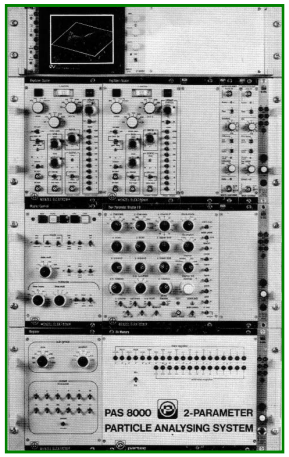
Application des techniques de cytométrie en flux et de fluorescence à la lutte contre le VIH-SIDA, la tuberculose, le paludisme et d'autres parasitoses

Par
Dr Leopold G. LEHMAN
Immunoparasitologue MSc, PhD

UN PEU D'HISTOIRE ! (1968 - 2008)

1968/69: ICP 11
Premier cytomètre commercialisé dans le monde. Développé par Prof. Goehde, distribué par Phywe.

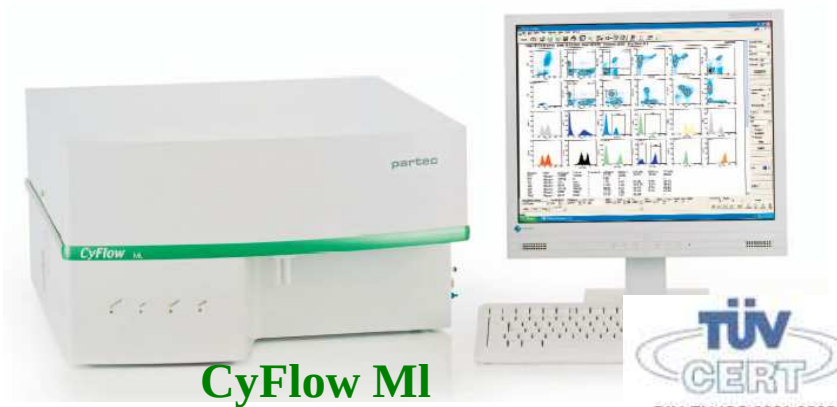


1973: PAS 8000
Développé et distribué par Partec

1975: ICP 22
Developpé par Partec.
Distribué par Phywe.

1977: ICP 22a
Développé par Partec.
Distribué par Ortho.

1990 PAS/PASIII



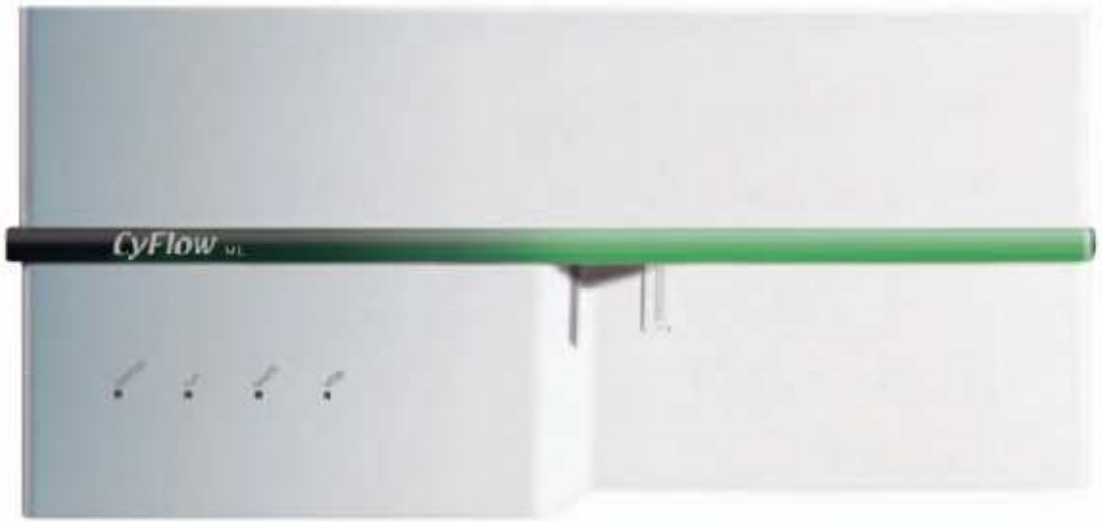
CyFlow Ml

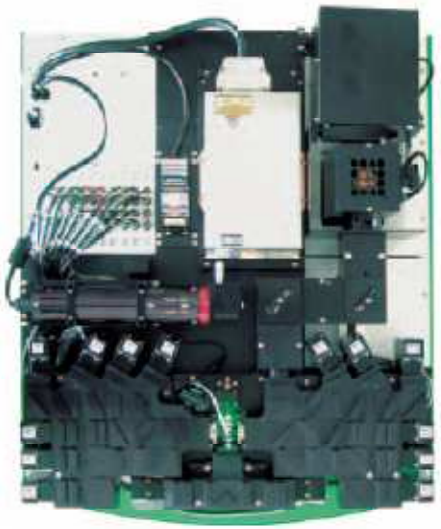
40 années d'expérience en cytometrie

TÜV CERT
DIN EN ISO 9001:2000
DIN EN ISO 13485:2003

LA CYTOMETRIE EN FLUX

CyFlow® Flow Cytometry System





Fluorescence

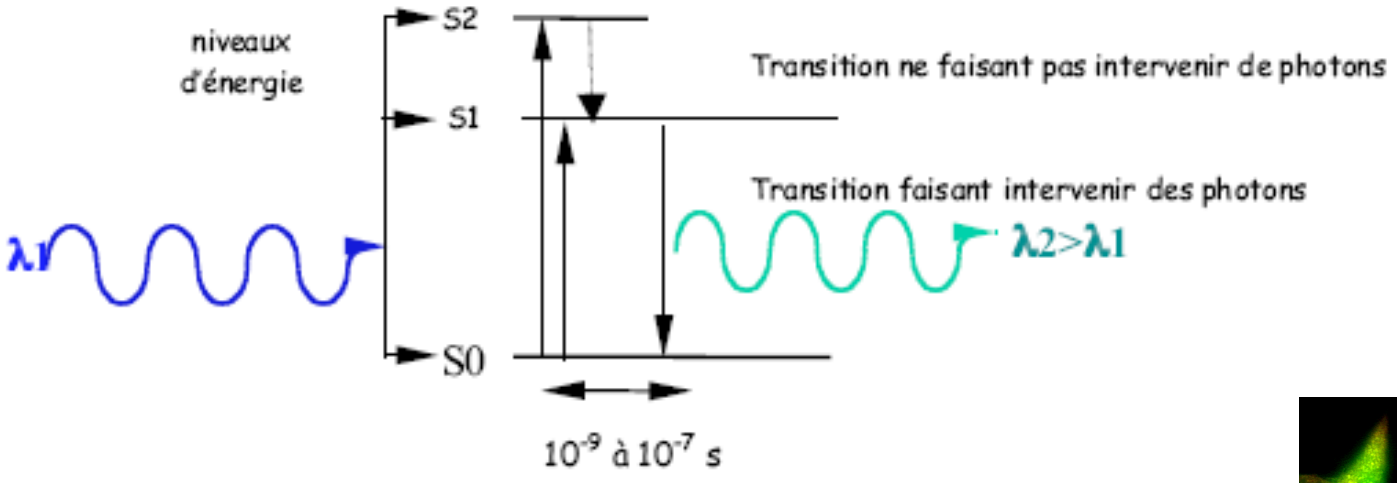
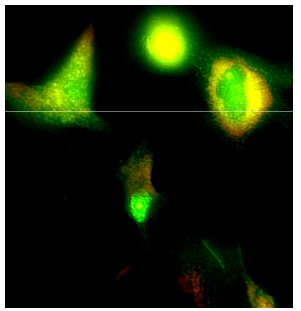


Figure 3 : principe de la fluorescence

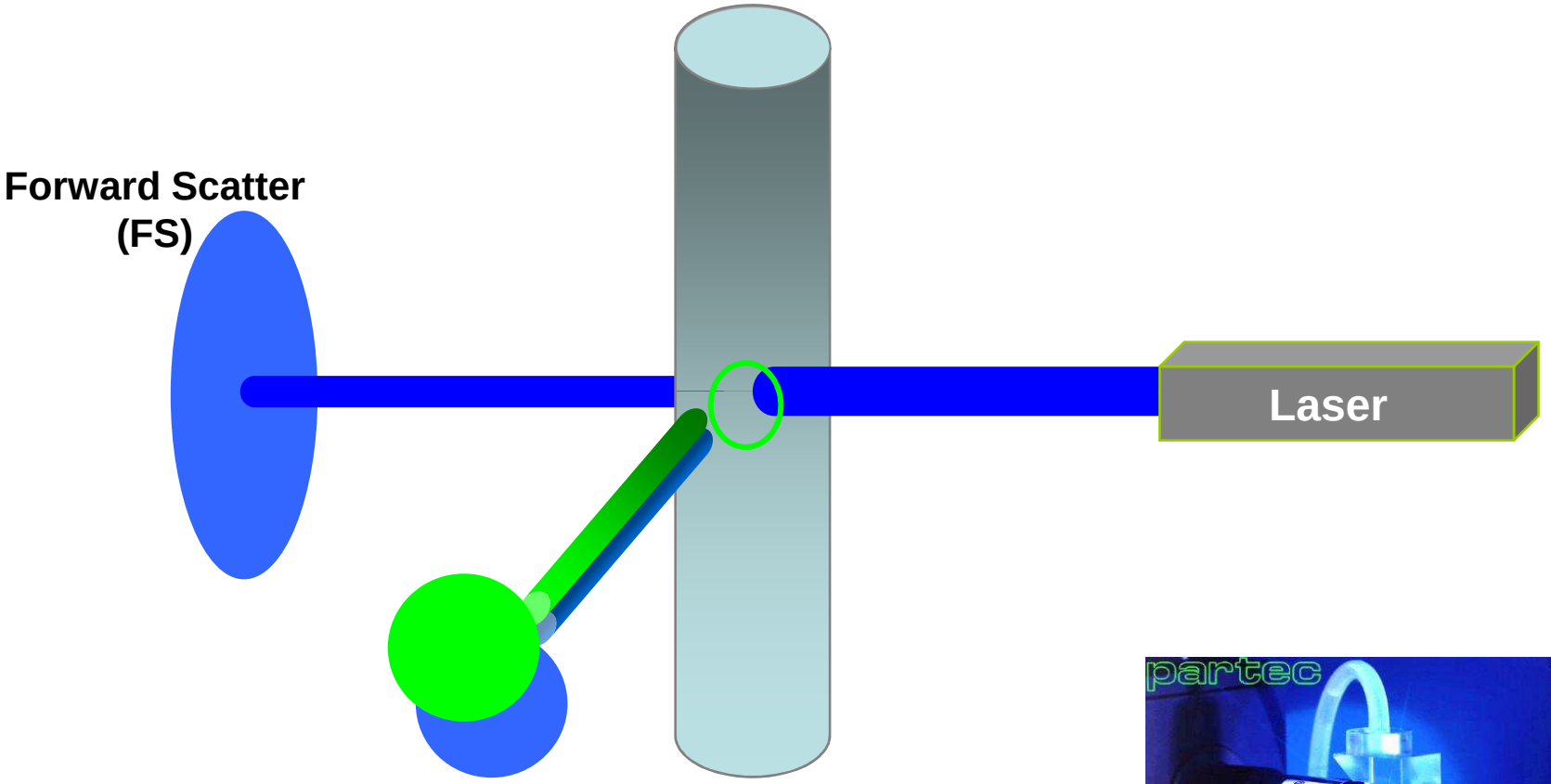


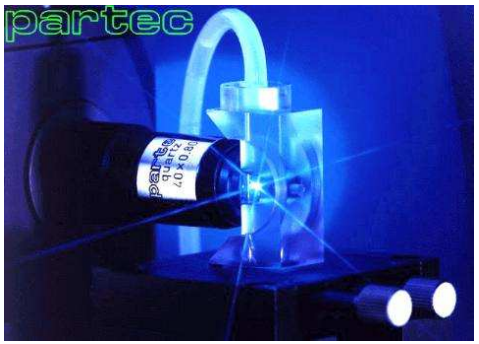
La fluorescence émise :

- **Fluorescence intrinsèque (Auto fluorescence)**

- **fluorescence induite par les différents fluorochromes est séparée par des jeux de filtres optiques choisis en fonction des spectres d'excitation et d'émission.**

CYTOMETRE EN FLUX - PRINCIPE DE BASE





Forward Scatter : FSC

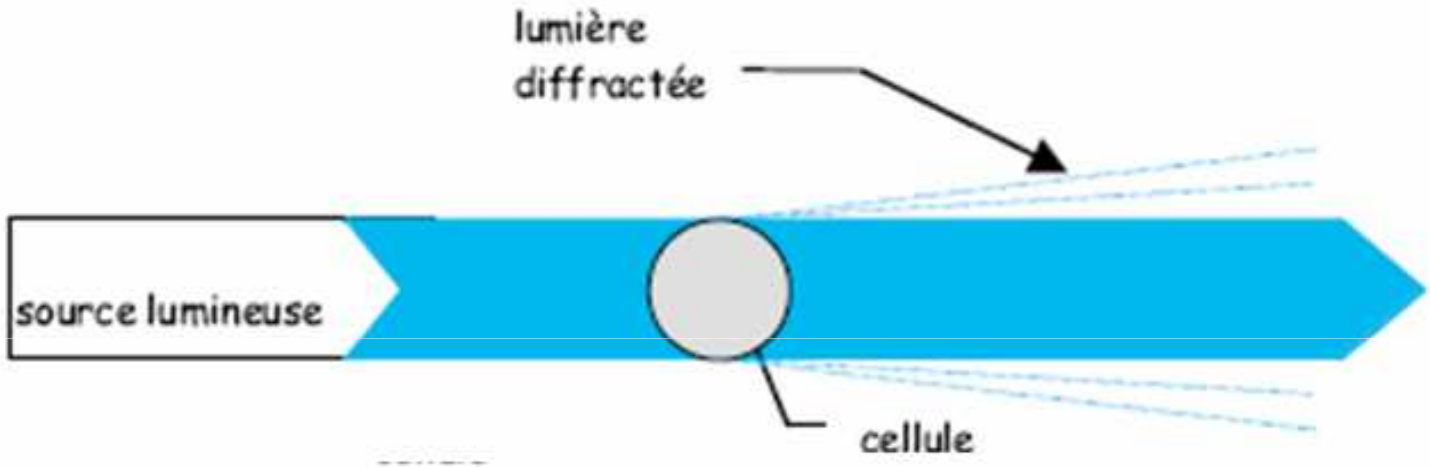


Figure 1: La lumière diffusée vers l'avant (FS) reflète la taille

Side Scatter : SSC

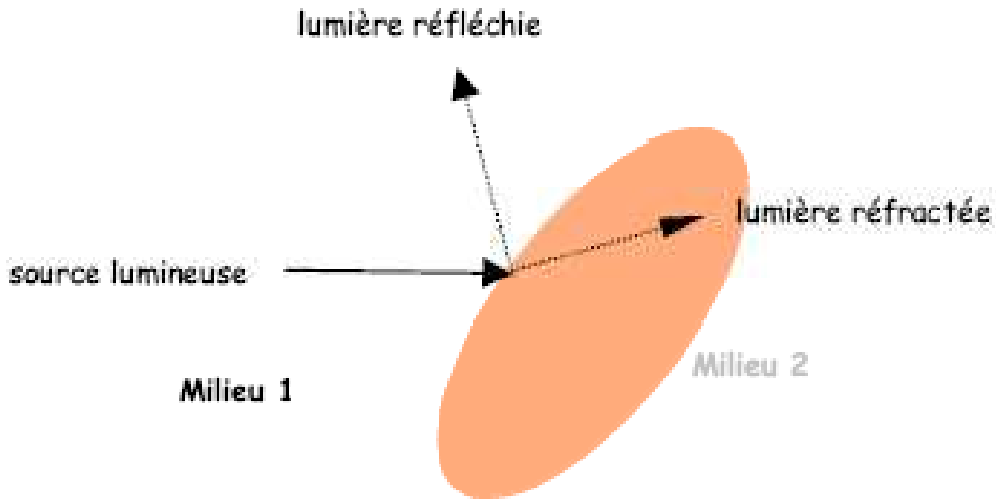
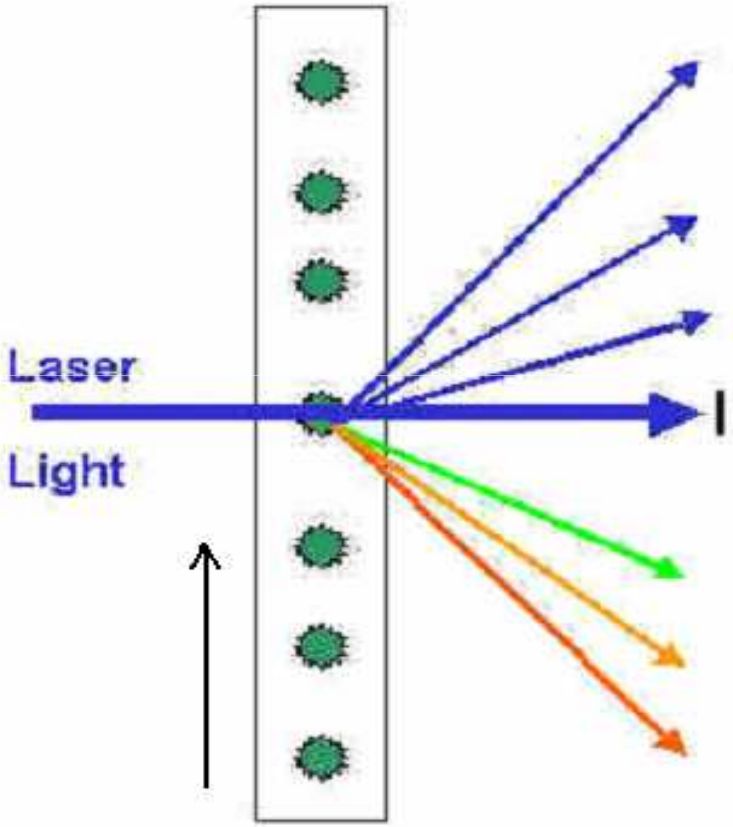


Figure 2: La lumière diffusée à 90° (SS) reflète la granulosité et le rapport nucléoplasmique

Signaux d'émission



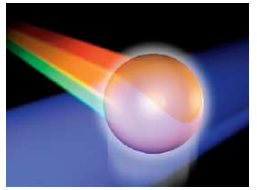
High angle scatter :
Reflection & refraction.
Cell structure. SSC

Low angle scatter : FSC
Diffraction. Cell size.

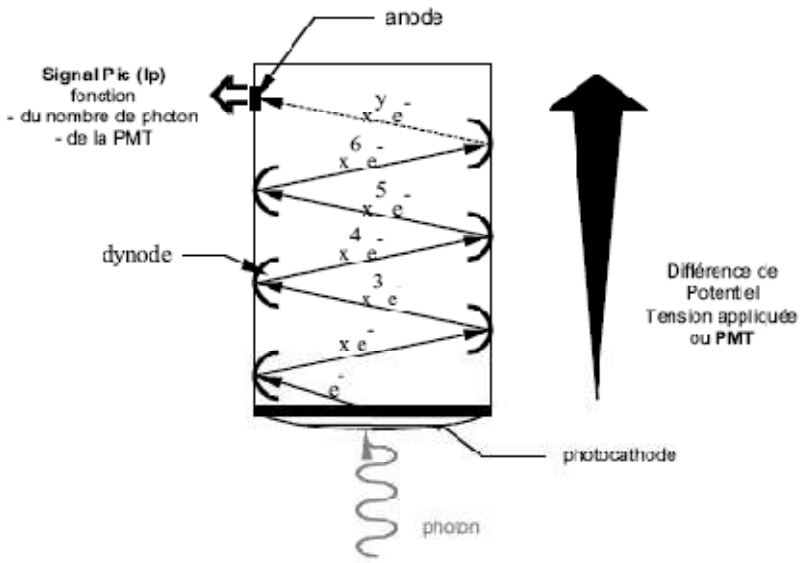
Direct beam stop.

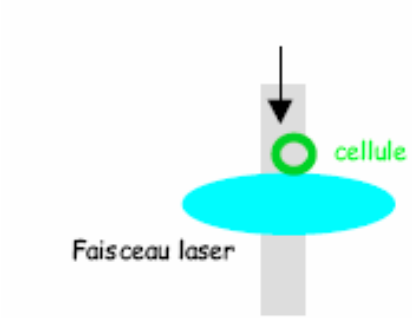
Fluorescence at longer wavelengths.

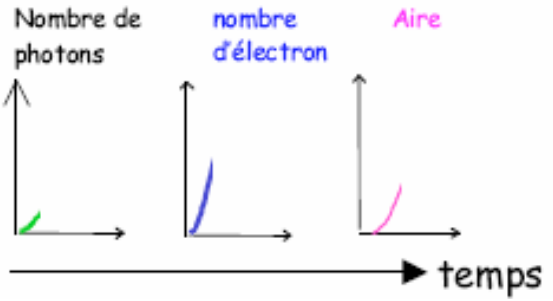
Intrinsic
(autofluorescence)
and extrinsic.

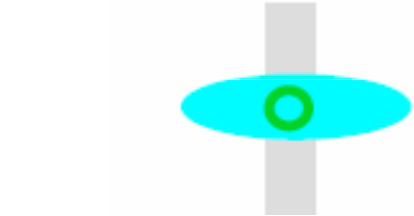


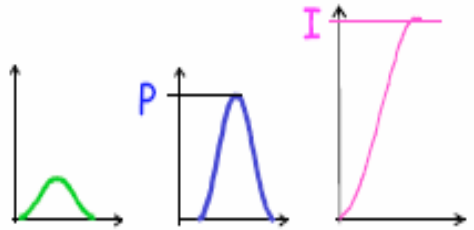
Obtention d'un signal électrique à partir d'un signal lumineux:



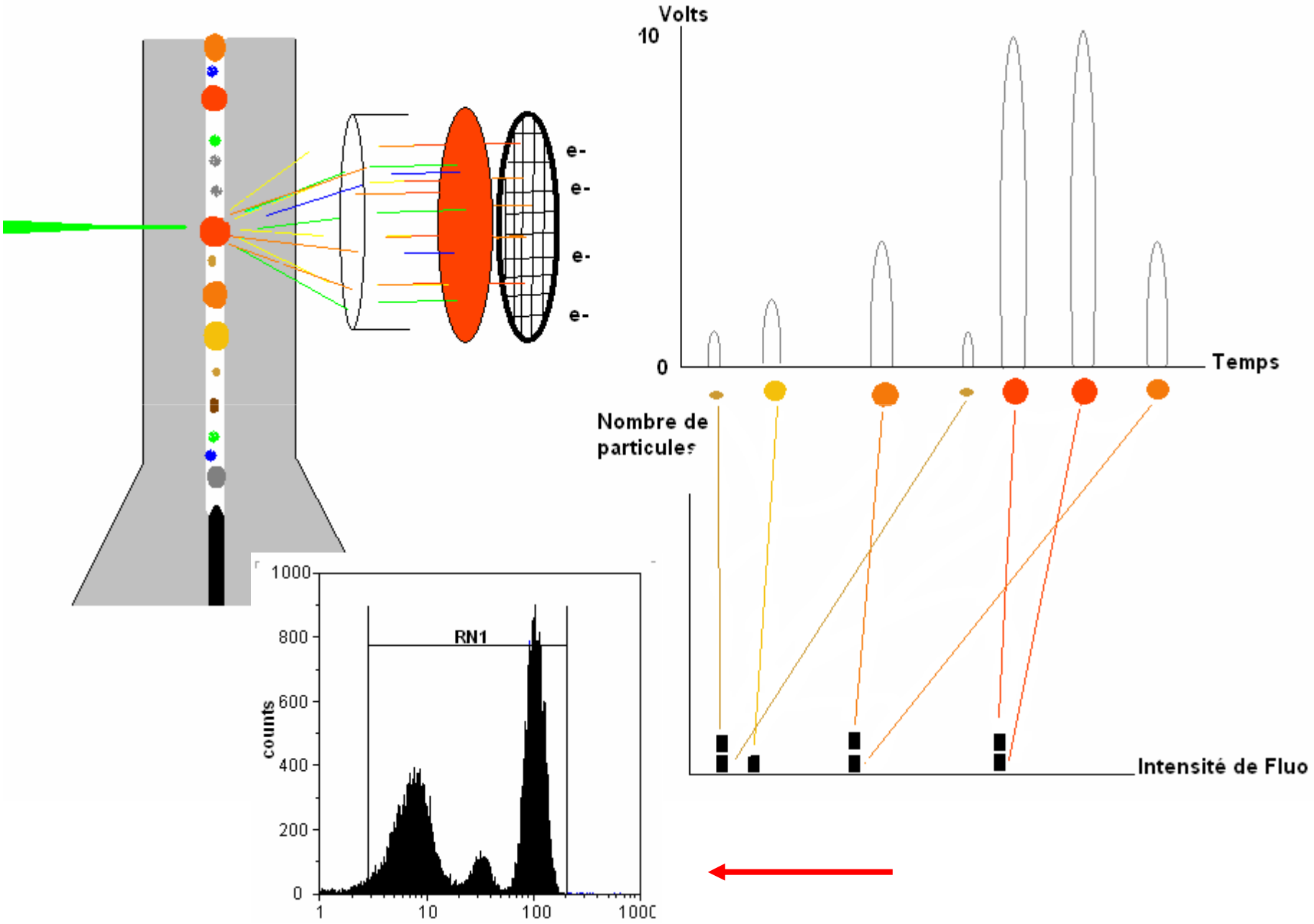




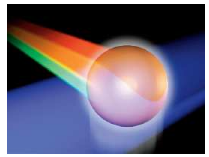




Enregistrement des signaux, classement des particules

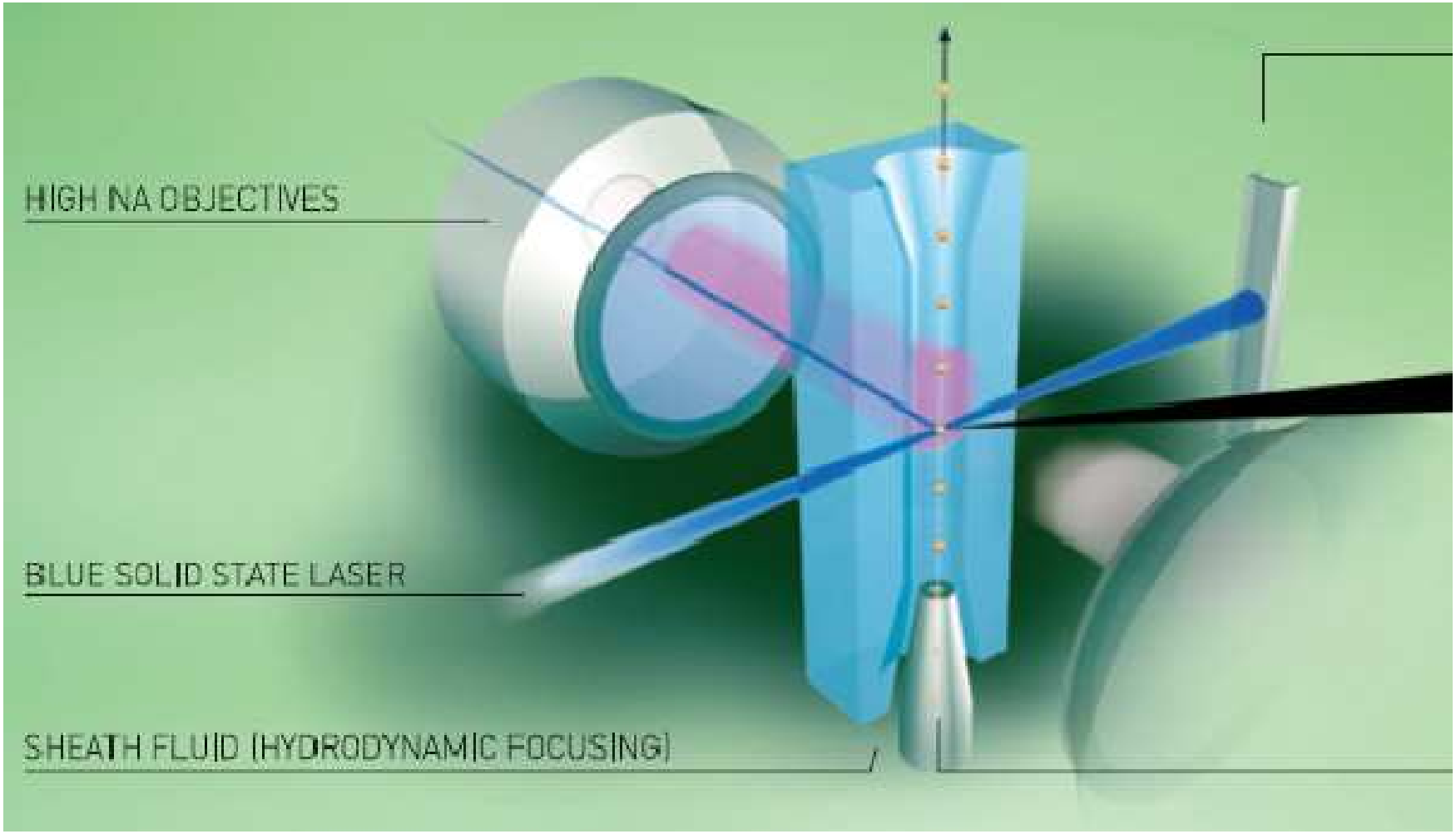


Source lumineuse d'excitation

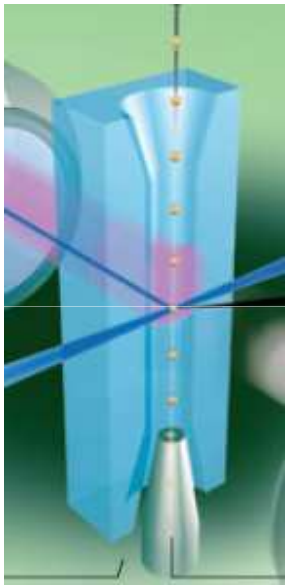


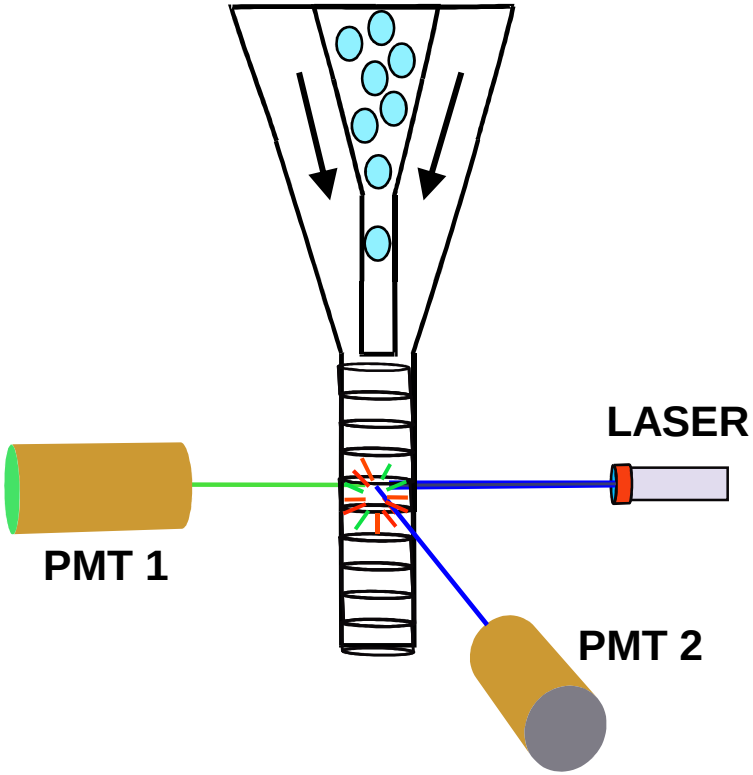
UV lamp 100 W	366 nm (200-600 nm)	VIOLET	INDO-1					
		BLUE	DAPI	Hoechst	Alexa® Fluor 350	AMCA		
		BLUEGREEN	MBB					
blue solid state laser 200 mW	488 nm	GREEN	FITC	GFP	Alexa Fluor® 488	SYTO 9		
		ORANGE	PE	eYFP				
		ORANGE RED	PE-Texas Red®		PI			
		RED	PE-Cy5	PE-Cy5.5	PerCP	PerCP-Cy5.5	ECD	EB
		INFRA RED	PE-Cy7					
red diode laser 25 mW	635 nm	RED	APC	APC-Cy5.5	SYTO 59-61	Alexa Fluor® 633	Alexa Fluor® 647	
		INFRA RED	APC-Cy7					
green solid state laser 100 mW	532 nm	ORANGE	PE	Alexa Fluor® 532	SYTO 80-83	CY3		
		RED	PE-Cy5	PE-Cy5.5	7-AAD	PI		
		INFRA RED	PE-Cy7					
violet diode laser 50 mW	405 nm	Alexa Fluor® 405	Pacific Blue	Cascade	CFP			
UV diode Laser 8 mW	375 nm	DAPI	Hoechst	IVDO 1	MBB	AMCA	Alexa Fluor® 350	

LA CELLULE DE MESURE
l'élément au cœur du système

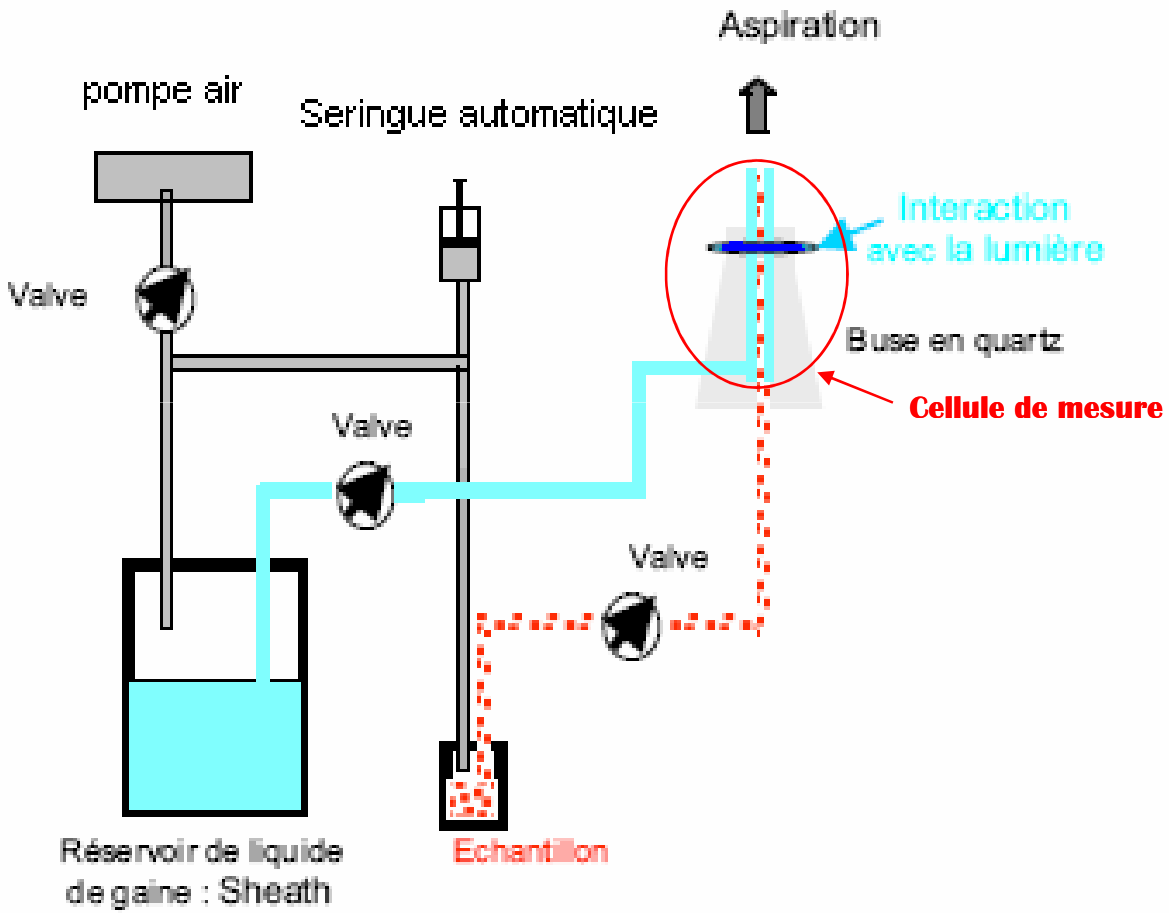


Focalisation hydrodynamique

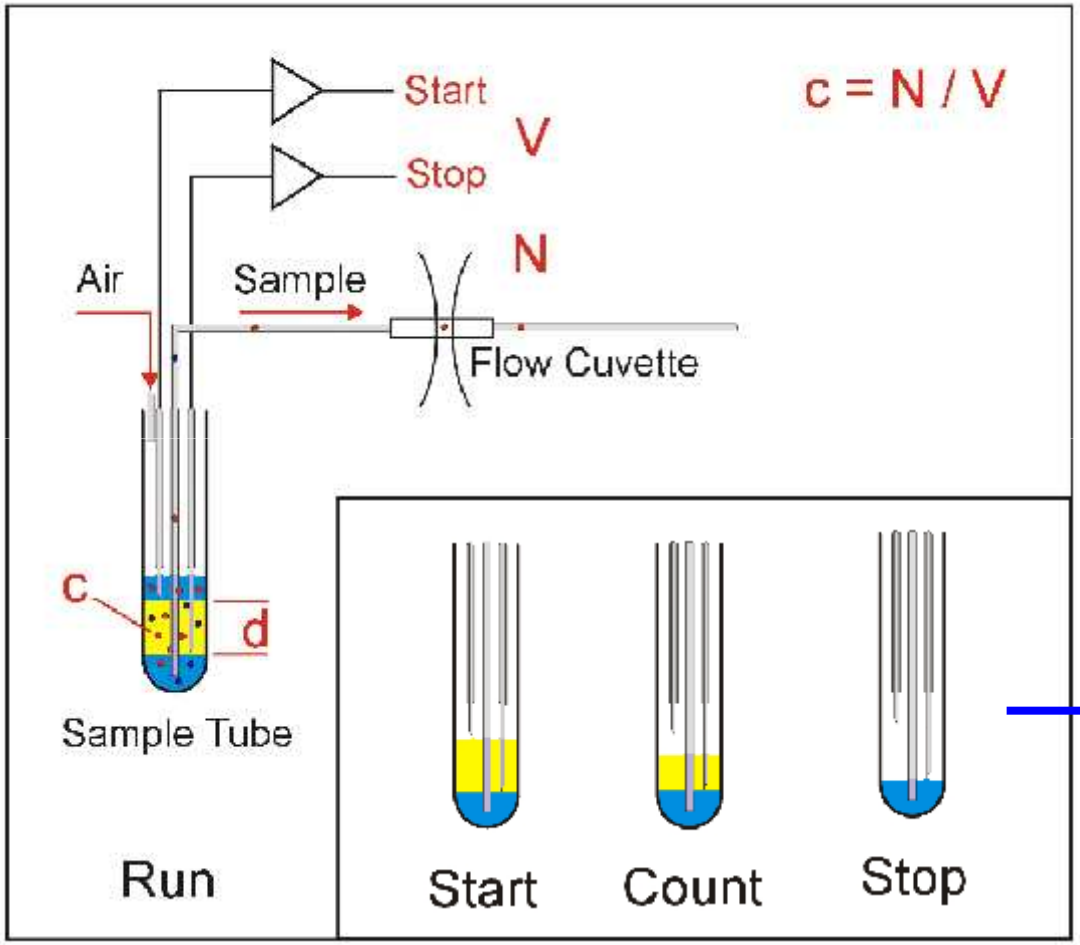




Système fluidique



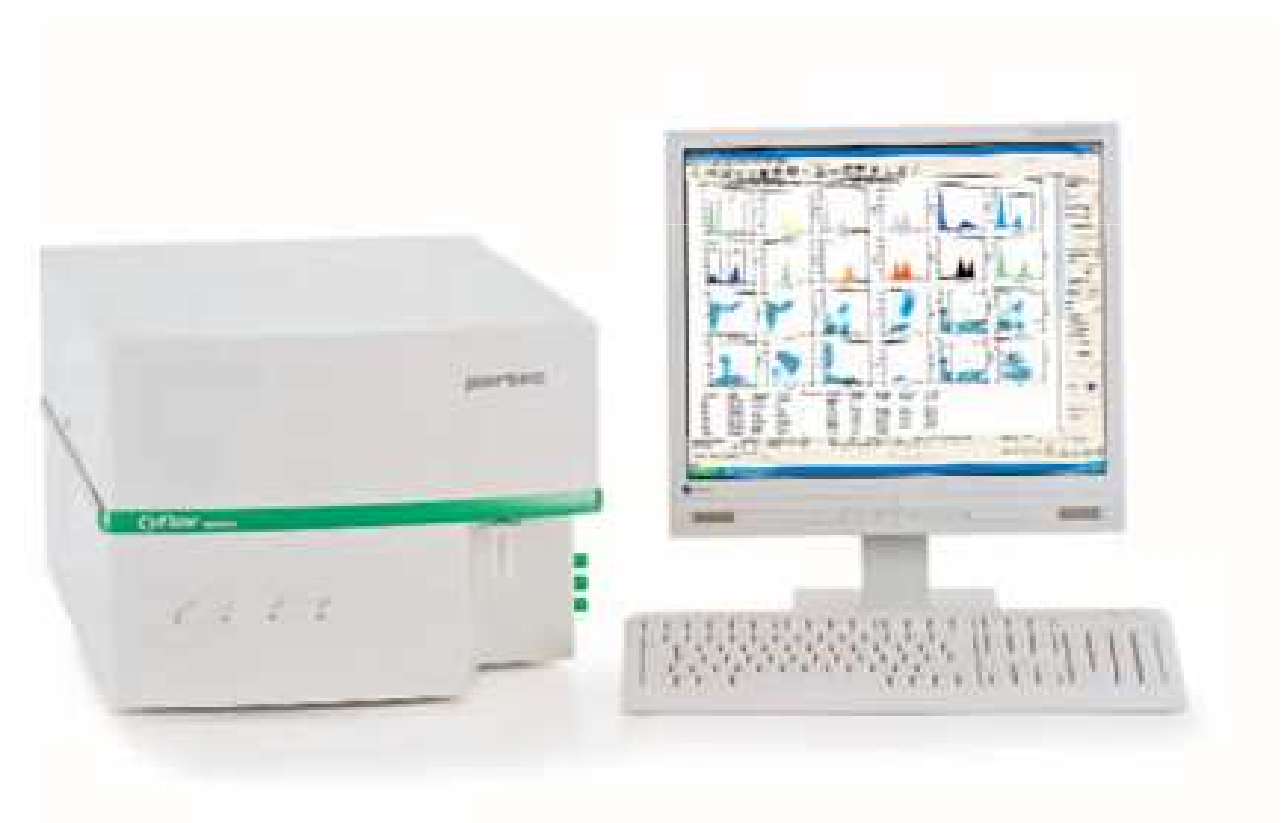
INJECTION DE L'ECHANTILLON, COMPTAGE VOLUMETRIQUE



CONCENTRATION EN PARTICULES/ml

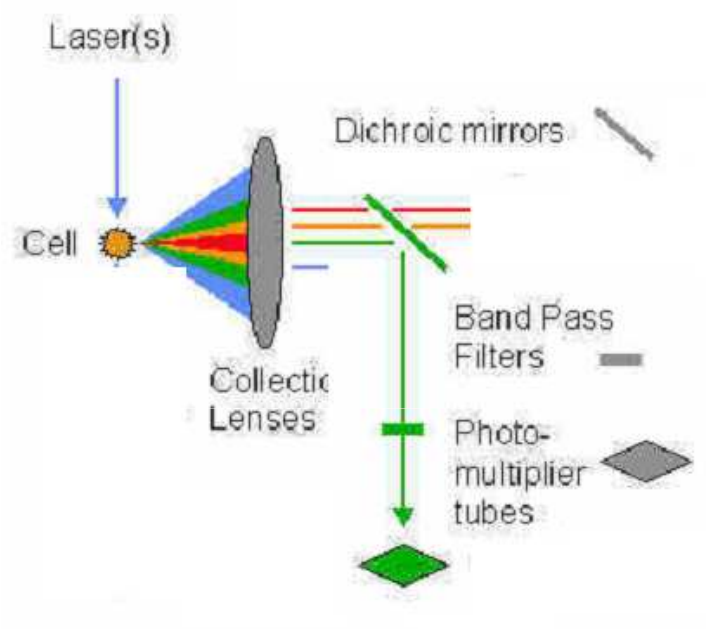
Cytomètre en flux pour la recherche

CyFlow® Space 6 paramètres (Code N° CY-S-3001)

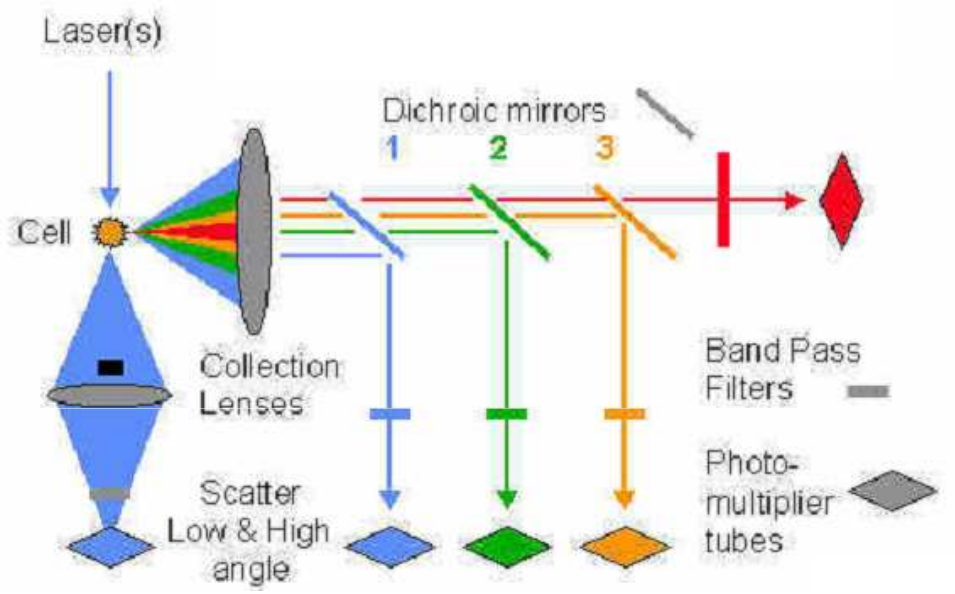


Parcours optique

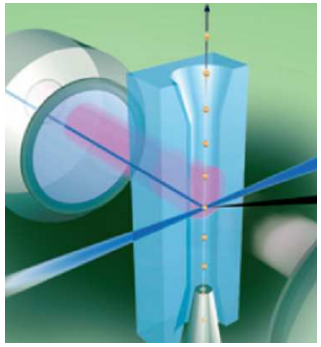
Système Mono-paramétrique

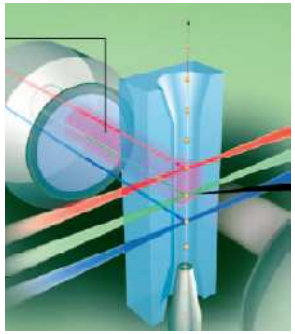


Système Multi-paramétrique



source d'excitation simple / sources multiples





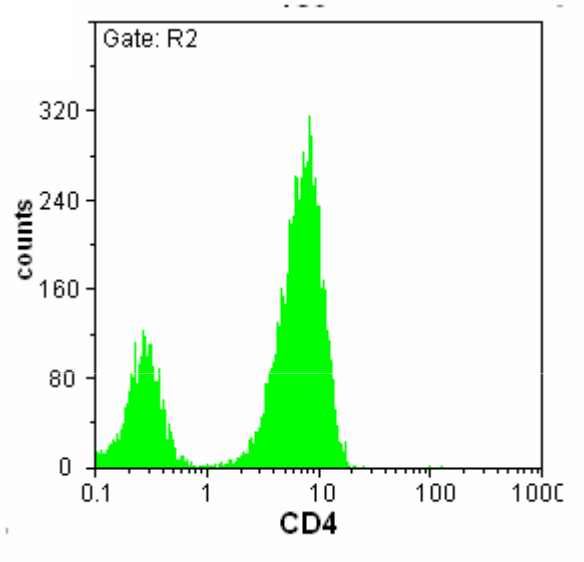
Avantage du multipara Large choix de marqueurs fluo.

Antigènes-Protéines			
AMCA	360	450	
APC	650	660	
APC-Cy7	(650-755)	767	Equivalent PharRed
Cascade Blue	399	423	
Cy3	(512-552)	(565-615)	
Cy5	(625-650)	670	Equivalent Far-Red
Cy7	743	767	
Fluorescéine (FITC)	495	519	FITC sensible au pH
Phycoérythrine-R (R-PE)	(495-565)	578	
PE-Cy5 (PC5)	495	670	Equivalent aka Cychrome, R670, Tri-Color, Quantum Red
PE-Cy7	(480-743)	767	
PerCP	490	675	
Red 613	(480-565)	613	Equivalent ECD
Rhodamine 640	590	625	
Sulforhodamine 101	570	610	
Texas Red	589	615	
TRICT	547	572	
TruRed	(490-675)	695	Equivalent PerCP-Cy5.5

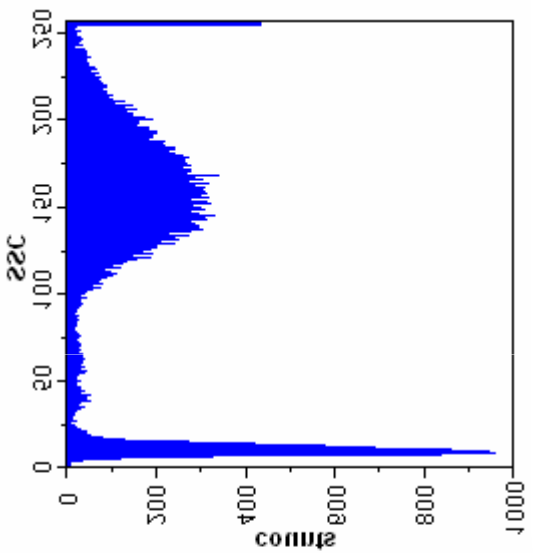
Système ouvert

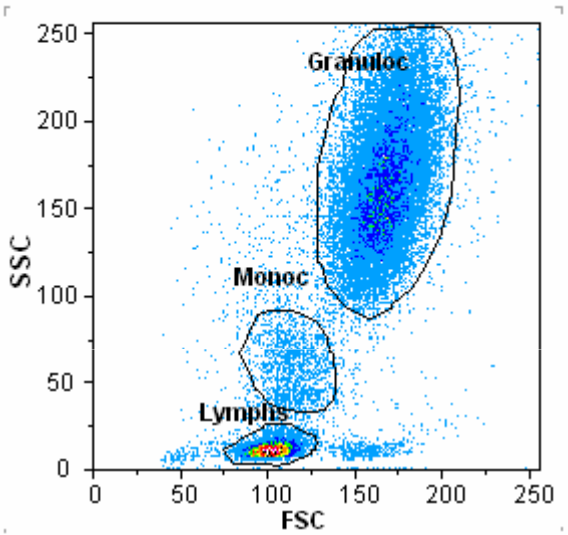
Acides Nucléiques			
7-AAD	546	647	ADN (bases CG)
Acridine Orange	503	530/640	ADN /ARN
Bromure d'Ethidium	370/530	620	ADN /ARN
Chromomycine A3	445	575	ADN (Bases CG)
DAPI	345	455	ADN (Bases AT)
Hoechst 33342	365	502	ADN (Bases AT)
Hoechst 33258	365	502	ADN (Bases AT)
Iodure de Propidium (IP)	370/536	617	Agent Intercalant
LDS 751	543/590	607/712	ADN (543ex/712em), ARN (590ex/607em)
Mithramycine	445	575	ADN
SYTOX Blue	431	480	ADN
SYTOX Green	504	523	ADN
SYTOX Orange	547	570	ADN
Thiazole Orange	510	530	ARN
Thioflavine	422	487	ARN
TOTO-1, TO-PRO-1	509	533	Colorant vital

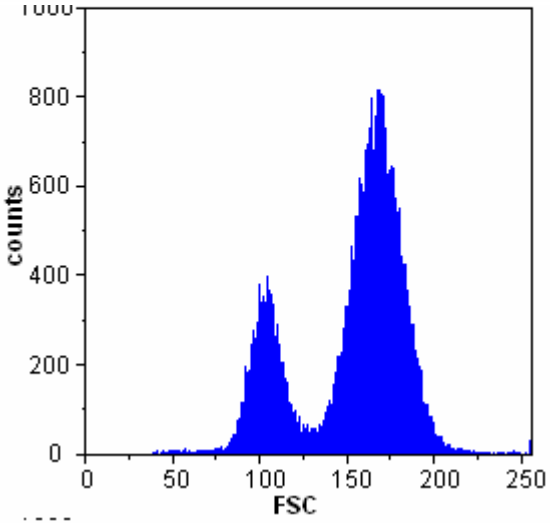
Représentation Mono ou Bi-paramétrique



Mono

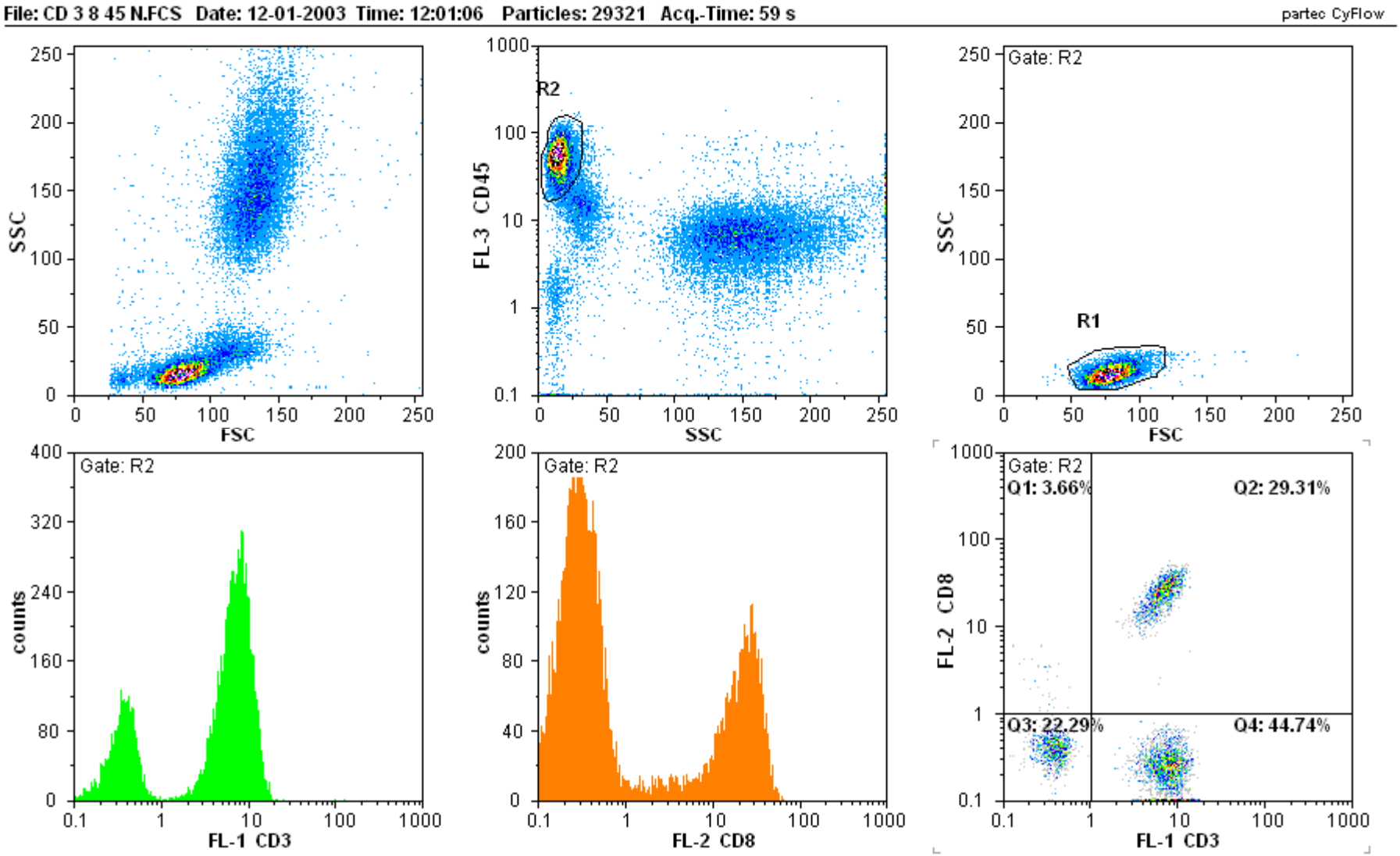




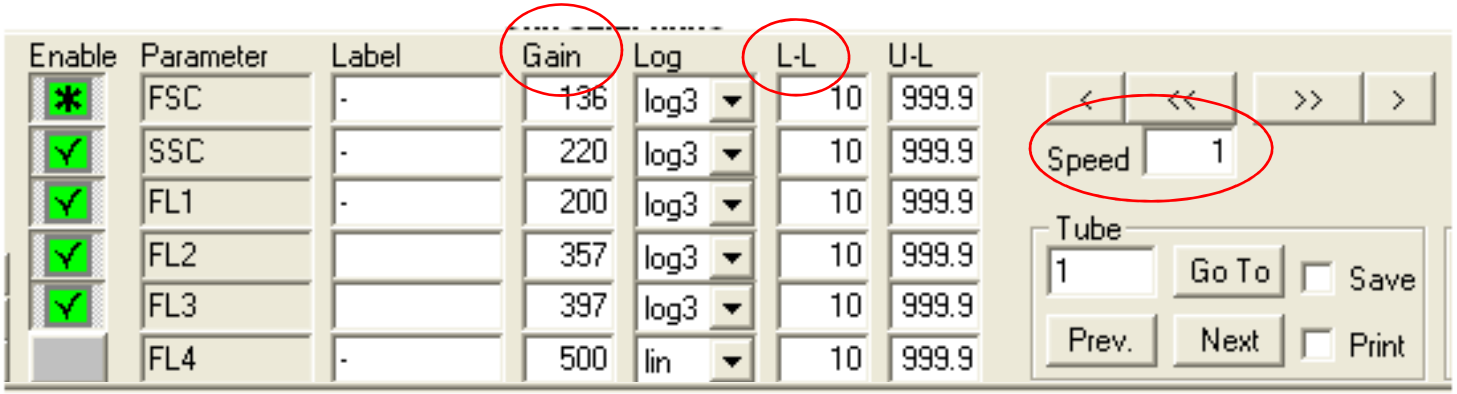


Bi

Marquage Trois couleurs CD45/CD3/CD8

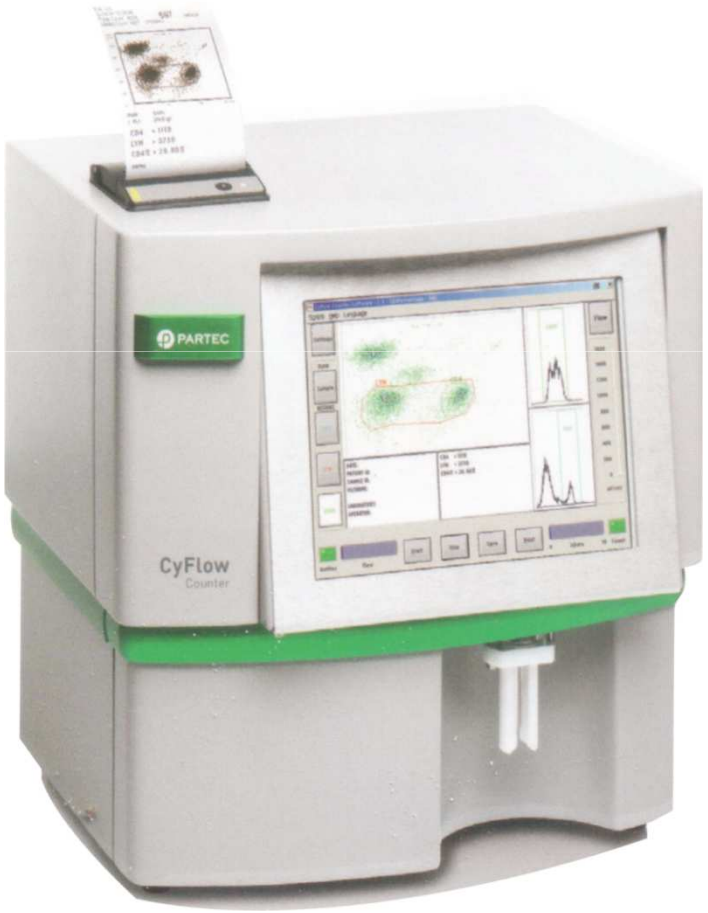


Possibilité de réglages des principaux paramètres d'analyse



- GAIN
- LOW LEVEL
- SPEED

La Solution pour le suivi des PVVS enfants et adultes

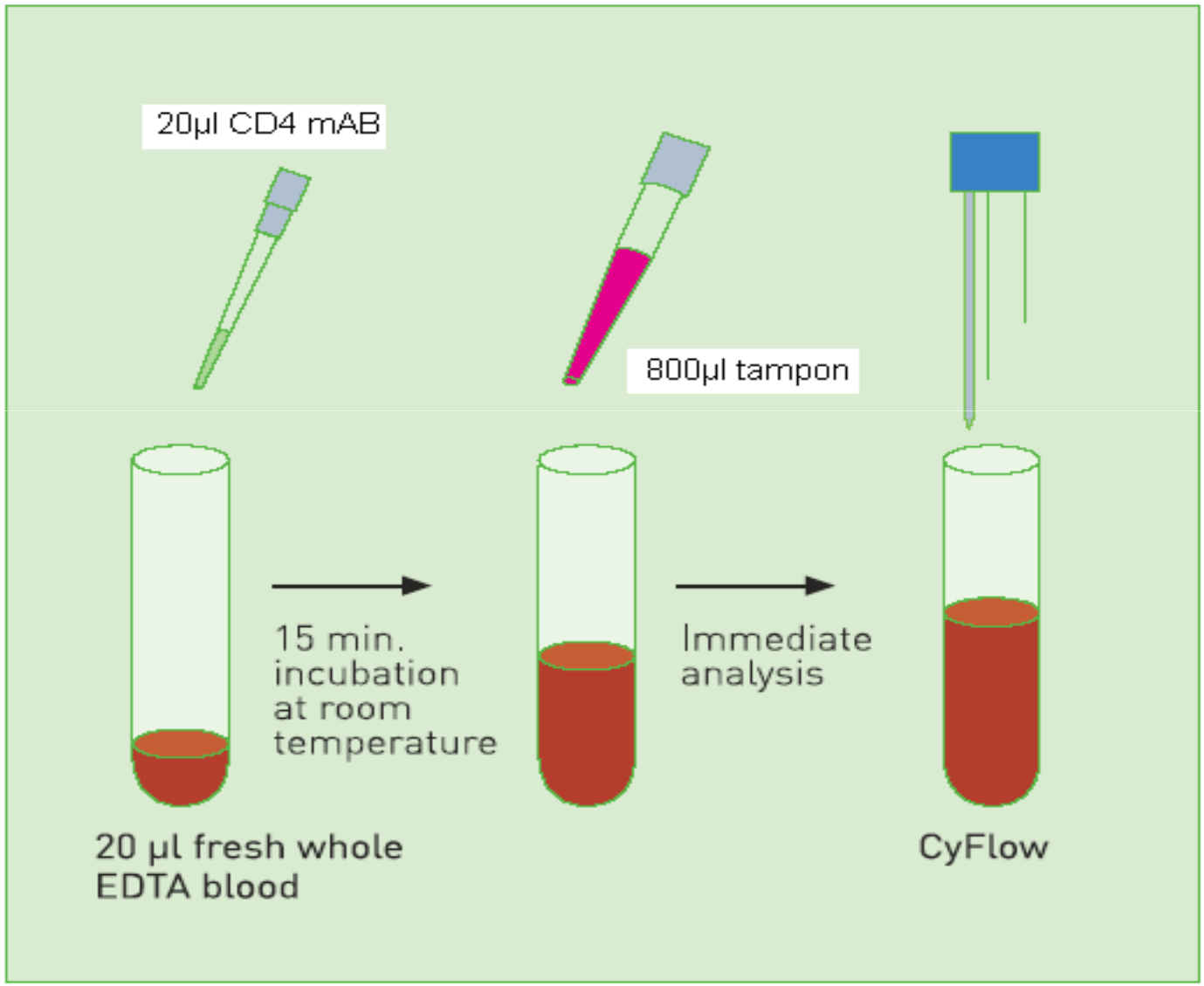


CyFlow® Counter

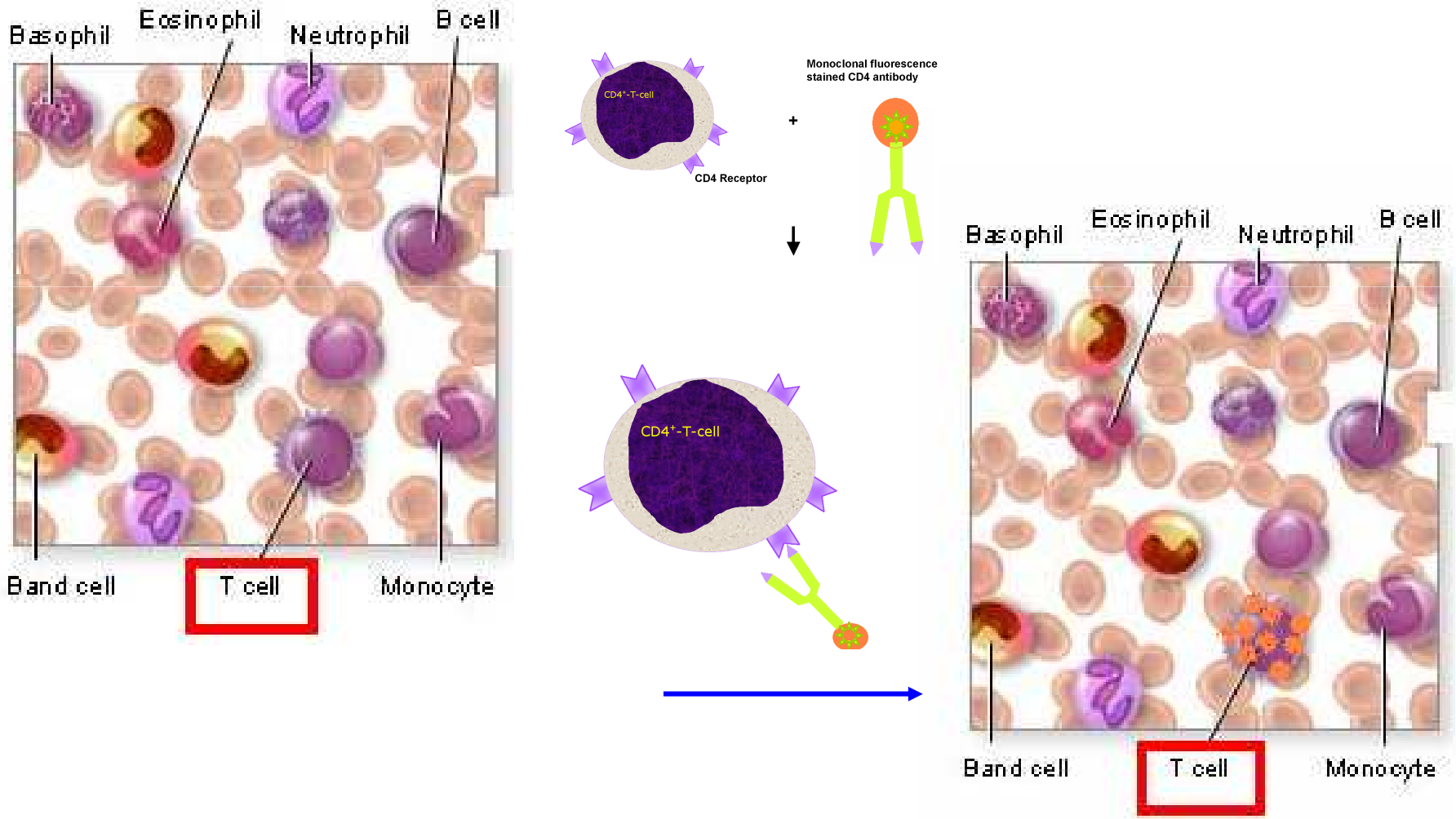
cytomètre en flux portable, mono-paramétrique pour analyse de CD4, CD4%. Equipé d'un laser solide d'excitation à 532 nm (Vert), puissance de 50mW. (Excitation compatible avec les fluochromes classiques du type : PE, PI, EB, PE-CY5).

Analyse sur un canal de fluorescence (orange/rouge).

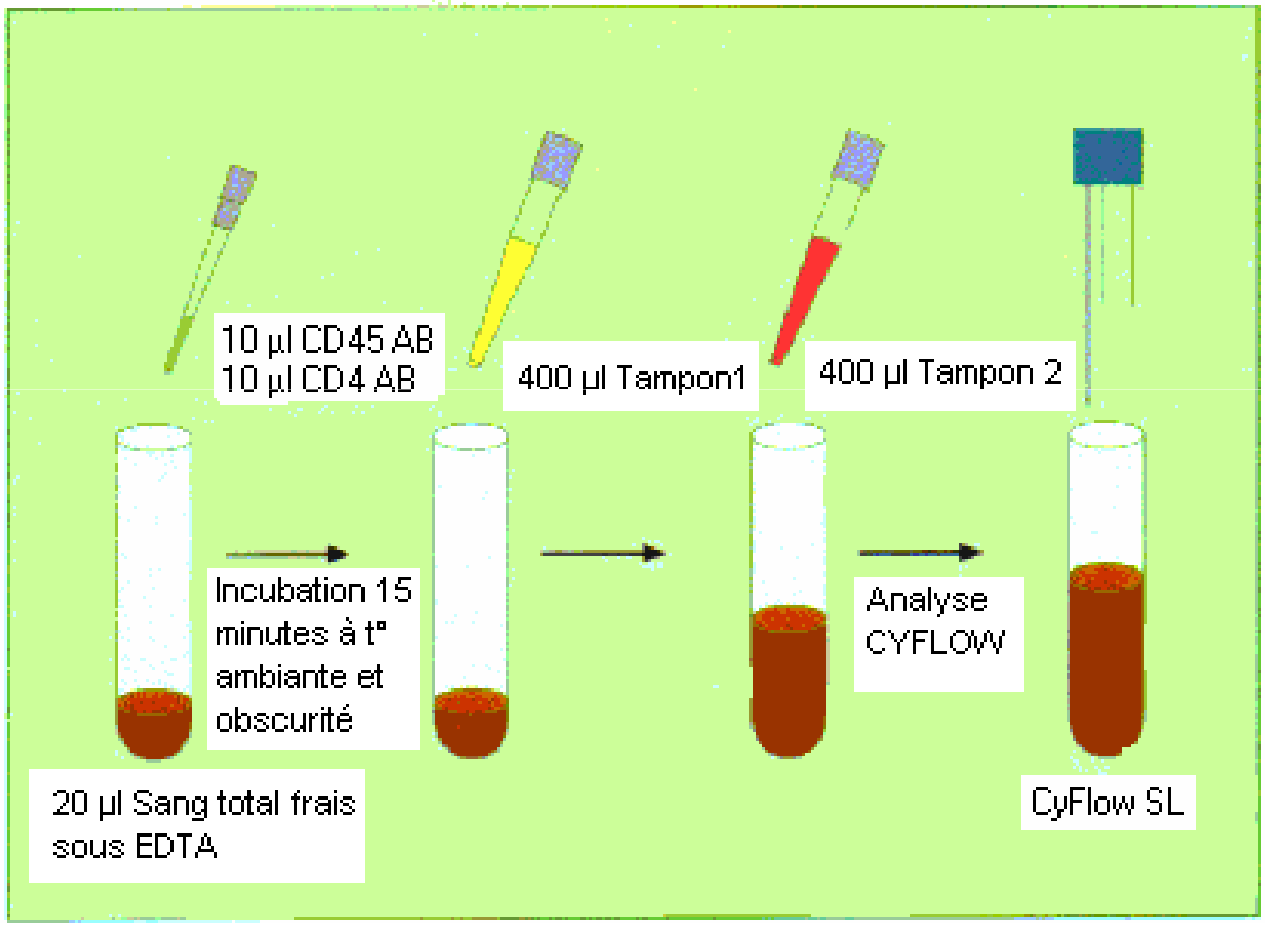
PROTOCOLE CD4: PREPARATION DES ECHANTILLONS



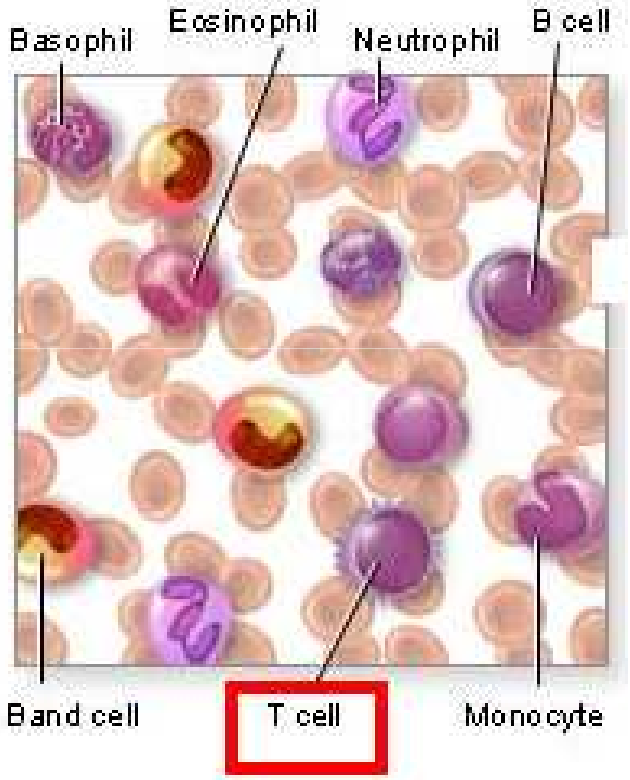
MARQUAGE DES CELLULLES – Anticorps Anti CD4

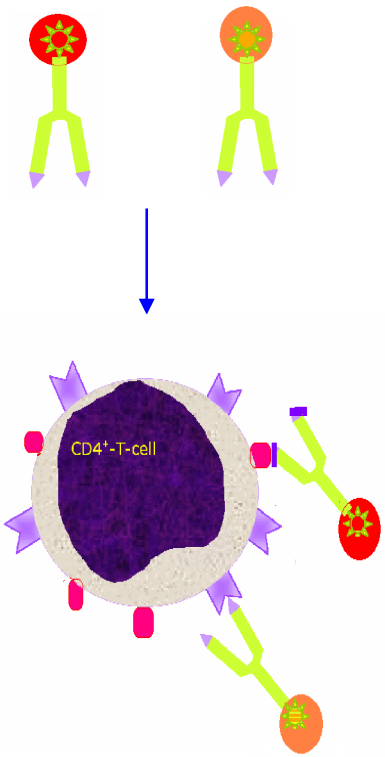


PREPARATION DES ECHANTILLONS PROTOCOLE CD4%

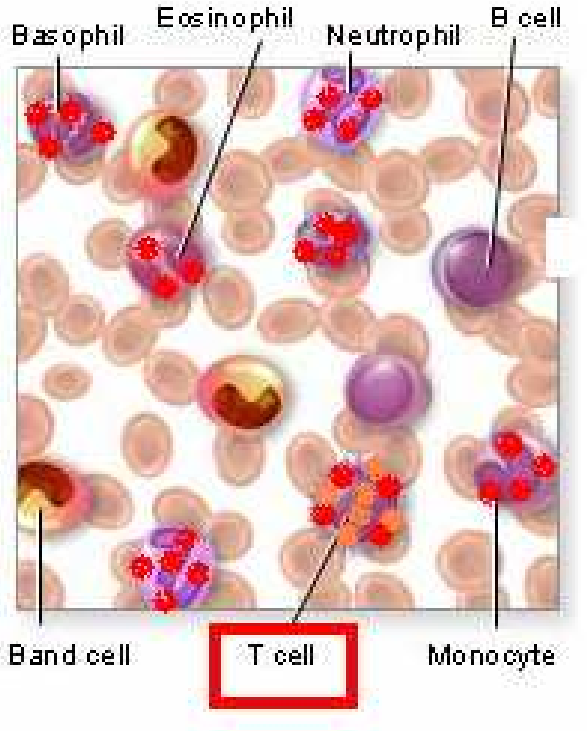


MARQUAGE DES LEUCOCYTES et LYMPHOCYTES CD4 / CD4%

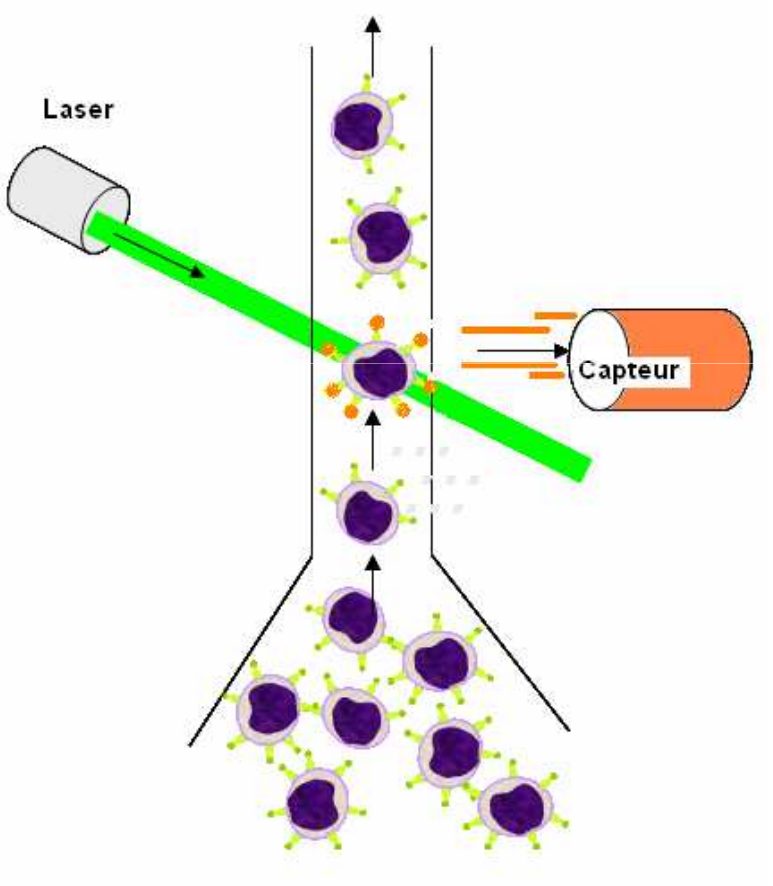




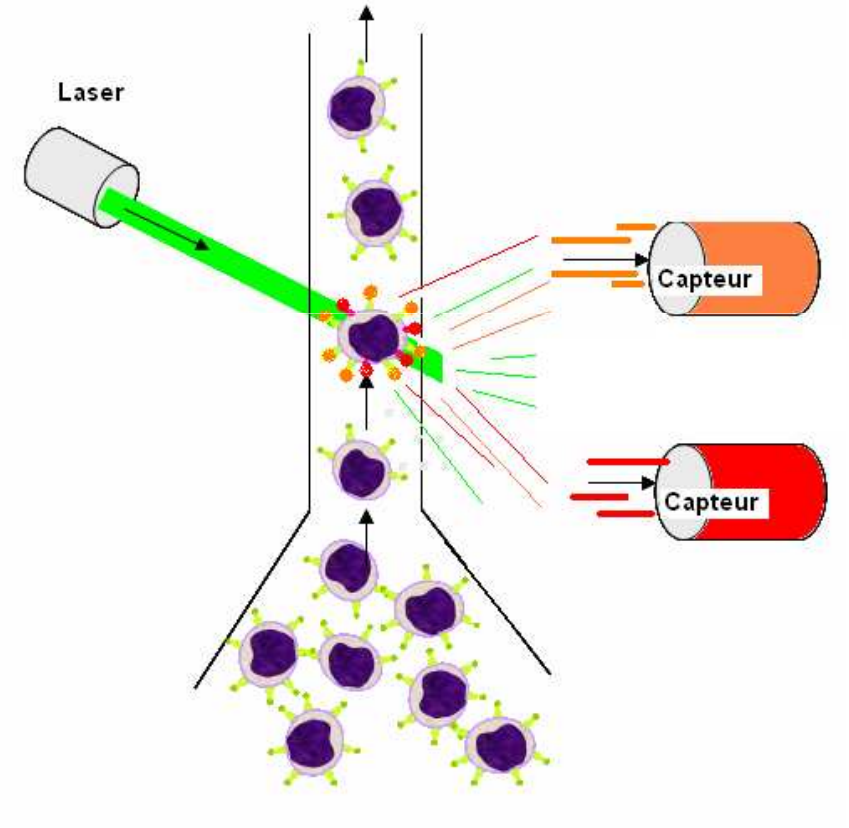
AC Anti CD4
Anti CD45



Analyse au cytomètre

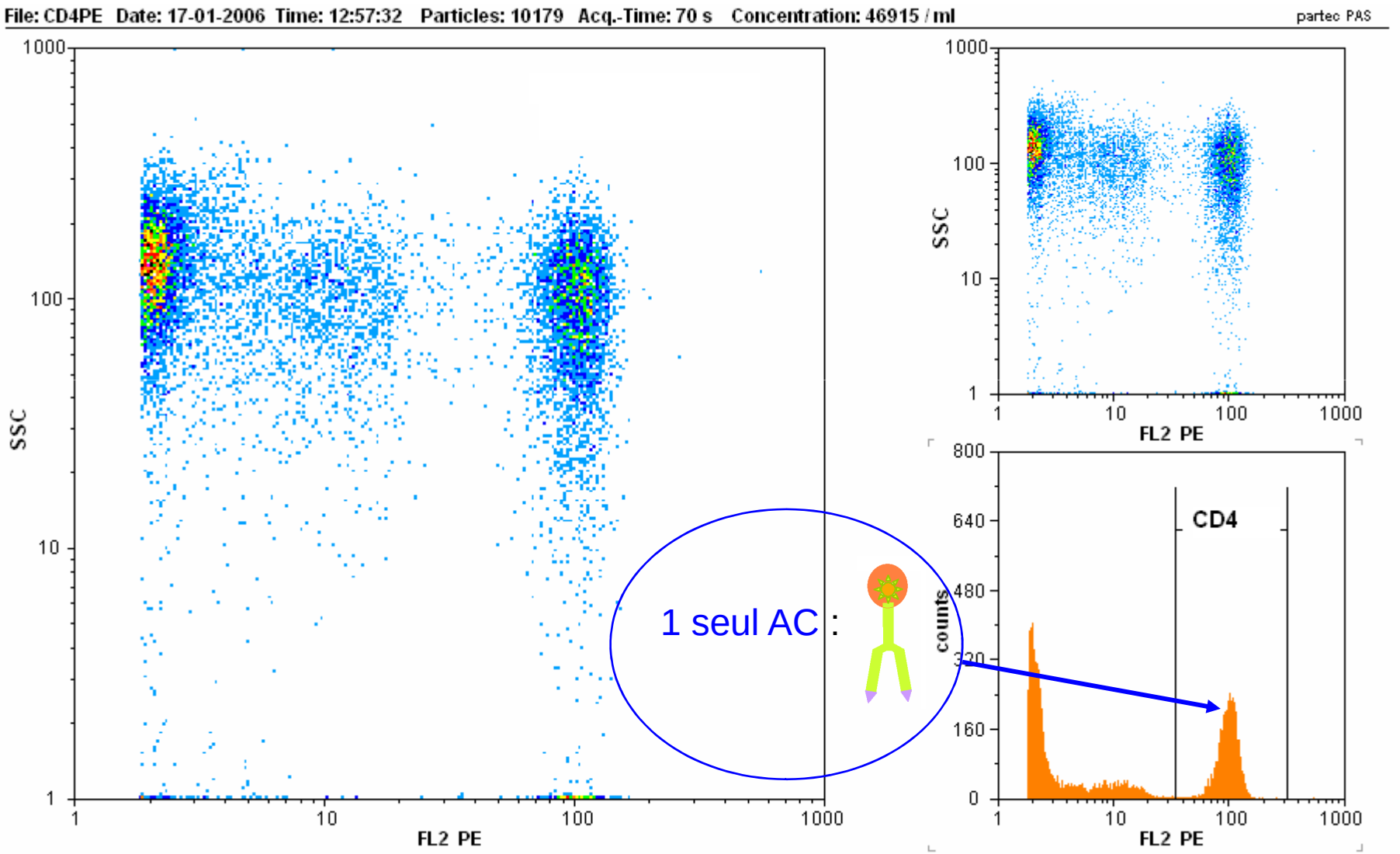


CD4/ 1 AC > 1 Couleur FL1 + FSC/SSC

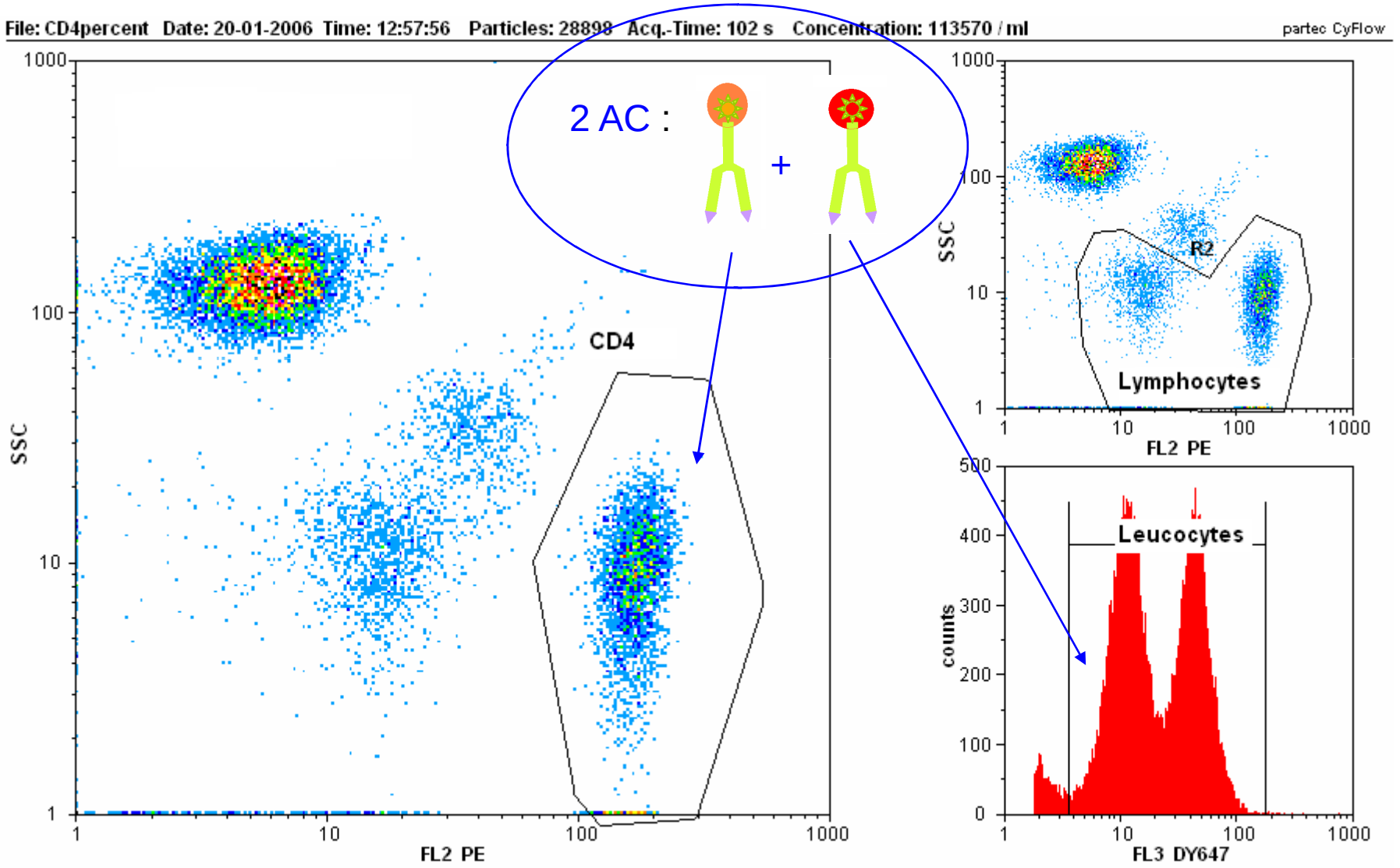


CD4%/ 2 AC > 2 Couleurs FL1/FL3 + FSC/ SSC

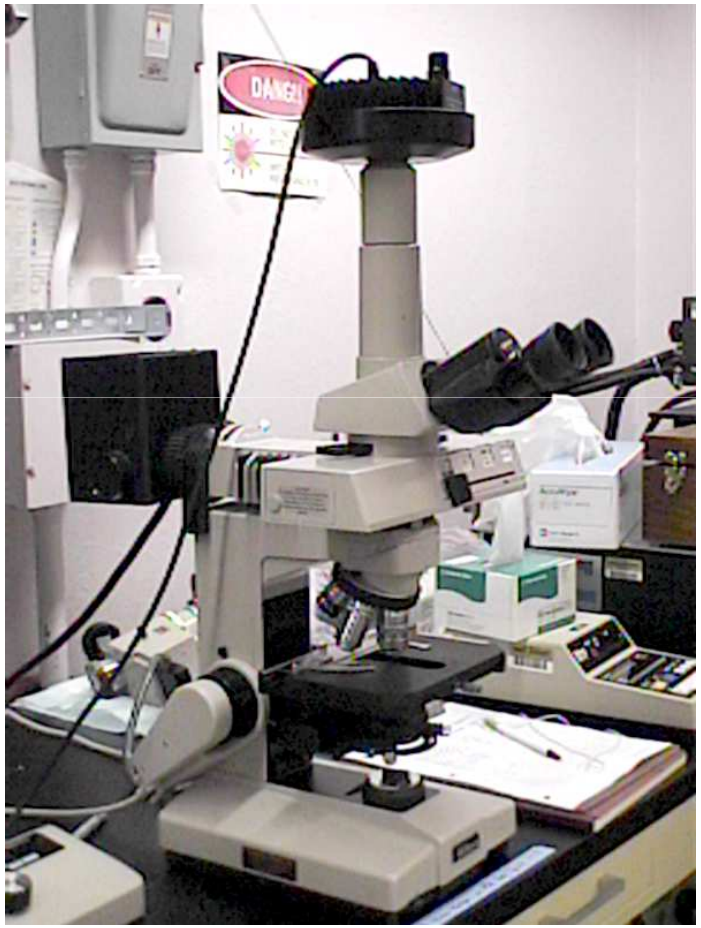
Histogrammes types CD4, comptage absolu



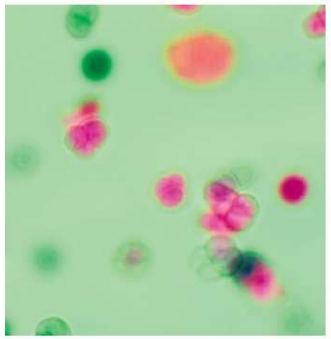
Histogrammes types CD4%

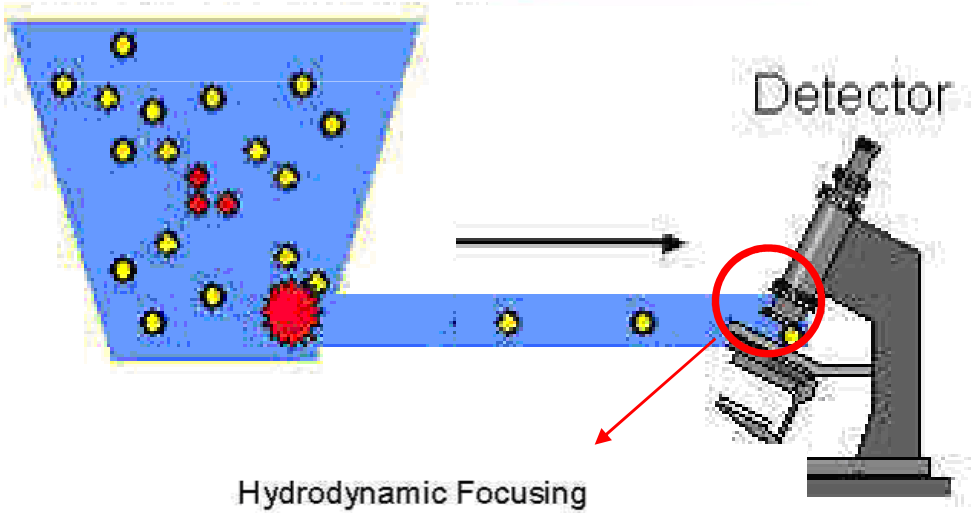


ANALOGIE ENTRE CYTOMETRIE ET MICROSCOPIE A FLUORESCENCE

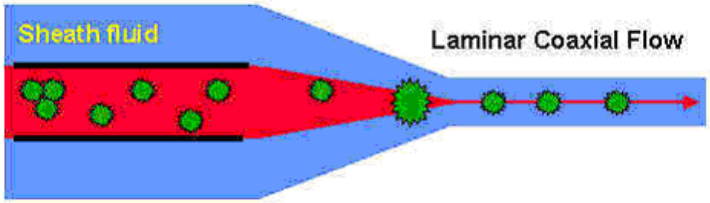


Echantillon liquide



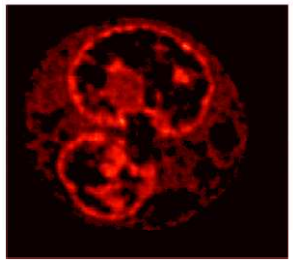


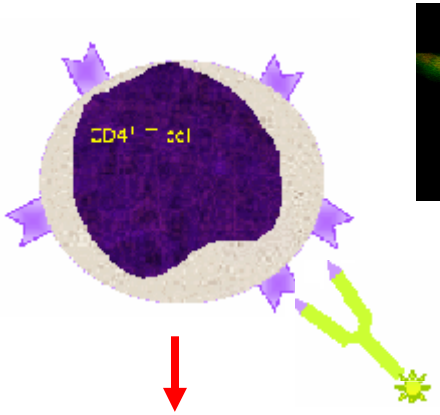
Hydrodynamic Focusing

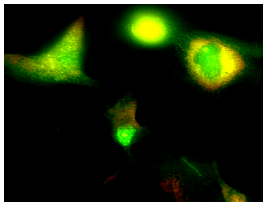


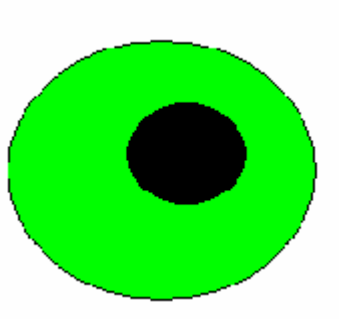
Différentes approches pour le marquage des cellules

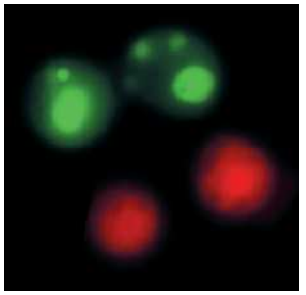
U937 CELL STAINED WITH PROPIDIUM IODIDE (without RNase)

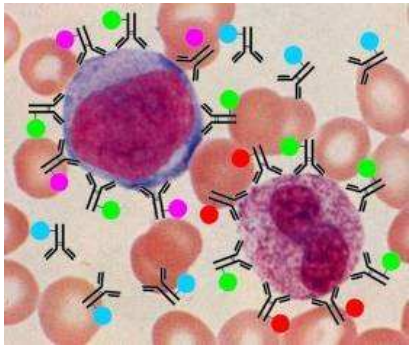




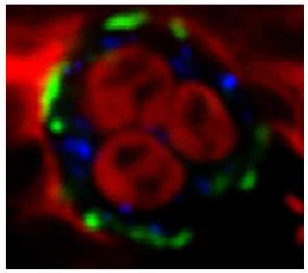


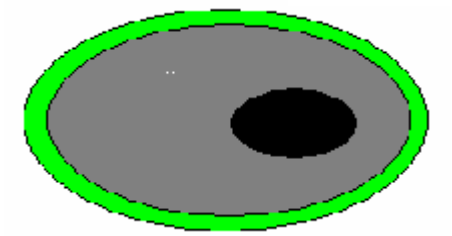


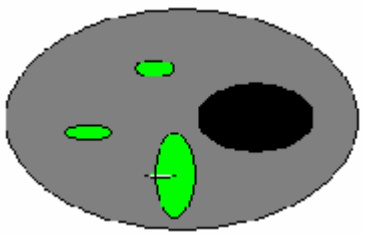


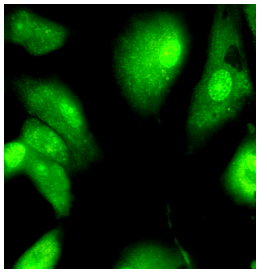


NEUTROPHIL STAINED WITH PROPIDIUM IODIDE (with RNase), anti-CD16-FITC, AND anti-IFNγ-Cy5









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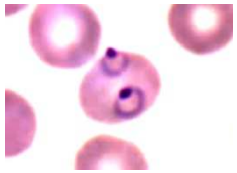
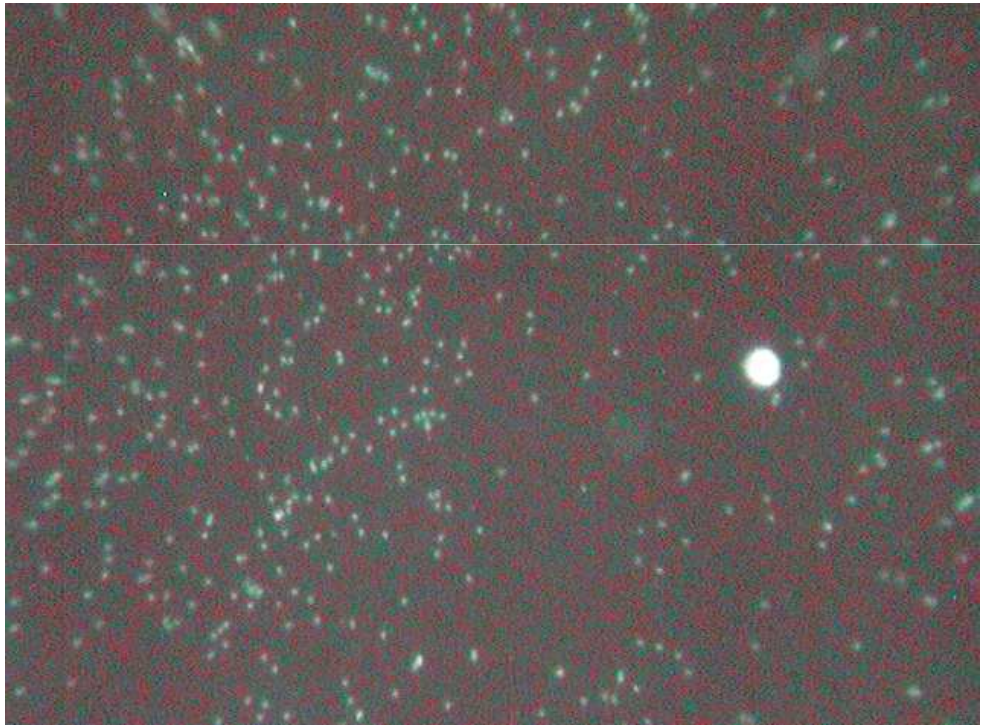
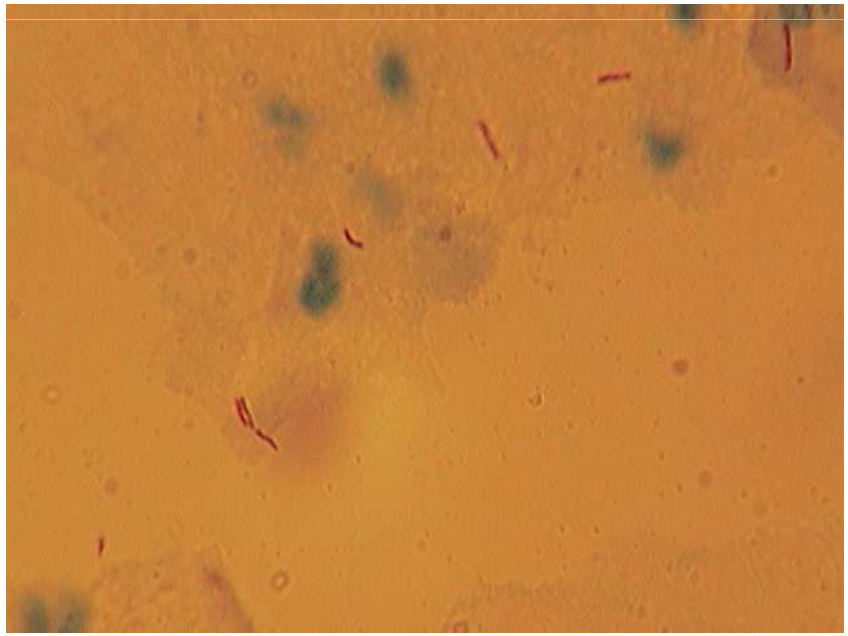




Auramine staining with CyScope®

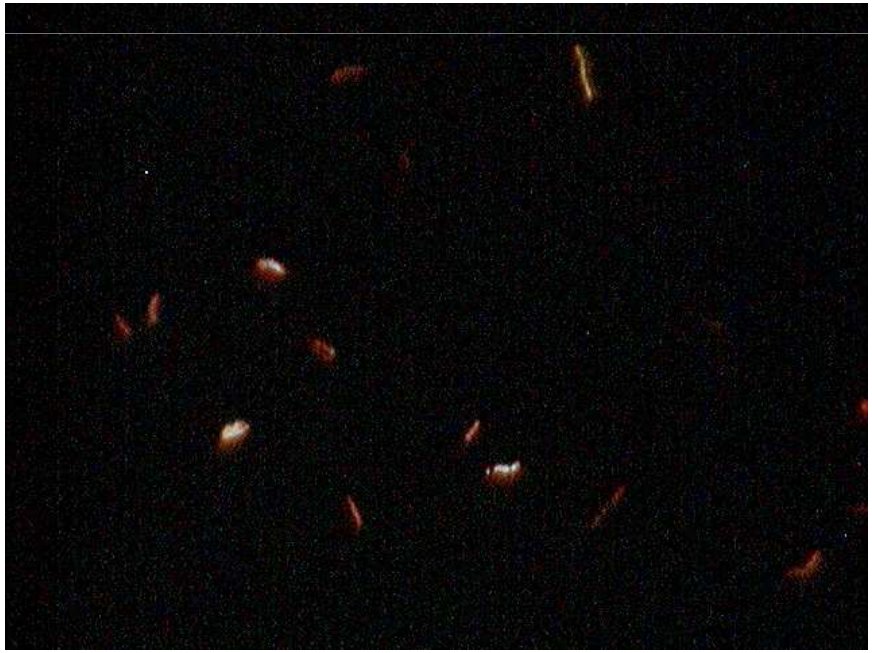
Ziehl Nielsen

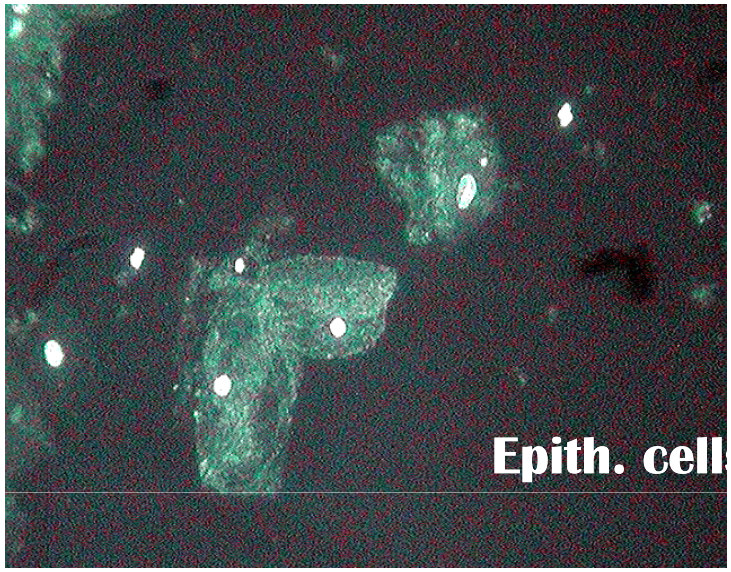
On CyScope®



Auramine

On CyScope®





OTHER BIOLOGICAL MATERIAL OBSERVED ON CyScope®