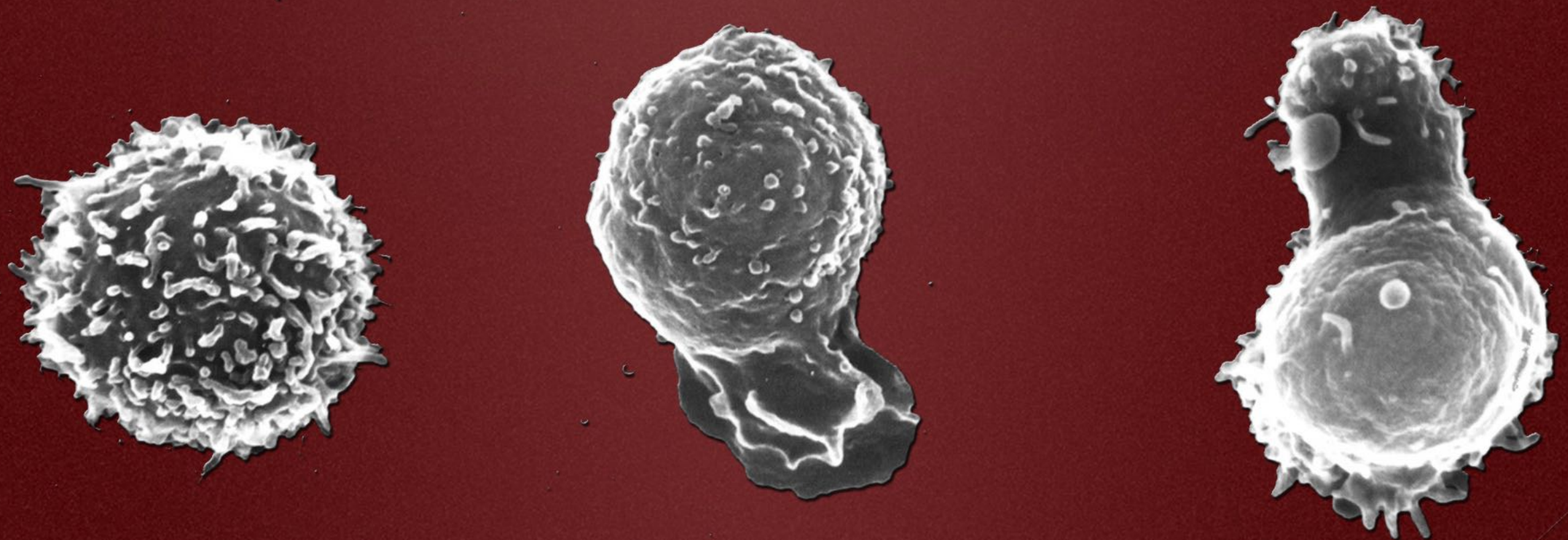


IMUNOFENOTIPARE PRIN CITOMETRIE IN FLUX



Obiectiv:

Analiza populațiilor de limfocite din sângele periferic.

Imunofenotipare

1. Specificarea markerilor de interes
2. Alegerea anticorpilor/fluorocromilor
3. Pregătirea probelor

Reactivi și materiale

1. Sânge colectat pe anticoagulant (EDTA)
2. Anticorpi conjugați cu fluorocromi (CD45-PerCP, CD3-Pacific blue,)
3. Soluție de liză pentru eritrocite
4. Soluție de spălare
5. Cutii de vârfuri sterile pentru pipete automate
6. Tuburi de centrifugă (5 mL)

Echipament

1. Citometru în flux
2. Pipete automate
3. Centrifugă



Markeri CD

CD (**cluster of differentiation**)

Molecule de la suprafata leucocitelor, care permit identificarea subpopulatiilor

±350 markeri

CD45+CD3+CD4+

CD45+CD3+CD8+

CD45+CD3-CD16+CD56+

CD45+CD3-CD19+

CD45+CD14+

Protocol de lucru

1. Se pregătesc reactivii și materiale necesare.
2. Pentru fiecare probă de sânge se pregătesc două tuburi de 5 mL:

(1)control
(2)marcat



3. Se pipetează 20 uL din fiecare anticorp în tubul (2).
4. Se pipetează 100 uL de sânge în fiecare tub.
5. Se incubează 20 min, RT, la întuneric.
6. Se adăuga 2 mL soluție de liză în fiecare tub și se incubează 10 min, RT, la întuneric.
7. Se centrifughează 5 min, la 1500 rpm.
8. Se decantează supernatantul.
9. Se adaugă 2 mL soluție de spălare în fiecare tub.
10. Se centrifughează 5 min, la 1500 rpm.
11. Se decantează supernatantul.
12. Se adaugă 500 uL soluție de spălare în fiecare tub.
13. Se achiziționează fiecare tub cu ajutorul citometrului.
14. Se analizează datele.

Analiza populațiilor de limfocite din sângele periferic

CD45



CD3



CD4



CD8



CD19

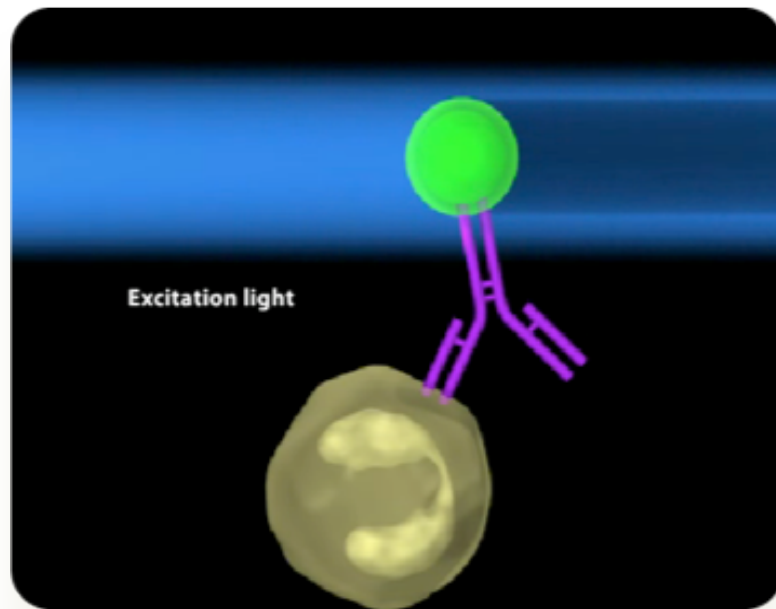


CD16



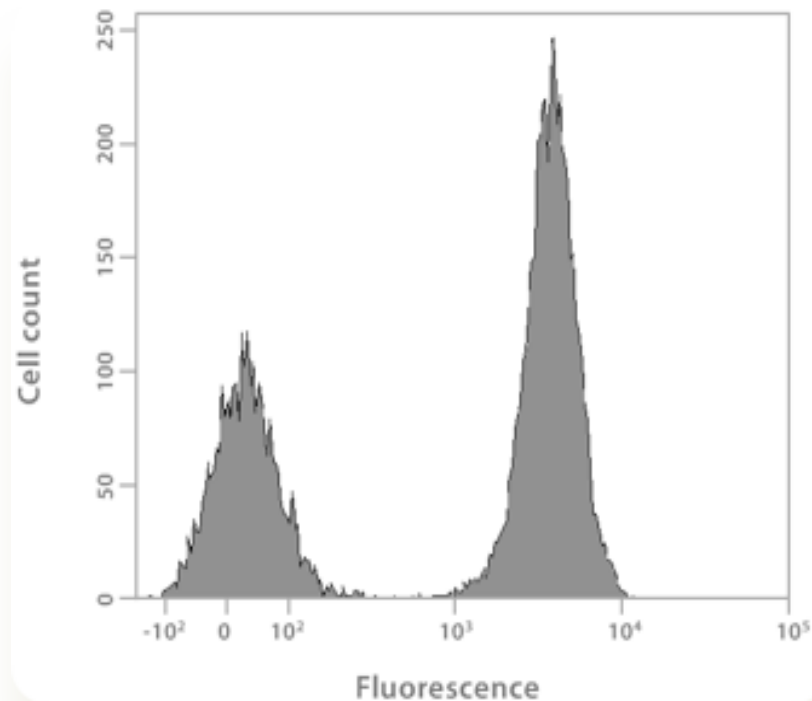
CD56



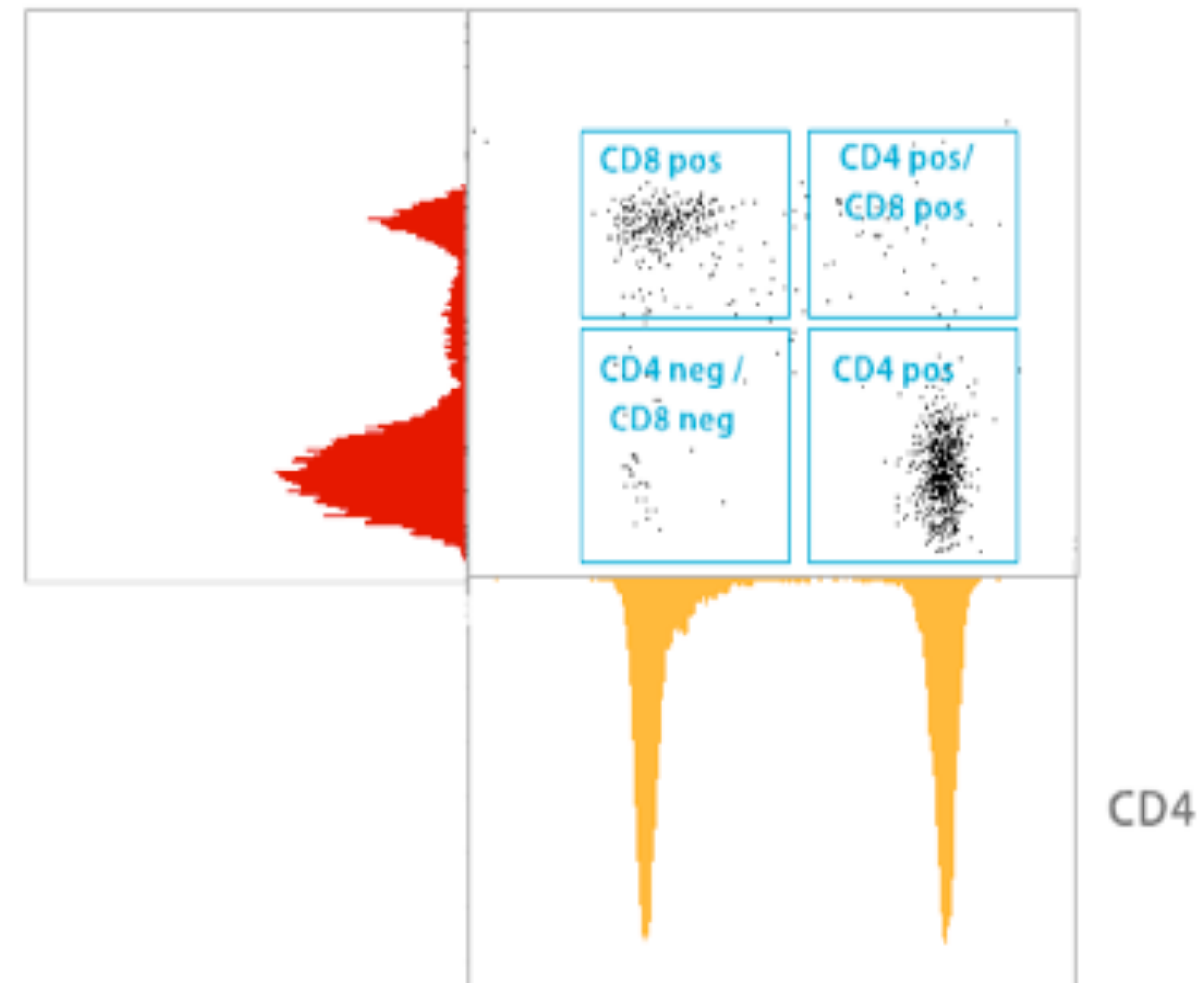
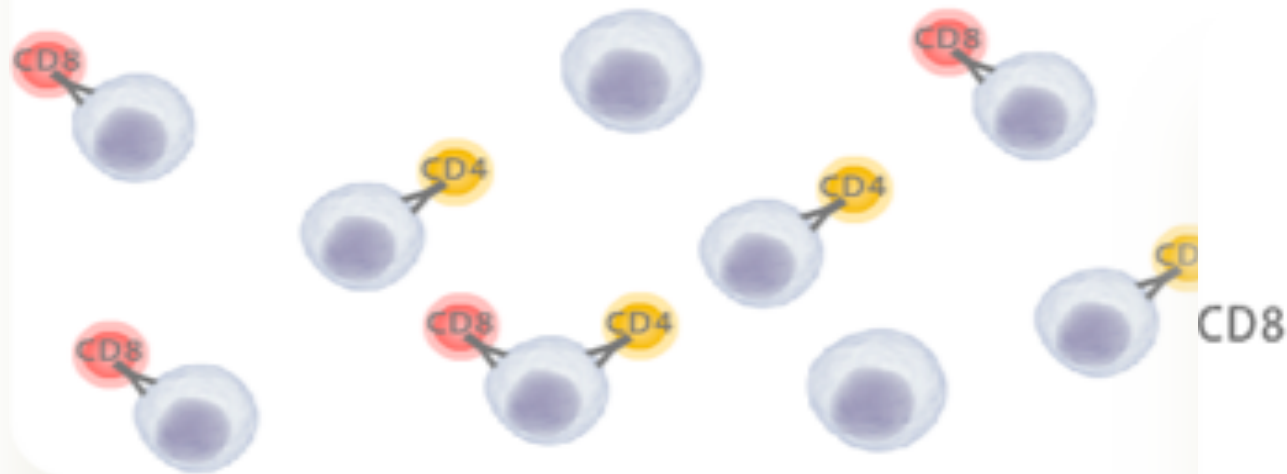
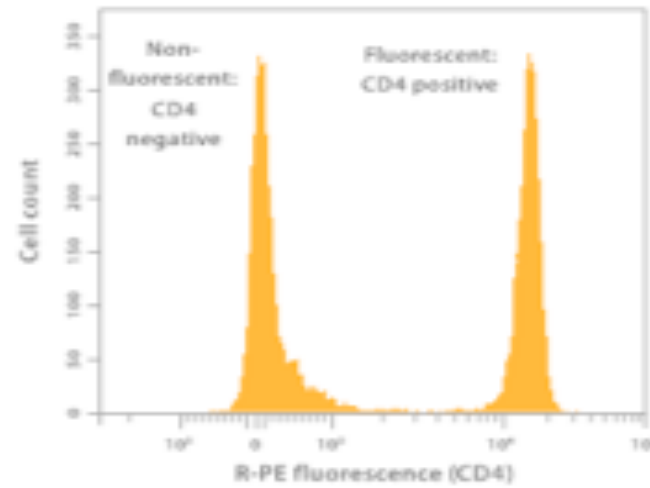
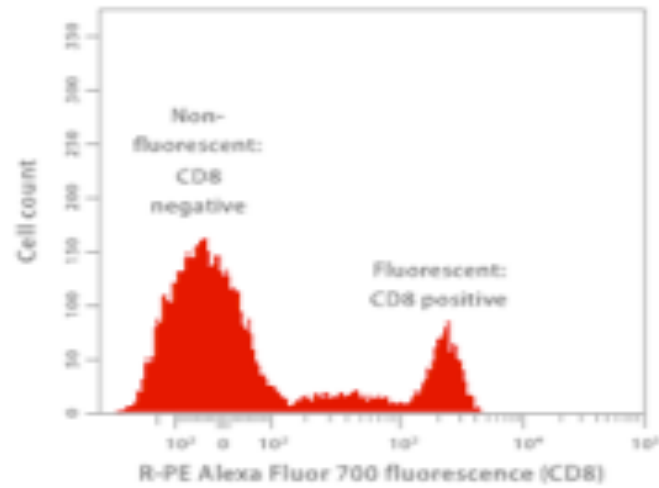


Anticorp conjugat cu fluorocrom

Fluorochrome	Fluorescence Emission Color	Em-Max (nm)
Brilliant Violet™ 421	Blue	421
BD Horizon™ V450	Blue	448
Pacific Blue™	Blue	452
BD Horizon™ V500	Green	500
Alexa Fluor® 488	Green	519
FITC	Green	519
PE	Yellow	578
BD Horizon PE-CF594	Orange	612
APC	Red	660
Alexa Fluor® 647	Red	668
PE-Cy™5	Red	667
PerCP	Red	678
PerCP-Cy™5.5	Far Red	695
Alexa Fluor® 700	Far Red	719
PE-Cy™7	Infrared	785
APC-Cy7	Infrared	785
BD APC-H7	Infrared	785



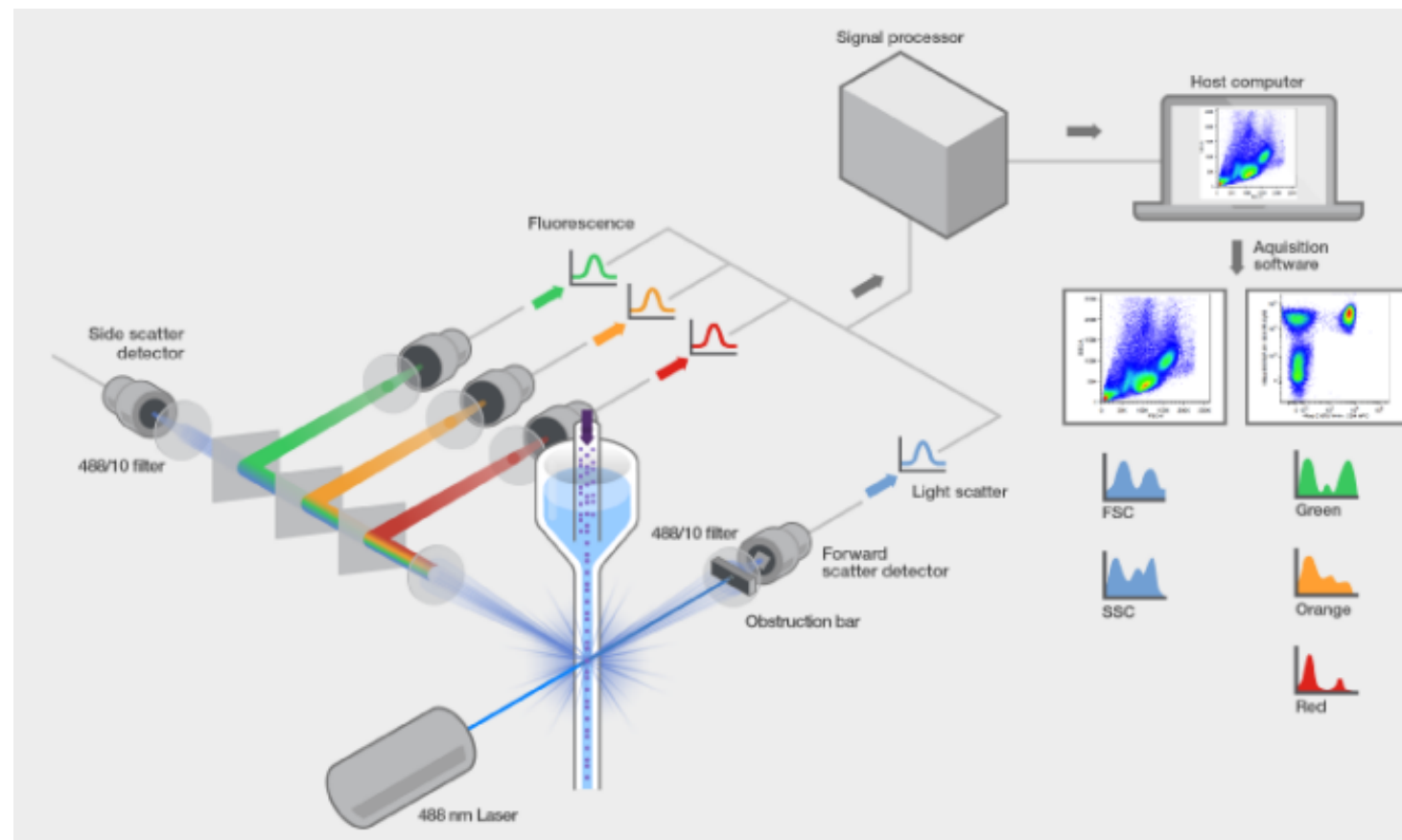
- 2 culori => determinarea a 4 populatii



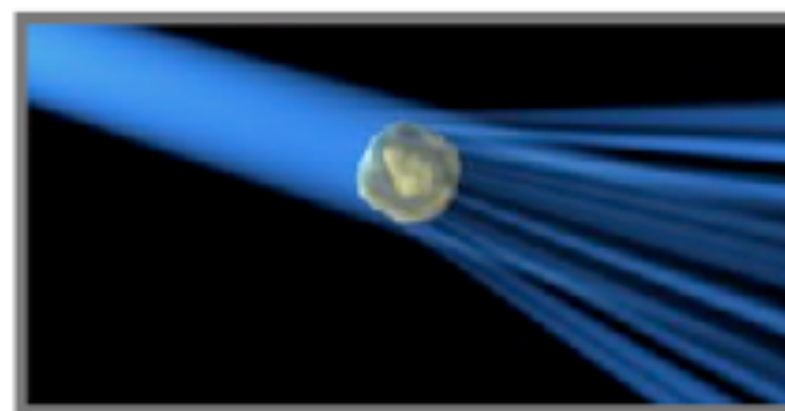
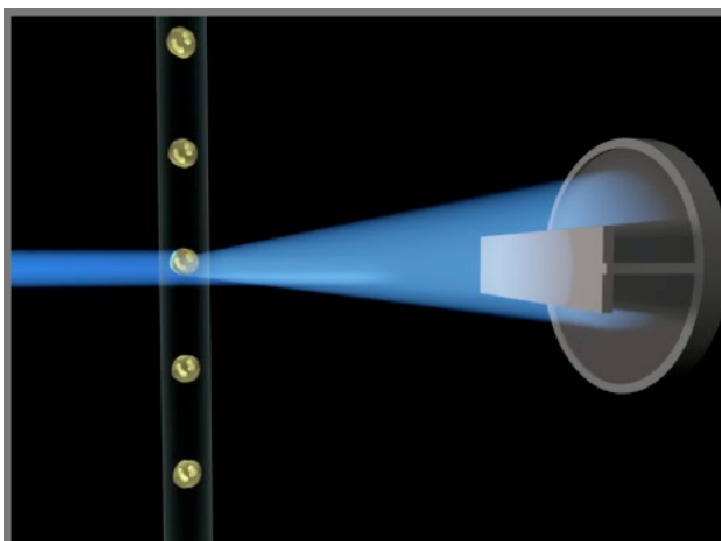
Cum funcționează citometrul?



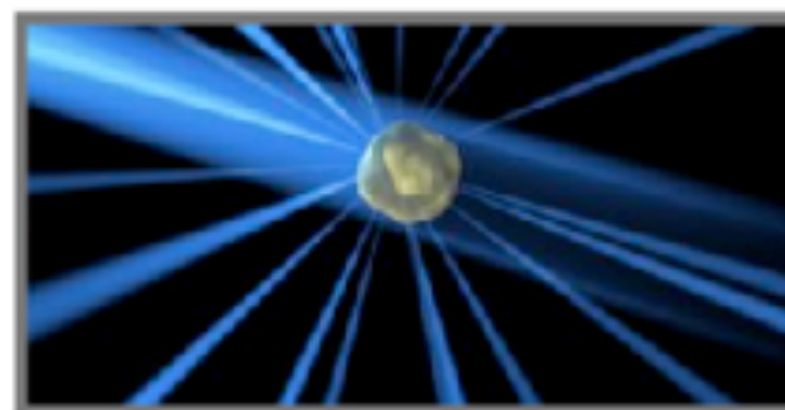
Analizează celulele în timp ce sunt iluminate de o rază laser.



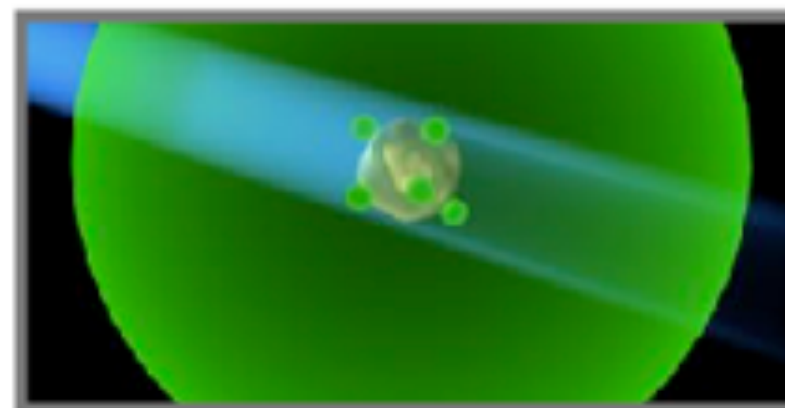
Parametrii determinați



Forward scatter

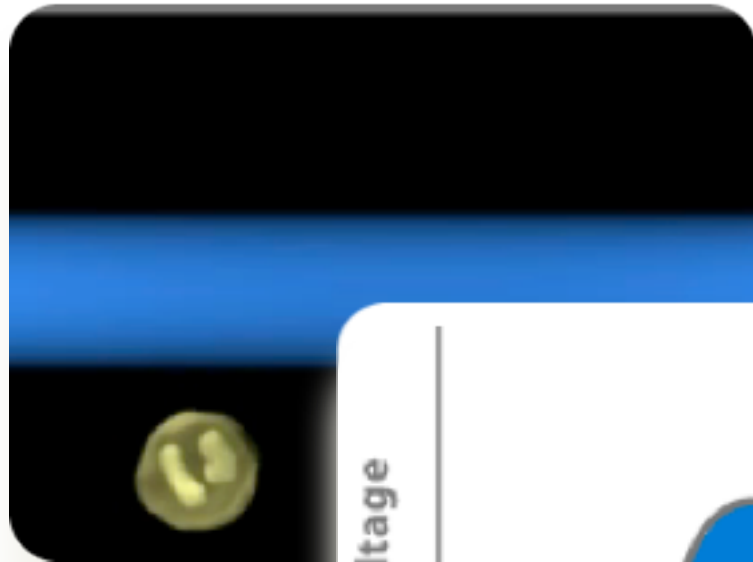


Side scatter

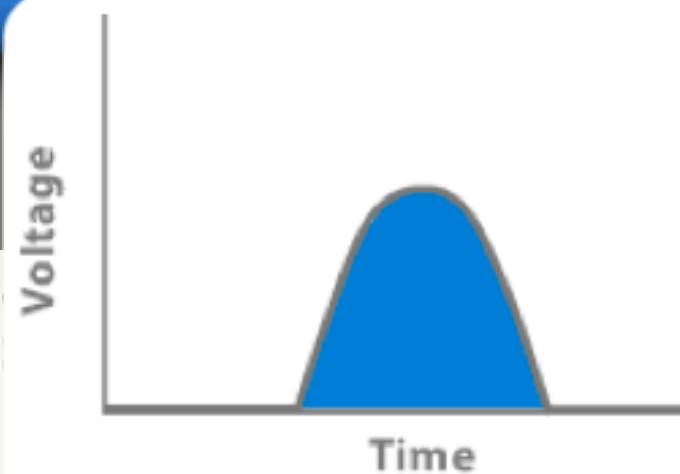


Fluorescence

Evenimente/celule -> date



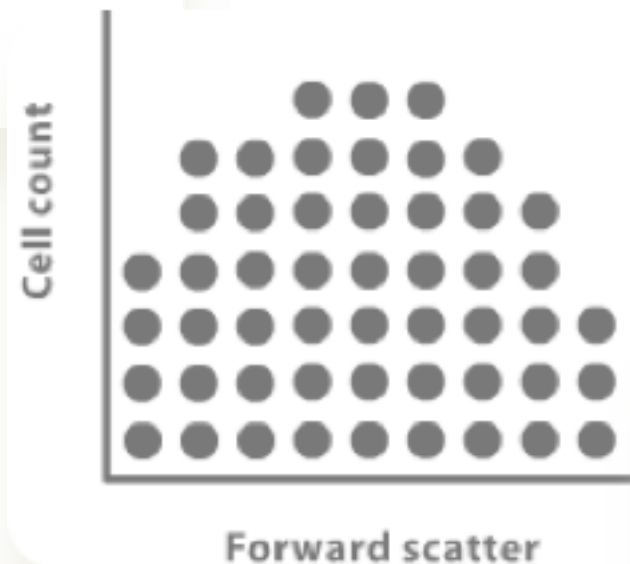
Celula trece prin dreptul unei laser



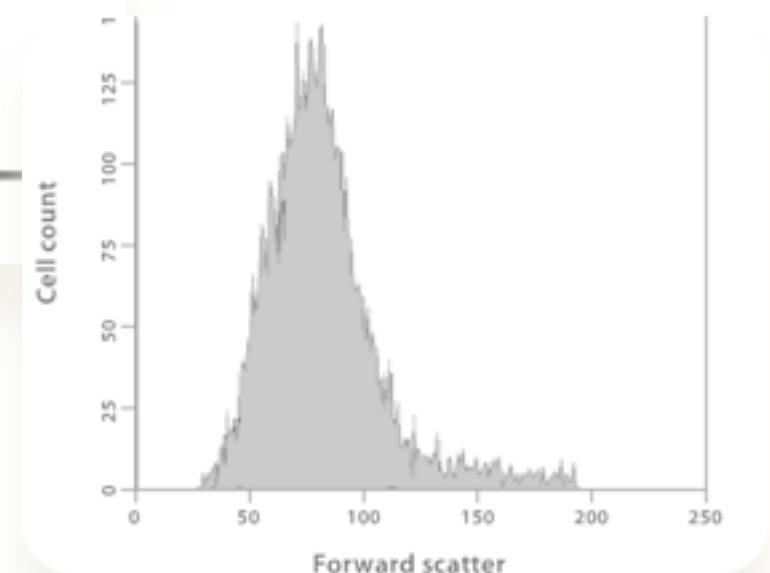
Semnalul luminos este convertit in semnal electric

Valoarea numerica(aria, inaltime, inaltimea, latimea peak-ului)

Valorile numerice sunt reprezentate grafic



Histograma finala



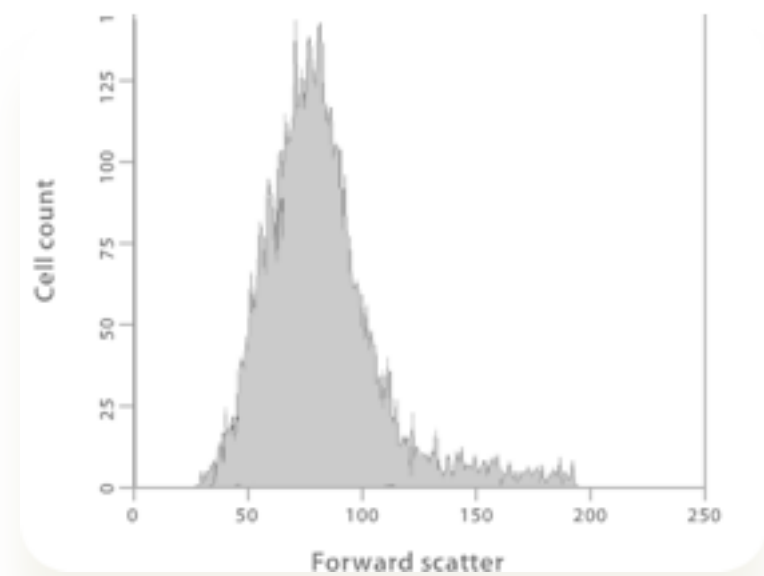
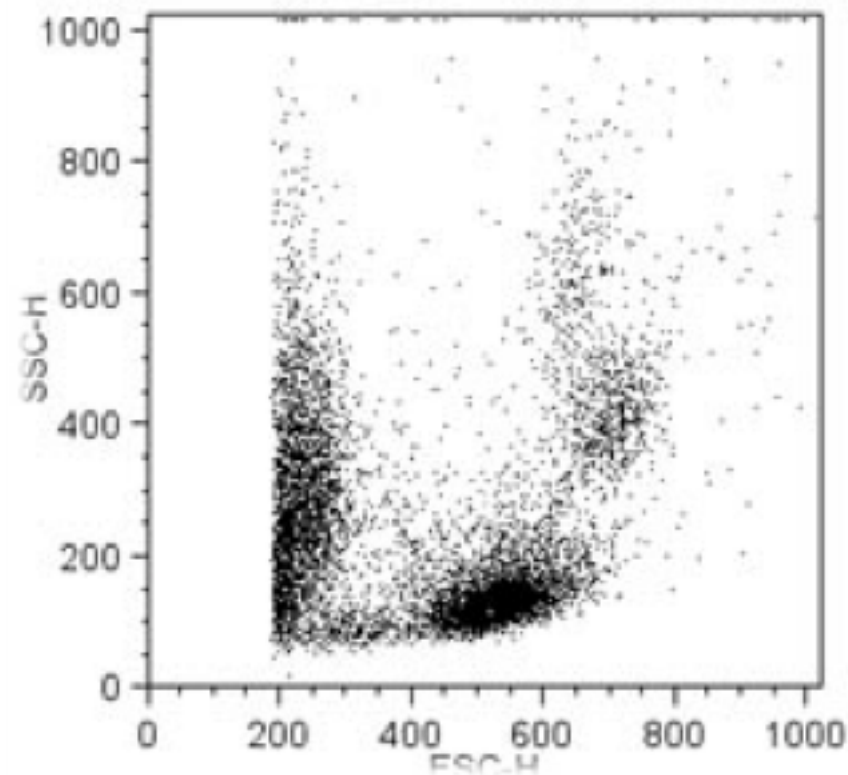
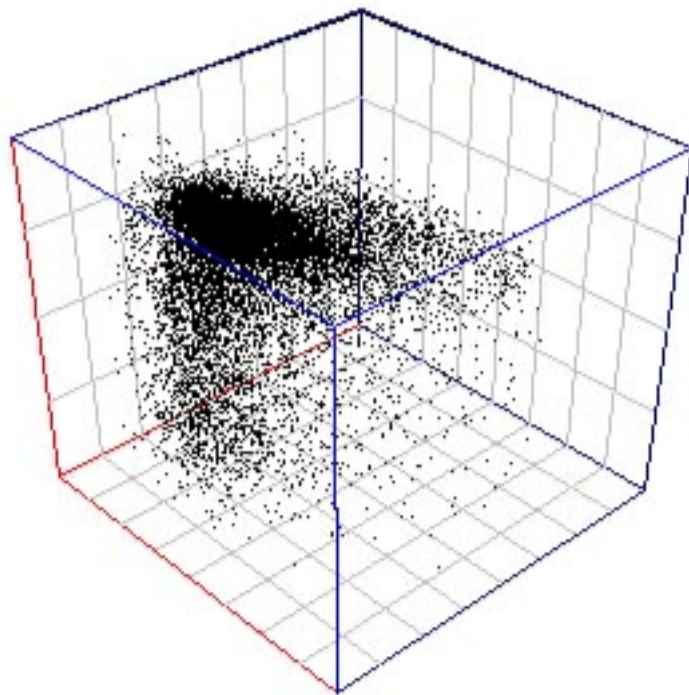
Ce este citometria in flux?

Flow cytometry

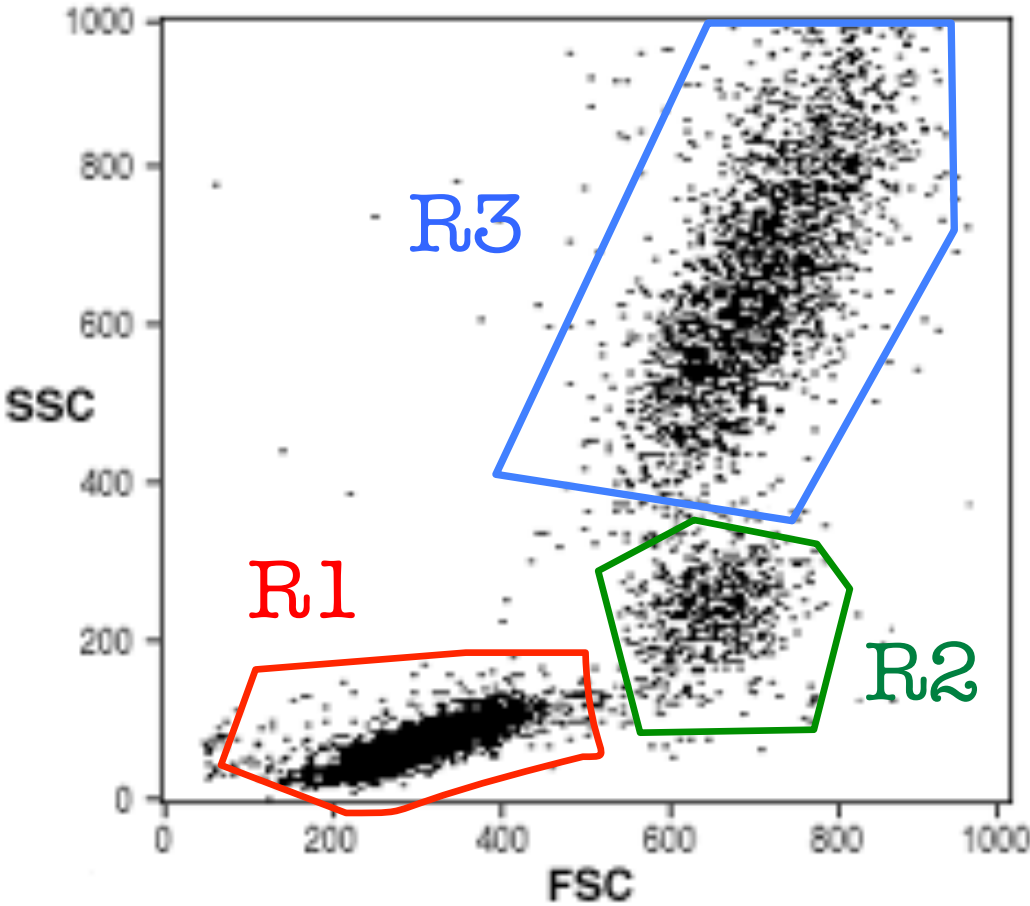
Analiza multiparametrica a unei singure celule, in cadrul unor populatii eterogene de celule

10.000 celule/secunda

30+ parametri



Citometrie...



Limfocyte

Monocyte

Neutrofile

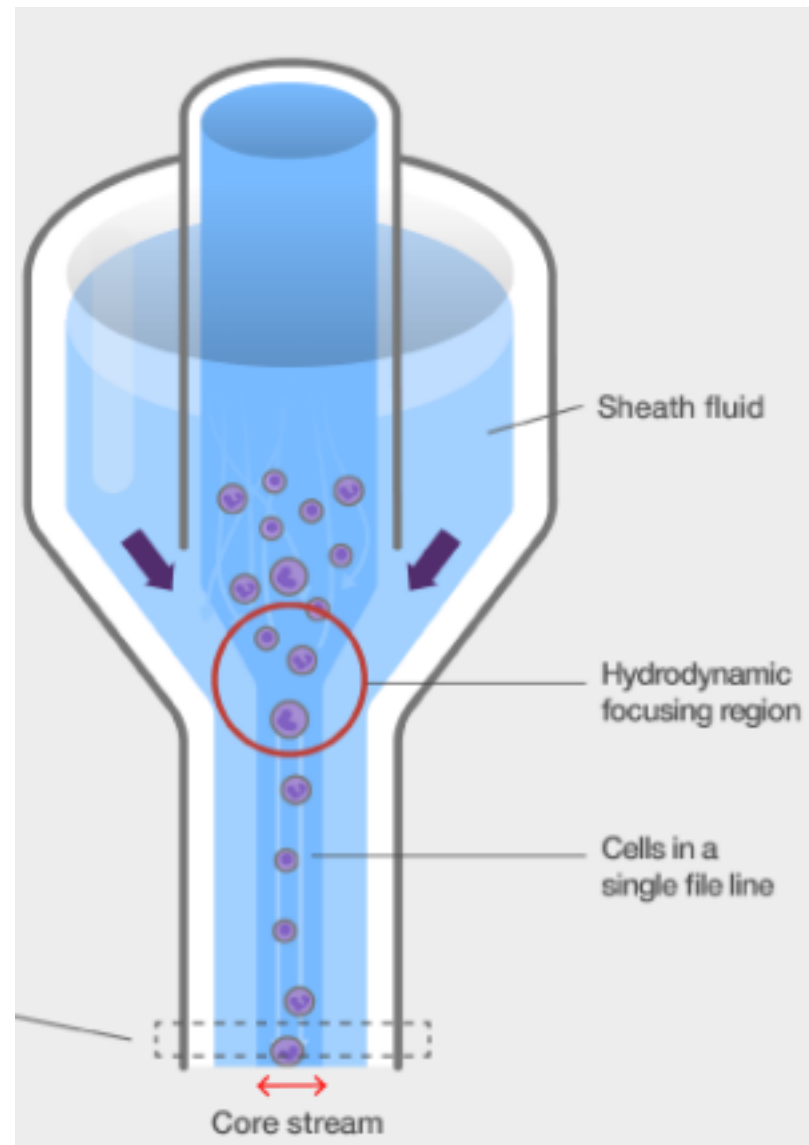
File: Leucocytes				
Total Events 10,000				
Region	Events	%Gated	XMean	YMean
R1	3978	39.78	793.1	662.6

File: Leucocytes				
Total Events 10,000				
Region	Events	%Gated	XMean	YMean
R2	4824	48.24	400.1	47.9

File: Leucocytes				
Total Events 10,000				
Region	Events	%Gated	XMean	YMean
R3	1198	11.98	731.2	258.6

...in flux

Focusarea hidrodinamica rezulta intr-un flux unicelular



Aplicații clinice ale citometriei

Diagnosticul leucemiilor acute

Imunofenotiparea leucemiilor/limfoamelor

Detectia bolii minime reziduale

Enumerarea de celule stem

Transplantul de organ

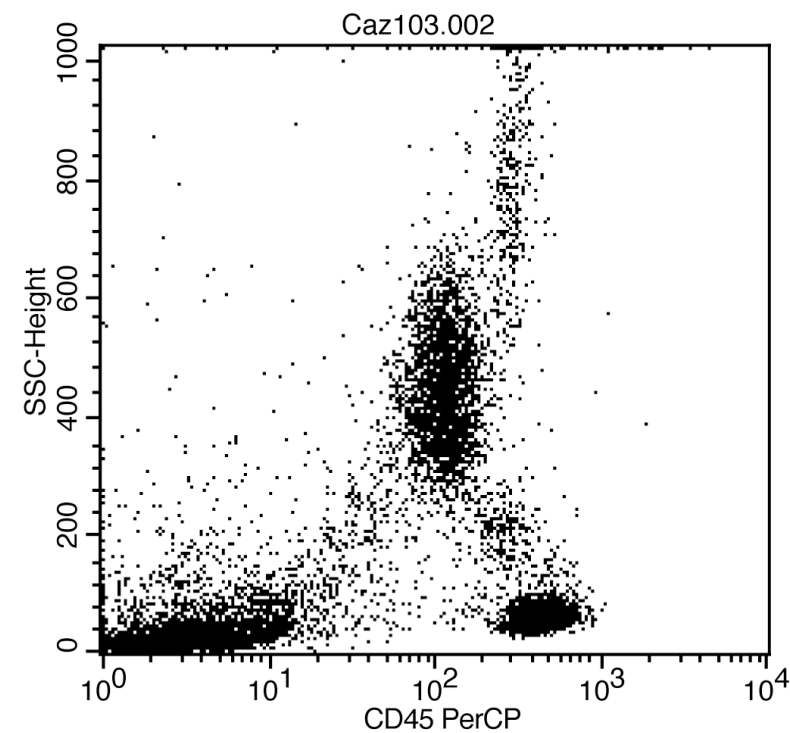
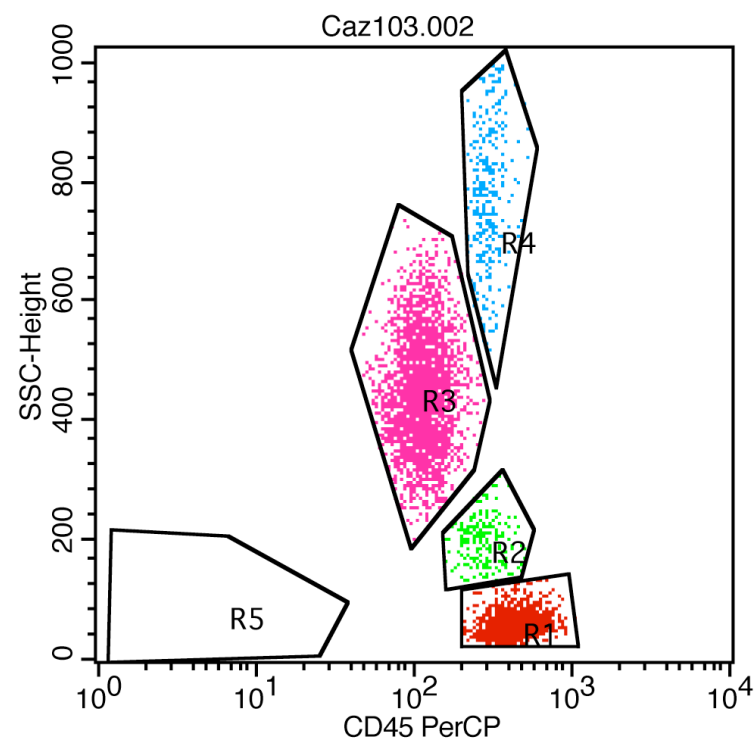
Determinarea autoanticorpilor

Monitorizarea progresiei infectiei cu HIV

Diagnosticul imunodeficientelor

Analiza datelor

Identificarea populațiilor de leucocite



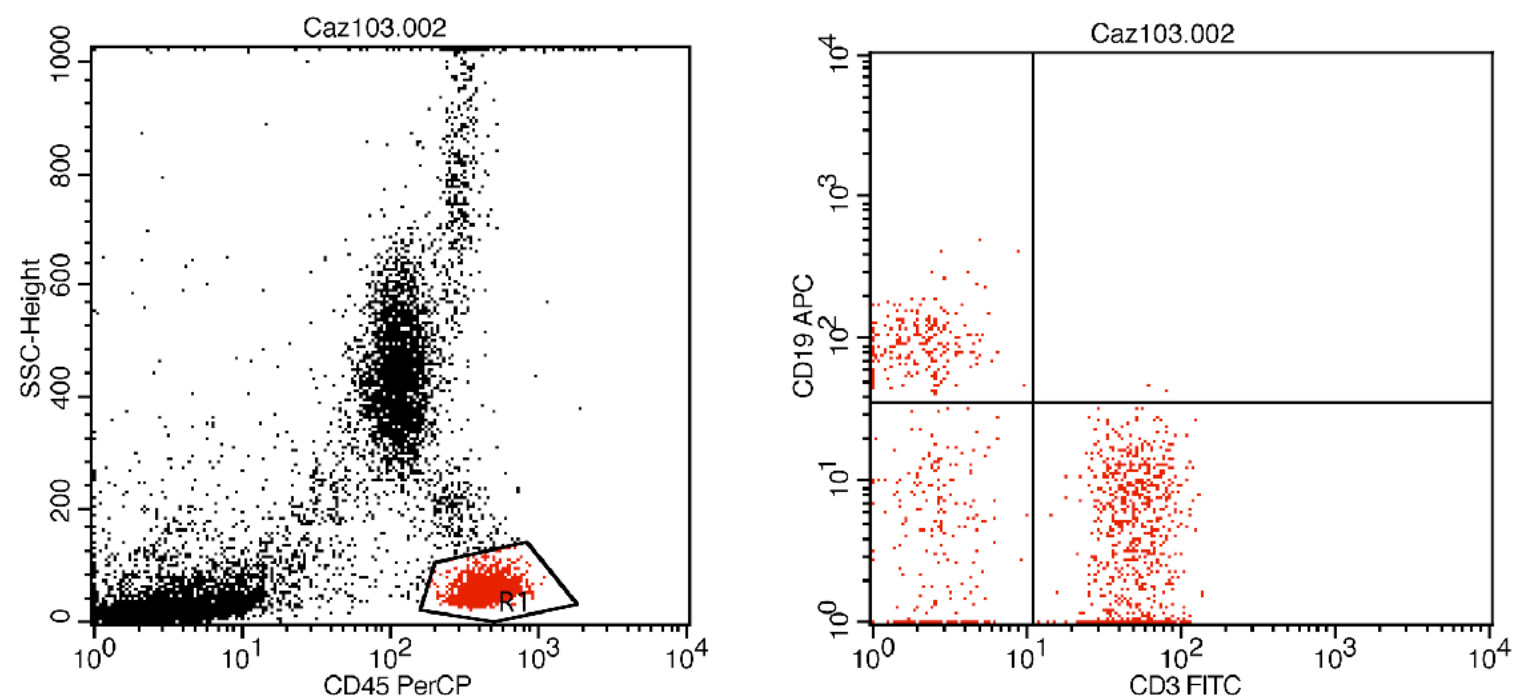
Region Statistics

File: Caz103.002
Sample ID: Florescu Paraschiva
Tube: CD3/CD16+56/CD45/CD19
Acquisition Date: 12-Jan-07
Gated Events: 4495
X Parameter: CD45 PerCP (Log)

Log Data Units: Linear Values
Patient ID: suspect artrita reumatoida
Panel: IMK Lymph
Gate: G6
Total Events: 10000
Y Parameter: SSC-Height (Linear)

Region	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean	Px,Py
R1	1324	29.45	13.24	456.55	440.92	60.28	57.65	5, 2
R2	198	4.40	1.98	280.77	271.81	200.18	196.74	5, 2
R3	2660	59.18	26.60	118.91	114.53	446.50	436.86	5, 2
R4	313	6.96	3.13	298.03	294.01	779.45	770.71	5, 2
R5	0	0.00	0.00	***	***	***	***	5, 2

Identificarea subpopulațiilor de limfocite



Quadrant Statistics

File: Caz103.002

Sample ID: Florescu Paraschiva

Tube: CD3/CD16+56/CD45/CD19

Acquisition Date: 12-Jan-07

Gated Events: 1325

X Parameter: CD3 FITC (Log)

Quad Location: 11, 35

Log Data Units: Linear Values

Patient ID: suspect artrita reumatoida

Panel: IMK Lymph

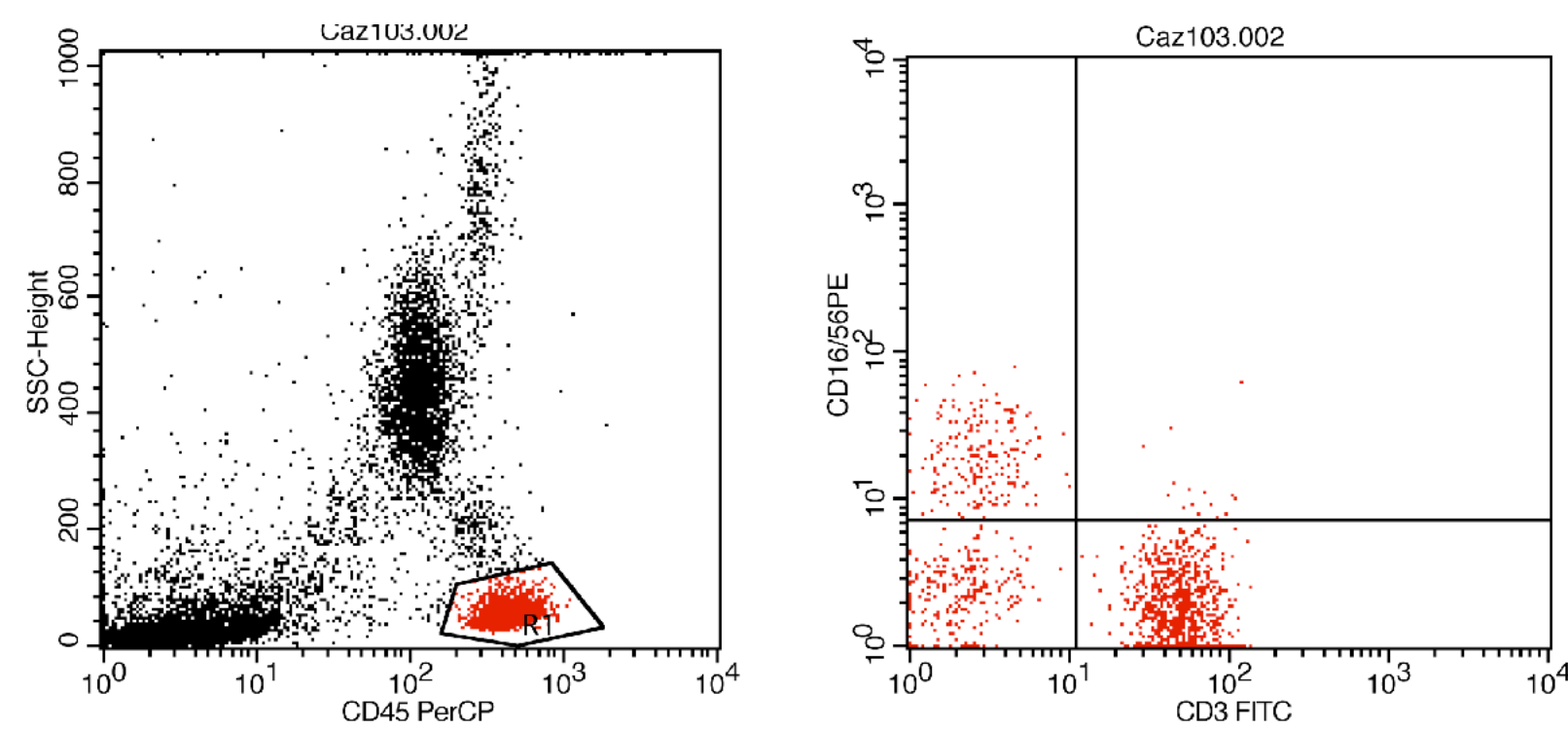
Gate: G1

Total Events: 10000

Y Parameter: CD19 APC (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	208	15.70	2.08	2.34	2.07	104.95	94.33
UR	2	0.15	0.02	69.88	69.16	44.98	44.91
LL	228	17.21	2.28	2.95	2.67	5.93	3.35
LR	887	66.94	8.87	52.92	49.20	5.57	3.21

Identificarea subpopulațiilor de limfocite



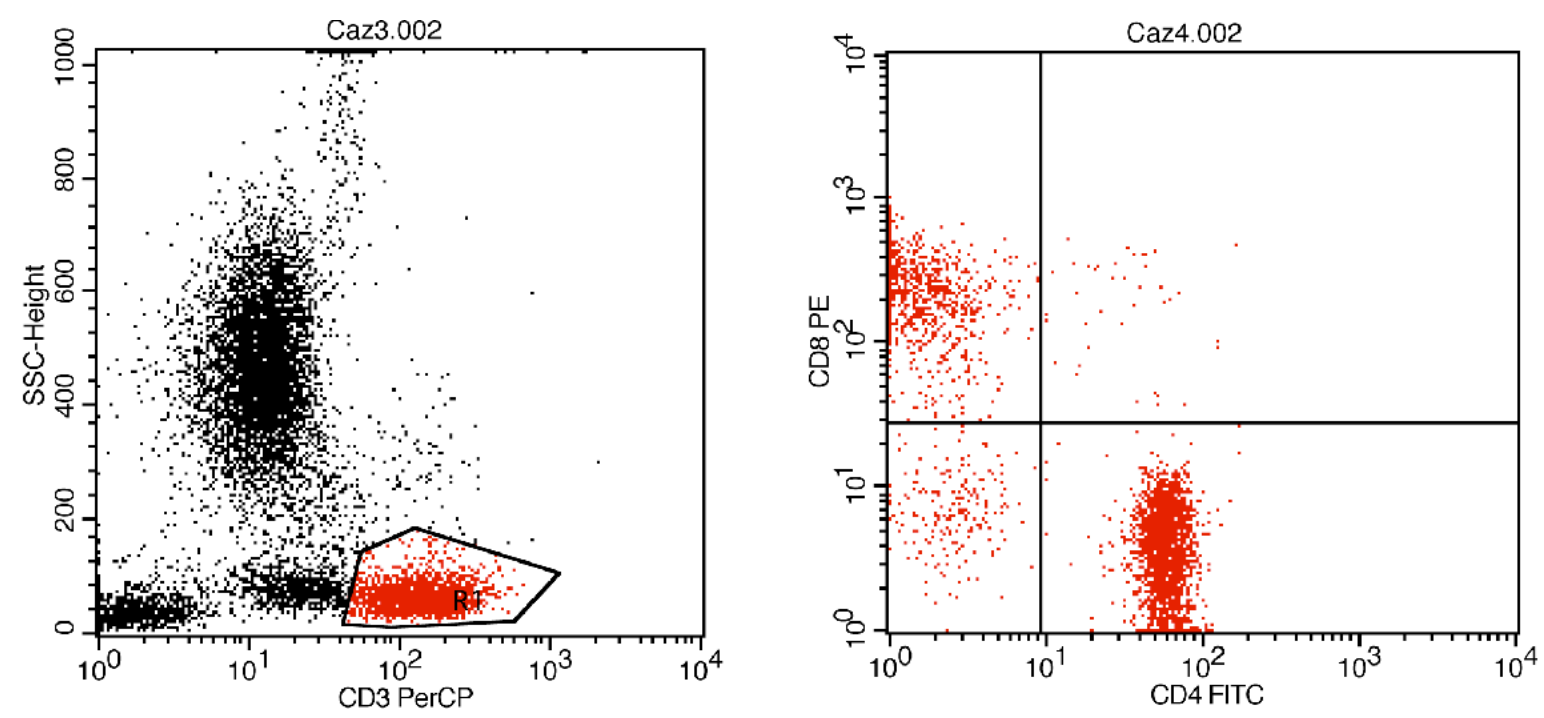
Quadrant Statistics

File: Caz103.002
Sample ID: Florescu Paraschiva
Tube: CD3/CD16+56/CD45/CD19
Acquisition Date: 12-Jan-07
Gated Events: 1325
X Parameter: CD3 FITC (Log)
Quad Location: 11, 7

Log Data Units: Linear Values
Patient ID: suspect artrita reumatoida
Panel: IMK Lymph
Gate: G1
Total Events: 10000
Y Parameter: CD16/56PE (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	202	15.25	2.02	3.03	2.73	23.20	20.03
UR	15	1.13	0.15	69.60	64.63	15.60	12.51
LL	234	17.66	2.34	2.34	2.09	2.55	2.31
LR	874	65.96	8.74	52.67	49.01	2.03	1.77

Identificarea subpopulațiilor de limfocite T



Quadrant Statistics

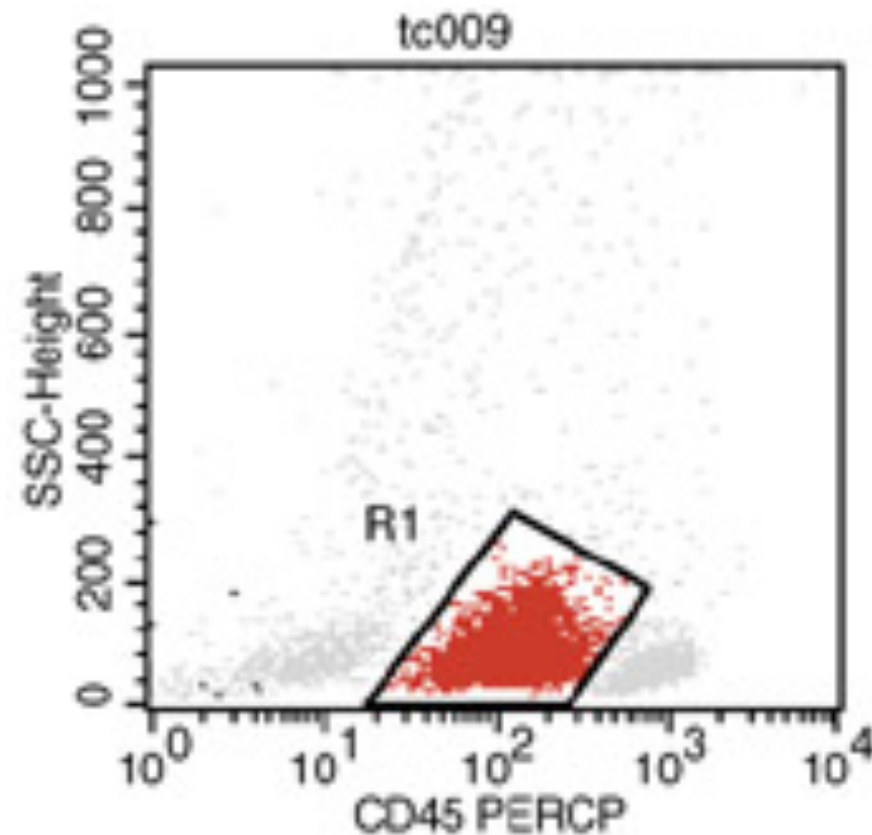
File: Caz4.002
Sample ID: DM
Tube: CD4/CD8/CD3/CD45
Acquisition Date: 19-Jan-10
Gated Events: 3631
X Parameter: CD4 FITC (Log)
Quad Location: 9, 28

Log Data Units: Linear Values
Patient ID: 45 ani
Panel: IMF
Gate: G1
Total Events: 20000
Y Parameter: CD8 PE (Log)

Quad	Events	% Gated	% Total	X Mean	X Geo Mean	Y Mean	Y Geo Mean
UL	1225	33.74	6.12	1.57	1.38	284.25	243.66
UR	39	1.07	0.19	40.87	31.63	230.85	177.79
LL	200	5.51	1.00	2.81	2.52	8.05	6.79
LR	2167	59.68	10.84	58.83	57.23	4.48	3.61

Diagnosticul leucemiilor

1. Identificarea populației leucemice (X).
2. Descrierea fenotipului populației (X)
3. Interpretarea datelor corelată cu analiza anatomo-patologică.



AML

MPO CD13
CD33 CD117

M0

M4-M5

M1-M3

M6

M7

CD19 cyCD22
CD79a CD10

B-ALL

cyCD3 CD2
CD7

T-ALL

