ESTRATEGIAS ACTUALES DE ANALISIS DE DATOS POR CMF: EL PROGRAMA INFINICYT

CANCER RESEARCH CENTER, UNIVERSITY & UNIVERSITY HOSPITAL of SALAMANCA (SPAIN)

EuroFlow

Curso Avanzado de Actualización en Oncohematología por citometria de flujo.
Buenos Aires, 30 de mayo de 2011

DIAGNOSTICS IN HEMATO-ONCOLOGY

1. Making the diagnosis
Normal ↔ reactive/regenerating ↔ malignant
Annually > 300,000 new patients with a hematological malignancy in developed countries

2. Classification of hematopoietic malignancies
- relation with prognosis
- relevance of risk-group definition in treatment protocols

→ Based on differentiation characteristics and particularly on chromosome aberrations, resulting in fusion gene transcripts or aberrantly (over) expressed genes

3. Evaluation of treatment effectiveness
Detection of minimal residual disease (MRD):
MRD-based risk-group stratification (treatment reduction or treatment intensification)
Annually > 400,000 follow-up samples in leukemia patients (ALL, AML, CML)

Prepared by J.J.M. van Dongen

APPLICATIONS OF FLOW CYTOMETRY

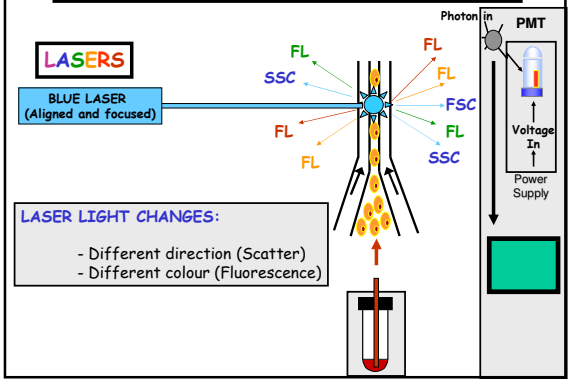
Flow cytometry: where can it be applied?

70s-90s

Hybridoma technology
Monoclonal antibodies
Fluorochrome-conjugates

From research laboratories to clinical diagnostics

GENERATION OF FLOW CYTOMETRY DATA



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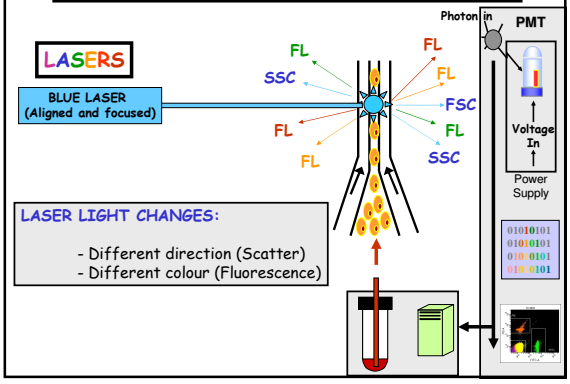
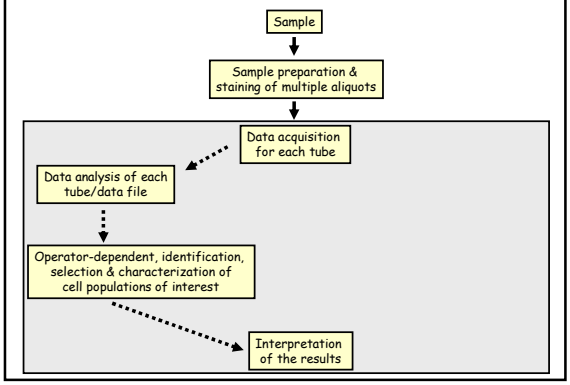


FLOW CYTOMETRY IMMUNOPHENOTYPING APPROACH



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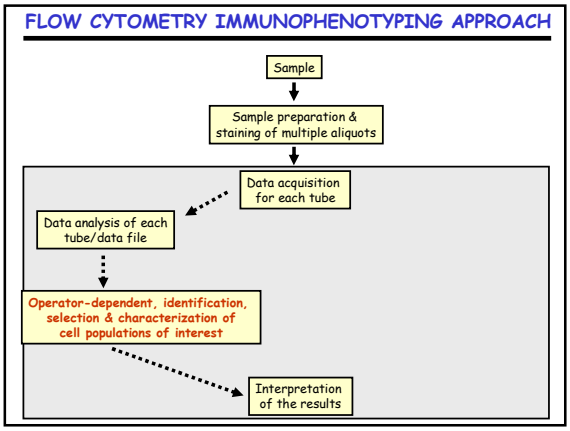
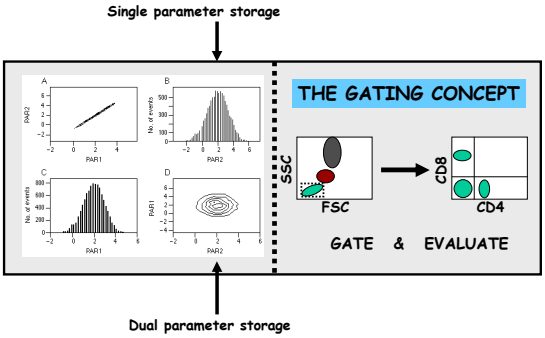


STORAGE & DISPLAY OF MULTIPARAMETER FLOW CYTOMETRY DATA



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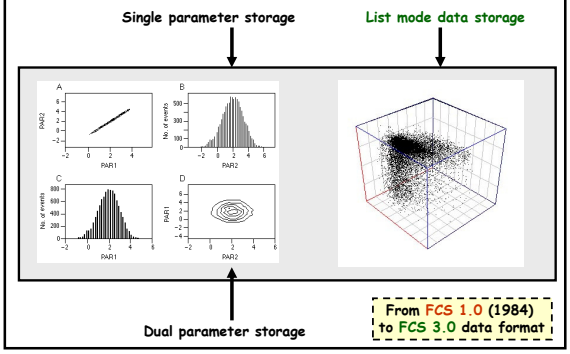
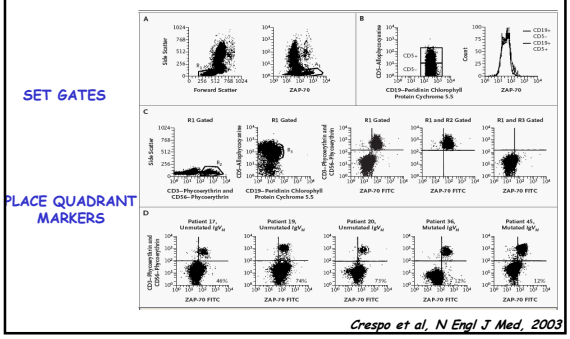
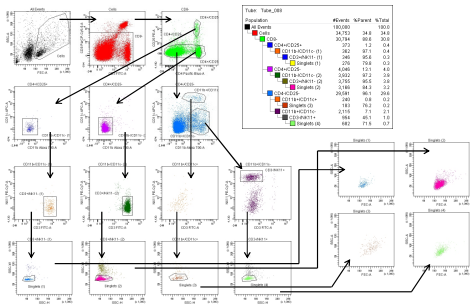


IMMUNOPHENOTYPIC CHARACTERIZATION OF CELL POPULATIONS



Crespo et al, N Engl J Med, 2003

BOOLEAN GATING STRATEGY

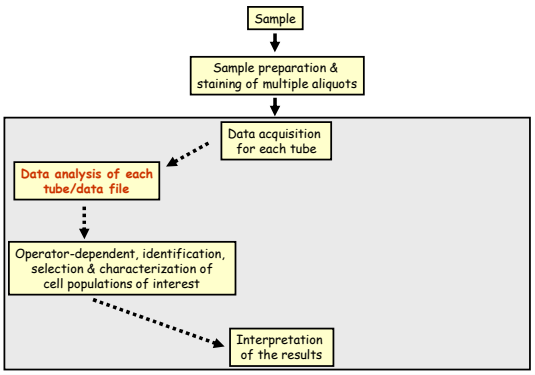


Prepared by A.Salvador

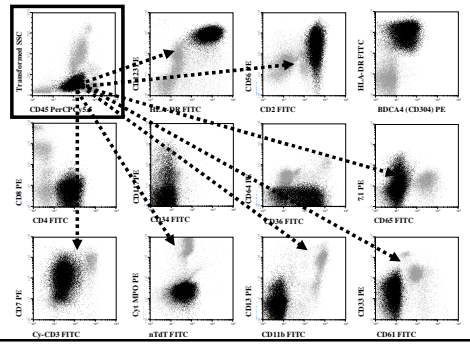
FLOW CYTOMETRY: TYPE OF INFORMATION

- **Identification** of cell populations
- **Enumeration** of cell numbers
- **Characterization** of cell populations

FLOW CYTOMETRY IMMUNOPHENOTYPING APPROACH



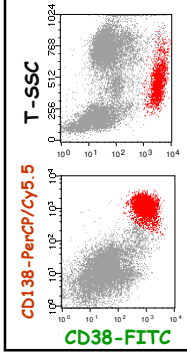
IMMUNOPHENOTYPIC FEATURES OF NEOPLASTIC CELLS



MDS/AML: 3-COLOR STAINING PANEL

- FITC	PE	PerCP/Cy5.5
- cCont.	cCont.	CD45
- nTdt	cMPO	CD45
- cCD3	CD7	CD45
- CD19	cCD79a	CD45
- sCont	sCont	CD45
- HLADR	CD117	CD45
- HLADR	CD123	CD45
- CD11b	CD13	CD45
- CD15	CD16	CD45
- CD36	CD64	CD45
- CD33	CD61	CD45
- CD71	GphA	CD45
- CD65	7.1	CD45
- CD2	CD56	CD45

MONOCLONAL GAMMOPATHIES: IDENTIFICATION OF CLONAL PLASMA CELLS



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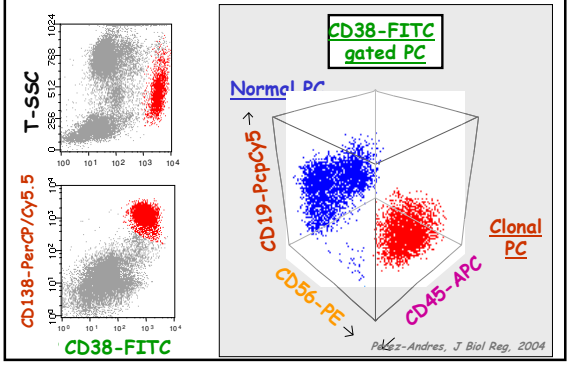


Do not forget....FCM language is COMPLEX

WIIYGIWYG

What Is In Your Gate Is What You Get

Prepared by A.Salvador

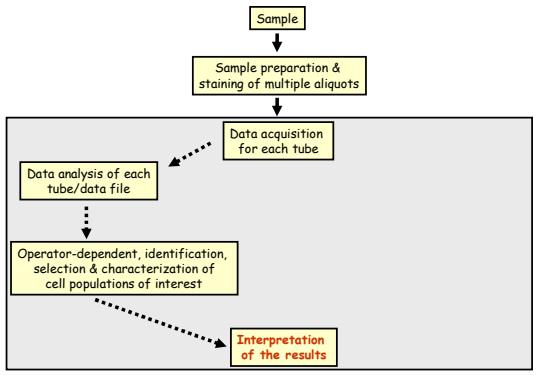
Do not forget....FCM language is COMPLEX

WINIYGIWYF

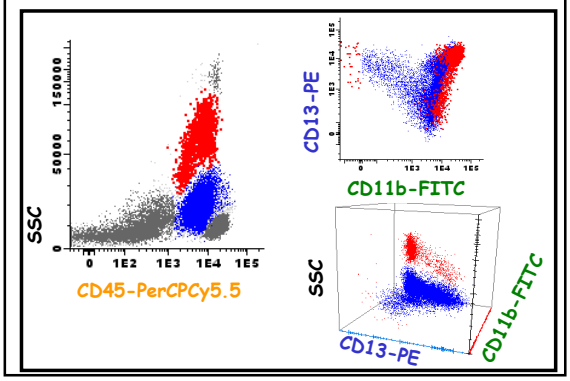
What Is Not In Your Gate Is What You Forget

Prepared by A.Salvador

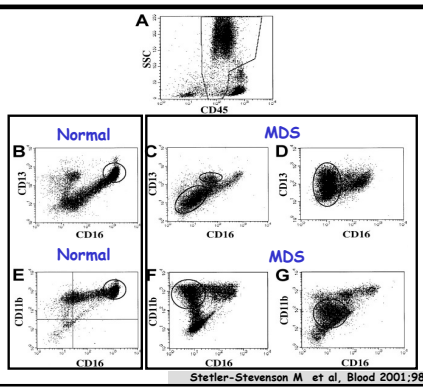
FLOW CYTOMETRY IMMUNOPHENOTYPING APPROACH



MDS: COEXISTENCE OF NORMAL & HYPOGRANULAR MATURING NEUTROPHILS

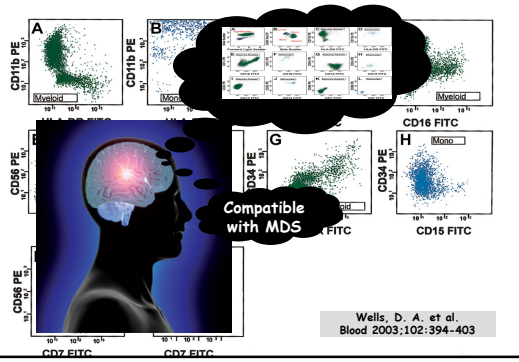


Immunophenotypic Myeloid Abnormalities in MDS



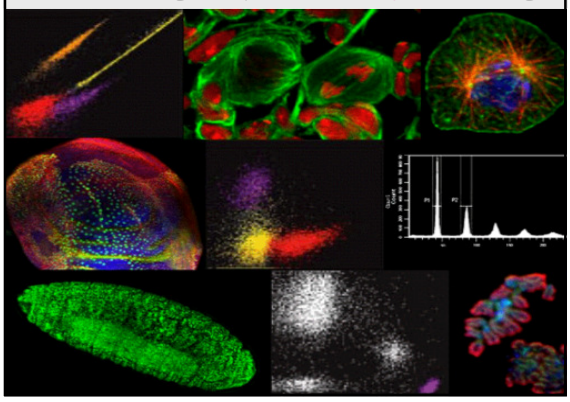
Stetler-Stevenson M et al, Blood 2001;98:979-987

Examples of various myeloid and monocytic aberrant antigenic patterns in MDS



Wells, D. A. et al. Blood 2003;102:394-403

MICROSCOPE VS FCM COLORFUL IMAGES



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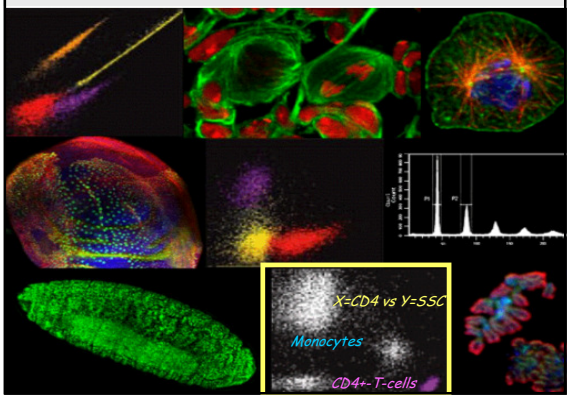


WHICH PROBLEMS ARE WE FACING ?

- Many reagents: costly and complex panels
- Need expertise in normal (reference) cell populations
- Time consuming
- Technical limitations
- Many (my) suboptimal strategies to reach a similar result
- Not standardized: reproducibly harmonized?
- Partial and more limited clinical utility than expected

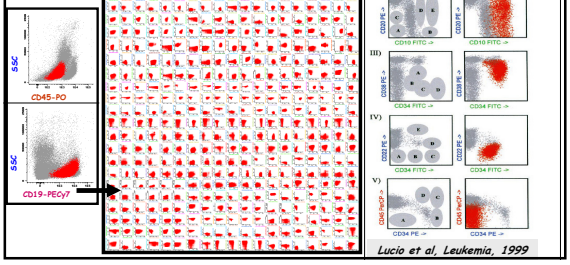
EuroFlow

IMMUNOPHENOTYPIC CHARACTERISTICS OF NORMAL vs LEUKEMIC B-CELLS

EuroFlow

CD19+ B-CELLS

8- COLOR flow cytometry: Bcp-ALL
EuroFlow panel (450 bivariate plots)



Lucio et al, Leukemia, 1999

REQUIRED DEVELOPMENTS IN FLOW CYTOMETRY

(status in 2005)

Immunobeads
- introduce combined cellular/immunobead assays
- special immunobead for leukemias

Novel antibodies
- test new (academic) antibodies for application in intracellular stainings
- development of new antibodies against oncoproteins and aberrant signalling pathways

Multicolor flow cytometry: ≥8 color comprehensive panels
- inclusion of solid state violet laser
- selection of appropriate fluorochromes
- compare conjugated antibodies (multiple companies)

Development of novel software for complex pattern recognition
- combining multiple tubes: calculate data & multivariate analyses
- mapping of diagnosis and follow-up leukemia samples against templates of reference "normal/control" samples

EuroFlow

FLOW CYTOMETRY IMMUNOPHENOTYPING APPROACH

Sample

Sample preparation & staining of multiple aliquots

Data acquisition for each tube

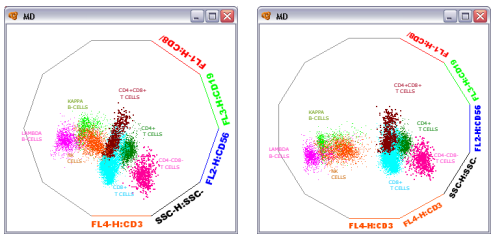
Data analysis of each tube/data file

Improved Data visualization
Data analysis
Data interpretation

Operator-dependent, identification, selection & characterization of cell populations of interest

Interpretation of the results

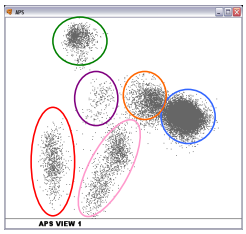
INCREASING DISCRIMINATION POWER
N-multiplication of individual parameters



Duplication of CD3 allows a better discrimination between CD8+ T-cells and NK-cells

EuroFlow

AUTOMATED SEPARATION AMONG DIFFERENT CELL POPULATIONS (APS view)



EuroFlow

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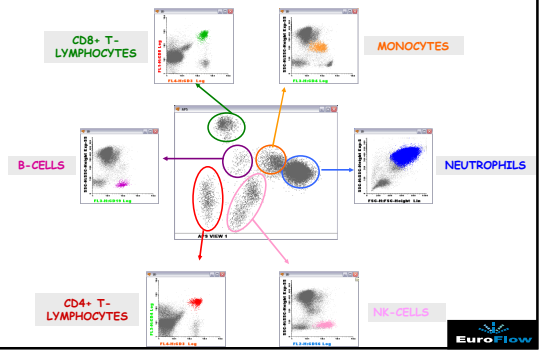

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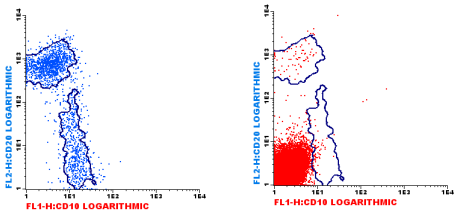
FCM DATA OVERLAYED ON REFERENCE IMAGES



EuroFlow

EuroFlow

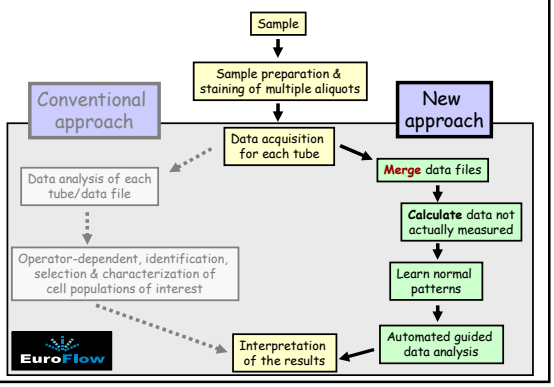
HOW TO SIMPLIFY DATA ANALYSIS

- **Construct reference data files** for normal and pathologic cell populations (e.g.: per disease category)

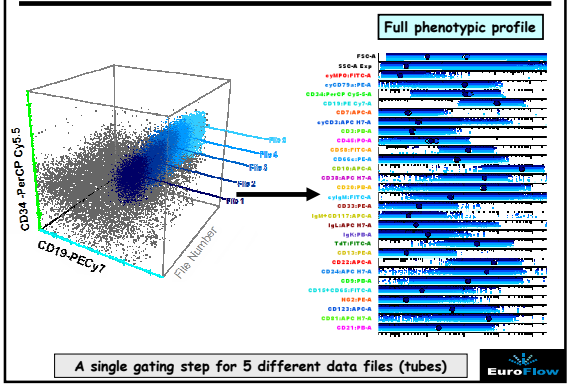
- Multi-n-dimensional **comparison of normal vs pathologic cell populations** (e.g.: at diagnosis and follow-up):

  - Automated PCA-guided approach for **homogeneous cell populations** (e.g. Blood lymphocyte subsets)
  - Maturation tools for **heterogeneous cell populations** (e.g. Maturing BM B-cell precursors)

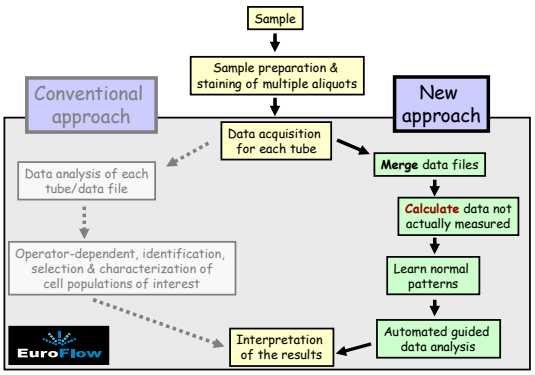
FLOW CYTOMETRY IMMUNOPHENOTYPING APPROACH



MERGED DATA FILES FOR SINGLE STEP GATING



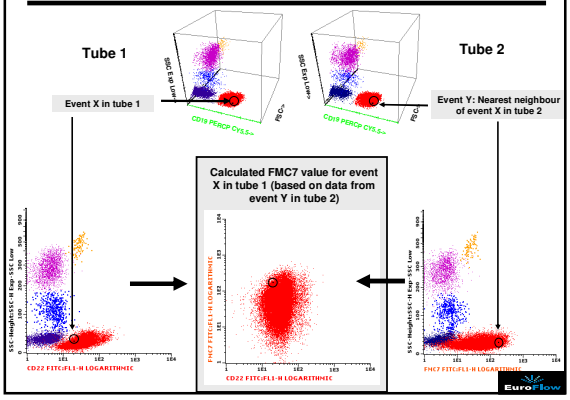
FLOW CYTOMETRY IMMUNOPHENOTYPING APPROACH



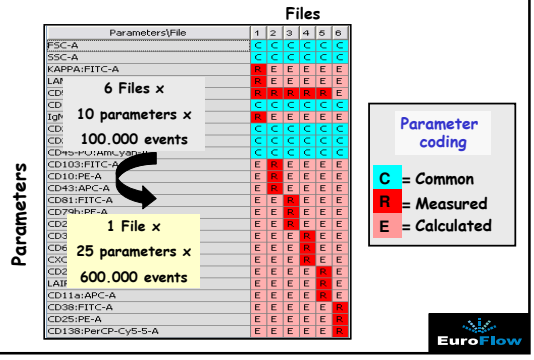
DATA IN A MERGED DATA FILE

PARAMETER	TUBE No 1	2	3	4	5	6	7	8	9
FSC-HEIGHT	C	C	C	C	C	C	C	C	C
SSC-HIGHT	C	C	C	C	C	C	C	C	C
CD11b-FITC	R								
CD13-PE	R								
CD45-PerCP	C	C	C	C	C	C	C	C	C
CD34-APC	C	C	C	C	C	C	C	C	C
CD2-FITC		R							
CD56-PE		R							
HLADR-FITC			R	R					
CD117-PE			R						
CD123-PE				R					
CD15-FITC					R				
CD16-PE					R				
CD22-FITC						R			
CD25-PE						R			
CD65-FITC							R		
7.1-PE							R		
CD61-FITC								R	
CD33-PE								R	
CD71-FITC									R
Glyphorin-PE									R

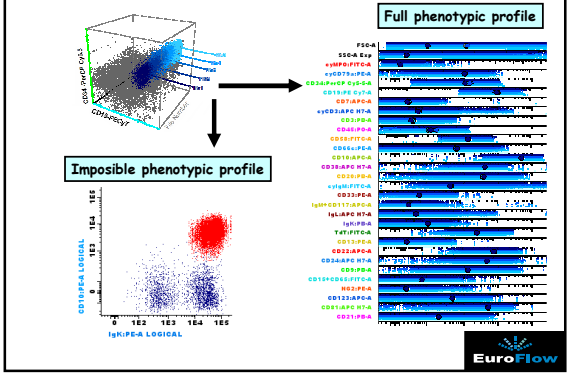
DATA CALCULATION IN THE INFINICYT™ SOFTWARE



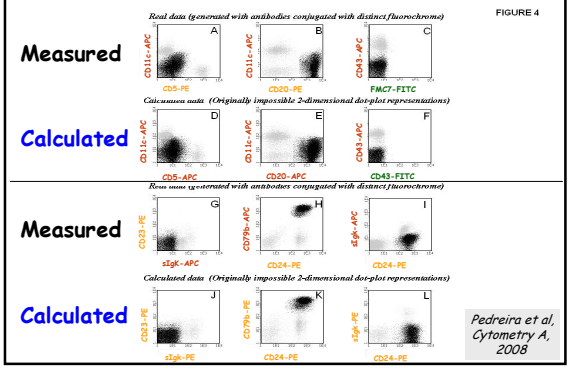
MERGED & CALCULATED LISTMODE DATA FILE



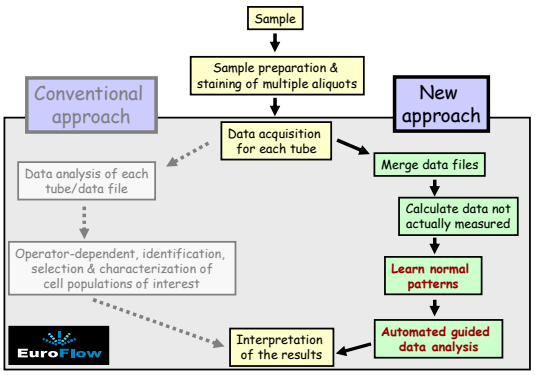
MERGED AND CALCULATED DATA FILE



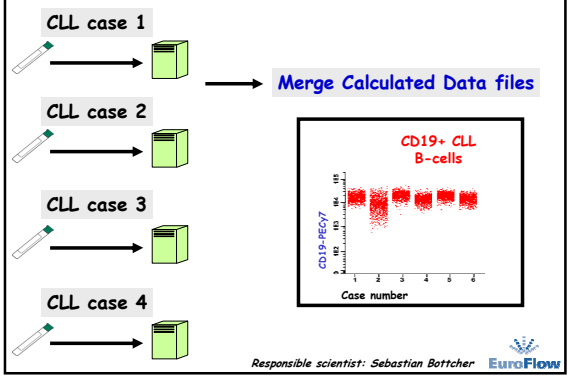
CALCULATED VS MEASURED DATA



FLOW CYTOMETRY IMMUNOPHENOTYPING APPROACH

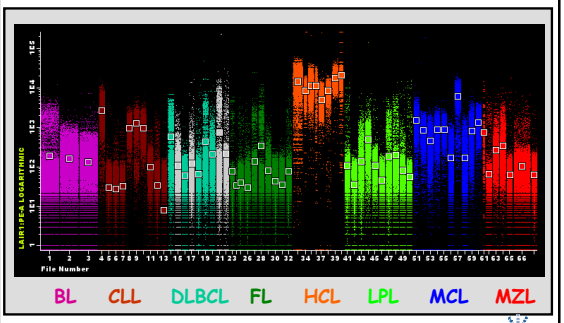


REFERENCE DATAFILES FOR CLL B-CELLS



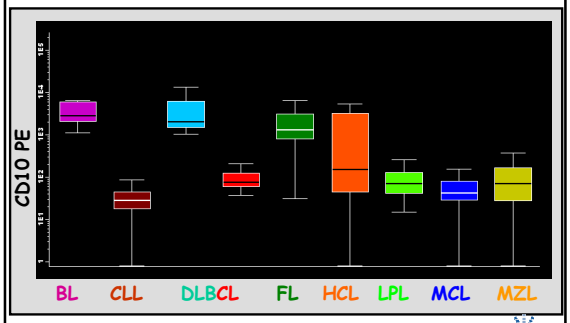
Responsible scientist: Sebastian Bottcher EuroFlow

Characterization markers: LAIR1(CD305)



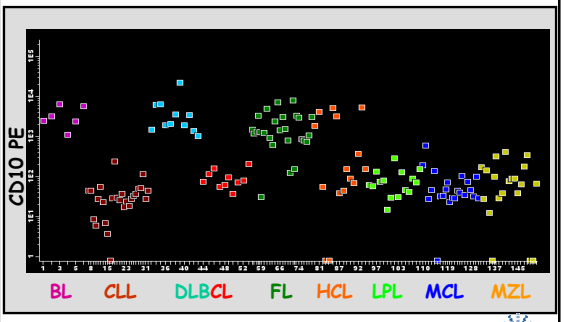
Responsible scientist: Sebastian Bottcher EuroFlow

Characterization markers: CD10



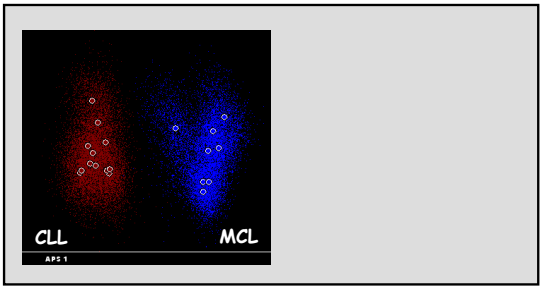
Responsible scientist: Sebastian Bottcher EuroFlow

Characterization markers: CD10



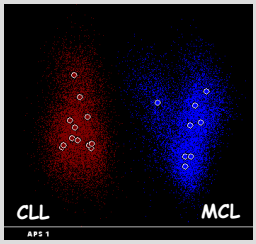
Responsible scientist: Sebastian Bottcher EuroFlow

B-CLPD panel: clinical utility



Responsible scientist: Sebastian Bottcher EuroFlow

B-CLPD panel: clinical utility

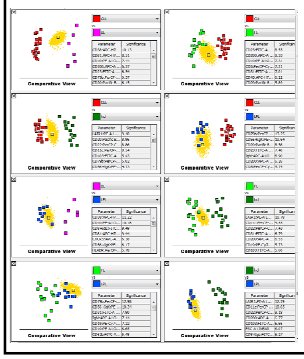


1	sIgM	14.02
2	CD200	13.76
3	CD79b	11.94
4	CD23	8.57
5	CD38	7.73

Responsible scientist: Sebastian Bottcher

EuroFlow

B-CLPD: Comparative analysis of "our case" vs multiple reference groups



Responsible scientists: Sebastian Bottcher

Costa et al, Leukemia, 2010

EuroFlow

HOW TO SIMPLIFY MRD STRATEGIES

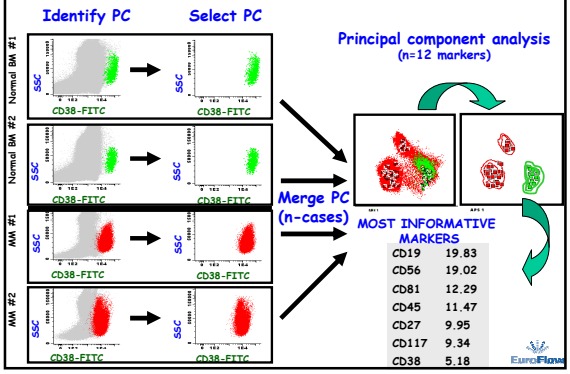
- Improve the design of MRD panels for a greater efficiency and higher reproducibility.

- Construct reference data files for normal and neoplastic cells (e.g.: per disease category)

- Multi-n-dimensional comparison of normal vs neoplastic cell populations (e.g.: at diagnosis and follow-up):

  - Automated PCA-guided approach for homogeneous cell populations (e.g. lymphoid)
  - Maturation tools for heterogeneous cell populations (e.g. myeloid)

CONSTRUCTION OF EuroFlow MRD PANELS: MM



Aim of Euroflow MRD approach for B-CLPD

- To find a marker combination to discriminate all neoplastic B-cells in every B-CLPD case vs all normal/reactive PB and BM mature B-cells/hematogones

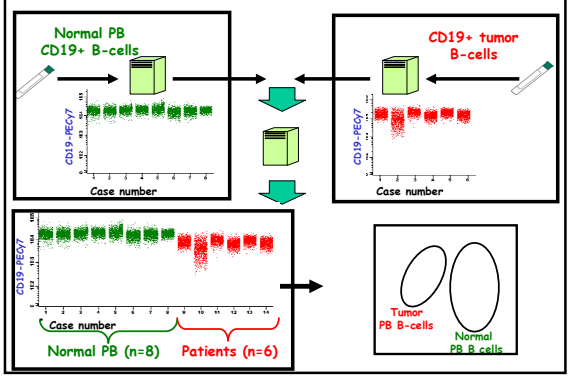
NOT an individualized approach per patient

↓

the approach should work without knowledge of the exact initial immunophenotype

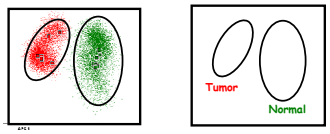
REFERENCE DATAFILES: NORMAL vs. TUMOR B-CELLS

EuroFlow



COMPARE A CASE VS NORMAL & NEOPLASTIC B-CELLS

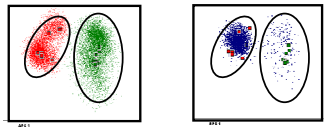
REFERENCE DATA FILES



COMPARE A CASE VS NORMAL & NEOPLASTIC B-CELLS

REFERENCE DATA FILES

DIAGNOSTIC SAMPLES vs. REFERENCE DATA FILES

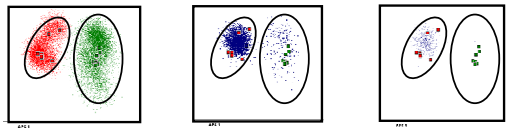


COMPARE A CASE VS NORMAL & NEOPLASTIC B-CELLS

REFERENCE DATA FILES

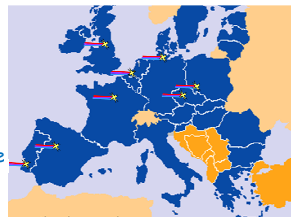
DIAGNOSTIC SAMPLES vs. REFERENCE DATA FILES

MRD SAMPLES vs. REFERENCE DATA FILES



UTILITY OF THE NEW SOFTWARE TOOLS
Standardization of FCM immunophenotyping

- How to get optimal and comparable measurements?
- Which are the most appropriate fluorochromes?
- What is the optimal sample preparation protocol?

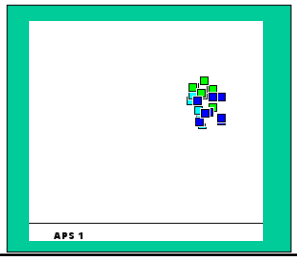


REPRODUCIBLE & OBJECTIVE RESULTS

Results of standardized experiments

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

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



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