

INFINICYT™ 2.0

CYTOGNOS NEXT GENERATION FLOW SOLUTION



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WHERE DOES INFINICYT COME FROM?


INDUSTRY




ACADEMIA

Licensed and developed



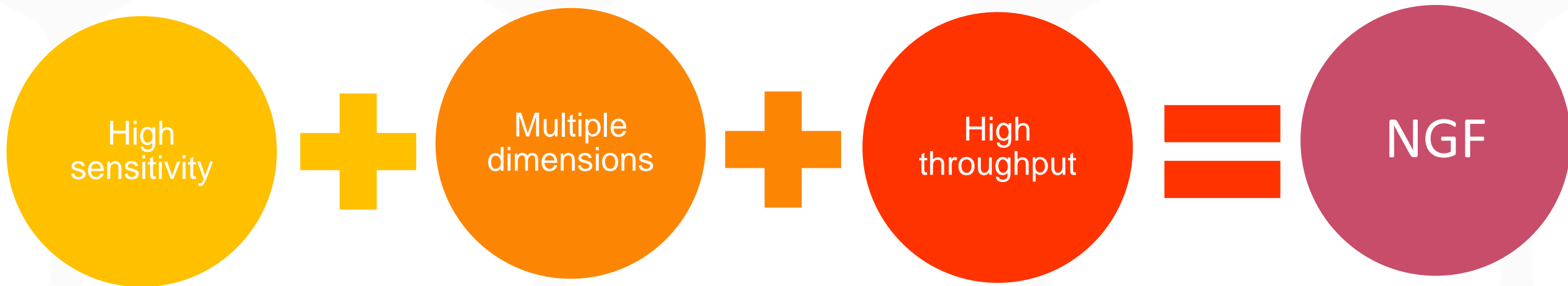
Based on USAL patents



Contribution and validation



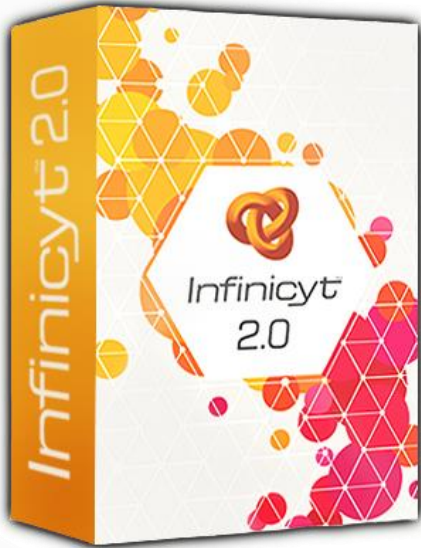
WHY IS INFINICYT NECESSARY?



“HIGHLY SENSITIVE MULTIDIMENSIONAL FLOW CYTOMETRY”

WHAT IS INFINICYT?

Analysis software of multicolor flow cytometry data.



- ✓ **Multivariate.** Multiparametric diagrams and analysis tools.
- ✓ **Standard.** EuroFlow™ Reference Databases.
- ✓ **Reproducible.** Automated analysis tools.
- ✓ **Efficient and sensitive.** Optimal performance when processing large files is essential in MRD studies.
- ✓ **Compatible with all types of cytometers and manufacturers.**

AGENDA

✓ Review of Infinicyt tools

- ✓ Data Analysis
- ✓ Data Integration
- ✓ Data Interpretation
 - EuroFlow™ Databases for sample comparison

AGENDA

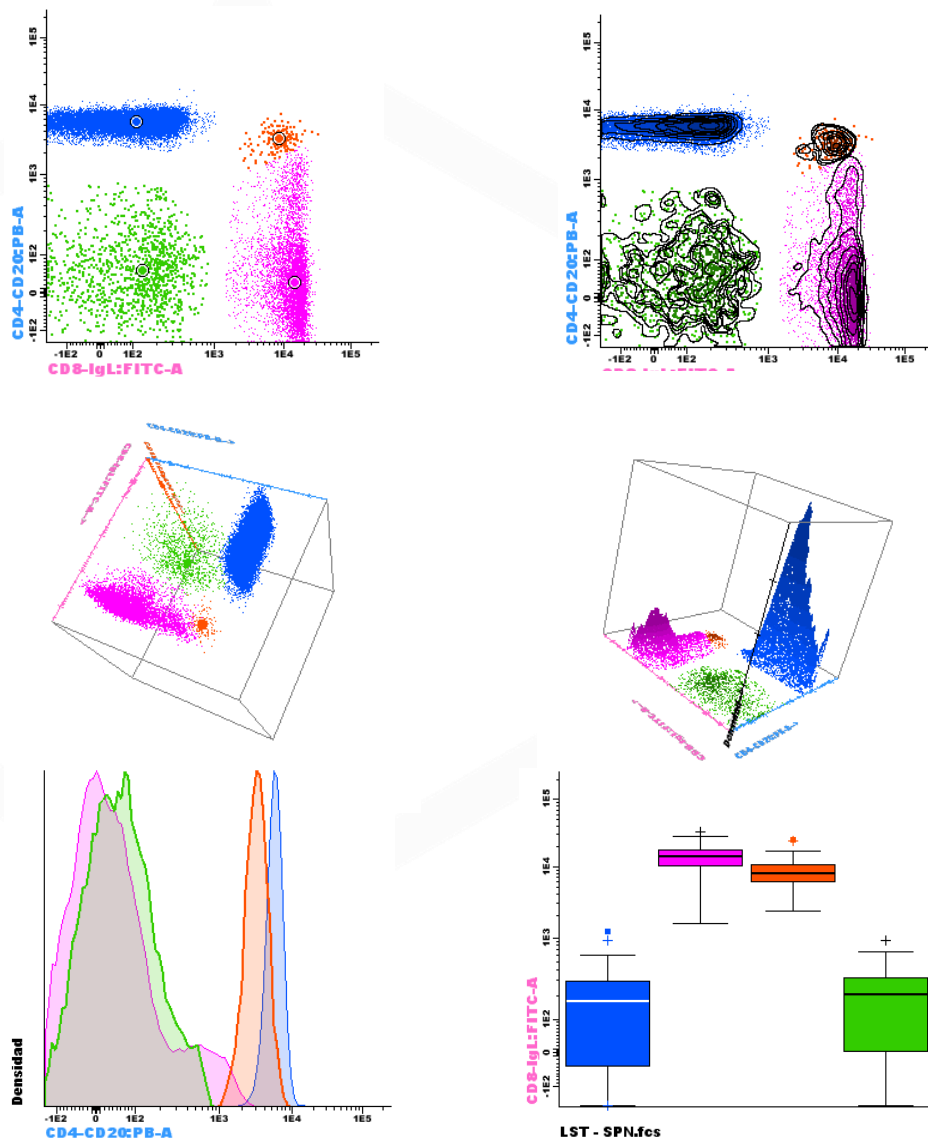


Data Analysis

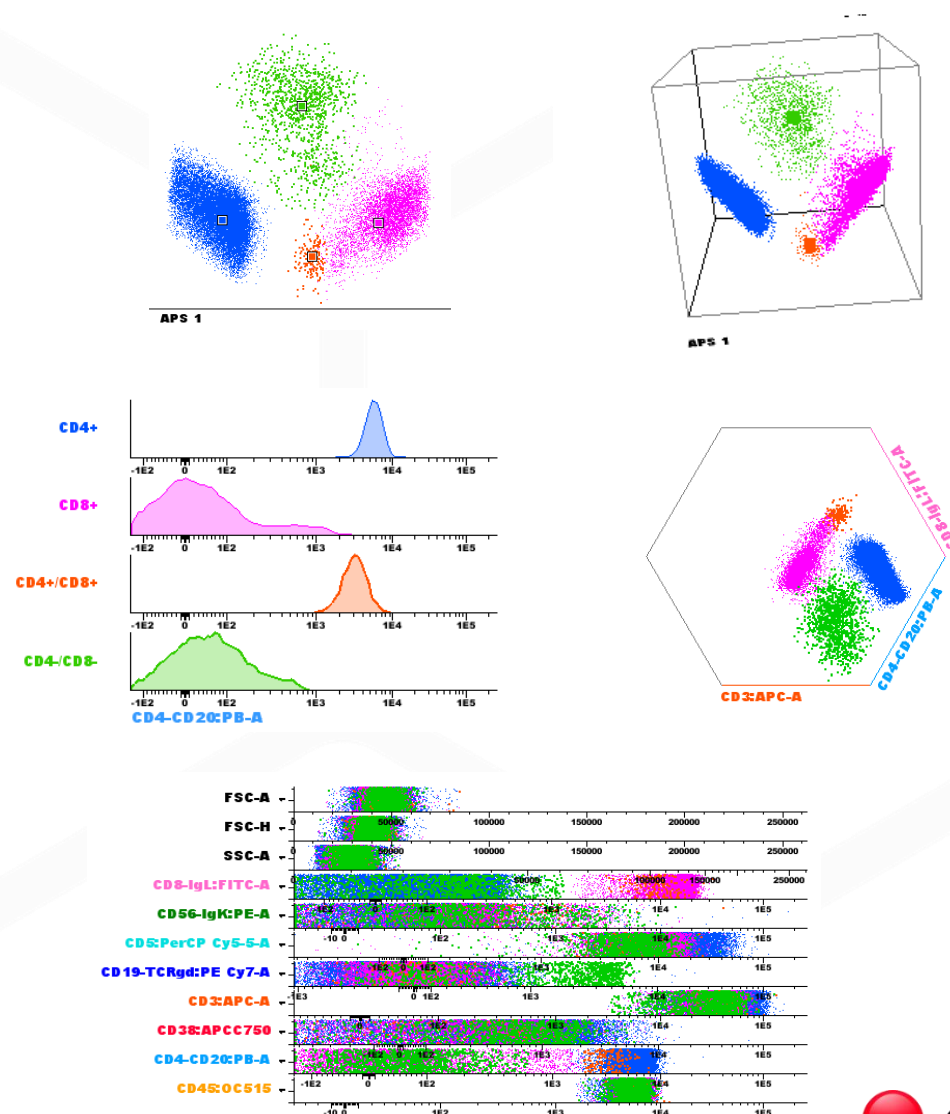
- ✓ **Classic and Multiparametric Diagrams**
- ✓ **Automatic Population Separator (APS) Diagram**
- ✓ **Population Tree and statistics**
- ✓ **Analysis Strategy**
- ✓ **Report**
- ✓ **Profile**

DIAGRAMS

Classic Diagrams

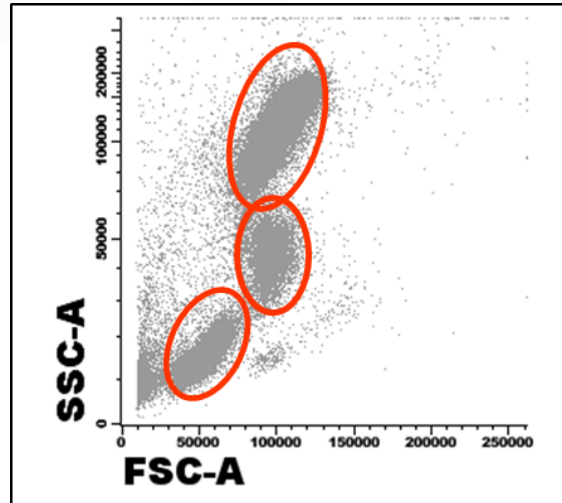


Multiparametric Diagrams



APS DIAGRAM

APS diagram takes into account all parameters to display the best separation of events in clusters.



CD8 CD5 CD4
FSC CD56 CD3
SSC CD19 IgK
CD20
CD45 CD38 IgL

→

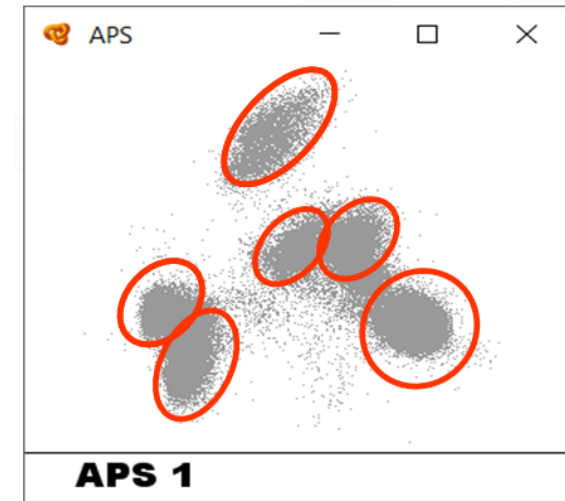


Diagram Info

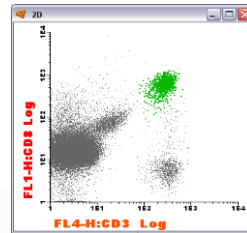
Parameter	Significance
CD19:PE Cy7-A LOGICAL	20.93
CD3:APC-A LOGICAL	16.31
CD5:PerCP Cy5-5-A LOGICAL	10.65
CD20+CD4:PB-A LOGICAL	10.53
SSC-A Exp-SSC Low	9.56
CD56+IgK:PE-A LOGICAL	9.37
CD8+IgL:FIT C-A LOGICAL	8.88
CD38:APC H7-A LOGICAL	5.92
CD45:PO-A LOGICAL	4.41
FSC-A LINEAR	3.43

OK

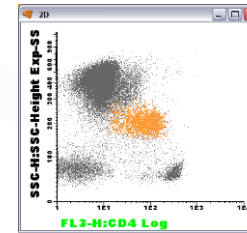
Exclusive tool!

APS DIAGRAM

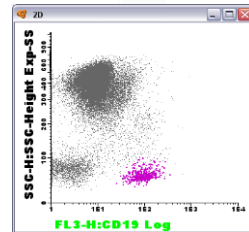
CD8+ T-LYMPHOCYTES



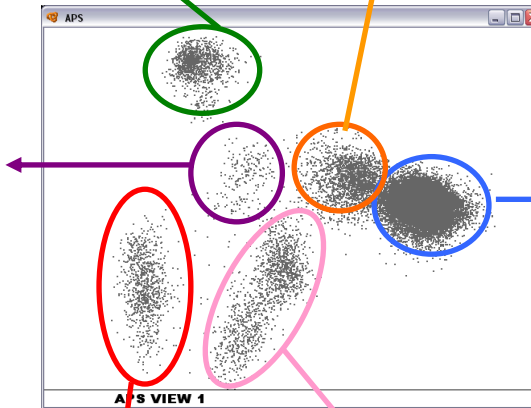
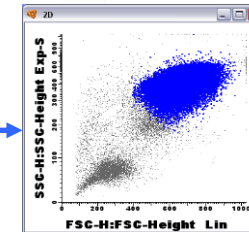
MONOCYTES



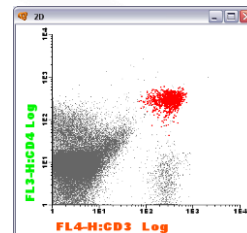
B-CELLS



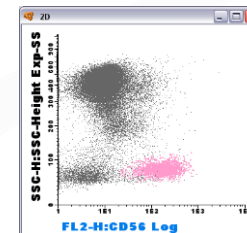
NEUTROPHILS



CD4+ T-LYMPHOCYTES



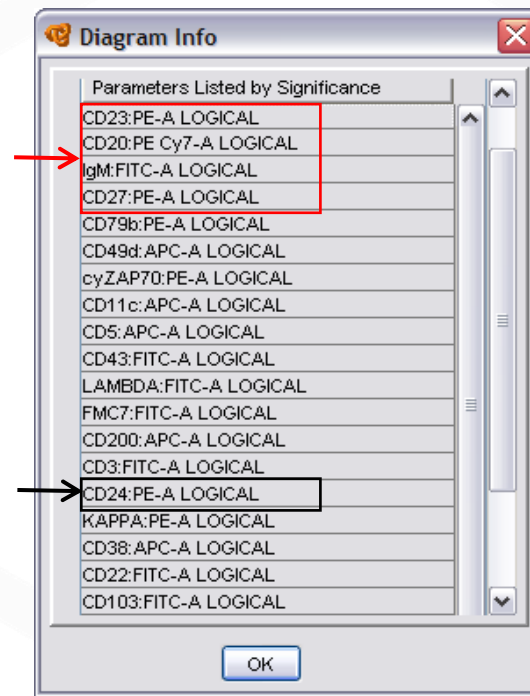
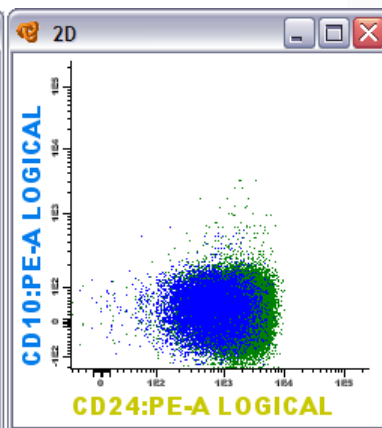
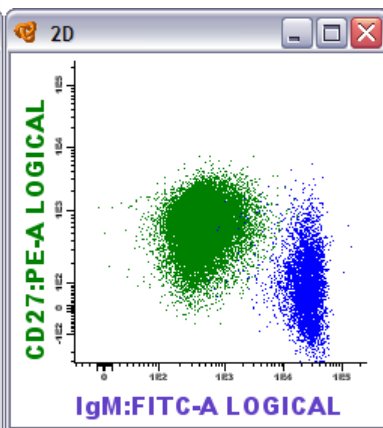
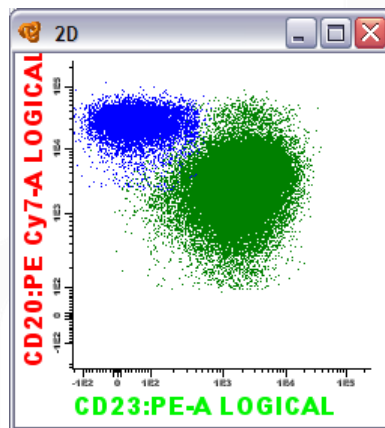
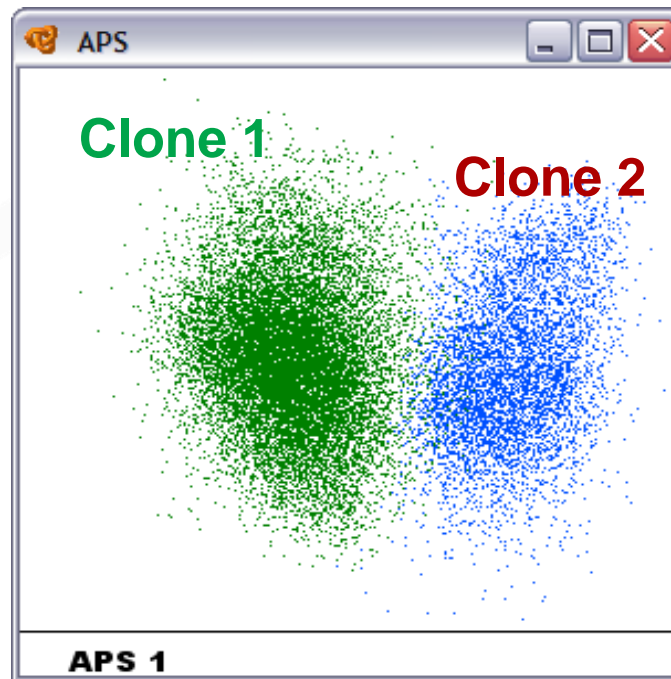
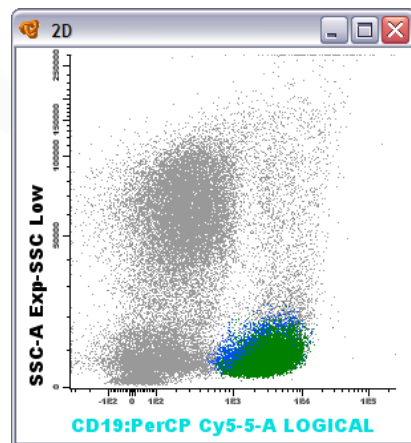
NK-CELLS



Exclusive tool!

APS DIAGRAM

B-cell compartment



Exclusive tool!

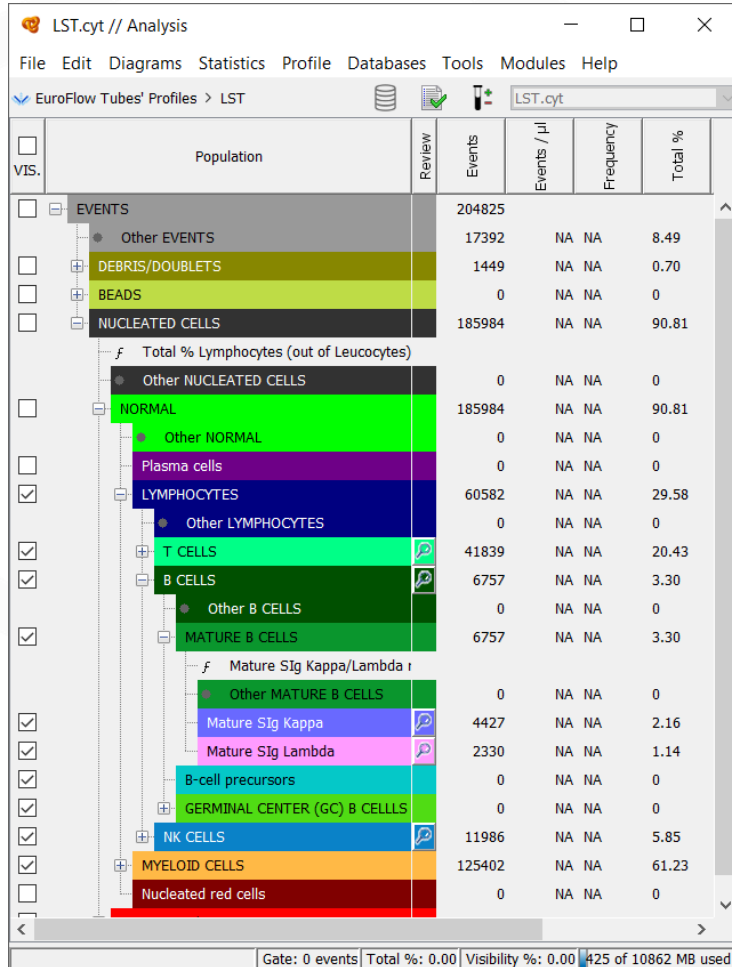
APPLICATIONS OF APS DIAGRAM

The APS diagram can be used for both clinical and research purposes.

- ✓ Additional multiparametric diagram for data analysis.
- ✓ Displays the events with the best cluster separation in a single diagram.
- ✓ Helps to identify the pathological population *versus* the normal counterpart.
- ✓ Aids to design your own panels.

POPULATION TREE AND STATISTICS

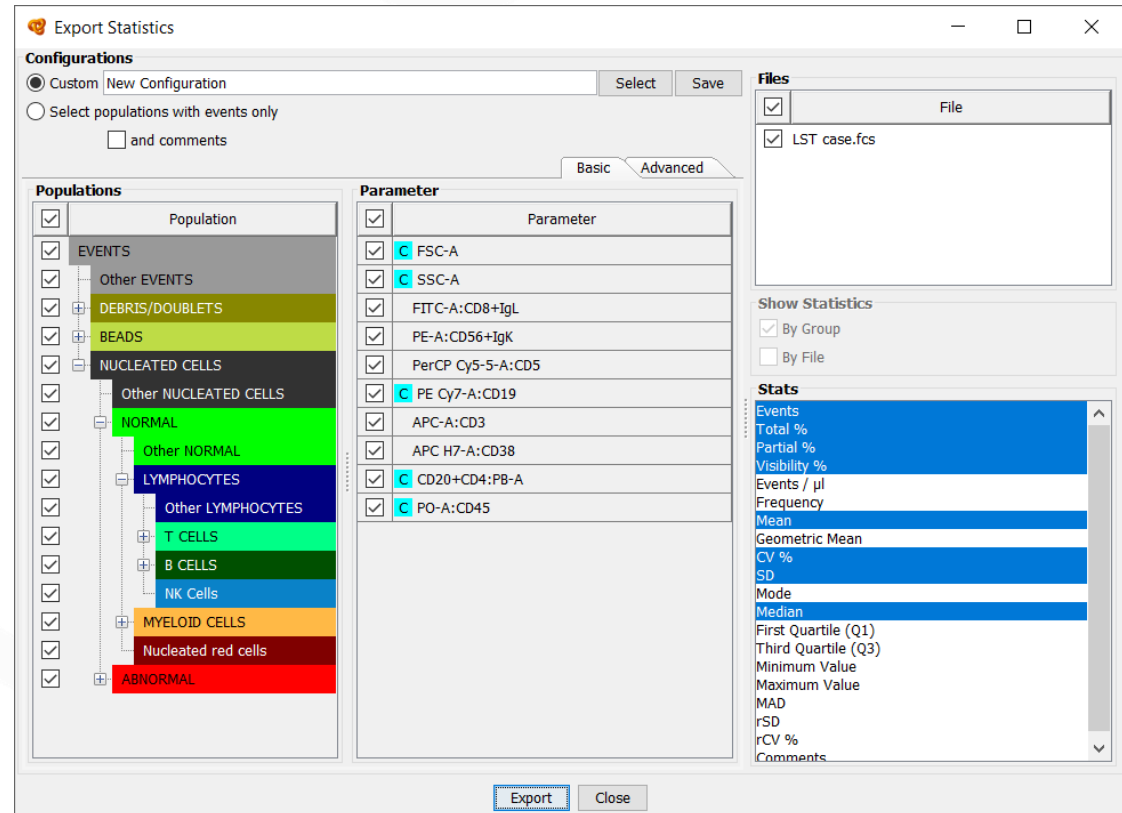
Infinicyt includes robust statistics which are less influenced by outlying events. Export data to other programs to perform further statistical analysis.



The screenshot shows the 'LST.cyt // Analysis' window. The 'Populations' pane on the left displays a hierarchical tree of cell populations. The main table lists the following populations with their respective event counts, volume, frequency, and total percentage:

Population	Events	Events / μ l	Frequency	Total %
EVENTS	204825			
Other EVENTS	17392	NA	NA	8.49
DEBRIS/DOUBLETS	1449	NA	NA	0.70
BEADS	0	NA	NA	0
NUCLEATED CELLS	185984	NA	NA	90.81
Total % Lymphocytes (out of Leucocytes)				
Other NUCLEATED CELLS	0	NA	NA	0
NORMAL	185984	NA	NA	90.81
Other NORMAL	0	NA	NA	0
Plasma cells	0	NA	NA	0
LYMPHOCYTES	60582	NA	NA	29.58
Other LYMPHOCYTES	0	NA	NA	0
T CELLS	41839	NA	NA	20.43
B CELLS	6757	NA	NA	3.30
Other B CELLS	0	NA	NA	0
MATURE B CELLS	6757	NA	NA	3.30
Mature S1g Kappa/Lambda				
Other MATURE B CELLS	0	NA	NA	0
Mature S1g Kappa	4427	NA	NA	2.16
Mature S1g Lambda	2330	NA	NA	1.14
B-cell precursors	0	NA	NA	0
GERMINAL CENTER (GC) B CELLS	0	NA	NA	0
NK CELLS	11986	NA	NA	5.85
MYELOID CELLS	125402	NA	NA	61.23
Nucleated red cells	0	NA	NA	0

At the bottom of the window, a status bar indicates: Gate: 0 events | Total %: 0.00 | Visibility %: 0.00 | 425 of 10862 MB used.



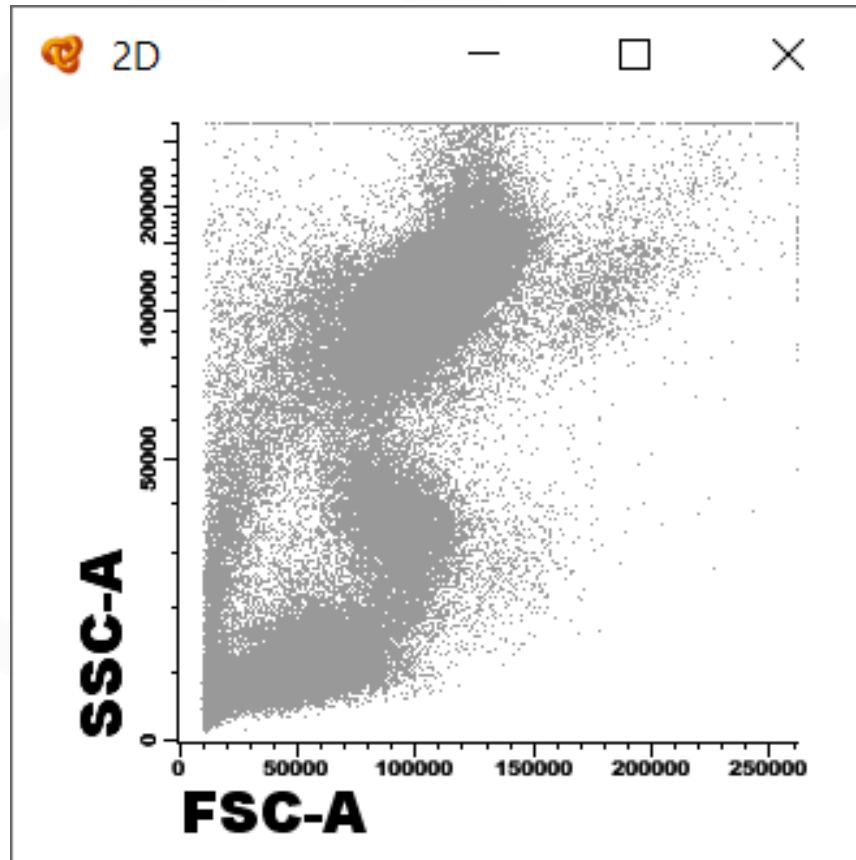
The 'Export Statistics' dialog box is shown with the 'Configurations' tab selected. The 'Custom' radio button is chosen. The 'Populations' list on the left includes all populations from the main window. The 'Parameter' list on the right includes the following parameters:

- FSC-A
- SSC-A
- FITC-A:CD8+IgI
- PE-A:CD56+IgK
- PerCP Cy5-5-A:CD5
- PE Cy7-A:CD19
- APC-A:CD3
- APC H7-A:CD38
- CD20+CD4:PB-A
- PO-A:CD45

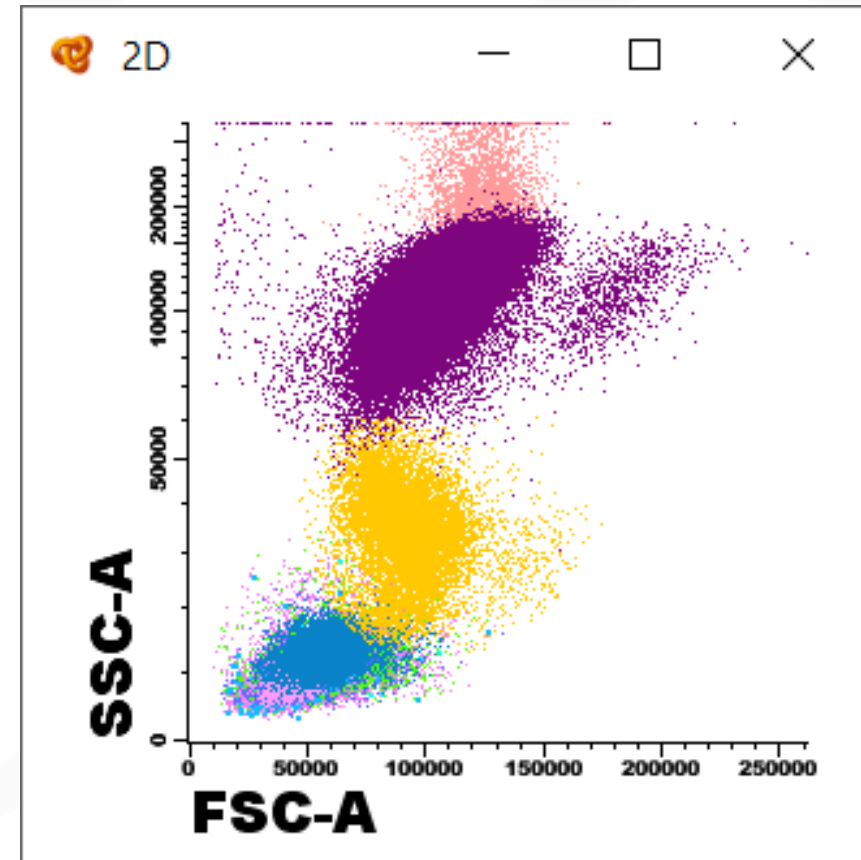
The 'Files' section on the right shows a list of files with 'LST case.fcs' selected. The 'Show Statistics' section has 'By Group' selected. The 'Stats' section lists various statistical parameters including Events, Total %, Partial %, Visibility %, Events / μ l, Frequency, Mean, Geometric Mean, CV %, SD, Mode, Median, First Quartile (Q1), Third Quartile (Q3), Minimum Value, Maximum Value, MAD, rSD, rCV %, and Comments. The 'Export' button is highlighted at the bottom.

ANALYSIS STRATEGY

Raw file

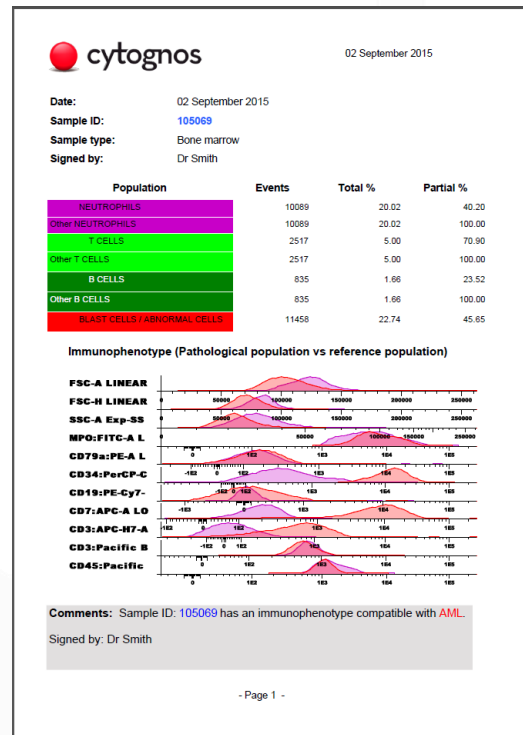
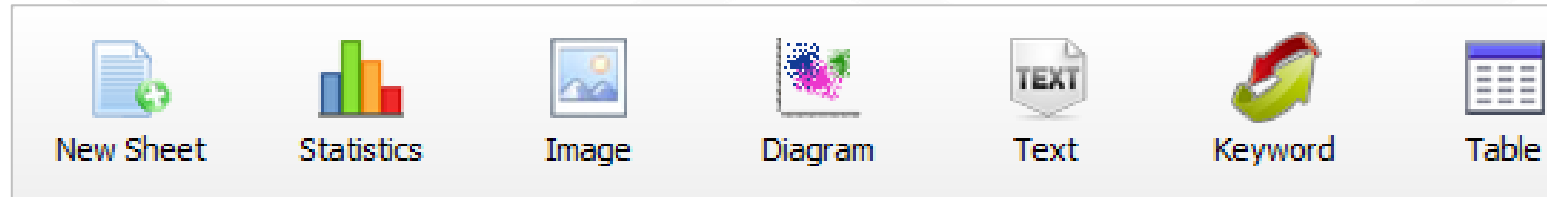


Analyzed file



ANALYSIS & DATA INTERPRETATION – REPORT

Additional functionality is added in 2.0 version of Infinicyt.



✓ Interface improvement

✓ New toolbar

✓ Keywords

Date automatically updated

Manager of Infinicyt and User Comments

✓ Automated Report with Change Controls

✓ Header and Footer improvements

✓ Compatible with LIS systems

PROFILE

PROFILE

=

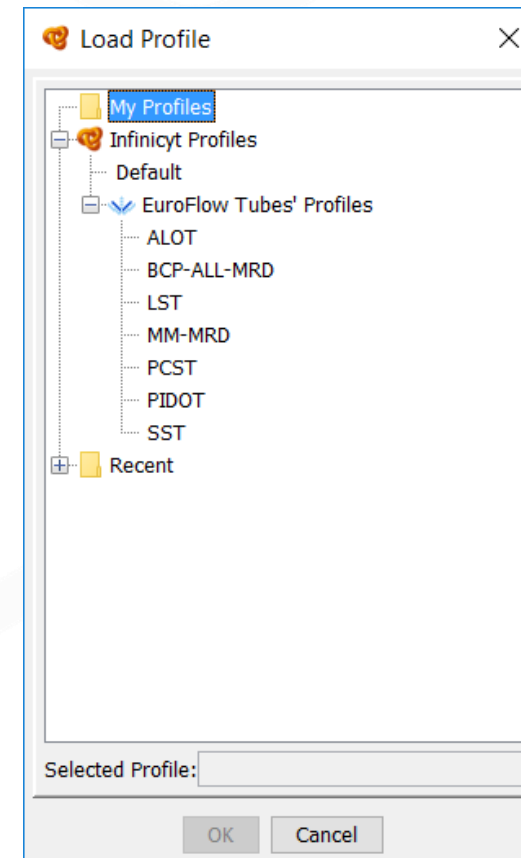
ANALYSIS TEMPLATE

CONFIGURATION OF WORKSPACE

- Diagrams and Statistics
- Population Tree
- Report

OTHER CONFIGURATIONS

- Compensation Matrix
- Analysis Strategy
- Reference Image
- Batch Analysis



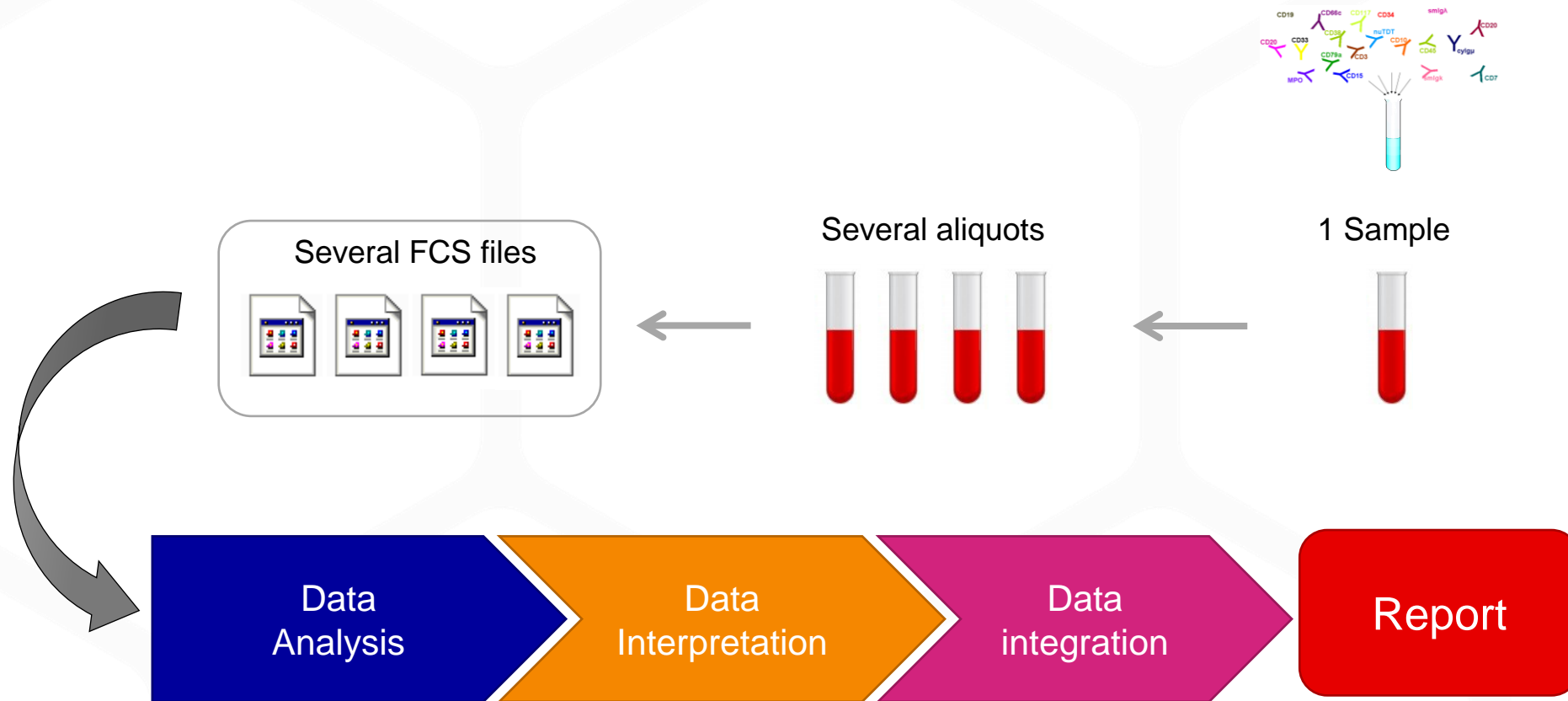
AGENDA

Data Integration

- ✓ **File Merge tool**
- ✓ **Calculate Data**

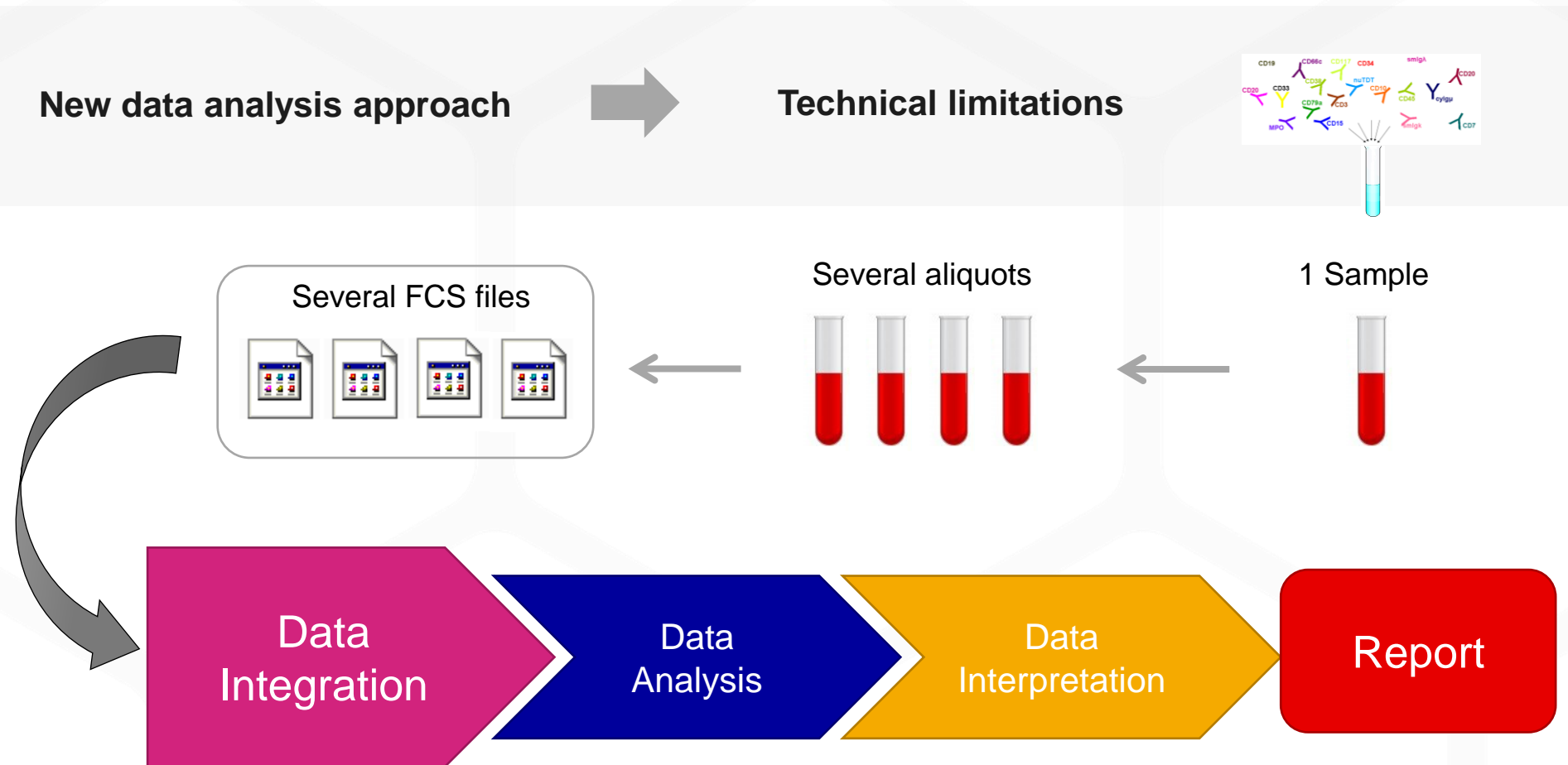
FILE MERGE TOOL

Technical limitations are derived from multicolor flow cytometry.



WHY SHOULD WE USE THE FILE MERGE TOOL?

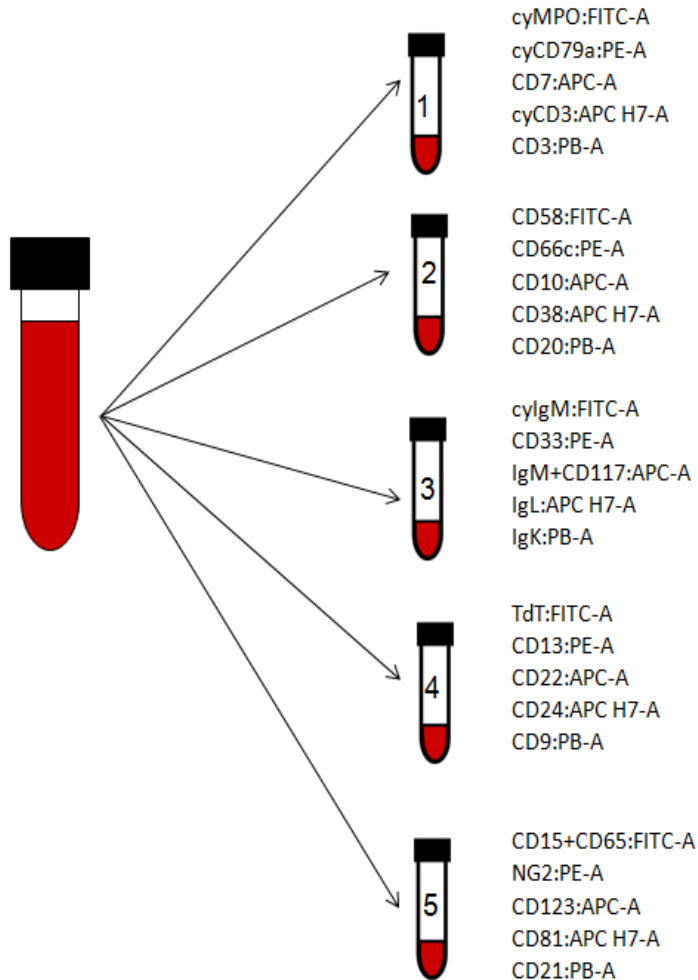
To overcome technical limitations derived from multicolor flow cytometry.



APPLICATIONS OF THE FILE MERGE TOOL

Patient sample

Aliquots



+

Common markers

FSC-A
SSC-A
CD34:PerCP Cy5-5-A
CD19:PE Cy7-A
CD45:PO-A

FILE
MERGE

SINGLE FILE

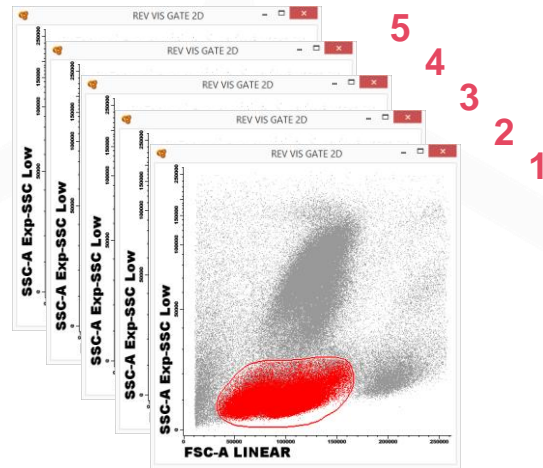
- 500.000 events
- All the marker's information

5 files / 100.000 events per tube

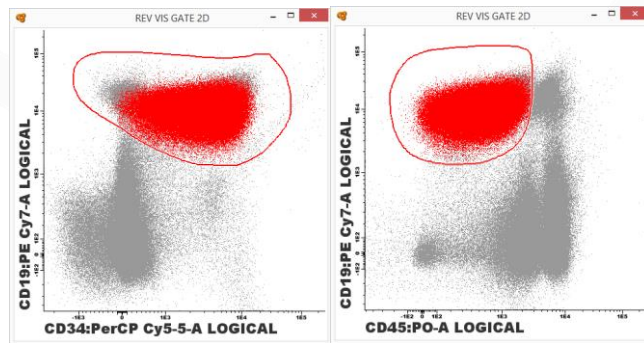
ALOT (Acute Leukemia Screening Tube)
BCP-ALL panel

APPLICATIONS OF THE FILE MERGE TOOL

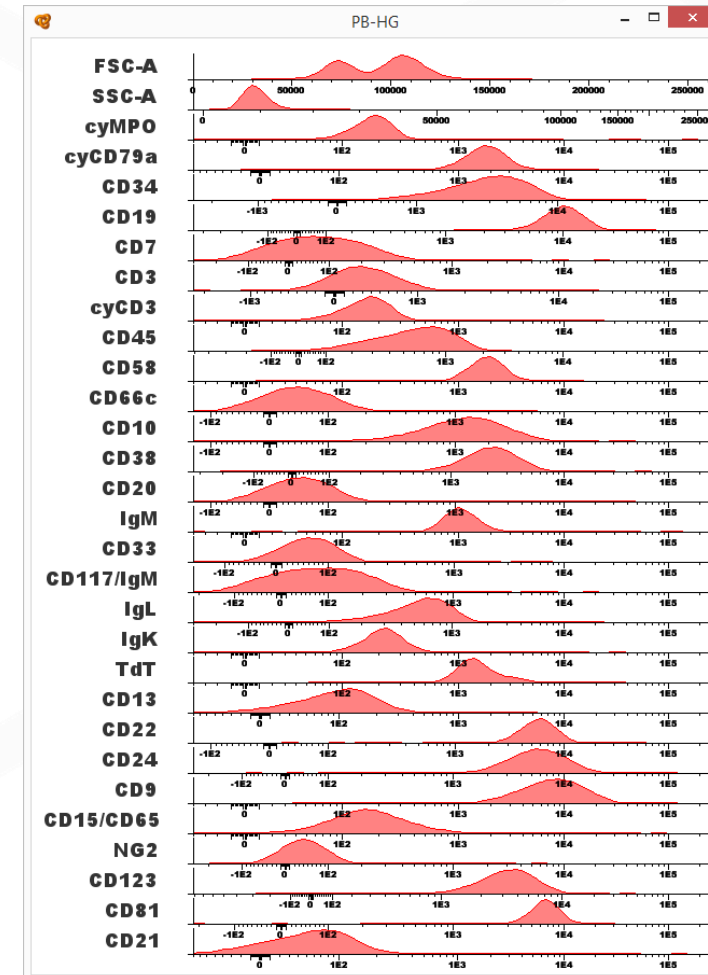
ALOT (Acute Leukemia Screening Tube)
BCP-ALL panel



File Merge + 3 gates => Global
analysis



Complete immunophenotype



CALCULATE DATA VS FILE MERGE

ALOT (Acute Leukemia Screening Tube)
BCP-ALL panel

File Merge

File Parameters		Info				
Parameter\File	1	2	3	4	5	
FSC-A	C	C	C	C	C	
SSC-A	C	C	C	C	C	
FITC-A:CD8+IgL	R	
PE-A:CD56 +IgK	R	
PerCP Cy5-5-A:CD5	R	
PE Cy7-A:CD19	C	C	C	C	C	
APC-A:CD3	R	
APC H7-A:CD38	R	
PO-A:CD45	C	C	C	C	C	
FITC-A:CD23	...	R	
PE-A:CD10	...	R	
PerCP Cy5-5-A:CD79b	...	R	
APC-A:CD200	...	R	
CD20:PB-A	C	C	C	C	C	
APC H7-A:CD43	...	R	
FITC-A:CD31	R	
PE-A:LAIR1	R	
PerCP Cy5-5-A:CD11c	R	
APC-A:IgM	R	
APC H7-A:CD81	R	
FITC-A:CD103	R	...	
PE-A:CD95	R	...	
PerCP Cy5-5-A:CD22	R	...	
APC-A:CXCR5	R	...	
APC H7-A:CD49d	R	...	
FITC-A:CD62L	R	
PE-A:CD39	R	
PerCP Cy5-5-A:HLADR	R	
APC-A:CD27	R	
APC H7-A	R	

Calculate
Data

File Parameters		Info				
Parameter\File	1	2	3	4	5	
FSC-A	C	C	C	C	C	
SSC-A	C	C	C	C	C	
FITC-A:CD8+IgL	R	D	D	D	D	
PE-A:CD56+IgK	R	D	D	D	D	
PerCP Cy5-5-A:CD5	R	D	D	D	D	
PE Cy7-A:CD19	C	C	C	C	C	
APC-A:CD3	R	D	D	D	D	
APC H7-A:CD38	R	D	D	D	D	
PO-A:CD45	C	C	C	C	C	
FITC-A:CD23	D	R	D	D	D	
PE-A:CD10	D	R	D	D	D	
PerCP Cy5-5-A:CD79b	D	R	D	D	D	
APC-A:CD200	D	R	D	D	D	
CD20:PB-A	C	C	C	C	C	
APC H7-A:CD43	D	R	D	D	D	
FITC-A:CD31	D	D	R	D	D	
PE-A:LAIR1	D	D	R	D	D	
PerCP Cy5-5-A:CD11c	D	D	R	D	D	
APC-A:IgM	D	D	R	D	D	
APC H7-A:CD81	D	D	R	D	D	
FITC-A:CD103	D	D	D	R	D	
PE-A:CD95	D	D	D	R	D	
PerCP Cy5-5-A:CD22	D	D	D	R	D	
APC-A:CXCR5	D	D	D	R	D	
APC H7-A:CD49d	D	D	D	R	D	
FITC-A:CD62L	D	D	D	D	R	
PE-A:CD39	D	D	D	D	R	
PerCP Cy5-5-A:HLADR	D	D	D	D	R	
APC-A:CD27	D	D	D	D	R	
APC H7-A	D	D	D	D	R	

AGENDA

Data Interpretation

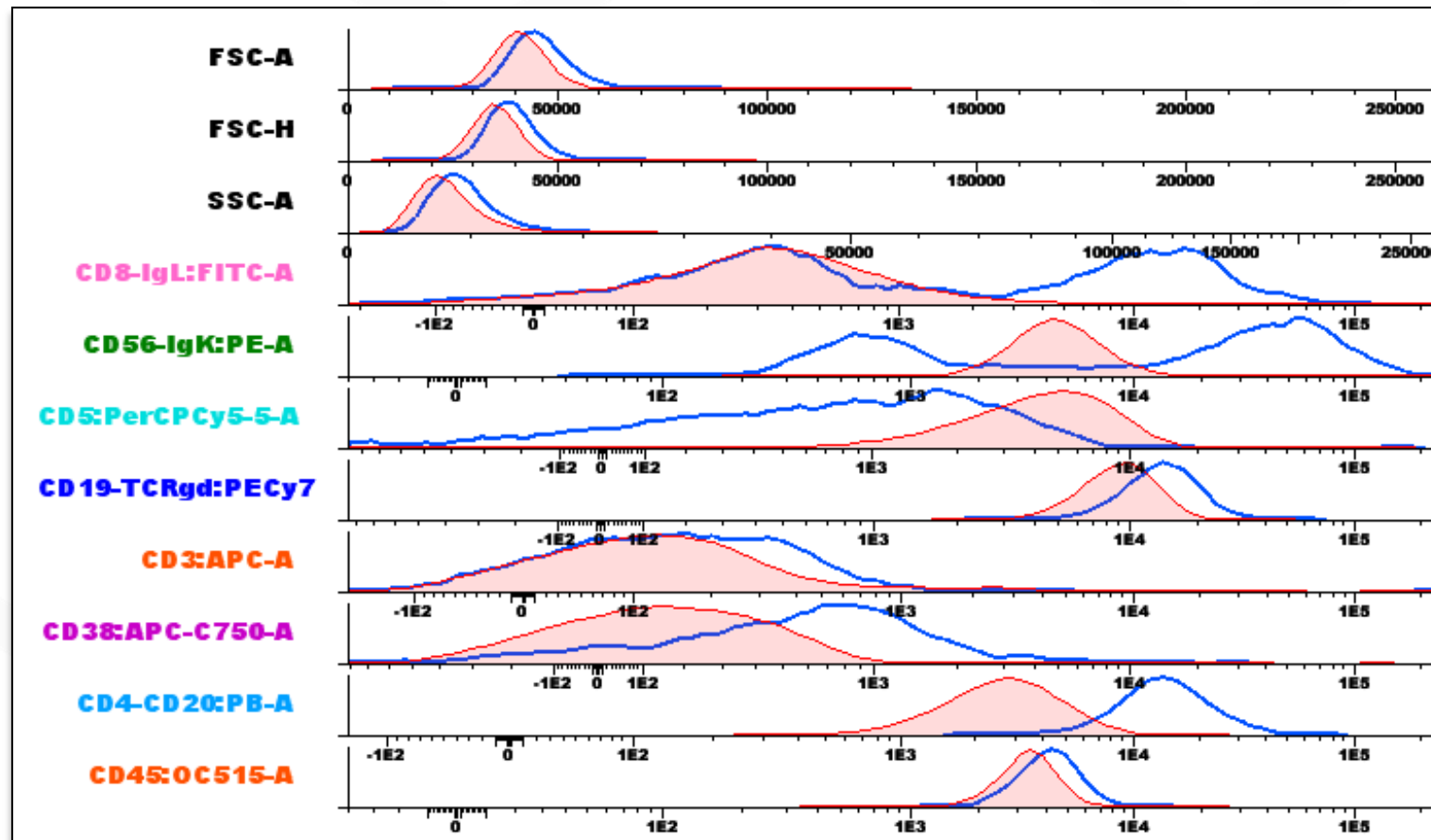
- ✓ **Reference Image**
- ✓ **Compass tool**
- ✓ **Maturation tool**
- ✓ **Report**
- ✓ **EuroFlow™ Databases for sample comparison**

REFERENCE IMAGE

New approach to data interpretation



Stored references helps with interpretation which will not only be experience-based



Normal pattern
(Reference Image)

Case study

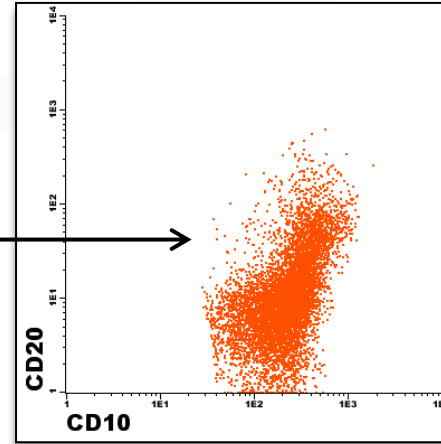
Patented tool!

APPLICATIONS OF THE REFERENCE IMAGE TOOL

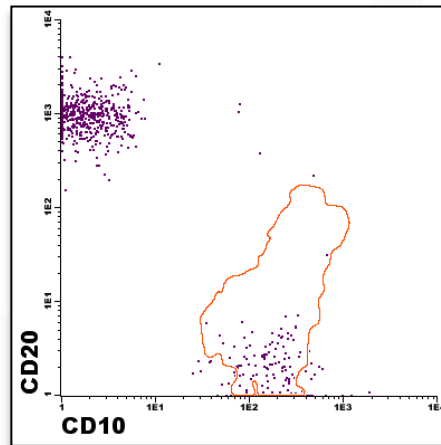
Follow-up of disease

B-ALL blast cells at diagnosis

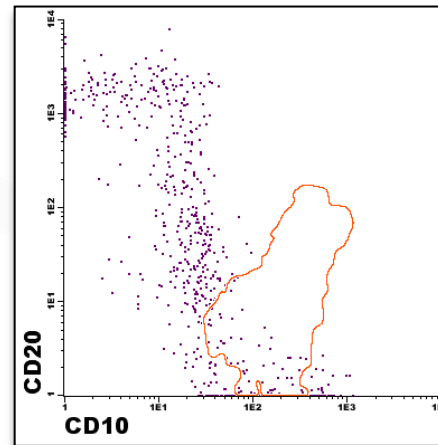
Reference Image



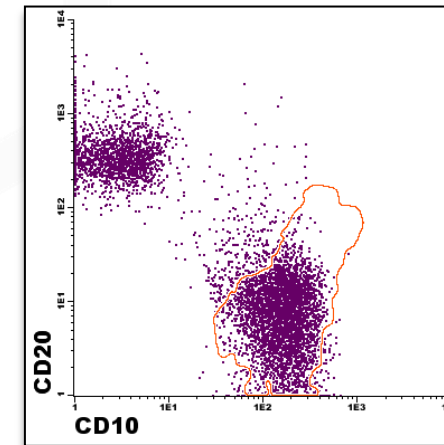
Day +14



Day +100



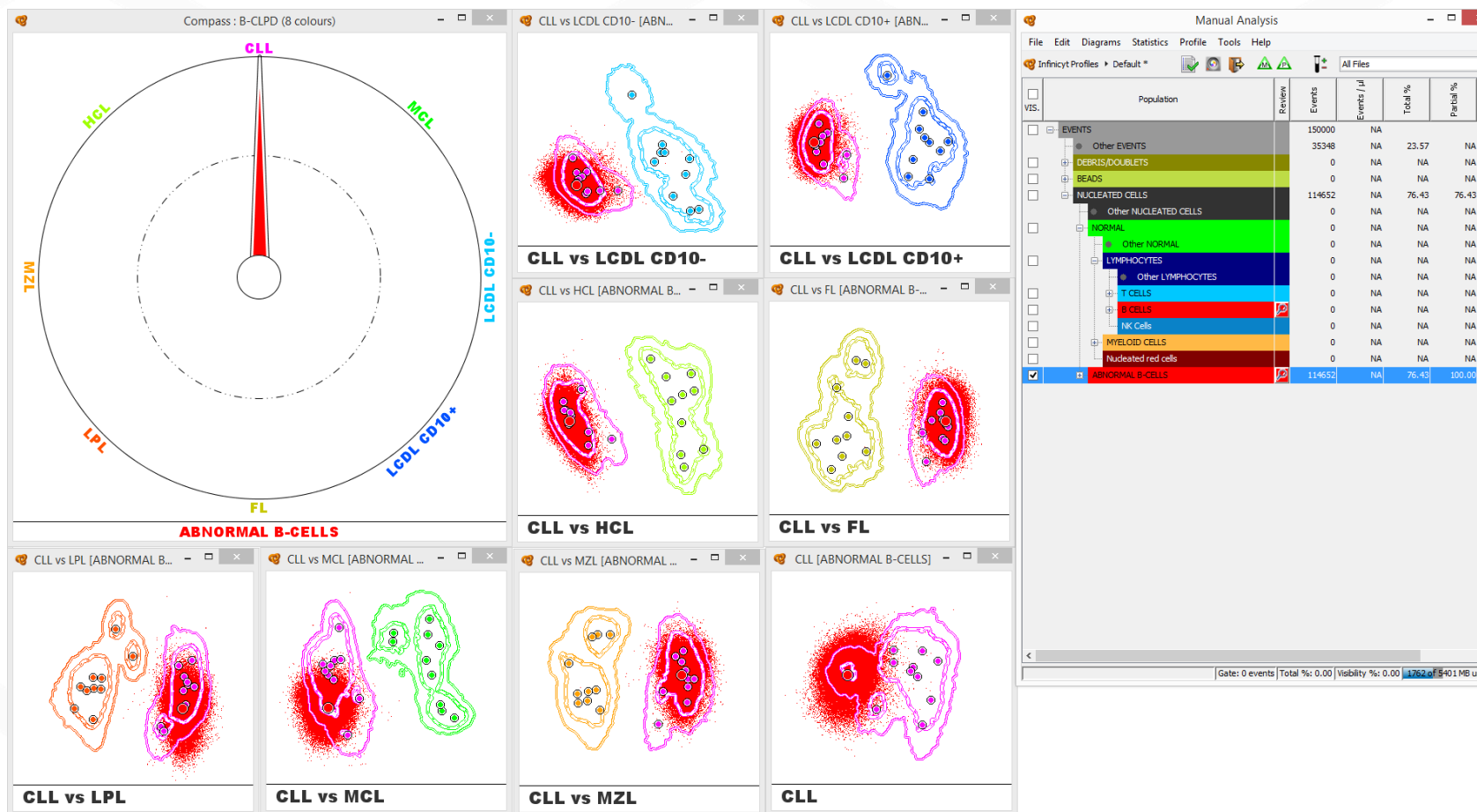
Month +24



Patented tool!

DATA INTERPRETATION – COMPASS TOOL

Study case vs Reference cases (Database)



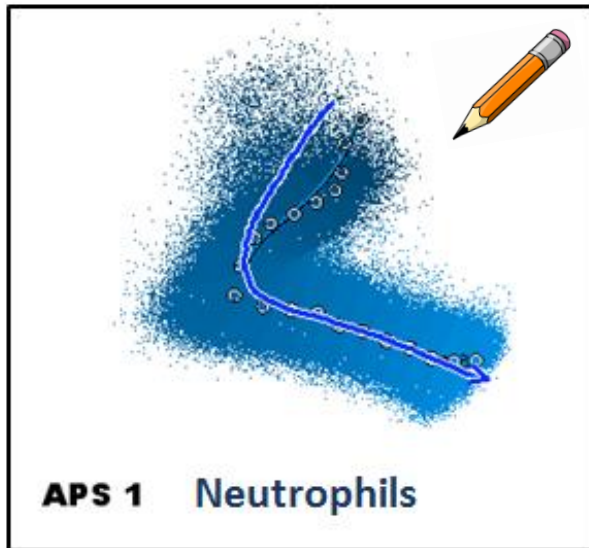
Exclusive tool!

Aids in diagnosis, **not** a diagnostic tool

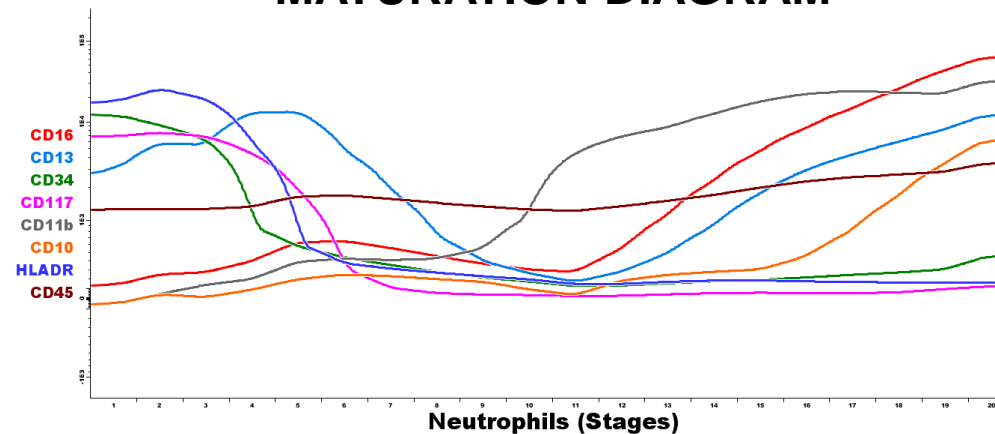
DATA INTERPRETATION – MATURATION TOOL

Assesment of maturation pathways

1. Identify the population of interest
2. Draw the Maturation Path on the APS Diagram
3. Assess the Maturation Diagram



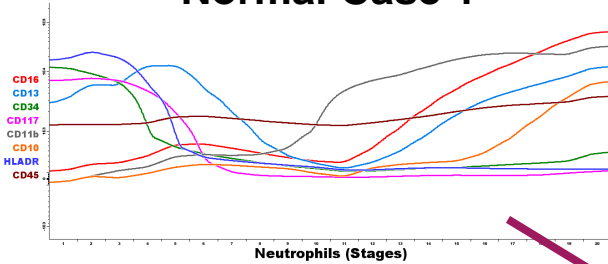
MATURATION DIAGRAM



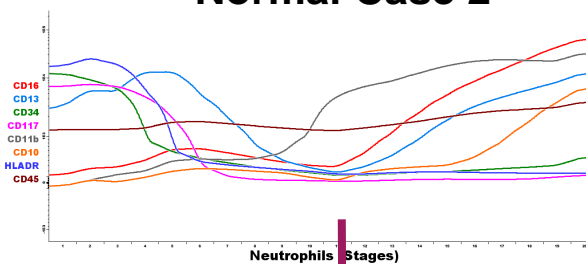
Exclusive tool!

DATA INTERPRETATION - MATURATION TOOL

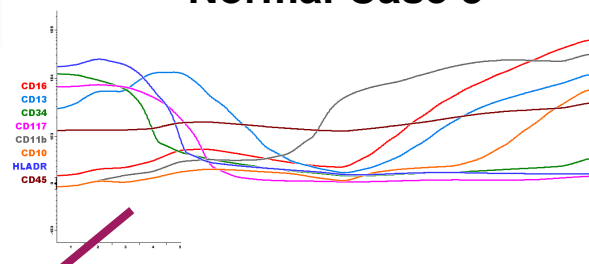
Normal Case 1



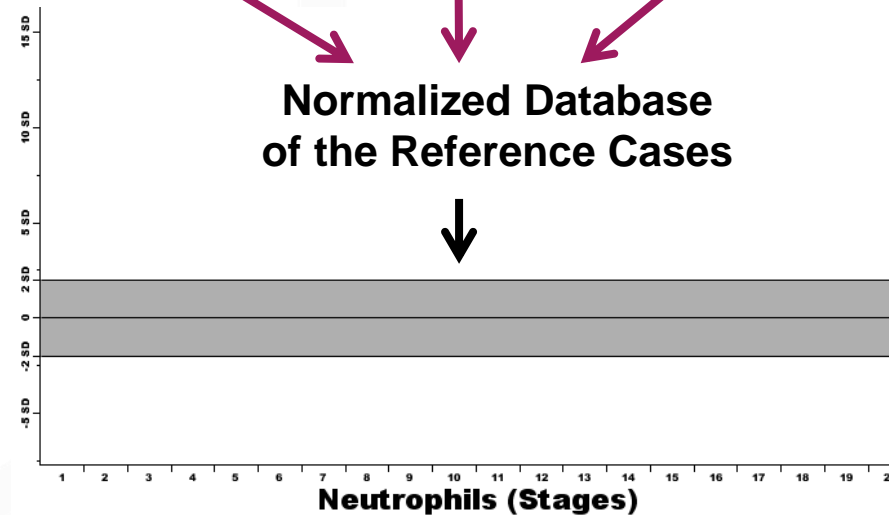
Normal Case 2



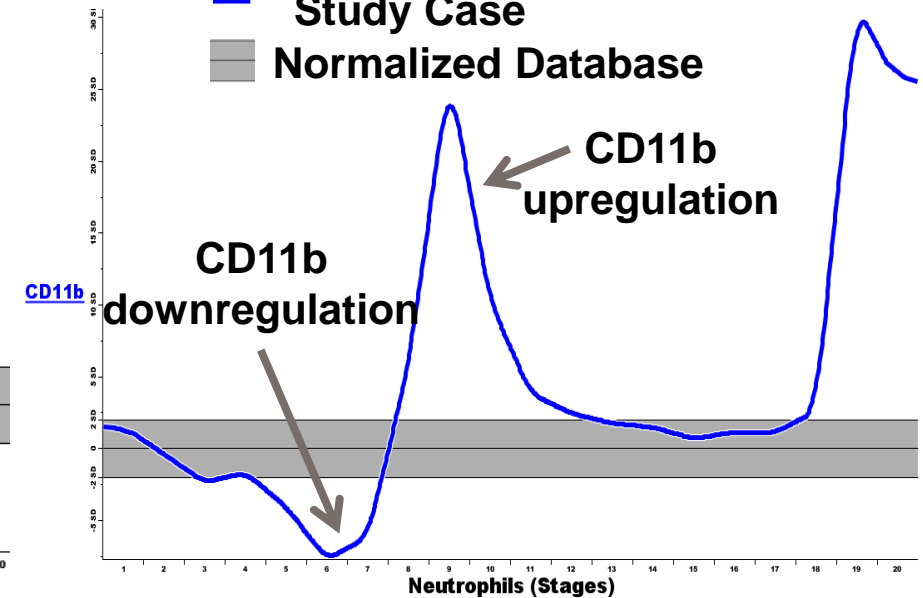
Normal Case 3



Normalized Database
of the Reference Cases

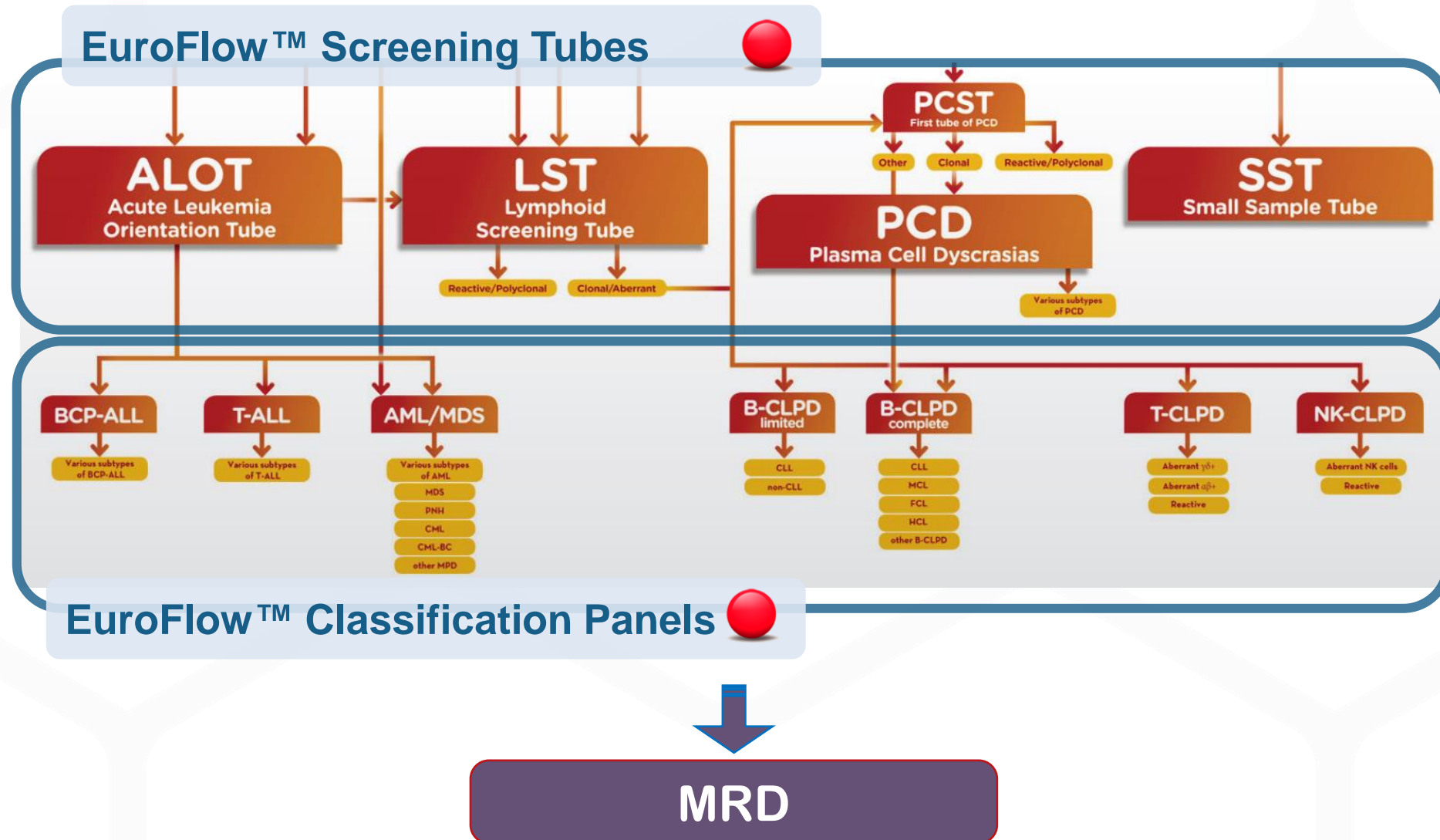


Study Case
Normalized Database

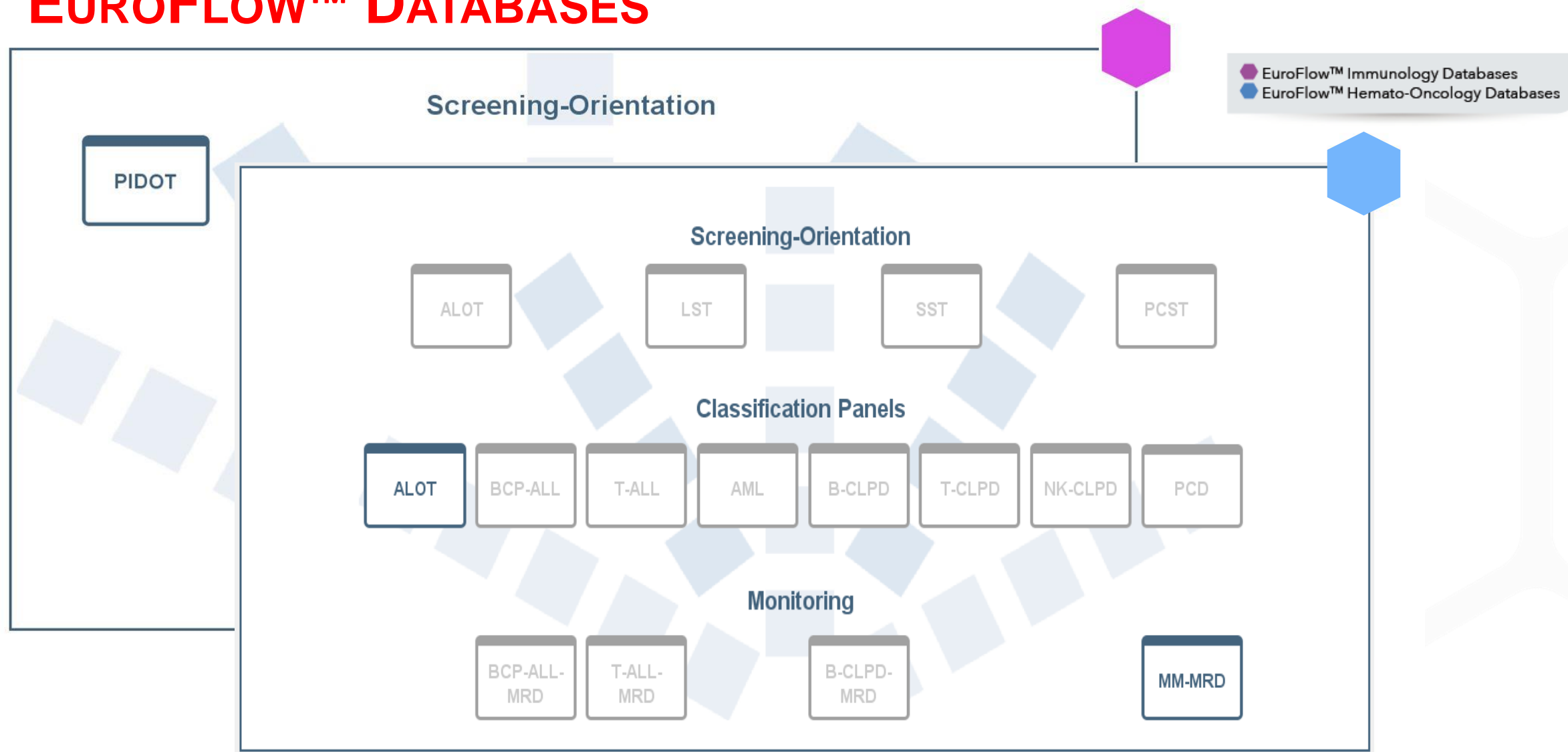


Exclusive tool!

EUROFLOW™ WORKFLOW



EUROFLOW™ DATABASES



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