Proyecto de Pasantía de Ingeniería Biológica

Análisis de datos de citometría de flujo

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Seminario de Procesamiento de Señales
Agosto-2015

OBJETIVO

Comprender la técnica de citometría de flujo y sus aplicaciones, principalmente aquellas que son desarrolladas en el Instituto Pasteur.

Reproducir las técnicas existentes y estudiar la posibilidad de realizar alguna mejora.

CITOMETRÍA DE FLUJO

Método analítico por el cual se mide la emisión de múltiples fluorescencias y la dispersión de luz de células o partículas microscópicas, alineadas secuencialmente mediante una corriente de flujo laminar, cuando son presentadas de una y a gran velocidad frente a un haz de luz láser de longitud de onda adecuada.

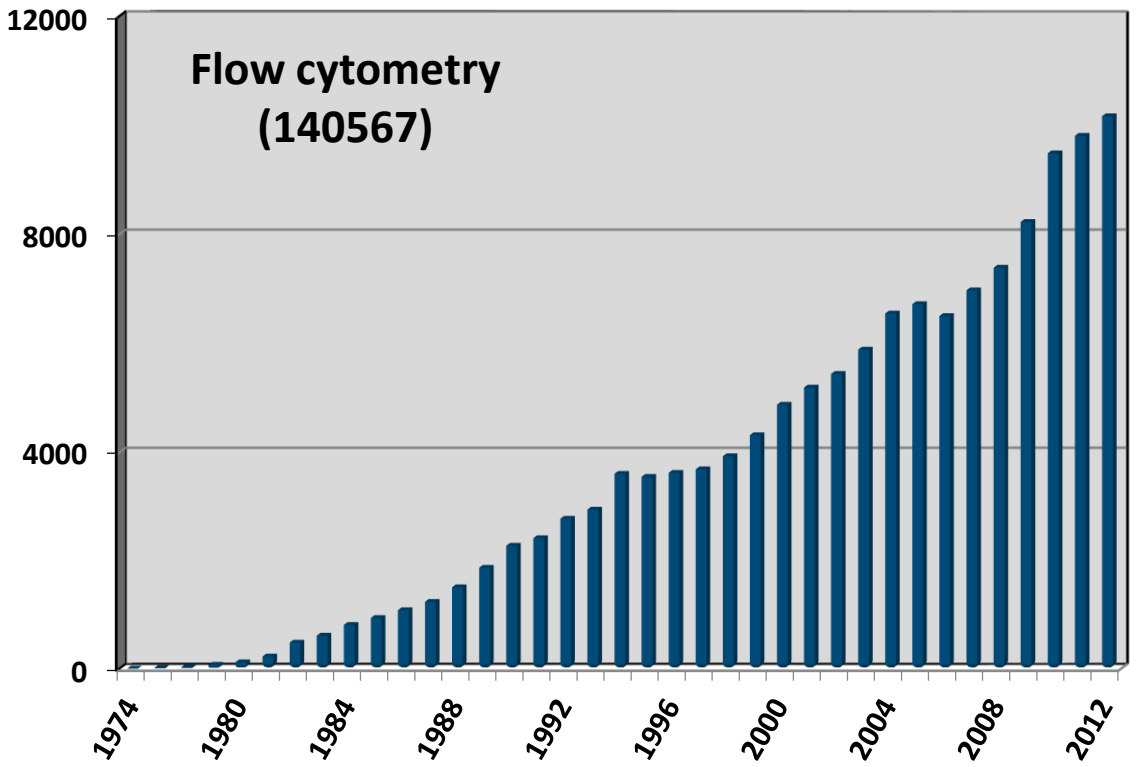
APLICACIONES CLÍNICAS GENERALES

- Diagnóstico basado en el análisis celular.
- Pronóstico basado en el análisis celular.
- Evaluación y monitoreo de tratamiento.
- Análisis de lesión y muerte celular.

CAMPOS DE APLICACIÓN DE LA CITOMETRÍA DE FLUJO

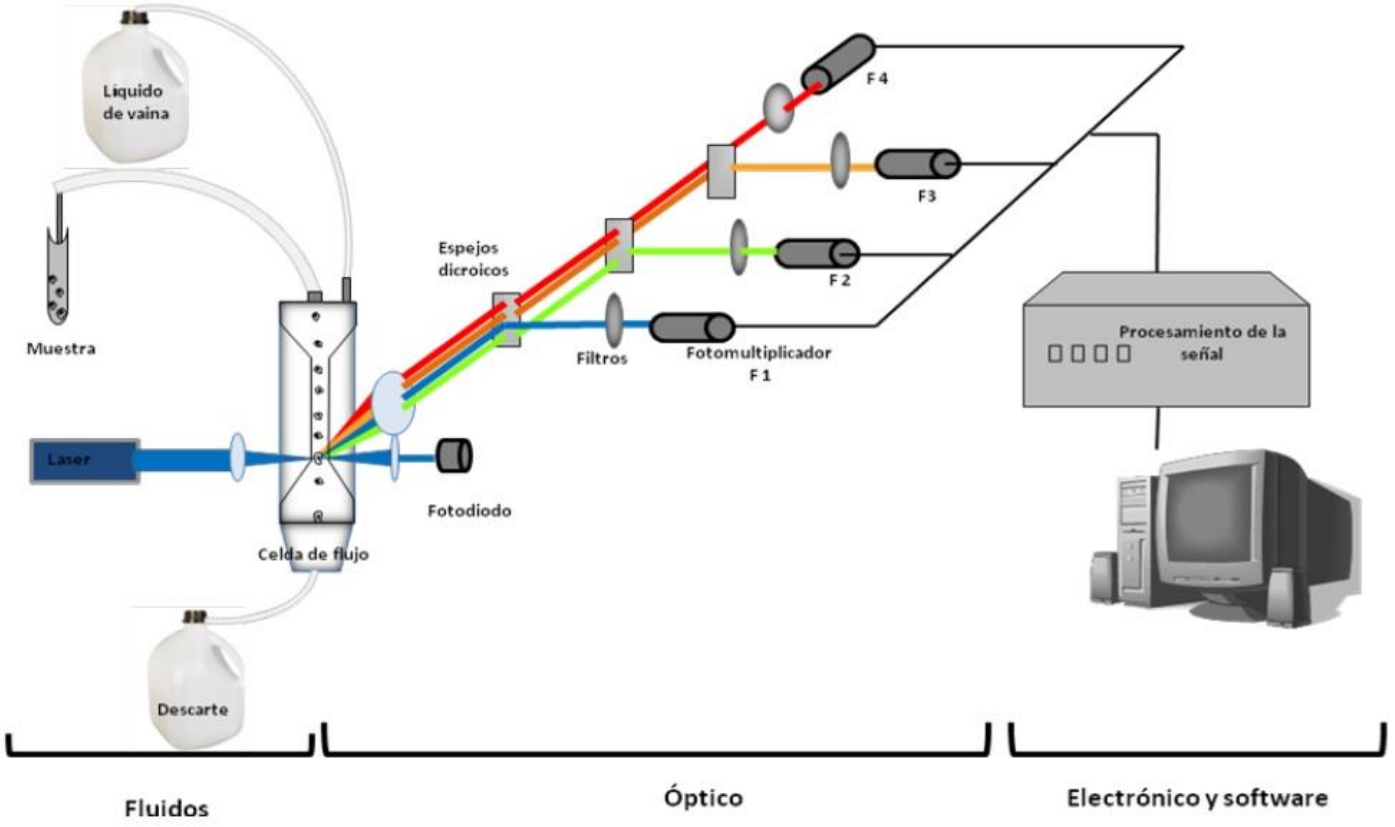
- Hematología
- Inmunología
- Patología
- Trasplantes
- Farmacología
- Investigación
- Toxicología
- Microbiología
- Parasitología
- Citogenética
- Virología
- Control de calidad

PUBLICACIONES ANUALES

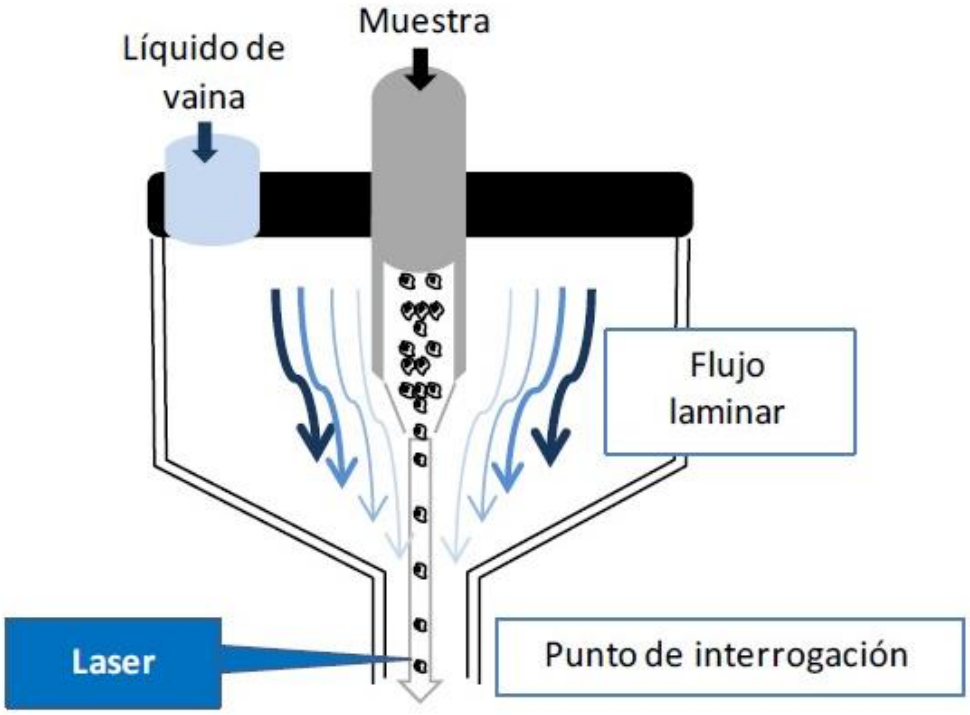


Fundamentos de Citometría de Flujo

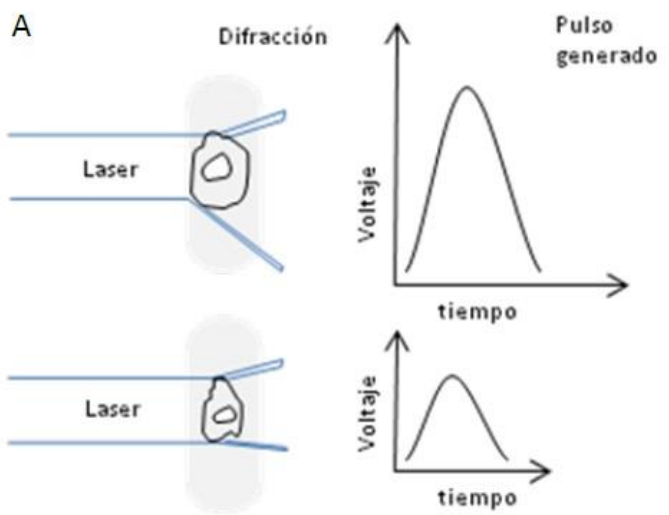
COMPONENTES PRINCIPALES DE UN CITOMETRO DE FLUJO



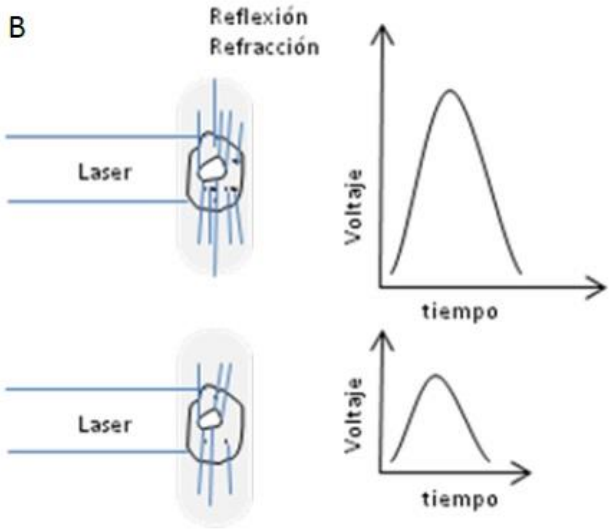
Enfoque hidrodinámico



Dispersión lumínica

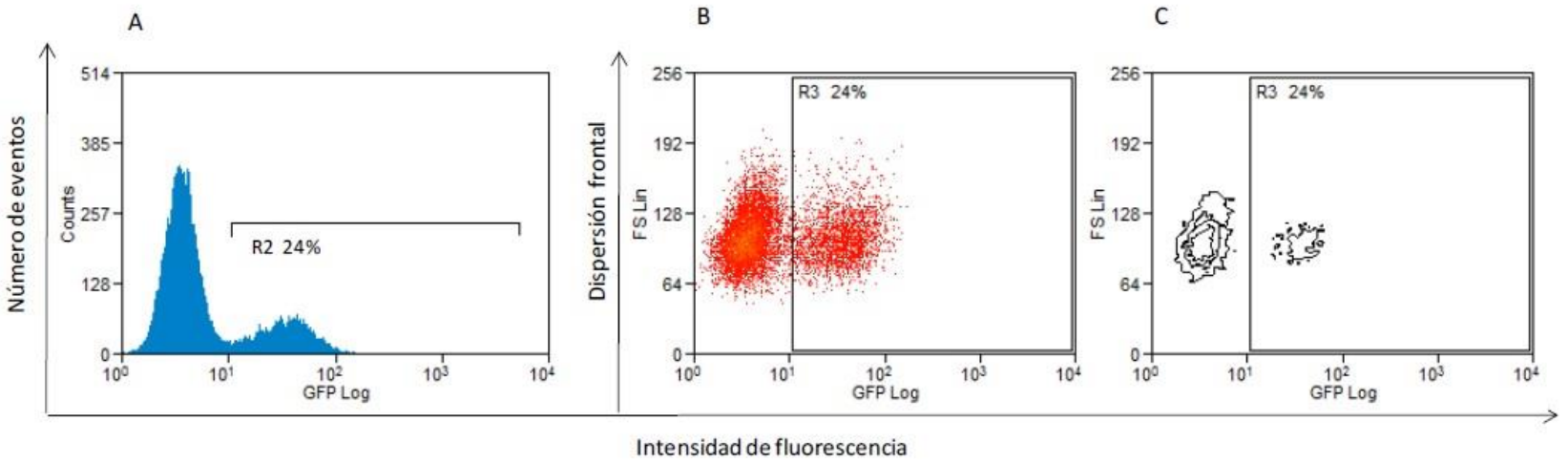


Luz difractada en dirección frontal



Luz refractada y reflejada en dirección lateral

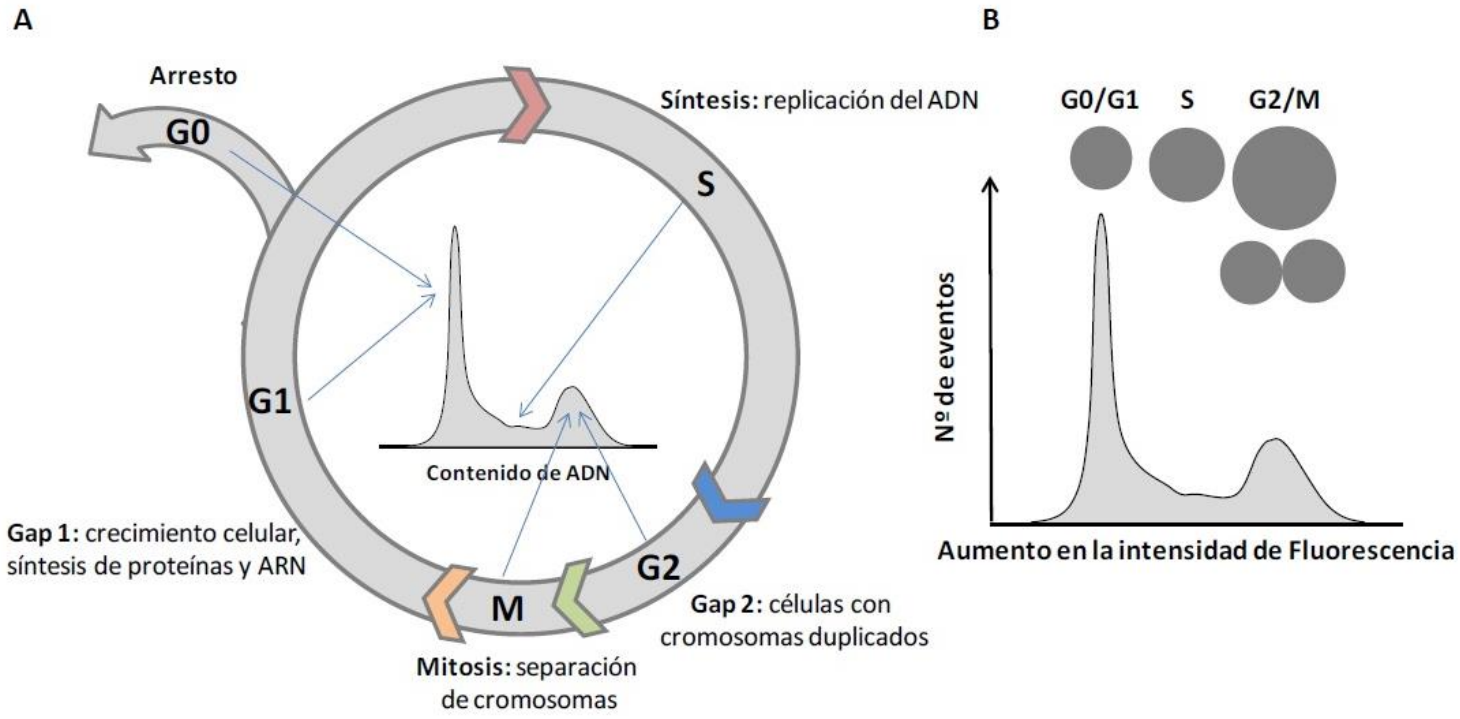
Representación gráfica de los datos



Análisis de la expresión de proteína verde fluorescente en una línea celular. Histograma (A), dot plot (B), contour plot (C).

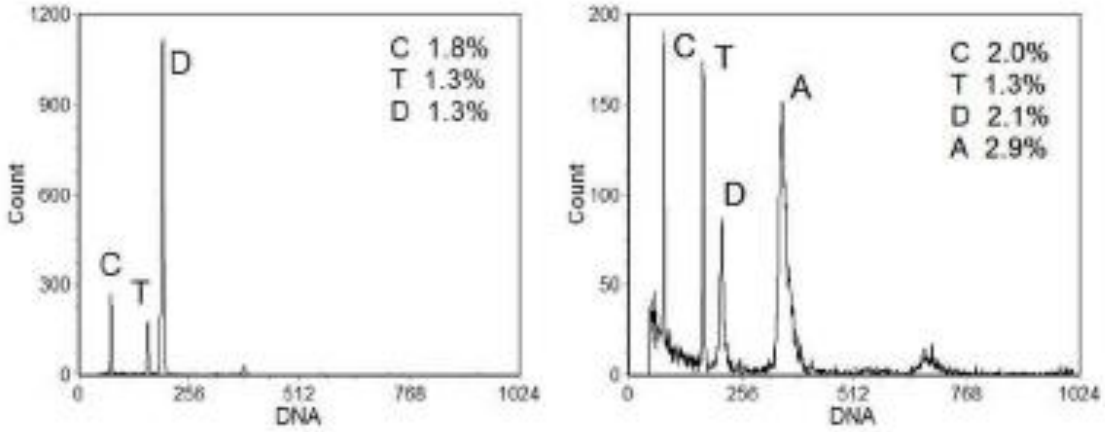
Análisis de Ciclo Celular

CICLO CELULAR Y CONTENIDO DE ADN

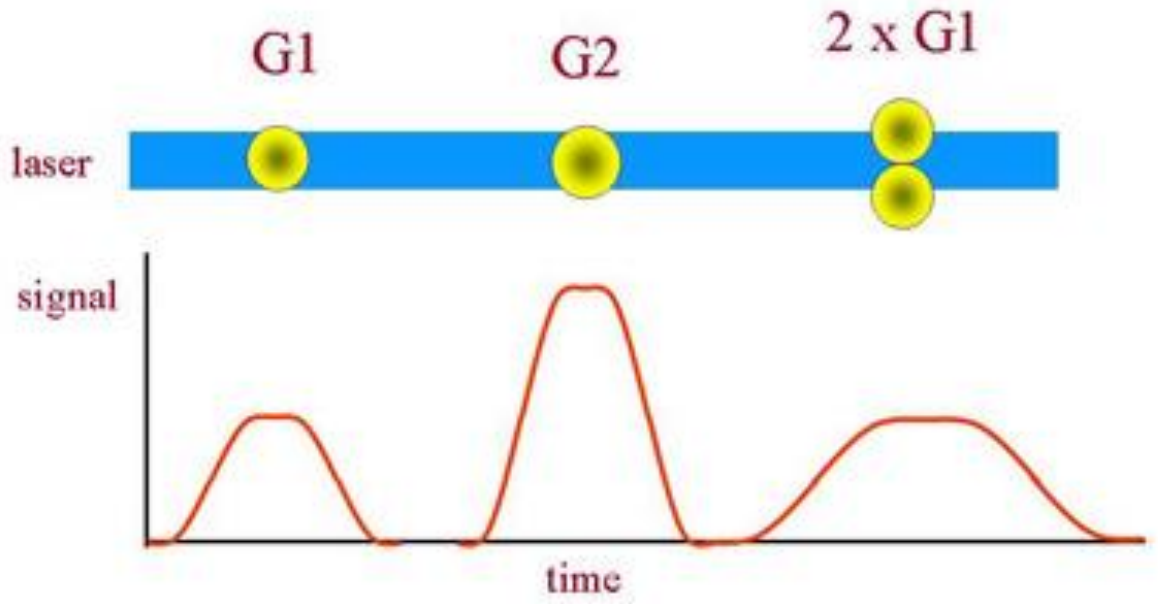


PLOIDÍA

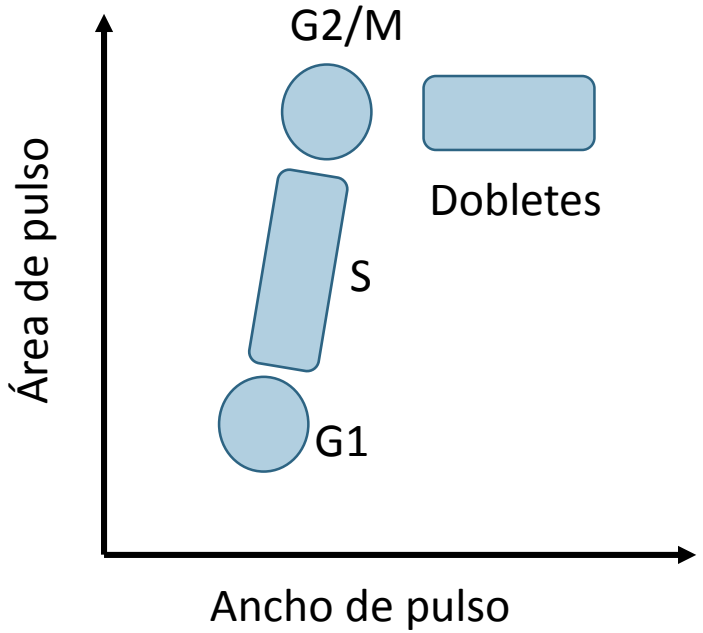
Nº de cromosomas:
n=haploide
2n=diploide
>2n=hiperploide
<2n=hipoploide

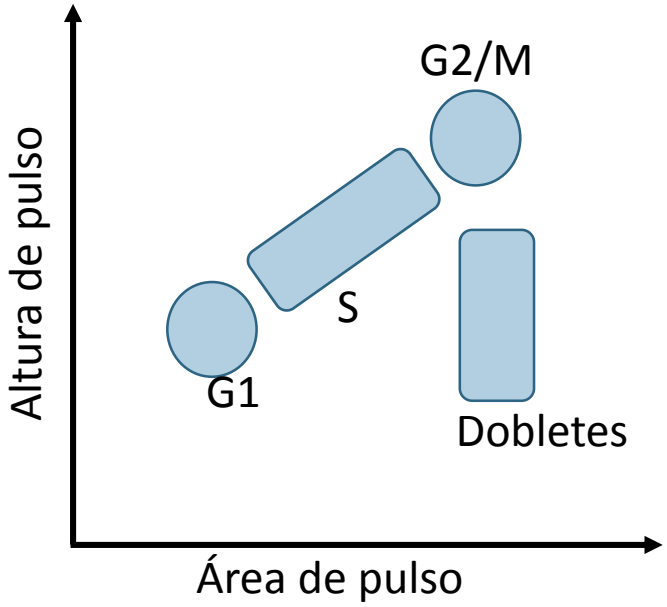


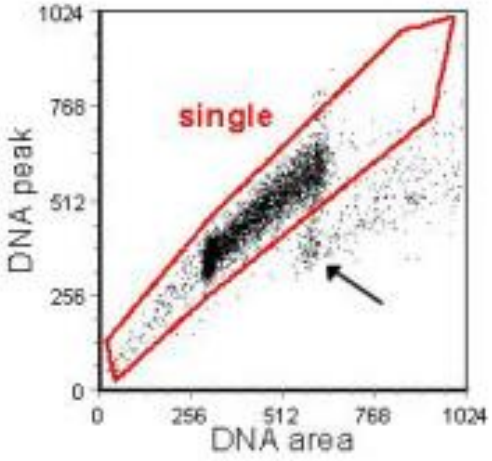
EXCLUSIÓN DE CÉLULAS PARA EL ANÁLISIS

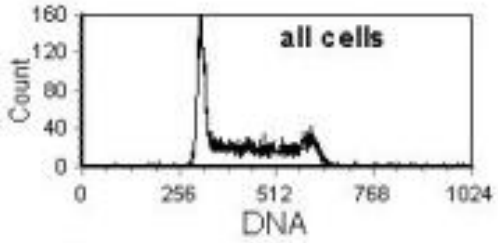


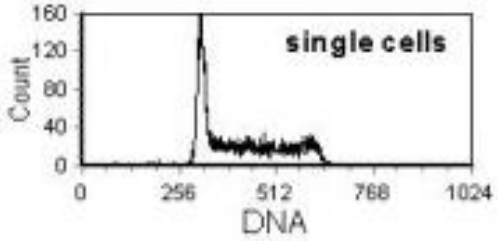
ANÁLISIS DE PULSO

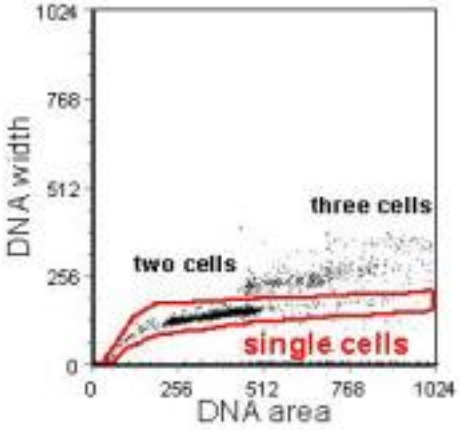


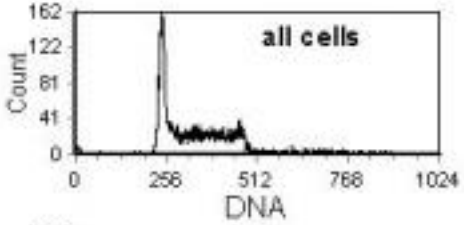


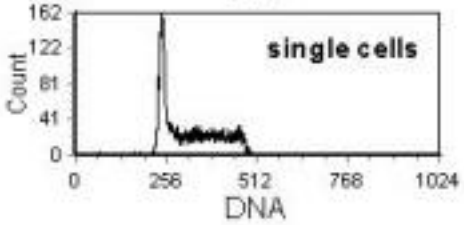

single
DNA peak
DNA area
0
256
512
768
1024

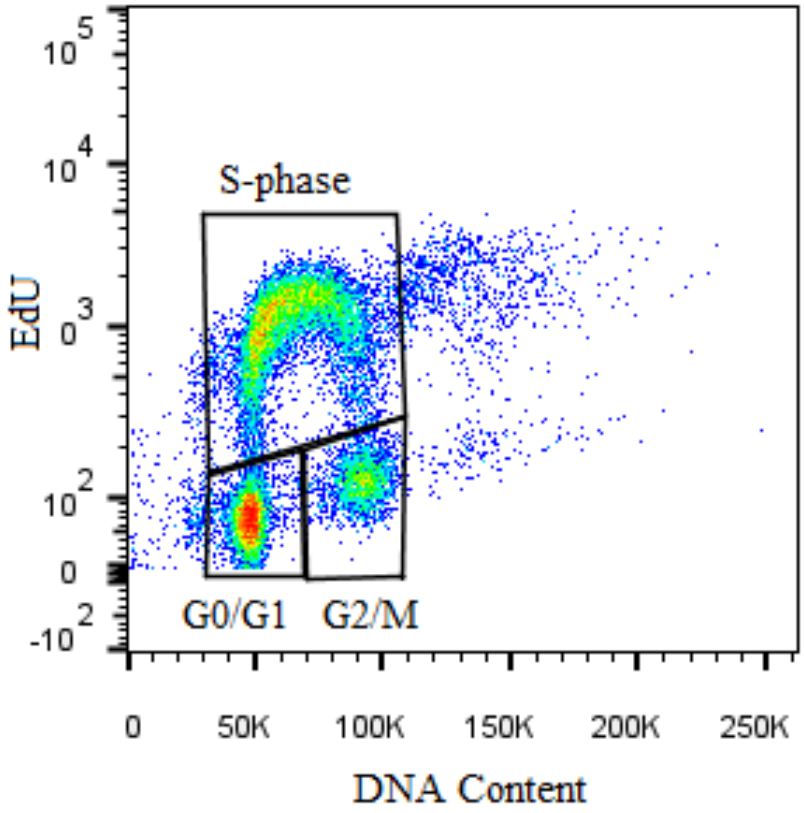

all cells
Count
DNA
0
40
80
120
160
256
512
768
1024


single cells
Count
DNA
0
40
80
120
160
256
512
768
1024

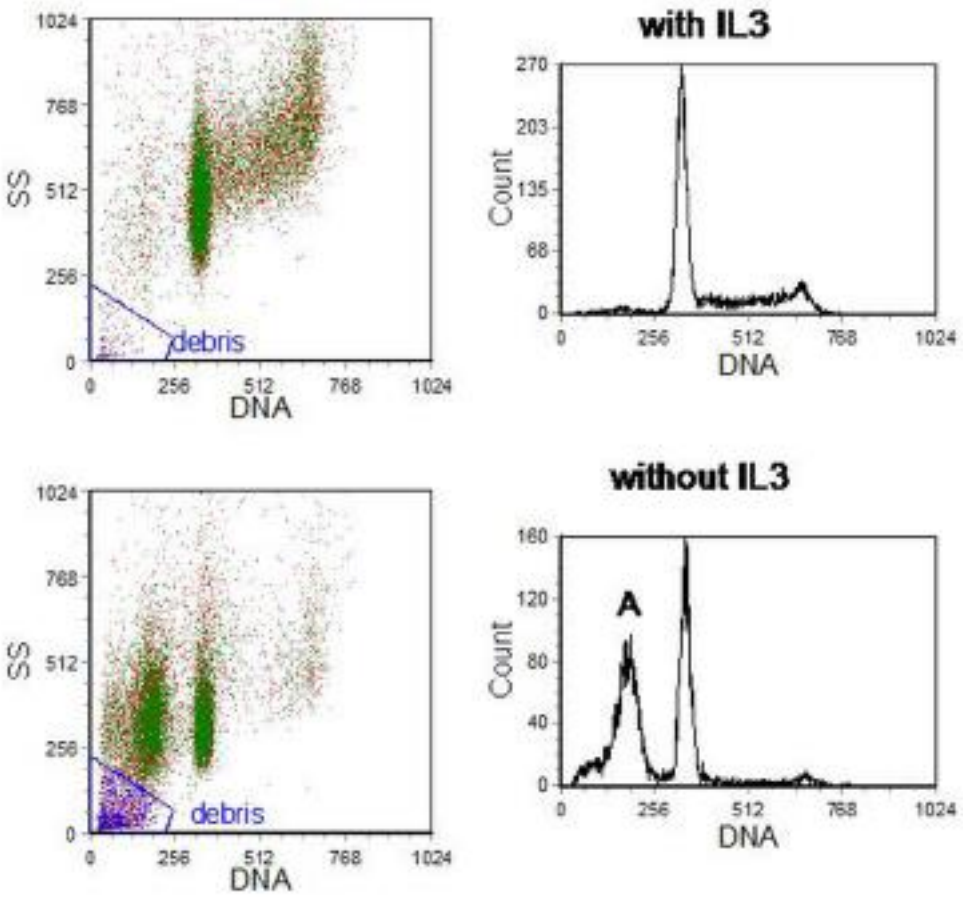

three cells
two cells
single cells
DNA width
DNA area
0
256
512
768
1024


all cells
Count
DNA
0
41
81
122
162
256
512
768
1024


single cells
Count
DNA
0
41
81
122
162
256
512
768
1024


S-phase
G0/G1
G2/M
EdU
DNA Content
10^5
10^4
10^3
10^2
0
-10^2
0
50K
100K
150K
200K
250K

DEGRADACIÓN DE ADN

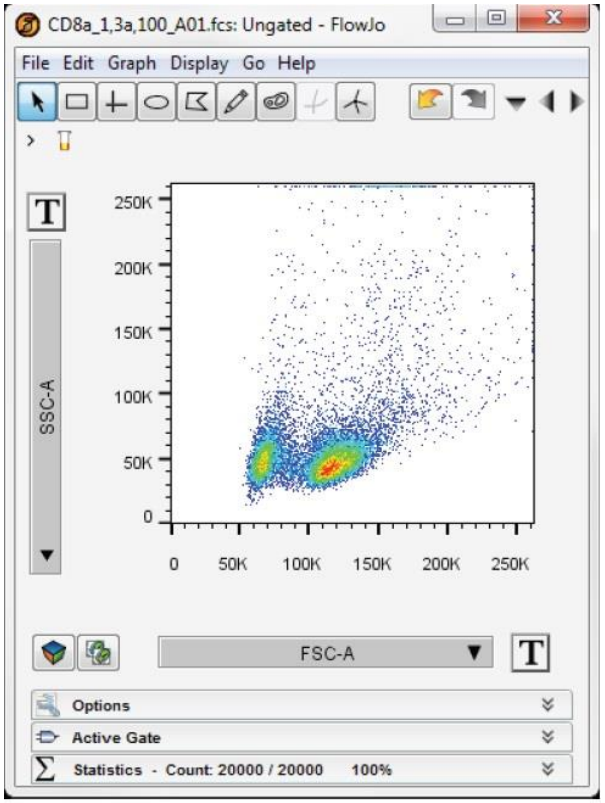


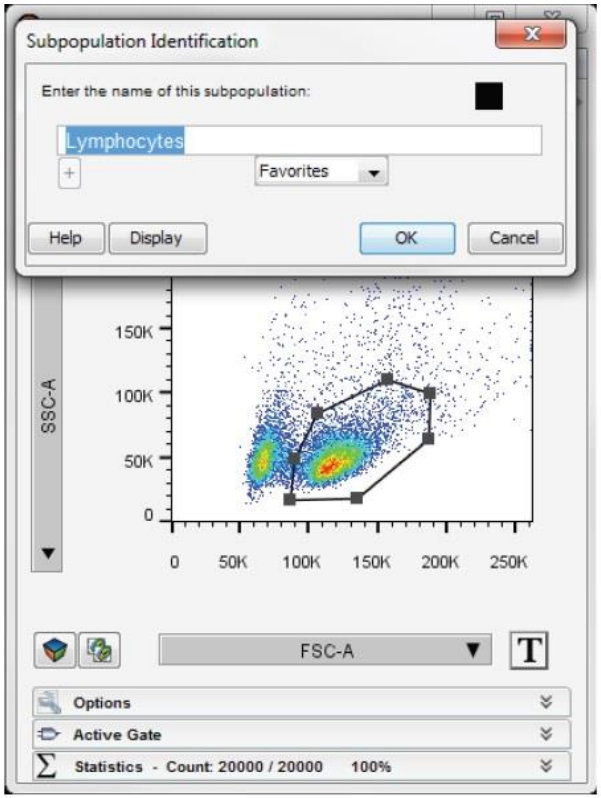
Análisis de datos del Instituto Pasteur

Objetivos

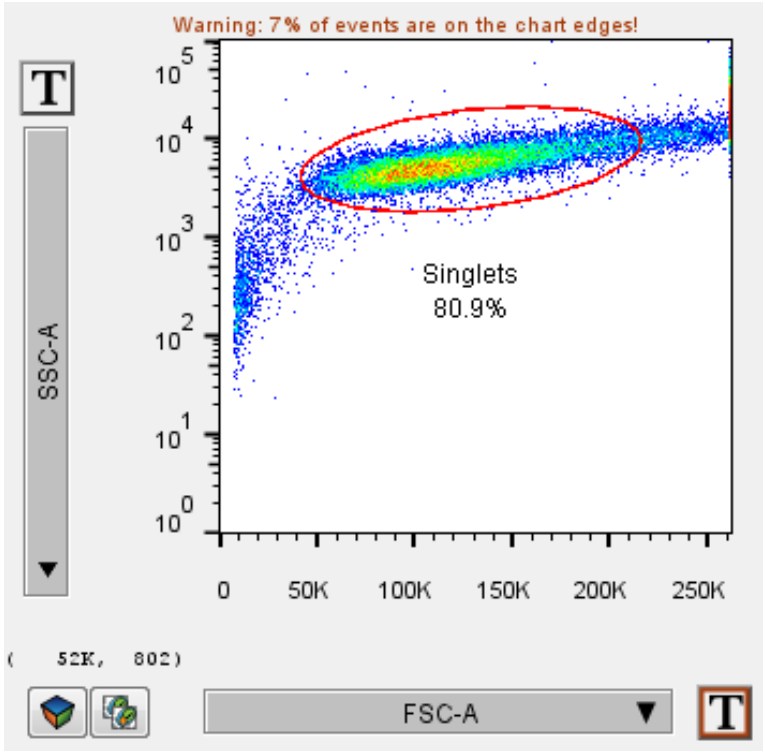
- Comprender el funcionamiento de la técnica.
- Comprender y tratar de reproducir los pasos llevados a cabo por el software de análisis.
- Comprender la problemática propuesta y plantear una posible solución.

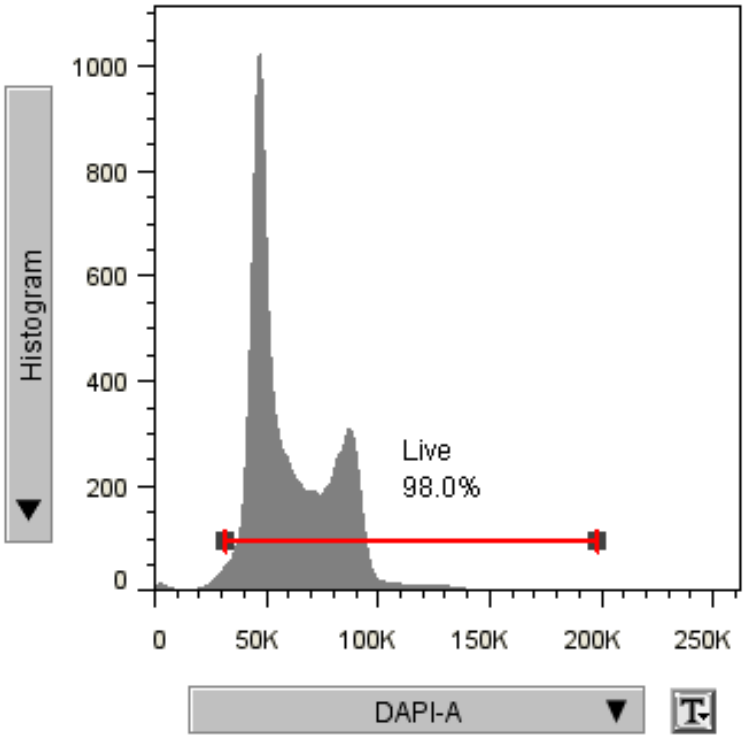
FLOWJO



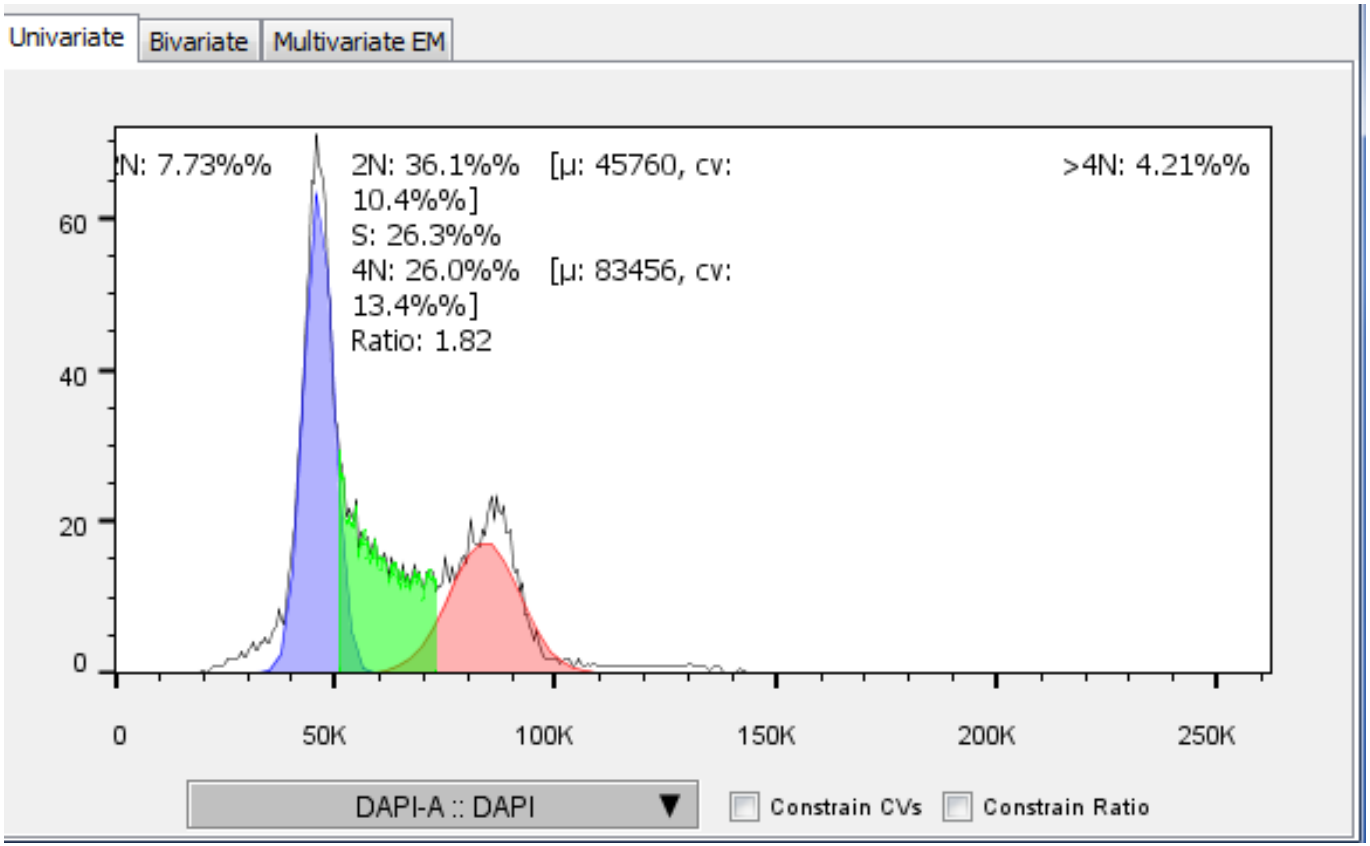


Name	Statistic	#Cells	TUBE NAME	Antibody Co...
CD8a_1,3a,100_A01.fcs		20000	1:100	100
Lymphocytes	67.1	13414		
CD8a_1,3a,200_A02.fcs		20000	1:200	200
CD8a_1,3a,400_A03.fcs		20000	1:400	400
CD8a_1,3a,800_A04.fcs		20000	1:800	800
CD8a_1,3a,1600_A05.fcs		20000	1:1600	1600

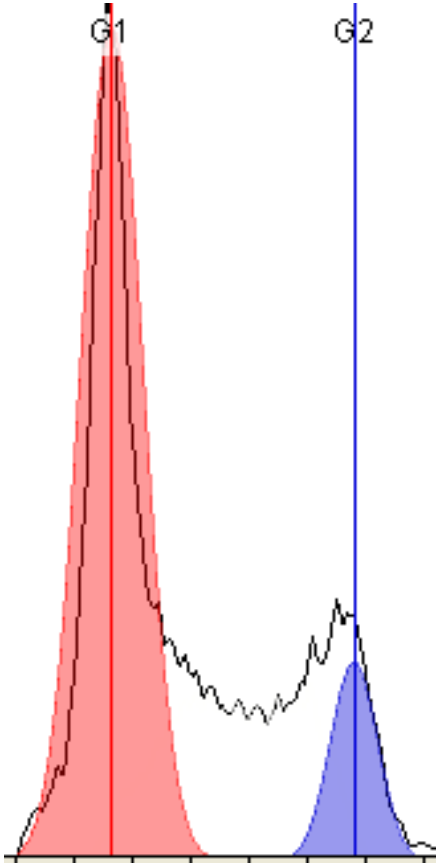

Warning: 7% of events are on the chart edges!
SSC-A
Singlets
80.9%
0
50K
100K
150K
200K
250K
(52K, 802)
FSC-A


Histogram
1000
800
600
400
200
0
Live
98.0%
0
50K
100K
150K
200K
250K
DAPI-A

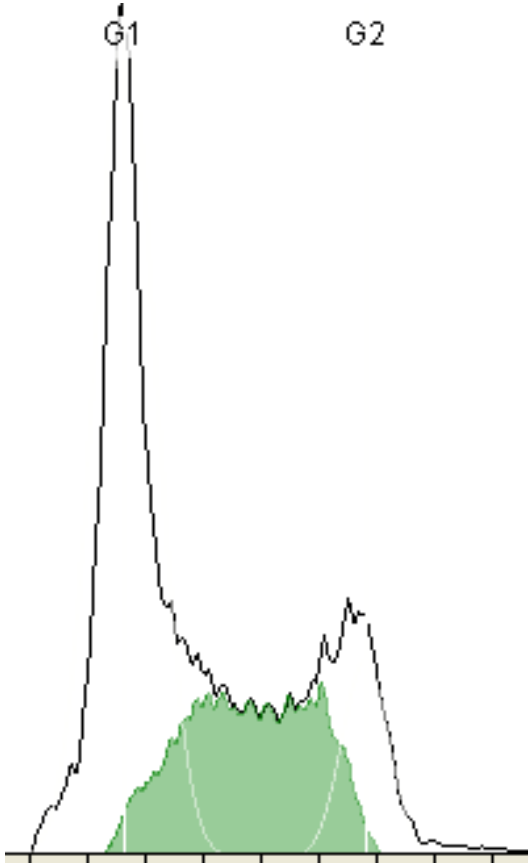
Análisis unidimensional



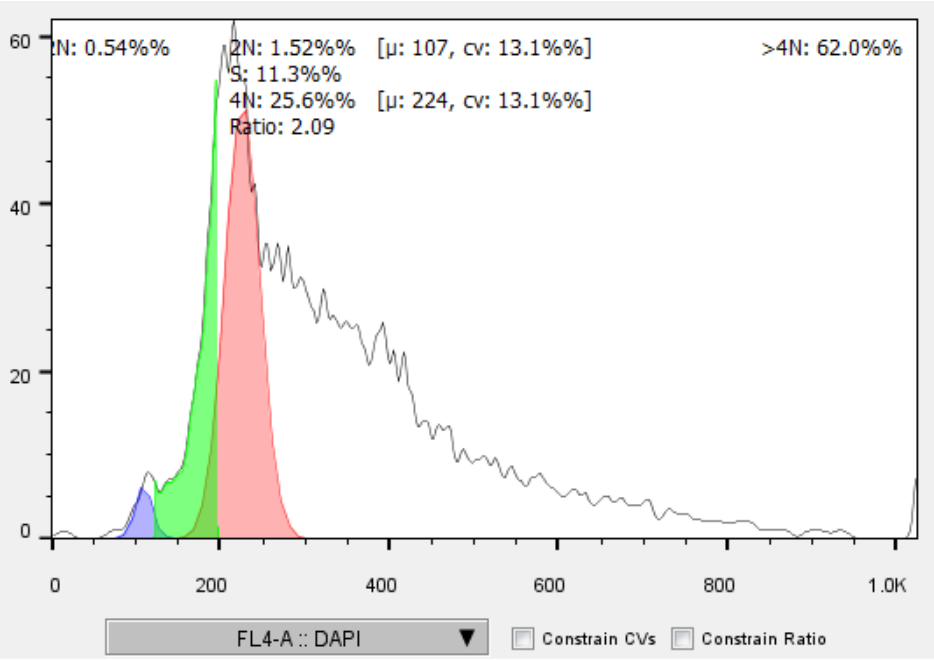
MODELO DE JAMES WATSON (1987)

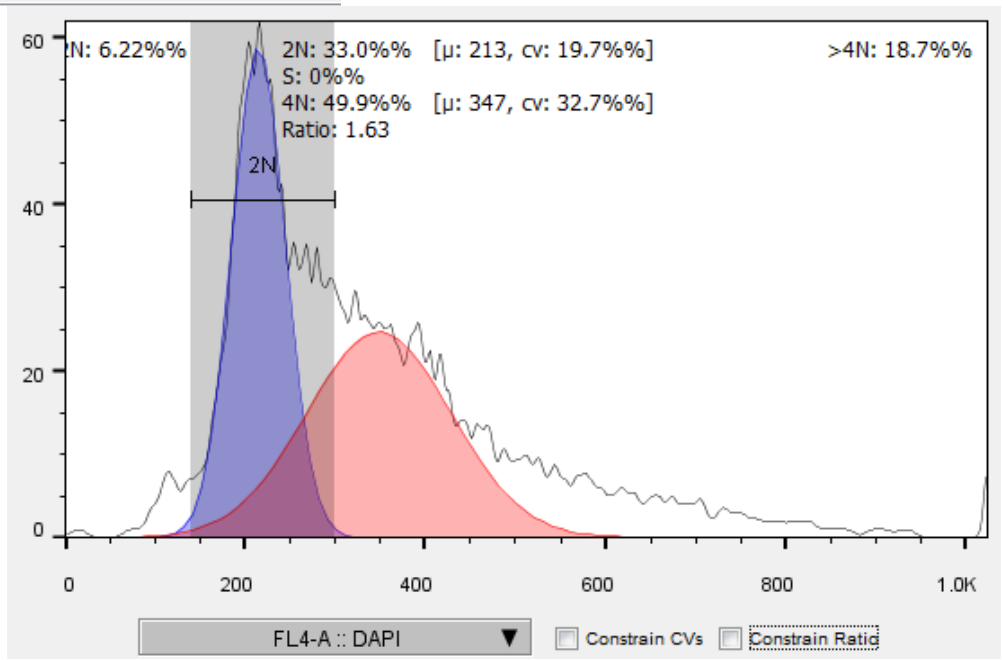


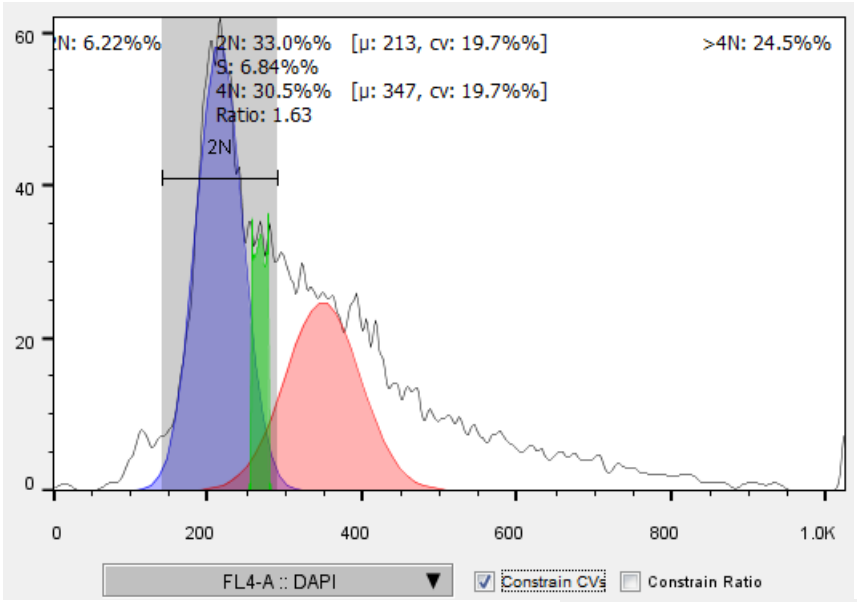
Ajuste de G0/G1 y G2/M

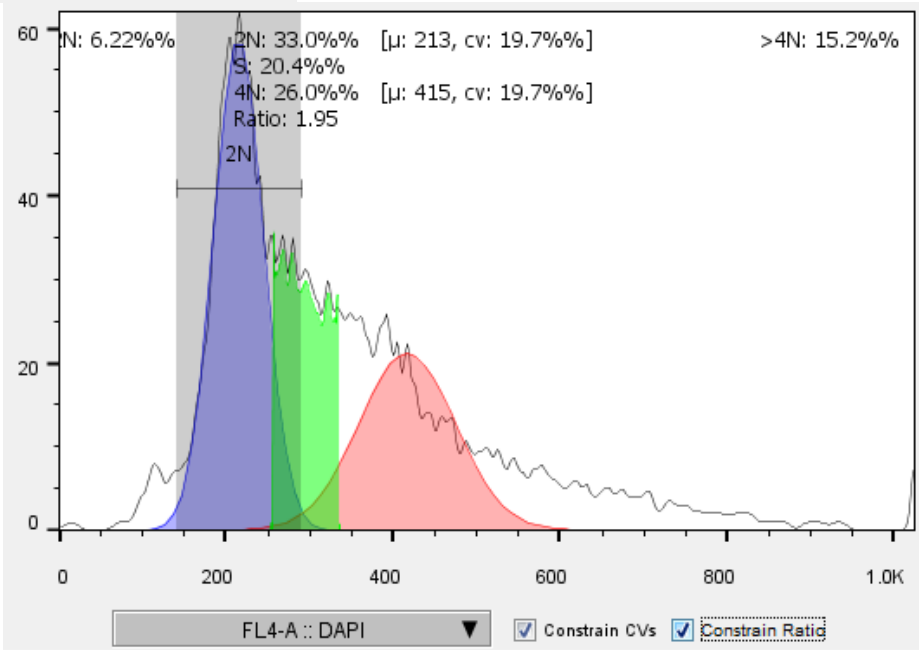


Ajuste de S

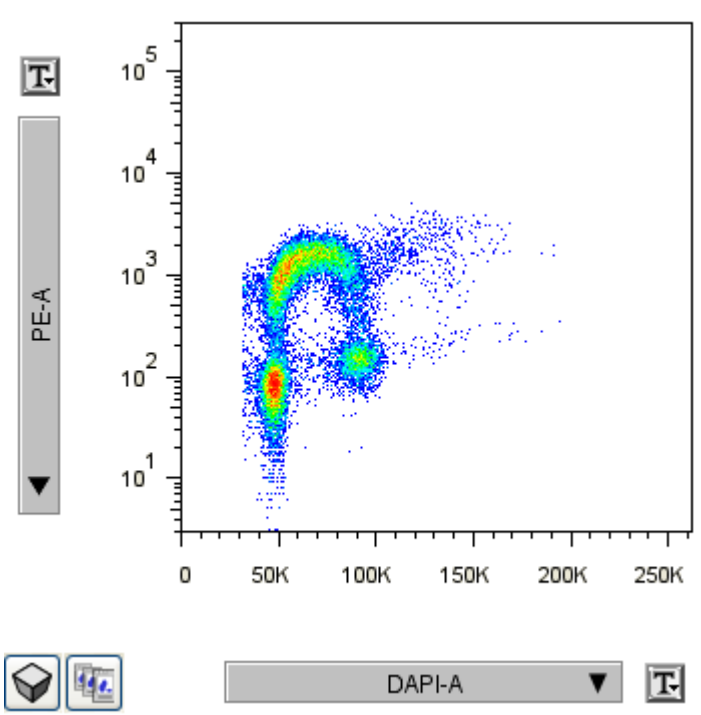

N: 0.54%%
2N: 1.52%% [μ: 107, cv: 13.1%%]
S: 11.3%%
4N: 25.6%% [μ: 224, cv: 13.1%%]
Ratio: 2.09
>4N: 62.0%%
60
40
20
0
0
200
400
600
800
1.0K
FL4-A :: DAPI
Constrain CVs
Constrain Ratio

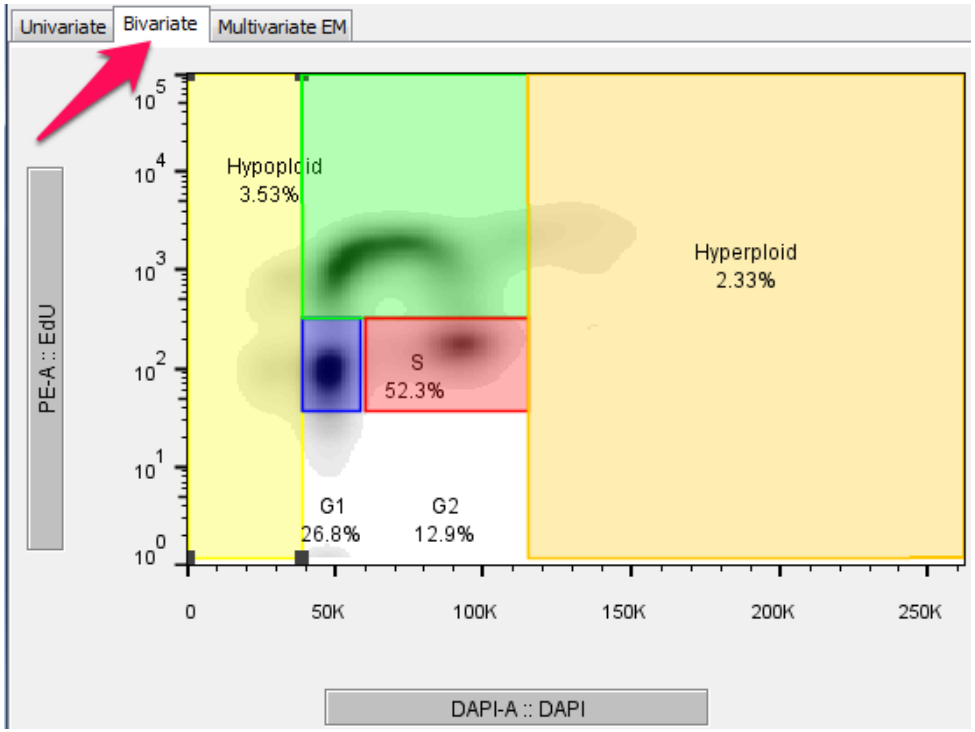

N: 6.22%%
2N: 33.0%% [μ: 213, cv: 19.7%%]
S: 0%%
4N: 49.9%% [μ: 347, cv: 32.7%%]
Ratio: 1.63
>4N: 18.7%%
2N
60
40
20
0
0
200
400
600
800
1.0K
FL4-A :: DAPI
Constrain CVs
Constrain Ratio


N: 6.22%%
2N: 33.0%%
[μ: 213, cv: 19.7%%]
>4N: 24.5%%
S: 6.84%%
4N: 30.5%%
[μ: 347, cv: 19.7%%]
Ratio: 1.63
2N
60
40
20
0
0
200
400
600
800
1.0K
FL4-A :: DAPI
Constrain CVs
Constrain Ratio

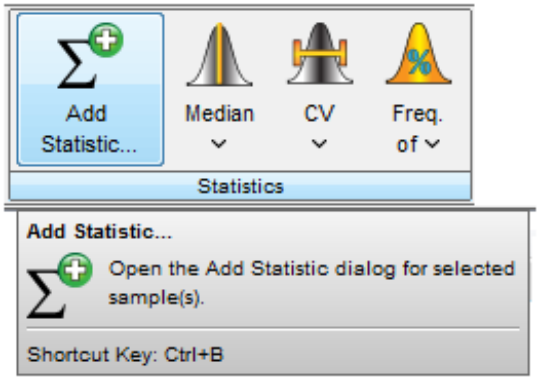

N: 6.22%%
2N: 33.0%%
[μ: 213, cv: 19.7%%]
>4N: 15.2%%
S: 20.4%%
4N: 26.0%%
[μ: 415, cv: 19.7%%]
Ratio: 1.95
2N
60
40
20
0
0
200
400
600
800
1.0K
FL4-A :: DAPI
Constrain CVs
Constrain Ratio

Análisis bidimensional



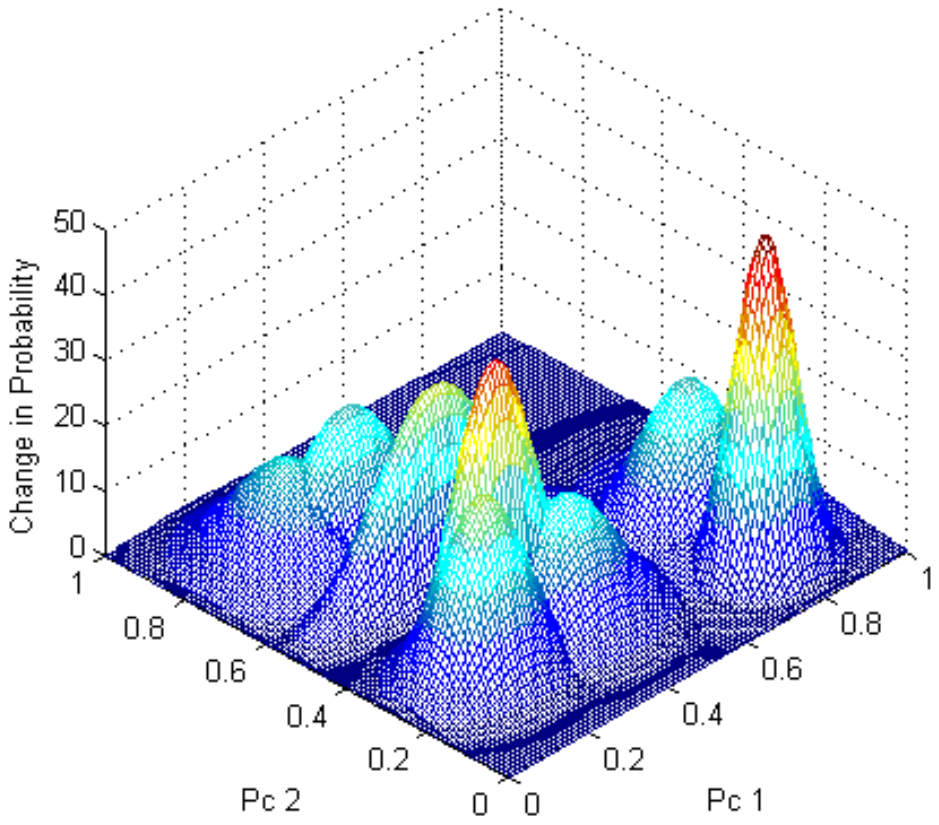


01h_Tube_1.fcs	
Singlets	83.1%
Live	99.7%
Cell Cycle	99.8%
1D: sub-2N	7.70%
1D: 2N	39.0%
1D: S	23.5%
1D: 4N	25.8%
1D: >4N	4.20%
2D: Hypoploid	3.53%
2D: G1	26.8%
2D: S	52.3%
2D: G2	12.9%
2D: Hyperploid	2.33%



2D: Hypoploid	3.53%
Σ CV : DAPI (DAPI-A)	17.5
Σ Mean : DAPI (DAPI-A)	29941
2D: G1	26.8%
Σ CV : DAPI (DAPI-A)	7.06
Σ Mean : DAPI (DAPI-A)	47147
2D: S	52.3%
Σ CV : DAPI (DAPI-A)	24.5
Σ Mean : DAPI (DAPI-A)	65061
2D: G2	12.9%
Σ CV : DAPI (DAPI-A)	9.39
Σ Mean : DAPI (DAPI-A)	89822
2D: Hyperploid	2.33%
Σ CV : DAPI (DAPI-A)	11.4
Σ Mean : DAPI (DAPI-A)	135086

Análisis multidimensional

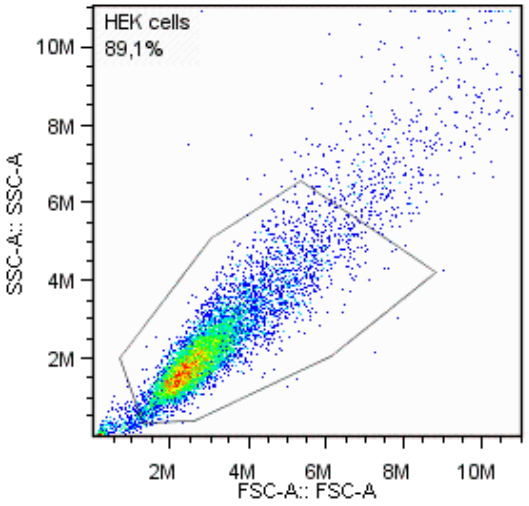


Expectation-Maximization

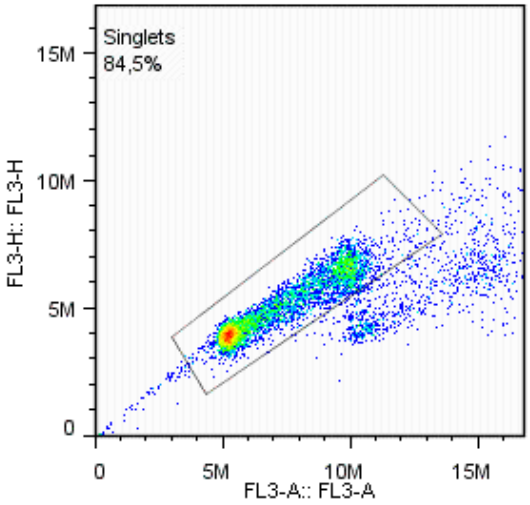
ANÁLISIS DE DATOS DEL INSTITUTO PASTEUR

Células: HEK (Human Embryonic Kidney 293 cells)
Tinción con IP: 50ug/mL y Metil Green 50ug/mL
Adquisición 24 horas post coloración.

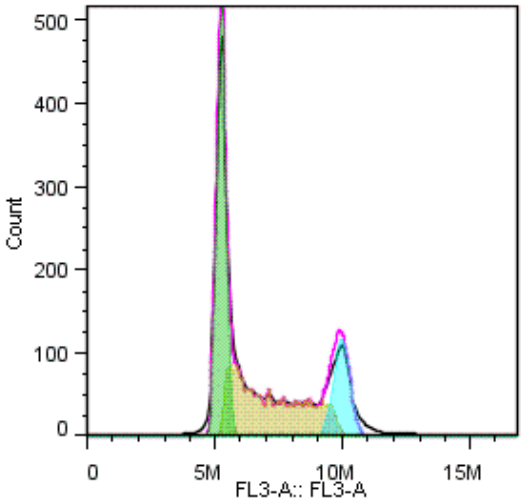
RESULTADOS PASTEUR – CASO CONTROL



A01 Et Et IP 50 HEK 1.fcs
Ungated
11388

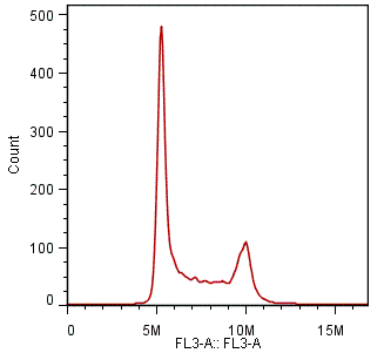


A01 Et Et IP 50 HEK 1.fcs
HEK cells
10142



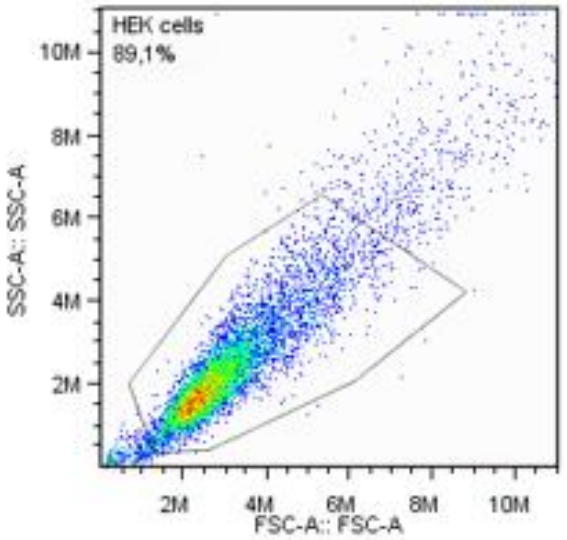
A01 Et Et IP 50 HEK 1.fcs
Cell Cycle
Watson
RMS = 1,94
Freq. G1 = 42,38
Freq. S = 40,19
Freq. G2 = 17,05
G1 Mean = 5233793
G2 Mean = 9944207
G1 cv = 4,96
G2 cv = 4,65
Freq. sub-G1 = 2,38
Freq. super-G2 = 1,24

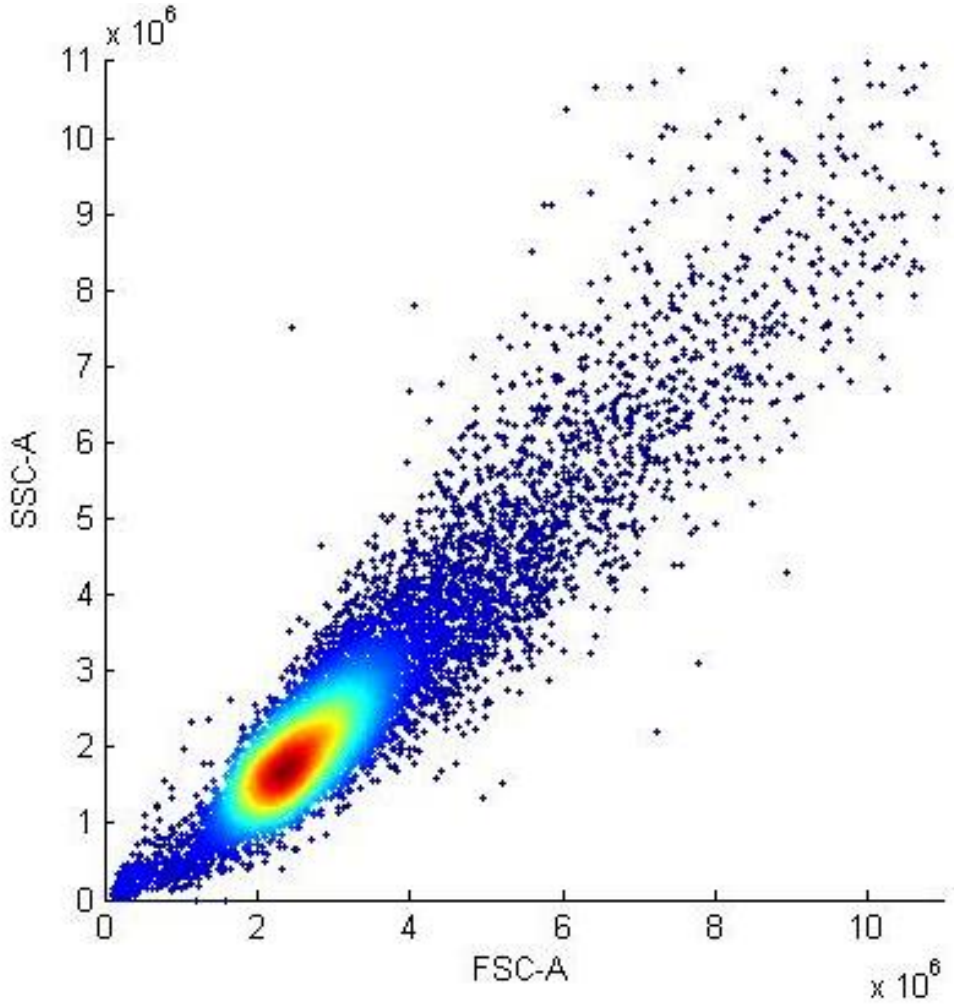
8565

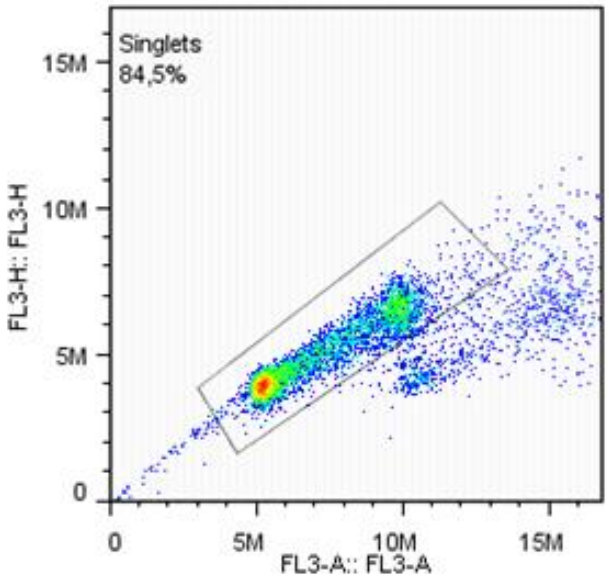


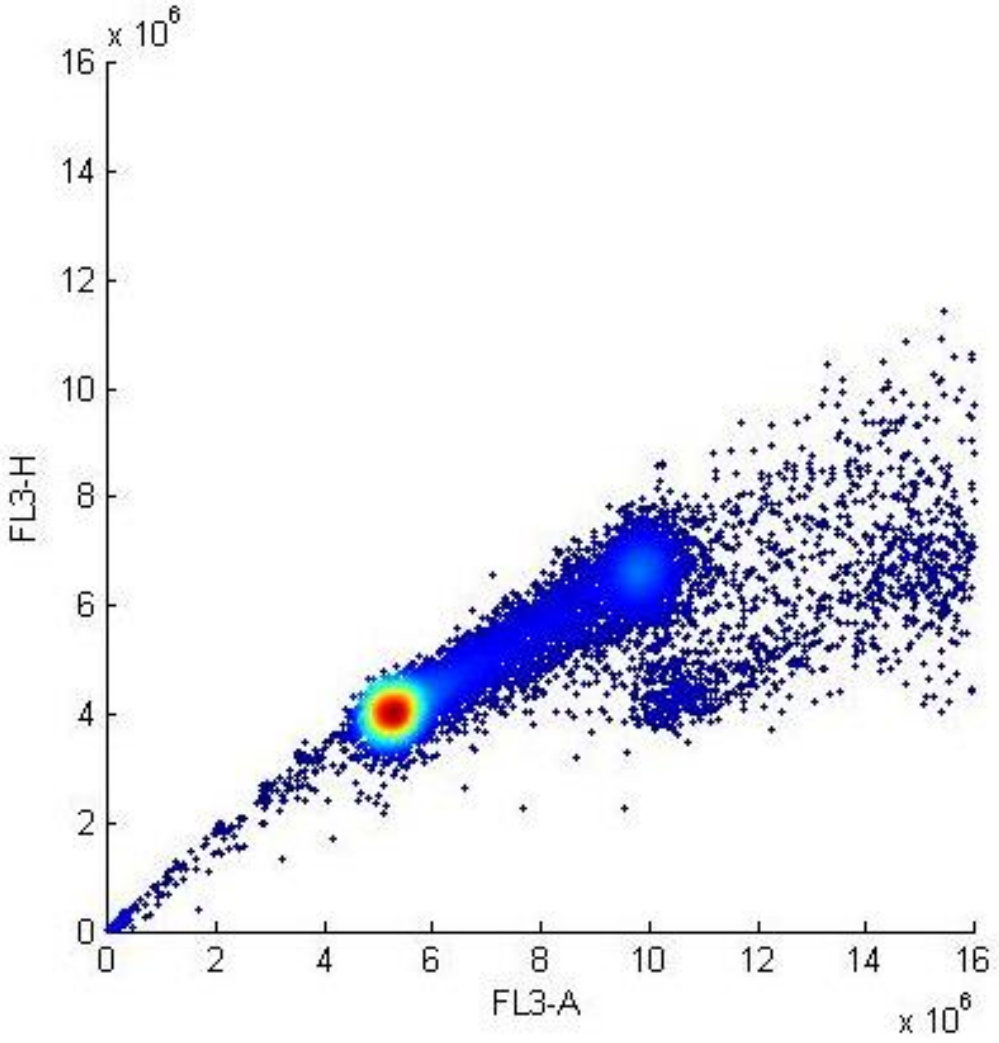
A01 Et Et IP 50 HEK 1.fcs
Singlets
8557

RESULTADOS HISTOGRAMAS 2D - CASO CONTROL

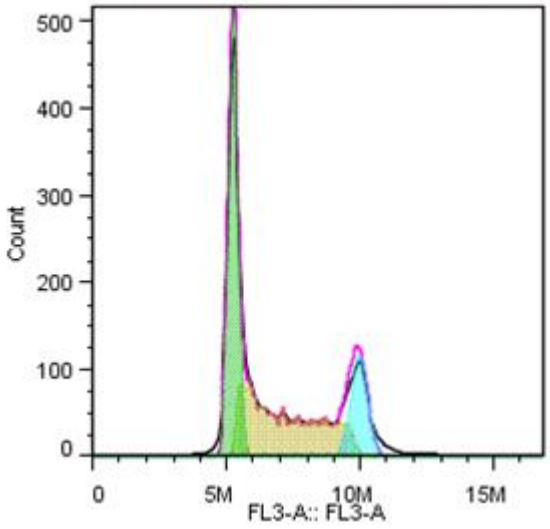


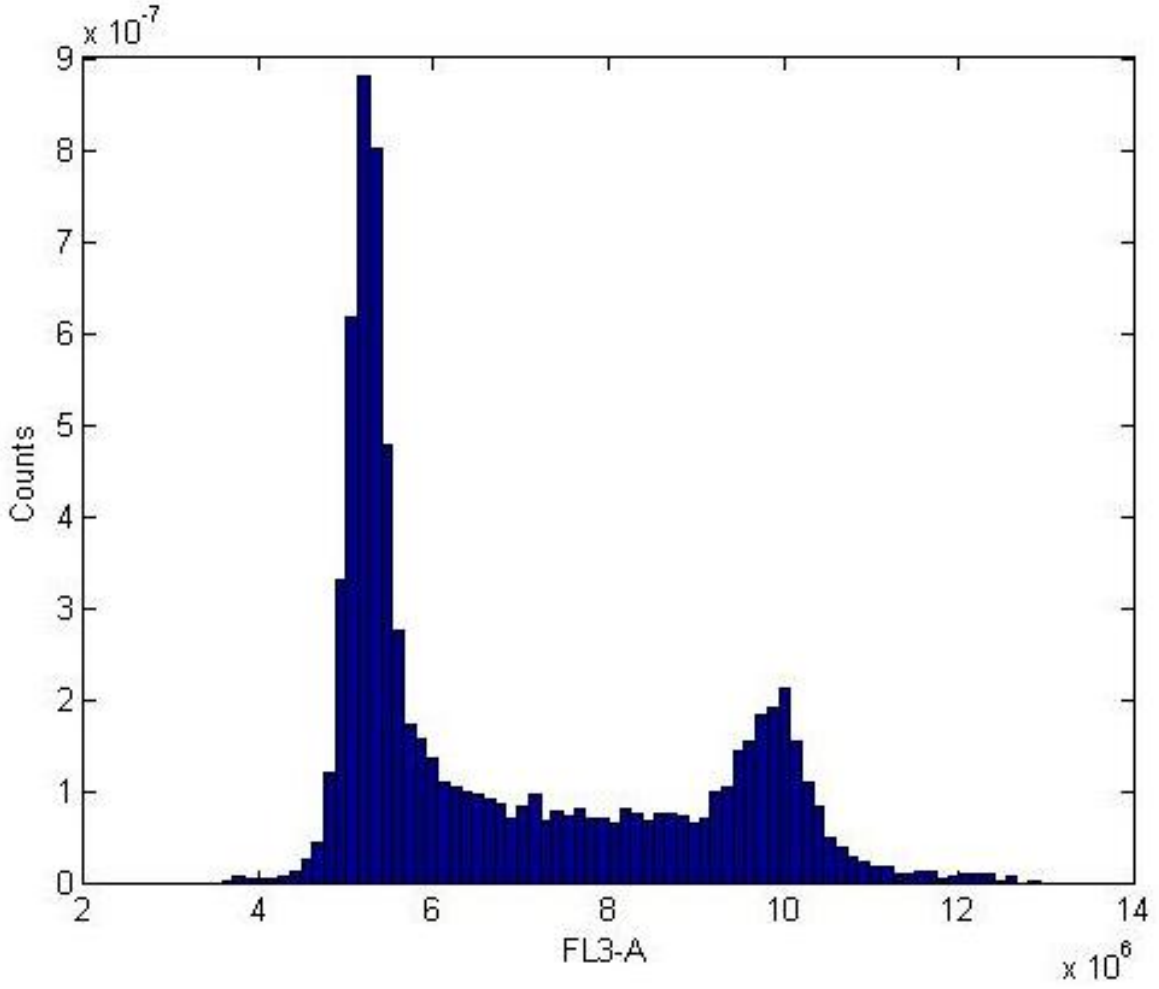



Singlets
84,5%
15M
10M
5M
0
FL3-H:: FL3-H
0
5M
10M
15M
FL3-A:: FL3-A

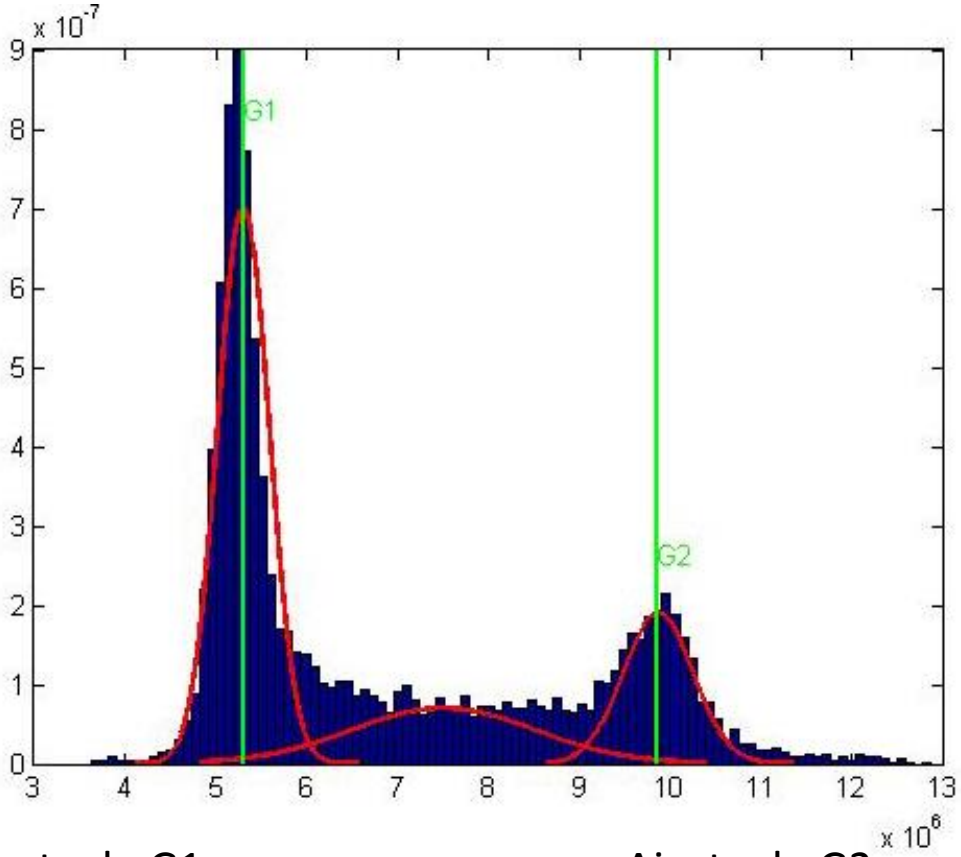

x 10^6
16
14
12
10
8
6
4
2
0
FL3-H
0
2
4
6
8
10
12
14
16
FL3-A
x 10^6

RESULTADOS HISTOGRAMAS 1D - CASO CONTROL





AJUSTE MANUAL DE GAUSIANAS



Ajuste de G1:

mu = 5.30542e+006

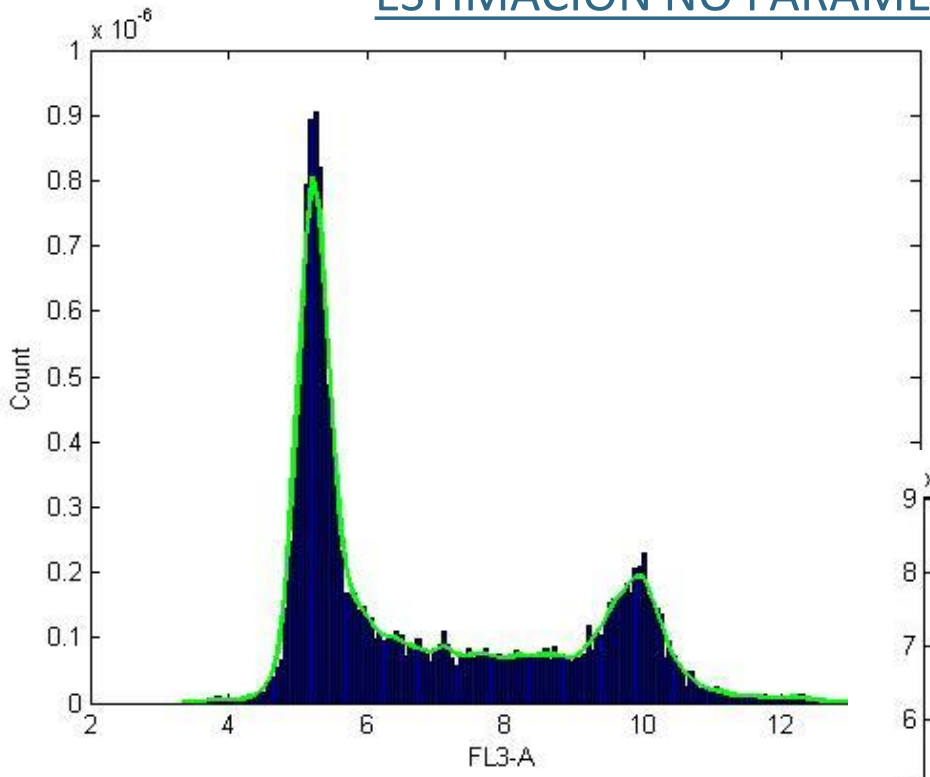
sigma = 281451

Ajuste de G2:

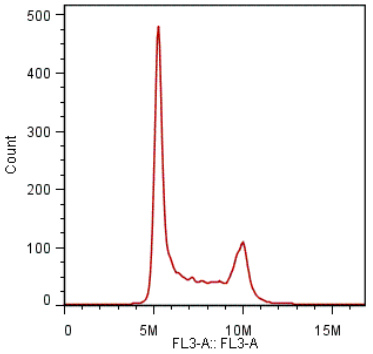
mu =9.87309e+006

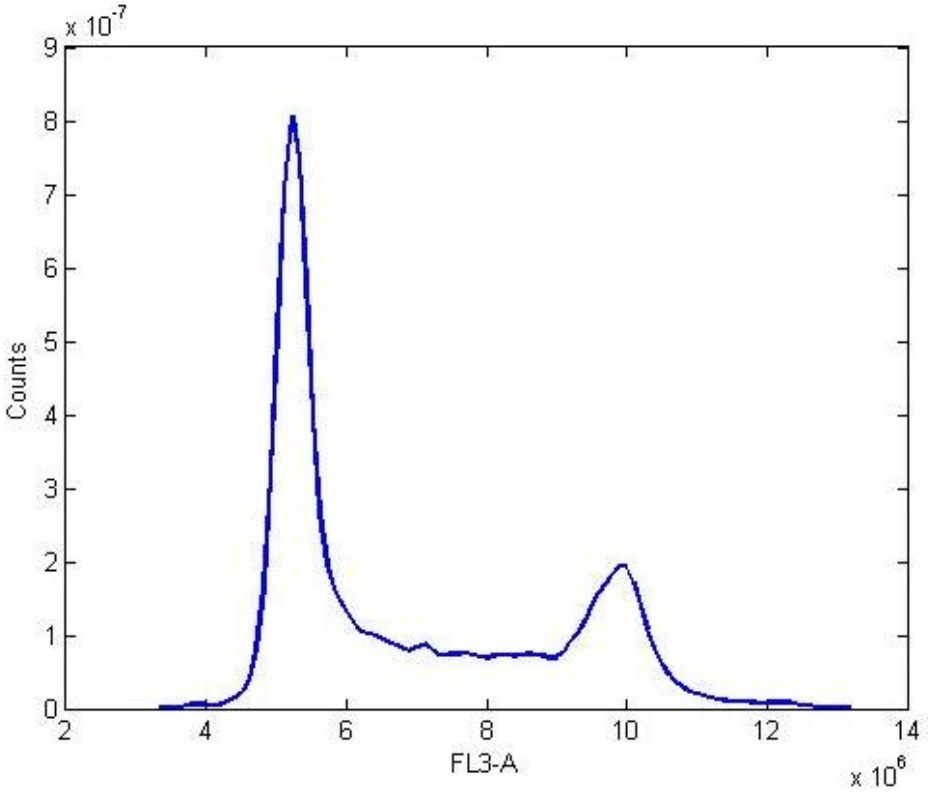
sigma = 385459

ESTIMACIÓN NO PARAMÉTRICA DE DENSIDADES

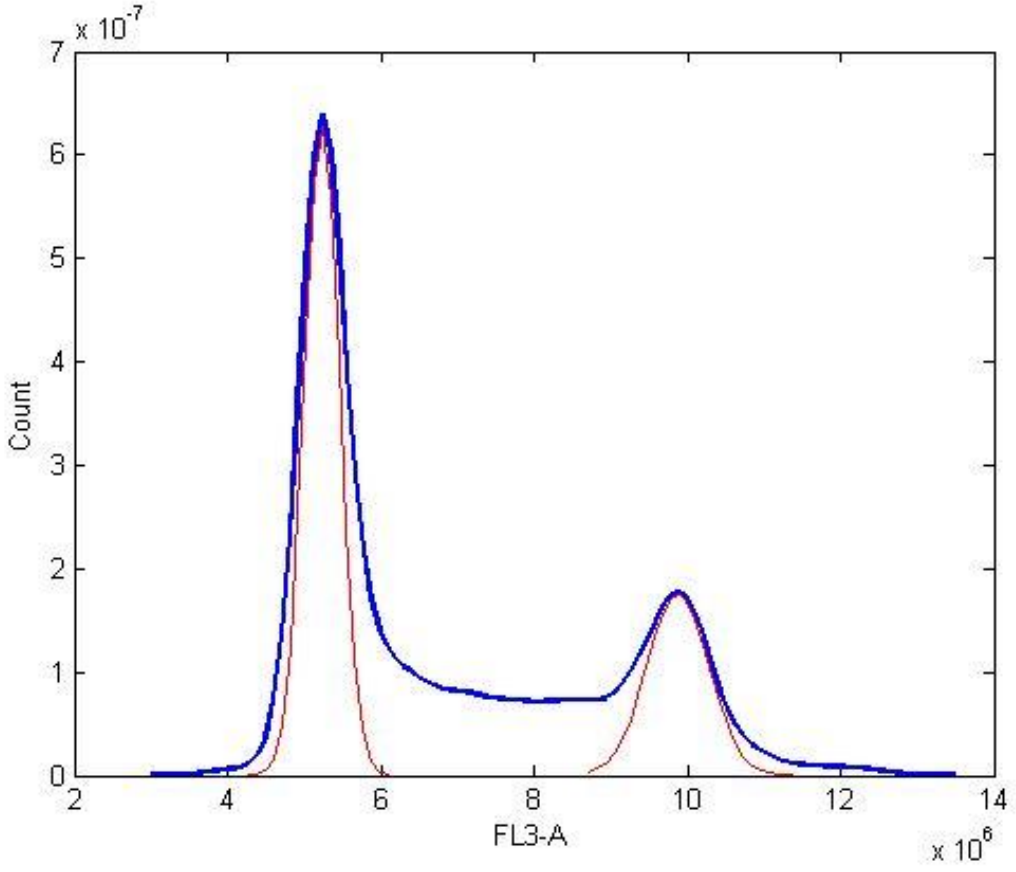


Ksdensity (MATLAB)

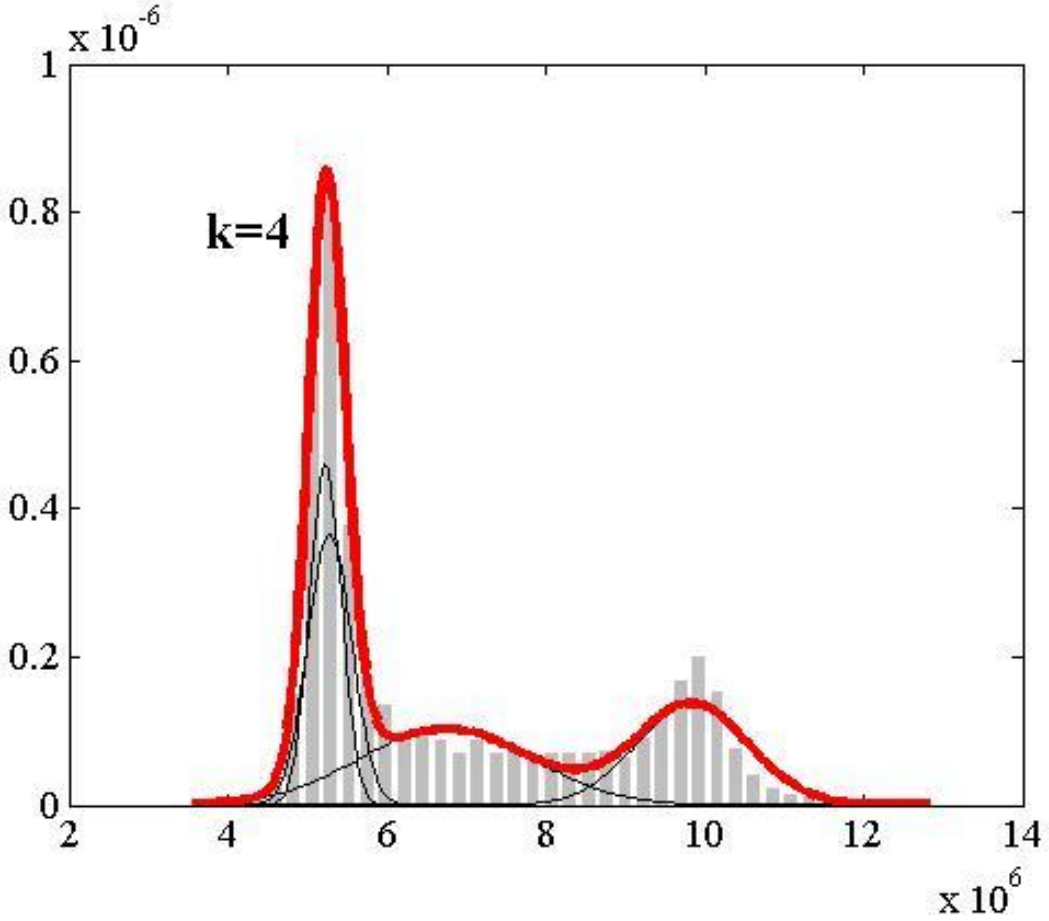




ESTIMACIÓN AUTOMÁTICA DE DENSIDADES de G1 Y G2

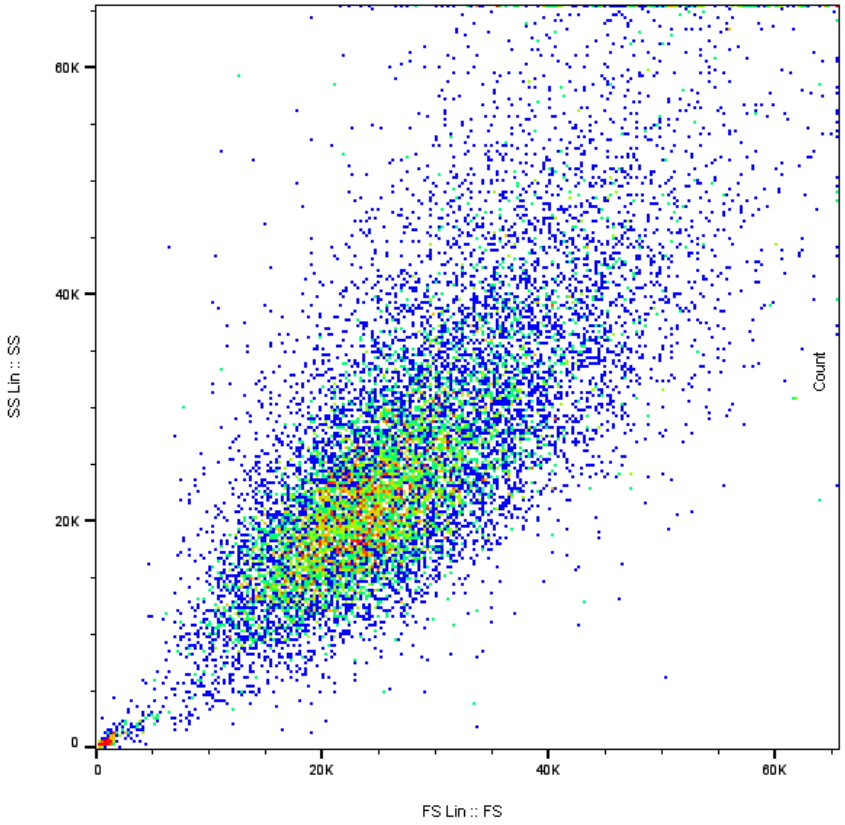


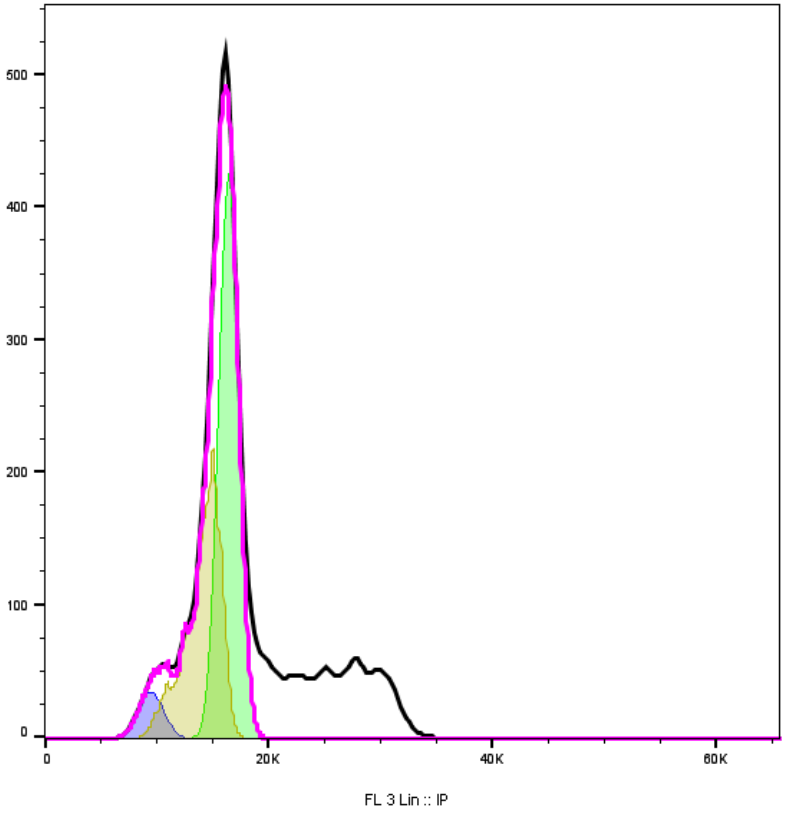
ESTIMACION GAUSSIAN MIXTURE MODEL

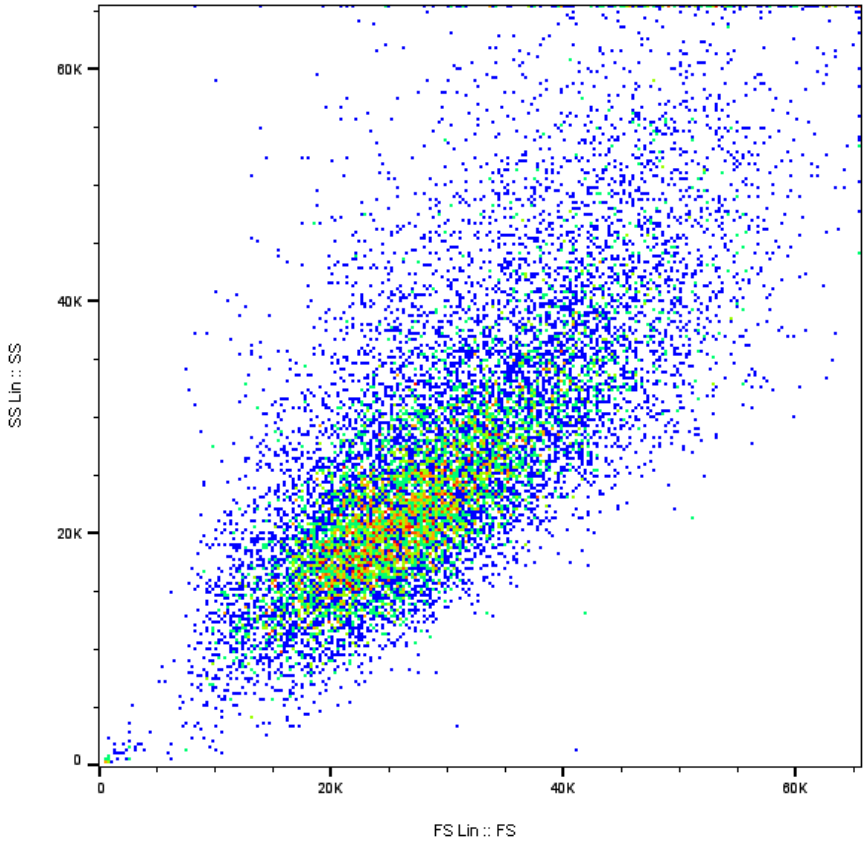


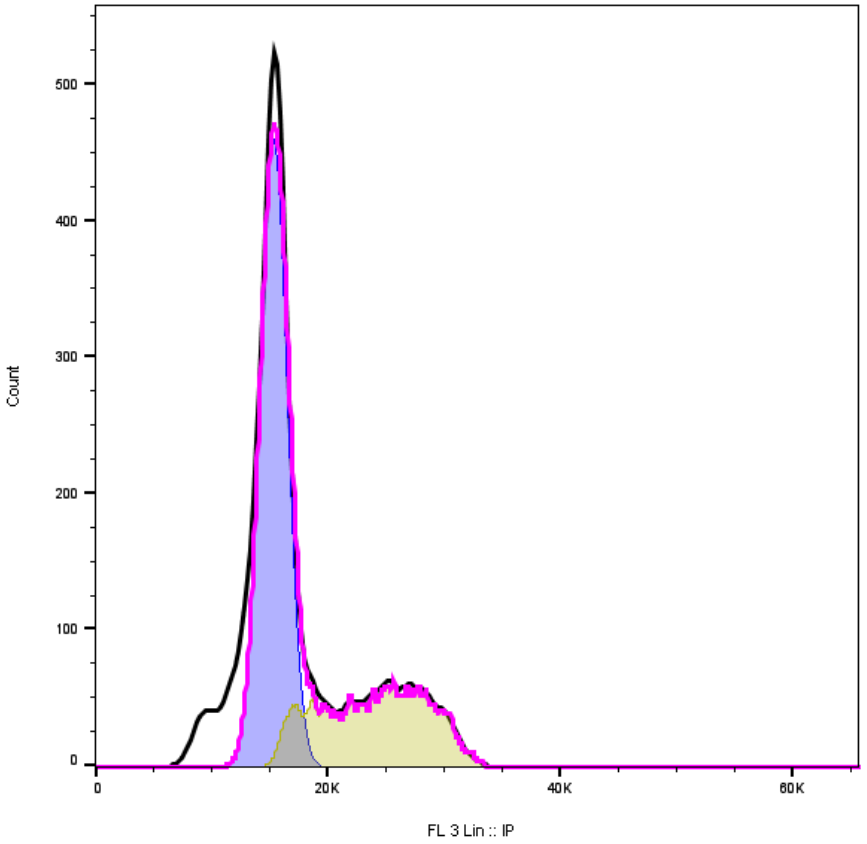
Expectation-Maximization

RESULTADOS PASTEUR – CASO TRATADO






60K
40K
20K
0
SS Lin :: SS
0
20K
40K
60K
FS Lin :: FS


500
400
300
200
100
0
Count
0
20K
40K
60K
FL 3 Lin :: IP

CONCLUSIONES

- Se logró comprender la técnica y sus aplicaciones.
- Se reprodujo la técnica existente (Algoritmo de Watson).
- Se espera aportar una solución en cuanto al modelado de Sub-G1.