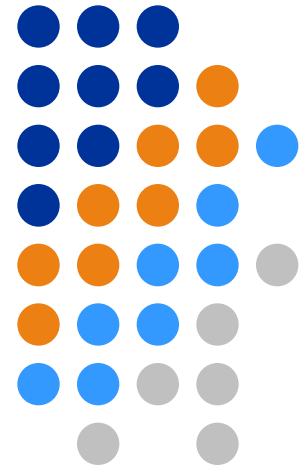


Richtlijn flowcytometrie bij MDS

Ingrid Lommerse



Waarom een richtlijn?



- Standaardisatie analyse
- Hoe krijgen we vergelijkbare analyses binnen verschillende centra?
 - Afname en bewerking
 - Instrument set-up
 - Gating



Afname en bewerking materiaal



- Heparine, EDTA of citraat ontstold beenmerg
Calcium afhankelijke antigenen
Bloedbijmenging
Binnen 24 uur na afname verwerken

- Bulklysis
Zonder fixatief
Indien noodzakelijk 2e lysisstap

Instrument setup



Wekelijkse calibratie van PMT waarden en compensatie settings

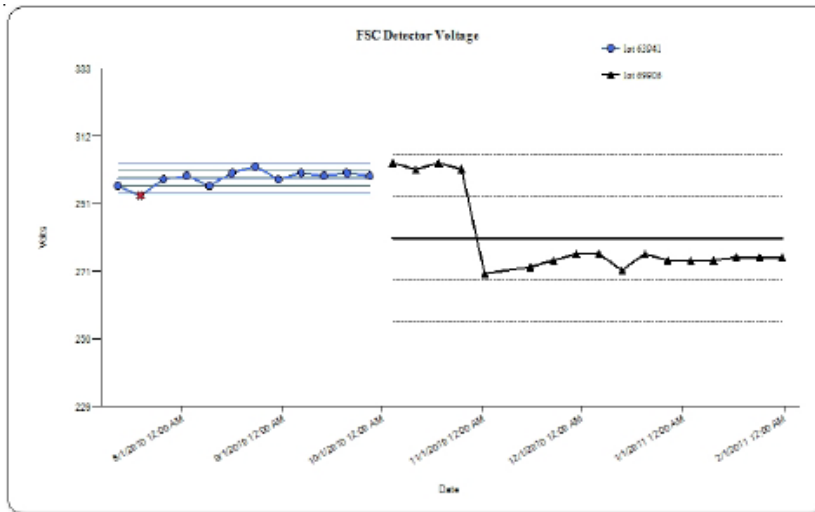
- Alignment beads of CST beads
- Compensatie settings zelf maken of software gebruiken
- Wat willen we zien?

Instrument setup

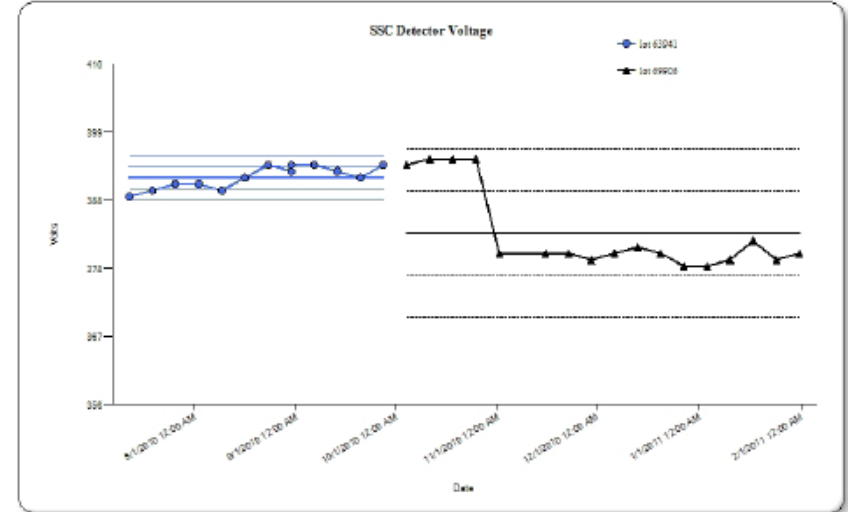
Wekelijkse calibratie – PMT voltages



FSC

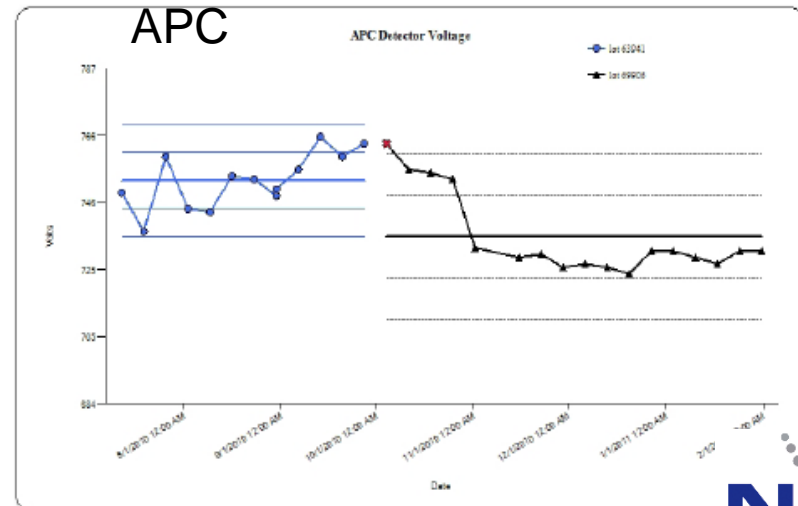
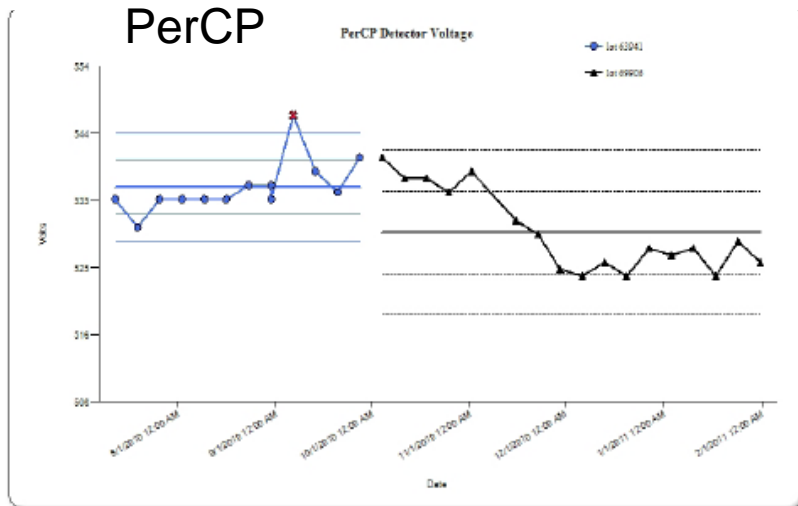
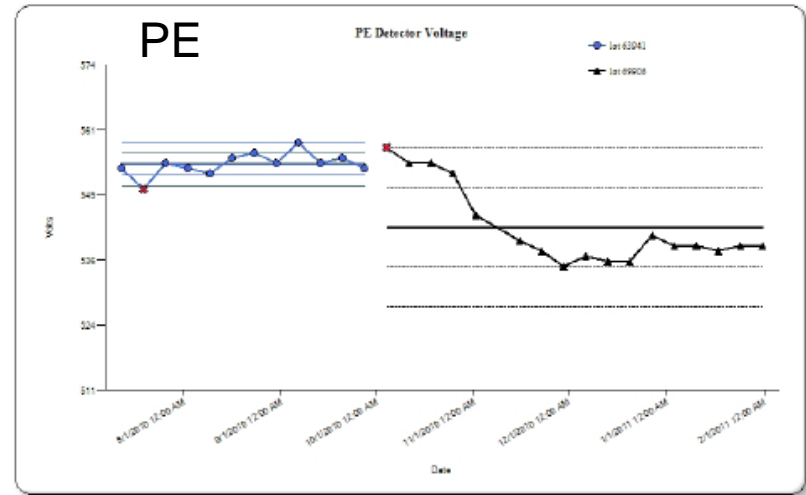
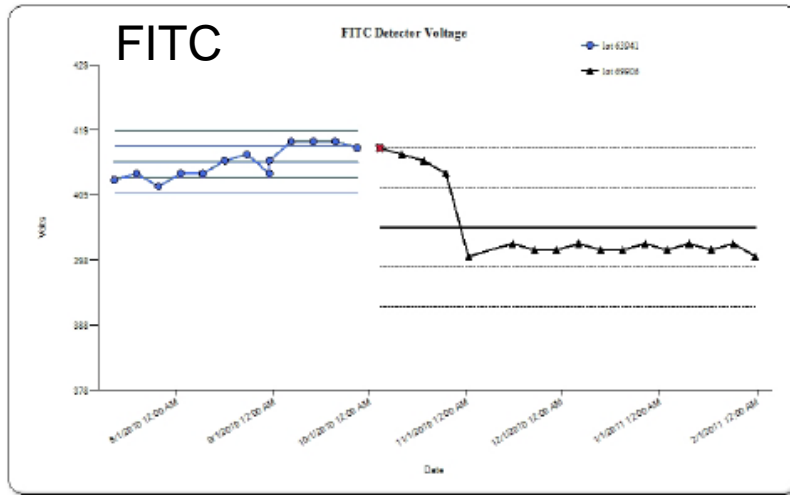
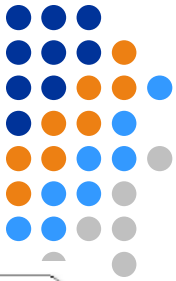


SSC



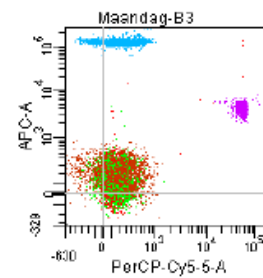
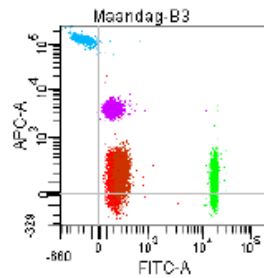
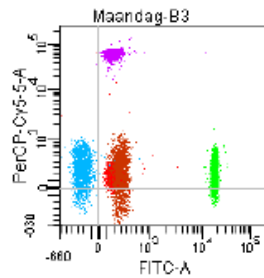
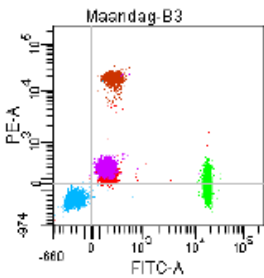
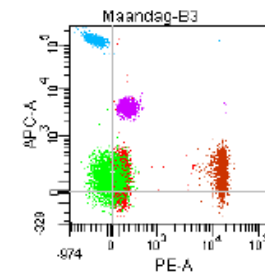
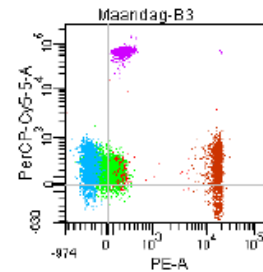
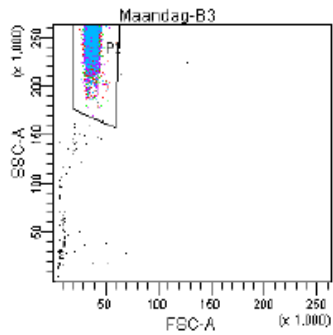
Instrument setup

Wekelijkse calibratie – PMT voltages



Instrument setup

Wekelijkse calibratie – controle compensatie



Well: B3

Population	#Events	%Parent	%Total
All Events	10,000	###	100.0
P1	9,531	95.3	95.3
FITC	1,997	21.0	20.0
PE	1,300	14.5	13.8
PerCP	1,856	19.5	18.6
APC	1,700	17.9	17.1

Instrument setup

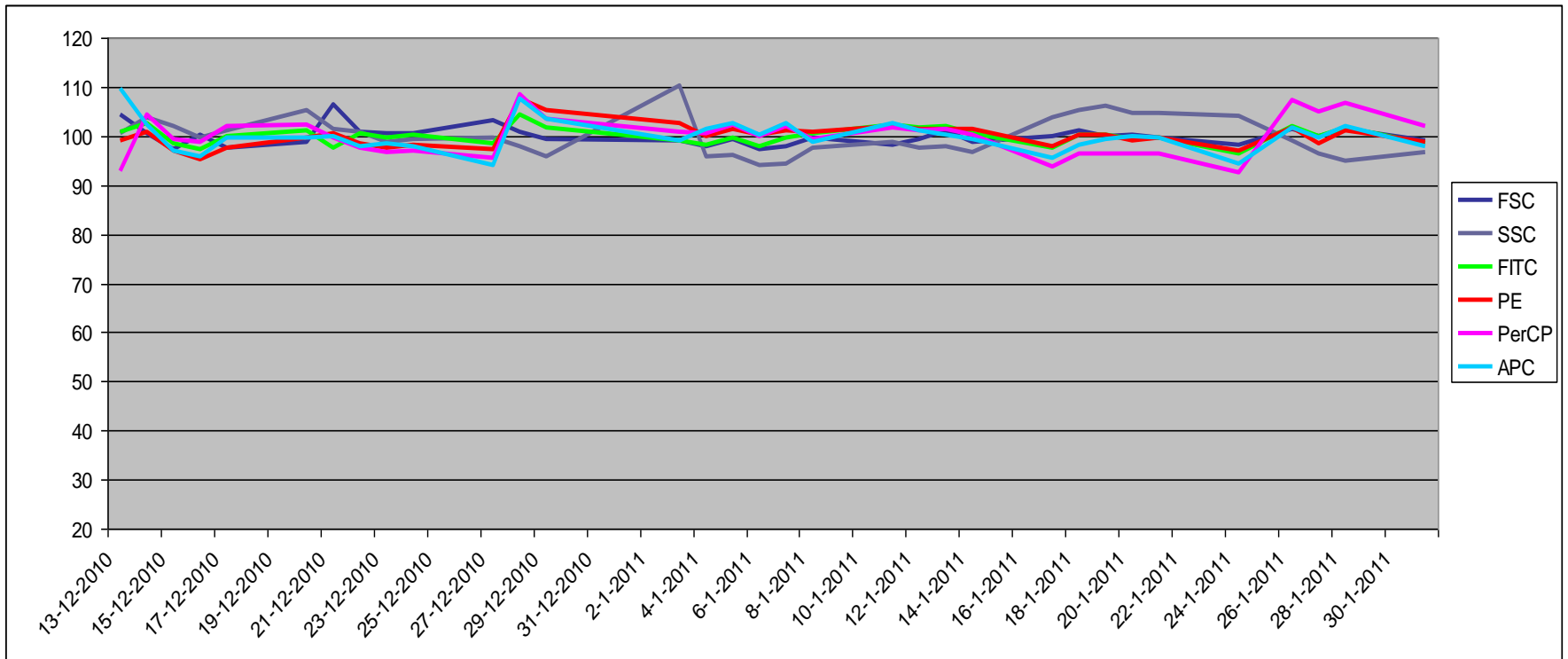


Dagelijkse controle PMT waarden

- 7-color beads
- Registratie van de gemeten fluorescentie intensiteiten
- Wat willen we zien?

Instrument setup

Dagelijkse controle



Antistofcombinaties



<i>Combinatie</i>				
1	CD45	PBS	PBS	PBS
2	CD45	CD16	CD11b	CD13
3	CD45	CD71	CD117	CD235a
4	CD45	CD15	CD11b	HLA-Dr
5	CD45	CD36	CD14	CD33
6	CD45	CD34	CD123	HLA-Dr
7	CD45	CD34	CD117	CD13+CD33
8	CD45	CD34	CD7	CD56
9	CD45	CD34	CD5	CD19
10	CD45	CD34	CD11b	CD15

Definiëren van subpopulaties

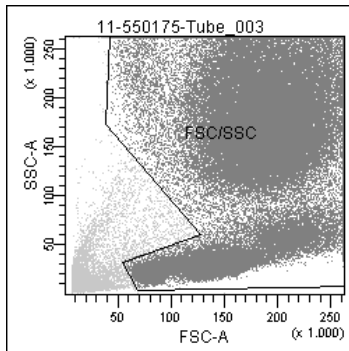


Erytroïde voorlopers	CD45-/dim; CD235a+; CD71+; SSClow
Myeloblasten	CD45dim; SSClow; CD34+; CD117+; CD13 en/of CD33+
Granulocyten	CD33+; SSChigh; backgating CD45/SSC
Monocyten	CD14+; backgating CD45/SSC
Lymfocyten	CD45bright; SSClow
Precursor B-cellen	CD45dim; SSClow; CD34+; CD19+
Plasmacytoïde DC's	CD123+; HLA-Dr++
Basofiele granulocyten	CD123+; HLA-Dr-; SSClow
Promyelocyten	CD34-; CD117+; SSChigh

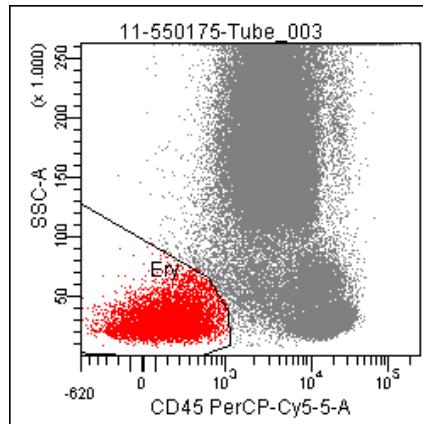
Gating erythroïde voorlopers



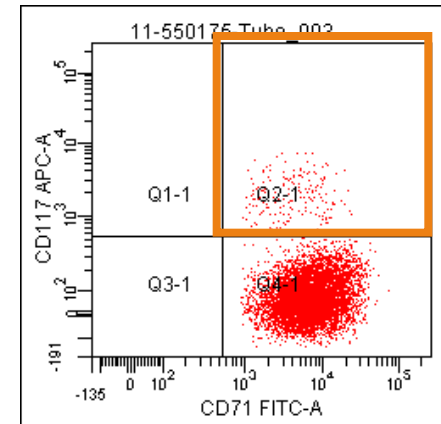
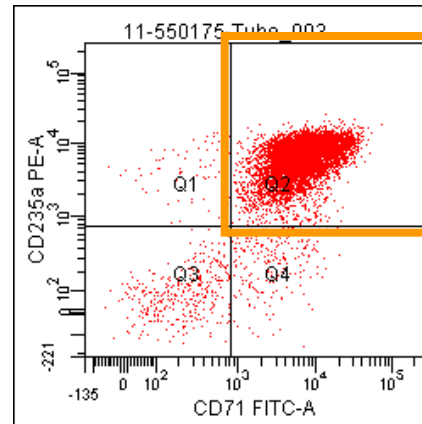
FSC/SSC gate



CD45^{-int} / SSC^{low} gate



CD235a⁺ / CD71⁺ gate



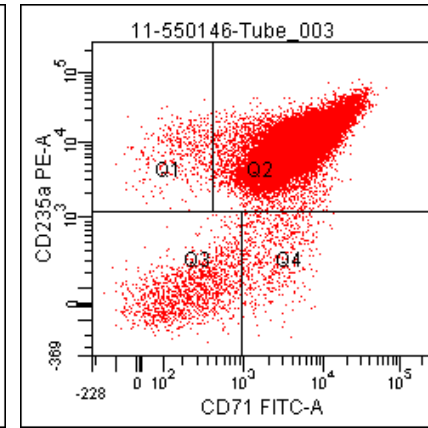
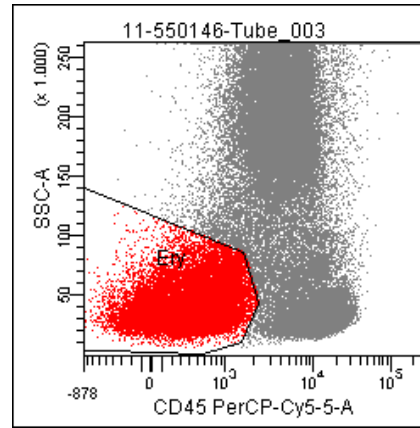
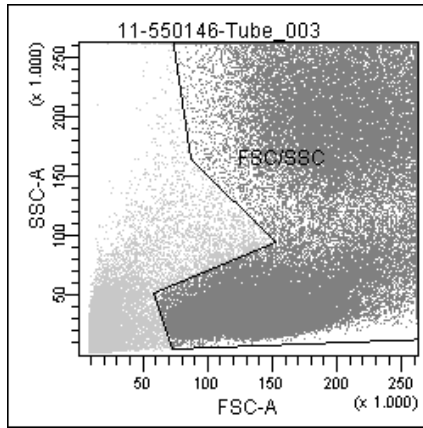
% NRBC

% CD117⁺
erythroïde
voorlopers

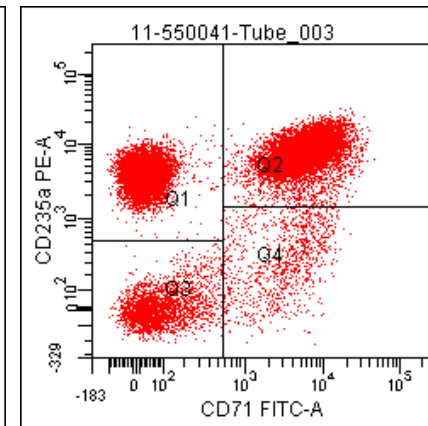
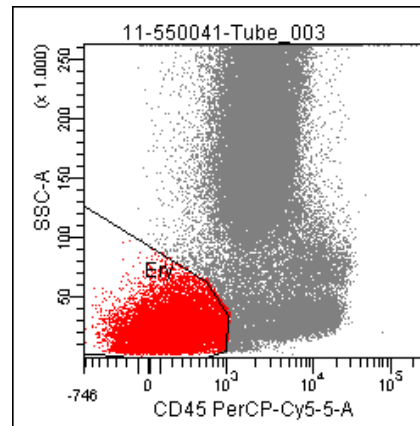
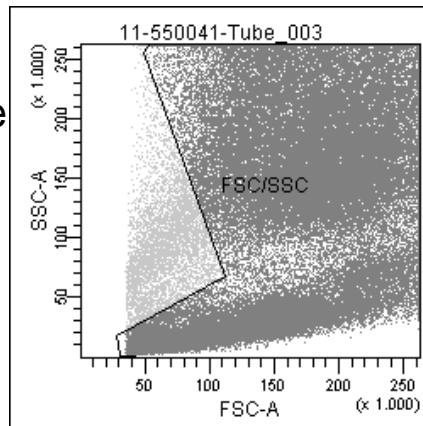
Gating erythroïde voorlopers – Problemen...



Veel
erythroblasten



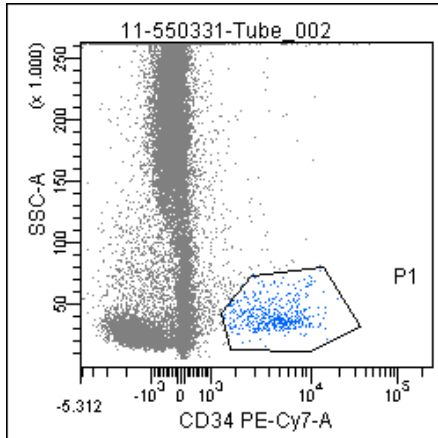
Niet gelyseerde
erythrocyten



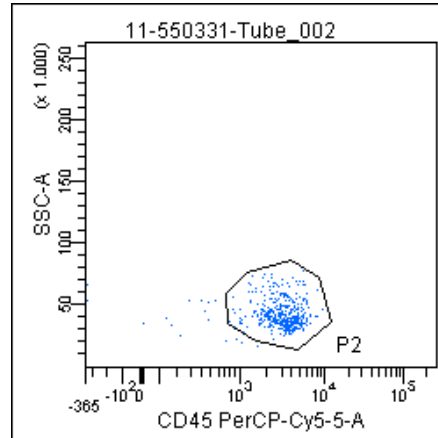
Gating myeloide blasten



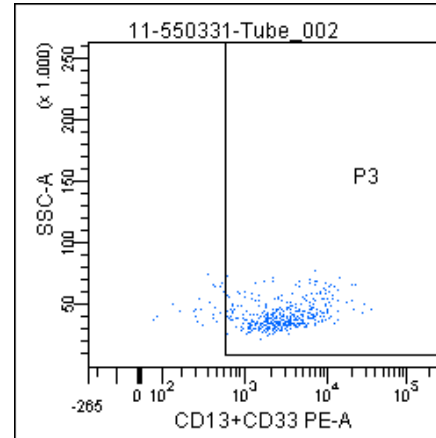
CD34⁺ /SSC^{low}



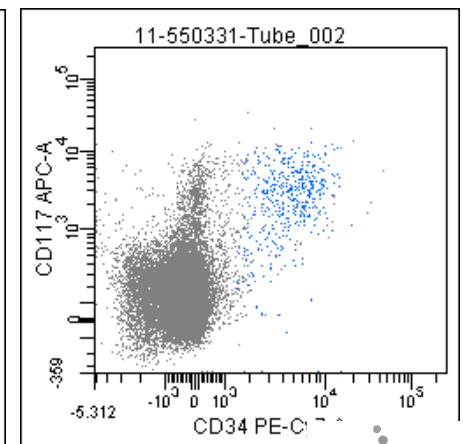
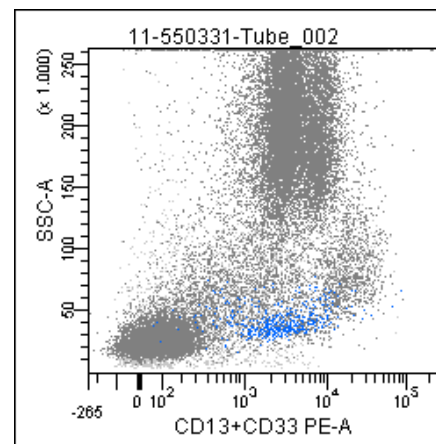
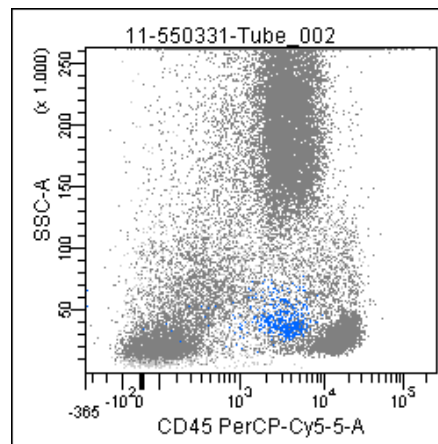
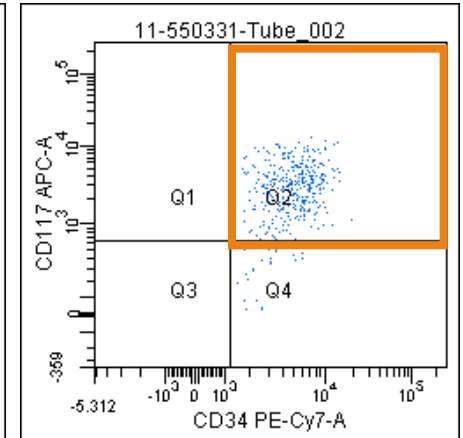
CD45^{dim} /SSC^{low}



CD13.CD33⁺



CD117⁺ / CD34⁺

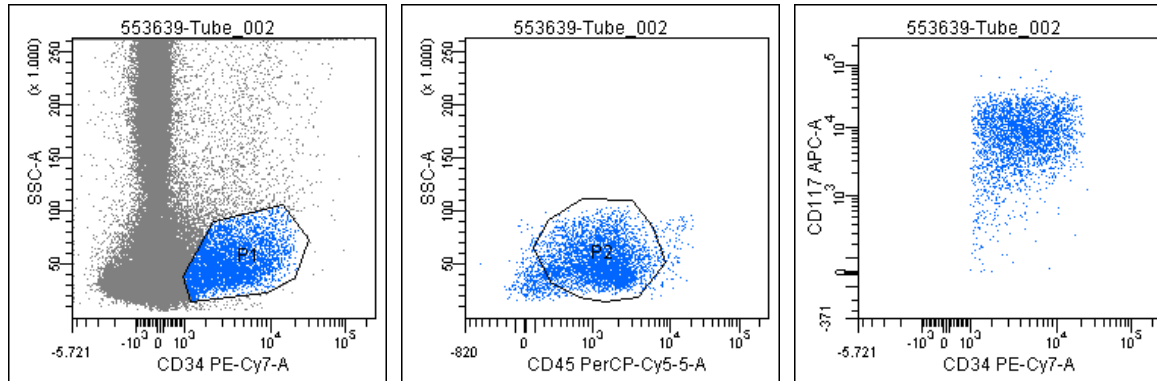


% myeloide
blasten

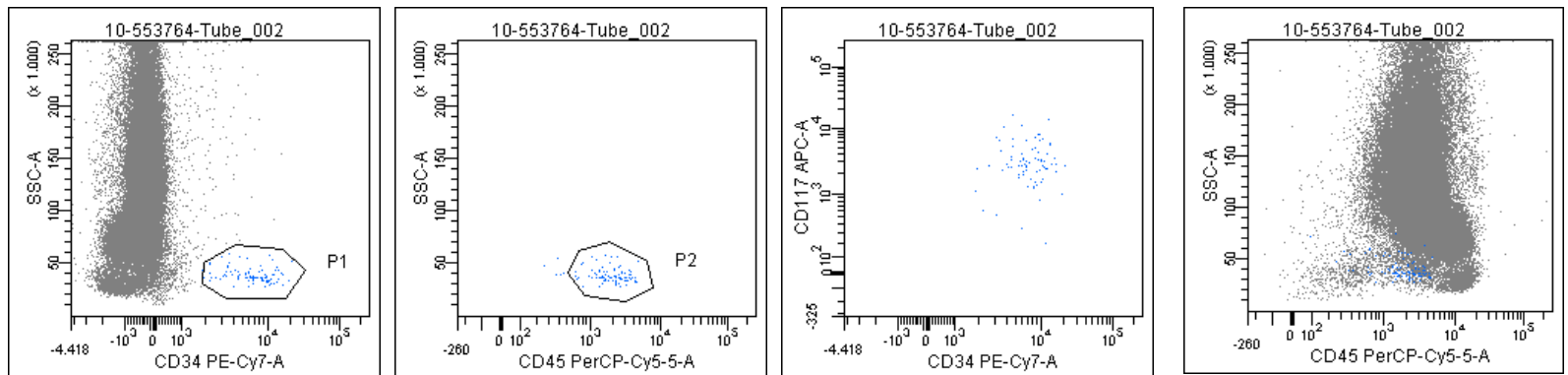
Gating myeloïde blasten – Problemen...



Heterogene
Populatie &
Veel ery's



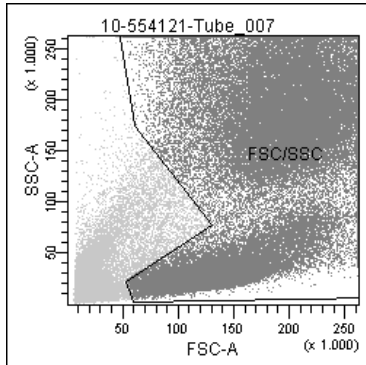
Laag aantal
blasten



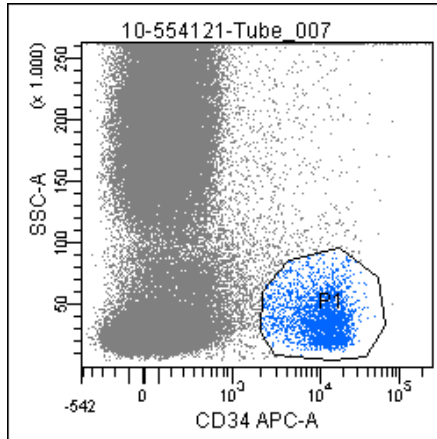
Gating precursor B-cellen



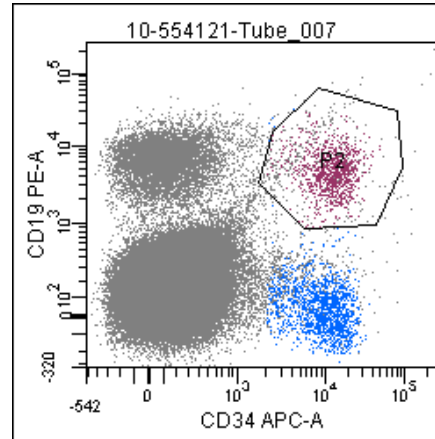
FSC / SSC



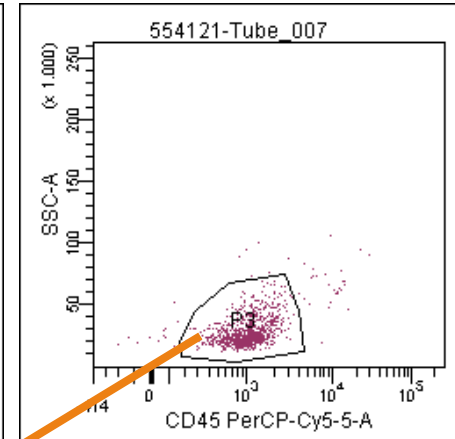
CD34+ / SSC^{low}



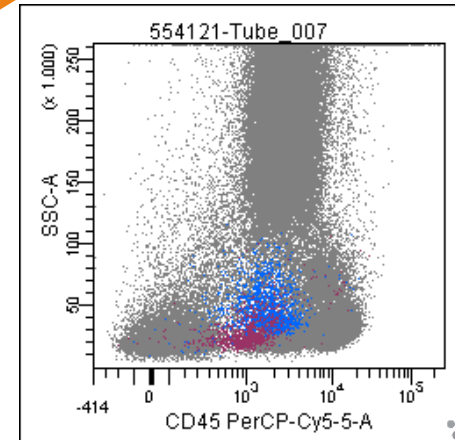
CD34+ / CD19+



CD45^{dim} / SSC^{low}



% precursor
B-cellen



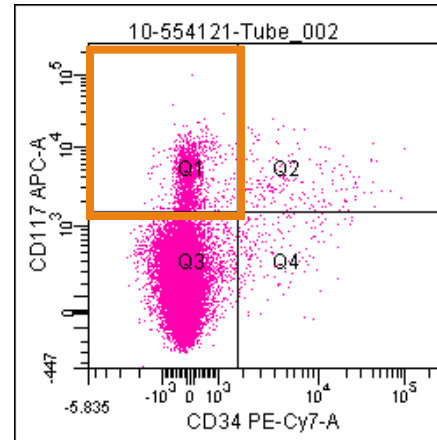
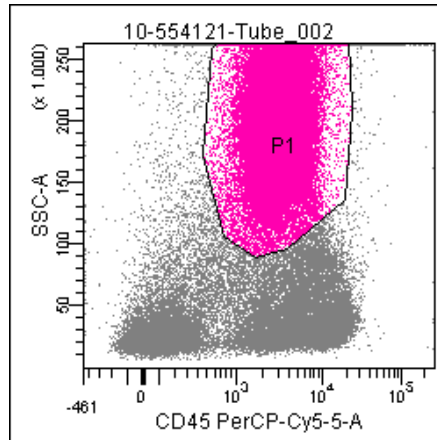
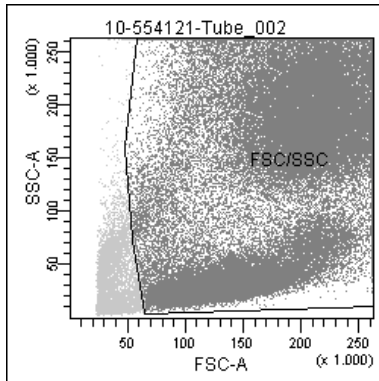
Gating promyelocyten



FSC /SSC

CD45^{dim} /SSC^{high}

CD34⁻ / CD117⁺



% promyelocyten

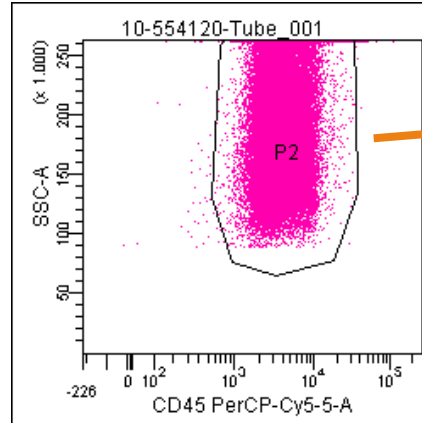
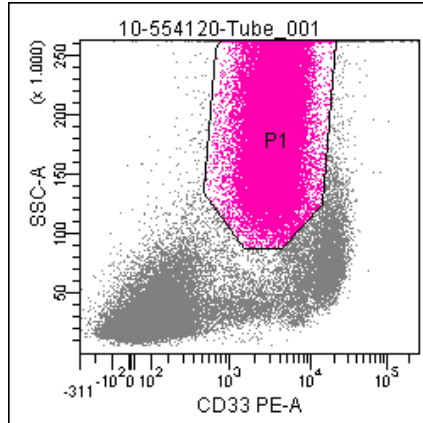
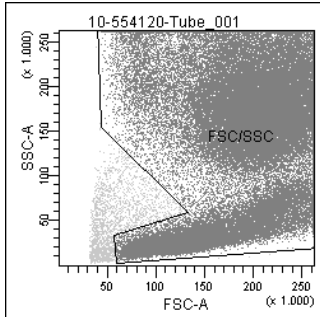
Gating granulocyten



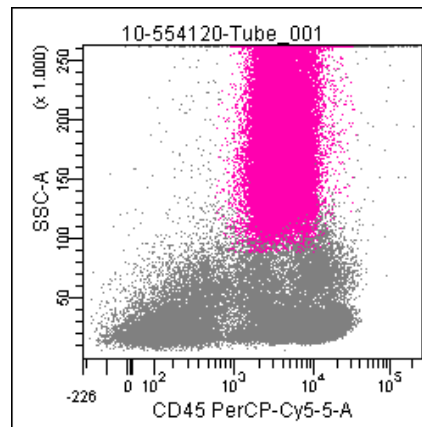
FSC / SSC

CD33+ / SSC^{high}

CD45^{dim} / SSC^{high}



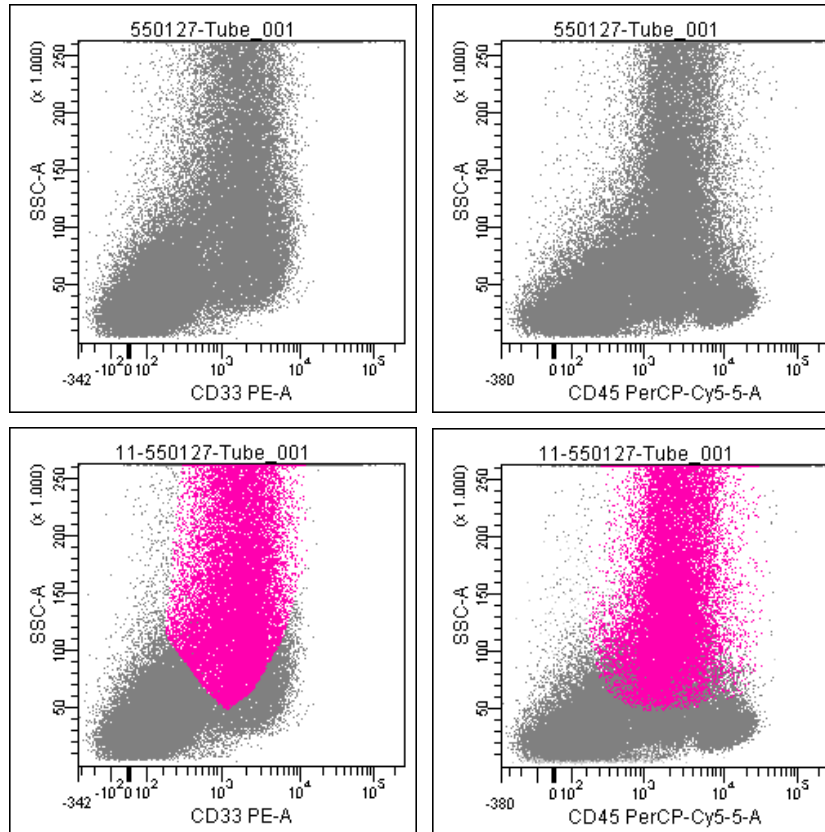
% granulocyten



Gating granulocyten – Problemen...



Hypogranulair
+
Zwak CD33+ mono's



Gating monocyten

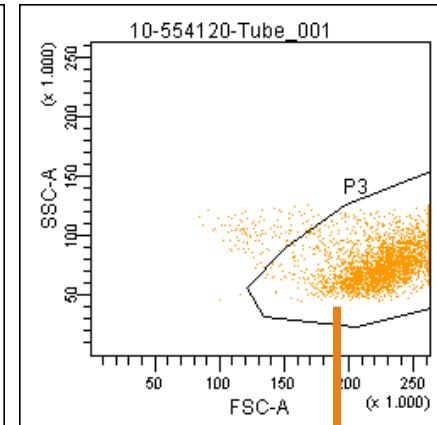
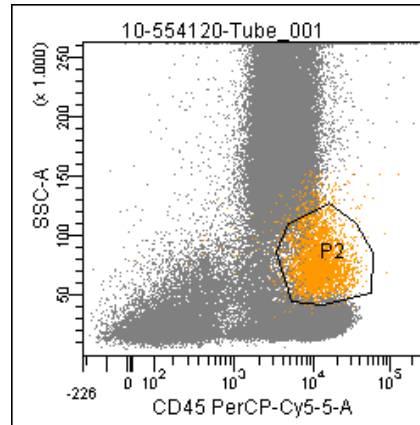
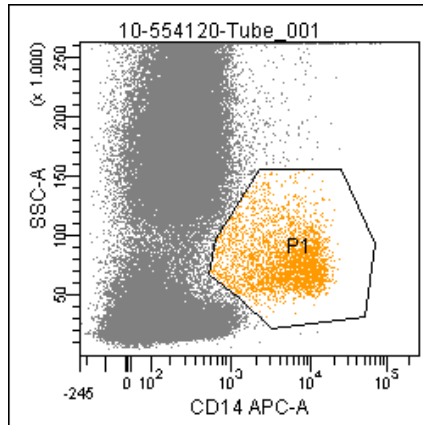
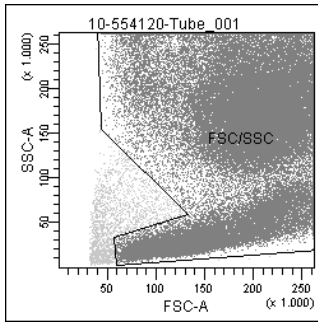


FSC / SSC

CD14⁺ / SSC^{int}

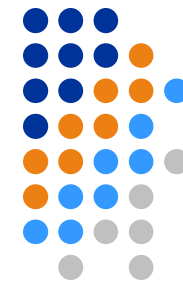
CD45^{dim/bright} / SSC^{int}

FSC / SSC

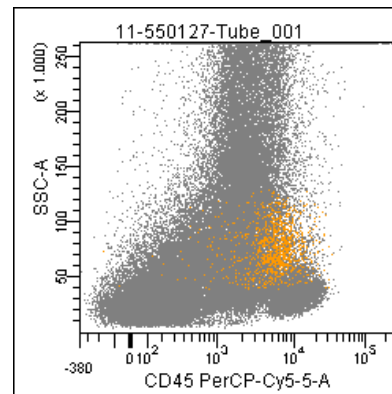
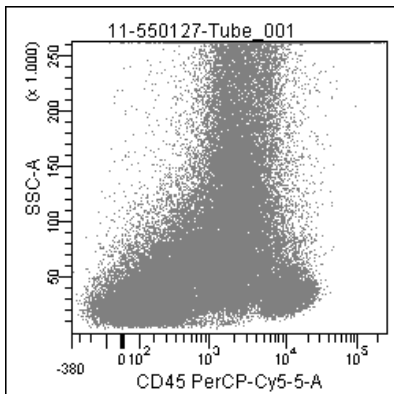
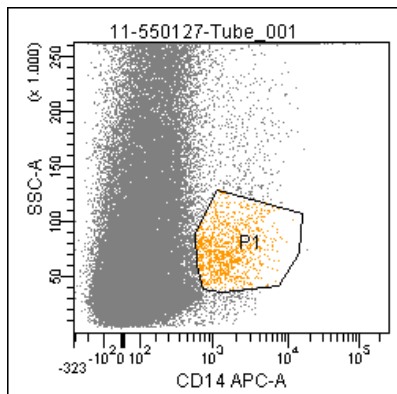


% monocyten

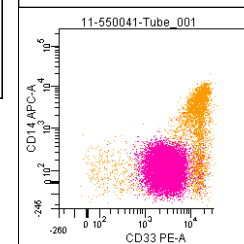
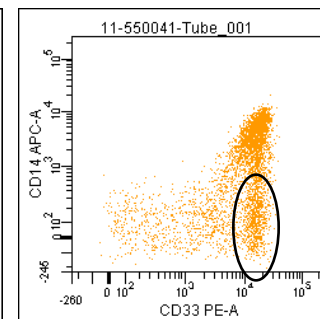
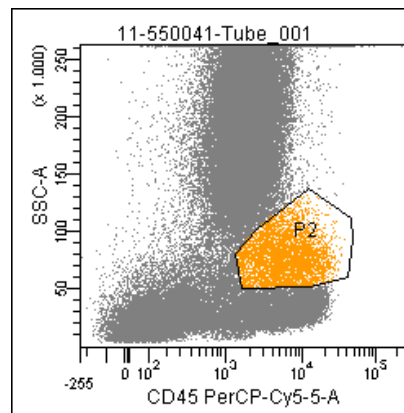
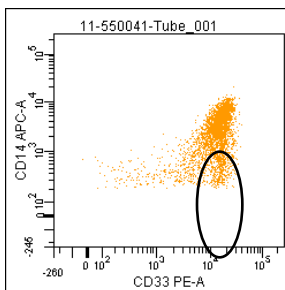
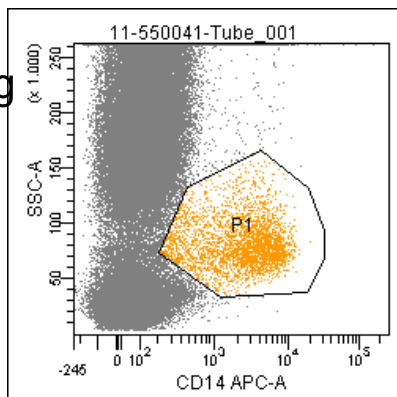
Gating monocyten – Problemen



Weinig mono's
+
Hypogranulariteit



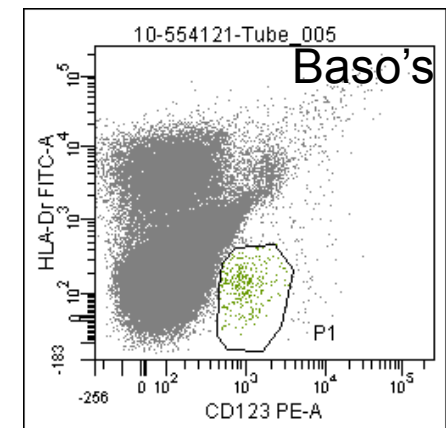
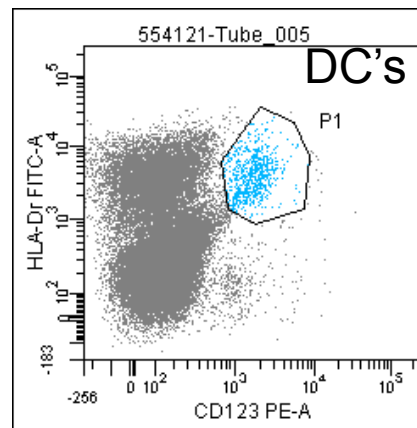
CD14 zwak+ /neg
monocyten
(onrijpe mono's)



Gating plasmacytoïde DC's & basofiele granulocyten



- Kunnen beide CD45^{dim} zijn
- Gate vervuiling bij de myeloblasten



Vertaling naar andere buizen



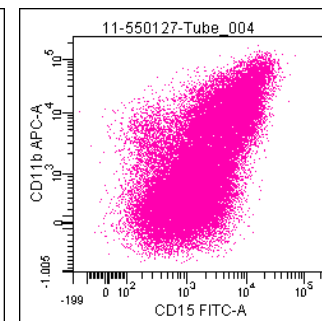
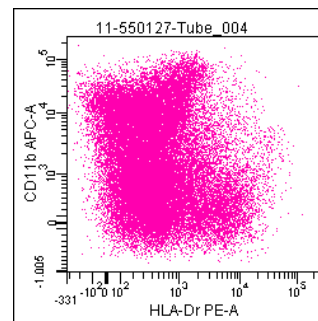
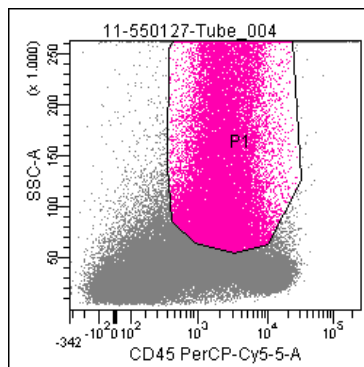
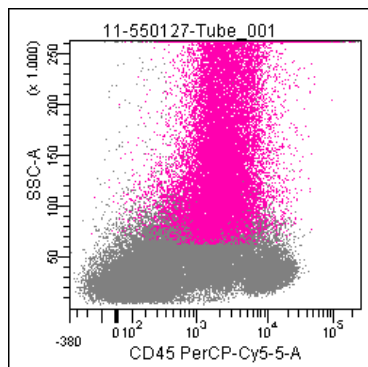
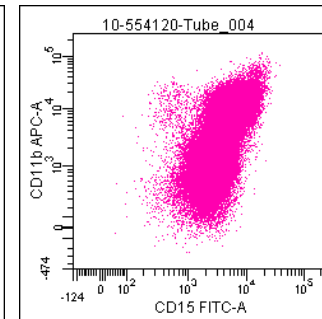
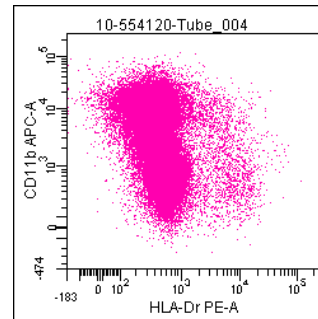
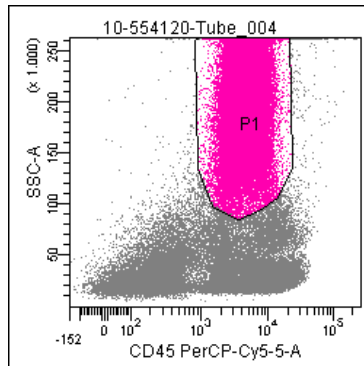
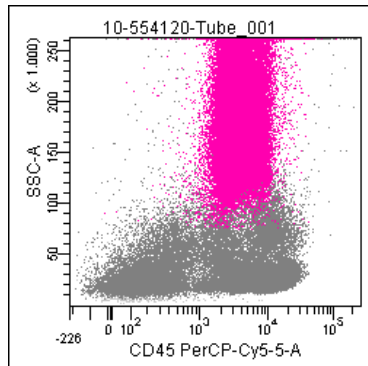
- Niet elke antistofcombinatie bezit de ‘specifieke merkers’
- Analyse van deze combinatie door middel van de CD45, FSC en SSC karakteristieken

Vertaling naar andere buizen – Granulocyten



Granulo's gegate
aan de hand
van de definitie

Vergelijkbare
populatie op CD45
expressie

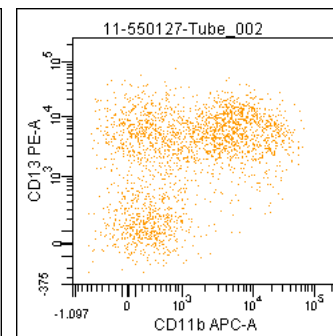
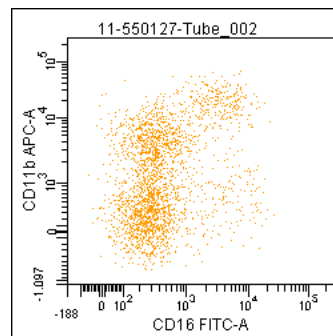
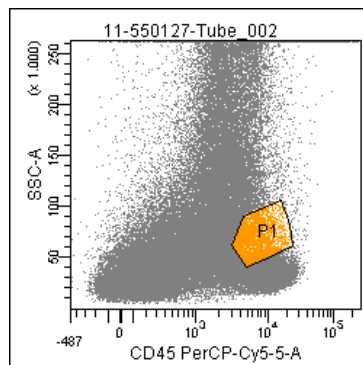
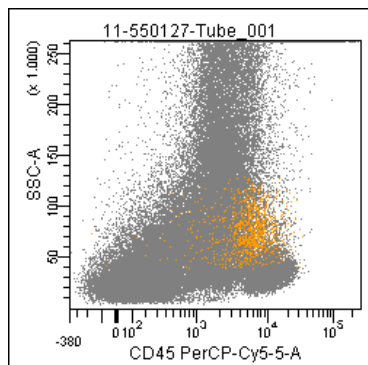
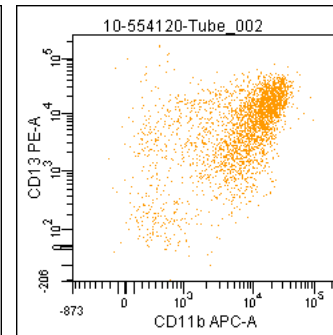
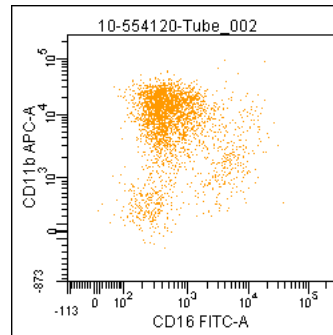
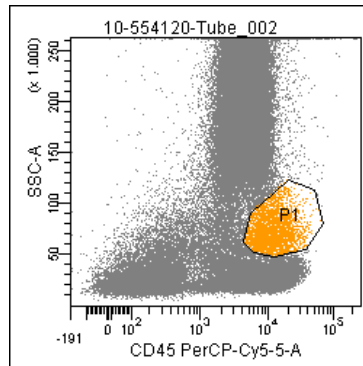
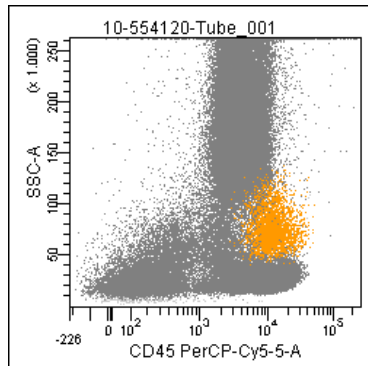


Vertaling naar andere buizen – Monocyten



Mono's gegate
aan de hand
van de definitie

Vergelijkbare
populatie op CD45
expressie





Vragen ?