Assessment of maturation pathways in Kaluza®: Applications in AML and MDS

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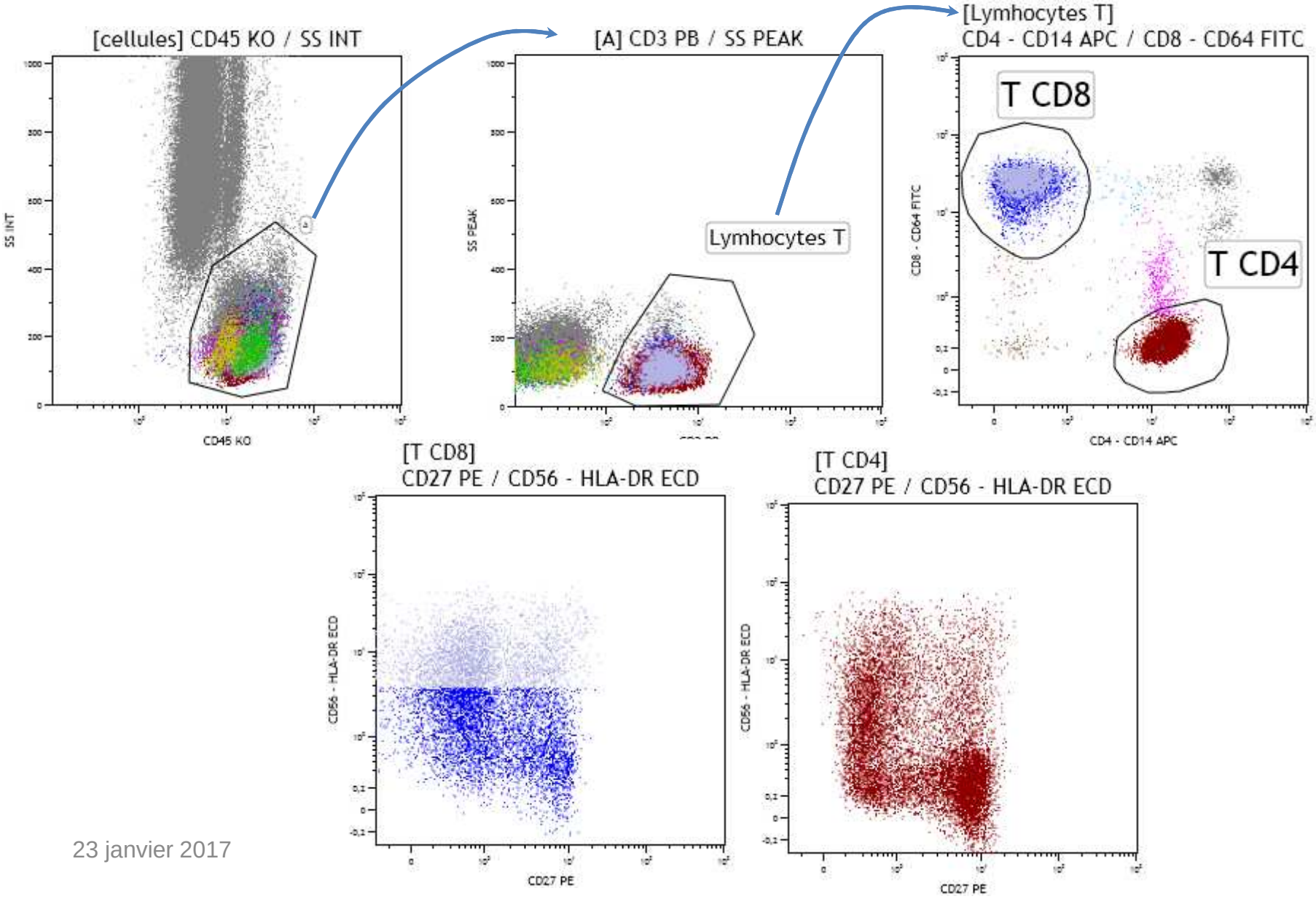
INTRODUCTION

- Flow cytometry is:
- Rapid and could used in emergency
- expensive
  - lymphocyte typing = ± € 33
  - Typing leukemia / immunodeficiency = ± 180 €
- Very sensitive (detection of rare events up to $1/10^5$ cel)
  - In addition to the molecular biology
  - Requires careful removal of artifacts
- Very specific if the phenotype of the cell is typical
  - Ex: residual disease 1/105 to 1/106 for B-CLL

MULTIPARAMETRIC FLOW CYTOMETRY AND ITS REPRESENTATION

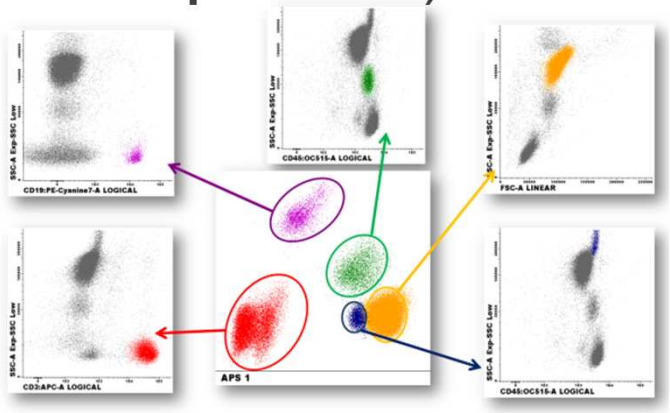
- 8-10 to twelve colors
  - 10 colors Navios BC
    - 5 on blue Laser (488nm)
    - 3 on red laser (633 nm)
    - 2 on violet laser (405 nm)
- Acquisition 200,000 event until 2 million in the analysis of rare populations.
- Historically, it uses the principle of sorting in cascade, on two-dimentional plot (dot plor or density plot

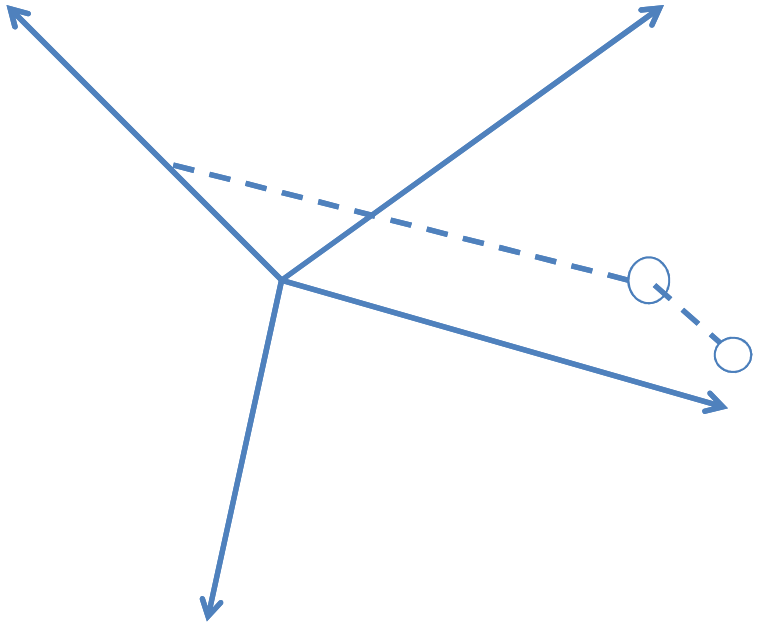
SORTING IN CASCADE



MULTI-VECTOR REPRESENTATION

- Infinycit® APS (Automatic Population Separator)
- Kaluza's Radar
  - no limit to the number of vectors
  - The position of each point depends on its vector coordinates..





INTEREST OF THE COMBINATION OF THE AXES

- The coincidence or opposition of axes allow for Boolean relationships within the radar plot
  - Two axes put together = "and"
  - Two axes in opposition = ", not" (and not)
- One can also use the combination of axes to eliminate
  - non-specific binding (arrows)
  - Partially fluorescent artifacts
- You can put lines with markers of immaturity in conflict with the axes of maturity markers.
  - Thus we can stretch into space, cell maturation pathways

BONE MARROW ROUTINE PANEL

- Myelodiff
  - maturation pathways analysis for blast, erythroid, myeloid and monocytic differentiation

FITC	PE	ECD	PC5.5	PC7	APC	AA700	AA750	PB	KO
CD64 CD71	CD36 CD294	CD33	CD117	CD16	CD13 CD14	CD34	CD38	CD15	CD45

B cell differentiation (κ/λ)-B blasts to memory B cells

FITC	PE	ECD	PC5.5	PC7	APC	AA700	AA750	PB	KO
LAMB	CD19	CD27	CD5	CD10	KAPPA	IGD	CD38	CD20	CD45

SLD (T sub-population ans plasmocytes)

FITC	PE	ECD	PC5.5	PC7	APC	AA700	AA750	PB	KO
CD8 CD64	CD27 cd138	CD56 – DR	CD5	CD19 *Tcrγ–δ*	CD4 CD14	CD34 CD16	CD38	CD3 CD20	CD45

REMOVAL OF ARTIFACTS, DEBRIS AND NON-CELLULAR EVENTS

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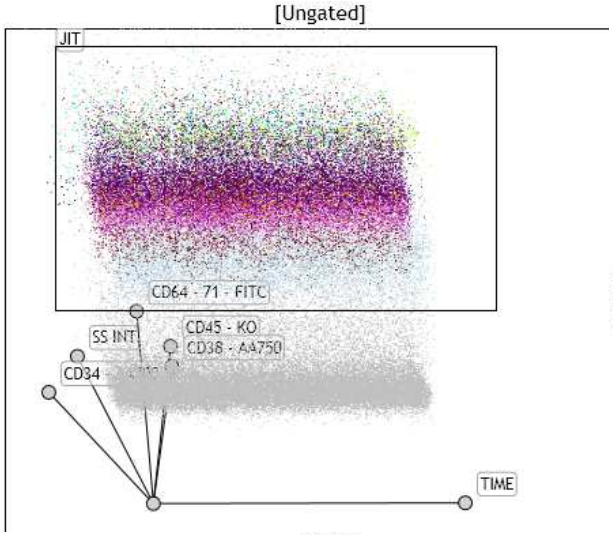
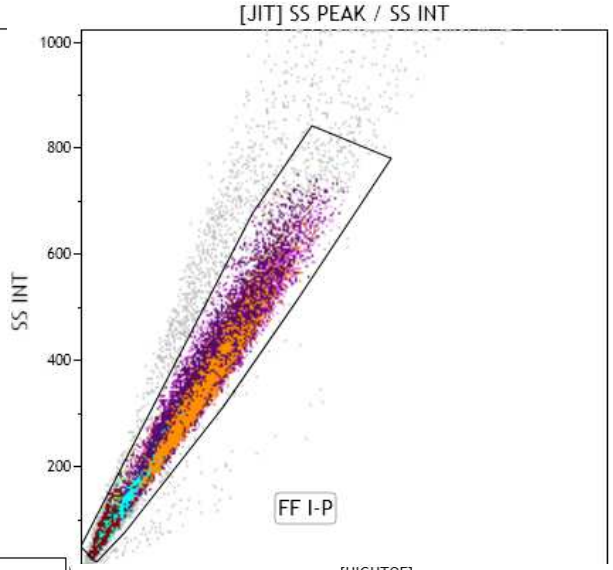
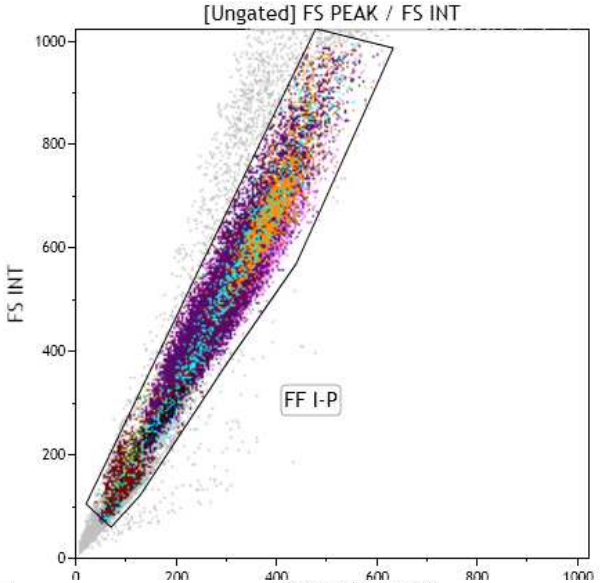
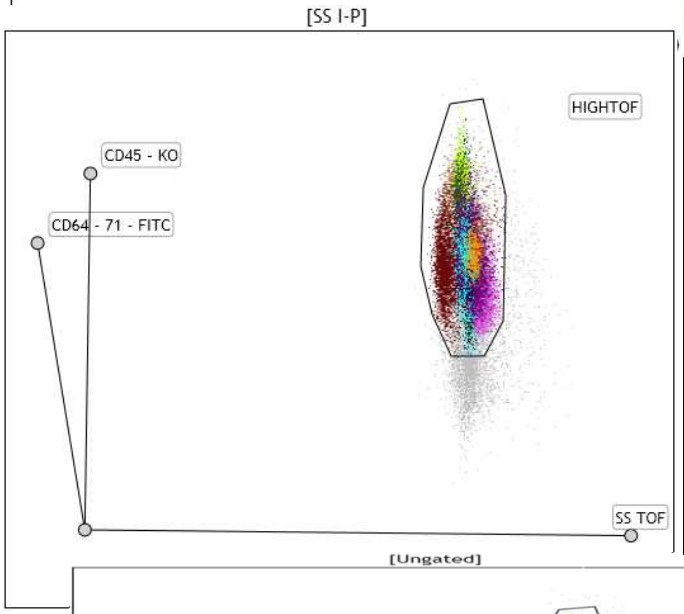
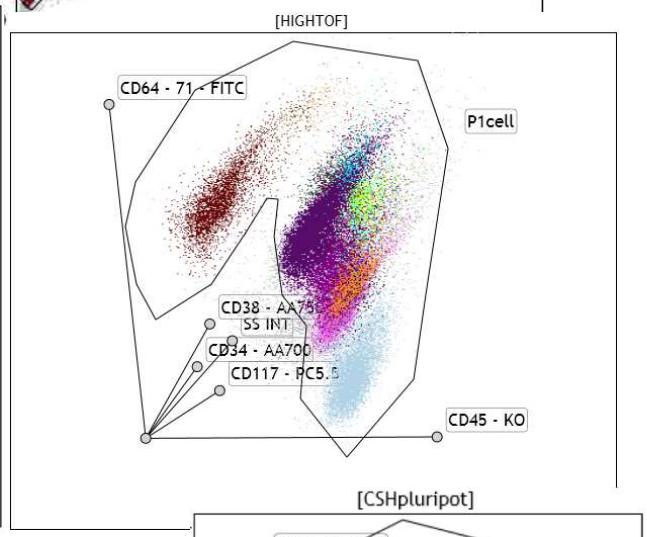
- Fluidic control with a time plot
- Removal of abnormally complex events
  - SSPeak versus SSInt
- Elimination of non-spherical cells
  - FSPeak versus FSInt
- Elimination of doublets and coincidences
  - FSTof vers SSTof
- Elimination of “Arrows” and multi-stained artifacts
  - Use of the “Fan” (Eventail) Procedures
- BACK Gating on all the debris removed
  - Control of the screening procedure

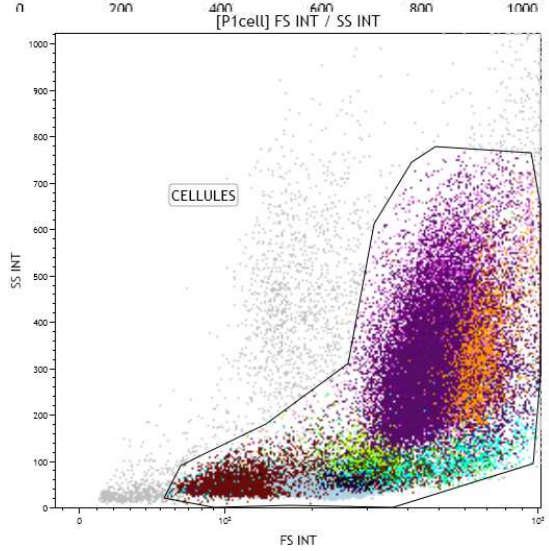

[Ungated]
JIT
CD64 - 71 - FITC
SS INT
CD45 - KO
CD38 - AA750
CD34
TIME

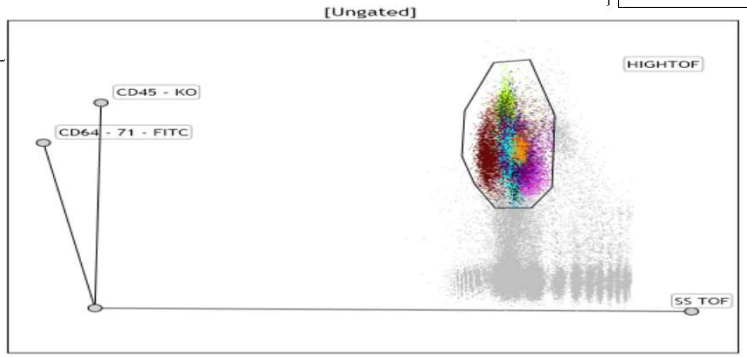

[JIT] SS PEAK / SS INT
SS INT
FF I-P

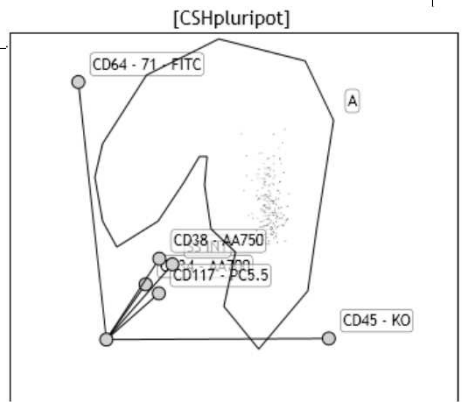

[Ungated] FS PEAK / FS INT
FS INT
FF I-P


[SS I-P]
HIGHTOF
CD45 - KO
CD64 - 71 - FITC
SS TOF


[HIGHTOF]
CD64 - 71 - FITC
P1cell
CD38 - AA750
SS INT
CD34 - AA700
CD117 - PC5.5
CD45 - KO


[P1cell] FS INT / SS INT
CELLULES
SS INT
FS INT


[Ungated]
HIGHTOF
CD45 - KO
CD64 - 71 - FITC
SS TOF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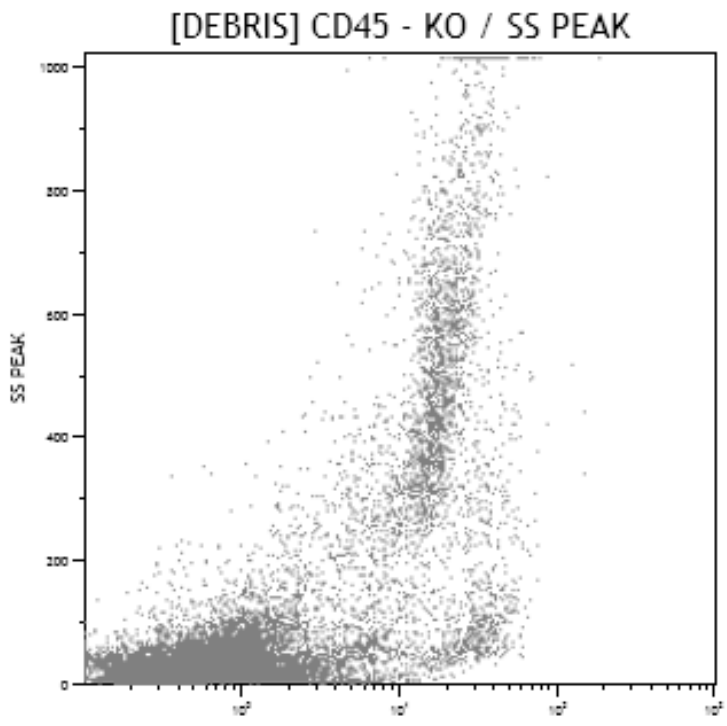
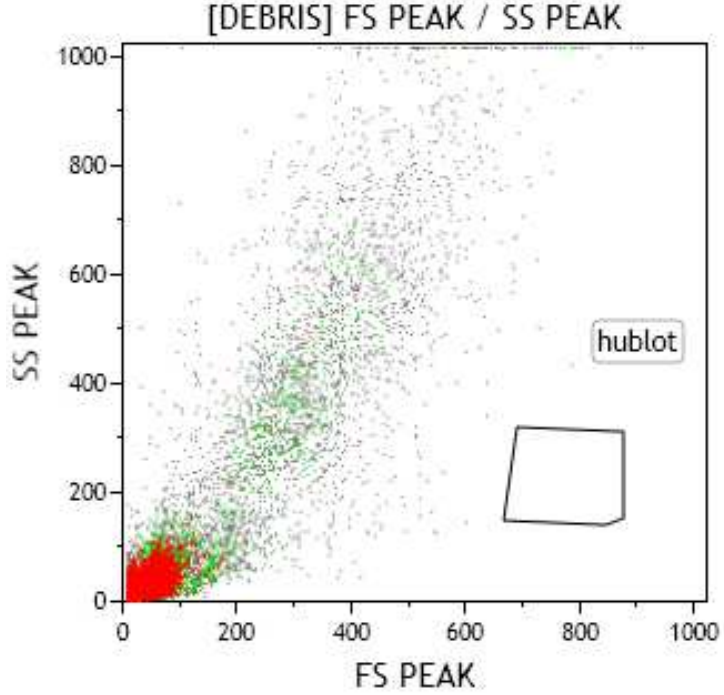
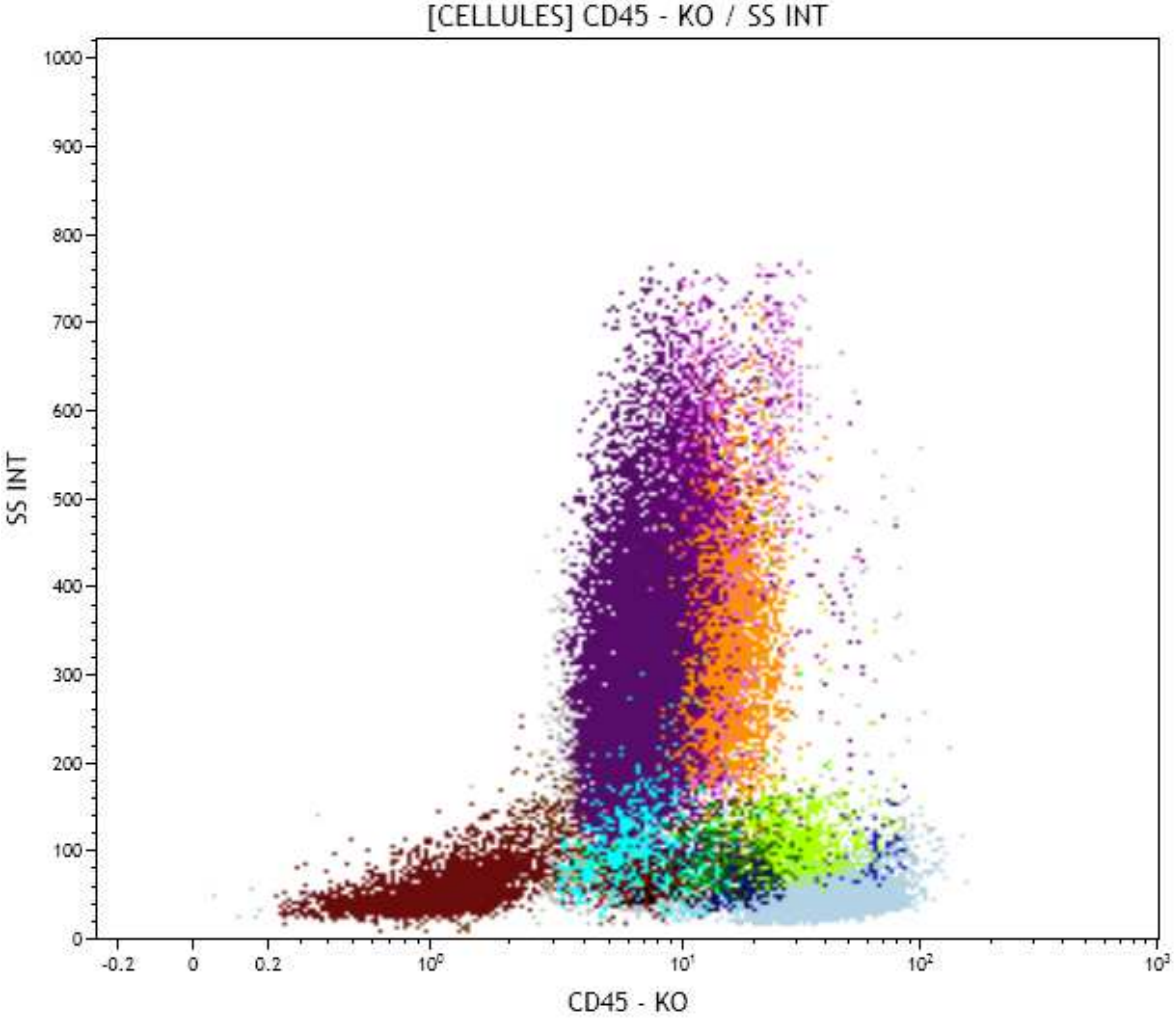
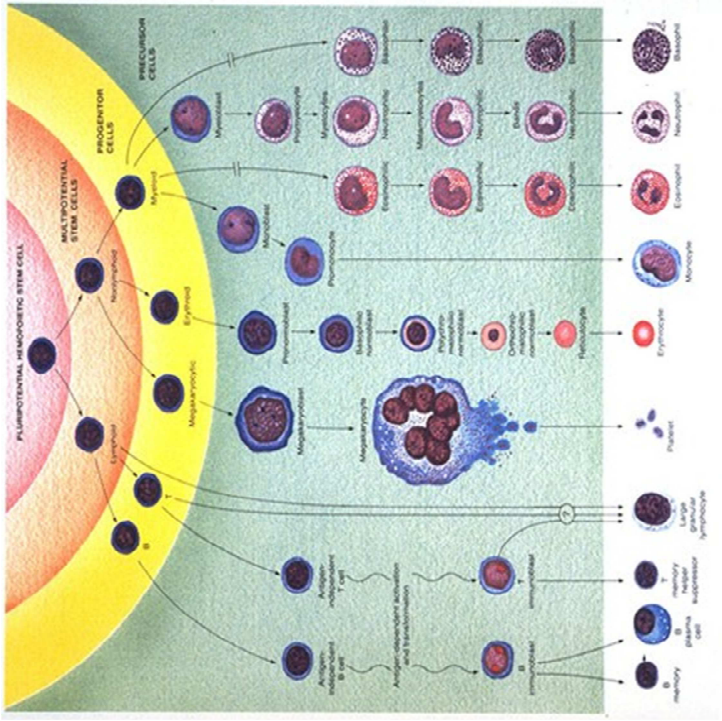
CD64 - 71 - FITC
A
CD38 - AA750
CD117 - PC5.5
CD45 - KO

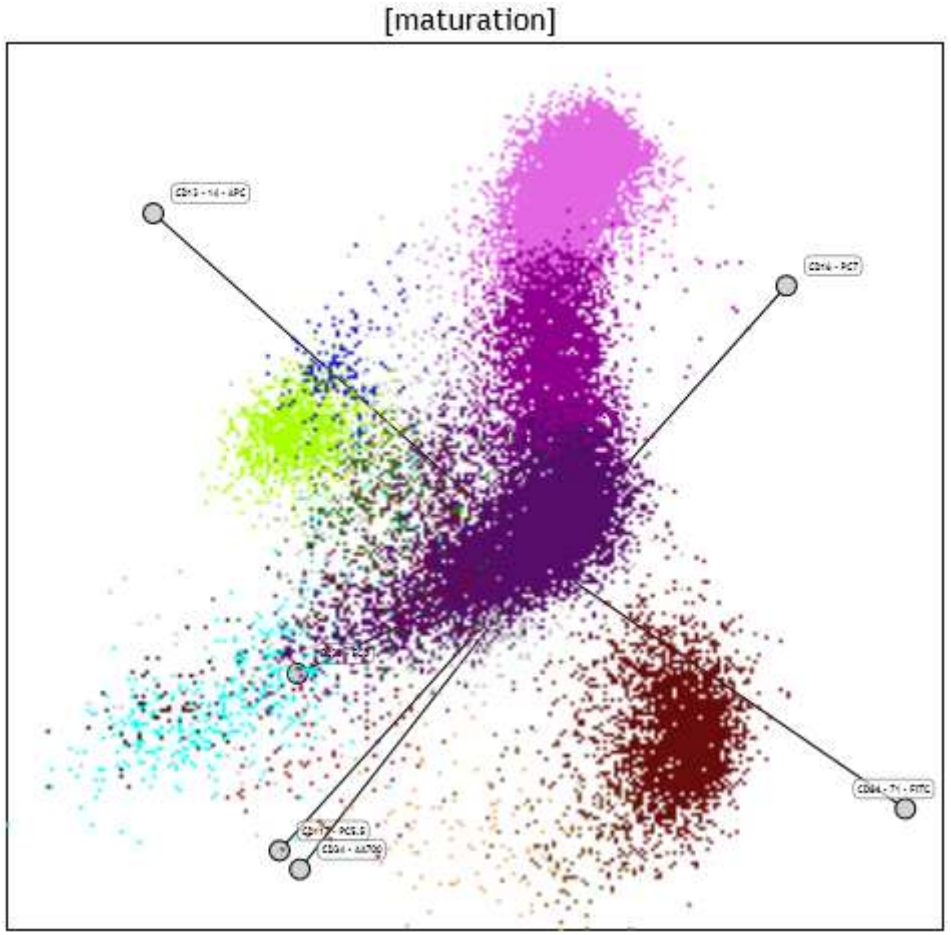

[DEBRIS] CD45 - KO / SS PEAK
SS PEAK


[DEBRIS] FS PEAK / SS PEAK
SS PEAK
FS PEAK
hublot


[CELLULES] CD45 - KO / SS INT
SS INT
CD45 - KO

MYELOID MATURATION OF HEMATOPOIETIC MARROW





WE MUST CREATE ENOUGH PLOT TO ORIENT AND WATCH THE MATURATION IN CYTOMETRIC SPACE

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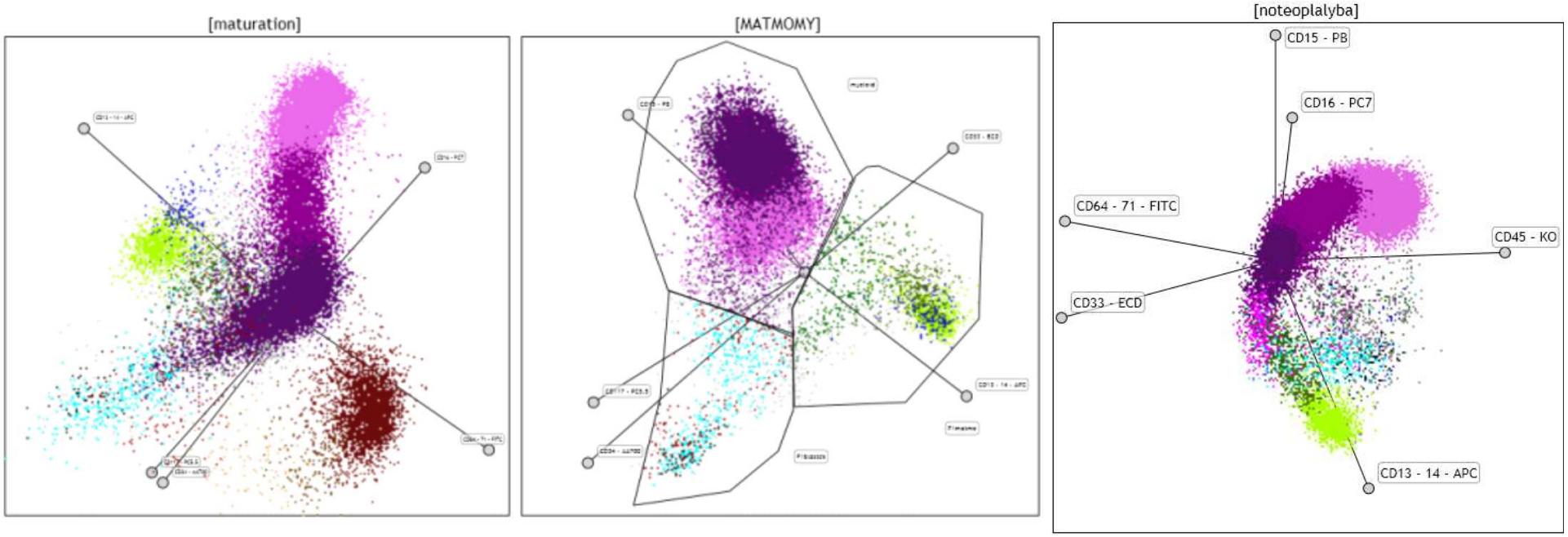
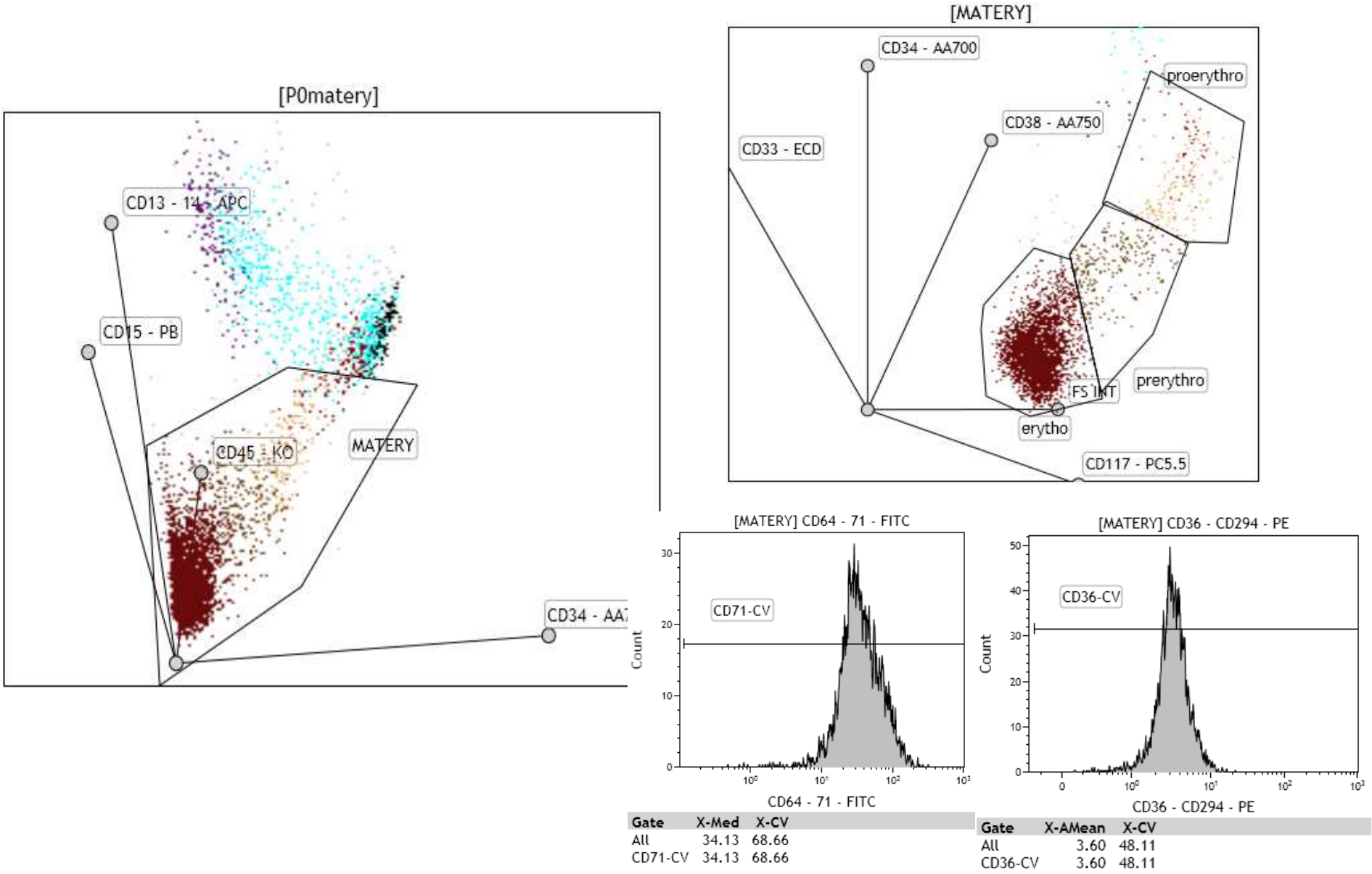
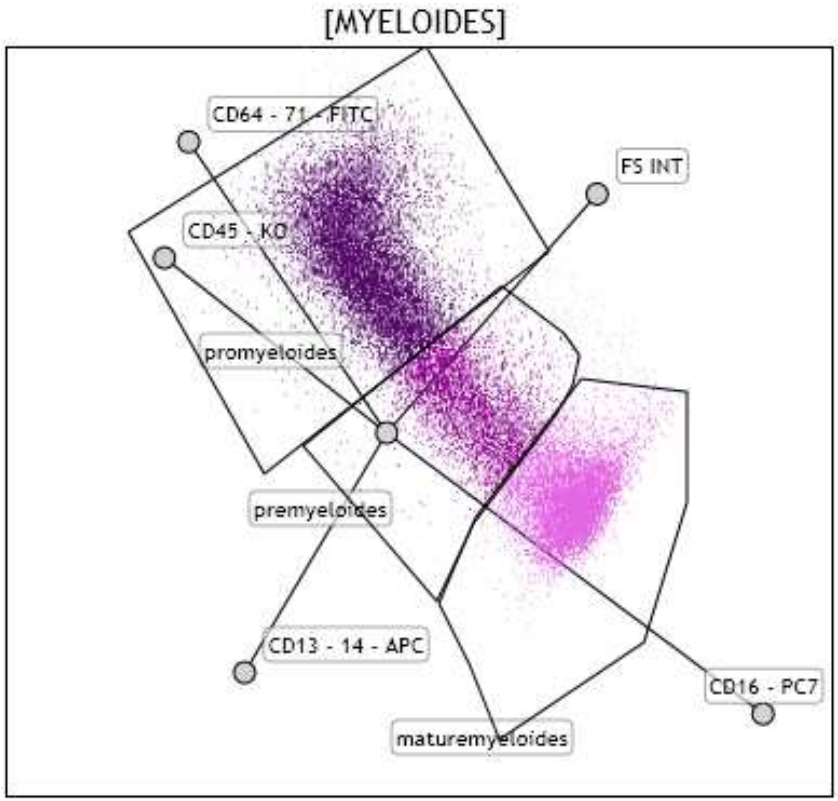
3 different viewpoints to observe the myeloid and monocytic maturation

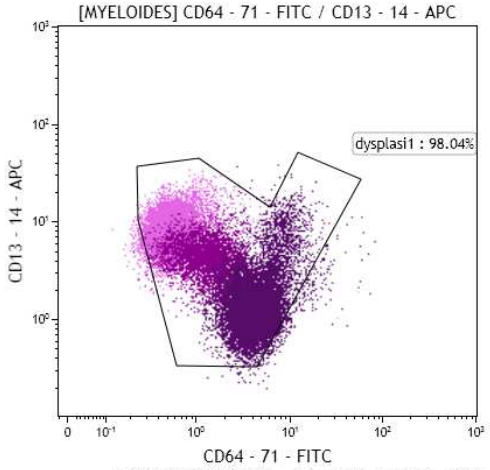


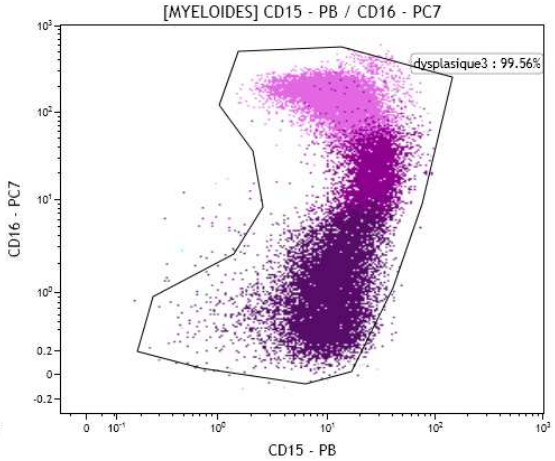
NORMAL ERYTHROID DIFFERENTIATION

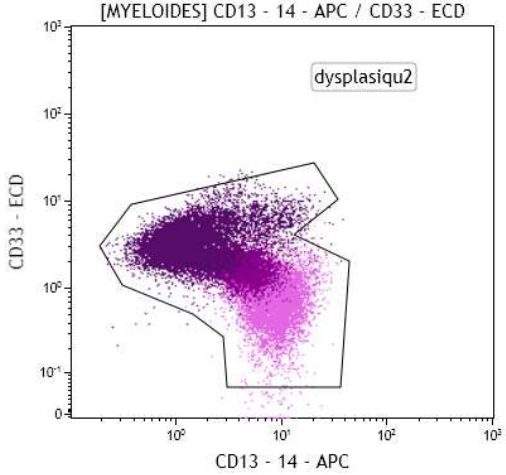


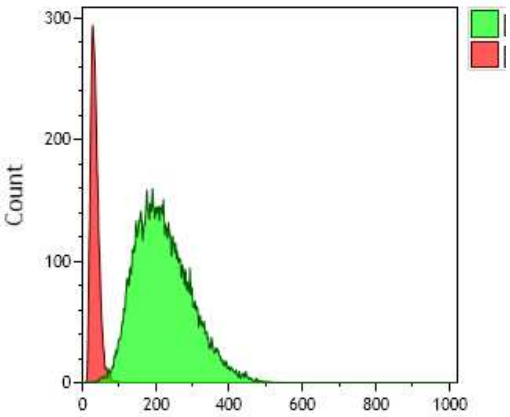
NORMAL MYELOID MATURATION PATHWAYS



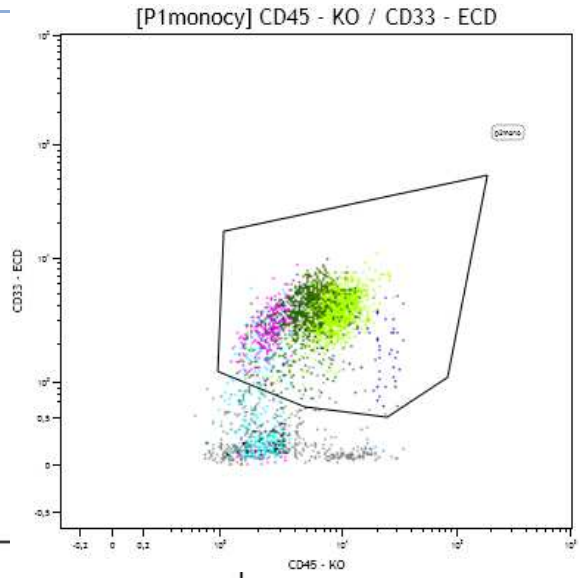


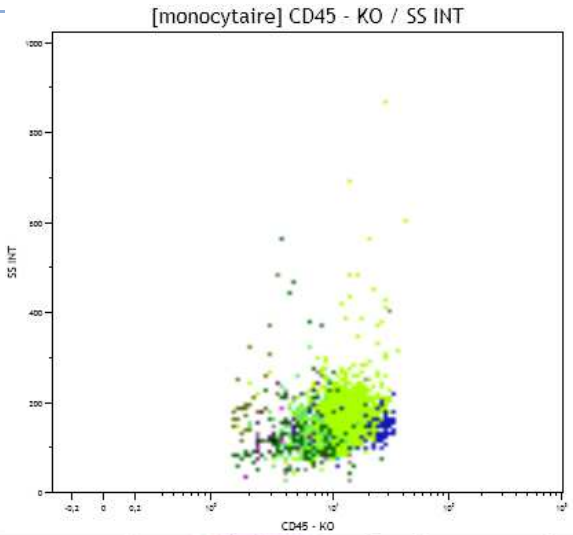


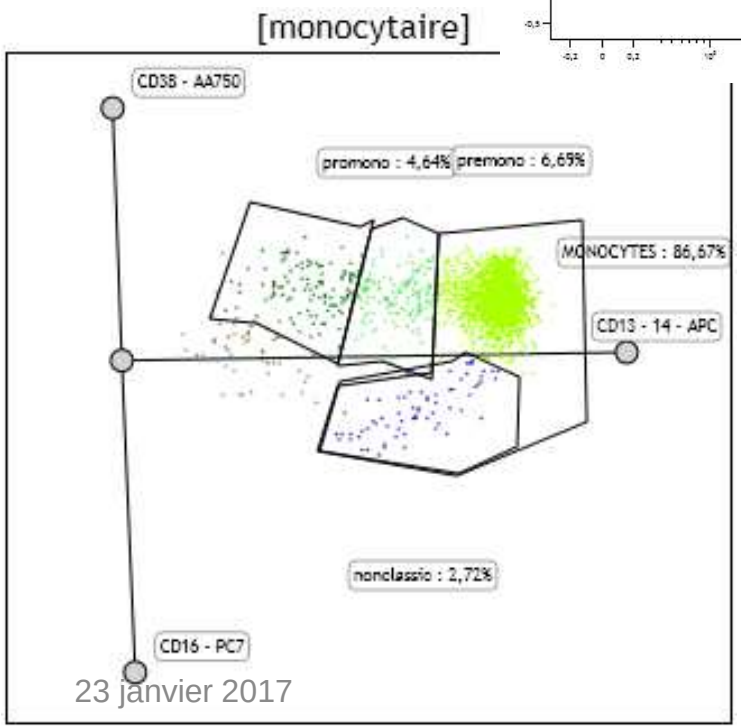


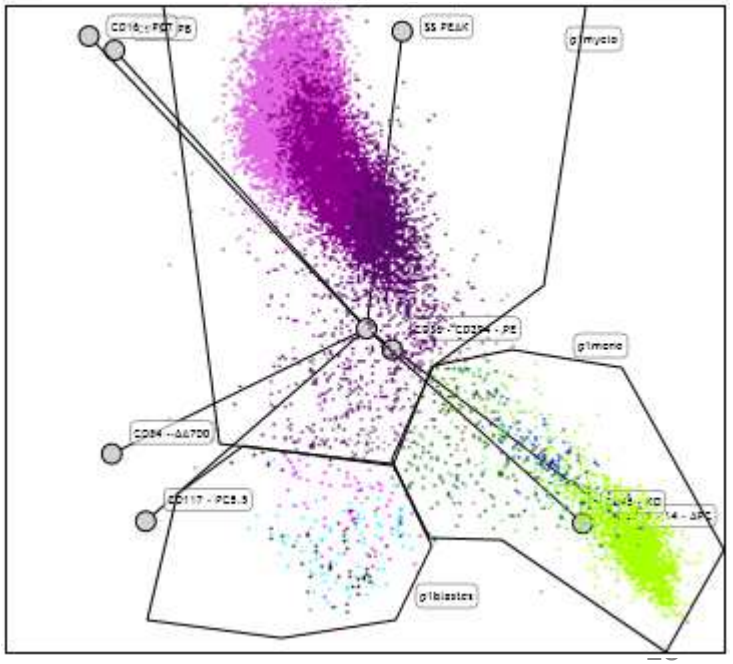


MATURATION MONOCYTAIRE









MYELODYSPLASIA

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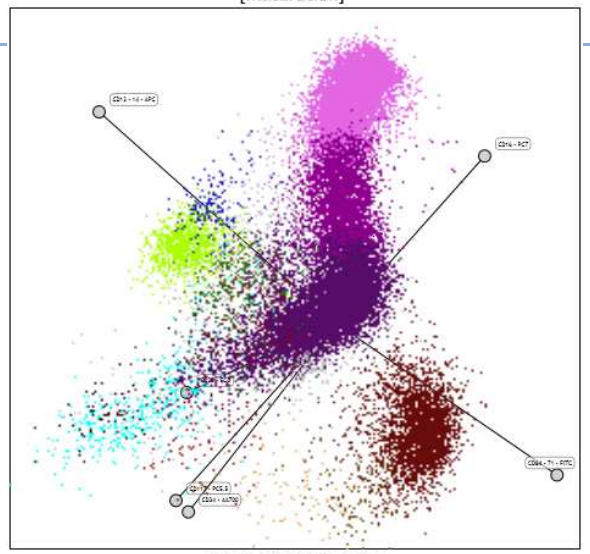
[maturation]



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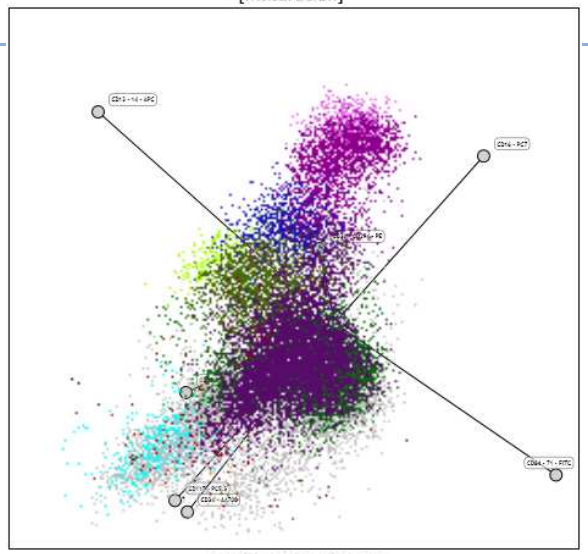

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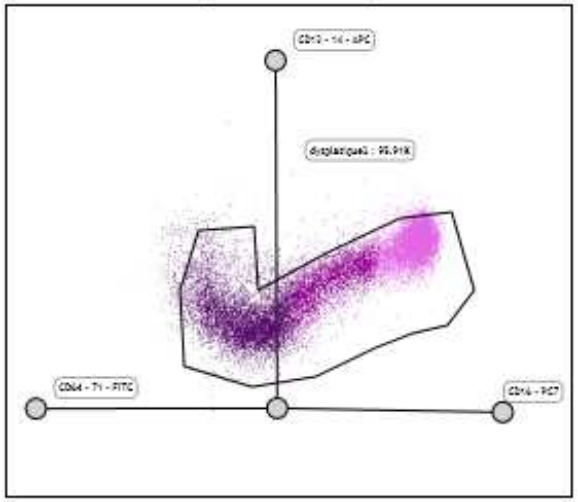

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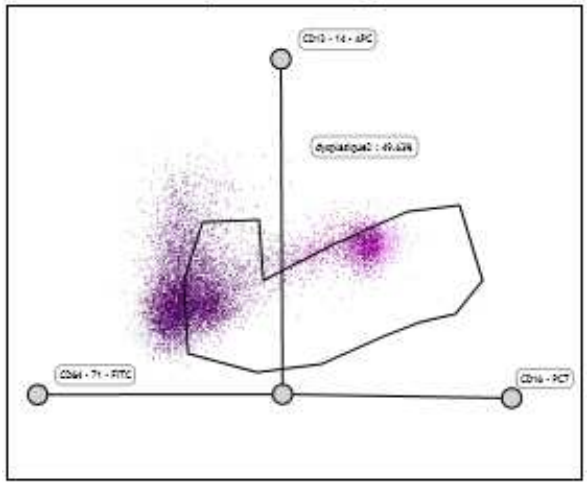

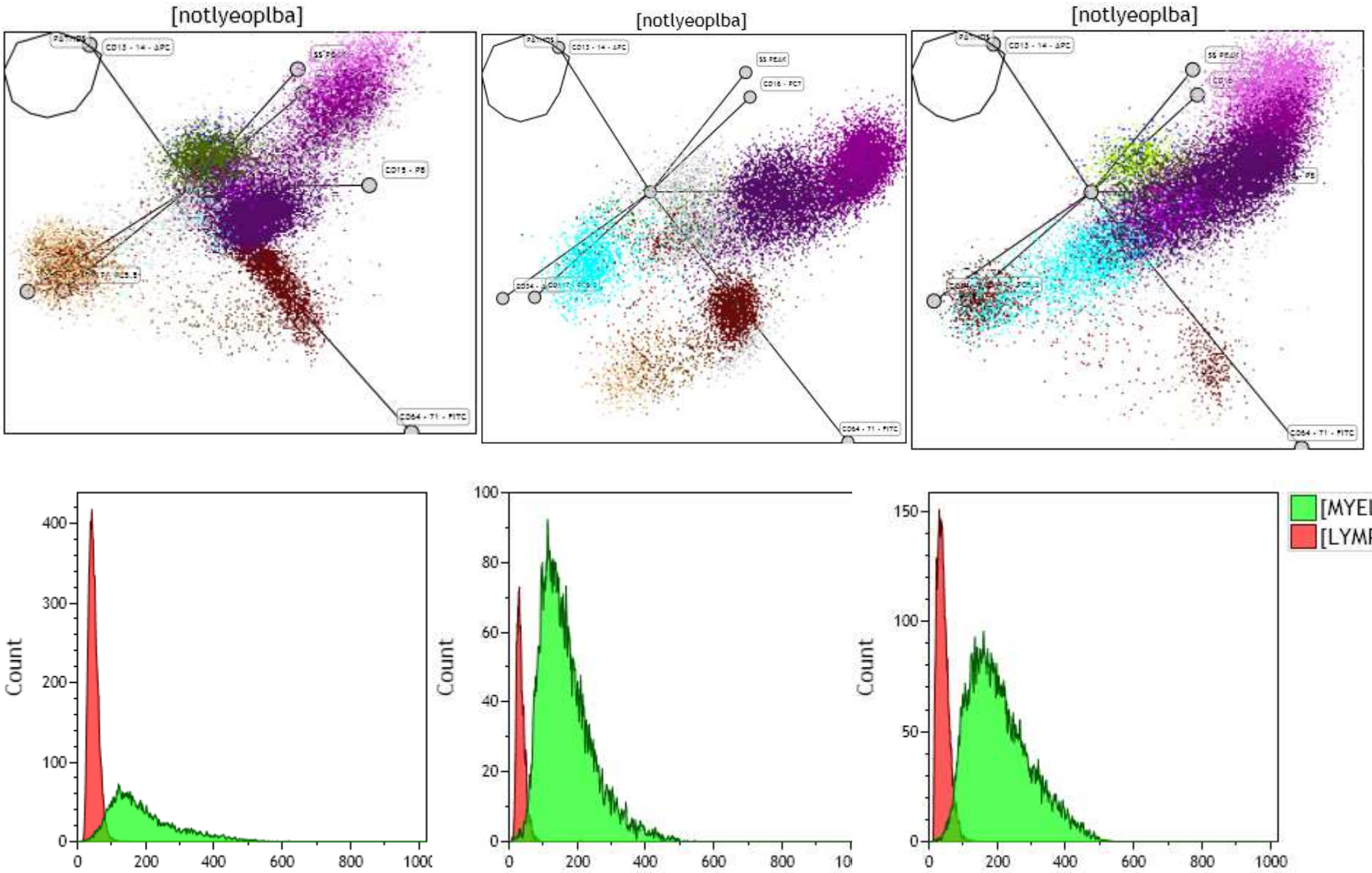
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Count
Count
Count
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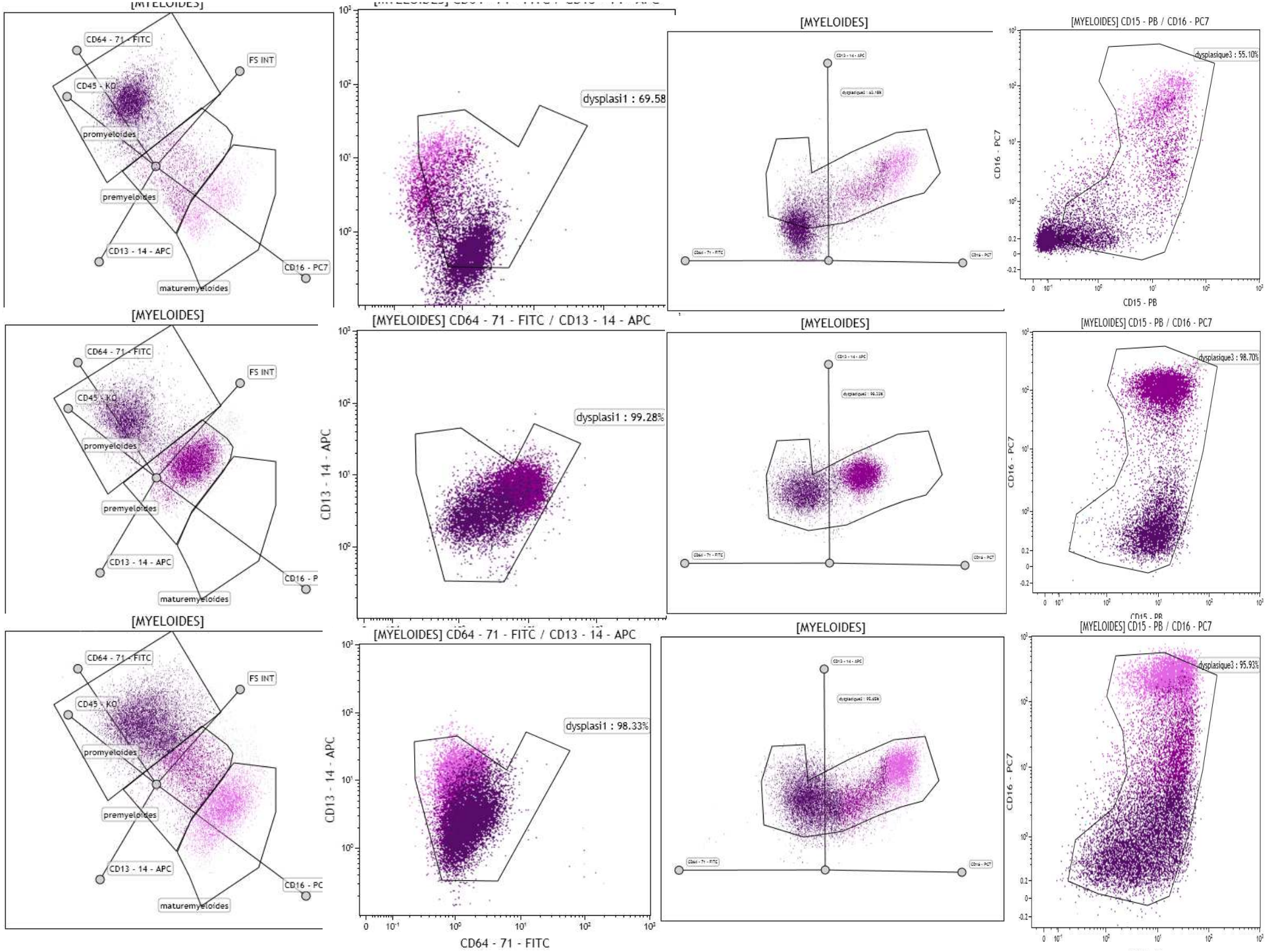
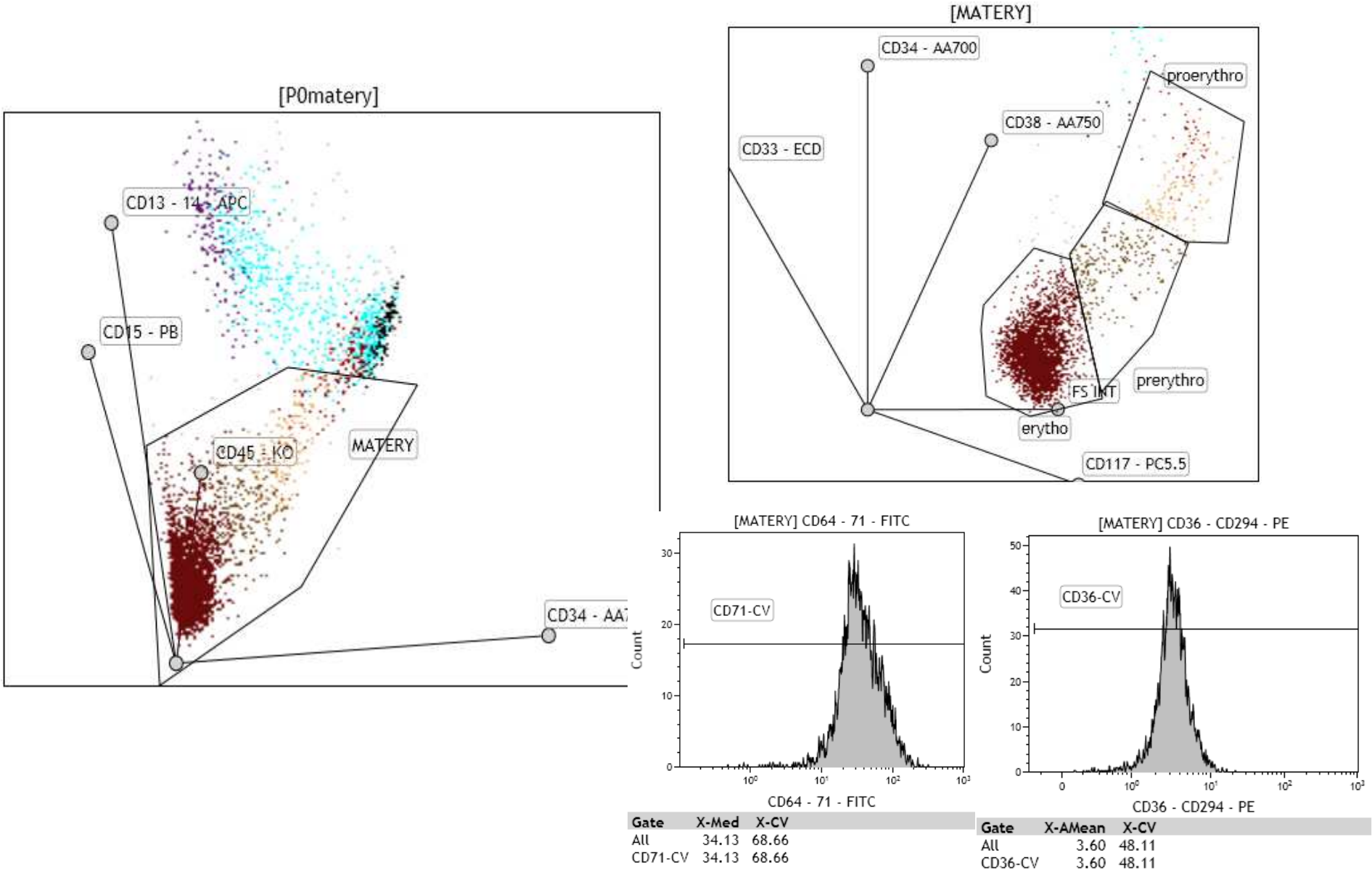
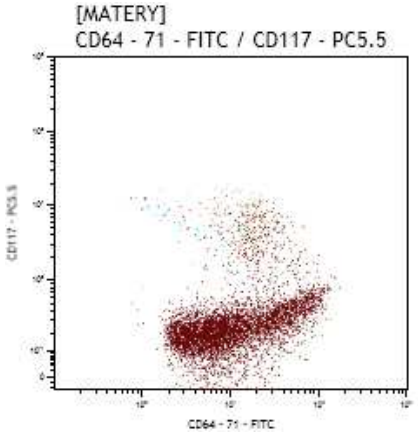
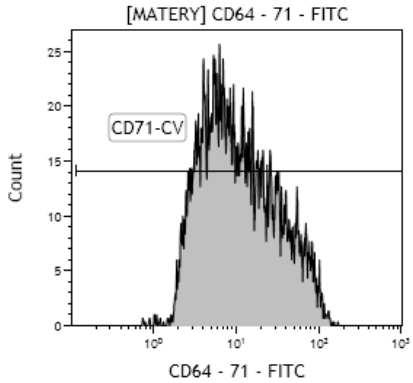

[MYELOIDES]
CD64 - 71 - FITC
FS INT
CD45 - KO
promyeloides
premyeloides
CD13 - 14 - APC
CD16 - PC7
matureMyeloides
dysplasi1 : 69.58
[MYELOIDES]
[MYELOIDES] CD15 - PB / CD16 - PC7
dysplasique3 : 55.10%
CD16 - PC7
CD15 - PB
[MYELOIDES]
[MYELOIDES] CD64 - 71 - FITC / CD13 - 14 - APC
dysplasi1 : 99.28%
CD13 - 14 - APC
[MYELOIDES]
[MYELOIDES] CD15 - PB / CD16 - PC7
dysplasique3 : 98.70%
CD16 - PC7
[MYELOIDES]
[MYELOIDES] CD64 - 71 - FITC / CD13 - 14 - APC
dysplasi1 : 98.33%
CD13 - 14 - APC
CD64 - 71 - FITC
[MYELOIDES]
[MYELOIDES] CD15 - PB / CD16 - PC7
dysplasique3 : 95.93%
CD16 - PC7

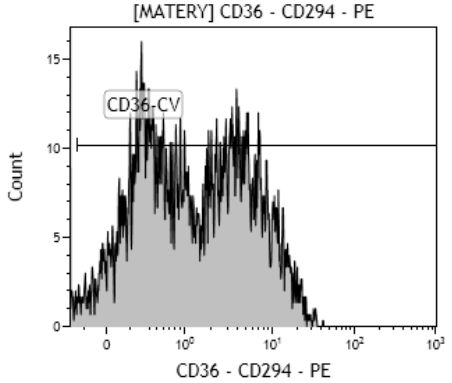
NORMAL ERYTHROID DIFFERENTIATION



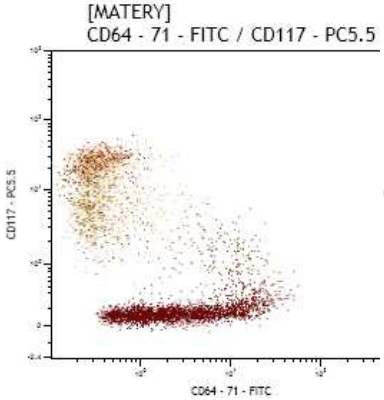


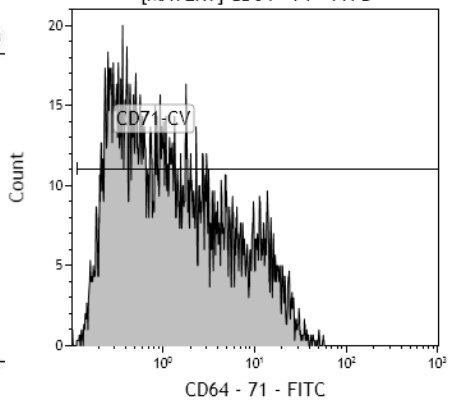


Gate	X-Med	X-CV
All	9.44	117.54
CD71-CV	9.44	117.54

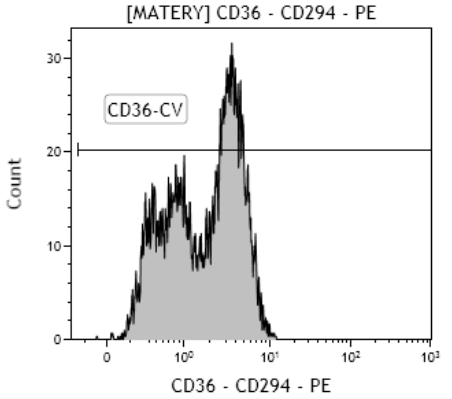


Gate	X-AMean	X-CV
All	3.14	146.15
CD36-CV	3.22	143.18

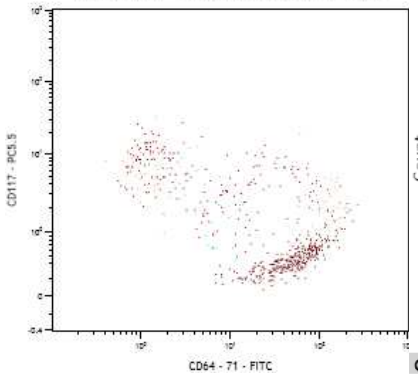


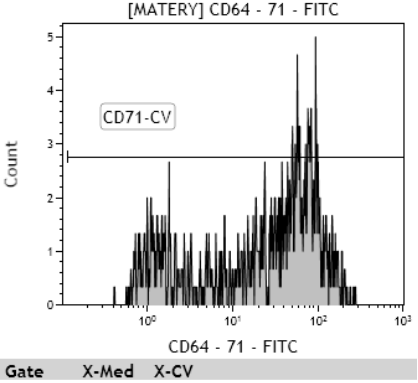


Gate	X-Med	X-CV
...	1.09	163.72
1-CV	1.09	163.70

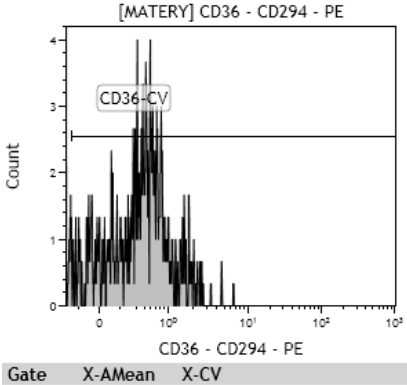


Gate	X-AMean	X-CV
All	2.44	78.11
CD36-CV	2.44	78.11



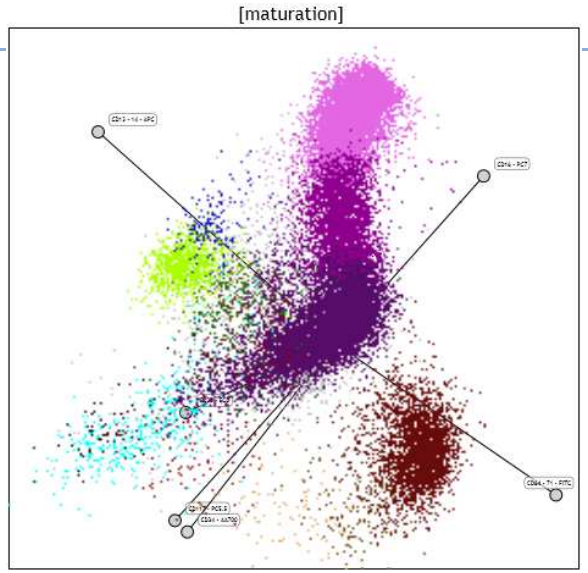


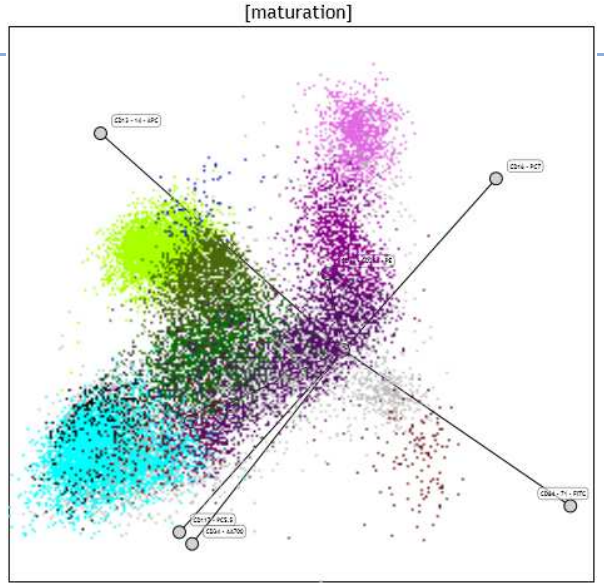
Gate	X-Med	X-CV
All	34.52	101.35
CD71-CV	34.52	101.35

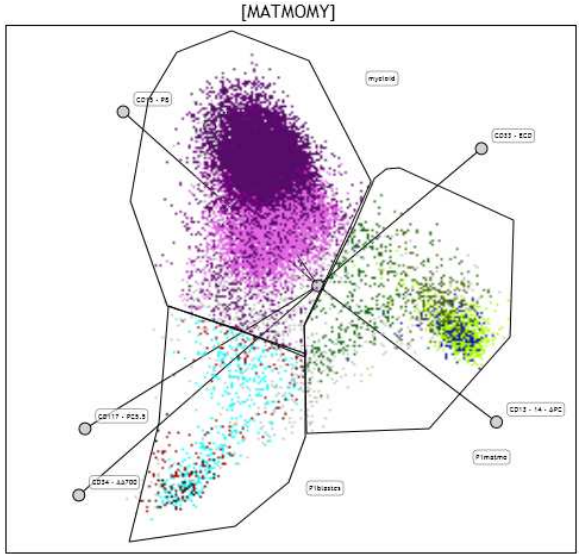


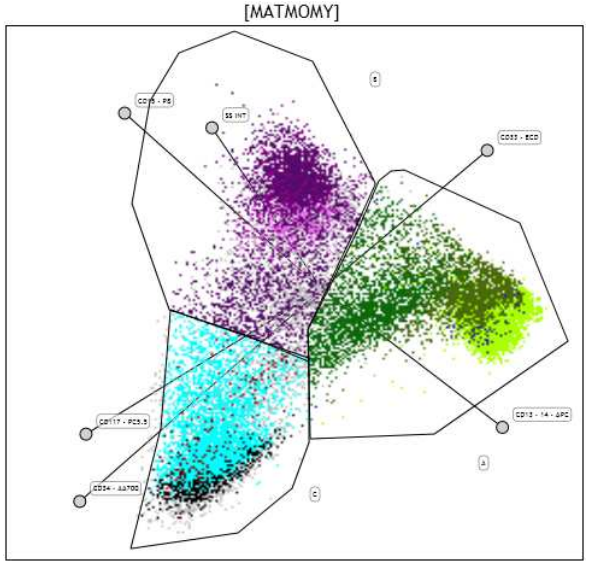
Gate	X-AMean	X-CV
All	-0.27	-569.21
CD36-CV	0.59	115.69

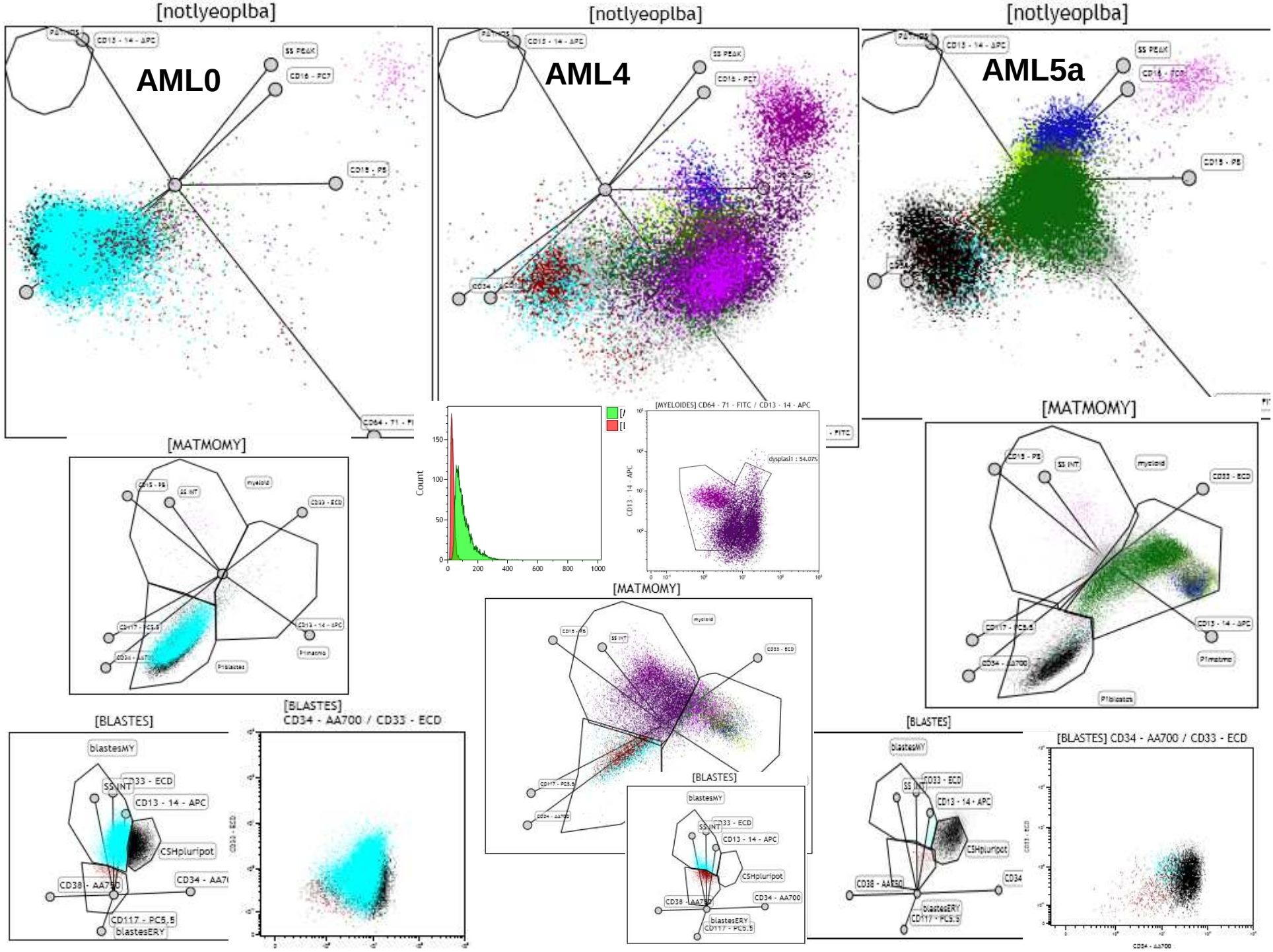
MYELOID LEUKEMIA







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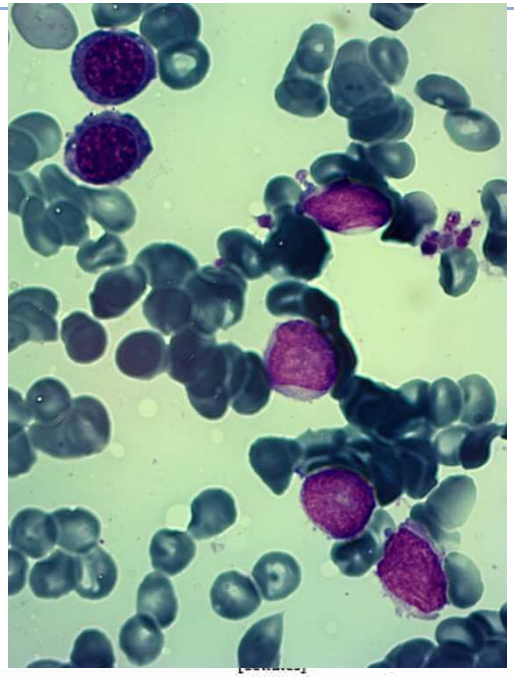
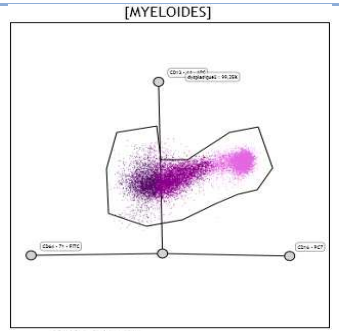
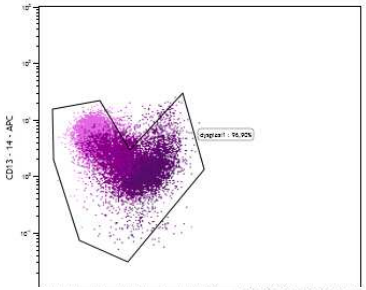
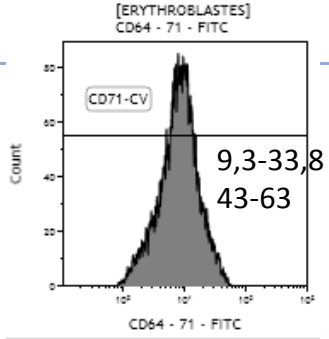
AML0
[notlyeoplba]
AML4
[notlyeoplba]
AML5a
[MATMOMY]
[MYELOIDES] CD64 - 71 - FITC / CD13 - 14 - APC
dysplasi1 : 54.07%
Count
[MATMOMY]
[MATMOMY]
[BLASTES]
[BLASTES]
CD34 - AA700 / CD33 - ECD
[BLASTES]
[BLASTES]
[BLASTES] CD34 - AA700 / CD33 - ECD

MDS-EB 12% BLASTS- MONOCYTIC EVOLUTION



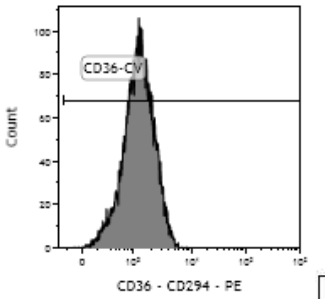




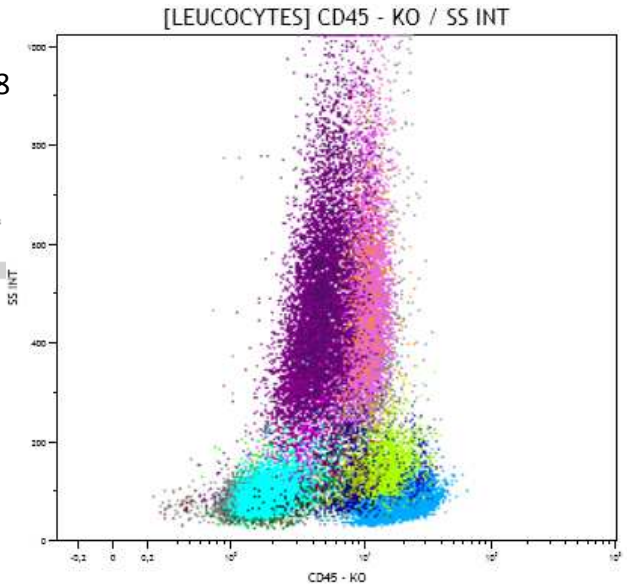


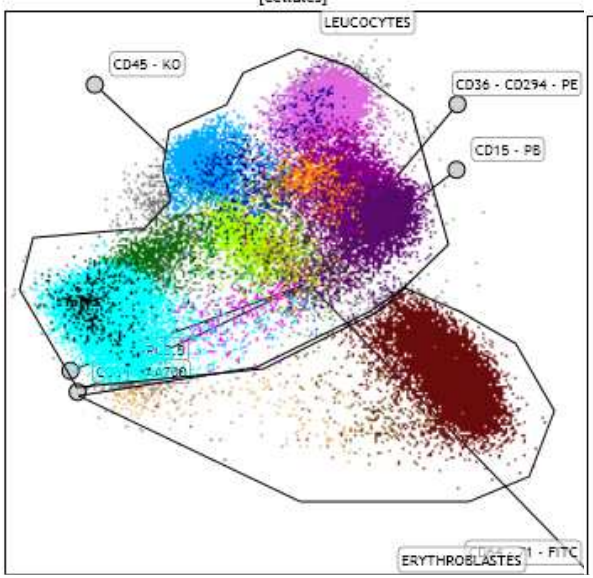
9,3-33,8
43-63

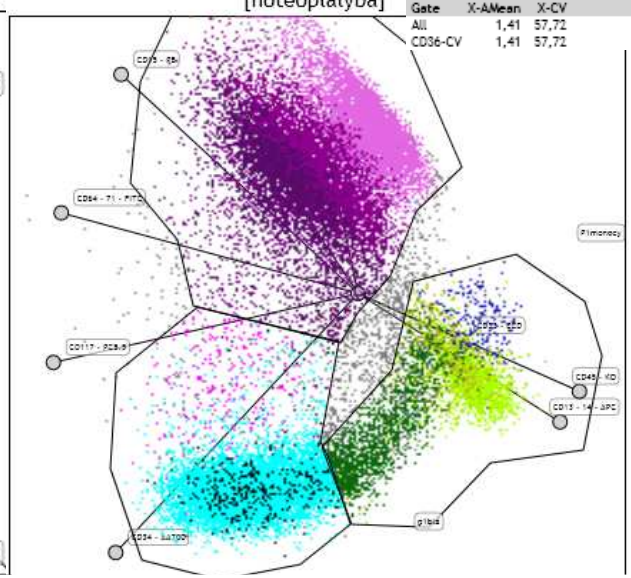
Gate	X-Med	X-CV
All	8,35	74,17
CD71-CV	8,35	74,17

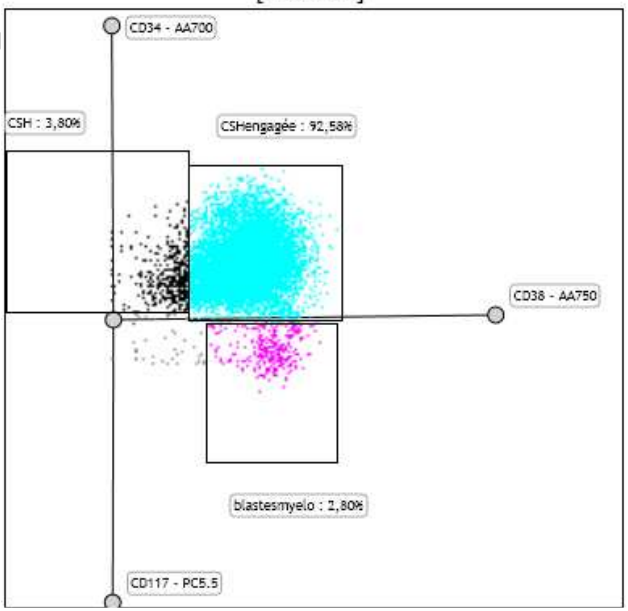


Gate	X-AMean	X-CV
All	1,41	57,72
CD36-CV	1,41	57,72

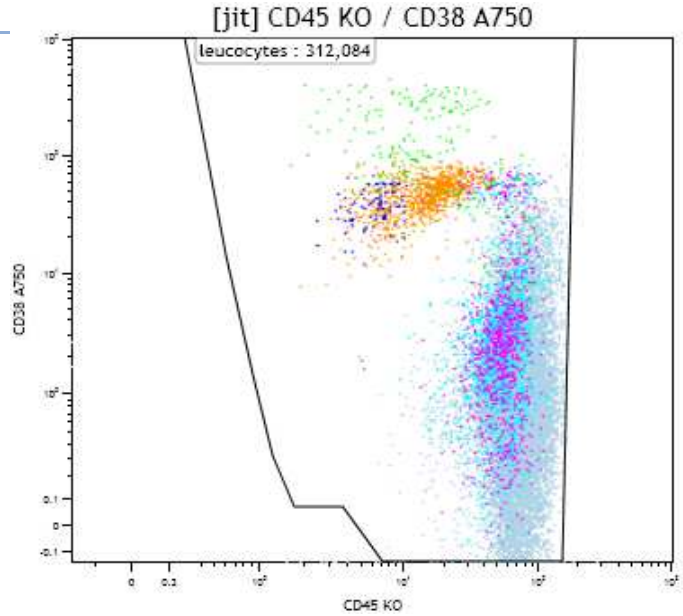


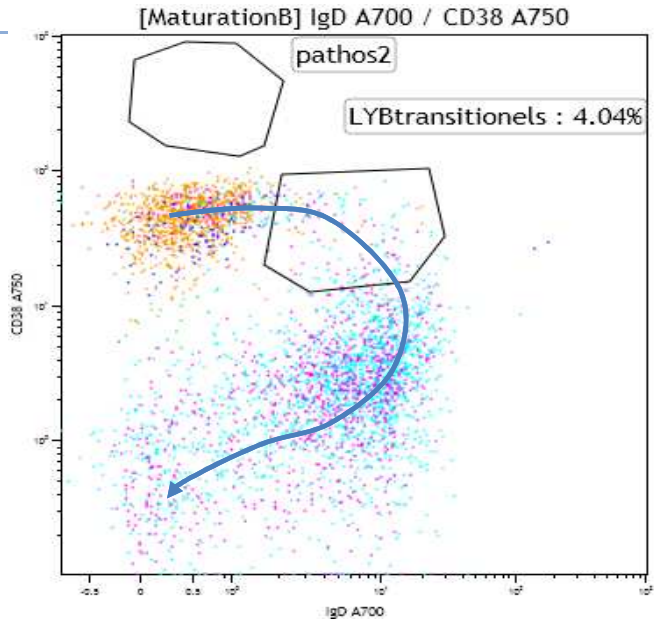


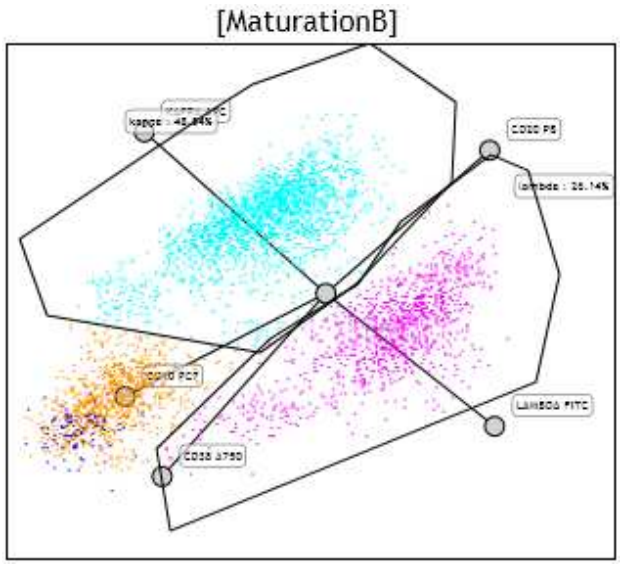


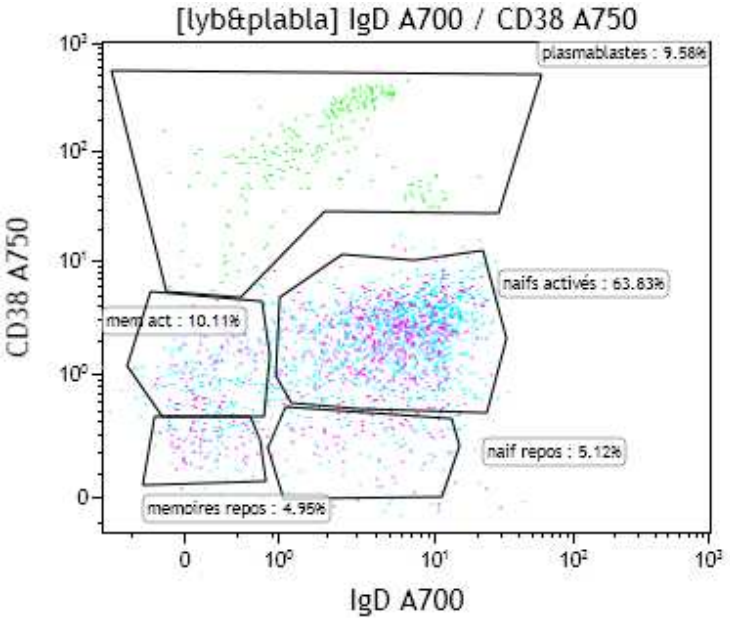


MATURATION LYMPHOÏDE









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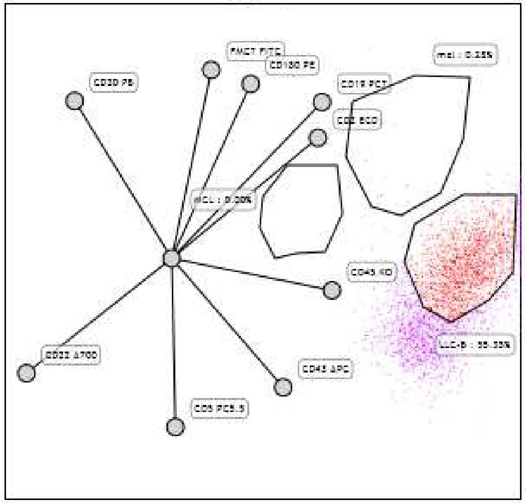

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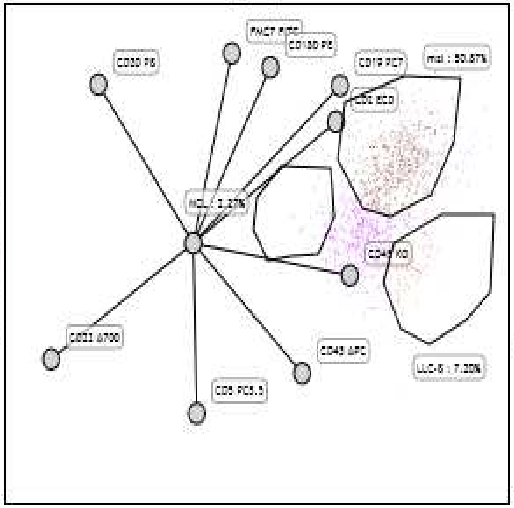

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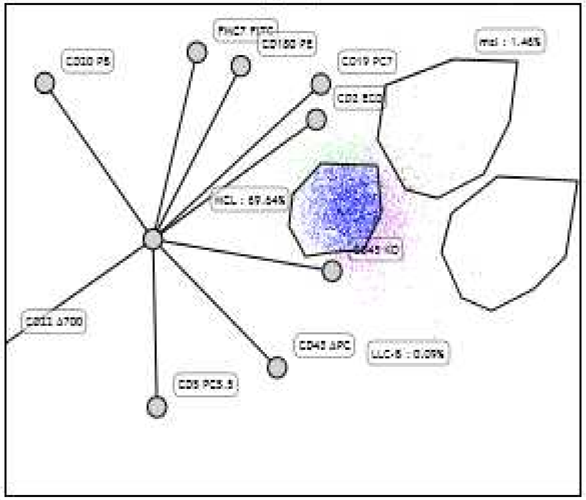

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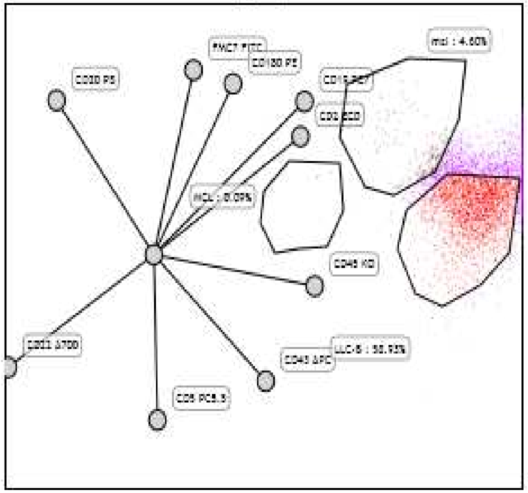

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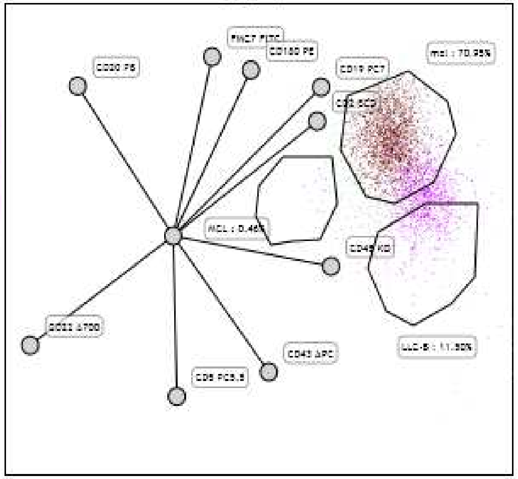

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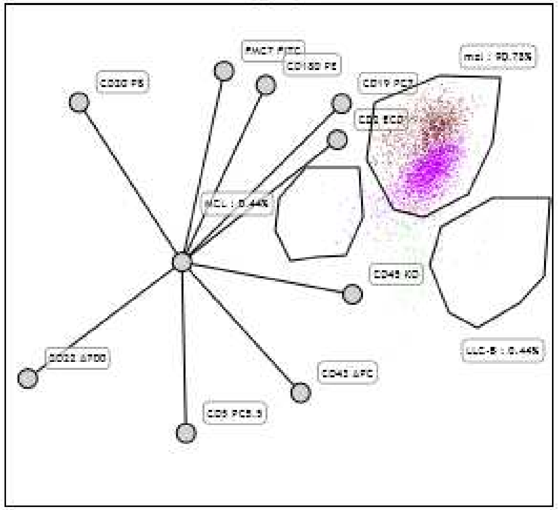


CONCLUSION : THE POSITIVE POINTS

- Multiaxial Vector Analysis using Kaluza allows multiparameter flow cytometry to give :
  - Significantly decrease the number of bi parametric plots
  - Having a dynamic view of evolutionary processes
  - Routes of maturation
  - activation pathways
- Position in a multidimensional space of "normal areas" and "areas of abnormality"
  - myelodysplasia
  - Lymphoma with stereotyped phenotype
    - The score Matutes Catovski in a stud ...
  - Each lymphoma has a different space

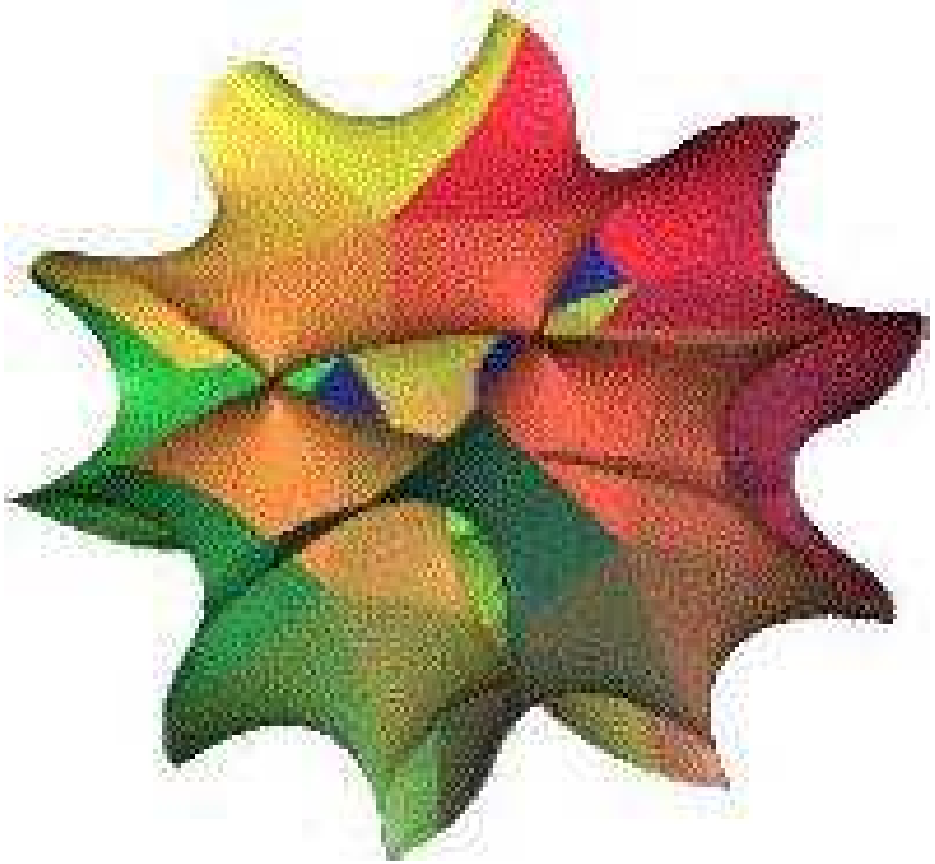
CONCLUSION: DIFFICULTIES

- Development of the analysis very complex.
  - Myélodiff ± 30 successive versions
  - always have the mink in space (loss of positive negative reference)
  - Sometimes the limit switching from one population to another population is not clear. => Never lose sight of the spatial representation of "normal" (repository)
- Hard to technicians to adapt to visual
  - They do not understand the internal logic of the analytical procedure
- Modfiing gatings is intellectually complex
  - Loss of a marker is difficult to “mind”
- Many windowing error by technologists
- Heavy workload for biologists

CONCLUSION : SHORTAGES

- He clearly lacks an independent statistical analysis of prior vision
  - Such as the APS from Infinicyt
- Very sensitive to the setup of the instrument
  - Waiting for instrument with daly auto-setup which compensates automatically for the variability of laser and PMT
- Requires internal control
  - streck or Eurobiocell

MERCI



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- Dr Jonathan Brauner

- Zoulikha Amraoui
- André Jerronez
- Renato Lovo
- Jacques Szylar