

9^ο Πανελλήνιο Συνέδριο Κυτταρομετρίας

με Διεθνή Συμμετοχή



Προκαταρκτικό
Επιστημονικό Πρόγραμμα

Δελφοί

27-29 Μαΐου 2016

**Ευρωπαϊκό
Πολιτιστικό Κέντρο
Δελφών**

DELPHI 27 V 2016

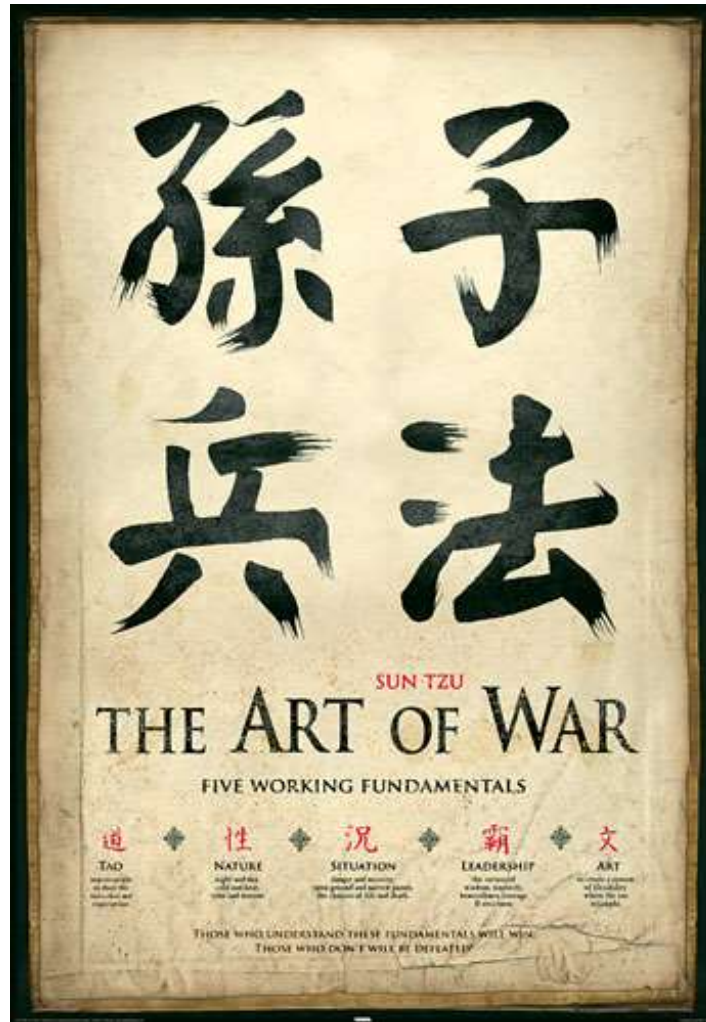
**CYTOMETRY STRATEGIES IN THE
DIAGNOSIS OF HEMATOLOGICAL
DISEASES**

**CLAUDIO ORTOLANI
UNIVERSITY OF URBINO - ITALY**

SUN TZU (544 b.C. – 496 b.C)



SUN TZU (544 b.C. – 496 b.C.)



THE ART OF CYTOMETRY

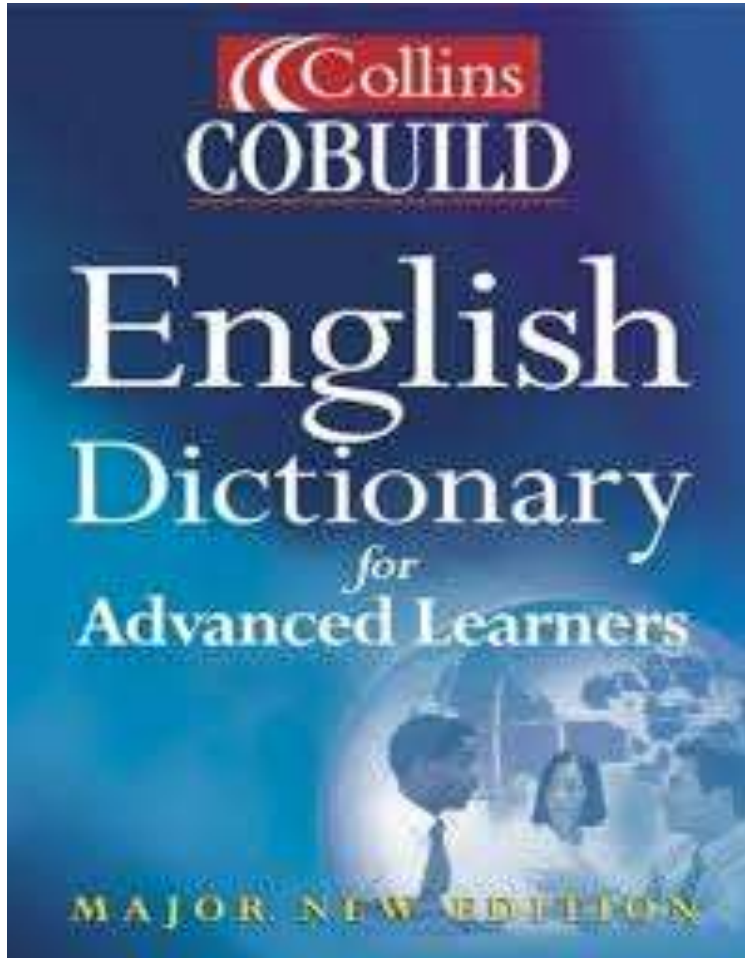
*"Strategy without tactics is
the slowest route to victory.*

*Tactics without Strategy is
the noise before defeat."*

Sun Tzu



WHAT ARE TACTICS?



Tactics are the methods that you choose in order to achieve what you want in a particular situation

OUR TACTIC

Given that what we want in a cytometric analysis is a better characterization of the events, our tactic is

**TO INCREASE THE DIMENSIONALITY OF
THE DATA-SET**

produced by our cytometer

DATA-SET DIMENSIONALITY

LIGHT SCATTER

FLUORESCENCE

TIME

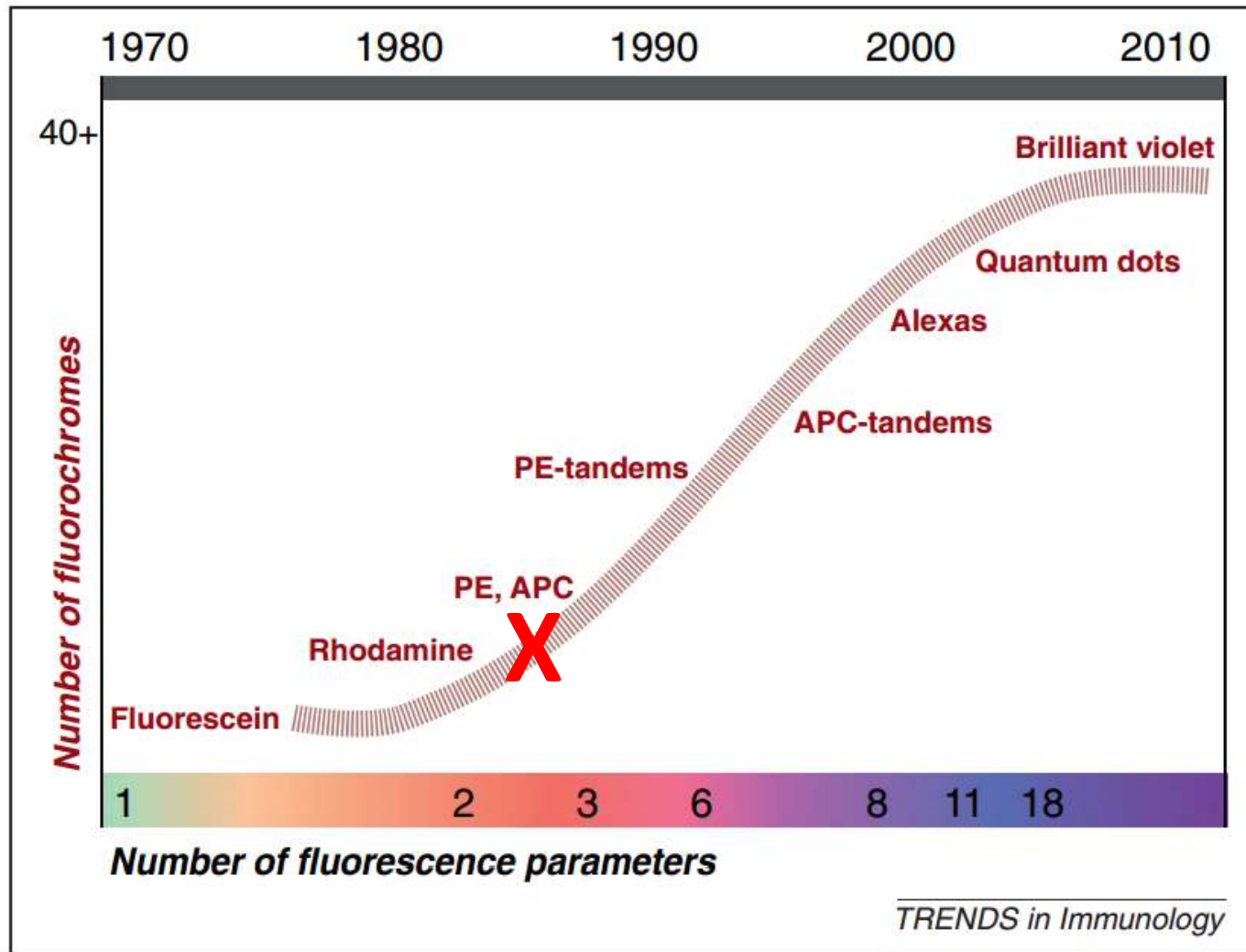
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000001	120072.891	85282	92271.49		365.12	855	150.48	1690.6	1134.293	14046.295	1002.2	611.3	772.56	56.3
000002	114715.906	93132	80724.37		697.1953	942.778968	392.16	754.68	946.2	10382	1746.96	526.64	1329.36	56.4
000003	100190.961	77214	85037.88		803.78	171	90.06	111.72	200.64	11.5999994	-58	90.4799957	104.399994	56.4
000004	22632.71	21503	68979.0859		894.13	6939.17969	861.839966	918.839966	10600.86	1984.75989	1919.79993	5932.23975	33840.68	56.4
000005	63358.793	56122	73986.71		168.3047	2770.2	289.56	491.34	4396.98	648.44	1056.76	2069.44	16647.16	56.4
					110.06	384.18	18.24	-69.54	-116.28	161.23999	-232	249.4	535.92	56.4
					303.7	200.64	74.1	26.22	108.299995	180.969991	23.1999989	134.56	174	56.6
					811.84	286.139984	116.28	403.56	131.099991	850.279968	184.439987	443.12	2495.16	56.7
					140.94	784.32	261.06	619.02	539.22	15896.64	1546.27991	317.84	981.36	56.9
					5237.836	5263.38	774.06	906.3	1827.41992	14578.88	1158.84	2364.07983	21484.36	56.9
000012	109337.586	86066	83256.43	87841.56	57068	100875.883	372.78	215.459991	616.74	121.979996	788.8	133.4	2679.59985	56.9
000013	57241.82	41342	90740.66	36006.9	19609	120340.063	448.88	114	1324.67993	3168.06	7376.44	1622.84	293.47998	56.9
000014	93475.96	73985	82801.11	44634.418	32515	89963.44	1062.48	344.28	8951.14	8632.08	10173.2	2836.2	599.72	56.9
000015	78883.0859	63852	80963.51	24814.3789	18146	89619.49	513	167.58	2663.64	4883.76	8822.96	2082.2	490.68	56.9
000016	43164.8125	38231	73993.6	17662.02	11267	102733.477	288.419983	48.02	90.06	196.08	361.919983	92.7999954	103.24	57
000017	96122.46	74597	84446.87	78084.3	54346	94162.0859	257.639984	137.94	499.32	12.54	606.68	114.84	534.76	57.1
000018	24501.06	22711	70701.4844	8467.92	6540	84855.3	-2.28	-19.38	261.06	12.54	-34.8	30.16	64.96	57.1
000019	54528.17	33584	106406.578	51127.86	31425	106626.789	1713.41992	348.84	793.44	-55.86	265.639984	-109.039993	370.039978	57.1
000020	102393.484	81523	82313.6953	28896.58	21044	89987.68	457.139984	190.38	862.98	1174.2	18474.16	2308.4	255.2	57.2
000021	47785.32	33095	94626.7344	41539.32	23941	113709.57	552.899963	218.88	510.72	809.399963	3782.76	2969.59985	396.72	57.3
000022	46327.18	41252	73598.8047	13447.4395	9885	89154.4141	314.639984	88.92	300.96	441.18	76.56	-102.079984	161.23999	57.3
000023	81313.9141	65595	81240.78	145574.578	100623	94813.08	5600.82	1210.67993	1557.24	438.9	1669.24	477.919983	2425.55961	57.3
000024	47461.62	42747	72764.0547	9957.899	7640	85418.97	62.7	78.659996	257.639984	207.48	1.16	-140.36	29	57.3
000025	55564.8523	48484	75106.9453	13725.6	10566	85133.54	277.02	83.22	287.28	387.6	160.08	15.08	89.32	57.5
000026	102682.625	80605	83486.24	123107.461	81102	99479.3047	264.48	158.459991	483.36	47.88	672.8	177.48	720.36	57.5
000027	69266.4141	52386	86653.76	37993.918	26382	94381.375	543.779968	263.34	1160.52	1804.62	22261.5586	2903.48	610.16	57.5
000028	86496.31	66554	85173.28	55927.2578	37172	98802.41	1013.45996	290.699982	1390.79993	730.74	12943.2793	1664.6	702.959961	57.5
000029	84842.97	69638	84511.43	79715.64	58639	89091.63	793.54	198.36	449.16	399	634.519958	295.8	1716.79993	57.5
000030	95869.6641	78443	80095.28	87172.375	66069	86469.13	305.52	140.22	541.5	142.5	698.32	505.759979	393.24	57.6
000031	88189.28	64806	89458.7856	147339.3	94026	102695.3	835.62	466.259979	1289.34	153.9	1213.36	99.7599945	783	57.6
000032	79618.57	67235	77606.65	19701.48	15805	81692.9	671.46	78.659996	5.7	376.199982	2390.76	-170.519989	801.56	57.6
000033	49620.69	43571	74635.46	17913.959	15483	75825.69	5820.84	679.44	621.3	2937.78	641.48	421.08	3521.76	57.6

WE HAVE TWO WAYS TO INCREASE DATA-SET DIMENSIONALITY

FIRST WAY (BY HARDWARE)

INCREASING THE NUMBER OF PARAMETERS
BY INCREASING
THE NUMBER OF PROBES

FLUORESCENT FLOW CYTOMETRY



From: roederer@drmr.com

Date: Sat, 3 May 2014 18:24:50 -0400

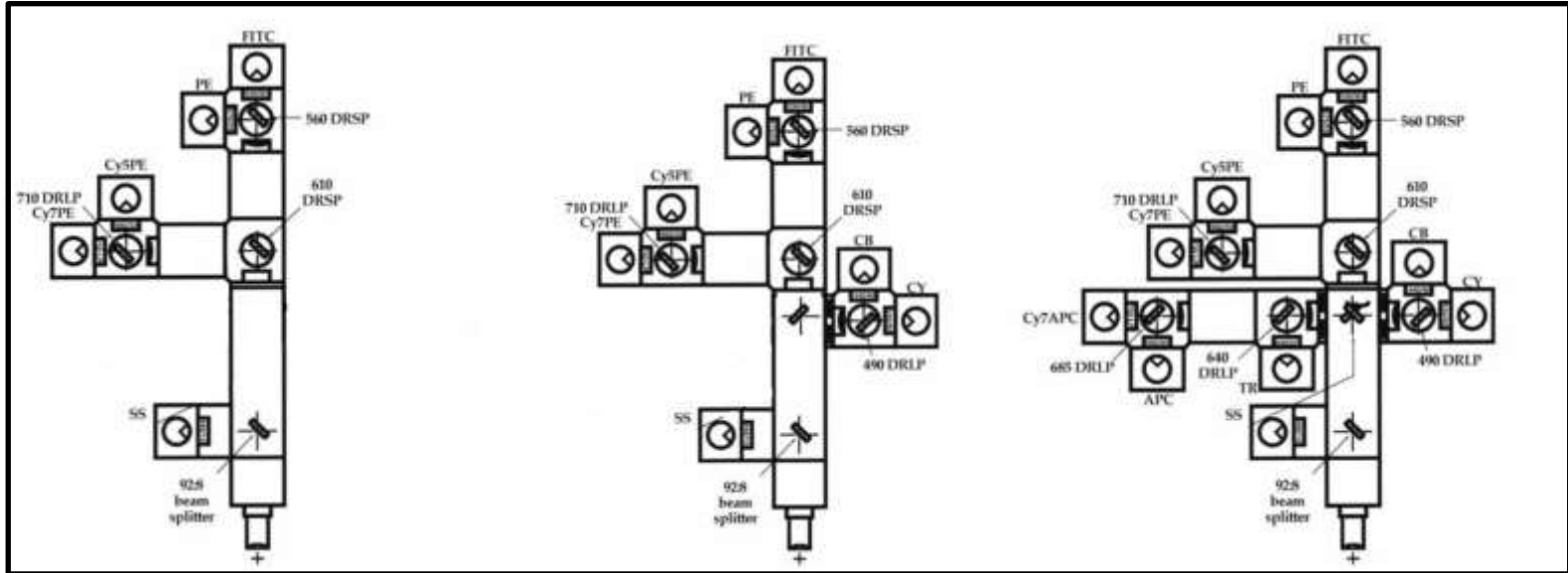
To: cytometry@lists.purdue.edu

Subject: [Cytometry] Splitting the rainbow

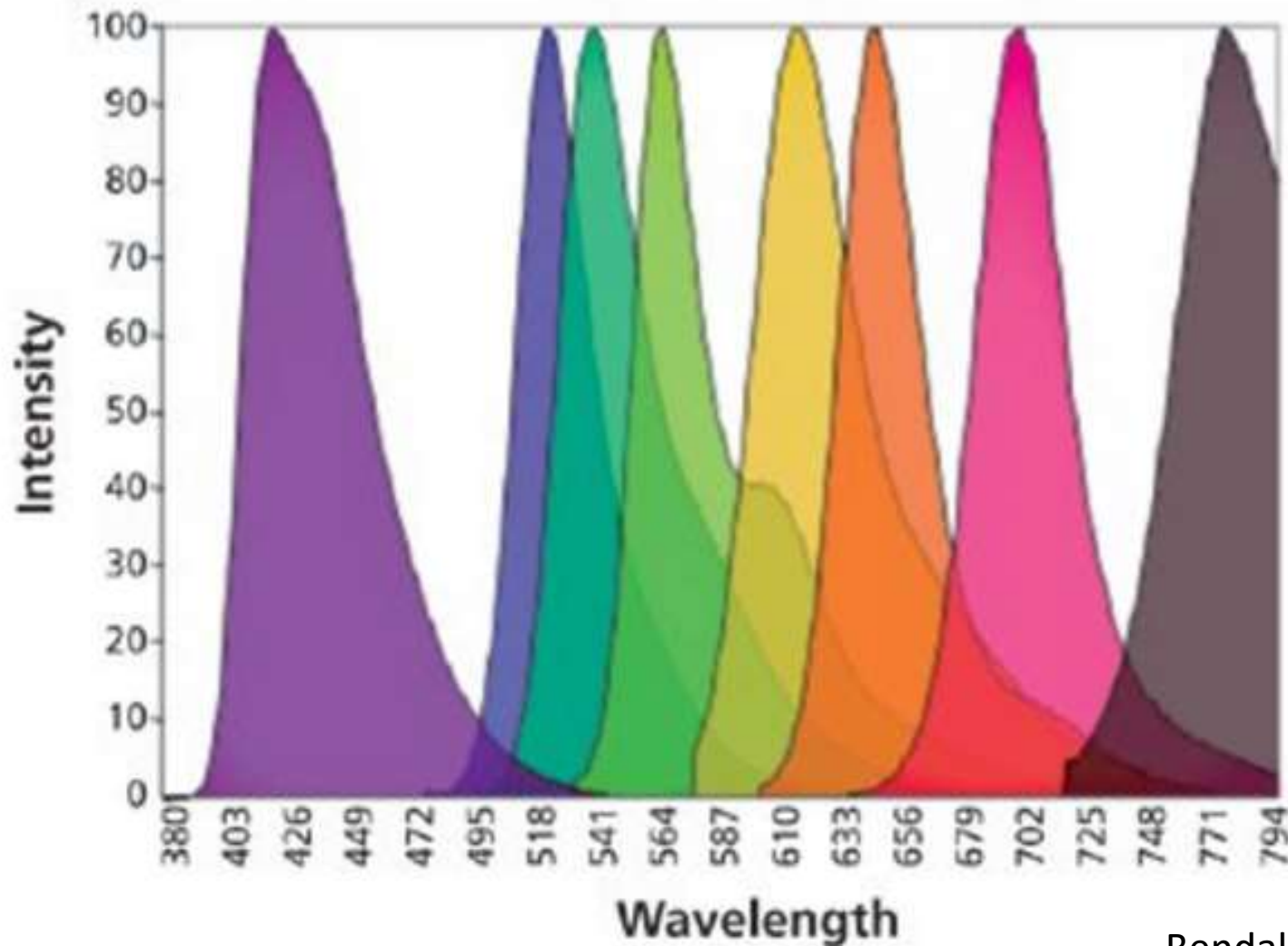
*I'm pleased to announce the dawn of a new era in fluorescence-based flow cytometry: A team headed by Pratip Chattopadhyay and Steve Perfetto in my lab [...] performed **the first 27-color fluorescence experiment** today. Despite virtually no optimization of the panel, this first attempt to break through the 18 color ceiling was a resounding success. **We are on track to exceed 30 colors** within the next few months.*

regards, Mr. ----

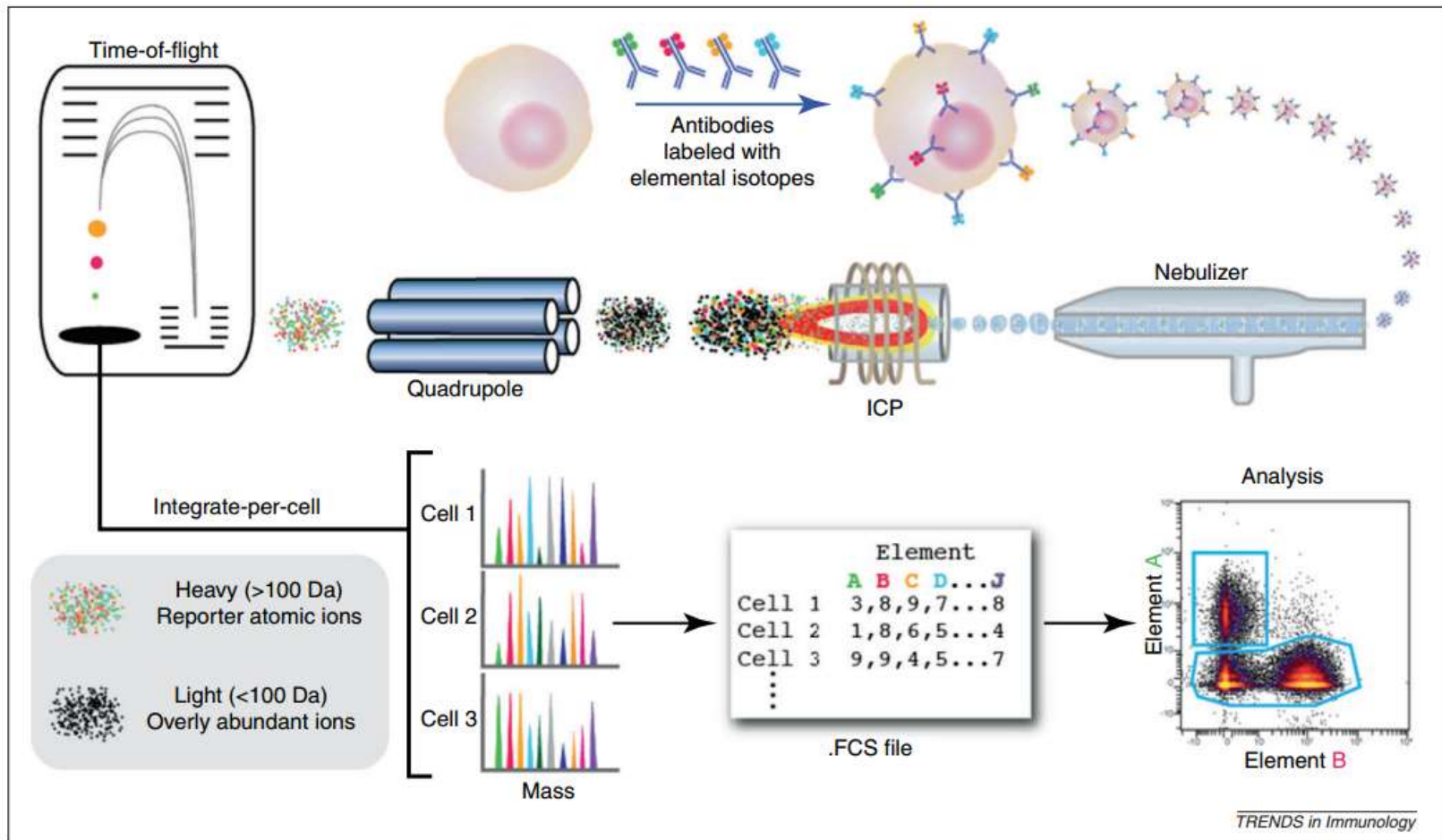
THE “SAGUARO EFFECT”



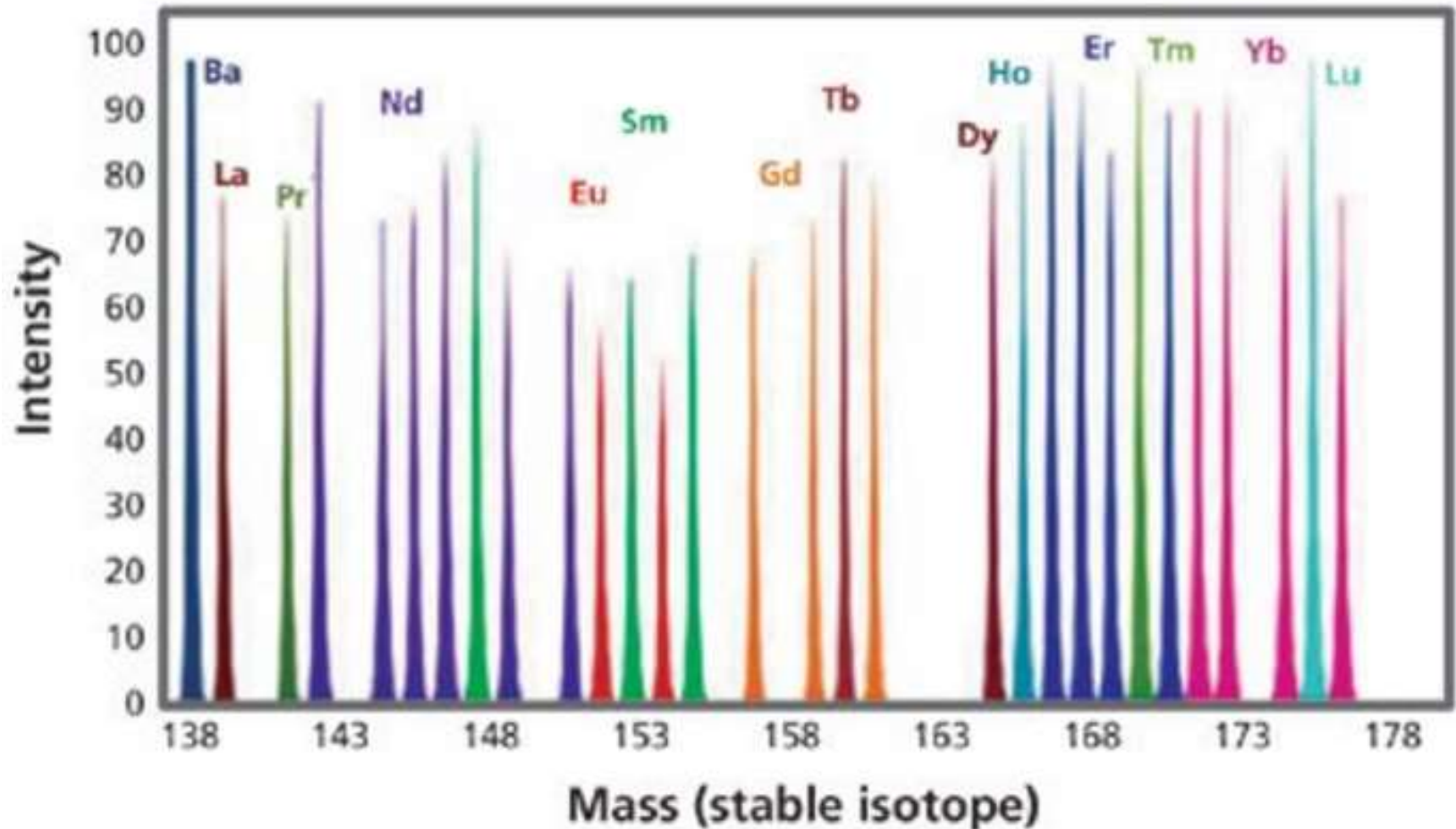
FLUORESCENT FLOW CYTOMETRY



... OR SOME OTHER MEANS ...



MASS FLOW CYTOMETRY



COMPARISON BETWEEN METHODS

	Fluorescence FCM	Mass FCM
Probe	Fluorochromes	Mass isotopes
Max Parameters	29 (2 + 27)	37
Sensitivity range	0,1 - 10	1 - 2
Sampling efficiency	> 95 %	< 30 %
Measured cells / sec	25.000	500 - 1000
Cells / h	25 - 60 million	2 million
Cost per probe	\$ 2,0 - 8,0	\$ 1,5 - 3,0
Sorting	Yes	No

MAIN DRAWBACKS

- FLUORESCENCE FCM
 - SPILLOVER (but please consider spectral compensation!)
 - AUTOFLUORESCENCE
- MASS FCM
 - LOW SENSITIVITY
 - LOW SPEED
 - NO USE OF FLUORESCENT PROBES COMMONLY USED IN CLASSIC FCM (INDO, CFSE, Rh123, and so on)
 - NO SORTING

WE HAVE TWO WAYS TO INCREASE DATA SET DIMENSIONALITY

SECOND WAY (BY SOFTWARE)

MERGING TOGETHER DIFFERENT
PARAMETERS OF CELLS SHARING THE
SAME EXPRESSION OF SOME OTHER
PARAMETERS

<http://www.ncbi.nlm.nih.gov/pubmed/25600947>
<https://www.bioconductor.org/packages/release/bioc/html/flowBin.html>

HOW DOES IT WORK?

A

FSC	SSC	A	B	C
15	45	97	124	12
26	3	50	100	70
100	34	12	45	77
69	90	23	84	72

B

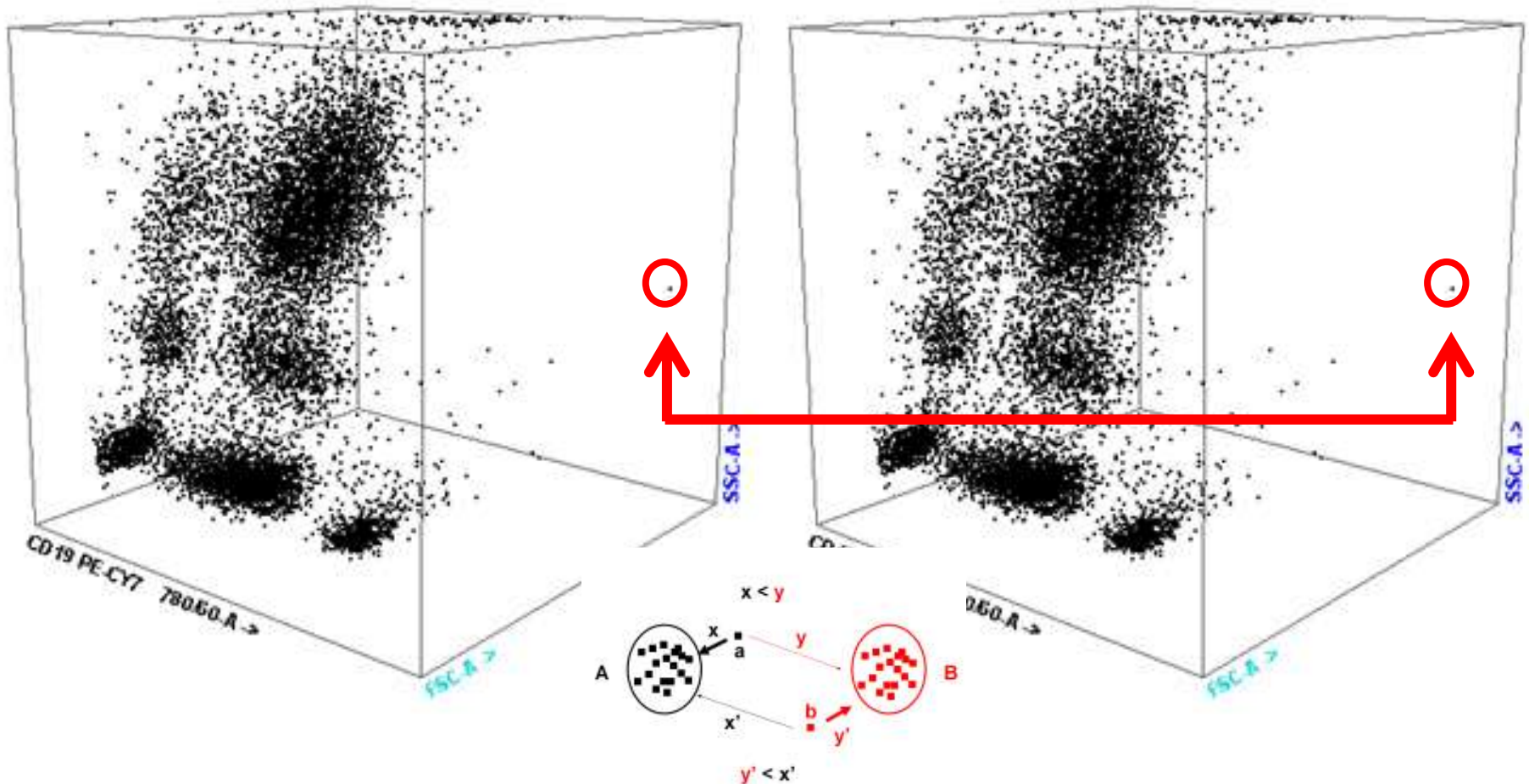
FSC	SSC	A	D	E
18	15	17	92	8
26	3	50	43	23
5	11	58	27	24
62	55	2	69	15

HOW DOES IT WORK?

FILE A

≠

FILE B



HOW DOES IT WORK?

A

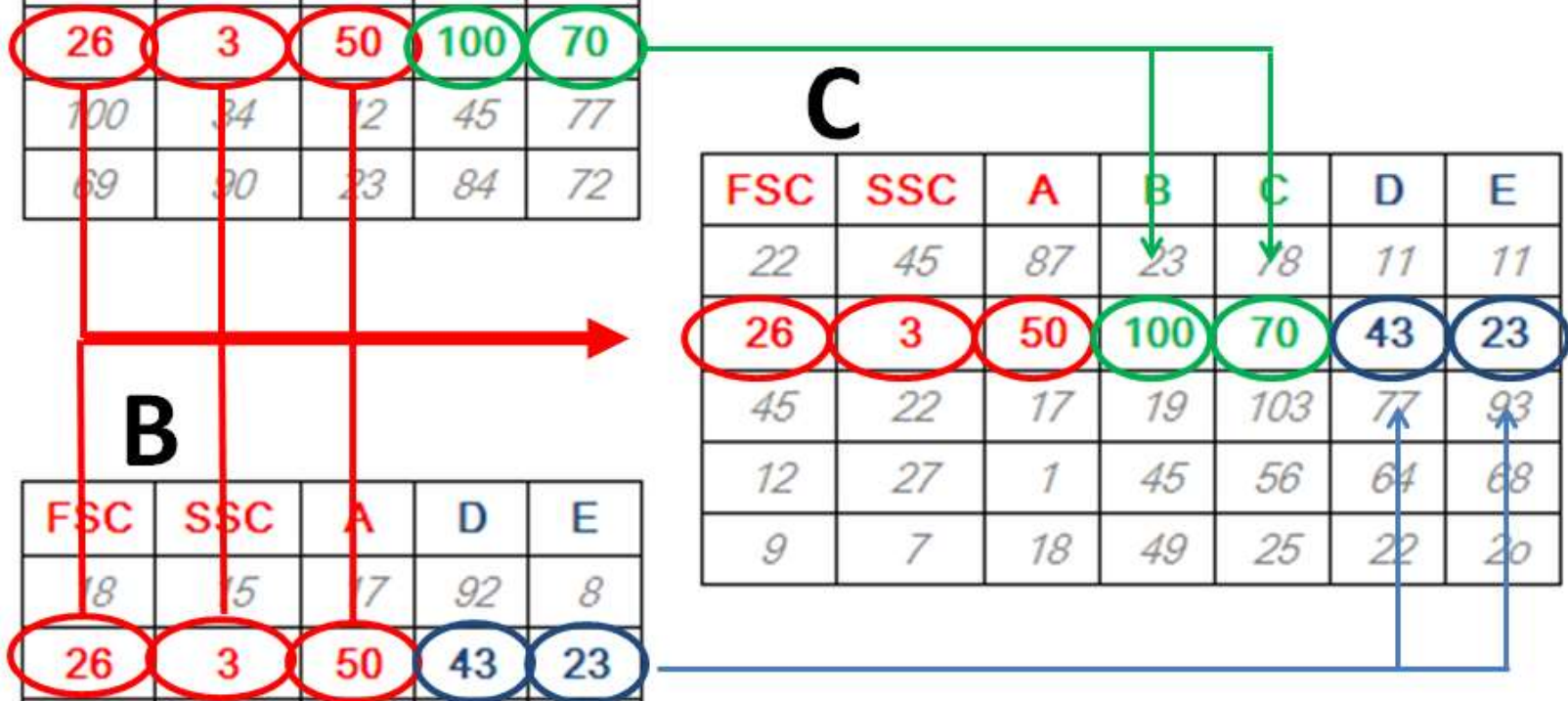
FSC	SSC	A	B	C
15	45	97	124	12
26	3	50	100	70
100	34	12	45	77
69	90	23	84	72

B

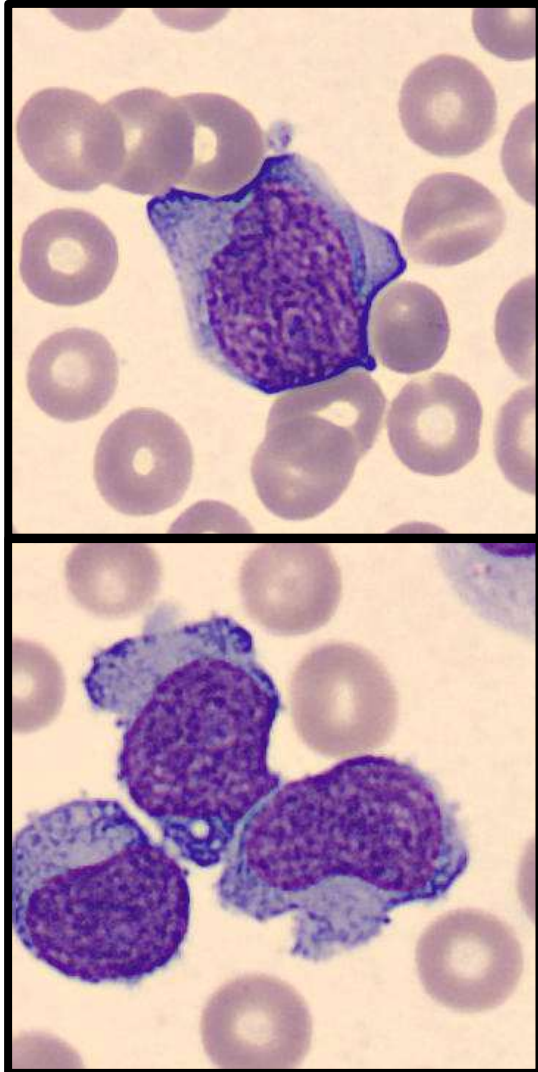
FSC	SSC	A	D	E
18	15	17	92	8
26	3	50	43	23
5	11	58	27	24
62	55	2	69	15

C

FSC	SSC	A	B	C	D	E
22	45	87	23	78	11	11
26	3	50	100	70	43	23
45	22	17	19	103	77	93
12	27	1	45	56	64	68
9	7	18	49	25	22	20



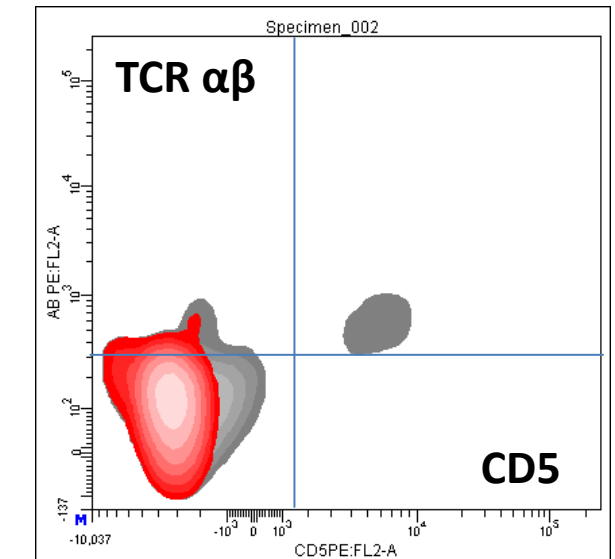
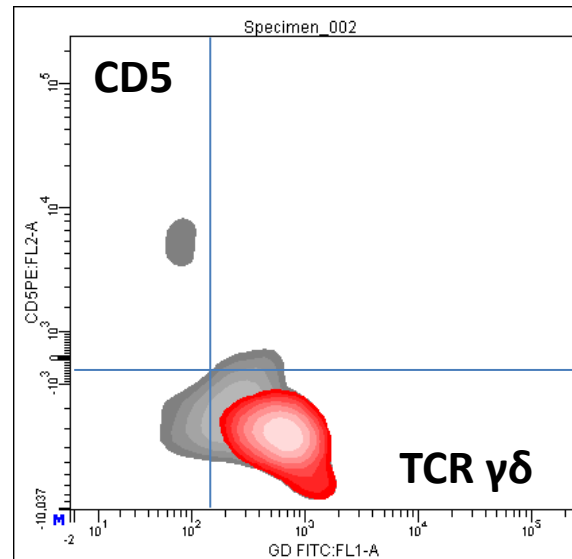
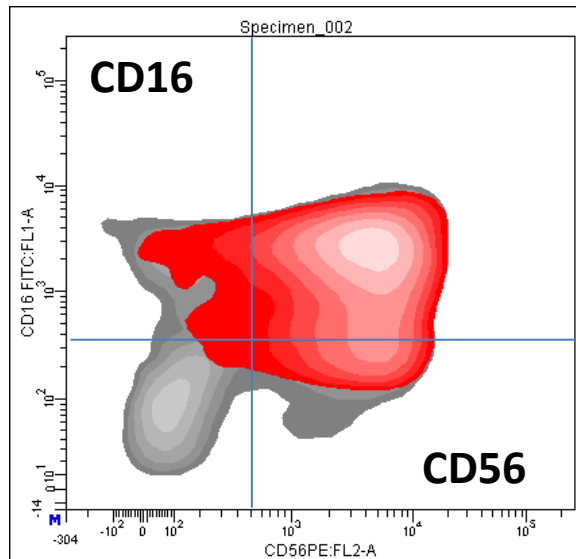
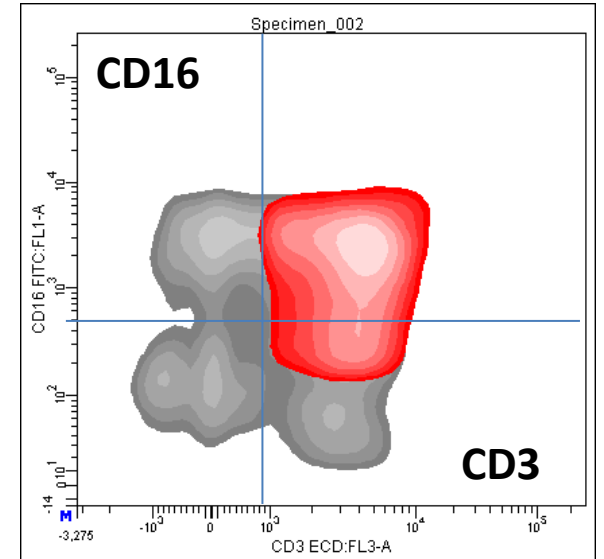
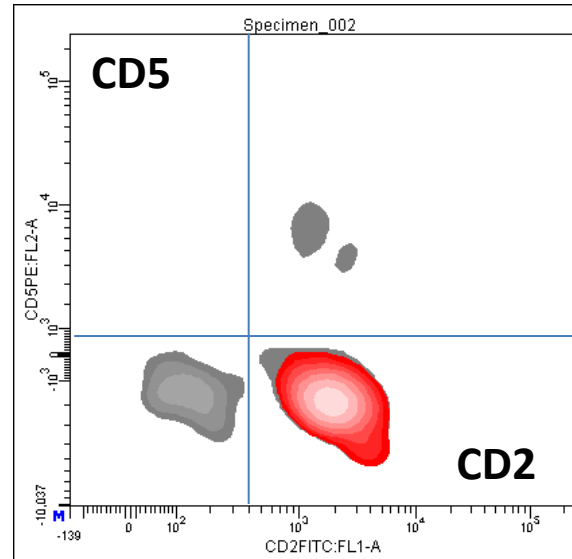
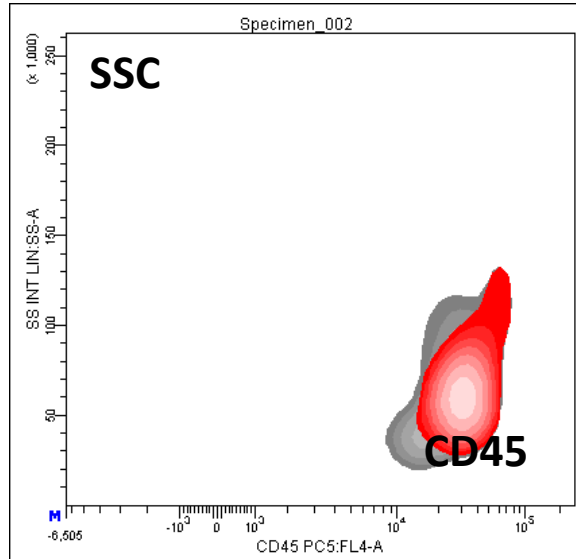
A PRACTICAL EXAMPLE OF DATA MERGING IN A CASE OF LEUKEMIZED HTSCL



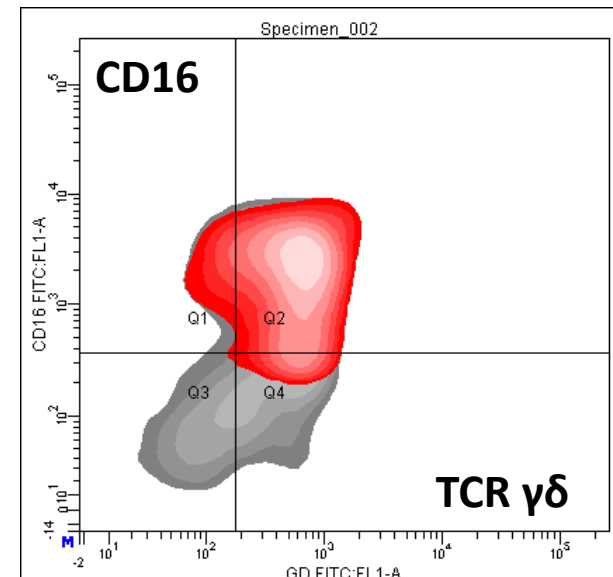
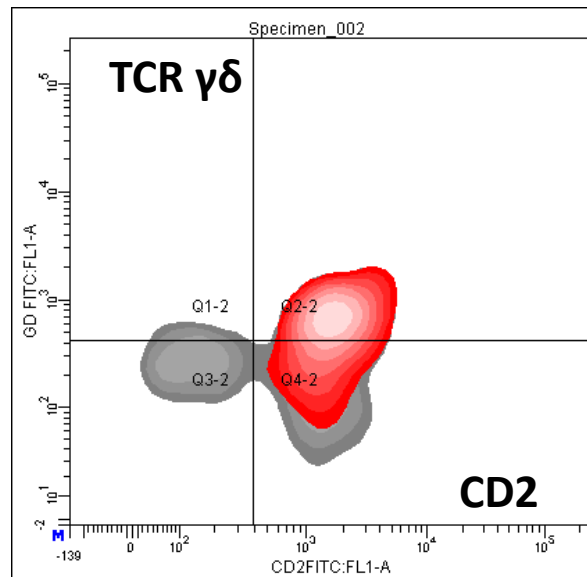
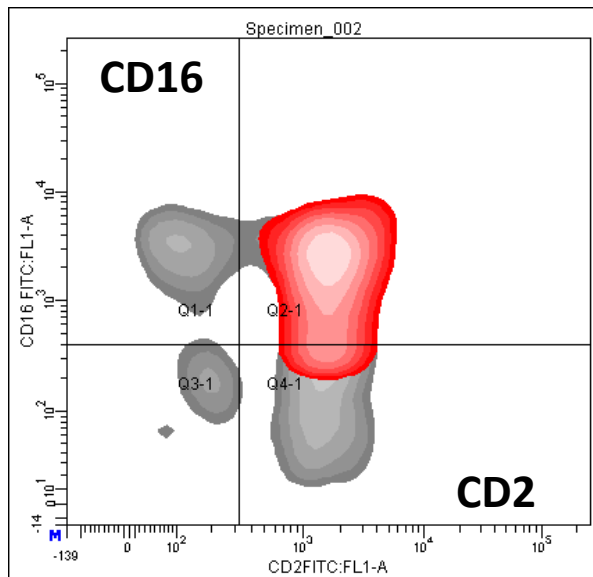
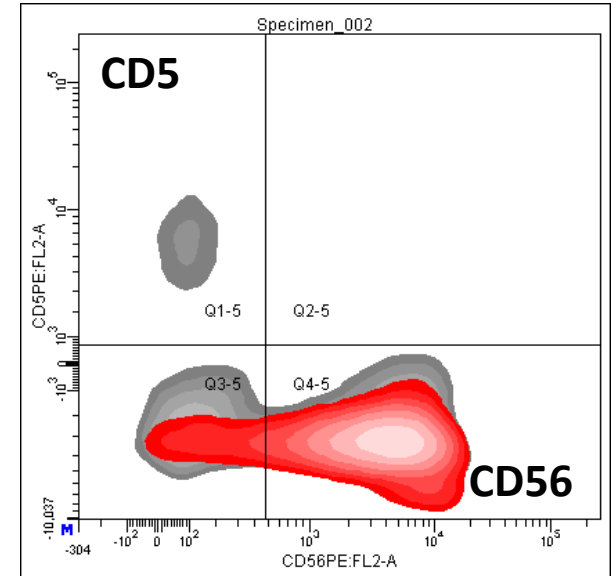
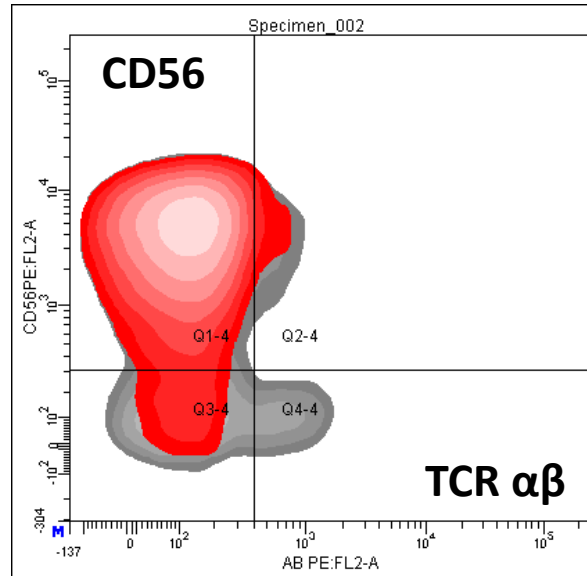
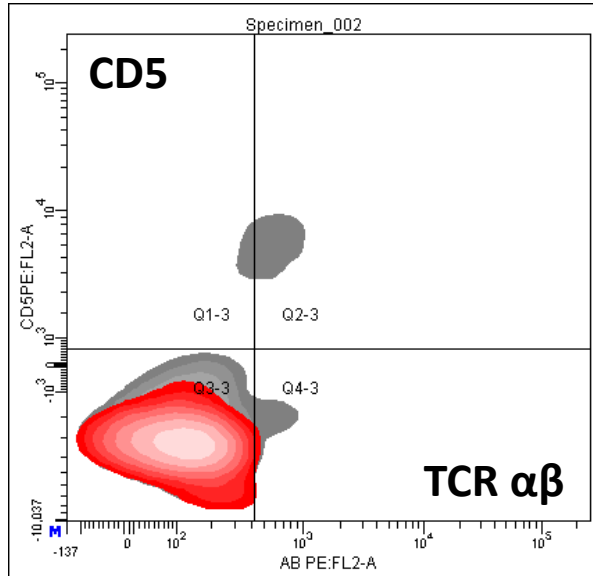
“BACKBONE” PARAMETERS IN RED
“PRIVATE” PARAMETERS IN BLACK

- 1st RUN: **FSC**, **SSC**, CD2, CD5, **CD3**, **CD45**
- 2nd RUN: **FSC**, **SSC**, CD16, CD56, **CD3**, **CD45**
- 3rd RUN: **FSC**, **SSC**, $\gamma\delta$, $\alpha\beta$, **CD3**, **CD45**

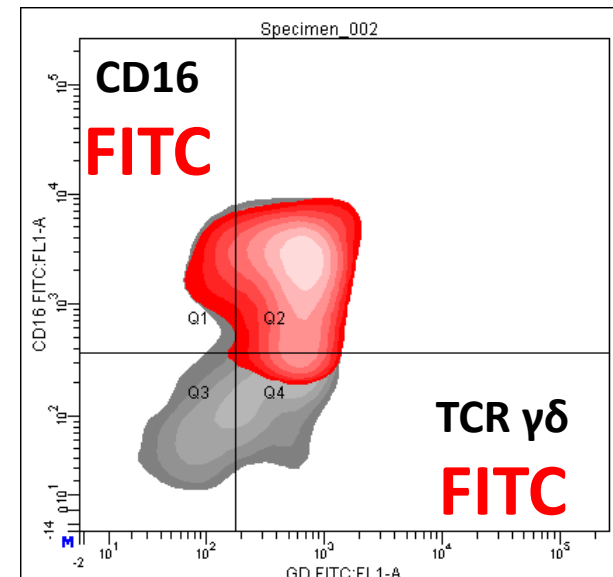
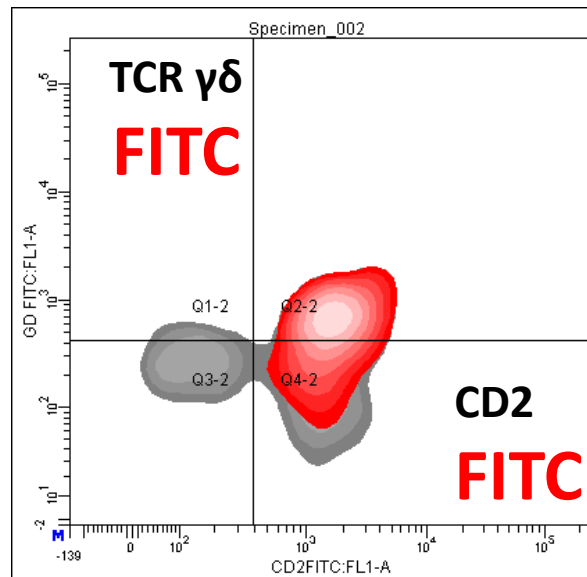
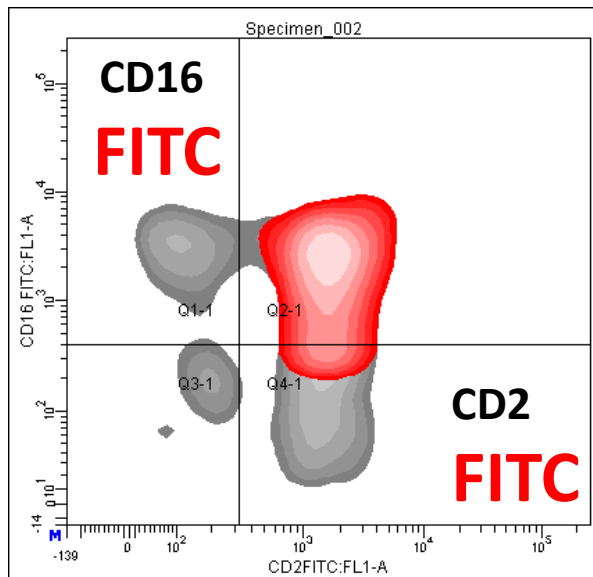
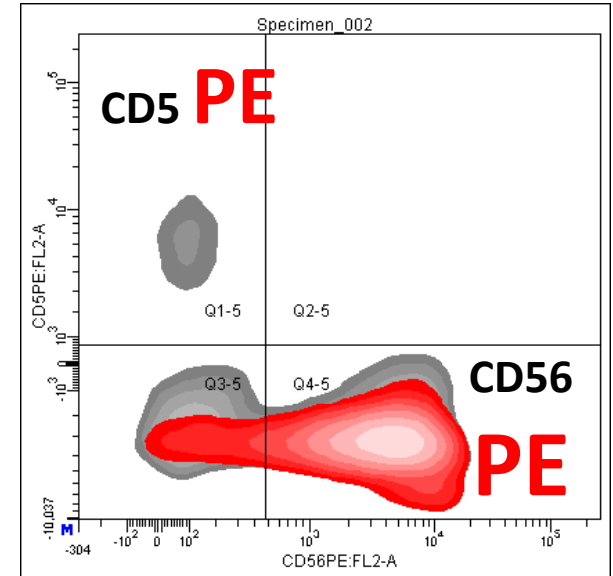
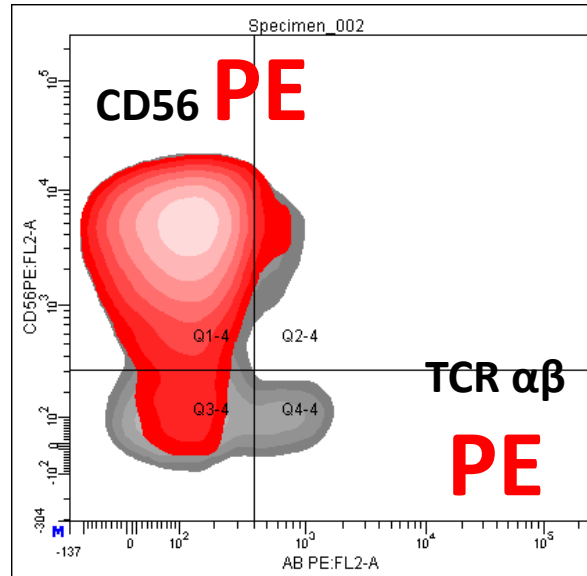
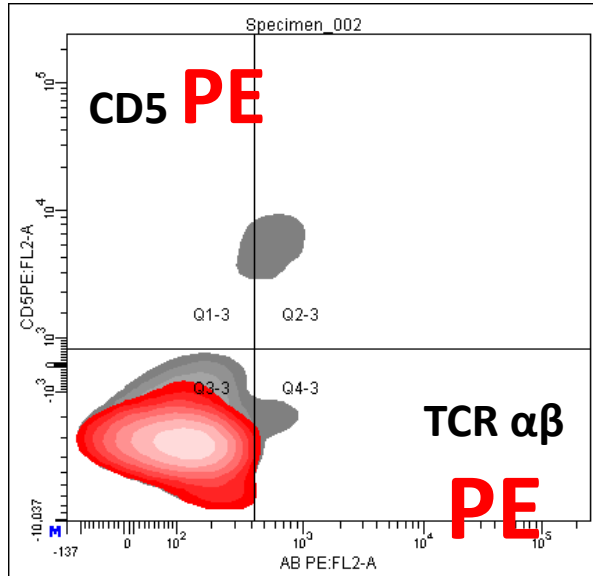
MERGING'S RESULTS



MERGING'S RESULTS



MERGING'S RESULTS



MAIN METHODOLOGICAL OBJECTIONS

STATISTICAL

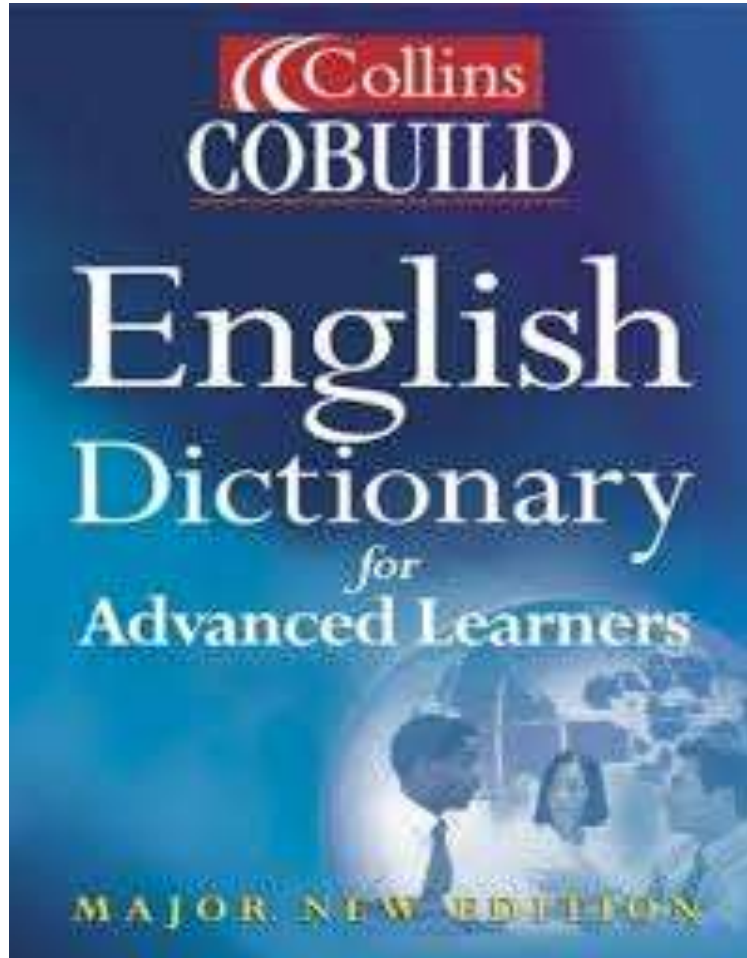
- The algorithms used to find the closest cell in two data files use the so-called “common Euclidean distance”, which is not the best for data with noise due to Poisson’s distribution

LOGICAL

- There is no mathematical or biological foundation to believe that the precise value of a cell in N dimensions means it is exactly the same cell as another cell with the same N values from another tube

**AND NOW,
WHAT
ABOUT
STRATEGY?**

WHAT IS STRATEGY?



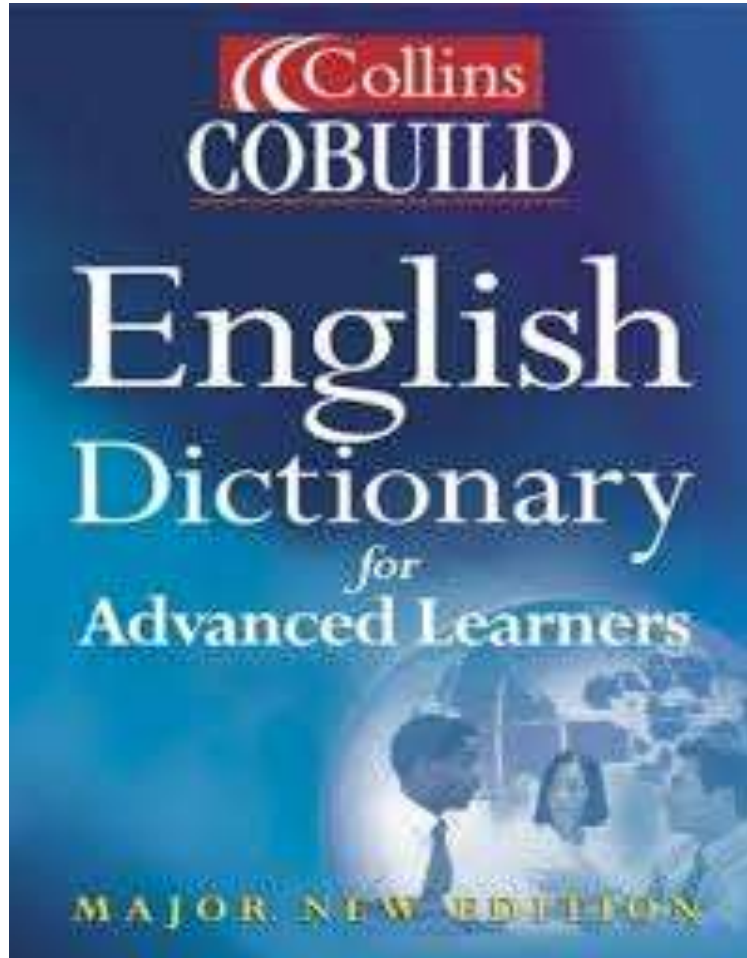
Strategy is a general plan or a set of plans intended to achieve something

What is “something” in our case?

It is diagnosis.

But, strictly speaking, what is
“diagnosis”?

DISAMBIGUATION



Diagnosis is [...] the discovery and naming of what is wrong with people who are ill or with things that do not work properly

WHAT IS WRONG WITH PEOPLE WHO ARE ILL?

- The disease itself
- The persistence of the disease over time
- The presence of the disease in a special compartment
- The resistance of the disease to the treatment
- The unwanted reactions to the therapy
- etc. etc.

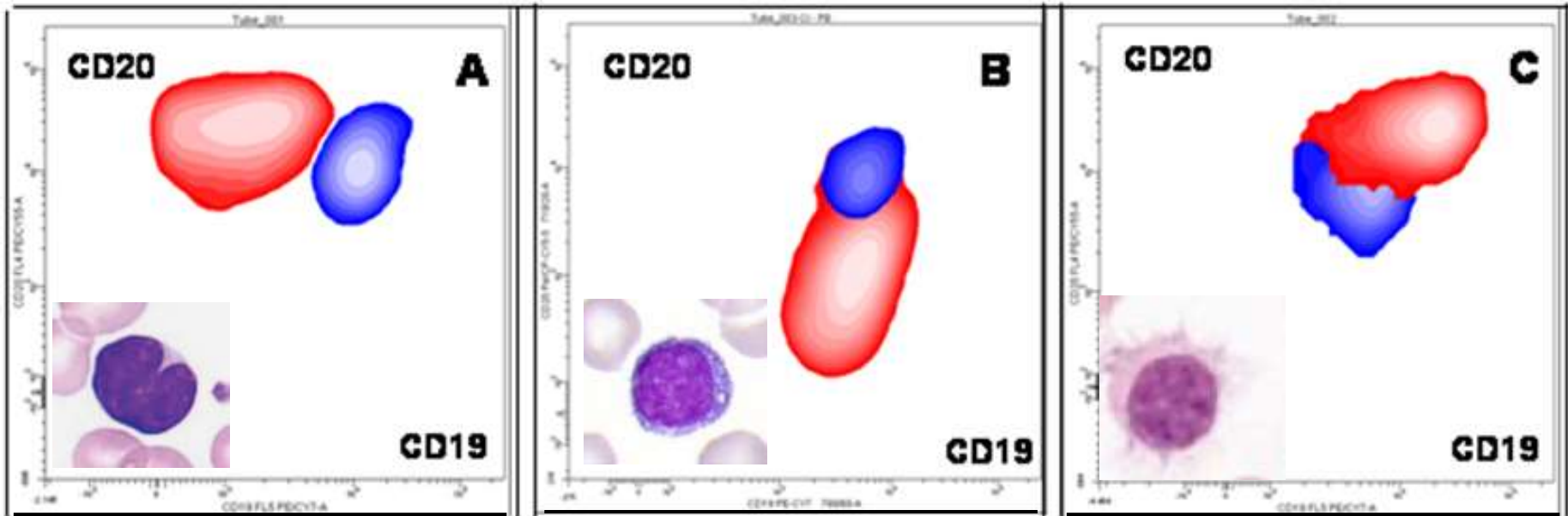
AND WHICH ARE OUR STRATEGIES TO GET BETTER CYTOMETRIC “DIAGNOSES”?

- Remembering that every cytometric diagnosis is an **image-driven diagnostic procedure**

THIS IS AN IMAGE DRIVEN DIAGNOSTIC PROCEDURE



THIS ALSO IS AN IMAGE DRIVEN DIAGNOSTIC PROCEDURE!



FCL

B-CLL

HCL

TYPICAL DISEASE-RELATED BEHAVIOUR OF THE EXPRESSION OF B-SPECIFIC ANTIGENS
FCL: FOLLICULAR LYMPHOMA; B-CLL: CHRONIC LYMPHATIC LEUKEMIA; HCL: HAIRY CELL
LEUKEMIA. RED: PATHOLOGICAL CELLS; BLUE: NORMAL RESIDUAL B LYMPHOCYTES

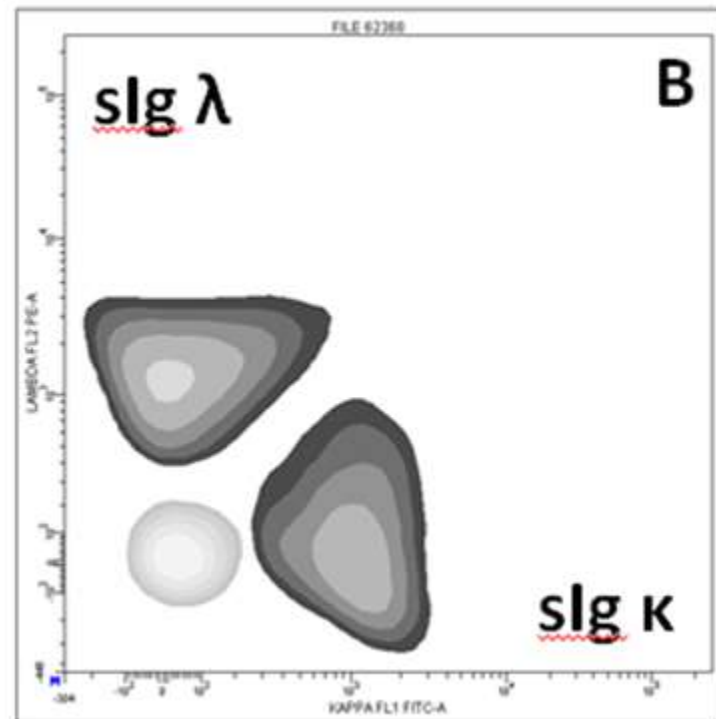
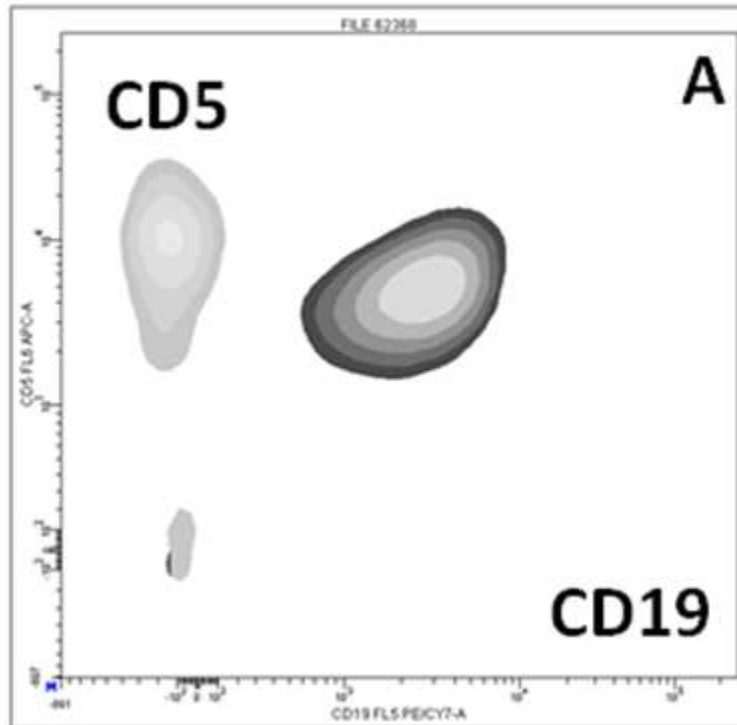
AND WHICH ARE OUR STRATEGIES TO GET BETTER CYTOMETRIC “DIAGNOSES”?

- Exploiting the increased data-set dimensionality **to ameliorate diagnostic accuracy**

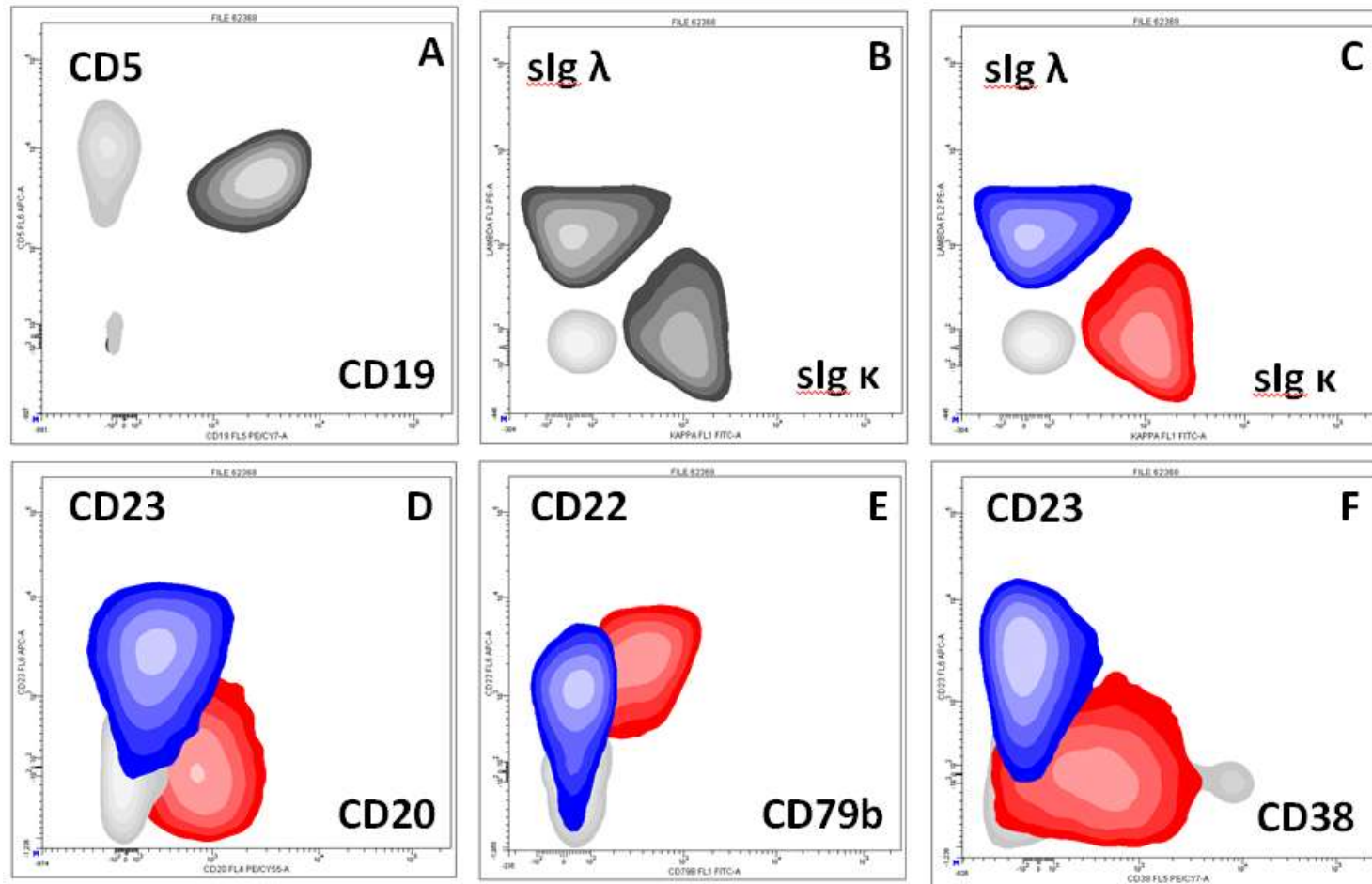
The more complex the analytical process employed, the greater the likelihood that flow cytometry will be able to identify and characterize an abnormal population in a heterogeneous sample.

Borowitz, 1997

THINGS ARE NOT ALWAYS AS THEY LOOK LIKE...



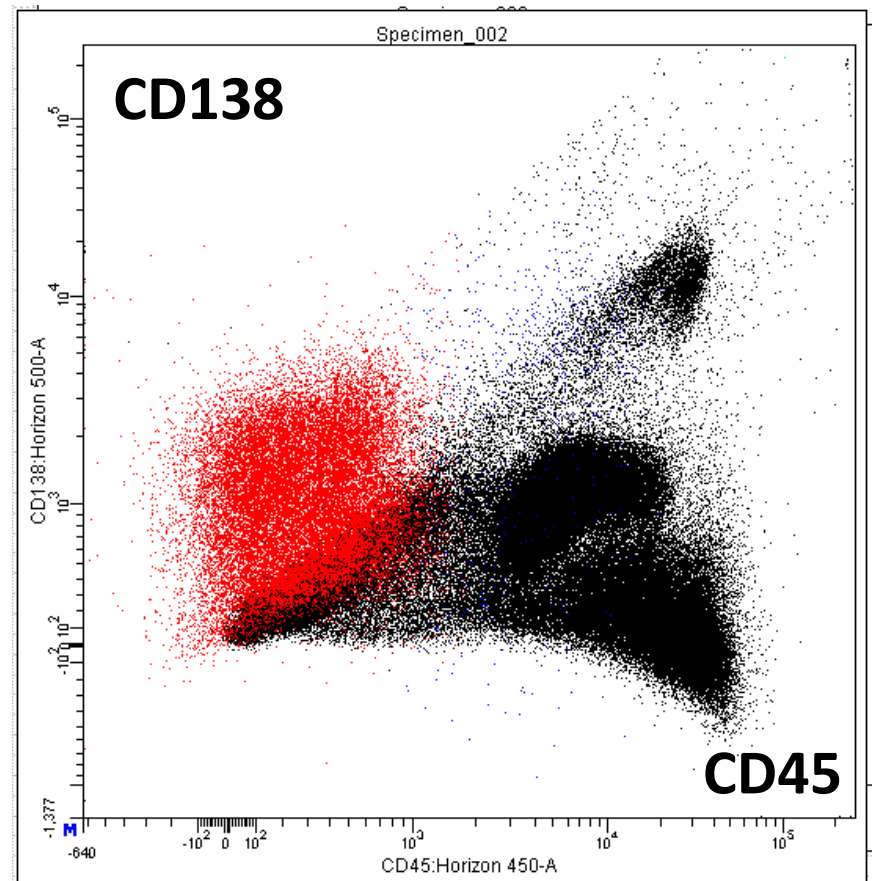
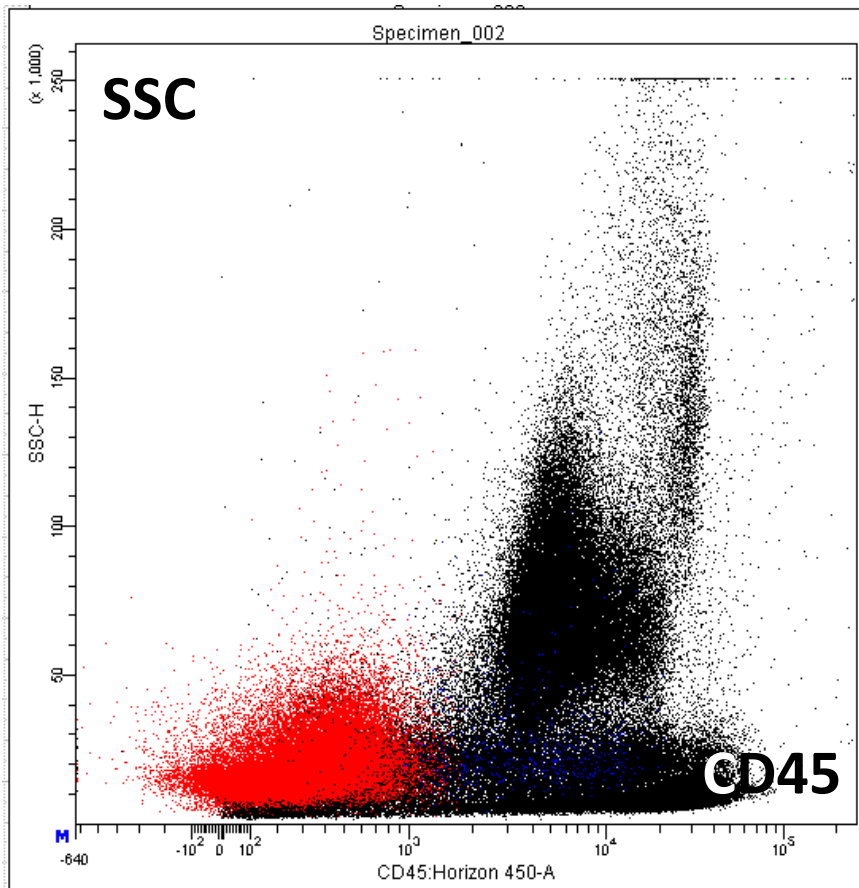
A CASE OF COMPOSITE LYMPHOMA



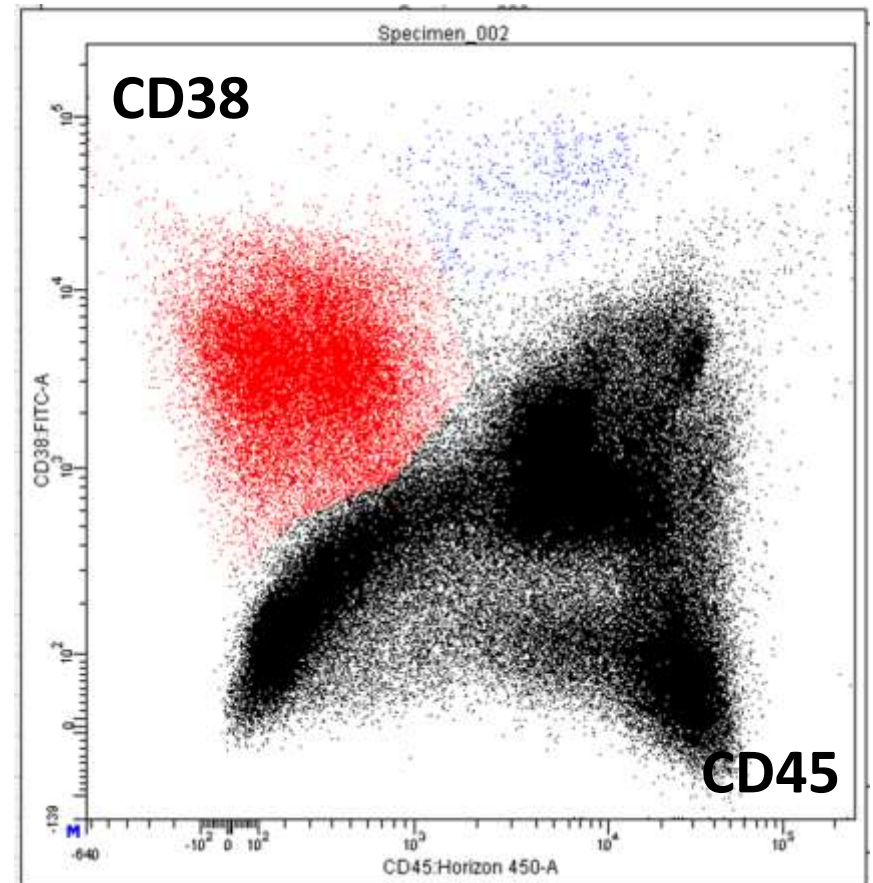
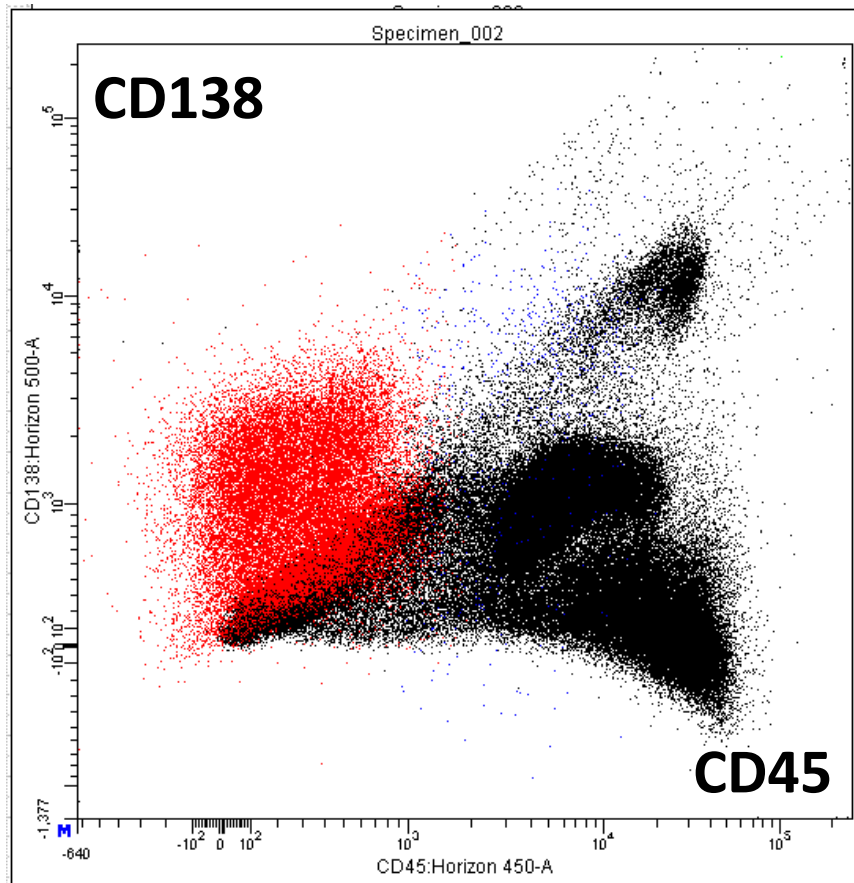
AND WHICH ARE OUR STRATEGIES TO GET BETTER CYTOMETRIC “DIAGNOSES”?

- Exploiting the increased data-set dimensionality **to ameliorate sensitivity**

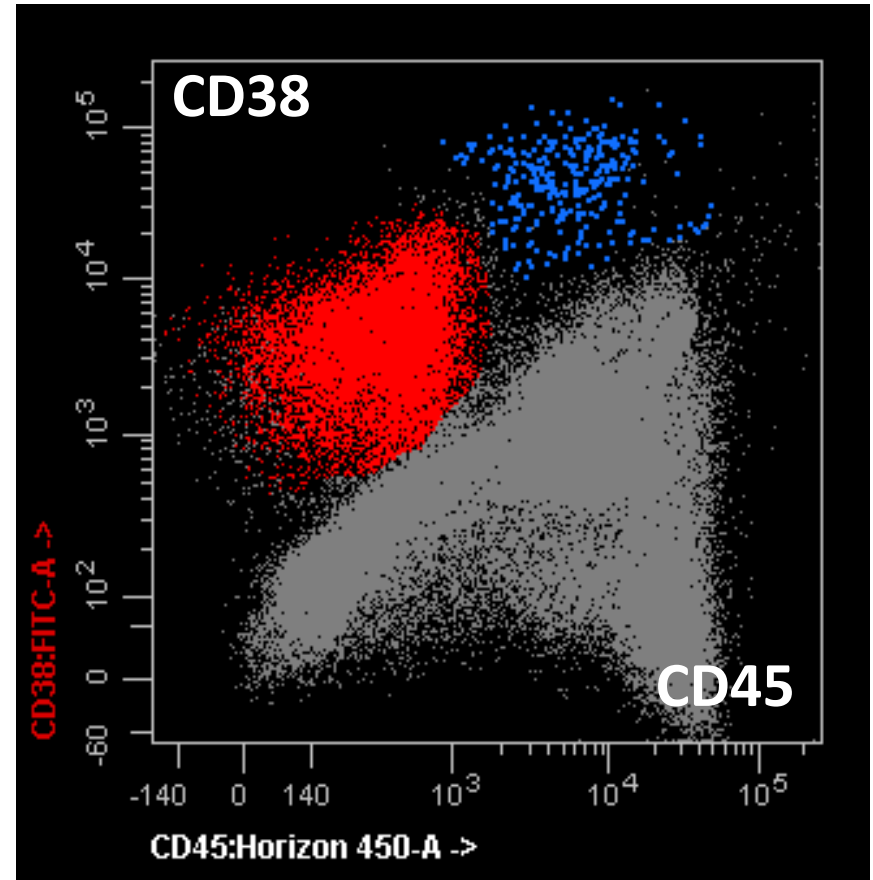
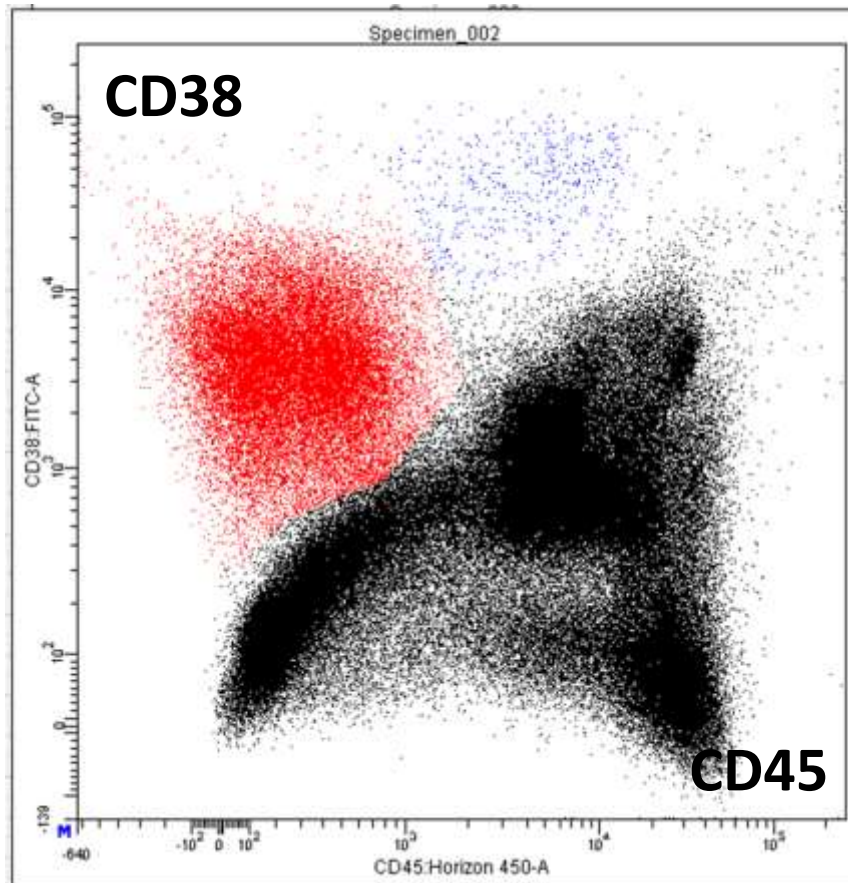
A TWELVE COLOR ANALYSIS OF A MINOR PLASMA CELL SUBSET



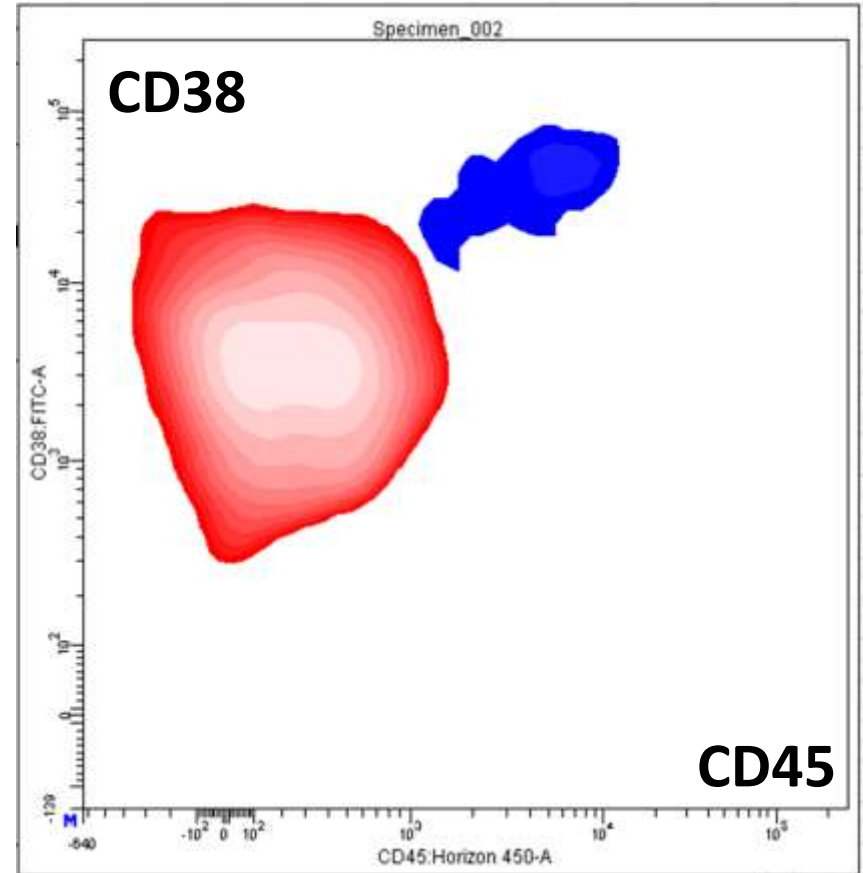
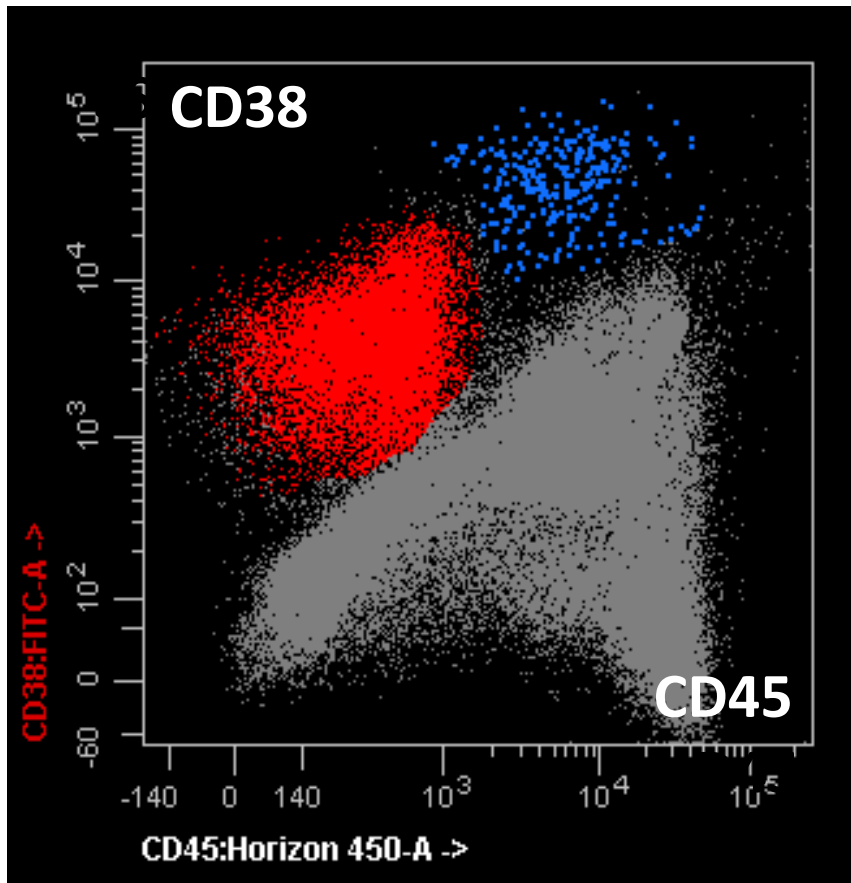
A TWELVE COLOR ANALYSIS OF A MINOR PLASMA CELL SUBSET



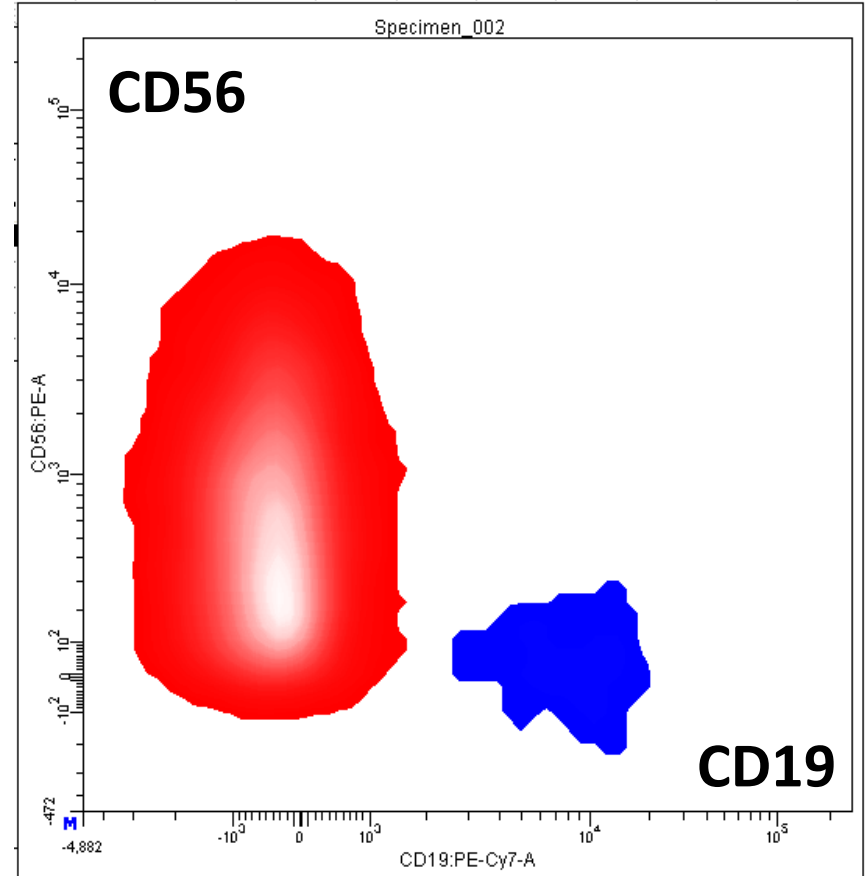
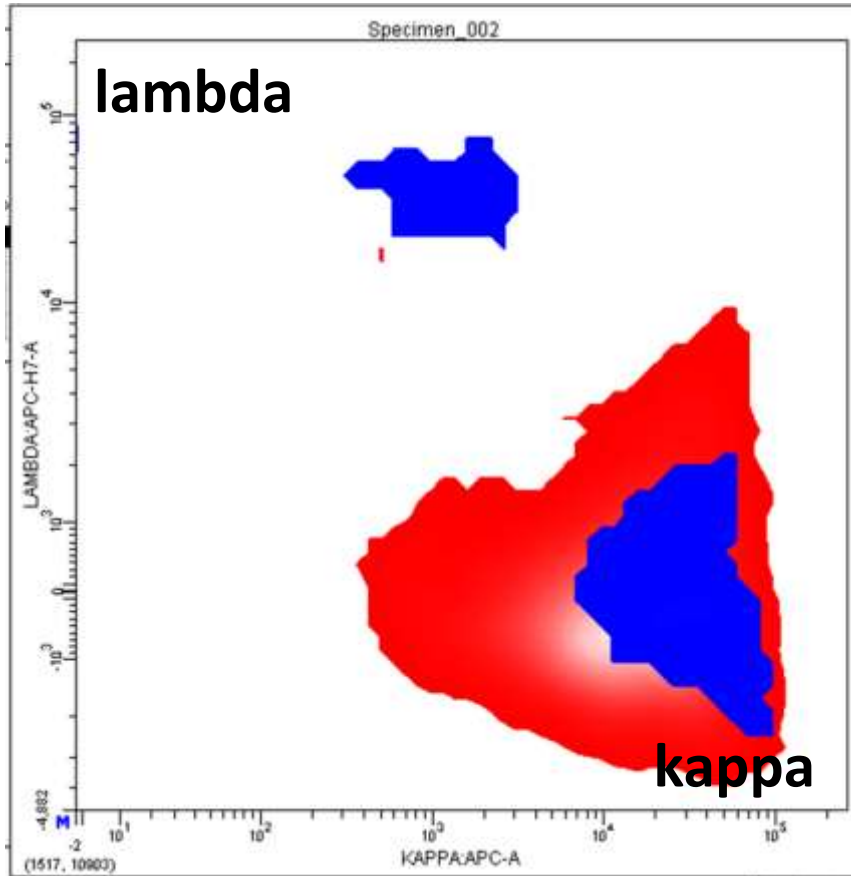
A TWELVE COLOR ANALYSIS OF A MINOR PLASMA CELL SUBSET (0.3%)



A TWELVE COLOR ANALYSIS OF A MINOR PLASMA CELL SUBSET (0.3%)



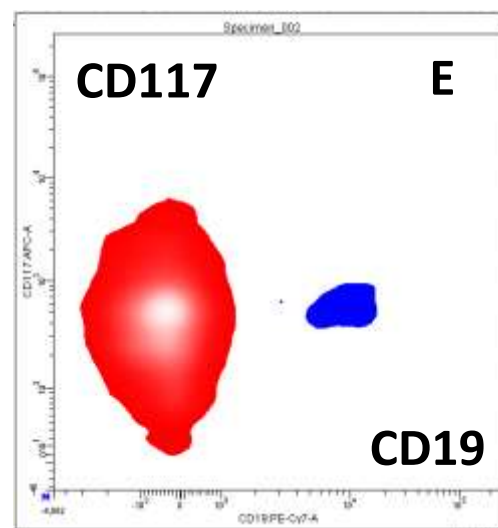
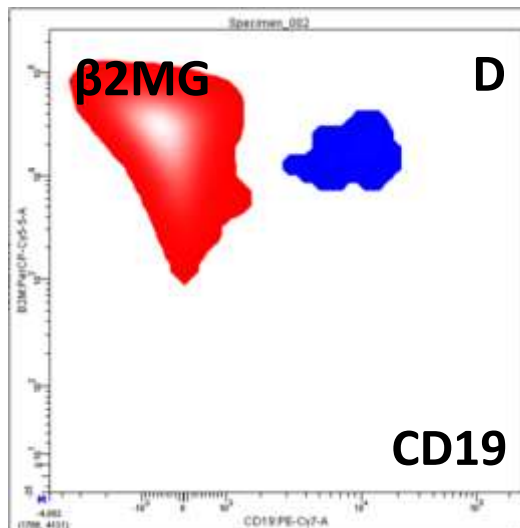
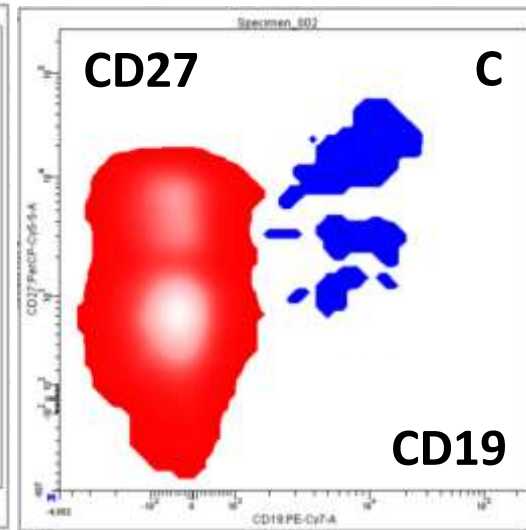
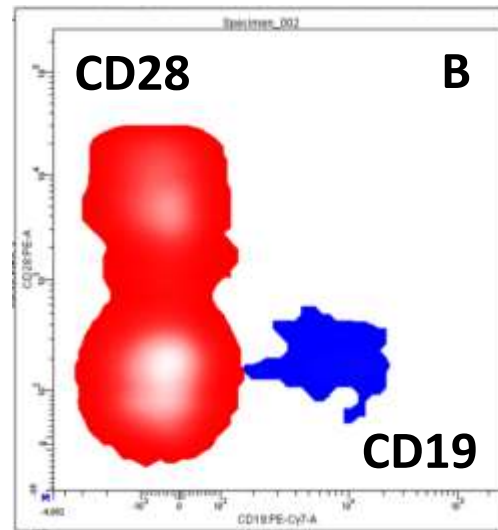
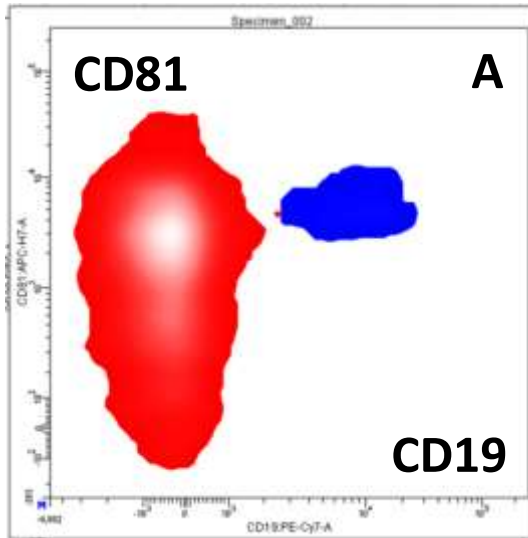
A TWELVE COLOR ANALYSIS OF A MINOR PLASMA CELL SUBSET (0.3%)



ANTIGENS WITH A PROGNOSTIC MEANING IN MULTIPLE MYELOMA

- **CD27:** Low CD27 expression in plasma cell dyscrasias correlates with high-risk disease
- **CD28:** CD28 expression correlates with tumor progression
- **CD81:** CD81 expression correlates with adverse prognosis
- **CD117:** CD117 expression is associated with a favorable outcome
- **β2-MG:** B2-microglobulin expression is significantly higher in clonal PC as compared to normal PC

ANTIGENS WITH A PROGNOSTIC MEANING IN MULTIPLE MYELOMA



A: CD81+

B: CD28 bimodal

C: CD27 bimodal

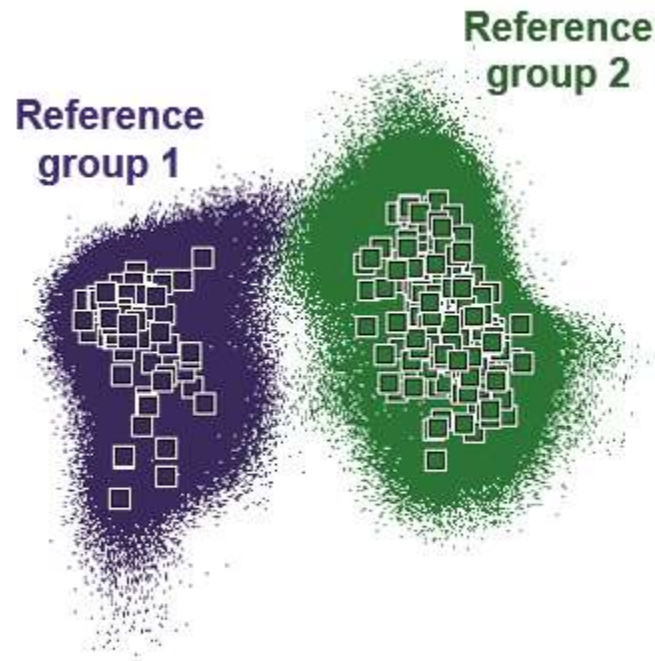
D: β 2MG bright

E: CD117 negative

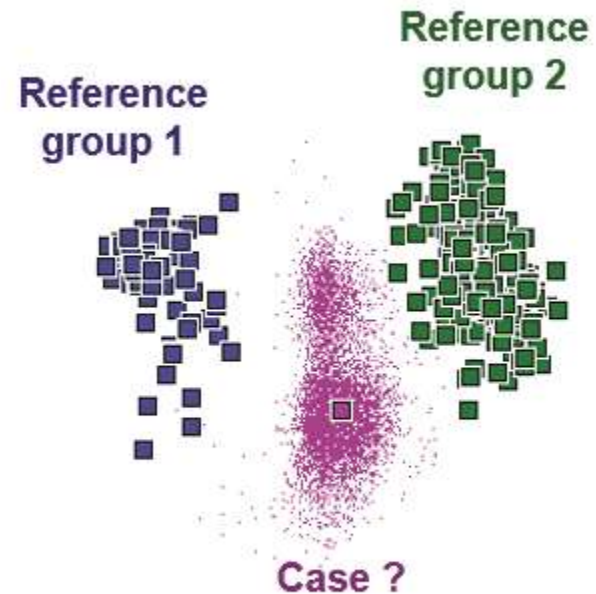
AND WHICH ARE OUR STRATEGIES TO GET BETTER CYTOMETRIC “DIAGNOSES”?

- Exploiting the increased data-set dimensionality **to allow better data analysis**
 - Comparing by PCA the behavior of a subset of yours with the behavior of a certified one
 - Feeding unsupervised algorithms
 - Applying a boolean approach to your data

COMPARING THE BEHAVIOR



APS 1



APS 1

FEEDING UNSUPERVISED ALGORITHMS

SUBJECTS

Microsoft Excel - DATASET8.xls

File Modifica Visualizza Inserisci Formato Strumenti Dati Finestra

Digitare una comando.

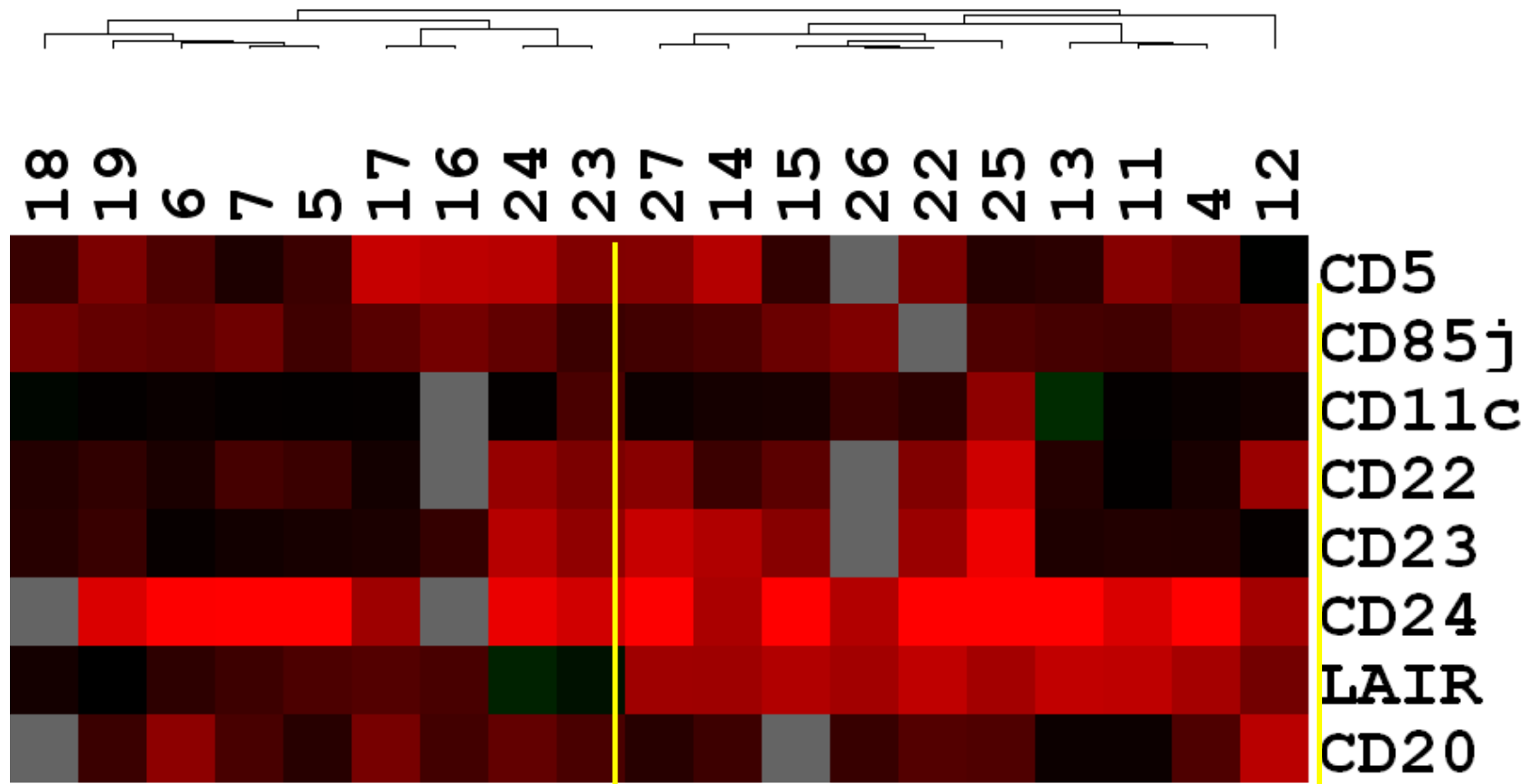
G4 10,4601604278075

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	AG RFI	4	5	6	7	11	12	13	14	15	16	17	18	19	22	23	24	25	26	27
2	CD5	13,55	3,92	5,83	1,97	21,70	0,97	2,72	62,17	3,11	75,76	93,72	3,65	16,94	16,05	19,54	66,28	2,36		20,18
3	CD79b	1,76	1,74	10,12	14,81	4,03		0,88		21,92		2,51	3,47	5,07	4,10	2,42	3,11	4,62		9,10
4	CD85j	7,46	4,28	8,58	12,58	4,46	10,46	4,88	5,52	11,58	14,28	7,43	14,22	9,74		3,82	9,25	5,97	18,39	4,45
5	CD11c	1,27	1,08	1,28	1,10	1,12	1,49	0,37	1,62	1,72		1,10	0,87	1,11	2,74	5,42	1,11	26,27	3,93	1,33
6	CD22	1,76	3,78	1,85	4,89	1,06	34,36	2,33	3,90	8,14		1,55	2,27	3,02	19,24	16,77	31,38	111,82		22,68
7	CD23	2,15	1,70	1,19	1,52	2,21	1,14	2,02	57,03	22,41	3,31	1,87	2,45	3,68	34,79	27,53	64,84	238,21		96,84
8	CD24	921,78	890,82	334,13	545,04	146,09	42,02	1516,74	50,19	686,03		37,99		149,25	467,86	119,72	212,85	643,72	59,96	333,13
9	LAIR	43,86	5,79	2,83	4,13	79,00	13,98	83,69	38,05	58,45	5,26	6,80	1,60	1,00	80,70	0,67	0,45	41,94	40,93	38,36
10	CD28	6,04	2,48	25,28	5,25	1,32	69,69	1,32	4,05		4,55	15,49		3,82	6,80	5,43	9,55	6,17	3,56	2,46
11	LC	17,91		33,48	49,07	64,57	348,98	0,86	12,28	74,36	13,70	33,86	52,82	22,21	190,59		25,05			15,59

Pronto MA NUM

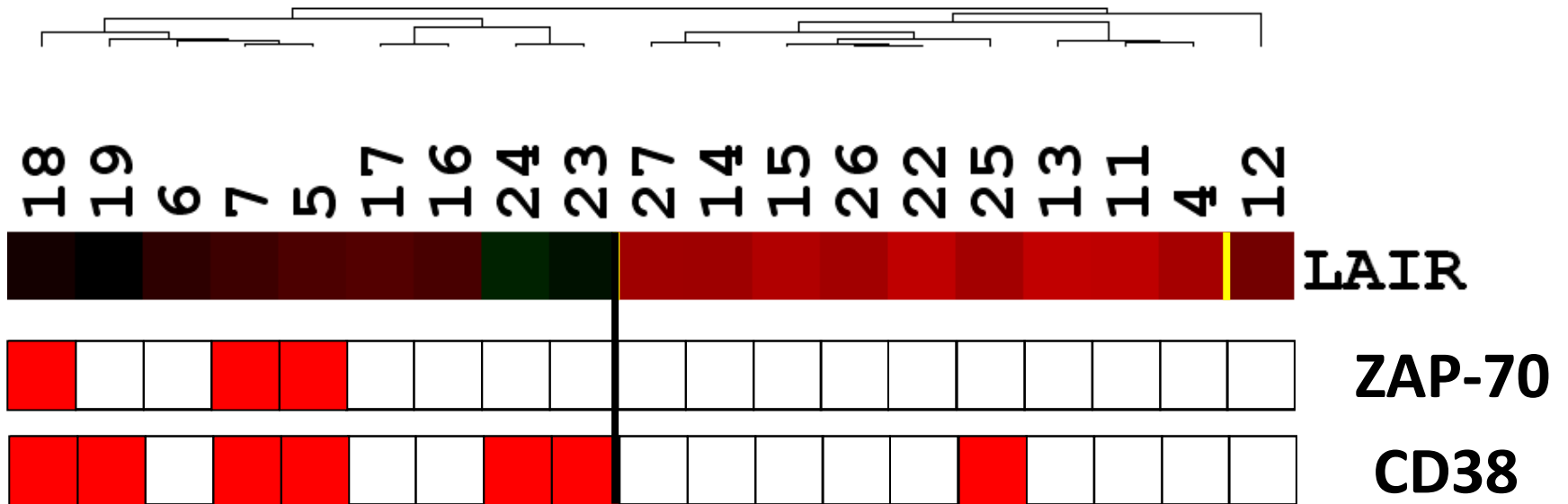
ANTIGENS EXPRESSION (MFI)

RESULTS OF A NOT SUPERVISED CLUSTER ANALYSIS BASED ON MFI OF CD5, CD11c, CD20, CD22, CD23, CD24, CD85j AND LAIR

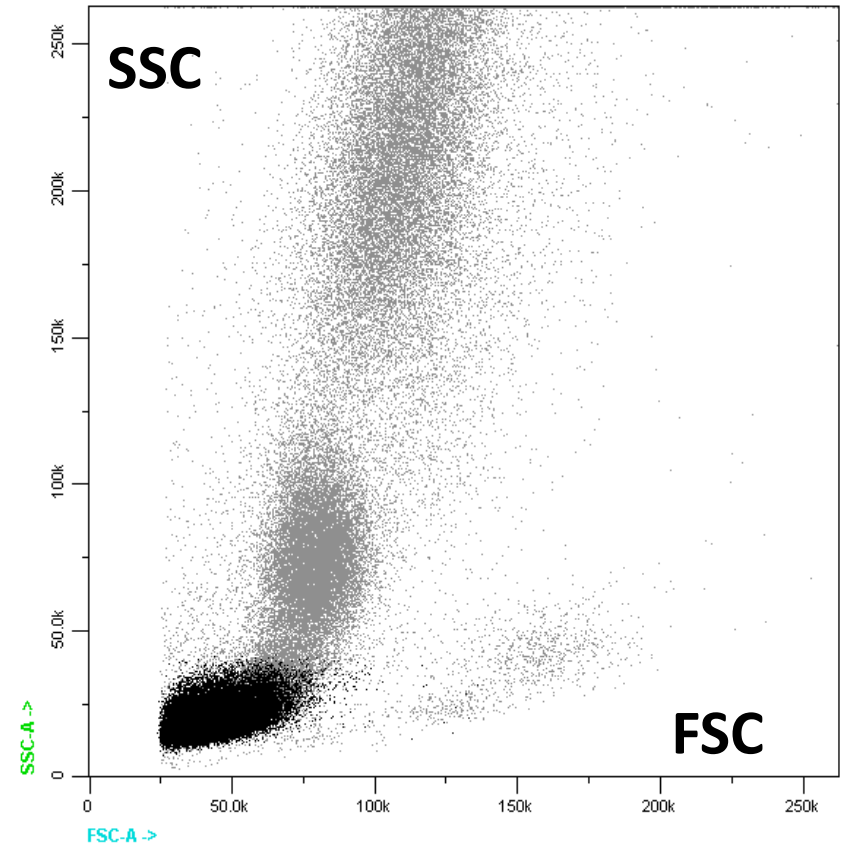
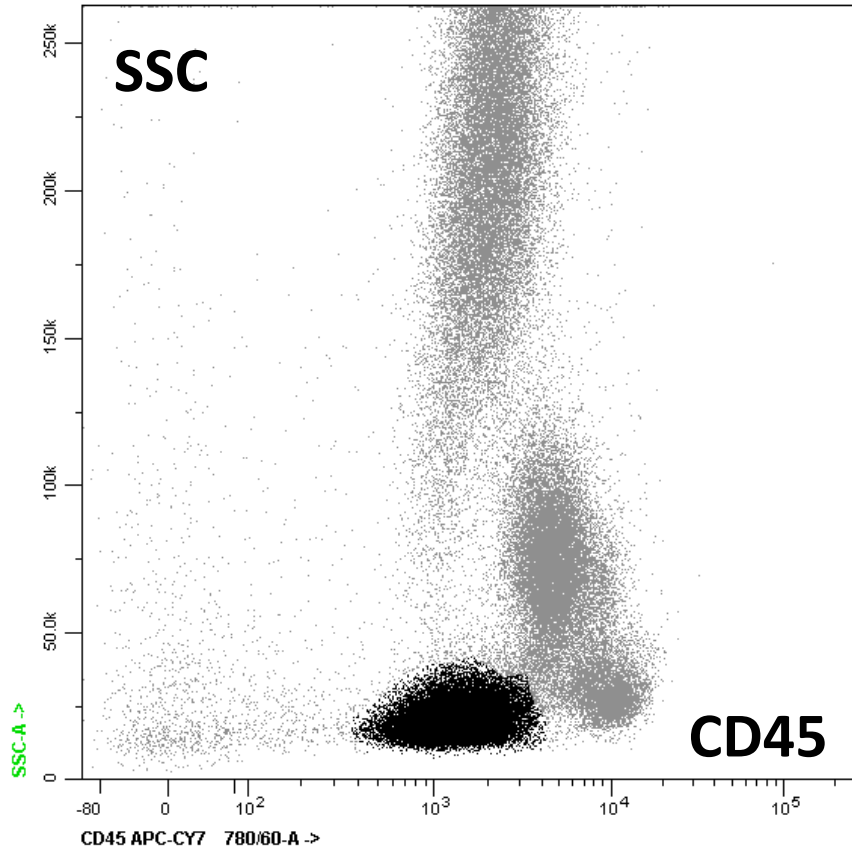


20 B-CLL PATIENTS

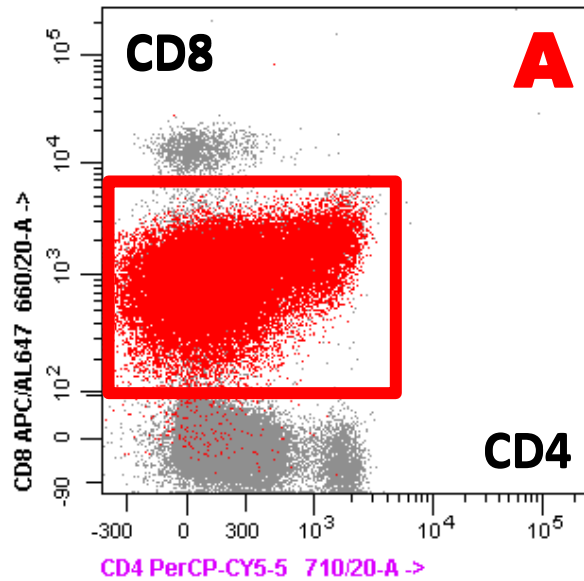
DISTRIBUTION OF TWO GENERALLY ACCEPTED PROGNOSTIC MARKERS IN THE TWO MAIN CLUSTERS



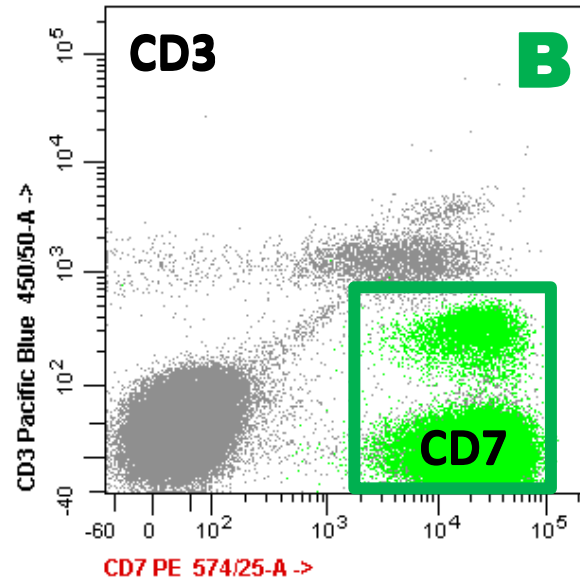
BOOLEAN APPROACH TO MRD IN A T-ALL CASE



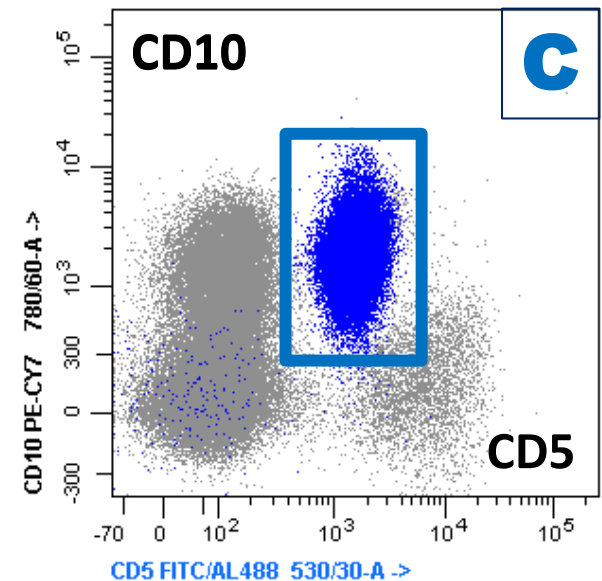
BOOLEAN APPROACH TO MRD IN A T-ALL CASE



**A: CD8 dim, CD4 neg
& CD8 dim, CD4 dim**



**B: CD7 pos, CD3 neg
& CD7 pos, CD3 dim**

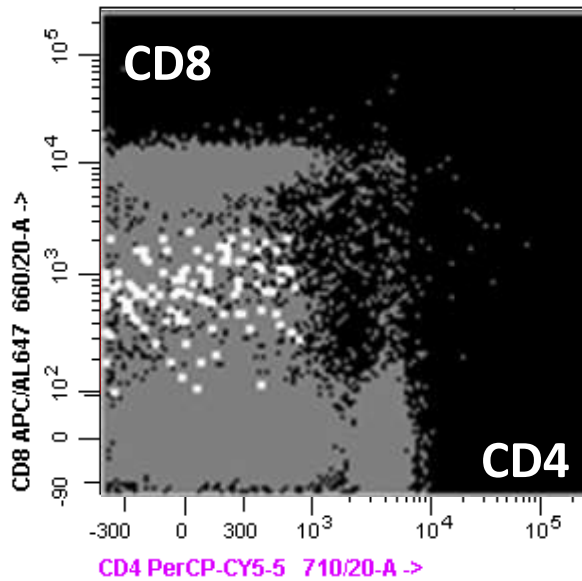


C: CD5 dim, CD10 pos

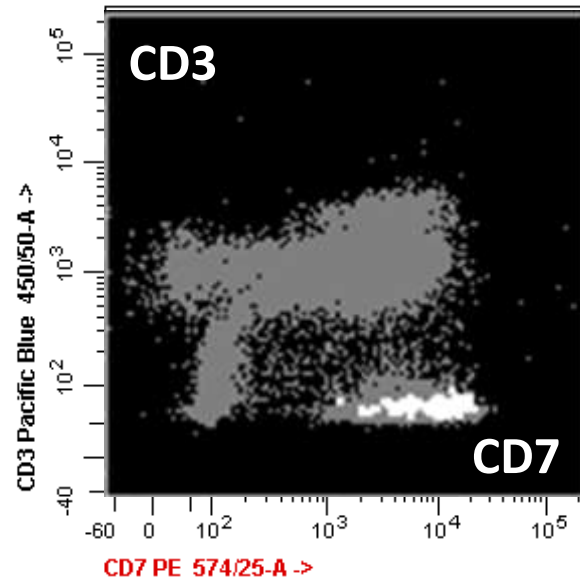
BOOLEAN APPROACH TO MRD IN A T-ALL CASE

A AND **B** AND **C**

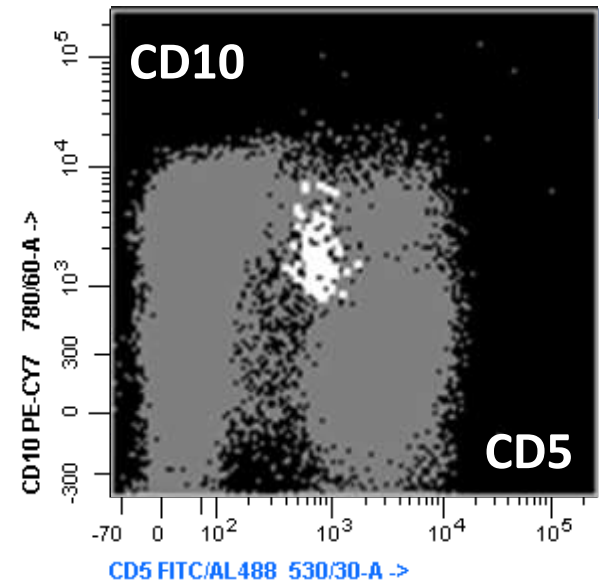
BOOLEAN APPROACH TO MRD IN A T-ALL CASE



**A: CD8 dim, CD4 neg
& CD8 dim, CD4 dim**

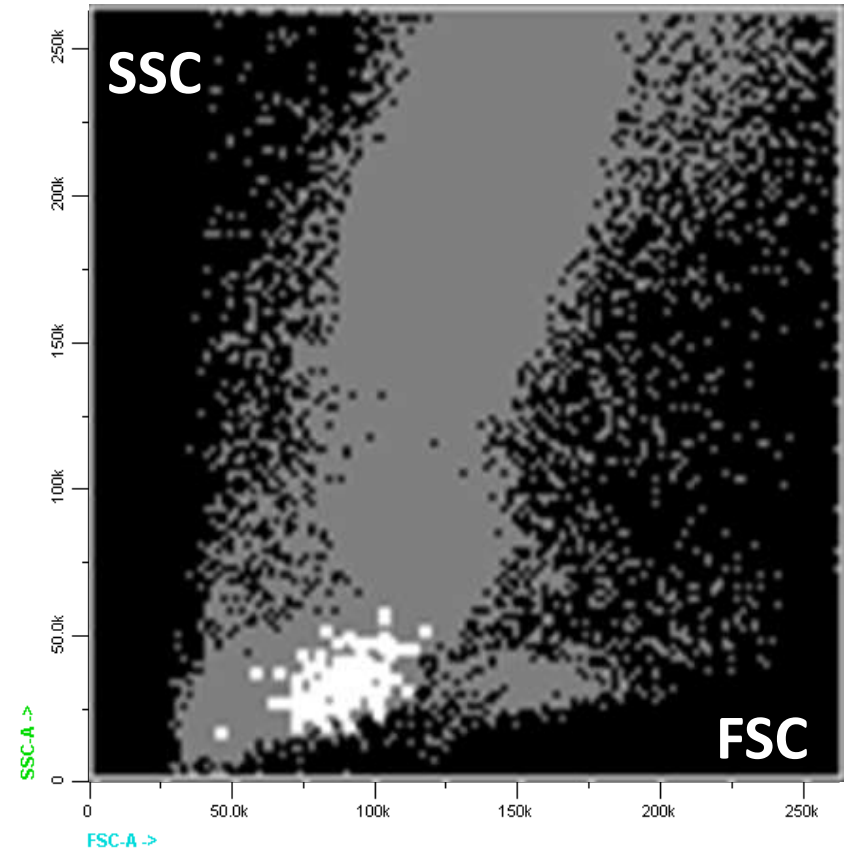
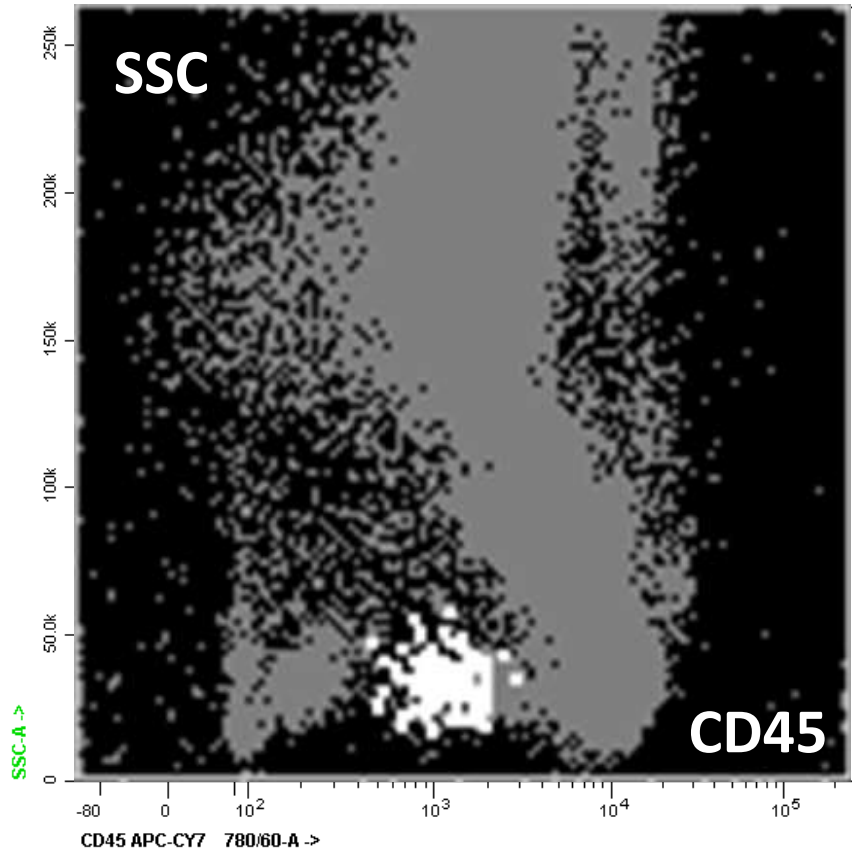


**B: CD7 pos, CD3 neg
& CD7 pos, CD3 dim**



C: CD5 dim, CD10 pos

BOOLEAN APPROACH TO MRD IN A T-ALL CASE



SENSITIVITY

- TOTAL ANALYZED EVENTS: 2×10^6
- ABNORMAL EVENTS FOUND: 116
- FREQUENCY OF ABN. EVENTS: **0,006**

BUT REMEMBER!



POWER IS NOTHING WITHOUT CONTROL

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AND SO, WHICH ARE OUR **FINAL** STRATEGIES TO KEEP THIS POWER UNDER OUR CONTROL??

- Promoting
 - Harmonization
 - Standardization
 - Creation of guidelines
 - Compliance with guidelines
 - Education

IN A WORD: QUALITY

*"...there is no definition...of quality
...you know it when you find it!"*

Robert Pirsig (1974)

Zen and the Art of Motorcycle Maintenance



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ΕΥΧΑΡΙΣΤΩ