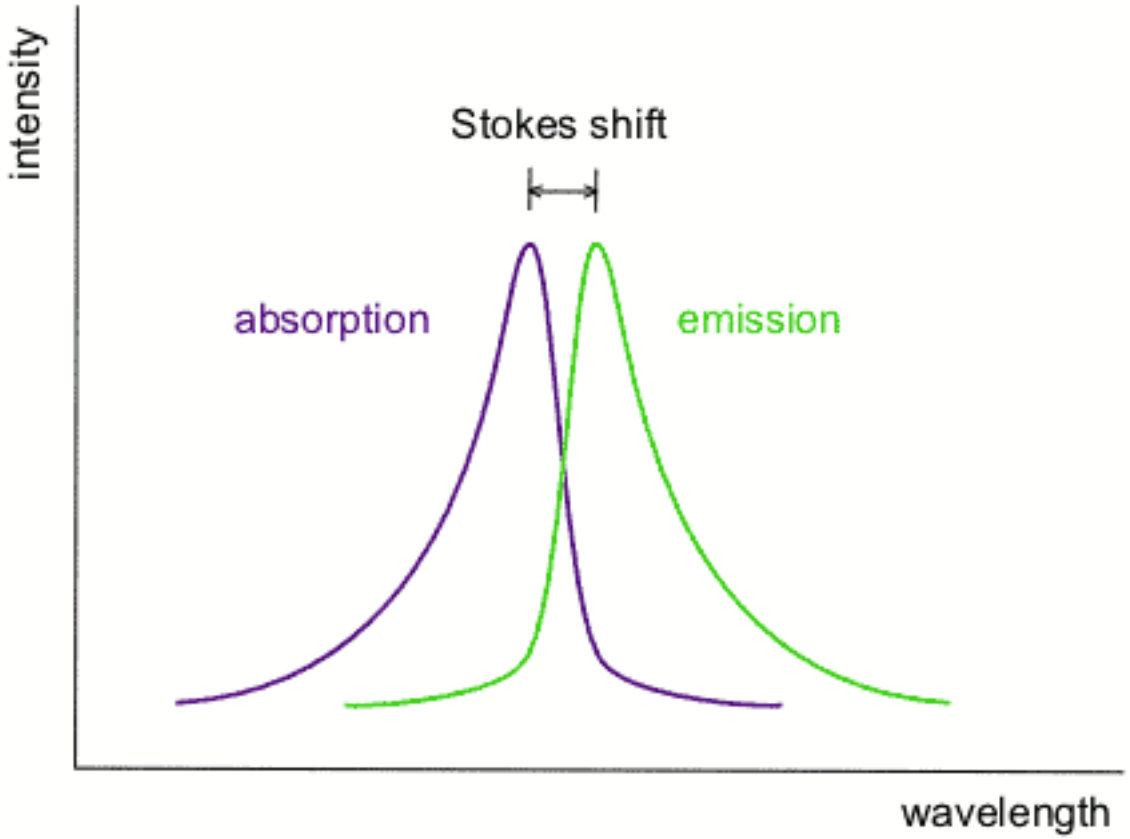

intensity
Stokes shift
absorption
emission
wavelength

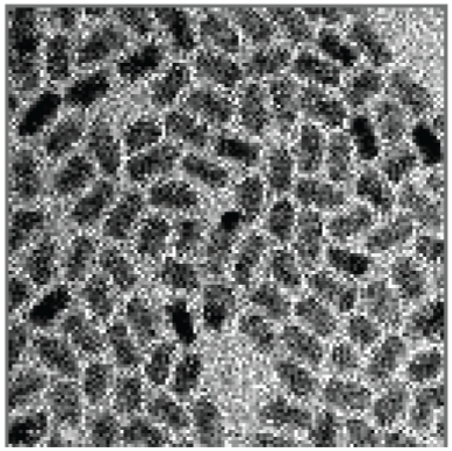
Instrument	Excitation Laser Line (nm)	Fluorescence Channel	Fluorochromes provided by BD Biosciences			
BD Accuri® C6	488	FL1 Green	FITC	Alexa Fluor® 488		
		FL2 Yellow	PE	PI		
		FL3 Red	7-AAD	PerCP	PerCP-Cy™5.5	PE-Cy™7
	640	FL4 Red	APC	Alexa Fluor® 647		
BD FACSCalibur™	488	FL1 Green	FITC	Alexa Fluor® 488		
		FL2 Yellow	PE	PI		
		FL3 Red	7-AAD	PE-Cy™5	PerCP	PerCP-Cy5.5	PE-Cy7
	635	FL4 Red	APC	Alexa Fluor® 647		
BD FACSVerse™*	488	Green	FITC	Alexa Fluor® 488		
		Yellow	PE	PI		
		Orange	BD Horizon™ PE-CF594[a]	PE-Texas Red®[a]		
		Red	7-AAD	PE-Cy5	PerCP	PerCP-Cy5.5
		Infrared	PE-Cy7			
	640[b]	Red	APC	Alexa Fluor® 647		
		Far Red	Alexa Fluor® 700[a]			
		Infrared	BD APC-H7	APC-Cy7		
	405[b]	Green	BD Horizon™ V500	AmCyan		
		Blue	BD Horizon™ V450	VPD450	Pacific Blue™	

BD FACSCanto™ II	488	Green	FITC	Alexa Fluor® 488		
		Yellow	PE	PI		
		Orange	BD Horizon PE-CF594[a]	PE-Texas Red®[a]		
		Red	7-AAD	PE-Cy5	PerCP	PerCP-Cy5.5
		Infrared	PE-Cy7			
	633	Red	APC	Alexa Fluor® 647		
		Far Red	Alexa Fluor® 700[a]			
		Infrared	BD APC-H7	APC-Cy7		
	405[b]	Green	BD Horizon V500	AmCyan		
		Blue	BD Horizon V450	VPD450	Pacific Blue™	
BD LSRFortessa™ and Special Order BD LSRFortessa (typical setup)[b]	488	Green	FITC	Alexa Fluor® 488		
		Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	7-AAD	PE-Cy5	PerCP	PerCP-Cy5.5
		Infrared	PE-Cy7			
	532[b] or 561[b]	Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	PE-Cy5			
		Infrared	PE-Cy7			
	640	Red	APC	Alexa Fluor® 647		
		Far Red	Alexa Fluor® 700			
		Infrared	BD APC-H7	APC-Cy7		
	405	Green	BD Horizon V500	AmCyan		
		Blue	BD Horizon V450	VPD450	Pacific Blue™	
	355	Blue	Hoechst 33342			

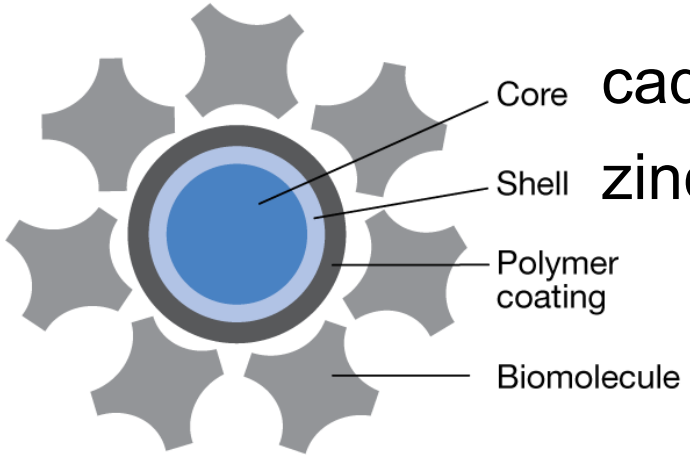
BD FACSAria™ III and Special Order BD FACSAria (typical setup)[b]	488	Green	FITC	Alexa Fluor® 488		
		Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	7-AAD	PE-Cy5	PerCP	PerCP-Cy5.5
		Infrared	PE-Cy7			
	561	Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	PE-Cy5			
		Infrared	PE-Cy7			
	640	Red	APC	Alexa Fluor® 647		
		Far Red	Alexa Fluor® 700			
		Infrared	BD APC-H7	APC-Cy7		
	405	Green	BD Horizon V500	AmCyan		
		Blue	BD Horizon V450	VPD450	Pacific Blue™	
	375[b]	Blue	Hoechst 33342			
BD Influx™	488	Green	FITC	Alexa Fluor® 488		
		Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	7-AAD	PE-Cy5	PerCP	PerCP-Cy5.5
		Infrared	PE-Cy7			
	532 or 561	Yellow	PE	PI		
		Orange	BD Horizon PE-CF594	PE-Texas Red®		
		Red	PE-Cy5			
		Infrared	PE-Cy7			
	640	Red	APC	Alexa Fluor® 647		
		Far Red	Alexa Fluor®700			
		Infrared	BD APC-H7	APC-Cy7		
	405	Green	BD Horizon V500	AmCyan		
		Blue	BD Horizon V450	VPD450	Pacific Blue™	
	375	Blue	Hoechst 33342			

Q-dots - structure

A



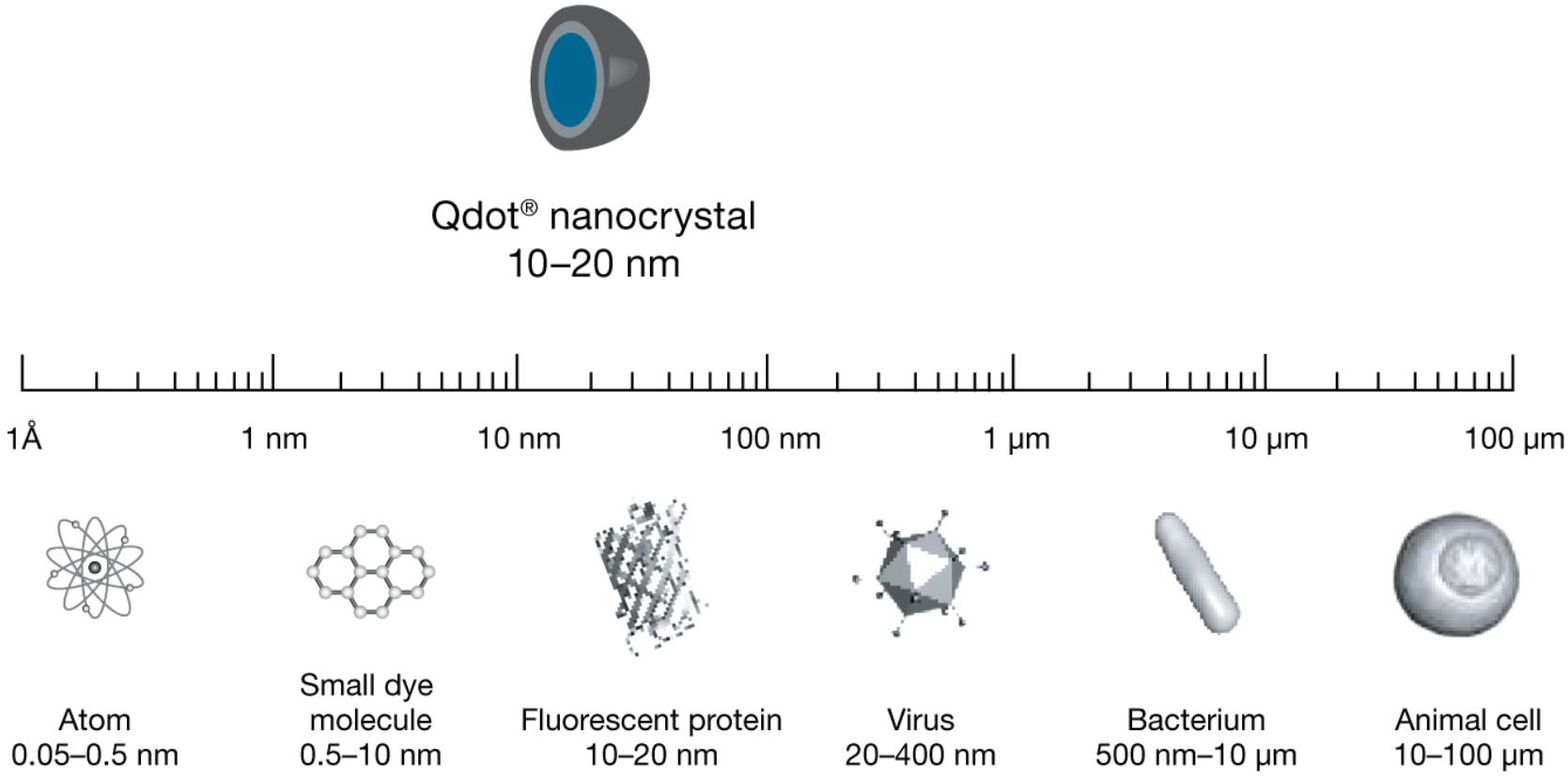
B



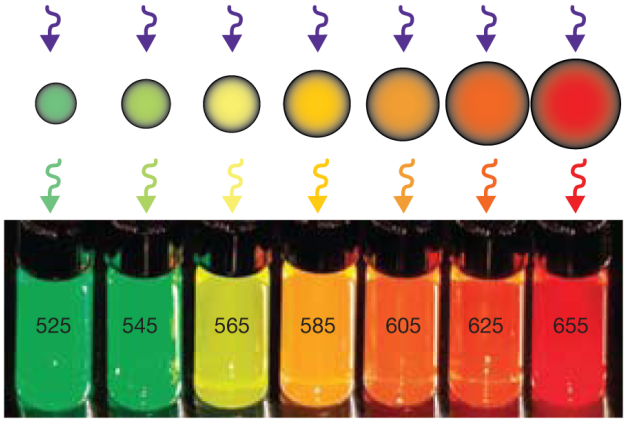
cadmium+selenium/tellurium

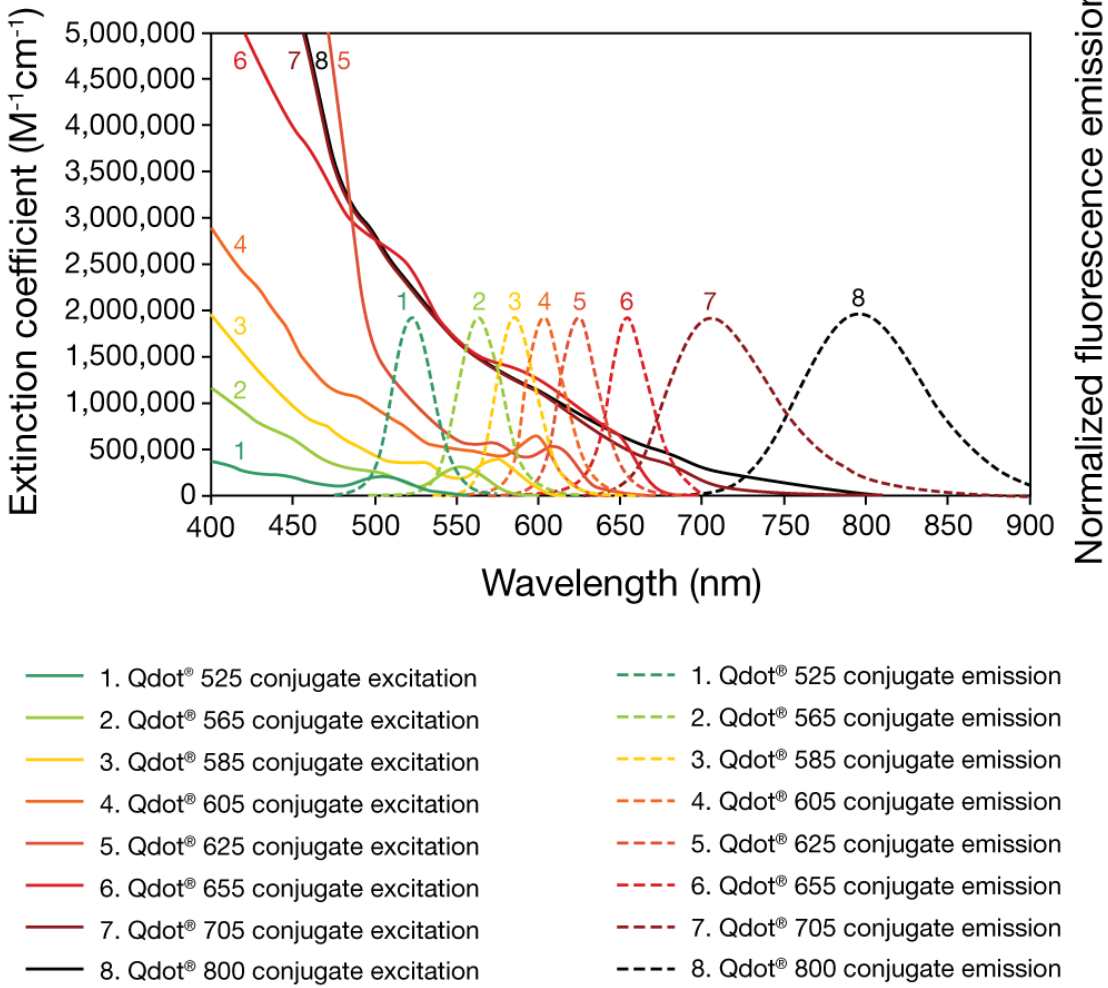
zinc-sulfide

Q-dots - size

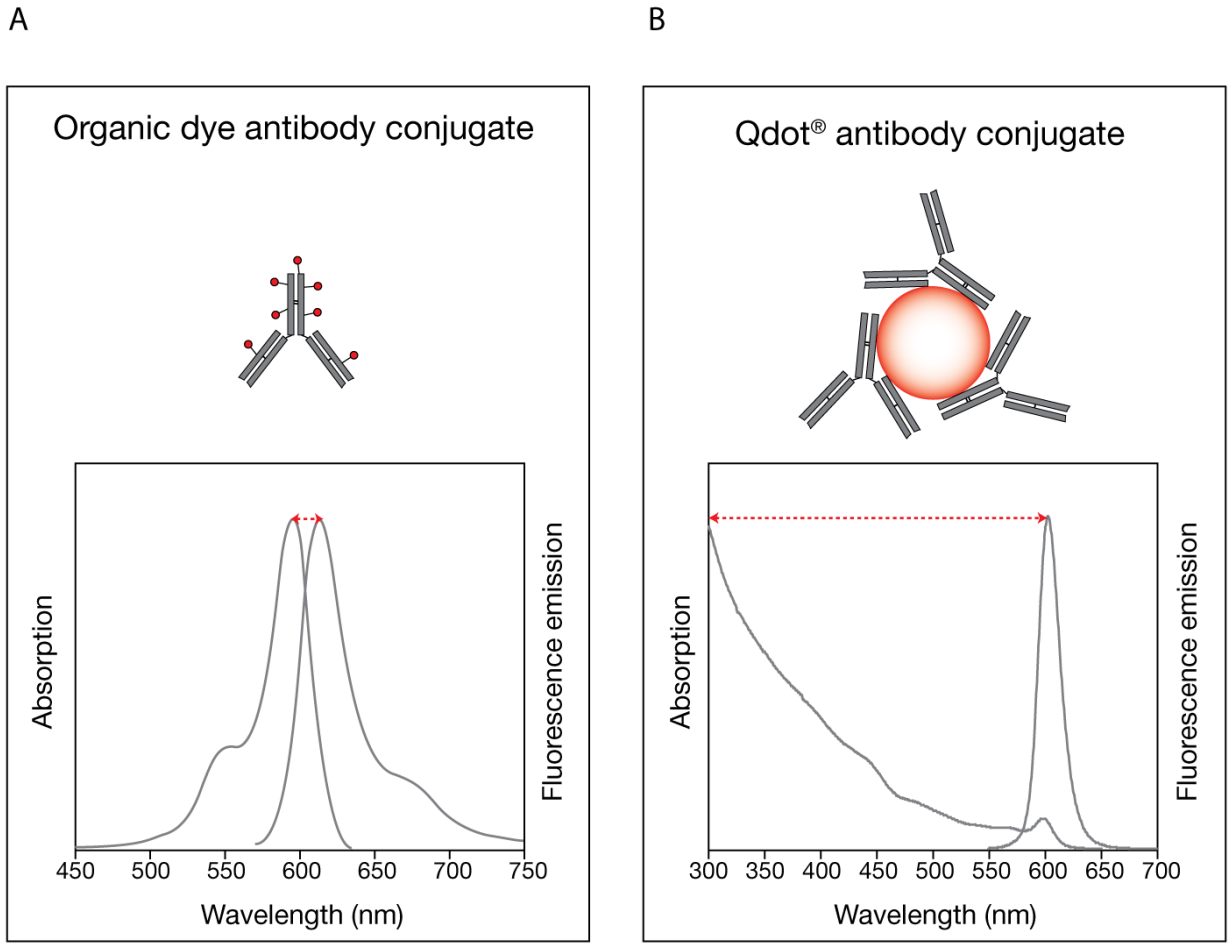

Qdot® nanocrystal
10–20 nm
1Å
1 nm
10 nm
100 nm
1 µm
10 µm
100 µm
Atom
0.05–0.5 nm
Small dye
molecule
0.5–10 nm
Fluorescent protein
10–20 nm
Virus
20–400 nm
Bacterium
500 nm–10 µm
Animal cell
10–100 µm

Q-dots - color


525
545
565
585
605
625
655


Extinction coefficient (M-1cm-1)
5,000,000
4,500,000
4,000,000
3,500,000
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
400
450
500
550
600
650
700
750
800
850
900
Wavelength (nm)
Normalized fluorescence emission
1. Qdot® 525 conjugate excitation
2. Qdot® 565 conjugate excitation
3. Qdot® 585 conjugate excitation
4. Qdot® 605 conjugate excitation
5. Qdot® 625 conjugate excitation
6. Qdot® 655 conjugate excitation
7. Qdot® 705 conjugate excitation
8. Qdot® 800 conjugate excitation
1. Qdot® 525 conjugate emission
2. Qdot® 565 conjugate emission
3. Qdot® 585 conjugate emission
4. Qdot® 605 conjugate emission
5. Qdot® 625 conjugate emission
6. Qdot® 655 conjugate emission
7. Qdot® 705 conjugate emission
8. Qdot® 800 conjugate emission

Q-dots vs. “traditional dyes”



New perspectives in flow cytometry. Multiplex arrays, CBA. Phospho-flow.

Immunology PhD course

2019.12.11.

Use of microbeads in multiplex measurements.

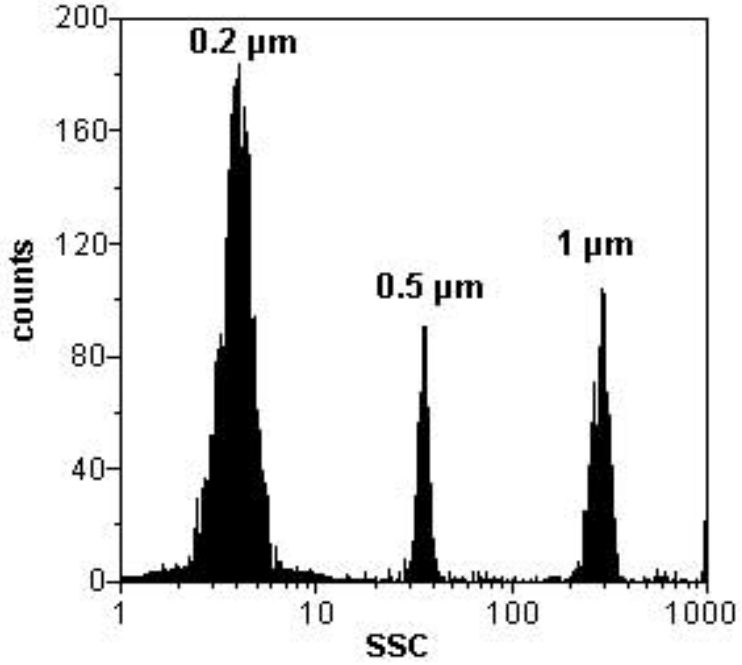
Multiplex technique:

Mixing of precisely identifiable beads

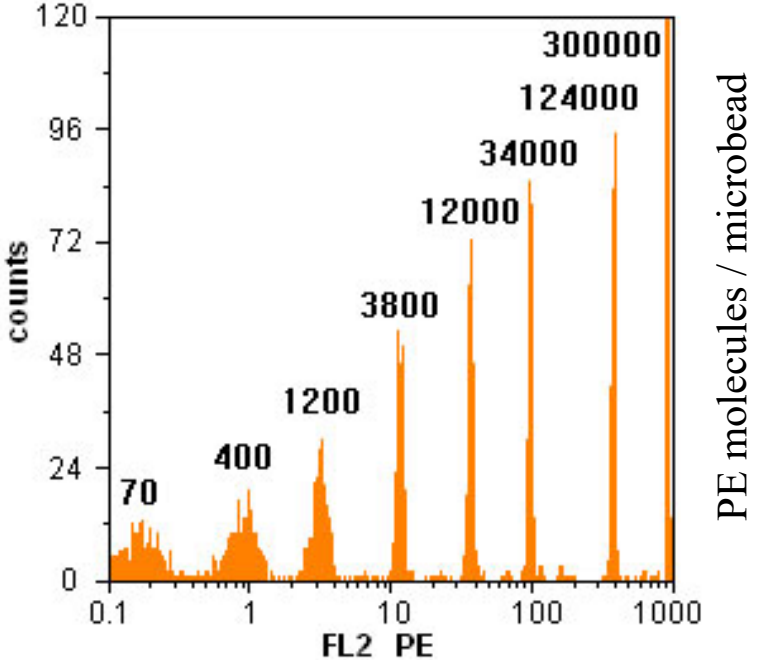
With a single measurement we gain a set of data

Identification of microbeads

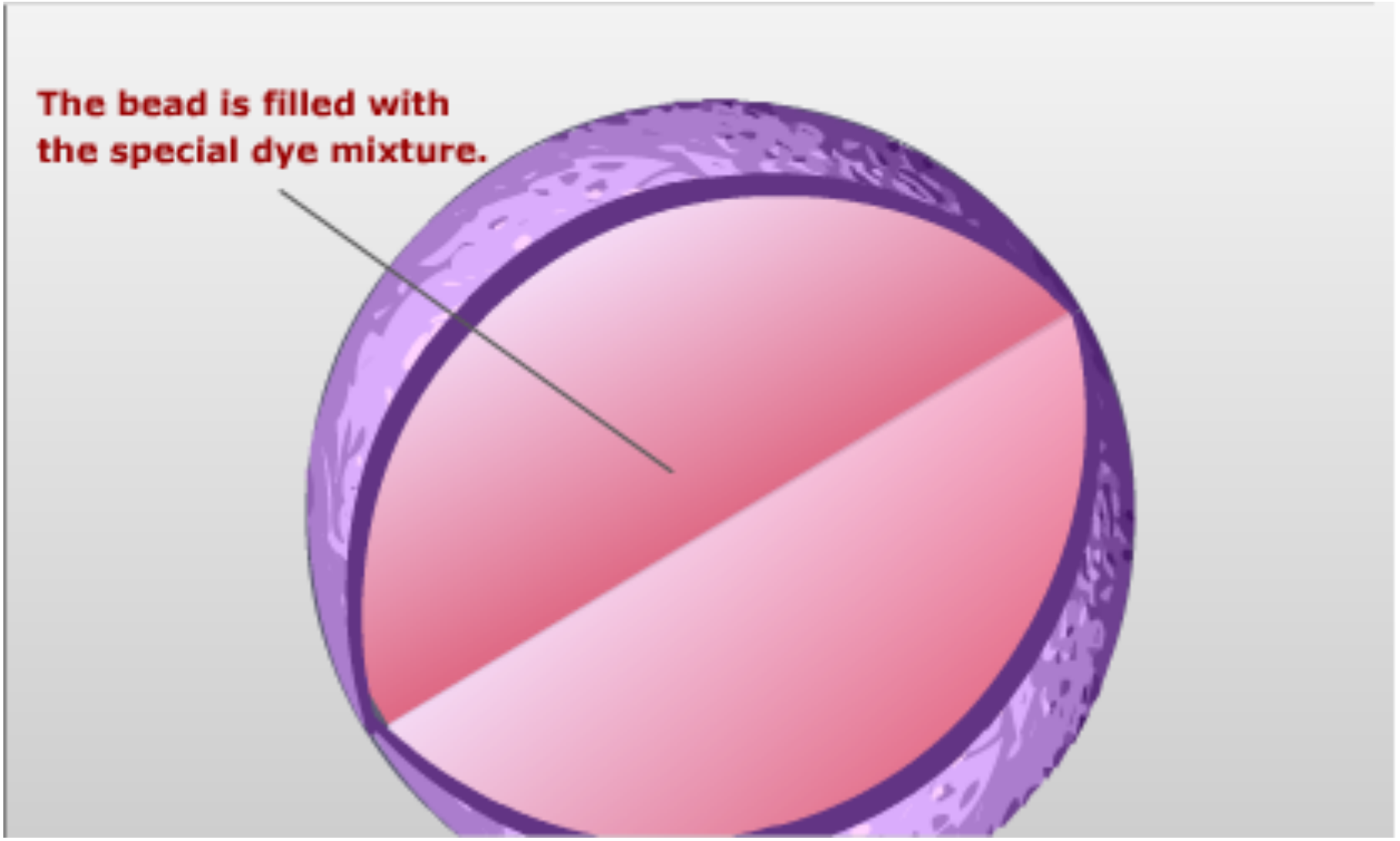
Size



Fluorescence

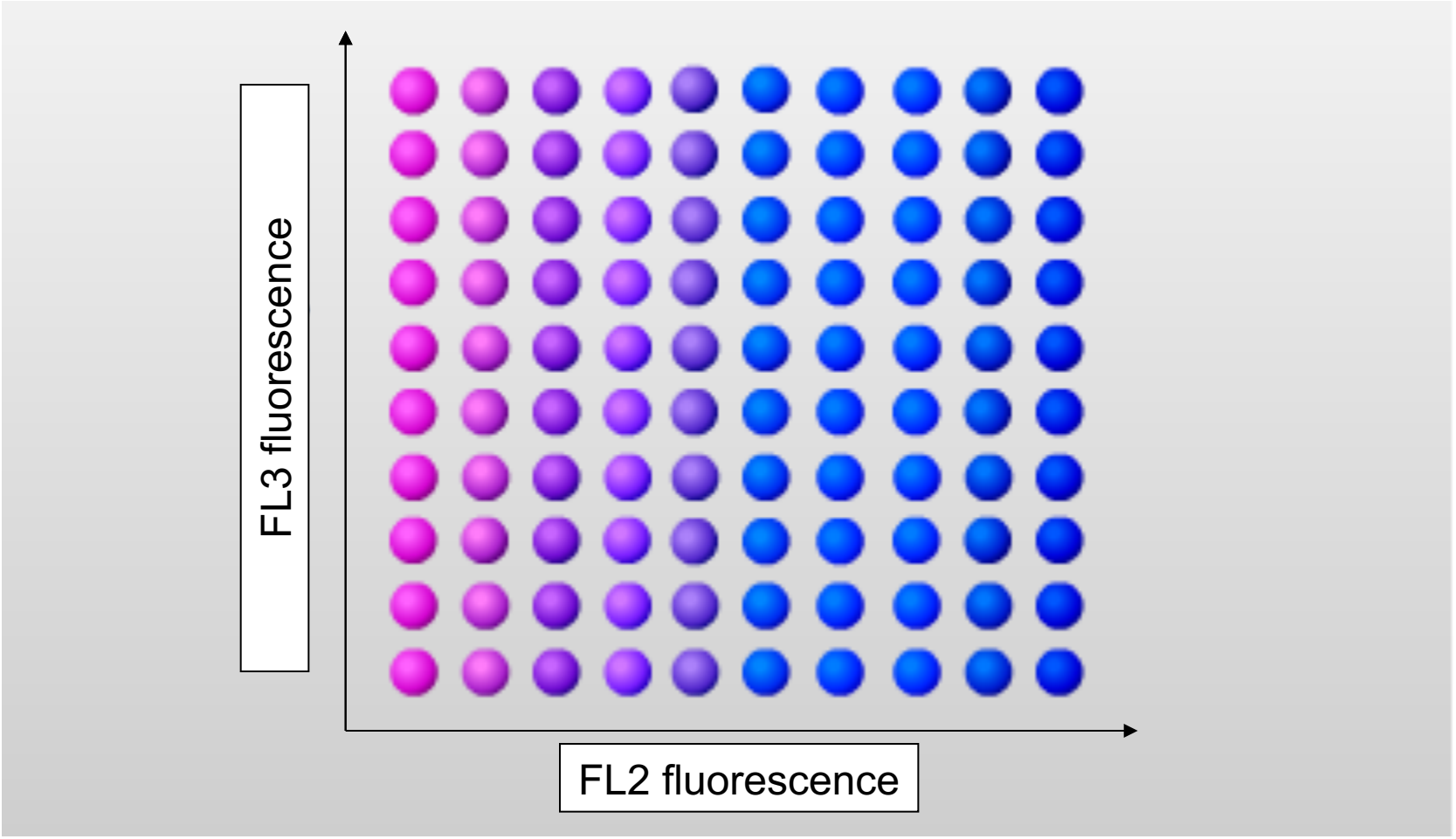


Luminex xMAP technology

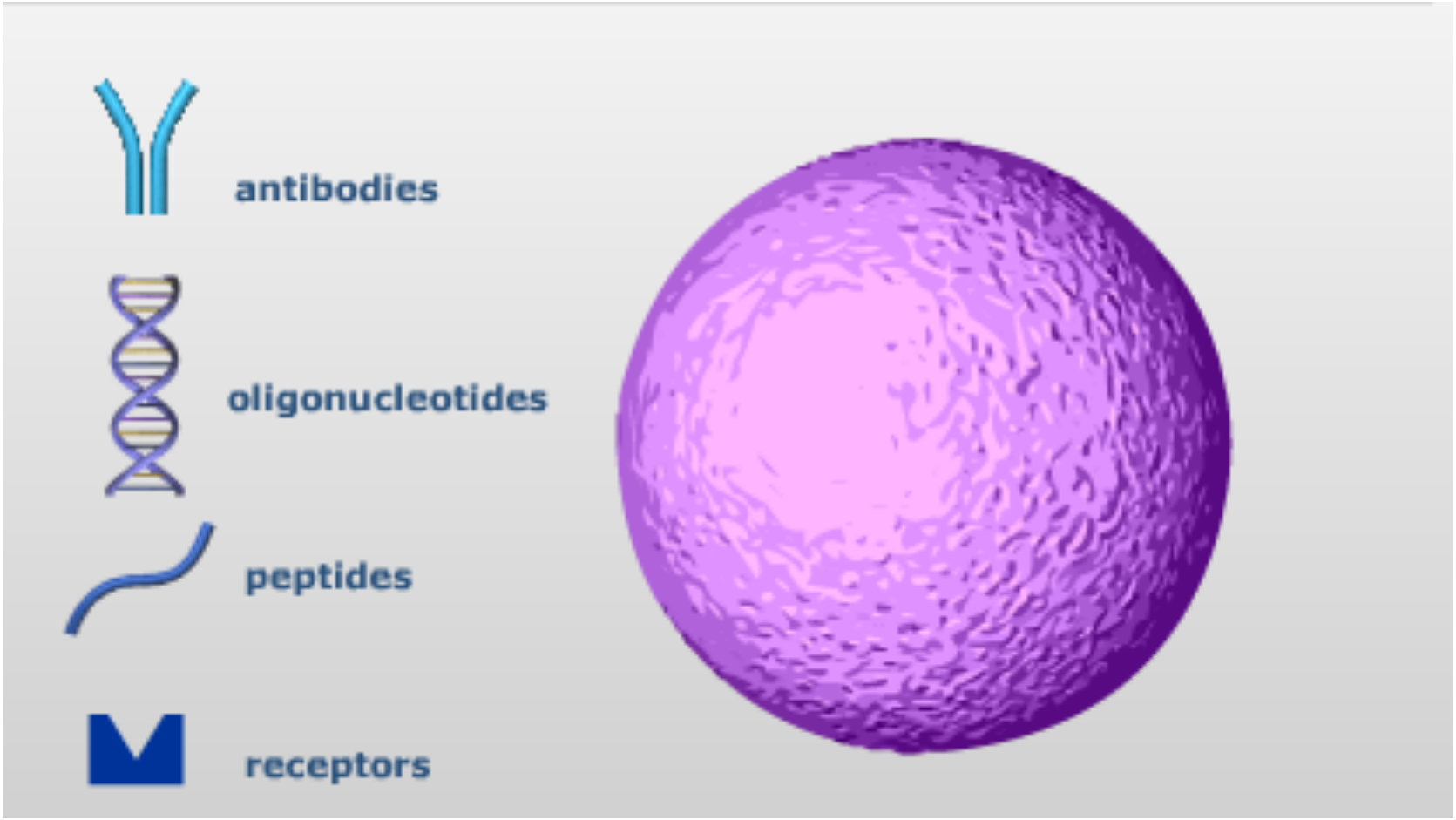


Luminex xMAP technológia

Different bead „clusters” can be mixed. Since all beads carry a unique identification mark, the detection system can detect which bead belongs to which cluster. Theoretically 100 measurements can be performed in one sample.

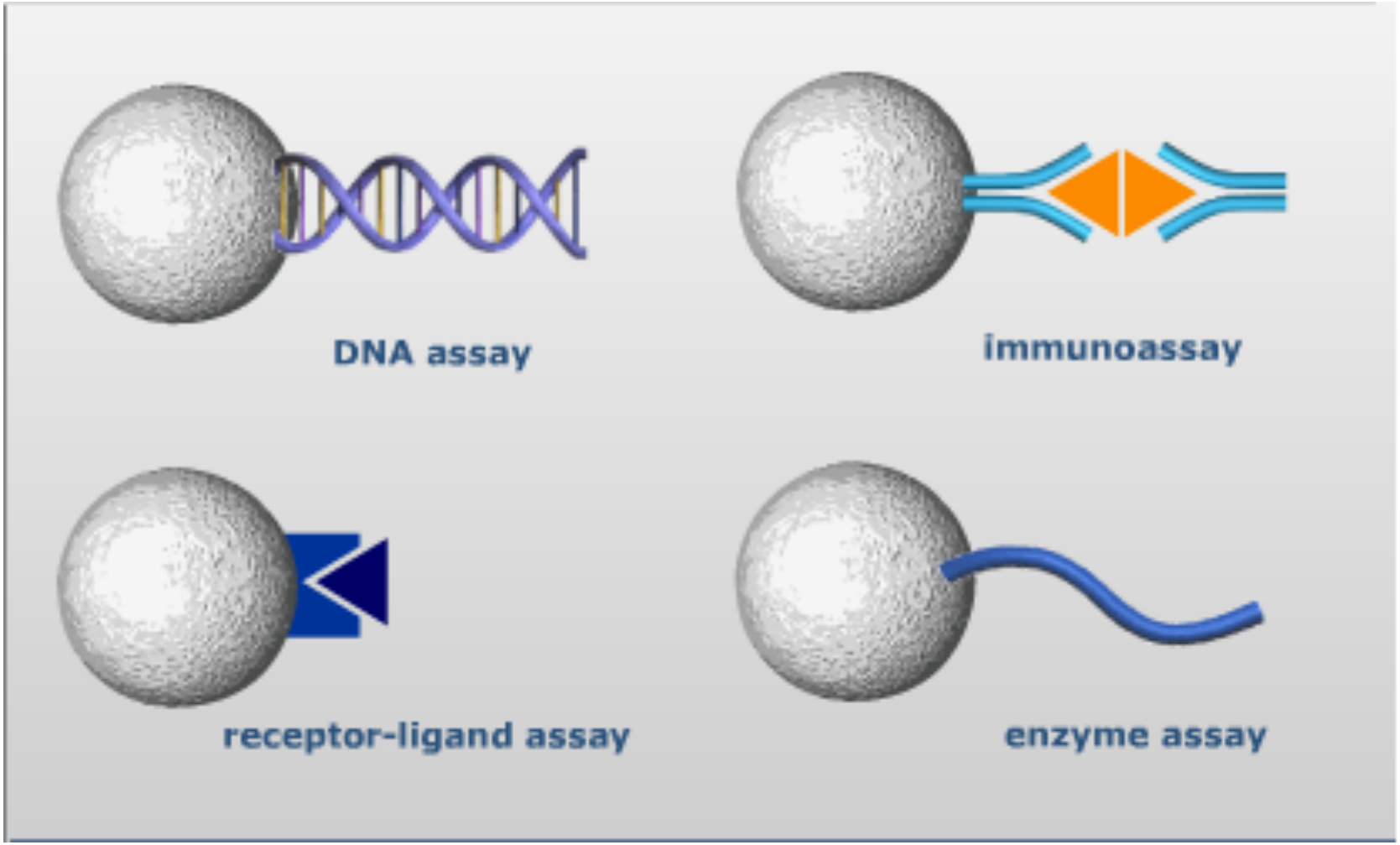


Binding molecules on the beads



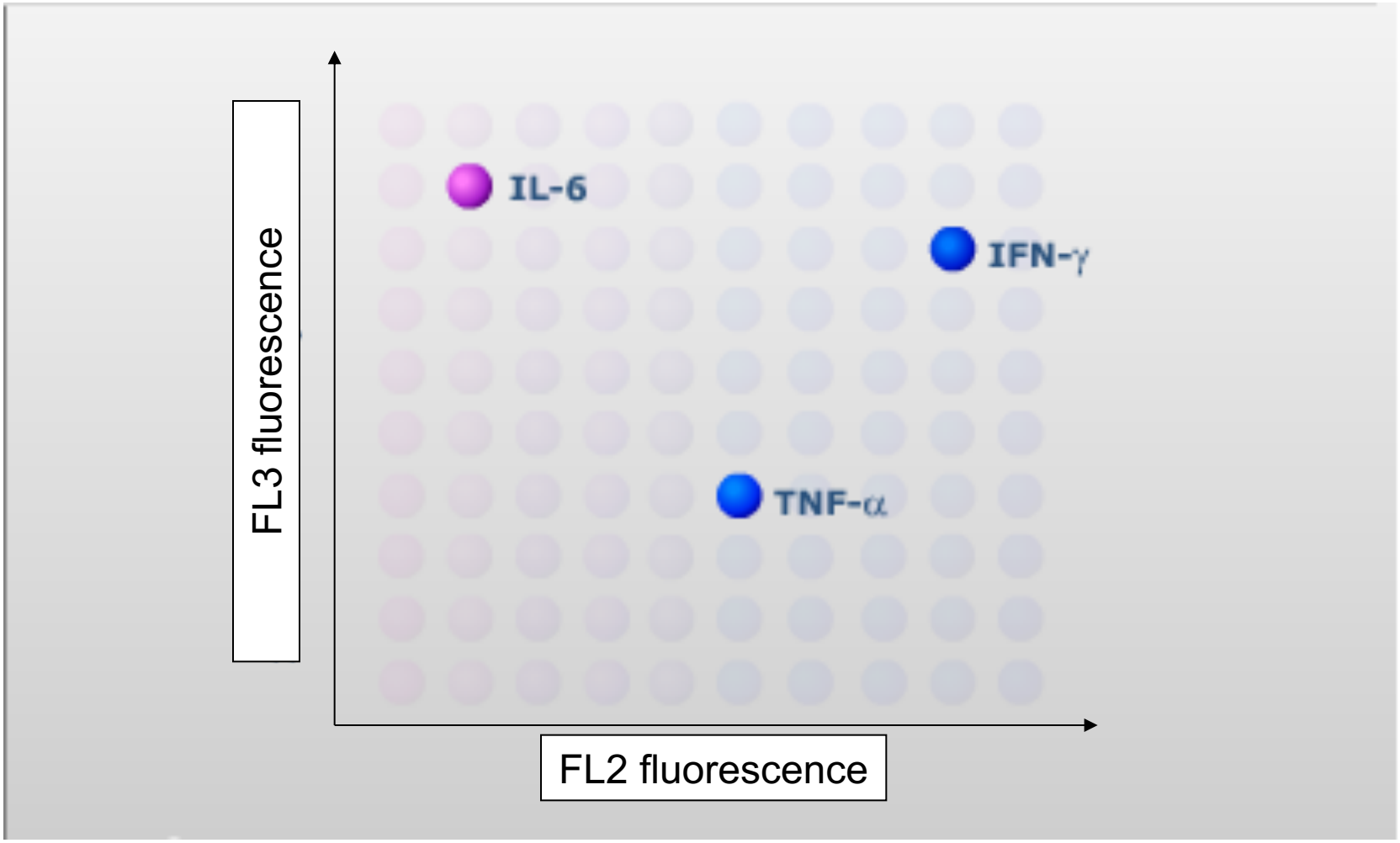
With simple chemical (covalent) binding different materials can be bound on the surface of microbeads.

Potential uses



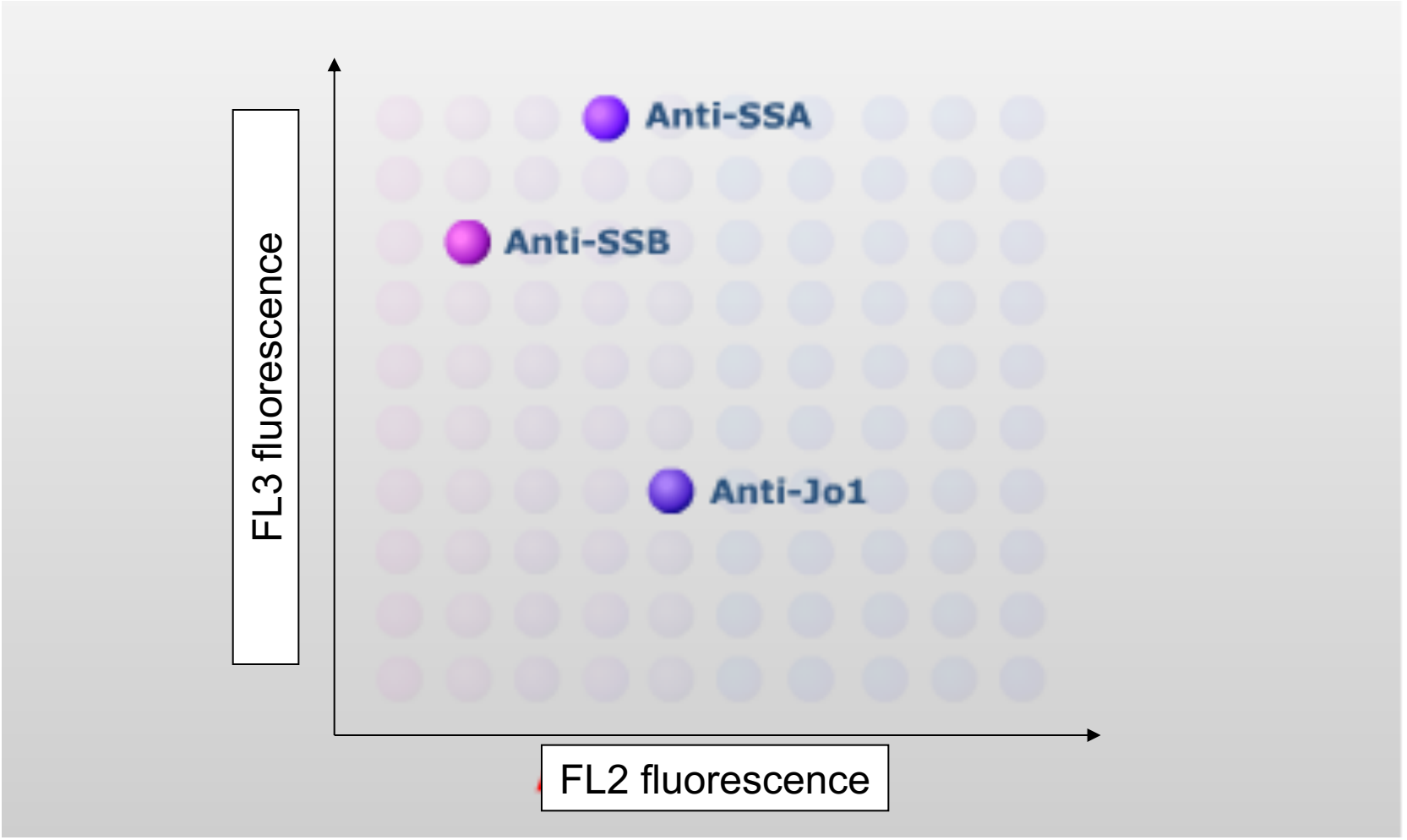
Luminex xMAP technology

Cytokines



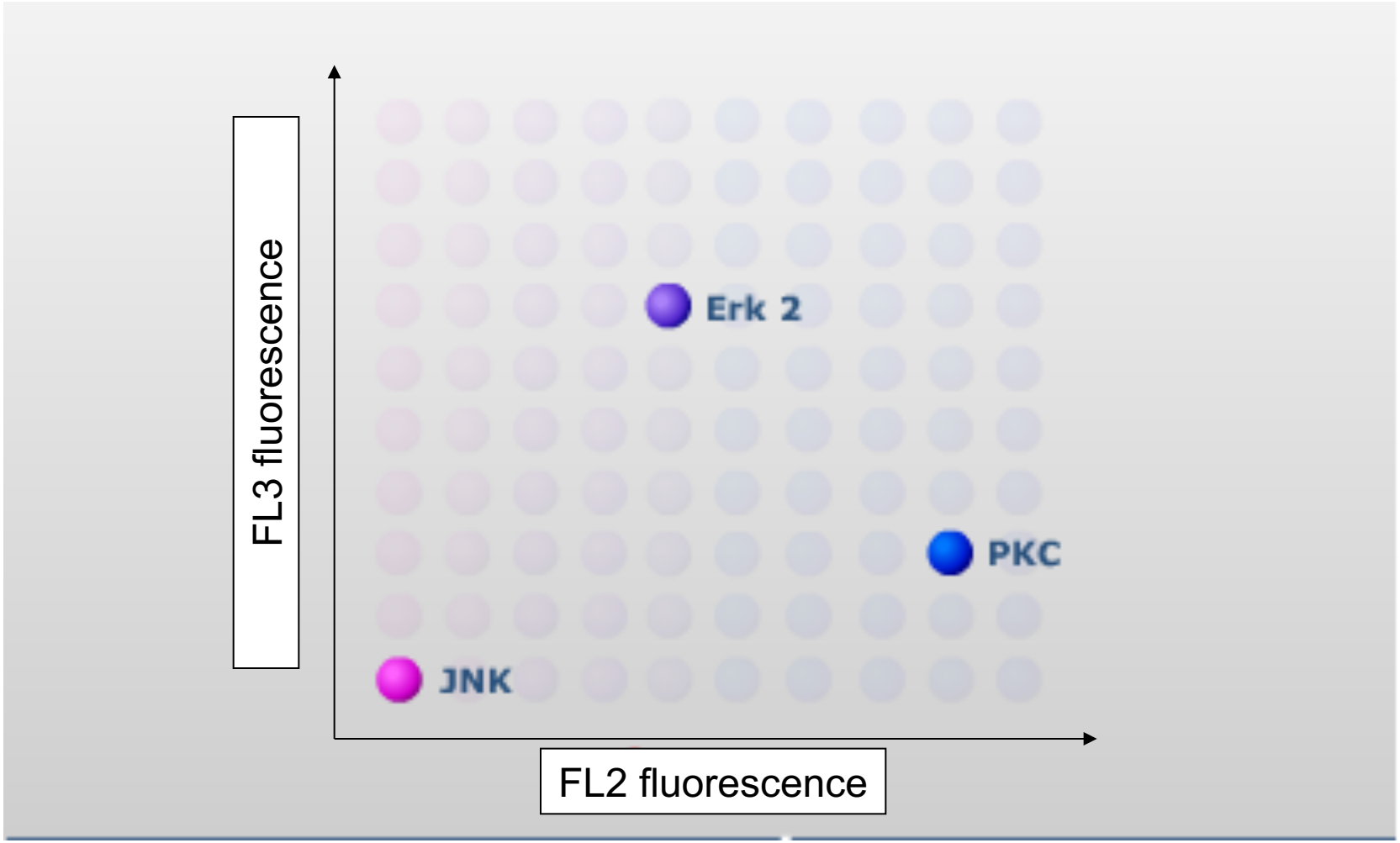
Luminex xMAP technology

Autoantibodies

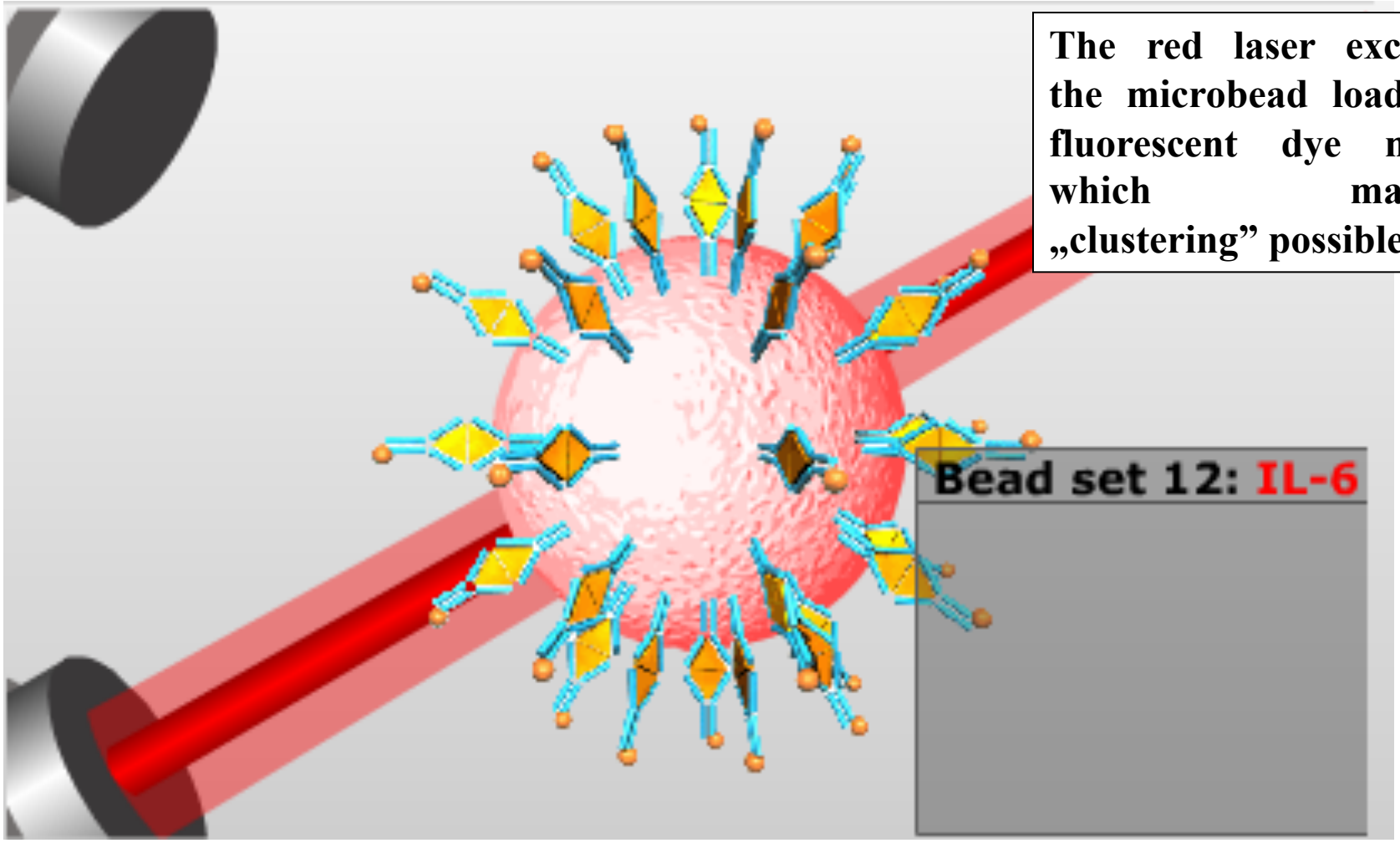


Luminex xMAP technology

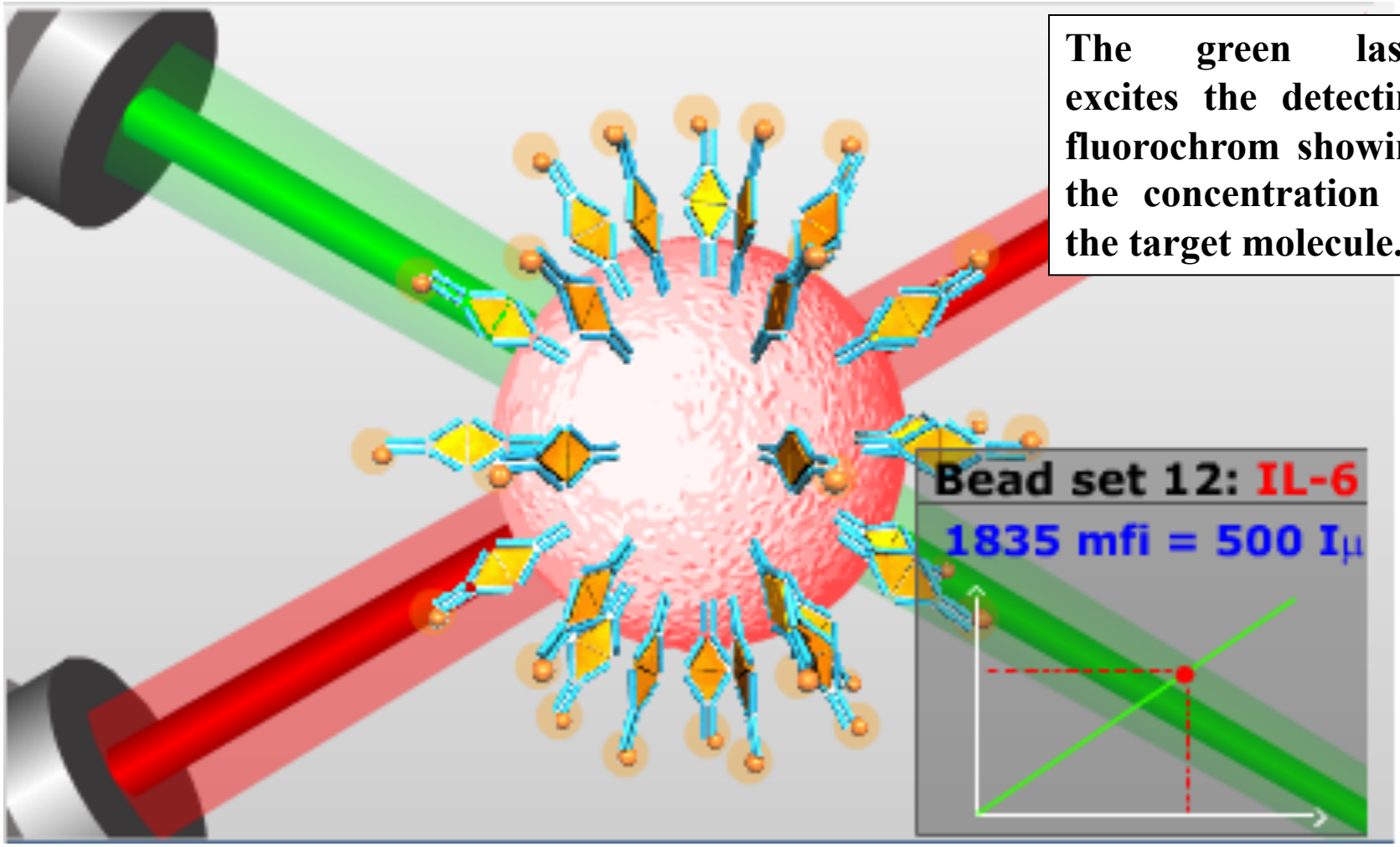
Kinases



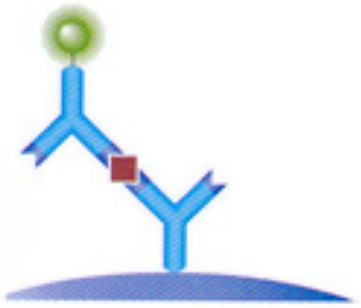
Identification of the microbead clusters



Detecting parameter

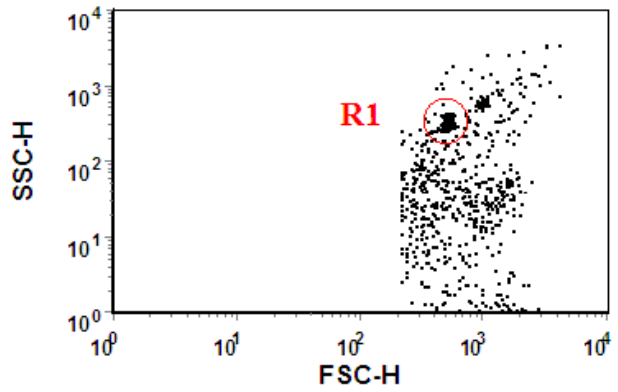


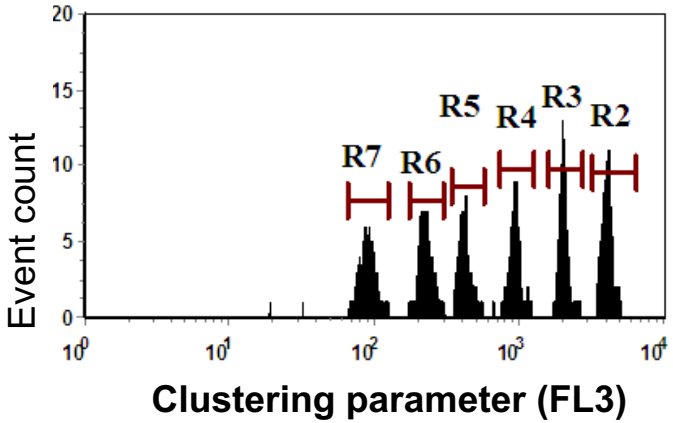
BD™ Cytometric Bead Array (CBA)

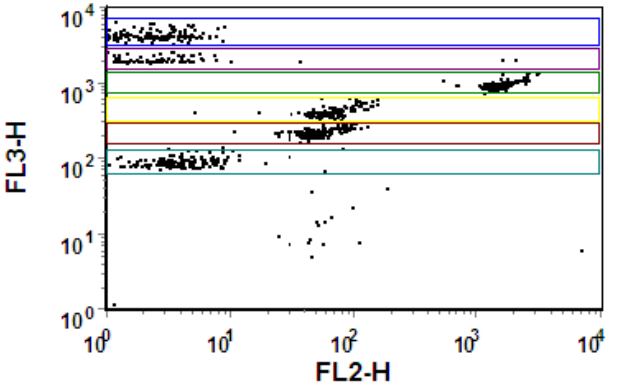


Detecting cytokine patterns with multiplex microbeads

Human inflammatory cytokine CBA kit



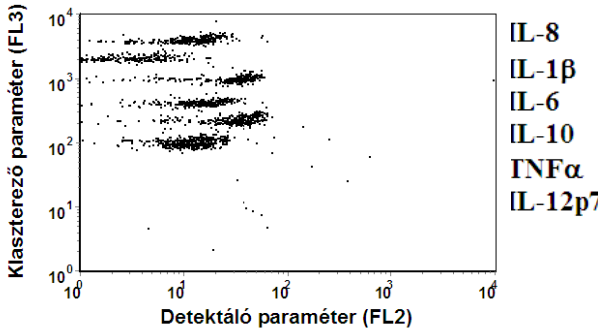




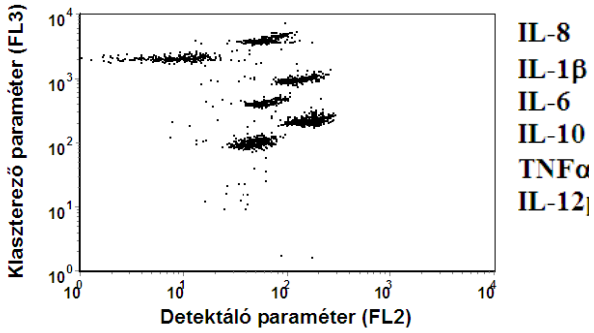
IL-8
IL-1β
IL-6
IL-10
TNFα
IL-12p70

Region #	# of Events	X Geometric Mean	X Arithmetic Mean
None	1201	26.83	322.22
1	1201	26.83	322.22
2	227	1.96	2.38
3	123	2.4	33.11
4	216	1536.98	1596.05
5	201	65.7	69.66
6	236	53.82	56.57
7	175	3.81	5.46

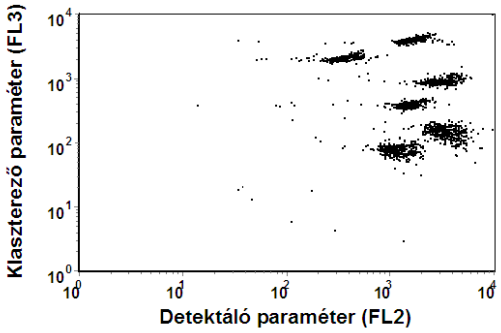
Calculating standard curve



20 pg/ml

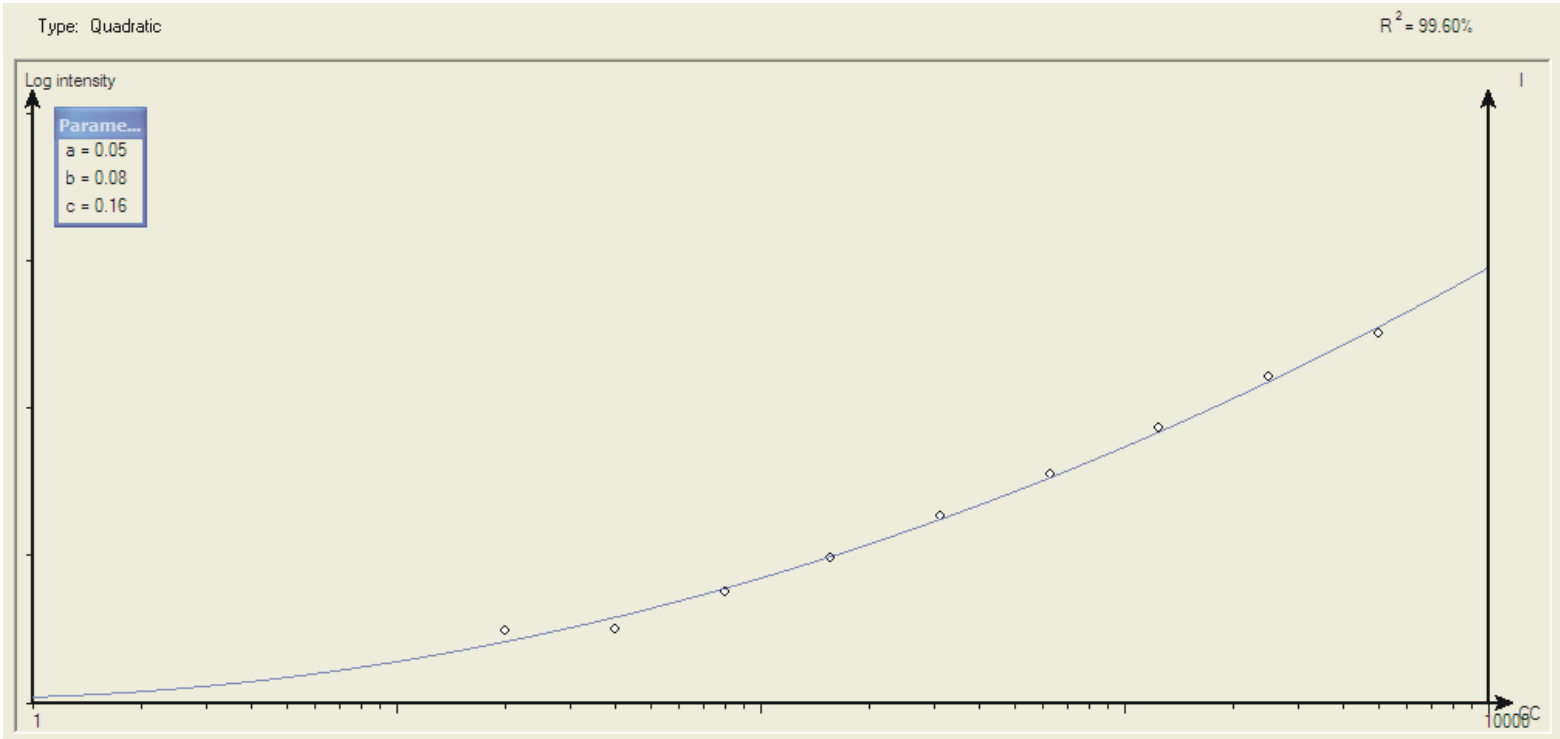


625 pg/ml



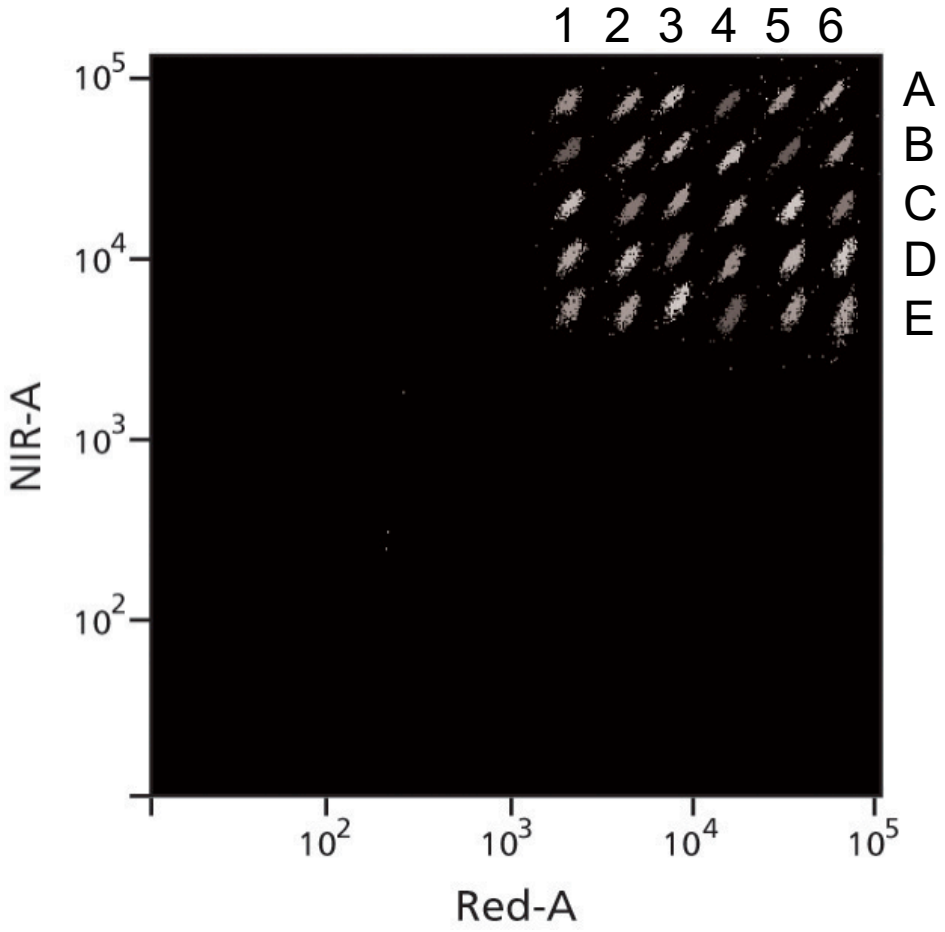
5000 pg/ml

FL2 MFI



CC 10.000

CBA - clustering



1. Clustering FL3/FL4
2. Gating - individual cytokines
3. Cytokine cc. - FL2

CBA – Potential uses

- Cytokines, chemokines and other soluble molecules
- Signaling
- Immunoglobulin isotype determination

CBA signaling reagents

TcR

- Phospho Itk (Y511) Flex Set (Bead C6)
- Phospho PLC-γ (Y783) Flex Set (Bead B7)
- Phospho Pyk2 (Y402) Flex Set (Bead D9)
- Phospho SLP-76 (Y145) Flex Set Bead (Bead D6)
- Phospho ZAP-70 (Y319) Flex Set (Bead B8)
- Total ZAP-70 Flex Set (Bead B8)

BcR

- Phospho BLNK (Y84) Flex Set (Bead C9)
- Phospho Btk (Y551) Flex Set (Bead D5)
- Phospho Syk (Y352) Flex Set (Bead B9)
- Total Syk Flex Set (Bead B9)

MAPK

- Phospho c-Jun (S63) Flex Set (Bead D8)
- Phospho ERK1/2 (T202/Y204) Flex Set (Bead C4)
- Phospho JNK1/2 (T183/Y185) Flex Set (Bead B5)
- Phospho MEK1/2 (S222) Flex Set (Bead A6)
- Phospho p38 (T180/Y182) Flex Set (Bead B6)
- Phospho Rsk (T573) Flex Set (Bead D7)
- Total JNK Flex Set (Bead B5)
- Total p38-α Flex Set (Bead B6)
- Phospho ERK1/2 (T202/Y204) Standard
- Phospho JNK1/2 (T183/Y185) Standard
- Phospho p38 (T180/Y182) Standard

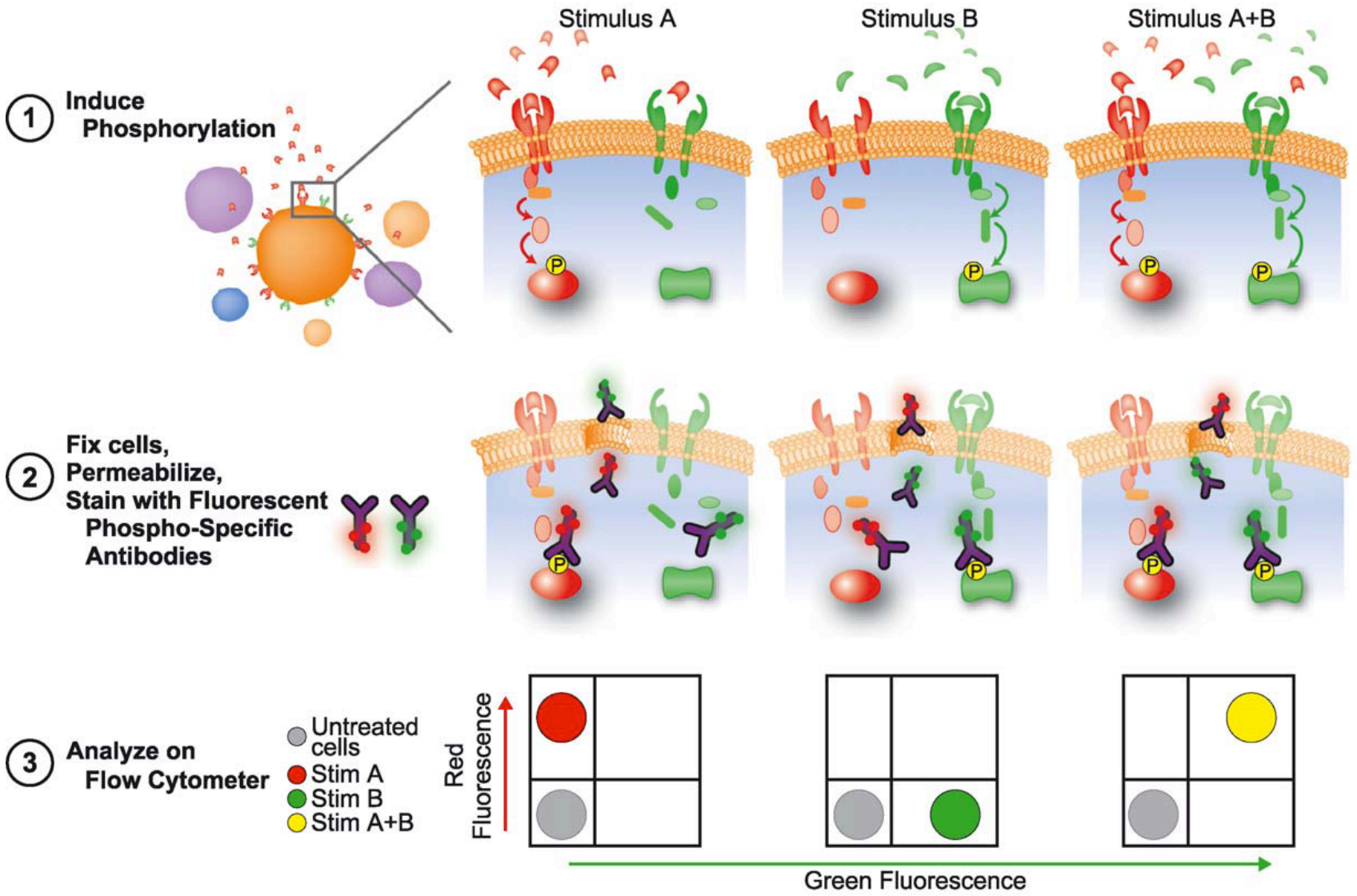
Akt

- Phospho Akt1 (S473) Flex Set (Bead A4)
- Phospho Akt1 (T308) Flex Set (Bead A4)
- Phospho Akt2 (S474) Flex Set (Bead A5)
- Phospho Akt2 (T309) Flex Set (Bead A5)
- Total Akt1 Flex Set (Bead A4)
- Total Akt2 Flex Set (Bead A5)

JAK/Stat

- Phospho Stat1 (Y701) Flex Set (Bead C5)
- Phospho Stat3 (Y705) Flex Set (Bead C8)
- Total STAT1 Flex Set (Bead C5)

Phospho-flow - Gary P. Nolan, Peter O. Krutzik



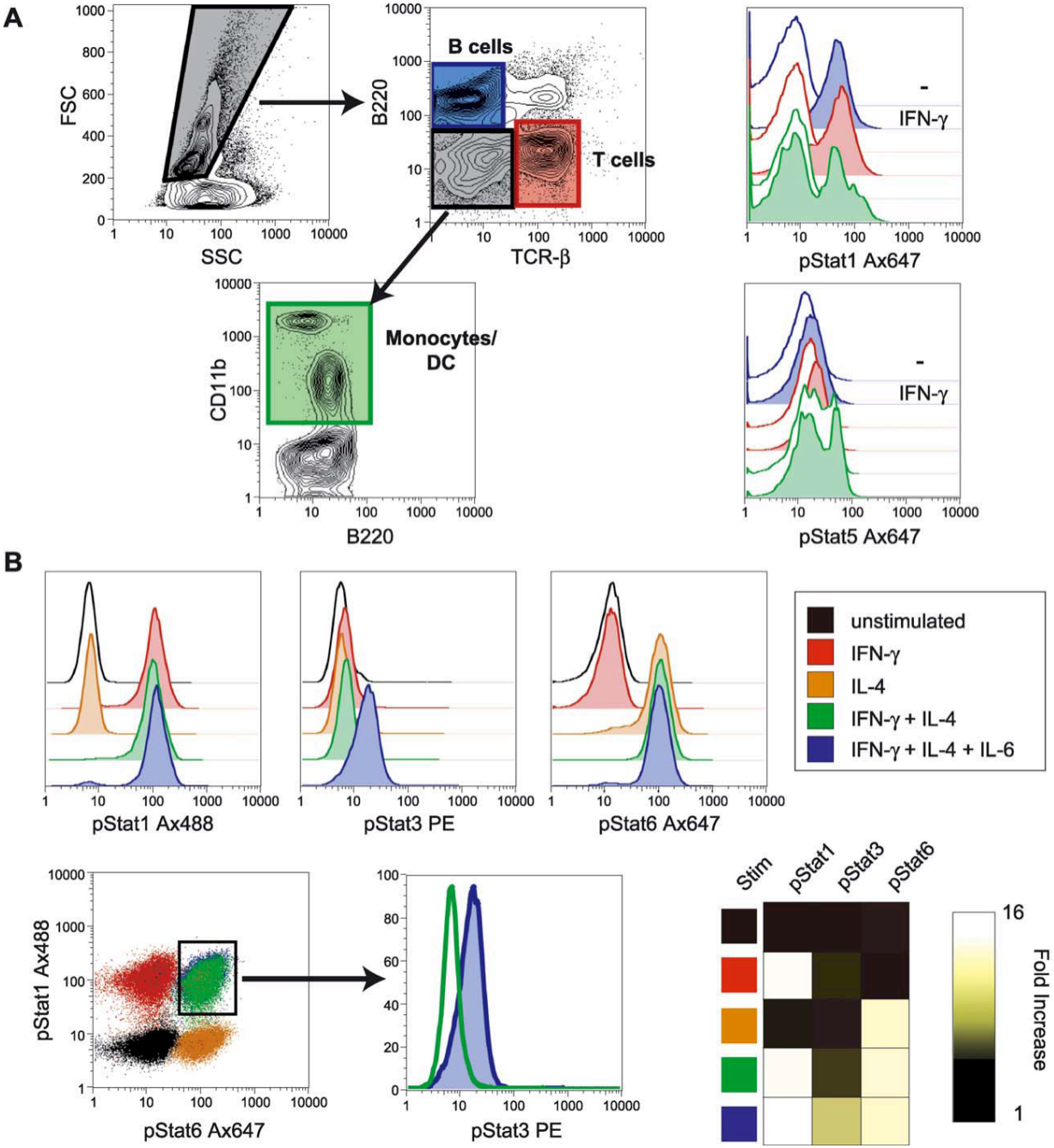
Krutzik et al. Clin. Immunol. 110 (2004) 206-221.

Phospho-flow

Table 2
Comparison of phospho-specific flow cytometry and traditional techniques (Western blotting)

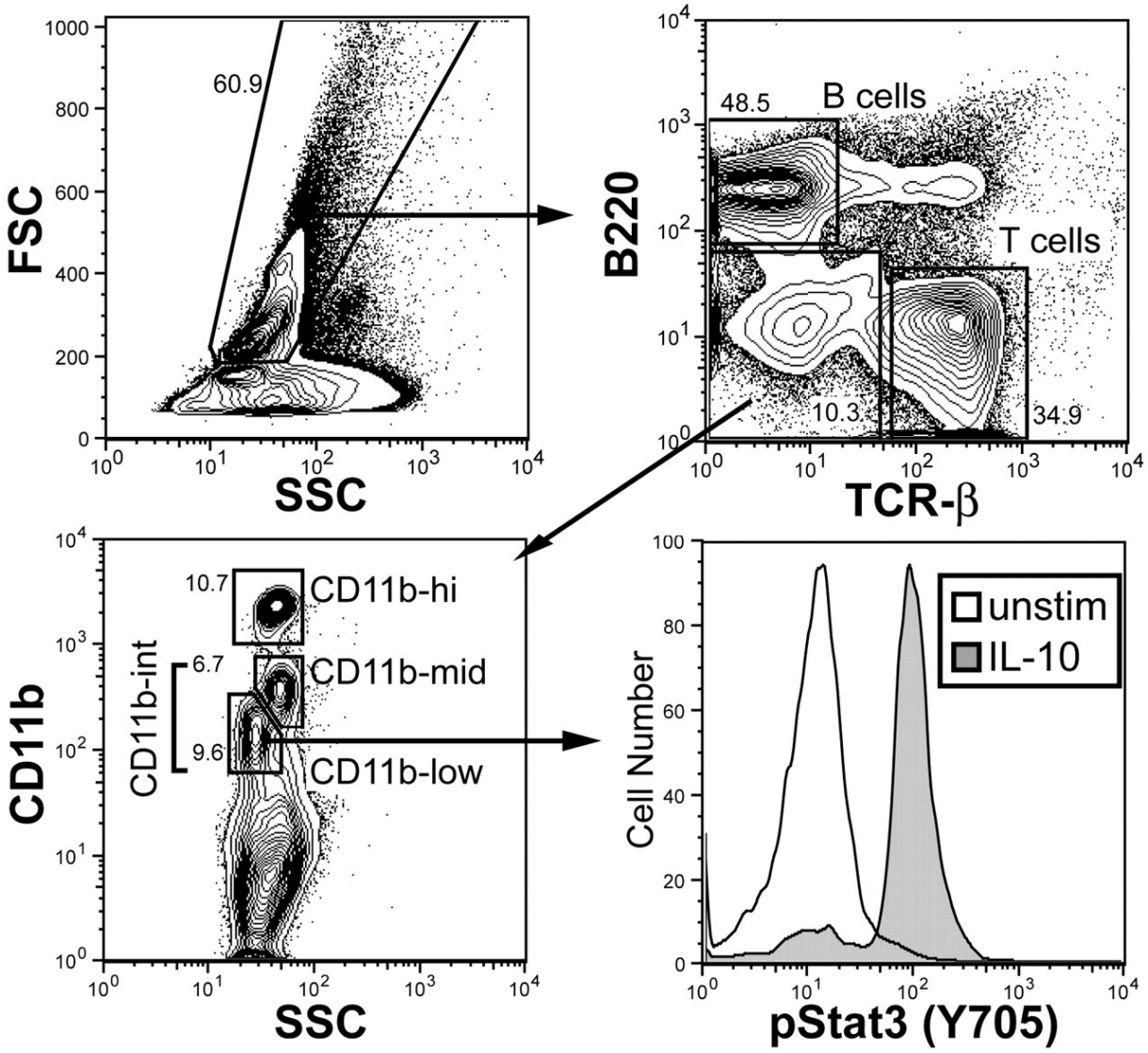
Western blot	Flow cytometry
Population analysis	**Single cell analysis**
Obtain average value of multiple cells	Collects data for each individual cell
Homogeneous sample	**Heterogeneous cell types**
Limited to cultured or purified cells	Complex primary samples, that is, immune cells
One parameter	**Multiparameter**
Obtain data sets individually	Correlate multiple markers simultaneously
Large number of cells	**Small number rare subsets**
Requires in vitro derived cultures of rare cells	Direct analysis of rare cell types (i.e., DC)
Time consuming for large sample sets	**Rapid and scalable**
Not amenable to large screening efforts	Performed in 96-well plates in parallel
Protein size and Ab specificity	**Ab must be validated**
Ab selectivity for target is clearly visible	Ab must have high affinity and selectivity

Krutzik et al. Clin. Immunol. 110 (2004) 206-221.



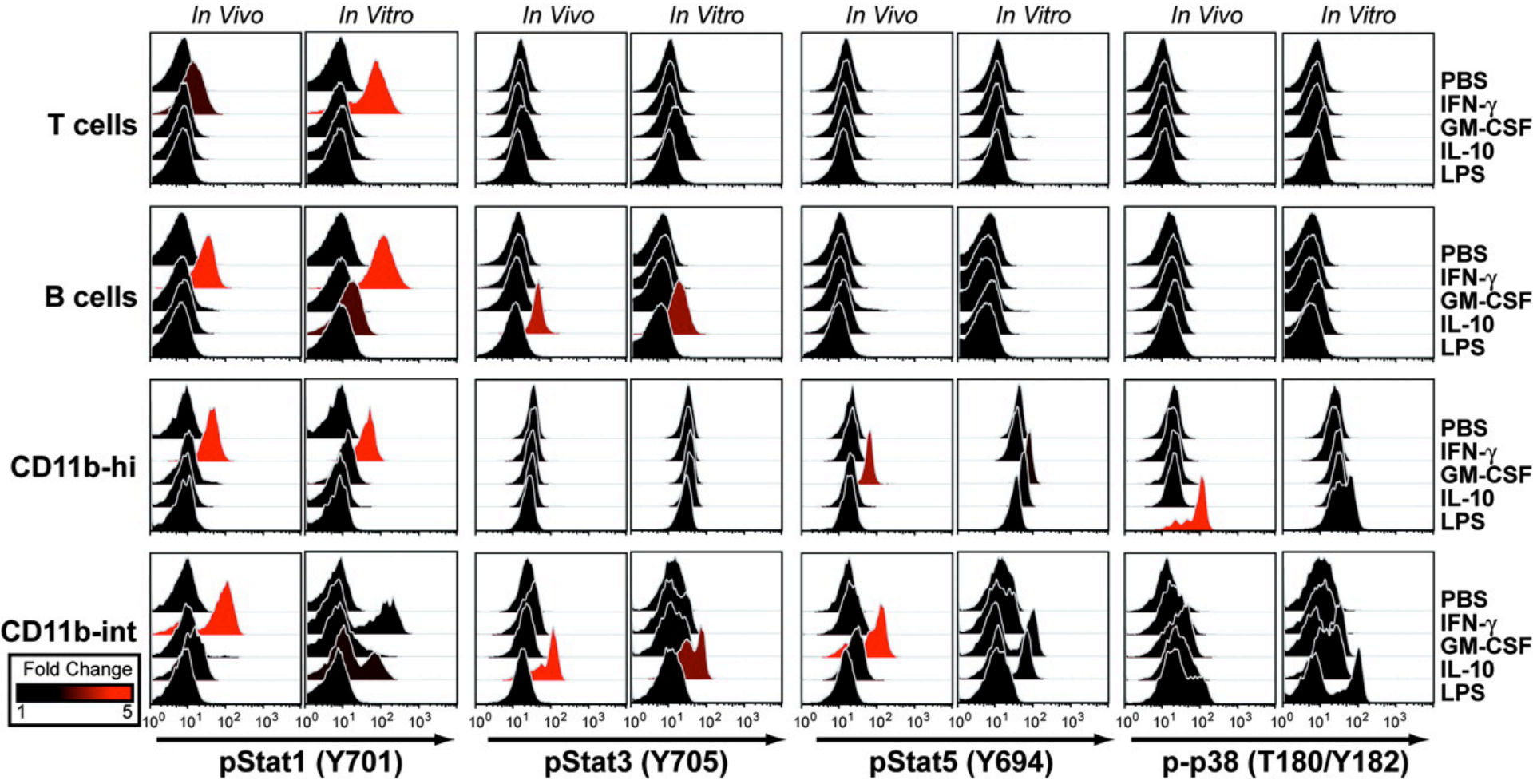
Krutzik et al. Clin. Immunol. 110 (2004) 206-221.

Phospho-flow



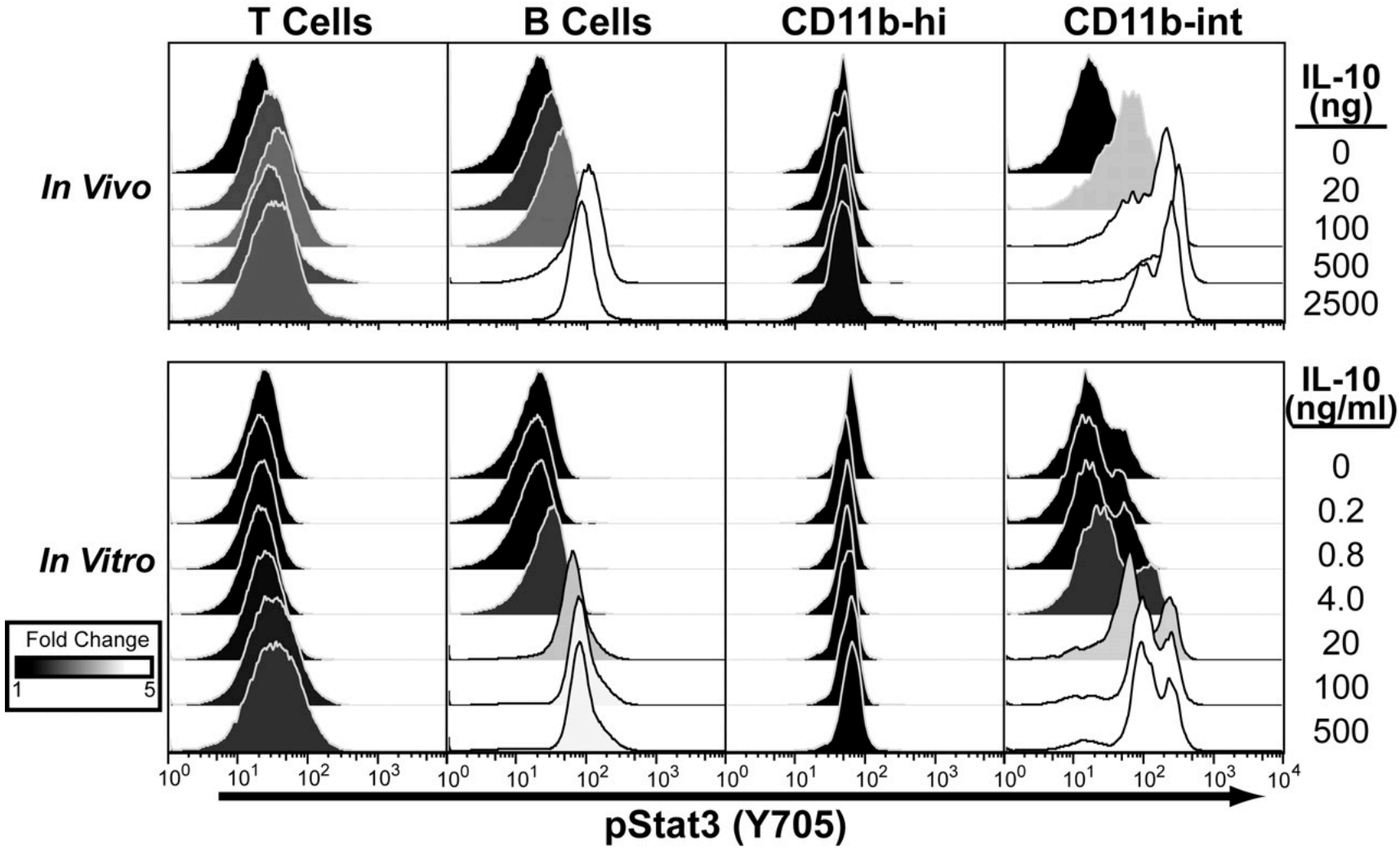
Krutzik et al. J. Immunol. 175 (2005): 2366-2373.

Phospho-flow



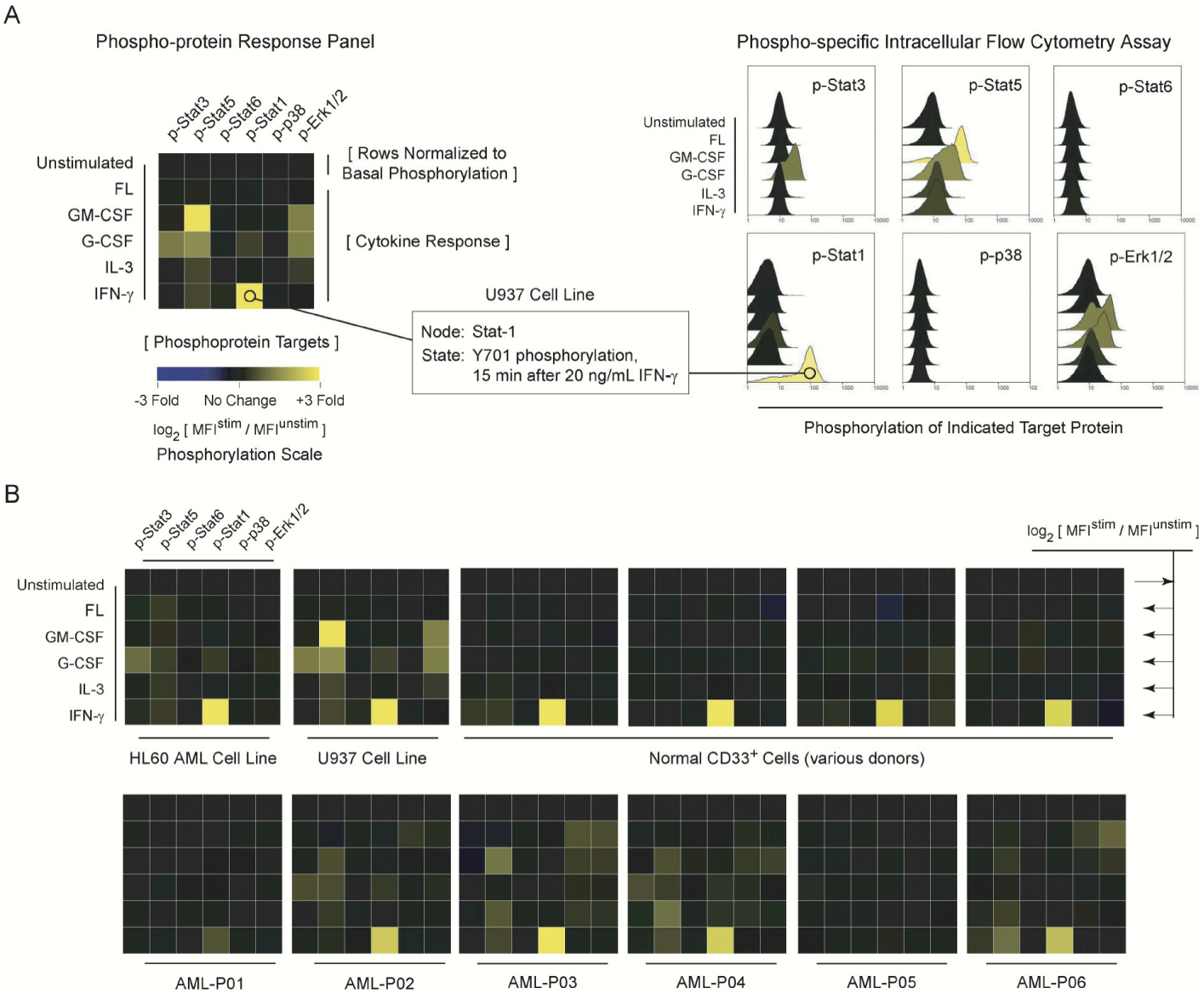
Krutzik et al. J. Immunol. 175 (2005): 2366-2373.

Phospho-flow



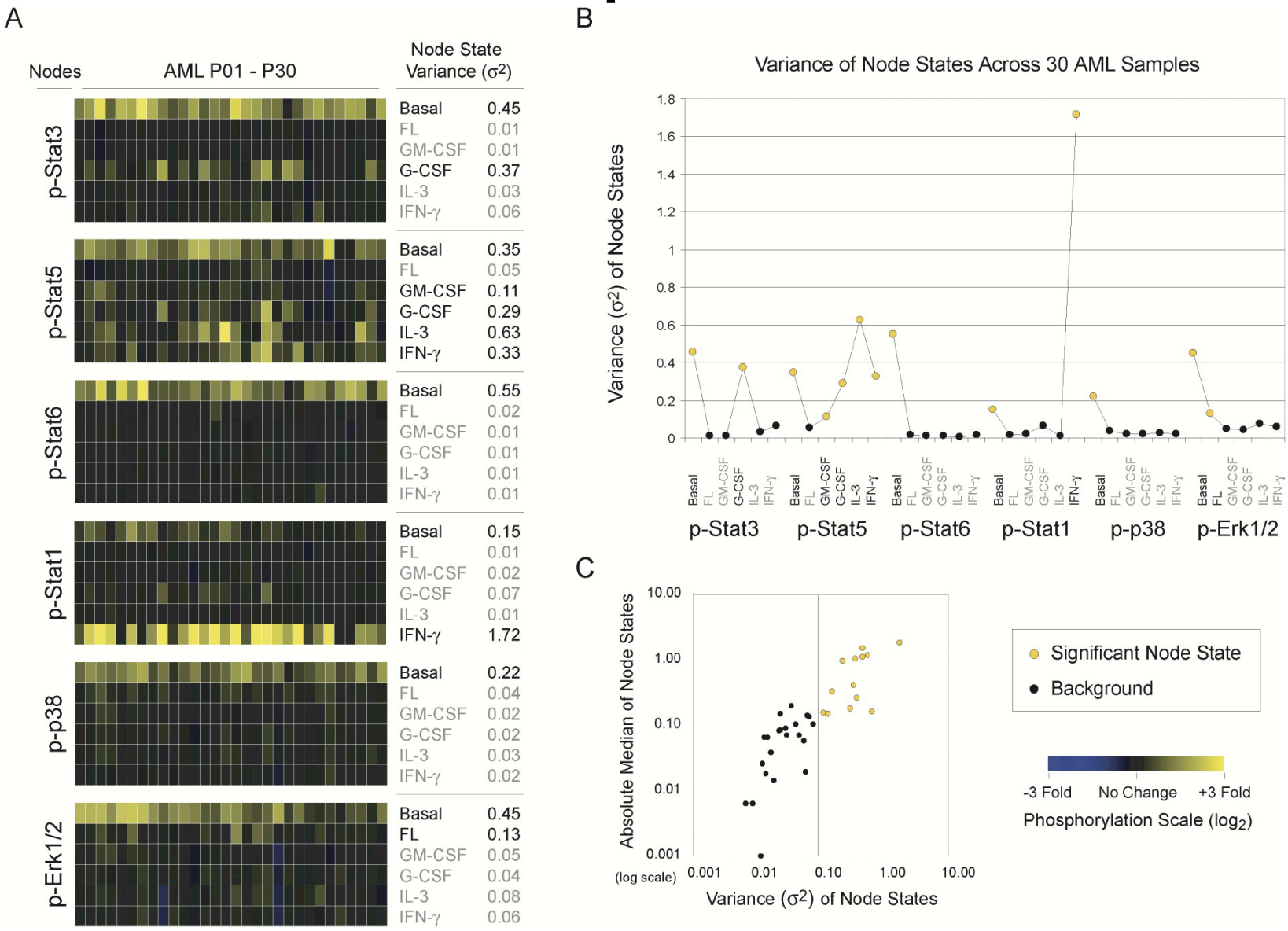
Krutzik et al. J. Immunol. 175 (2005): 2366-2373.

Phospho-flow



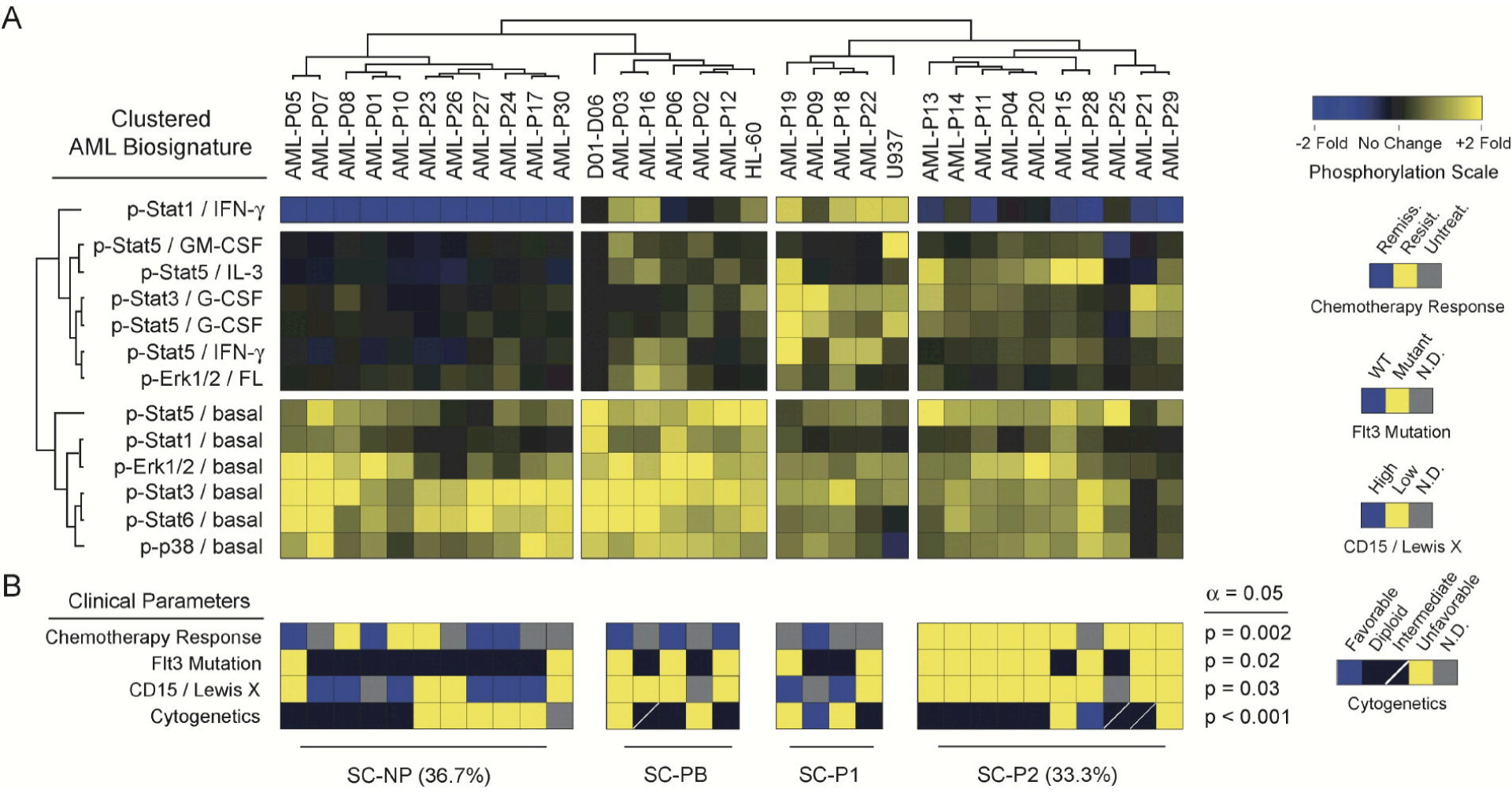
Irish JM et al. Cell 118 (2004): 217-228.

Phospho-flow



Irish JM et al. Cell 118 (2004): 217-228.

Phospho-flow



Irish JM et al. Cell 118 (2004): 217-228.