



Voorbeeld analyse in de praktijk

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Afdeling Immunologie, Erasmus MC, Rotterdam

MDS Workshop – Amsterdam, 5 September 2012



Analyse in de praktijk

- Wat meten?
- Wat analyseren?
- Hoe analyseren?
- Hoe interpreteren?
- Hoe rapporteren? → niet nader besproken

Analyse in de praktijk

- Wat meten?
- Wat analyseren?
- Hoe analyseren?
- Hoe interpreteren?
- Hoe rapporteren?

Proposed core markers

<i>General core markers</i>	<i>Erythroid</i>	<i>Progenitors</i>	<i>Maturing neutrophils</i>	<i>Monocytes</i>
CD45	CD45	CD45	CD45	CD45
-	CD71	-	-	-
-	CD235a	-	-	-
CD34	-	CD34	CD34	CD34
CD117	CD117	CD117	CD117	CD117
HLA-DR	-	HLA-DR	HLA-DR	HLA-DR
CD11b	-	CD11b	CD11b	CD11b
CD13	-	CD13	CD13	CD13
CD16	-	-	CD16	CD16
CD33	-	-	CD33	CD33
CD14	-	-	CD14	CD14
-	CD36	-	-	CD36
-	-	-	CD64	CD64
CD7	-	CD7	-	-
CD56	-	CD56	CD56	CD56
CD19	-	CD19	-	-
-	-	CD5	-	-
-	-	-	-	CD2
-	-	CD15	CD15	-
-	-	-	CD10	-

Erasmus MC panel (EuroFlow AML/MDS panel)

Backbone:

	<i>PCP-Cy5.5</i>	<i>PC7</i>	<i>PB</i>	<i>PO</i>
	CD34	CD117	HLA-DR	CD45

Antibody combinations:

	<i>FITC</i>	<i>PE</i>	<i>APC</i>	<i>APC-H7</i>
Myeloid	CD16	CD13	CD11b	CD10
Monocytic	CD35	CD64	IREM2	CD14
Erythroid	CD36	CD105	CD33	CD71
precursor-B	TdT	CD56	CD7	CD19

Proposed core markers

<i>General core markers</i>	<i>Erythroid</i>	<i>Mast cells/turing trophils</i>		<i>Monocytes</i>
CD45	CD45	CD45	CD45	CD45
-	CD71	-	-	-
-	<u>CD235a</u>	-	-	-
CD34	-	CD34	CD34	CD34
CD117	CD117	CD117	CD117	CD117
HLA-DR	-	HLA-DR	HLA-DR	HLA-DR
CD11b	-	CD11b	CD11b	CD11b
CD13	-	CD13	CD13	CD13
CD16	-	-	CD16	CD16
CD33	-	-	CD33	CD33
CD14	-	-	CD14	CD14
-	CD36	-	-	CD36
-	-	-	CD64	CD64
CD7	-	CD7	-	-
CD56	-	CD56	CD56	CD56
CD19	-	CD19	-	-
-	-	<u>CD5</u>	-	-
-	-	-	-	<u>CD2</u>
-	-	<u>CD15</u>	<u>CD15</u>	-
-	-	-	CD10	-

Does not work on whole PB → CD105

Priority/costs

Analyse in de praktijk

- Wat meten?
- Wat analyseren?
- Hoe analyseren?
- Hoe interpreteren?
- Hoe rapporteren?

Recommended minimal requirements

Bone marrow subset	Recommended analyses	Aberrancy
Immature myeloid and monocytic progenitors	Percentage of cells in nucleated cell fraction ^a Expression of CD45 Expression of CD34 Expression of CD117 Expression of HLA-DR Expression of CD13 and CD33 Asynchronous expression of CD11b, <u>CD15</u> Expression of <u>CD5</u> , CD7, CD19, CD56 ^b	Increased percentage Lack of/decreased/increased Lack of/decreased/increased Homogenous under/overexpression Lack of/increased expression Lack of/decreased/increased Presence of mature markers Presence of lineage infidelity markers
Maturing neutrophils	Percentage of cells as ratio to lymphocytes SSC as ratio vs SSC of lymphocytes Relationship of CD13 and CD11b Relationship of CD13 and CD16 Relationship of <u>CD15</u> and CD10	Decreased Decreased Altered pattern ^c Altered pattern ^c Altered pattern ^c ; for example, lack of CD10 on mature neutrophils
Monocytes	Percentage of cells Distribution of maturation stages Relationship of HLA-DR and CD11b Relationship of CD36 and CD14 Expression of CD13 and CD33 Expression of CD56 ^b	Decreased/increased Shift towards immature Altered pattern ^c Altered pattern ^c (Homogenous) under/overexpression Presence of lineage infidelity marker
Progenitor B cells	Enumeration as fraction of total CD34+ based on CD45/CD34/SSC in combination with CD10 or CD19	Decreased or absent
Erythroid compartment ^d	Percentage of nucleated erythroid cells Relationship CD71 and CD235a / CD105 Expression of CD71 Expression of CD36 Percentage of CD117-positive precursors	Increased Altered pattern ^c Decreased Decreased Increased

Analyse in de praktijk

- Wat meten?
- Wat analyseren?
- Hoe analyseren? }
 → Afhankelijk van antistofpanel, software
- Hoe interpreteren?
- Hoe rapporteren?

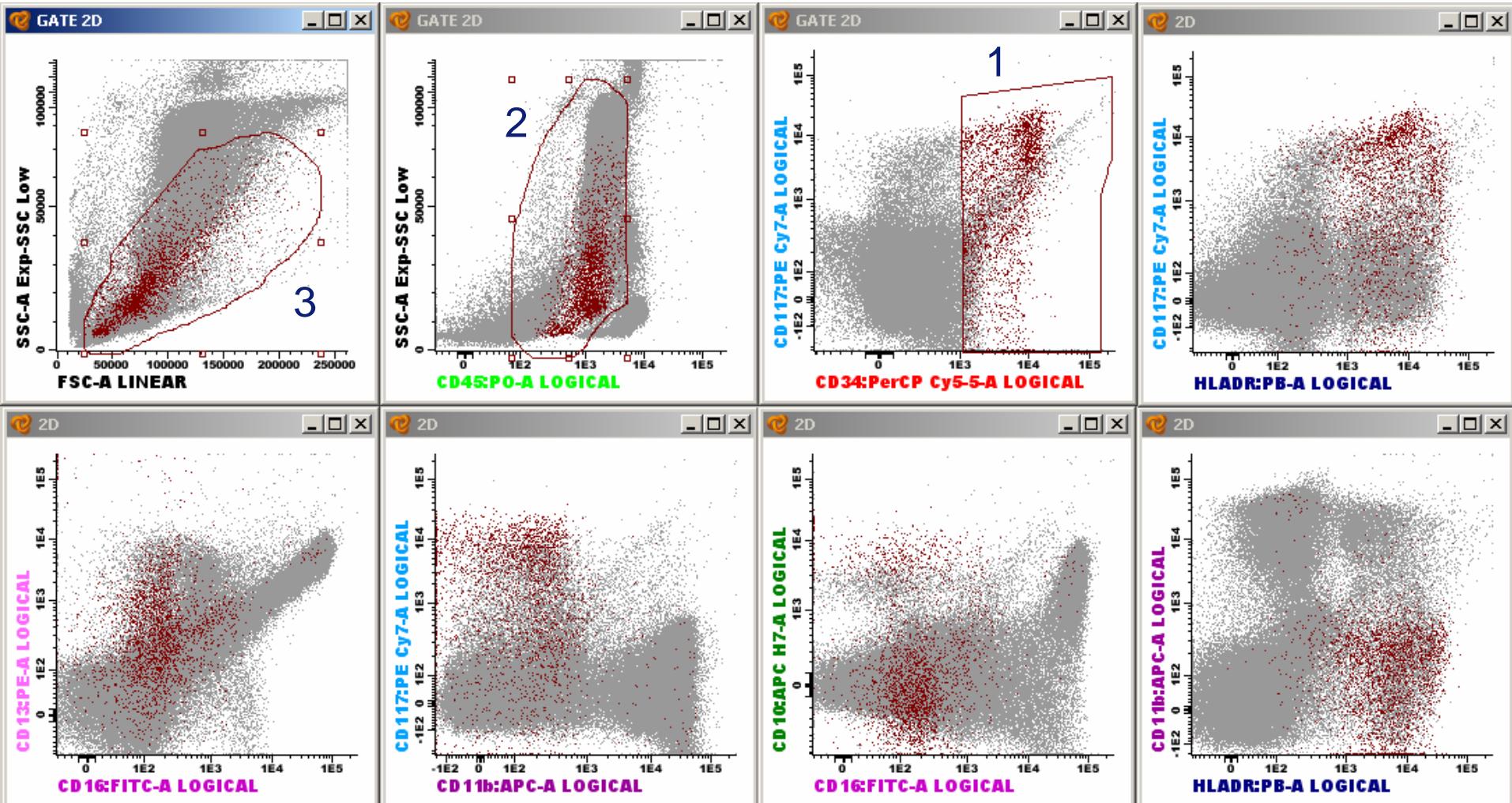
Normal myeloid differentiation

- Myeloid precursors
- Granulocytic differentiation
- Monocytic differentiation
- Erythroid differentiation

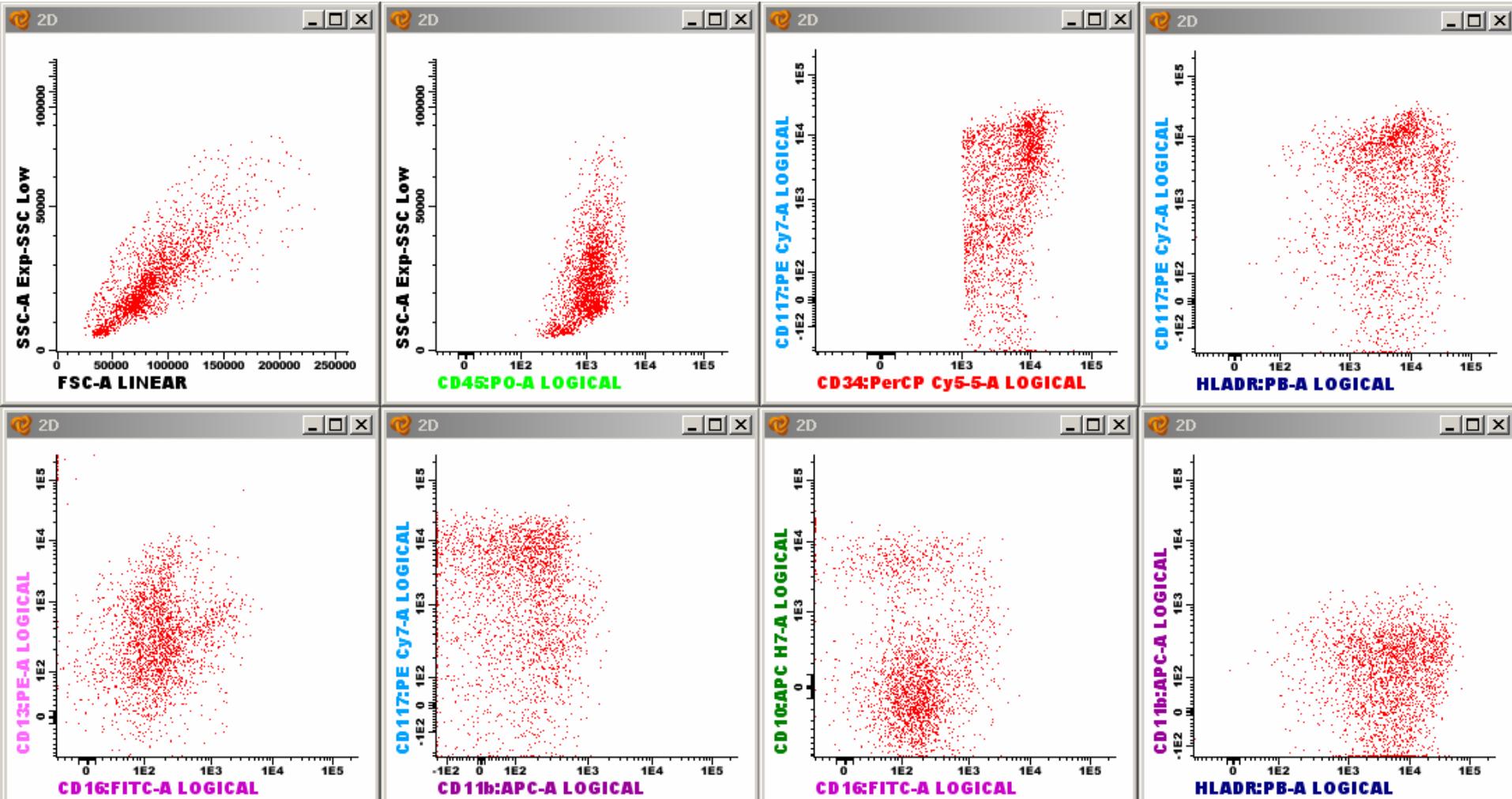
Tube	PacB	PacO	FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7	Aim
1	HLADR	CD45	CD16	CD13	CD34	CD117	CD11b	CD10	Diagnosis and classification, neutrophilic maturation, PNH
2	HLADR	CD45	CD35	CD64	CD34	CD117	CD300e (IREM2)	CD14	Diagnosis and classification, monocytic maturation, PNH
3	HLADR	CD45	CD36	CD105	CD34	CD117	CD33	CD71	Diagnosis and classification, erythroid maturation
4	HLADR	CD45	TdT	CD56	CD34	CD117	CD7	CD19	Aberrant expression of lymphoid markers, abnormal B lymphoid maturation

+FSC+SSC → uniform gating in all tubes

Tube 1 – CD34+ cells

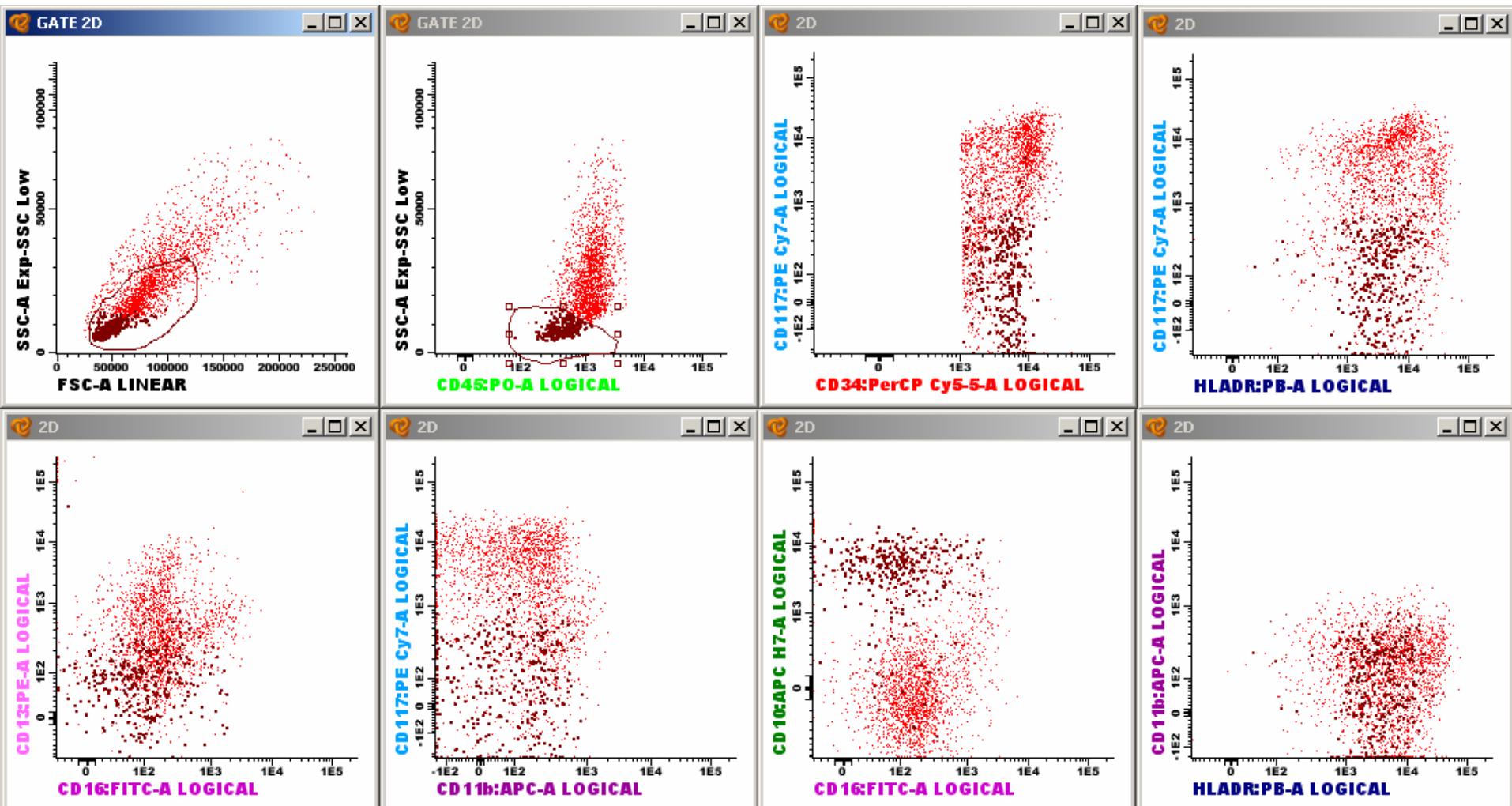


Tube 1 – CD34+ cells



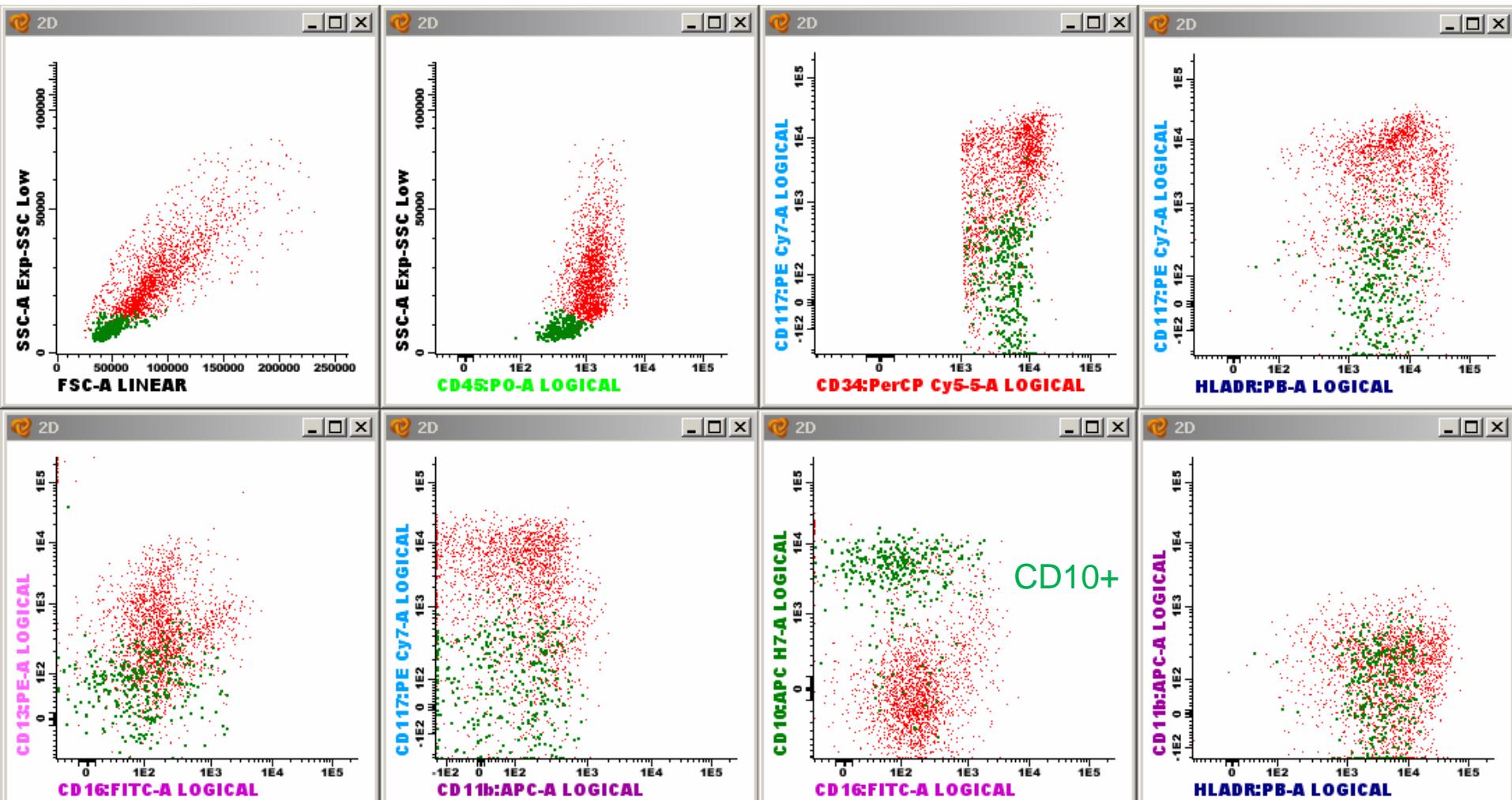
Tube 1 – CD34+ cells

- B-cell precursors



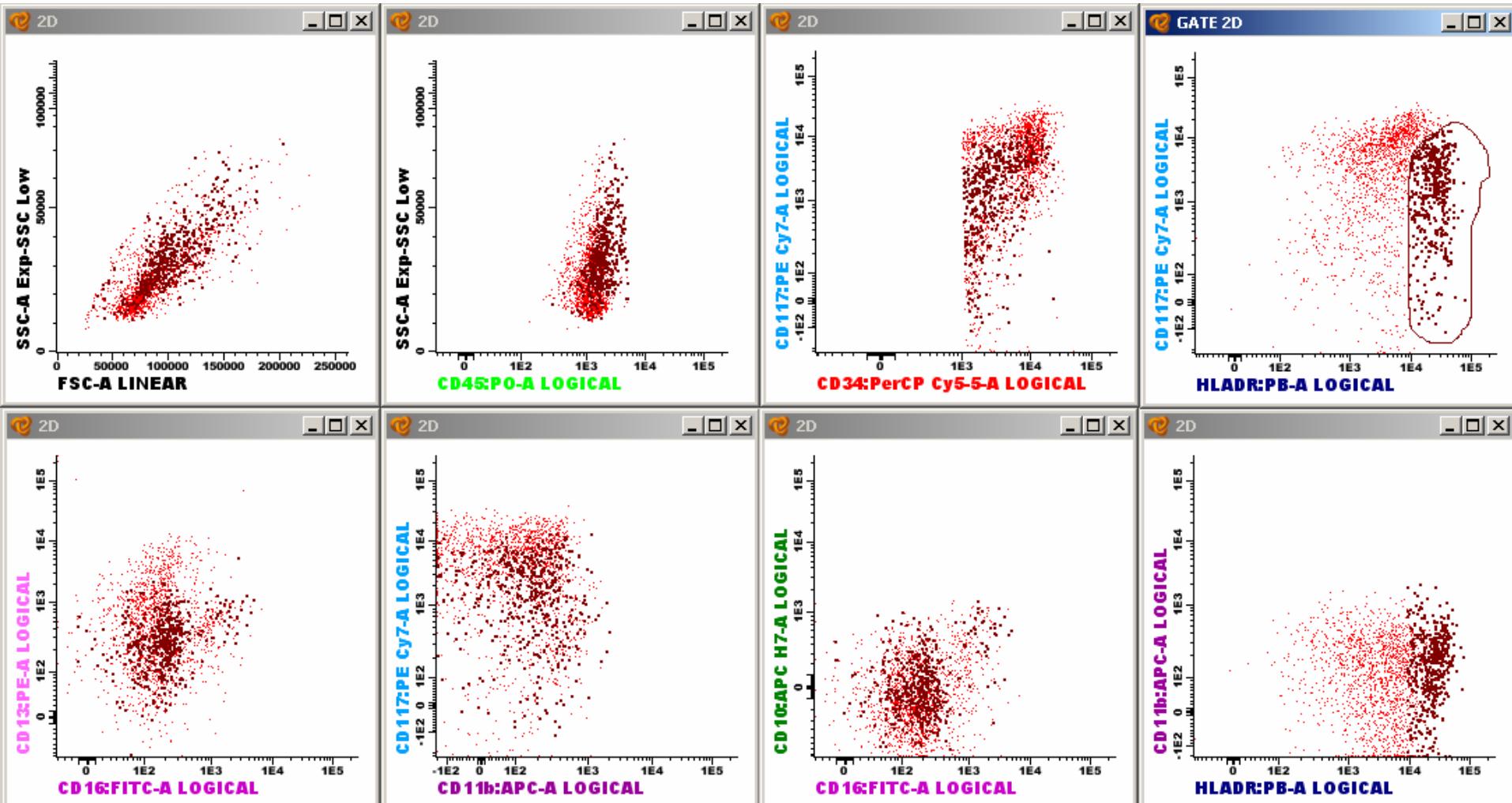
Tube 1 – CD34+ cells

- B-cell precursors



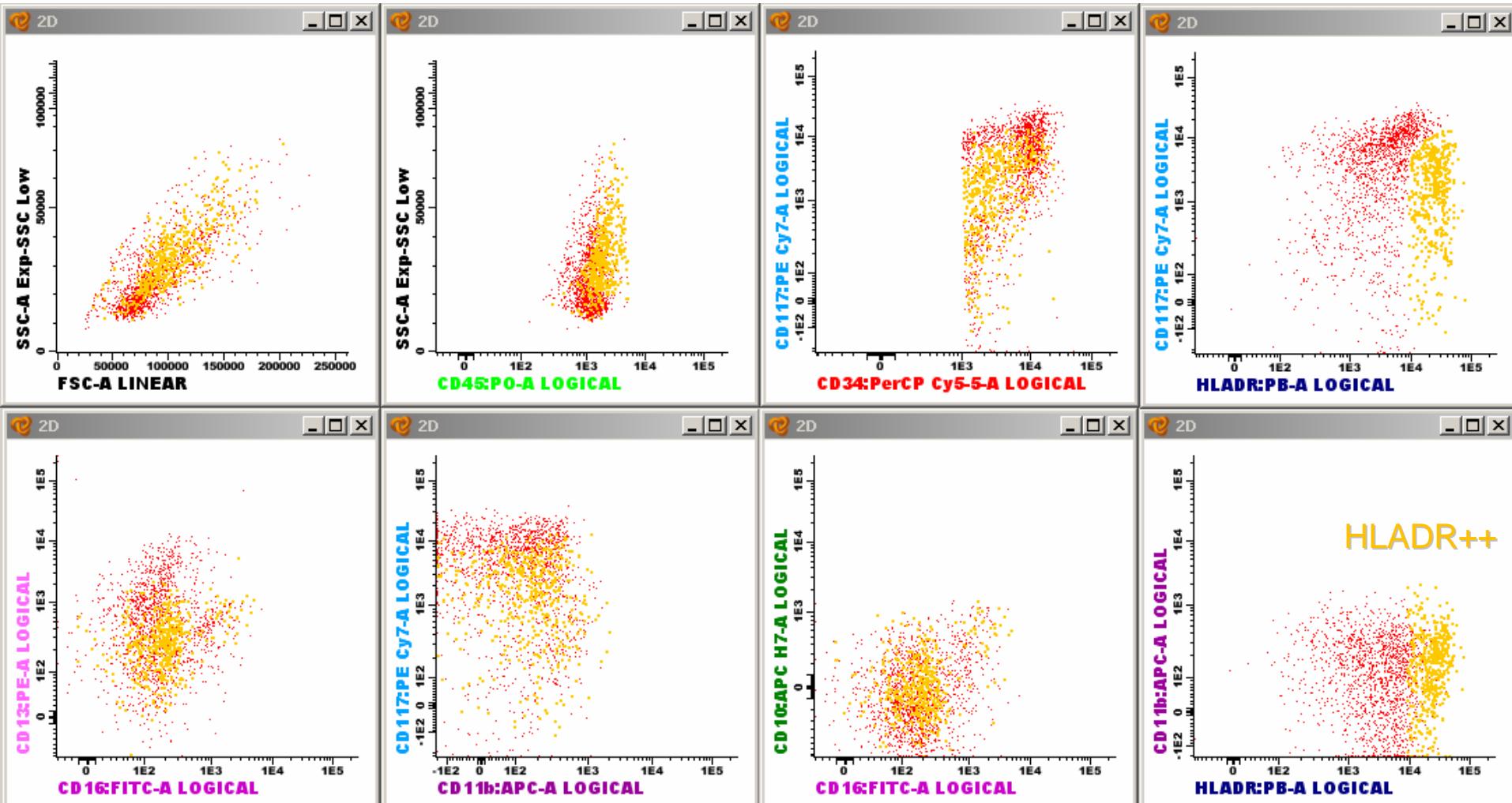
Tube 1 – CD34+ cells

- Monocytic precursors

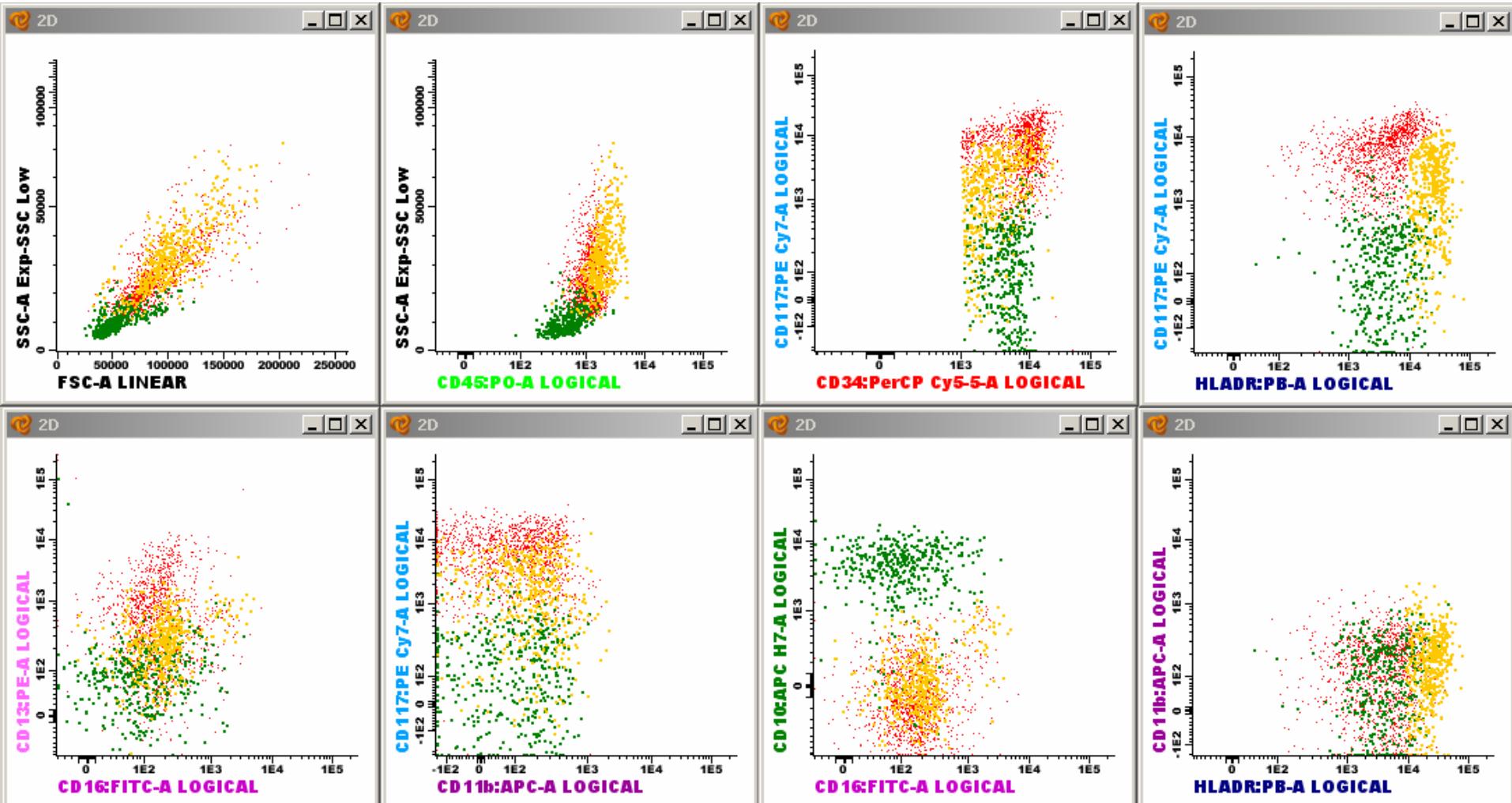


Tube 1 – CD34+ cells

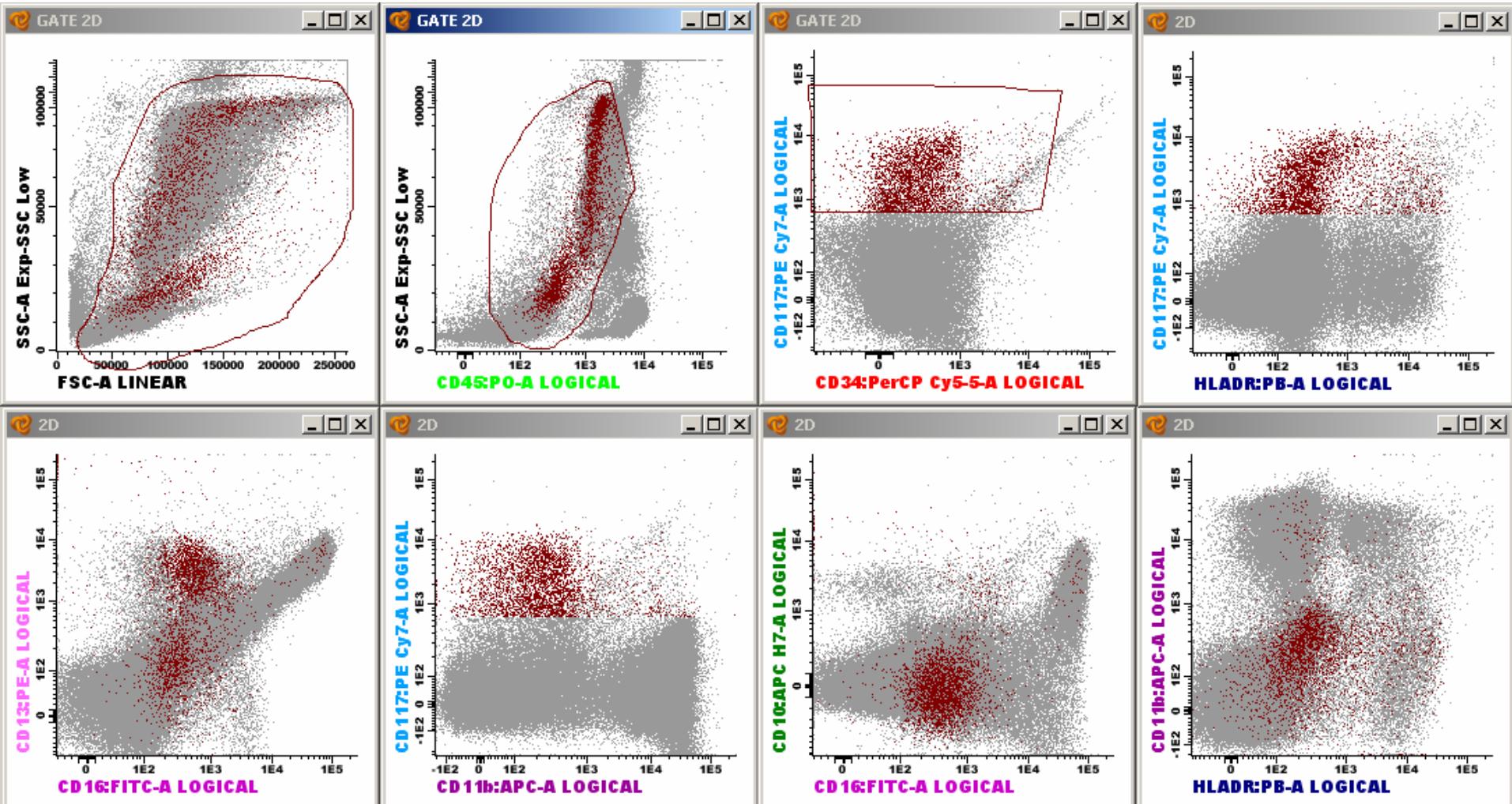
- Monocytic precursors



Tube 1 – CD34+ cells

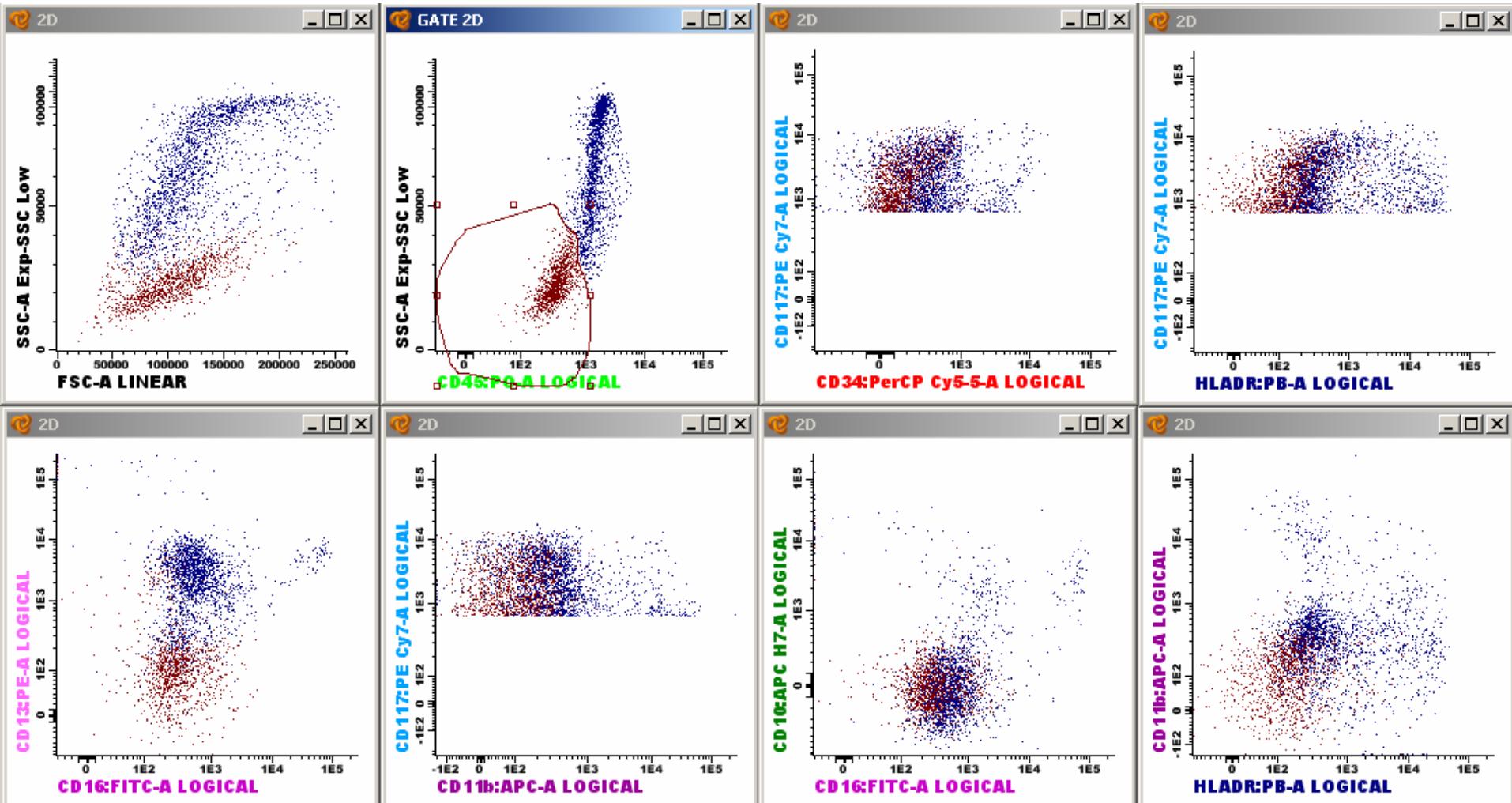


Tube 1 – CD34-/CD117+ cells



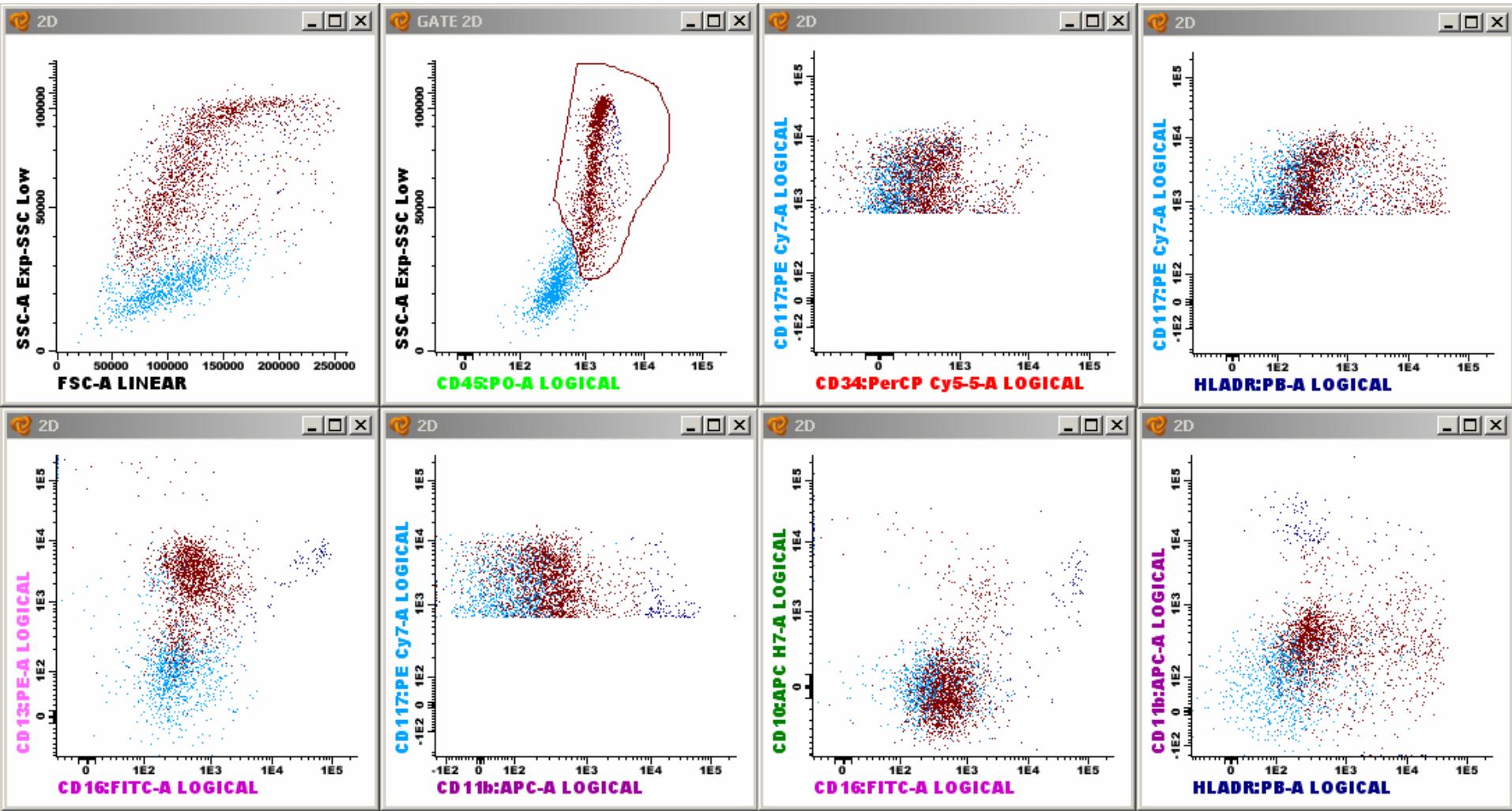
Tube 1 – CD34-/CD117+ cells

- Erythroid precursors

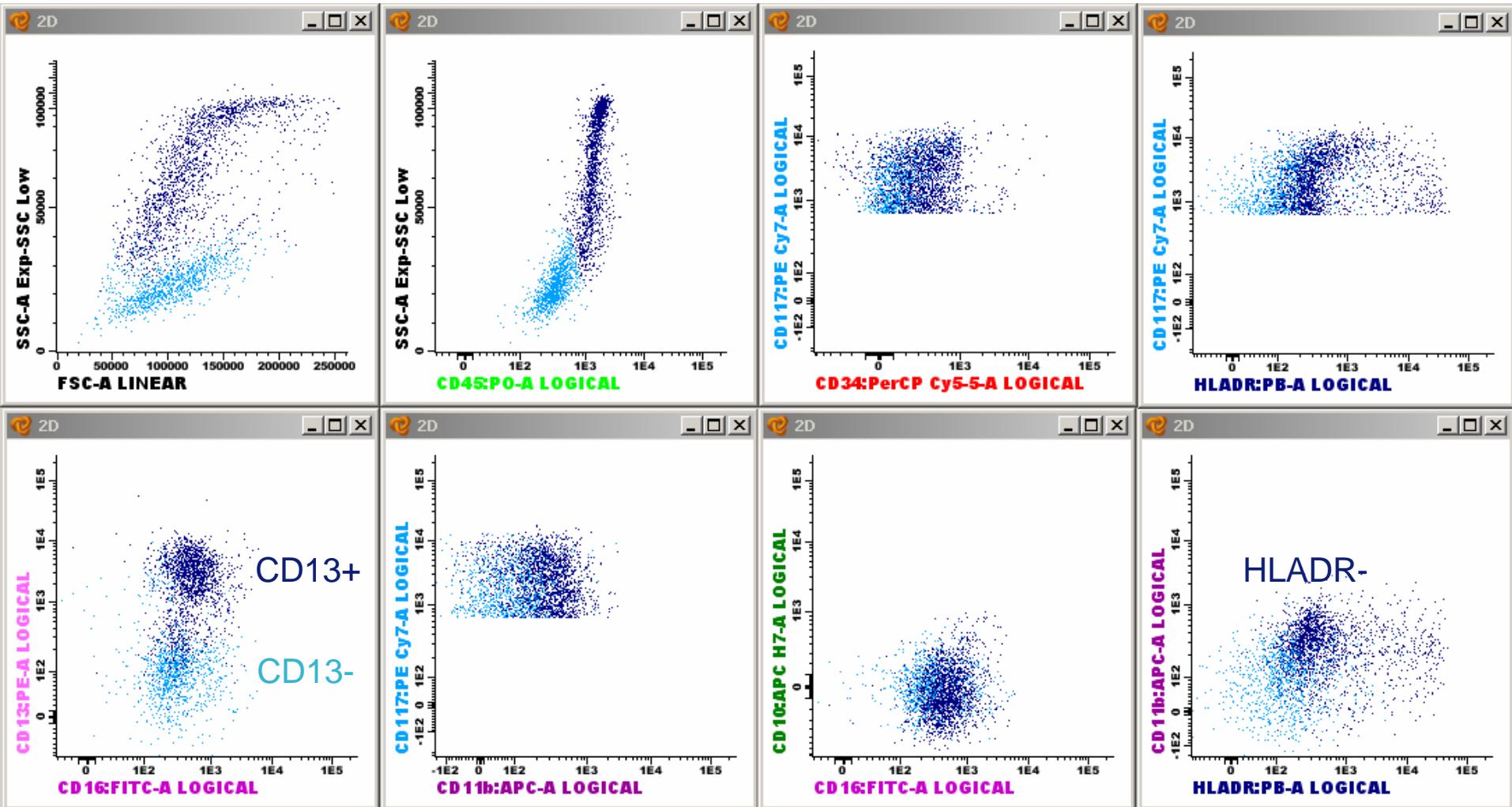


Tube 1 – CD34-/CD117+ cells

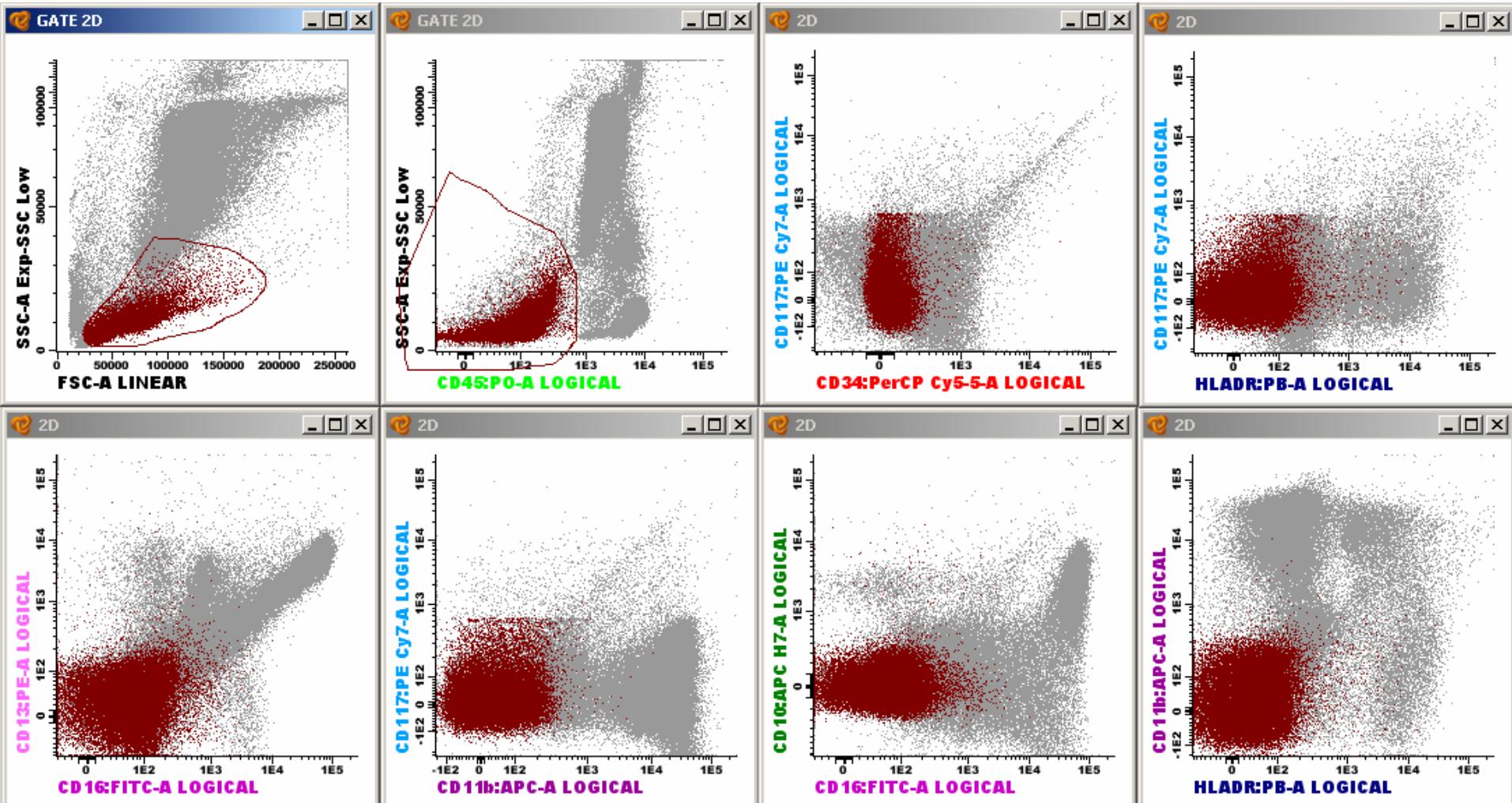
- Promyelocytes



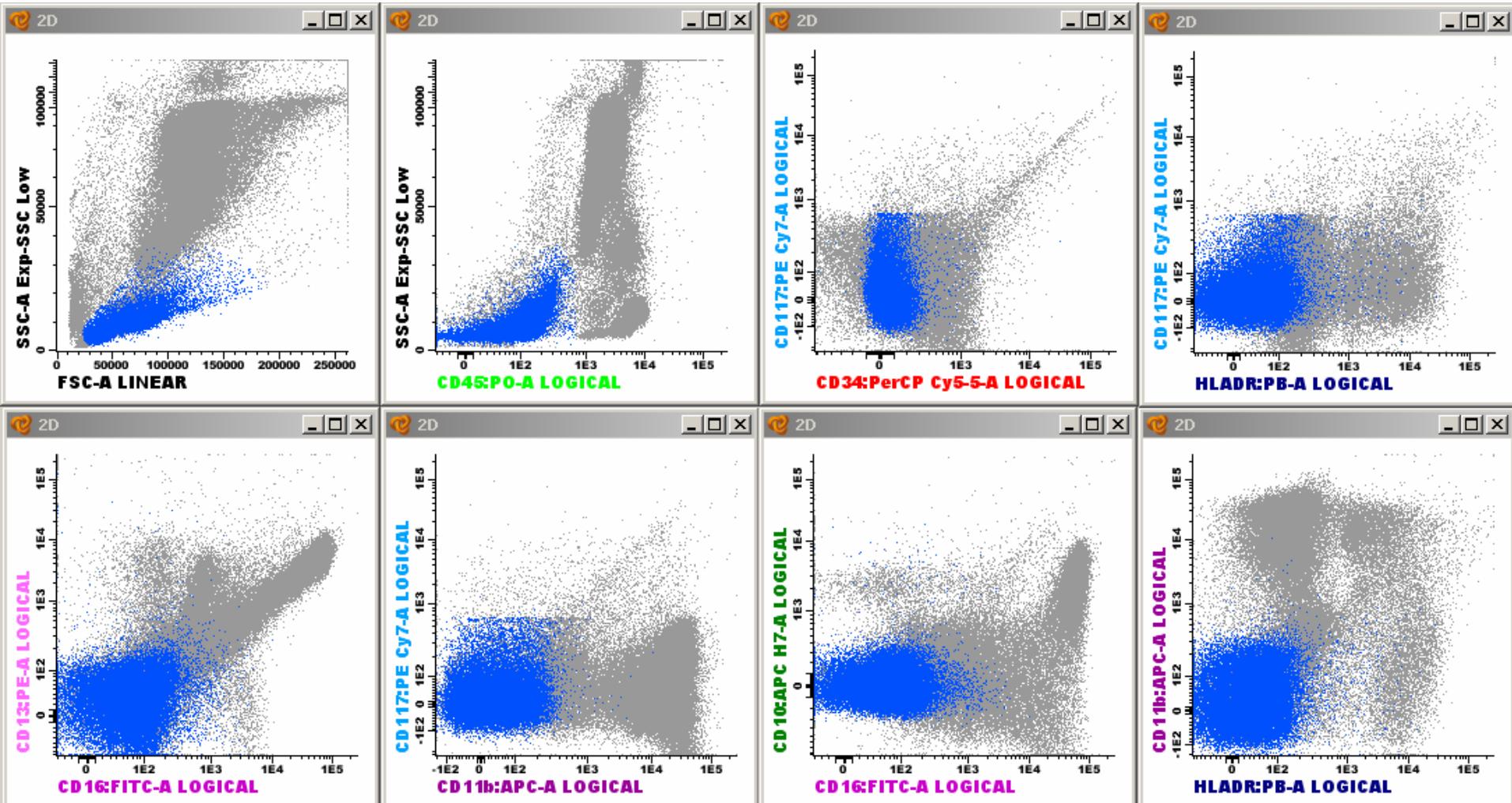
Tube 1 – CD34-/CD117+ cells



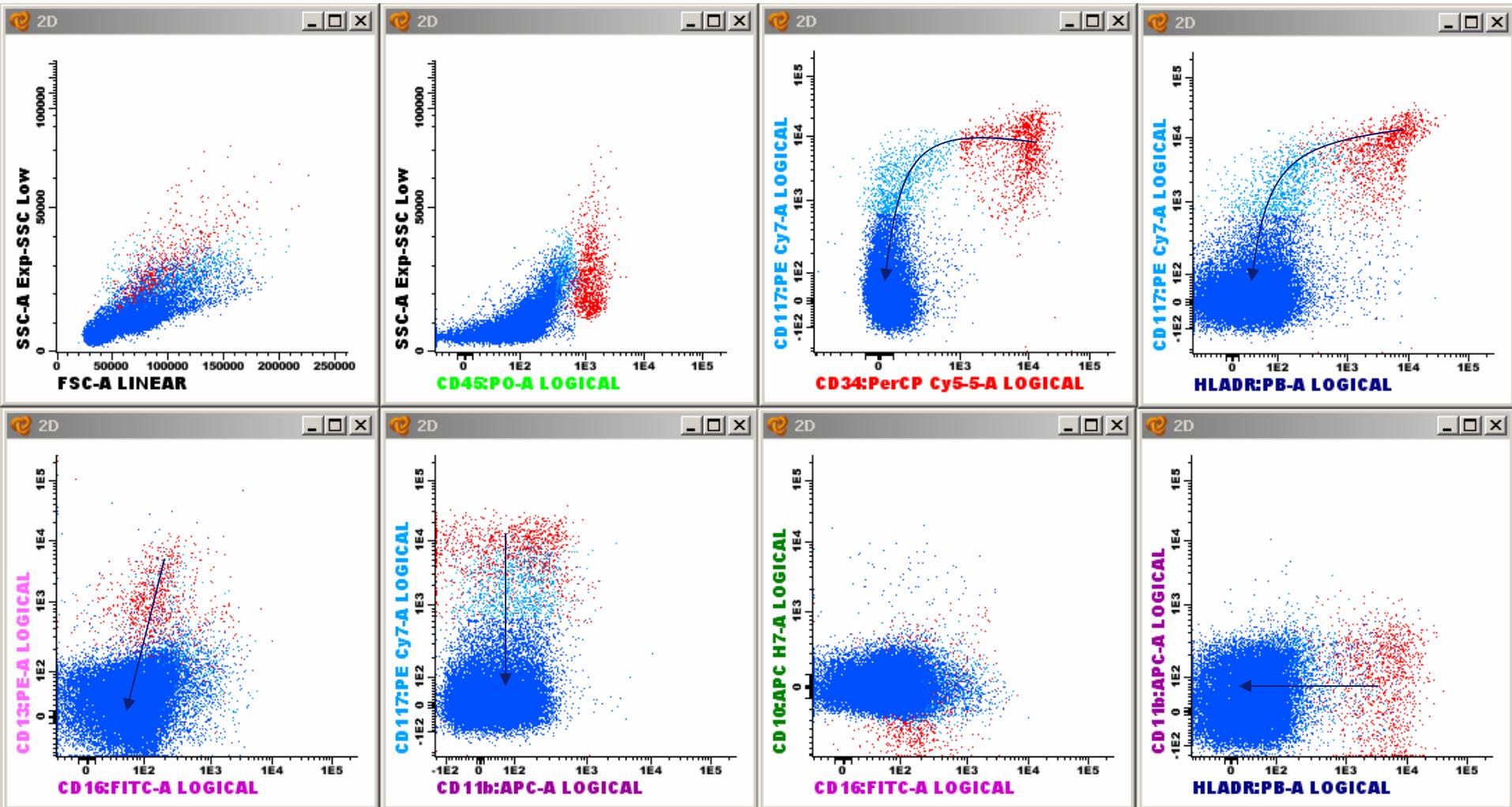
Tube 1 – Erythroid cells



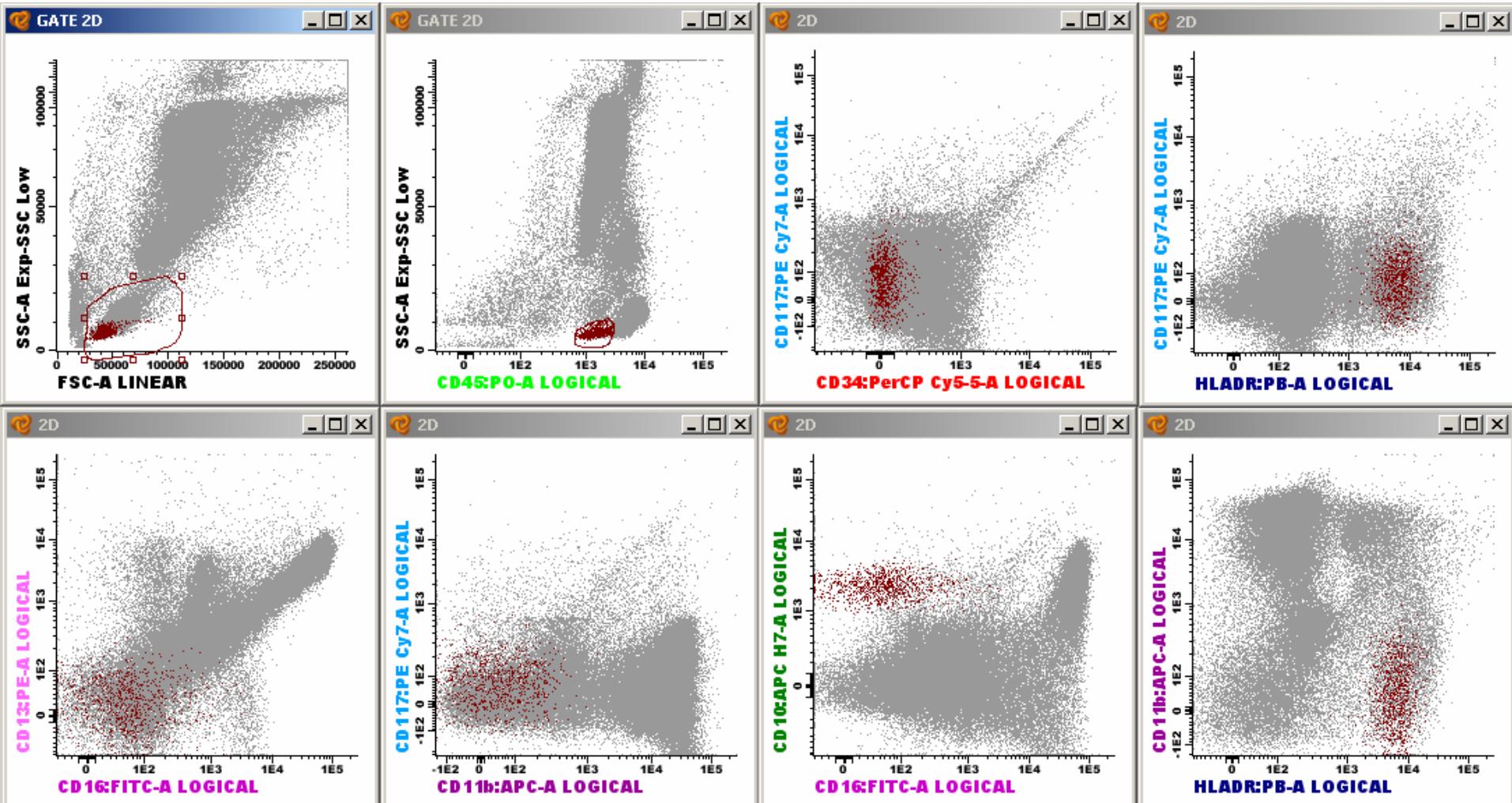
Tube 1 – Erythroid cells



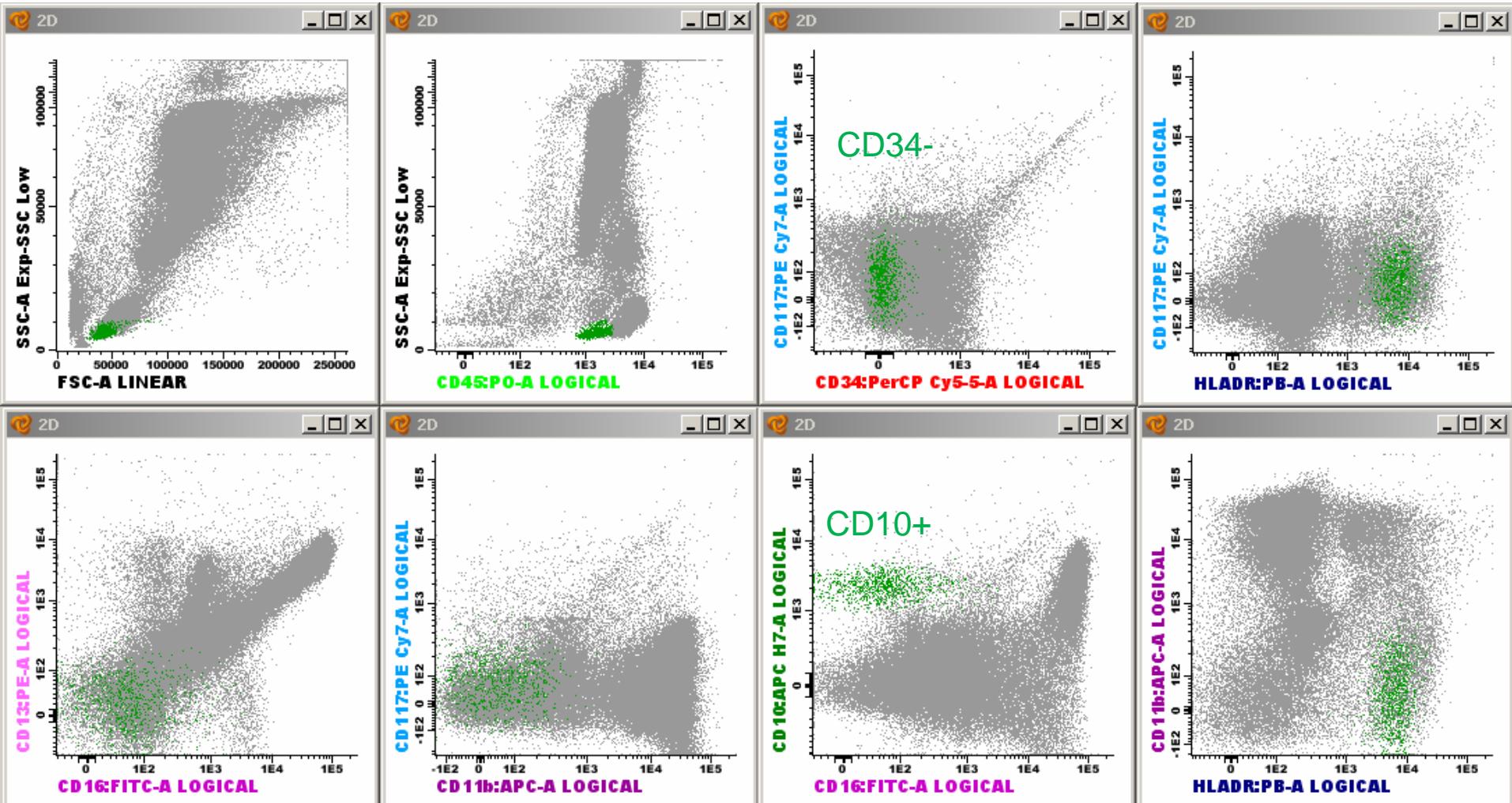
Tube 1 – Erythroid differentiation



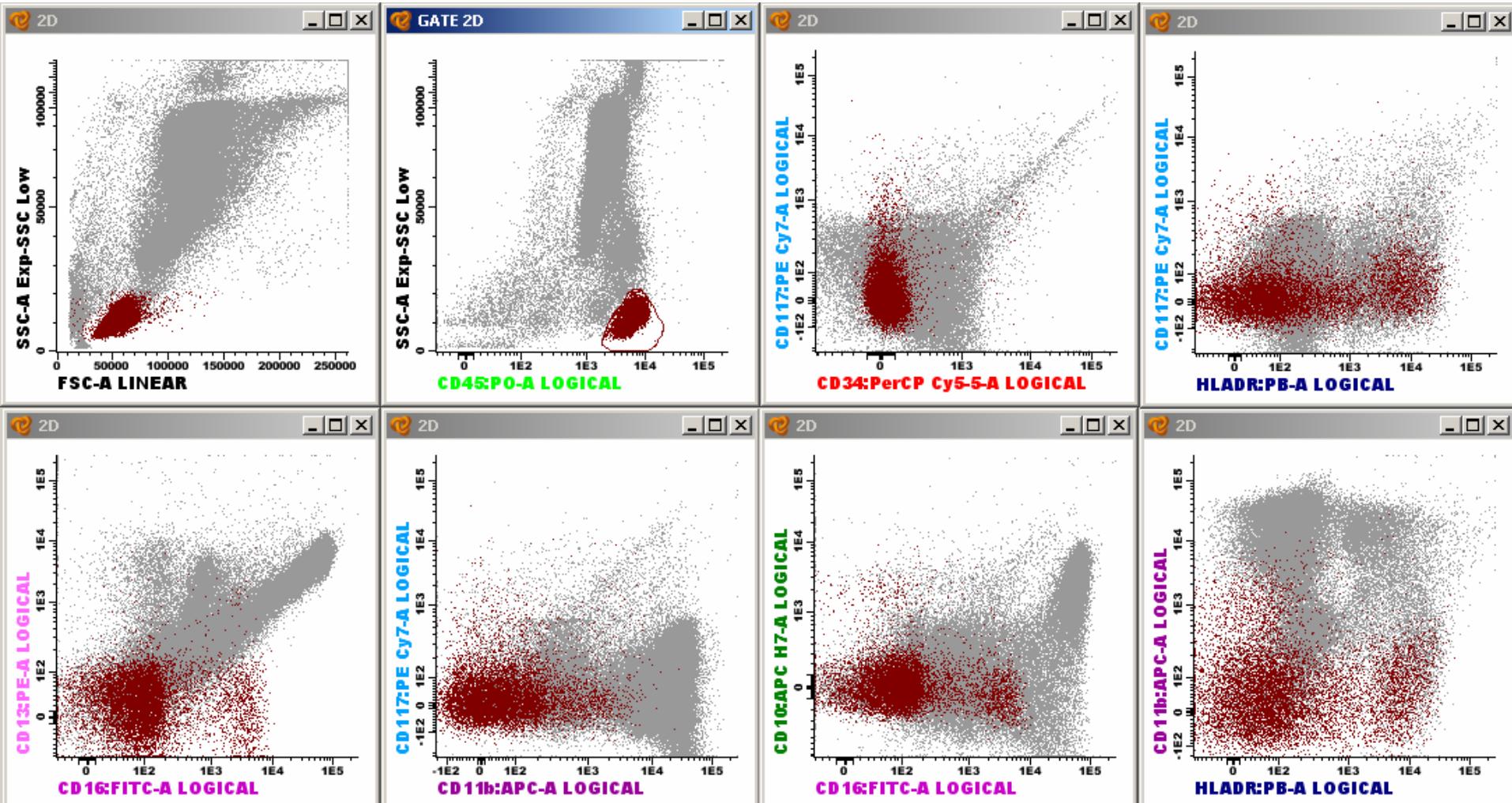
Tube 1 – B-cell precursors



Tube 1 – B-cell precursors

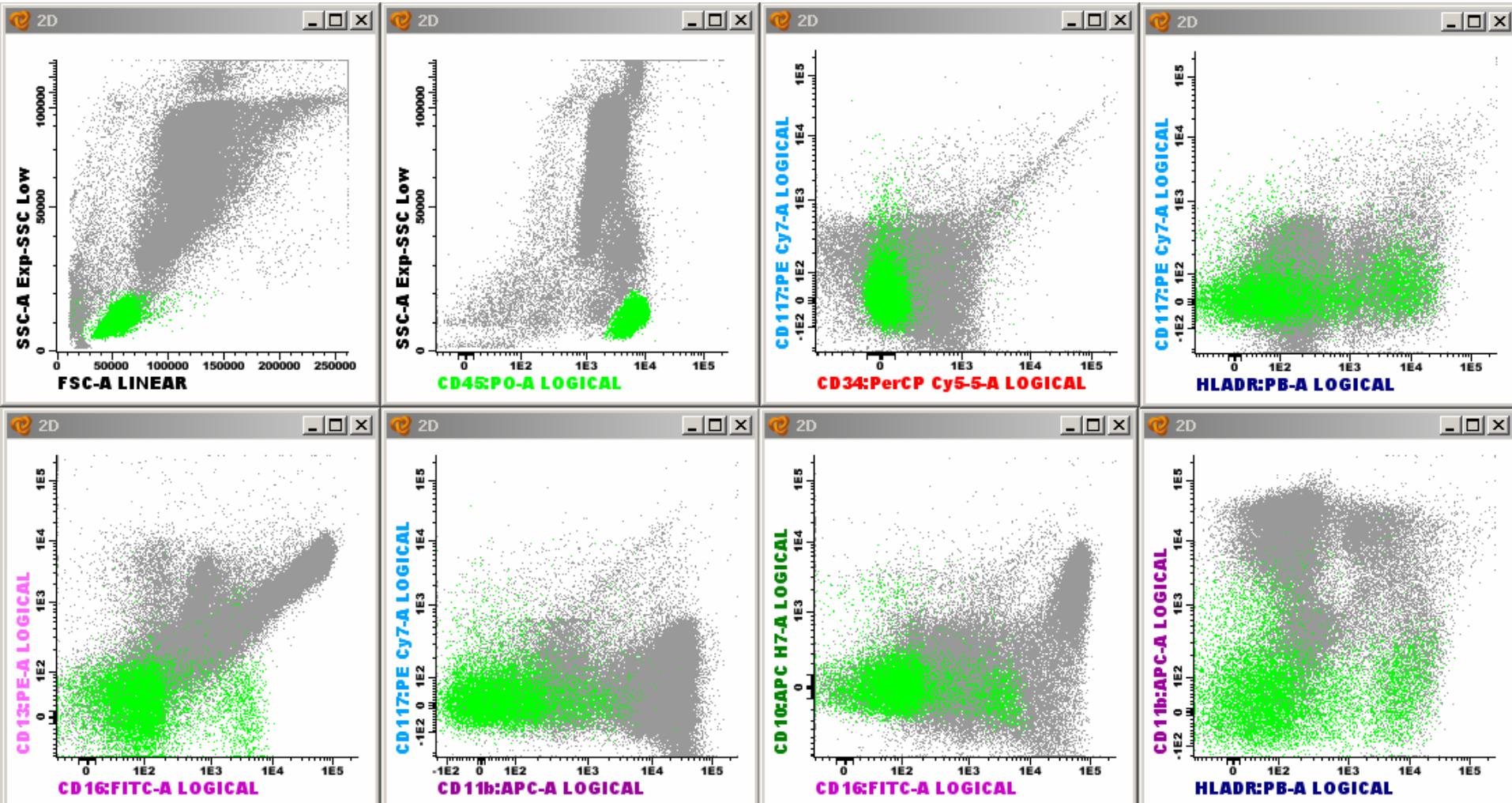


Tube 1 – Lymphocytes



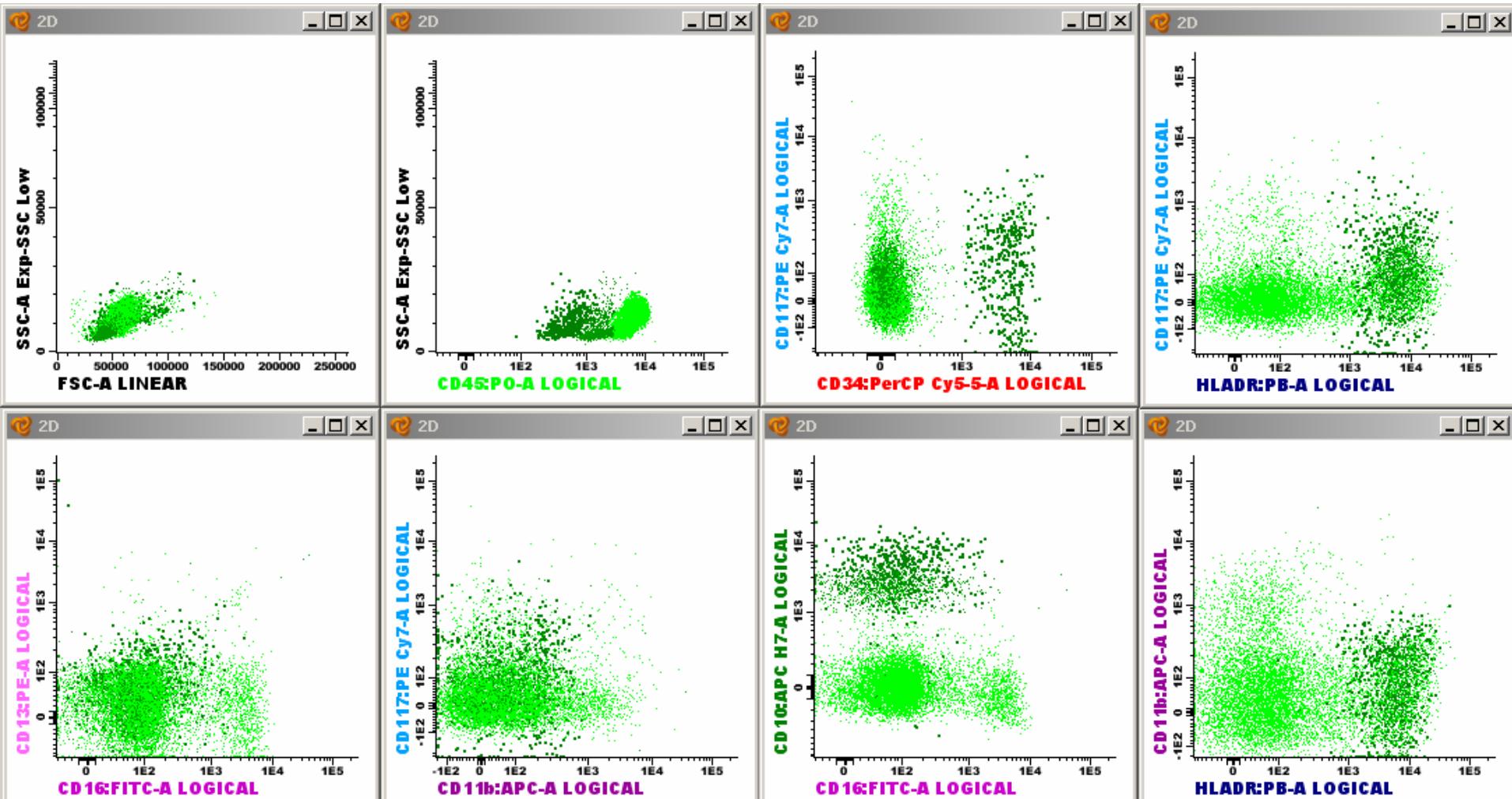


Tube 1 – Lymphocytes

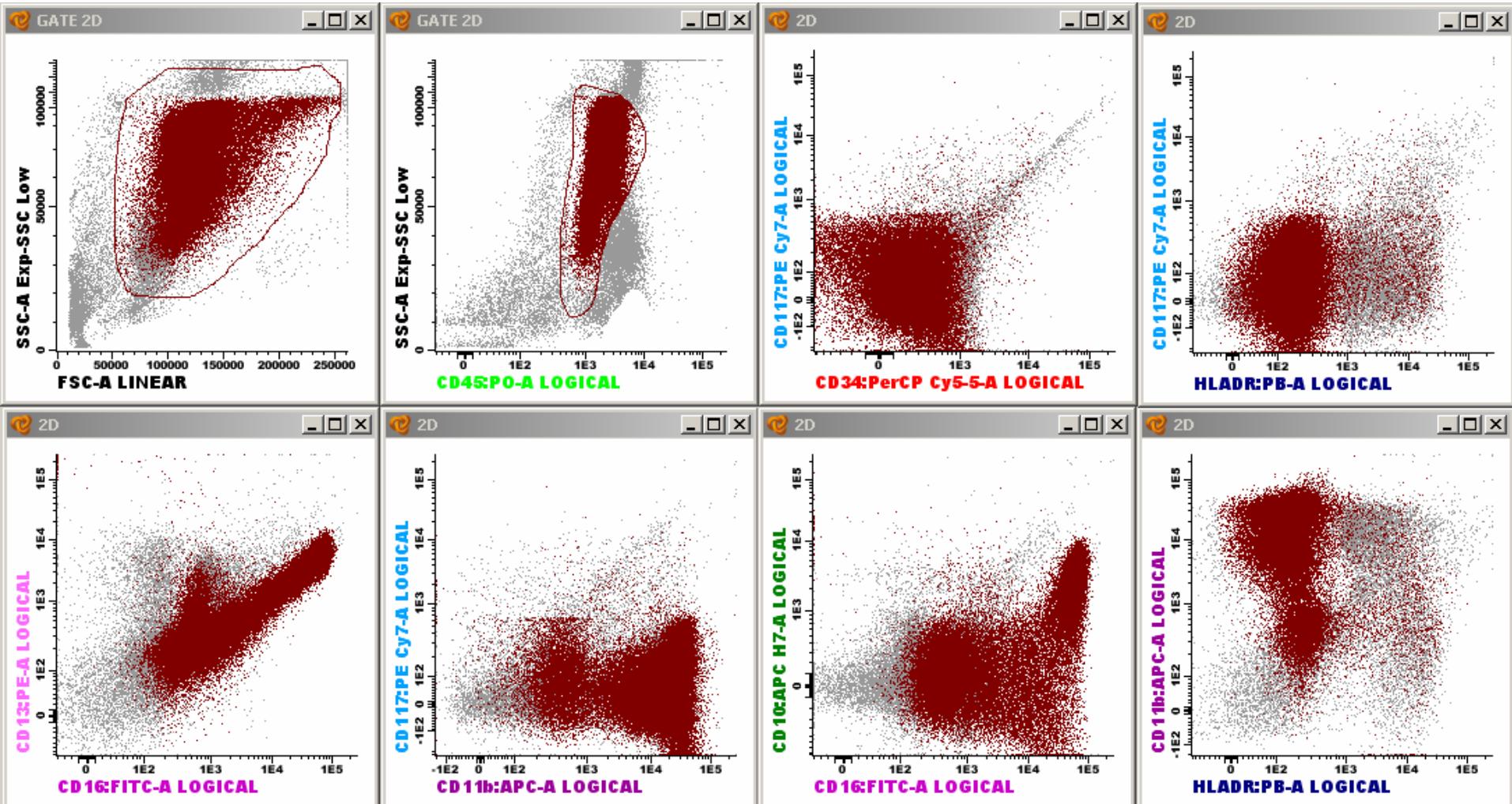


Tube 1 – Lymphocytes

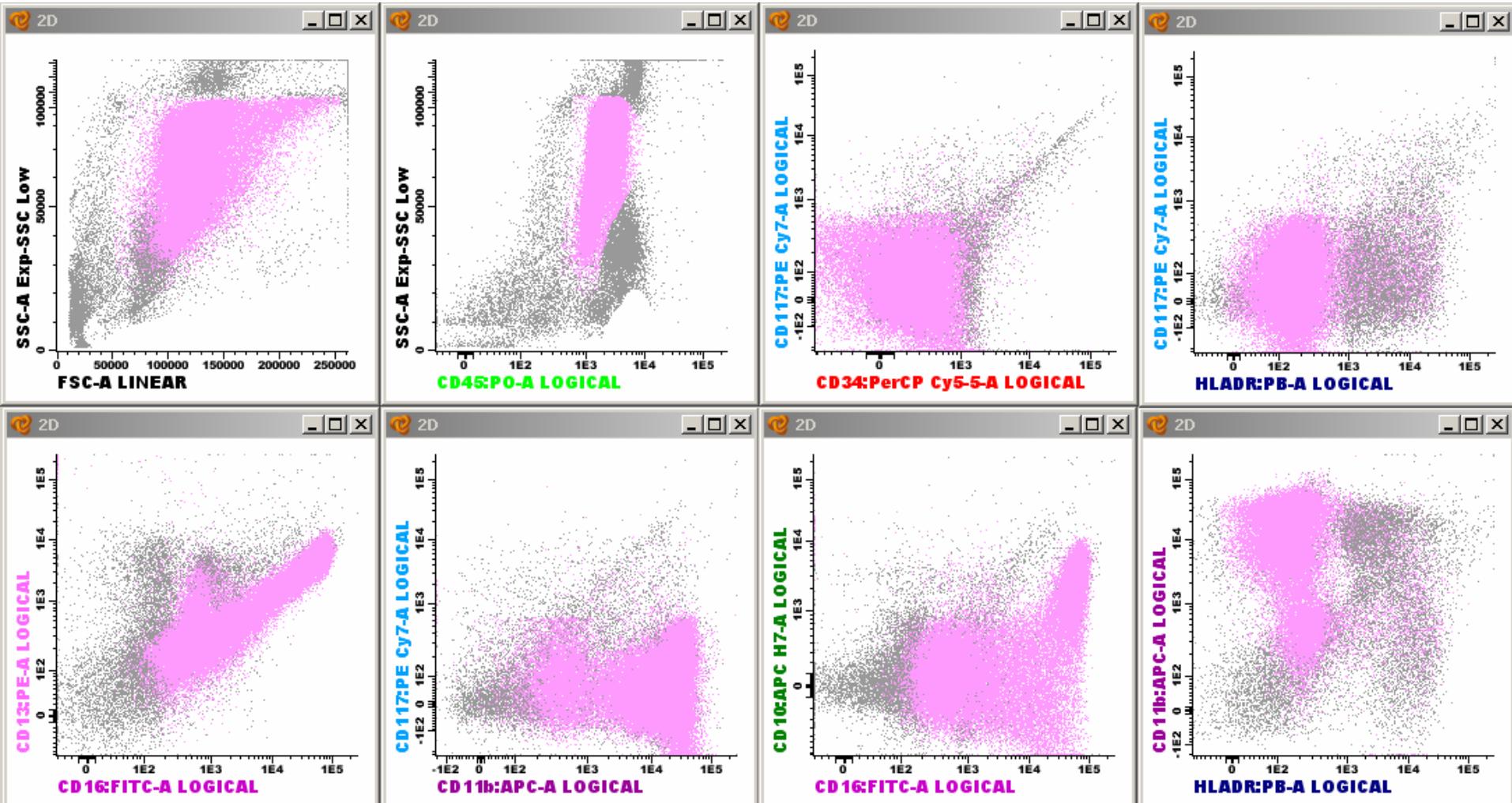
- B-cell precursors and mature lymphocytes



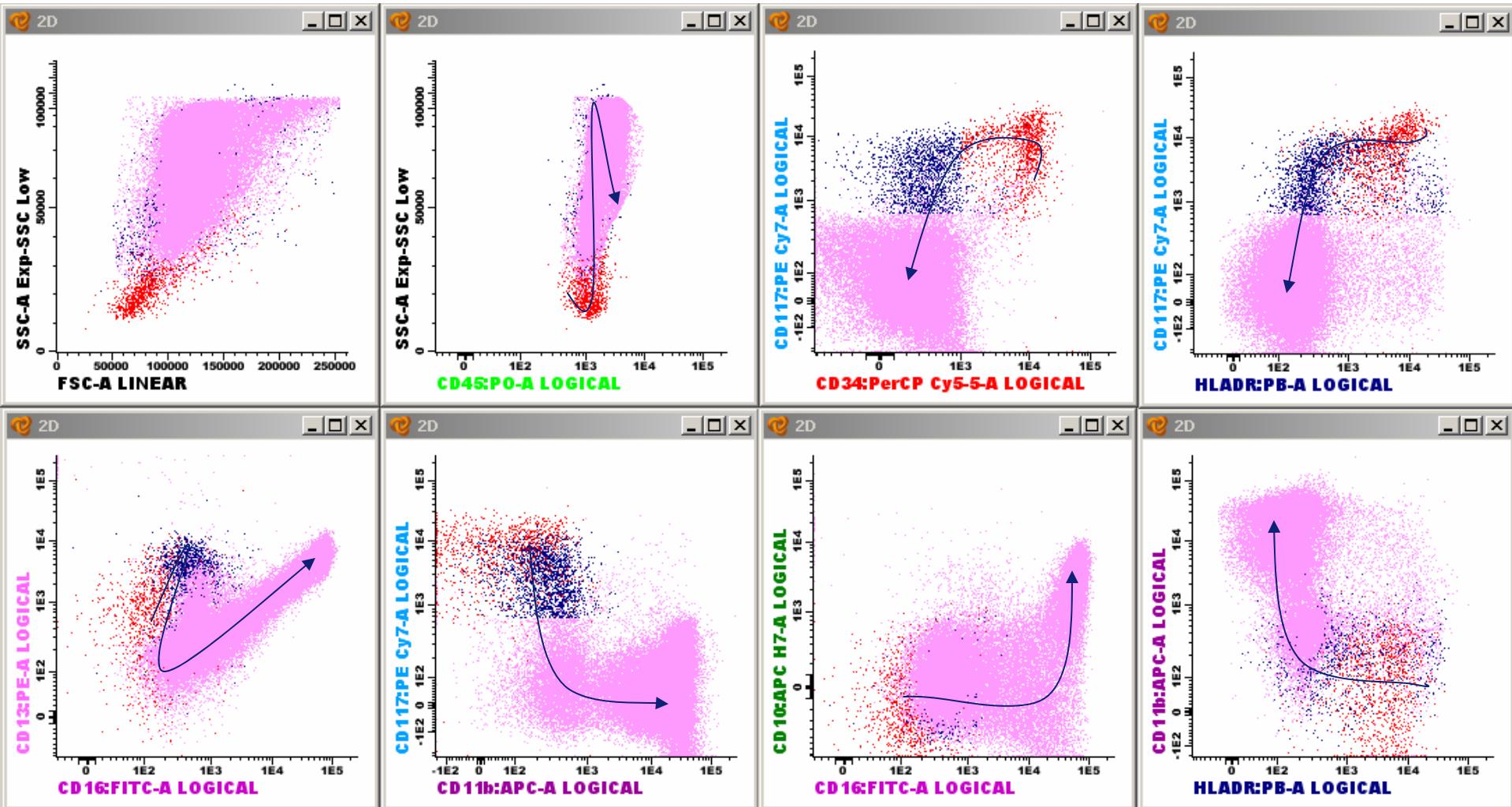
Tube 1 – Neutrophils



Tube 1 – Neutrophils

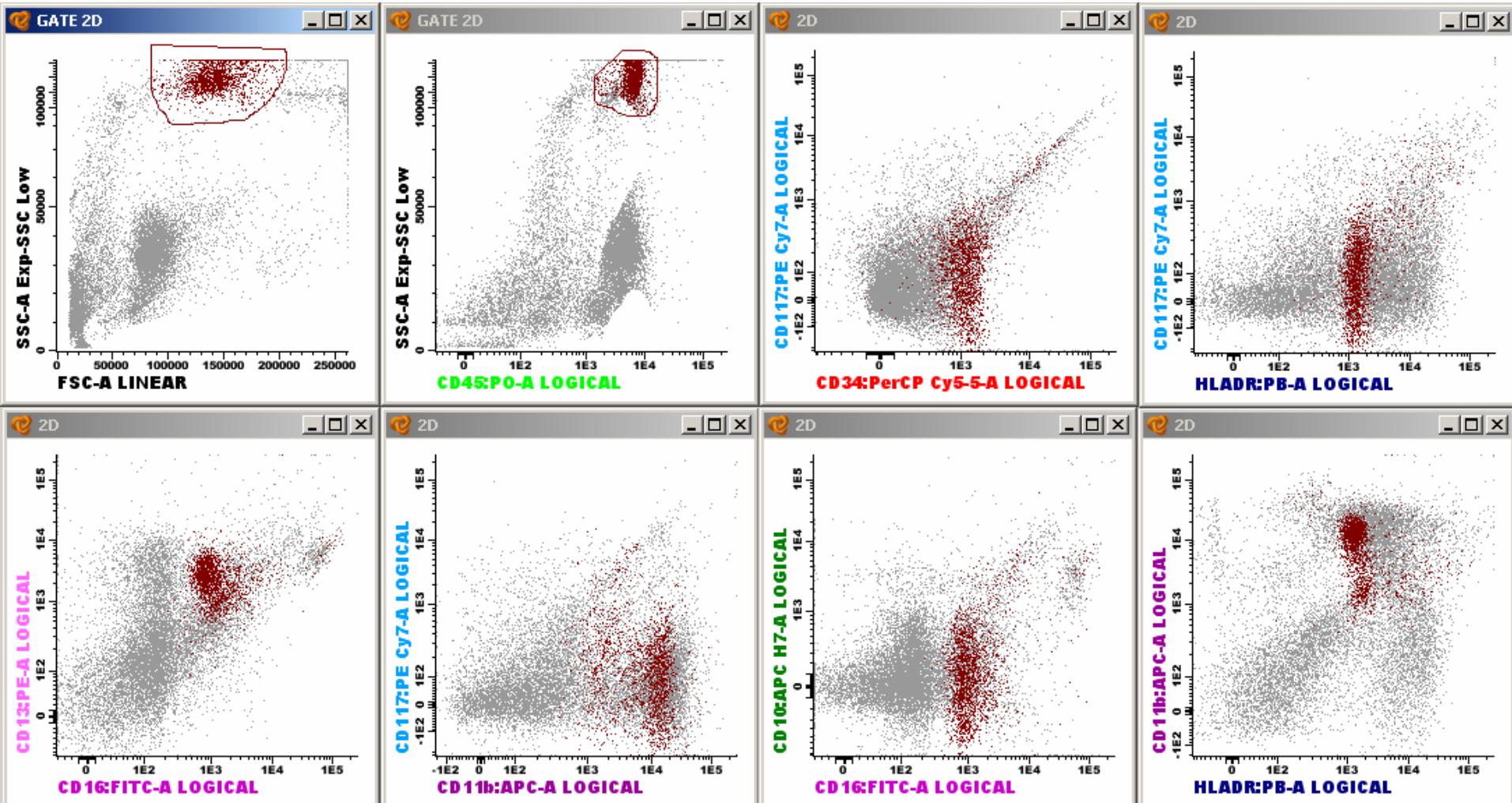


Tube 1 – Neutrophilic differentiation

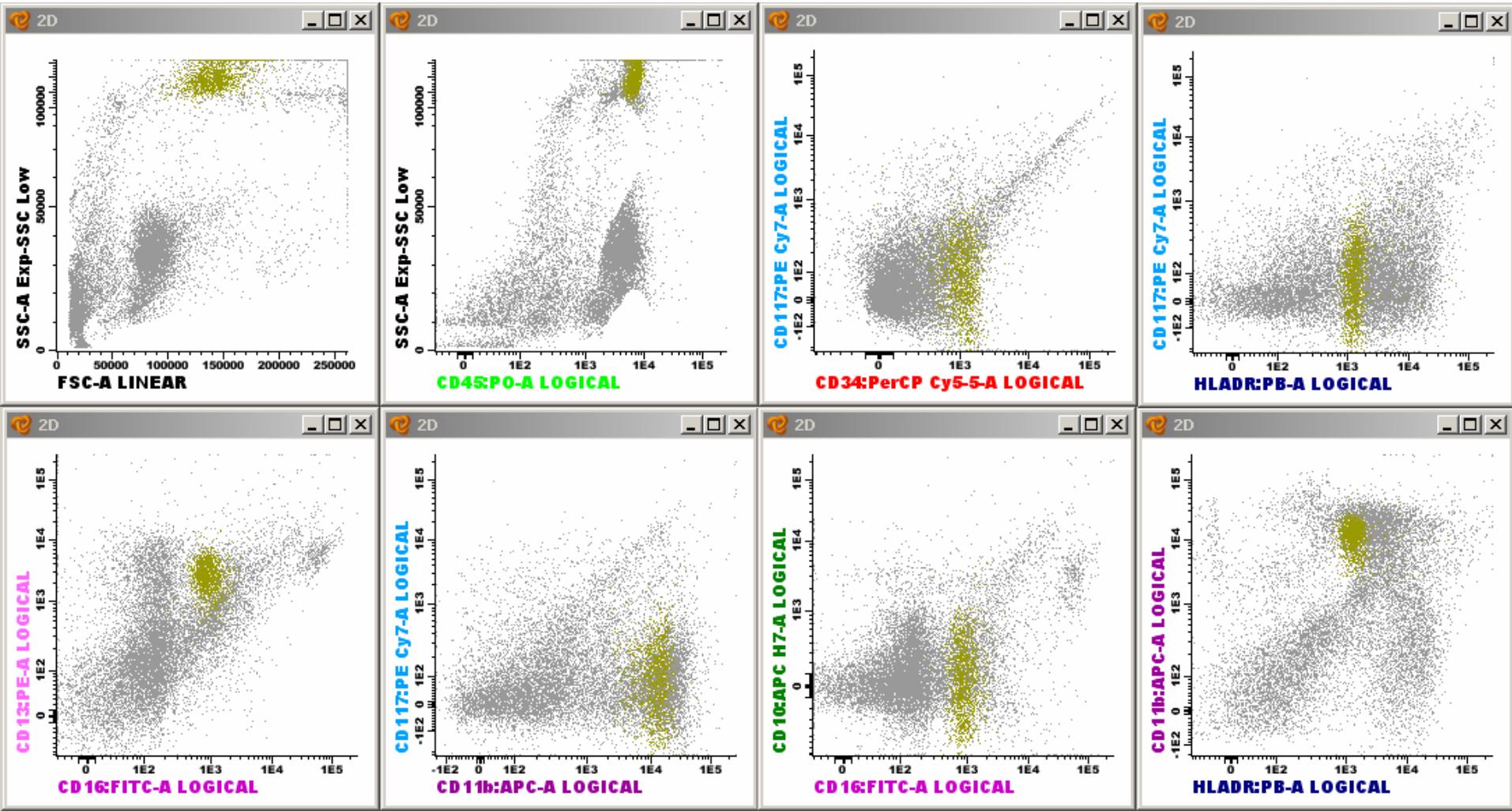




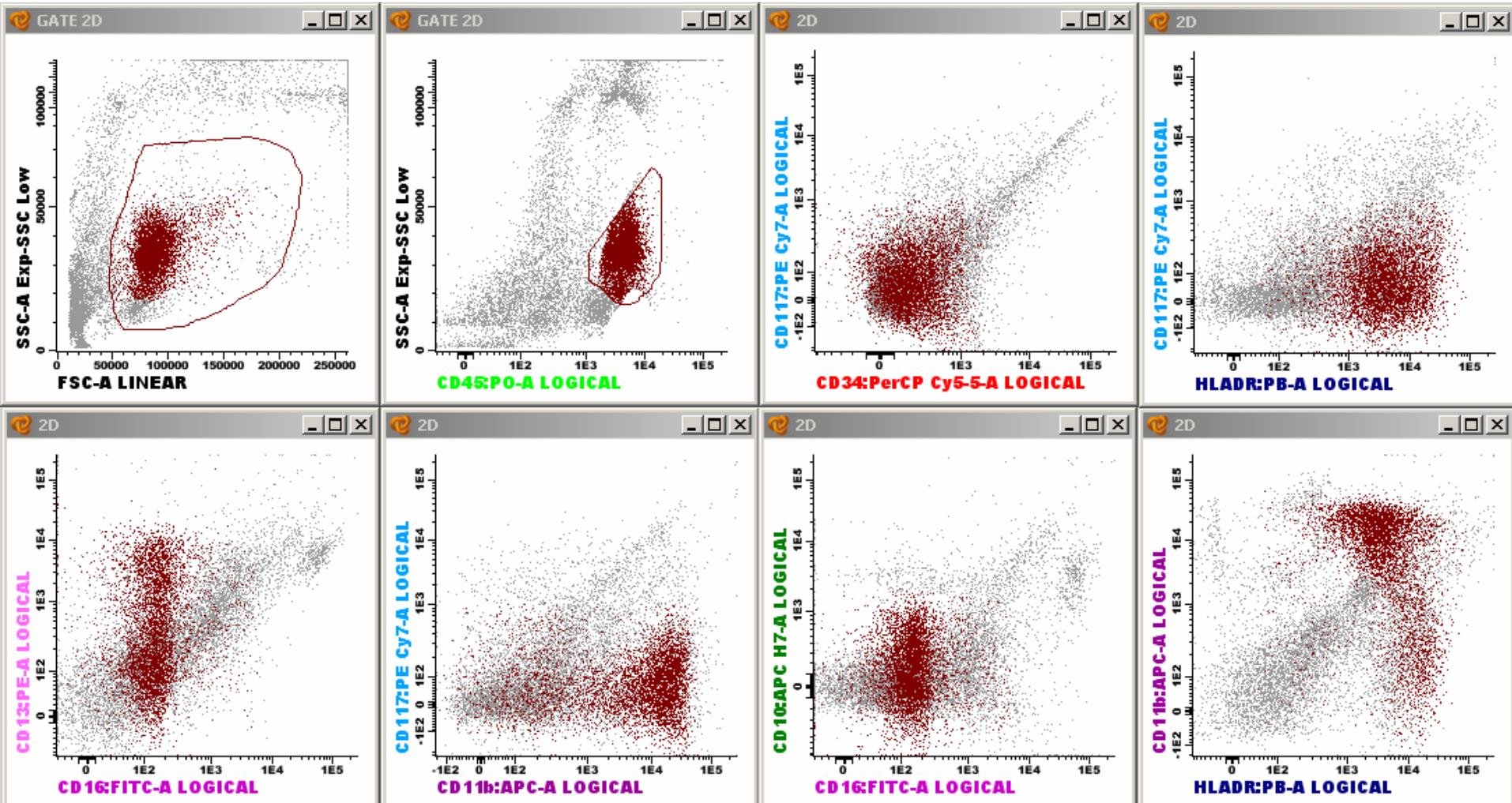
Tube 1 – Eosinophils



Tube 1 – Eosinophils

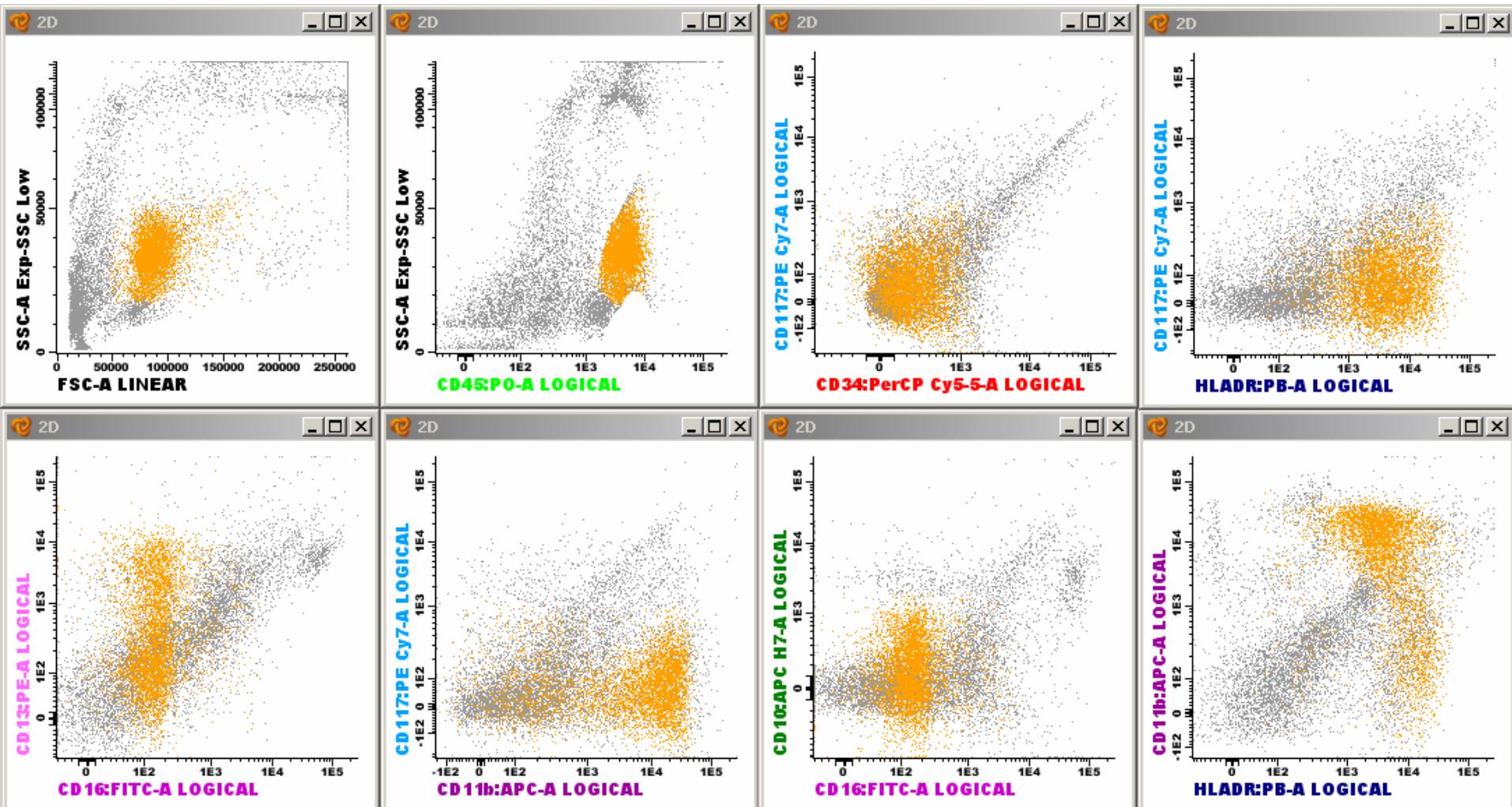


Tube 1 – Monocytes

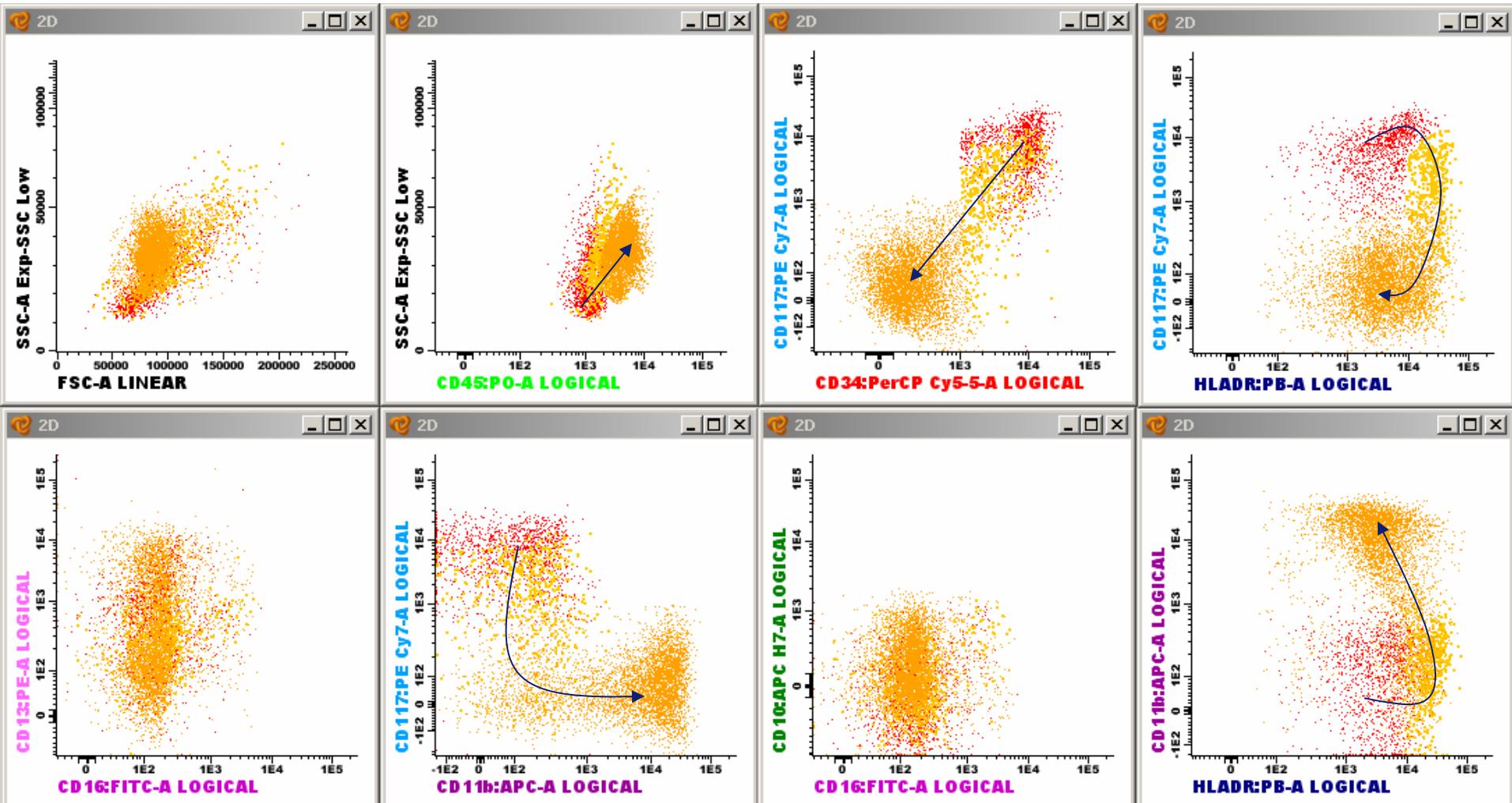




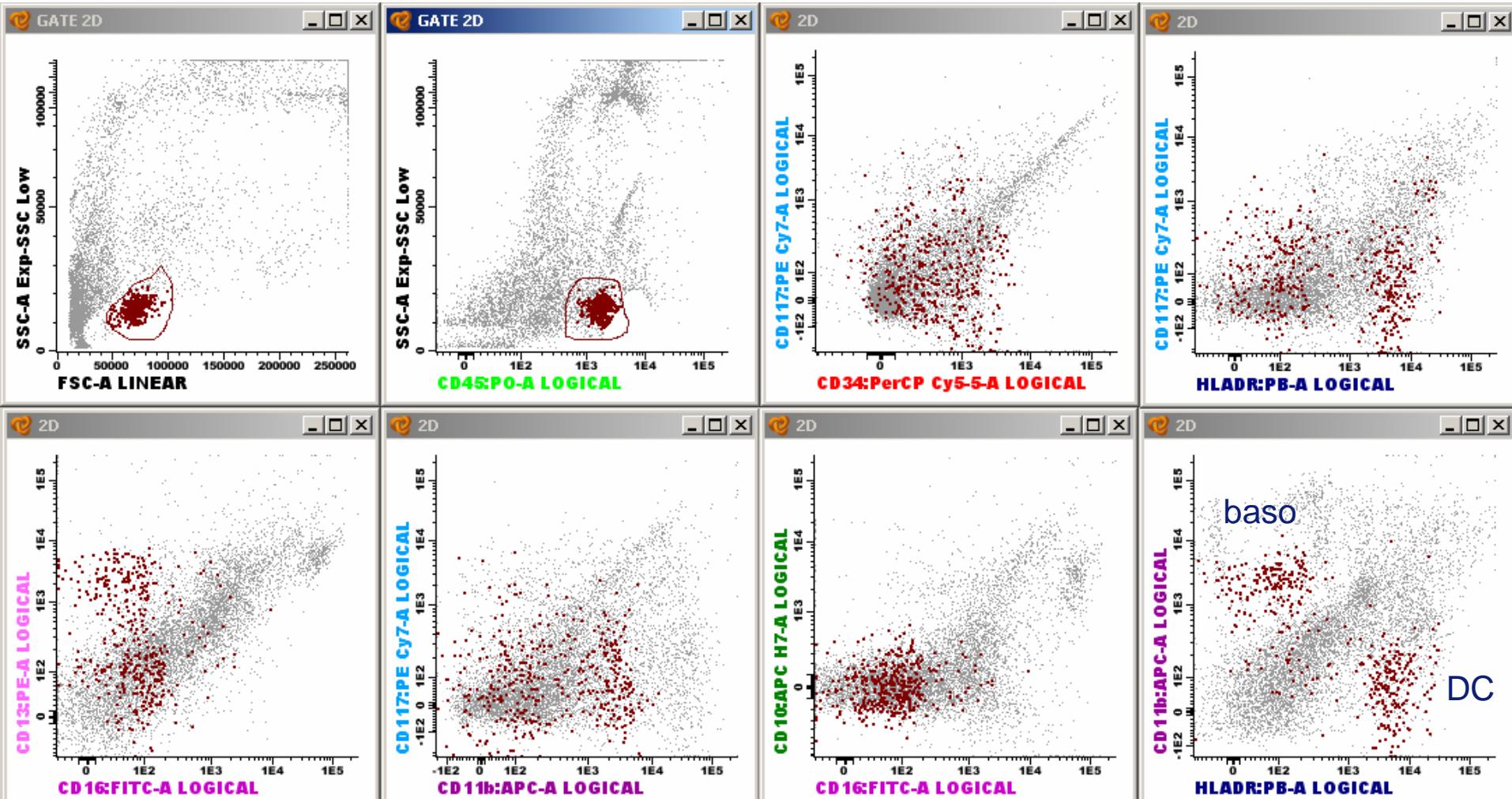
Tube 1 – Monocytes



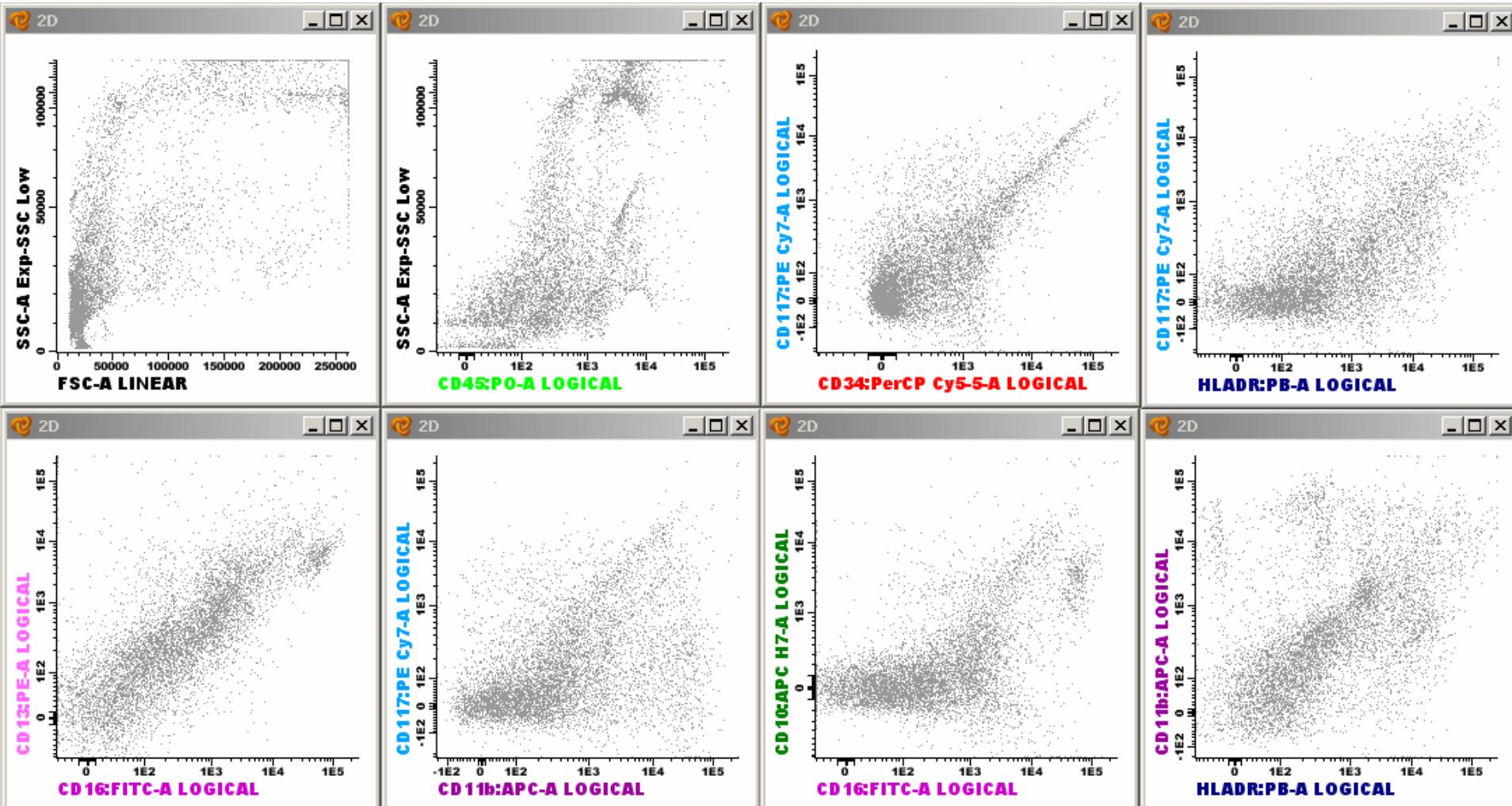
Tube 1 – Monocytic differentiation



Tube 1 – DC/Basophils



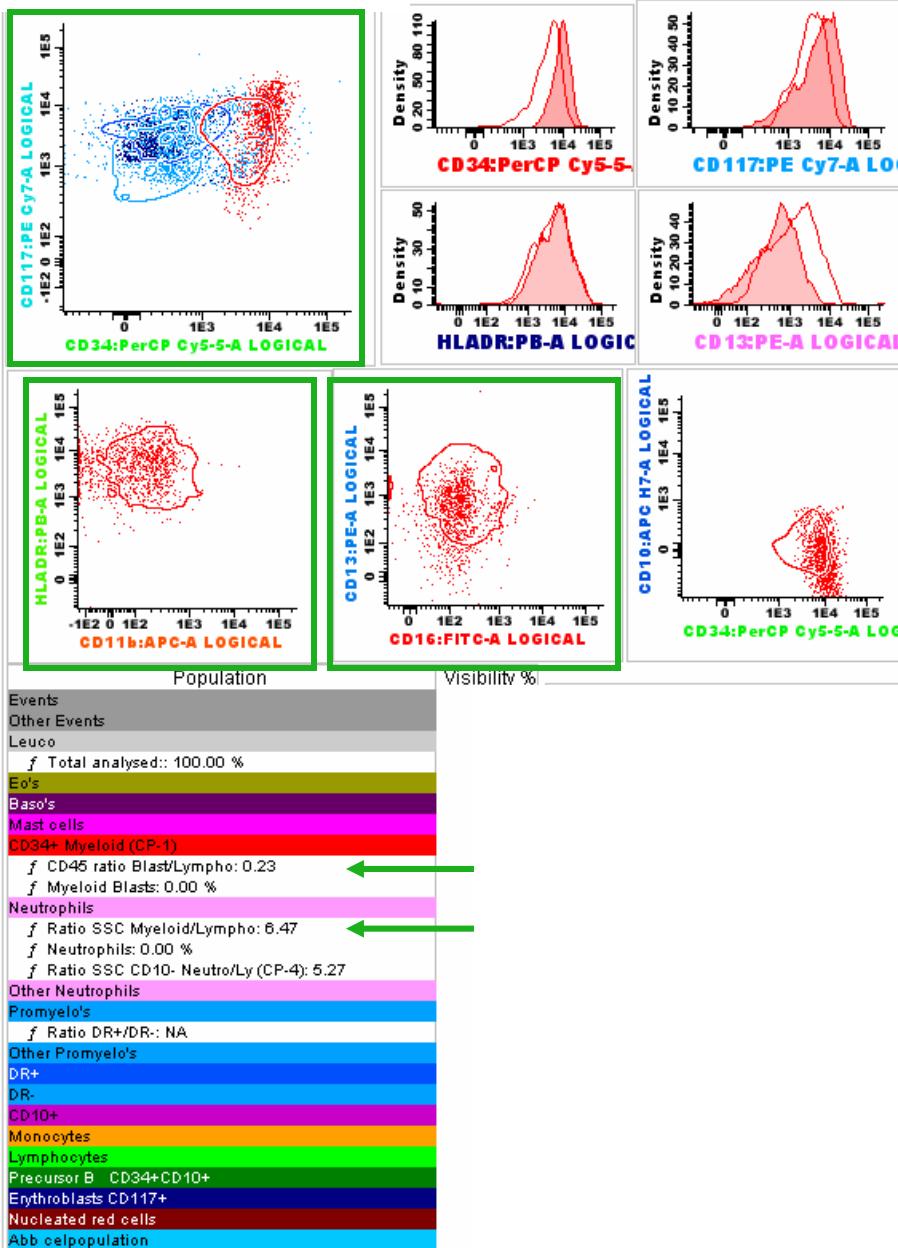
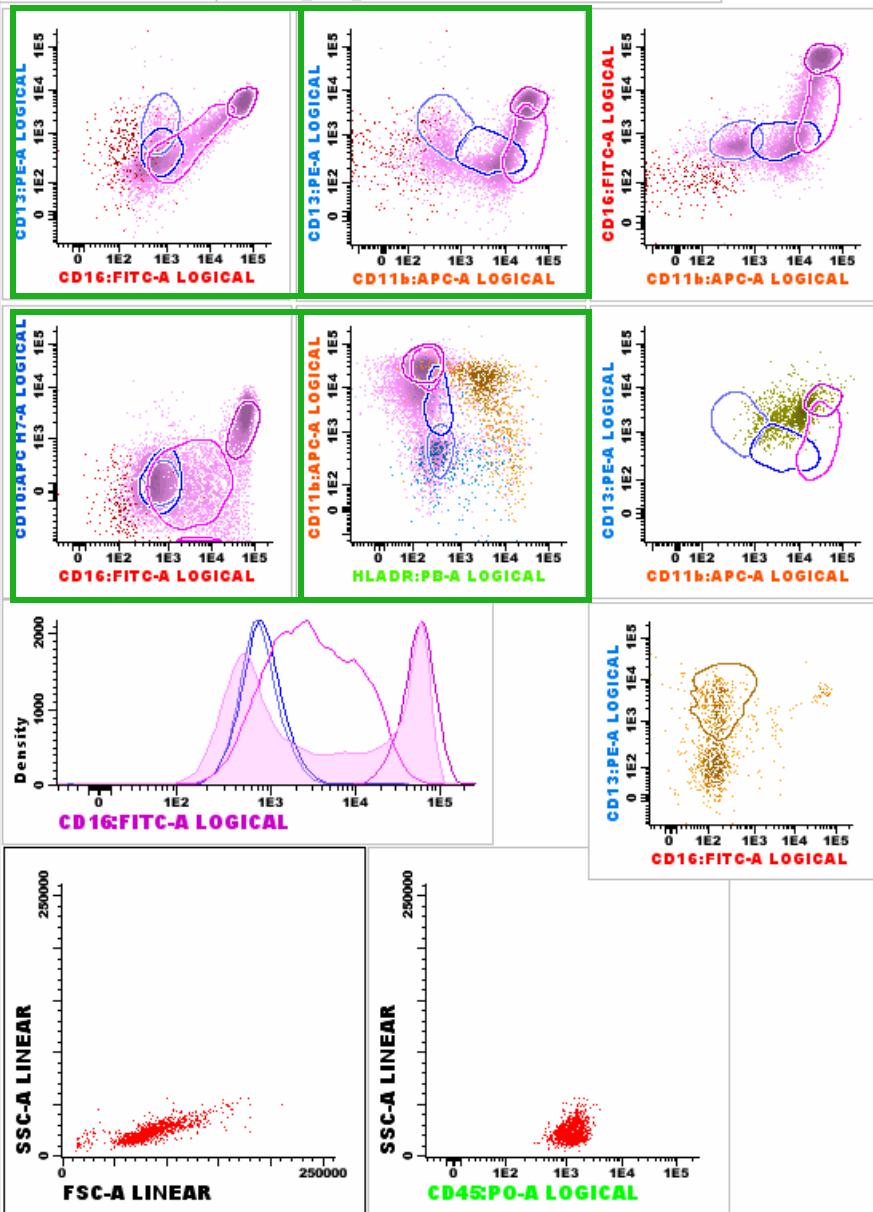
Tube 1 – Debris



Report – Tube 1

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Recommended minimal requirements

Bone marrow subset	Recommended analyses	Aberrancy
Immature myeloid and monocytic progenitors	Percentage of cells in nucleated cell fraction ^a Expression of CD45 Expression of CD34 Expression of CD117 Expression of HLA-DR Expression of CD13 and CD33 Asynchronous expression of CD11b, CD15 Expression of CD5, CD7, CD19, CD56 ^b	Increased percentage Lack of/decreased/increased Lack of/decreased/increased Homogenous under/overexpression Lack of/increased expression Lack of/decreased/increased Presence of mature markers Presence of lineage infidelity markers
Maturing neutrophils	Percentage of cells as ratio to lymphocytes SSC as ratio vs SSC of lymphocytes Relationship of CD13 and CD11b Relationship of CD13 and CD16 Relationship of CD15 and CD10	Decreased Decreased Altered pattern ^c Altered pattern ^c Altered pattern ^c ; for example, lack of CD10 on mature neutrophils
Monocytes	Percentage of cells <u>Distribution of maturation stages</u> Relationship of HLA-DR and CD11b Relationship of CD36 and CD14 Expression of CD13 and CD33 Expression of CD56 ^b	Decreased/increased Shift towards immature Altered pattern ^c Altered pattern ^c (Homogenous) under/overexpression Presence of lineage infidelity marker
Progenitor B cells	Enumeration as fraction of total CD34+ based on CD45/CD34/SSC in combination with CD10 or CD19	Decreased or absent
Erythroid compartment ^d	Percentage of nucleated erythroid cells Relationship CD71 and CD235a Expression of CD71 Expression of CD36 Percentage of CD117-positive precursors	Increased Altered pattern ^c Decreased Decreased Increased

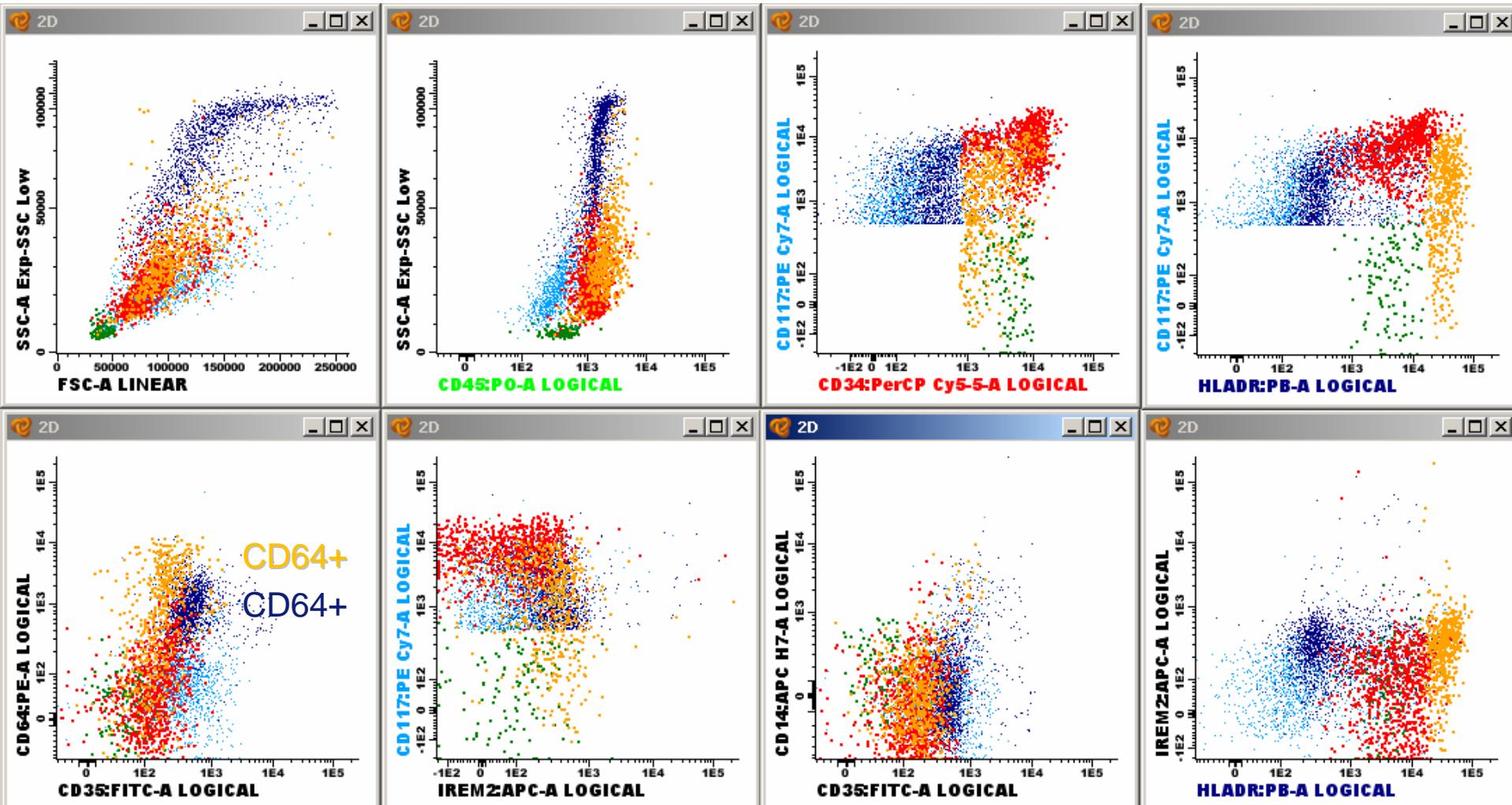
Normal myeloid differentiation

- Myeloid precursors
- Granulocytic differentiation
- Monocytic differentiation
- Erythroid differentiation

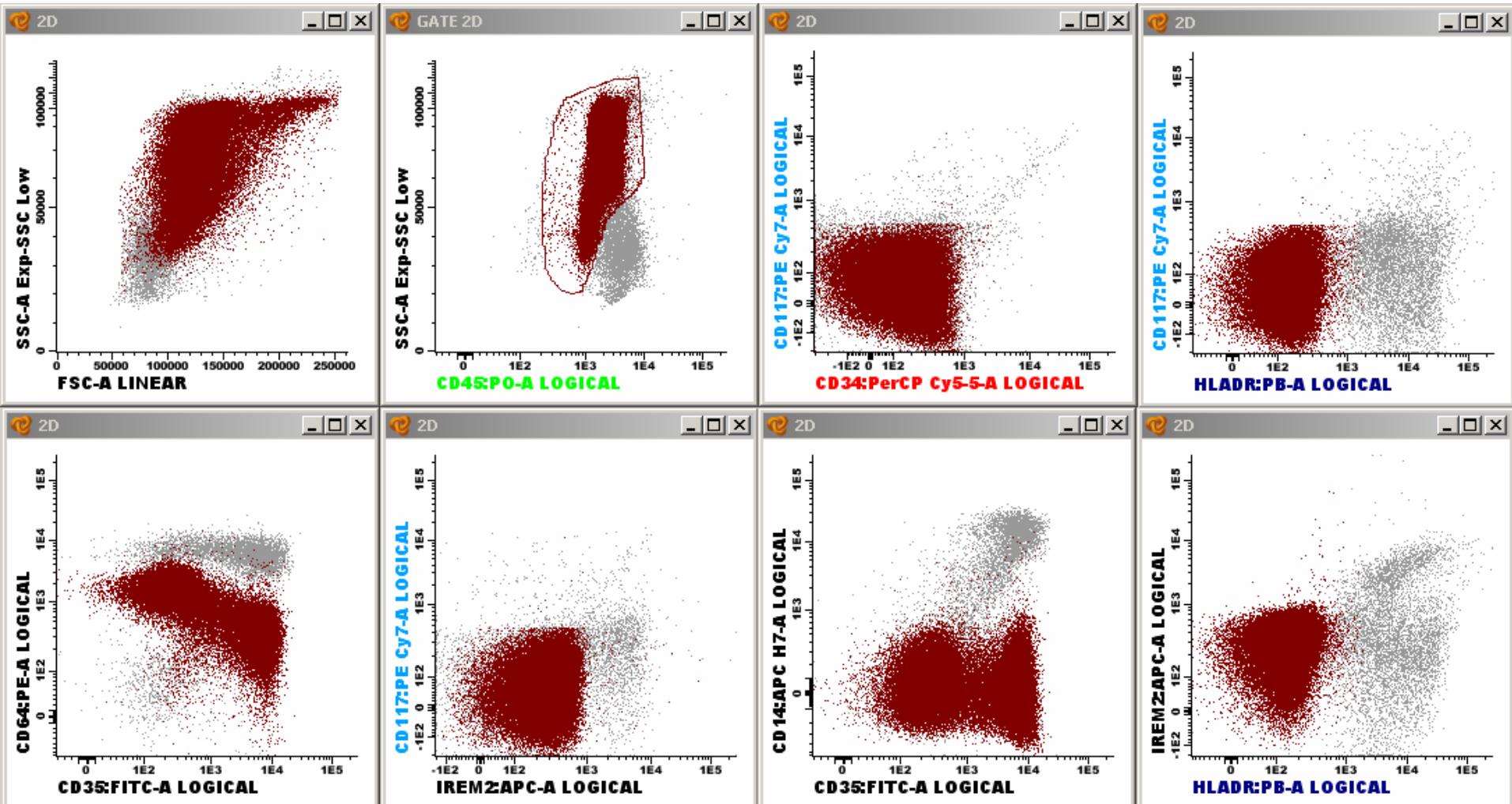
Tube	PacB	PacO	FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7	Aim
1	HLADR	CD45	CD16	CD13	CD34	CD117	CD11b	CD10	Diagnosis and classification, neutrophilic maturation, PNH
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3	HLADR	CD45	CD36	CD105	CD34	CD117	CD33	CD71	Diagnosis and classification, erythroid maturation
4	HLADR	CD45	TdT	CD56	CD34	CD117	CD7	CD19	Aberrant expression of lymphoid markers, abnormal B lymphoid maturation

Tube 2 – Precursors

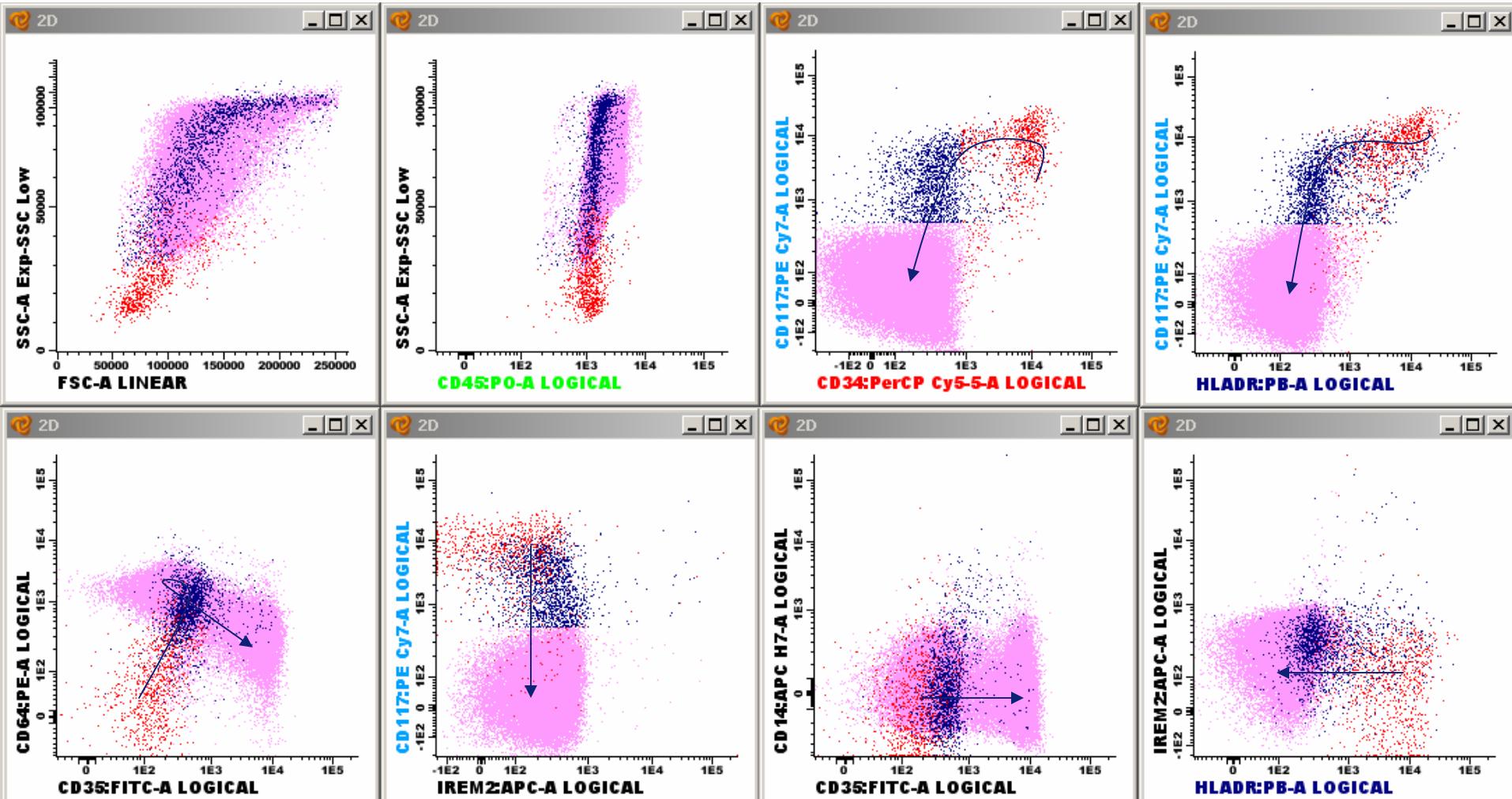
- CD34+ and/or CD117+ (back bone gating)



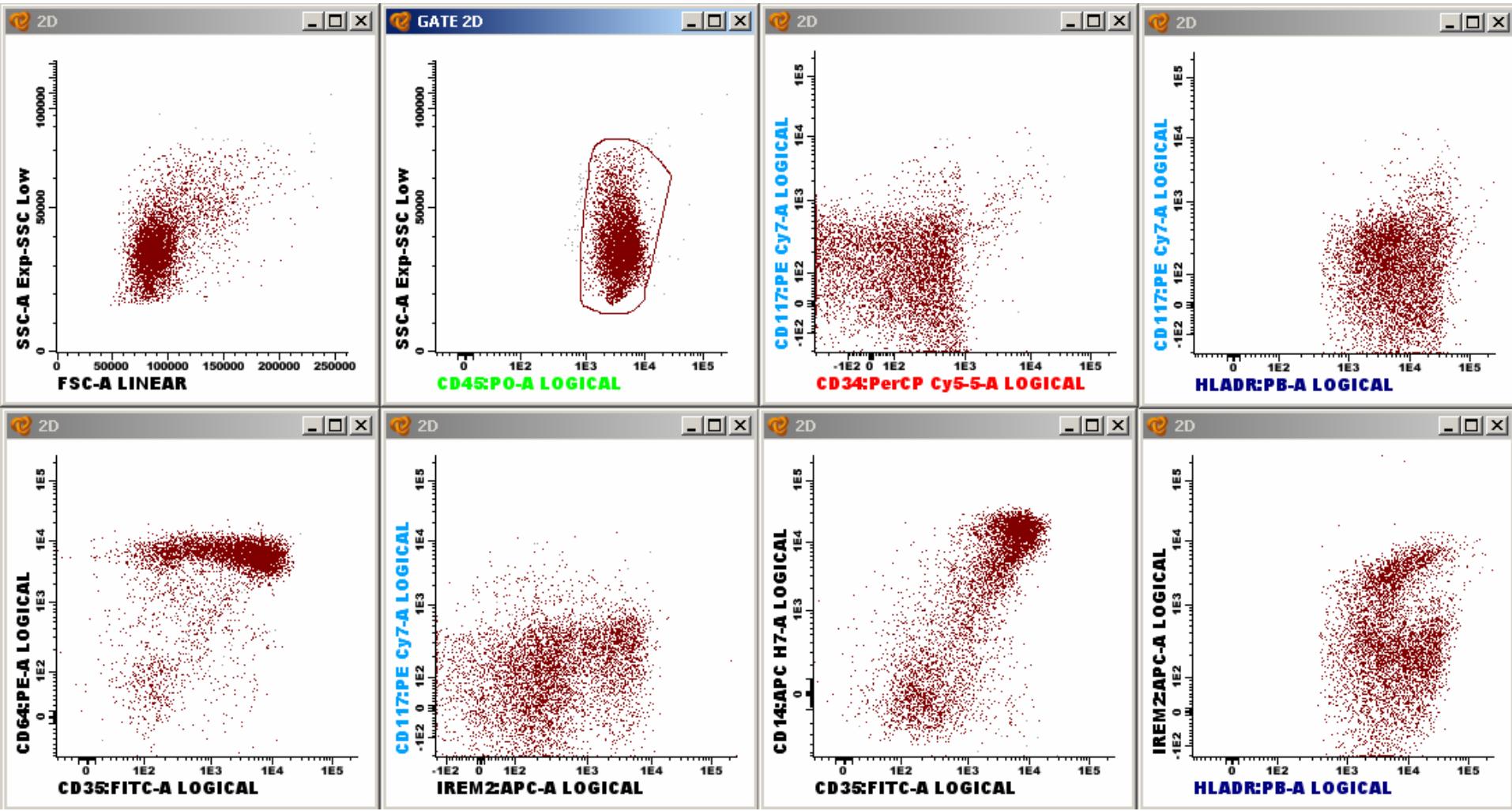
Tube 2 – Neutrophils



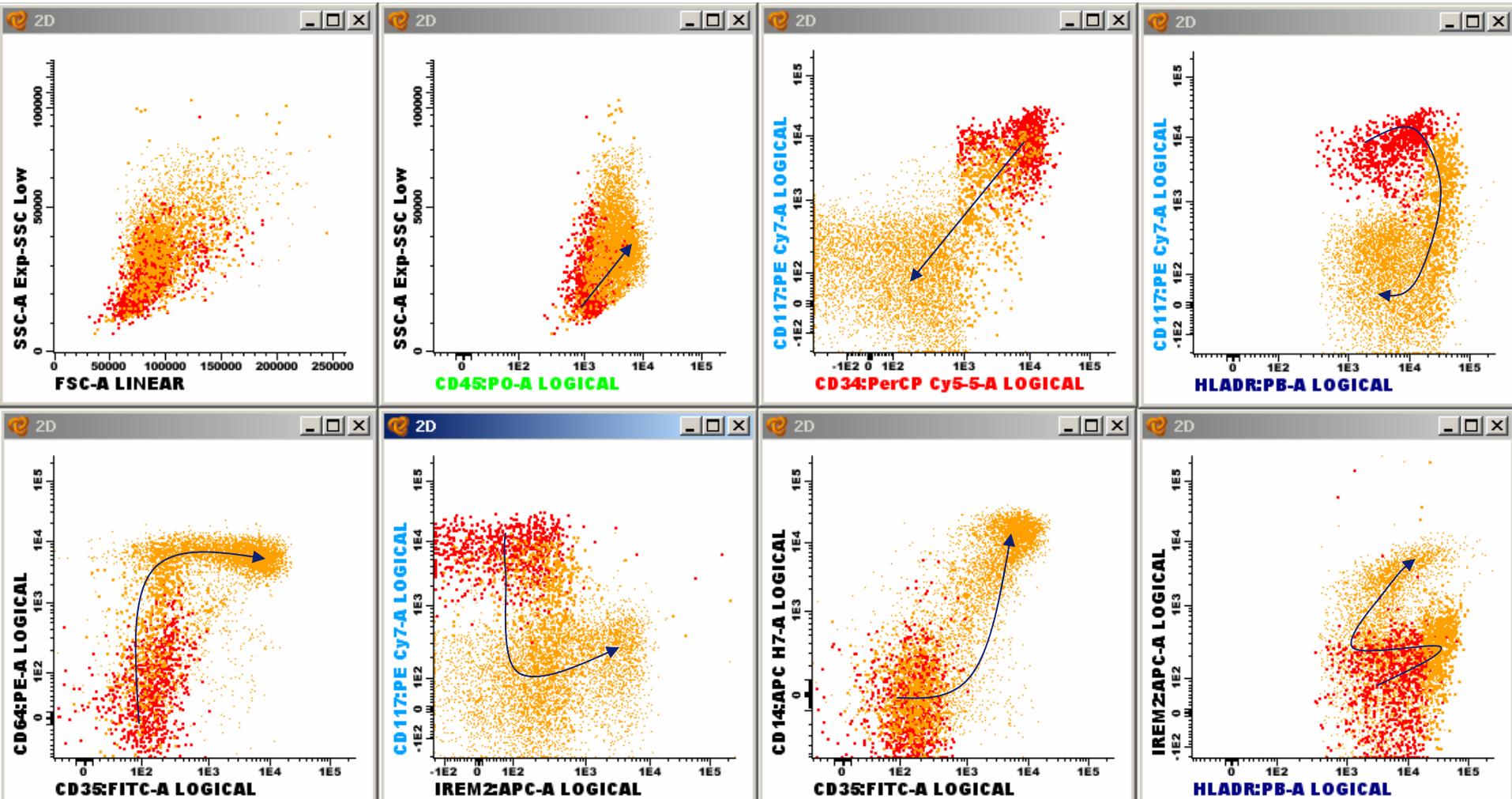
Tube 2 – Neutrophilic differentiation



Tube 2 – Monocytes

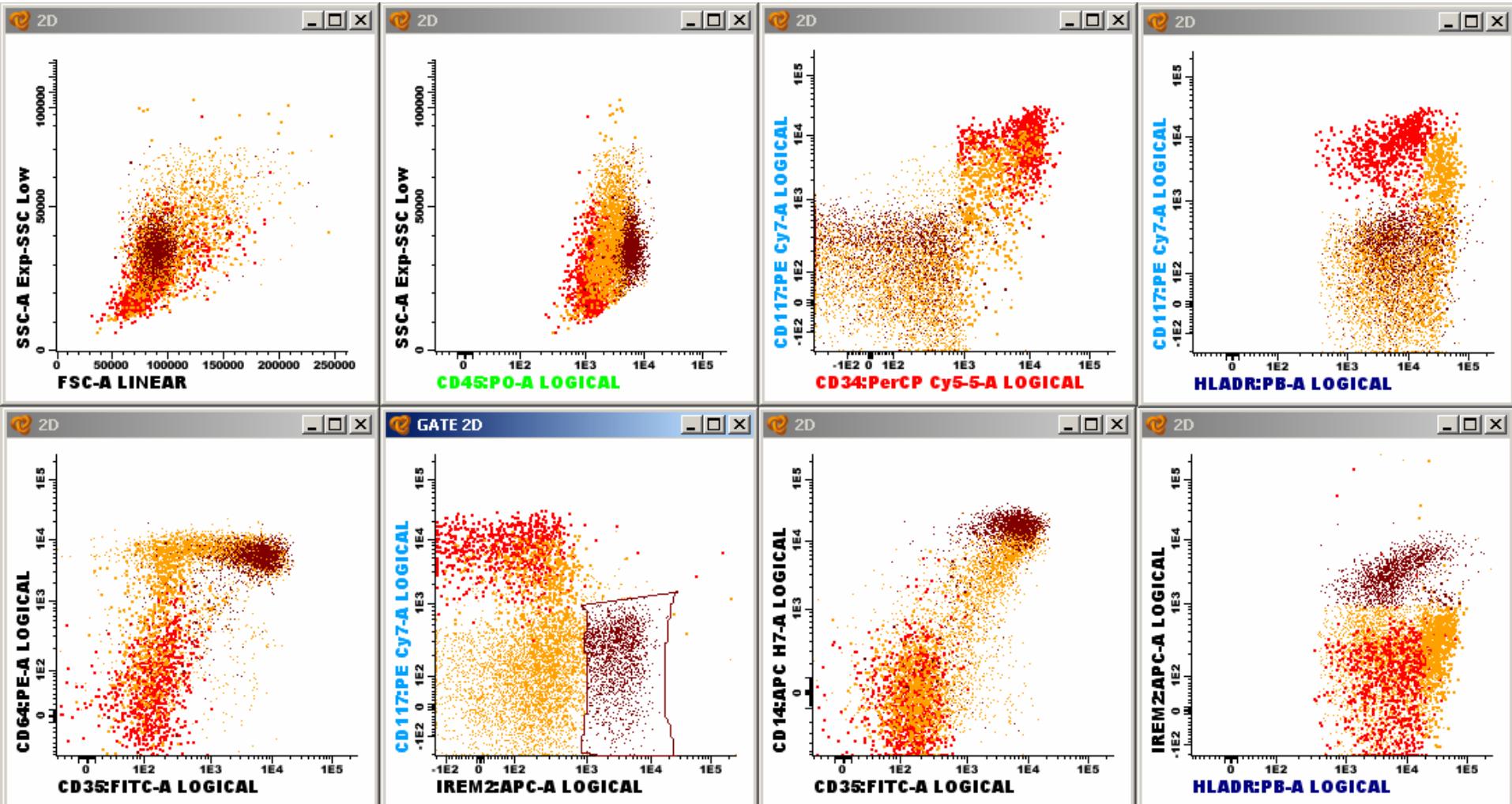


Tube 2 – Monocytic differentiation

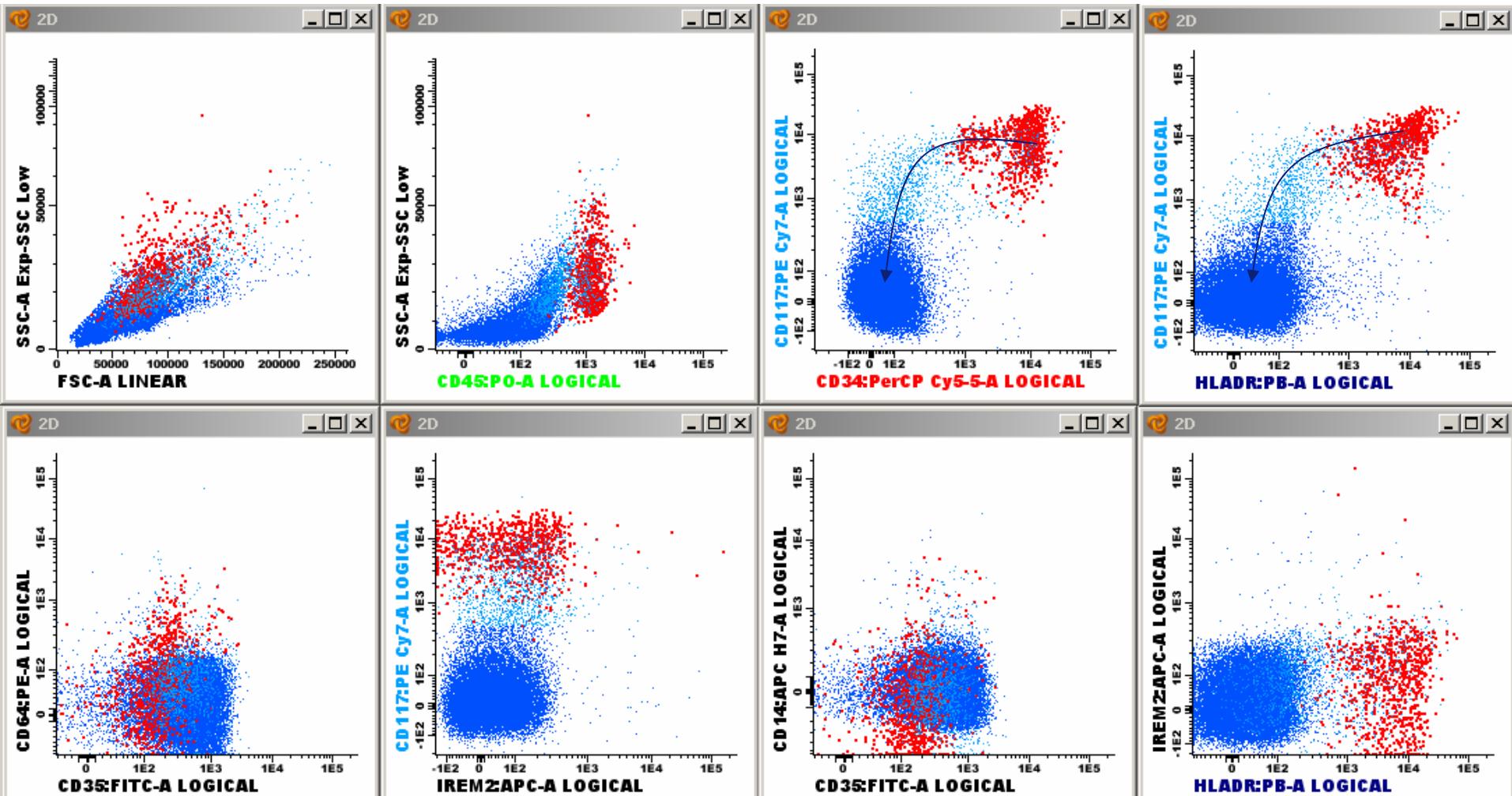


Tube 2 – Monocytic differentiation

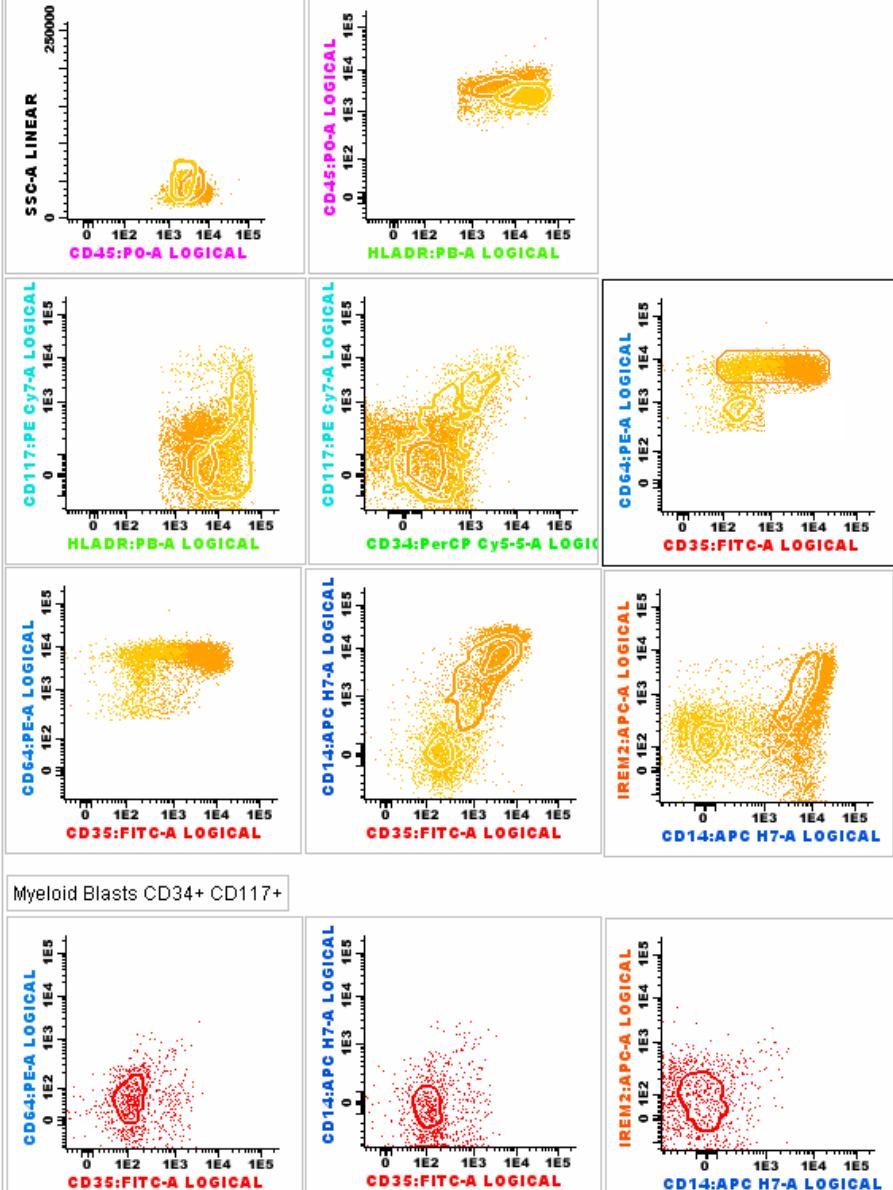
- Most mature monocytic stages



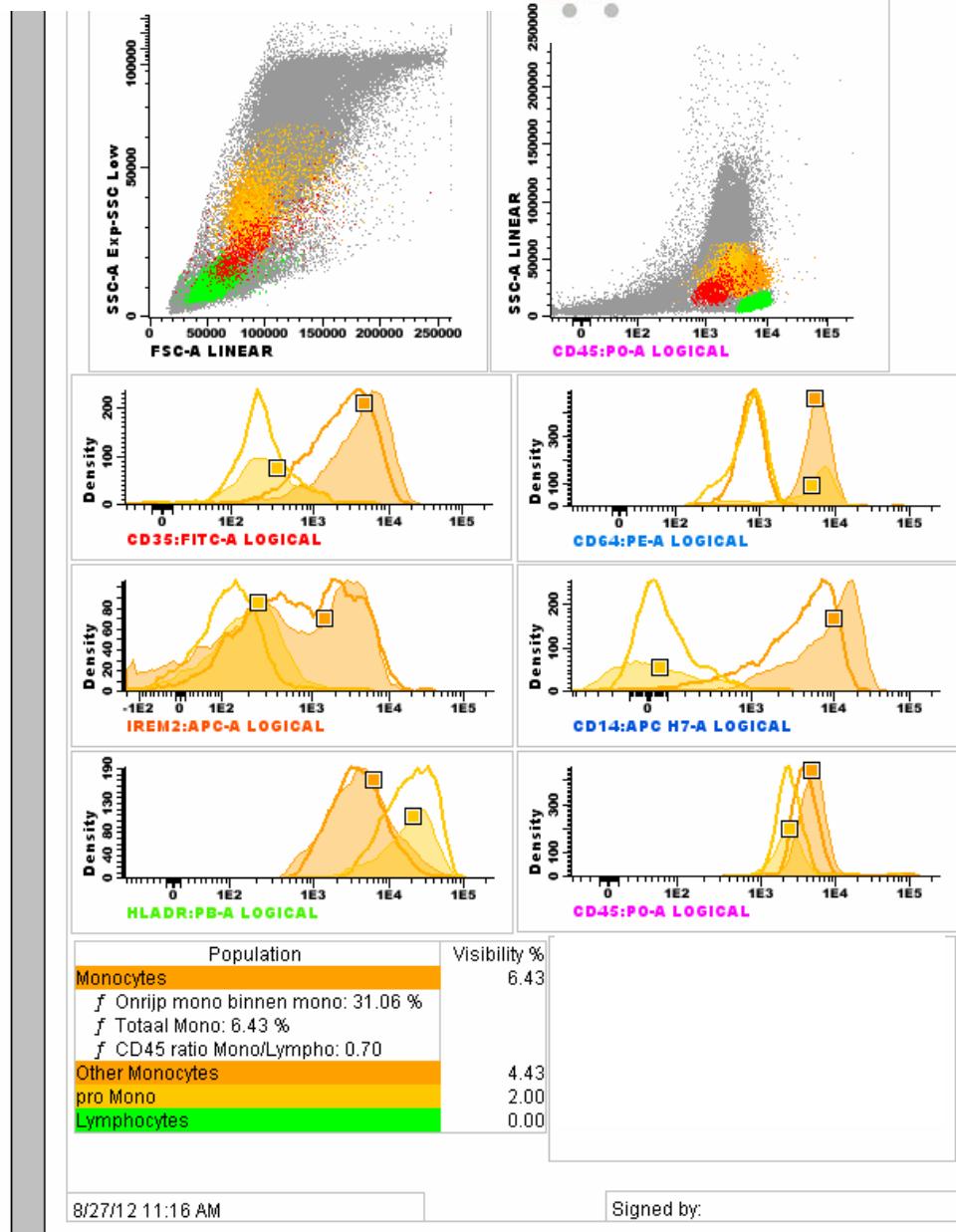
Tube 2 – Erythroid differentiation



Report – Tube 2

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Myeloid Blasts CD34+ CD117+



Recommended minimal requirements

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Maturing neutrophils	Percentage of cells as ratio to lymphocytes SSC as ratio vs SSC of lymphocytes Relationship of CD13 and CD11b Relationship of CD13 and CD16 Relationship of CD15 and CD10	Decreased Decreased Altered pattern ^c Altered pattern ^c Altered pattern ^c ; for example, lack of CD10 on mature neutrophils
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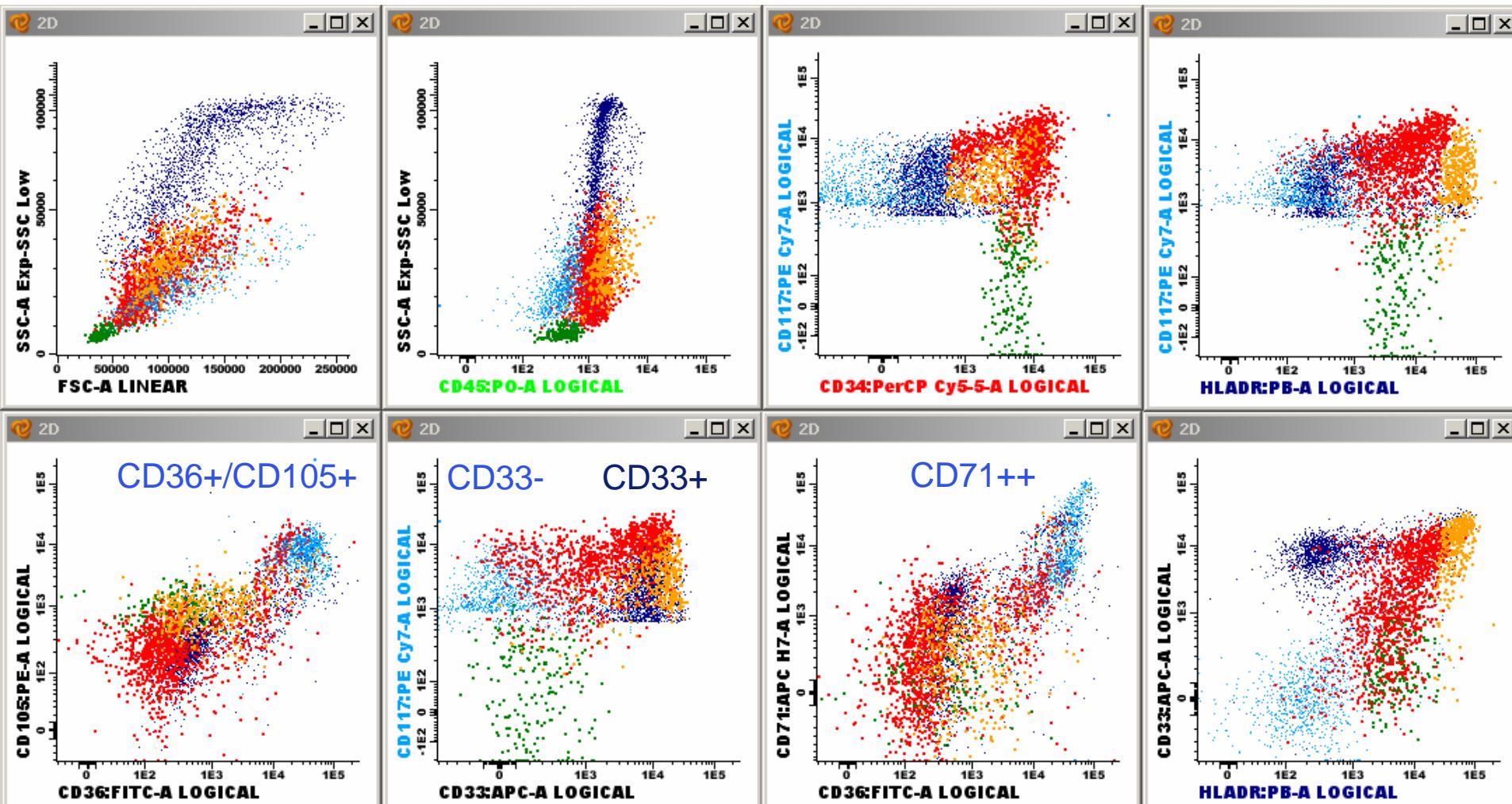
Normal myeloid differentiation

- Myeloid precursors
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Tube	PacB	PacO	FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7	Aim
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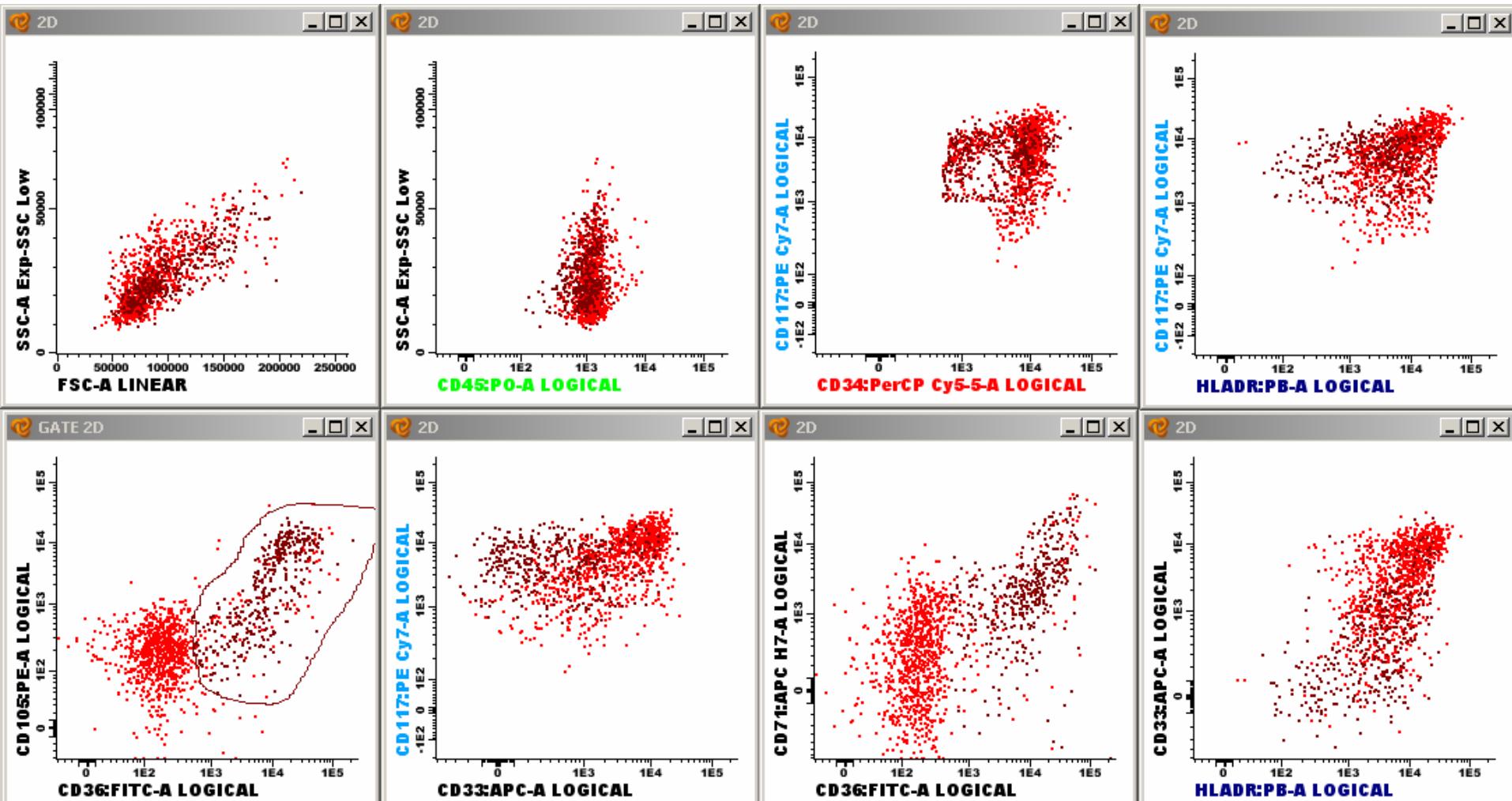
Tube 3 – Precursors

- CD34+ and/or CD117+ (back bone gating)

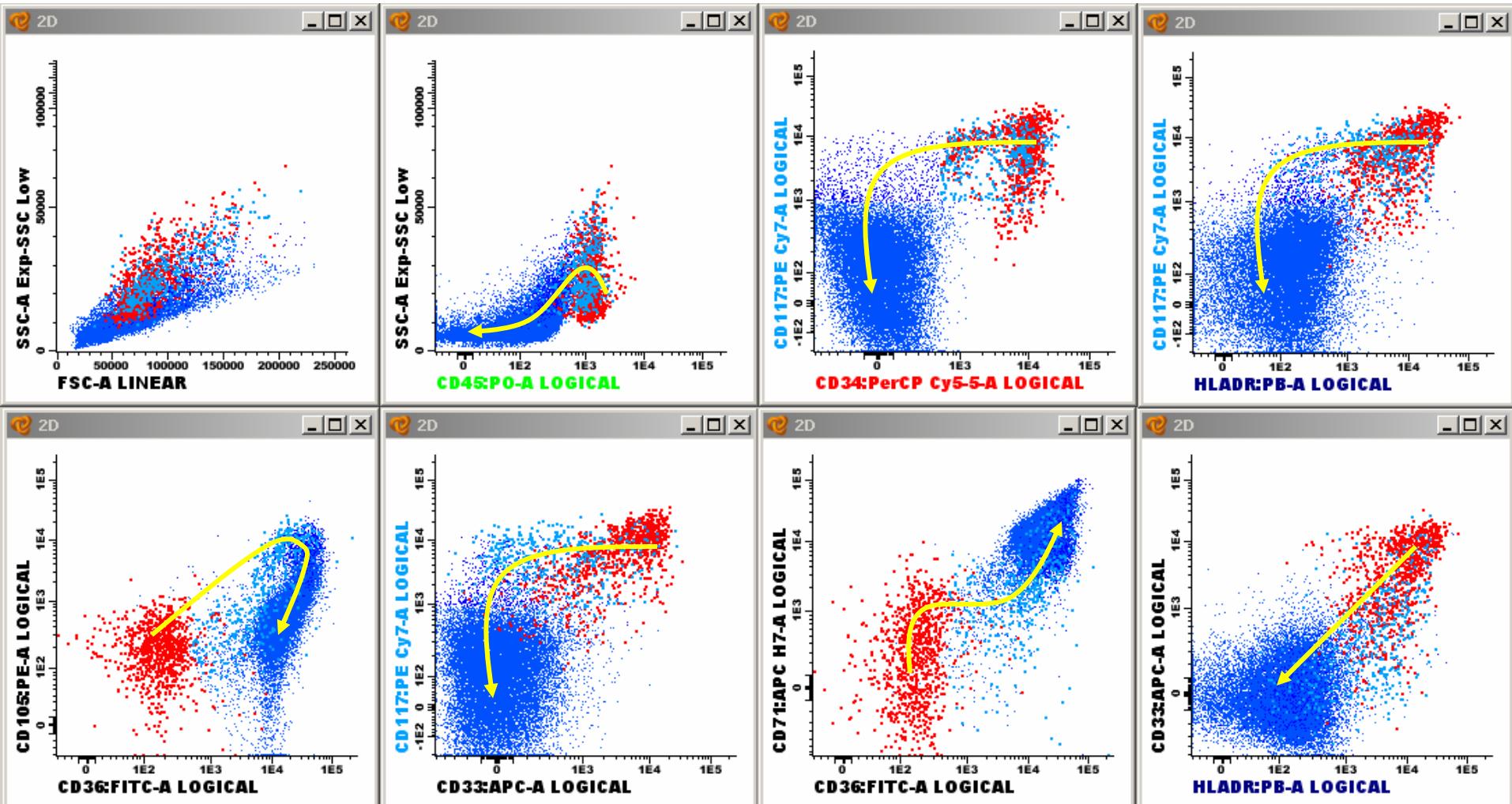


Tube 3 – Erythroid precursors

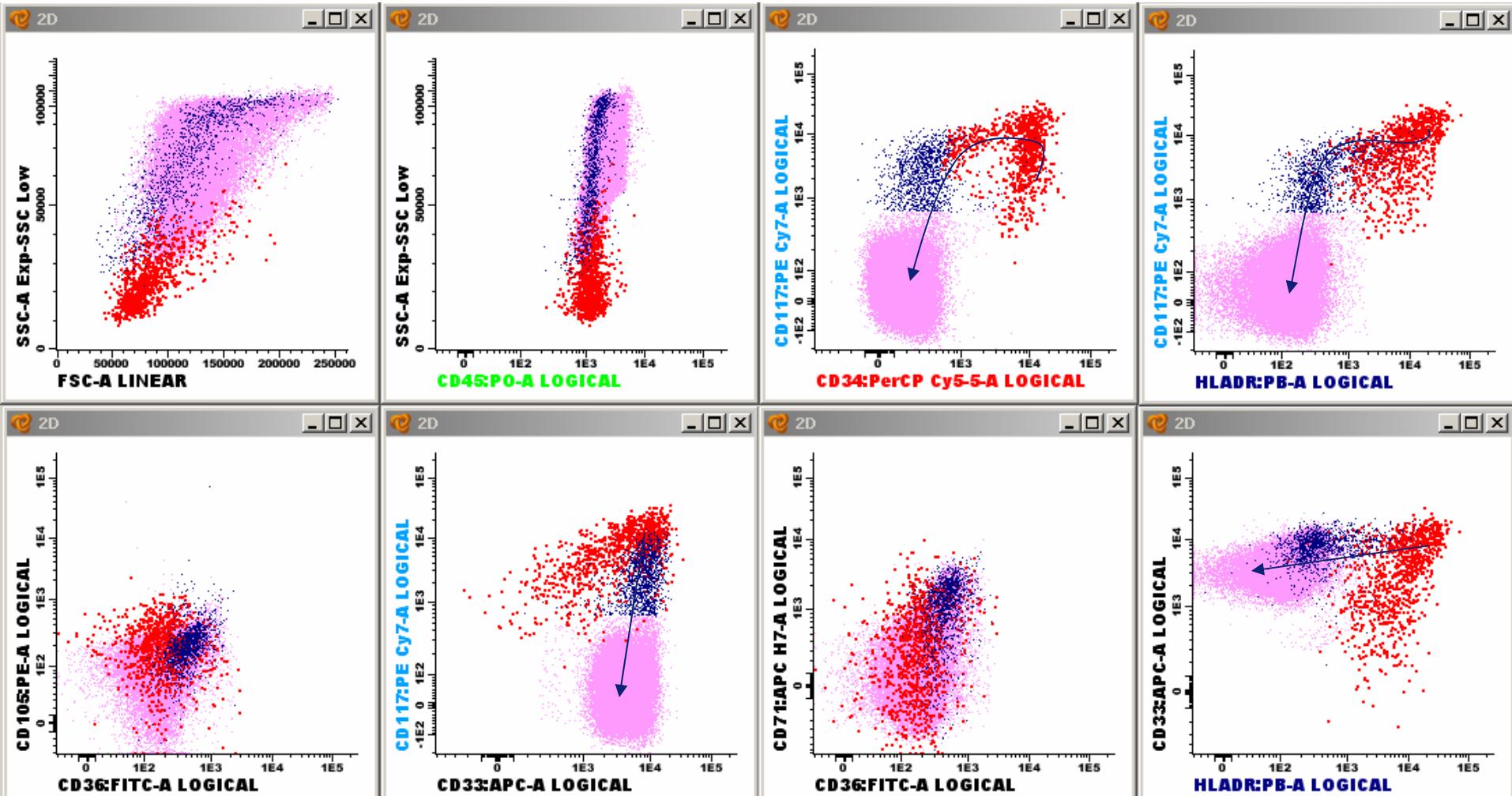
- CD34+ cells



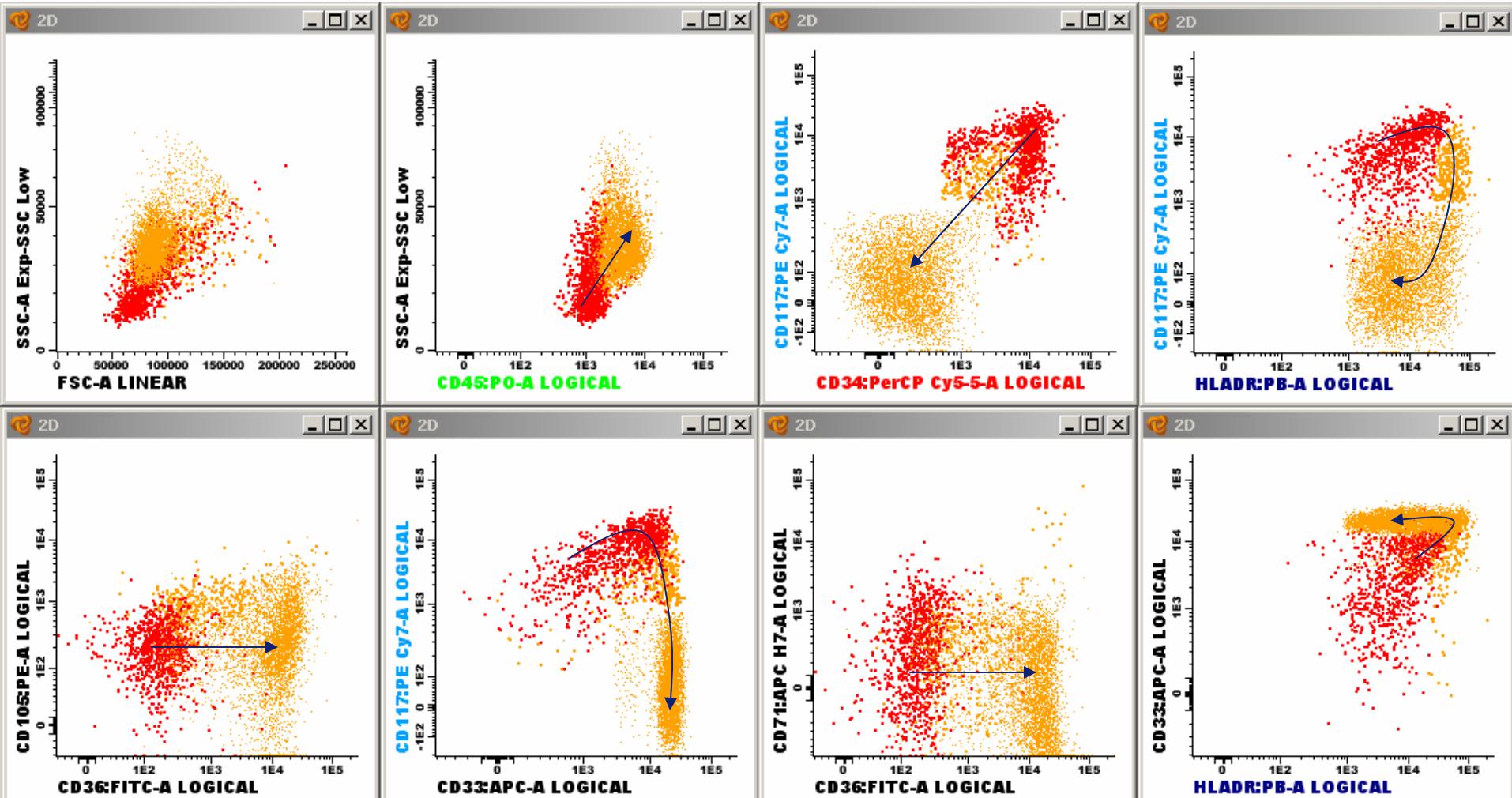
Tube 3 – Erythroid differentiation



Tube 3 – Neutrophilic differentiation



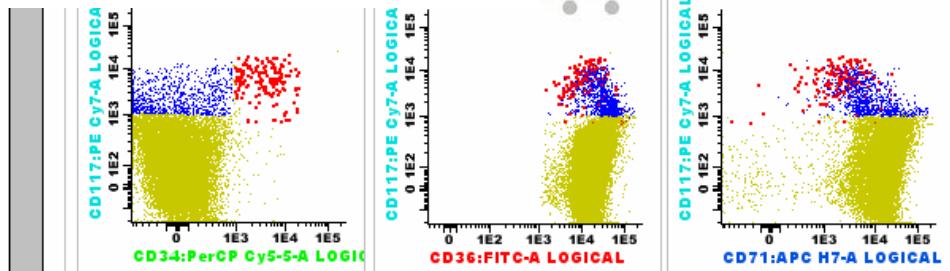
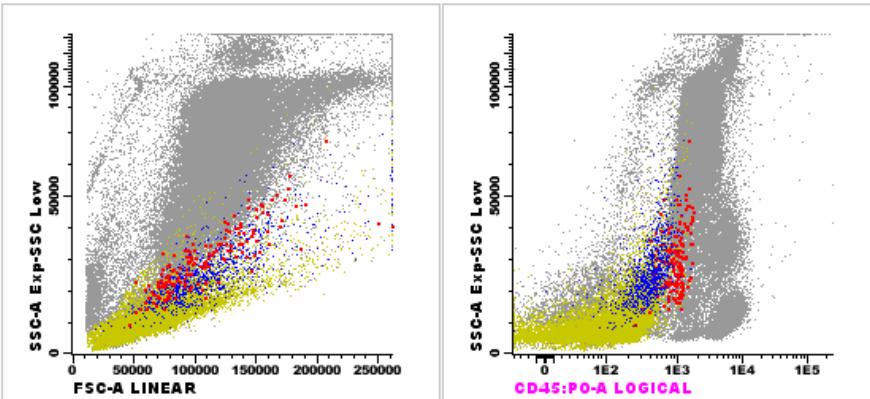
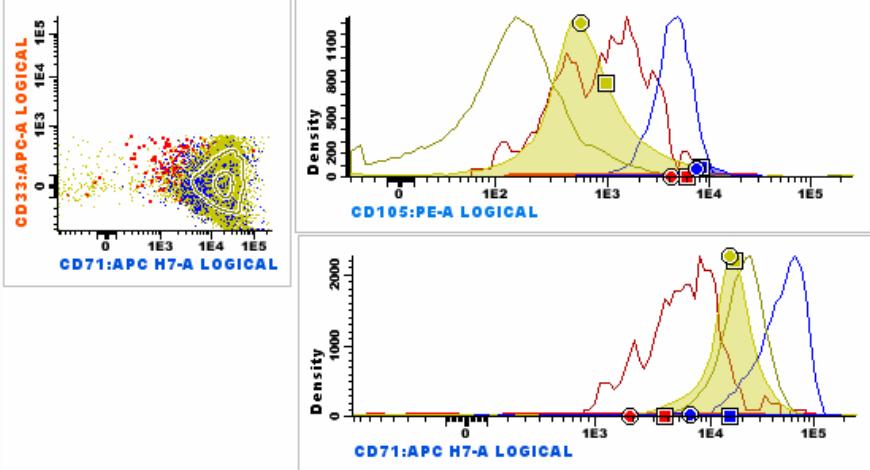
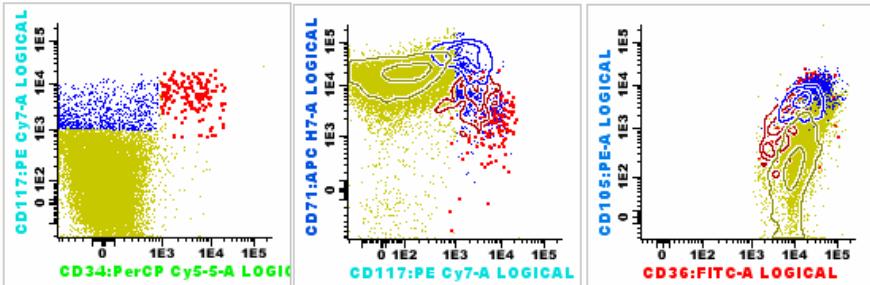
Tube 3 – Monocytic differentiation



Report – Tube 3

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Population	Visibility %
Events	0.00
Other Events	0.00
Nucleated red cells	100.00
f Total Red cells: 100.00 %	
f CD34+ 117+ within nucleated red cells: 0.74 %	
f CD34- 117+ within nucleated red cells: 3.90 %	
Other Nucleated red cells	
CD34+ 117+ 36+	95.36
CD34- 117+ 36+	0.74
CD34- 117+ 36+	3.90

Recommended minimal requirements

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Immature myeloid and monocytic progenitors	Percentage of cells in nucleated cell fraction ^a Expression of CD45 Expression of CD34 Expression of CD117 Expression of HLA-DR Expression of CD13 and CD33 Asynchronous expression of CD11b, CD15 Expression of CD5, CD7, CD19, CD56 ^b	Increased percentage Lack of/decreased/increased Lack of/decreased/increased Homogenous under/overexpression Lack of/increased expression Lack of/decreased/increased Presence of mature markers Presence of lineage infidelity markers
Maturing neutrophils	Percentage of cells as ratio to lymphocytes SSC as ratio vs SSC of lymphocytes Relationship of CD13 and CD11b Relationship of CD13 and CD16 Relationship of CD15 and CD10	Decreased Decreased Altered pattern ^c Altered pattern ^c Altered pattern ^c ; for example, lack of CD10 on mature neutrophils
Monocytes	Percentage of cells Distribution of maturation stages Relationship of HLA-DR and CD11b Relationship of CD36 and CD14 Expression of CD13 and CD33 Expression of CD56 ^b	Decreased/increased Shift towards immature Altered pattern ^c Altered pattern ^c (Homogenous) under/overexpression Presence of lineage infidelity marker
Progenitor B cells	Enumeration as fraction of total CD34+ based on CD45/CD34/SSC in combination with CD10 or CD19	Decreased or absent
Erythroid compartment ^d	Percentage of nucleated erythroid cells Relationship CD71 and CD235a Expression of CD71 Expression of CD36 Percentage of CD117-positive precursors	Increased Altered pattern ^c Decreased Decreased Increased

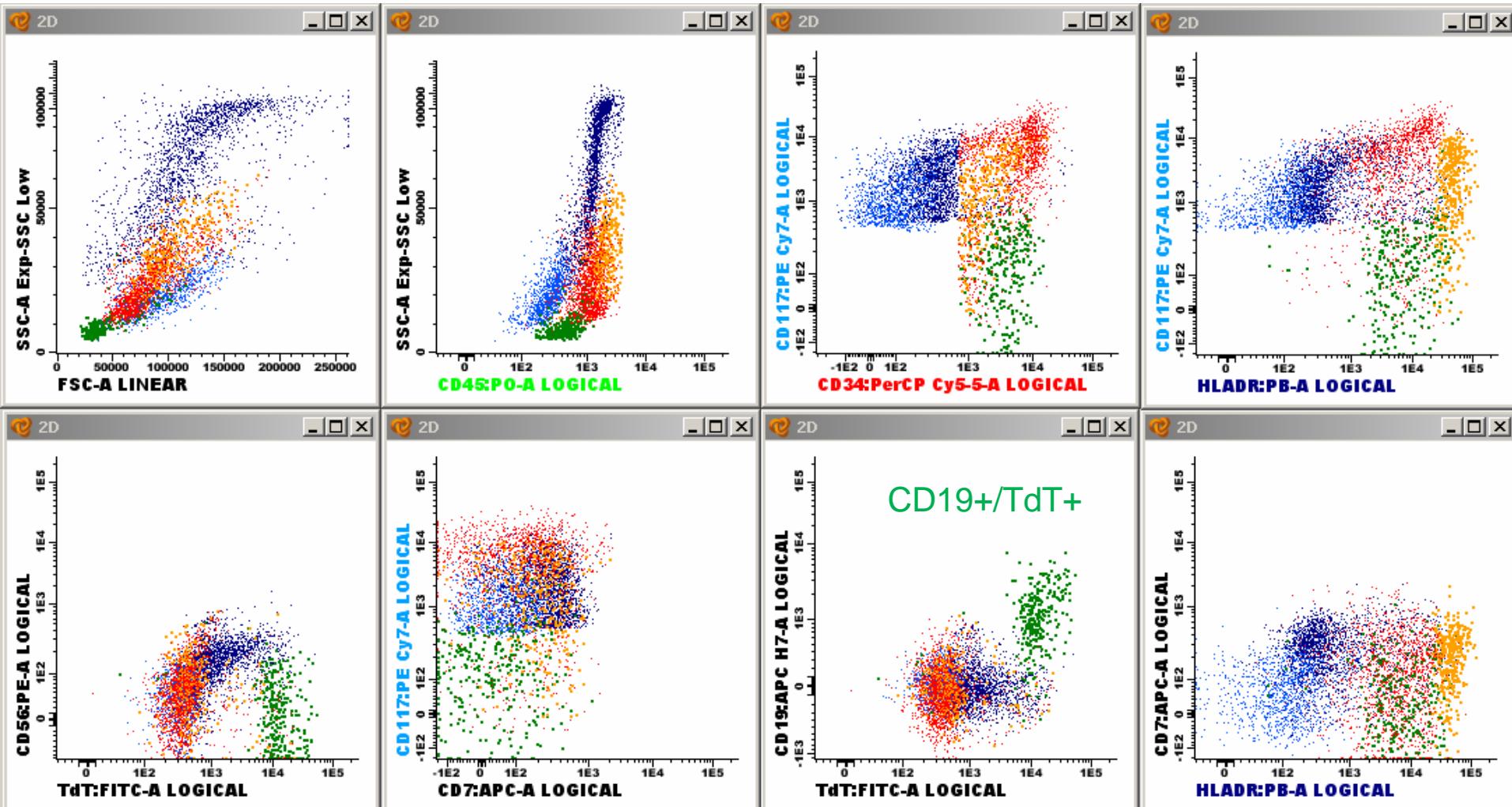
Normal myeloid differentiation

- Myeloid precursors
- Granulocytic differentiation
- Monocytic differentiation
- Erythroid differentiation

Tube	PacB	PacO	FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7	Aim
1	HLADR	CD45	CD16	CD13	CD34	CD117	CD11b	CD10	Diagnosis and classification, neutrophilic maturation, PNH
2	HLADR	CD45	CD35	CD64	CD34	CD117	CD300e (IREM2)	CD14	Diagnosis and classification, monocytic maturation, PNH
3	HLADR	CD45	CD36	CD105	CD34	CD117	CD33	CD71	Diagnosis and classification, erythroid maturation
4	HLADR	CD45	TdT	CD56	CD34	CD117	CD7	CD19	Aberrant expression of lymphoid markers, abnormal B lymphoid maturation

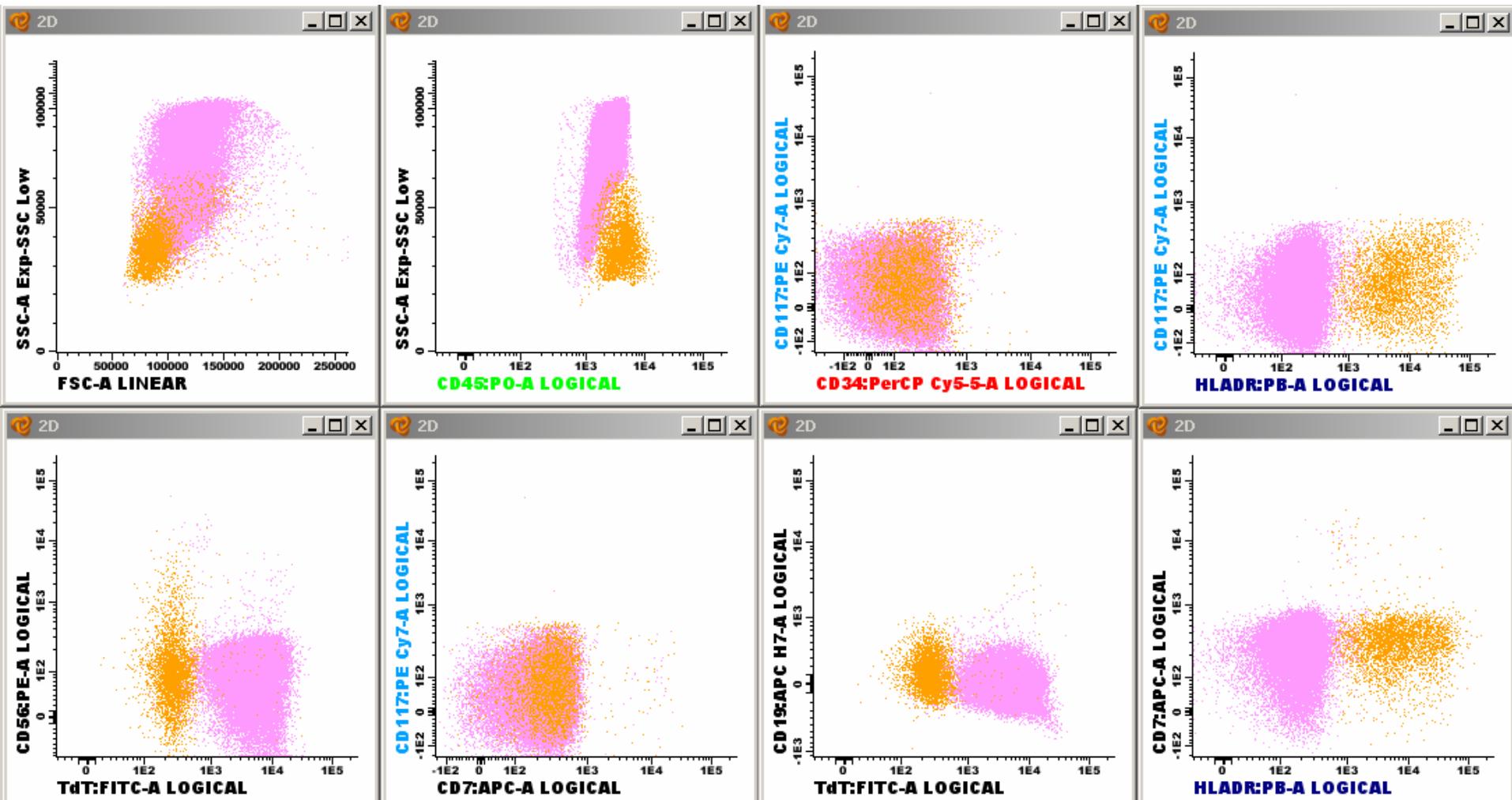
Tube 4 – Aberrant expressions

- CD34+ and/or CD117+ (back bone gating)

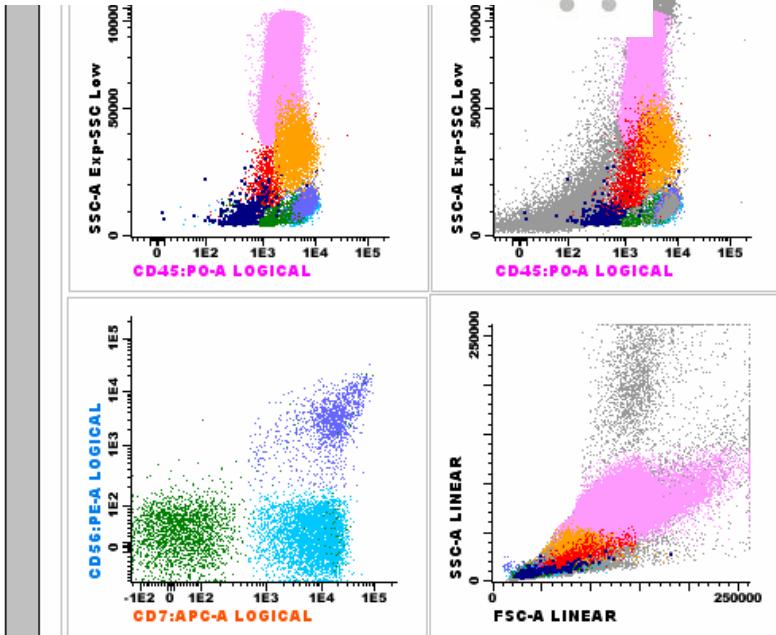
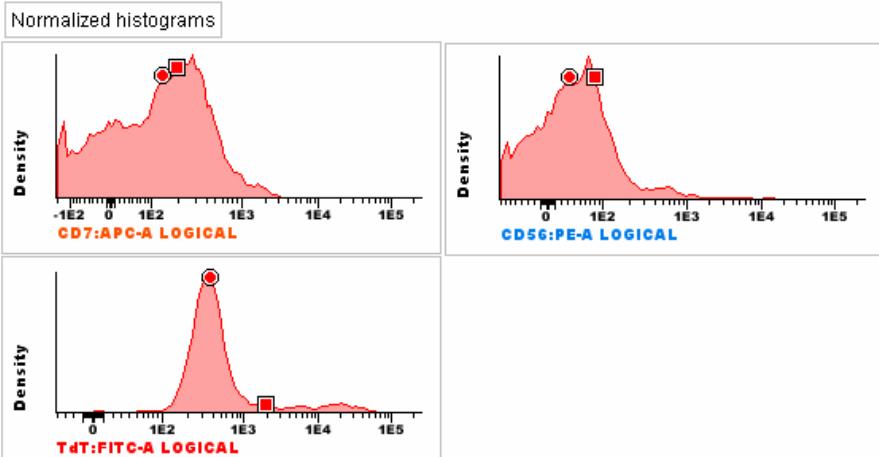
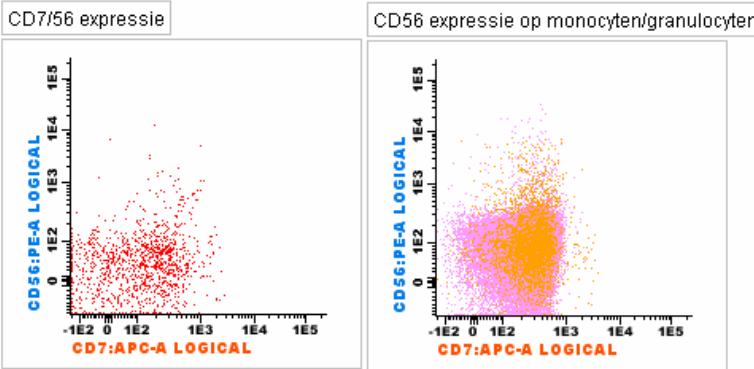
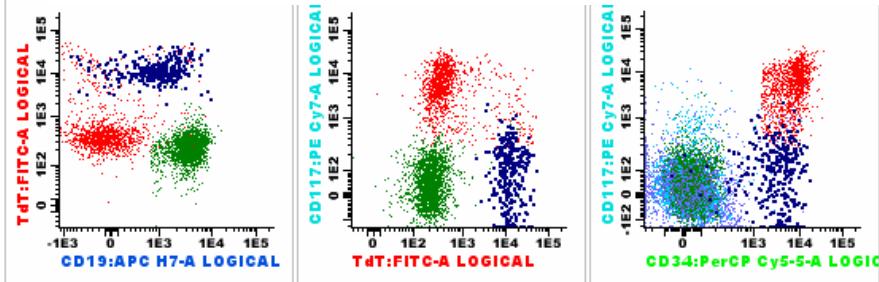


Tube 4 – Aberrant expressions

- Neutrophils & monocytes



Report – Tube 4



Population	Events	Visibility %
Events	100000	100.00
Other Events	32478	40.99
Leuco	0	NA
Monocytes	3791	0.00
Lymphocytes	7798	0.00
f CD45 Ratio Ly/Myelo Blasts (CP-3): 4.25		
T-cell	4395	0.00
mature B-cell CD34+	2038	0.00
Nk cell	1365	0.00
CD34+ totalt	1541	0.00
CD34+ CD117+	1200	0.00
f Lymphoid blasts binnen CD34+: 77.87 %		
f Myeloid blasts: 0.00 %		
f Myeloid blasts CD7 (within Myel.BI): 0.00 %		
f Myeloid blasts CD56 (within Myel.BI): 0.00 %		
f Myeloid blasts TdT (within Myel.BI): 0.00 %		
CD7	0	NA
CD56	0	NA
TdT+	0	NA
CD34+ TdT++ (B-cells)	341	0.00
f Ly blasts binnen CD34+ (CP-2): 22.13 %		
f Lymphoid blasts: 0.00 %		

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Signed by:

Recommended minimal requirements

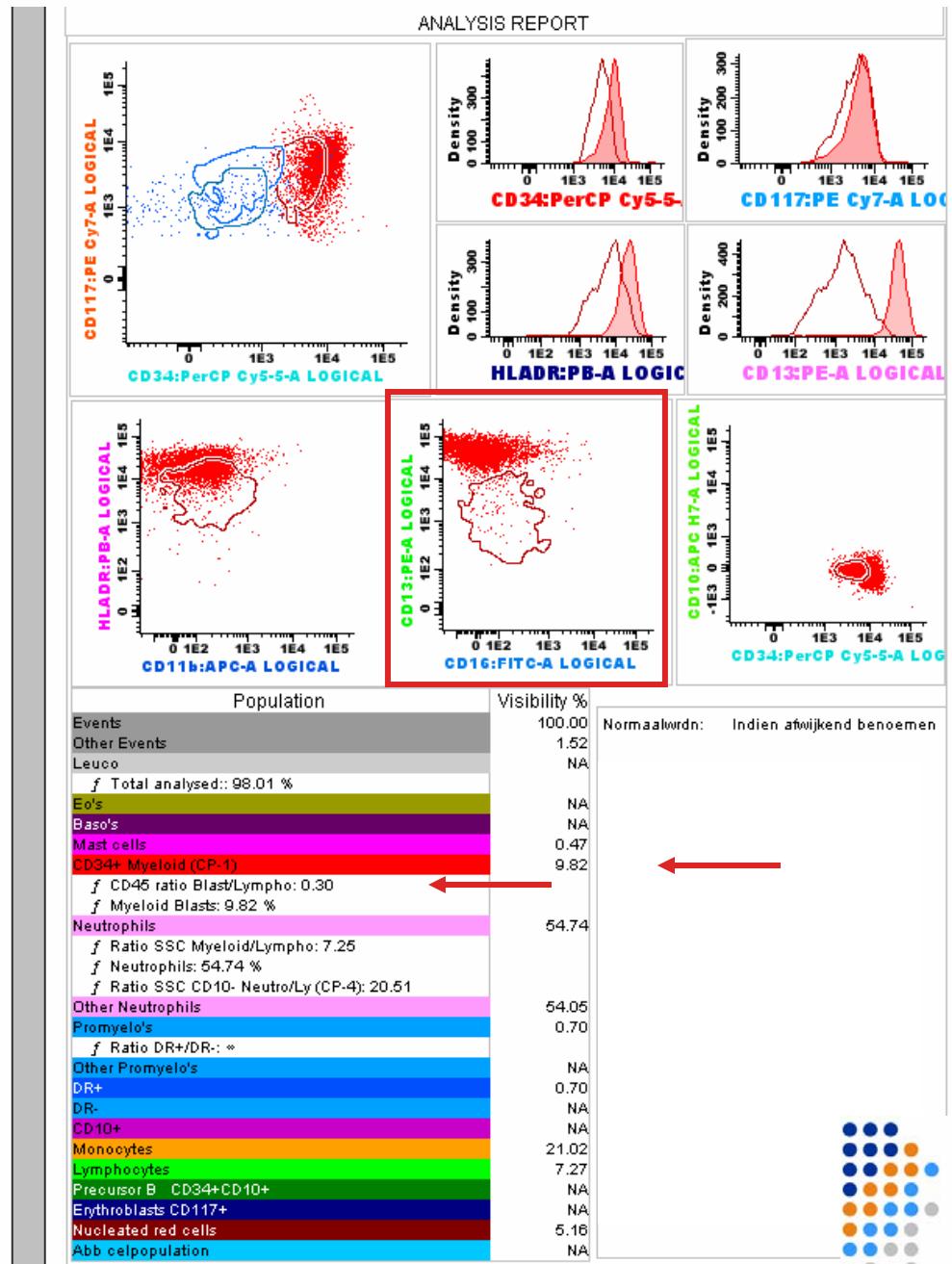
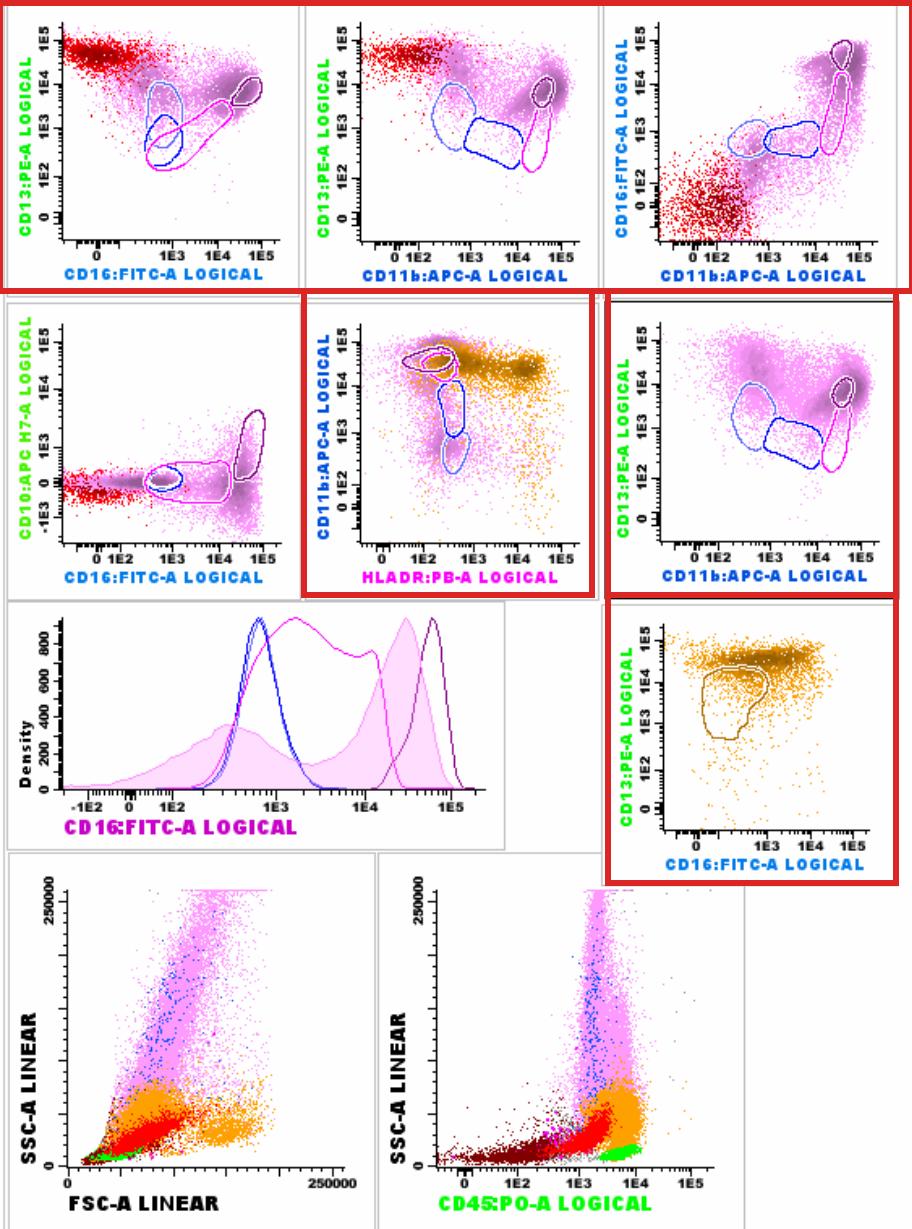
Bone marrow subset	Recommended analyses	Aberrancy
Immature myeloid and monocytic progenitors	Percentage of cells in nucleated cell fraction ^a Expression of CD45 Expression of CD34 Expression of CD117 Expression of HLA-DR Expression of CD13 and CD33 Asynchronous expression of CD11b, CD15 Expression of CD5, CD7, CD19, CD56^b	Increased percentage Lack of/decreased/increased Lack of/decreased/increased Homogenous under/overexpression Lack of/increased expression Lack of/decreased/increased Presence of mature markers Presence of lineage infidelity markers
Maturing neutrophils	Percentage of cells as ratio to lymphocytes SSC as ratio vs SSC of lymphocytes Relationship of CD13 and CD11b Relationship of CD13 and CD16 Relationship of CD15 and CD10	Decreased Decreased Altered pattern ^c Altered pattern ^c Altered pattern ^c ; for example, lack of CD10 on mature neutrophils
Monocytes	Percentage of cells Distribution of maturation stages Relationship of HLA-DR and CD11b Relationship of CD36 and CD14 Expression of CD13 and CD33 Expression of CD56^w	Decreased/increased Shift towards immature Altered pattern ^c Altered pattern ^c (Homogenous) under/overexpression Presence of lineage infidelity marker
Progenitor B cells	Enumeration as fraction of total CD34+ based on CD45/CD34/SSC in combination with CD10 or CD19	Decreased or absent
Erythroid compartment ^d	Percentage of nucleated erythroid cells Relationship CD71 and CD235a Expression of CD71 Expression of CD36 Percentage of CD117-positive precursors	Increased Altered pattern ^c Decreased Decreased Increased

Patient met MDS?

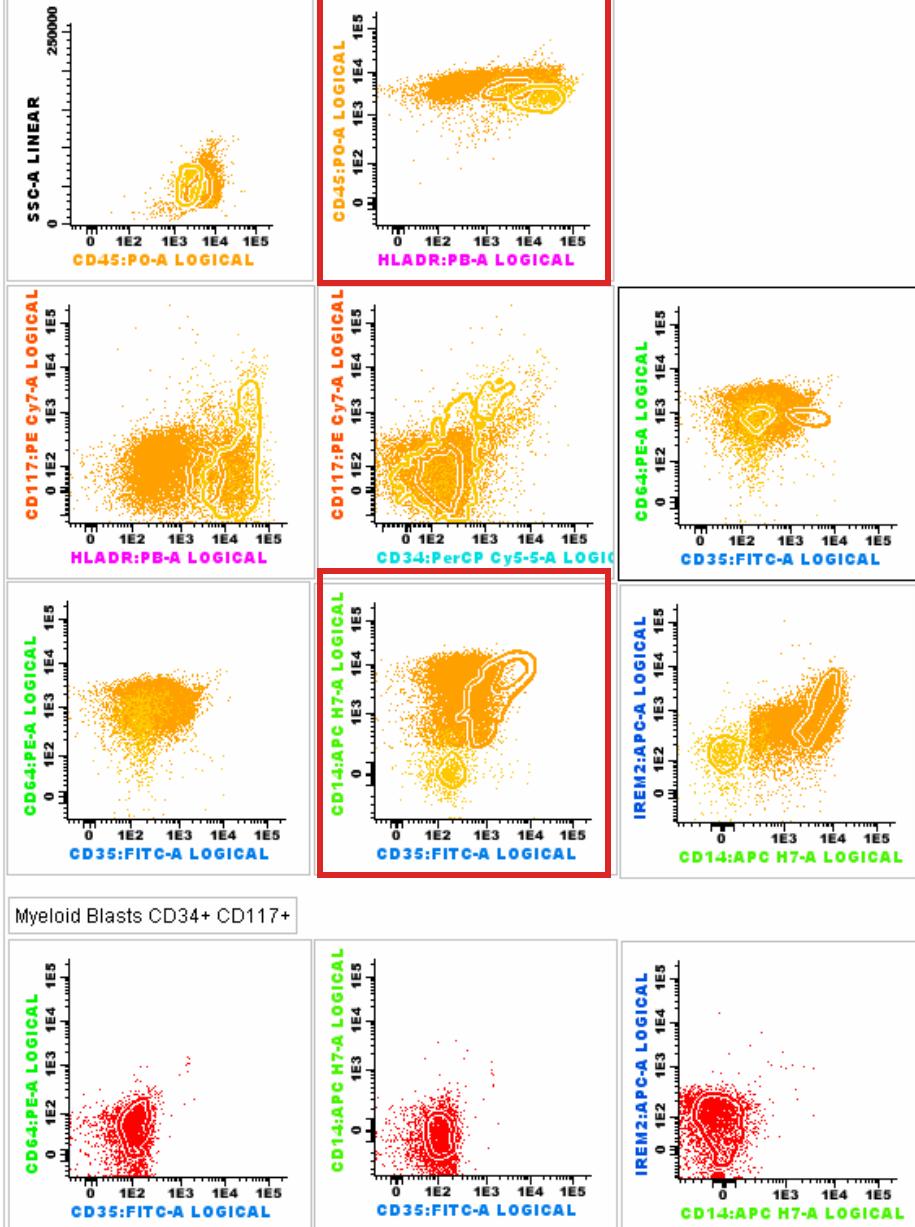
- 71-jarige vrouw
- Relatief blanco voorgeschiedenis
- Pijnlijke knie, nachtweten en gewichtsverlies

- In verband met leukocytose, anemie en trombopenie vond een beenmergevaluatie plaats

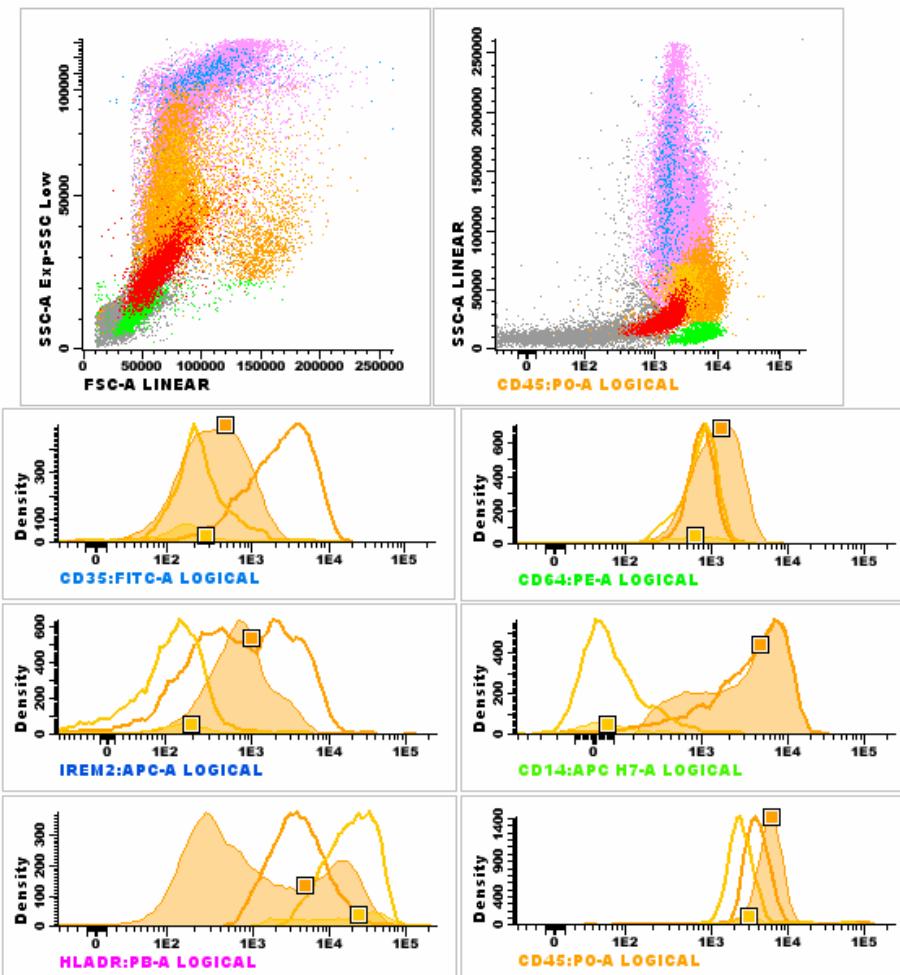
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 Experiment name: 1037790 AML Dx 020412
 File BM_Tube_001.fcs



Acquisition date: 02-APR-2012
Experiment name: 1037790 AML Dx 020412
File name: BM_Tube_020412.os



ANALYSIS REPORT

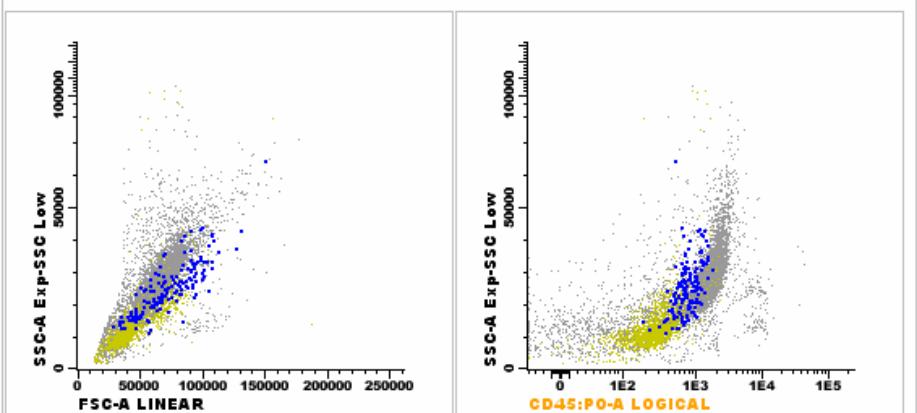
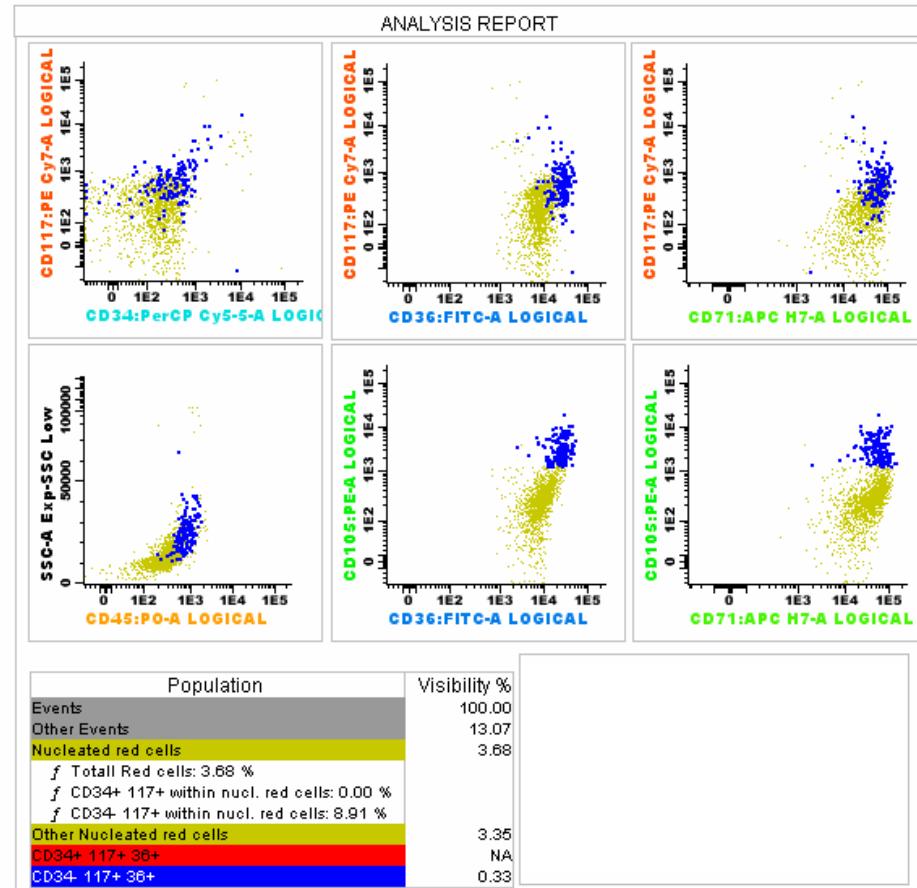
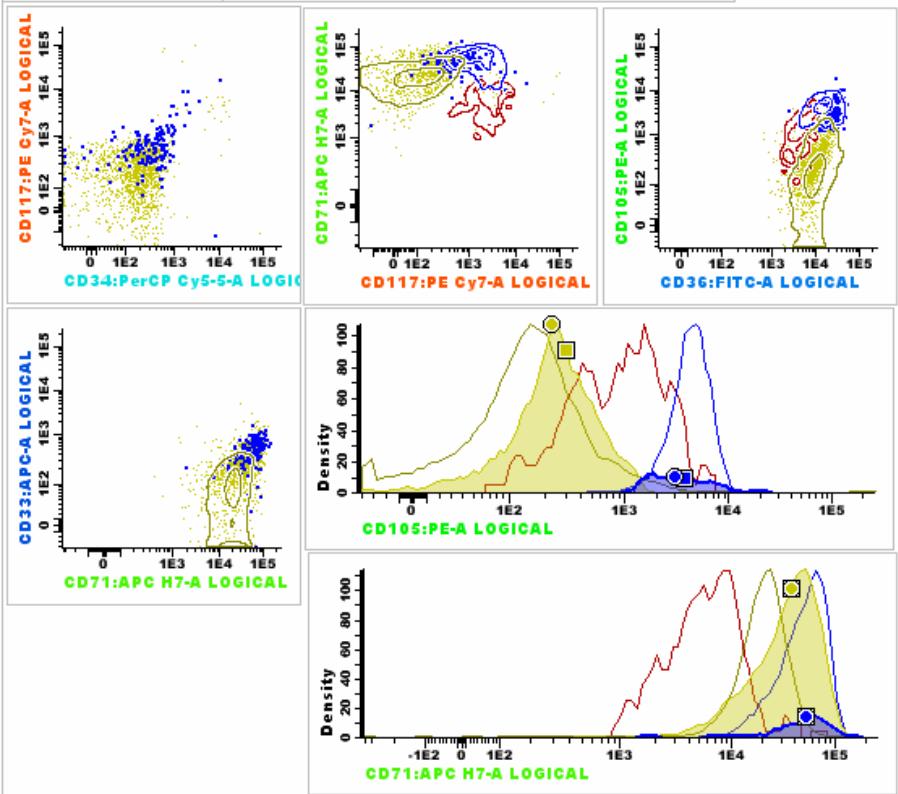


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Acquisition date: 02-APR-2012
 Experiment name: I037790 AML Dx 020412
 File: BM_Tube_003.fcs

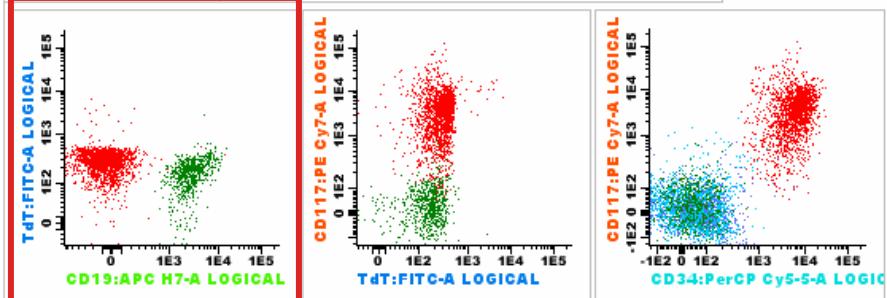


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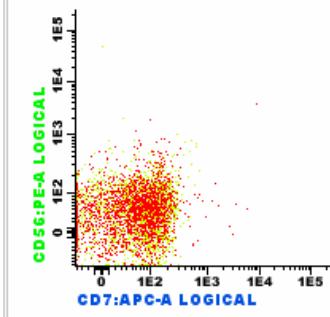
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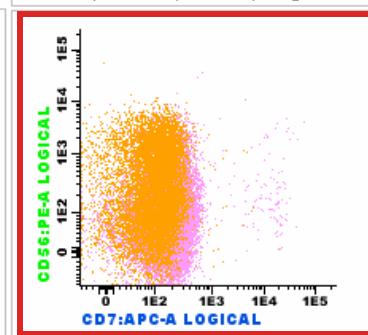
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 Experiment name: 1037790 AML Dx 020412
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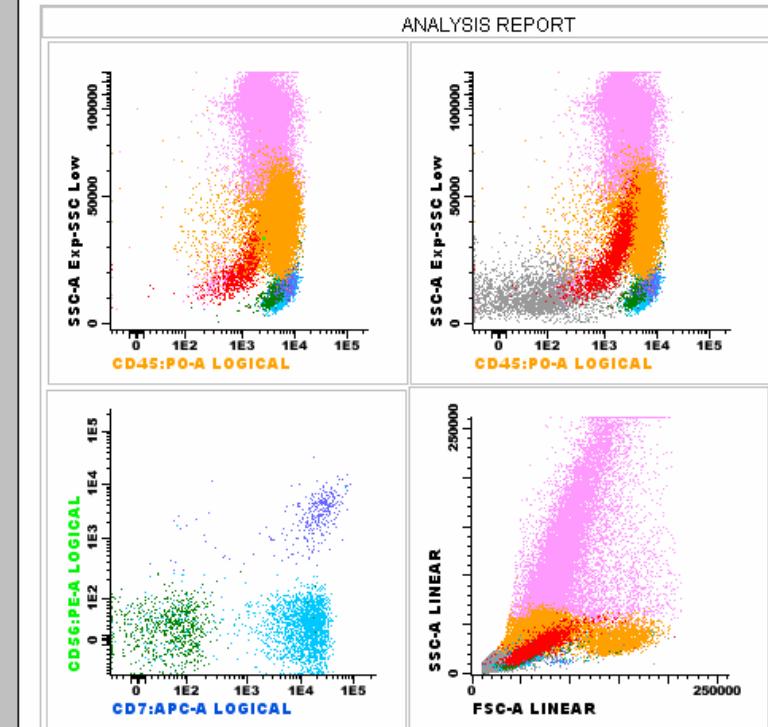
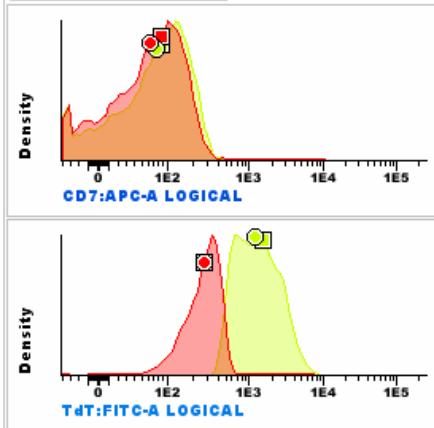
CD7/56 expressie



CD56 expressie op monocyten/granulocyten



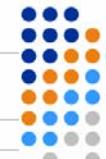
Normalized histograms



Population	Events	Visibility %	Normaalwaarden: Afwijkend benoemen
Events	50000	100.00	
Other Events	3820	8.37	
Leuco	0	NA	
Monocytes	11558	25.31	
Lymphocytes	3324	7.28	
f CD45 Ratio Ly/Myelo Blasts (CP-3): 3.54			
T-cell	2286	5.01	
mature B-cell CD34+	709	1.55	
Nk cell	328	0.72	
CD34+ total	4063	8.90	
CD34+ CD117+	4063	8.90	
f Lymphoid blasts binnen CD34+: 100.00 %			
f Myeloid blasts: 8.90 %			
f Myeloid blasts CD7 (within Myel.BI): 0.00 %			
f Myeloid blasts CD56 (within Myel.BI): 0.00 %			
f Myeloid blasts TdT (within Myel.BI): 53.14 %			
CD7	0	NA	
CD56	0	NA	
TdT+	2159	4.73	
CD34+ TdT++ (B-cells)	0	NA	
f Ly blasts binnen CD34+ (CP-2): 0.00 %			
f Lymphoid blasts: 0.00 %			

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Signed by:



Conclusion

- Immunophenotyping:
 - Increased % blasts
 - Increased % monocytes
 - Decreased % B-cell precursors
 - Abnormal granulocytic differentiation
 - Abnormal monocytic differentiation

→ Compatible with MDS

Morphological data

Onderzoek	Cytologie beenmerg	Status: Definitief 
Labnr	20120067203101	Aanvraag: 16-04-2012 Autorisatie: 20-04-2012
Resultaten		
Beenmergbrokjes	voldoende	
Celrijkdom	toegenomen	
Erytropoiese	in alle uitrijppingsstadia aanwezig , normoblastair, <u>dysplasiescore: 4% (4/90)</u>	
Granulopoiese/monocyten	volledige uitrijping , lichaampjes van Döhle +, hypogranulatie +++, (pseudo) Pelger Huet +, bizarre vormen +, <u>dysplasiescore: 100% (90/90)</u>	
Lymfocyten	<u>geen afwijkingen</u>	
Plasmacellen	<u>geen afwijkingen</u>	
Megakaryopoiese	aantal pgv: 0 - 2, micromegakaryocyten +, een/twee lobbige[n] +, dysplasie niet te scoren	
Overig	toename macrofagen ++, mitoses: toename	
IJzerkleuring	<u>weefselijzer verminderd, geen ringsideroblasten,</u>	
Sudan black kleuring	meerdere verspreid positief, te weinig blasten voor kwantitatieve beoordeling	

Differentiatie

	Bloed		Beenmerg	
blast	8	%	<u>8,3</u>	%
promyelocyt	3	%	11,7	%
myelocyt	5	%	12,1	%
metamyelocyt	3	%	7,6	%
staaf	3	%	7,3	%
segment	53	%	25,3	%
eosinofiel	0	%	0,1	%
basofiel	0	%	0,1	%
monocyt	15	%	12,2	%
promonocyt	4	%	2,9	%
lymfocyt	6	%	4,9	%
plasmacel			0,3	%
erytroblast	0		7,2	%

Cytogenetics: +21

Conclusion

- Immunophenotyping:
 - Increased % blasts
 - Increased % monocytes
 - Decreased % B-cell precursors
 - Abnormal granulocytic differentiation
 - Abnormal monocytic differentiation
 - Morphology:
 - Increased % blasts
 - Abnormal granulocytic/monocytic differentiation
 - Cytogenetics: +21
- 
- CMMOL

A dense crowd of colorful smiley faces, each with a question mark for an eye, set against a white background.

vragen?

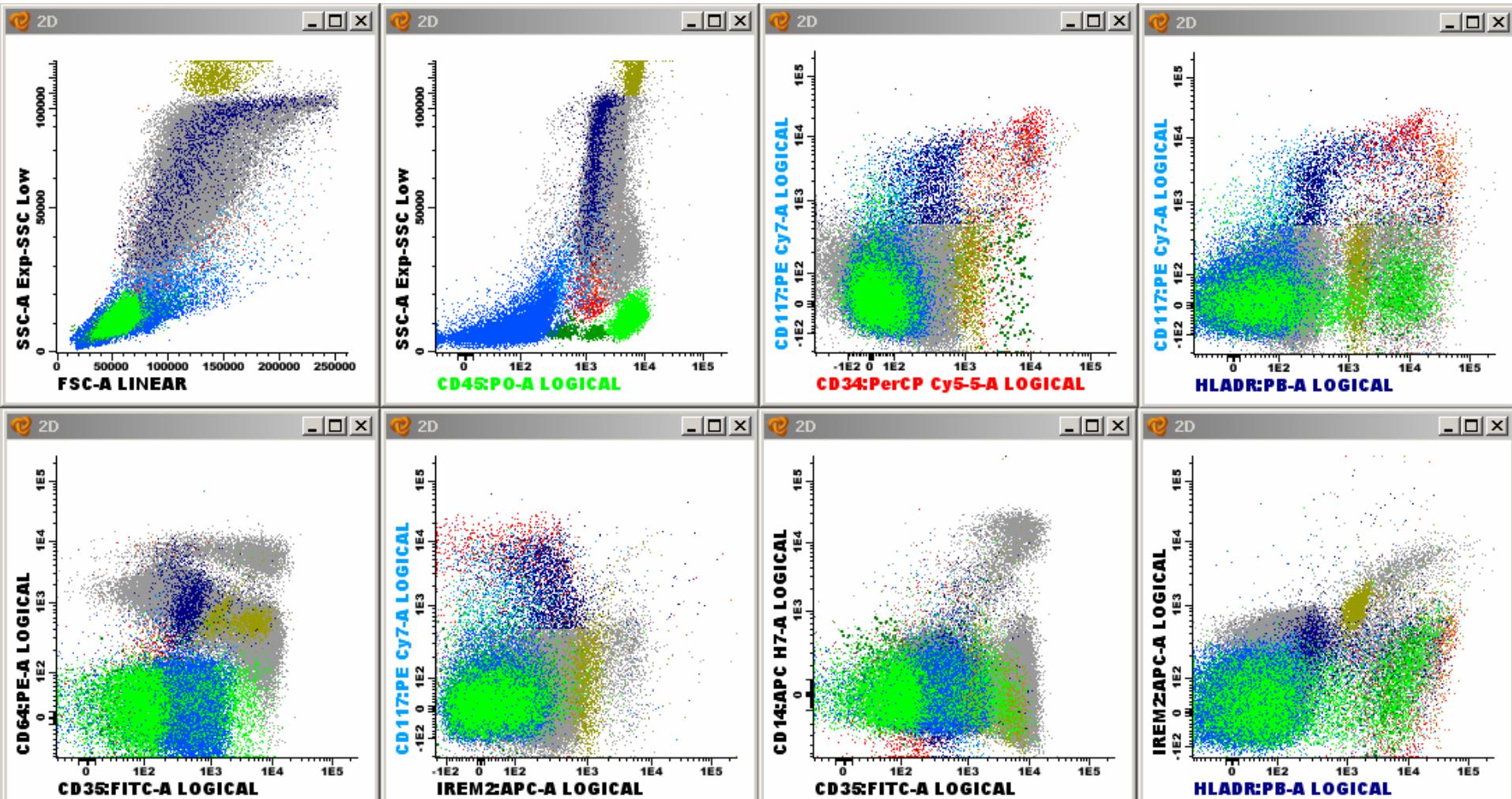
Proposed markers for flow cytometry in MDS

Table 4. Proposed marker combinations for flow cytometric analysis of dysplasia in myelodysplastic syndromes.*

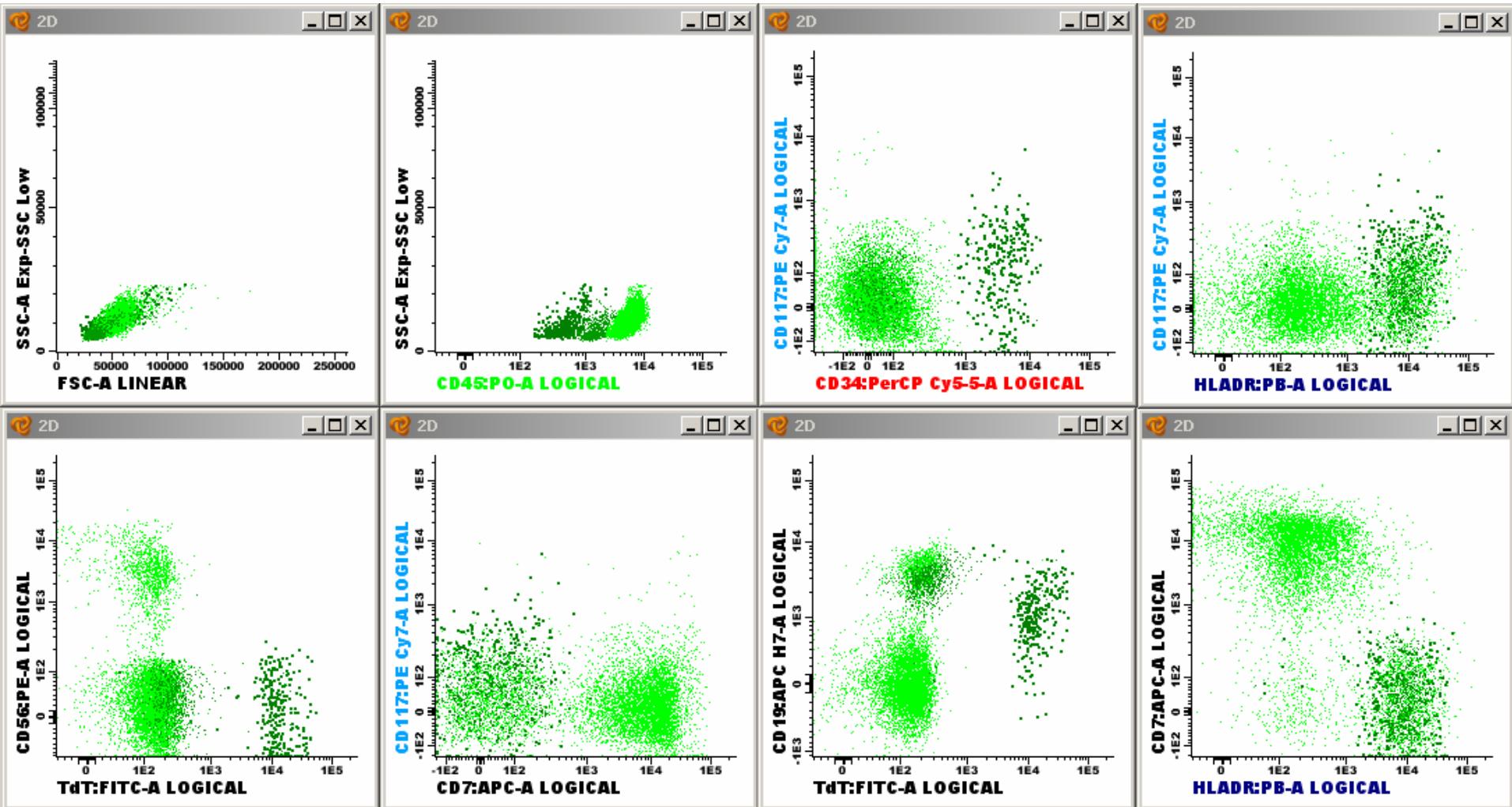
	Erythroid	Immature myeloid	Immature lymphoid	Maturing granulocytes	Monocytes	Mature lymphoid
CD71, CD235a, CD117	X					
CD34, CD117		X				
CD11b, CD117				X		
CD11b, HLA-DR		X		X		X
CD117, HLA-DR		X				
CD34, CD15		X		X		
CD34, CD5		X		X		X
CD34, CD7, (CD13 ^b)		X		X		X
CD34, CD56		X		X		X
CD34, CD19		X	X	X		X
CD10, CD19				X		
CD11b, CD13, CD16				X		
CD64, CD14					X	
CD33, CD14		X		X		X
CD33, CD36	(x)	CD36	X			X

Tube 2 – Monocytic differentiation

- CD34+, CD117+, lymphocytes, eosinophils, erythroid cells (Backbone gating)



Aberrant expressions – Lymphocytes



Aberrant expressions – Precursors

