

Curso: Aspectos teóricos y aplicaciones de la citometría de flujo

Introducción teórica a la citometría de flujo.

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Tecnologia (UAT), VHIR

Organitza:



Participa:

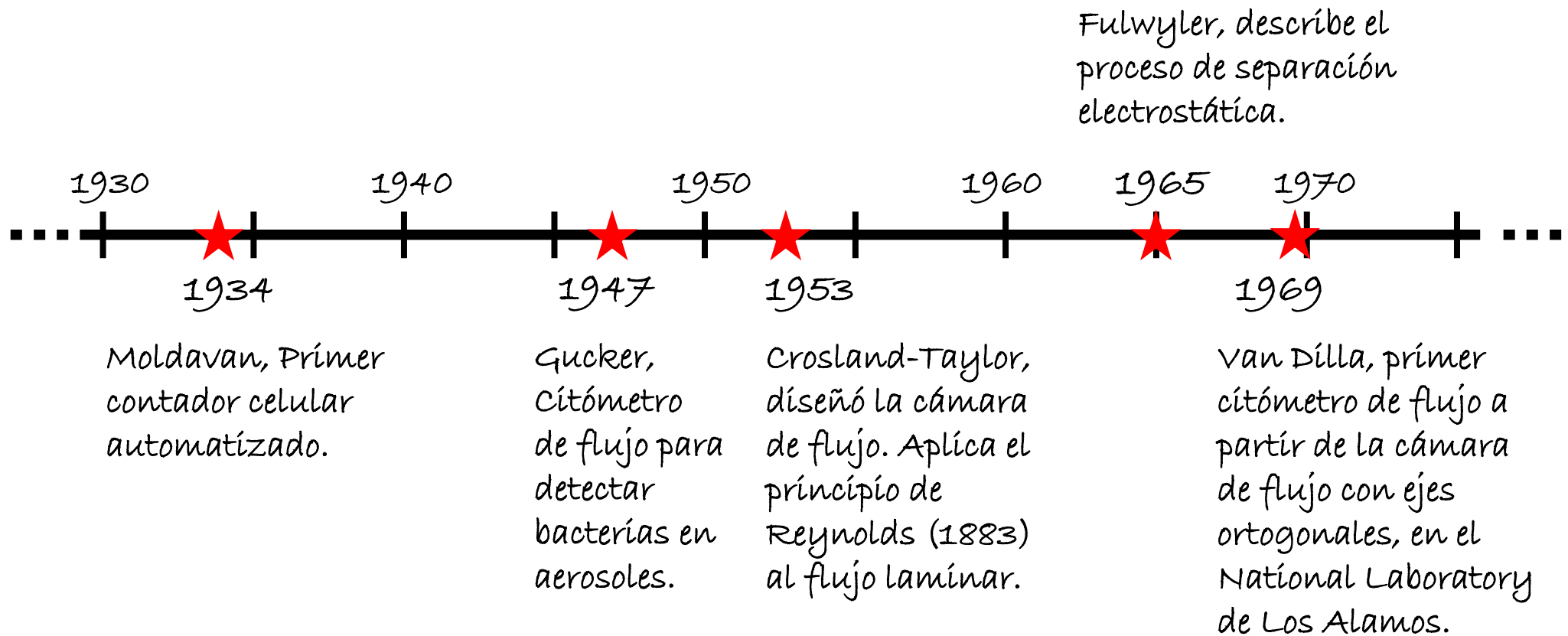


Qué es la Citometría de flujo?

La citometría es una técnica que permite medir simultáneamente múltiples características físicas y químicas de células o partículas en suspensión que atraviesan un haz de luz (laser), y ofrece la posibilidad de separar las células (sorting) en función de sus características.



Historia de la citometría de flujo



- 1970 Se construyen los primeros citómetros de flujo.
- 1980- Actualidad se han producido muchos adelantos en las aplicaciones de la citometría de flujo en el campo de la investigación clínica-biológica, sobretudo a partir del desarrollo de los anticuerpos.

Componentes de un citómetro de flujo

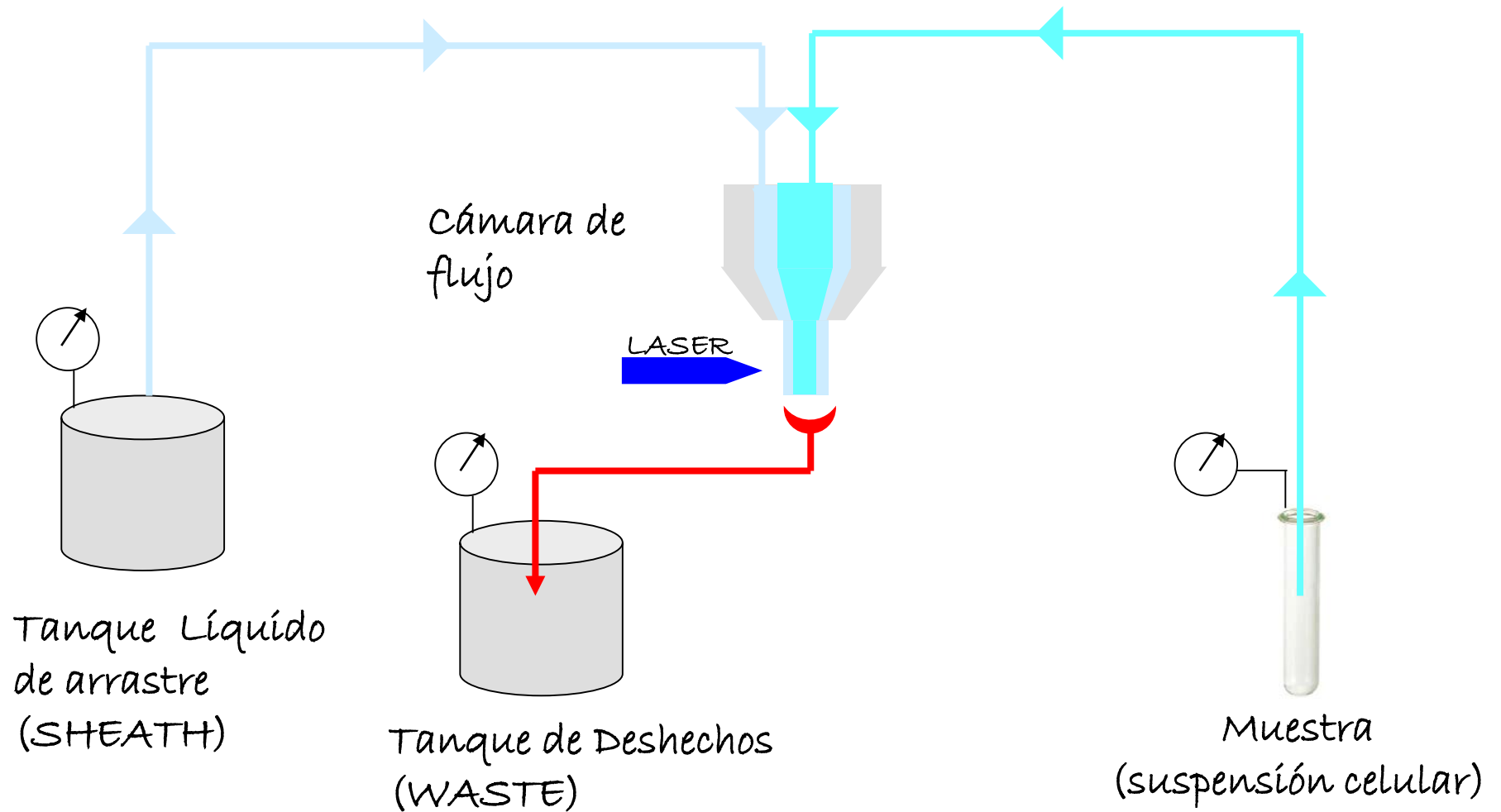
Fluídica

- Flujo laminar (sistema presurizado).
- Cámara de flujo (enfoque hidrodinámico).

Óptica

Electrónica

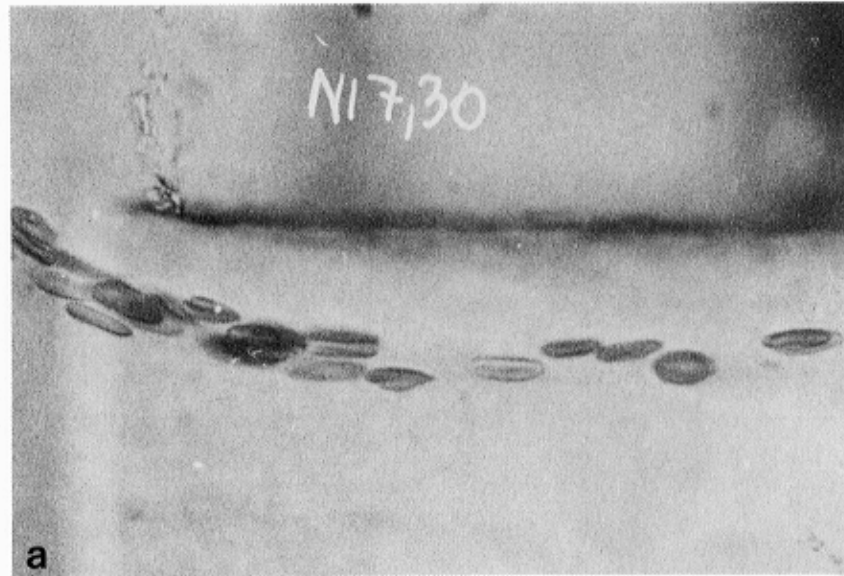
Informática



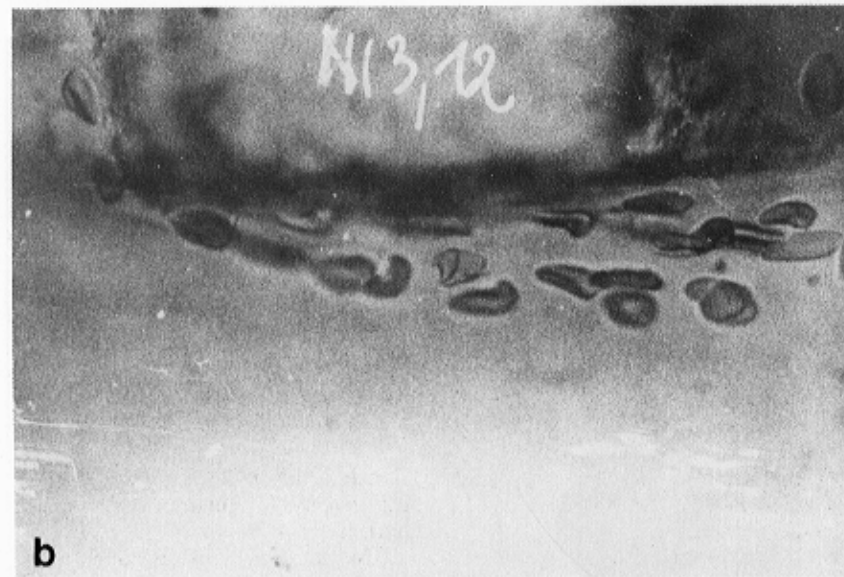


Fluídica (II)

Flujo laminar

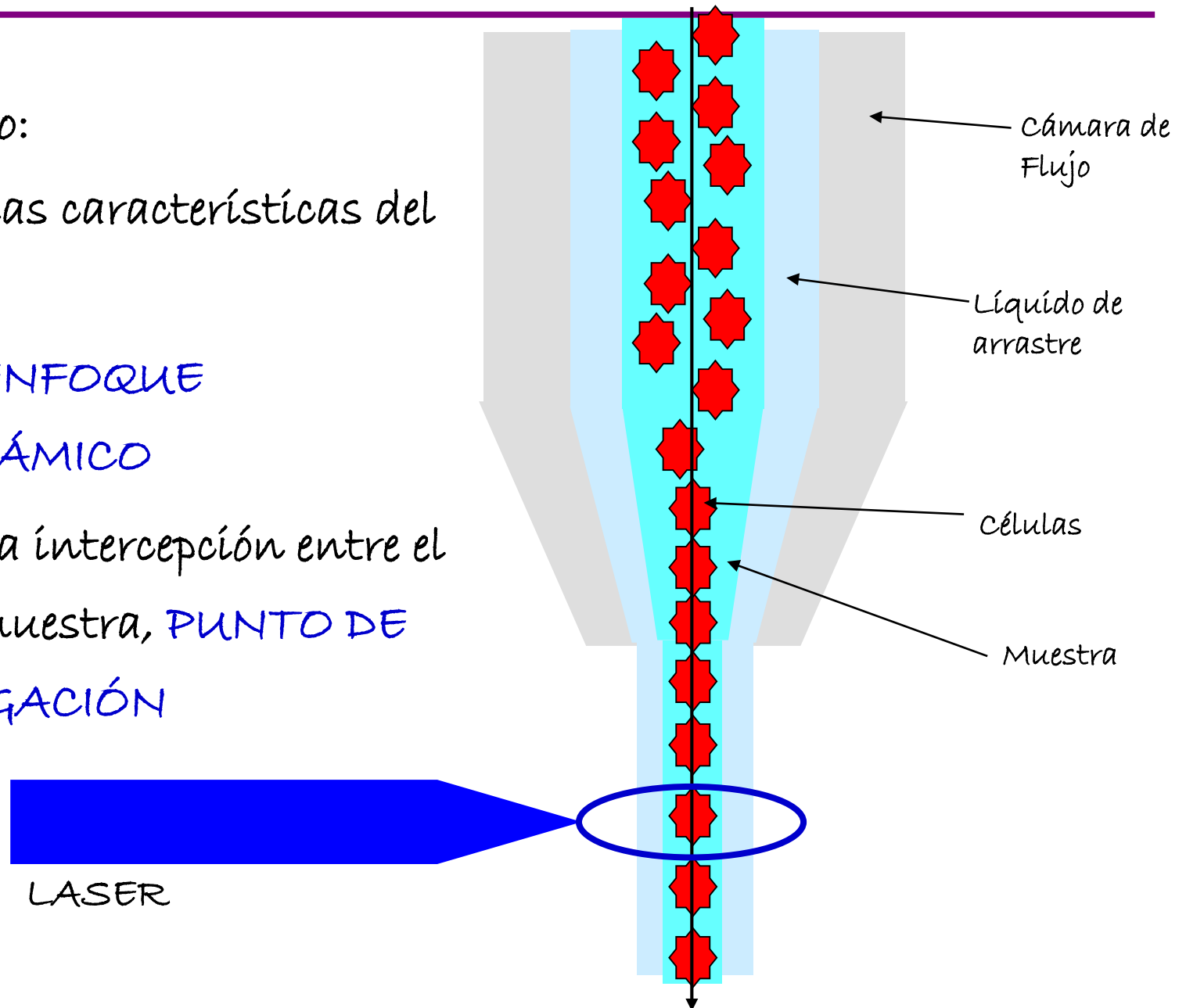


Flujo turbulento



La cámara de flujo:

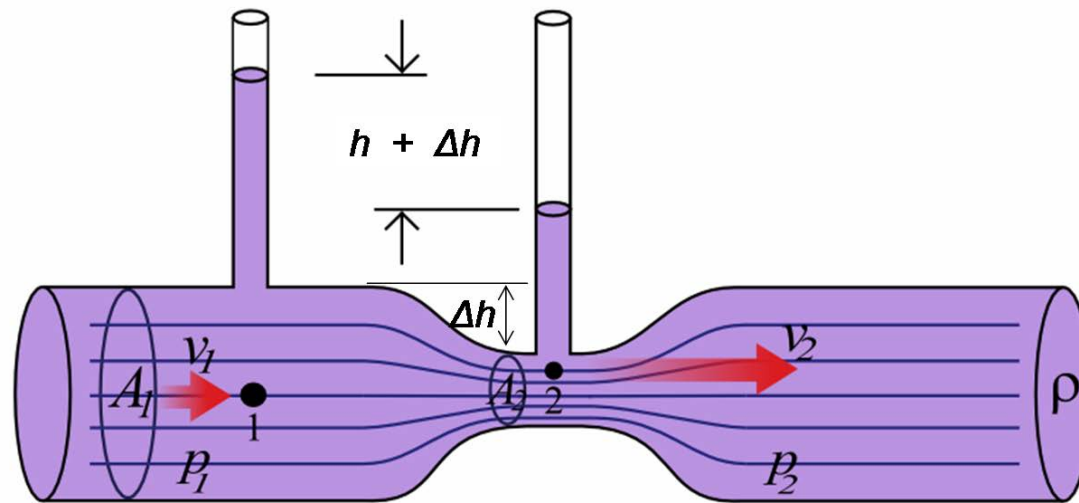
- determina las características del flujo
- permite el ENFOQUE HIDRODINÁMICO
- se produce la intercepción entre el láser y la muestra, PUNTO DE INTERROGACIÓN





Fluídica (IV)

- ◆ Enfoque hidrodinámico permite el paso individualizado de las células a través de la cámara de flujo.
- ◆ La disminución de la presión y el aumento de la velocidad en el centro del flujo provoca la focalización (Efecto Venturi 1797, explicado por el principio de Bernoulli, 1738).



- La velocidad del líquido de arrastre viene determinada por la presión a la que está sometido, no modificable en la mayoría de citómetros analizadores.
- La diferencia de presiones entre el líquido de arrastre y la muestra, determina la velocidad del paso de células por la cámara de flujo (low/med/high).

Componentes de un citómetro de flujo

Fluídica

- Flujo laminar (sistema presurizado).

- Cámara de flujo (enfoco hidrodinámico).

Óptica

- LASER (Light Amplification by Stimulated Emission of Radiation).

- Luz dispersada y fluorescencia.

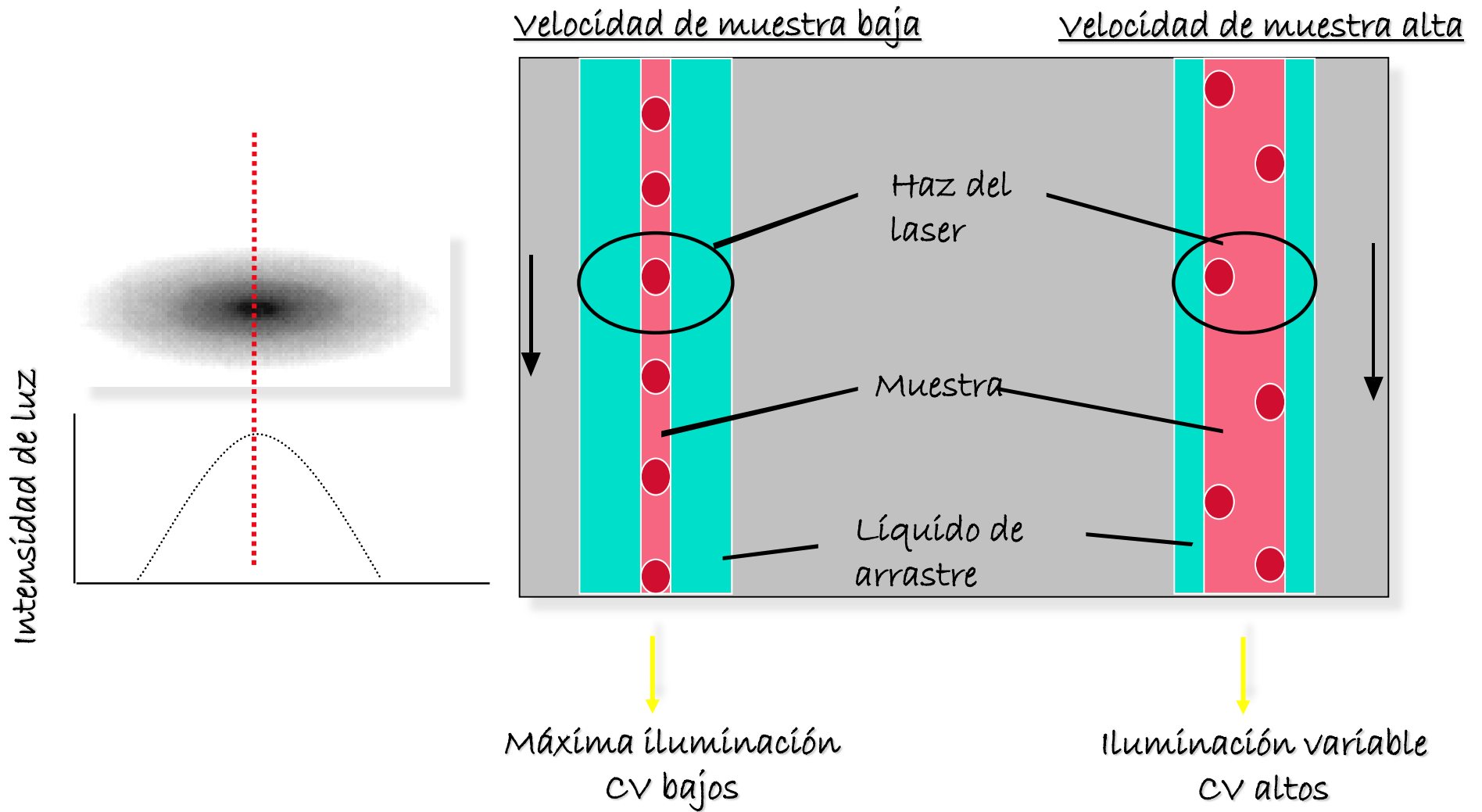
- Sistema de filtros para la recolección de la señal.

Electrónica

Informática

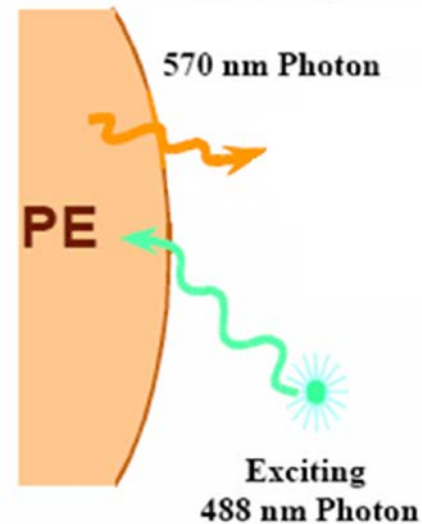


Óptica (I)



Óptica (II)

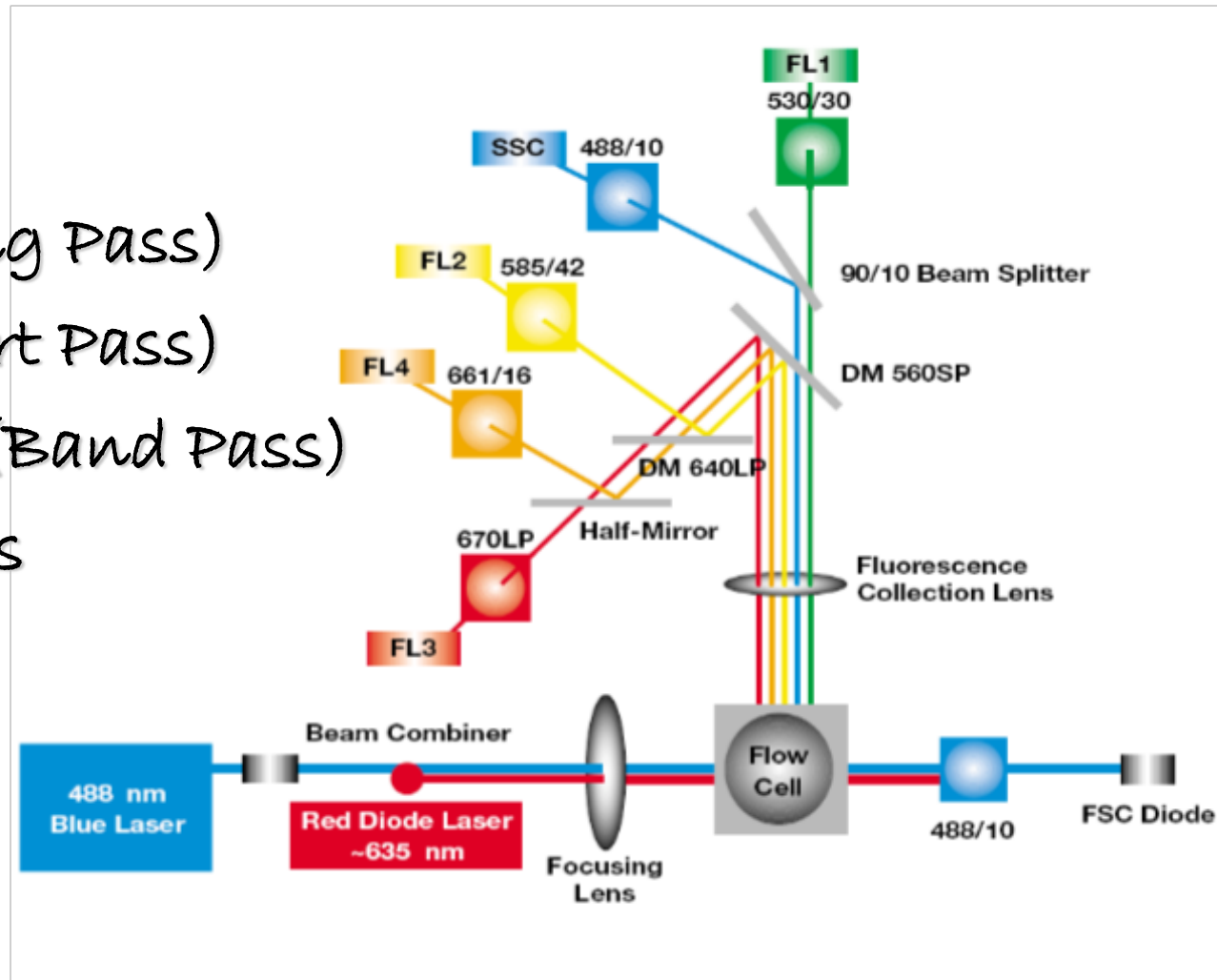
- Cada célula (evento) que atraviesa la fuente de luz (láser) genera una señal (dispersión de la luz, fluorescencia) que es recogida por espejos y filtros ópticos.
- La especificidad de la detección está controlada por la selección de longitudes de onda por parte de espejos y filtros ópticos.



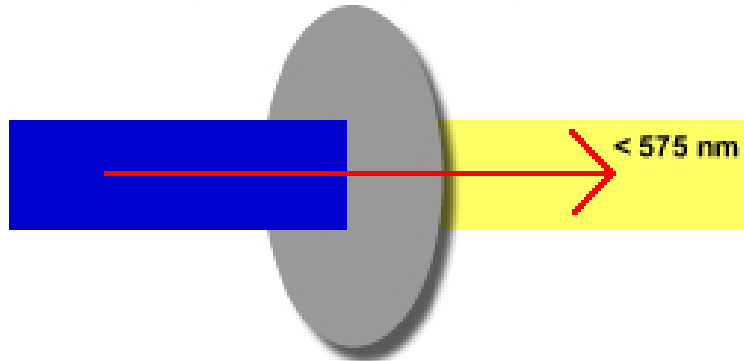
• Los filtros ópticos absorben o reflejan determinadas longitudes de onda.

• Tipos de filtros:

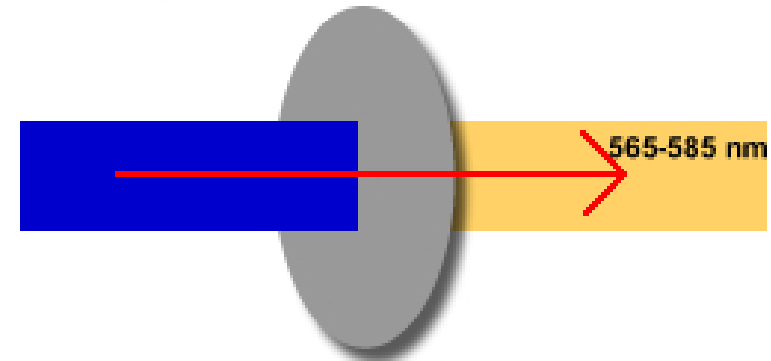
- Paso largo (Long Pass)
- Paso corto (Short Pass)
- Paso de banda (Band Pass)
- Espejos Dicroicos
- Neutros



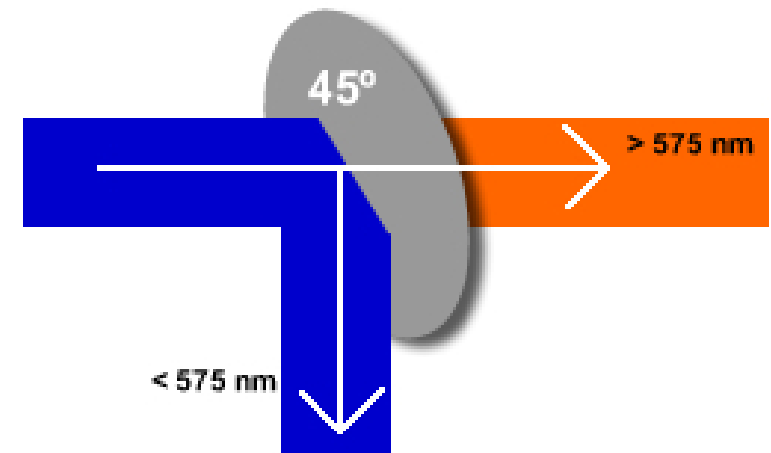
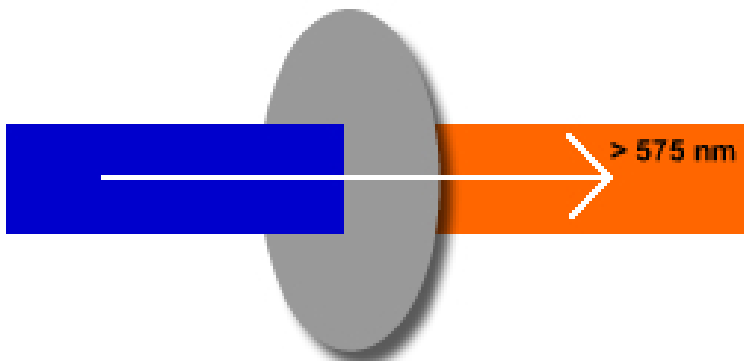
575 nm Short Pass Filter



575/20 nm Band Pass Filter



575 nm Long Pass Filter



575 nm Long Pass Dichroic Mirror

Combinando los distintos filtros podemos detectar los distintos fluorocromos que presente la muestra simultáneamente.

Qué información da un citómetro de flujo sobre una célula

1. Tamaño celular (Forward Scatter, FSC).
2. Complejidad o granulosidad celular (Side Scatter, SSC).
3. Fluorescencia (FL).

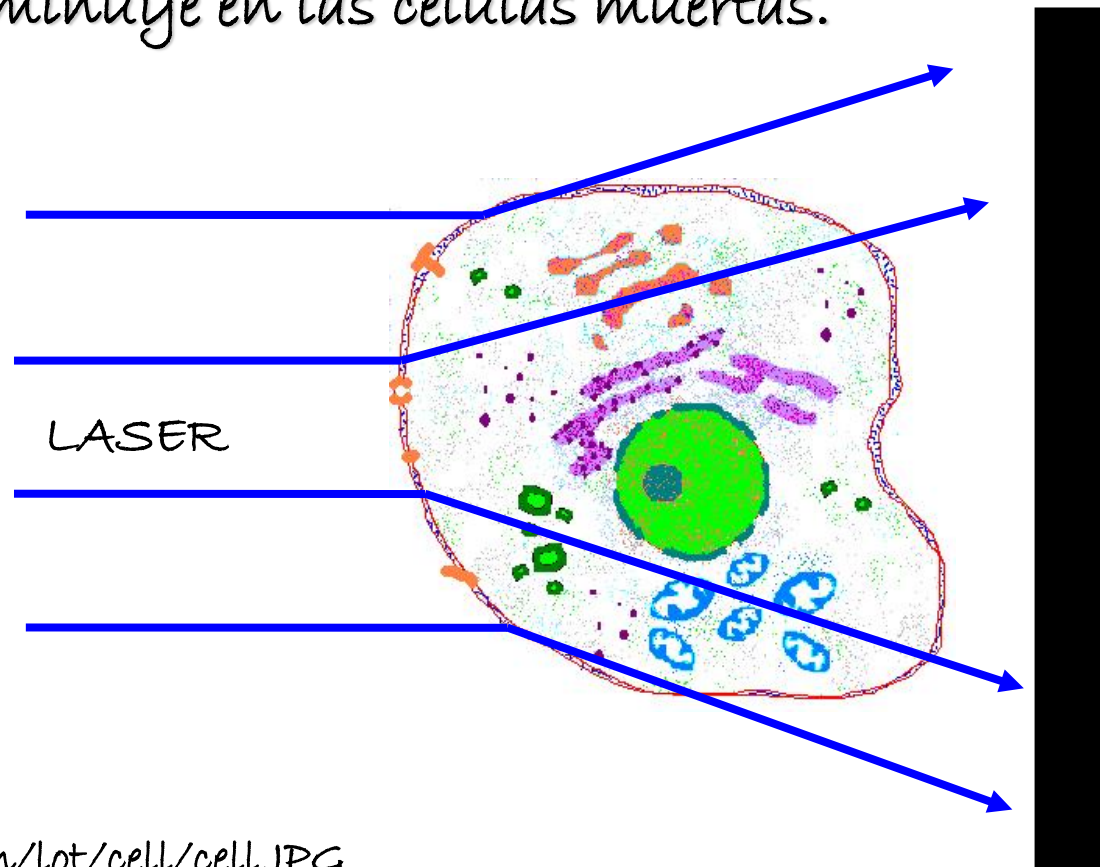
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1. Tamaño celular (FSC).
2. Complejidad o granulosidad celular (SSC).
3. Fluorescencia (FL).

1. Tamaño celular (FSC)

Difracción. Recogida entre 0.5 y 2° . Proporcional al tamaño celular.

Refracción. Entre $2-15^\circ$. Información sobre la estructura celular externa. Depende del índice de refracción entre el medio y las células. Disminuye en las células muertas.

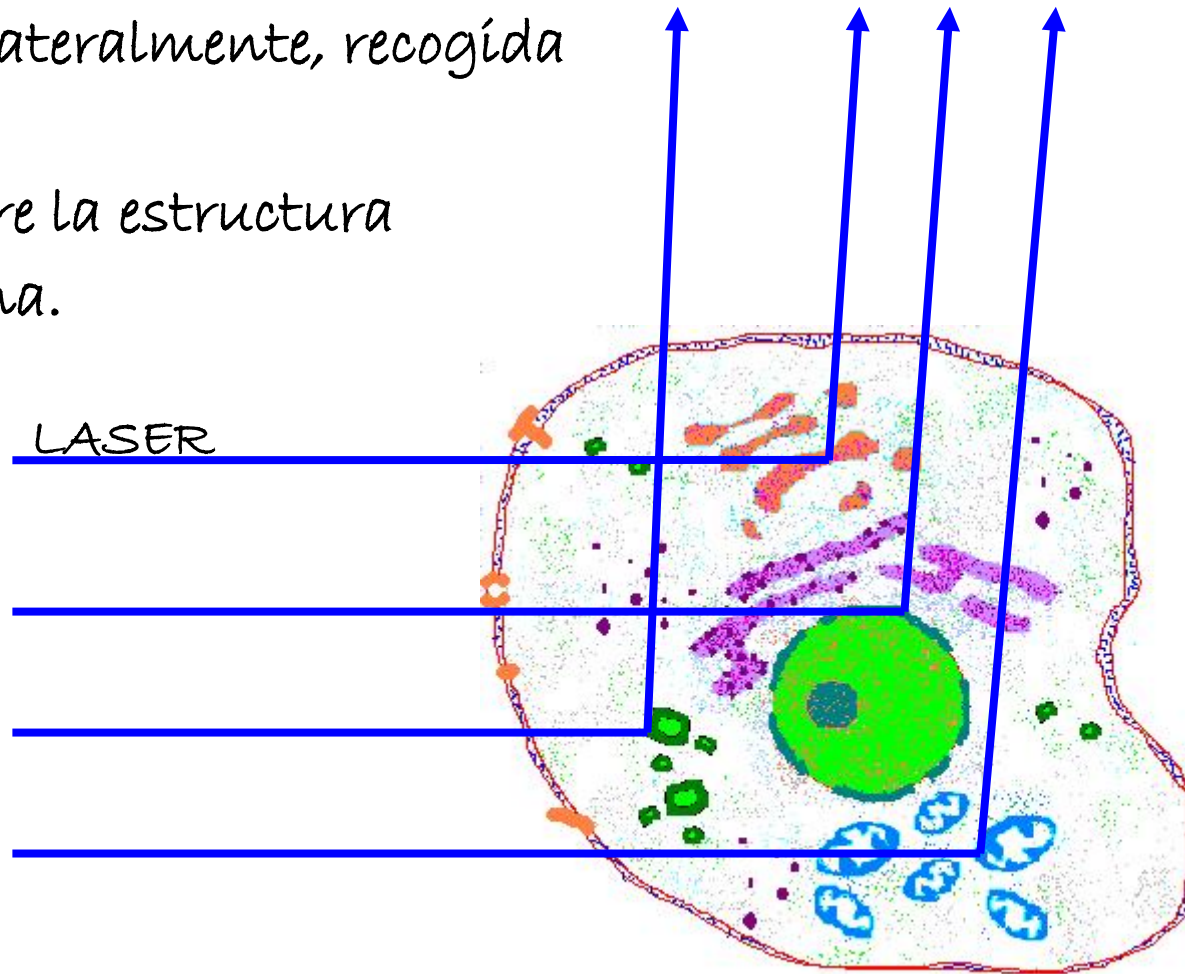


Qué información da un citómetro de flujo sobre una célula

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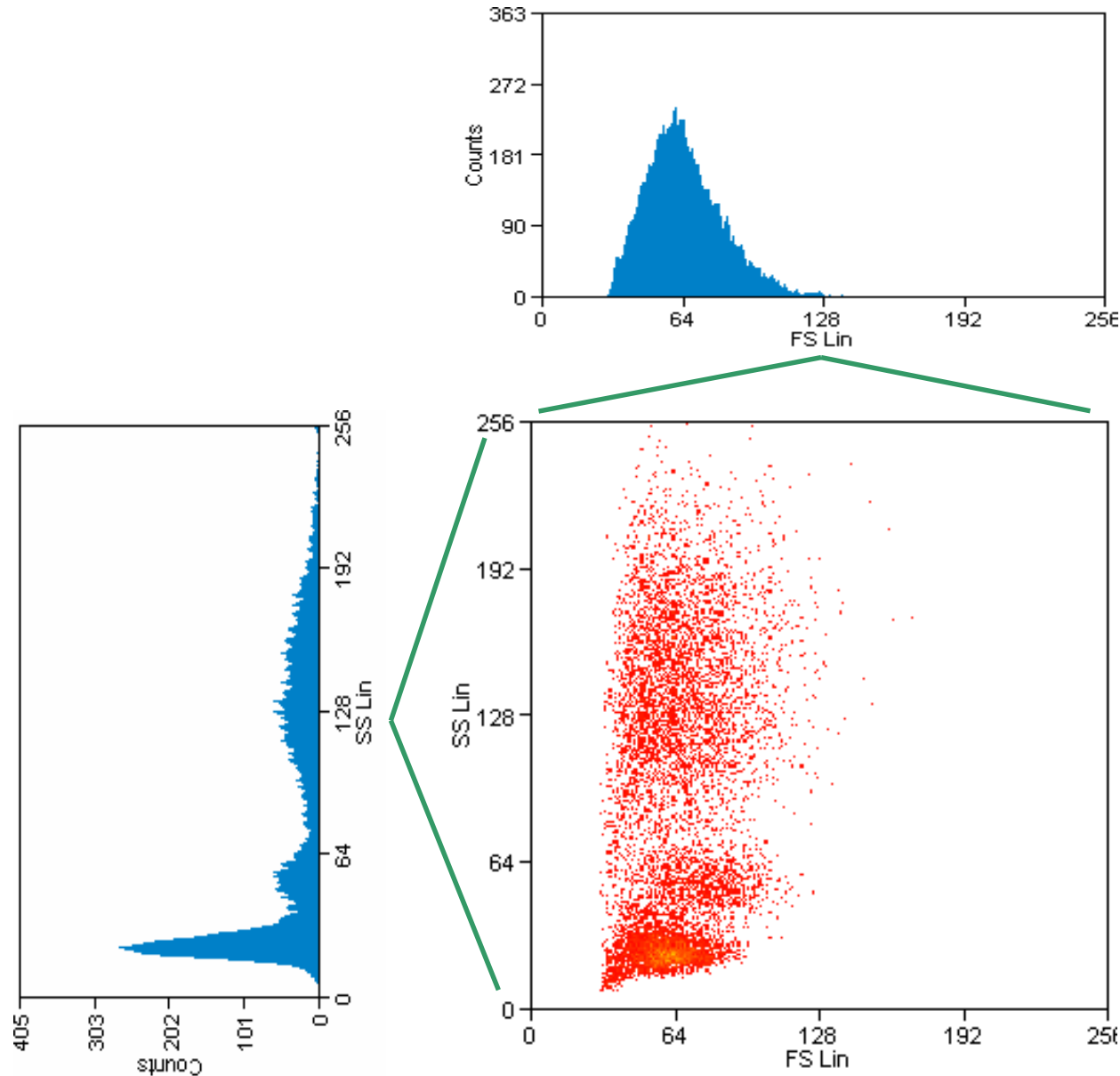
2. Complejidad o granulosis celular (SSC)

Luz dispersada lateralmente, recogida a 90° .
Nos informa sobre la estructura celular interna.





2. Complejidad o granulosis celular (SSC)



Qué información da un citómetro de flujo sobre una célula

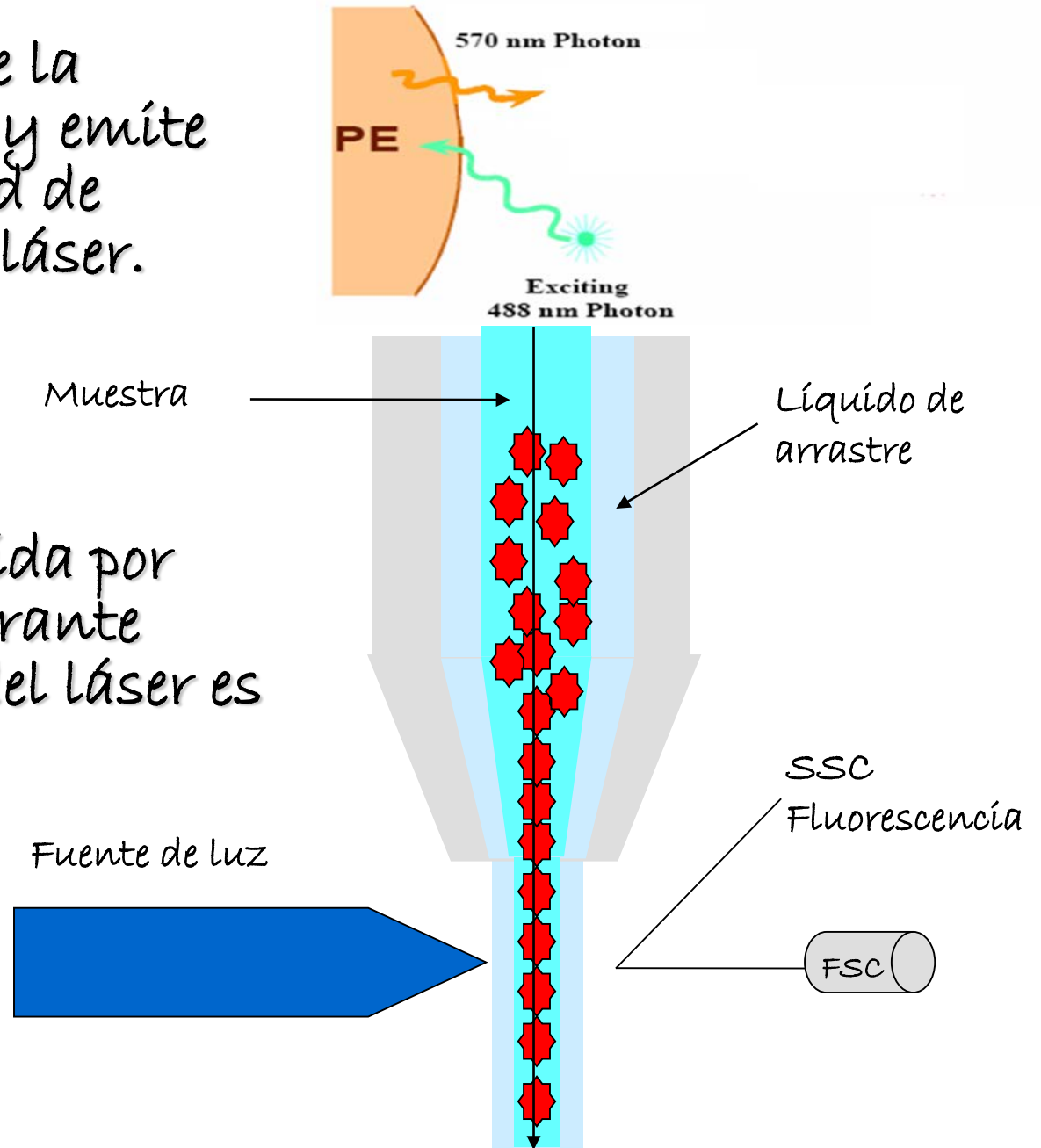
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3. Fluorescència (FL).

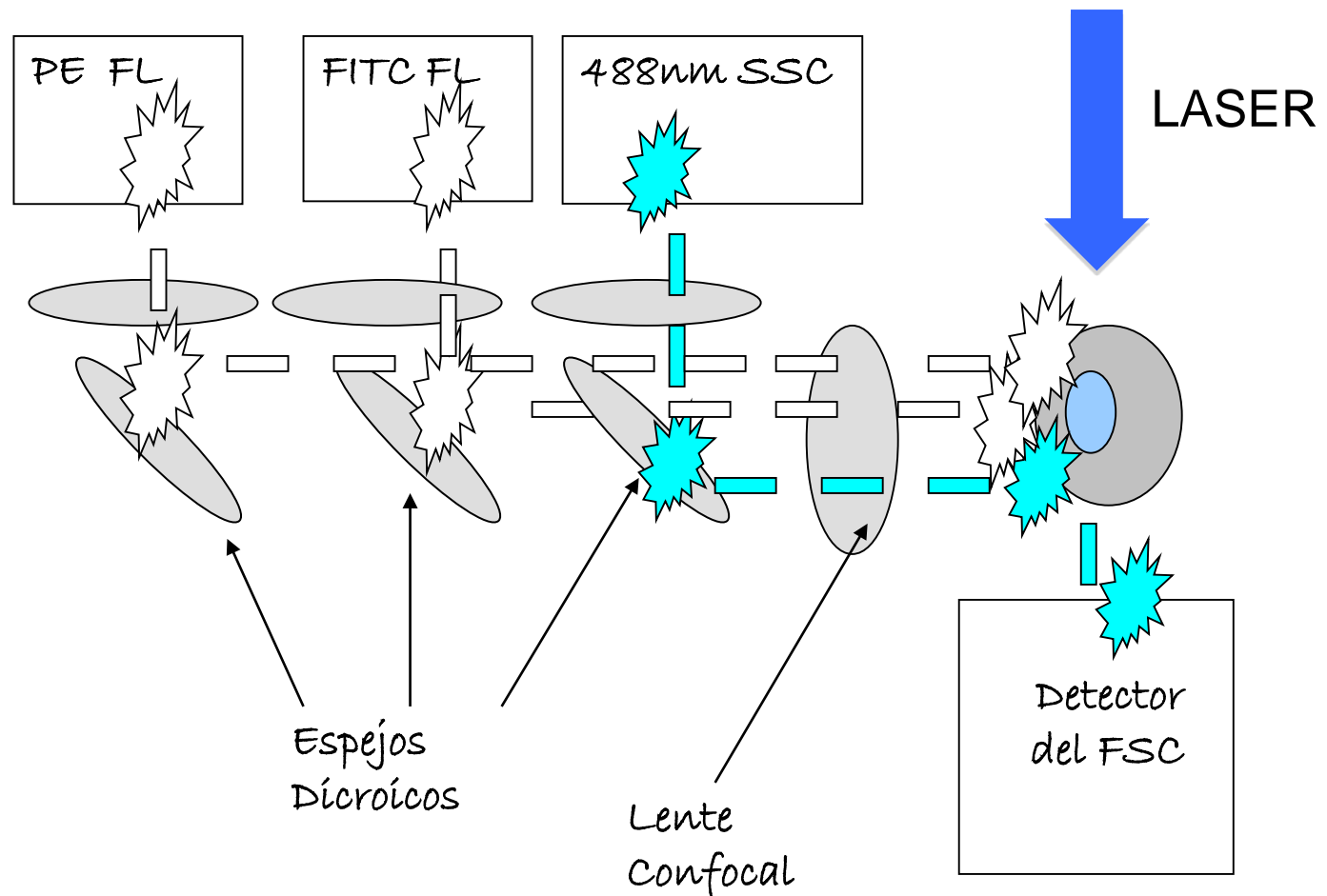
• El fluorocromo absorbe la energía del láser, vibra y emite fotones de una longitud de onda mayor que la del láser.

• La fluorescencia emitida por cada fluorocromo o colorante debida a la excitación del láser es recogida a 90°.



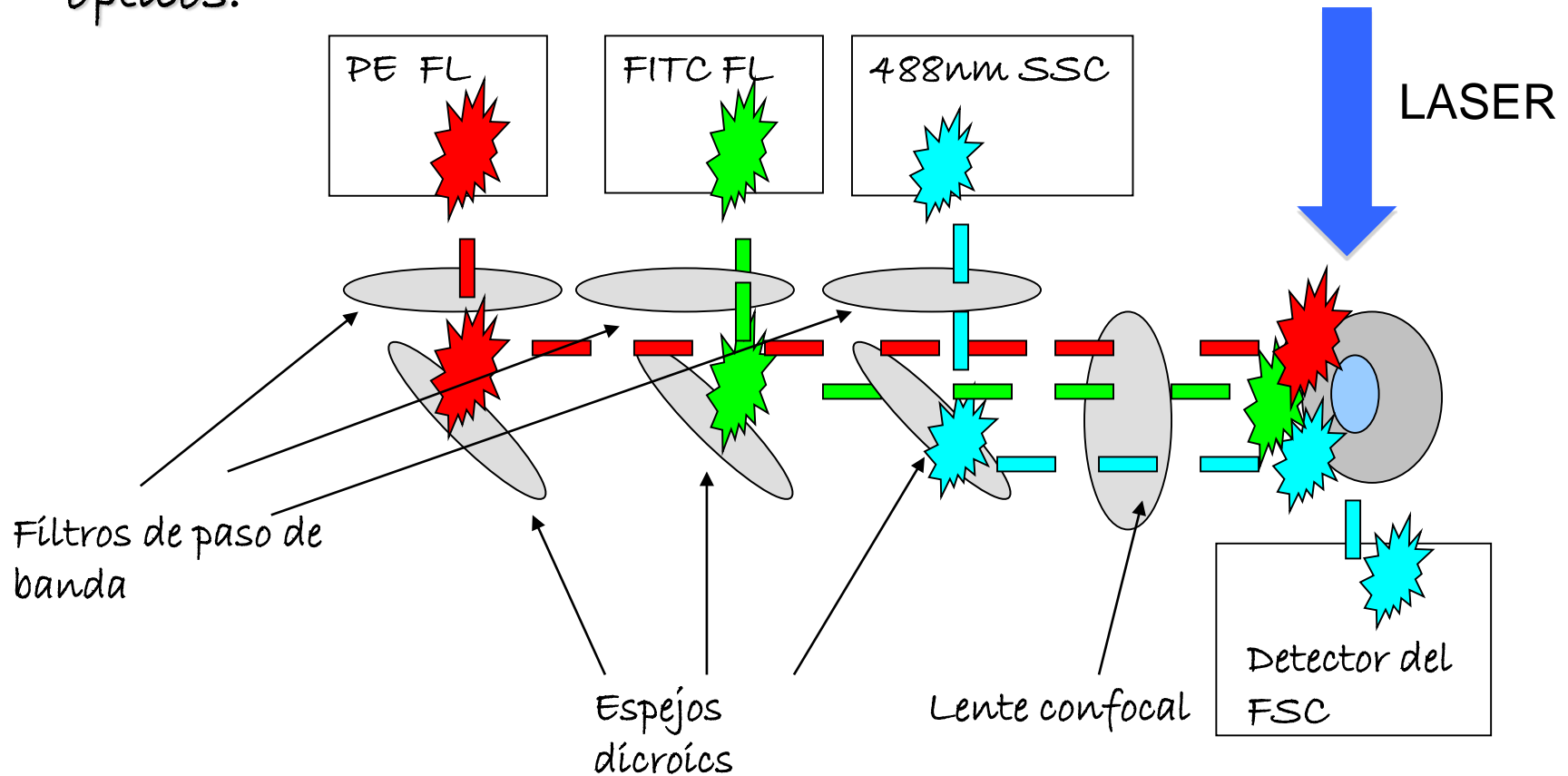
3. Fluorescència (FL).

Las células negativas también son detectadas.



3. Fluorescència (FL).

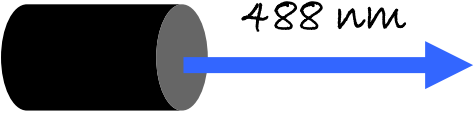











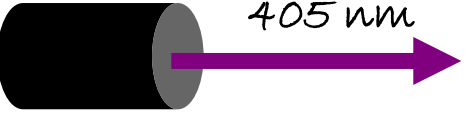



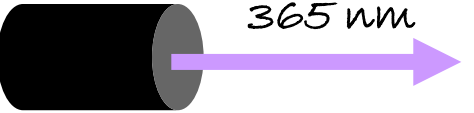



La especificidad de la detección está controlada por la selección de las longitudes de onda mediante espejos y filtros ópticos.



3. Fluorescència (FL).

EXITACIÓ

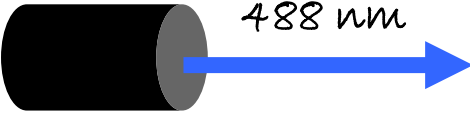










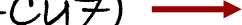








EMISIÓ

 <p>488 nm</p>	Fluoresceïna (FITC)		519 nm
	Ficoeritrina (PE)		578 nm
	Ficoeritrina-Cianina5 (PECy5)		670 nm
	Peridínin-clorofil·la (PerCP)		675 nm
 <p>561 nm</p>	Ficoeritrina (PE)		578 nm
	Ficoeritrina-Cianina5 (PECy5)		670 nm
 <p>633 nm</p>	Aloficocianina (APC)		660 nm
	Aloficocianina H7 (APC-H7)		785 nm
	Aloficocianina-Cianina7 (APC-Cy7)		767 nm
 <p>405 nm</p>	Alexa405		421 nm
	Alexa430		540 nm
	Pacífic Blue		455 nm
 <p>365 nm</p>	Hoechst33342		460 nm
	DAPI		461 nm
	Indo1		401 nm

3. Fluorescència (FL).

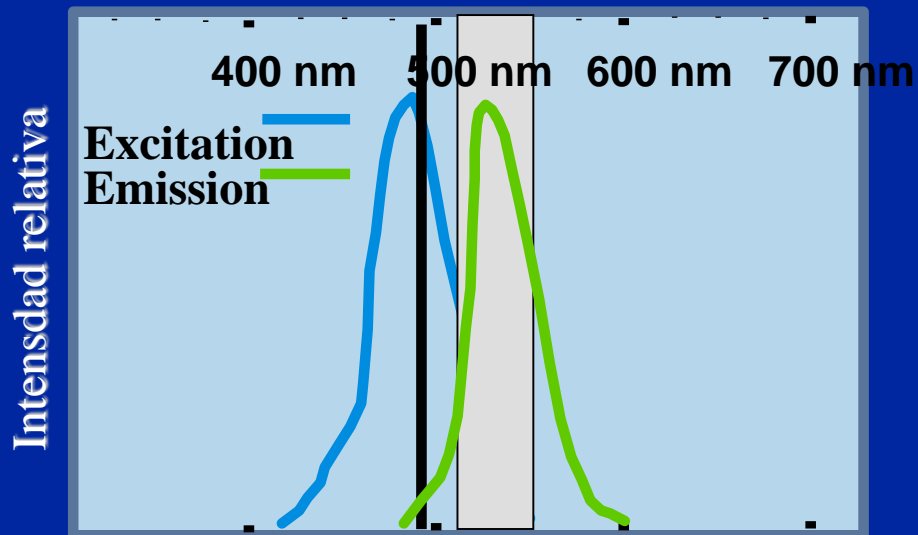
EXITACIÓ

EMISIÓ

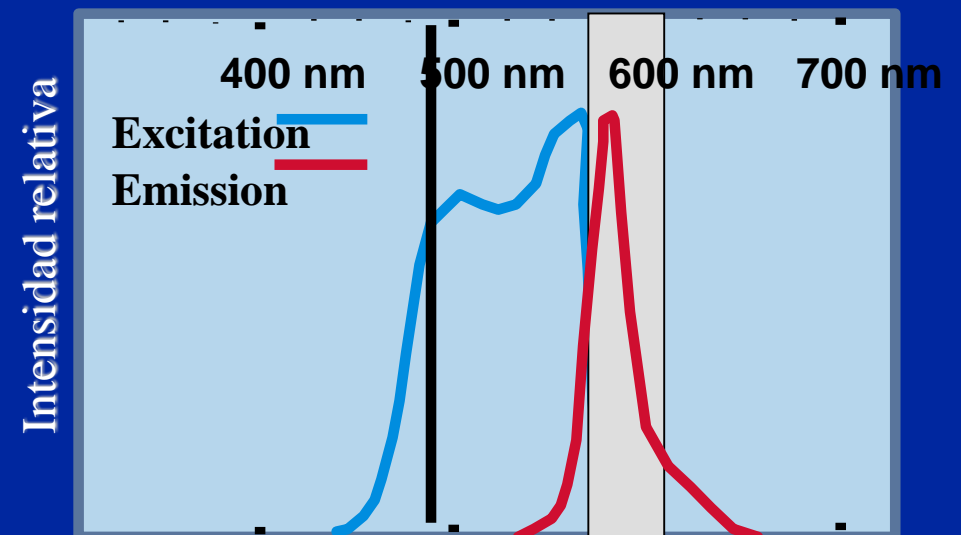
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3. Fluorescència (FL).

Ensayo multicolor: combinación de 2 o más fluorocromos



Fluoresceína (FITC)

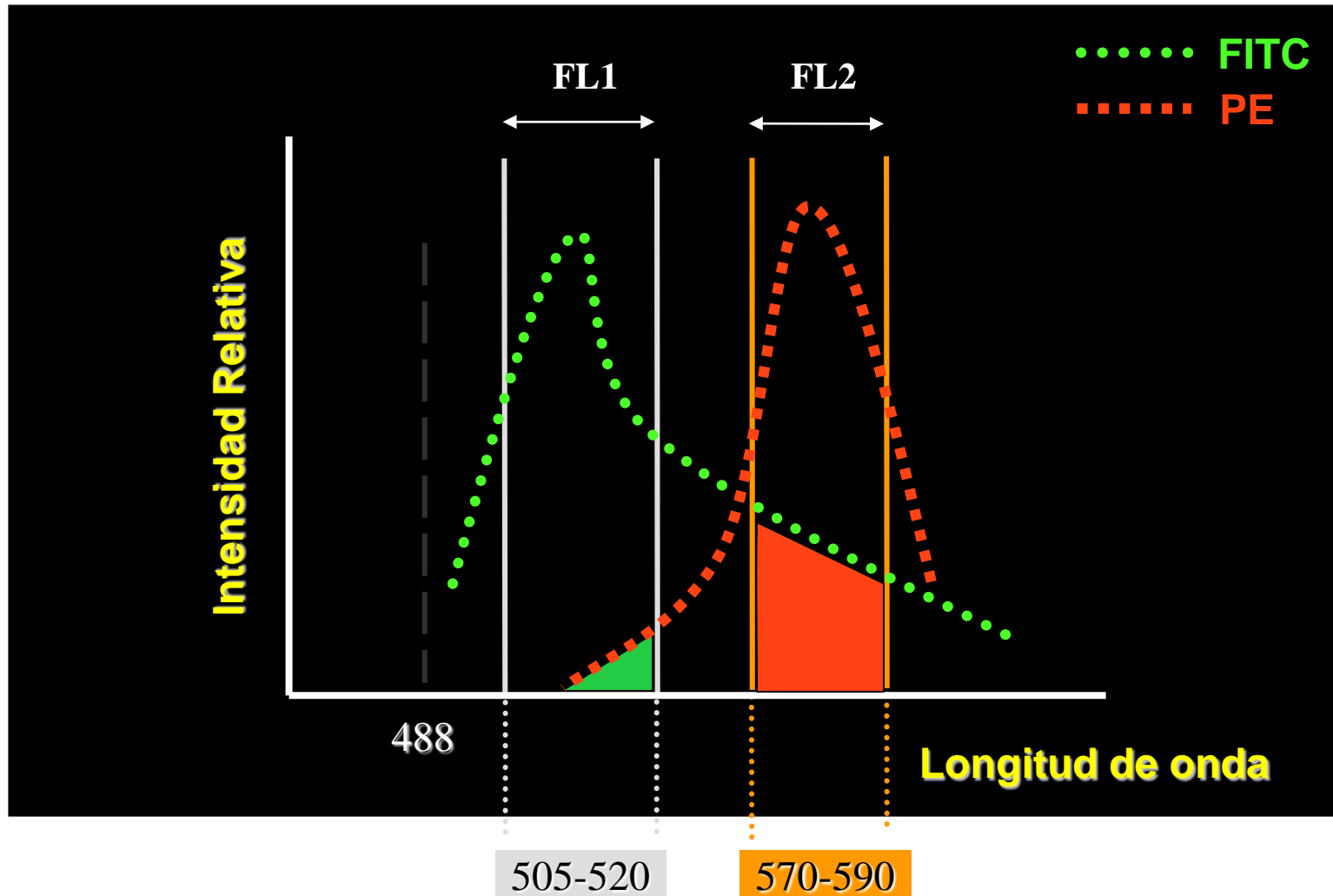


Ficoeritrina (PE)



3. Fluorescència (FL).

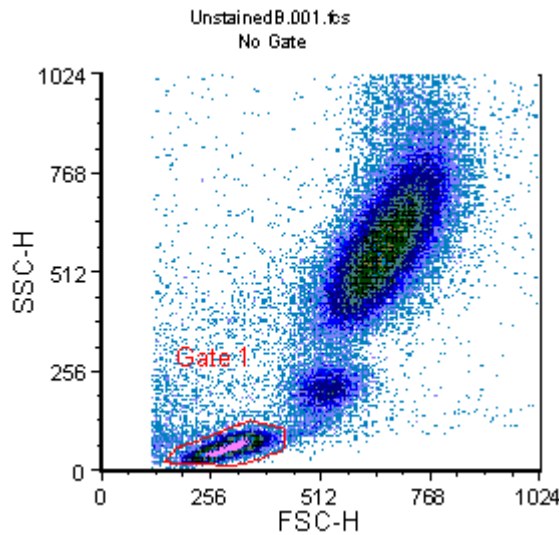
Solapamiento y Compensación



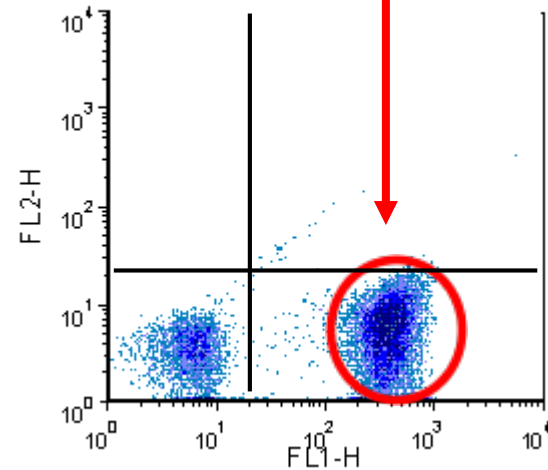
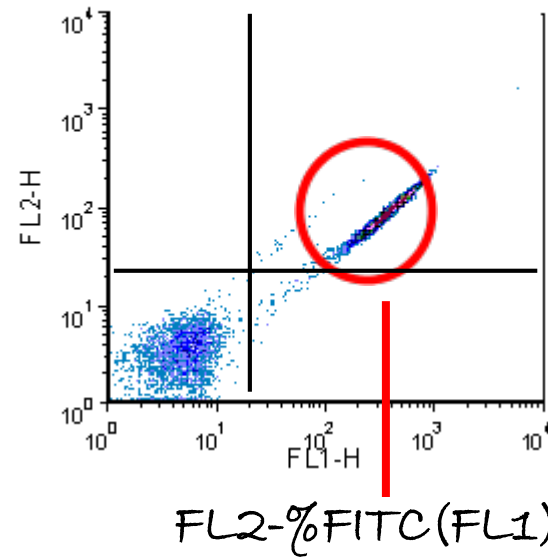


3. Fluorescència (FL).

Ejemplo



Seleccionamos la población de interés



FCS Filename	Gate	Y Arithmetic Mean
CD3 FITC.002	UL	32,01
CD3 FITC.002	UR	87,85
CD3 FITC.002	LL	3,42
CD3 FITC.002	LR	21,19

Solamente deberíamos detectar fluorescencia en el canal del FITC (FL1).

FCS Filename	Gate	Y Arithmetic Mean
CD3 FITC.002	UL	0,0
CD3 FITC.002	UR	75,81
CD3 FITC.002	LL	3,31
CD3 FITC.002	LR	3,44

Una vez compensada solamente tenemos fluorescencia en el detector que nos interesa.

3. Fluorescència (FL).

Cómo compensamos?

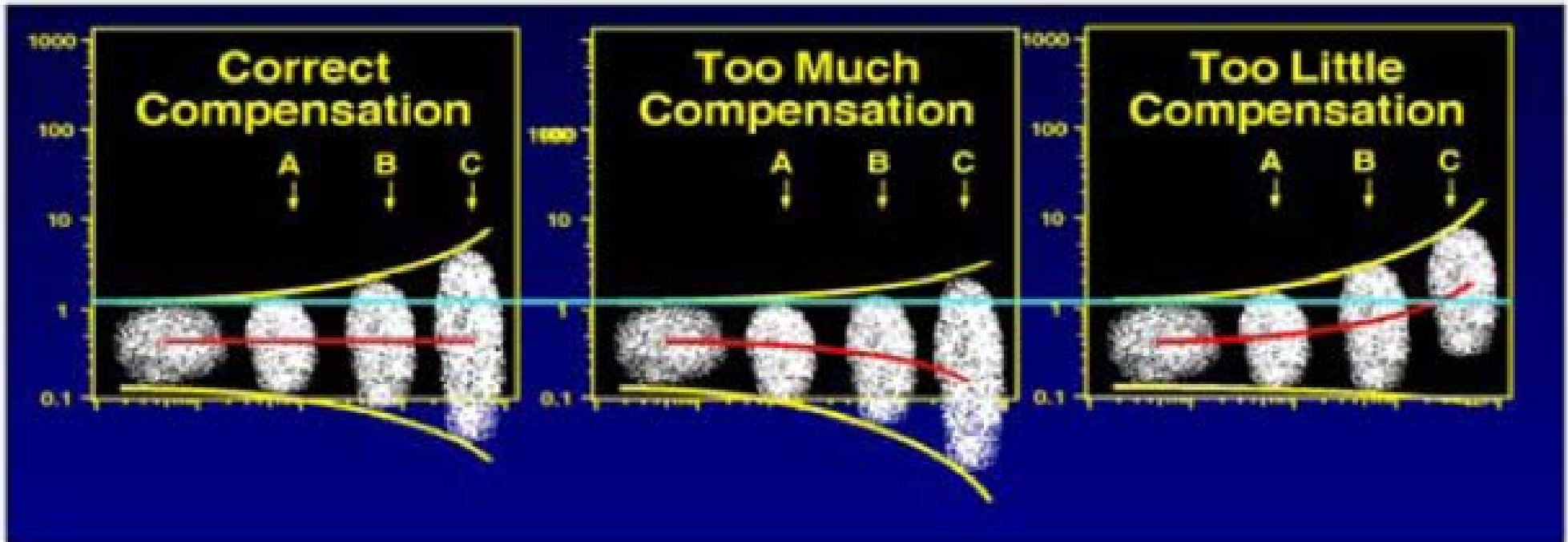
- Para compensar lo que hacemos es restar señal de un detector a otro.
- Se necesitan controles positivos con una sola fluorescencia conocida.
- Los equipos analógicos restan la señal antes de procesarla y por lo tanto, no se puede recuperar.
- Los equipos digitales procesan la señal entera y después aplican matrices.

$$\%FL2 \text{ compensación} = \frac{\text{Median FL2pos} - \text{Median FL2neg} \times 100}{\text{Median FL1pos} - \text{Median FL1neg}}$$



3. Fluorescència (FL).

Comprovación de la compensación



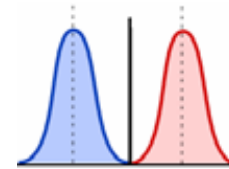
3. Fluorescència (FL).

Qué fluorocromos escogemos y porqué?

Fluorocromos más brillantes para los marcajes "dím" EVITANDO el solapamiento de los espectros con la población brillante!

Com se define y se mide el brillo de un fluorocromo?

Por su capacidad de discriminar Células DIM de las Negativas así como las Células Negativas del Background.



Esta capacidad está influenciada por el CV, el ruido electrónico, background, autofluorescencia celular....

Por lo tanto, una medida de Sensibilidad de resolución de un fluorocromo es el **ÍNDICE DE TINCIÓN**.

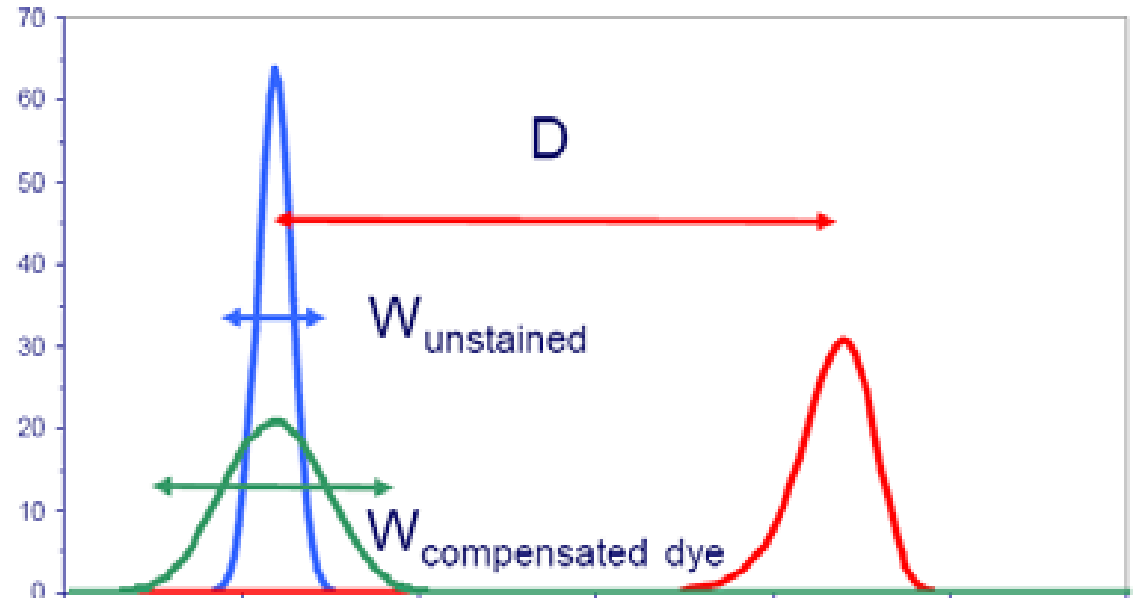
3. Fluorescència (FL).

Índice de Tinción (Stain Index)

Es una medida del brillo

D = Distancia de la población positiva de la negativa

W = Anchura de la población negativa



$$SI_{\text{parametric}} = \frac{\text{mean}_{\text{positive}} - \text{mean}_{\text{negative}}}{2 \times SD_{\text{negative}}}$$

1. Interesan fluorocromos con elevados índices de Tinción.
2. Interesa MINIMIZAR las compensaciones.

3. Fluorescència (FL).

Table 1. Stain index of various anti-CD4 fluorochrome conjugates on a BD LSR II flow cytometer.

Reagent	Filter	Stain Index
PE	585/40	356.3
¹ Alexa Fluor® 647	660/20	313.1
¹ APC	660/20	279.2
PE-Cy7	780/60	278.5
² PE-Cy5	695/40	222.1
² PerCP-Cy5.5	695/40	92.7
³ Alexa Fluor® 488	530/30	75.4
³ FITC	530/30	68.9
² PerCP	695/40	64.4
APC-Cy7	780/60	42.2
Alexa Fluor® 700	720/45	39.9
Pacific Blue™	440/40	22.5
AmCyan	525/50	20.2

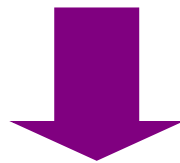
^{1,2,3} Fluorochromes listed with the same superscript number are read in the same detector, and thus would not normally be used in combination.

3. Fluorescència (FL).

Ejemplo: Estudiamos la expresión de CD62L (débil) en la población CD8 (alta).

 CD8 FITC/CD62L PE

- El FITC tiene un elevado grado de solapamiento con el PE.
- Compromete la resolución en el canal del PE.
- Menor Sensibilidad de resolución en la población problema (CD62L).



1. Escoger otro fluorocromo para el CD8 con menos solapamiento en PE (ex. PerCP-Cy5.5 ó APC).
2. Escoger otro fluorocromo para el CD62L igual de brillante, que no se solape con el FITC (ex. APC)

3. Fluorescència (FL).

Tándems

- 2 fluorocromos unidos químicamente:

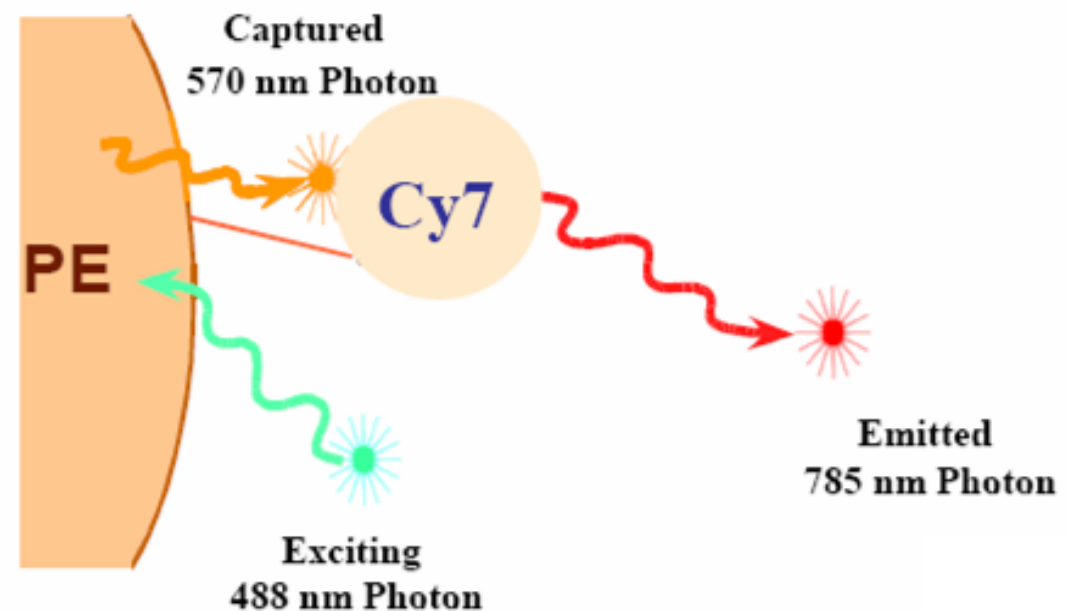
PE-Cy5

PE-Cy7

APC-Cy5

APC-Cy7

PerCP-Cy5.5...



Inconvenientes:

- Pueden degradarse a consecuencia de: luz, fijación, elevadas temperaturas...
- Emitiendo en el detector del fluorocromo parental:
Ej. APC-Cy7 al degradarse emitirá en APC y PE-Cy7 en PE dando Falsos positivos

3. Fluorescència (FL).

Tándems

Prevencción:

- Minimizar la exposición a la luz, calor y evitar fijaciones con formaldehído.
- Sí no se pueden evitar las fijaciones: usar soluciones estabilizantes.



Alternativa: APC-H7

- Más estable a la luz y temperatura.
- Más estable a la fijación con formaldehído.
- Bajo solapamiento en otros detectores.

3. Fluorescència (FL).

Controles para un experimento de citometría:

- control de autofluorescència: células sin marcar.
- control isotópico
- Controles compensación: Single color, FMOs

3. Fluorescència (FL).

Controles para un experimento de citometría:

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3. Fluorescència (FL).

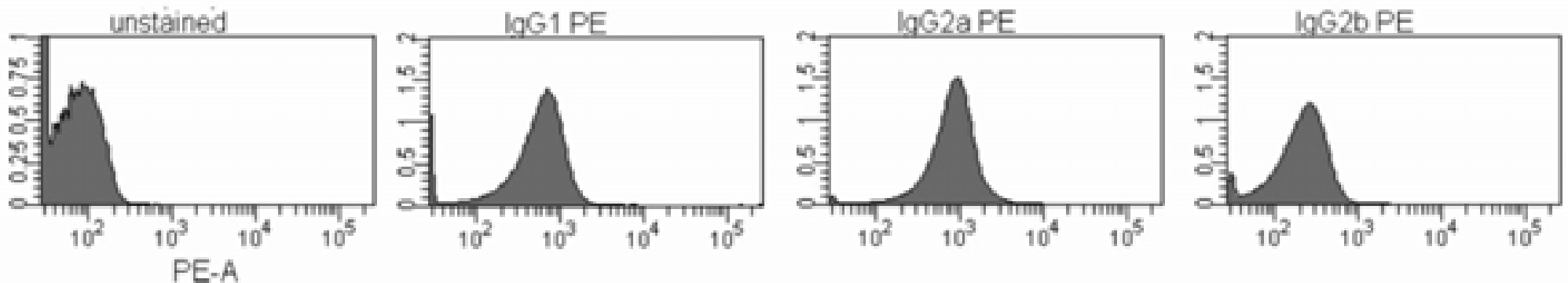
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3. Fluorescència (FL).

Controles Isotípicos:

- Tenemos en cuenta la tinción inespecífica de un anticuerpo con un ISOTIP determinado conjugado a un fluorocromo en concreto.
Ex. Mouse IgG1 FITC
- Distintos isotipos presentan distinto background:



3. Fluorescència (FL).

Controles para un experimento de citometría:

- control de autofluorescència: células sin marcar.
- control isotópico
- Controles compensación: Single color, FMOs

3. Fluorescència (FL).

Controles de compensación: se realizan para aquellos experimentos en los que se combinan 2 o más fluorescencias.

- Single Color: cada fluorocromo por separado.
- FMOS



3. Fluorescència (FL).

FMO: "Fluorescence Minus One"

- Los controles FMO contienen todos los anticuerpos de la muestra menos 1.
- El detector en el cual no hay anticuerpo es el que el FMO proporciona el control negativo:

Ejemplo.

CD3 CD4 CD8 CD25 CD127 CD45.

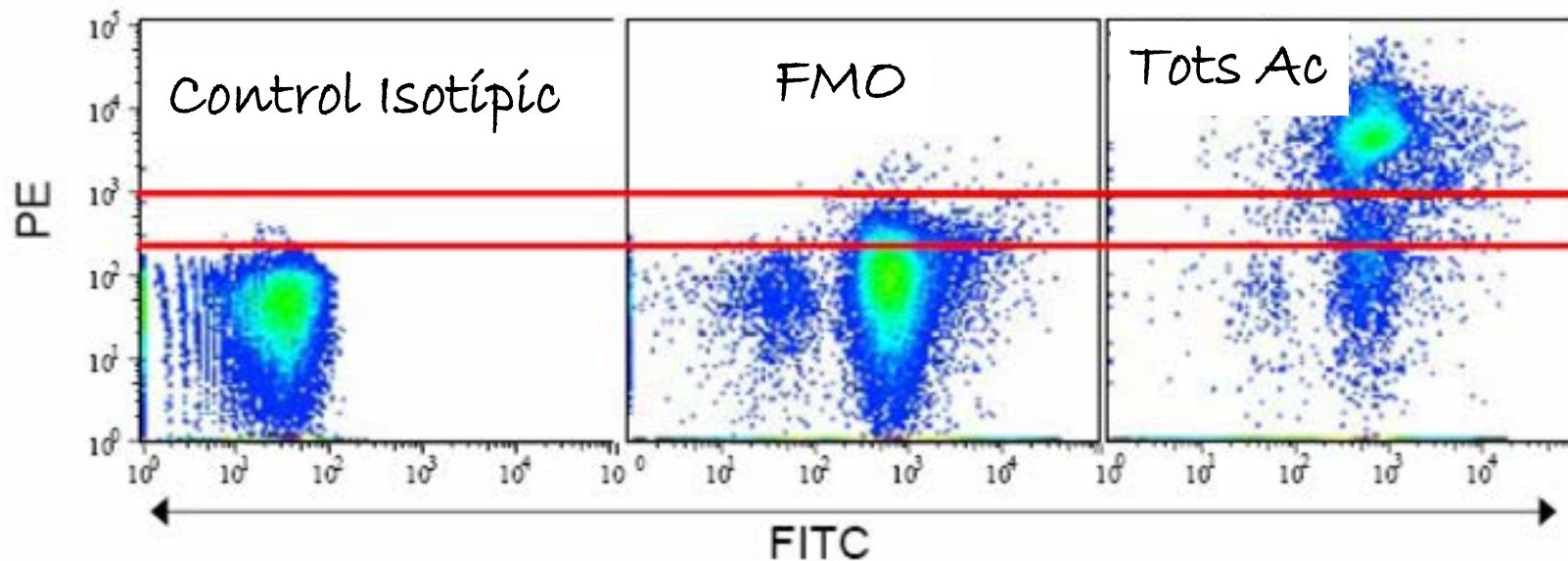
El FMO para situar la región de positividad para el CD25 sería:

- CD3 CD4 CD8 --- CD127 CD45

3. Fluorescència (FL).

FMO: "Fluorescence Minus One"

Ejemplo: CD3 FITC / CD4 PE / CD8 PE-Cy5 / CD45RO PE-Cy7.

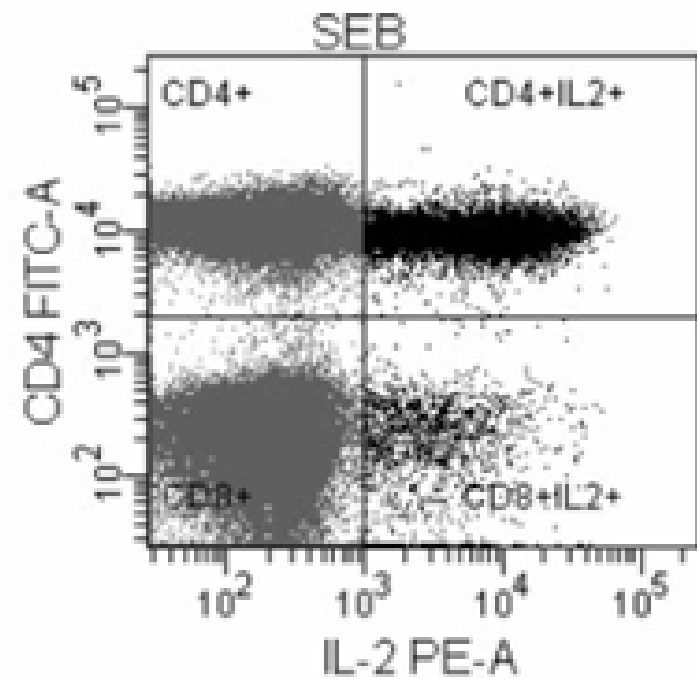
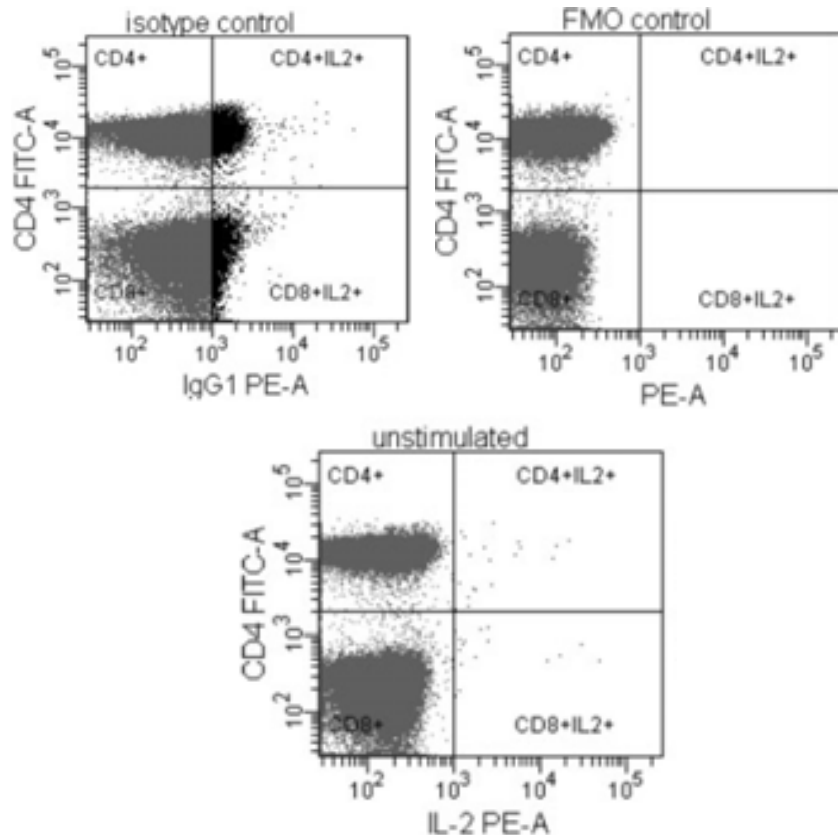




3. Fluorescència (FL).

Ejemplo. CD4 FITC / IL-2 PE

¿Dónde situamos el cuadrante de positividad para la IL-2 PE?



Dades procedents Joseph Trotter,
Cytometry Part A 69A:1037-1042 (2006)

3. Fluorescència (FL).

Otros aspectos a considerar:

- Unión inespecífica:

ex. PE-Cy5 se pueden unir inespecíficamente a las Fc expresadas en linfocitos B, monocitos y células dendríticas.

Solución:

- Células de Rata o Ratón: usar bloqueo de Fc comerciales.
 - Células humanas: Cocktails de IgGs, suero humano.
 - Usar otros tandems de baja o nula afinidad por los receptores Fc: PE-TexasRed y PE-Cy7.
- Efecto del tamaño del fluorocromo en marcajes intracelulares: No influye! Recomendación usar fluorocromos brillantes.

3. Fluorescència (FL).

Creación de paneles multicolor

1. Seleccionar los fluorocromos de acuerdo al instrumento que vamos a usar.
2. Adecuar el brillo de los fluorocromos al nivel de expresión del antígeno.
 - Fluorocromos Brillantes - Antígenos poco expresados
 - Fluorocromos débiles - Antígenos muy expresados
3. Minimizar el solapamiento de espectros de emisión en marcadores que se expresan en la misma célula.
4. Evitar combinaciones que generen falsos positivos en caso de degradación del fluorocromo.
5. Intentar, dentro de lo posible, usar fluorocromos excitados por el LASER rojo en antígenos que se expresen en células con alta autofluorescencia.

Componentes de un citómetro de flujo

Fluídica

- Flujo laminar (sistema presurizado).
- Cámara de flujo (enfoco hidrodinámico).

Óptica

- LASER.
- Luz dispersada y fluorescencia.
- Sistema de filtros para la recolección de la señal.

Electrónica

- Amplificación mediante PMTs.
- Conversión a valores digitales.

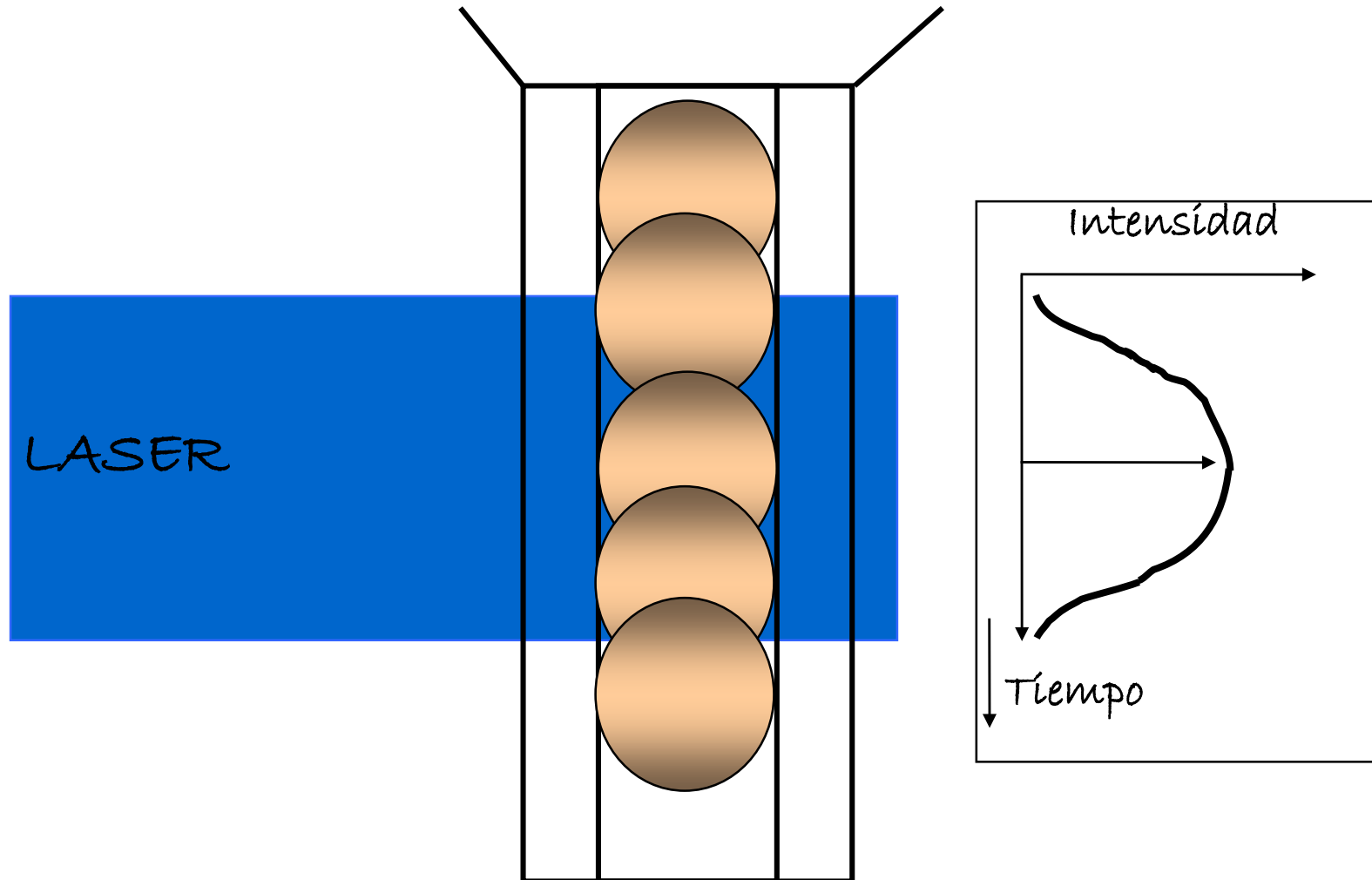
Informática



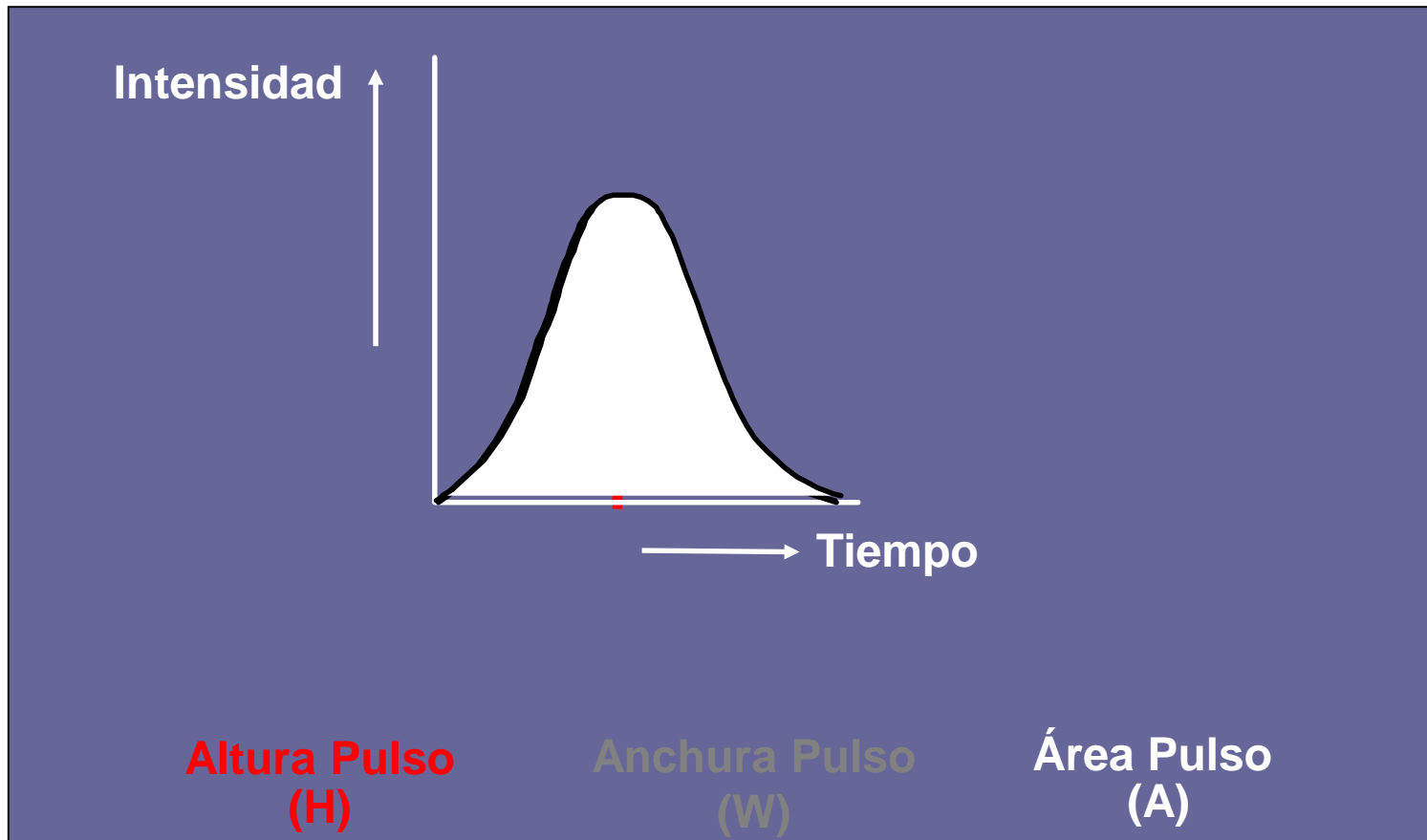
Electrónica (I)

La fluorescencia generada por cada célula es recogida por diversos detectores fotomultiplicadores de la señal (PMTs).

PMTs convierten la señal luminosa recibida en pulsos eléctricos.

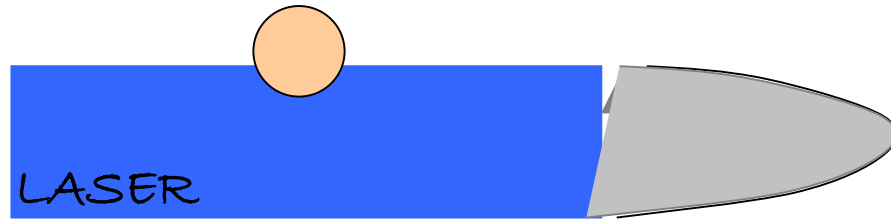


De cada señal podemos obtener la altura, anchura y área.

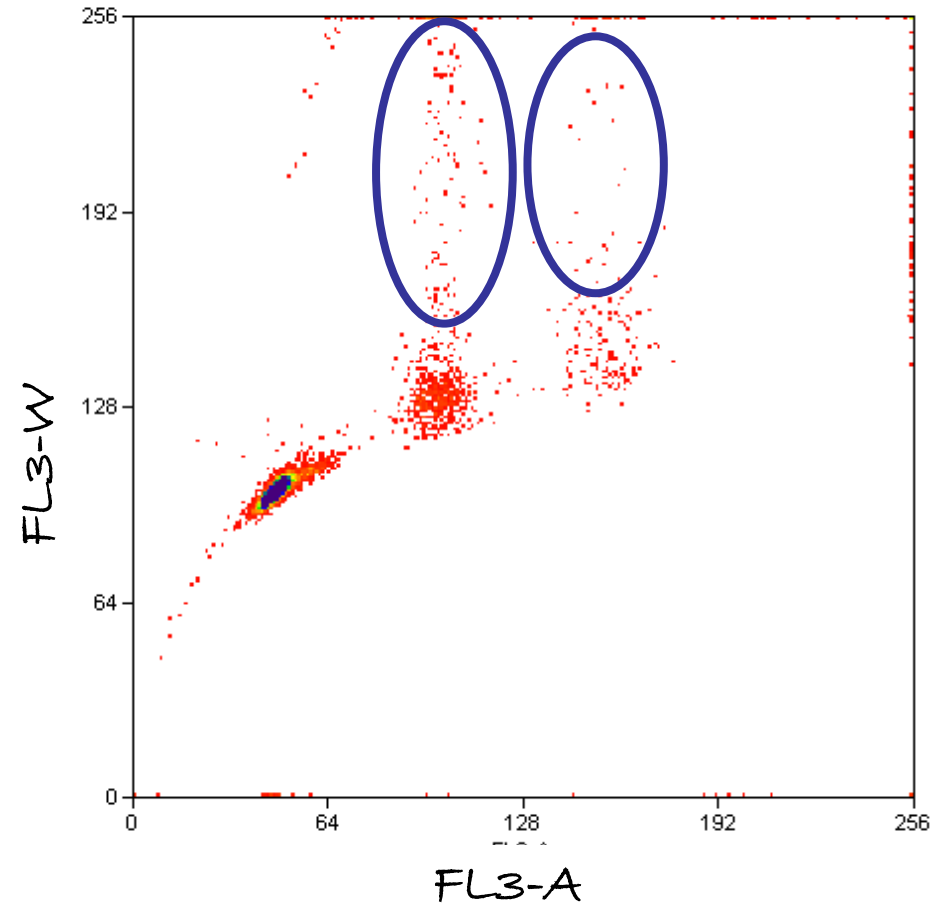
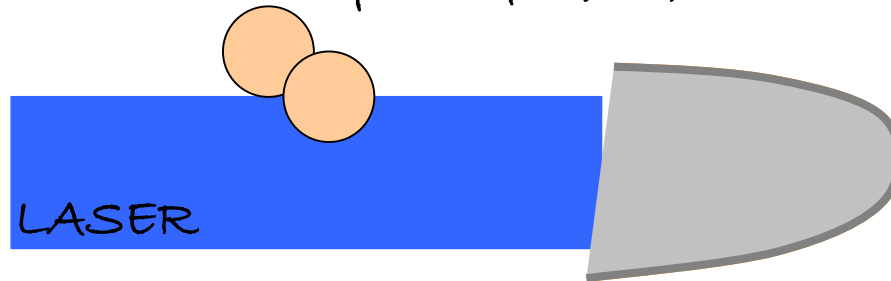




1 Células en fase G₂/M (4n)



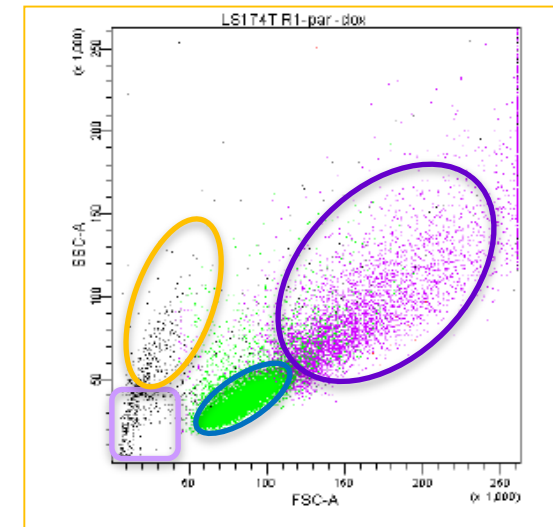
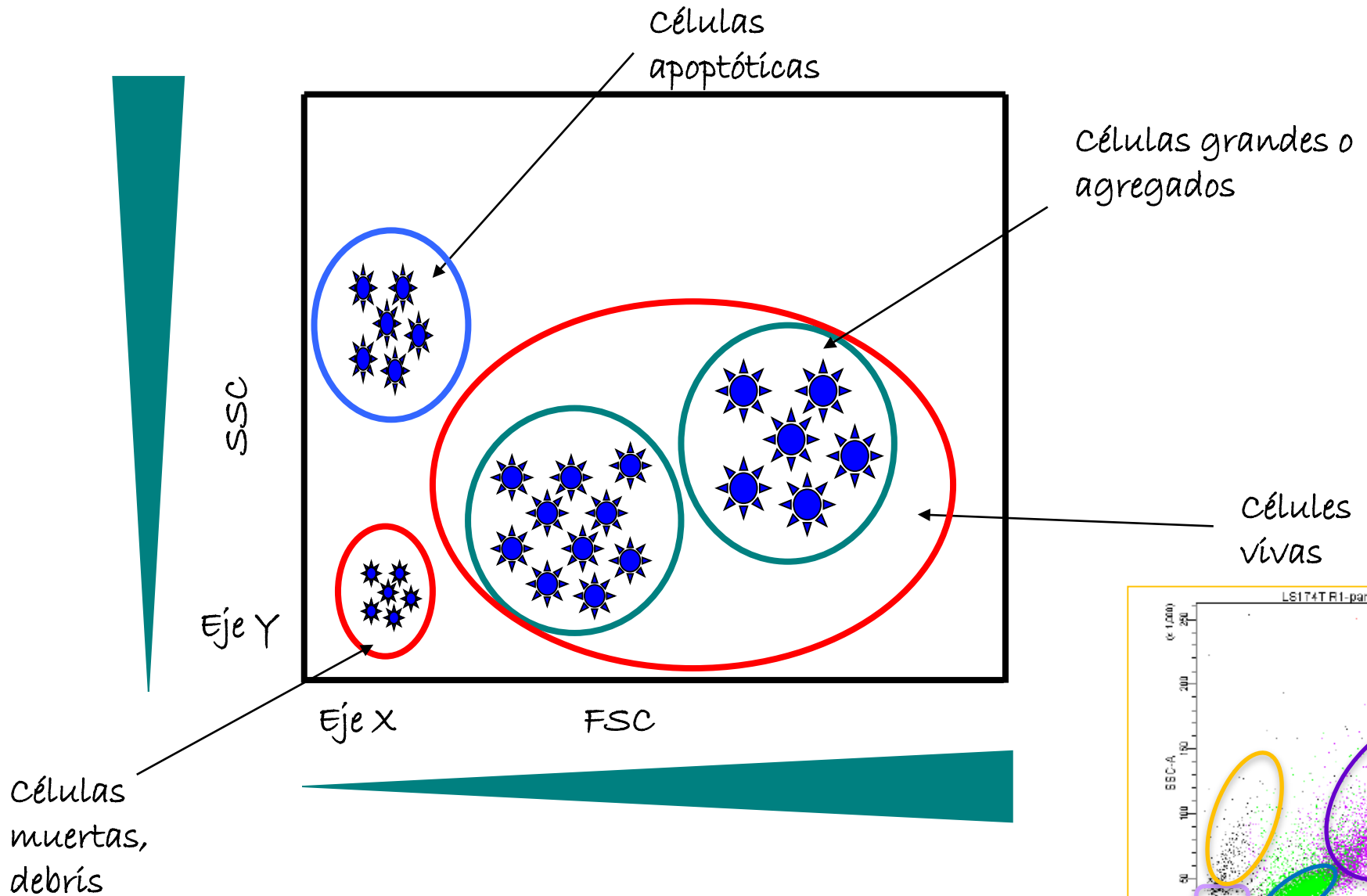
2 Células en fase G₁ (2n)



- ◆ Estas señales eléctricas son amplificadas y digitalizadas mediante los ADCs (Analog to Digital Converters).
- ◆ A cada señal de fluorescencia generada por cada evento se le asigna un canal de intensidad de fluorescencia, en función de la señal detectada por los PMTs, en un histograma de 1 o 2 parámetros.
- ◆ Cada evento está correlacionado de manera individual con todos los parámetros analizados (FSC, SSC, y fluorescencias).

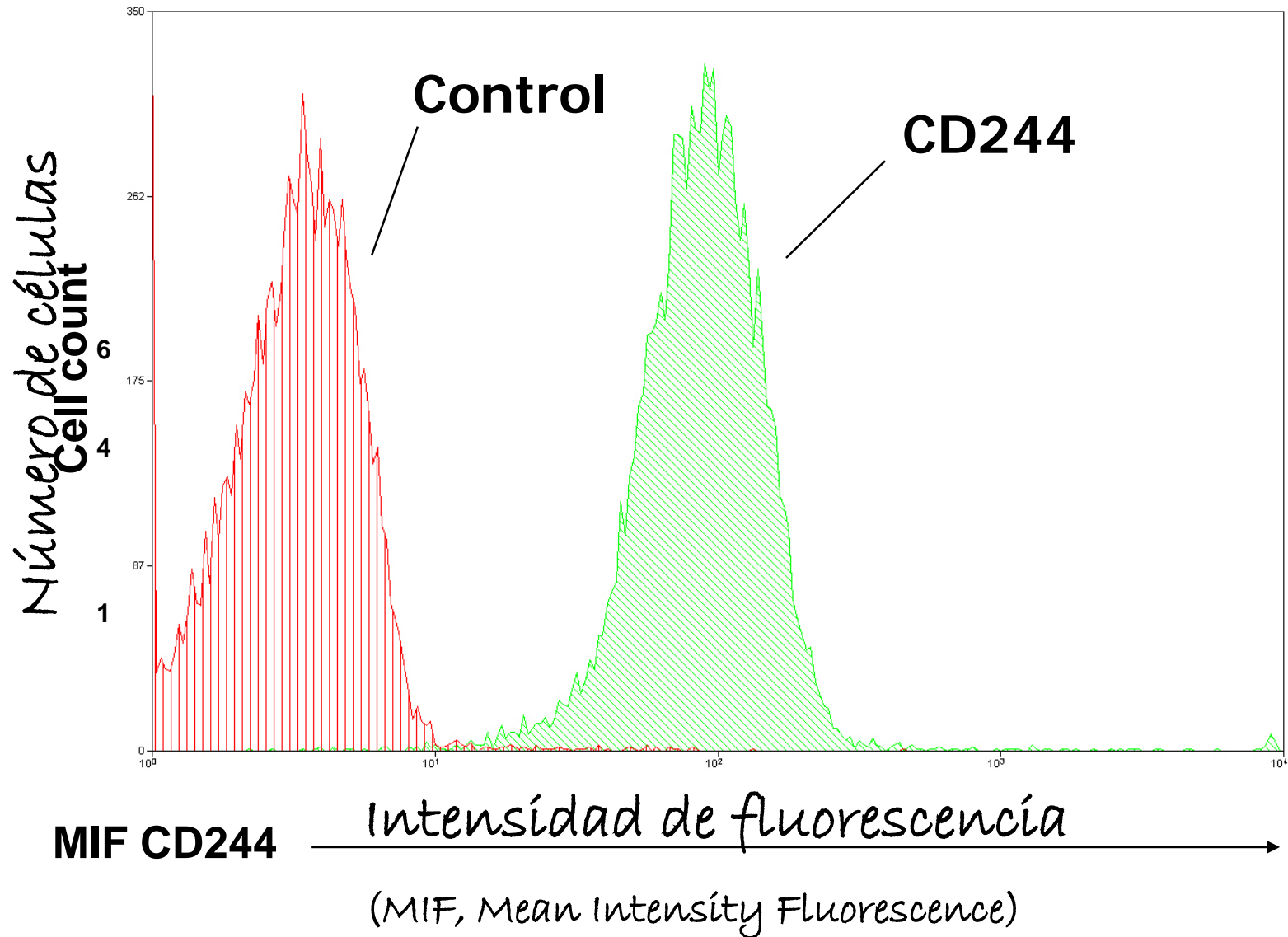


Electrónica (V)

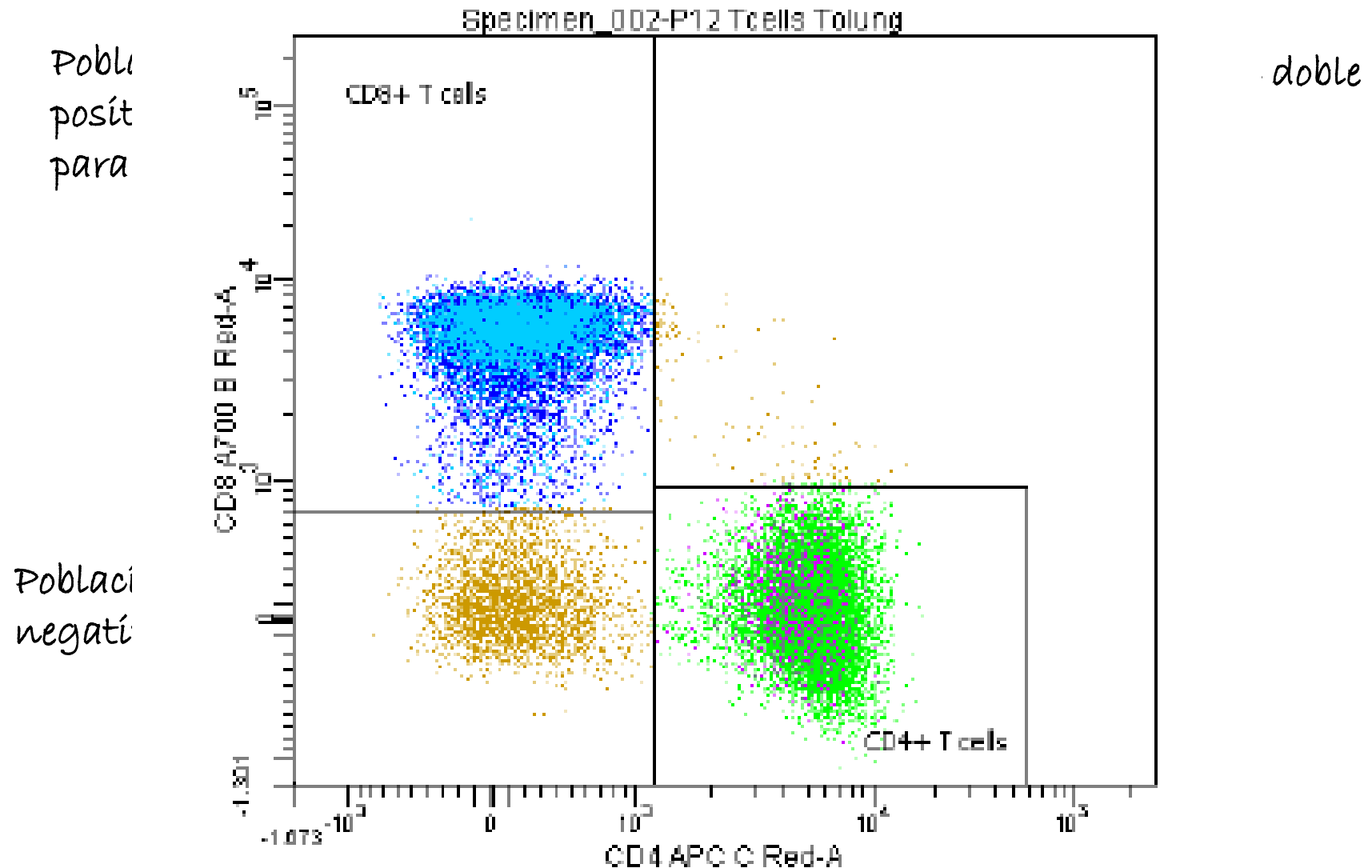


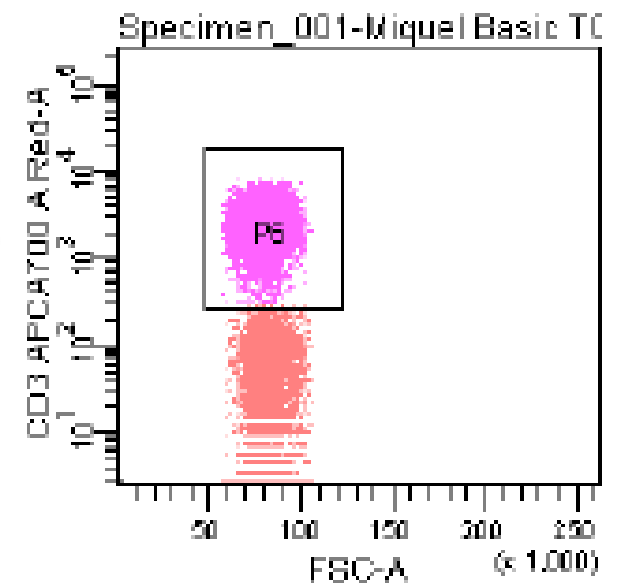
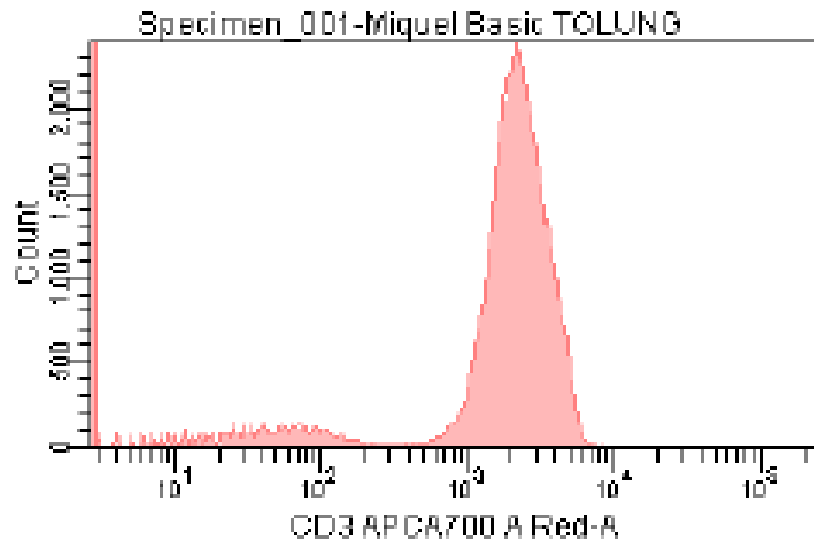
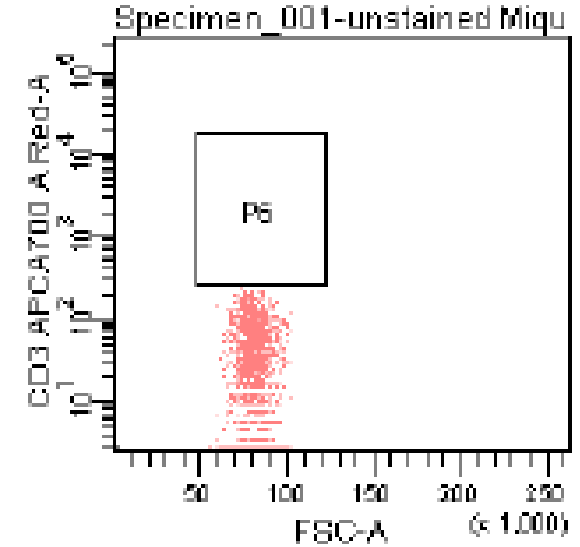
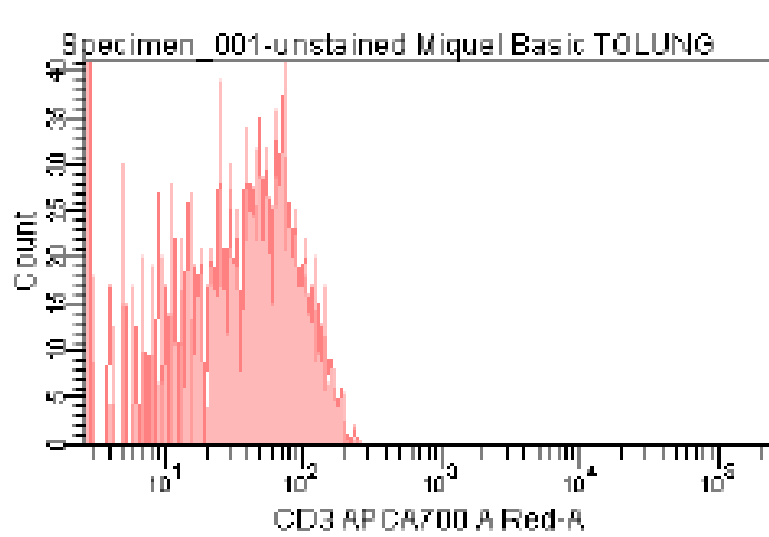


- Histograma uniparamétrico.

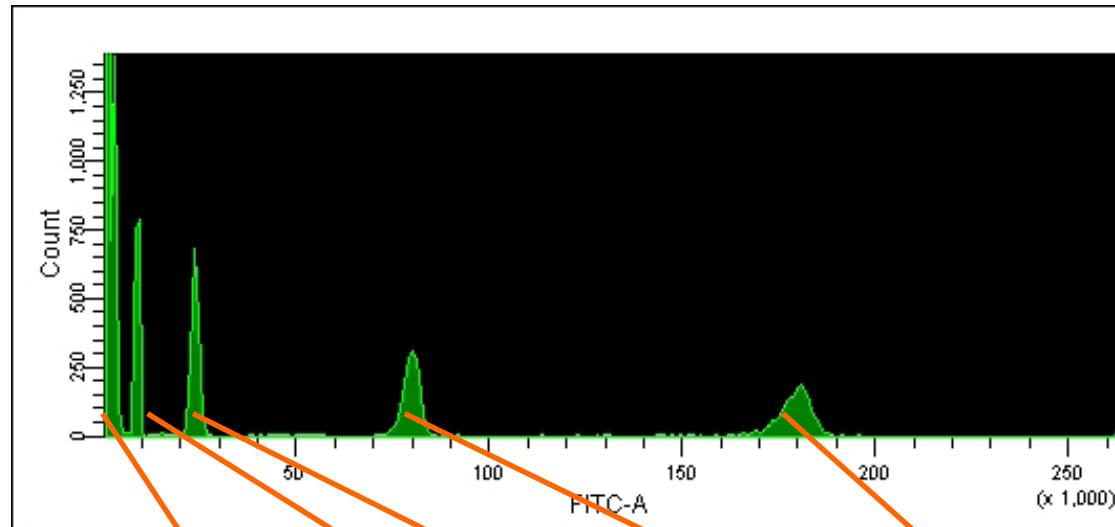


- Histograma bípamétrico.

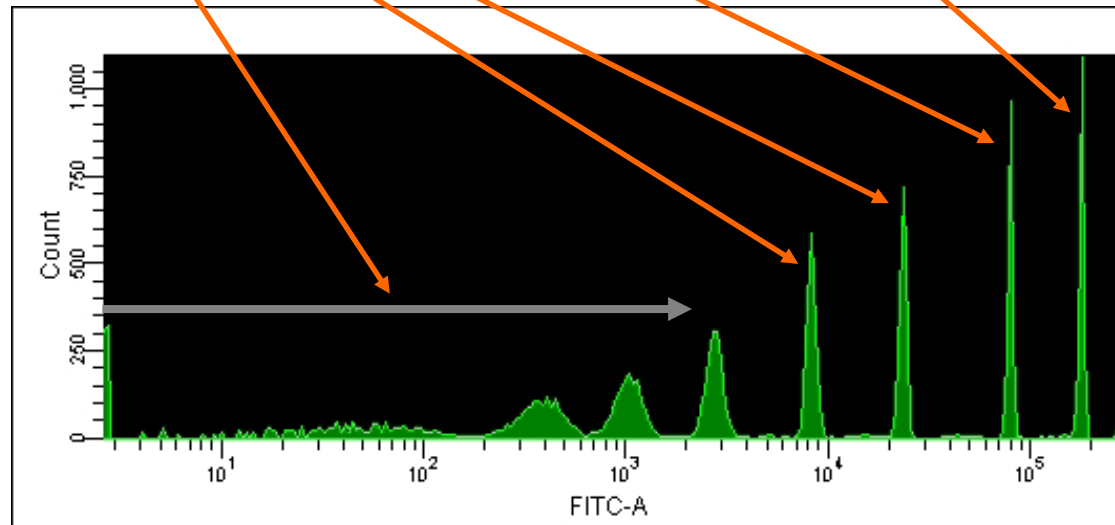




Escala
lineal



Escala
logarítmica



Componentes de un citómetro de flujo

Fluídica

- Flujo laminar (sistema presurizado).
- Cámara de flujo (enfoque hidrodinámico).

Óptica

- LASER.
- Luz dispersada y fluorescencia.
- Sistema de filtros para la recolección de la señal.

Electrónica

- Amplificación mediante PMTs.
- Conversión a valores digitales.

Informática

- Análisis en un sistema informático.

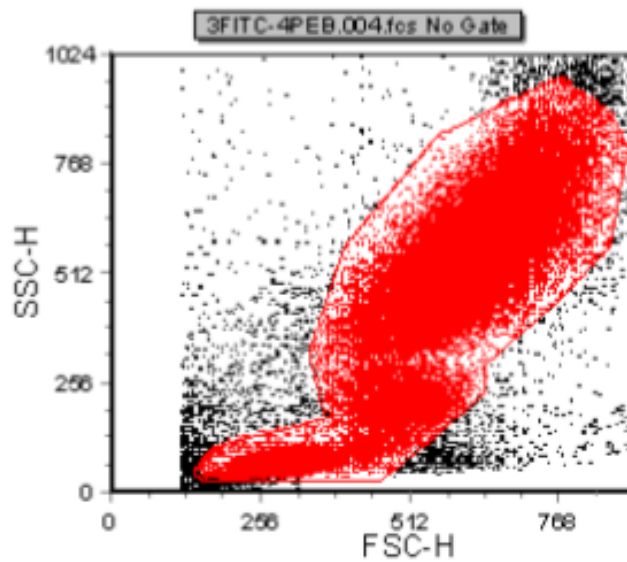
El citómetro nos da datos de la muestra que adquirimos:

- Formato Estándar (FCS 2.0, FCS 3.0).
- Archivo de Texto.
- Información de cada célula.
- Necesidad de compartir y comparar datos entre diferentes laboratorios y equipos.

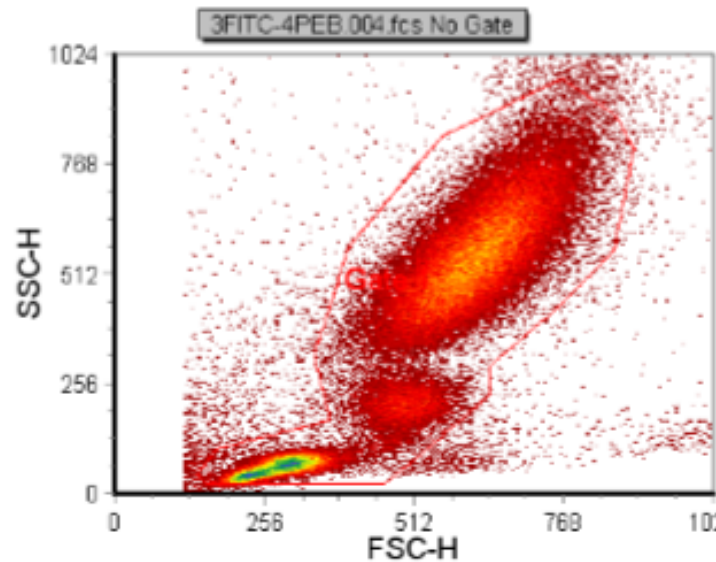
Cell #	FSC-A	SSC-A	FITC-A	PE-A	PerCP-Cy5-5	APC-A
1	91825	117731	3260	1064	95	96
2	91261	125190	115	136	3214	1018
3	93022	112909	70	116	114	17308
4	93433	128917	95	110	150	11776
5	100540	115243	84	7888	890	92
6	88810	151105	62	136	2957	590
7	96170	126448	3260	1141	67	3
8	93497	124832	89	85	43	58
9	91282	100535	31	50	98	12314
10	93755	120241	152	184	113	119
11	95665	102580	96	86	156	151
12	84486	124264	97	163	3198	812
13	99302	134495	142	8425	757	99
14	97278	110935	28	47	80	12283

- Distinguímos 3 tipos de archivos:
 - Documento o Protocolo o Plantilla: es exclusivo del software. Nos permite visualizar los datos y escoger la información que deseamos guardar.
 - Instrument Settings: es un archivo de la configuración de los detectores. Nos permite guardar las condiciones de adquisición para poder adquirir muestras en las mismas condiciones.
 - Data: archivo FCS. Contiene la información sobre la muestra y es estándar. Eso nos permite analizar estos datos con otros softwares distintos al de adquisición.

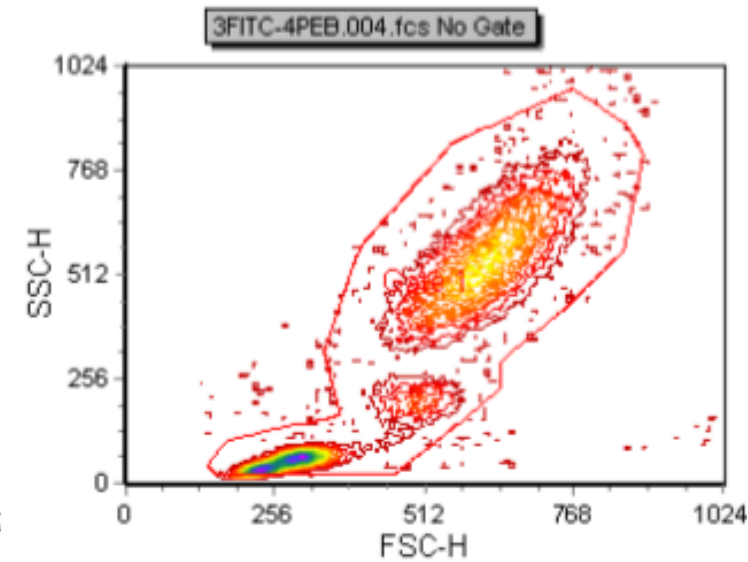
- Las diferentes formas de representar los datos en el software son, en general:
 - Gráficos de puntos biparamétricos:



Dispersión

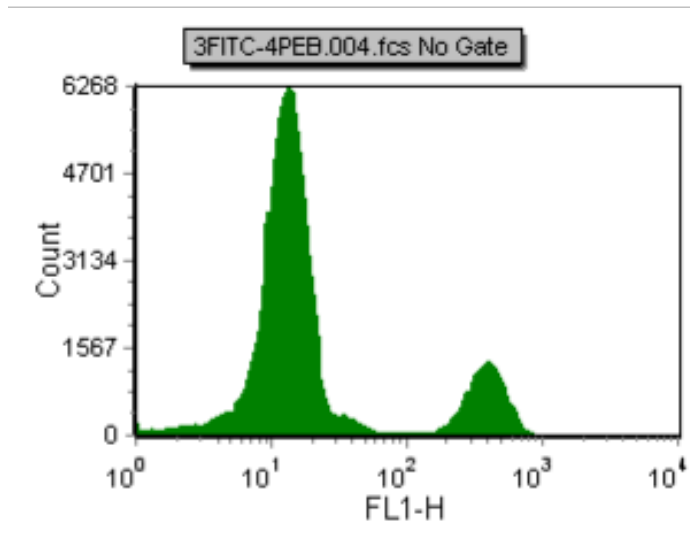


Densidad

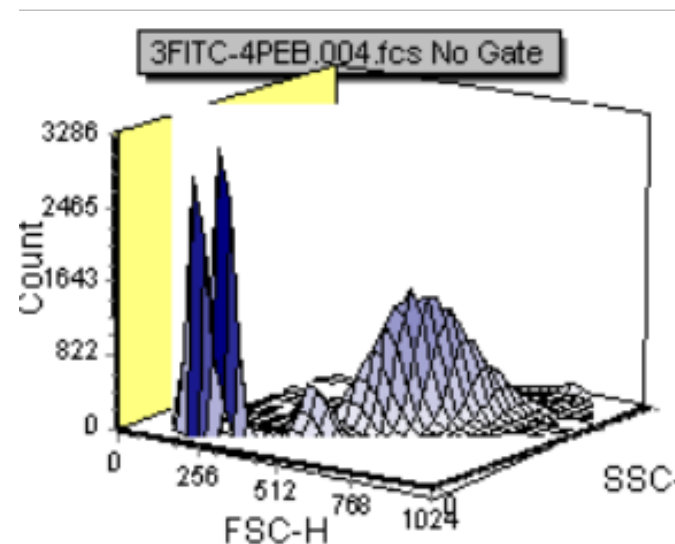


Contorno

Gráficos monoparamétricos:



Gráficos especiales:

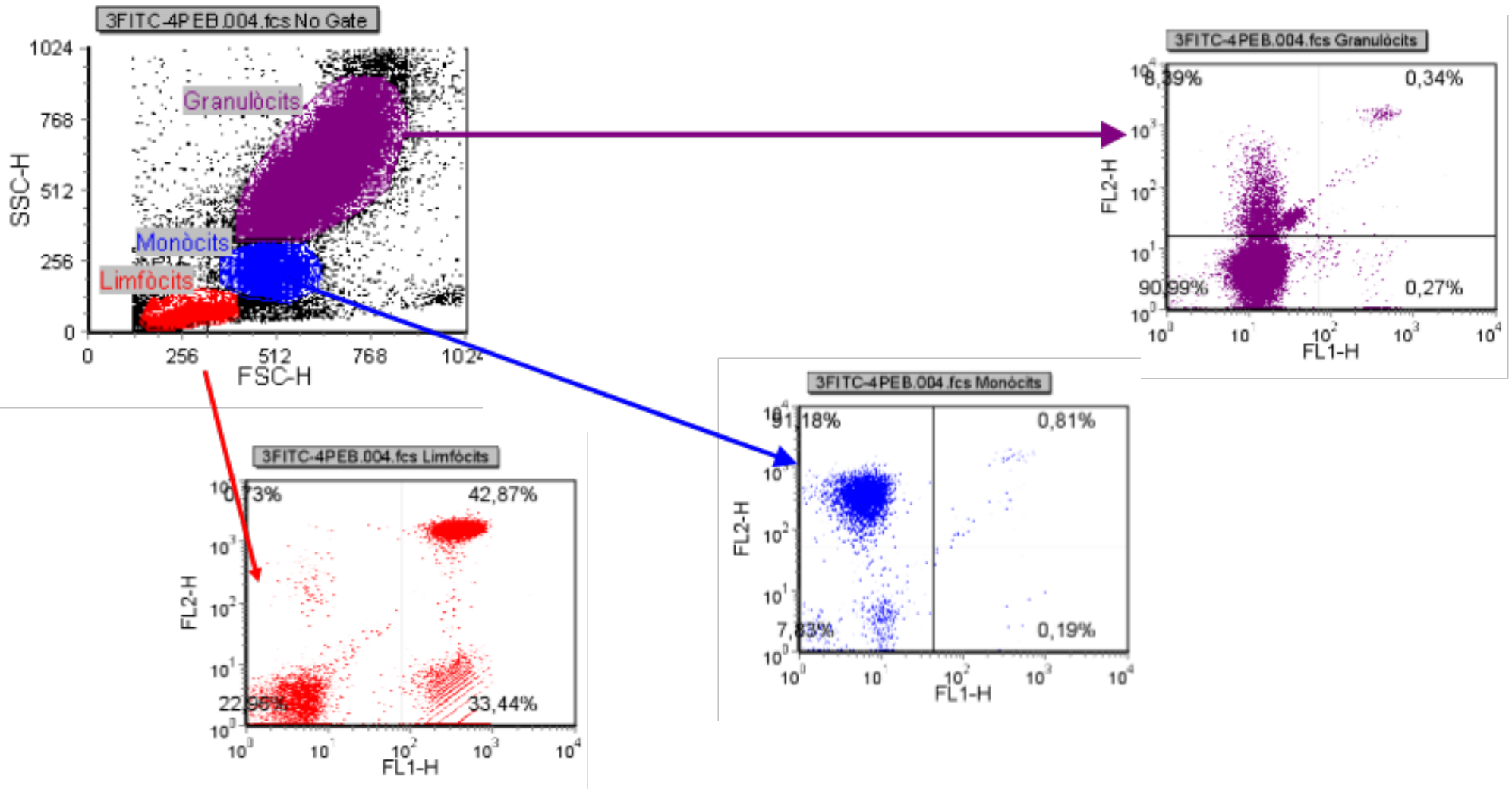




Software (IV)

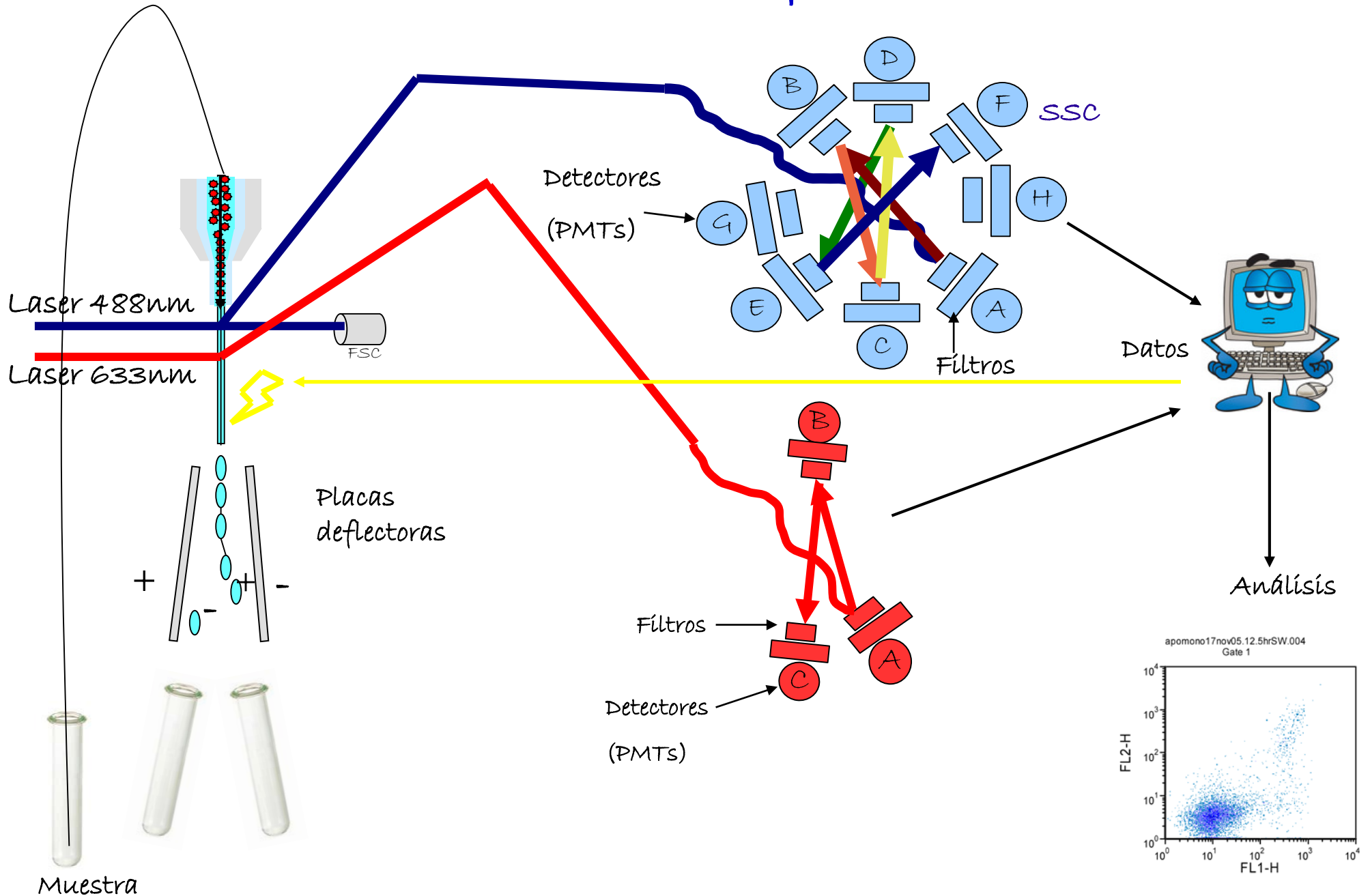
¿Cómo estudiamos la población que nos interesa?

- Utilizamos Regiones y "Gates". Eso nos permite estudiar poblaciones concretas.





Esquema de un citómetro- separador





- Inmunofenotipaje
- Proliferación celular
- Ciclo celular (ploídías)
- Apoptosis
- Esayos funcionales (potencial de membrana mitocondrial, extrusión sondas fluorescentes, metabolismo oxidativo...)
- Análisis de marcadores de superficie
- Análisis de proteínas intracelulares
- Comprobación eficiencia de transfección, análisis de proteínas fluorescentes.
- Estudio de microorganismos
- Fosforilación de proteínas
- Sorting de poblaciones puras con características específicas de interés.

Aspectos a tener en cuenta en el planteamiento de un experimento de citometría de flujo

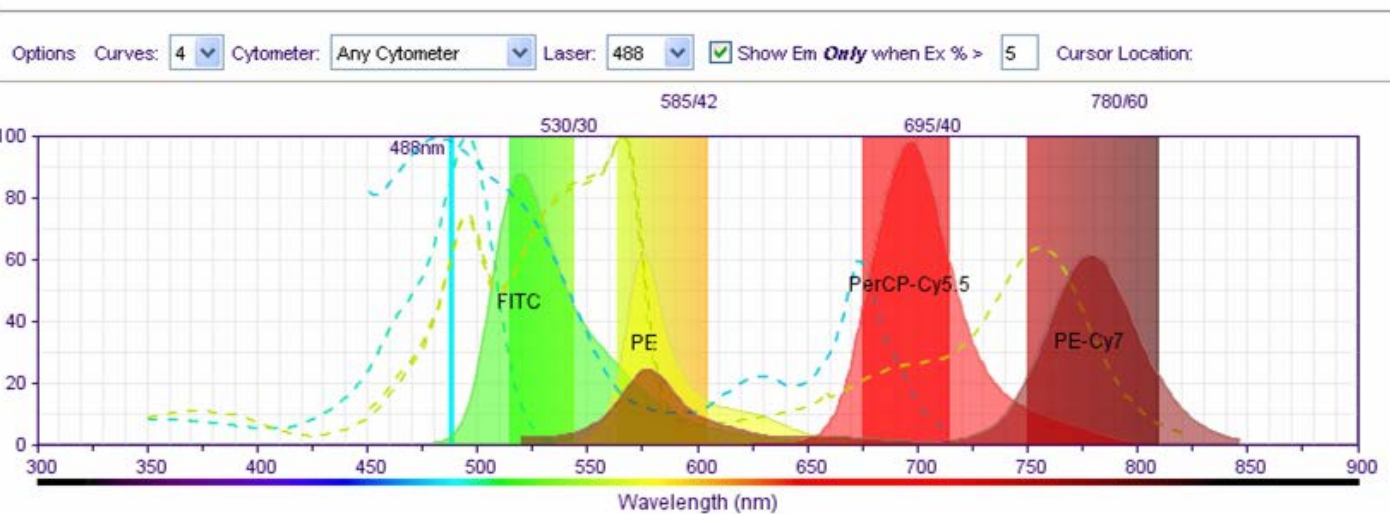
- Qué fluorocromos podemos medir?
- Qué fluorocromos queremos medir?
- Cuántos queremos medir simultáneamente?
- Cómo interaccionan entre ellos?

Aspectos a tener en cuenta en el planteamiento de un experimento de citometría de flujo

<http://www.invitrogen.com/site/us/en/home/support/Research-Tools/Fluorescence-SpectraViewer.html>

<http://www.bdbiosciences.com/us/s/spectrumviewer>

Fluorescence Spectrum Viewer A Multicolor Tool



Fluorochrome	%	<input checked="" type="checkbox"/> Ex	<input checked="" type="checkbox"/> Em	<input checked="" type="checkbox"/> Filters	FITC	PE	PerCP-C...	PE-Cy7
FITC	88	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	530/30 <input checked="" type="checkbox"/>	--	--	--	--
PE	62	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	585/42 <input checked="" type="checkbox"/>	--	--	--	--
PerCP-Cy5.5	98	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	695/40 <input checked="" type="checkbox"/>	--	--	--	--
PE-Cy7	62	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	780/60 <input checked="" type="checkbox"/>	--	--	--	--



Aspectos a considerar

- El equipo da valores absolutos.....pero no sabe si una muestra presenta fluorescencia o no. Es necesario referenciarla a un control.
- Aumentar la velocidad de paso de la muestra (aumentando el diferencial de presiones) dificulta la resolución de las poblaciones.
- El software nos permite ver los datos de una forma estadística. Nos muestra los datos de manera que los podamos interpretar.
- Antes de diseñar un experimento preguntad al personal de la UAT.

UAT - Plataforma de Citómica

En la UAT, disponemos de 2 citómetros FacsCalibur de BD. Los citómetros disponen de 2 LASERS.

- LASER Azul que emite luz a 488nm de longitud de onda.
- LASER Rojo que emite luz a 633nm.

Detección de 4 fluorescencias simultáneamente. 3 en el LASER Azul y 1 en el LASER Rojo.



WAT - Plataforma de Cítomica

Disponemos de 1 citómetro Fortessa de BD. Equipado con 4 LASERes.

- LASER **Azul** que emite luz a 488nm de longitud de onda.
- LASER **Rojo** que emite luz a 633nm de longitud de onda.
- LASER **Violeta** que emite luz a 405nm de longitud de onda.
- LASER **Amarillo-verde** que emite luz a 561nm de longitud de onda.

Detección de 16 fluorescencias simultáneamente. 2 en el azul, 5 en el Amarillo-verde, 3 en el Rojo y 6 en el Violeta.





UCTS - Plataforma de Citómica

- También disponemos de 1 separador celular que disponen de 4 LASERES (488 nm, 633 nm, 405 nm, 561 nm) y 13 detectores de fluorescencia.



- ◆ Se realizarán unas prácticas para aprender a usar el citómetro en sus aspectos más básicos:
 - Encender el citómetro
 - Crear un documento de trabajo
 - Pasar muestras
 - Compensar
 - Obtener datos
 - Apagar el citómetro
 - Análisis de datos básico FCSExpress

Responsable UAT:

Dra Rosa Prieto email: rosa.prieto@vhir.org

Responsable Plataforma de Citòmica:

Dra Irene Sales email: irene.sales@vhir.org

Email plataforma: citom@vhir.org

Telf: 934894179 (ext.4179)

REVEALING A NEW
HORIZON

Multicolor Flow Cytometry

Alberto Crespo Guardo, PhD.

Application Specialist

BD Biosciences

Master of Immunology

March 25th, 2019

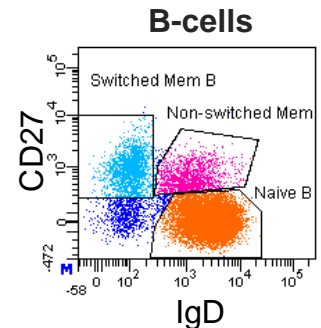
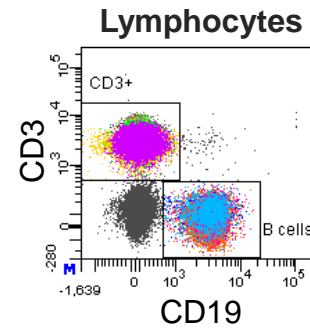
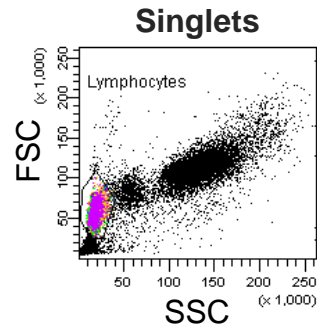


Agenda

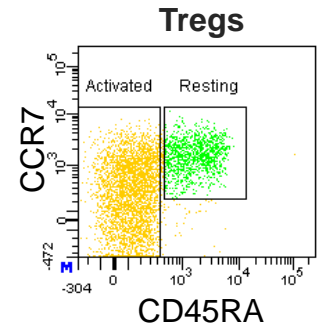
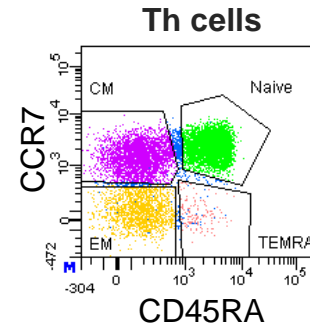
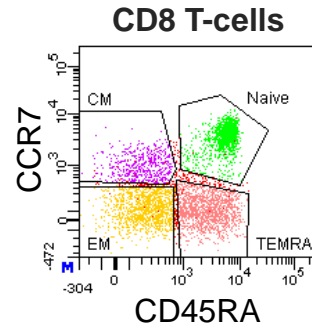
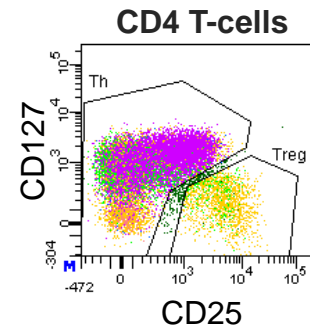
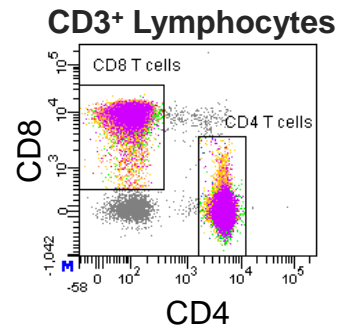
- Normas básicas para el diseño de paneles (60 min)
- Ejercicio práctico: diseño de un panel (45 min)
- Uso de la herramienta GPS (Guided Panel Solution) (15 min)

Previous considerations

e.g. Defining T- and B-cell populations



Marker
CD45RA
CD3
CD4 + IgD
CD19
CD25
CD127
CCR7
CD27
CD8



Menganito et al., 2019



Previous considerations

e.g. Defining T- and B-cell populations

Menganito et al., 2019

Fluorochrome	Marker
BD Horizon™ V450	CD45RA
BD Horizon™ V500	CD3
FITC	CD4 + IgD
PerCP-Cy™5.5	CD19
PE-Cy™5	CD25
PE-Cy™7	CD127
Alexa Fluor® 647	CCR7
Alexa Fluor® 700	CD27
APC-H7	CD8

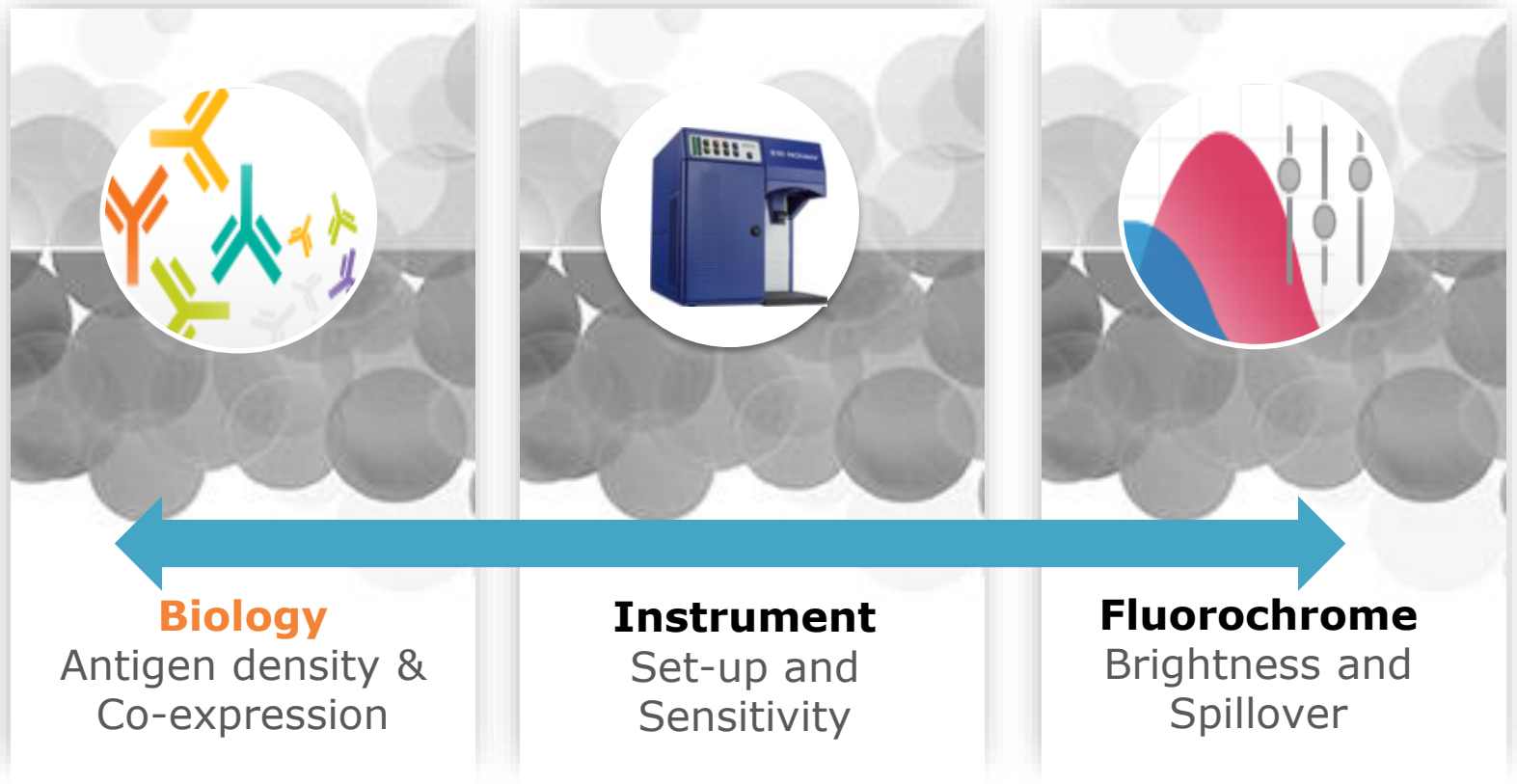
Scenario 1:
Have you checked
your instrument
and its
configuration?

Marker	Fluorochrome
CCR7	BD Horizon™ V450
CD4 + IgD	BD Horizon™ V500
CD3	FITC
	???
CD25	PE-Cy™5
CD27	PE-Cy™7
	???
CD127	Alexa Fluor® 700
CD45RA	APC-H7

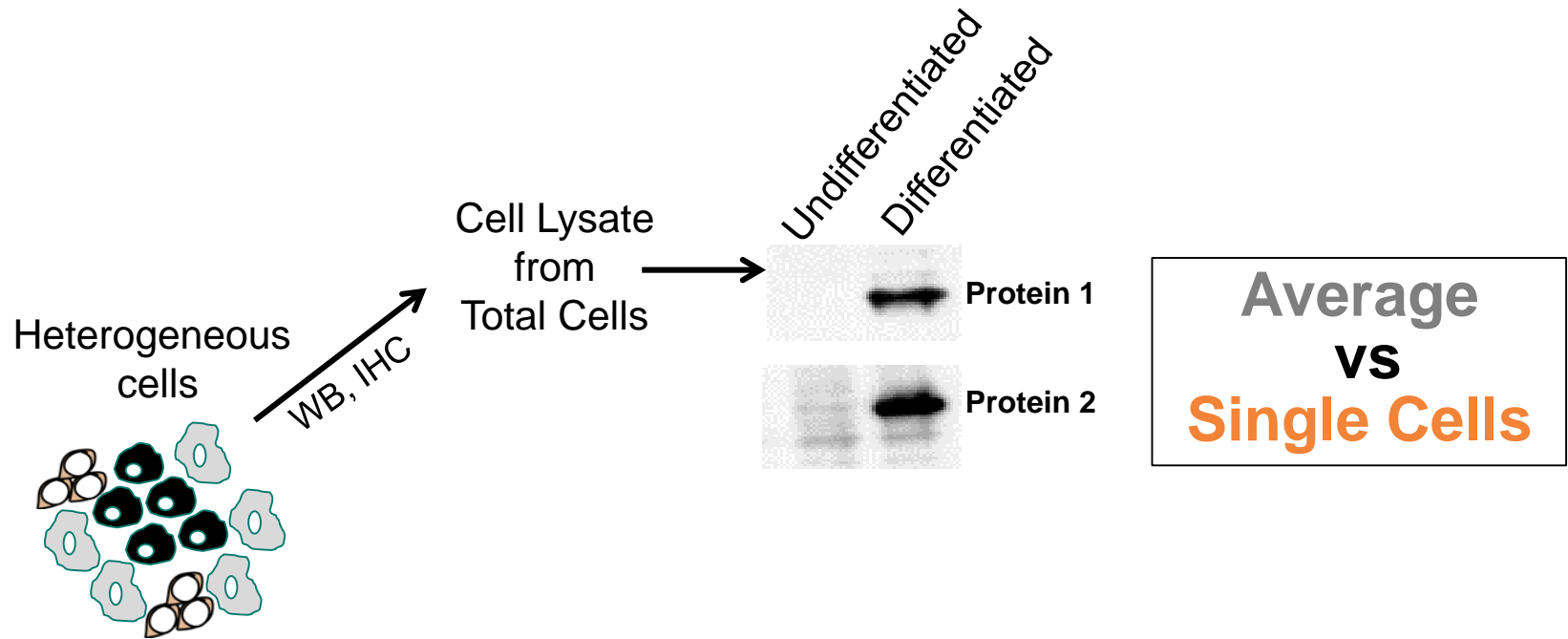
Scenario 2:
You will save
ab_s but... have
you checked
how it works?

Elements of multicolor flow cytometry

Considerations in designing panels



The power of flow cytometry: enables single-cell protein expression analysis & quantitation

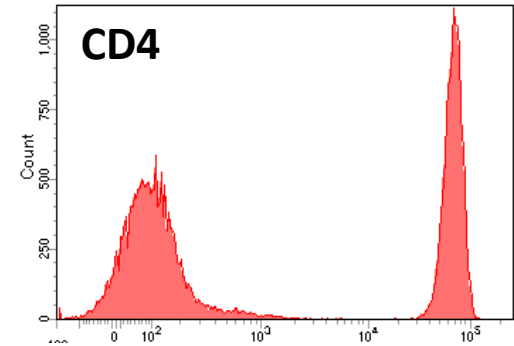


Leucocyte antigen expression

- **Primary**

Well characterized, easily classified as positive or negative, typically define broad subsets or lineages

- Examples: CD3, CD4



How to know the expression levels of your cells?

- Previous lab work
- Scientific literature
- Testing
- Asking to BD: “Our scientific support can help you!” (help.biosciences@europe.bd.com or Applications_spain@bd.com)



Defining the human antigen proteome

- Assess the density of surface antigens of all known CD markers on normal blood cells
- Create an antigen density and expression resource for use in multicolor panel design

Cell	Antigen	Molecules per Cell	Reference
T cell	TCR	100,000	Cho, B. et al. 2000. <i>PNAS</i> . 98:1723.
	CD2	55,000	Ginaldi, L. et al. 1996. <i>J Clin Pathol</i> . 49:539.
	CD3	124,000	Ginaldi, L. et al. 1996. <i>Br J Haematol</i> . 93:921.
	CD5	90,000	Ginaldi, L. et al. 1996. <i>J Clin Pathol</i> . 49:539.
	CD7	20,000	Ginaldi, L. et al. 1996. <i>Br J Haematol</i> . 93:921.
	CD45	>200,000	Glattling, G. et al. 2006. <i>J Nucl Med</i> . 47:1335.
CD4+ T cell	CD4	100,000	Davis, K. et al. 1998. <i>Cytometry</i> . 33:197.
	CD28	20,000	Bryl, E. et al. 2005. <i>Arthritis Rheum</i> . 52:2996.
	CCR5	4,000-24,000	Reynes, J. et al. 2006. <i>J Infect Dis</i> . 181:927.
CD8+ T cell	CD8	90,000	Takada, S. et al. 1987. <i>J Immunol</i> . 139:3231.
	CD28	15,000	Bryl, E. et al. 2005. <i>Arthritis Rheum</i> . 52:2996.
B cell	CD19	18,000	Ginaldi, L. et al. 1998. <i>Pathobiology</i> . 66:17.
	CD20	109,000	Ginaldi, L. et al. 1998. <i>Pathobiology</i> . 66:17.
	CD21	210,000	Ginaldi, L. et al. 1998. <i>Pathobiology</i> . 66:17.
	CD22	14,000	Ginaldi, L. et al. 1998. <i>Pathobiology</i> . 66:17.
	HLA-DR	85,000	Ginaldi, L. et al. 1998. <i>Pathobiology</i> . 66:17.
	CD11a	10,000	Unternaehrer, J. et al. 2007. <i>PNAS</i> . 104:234.
	CD40	2,000	Unternaehrer, J. et al. 2007. <i>PNAS</i> . 104:234.
	CD86	16,000	Unternaehrer, J. et al. 2007. <i>PNAS</i> . 104:234.
	CD80	2,000	Unternaehrer, J. et al. 2007. <i>PNAS</i> . 104:234.
	Dendritic cell	CD11a	27,000
CD40		17,000	Unternaehrer, J. et al. 2007. <i>PNAS</i> . 104:234.
CD80		132,000	Unternaehrer, J. et al. 2007. <i>PNAS</i> . 104:234.
CD86		208,000	Unternaehrer, J. et al. 2007. <i>PNAS</i> . 104:234.
Monocyte	CD14	110,000	Antal-Szalmas, P. et al. 1997. <i>J Leukoc. Biol</i> . 61:721.
	CD32	21,000	Antal-Szalmas, P. et al. 1997. <i>J Leukoc. Biol</i> . 61:721.
	CD64	13,000	Antal-Szalmas, P. et al. 1997. <i>J Leukoc. Biol</i> . 61:721.
Neutrophil	CD14	3,500	Antal-Szalmas, P. et al. 1997. <i>J Leukoc. Biol</i> . 61:721.
	CD16	225,000	Antal-Szalmas, P. et al. 1997. <i>J Leukoc. Biol</i> . 61:721.
NK cell	CD56	10,000	Ginaldi, L. et al. 1996. <i>J Clin Pathol</i> . 49:539.
Red Blood Cell	Glycophorin A	340,000	Antal-Szalmas, P. et al. 1997. <i>J Leukoc. Biol</i> . 61:721.
Basophil	CD23	15,000	MacGlashan, D. et al. 2000. <i>J Leuk Biol</i> . 68:479.

BD Biosciences

Density of Common Human Surface Markers

Subset	Antigen	Density molecules/cell
Lymphocytes	CD3	32,000
	CD4	36,400
	CD8	65,500
	CD19	7,800
T Cells (CD3 ⁺ CD4 ⁺ Lymphocytes)	CD25	600
	CD25 ^{hi}	3,400
	CD27	10,900
	CD28	7,700
	CD45RA	33,400
	CD45RO	12,600
	CD122	5,300
	CD127	2,000
	CD132	400
	CD194 (CCR4)	2,500
CD197 (CCR7)	2,000	
B Cells (CD19 ⁺ Lymphocytes)	CD20	24,600
	CD24 ^{mid}	3,000
	CD24 ^{hi}	16,100
	CD27	3,200
	CD38 ^{mid}	2,800
	CD38 ^{hi}	15,900
	CD138	400
	IgD ^{mid}	4,900
	IgD ^{hi}	23,800
	IgG	28,100
IgM	3,800	

Antigen density determined on human whole blood samples using PE conjugated antibodies to each antigen and BD Quantibrite™ beads for quantitation.

Elements of multicolor flow cytometry

Considerations in designing panels



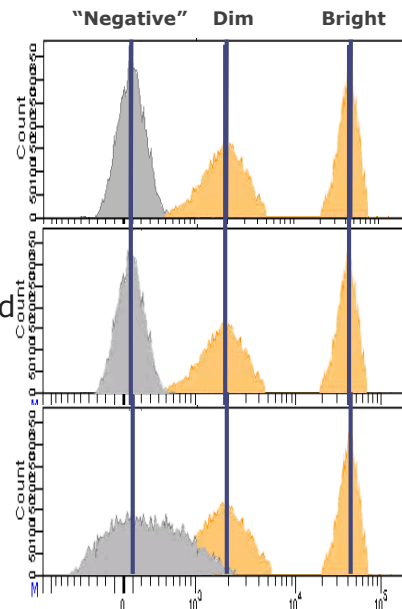
Resolution vs background & spread

- Resolution: The degree to which a flow cytometer can distinguish dimly stained cells from unstained cells.

Negative population has low background; populations well resolved.

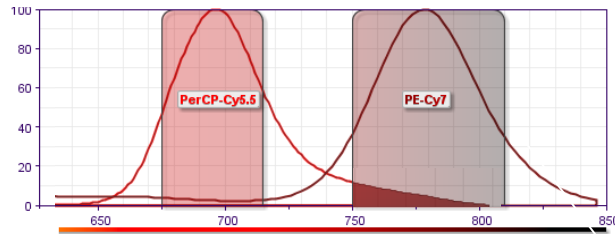
Negative population has high background; populations not resolved.

Negative population has low background but high rSD (spread); populations not resolved.



The ability to resolve populations is a function of both **background** and **spread** of the negative population.

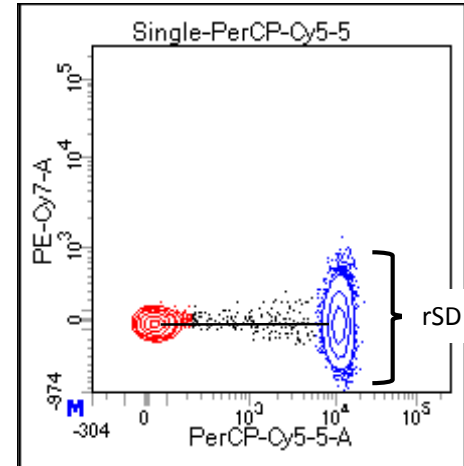
Fluorescence spillover



Fluorochromes spill over into other detectors; for example, PerCP-Cy5.5 spills into the PE-Cy7 detector.

This fluorescence spillover contributes to:

- Increased background (MFI)
- Spread (measured as rSD)

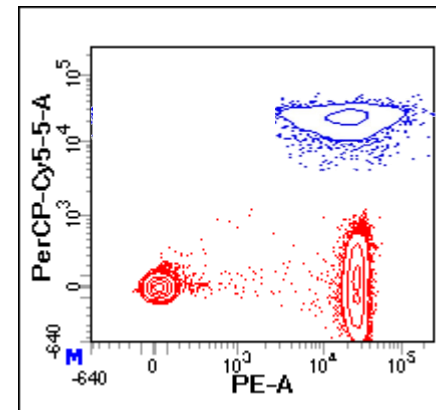
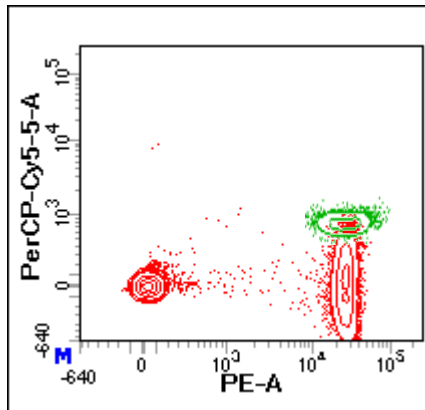
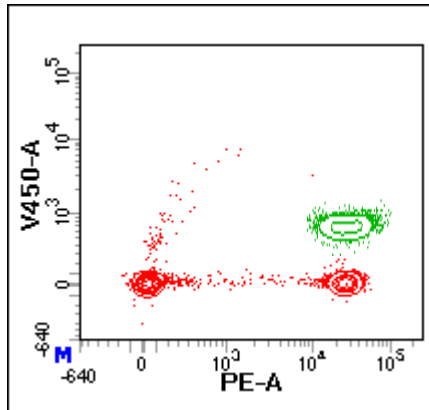
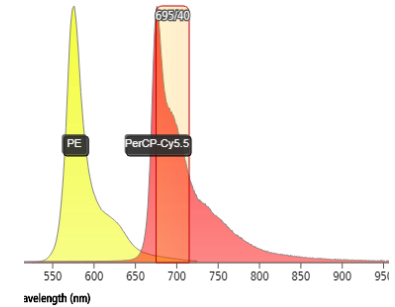


This "background" is subtracted in the process called compensation.

	Negative		Positive	
	MFI	rSD	MFI	rSD
No comp	12	29	3,098	291
Comp	4	29	3	289

Spread affects the panel resolution

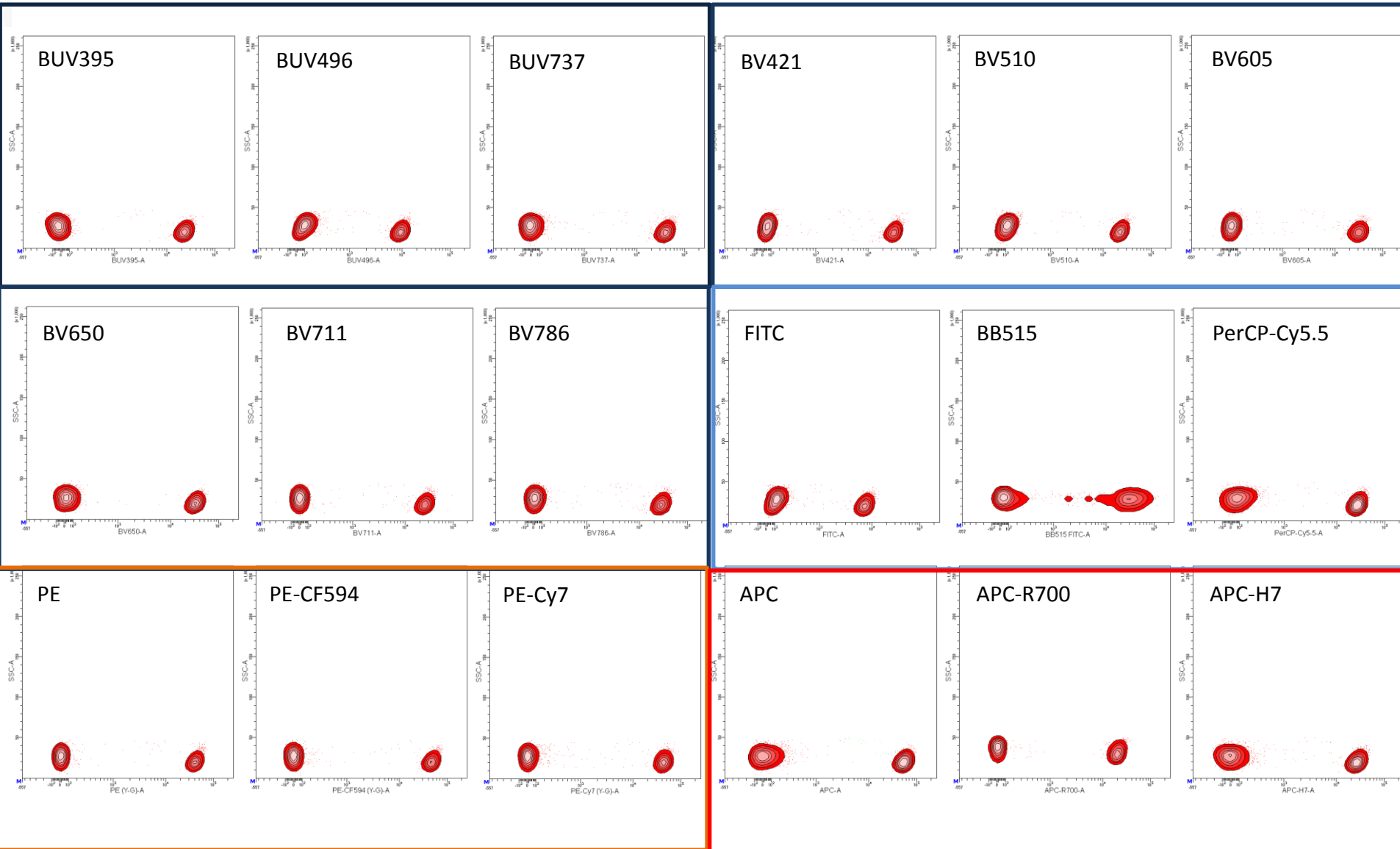
- CD4 PE causes spread in PerCP-Cy5.5 channel



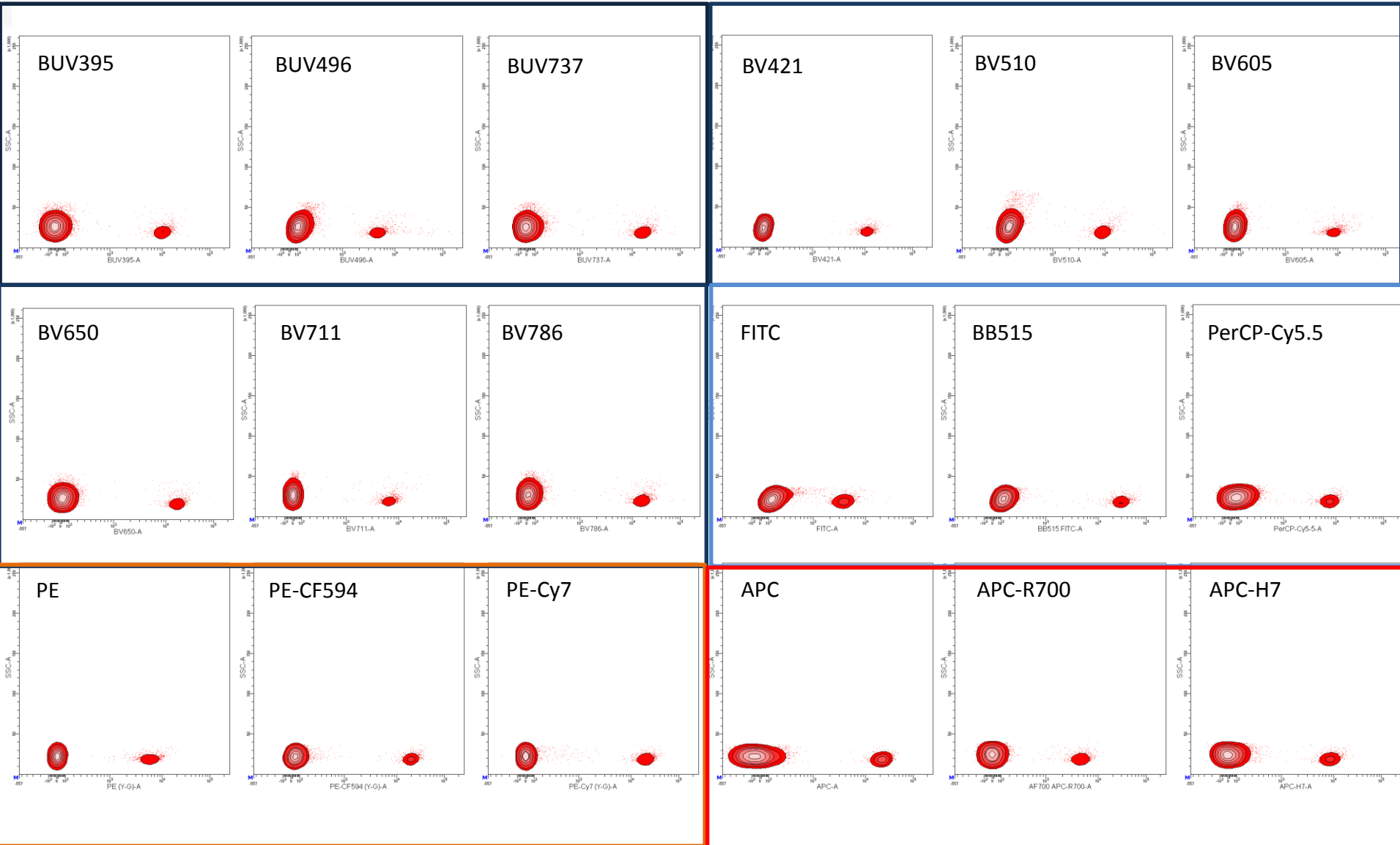
Assessing resolution using CD4, CD19 and CD127

- CD4, CD19 and CD127 are receptors known to be expressed at high (45,000 receptors), medium (10,000 receptors) and low (2,500 receptors) density, respectively, on normal human lymphocytes
- Blood cells are stained with antibody conjugated with different fluorochromes compatible with the instrument configuration

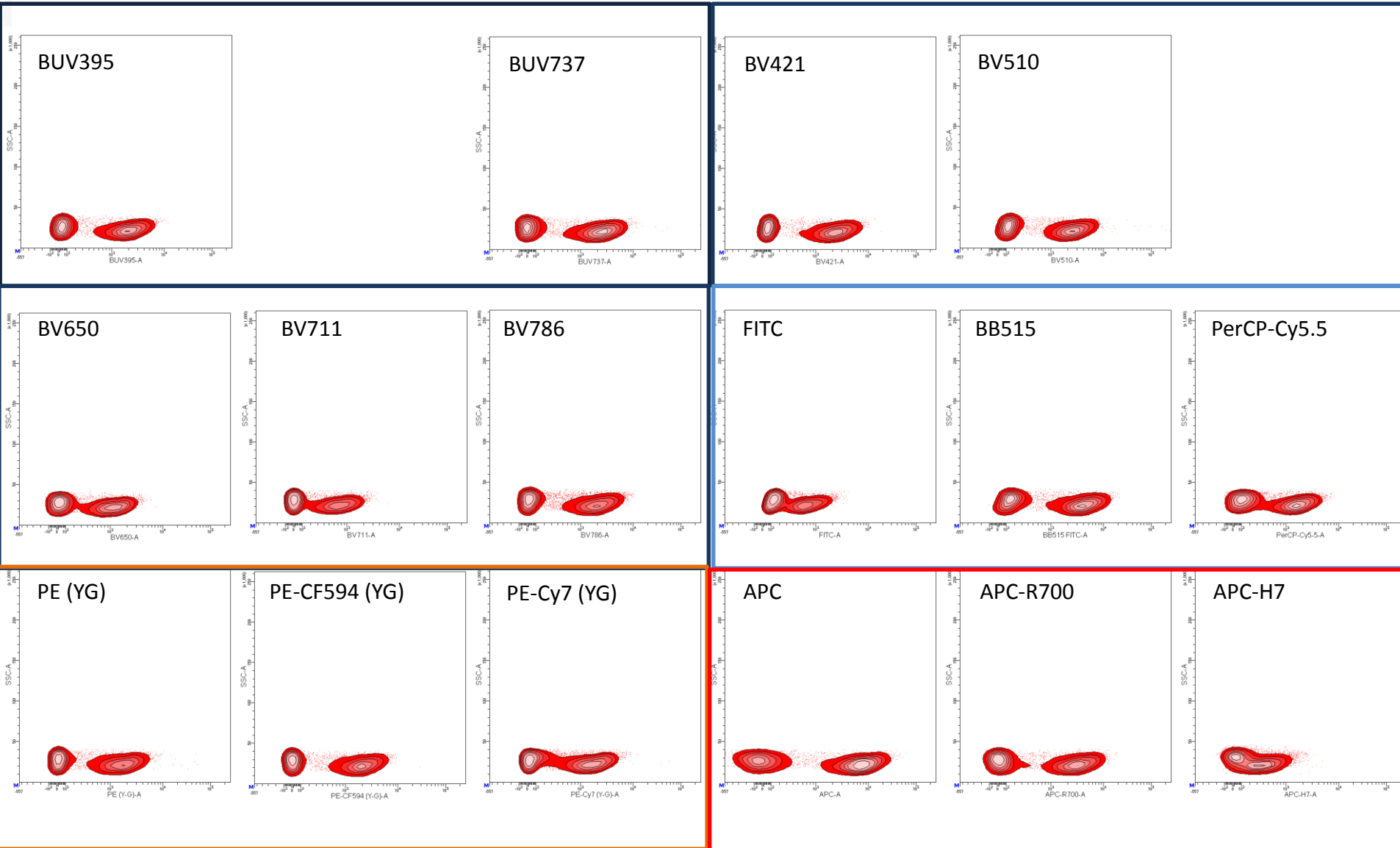
Resolution: CD4 (high)



Resolution: CD19 (medium)



Resolution: CD127 (low)

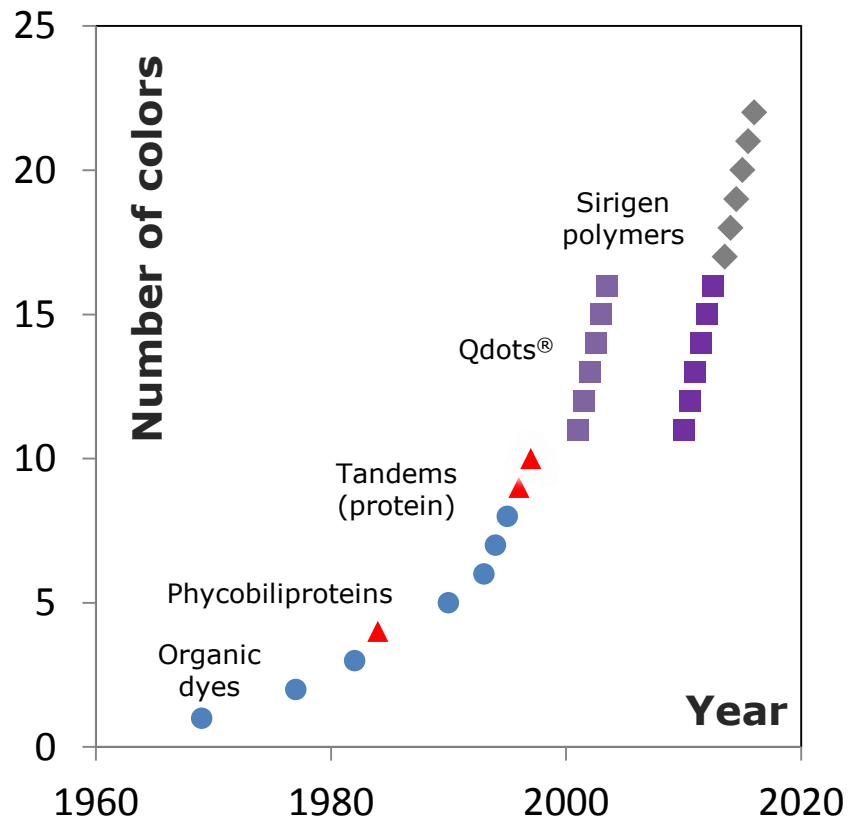


Elements of multicolor flow cytometry

Considerations in designing panels



Evolution of fluorochromes



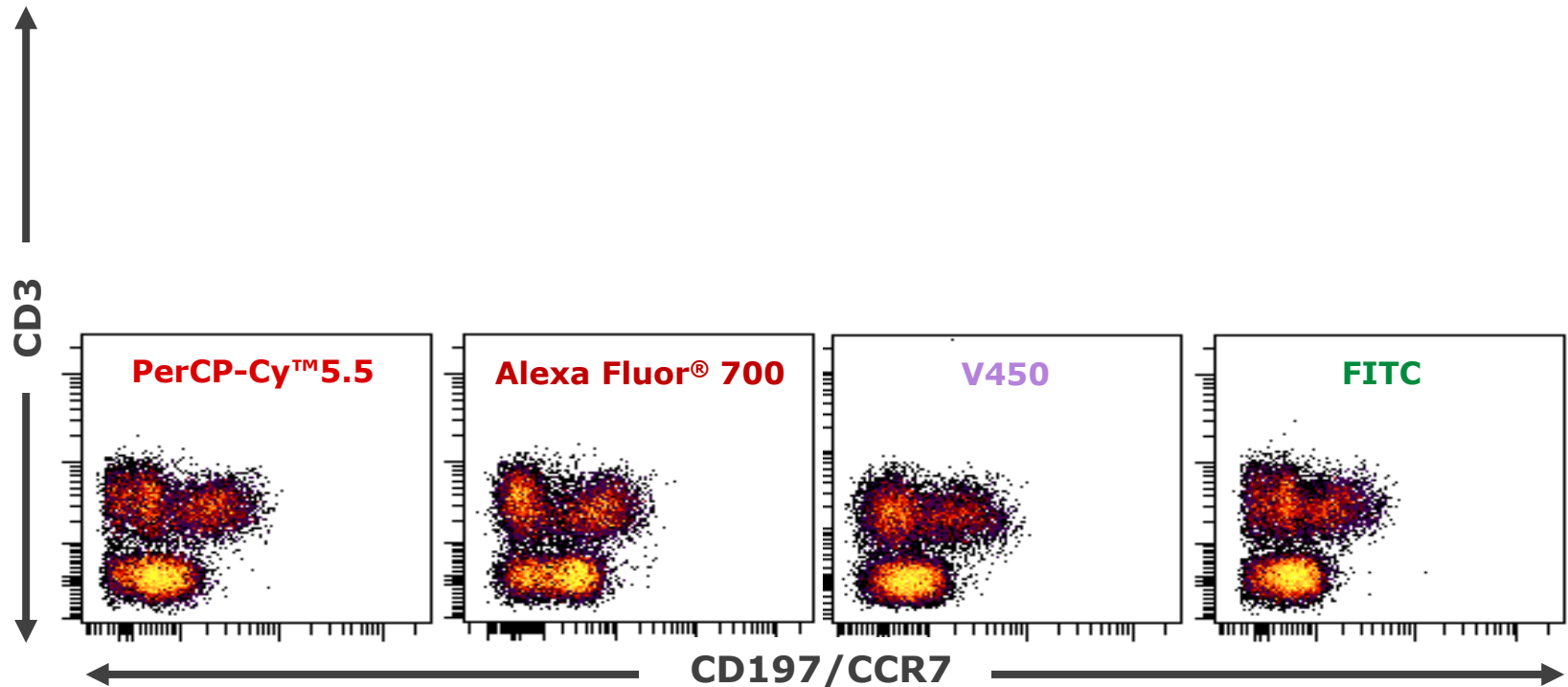
- The development of new fluorescent dye choices, plus development of monoclonal antibody reagents, has driven major advances in flow cytometry.

Fluorochrome choices

		Emission							
		UV	Blue	Green	Yellow	Orange	Red	Dark Red	
Laser	Ultraviolet (355 nm)	BUV395		BUV496	BUV563		BUV661	BUV737	BUV805
	Violet (405 nm)		BV421 V450	BV480 BV510 V500		BV605	BV650	BV711	BV786
	Blue (488 nm)			BB515 FITC Alexa Fluor® 488	PE	PE-CF594	PE-Cy™ 5	BB700 PerCP PerCP- Cy5.5	PE-Cy™ 7
	Yellow/Green (561 nm)				PE	PE-CF594	PE-Cy5	PE-Cy5.5	PE-Cy7
	Red (640 nm)						APC Alexa Fluor® 647	APC-R700 Alexa Fluor® 700	APC-H7 APC-Cy7

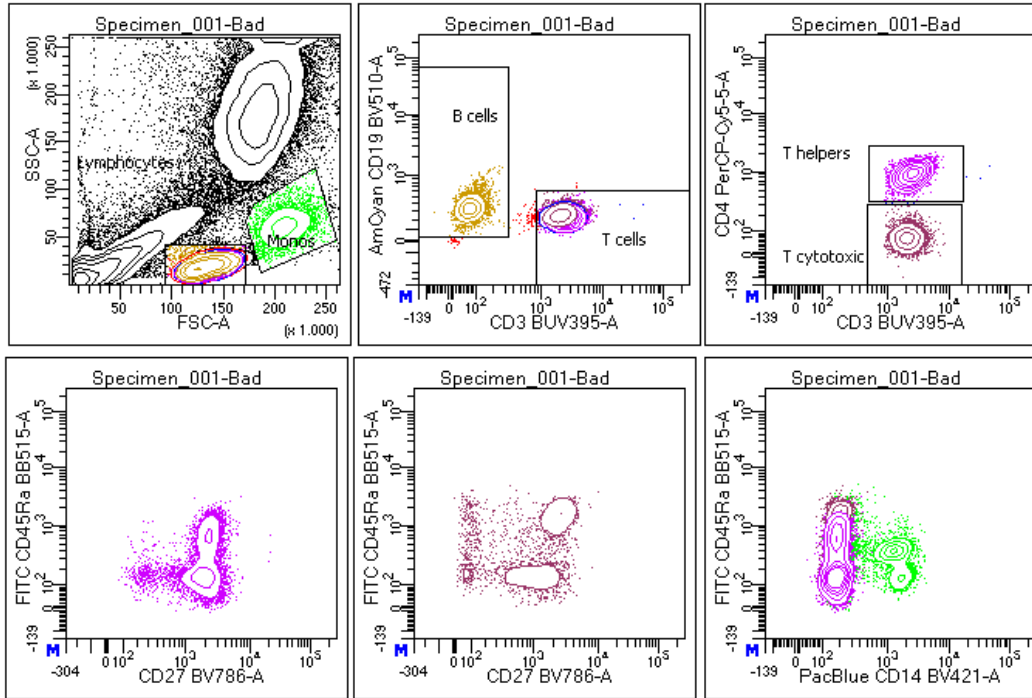
Choice of fluorochromes depends on the available instrument configuration and the total number of markers being used in an experiment.

Fluorochromes reveal biology



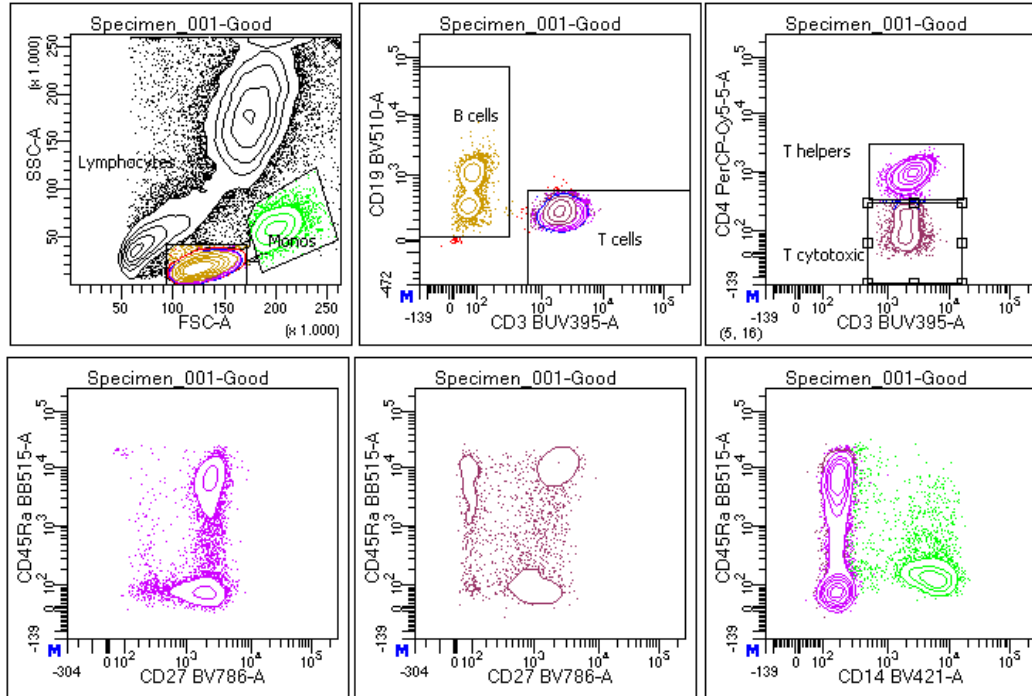
- The proper choice of fluorochrome helps us understand more about the biology of the experiment
- Bright dyes are important when looking at dim antigens

What about using the “vintage” fluorochromes?



BUV395	CD3
PacBlue	CD14
AmCyan	CD19
BV786	CD27
FITC	CD45Ra
PerCP-Cy5.5	CD4

What about using the “vintage” fluorochromes?



BUV395	CD3	BUV395
PacBlue	CD14	BV421
AmCyan	CD19	BV510
BV786	CD27	BV786
FITC	CD45Ra	BB515
PerCP-Cy5.5	CD4	PerCP-Cy5.5

New BD Horizon Brilliant™ dyes

- Seven fluorochromes excited by the violet laser
 - Base polymers: BV421, BV510 and **BV480^{new}**
 - Tandems: BV605, BV650, BV711 and BV786
- Six fluorochromes excited by the 355-nm UV laser
 - Base polymer: BUV395
 - Tandems: BUV496, BUV563, BUV661, BUV737, BUV805
- Two fluorochromes excited by the blue laser
 - BB515 brighter alternative to FITC
 - BB700 brighter alternative to PerCP-Cy5.5
- ... and more

Fluorochrome characterization

- Fluorochromes have intrinsic properties relevant to panel design
 - Brightness
 - Spillover

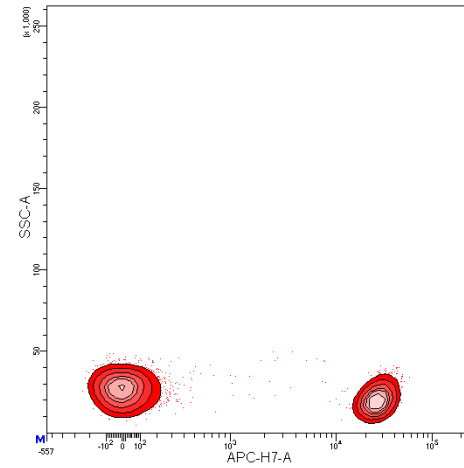
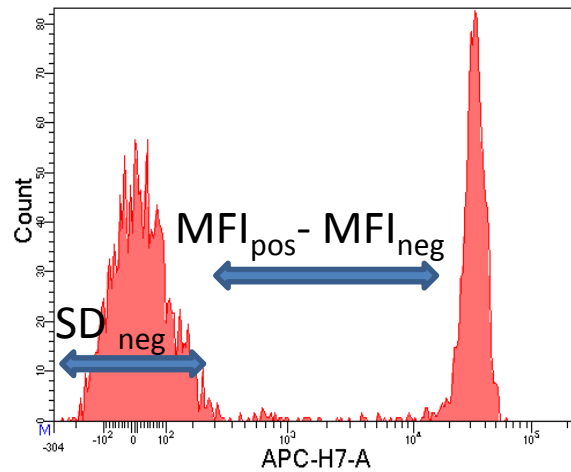
Fluorochrome characterization: Brightness

- Blood cells are stained with anti-CD4 conjugated with fluorochromes compatible with the instrument configuration
- Samples are acquired using optimized Instrument Settings
- Stain index is calculated and fluorochromes are ranked by brightness

Sensitivity measured by Stain Index

$$\text{Stain Index} = (\text{MFI}_{\text{Pos}} - \text{MFI}_{\text{Neg}}) / (2 \times \text{rSD}_{\text{Neg}})$$

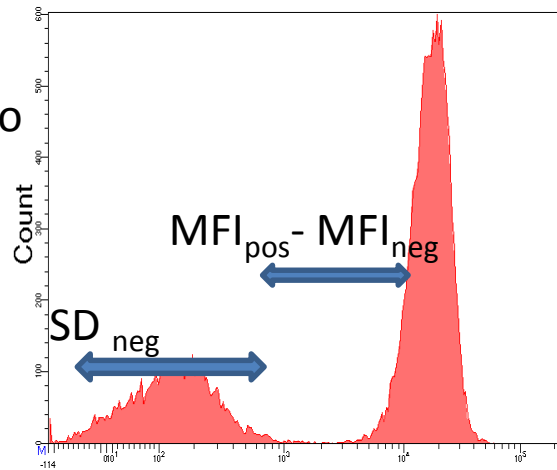
SI: 360



Sensitivity measured by Stain Index

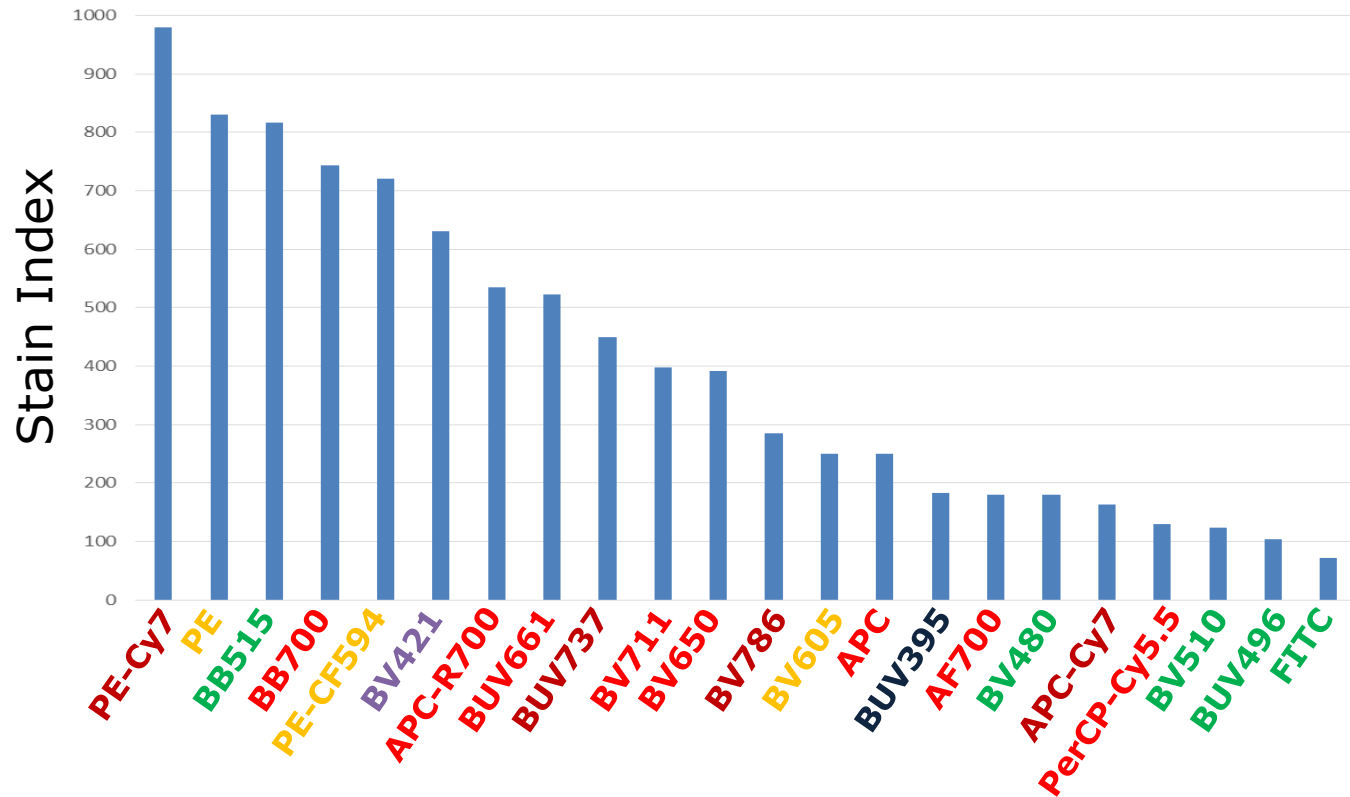
$$\text{Stain Index} = (\text{MFI}_{\text{Pos}} - \text{MFI}_{\text{Neg}}) / (2 \times \text{rSD}_{\text{Neg}})$$

SI: 135
ha bajado



Fluorochrome characterization: Brightness

Specific fluorochrome ranking



- Ranks each individual fluorochrome for a true understanding of brightest to dimmest on a specific instrument
- This data can be paired with antigen density data for optimal fluorochrome-marker selection

Fluorochrome characterization: Brightness

Antigen/fluorochrome combinations

	Very Bright	Bright	Moderate	Dim
Ultraviolet (355 nm)		BD Horizon BUV661 BD Horizon BUV737 BD Horizon BUV563	BD Horizon BUV395 BD Horizon BUV496	BD Horizon BUV805
Violet (405 nm)	BD Horizon BV421 BD Horizon BV650 BD Horizon BV711	BD Horizon BV480 BD Horizon BV605 BD Horizon BV786	BD Horizon BV510	BD Horizon V450 BD Horizon V500
Blue (488 nm)	BD Horizon BB515 BD Horizon PE-CF594 PE-Cy5	PE PE-Cy7	FITC Alexa Fluor® 488 PerCP-Cy5.5	PerCP
Yellow/Green (561 nm)	PE BD Horizon PE-CF594 PE-Cy5 PE-Cy7			
Red (640 nm)		APC Alexa Fluor® 647 BD Horizon APC-R700		Alexa Fluor® 700 APC-H7 APC-Cy7

Basic concept of panel design:

“for low expressed antigens use brightest available fluorochrome”.

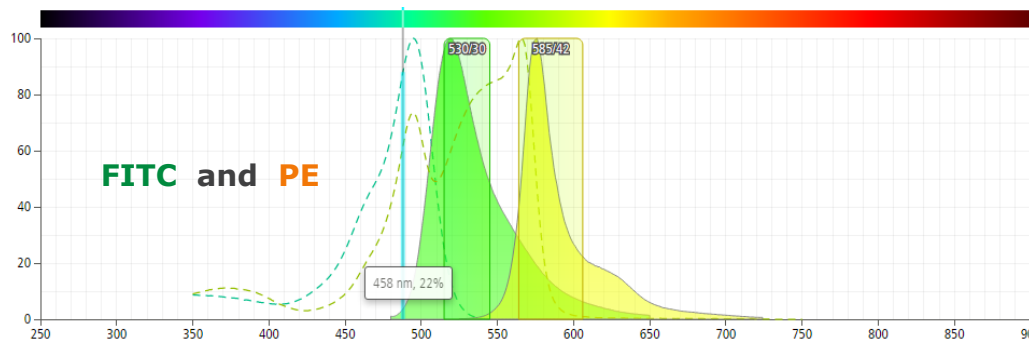


Fluorochrome characterization: Spillover

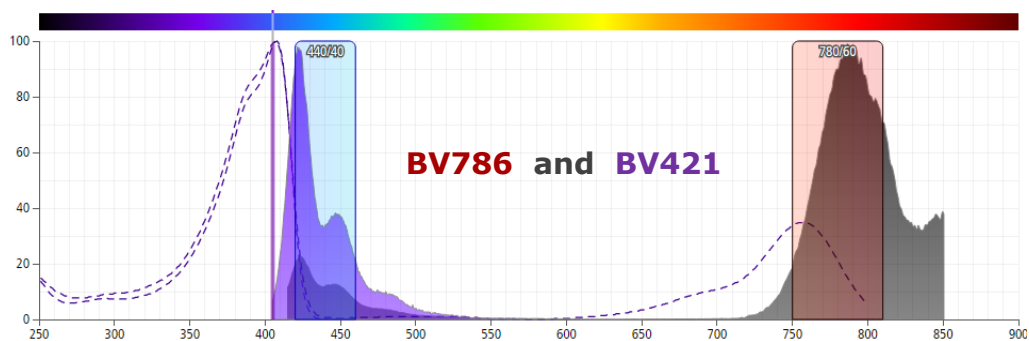
- Fluorescence spillover is an important factor in creating a panel design with good **resolution** of populations of interest
-
- Incorrect or poor calculation of spillover values (SOVs) negatively impacts the quality of data obtained from an assay

What are some sources of spillover?

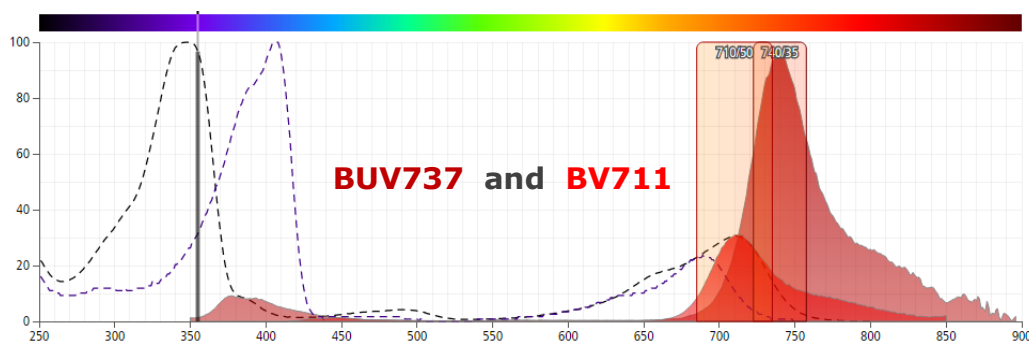
Adjacent detectors



Residual base fluorescence



Similar emission spectra (cross-laser)

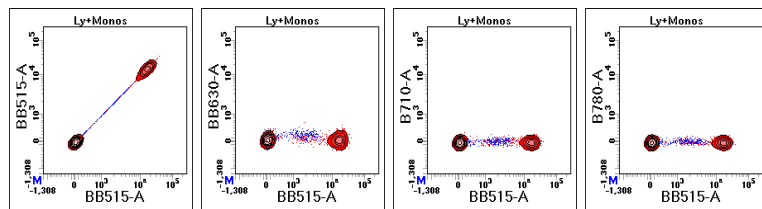


Fluorochrome characterization: Spread analysis

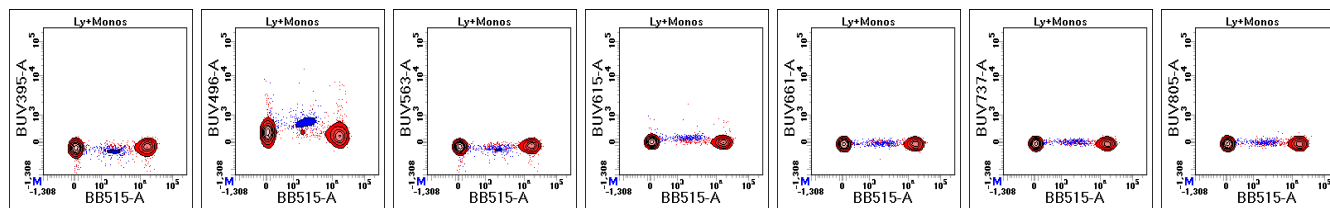
- Blood cells are stained with anti-CD4 conjugated with fluorochromes compatible with the instrument configuration
- Samples are acquired using optimized instrument settings
- For each fluorochrome, an $n \times n$ analysis is performed to visually assess the spread introduced into each detector

CD4s by BB515

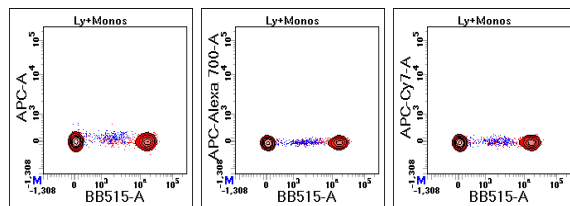
B



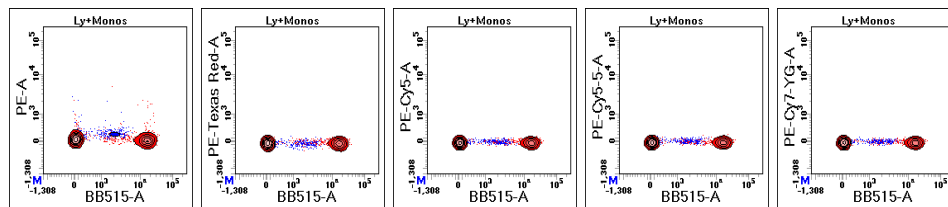
UV



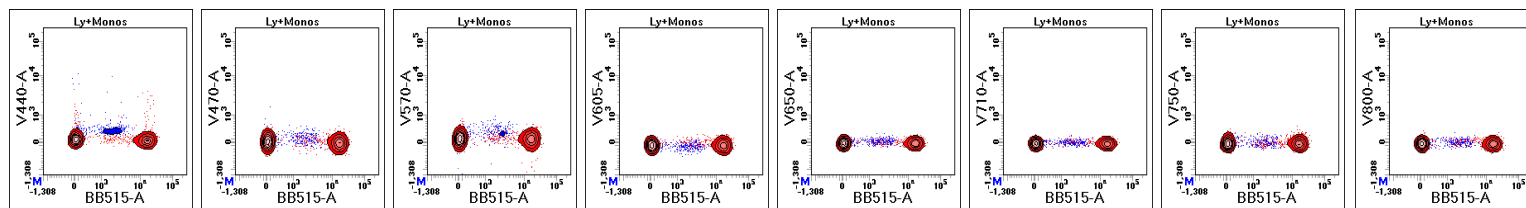
RED



G

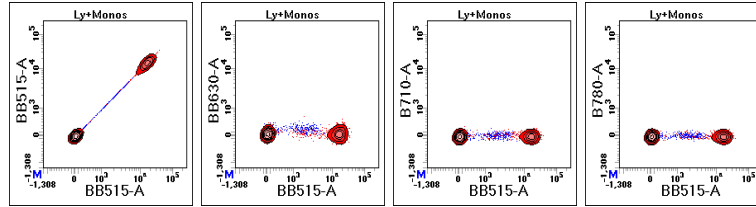


V

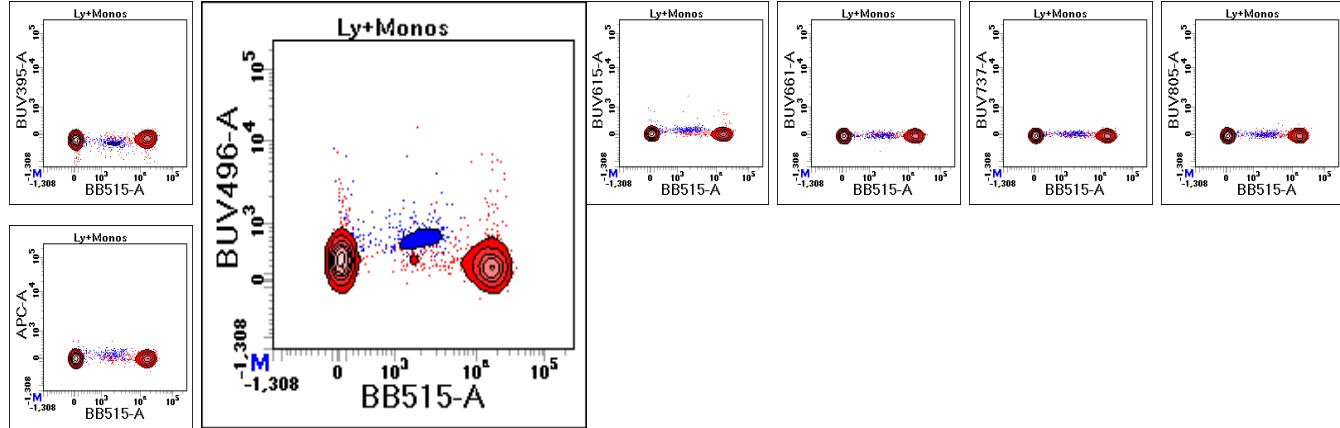


CD4s by BB515

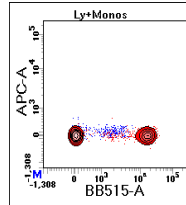
B



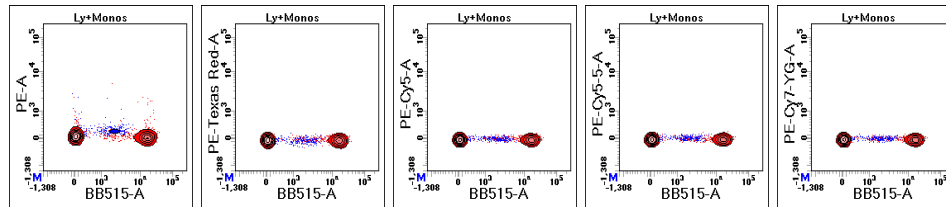
UV



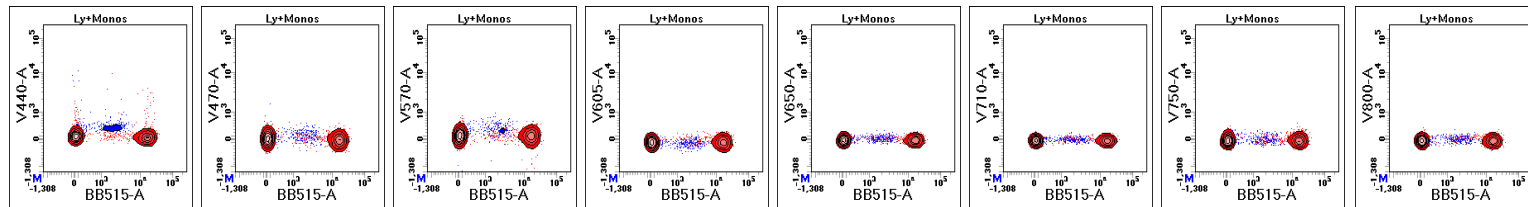
RED



G

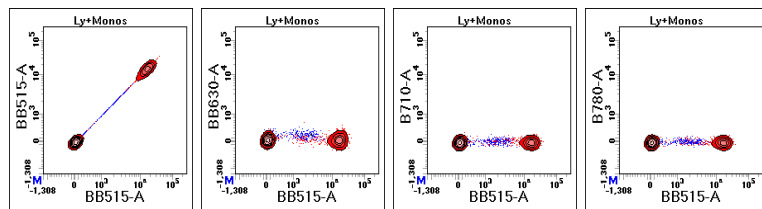


V

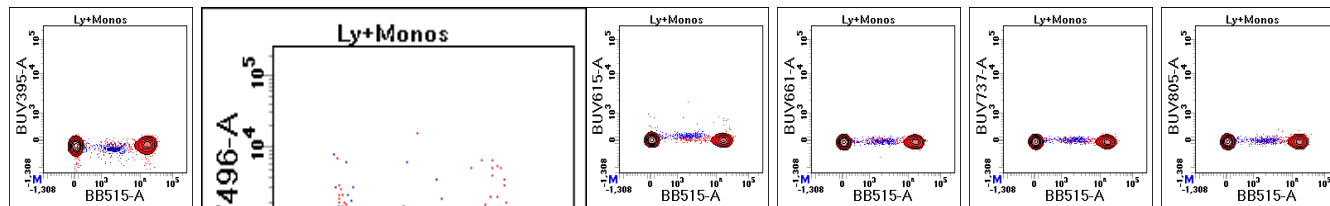


CD4s by BB515

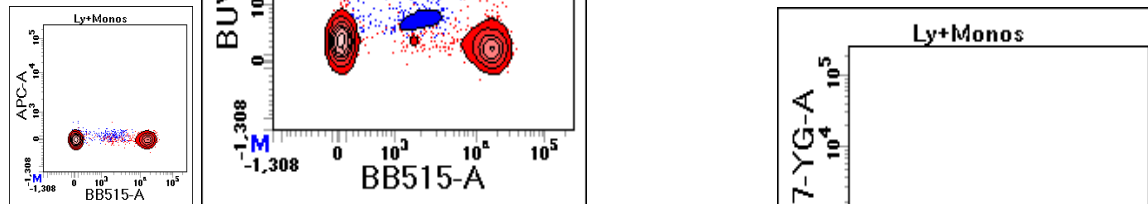
B



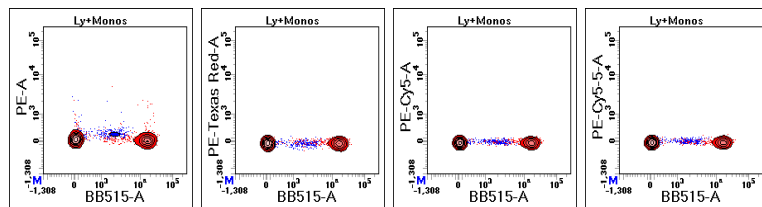
UV



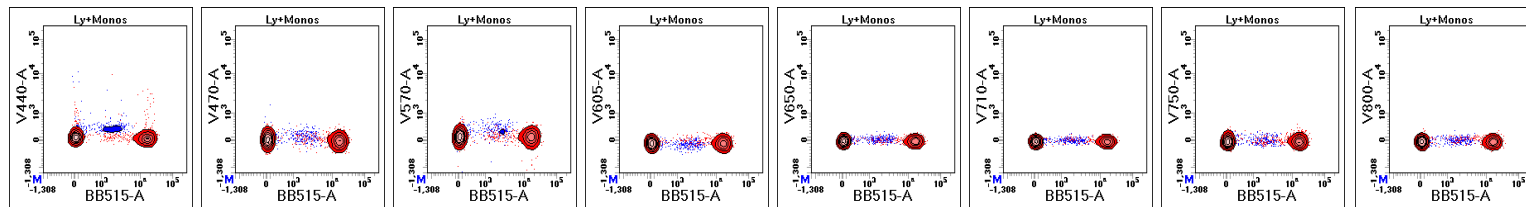
RED



G



V



Do I have to know this?

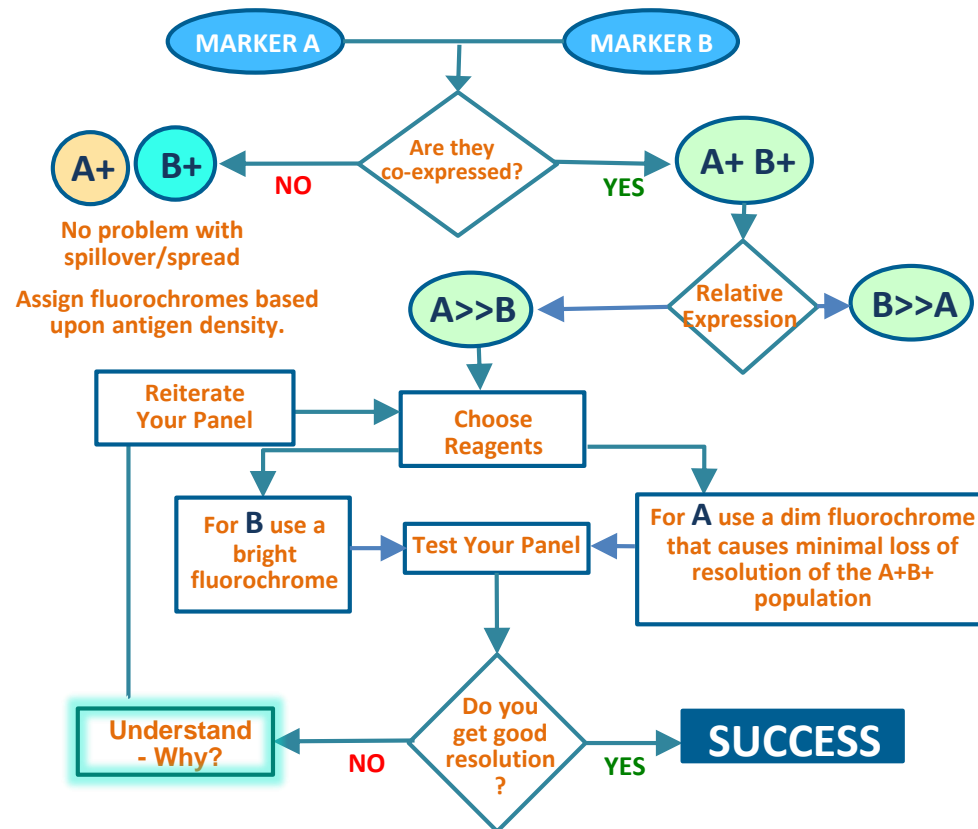
RIM (Resolution impact matrix)

- The loss of resolution in different cytometer configurations can be measured
- It depends on optical filters in the cytometer (optical configuration)



Primary fluorochrome (dim antigen)	Secondary fluorochrome (bright antigen)													
	BB515	FITC	PE	PE-CF594	PerCP-Cy5.5	BUV395	BUV737	BV421	BV480	BV510	BV605	BV650	BV711	BV786
BB515	Grey	Grey	Green	Green	Green	Green	Green	Green	Yellow	Green	Green	Green	Green	Green
FITC	Green	Green	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
PE	Orange	Yellow	Grey	Yellow	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
PE-CF594	Orange	Yellow	Red	Grey	Green	Green	Green	Green	Green	Green	Yellow	Green	Green	Green
PerCP-Cy5.5	Yellow	Yellow	Red	Red	Grey	Green	Green	Green	Green	Green	Yellow	Yellow	Orange	Green
BB700	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
BUV395	Green	Green	Green	Green	Green	Grey	Yellow	Green	Green	Green	Green	Green	Green	Green
BUV737	Green	Green	Yellow	Yellow	Green	Green	Grey	Green	Green	Yellow	Orange	Orange	Red	Orange
BV421	Green	Green	Green	Green	Green	Green	Green	Green	Orange	Green	Green	Green	Yellow	Yellow
BV480	Green	Green	Green	Green	Green	Green	Green	Green	Orange	Grey	Green	Green	Green	Green
BV510	Green	Green	Green	Green	Green	Green	Green	Green	Yellow	Grey	Green	Green	Green	Green
BV605	Green	Green	Orange	Orange	Green	Green	Green	Green	Orange	Orange	Grey	Orange	Green	Green
BV650	Green	Green	Yellow	Yellow	Yellow	Green	Green	Green	Orange	Orange	Grey	Orange	Orange	Yellow
BV711	Green	Green	Yellow	Yellow	Orange	Green	Yellow	Green	Yellow	Yellow	Red	Red	Grey	Yellow
BV786	Green	Green	Yellow	Yellow	Orange	Green	Red	Green	Yellow	Orange	Orange	Red	Red	Grey

The panel design process



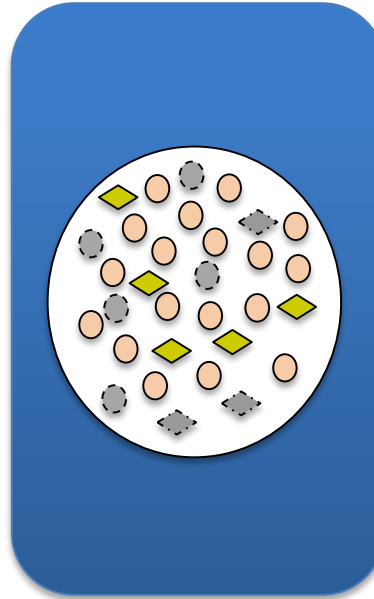
Other factors to take into account in Panel design...

- We will apply the principles of panel design to achieve optimal resolution of key memory T-cell populations:
 - Design panels on multiple instruments with varying capabilities

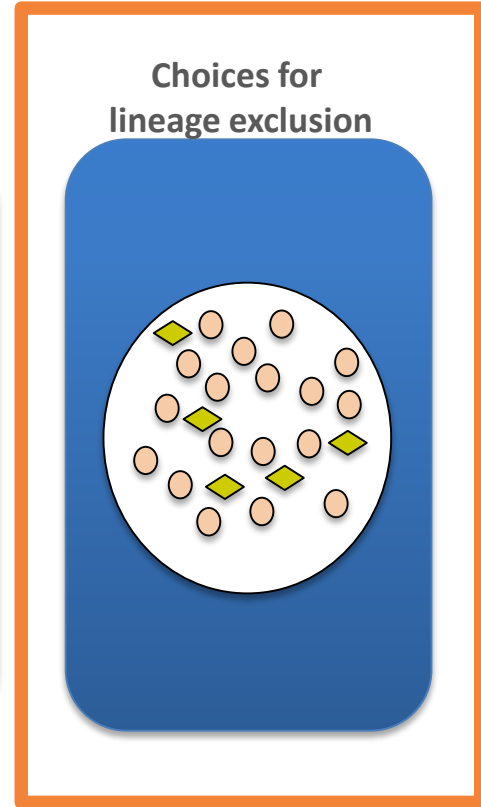
Exclude unwanted cells

- ☠ Dead cells
- ◆ Lineage cells
- Cells of interest

Choices for
dead-cell exclusion

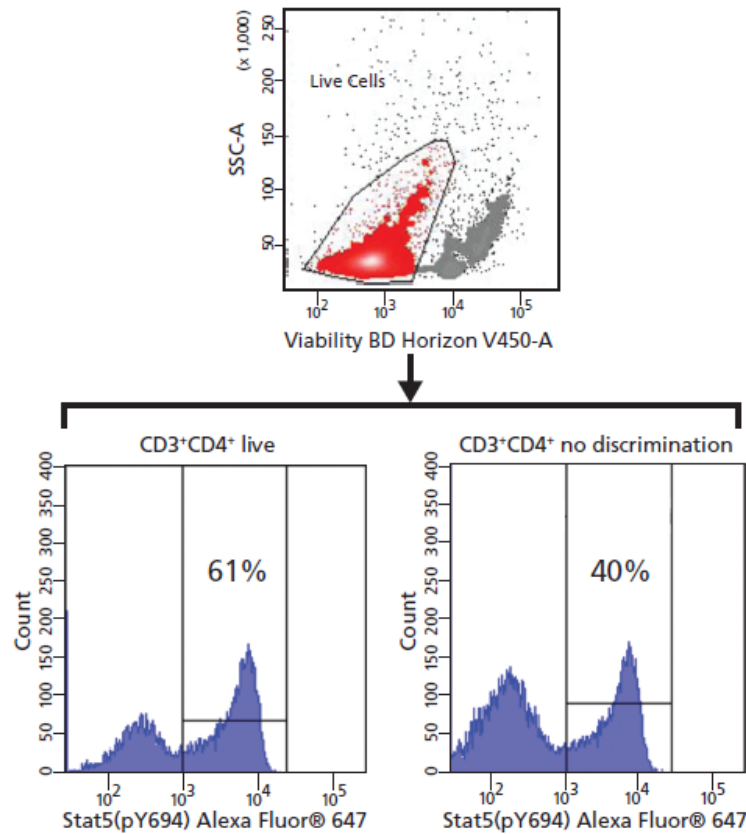


Choices for
lineage exclusion

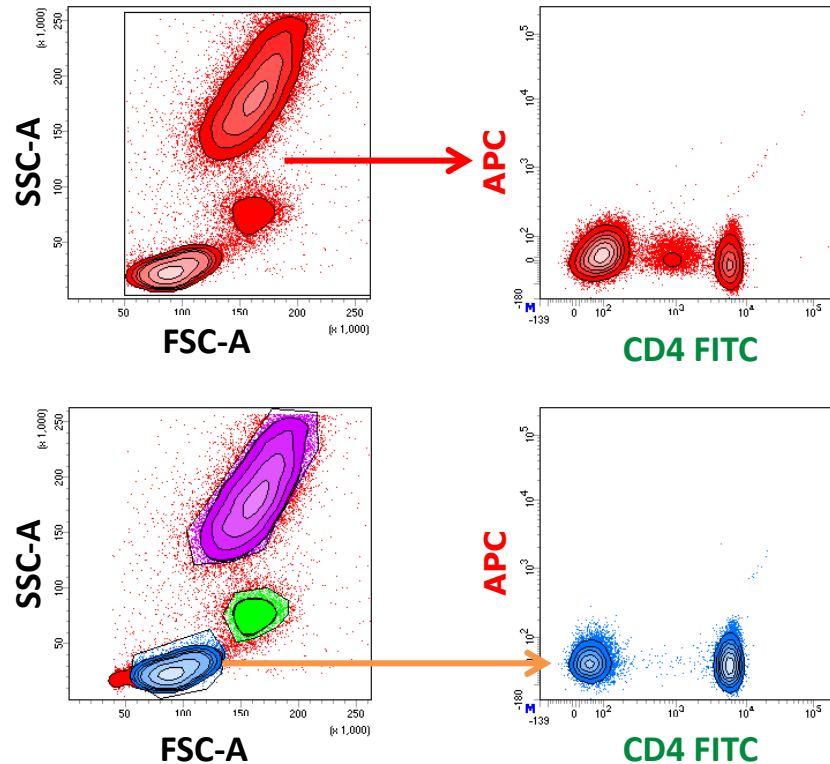


Viability Staining Improves Results

- Gating out dead cells leads to more accurate statistics

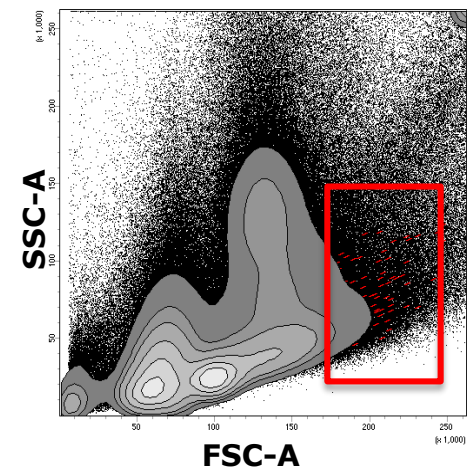
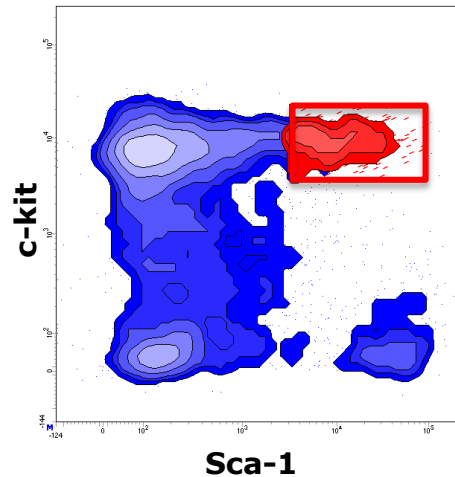
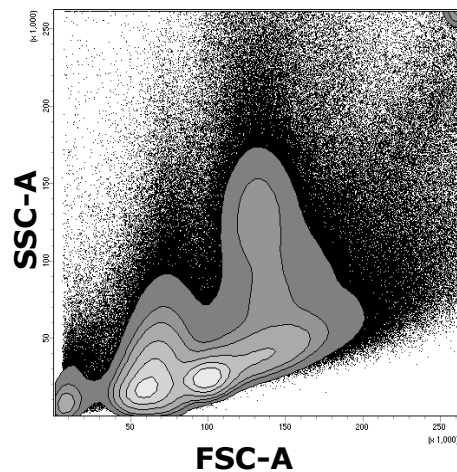


Lineage exclusion by light scatter



- In peripheral blood, different cell lineages can be easily discriminated based on light scatter.

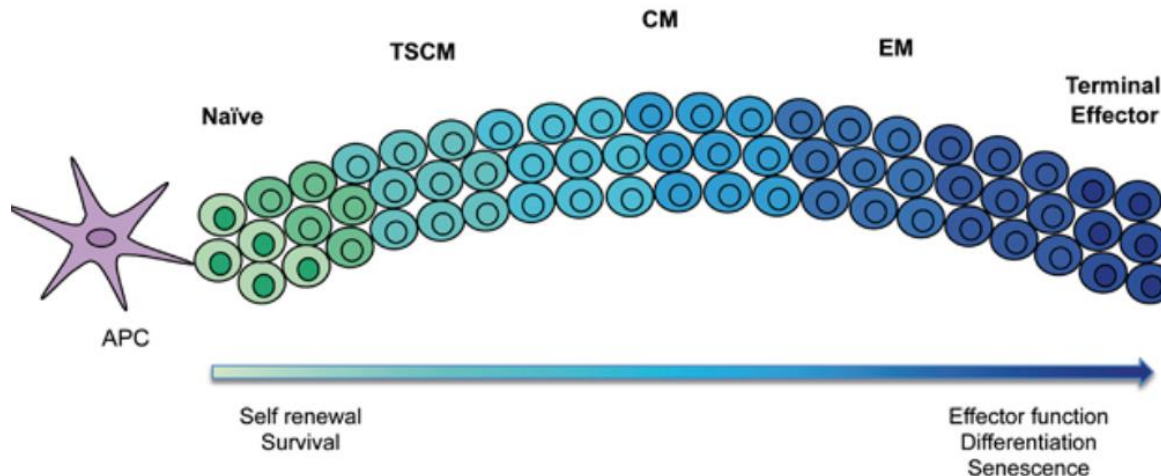
Lineage Exclusion by Light Scatter is not Sufficient for Rare Population Detection



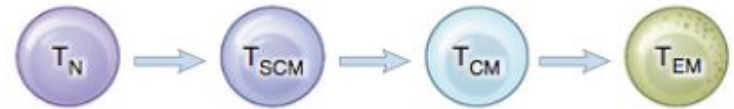
- In samples such as mouse bone marrow, detection of rare stem cells is confounded by the overwhelming presence of lineage cells
- The use of lineage markers is needed to clearly detect rare populations of interest.

Experimental goal

- Use best panel design practices to optimize panels for identification of different T-cell subsets



Panel design: Know the biology

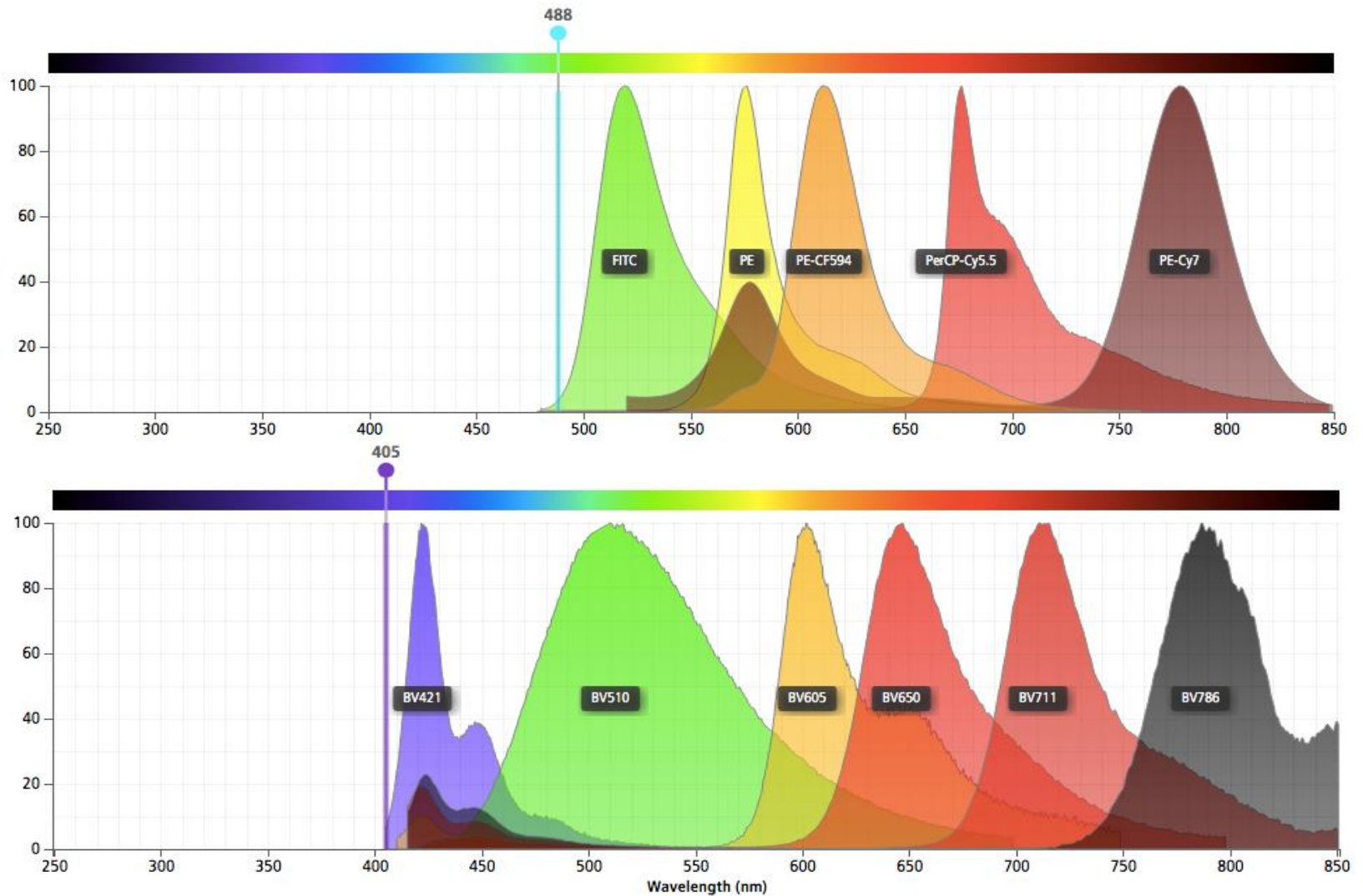


Marker	Ag Density	Class	T _N	T _{SCM}	T _{CM}	T _{EM}
CD95	Low	Tertiary	-	+	++	+
CD183/CXCR3	Low	Tertiary	+	+	++	+
CD197/CCR7	Low	Primary	+	+	+	-
CD27	Medium	Primary	+	+	+	-
CD45RO	Low-to-Hi	Secondary	-	-	++	++
CD45RA	Low-to-Hi	Secondary	+++	+++	+	+
Lin/LD	Med-to-Hi	Primary	+++	+++	+++	+++
CD3	High	Primary	+++	+++	+++	+++
CD8	High	Primary	+++	+++	+++	+++

Different systems configurations...

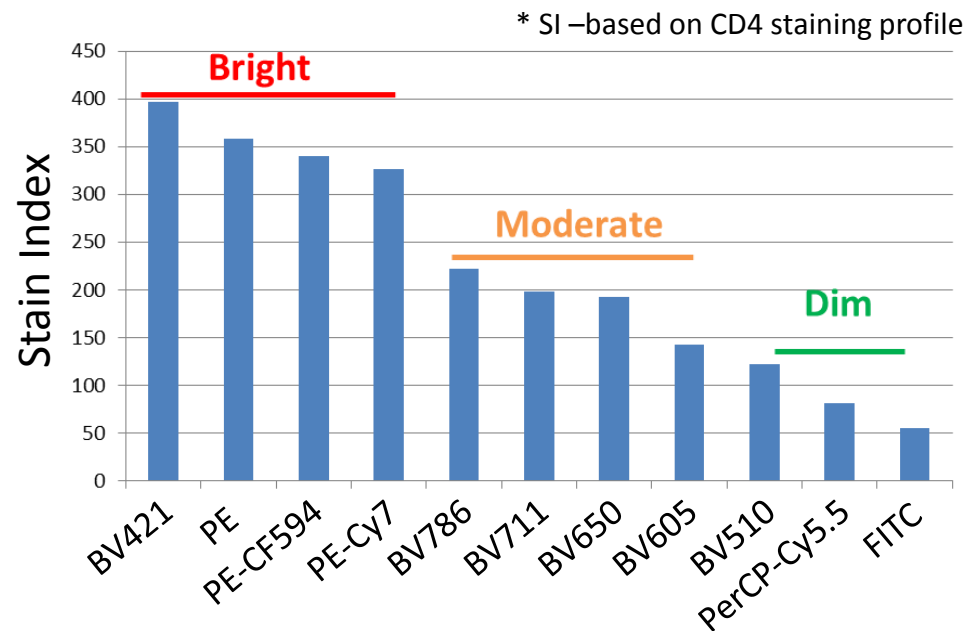
- Laser sources
- Filters

2-Laser system: Blue/Violet configuration



2-Laser system: Instrument characterization

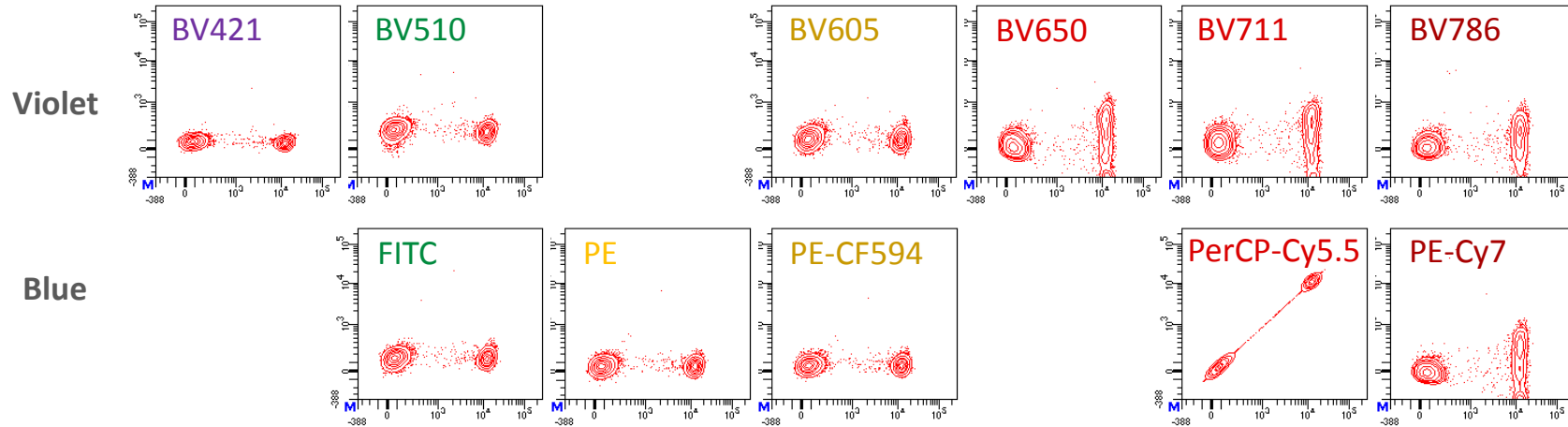
X-20 2-Laser configuration	
405 nm	BV421
	BV510
	BV605
	BV650
	BV711
	BV786
488 nm	FITC
	PE
	PE-CF594
	PerCP-Cy5.5
	PE-Cy7



Spread Analysis: PerCP-Cy5.5



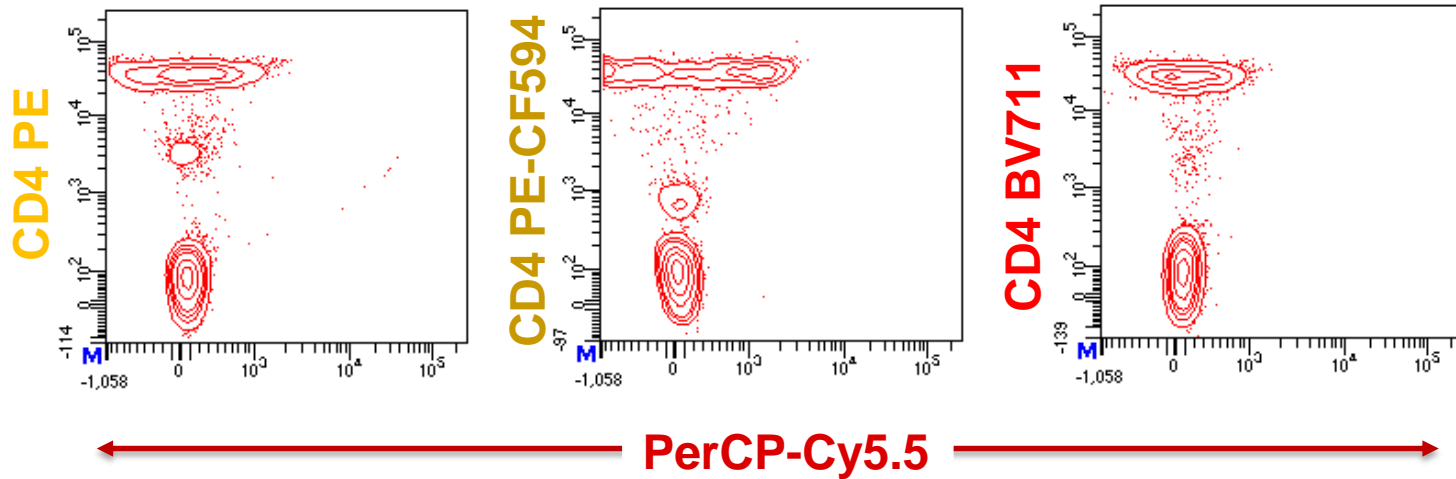
Laser



← CD4 PerCP-Cy5.5 →

- PerCP-Cy5.5 is a dim fluorochrome with significant impact on other fluorochromes (adjacent and cross-laser)

Spread Analysis: Fluorochromes impacting PerCP-Cy5.5

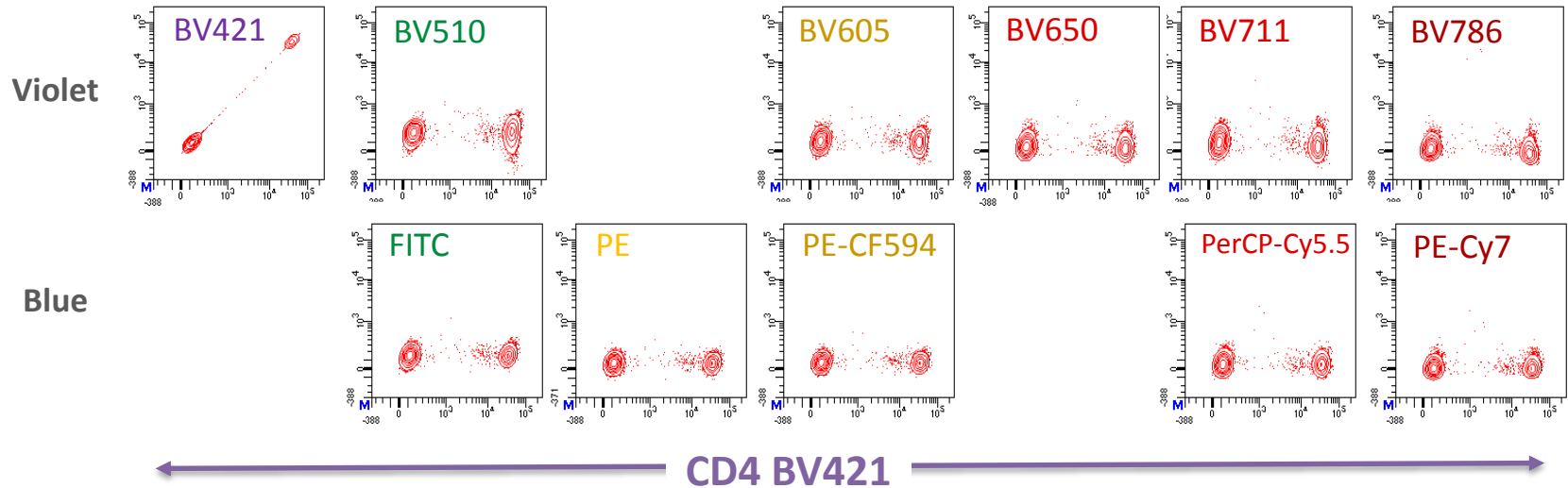


- PerCP-Cy5.5 is a dim fluorochrome with significant impact on other fluorochromes (adjacent and cross-laser)
- PerCP-Cy5.5 is also impacted by other fluorochromes
- The viability dye 7-AAD is detected in the same channel
- PerCP-Cy5.5 is a good choice for a dump channel

Spread Analysis: BV421

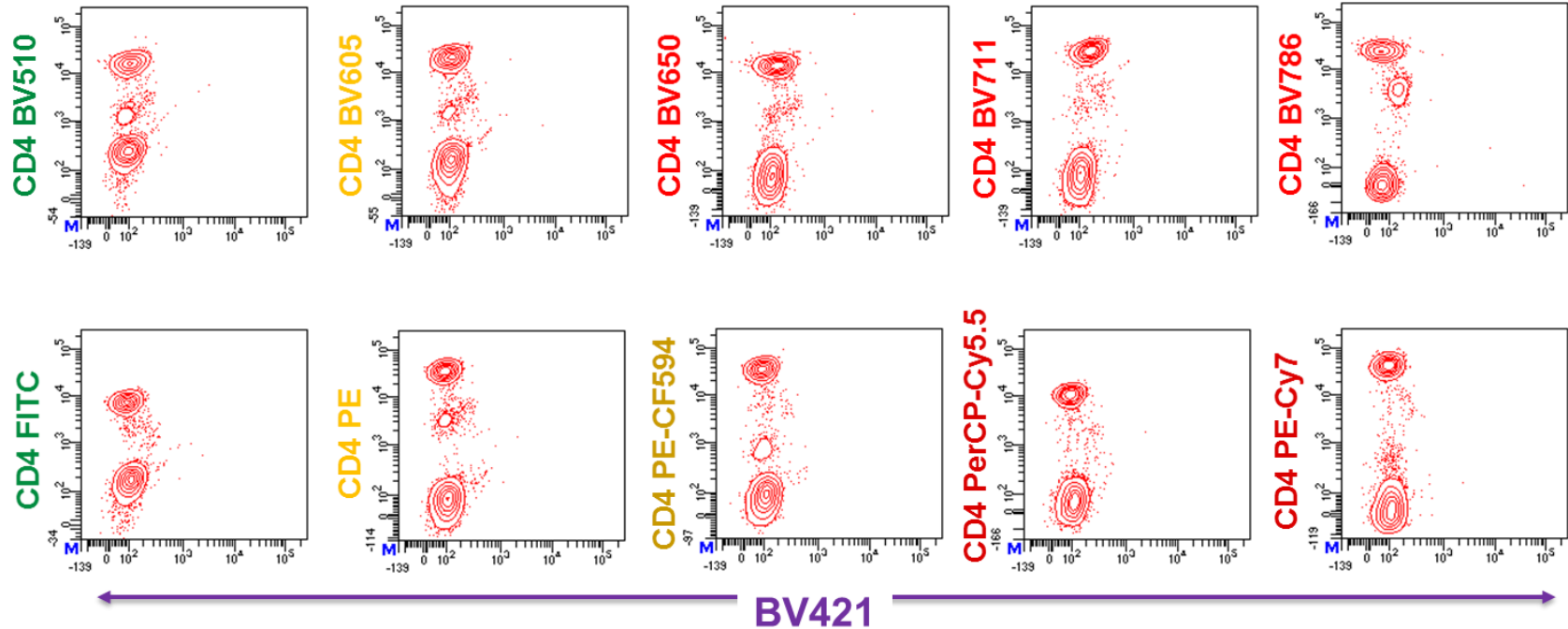


Laser



- BV421 is a bright fluorochrome with minimal impact on other fluorochromes

Spread Analysis: Fluorochromes impacting BV421












- BV421 is a bright fluorochrome with minimal impact on other fluorochromes
- BV421 is minimally impacted by other fluorochromes
- BV421 is a good choice for critical markers expressed at low levels (CD183/CXCR3, CD95)

2-Laser system panel design

ANTIGEN

Marker	Ag Density
CD95	Low
CD183/CXCR3	Low
CD197/CCR7	Low
CD27	Medium
CD45RO	Low-to-High
CD45RA	Low-to-High
*Lin/LD	Med-to-High
CD3	High
CD8	High

ASSIGNMENT

Fluorochrome	Laser
PE	
BV605	
BV786	
BV421	
PE-CF594	
PE-Cy7	
FITC	
PerCP-Cy5.5	
BV510	

* Lineage markers – CD4, CD19, CD14

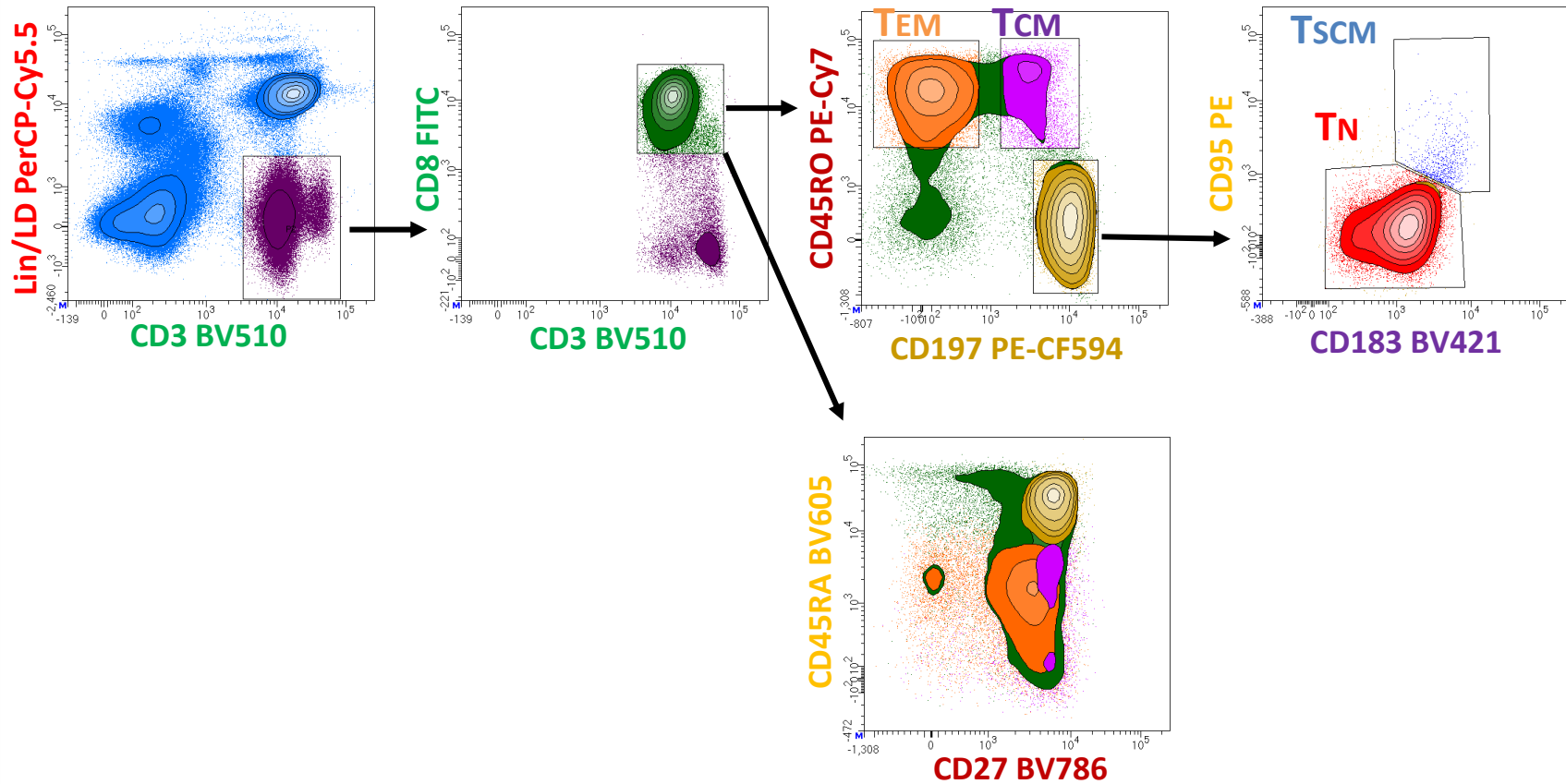
2-Laser system panel design

ANTIGEN

ASSIGNMENT

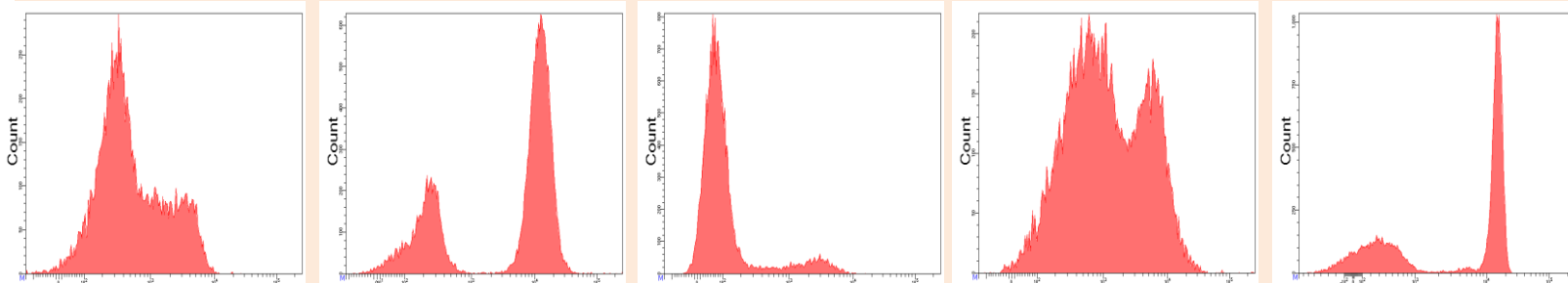
Marker	Ag Density	Fluorochrome	Laser
CD95	Low	PE	●
CD45RA	Low-to-High	BV605	●
CD27	Medium	BV786	●
CD183/CXCR3	Low	BV421	●
CD197/CCR7	Low	PE-CF594	●
CD45RO	Low-to-High	PE-Cy7	●
CD8	High	FITC	●
*Lin/LD	Med-to-High	PerCP-Cy5.5	●
CD3	High	BV510	●

2-Laser panel



Panel review: Maintained resolution

Single Stain



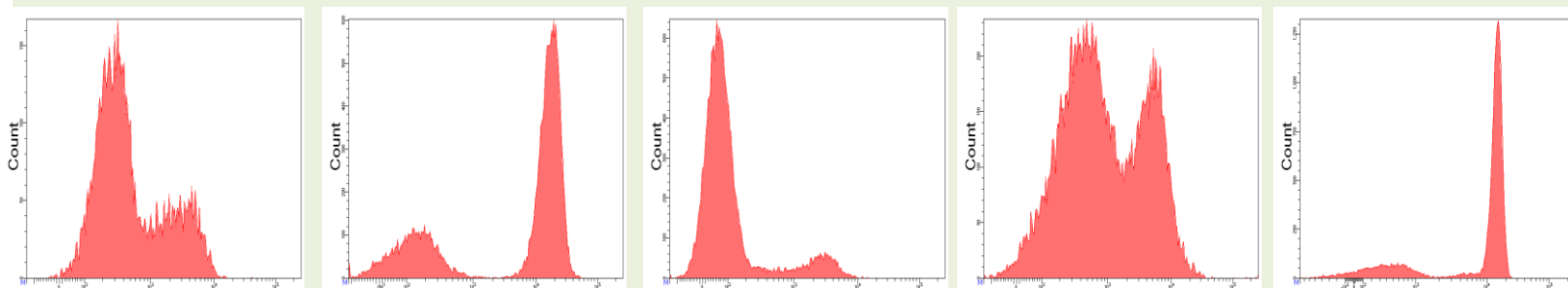
CXCR3
BV421

CD3
BV510

CD8
FITC

CD95
PE

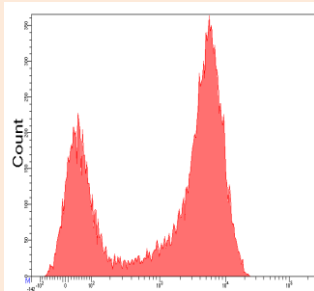
CD4/CD19/CD14
PerCP-Cy5.5



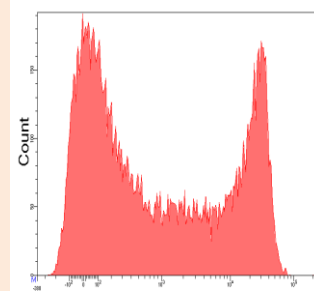
Panel

Panel review: Loss of resolution

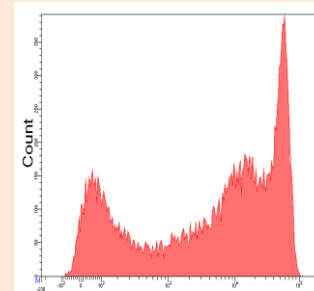
Single Stain



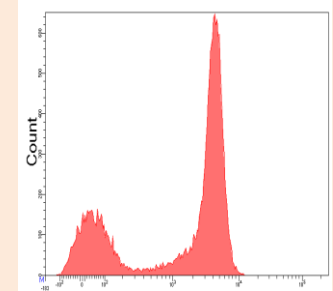
CCR7
PE-CF594



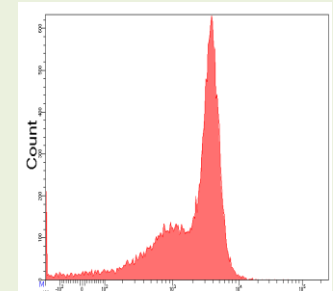
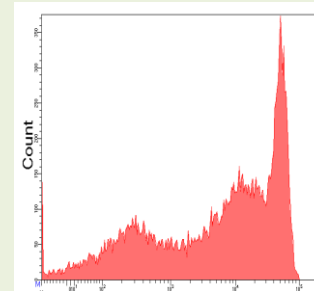
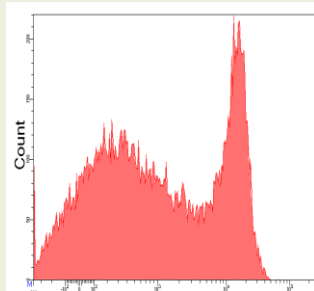
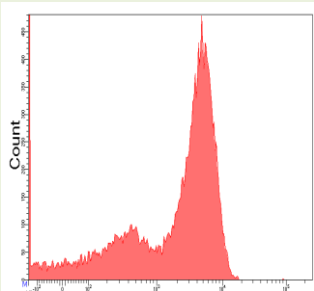
CD45RO
PE-Cy7



CD45RA
BV605



CD27
BV786



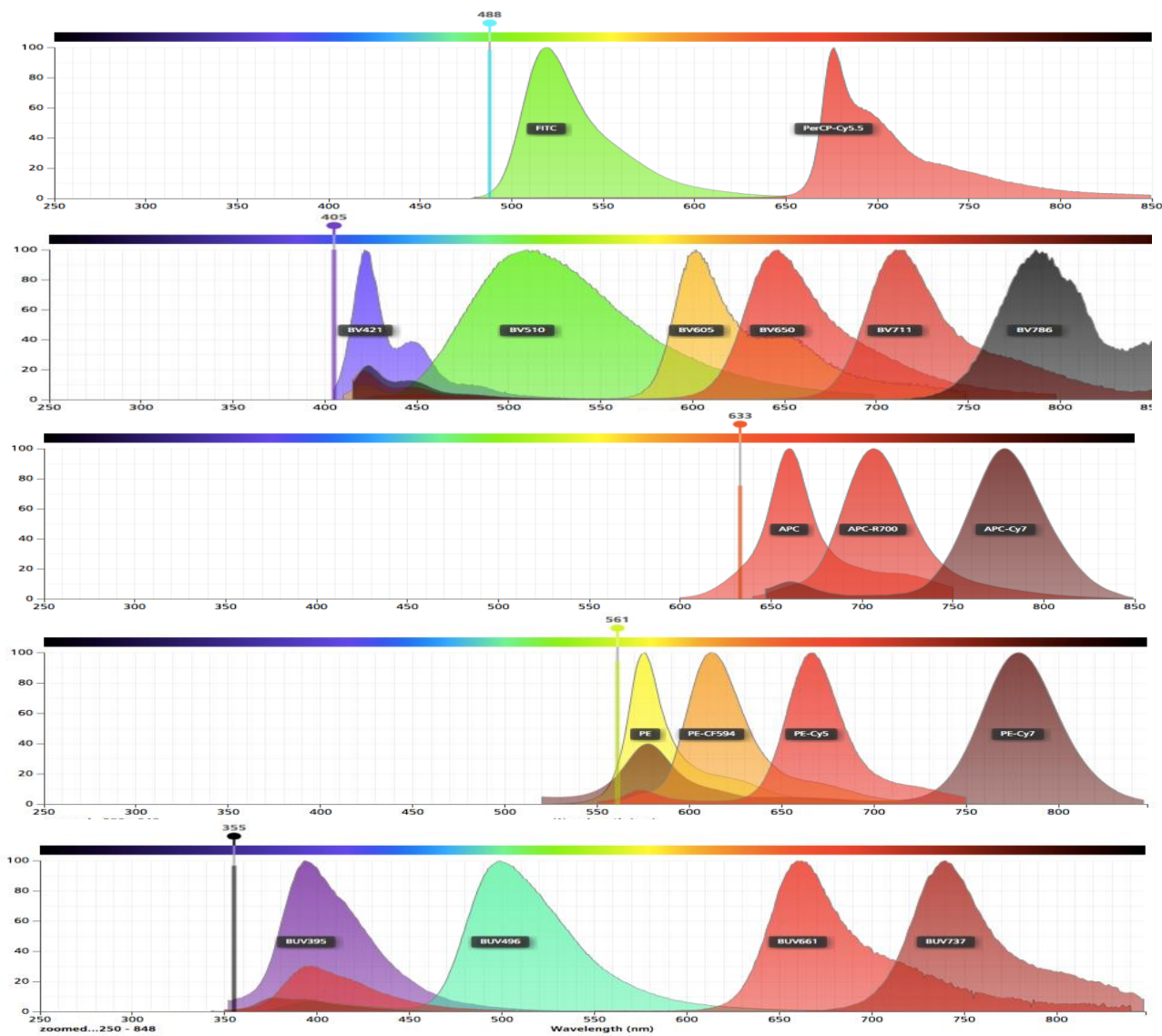
Panel

2-Laser configuration panel review

Marker	Ag Density	Fluorochrome	Resolved	Impacted
CD95	Low	PE	√	
CD183/CXCR3	Low	BV421	√	
CD197/CCR7	Low	PE-CF594		X
CD27	Medium	BV786		X
CD45RO	Low-to-Hi	PE-Cy7		X
CD45RA	Low-to-Hi	BV605		X
Lin/LD	Med-to-Hi	PerCP-Cy5.5	√	
CD3	High	BV510	√	
CD8	High	FITC	√	

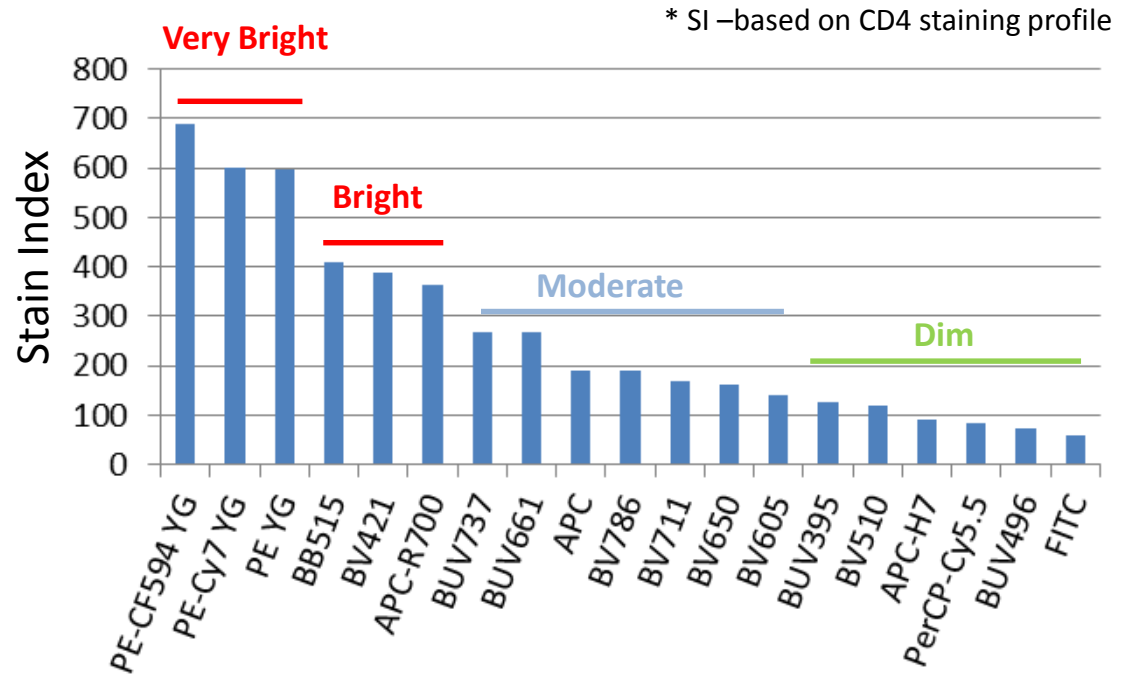
- PE and BV421 allowed for good resolution of T_{SCM}
- BV510 and PE-CF594 impact the resolution of BV605
- BV605 is impacting the resolution of BV786

5-Laser system: B/V/R/YG/UV configuration



5-Laser system: Instrument characterization

X-20 5-Laser Configuration	
355 nm	BUV395
	BUV496
	BUV661
	BUV737
405 nm	BV421
	BV510
	BV605
	BV650
	BV711
	BV786
488 nm	FITC
	PerCP-Cy5.5
561 nm	PE
	PE-CF594
	PE-Cy7
640 nm	APC
	APC-R700
	APC-H7

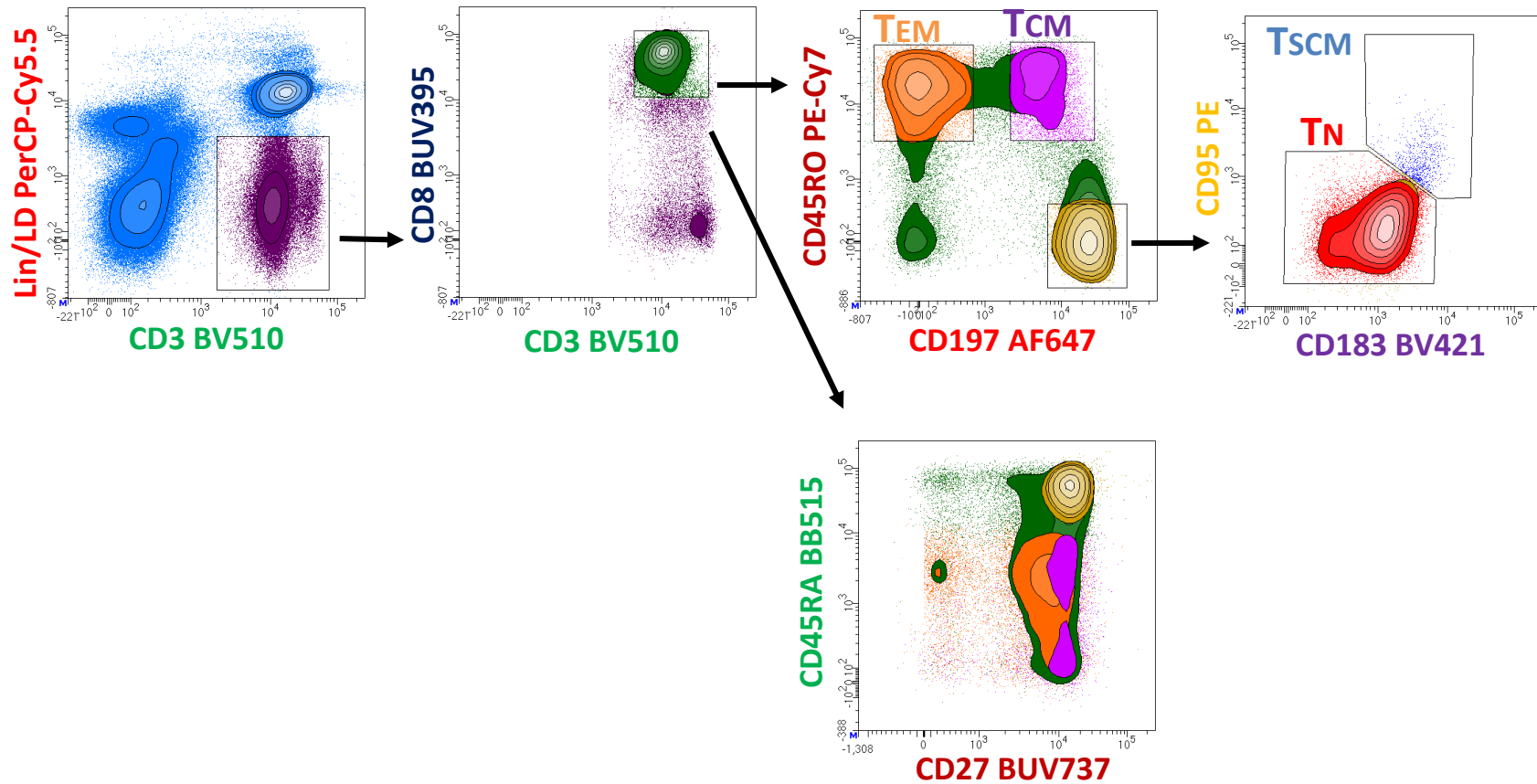


5-Laser configuration: Panel design

5-Laser System

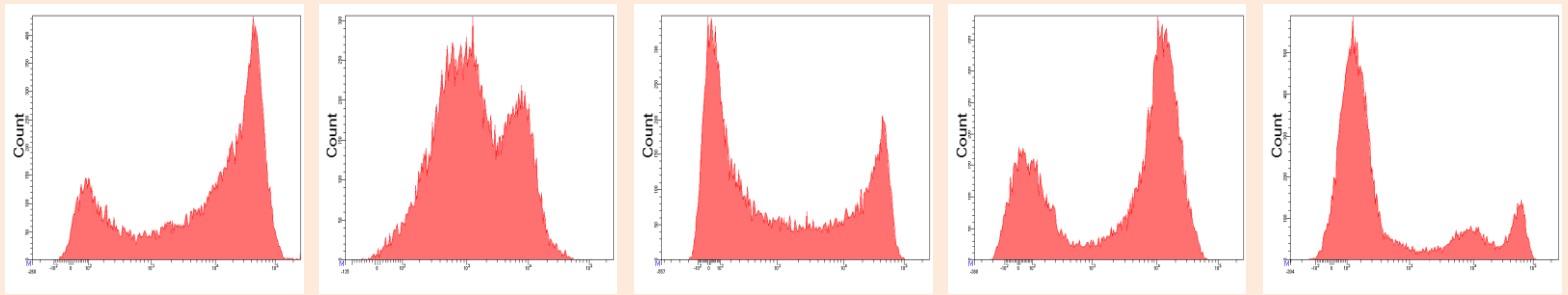
Markers	Ag Density	Fluorochrome	
CD95	Low	PE-YG (-B)	●
CD183/CXCR3	Low	BV421	●
CD197/CCR7	Low	AF647	●
CD27	Medium	BUV737 (BV786)	●
CD45RO	Low-to-Hi	PE-Cy7-YG (-B)	●
CD45RA	Low-to-Hi	BB515 (FITC)	●
Lin/LD	Med-to-Hi	PerCP-Cy5.5	●
CD3	High	BV510	●
CD8	High	BUV395 (APC-H7)	●

5-Laser panel



Panel review: Maintained resolution

Single Stain



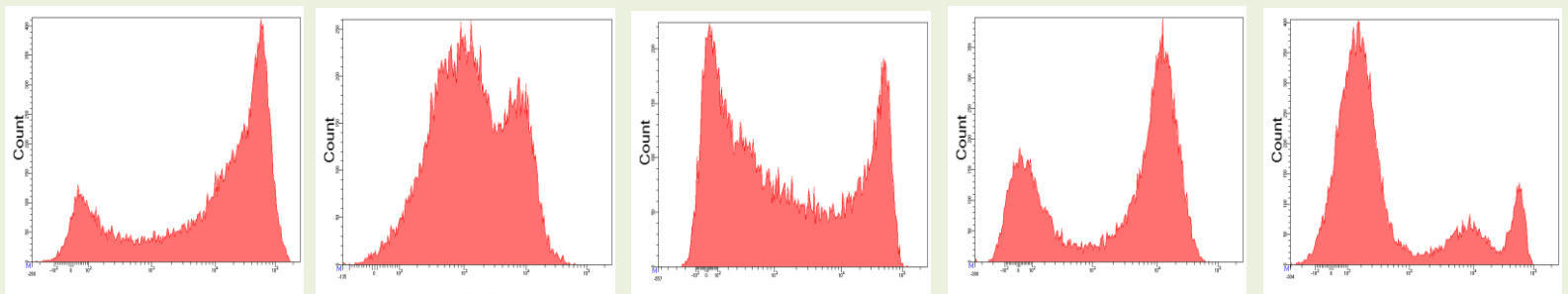
CD45RA
BB515

CD95
PE

CD45RO
PE-Cy7

CD197
AF647

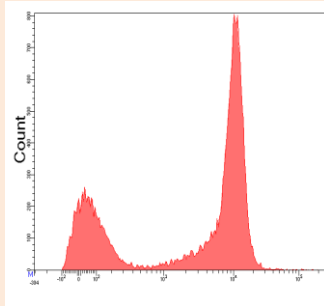
CD8
BUV395



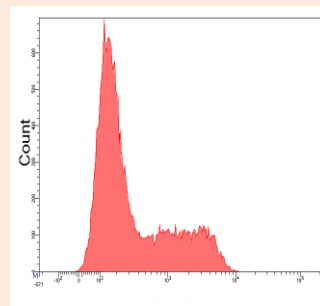
Panel

Panel review: Maintained resolution

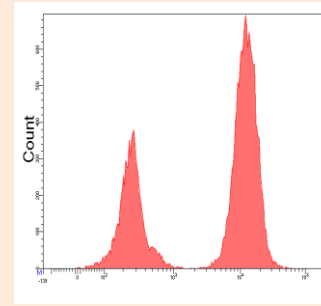
Single Stain



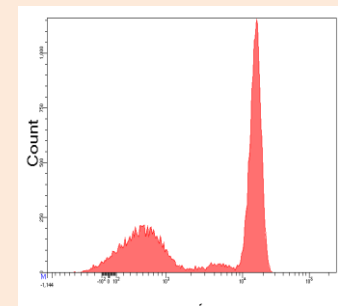
CD27
BUV737



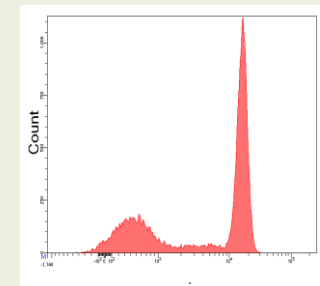
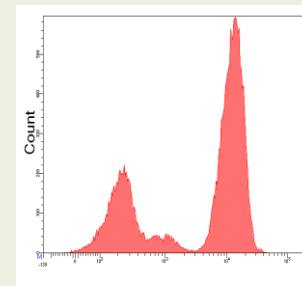
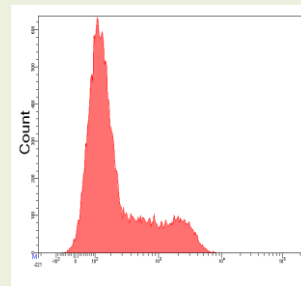
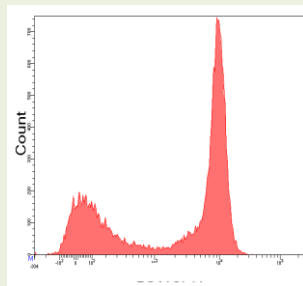
CD183
BV421



CD3
BV510



CD4/19/14
PerCP-Cy5.5



Panel

5-Laser configuration panel review

Marker	Ag Density	Fluorochrome	Resolved	Impacted
CD95	Low	PE	✓	
CD183/CXCR3	Low	BV421	✓	
CD197/CCR7	Low	AF647	✓	
CD27	Medium	BUV737	✓	
CD45RO	Low-to-Hi	PE-Cy7	✓	
CD45RA	Low-to-Hi	BB515	✓	
Lin/LD	Med-to-Hi	PerCP-Cy5.5	✓	
CD3	High	BV510	✓	
CD8	High	BUV395	✓	

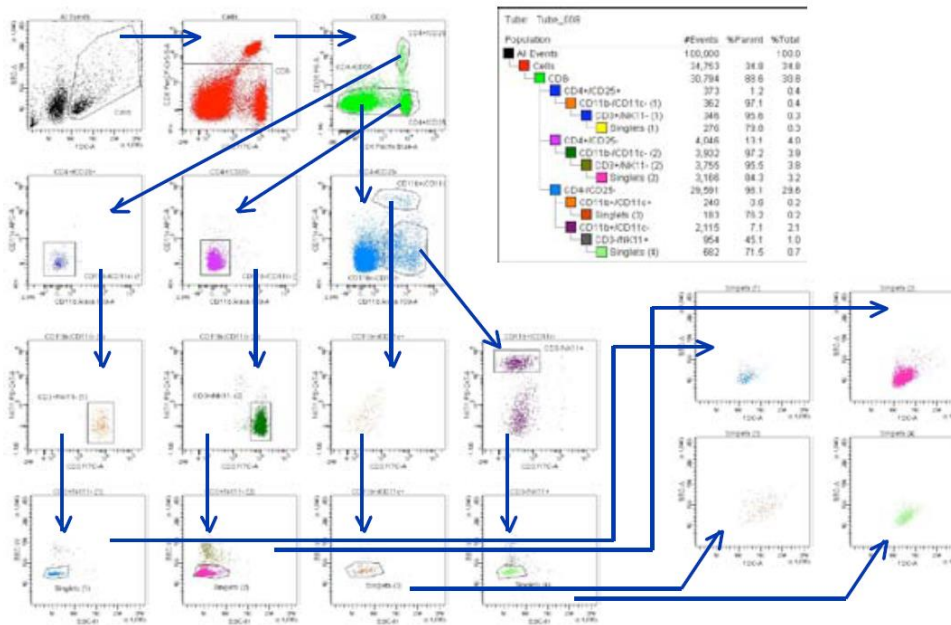


Designing a panel

Departamento de aplicaciones




Barcelona 2019

Our aim: design (correctly) a simple panel



A. Cytometer configuration

- Cytometer: FACSCantoII 3L 8C

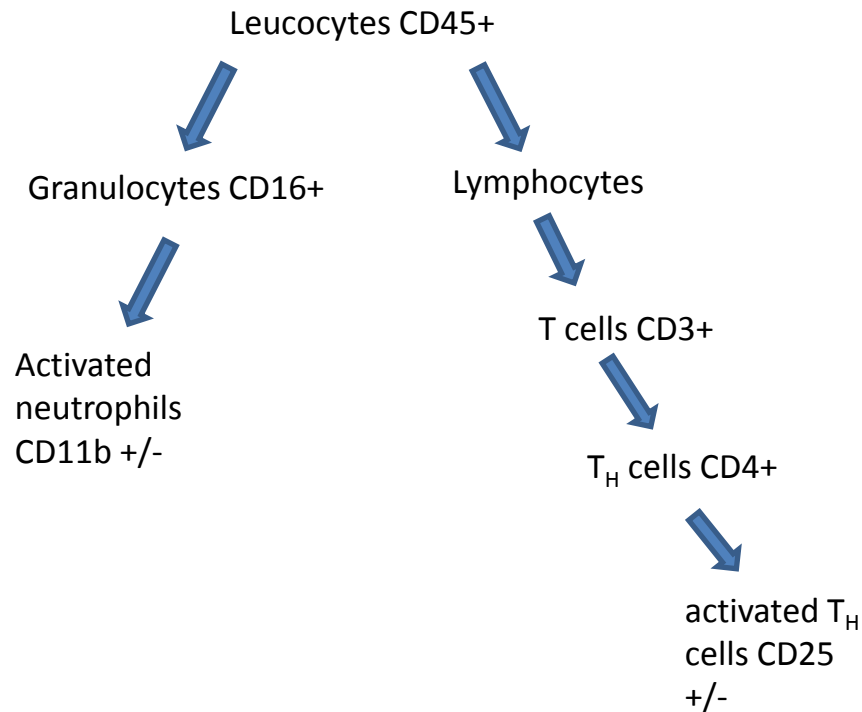
	Blue laser <ul style="list-style-type: none">• FITC/BB515• PE• PerCP-Cy5.5
	Red laser <ul style="list-style-type: none">• APC• APC-H7
	Violet laser <ul style="list-style-type: none">• BV421• BV510

B. Fluorochrome brightness

Fluorochoime		Brightness
405 nm	BV421	High
	BV510	Dim
488 nm	FITC	Dim
	PE	High
	PerCP-Cy5.5	Dim
	PE-Cy7	High
640 nm	APC	High
	APC-H7	Dim

C. Markers: their expression levels & coexpression

- Hierarchy and population of interest

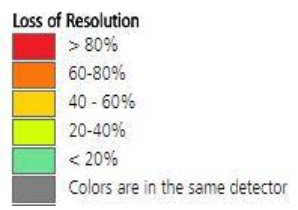


- Expression levels:

Marker	Ag density
CD3	High
CD4	High
CD25	Low
CD11b	Low
CD16	Medium
CD45	High

D. Spread/coexpression issues (Resolution Impact Matrix)

Primary fluorochrome (dim antigen)	Secondary fluorochrome (bright antigen)							
	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	BV421	BV510
FITC	Grey	Yellow	Green	Green	Green	Green	Green	Yellow
PE	Yellow	Grey	Green	Orange	Green	Green	Green	Green
PerCP-Cy5.5	Yellow	Red	Grey	Orange	Yellow	Green	Green	Green
PE-Cy7	Green	Orange	Red	Grey	Green	Yellow	Green	Green
APC	Green	Green	Yellow	Green	Grey	Yellow	Green	Green
APC-H7	Green	Green	Yellow	Orange	Orange	Grey	Green	Green
BV421	Green	Green	Green	Green	Green	Green	Grey	Green
BV510	Green	Green	Green	Green	Green	Green	Yellow	Grey



Matching rules

Marker	Ag density
CD3	High
CD4	High
CD25	Low
CD11b	Low
CD16	Medium
CD45	High



- 1) Ag. Density <-> Fluorochrome brightness
- 2) Coexpression in populations of interest

Fluorochrome	Brightness
BV421	High
BV510	Dim
FITC	Dim
PE	High
PerCP-Cy5.5	Dim
PE-Cy7	High
APC	High
APC-H7	Dim

Matching rules

Marker	Ag density
CD45	High
CD3	High
CD4	High
CD16	Medium
CD25	Low
CD11b	Low



Fluorochoime	Brightness
BV421	High
PE	High
APC	High
PE-Cy7	High
PerCP-Cy5.5	Moderate
FITC	Moderate
BV510	Moderate
APC-H7	Dim



Designing a panel

Soluciones panel

Matching rules

Marker	Ag density
CD45	High
CD3	High
CD4	High
CD16	Medium
CD25	Low
CD11b	Low



Fluorochoime	Brightness
BV421	High
PE	High
APC	High
PE-Cy7	High
PerCP-Cy5.5	Moderate
FITC	Moderate
BV510	Moderate
APC-H7	Dim



	A	B	C
CD45	FITC	PerCP-Cy5.5	FITC
CD3	PerCP-Cy5.5	FITC	PerCP-Cy5.5
CD4	BV510	BV510	BV510
CD16	APC	APC	PE-Cy7
CD25	PE	PE	PE
CD11b	BV421	BV421	BV421





GPS

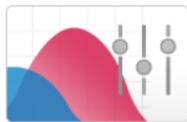
Departamento de aplicaciones

Barcelona 2019

Some useful tools

Selection

We've put together a variety of tools and information to help you design your next multicolor assay.



Fluorescence Spectrum Viewer

Find fluorochromes for a multicolor experiment based on flow cytometer and compensation trade-offs.

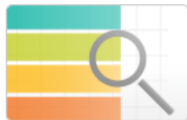
[Launch the Spectrum Viewer »](#)



BD Horizon™ Guided Panel Solution (GPS)

A guided workflow to help streamline and standardize the panel design process.

[Use the Guided Panel Solution »](#)



Absorption and Emission Spectra

View the range of emission and learn more about each fluorochrome in the BD product line.

[See Absorption & Emission Spectra »](#)



Multicolor Antibody Reagents Catalog

Choose from our extensive portfolio of high-quality fluorescent-conjugated reagents to build your multicolor panels.

[Download the Multicolor Catalog »](#)



BD FACSelect™ Buffer Compatibility Resource

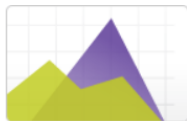
Navigate buffer choices to select the right combination for your intracellular and surface marker experiments.

[Use Buffer Compatibility Resource »](#)



Example Human and Mouse Panels

[Human Panels »](#)
[Human Small Size Panels »](#)
[Mouse Panels »](#)
[Mouse Small Size Panels »](#)



Fluorochrome Specifications Chart and Guides

[Fluorochrome Specifications Chart »](#)
[Fluorochrome/Laser Ref. Poster »](#)

[Fluorescence Spectrum Viewer](#)
[Multicolor Antibody Catalog](#)
[BD Horizon Brilliant Dyes Booklet](#)
[All Resources →](#)

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[Training](#)

<https://www.bdbiosciences.com/us/tools/s/gps>

Some useful tools

BD Horizon™ Guided Panel Solution

<https://www.bdbiosciences.com/us/tools/s/gps>


Aplicaciones Login InterQC Login InterQC admin Citi Commercial Card https://merchants.g... Guided Panel Solu... spectrumviewer | BD Infinicyt™ Support: T SharePoint South Region BDB -

BD Biosciences [Sign In or Create an Account](#)

INSTRUMENTS REAGENTS CUSTOM REAGENTS APPLICATIONS SUPPORT

Home / Gps

BD HORIZON™ GUIDED PANEL SOLUTION



Panel design can be a difficult and time-consuming process but is essential to obtaining good data. The BD Horizon™ Guided Panel Solution (GPS) provides a guided workflow for reagent selection based on the principles of panel design. This tool will help you streamline the panel design process and avoid reagent selections that may negatively affect population resolution.

[CREATE ACCOUNT](#) [START BD HORIZON GPS](#)

Overview **User Guide**

There are many factors to consider when designing a panel such as instrument configuration, fluorochrome brightness, spectral overlap, antigen density and co-expression and reagent availability. This can make panel design an overwhelming, iterative and time-consuming process. BD has created an online tool that organizes the principles of panel design into a guided workflow to help streamline and standardize the panel design process. Additionally, the tool has features to assess the reagent selections to help avoid selections that may negatively affect population resolution.

How the Tool Works

[CONTACT US](#)

Key Resources

- [Quick Reference Guide](#)
- [Spectrum Viewer](#)
- [BD Horizon™ Tour Videos](#)
- [Multicolor Resources and Tools](#)

More Information

- [Ask BD](#)
- [Sign Up for Email Updates](#)

Help

- [Ordering Support](#)
- [Training](#)

ES 11:19 03/01/2019

<https://www.bdbiosciences.com/us/tools/s/gps>

Some useful tools

BD Biosciences

Alberto Crespo

BD Horizon™ Guided Panel Solution

Help

MY PANELS

CONTACT US

NEW PANEL

Name	Description	Actions	Last Modified
Sample Panel 1: 6-Color Treg Panel – No Rules	6-color panel to identify regulatory T-cell (Treg) subsets: Minimal attention to fluorochrome brightness or antigen density.		
Sample Panel 2: 6-Color Treg Panel – Some Rules	6-color panel to identify regulatory T-cell (Treg) subsets: Refined panel with focus on fluorochrome assignment based on the expression of antigens.		
Sample Panel 3: 6-Color Treg Panel – Best Practices	6-color panel to identify regulatory T-cell (Treg) subsets: Use of best practices to further optimize the panel to maximize the resolution of Tregs: – Antigen density and co-expression, – Fluorochrome brightness, – Spread due to spillover		
Tscm	stem cell memory		noviembre 27, 2018 17:27

Edit

Page 1 of 1 < Prev 1 Next >

Version: 1.0
BD Horizon™ Guided Panel Solution provides a guided approach for reagent selection and helps users avoid some common mistakes. It does not give recommendations for specific reagents or guarantee optimal panel performance.

<https://bdbiosciences.azurewebsites.net/PanelDesignTools/Start/d9e42c16-abdf-44f6-8e04-83f4f3dfe6c0> property of Becton, Dickinson and Company.

<https://www.bdbiosciences.com/us/tools/s/gps>

Some useful tools

The screenshot shows a web browser window displaying the BD Biosciences Panel Design Tools interface. The browser's address bar shows the URL: <https://bdbiosciences.azurewebsites.net/PanelDesignTools/Start/d9e42c16-abdf-44f6-8e04-83f4f3dfe6c0>. The page header includes the BD Biosciences logo and the user name "Alberto Crespo". A navigation bar contains "BD Horizon™ Guided Panel Solution" and a "Help" icon. Below this, the breadcrumb "MY PANELS / TSCM / Describe Panel" is visible. The main content area is titled "TSCM" and features a "CONTACT US" button. A series of tabs at the top of the form include "Describe Panel", "Define Markers", "Define Populations", "Choose Cytometer", "Design Panel", and "Review Panel". The "Describe Panel" tab is active, showing a form with a "Name" field containing "Tscm" and a "Description" field containing "stem cell memory". "Save" and "Continue >" buttons are located at the bottom right of the form area. The Windows taskbar at the bottom shows the system tray with the time 11:28 and date 03/01/2019.

<https://www.bdbiosciences.com/us/tools/s/gps>

Some useful tools

The screenshot displays the 'BD Horizon™ Guided Panel Solution' web application. The browser address bar shows the URL: <https://bdbiosciences.azurewebsites.net/PanelDesignTools/Markers/d9e42c16-abdf-44f6-8e04-83f4f3dfe6c0>. The page title is 'BD Horizon™ Guided Panel Solution' and the breadcrumb navigation is 'MY PANELS / TSCM / Define Markers'. A 'CONTACT US' button is visible in the top right corner.

The main content area is titled 'TSCM' and features a navigation bar with the following steps: Describe Panel, Define Markers (current step), Define Populations, Choose Cytometer, Design Panel, and Review Panel. Navigation buttons '< Back', 'Save', and 'Continue >' are located to the right of the navigation bar.

The 'Define Markers' section includes a 'Select Target Species' section with radio buttons for Human (selected), Mouse, Non-Human Primate, and Rat. Below this is the 'Add Markers' section, which contains a table with columns for Marker, Type, and Clone.

Marker	Type	Clone
CD3	1°	L78,SK7 (als...
CD4	Primary 1°	
CD8	Secondary 2°	
CD95	Teritary 3°	Clone
CD27	3°	Clone
CD45RA	1°	Clone
ccr7	2°	Clone

The 'Type' dropdown menu is open, showing options: Primary 1°, Secondary 2°, and Teritary 3°. A plus sign (+) is located at the bottom left of the 'Add Markers' section.

<https://www.bdbiosciences.com/us/tools/s/gps>

Some useful tools

The screenshot displays the BD Horizon™ Guided Panel software interface, specifically the 'Define Populations' step. The interface is organized into several sections:

- Navigation Bar:** Includes tabs for 'Describe Panel', 'Define Markers', 'Define Populations', 'Choose Cytometer', 'Design Panel', and 'Review Panel'. Action buttons for '< Back', 'Save', and 'Continue >' are also present.
- Step 1: Create Population Tree:** A list of markers with plus (+) and minus (-) buttons for selection. The markers listed are: All Cells, CD3, CD4, CD8, CD95, CD27, CD45RA, and ccr7.
- Step 2: Define Critical Populations:** A population tree diagram showing the hierarchy of cell populations. The tree starts with 'All Cells' (red circle) which branches into 'CD3' (red circle) and 'CD8' (red circle). 'CD3' further branches into 'CD4' (red circle) and 'CD45RA' (blue circle). 'CD4' branches into 'CD45RA' (blue circle), 'CCR7' (red circle), 'CD27' (red circle), and 'CD95 (Tscm CD4)' (blue circle). 'CD8' branches into 'CD45RA' (red circle), 'CCR7' (red circle), 'CD27' (red circle), and 'CD95 (Tscm CD8)' (blue circle). A red 'X' is visible next to the 'CD45RA' node under 'CD3'.

The Windows taskbar at the bottom shows the system time as 11:32 on 03/01/2019.

<https://www.bdbiosciences.com/us/tools/s/gps>

Some useful tools

BD Horizon™ Guided Panel | Define Populations - BD

Es seguro | <https://bdbiosciences.azurewebsites.net/PanelDesignTools/Populations/d9e42c16-abdf-44f6-8e04-83f4f3dfe6c0>

Aplicaciones | Login InterQC | Login InterQC admin | Citi Commercial Carc | <https://merchants.ga> | Guided Panel Soluti | spectrumviewer | BD | Infinicyt™ Support: T | SharePoint | South Region BDB

Describe Panel | Define Markers | Define Populations | Choose Cytometer | Design Panel | Review Panel | < Back | Save | Continue >

Step 1: Create Population Tree | Step 2: Define Critical Populations

All Cells/CD3/CD4/CD45RA

CD45RA

Name this Population*

Check if this is a critical population

Coexpressions

Marker	Type	Expression Level
CD3	1°	+
CD4	1°	+
CD45RA	1°	+

All Cells | CD3 | CD4 | CD45RA | CCR7 | CD27 | CD95 (Tscm CD4)

All Cells | CD3 | CD8 | CD45RA | CCR7 | CD27 | CD95 (Tscm CD8)

ES | 11:32 | 03/01/2019

<https://www.bdbiosciences.com/us/tools/s/gps>

Some useful tools

The screenshot shows the BD Horizon™ Guided Panel web application. The browser address bar displays the URL: <https://bdbiosciences.azurewebsites.net/PanelDesignTools/Cytometer/d9e42c16-abdf-44f6-8e04-83f4f3dfe6c0>. The page content includes:

- Cytometer Selection:** A dropdown menu showing various cytometer models, with "BD FACSCelesta™" selected.
- Configuration:** A dropdown menu showing the selected configuration "4-Blue 3-Red 5-Violet".
- Resolution Impact:** Buttons for "View", "Create RIM", and "Delete".
- Filter + Detector Table:** A table with columns for Filter + Detector, Fluorochrome, Relative Brightness, and Add Fluorochrome. The table contains four rows of configurations.
- Red 640 nm Table:** A table with columns for Filter + Detector, Fluorochrome, Relative Brightness, and Add Fluorochrome. It contains one row of configuration.

Filter + Detector	Fluorochrome	Relative Brightness	Add Fluorochrome
530/30 FITC	FITC	2	-
575/25 PE	BB515	4	+
610/20 PE-CF594	PE	3	-
695/40 PerCP-Cy5.5	PE-CF594	4	+
+	PerCP	1	-

Filter + Detector	Fluorochrome	Relative Brightness	Add Fluorochrome
670/30	APC	3	-

<https://www.bdbiosciences.com/us/tools/s/gps>

Some useful tools

The screenshot displays the BD Horizon™ Guided Panel web application. The browser address bar shows the URL: <https://bdbiosciences.azurewebsites.net/PanelDesignTools/Cytometer/d9e42c16-abdf-44f6-8e04-83f4f3dfe6c0>. The page content includes:

- Cytometer:** A dropdown menu set to "BD FACSCelesta™".
- Configuration:** A dropdown menu set to "4-Blue 3-Red 5-Violet".
- Resolution Impact:** A section with "View", "Create RIM", and "Delete" buttons.
- Lasers:** Two sections for laser configurations, each with a table of filter/detector, fluorochrome, and relative brightness settings.

Blue 488 nm Laser Configuration:

Filter + Detector	Fluorochrome	Relative Brightness	Add Fluorochrome
530/30 FITC	FITC	2	-
	BB515	4	+
575/25 PE	PE	3	-
			+
610/20 PE-CF594	PE-CF594	4	-
			+
695/40 PerCP-Cy5.5	PerCP	1	-
			+

Red 640 nm Laser Configuration:

Filter + Detector	Fluorochrome	Relative Brightness	Add Fluorochrome
670/30 APC	APC	3	-

<https://www.bdbiosciences.com/us/tools/s/gps>

Some useful tools

Cytometer Name: BD FACSCelesta™, Configuration Name: 4-Blue 3-Red 5-Violet

Secondary fluorochrome (bright antigen)

	FITC	BB515	PE	PE-CF594	PerCP	APC	APC-R700	APC-Cy7	BV421	BV510	BV480	BV605	BV650	BV786
FITC	Grey	Grey	Yellow	Yellow	Grey	Green	Green	Green	Green	Green	Green	Green	Green	Green
BB515	Grey	Grey	Orange	Orange	Grey	Green	Green	Green	Green	Yellow	Yellow	Green	Green	Green
PE	Yellow	Green	Grey	Red	Grey	Green	Green	Green	Green	Green	Green	Orange	Yellow	Green
PE-CF594	Green	Green	Yellow	Grey	Grey	Yellow	Green	Green	Green	Green	Green	Green	Orange	Yellow
PerCP	Green	Green	Green	Green	Grey	Green	Green	Green	Green	Green	Green	Green	Green	Green
APC	Green	Green	Green	Green	Grey	Grey	Red	Yellow	Green	Green	Green	Green	Orange	Green
APC-R700	Green	Green	Green	Green	Grey	Red	Grey	Orange	Green	Green	Green	Green	Green	Yellow
APC-Cy7	Green	Green	Green	Green	Grey	Red	Orange	Grey	Green	Green	Green	Green	Green	Orange
BV421	Yellow	Green	Green	Green	Grey	Green	Green	Green	Grey	Yellow	Yellow	Green	Green	Green
BV510	Yellow	Green	Green	Green	Grey	Green	Green	Green	Green	Grey	Grey	Orange	Orange	Yellow
BV480	Orange	Yellow	Green	Green	Grey	Green	Green	Green	Yellow	Grey	Grey	Orange	Yellow	Green
BV605	Green	Green	Green	Yellow	Grey	Green	Green	Green	Green	Green	Green	Grey	Red	Orange
BV650	Green	Green	Green	Green	Grey	Red	Orange	Green	Yellow	Green	Green	Orange	Grey	Red
BV786	Green	Green	Green	Green	Grey	Green	Yellow	Yellow	Orange	Green	Green	Green	Green	Grey

Primary fluorochrome (dim antigen)

Loss of Resolution

- > 80% (Red)
- 60-80% (Orange)
- 40 - 60% (Yellow)
- 20-40% (Light Green)
- < 20% (Green)
- Colors are in the same detector (Grey)
- Resolution Impact is not available (White)

Some useful tools

The screenshot displays the BD Horizon™ Design Panel tool interface. At the top, there are browser tabs for 'BD Horizon™ Guided Panel', 'Design Panel - BD Biosci', 'Analyze Resolution Impa', and 'Resolution Impact'. The address bar shows the URL: <https://bdbiosciences.azurewebsites.net/PanelDesignTools/DesignPanel/d9e42c16-abdf-44f6-8e04-83f4f3dfe6c0>. The browser's address bar also shows 'Es seguro' and a star icon.

The main interface is divided into several sections:

- Markers:** A list of markers including CD3, CD4, CD45RA, ccr7, CD27, CD95, and CD8.
- Fluorochromes:** A list of fluorochromes including BB515, BV421, BV605, BV786, PE, BV510, FITC, APC-Cy7, and PerCP.
- Laser:** A list of lasers including Blue and Violet.
- Relative Brightness:** A list of relative brightness levels including Dim, Moderate, Bright, and Very Bright.

Instructions: Match the following **Markers** to the appropriate **Fluorochromes** based on the co-expression levels listed in the preceding list.

Buttons: View Resolution Impact, Analyze Resolution Impact

Analyze Resolution Impact: Tscm

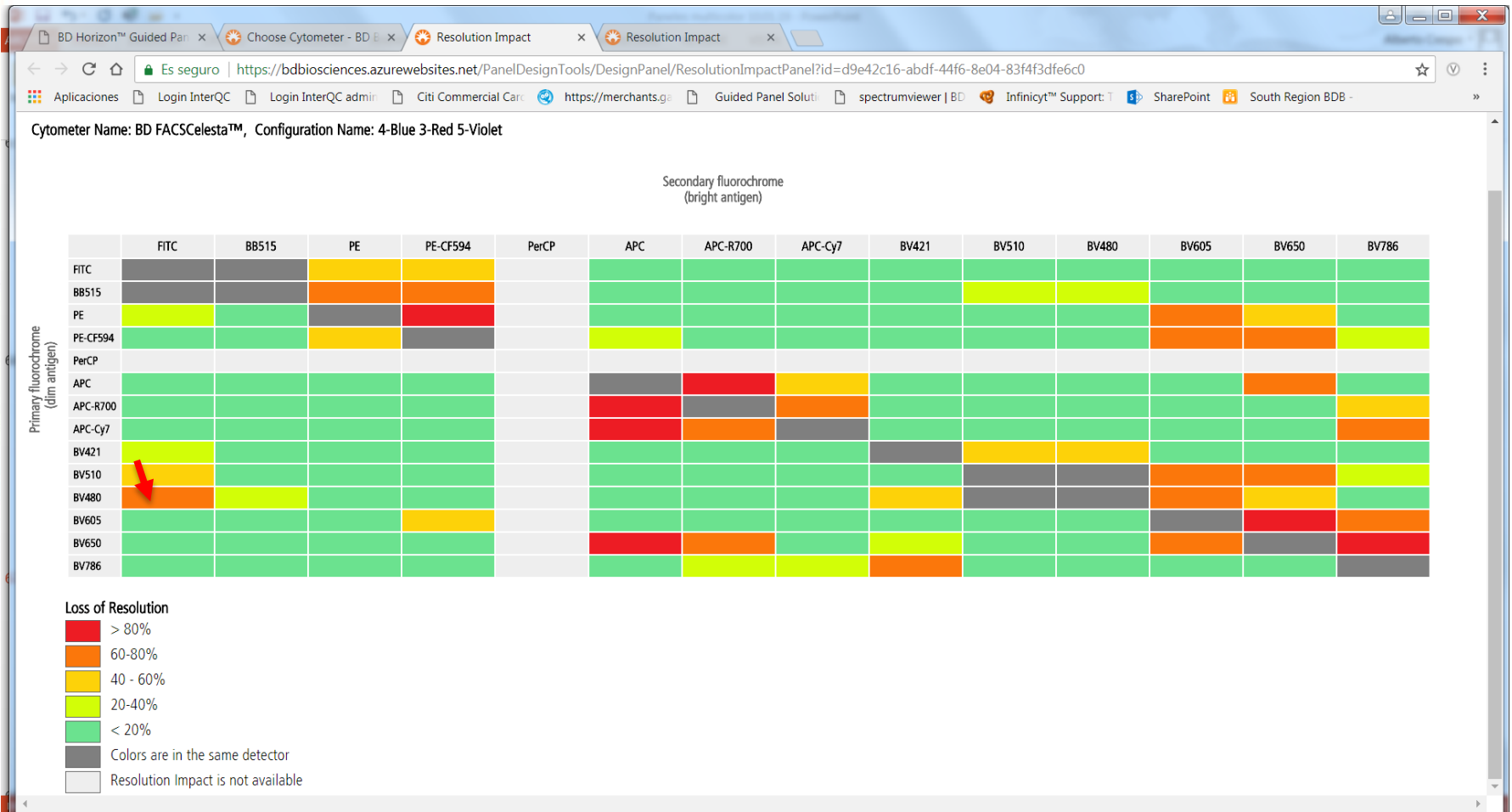
Potential loss of resolution of Dim Antigen due to spread from Bright Antigen:

Subpopulation	Dim Antigen		Resolution Loss	Bright Antigen	
Tscm CD8	BV480	CD95	60-80%	FITC	CD8

Relative Brightness Key: ① Dim ② Moderate ③ Bright ④ Very Bright

<https://www.bdbiosciences.com/us/tools/s/gps>

Some useful tools



Some useful tools

The screenshot displays the BD Horizon™ Design Panel tool interface. At the top, there are browser tabs for 'BD Horizon™ Guided Panel', 'Design Panel - BD Biosciences', 'Analyze Resolution Impact', and 'Resolution Impact'. The address bar shows the URL: <https://bdbiosciences.azurewebsites.net/PanelDesignTools/DesignPanel/d9e42c16-abdf-44f6-8e04-83f4f3dfe6c0>. The browser's address bar also shows 'Es seguro' and various icons for applications like 'Login InterQC', 'Citi Commercial Car', 'https://merchants.ga', 'Guided Panel Solu', 'spectrumviewer | BD', 'Infinicyt™ Support: T', 'SharePoint', and 'South Region BDB'.

The main content area shows a table with columns for 'Markers', 'Fluorochromes', 'Laser', and 'Relative Brightness'. The 'Markers' column lists CD3, CD4, CD45RA, ccr7, CD27, CD95, and CD8. The 'Fluorochromes' column lists BB515, BV421, BV650, PE-CF594, APC, APC-R700, BV480, BV605, BV786, PE, BV510, FITC, APC-Cy7, and PerCP. The 'Laser' column lists Blue, Violet, and Red. The 'Relative Brightness' column lists 4, 3, 2, and 1. The table is organized into four groups based on relative brightness. The first group (Relative Brightness 4) includes markers CD27 and ccr7, fluorochromes BB515, BV421, BV650, and PE-CF594, and a Blue laser. The second group (Relative Brightness 3) includes markers CD45RA and CD95, fluorochromes APC, APC-R700, BV480, BV605, BV786, and PE, and a Red laser. The third group (Relative Brightness 2) includes marker CD8, fluorochromes FITC and BV510, and a Violet laser. The fourth group (Relative Brightness 1) includes markers CD4 and CD3, fluorochromes APC-Cy7 and PerCP, and a Red laser. There are red exclamation mark icons next to FITC, APC-Cy7, and PerCP. To the right of the table are two buttons: 'View Resolution Impact' and 'Analyze Resolution Impact'.

Match the following **Markers** to the appropriate **Fluorochromes** based on the co-expression levels listed in the preceding list.

Markers	Fluorochromes	Laser	Relative Brightness
CD27	BB515	Blue	4
CCR7	BV421	Violet	
	BV650	Violet	
	PE-CF594	Blue	
CD45RA	APC	Red	3
	APC-R700	Red	
CD95	BV480	Violet	
	BV605	Violet	
	BV786	Violet	
	PE	Blue	
CD8	FITC	Violet	2
	BV510	Blue	
CD4	APC-Cy7	Red	1
CD3	PerCP	Blue	

Relative Brightness Key: ① Dim ② Moderate ③ Bright ④ Very Bright

<https://www.bdbiosciences.com/us/tools/s/gps>

Some useful tools

The screenshot displays the BD Horizon™ Design Panel tool interface. The browser address bar shows the URL: <https://bdbiosciences.azurewebsites.net/PanelDesignTools/DesignPanel/d9e42c16-abdf-44f6-8e04-83f4f3dfe6c0>. The interface includes a 'Markers' list on the left, a central table for marker assignment, and a 'Resolution Impact' analysis section.

Markers List:

Markers
CD3
CD4
CD45RA
ccr7
CD27
CD95
CD8

Match the following Markers to the appropriate Fluorochromes based on the co-expression levels listed in the preceding list.

Resolution Impact Analysis Table:

Marker	Fluorochromes	Laser	Relative Brightness
CD27	BB515	Blue	4
ccr7	BV421	Violet	
	BV650	Violet	
CD45RA	BV605	Violet	2
CD27	BV786	Violet	
CD95	PE	Blue	
CD8	BV510	Violet	1
	FITC	Blue	
CD4	APC-Cy7	Red	1
CD3	PerCP	Blue	

Analyze Resolution Impact: Tscm

Your panel has generated no warnings.

Relative Brightness Key: ① Dim ② Moderate ③ Bright ④ Very Bright

<https://www.bdbiosciences.com/us/tools/s/gps>

Some useful tools

Please review the grid below, select the appropriate reagents and click the **Exit** button to complete work on this panel.

Export
Add to Shopping List
Add to Cart

Specificity	Fluorochrome	Cat. No.	Description	Clone	Status	TDS
CD3	PerCP	No matches available. Contact BDB Custom Orders group for possible custom order conjugate : bdb_custom_orders@bd.com				
CD4	APC-Cy7	<input checked="" type="checkbox"/> 341095	APC-Cy™7 Mouse Anti-Human CD4 100 Tests	SK3 (also know...	RUO_GMP	Download
		<input type="checkbox"/> 341105	CD4 APC-Cy™7 100 Tests	SK3 (also know...	ASR	Download
		<input type="checkbox"/> 557871	BD Pharmingen™ APC-Cy™7 Mouse Anti-Human CD4 100 Tests	RPA-T4	RUO	Download
		<input type="checkbox"/> 561839	BD Pharmingen™ APC-Cy™7 Mouse Anti-Human CD4 25 Tests	RPA-T4	RUO	Download
		<input type="checkbox"/> 566319	BD Pharmingen™ APC-Cy™7 Mouse Anti-Human CD4 25 Tests	SK3 (also know...	RUO	Download
CD27	BB515	<input checked="" type="checkbox"/> 564642	BD Horizon™ BB515 Mouse Anti-Human CD27 100 Tests	M-T271	RUO	Download
		<input type="checkbox"/> 564643	BD Horizon™ BB515 Mouse Anti-Human CD27 25 Tests	M-T271	RUO	Download
ccr7	BV421	No matches available. Contact BDB Custom Orders group for possible custom order conjugate : bdb_custom_orders@bd.com				
CD45RA	APC	<input checked="" type="checkbox"/> 550855	BD Pharmingen™ APC Mouse Anti-Human CD45RA 100 Tests	HI100	RUO	Download
		<input type="checkbox"/> 561884	BD Pharmingen™ APC Mouse Anti-Human CD45RA 25 Tests	HI100	RUO	Download
CD95	BV480	No matches available. Contact BDB Custom Orders group for possible custom order conjugate : bdb_custom_orders@bd.com				
CD8	BV786	<input checked="" type="checkbox"/> 563823	BD Horizon™ BV786 Mouse Anti-Human CD8 100 Tests	RPA-T8	RUO	Download
		<input type="checkbox"/> 563824	BD Horizon™ BV786 Mouse Anti-Human CD8 25 Tests	RPA-T8	RUO	Download
		<input type="checkbox"/> 740988	BD OptiBuild™ BV786 Mouse Anti-Human CD8 50 µg	HIT8a	RUO	Download
		<input type="checkbox"/> 743069	BD OptiBuild™ BV786 Mouse Anti-Human CD8 50 µg	G42-8	RUO	Download

Notes

Type your notes here

Summary

- Multicolor flow cytometry has become more accessible with advances in system capability and fluorochrome availability
- When designing a panel it is important to have:
 - Information about cell biology
 - Information about available flow cytometers
 - Familiarity with excitation and detection optics to understand what fluorochromes are usable
 - Information about spectral characteristics of usable fluorochromes
 - Fluorochrome resolution ranking
 - Impact of fixation method on fluorochrome brightness

REVEALING A NEW
HORIZON

Thank you!

