Ejemplos prácticos de la estrategia de calibración: reproductibilidad entre los grupos EuroFlow

Departamento de Medicina, Centro de Investigación del Cáncer y Servicio de Citometría.
Universidad de Salamanca, Salamanca, España.
EuroFlow™ Consortium

Curso avanzado de Actualización en Onco Hematología. Buenos Aires, Mayo, 30 a Junio, 1, 2011

Results of synchronized experiments

- **Synchronized data acquisition in 8 centers**

Antibody combination:

PacB	PacO	FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7
CD20	CD45	CD8	CD27	CD4	CD19	CD14	CD3

- **Number of samples tested per center:**
  - 1 stabilized blood tested in all centers
  - 3-4 healthy donors locally recruited

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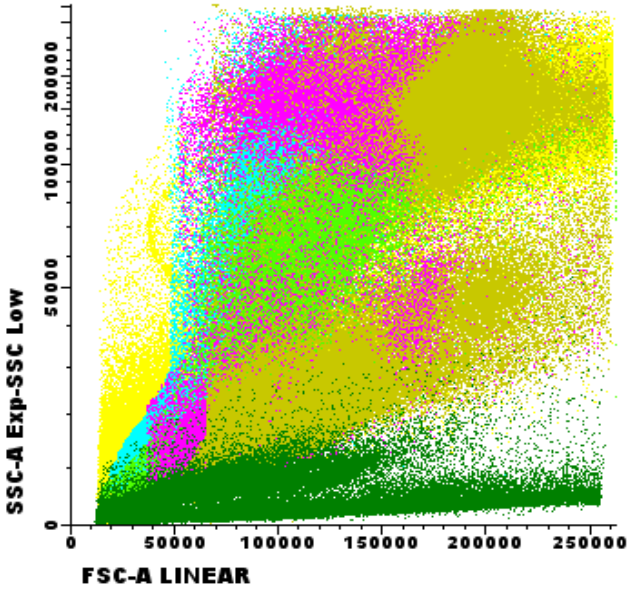
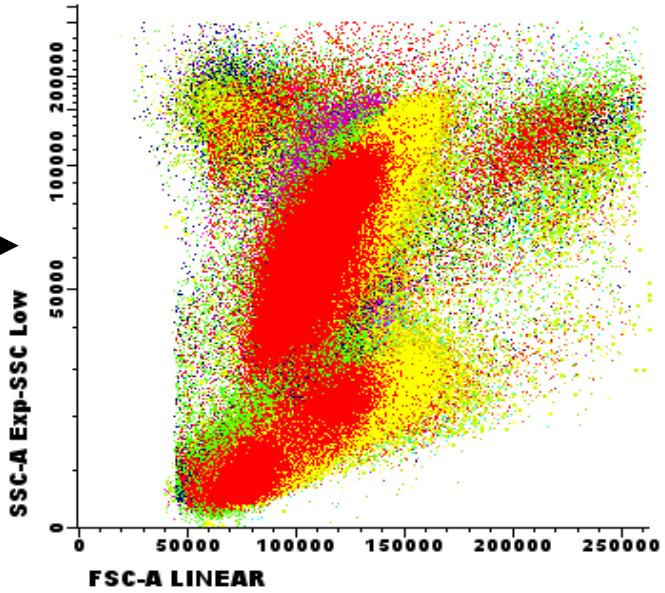
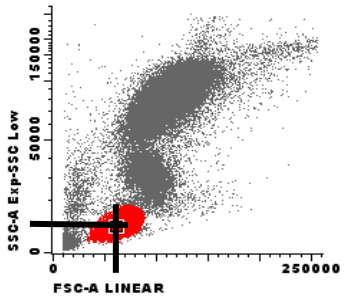
Synchronized light scatter experiments

"Local" settings



EuroFlow settings





7 different normal PB samples acquired in 7 different centers

Normal PB samples processed according to EuroFlow sample preparation protocol

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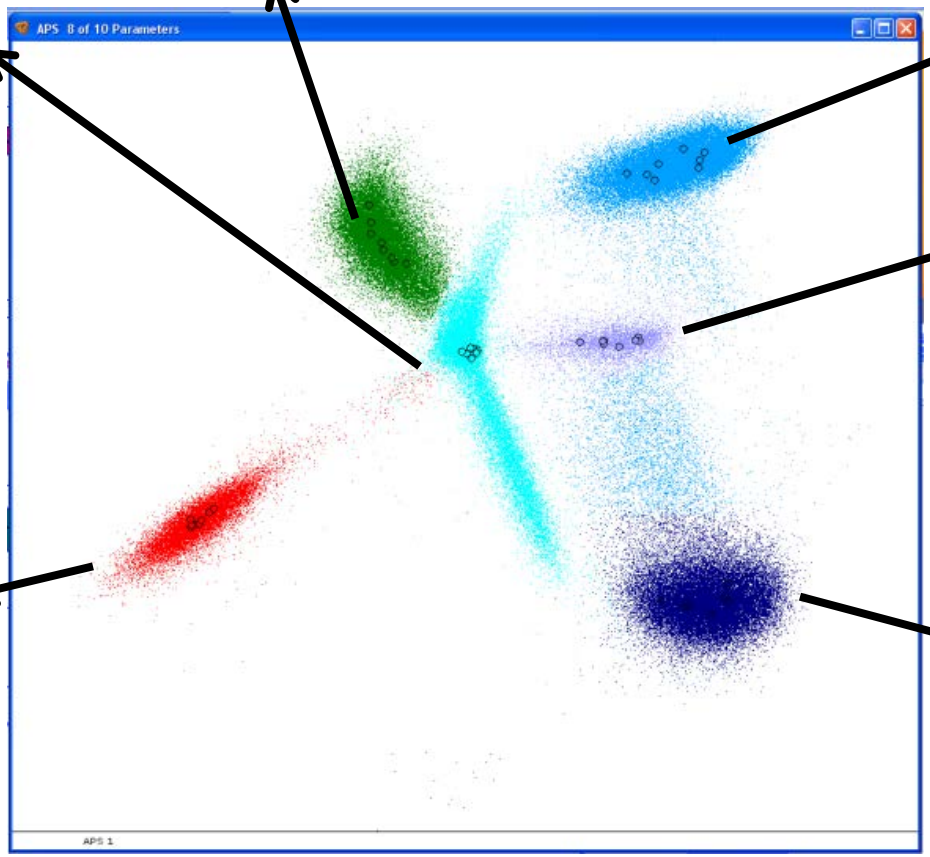
Results of synchronized experiments

APS view of 8 merged data files from different centers (n=8)



CD14+ Monocytes

CD8+ CD3- T cells

CD3+ CD4+ T-cells

CD3+ CD8- CD4- T-cells

CD19+ CD20+ B-cells

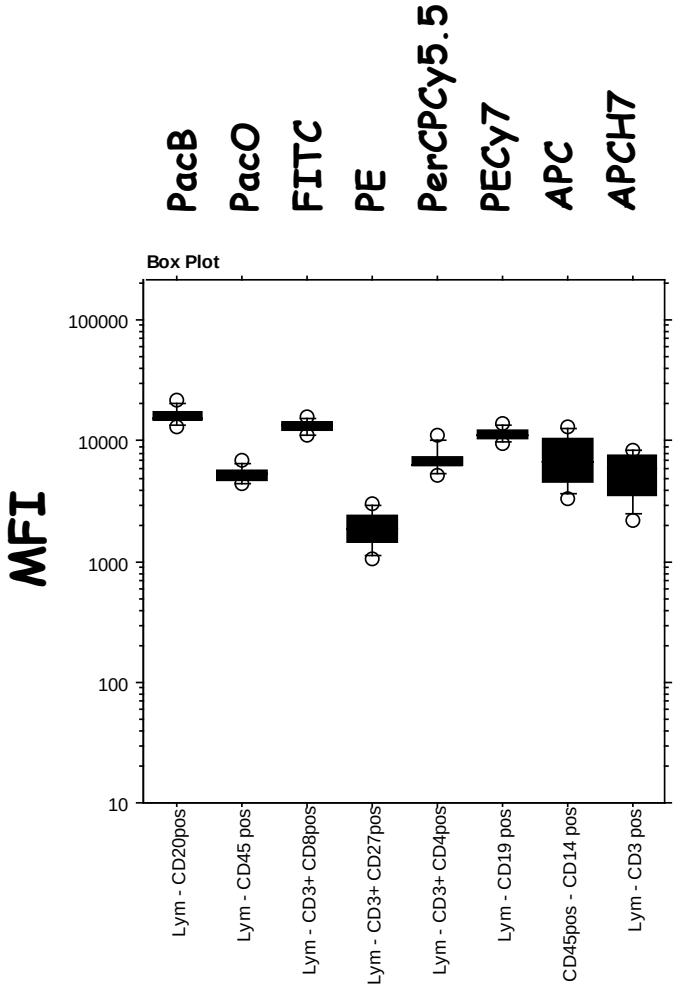
CD3+ CD8+ T cells

Stabilized normal PB

EuroFlow

Results of synchronized experiments

Stabilized donor



Interlaboratory MFI CV of gated cell subsets

Cell subset	CV
Lym - CD20pos	15,25%
Lym - CD45 pos	13,90%
Lym - CD19 pos	11,13%
Lym - CD3 pos	38,67%
CD45pos - CD14 pos	43,77%
Lym - CD3+ CD4pos	24,68%
Lym - CD3+ CD8pos	11,35%
Lym - CD3+ CD27pos	32,09%

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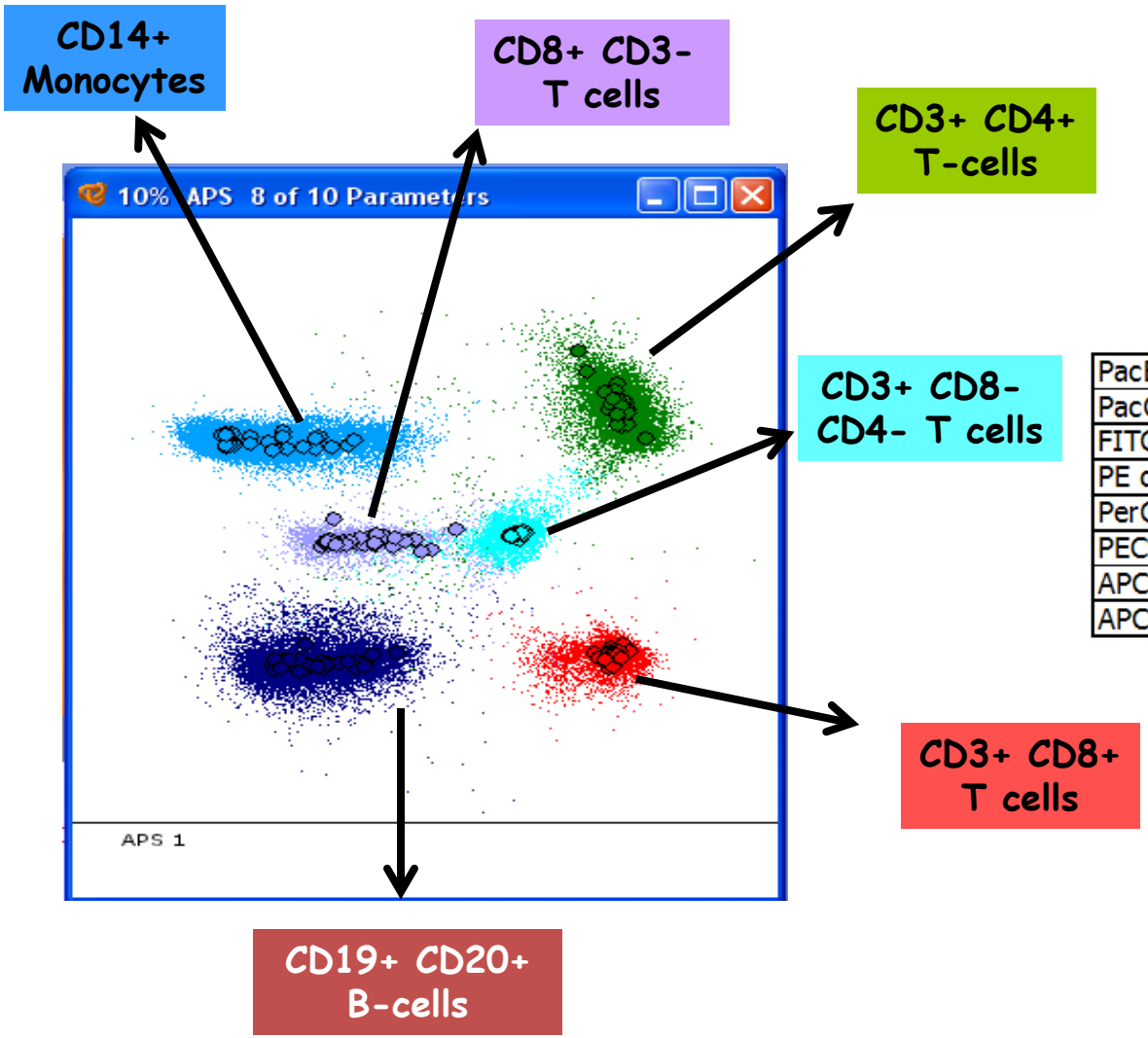
Results of synchronized experiments

APS view of 30 merged data files from different centers



Local normal donors

CV MFI subset

PacBlue channel	Lym - CD20pos	16,90%
PacOr channel	Lym - CD45 pos	15,52%
FITC channel	Lym - CD3+ CD8pos	16,93%
PE channel	Lym - CD3+ CD27pos	27,95%
PerCPCy55 chann	Lym - CD3+ CD4pos	28,39%
PECy7 channel	Lym - CD19 pos	15,40%
APC channel	CD45pos - CD14 pos	22,68%
APC-H7 channel	Lym - CD3 pos	**48,35%**

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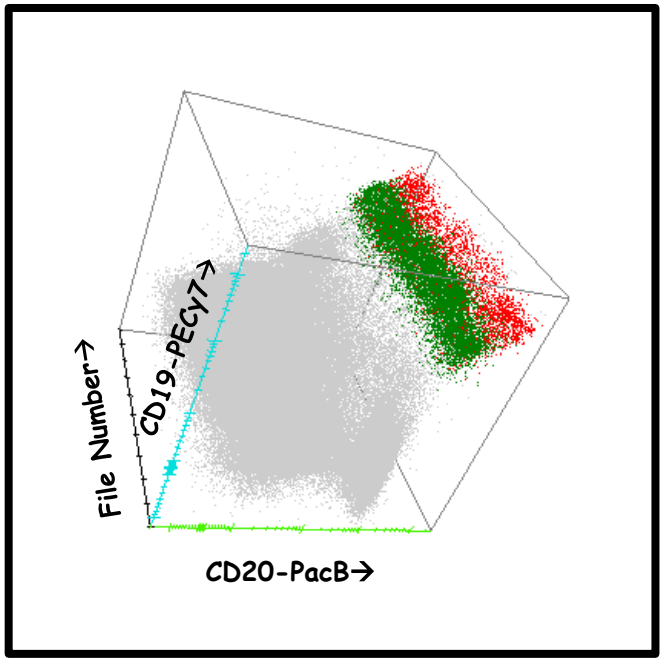
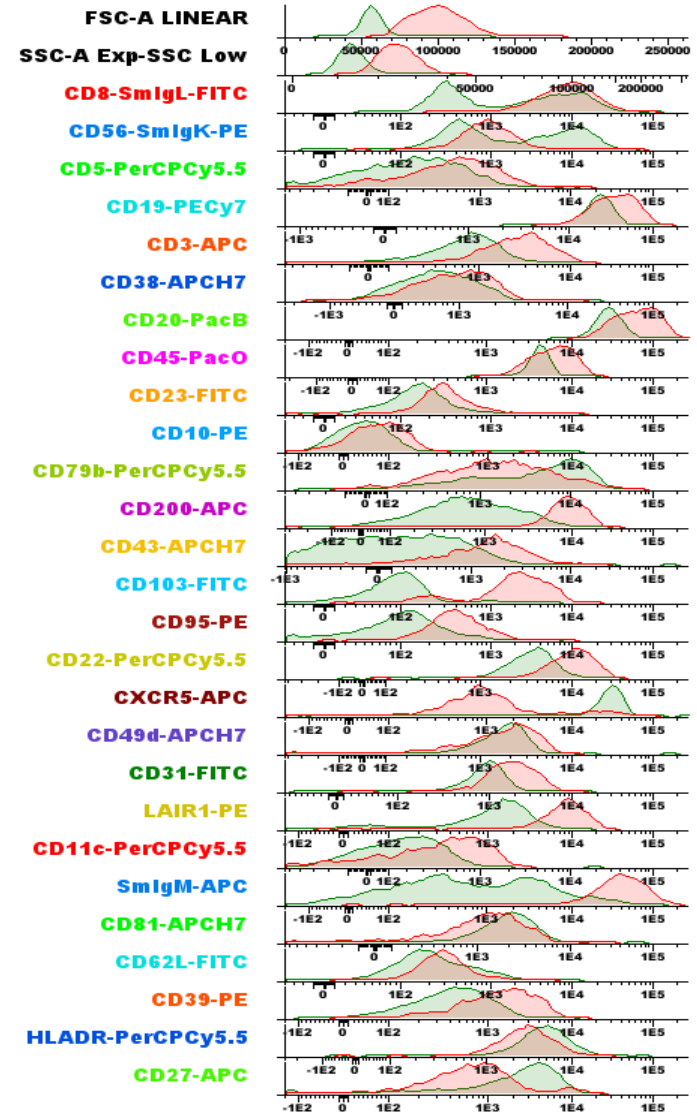
MERGED DATA FILES FOR SINGLE STEP GATING

Full phenotypic profile





A single gating step for 5 different data files (tubes)

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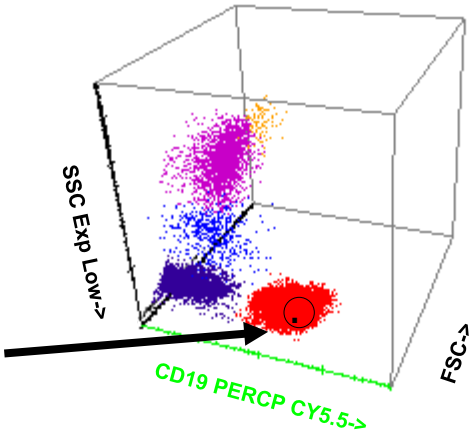
DATA CALCULATION IN THE INFINICYT™ SOFTWARE

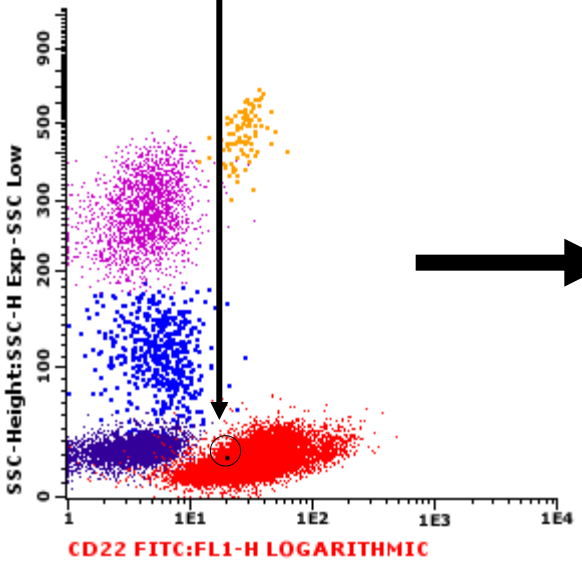
Tube 1



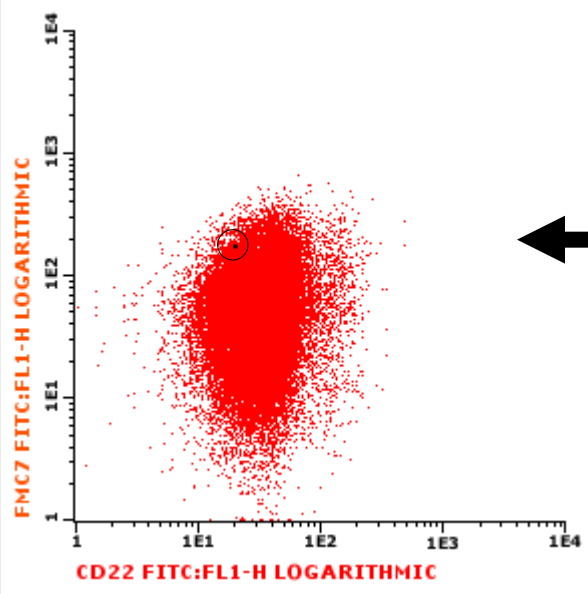
Tube 2

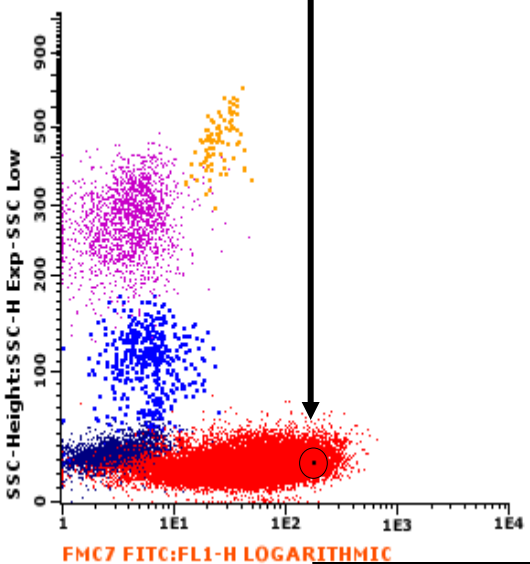
Event X in tube 1

Event Y: Nearest neighbour of event X in tube 2



Calculated FMC7 value for event X in tube 1 (based on data from event Y in tube 2)





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MERGED & CALCULATED LISTMODE DATA FILE

Files

Parameters

Parameters\File	1	2	3	4	5	6
FSC-A	C	C	C	C	C	C
SSC-A	C	C	C	C	C	C
KAPPA:FITC-A	R	E	E	E	E	E
LAI	R	E	E	E	E	E
CD	R	R	R	R	R	E
CD	C	C	C	C	C	C
IgM	R	E	E	E	E	E
CD	C	C	C	C	C	C
CD	C	C	C	C	C	C
CD45-PO:AmCyan-A	C	C	C	C	C	C
CD103:FITC-A	E	R	E	E	E	E
CD10:PE-A	E	R	E	E	E	E
CD43:APC-A	E	R	E	E	E	E
CD81:FITC-A	E	E	R	E	E	E
CD79b:PE-A	E	E	R	E	E	E
CD2	E	E	R	E	E	E
CD3	E	E	E	R	E	E
CD6	E	E	E	R	E	E
CXC	E	E	E	R	E	E
CD2	E	E	E	E	R	E
LAIP	E	E	E	E	R	E
CD11a:APC-A	E	E	E	E	R	E
CD38:FITC-A	E	E	E	E	E	R
CD25:PE-A	E	E	E	E	E	R
CD138:PerCP-Cy5-5-A	E	E	E	E	E	R

6 Files x

10 parameters x

100.000 events

1 File x

25 parameters x

600.000 events

Parameter coding

- C = Common
- R = Measured
- E = Calculated

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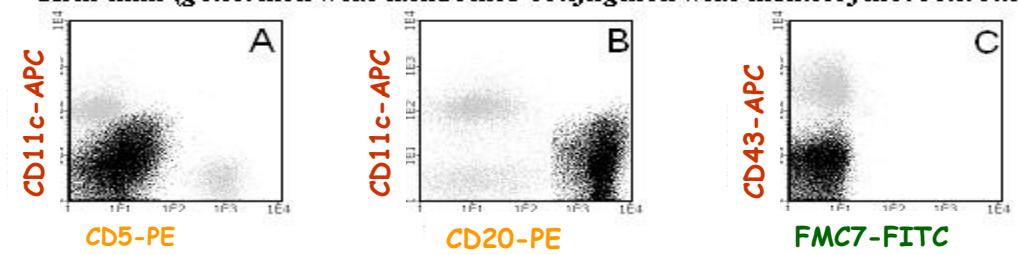
CALCULATED VS MEASURED DATA

FIGURE 4

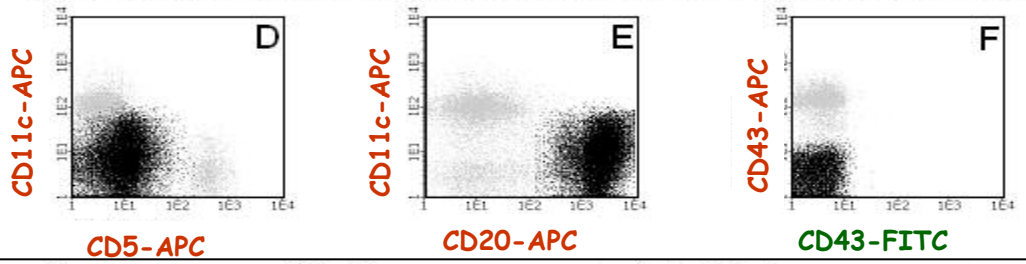
Measured

Real data (generated with antibodies conjugated with distinct fluorochrome)



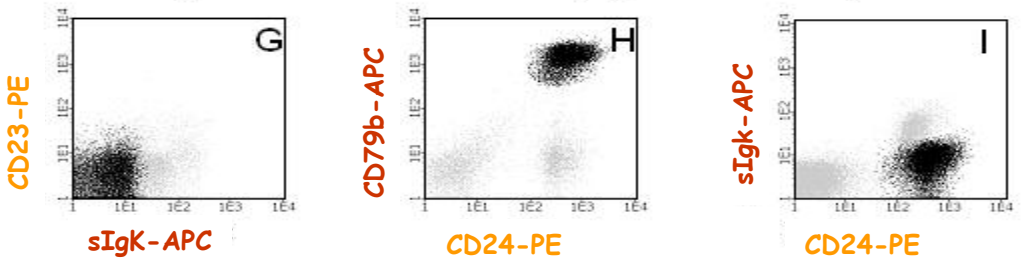
Calculated

Calculated data (Originally impossible 2-dimensional dot-plot representations)



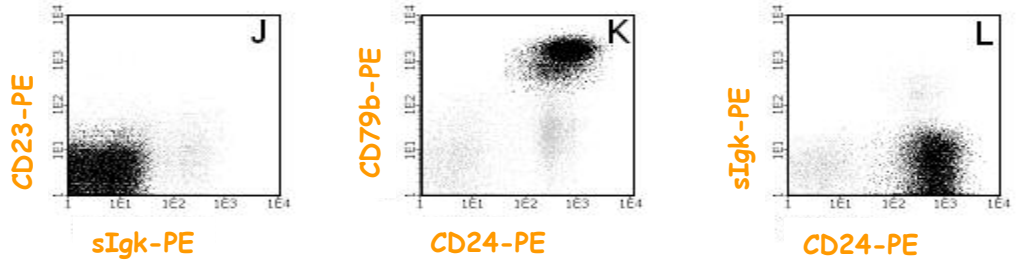
Measured

Real data (generated with antibodies conjugated with distinct fluorochrome)



Calculated

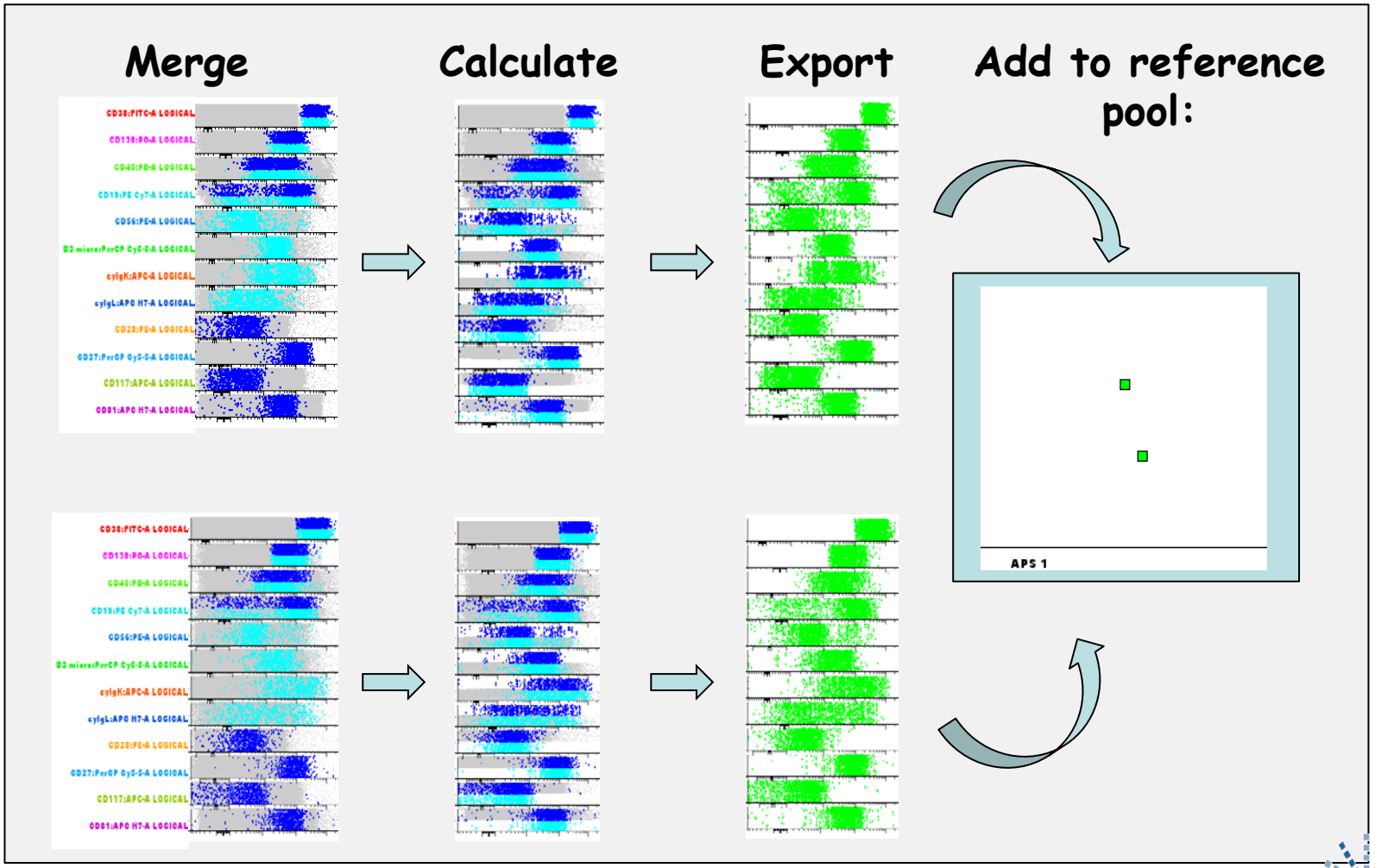
Calculated data (Originally impossible 2-dimensional dot-plot representations)



Pedreira et al, Cytometry A, 2008

Generation of reference data files

PCD panel example, normal-PC populations reference pool



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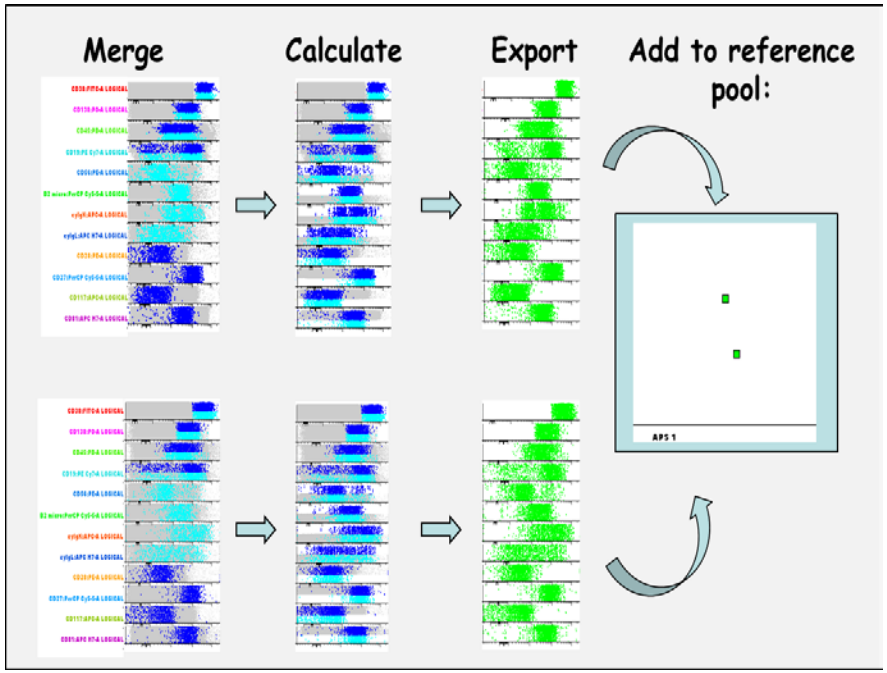
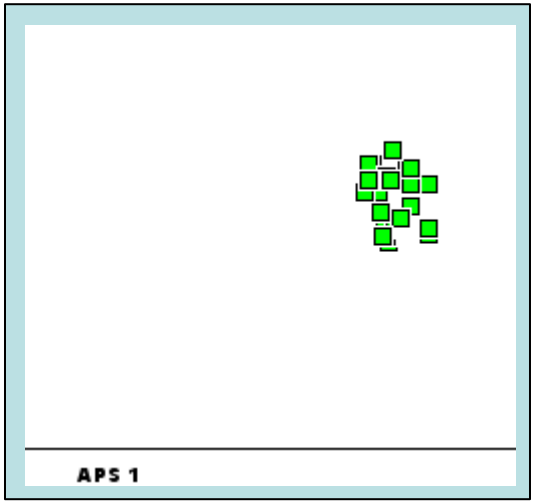
Generation of reference data files

PCD panel example, normal-PC populations reference pool



x n



Normal BM PC from 24 samples from different centres, acquired over a one year period.

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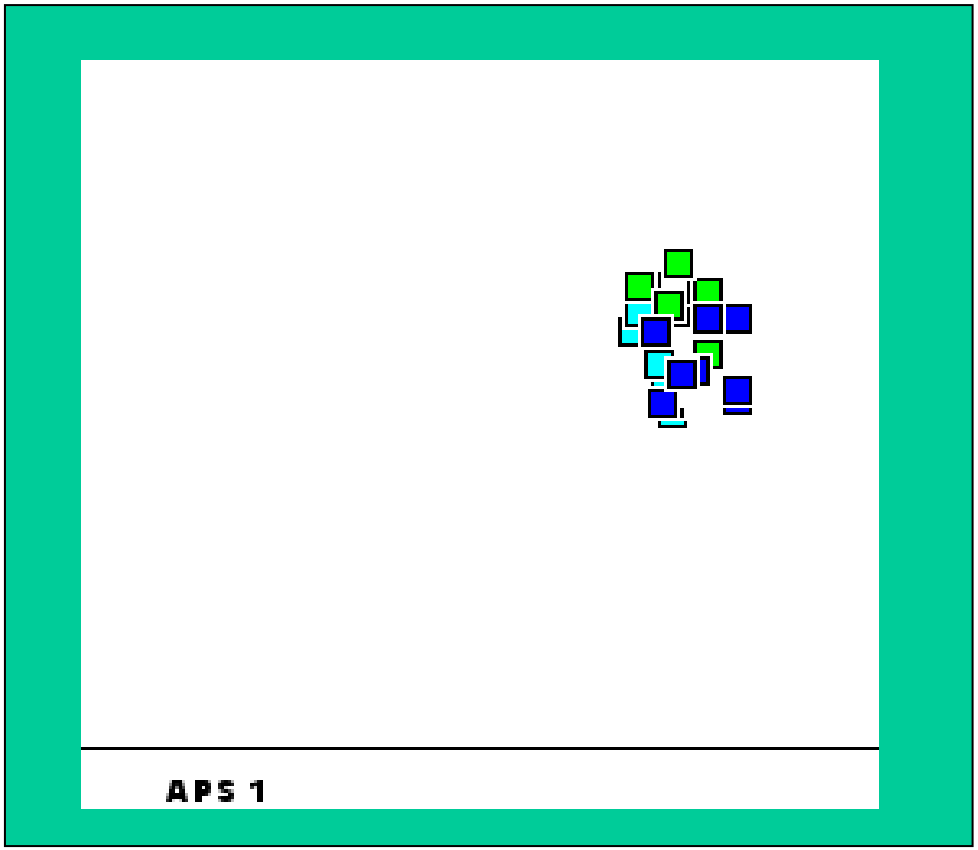
Results of standardized experiments

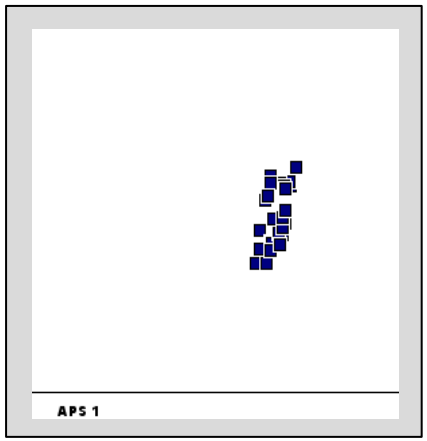
EuroFlow Panel for Plasma Cell Disorders:
Can we identify the center?



EuroFlow

Results of standardized experiments

Case *versus* normal reference pool

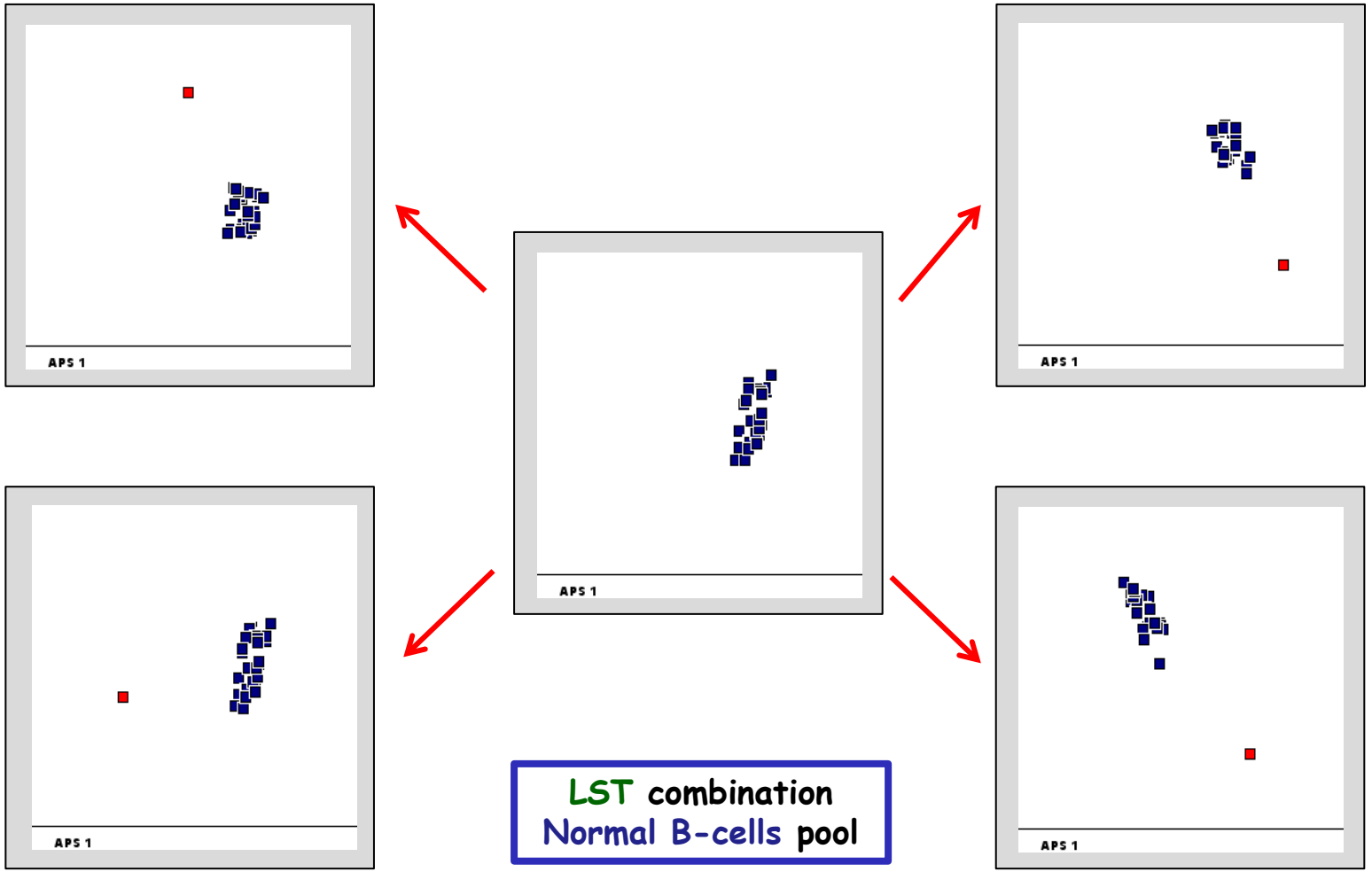


LST combination
Normal B-cells pool

EuroFlow

Results of standardized experiments

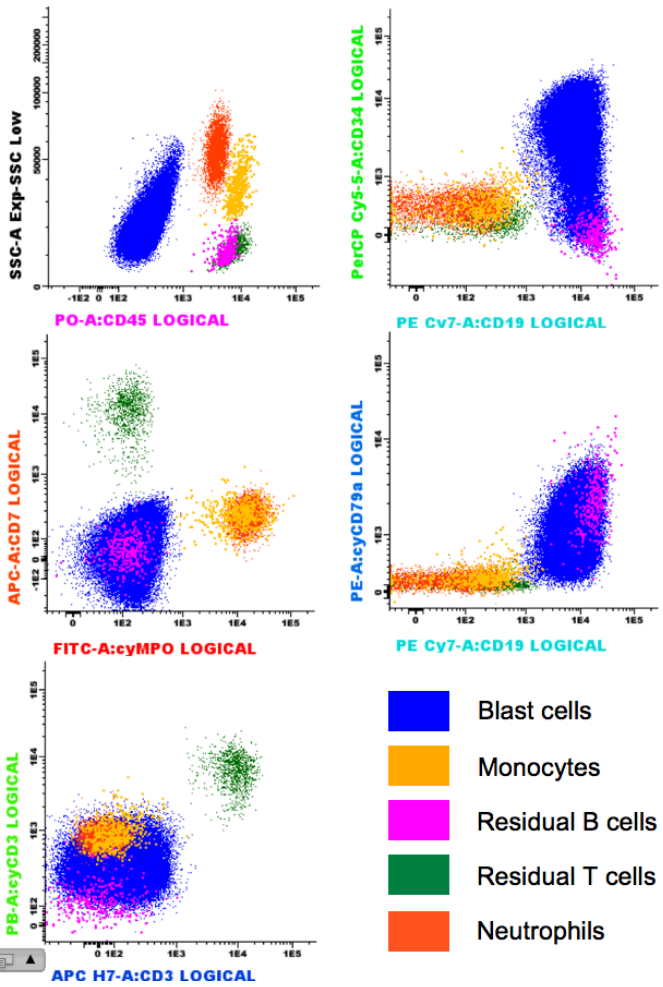
Case *versus* normal reference pool

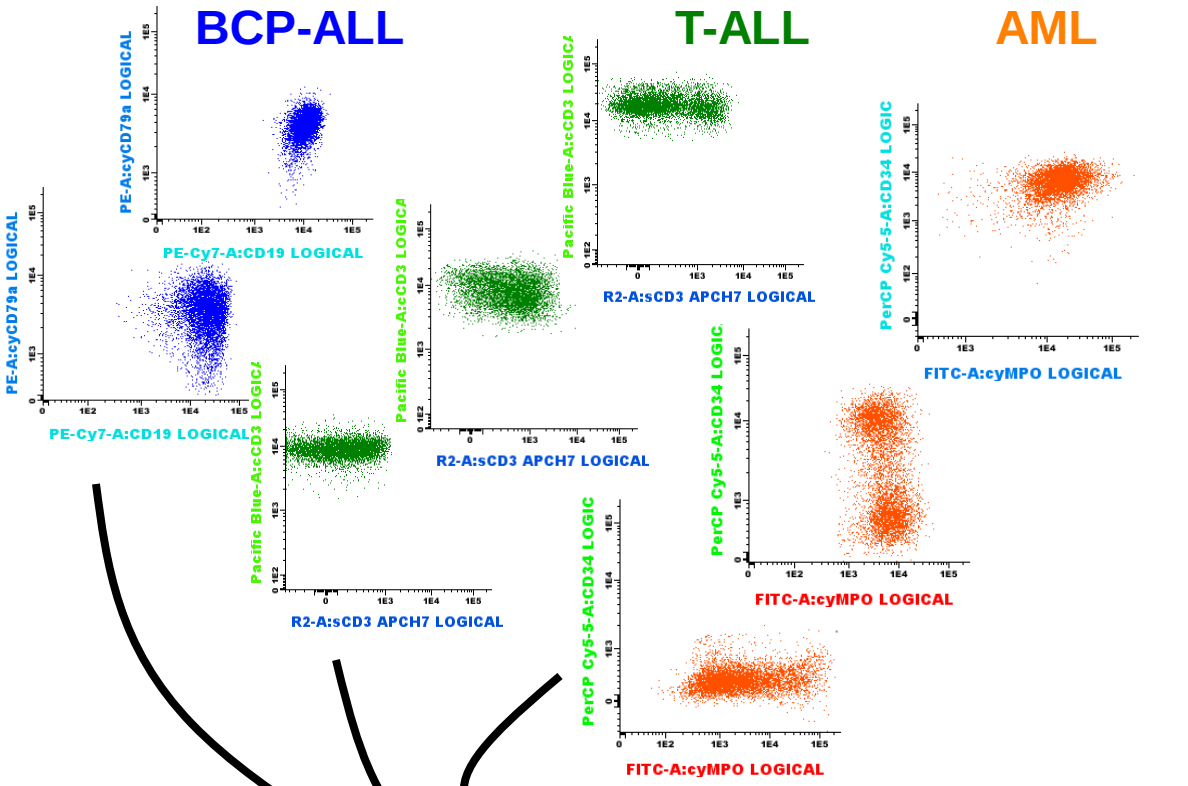


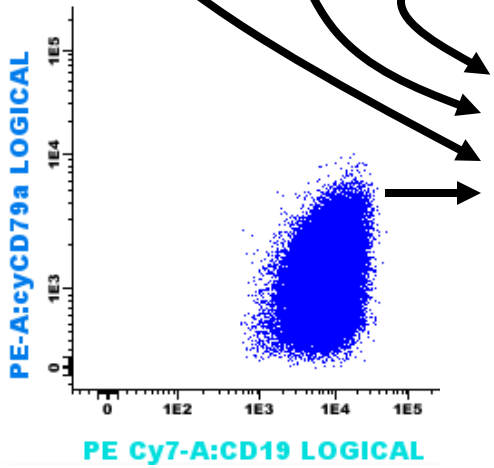
LST combination
Normal B-cells pool

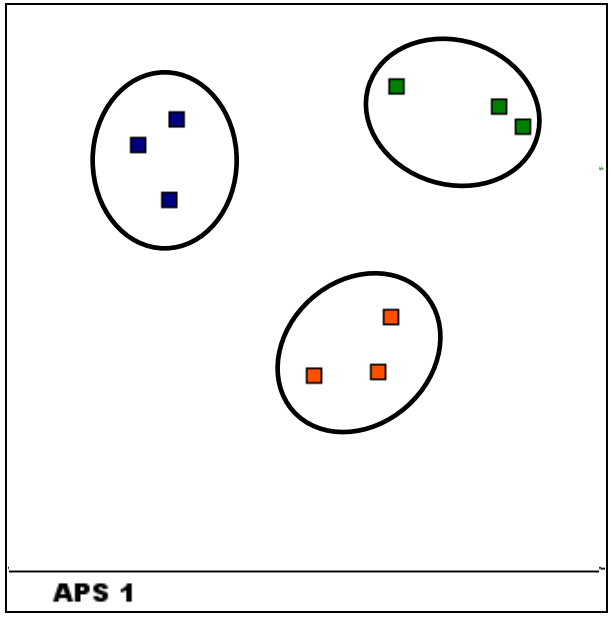
EuroFlow

ALOT: Reference datafile

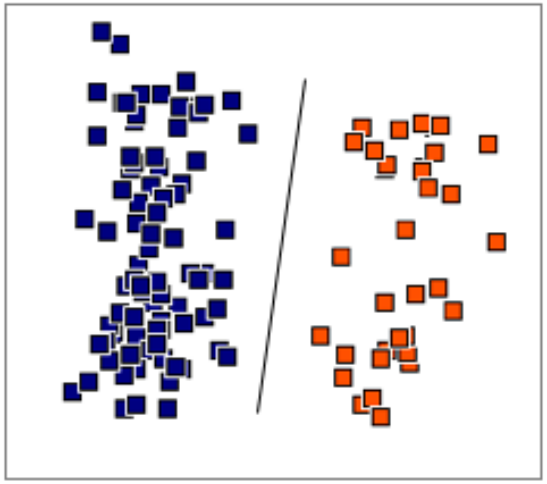


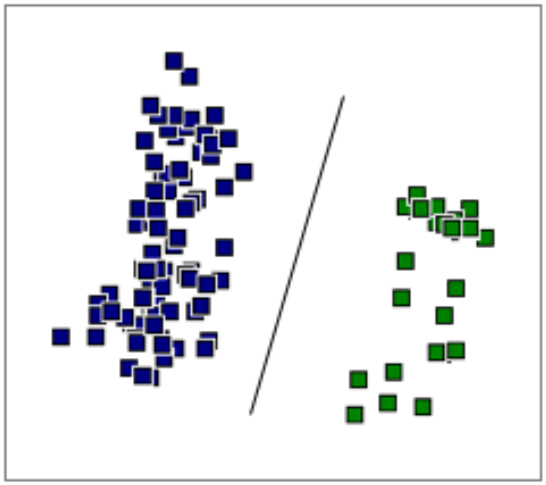


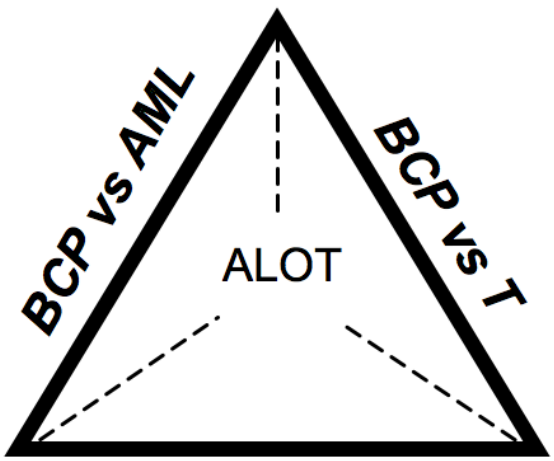


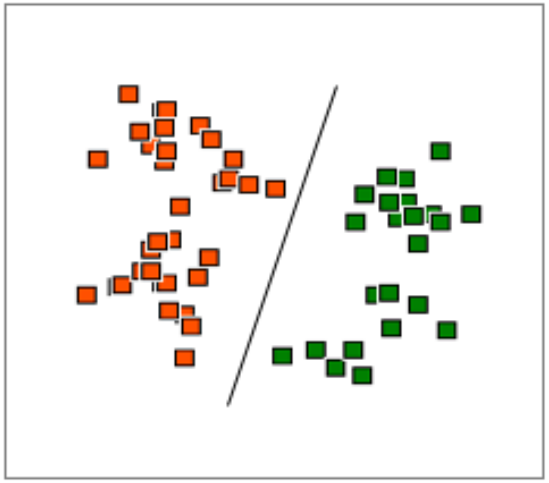


ALOT. Reference groups









BCP-ALL cases (n=89)

T-ALL cases (n=27)

AML cases (n=36)

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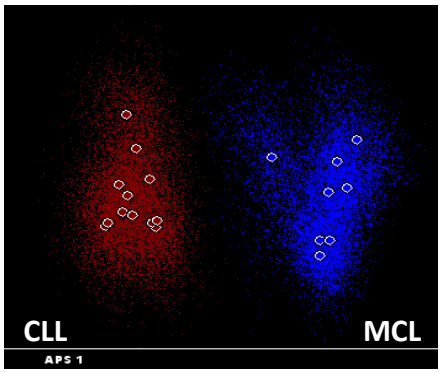
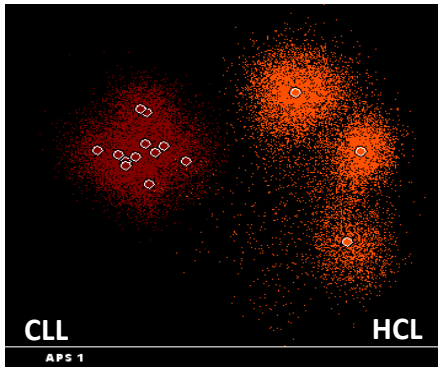
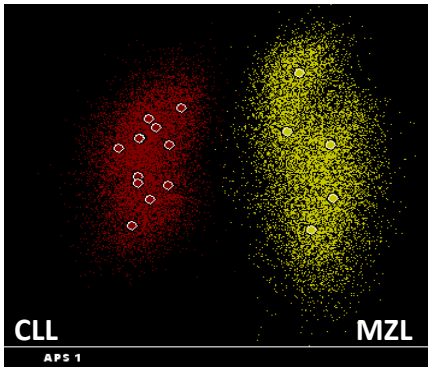
Responsible scientist:
Ludovic Lhermitte

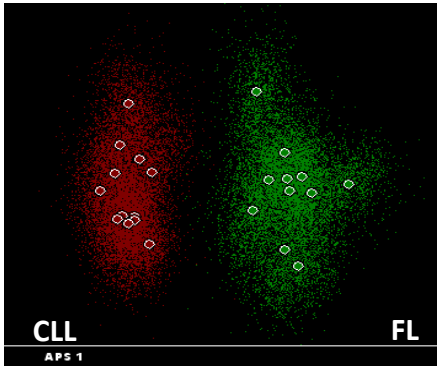
BCLPD classification panel

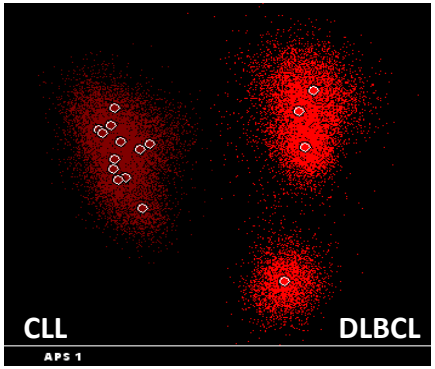
BCLPD disease category reference groups











Responsible scientist: Sebastian Bottcher

EuroFlow

Quality Control. December 2010

- Synchronized data acquisition in 9 centers / 10 instruments

Antibody combination:

PacB	PacO	FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7
CD20 & CD4	CD45	CD8 & anti-Igλ	CD56 & anti-Igκ	CD5	CD19	SmCD3	CD81

30 peripheral blood samples from healthy donor

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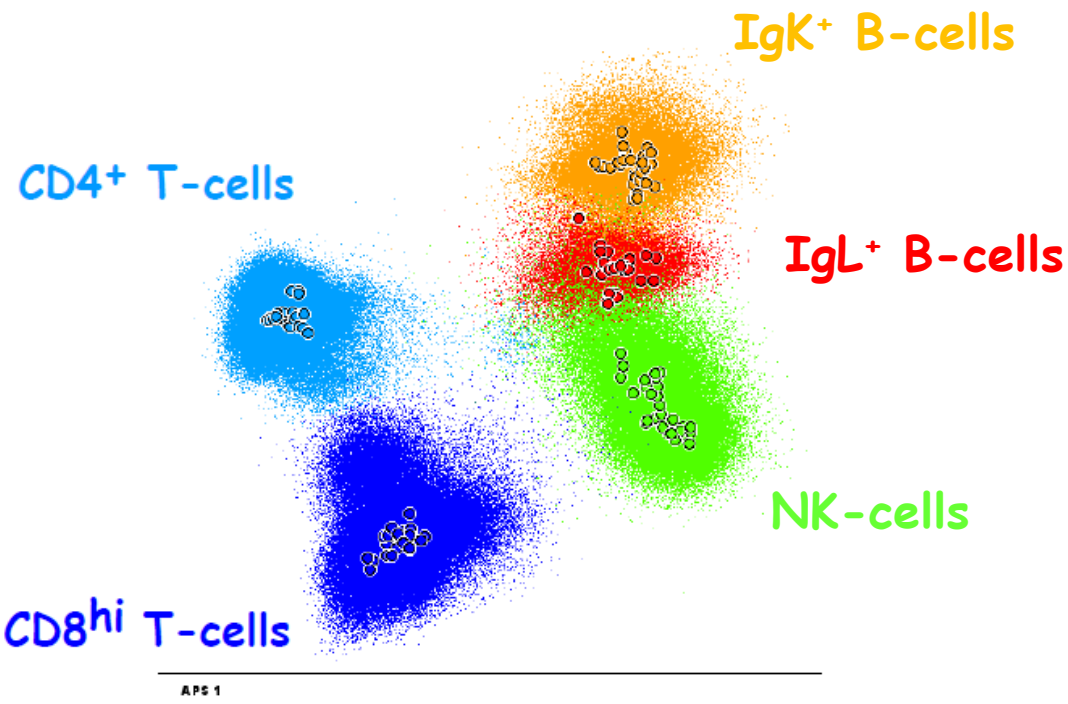
Quality Control. December 2010

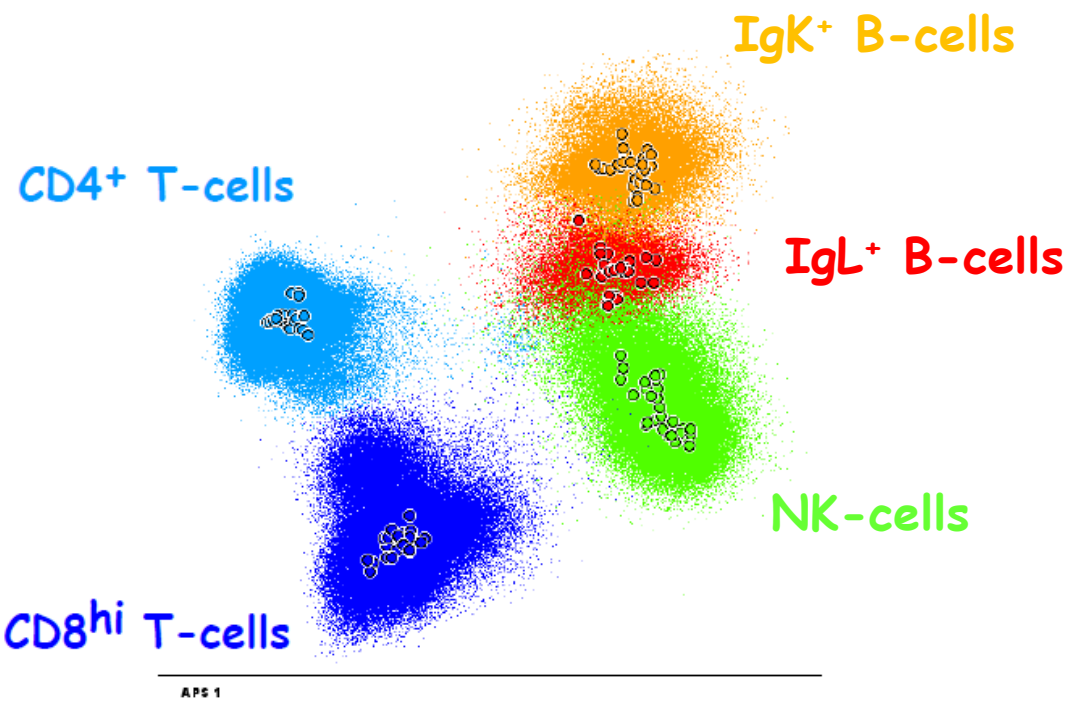
Clustering of normal lymphoid (sub)populations



EuroFlow

Quality Control. December 2010

Clustering of normal lymphoid (sub)populations

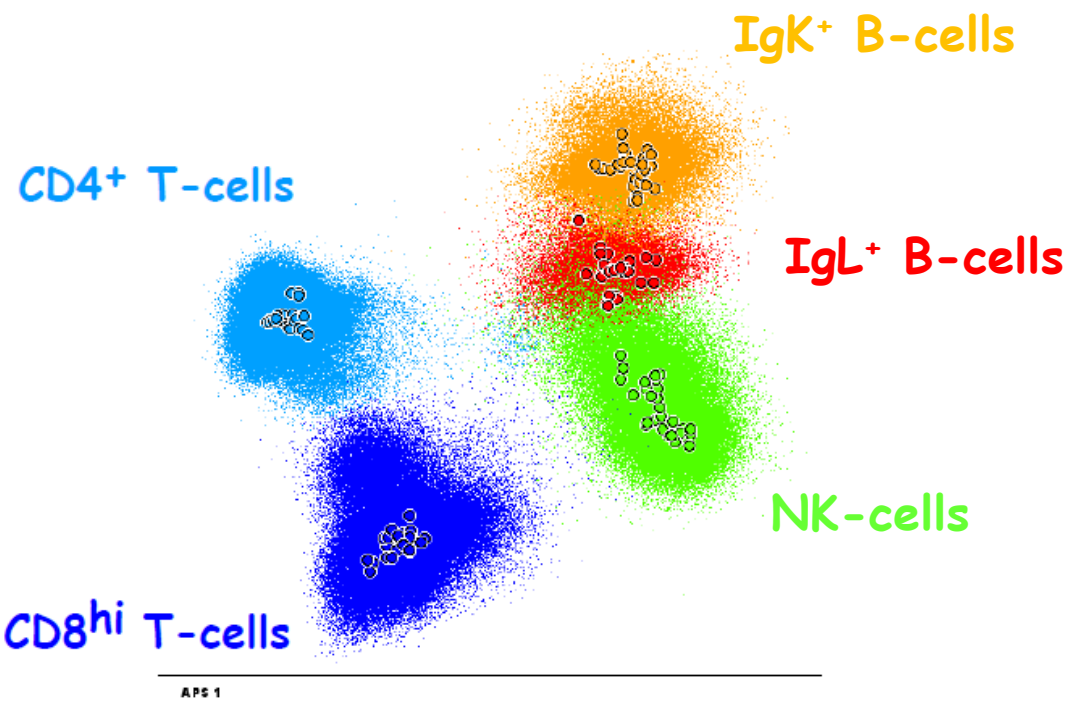


Fluorescence Channel	Target	CV
Violet-1	CD20	30%
	CD4	19%
Violet-2	CD45	23%
Blue-1	CD8	16%
	IgL	87%
Blue-2	CD56	54%
	IgK	77%
Blue-3	CD5	23%
Blue-4	CD19	32%
Red-1	CD3	30%
Red-2	CD81	50%

EuroFlow

Quality Control. December 2010

Clustering of normal lymphoid (sub)populations



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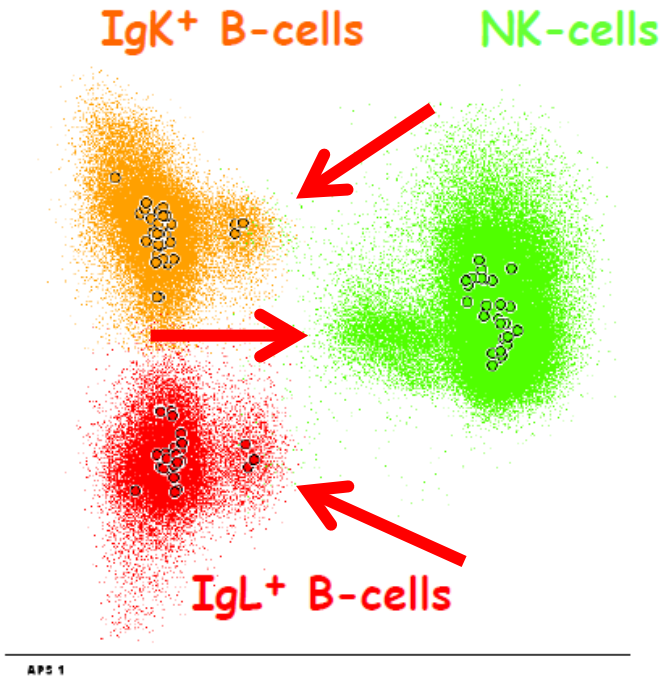
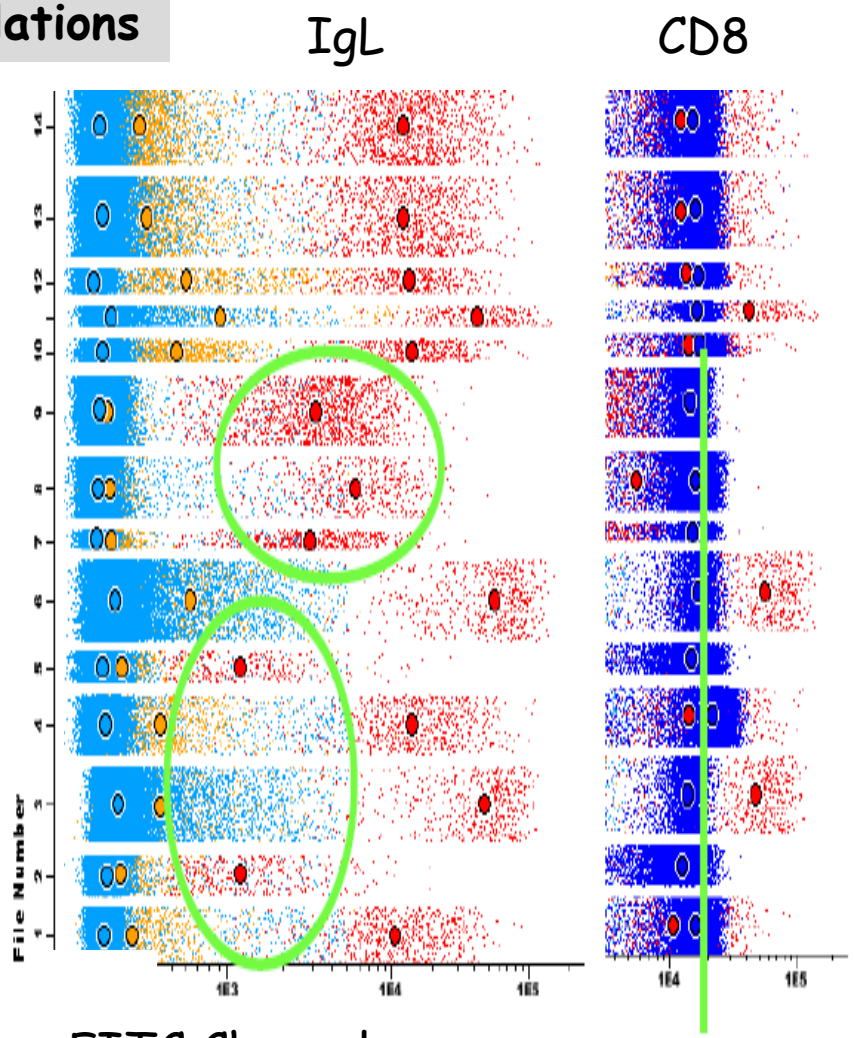
EuroFlow

Quality Control. December 2010

Clustering of normal lymphoid (sub)populations

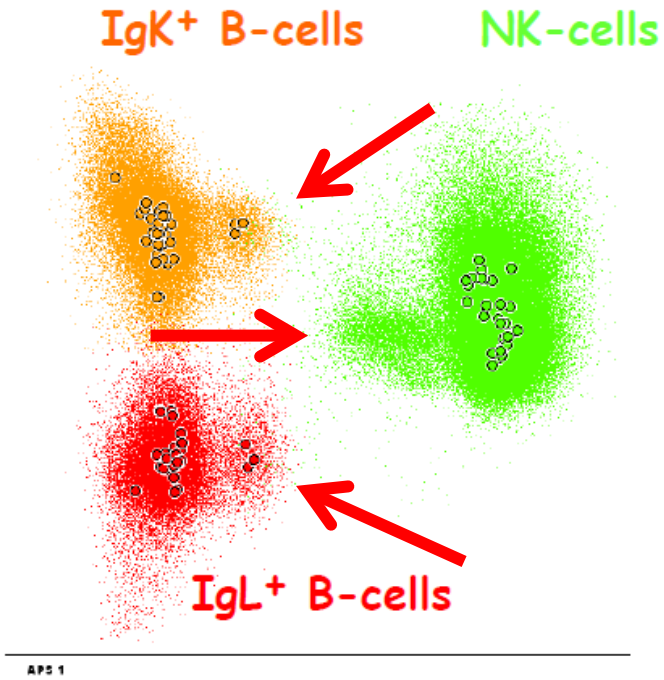


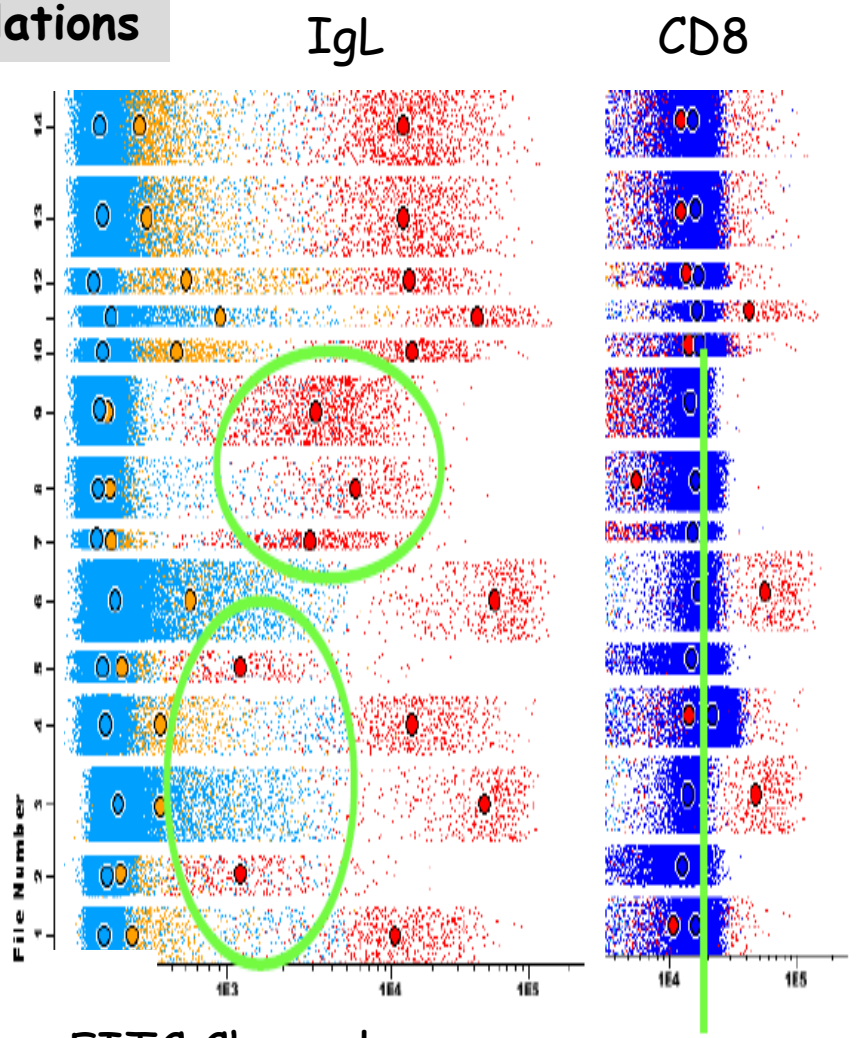


EuroFlow

Quality Control. December 2010

Clustering of normal lymphoid (sub)populations



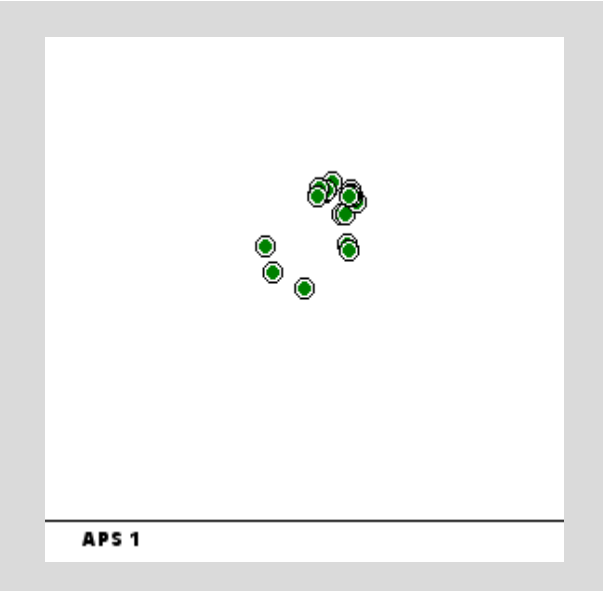


Sample preparation- and/or patient- related issues

EuroFlow

Quality Control

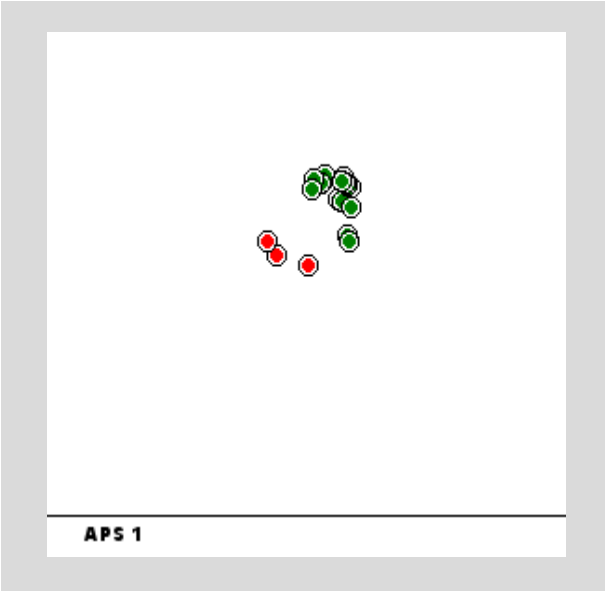
Clustering of normal CD4+ T-cells

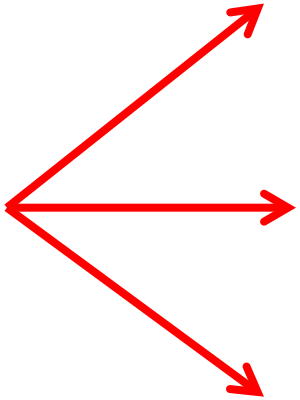


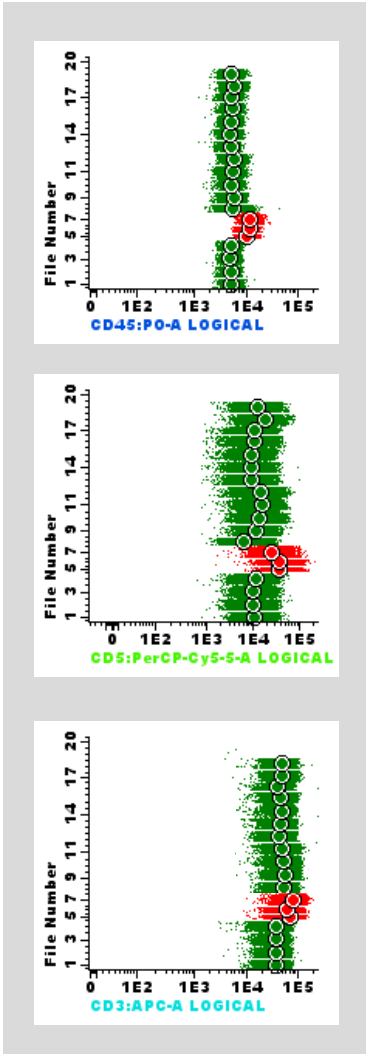
EuroFlow

Quality Control

Clustering of normal CD4+ T-cells







VIOLET laser

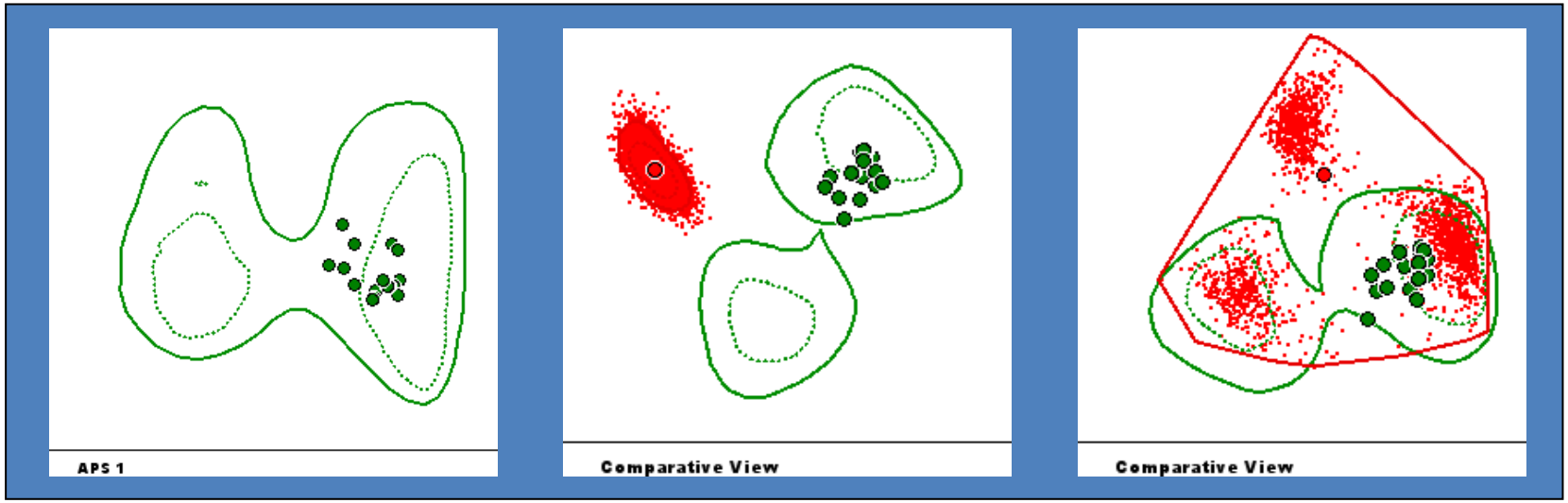
BLUE laser

RED laser

EuroFlow

Results of SOPs implementation

EuroFlow LST combination: B-cells



22 healthy donors

One sample with a unique abnormal B-cell population

One sample with normal and abnormal B-cell populations

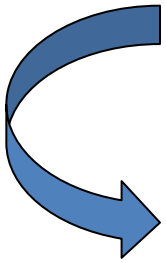
8 different centers/instruments, over 4 years

EuroFlow

Results of synchronized experiments

- Optimal set up & close monitoring of flow cytometers

- Evidence-based recommendations regarding reagents, fluorochromes & sample preparation procedures



Generation of standardized & highly reproducible results

EuroFlow

Which problems are we facing?

- Many reagents (costly and complex)
- Need expertise (reference profiles)
- Time consuming
- Technical limitations
- Many (my) strategies (suboptimal)
- Not standardized (reproducible?)
- Limited clinical utility

Which are we solving?

- No redundancy
- Computer expertise
- Fast
- Real multi-D FCM
- Harmonized
- Reproducible
- Higher clinical utility

THANKS