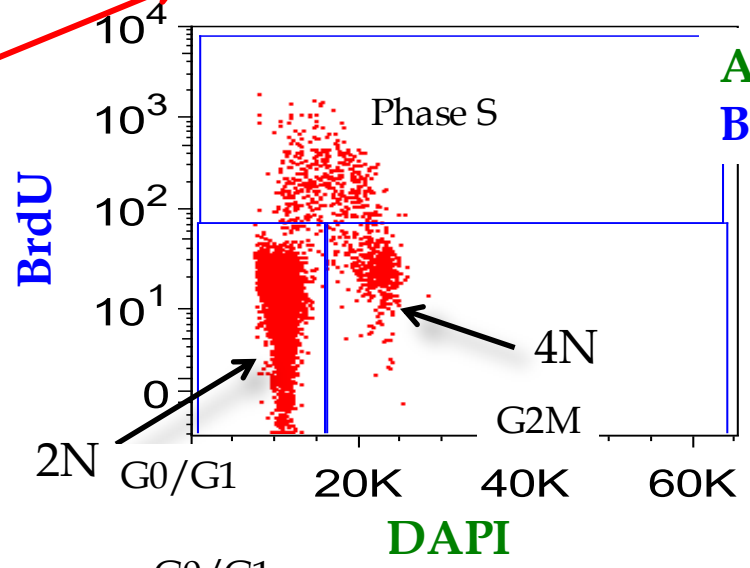
Histogramme



ADN = IODURE DE PROPIDIUM

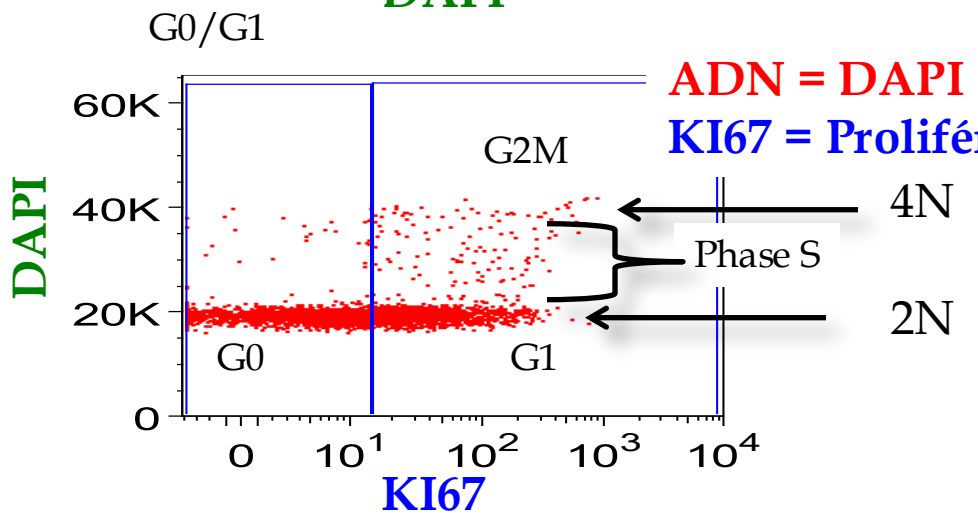


DOT Plot



ADN = DAPI

BrdU = Thymidine



ADN = DAPI

KI67 = Prolifération

Apoptose

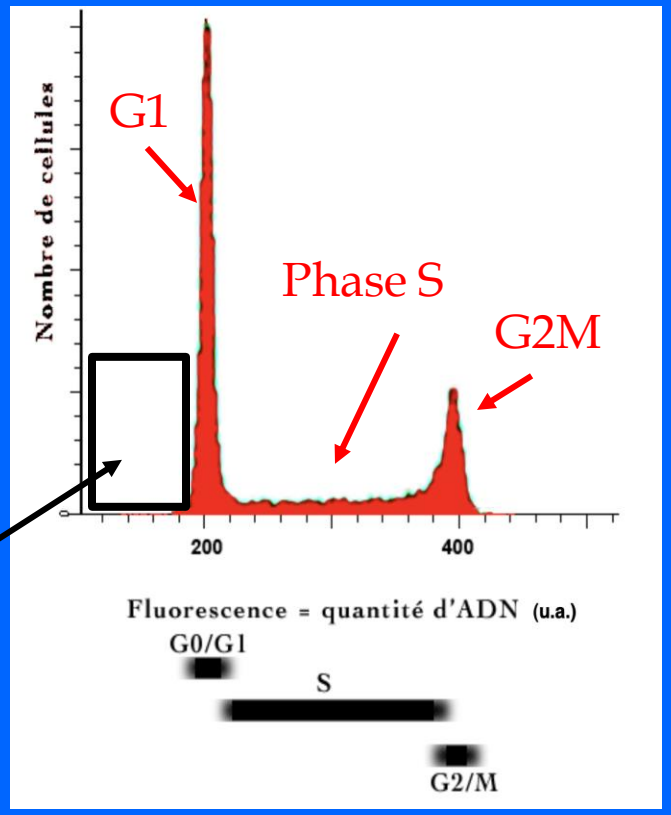
Apoptose

- Mitochondrie et lysosomes
  - Diminution du potentiel de membrane
- Membrane plasmique
  - Exposition du Phosphatidylserine à l'extérieur
- Mobilisation du Ca^{++} intracellulaire
  - Condensation de la chromatine
- Activation des endonucléases
  - dégradation de l'ADN par fragmentation

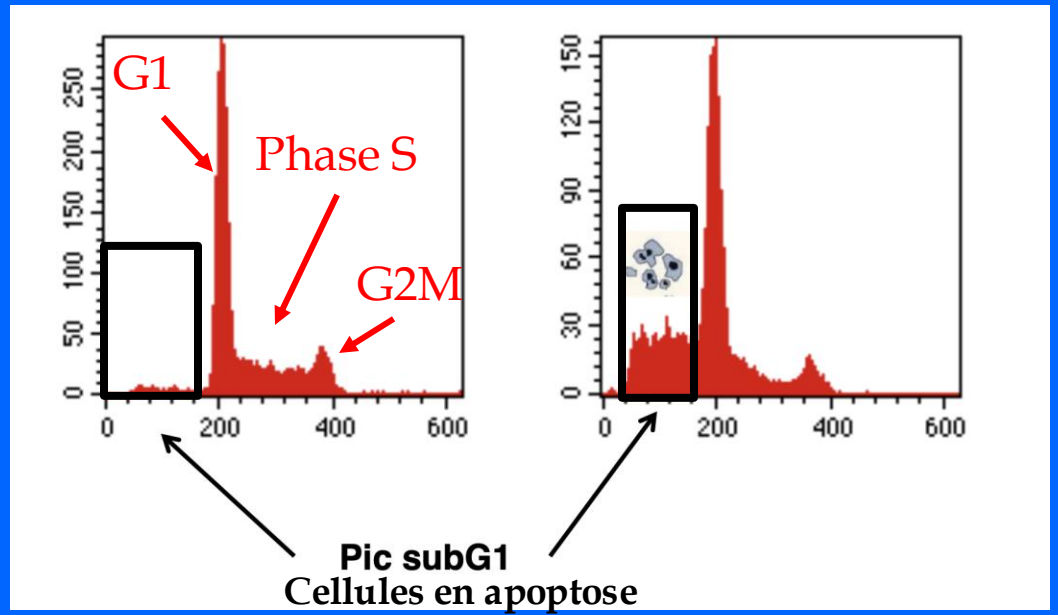
Nécrose

- Rupture des mitochondries
- Rupture de la membrane plasmique
- Libération du cytoplasme avec les protéases notanment
- ADN est dégradé par les différentes nucléases

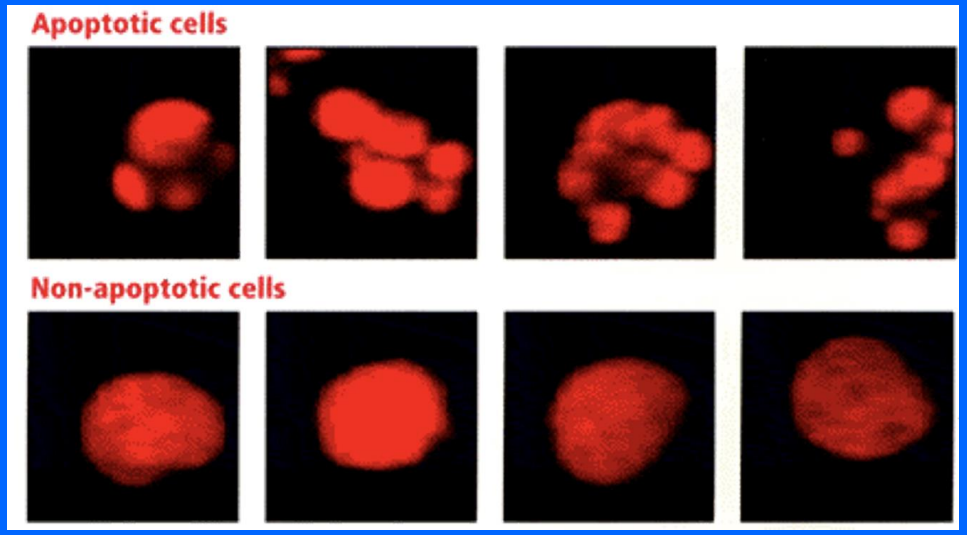
Dégradation ADN



Pas d'apoptose
Pas de subG1



Pic subG1
Cellules en apoptose



Marquage Annexin V

Phosphatidyl serine

Principe:

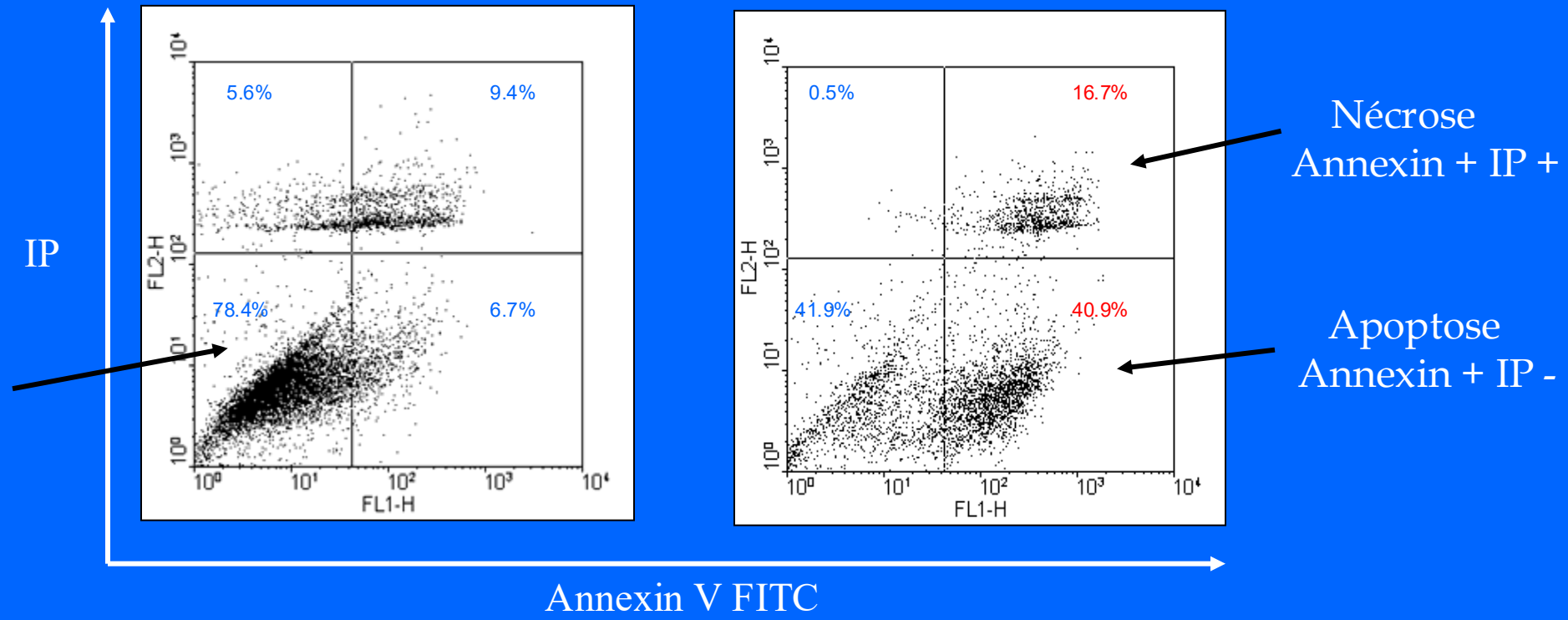
Les cellules en apoptose précose expriment la phosphatidyl sérine à l'extérieur de la membrane cytoplasmique. L'Annexine couplée à un fluorochrome est une protéine possédant une forte affinité pour la phosphatidyl sérine.

Le marquage Annexin V (Fitc) associé à un marqueur ADN (IP) permet de détecter à la fois les cellules:

- en apoptose
- en nécrose

Contrôle

Après UV



Cellules vivantes
Annexin - IP -

Nécrose
Annexin + IP +

Apoptose
Annexin + IP -

CYTOMETRIE EN FLUX

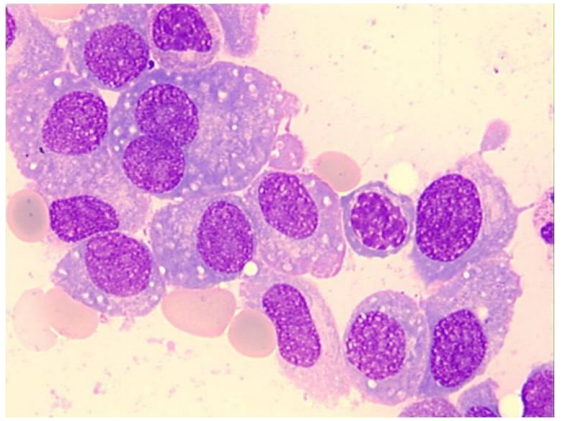
6. ***Applications cliniques:***

Hémopathie maligne : myélome multiple

- Détection des cellules tumorales au diagnostic et en rechute
- Evaluation de la maladie résiduelle:
  - ↳ Détection des cellules tumorales restantes post-traitement (chimiothérapie)
- Evaluation de la prolifération des cellules tumorales:
  - ↳ (Cycle cellulaire : % des cellules en Phase de Synthèse)

Multiple Myeloma

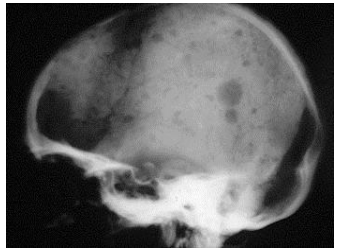
B cell neoplasia characterized by the accumulation of malignant plasma cells within the bone marrow



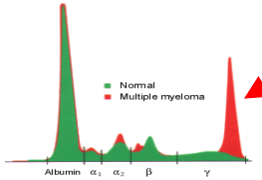
Second most common hematological

Incidence increases with age: 80% of patients

C - Calcemia
R - Renal failure
A - Anemia
B - Bone lesions



- Monoclonal Immunoglobulin



Monoclonal Ig (IFE – Blood)

Normal Plasma Cells:
* Polyclonality
* Light Chains
Kappa 60% / Lambda 40%

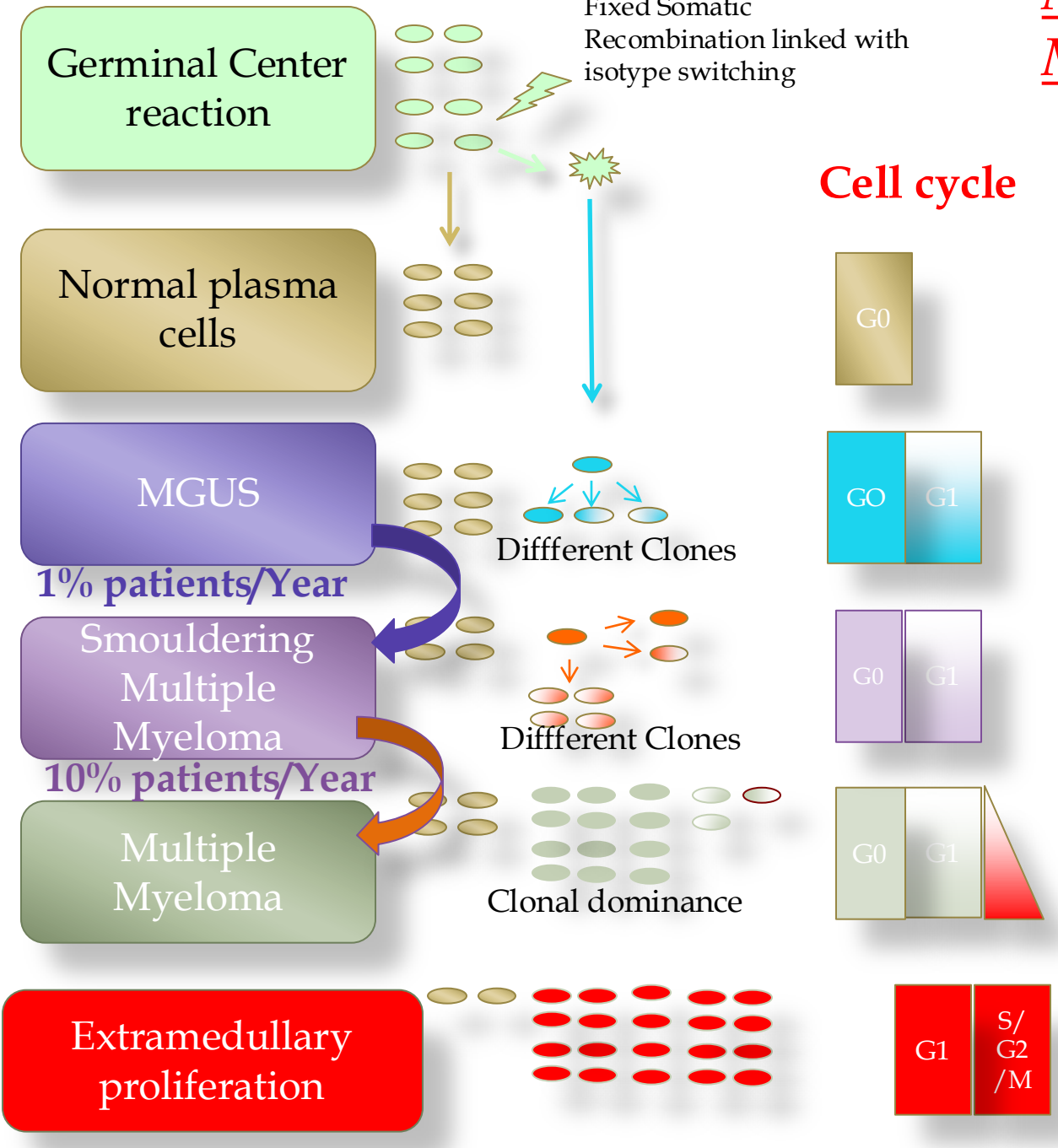
- Clinical course: relapsing and remitting disease

In spite of the progress in survival with novel agents, MM remains without definitive treatment for the majority of the patients

With current treatment:

- 5-years survival 50% - 80%
- Potentially cured ≈10 - 20%

MULTIPLE MYELOMA



Cell cycle

Quiescent cells?
Long lived: several years

Bone marrow or MALT
10^9 PCs, 1000 specificity
10^6 PCs/specificity

Block in G1.
Oncogene induced senescence

Bone marrow
50% normal PCs
50% monoclonal PCs

Block in G1.
Oncogene induced senescence

Bone marrow
Normal PCs
Monoclonal PCs (>10%)

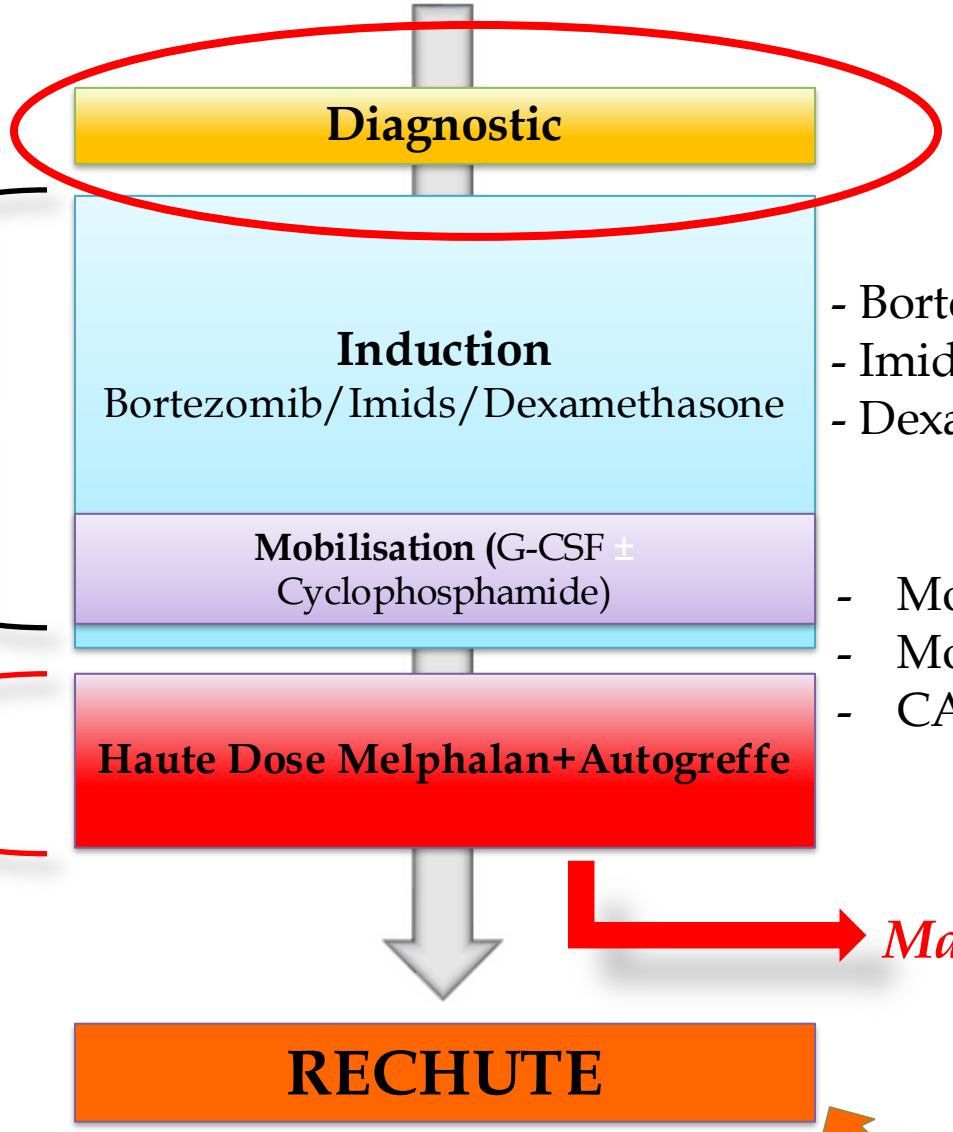
Heterogeneous disease
Clonal dominance
From 0% to 4% MMCs in S phase.
Strong prognosis factor
Median S-PHASE = 0.4%

End organ damage
CRAB. Fully dependent on the environment

Highly proliferating

Less dependent on the environment

Multiple Myeloma



Diagnosis and Risk stratification in multiple myeloma

Chimiothérapie

- Bortezomib : Proteasome inhibitor
- Imids: Immunomodulatory agents
- Dexamethasone : Corticoid

Recent therapies:

- Monoclonal Antibody (Anti-CD38)
- Monoclonal Antibody (Anti-CD3 /Anti-GPRC5D)
- CAR-T Cells (Chimeric Antigen receptor) BCMA(B Cells Maturation Antigen)

Autogreffe

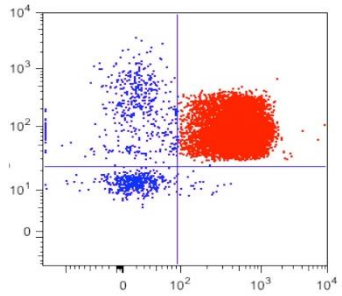
Maladie Résiduelle

Why do Multiple Myeloma Patients Relapse?

LABORATOIRE SUIVI DES THERAPIES INNOVANTES (STI)

Bone marrow

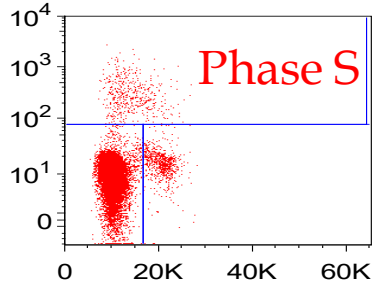
Cytométrie en Flux



Phénotypage Plasmocytes

Plasmocytes Tumoraux

Plasmocytes Normaux



Prolifération Plasmocytes

Plasmocytes Tumoraux

Plasmocytes Normaux

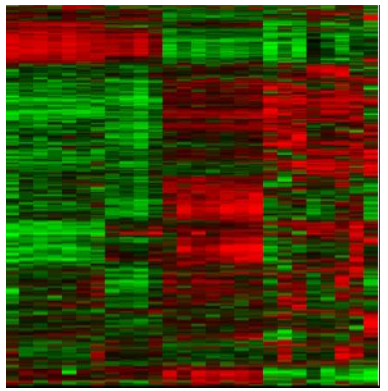
Phase S = Phase de Synthèse

Purification (Trieurs)

Billes Magnétiques

Cytométrie en flux

Puce ADN



Transcriptome Plasmocytes:

Plasmocytes Tumoraux

ARN messager ➡ Gènes

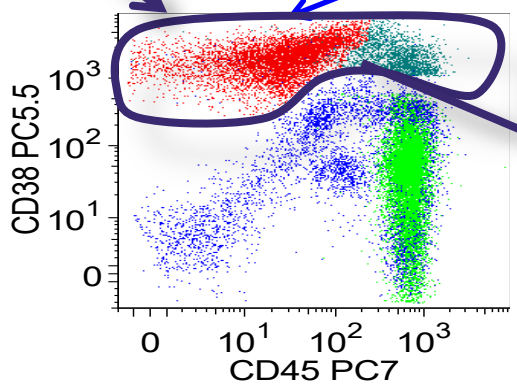
Score de Risque = RS Score

RNA-Seq (2022)

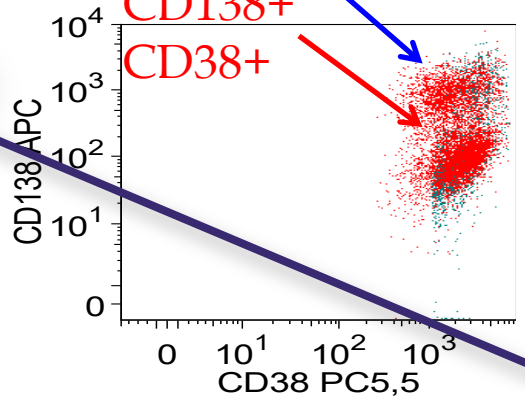
CYTOMETRIE:Diagnostic

= Tumor cells (Kappa, CD138+,CD38+,CD45-,CD19-,CD20-)

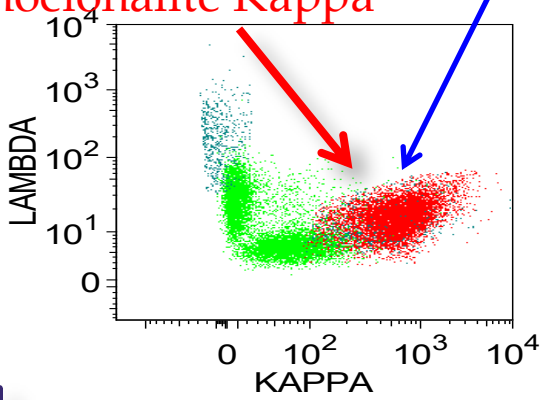
Plasmocytes

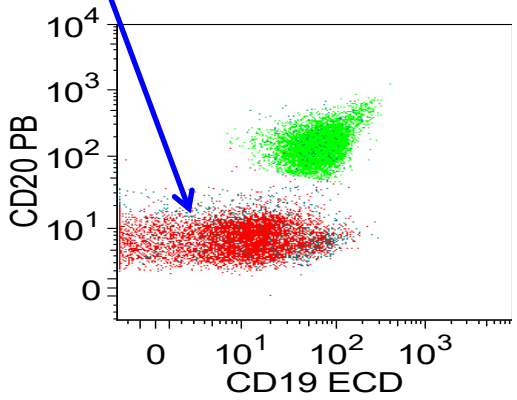


CD138+
CD38+



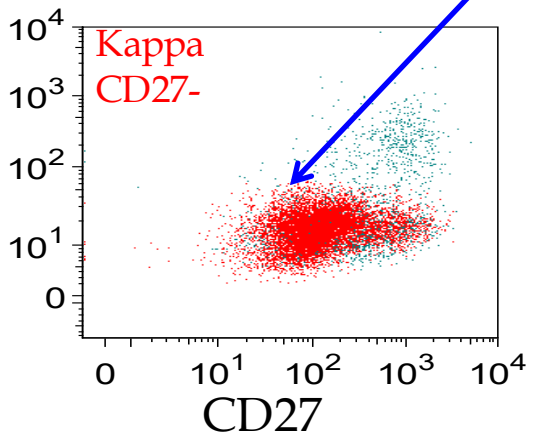
Monoclonalité Kappa

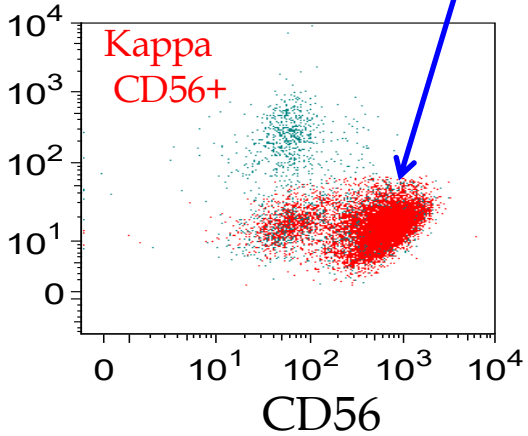


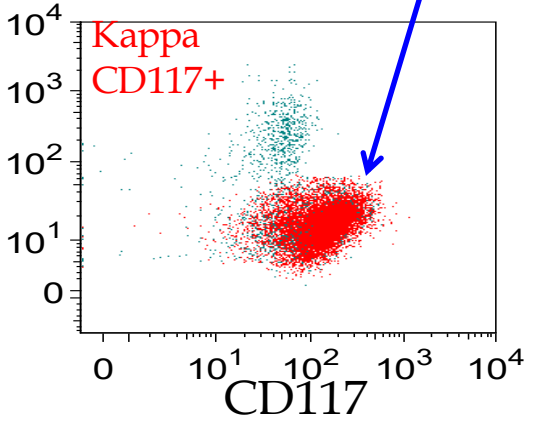


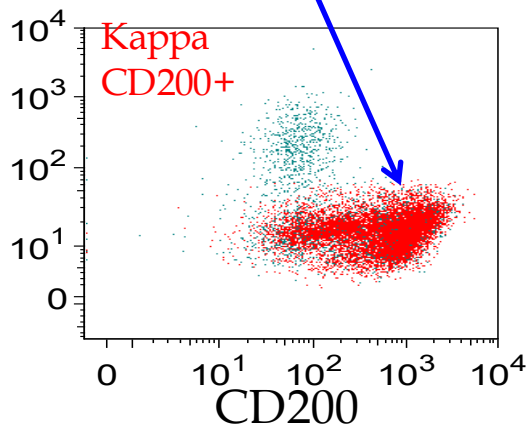
= Tumor cells (Kappa, CD27-, CD56+, CD117+, CD200+)

Kappa Lambda



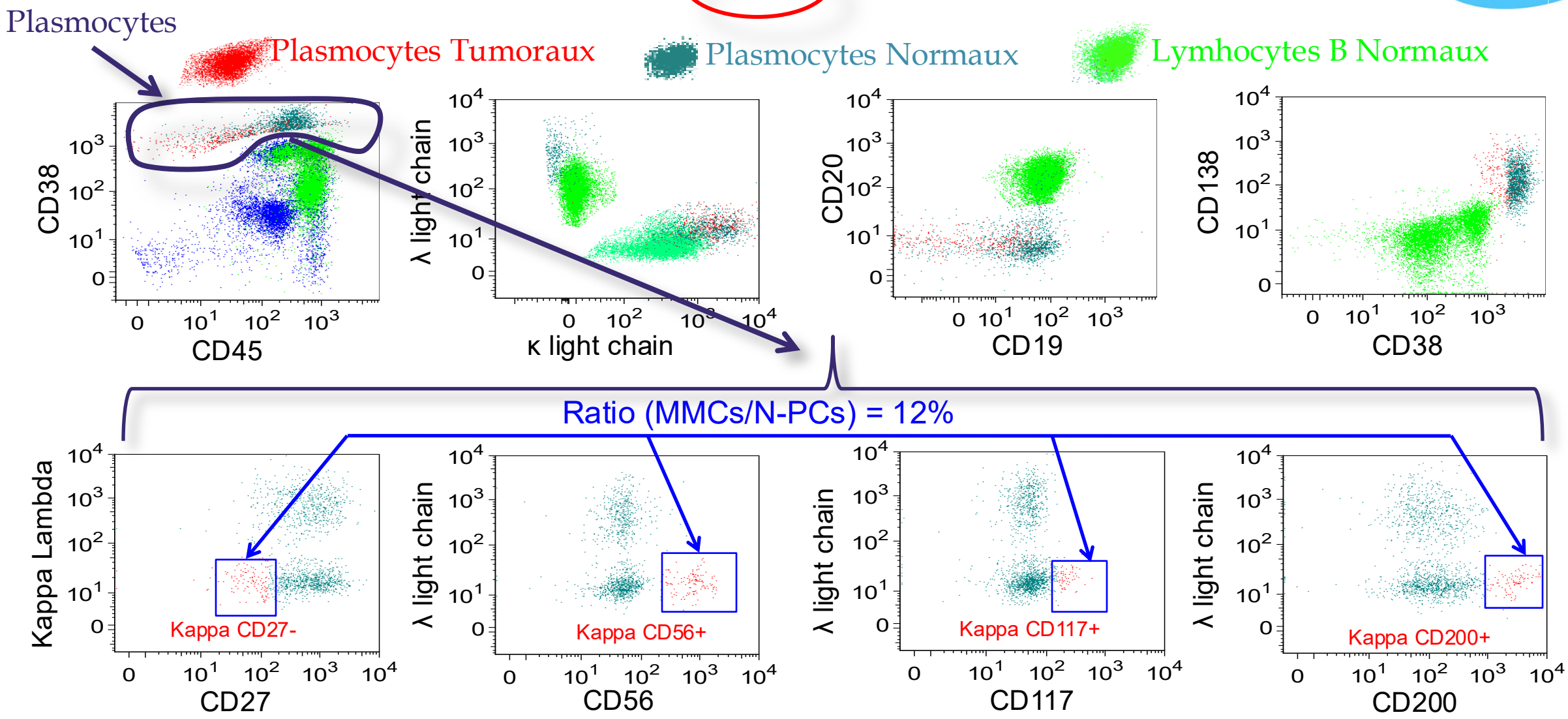






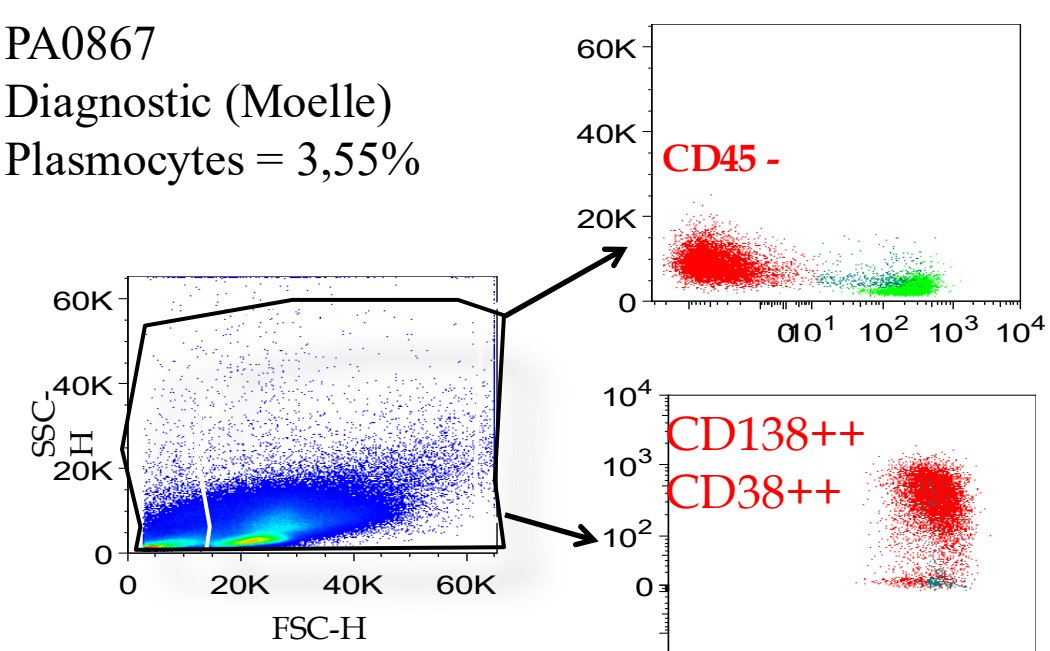
CYTOMETRIE: Maladie résiduelle

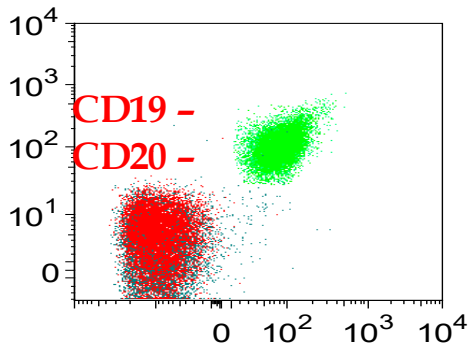
PA0773 Post allogreffe (6mois) Plasmocytes= 0,13%

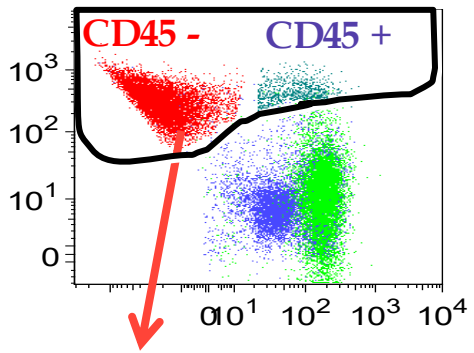


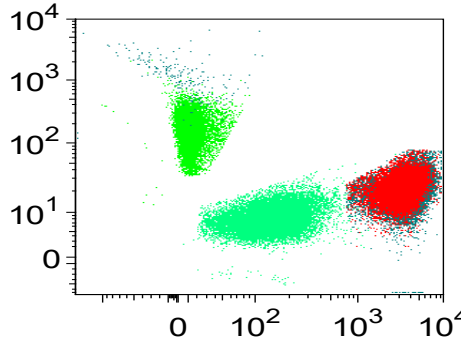
« Strategy of gating » Multicolor Flow Cytometry

PA0867
Diagnostic (Moelle)
Plasmocytes = 3,55%









Kappa Monoclonal

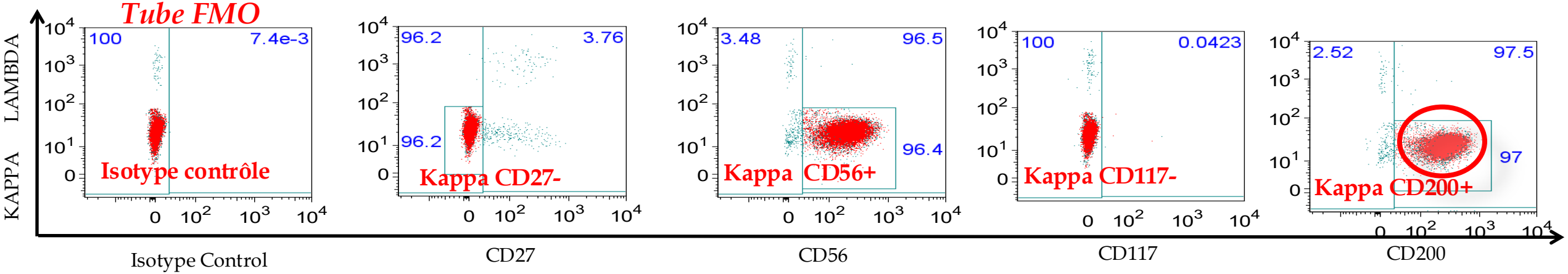
Plasmocytes Normaux

Plasmocytes Tumoraux

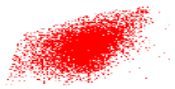
Lymphocytes B normaux

Plasmocytes tumoraux (Kappa, CD138+, CD38+, CD45-, CD19-, CD20-,CD27-, CD117-,CD200+)

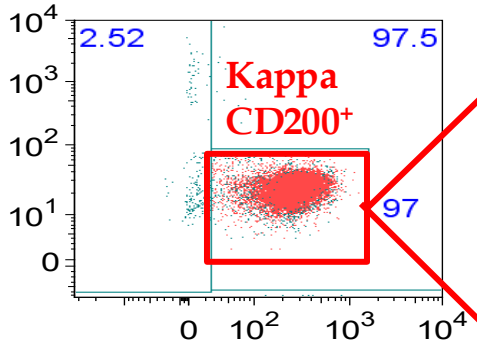
Tube FMO



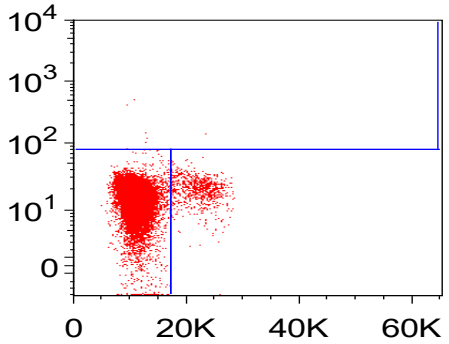
Cycle cellulaire : comparaison de deux méthodes

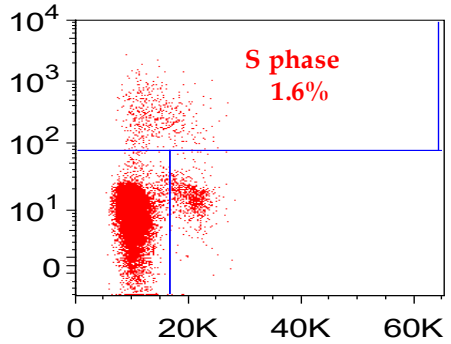


Tumor cells



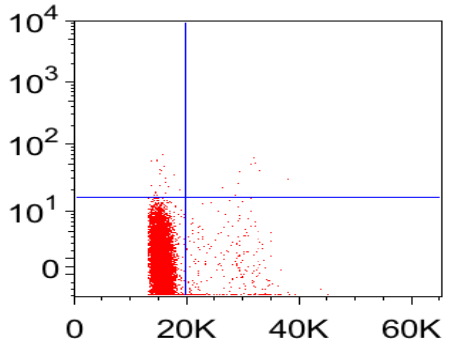
Incorporation BrdU / DAPI

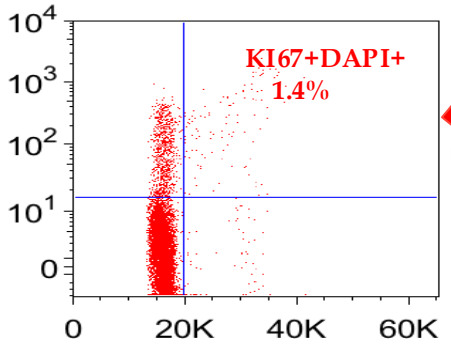




Phase S

KI67 /DAPI Staining





Cytométrie multicouleur + cycle cellulaire:

➡ **méthode sensible (1 cellule tumorale/100000 leucocytes) associée au cycle cellulaire**

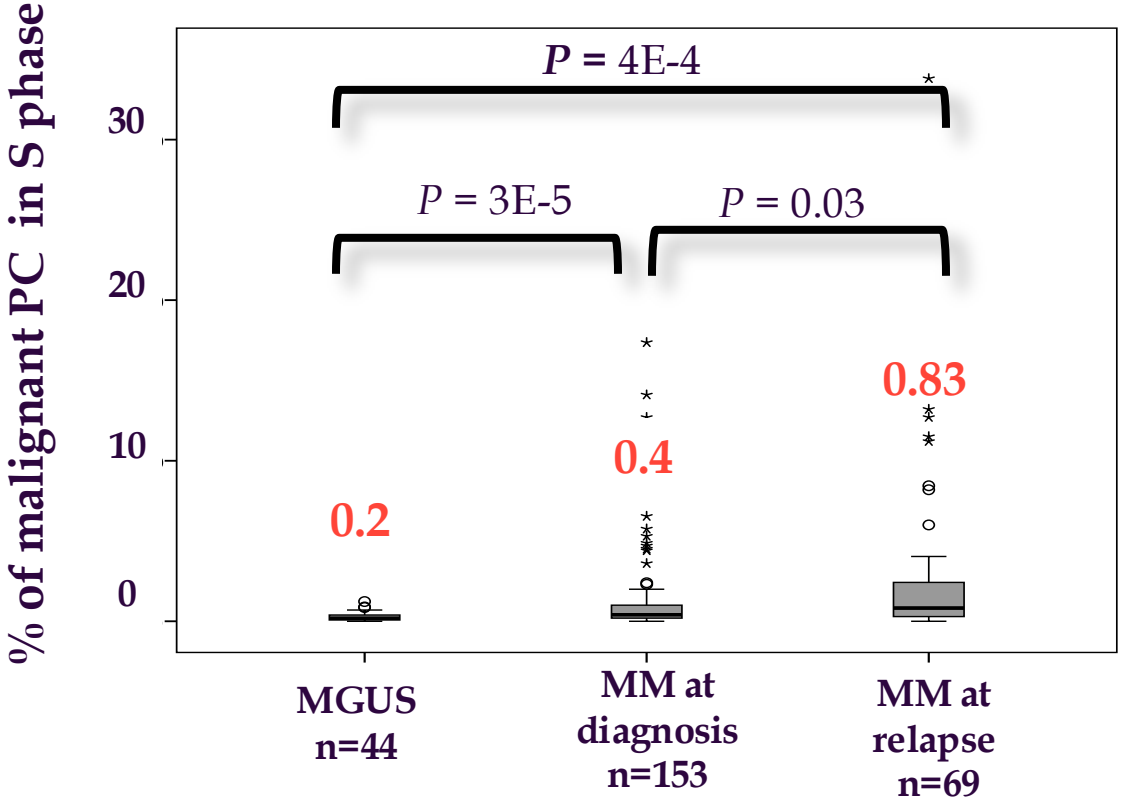
Cycle cellulaire en cytométrie en flux des patients au diagnostic et en Rechute

BrdU incorporation in multiparameter flow cytometry : A new cell cycle assessment approach in multiple myeloma

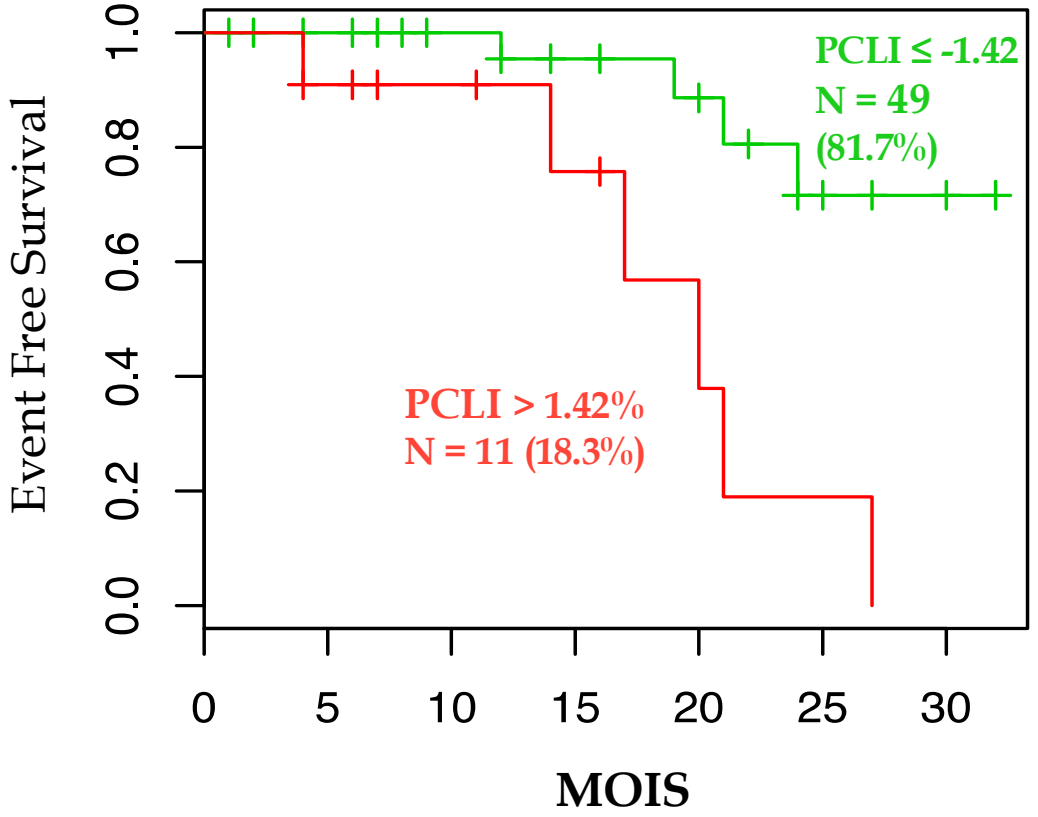
Guilhem Requirand[1,2], Nicolas Robert[1,2], Stéphanie Boireau[1,2], Laure Vincent[6], Anja Seckinger[4,5], Salaheddine Bouhya[6], Patrice Ceballos[6], Guillaume Cartron[3,6], Dirk Hose[4,5], Bernard Klein[1,2,3], Jérôme Moreaux[1,2,3]

Cytometry: Part B - Clinical Cytometry

Incorporation BrdU /DAPI



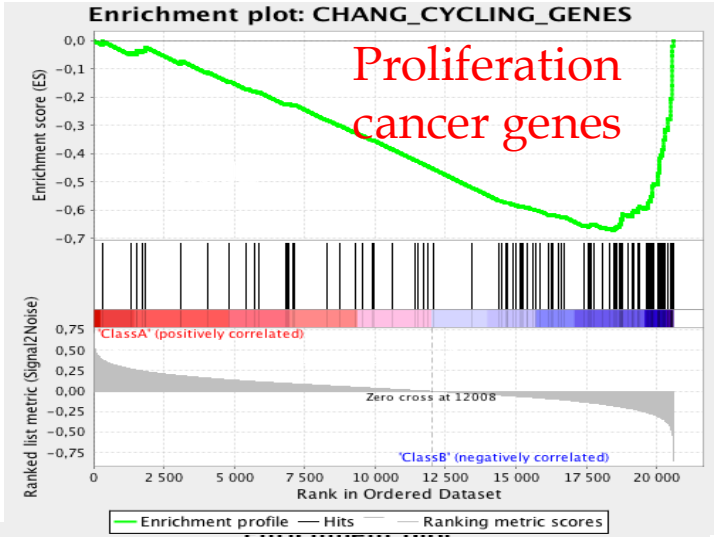
Incorporation BrdU/DAPI

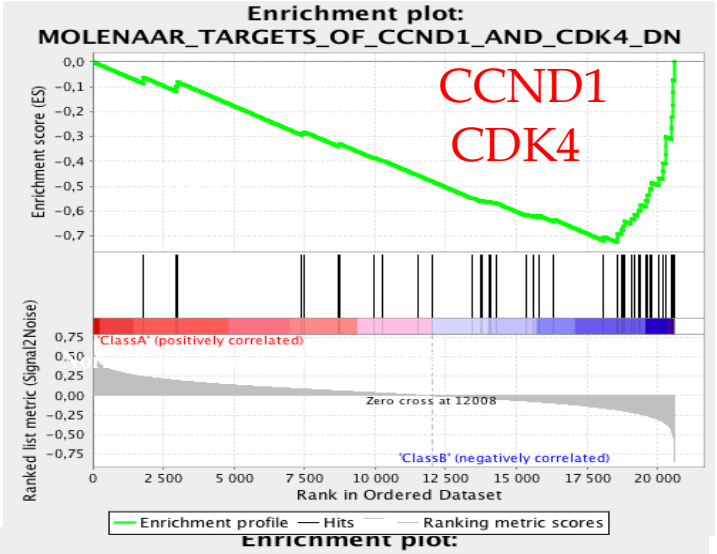


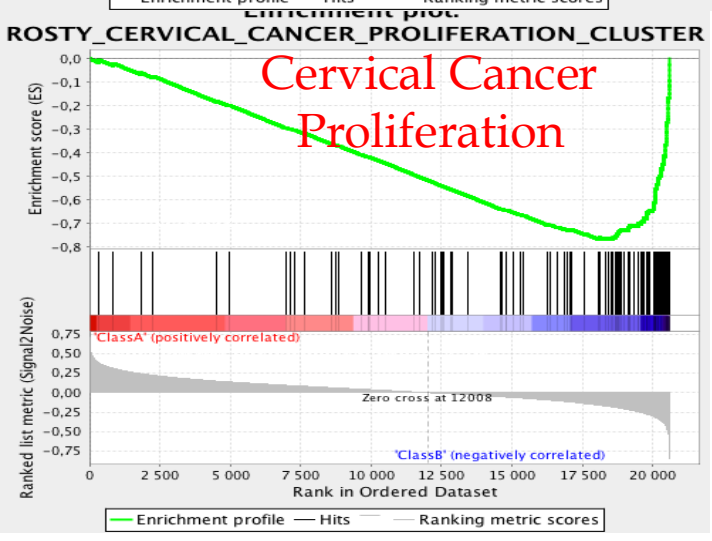
Cycle Cellulaire est un Puissant facteur pronostique

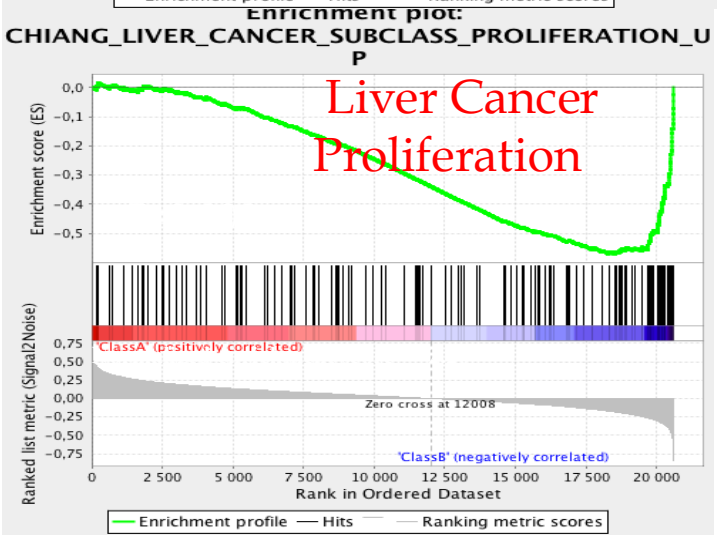
Gene Set Expression Analysis (GSEA)
Patients au diagnostic

Patients
Phase S > 1,42%

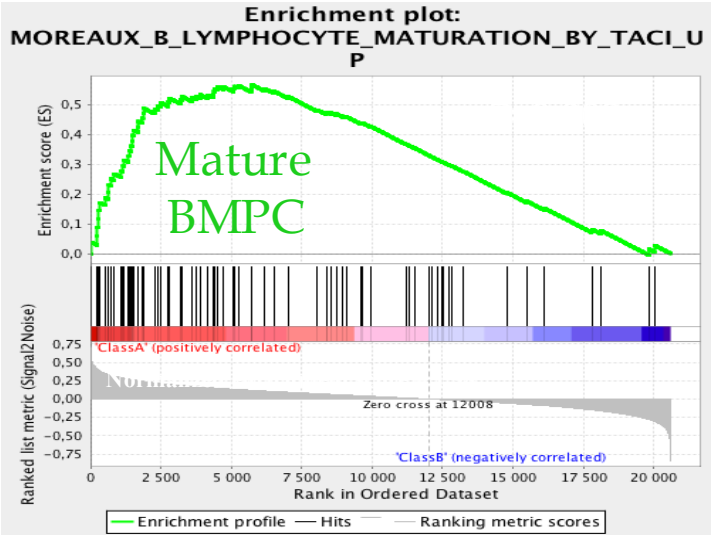




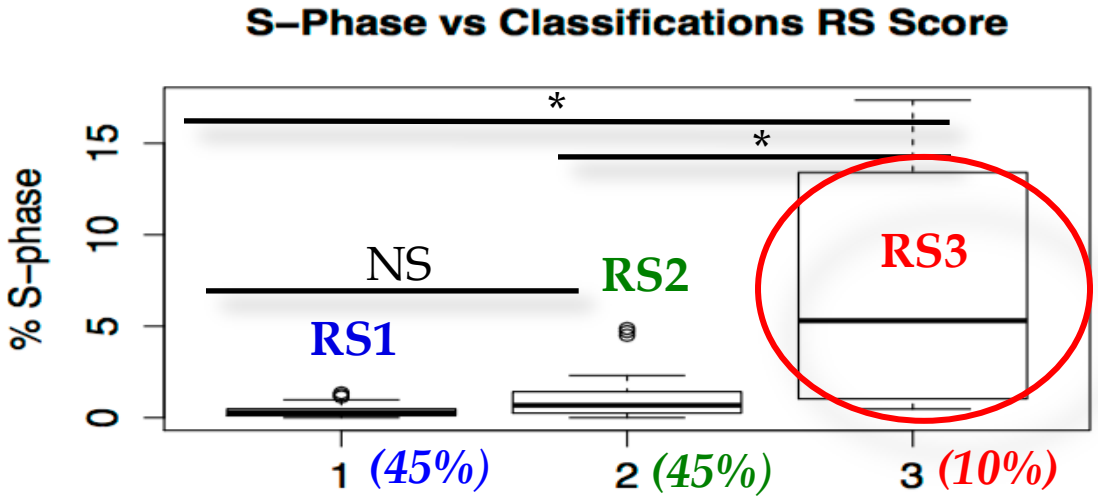




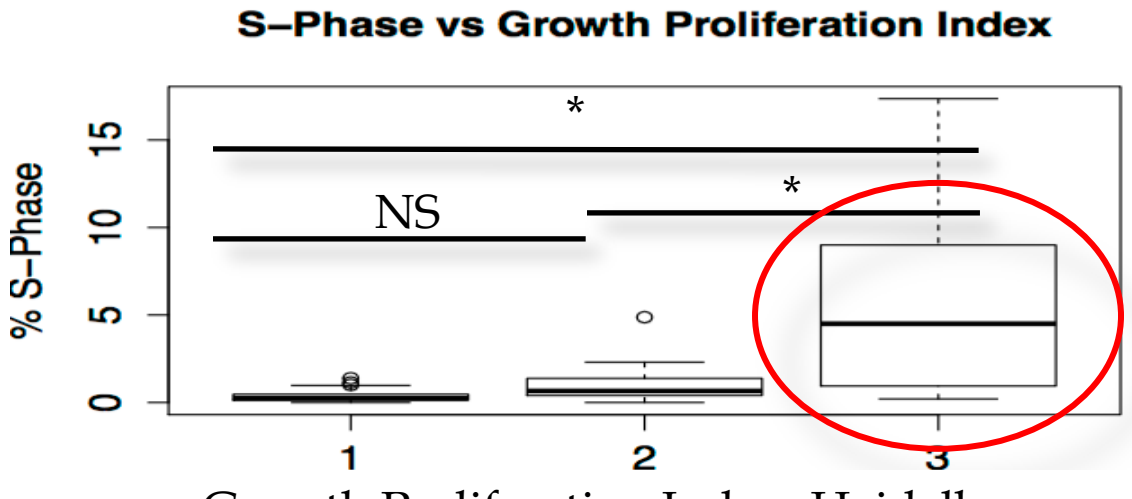
Patients
Phase S ≤ 1,42%



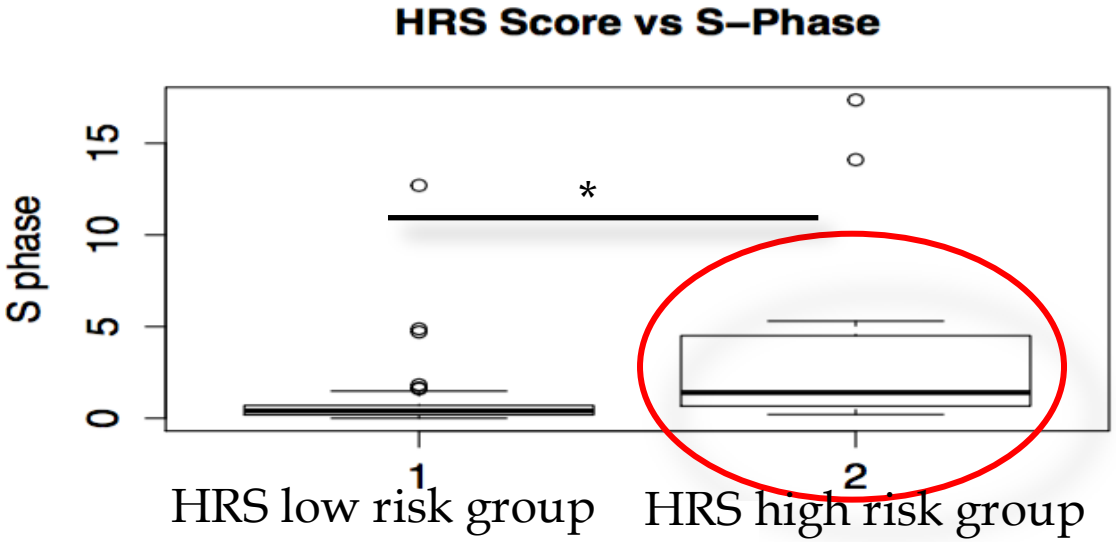
Corrélation entre Cycle Cellulaire et Gene-based Risk Score
Incorporation Brdu/DAPI Patients Au diagnostic (N=87)



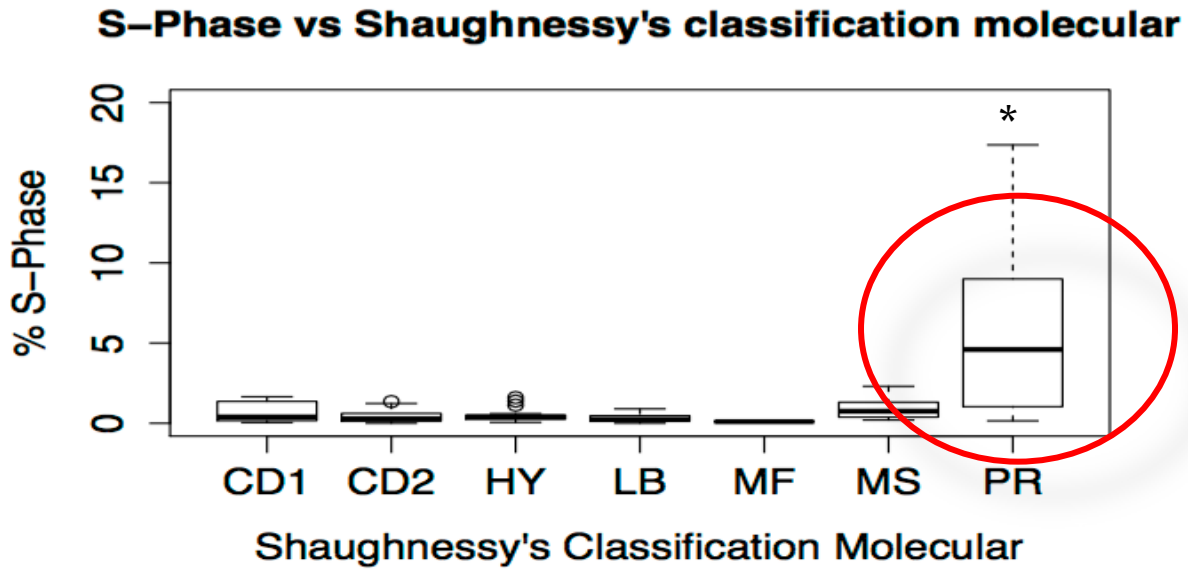
Classification RS Score - STI (Montpellier)



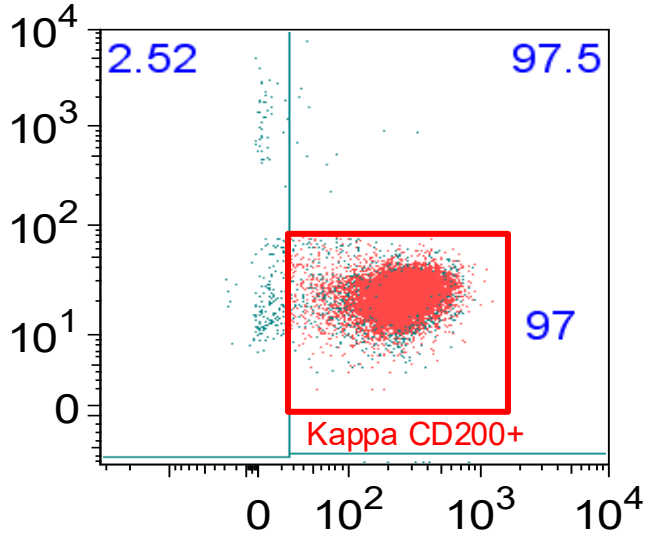
Growth Proliferation Index -Heidelberg

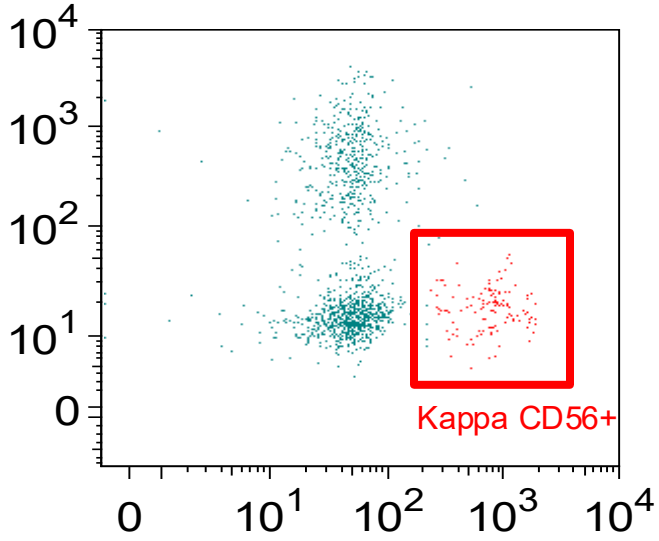


HRS low risk group HRS high risk group



Intérêt du cycle cellulaire en multicouleurs ??





Contexte Diagnostic

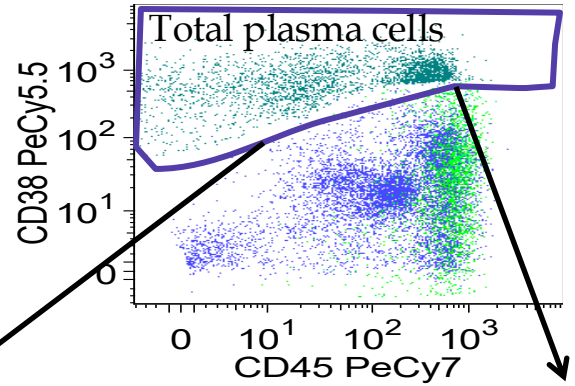
Quel intérêt?

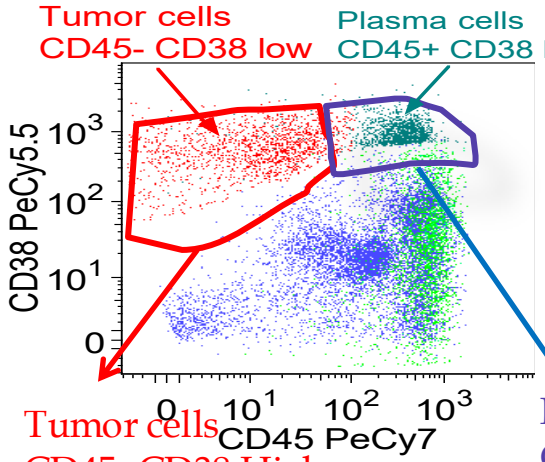
Contexte Maladie Résiduelle (MRD

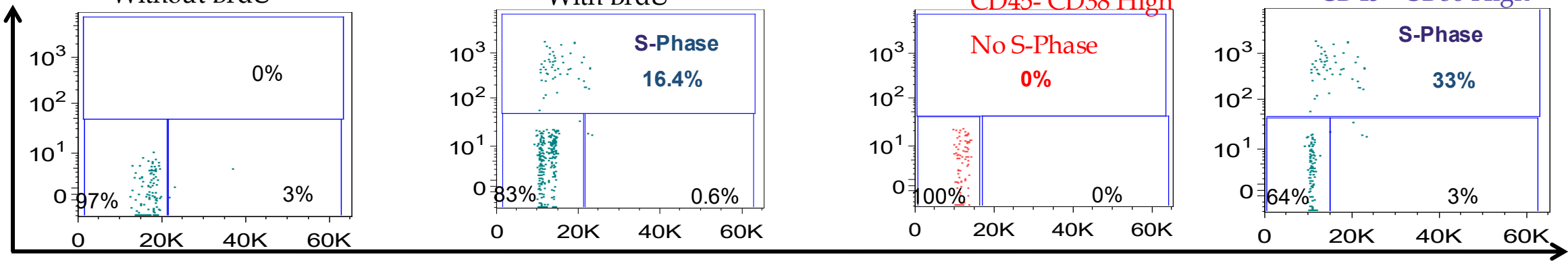
Analyse fine du cycle cellulaire
sur un faible pourcentage de cellules tumorales
au sein de la population plasmocytaire normale

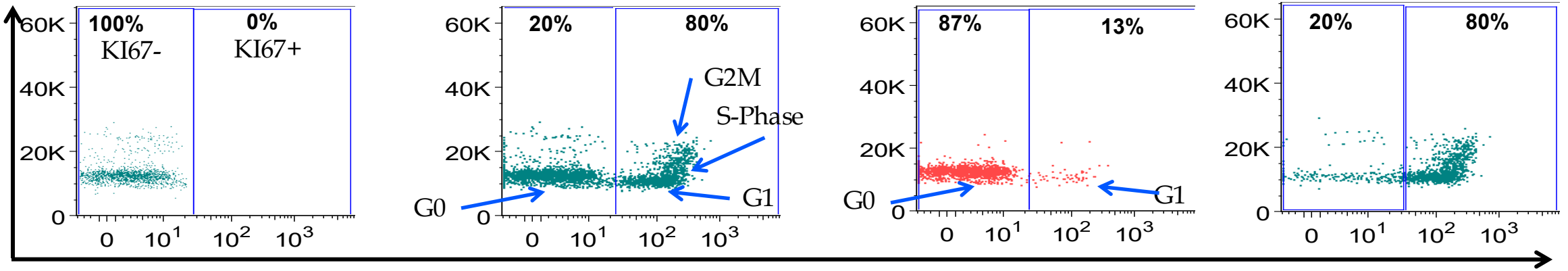
PA0803
Diagnostic
Plasmocytes = 0,2%

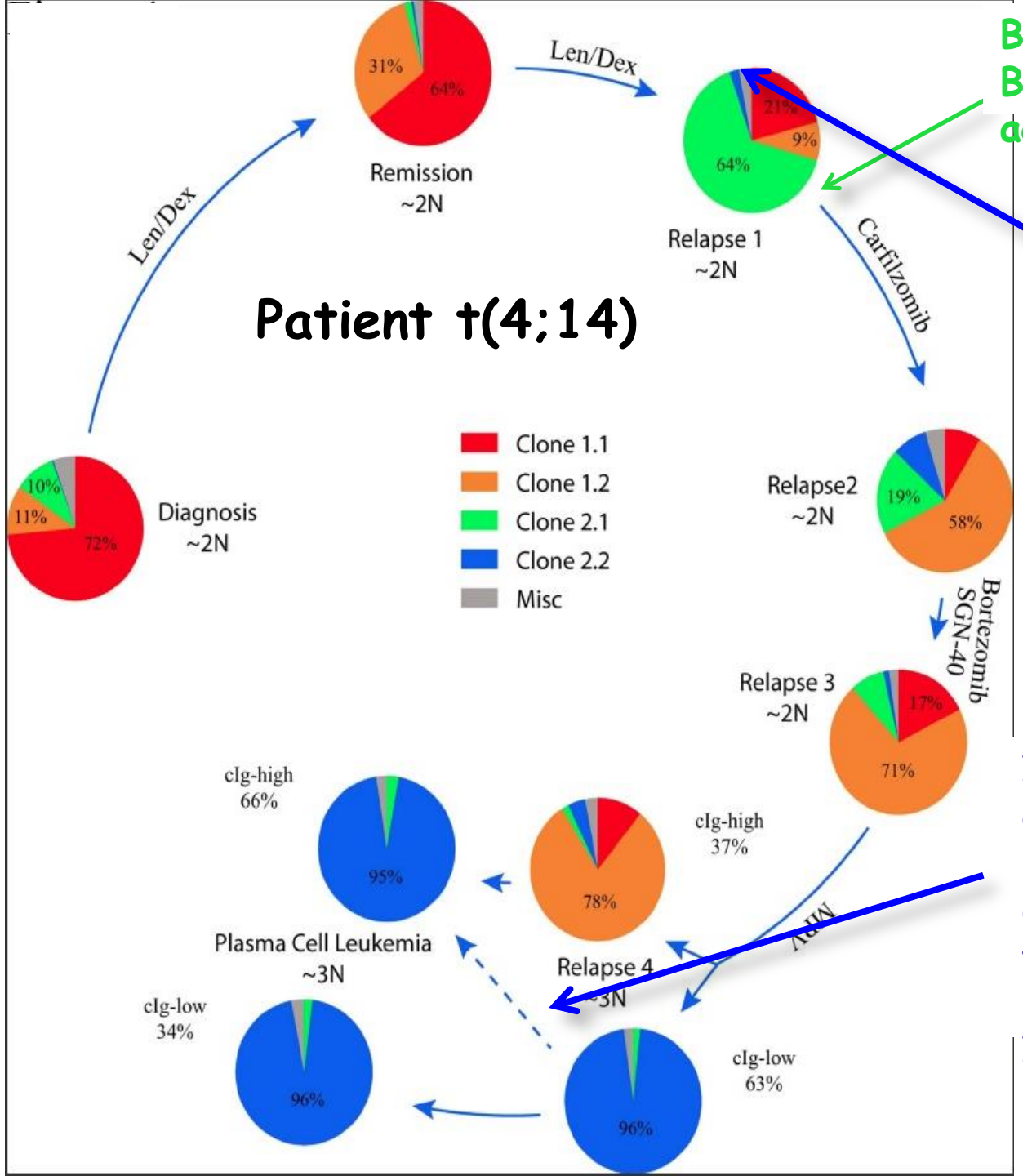
RS1











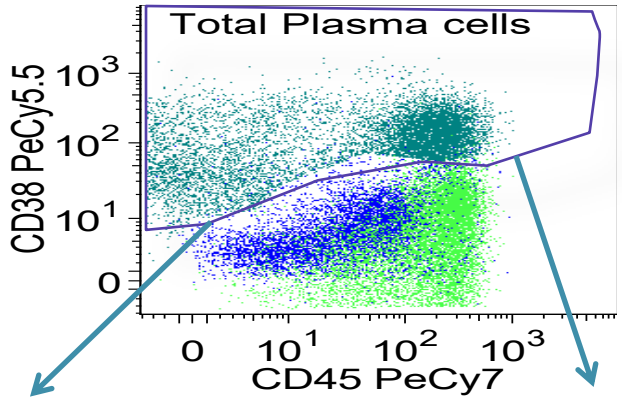
Biallellic deletion of BIRC2/3: NF-kappa B activation

Independent Biallellic deletion of BIRC2/3: NF-kappa B activation

Independent Biallellic deletion of BIRC2/3: NF-kappa B activation, additional complexity, trisomy

Induced by melphalan?

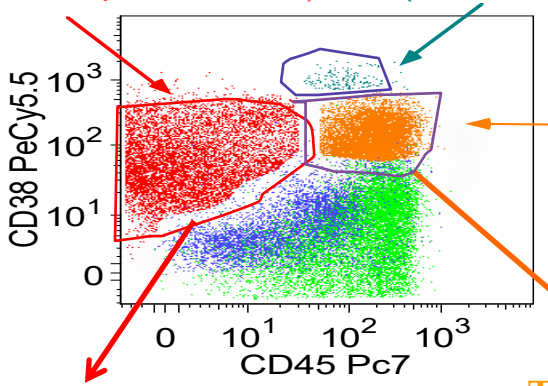
Moelle Osseuse
Diagnostic Stade III B
Total Plasmocytes = 1 %



Phase S = 17%

Tumor cells
CD45- CD38 Low
(84% of total plasma cells)

Normal plasma cells
CD45+ CD38 high
(5% of total plasma cells)



Tumor cells
CD45+ CD38 low
(11% of total plasma cells)

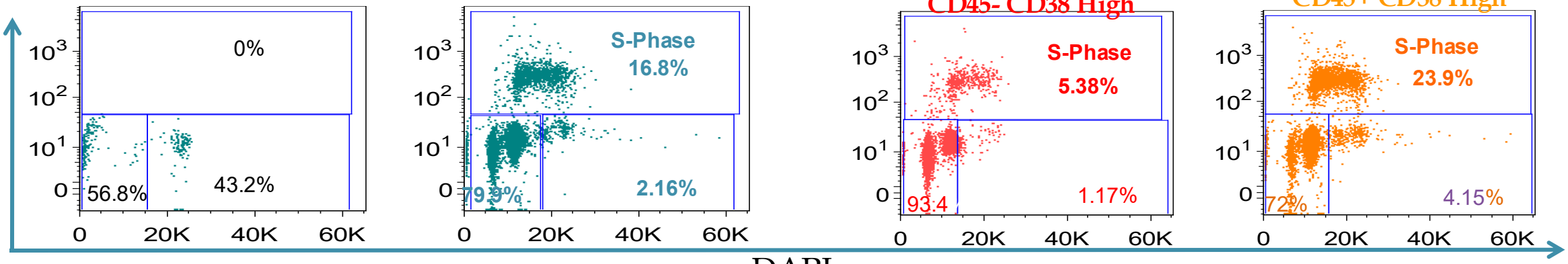
Without BrdU

With BrdU

Tumor cells
CD45- CD38 High

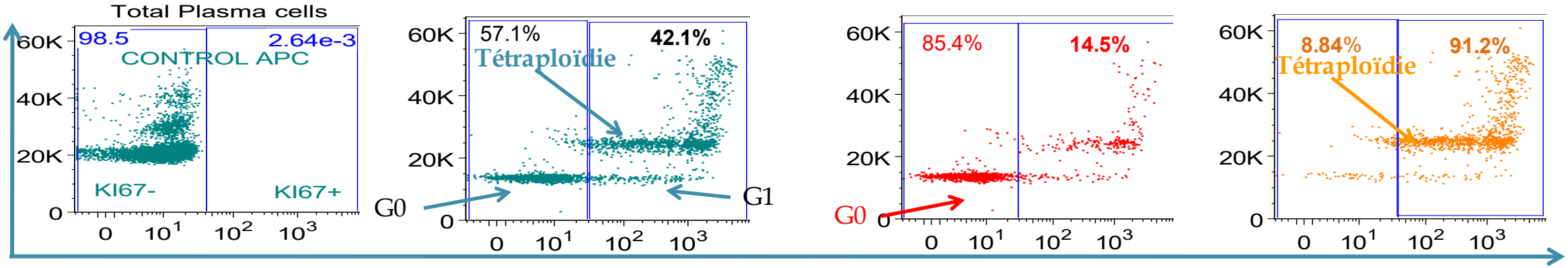
Tumor cells
CD45+ CD38 High

BrdU



DAPI

DAPI



KI67

Analyse du Transcriptome

1. Signatures de gènes:

• Score de Haut Risque (HRS)
• Index de Prolifération (GPI)
•Score de Risque (RS)
BMC; T. Rème; 2008

2. Translocations:
• t(4;14) NEG
• t(11;14) POS

3. Délétion del17p

4. Classification moléculaire:
7 groupes
• Mauvais pronostic
PR / MS
•Sans mauvais pronostic
LB / HY
CD1/ CD2 / MF
John D. Shaughnessy Jr
(Blood. 2006;108:2020-2028)

Analyse du transcriptome des cellules plasmocytaires tumorales par puces Affymetrix (Maj 27-05-2011)

Conclusions :

Patiente avec des signatures de très mauvais pronostic. HMS de très mauvais pronostic, signature de prolifération et PCLI de 17,36%.

- SIGNATURES ASSOCIEES A UN MAUVAIS PRONOSTIC : OUI

■ Translocation t(4;14) : NON (15% des patients)

■ Délétion 17p : non disponible (1) (11% des patients)

■ Score de risque (HMS) (7) : 3 **RS3**

Score 3 = mauvais pronostic, Score 2 = pronostic intermédiaire, Score 1 = Bon pronostic

■ Score de haut risque (HRS) : OUI (2).

Score : 2.39 (13% des patients avec score ≥ 0,66) (range : -1,29 à + 3)

■ Index de prolifération (3)

Growth proliferation index : 3
Score 3 = mauvais pronostic, Score 2 = pronostic intermédiaire, Score 1 = Bon pronostic

Rappel PCLI par cytométrie : 17.36 % (Limite normale : < 1%)

■ Classification moléculaire du MM (7 groupes)(4) : PR

Groupes de mauvais pronostic : PR/MS
Groupes sans mauvais pronostic : LB/HY/CD1/CD2/MF

- SIGNATURES SANS VALEUR PRONOSTIQUE :

■ Translocation t(11 ;14) : OUI (5) (16% des patients)

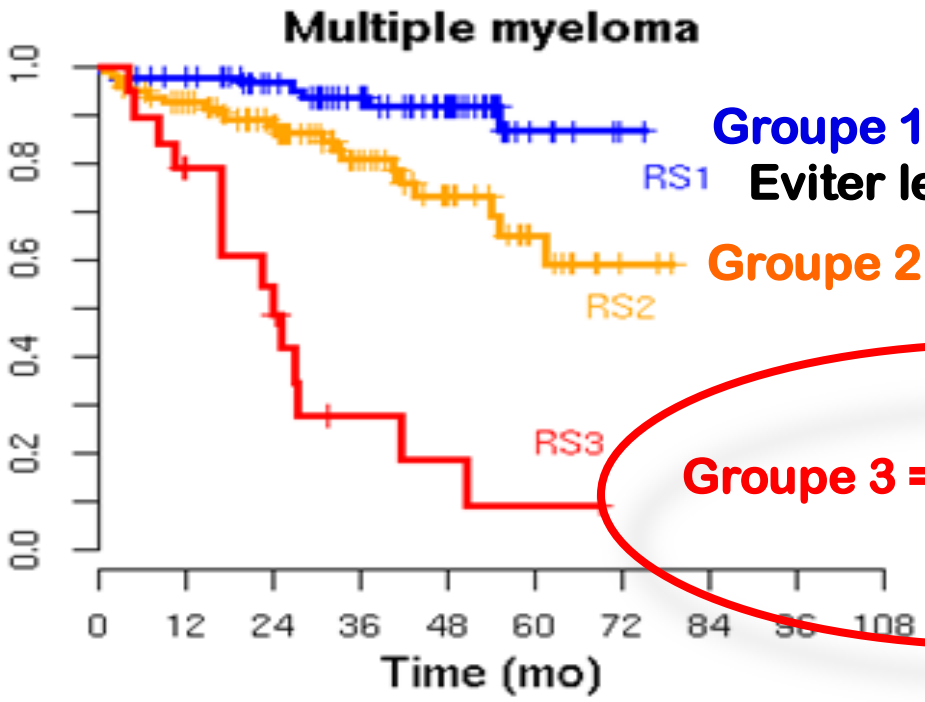
Mauvais pronostic

PA0813 E12005
Plasmocytes = 1 %

Groupe Prolifération

Applications en biologie hospitalière

Evaluation de la prolifération des cellules tumorales :
Evaluation de la réponse au traitement



Groupe 1 = 45% Phase S < 0,5%
Eviter le « surtraitement »?

Groupe 2 = 45% 0,5% ≤ Phase S ≤ 3%

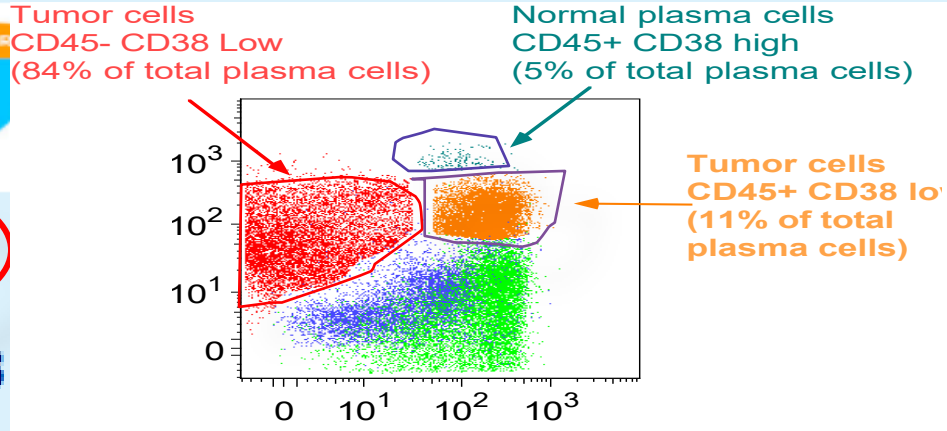
Groupe 3 = 10% Phase S > 3%

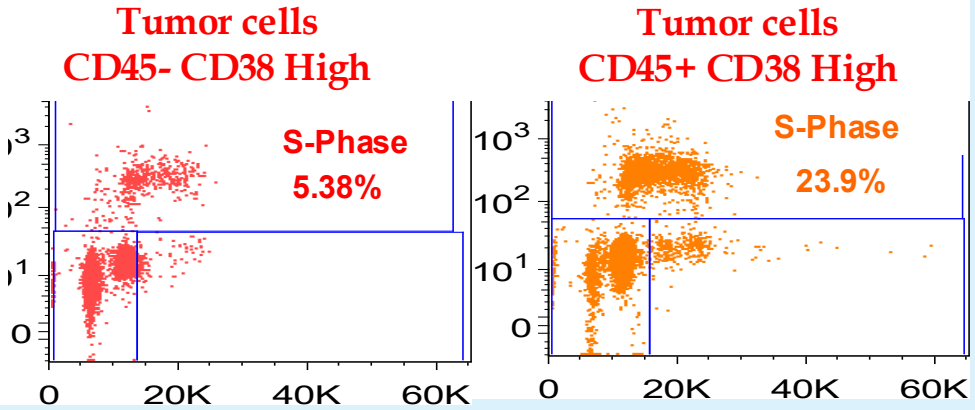
	% patients	% survival at 48 months	Median Overall survival
Rs1	45%	81	NR
Rs2	45%	70	88
Rs3	**10%**	**38**	**31**

→ Protocole Allogreffe

PA0813

Prélèvements



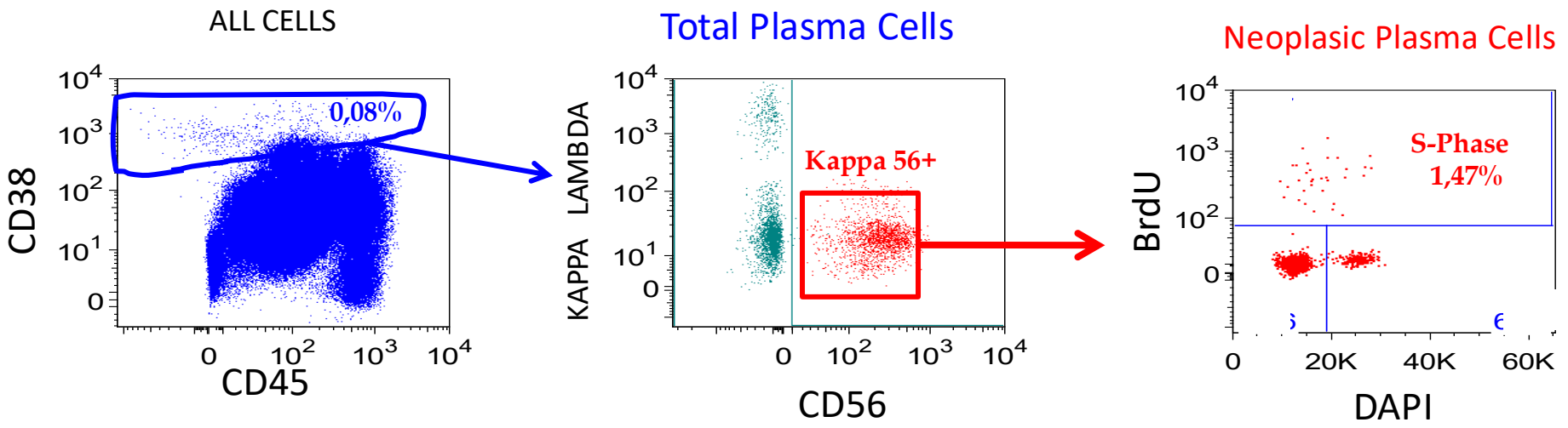


Date	Echantillon	Stade	RS score	%CD138 Ech1	MMC /mm³	NPC /mm³	%CD138 Ech2	MMC /mm³	NPC /mm³	Projet
04-01-2012	E12005	Diagnostic (2012/01/04)					Patient	Groupe3	0.03	0.96	1.76	
16-10-2012	E12193	HDT + AutoGraft (2012/06/01)			0	0.15			une donnée	
30-04-2013	E13081				0	1.1			une donnée	
16-07-2013	E13141	AlloGraft (2013/01/22)			0	4.61	Aucune donnée	Aucune donnée	Aucune donnée	
30-10-2013	E13208	Suivi MM		0.05	0	3.25	Aucune donnée	Aucune donnée	Aucune donnée	
05-02-2014	E14033	Suivi MM		0.43	0	28.6	Aucune donnée	Aucune donnée	Aucune donnée	
17-06-2014	E14111	Suivi MM	1 Year	0.03	0	1.7	Aucune donnée	Aucune donnée	Aucune donnée	
16-09-2014	E14154	Suivi MM		0.06	0	3.46	Aucune donnée	Aucune donnée	Aucune donnée	
09-12-2014	E14200	Suivi MM		0.05	0	3.4	Aucune donnée	Aucune donnée	Aucune donnée	
02-06-2015	E15097	Suivi MM	2 Years	0.09	0	4.75	Aucune donnée	Aucune donnée	Aucune donnée	
02-11-2015	E15176	Suivi MM		0.06	0	2.6	Aucune donnée	Aucune donnée	Aucune donnée	
09-05-2016	E16111	Suivi MM	3 Years	0.03	0	3.06	Aucune donnée	Aucune donnée	Aucune donnée	
10-10-2016	E16203	Suivi MM		0.23	0	28.27	Aucune donnée	Aucune donnée	Aucune donnée	
04-05-2017	E17124	Suivi MM	4 Years	0.07	0	4.44	Aucune donnée	Aucune donnée	Aucune donnée	

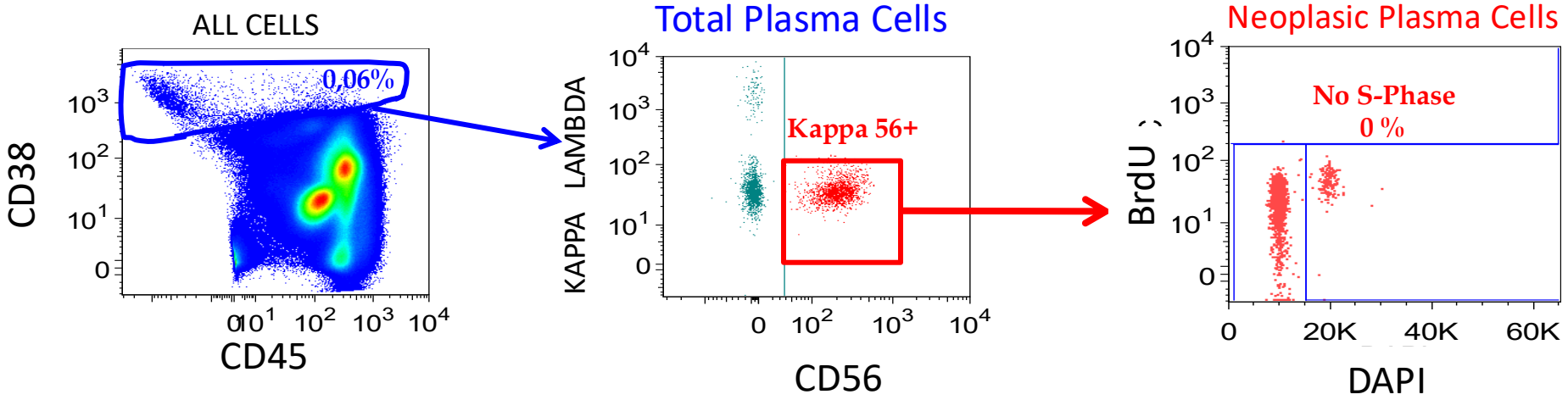
No Tumor Cells

Allograft

Cycle cellulaire en multicouleurs : Maladie Résiduelle (Allogreffe)

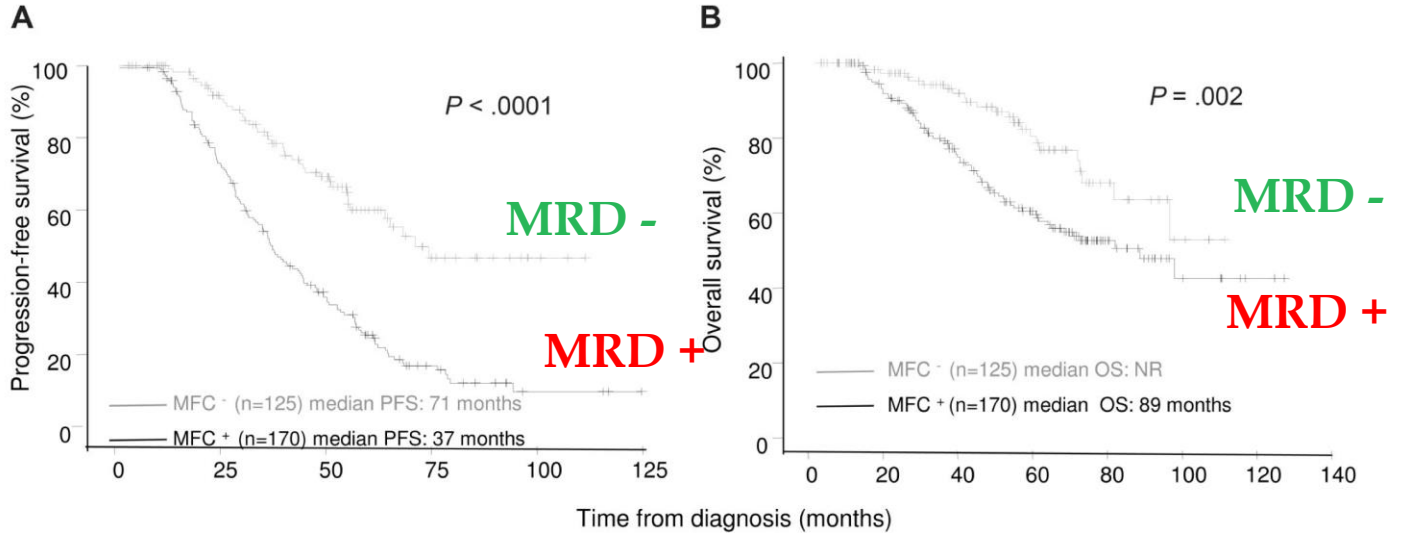


HDT + Post autograft
+ Post allograft
(4 years)

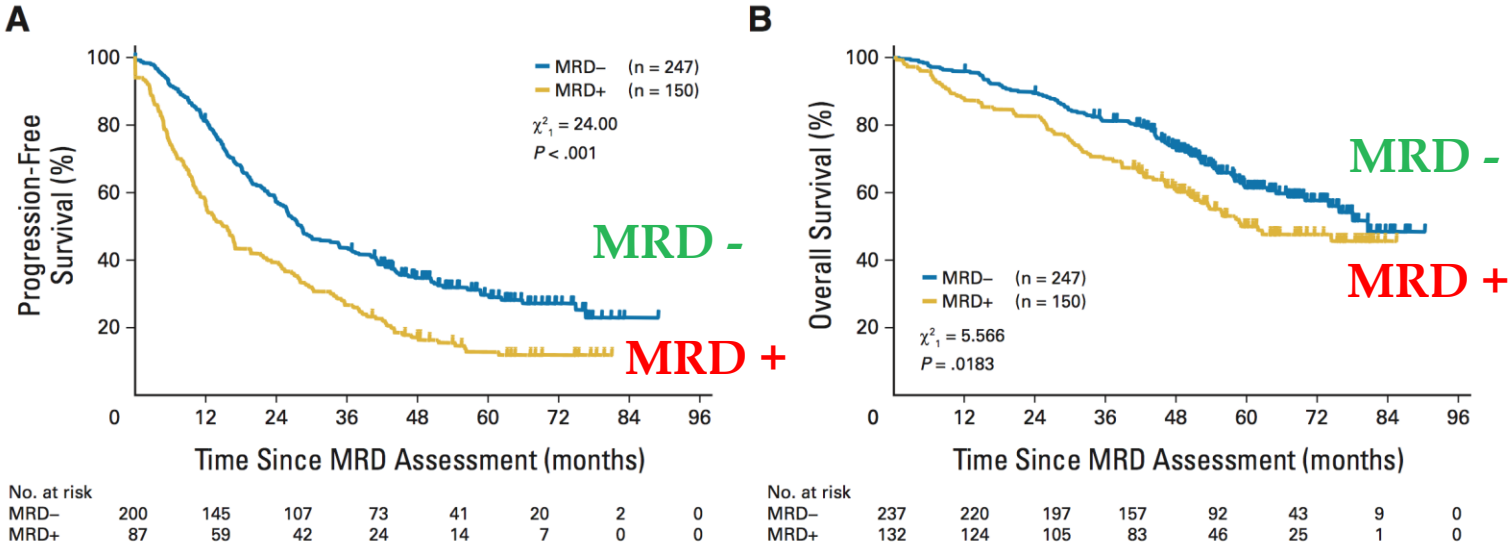


HDT + Post autograft
+ Post allograft
(1,5 years)

Impact on survival of MRD in MM patients



Paiva et al
Blood 2008



Rawstron et al
JCO 2013

3 months after HDT + Autograft

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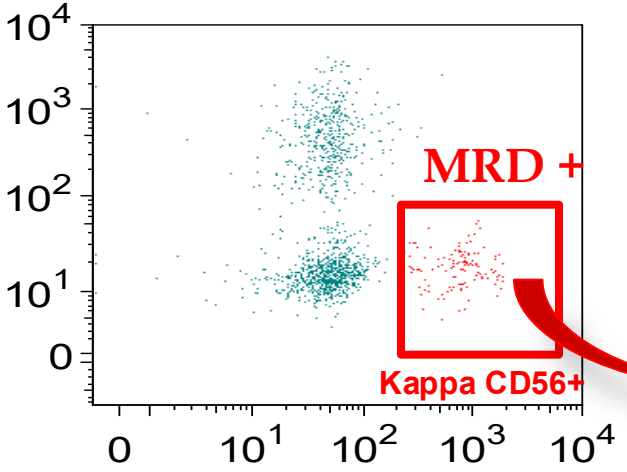
Comparison of Immunofixation, Serum Free Light Chain, and Immunophenotyping for Response Evaluation and Prognostication in Multiple Myeloma

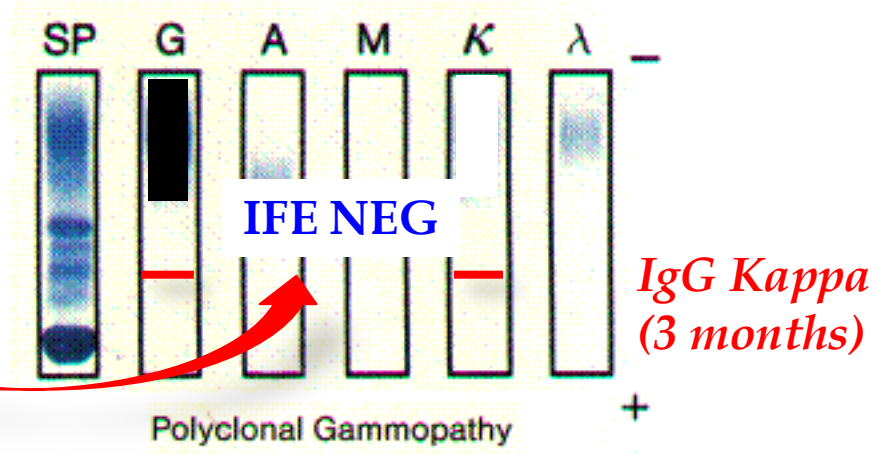
Bruno Paiva, Joaquin Martinez-Lopez, Maria-Belen Vidriales, Maria-Victoria Mateos, Maria-Angeles Montalban, Elena Fernandez-Redondo, Lourdes Alonso, Albert Oriol, Ana-Isabel Teruel, Raquel de Paz, José-Garcia Laran˜a, Enrique Bengoechea, Alejandro Martin, Joaquin Diaz Mediavilla, Luis Palomera, Felipe de Arriba, Joan Blad´e, Alberto Orfao, Juan-Jose Lahuerta, and Jesus F. San Miguel

MFC-positive patients who were immunofixation negative (n =20) showed a tendency toward early reappearance of the M-component (median, 3 months)

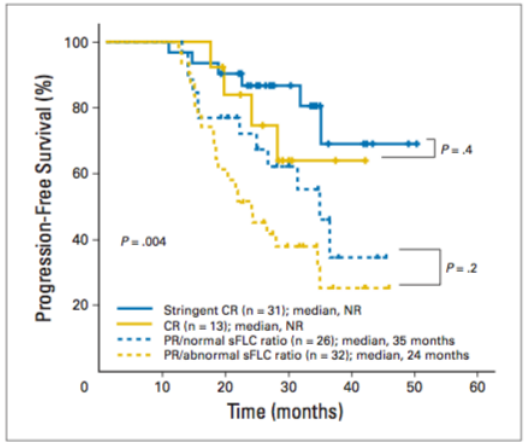
Bone Marrow



BLOOD



PFS (Post Autograft)



CONCLUSIONS

La cytomètrie en flux :

Rapidité d'analyse

Utilisable pour divers types cellulaires
(humaines, animales, végétales, …)

Grande sensibilité (détection d'événements rares)

Intérêt dans divers domaines
(médical, recherche scientifique, industriel,…)

Standardisation des protocoles et reproductibilité (assurance qualité)

Un cytométre rend toujours un résultat !!!!