# Workshop Masterclass Flowcytometrie in MDS

## Technische Aspecten

Sanquin, Amsterdam 5 september 2012

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# Haematologica 2009;94(8) Loosdrecht et al

- Standardization of flow cytometry in myelodysplastic syndromes; report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes.

Minimale variatie in/bij:

- Monster verwerking
- Antistof combinaties
- Data acquisitie
- Data interpretatie

# Haematologica 2009;94(8) Loosdrecht et al

- Monster verwerking
  - Beenmerg (bij voorkeur)
  - Anticoagulans: heparine (bij voorkeur)
  - Verwerking binnen 24 uur (bij voorkeur)
  - Bewaren bij kamertemperatuur

# Haematologica 2009;94(8) Loosdrecht et al

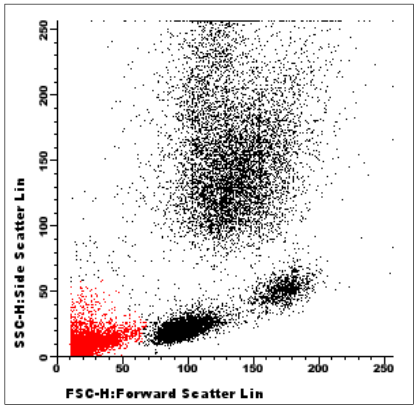
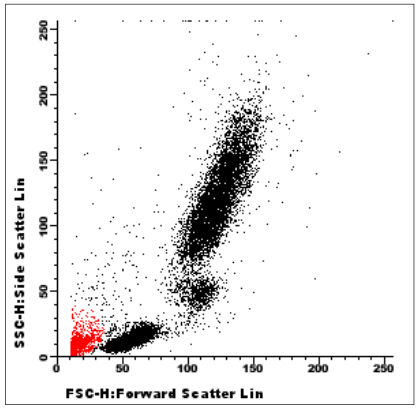
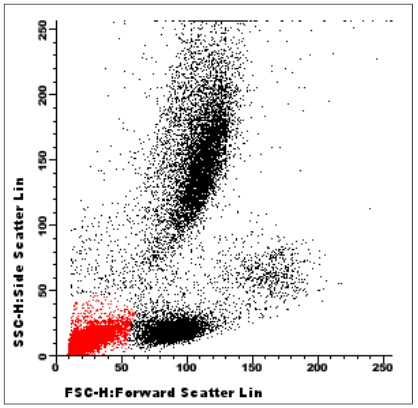
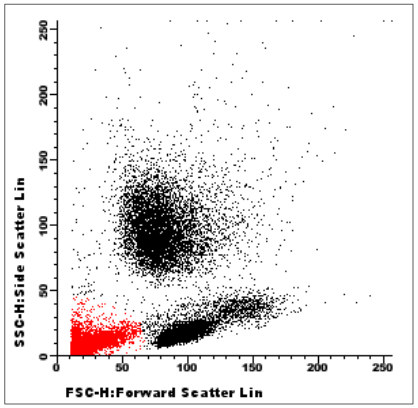
- Lysis rode bloedcellen
  - Ammoniumchloride (in huis of commercieel)
  - Bulklysis (géén ficoll scheiding)
    - 3 ml BM aanvullen tot 50 ml met NH4Cl, 10 min bij KT op menger

    Voordelen:
    - Identieke celsuspensie én
    - Fixed celconcentraties in de verschillende buizen
  - Wasbuffers en/of lyseervloeistoffen met fixatieven (bv PFA) niet gebruiken voor labelen
  - Fixatief beïnvloedt scatter

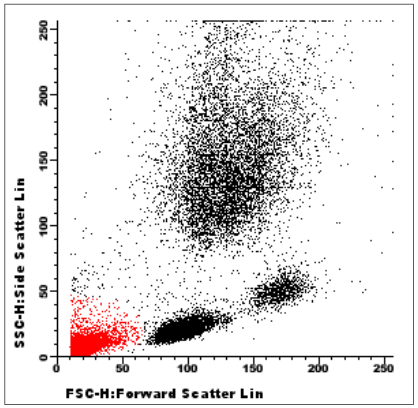
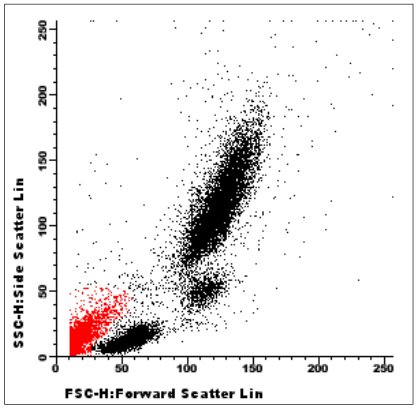
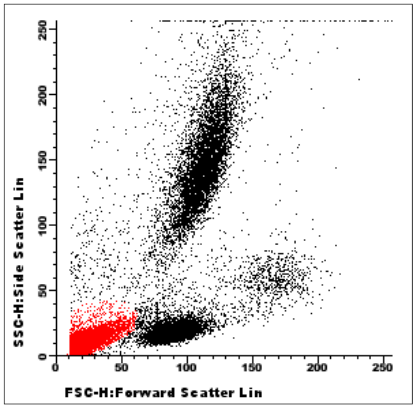
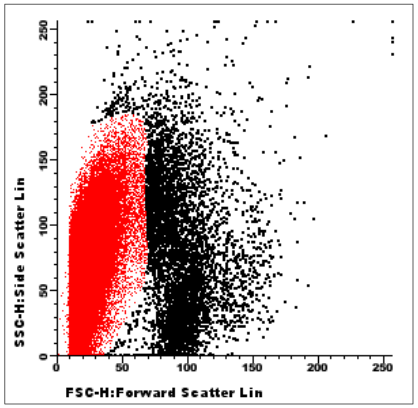
  - Wassen met PBS + 0,5% bovine of humane serum albumine

# Anticoagulans en Lyseer vloeistoffen

## EDTA vers

| NH4Cl home made | FACS Lysing Sol. BD | QuickLysis Cytognos | VersaLyse Coulter |
|---|---|---|---|
|  |  |  |  |
| 53% | 9% | 62% | 61% |

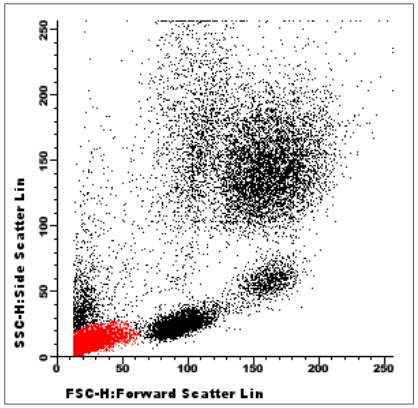
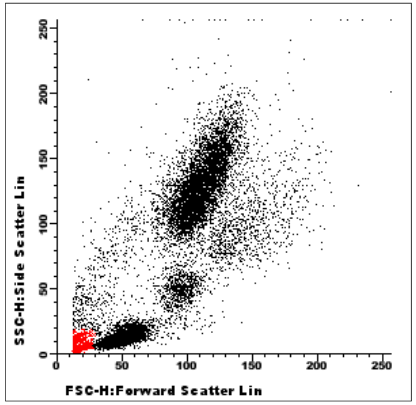
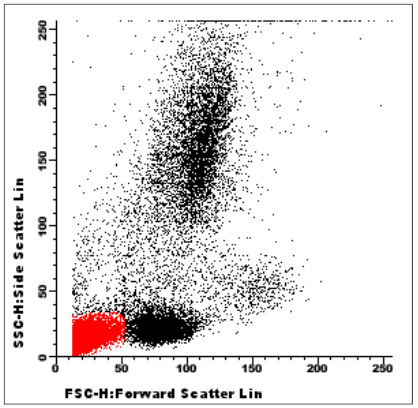
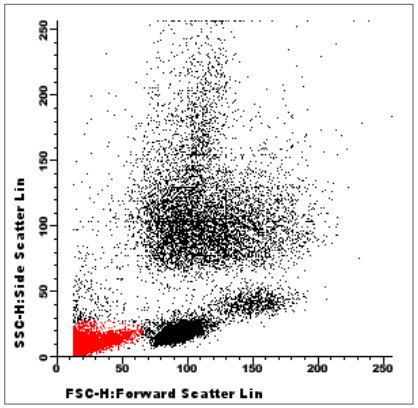
## Heparine vers

| | | | |
|---|---|---|---|
|  |  |  |  |
| 40% | 18% | 60% | 97% |

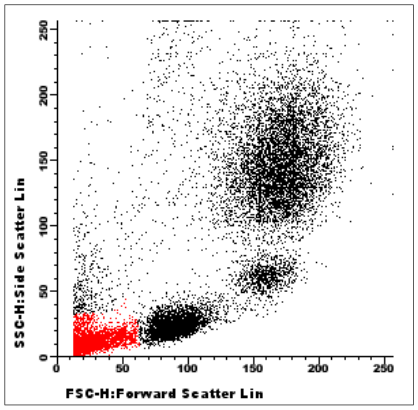
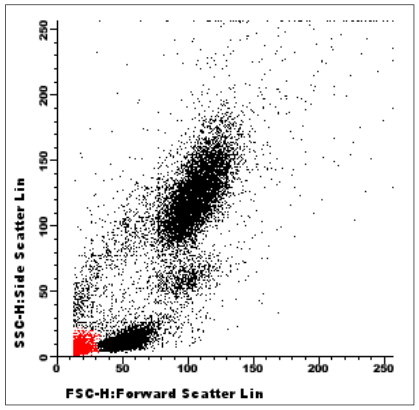
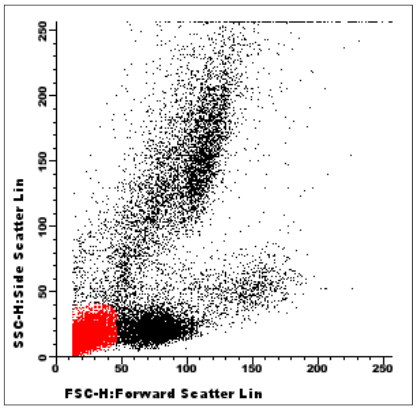
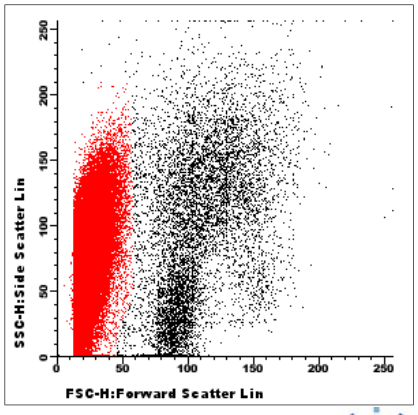
EuroFlow

# Anticoagulans en Lyseer vloeistoffen

## EDTA 24 uur

| NH4Cl home made | FACS Lysing Sol. BD | QuickLysis Cytognos | VersaLyse Coulter |
|---|---|---|---|
|  |  |  |  |
| 40% | 6% | 60% | 55% |

## Heparine 24 uur

| | | | |
|---|---|---|---|
|  |  |  |  |
| 46% | 6% | 59% | 97% |

EuroFlow

# Haematologica 2009;94(8) Loosdrecht et al

- Antistof labelingen
  - Label min. 500.000 cellen (50 ul van een $10x10^6$/ml suspensie)
  - Optimale titers van antistoffen gebruiken
  - Incubatie: 15 min bij kamertemperatuur in het donker
  - Wassen met PBS/BSA 0,5%
  - In het geval van intracellulaire kleuringen eerst de membraanlabeling uitvoeren gevolgd door de intracellulaire labeling.
  - Gebruik alleen bekende en geevalueerde fix/perm vloeistoffen
  - Meet bij voorkeur direct (<1 uur), of fixeer met 0.5% PFA in PBS (pH 7,4)
  - Meet tenminste 100.000 events

# Haematologica 2009;94(8) Loosdrecht et al

- Aanbevolen antistof combinaties

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

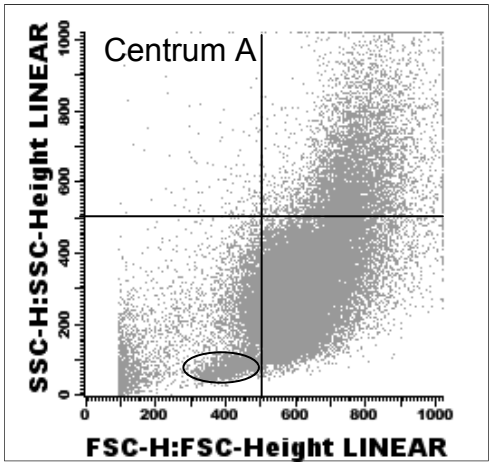
| | Erythroid | Immature myeloid | Immature lymphoid | Maturing granulocytes | Monocytes | Mature lymphoid |
|---|---|---|---|---|---|---|
| CD71, CD235a, CD117 | X | | | | | |
| CD105 | (x) | | | | | |
| CD34,CD117 | (x) | X | | | | |
| CD11b, CD117 | | | | X | | |
| CD11b, HLA-DR | | X | | X | X | |
| CD117, HLA-DR | | X | | | | |
| CD123, HLA-DR | | (x)† | | (x)‡ | | |
| CD34, CD15 | | X | | X | | |
| CD34, CD5 | | X | | X | X | |
| CD34, CD7, ($CD13^{s}$) | | X | | X | X | |
| CD34, CD56 | | X | | X | X | |
| CD34, CD19 | | X | X | X | X | |
| CD10, CD19 | | | (x) | X | | |
| CD10, CD38 | | | (x) | | | |
| CD11b, CD13, CD16 | | | | X | | |
| CD65 | | | | (x) | | |
| CD64, CD14 | | | | | X | |
| CD64, CD36 | | | | | (x) | |
| CD33, CD14 | | X | | X | X | |
| CD33, CD36 | (x) CD36 | X | | | X | |
| TdT | | (x) | (x) | | | |
| CD79a | | | (x) | | | |
| CD19 κ,λ | | | | | | (x)** |
| CD3, CD4, CD8 | | | | | | (x)** |

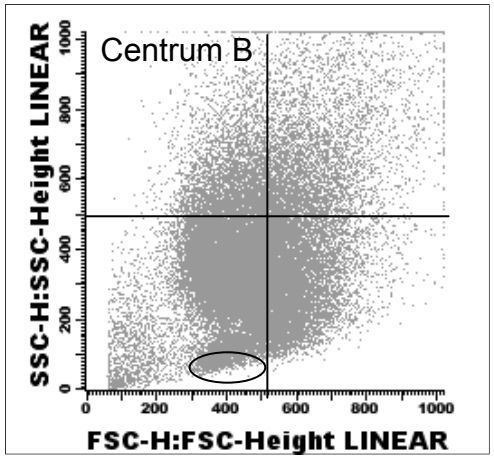
*CD45 is ubiquitous in every combination. X: minimally required, (x): supplementary. †Analysis of plasmacytoid DC (precursors), ‡analysis of basophils, §CD7 expression on myeloblasts can be normal, e.g. monocytic/dendritic precursors are CD13dim/CD7dim, **to complete immunological differential.

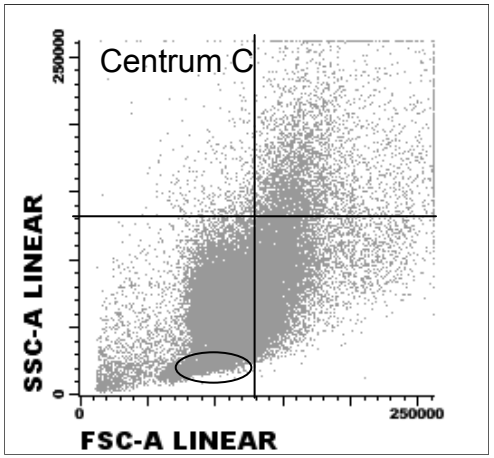
# Valkuilen

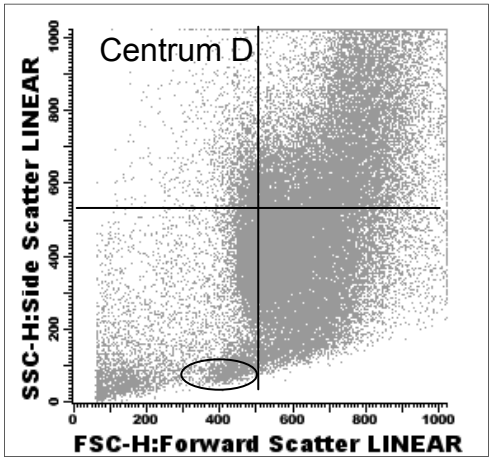
- Experimenteel
  - Instrument Setup dia 10 - 24
    - Scatter
    - Fluorescentie
  - Procedure dia 26 - 28
  - Antistoffen dia 30 - 39
    - Fluorochromen
    - Clonen
    - Tandem Fluorochromen
    - Data Spread
  - Fluorescentiepatronen dia 41 - 49

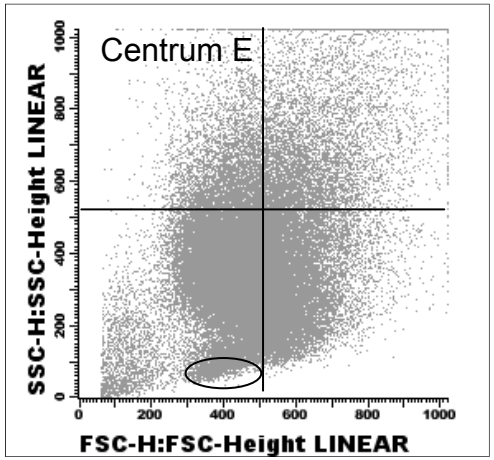
# Discussie

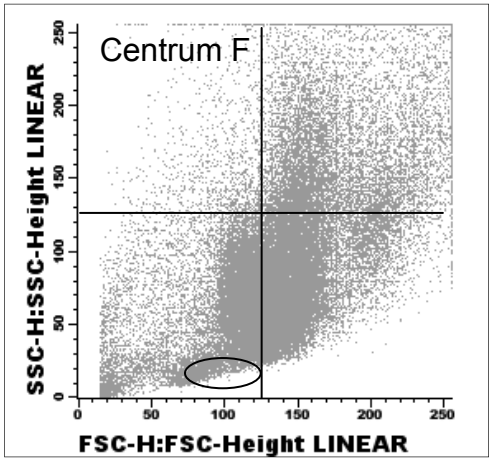


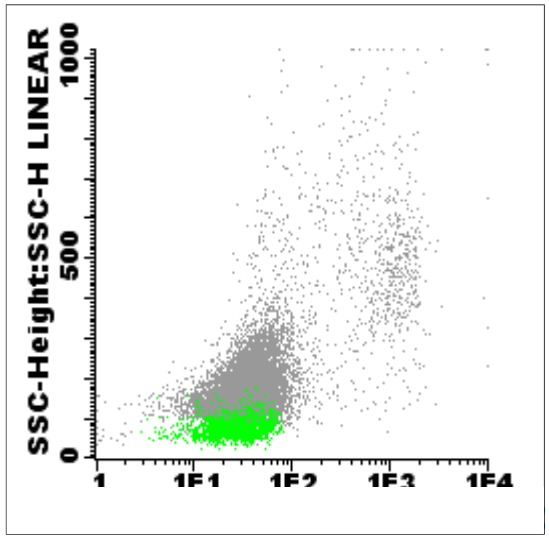



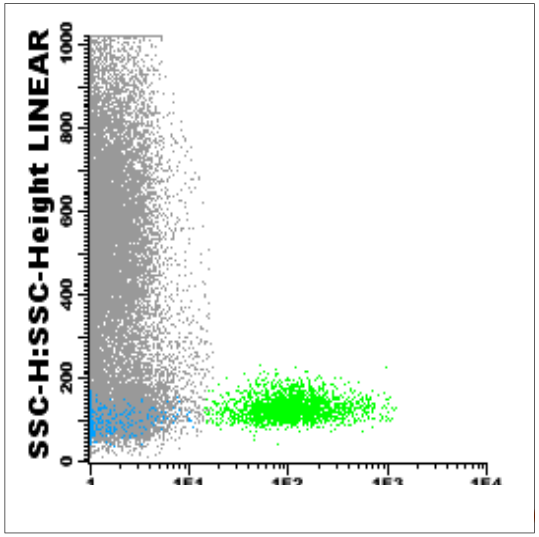



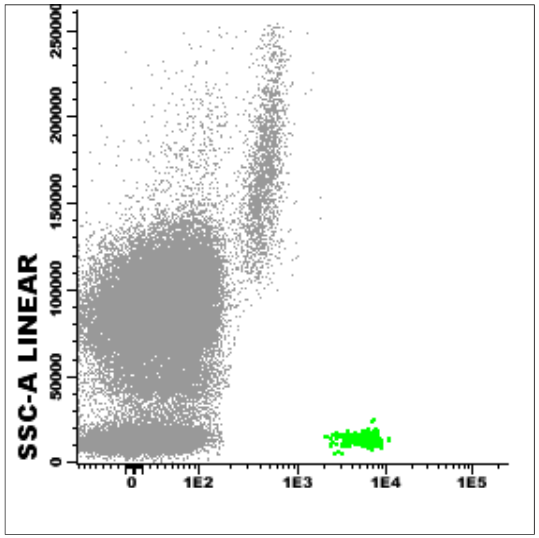



# Discussie

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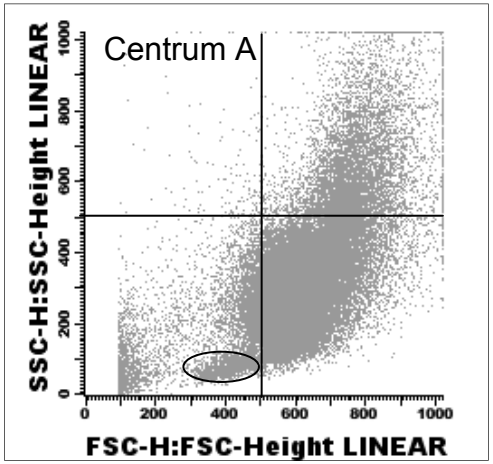
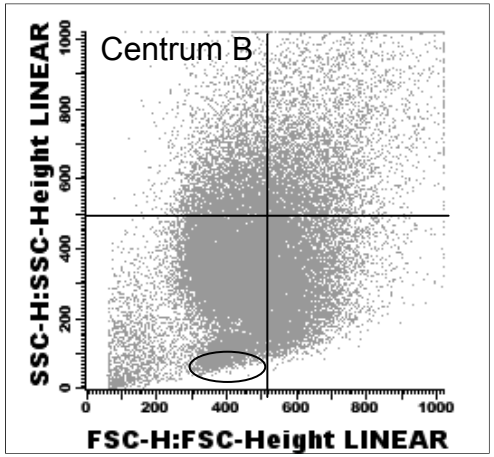
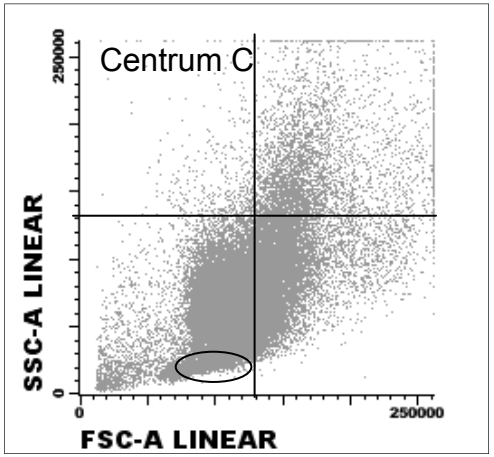
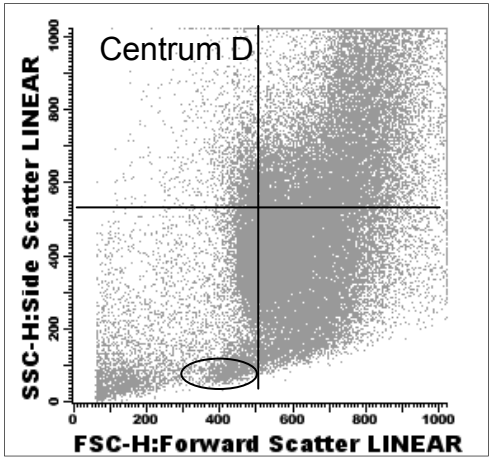
# Instrument Setup

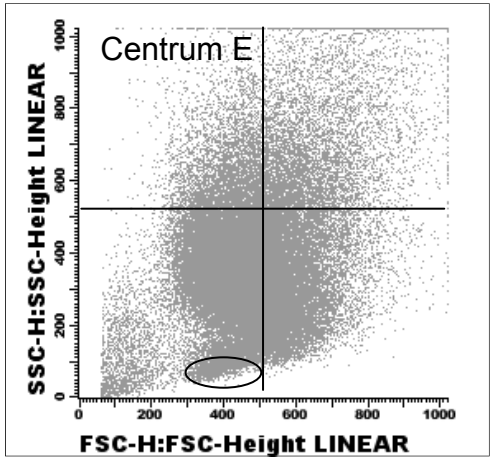
- Scatterpatronen
  - 1 BM monster
  - 6 centra

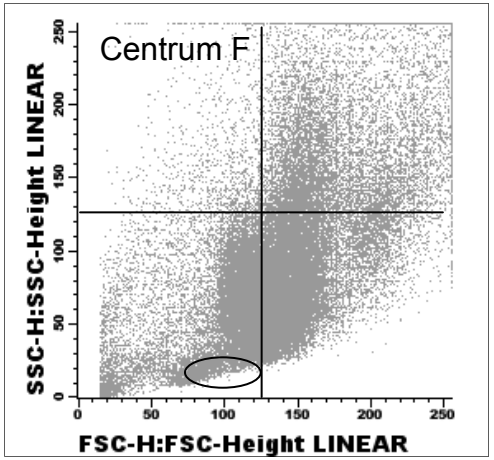


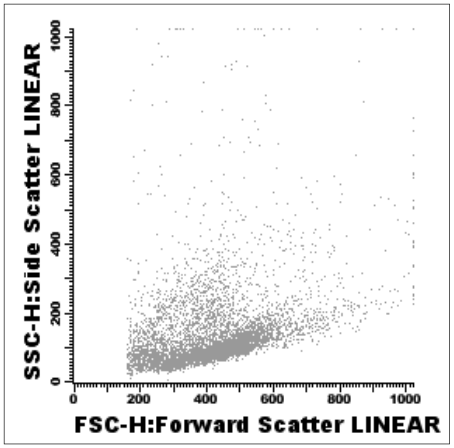



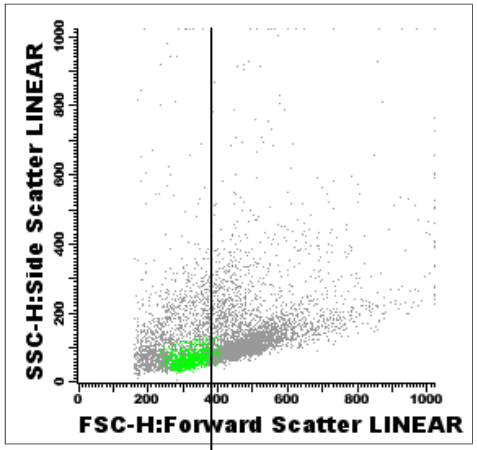



# Instrument Setup

- Scatter
  - 1 centrum
  - 2 monsters zelfde patient, 2 tijdstippen

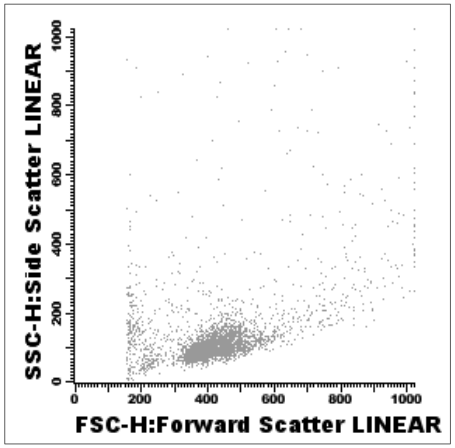
t = 1

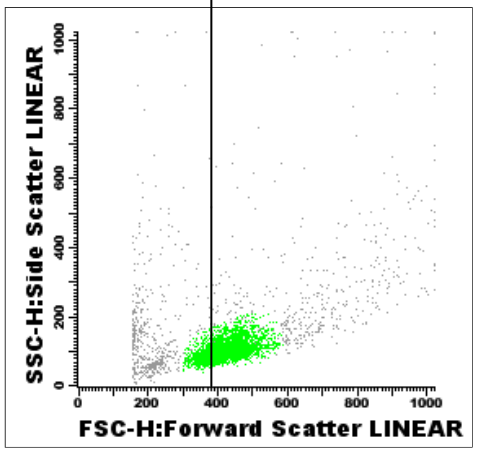



Lymfocyten

t = 2

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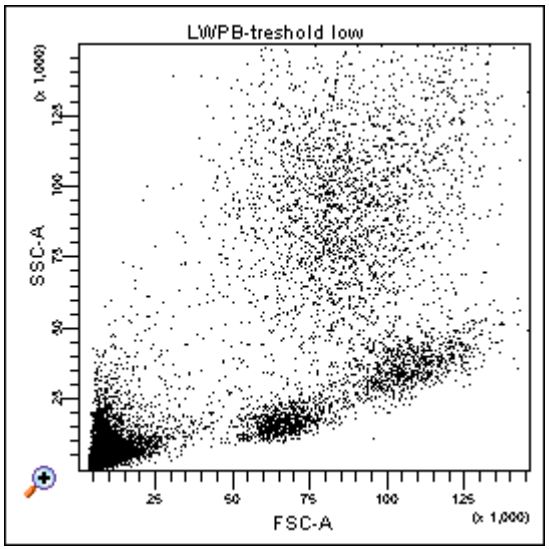
# Instrument Setup

- Treshold

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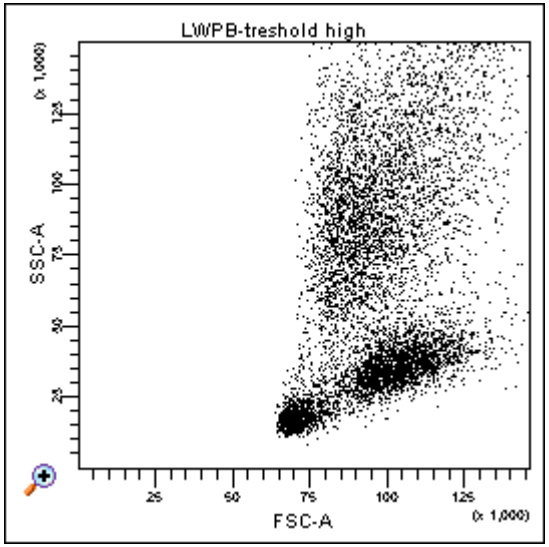


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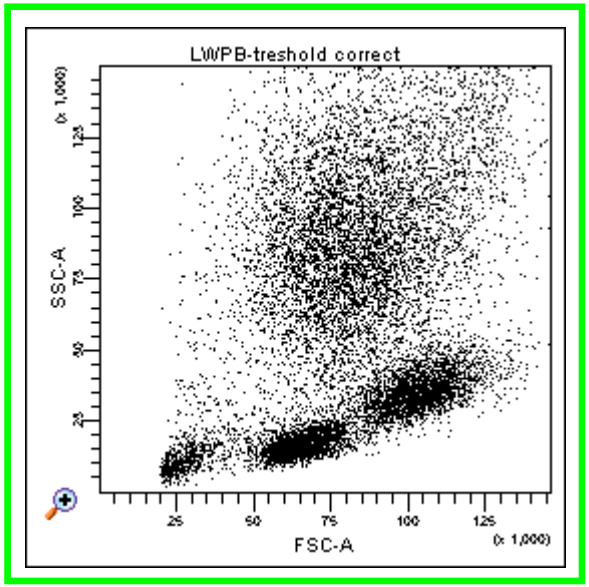


correct

- Debris zoveel mogelijk excluderen
- Leukocyten plus kernhoudend rood zoveel mogelijk includeren

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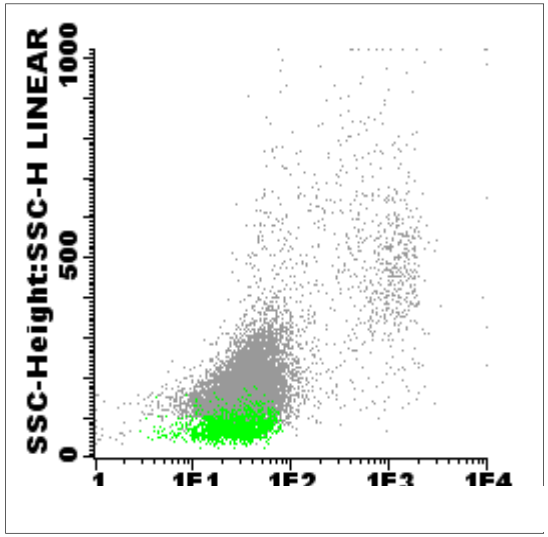
# Instrument Setup

- Fluorescentie
  - PMT instelling
    - Sterk signaal
    - Lage achtergrond
    - Optimaal scheidend vermogen

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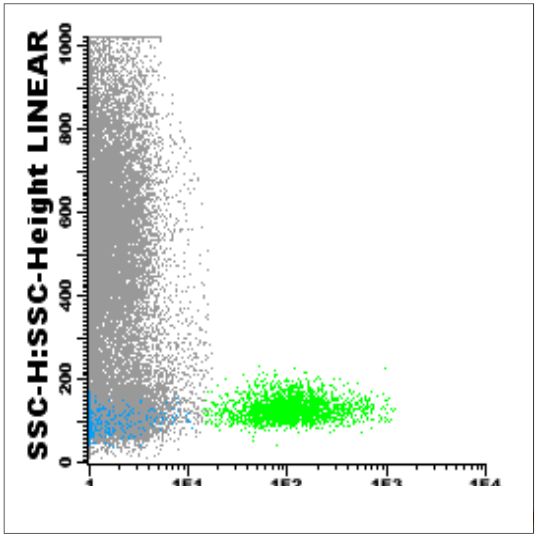


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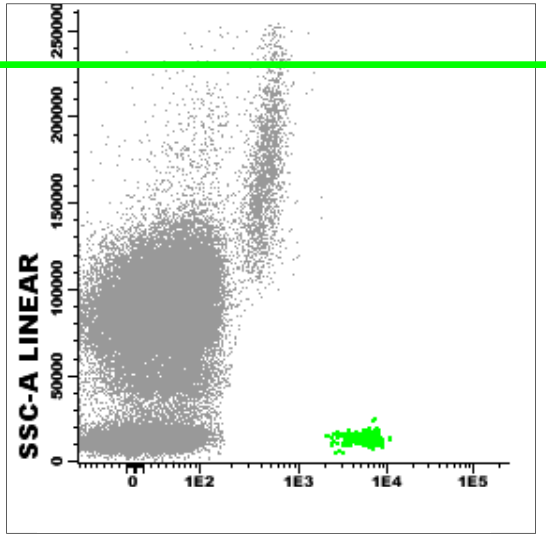


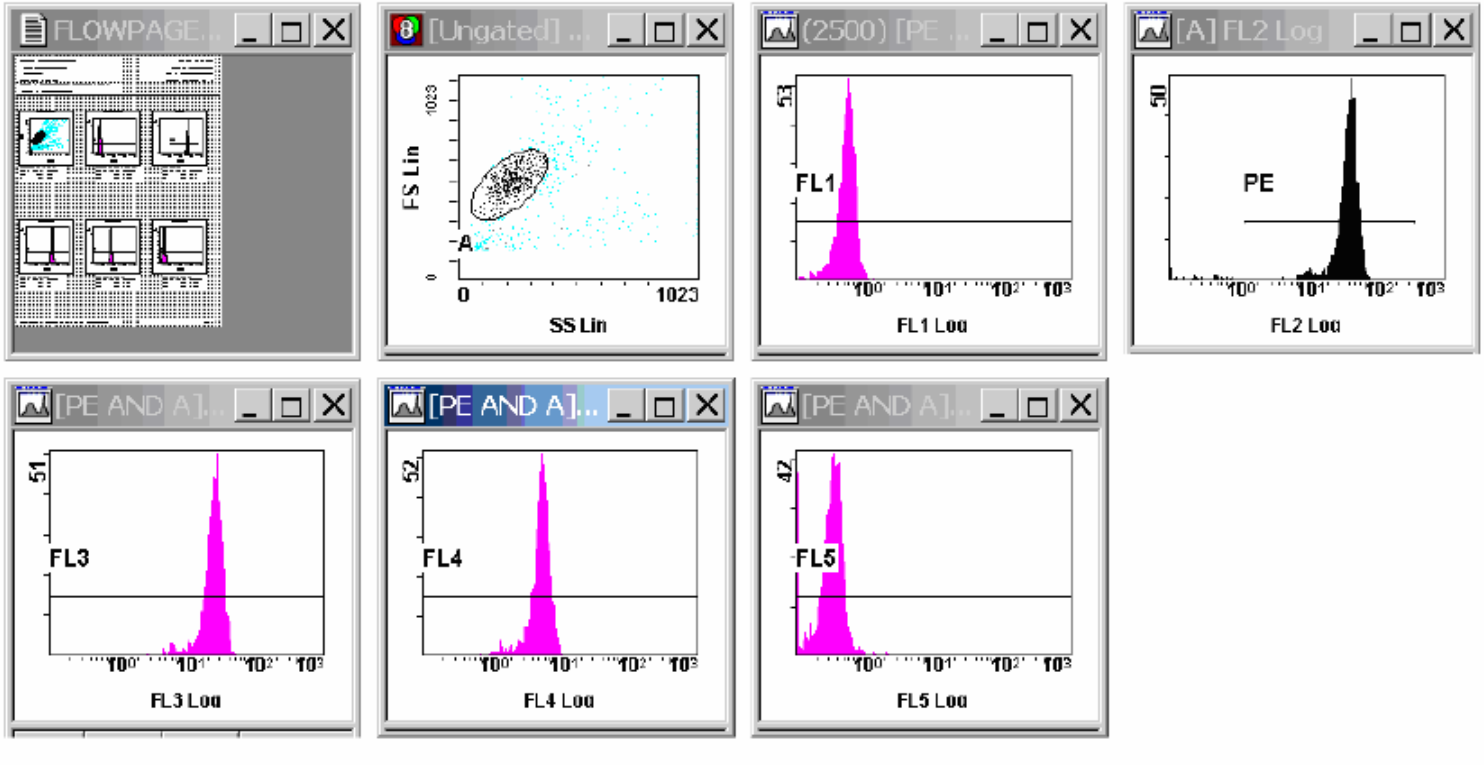

correct

# Fluorescence - PMT

- Fluorescentie
  - PMT instelling
    - Cellen (Coulter)
    - CST: Cytometer Setup & Tracking Beads (BD)
    - Af fabriek (Accuri C6)
    - Target MFI for 8-pk Rainbow Beads

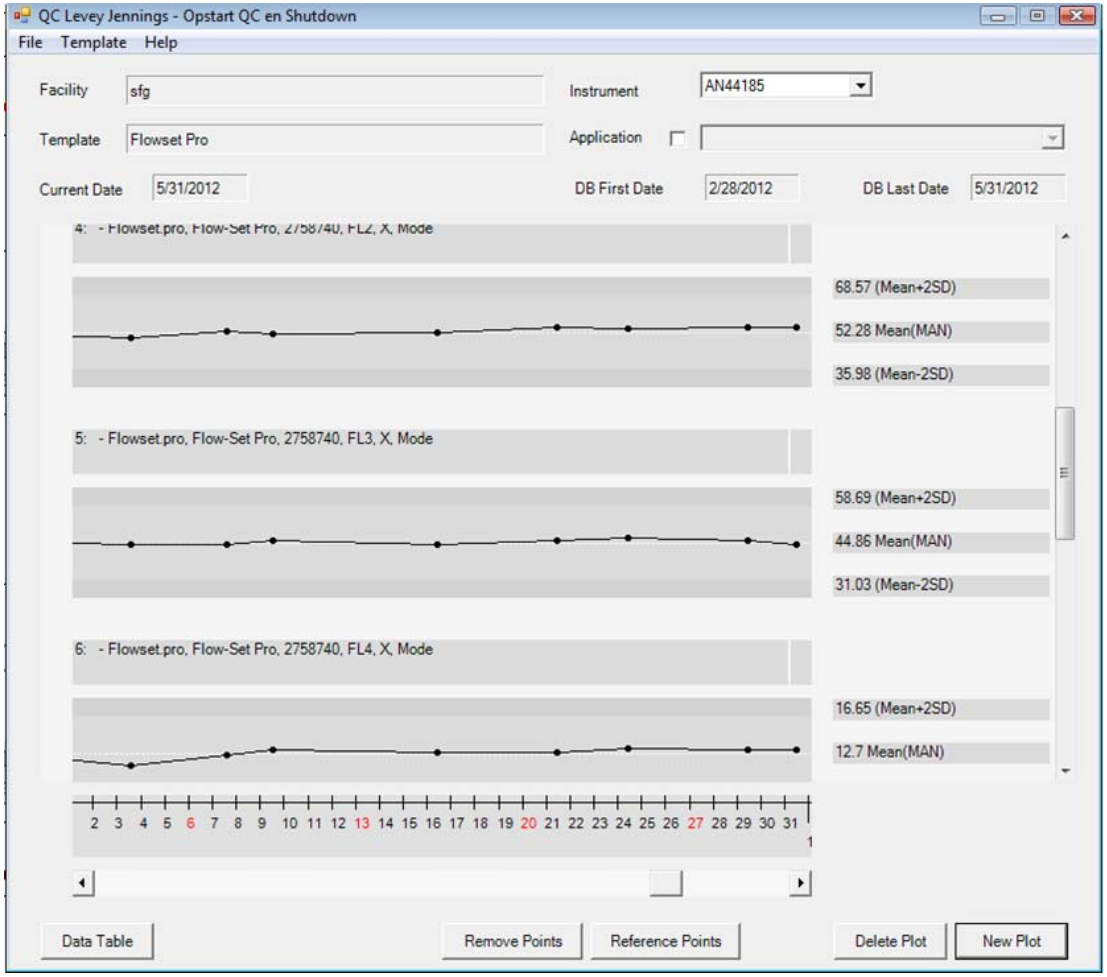
# Fluorescence - PMT

- Cellen



# Flow-Set Pro Fluorophores

- Performance check

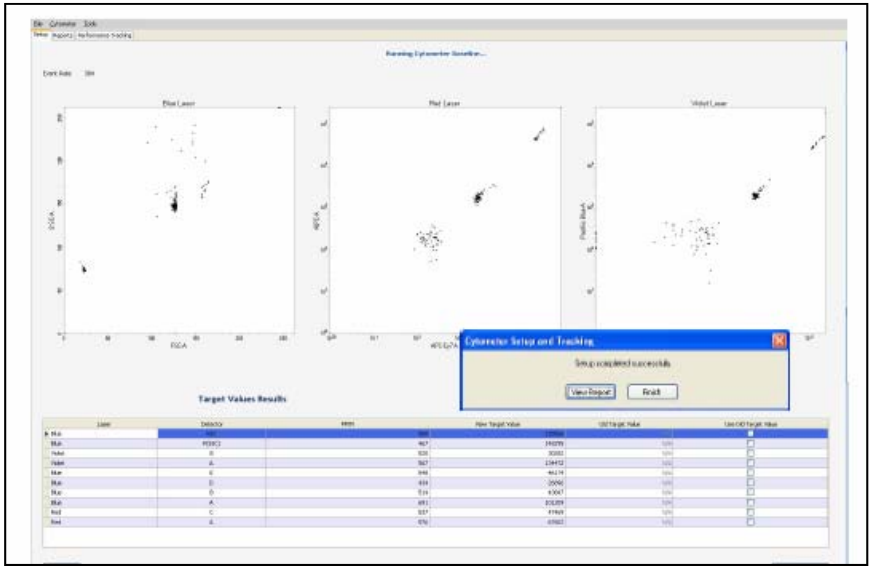


# CST beads

- CST: Cytometer Setup & Tracking Beads

## Setup Beads

| Bead | Baseline (TS/AS&Admin) | Daily (Admin&Operator) |
|---|---|---|
| Dim | • Background (B)<br>• PMT voltages | • Background (B)<br>• PMT voltages<br>• rCV* |
| Mid | • Sensitivity (Q)<br>• Linearity | • Sensitivity (Q) |
| Bright | • Linearity<br>• Laser Delay<br>• Mean Target values | • Laser Delay<br>• Area Scaling<br>• Adjust PMT voltages to target MFI |



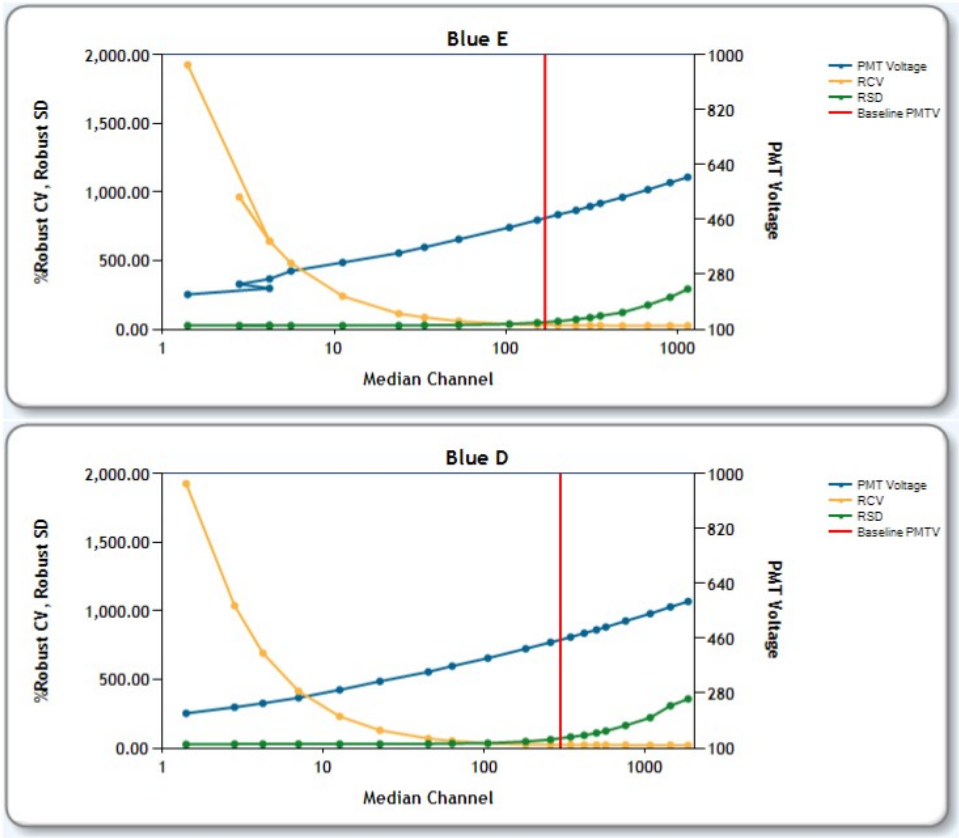
# CST beads

- Baseline Report

**Cytometer Baseline Report**

| | | | |
|---|---|---|---|
| Cytometer: | FACSCantoII | User: | Administrator |
| Cytometer Name: | FACSCantoII ErasmusMC | Institution: | N/A |
| Serial Number: | V96300046 | Software: | BD FACSDiva 6.1.1 |
| Input Device: | Manual | Date: | 04/28/2009 03:12 PM |
| Cytometer Configuration: | EuroFlow config 3-laser, 8-color (4-2-2) | | |

**PMTV Optimization Results**

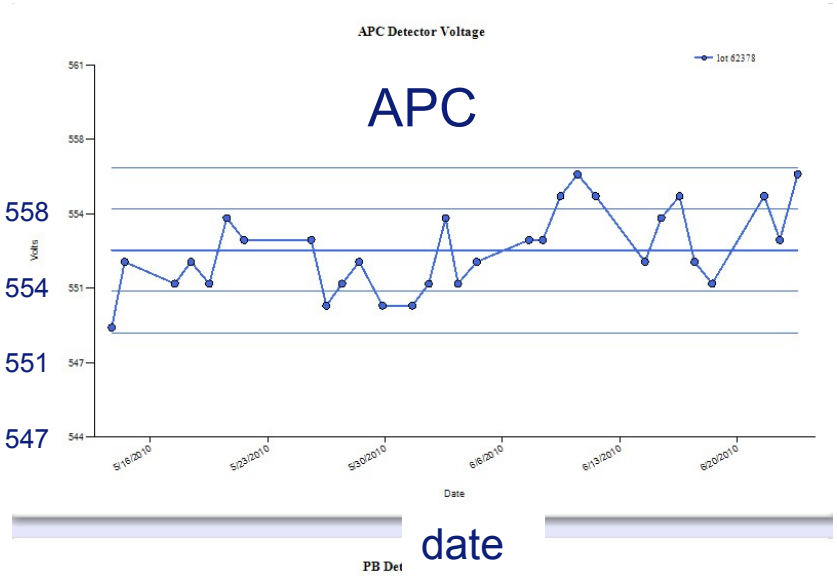



→ Optimal PMT to maximize resolution between populations

# CST beads

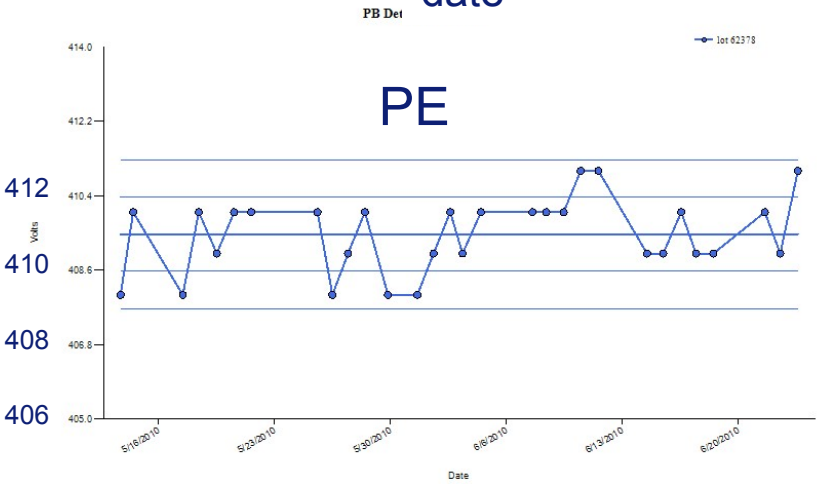
- Performance check

PMT




date

PMT




date

Adjustment of PMT

Always acquire CST beads and accept CST settings because of correct time delay and area scaling. This is independent of your instrument

# Fluorescence - PMT

## Alternatief voor CST beads:

- Target MFI for 8-pk Rainbow beads

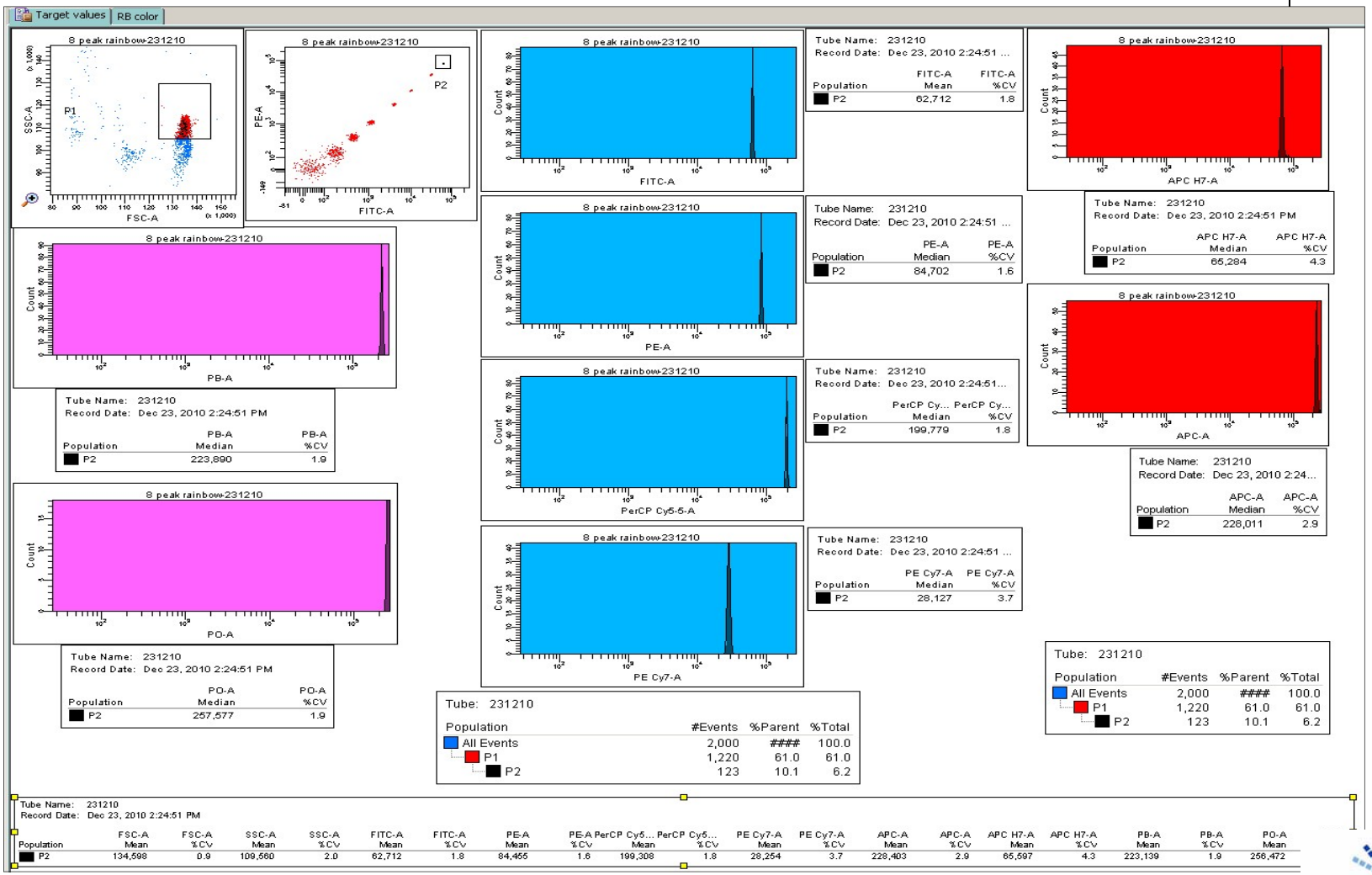
| 2010 | Table 3. | | Target MFI | |
|---|---|---|---|---|
| NEW MASTER LOT | | Lower MFI (-15%) | Rainbow 8-peak (DiVa) | Upper MFI (+15%) |
| Lot: 41610 | Pacific Blue | 183,049 | 215,352 | 247,655 |
| | Pacific Orange | 185,222 | 217,908 | 250,594 |
| | FITC | 55,491 | 65,283 | 75,075 |
| | PE | 72,120 | 84,847 | 97,574 |
| | PerCP | 194,495 | 228,818 | 262,000 |
| | PC7 | 25,385 | 29,865 | 34,345 |
| | APC | 214,200 | 252,000 | 289,800 |
| | APC-H7 | 86,784 | 102,099 | 117,414 |

Batches/Lots prepared from Masterlot 41610 at Spehrotech are labeled as "EAB01" where "E" means "Euroflow"
If you have any doubts, ask Brian Shah (bshah@spherotech.com) or Marta M. Ayuso (mmartin@cytognos.com)

EuroFlow

# 8-pk Rainbow beads

- Adjust PMT to reach target MFI

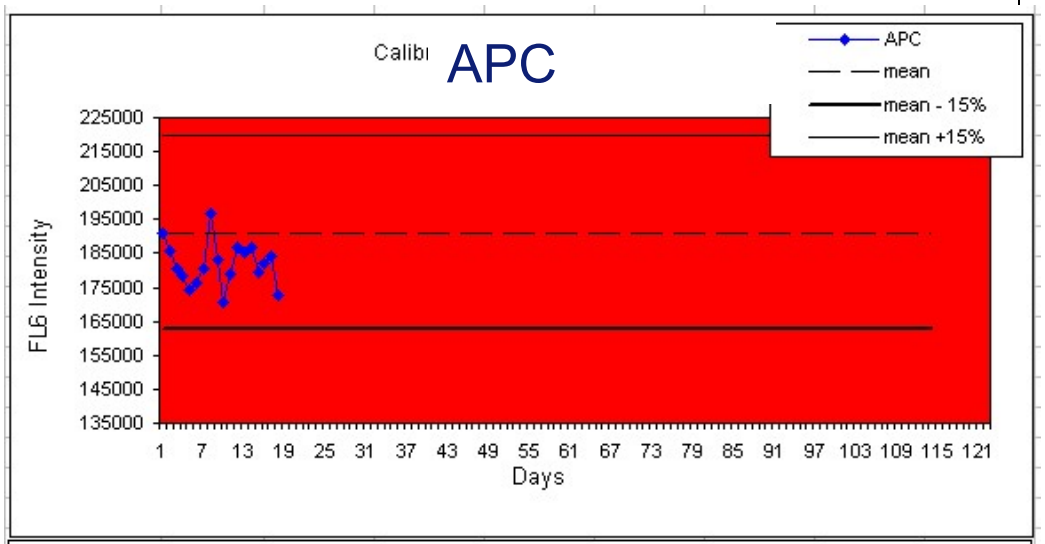



EuroFlow

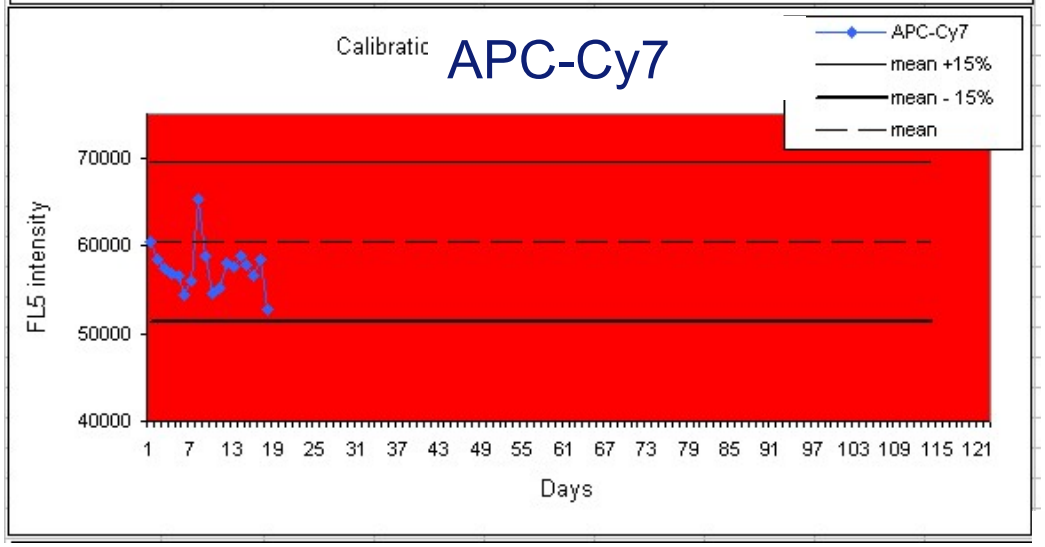
# 8-pk Rainbow beads

- Daily performance check

MFI




MFI

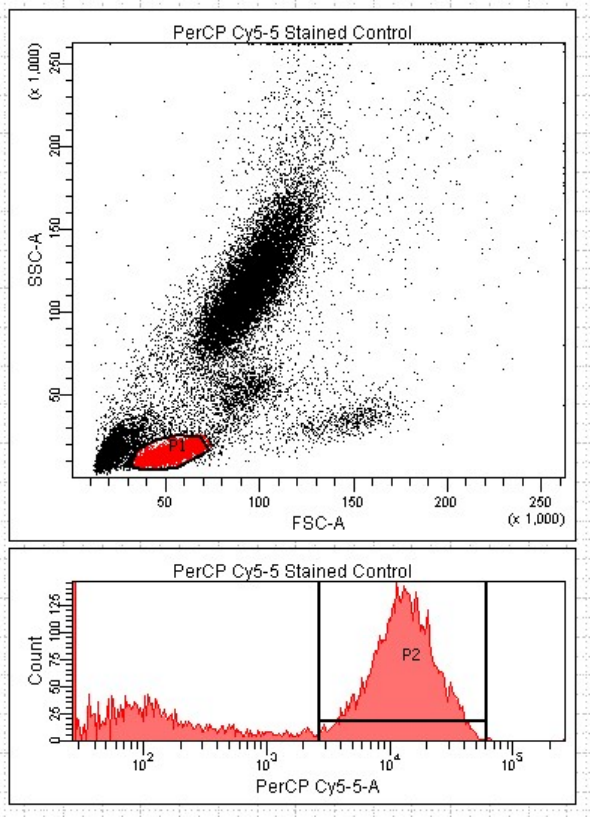



No adjustment of PMT

EuroFlow

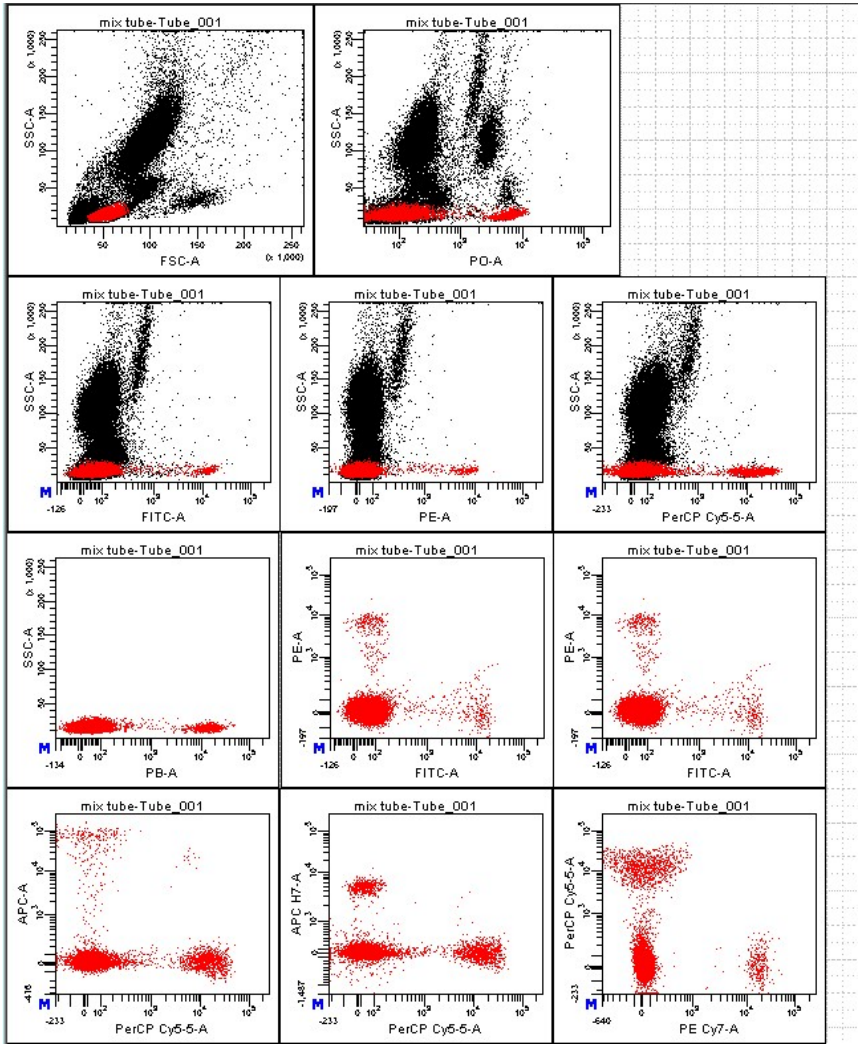
# Compensation controls

- SST: single stained tubes
  - Cells, CompBeads or a combination of both
  - PMT: CST or EF (8-pk Rainbow beads)



# Compensation Matrix

- Mixture of SST's



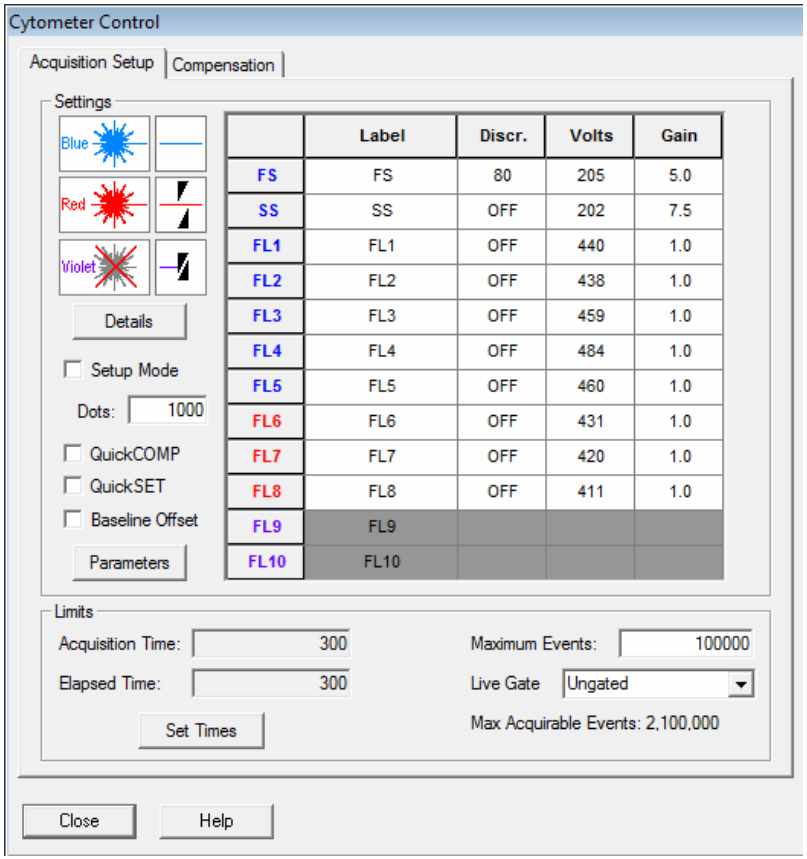

Correct compensation matrix

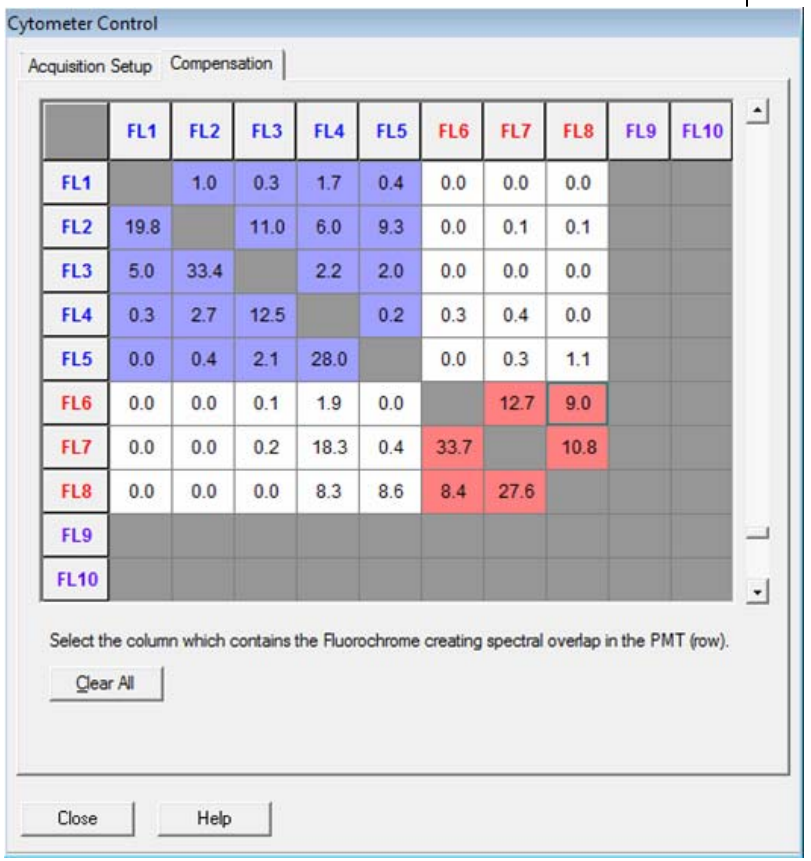
Opmerking: generic vs specific

→ Instrument Settings: Scatter, PMT and compensation

# Advanced Digital Compensation

- Automatische compensatie



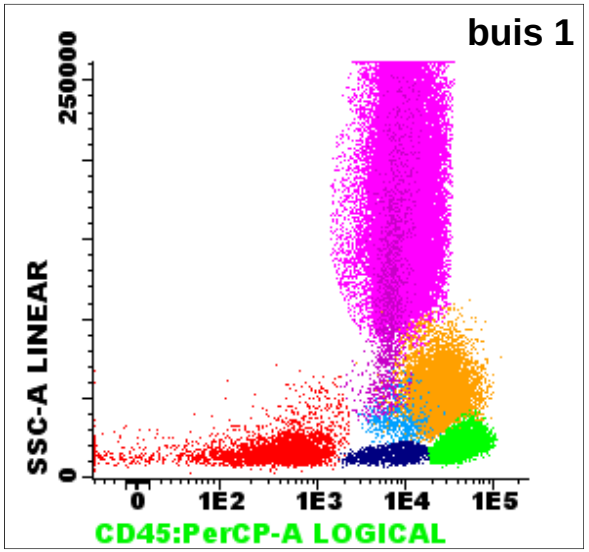

Correct compensation matrix

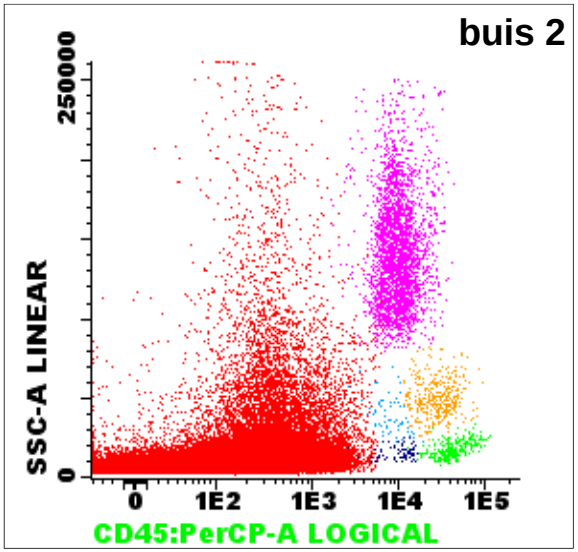
# Valkuilen

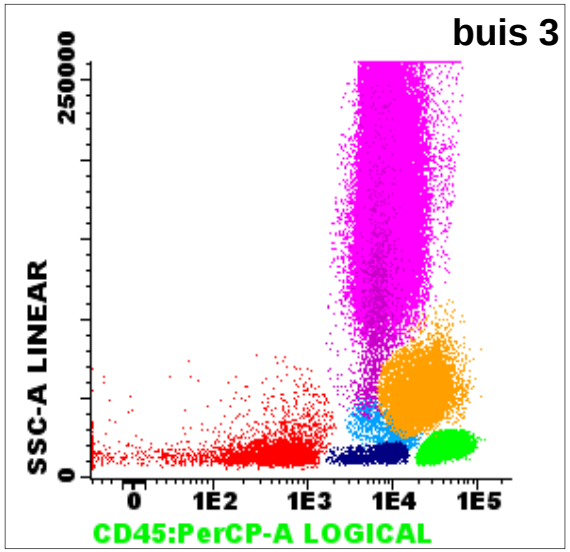
- Experimenteel
  - Instrument Setup
    - Scatter
    - Fluorescentie
  - Procedure
  - Antistoffen
    - Fluorochromen
    - Clonen
    - Tandemfluorochromen
    - Data Spread
  - Fluorescentiepatronen

- Data interpretatie

# Discussie

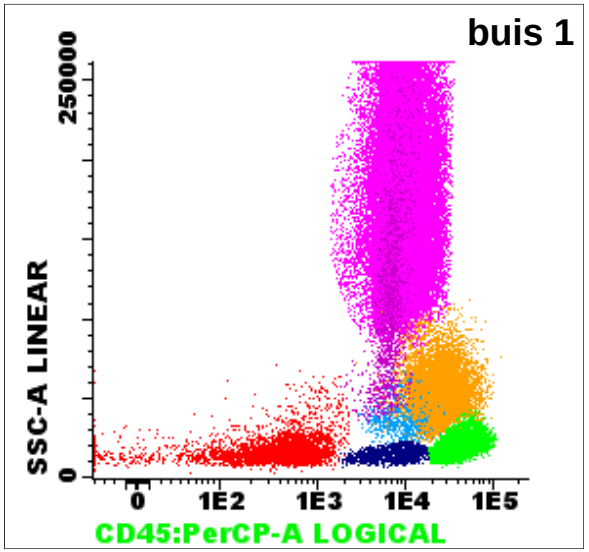

buis 1
SSC-A LINEAR
250000
0
0
1E2
1E3
1E4
1E5
CD45:PerCP-A LOGICAL

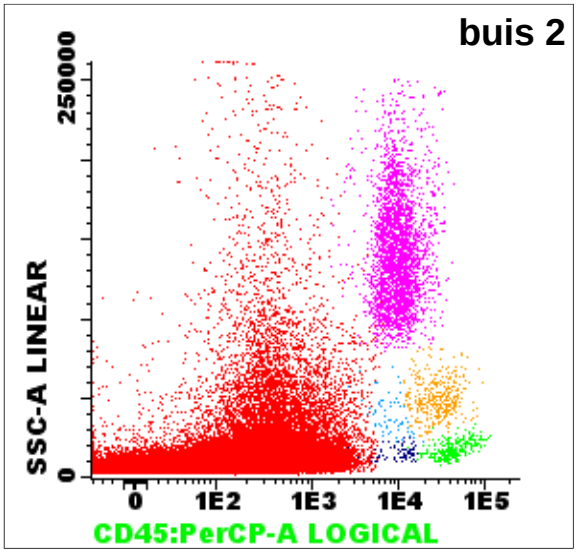

buis 2
SSC-A LINEAR
250000
0
0
1E2
1E3
1E4
1E5
CD45:PerCP-A LOGICAL

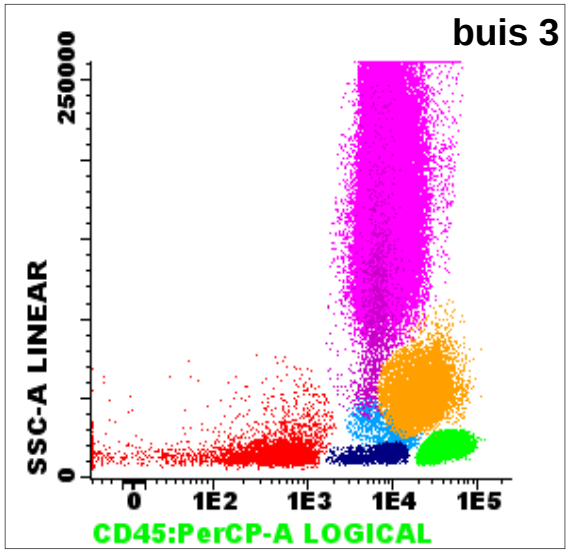

buis 3
SSC-A LINEAR
250000
0
0
1E2
1E3
1E4
1E5
CD45:PerCP-A LOGICAL

# Procedure

- Stain-Lyse-Wash
- Buis per buis lysis




Buis tot buisvariaties door:

- Kans op het meepipetteren van een stolsel is groter
- Ongelijke erylysis
- Wegvangen van moabs door niet gelyseerd rood (bv CD235a)
- Geen constant aantal leucocyten per buis dus detectielimiet ongelijk

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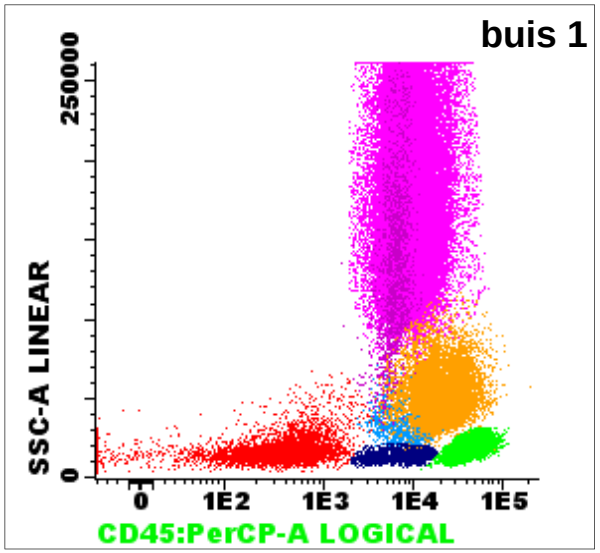
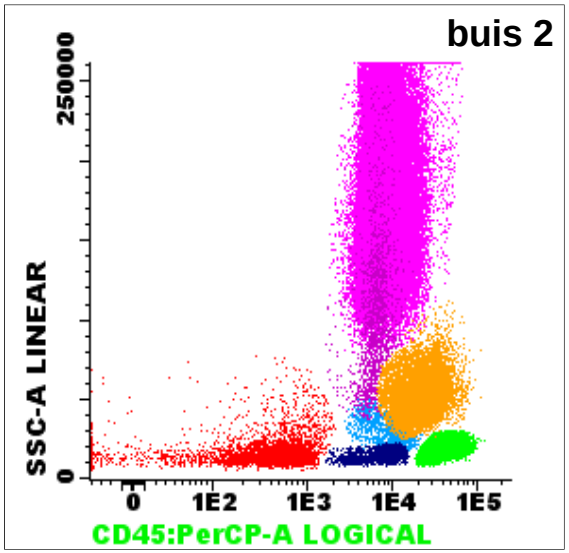
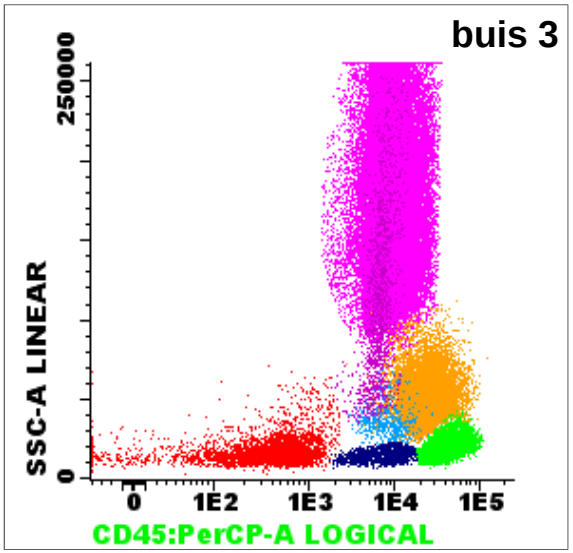
# Procedure

- Lyse-Stain-Wash
- Bulklysis




Nagenoeg geen buis tot buisverschillen

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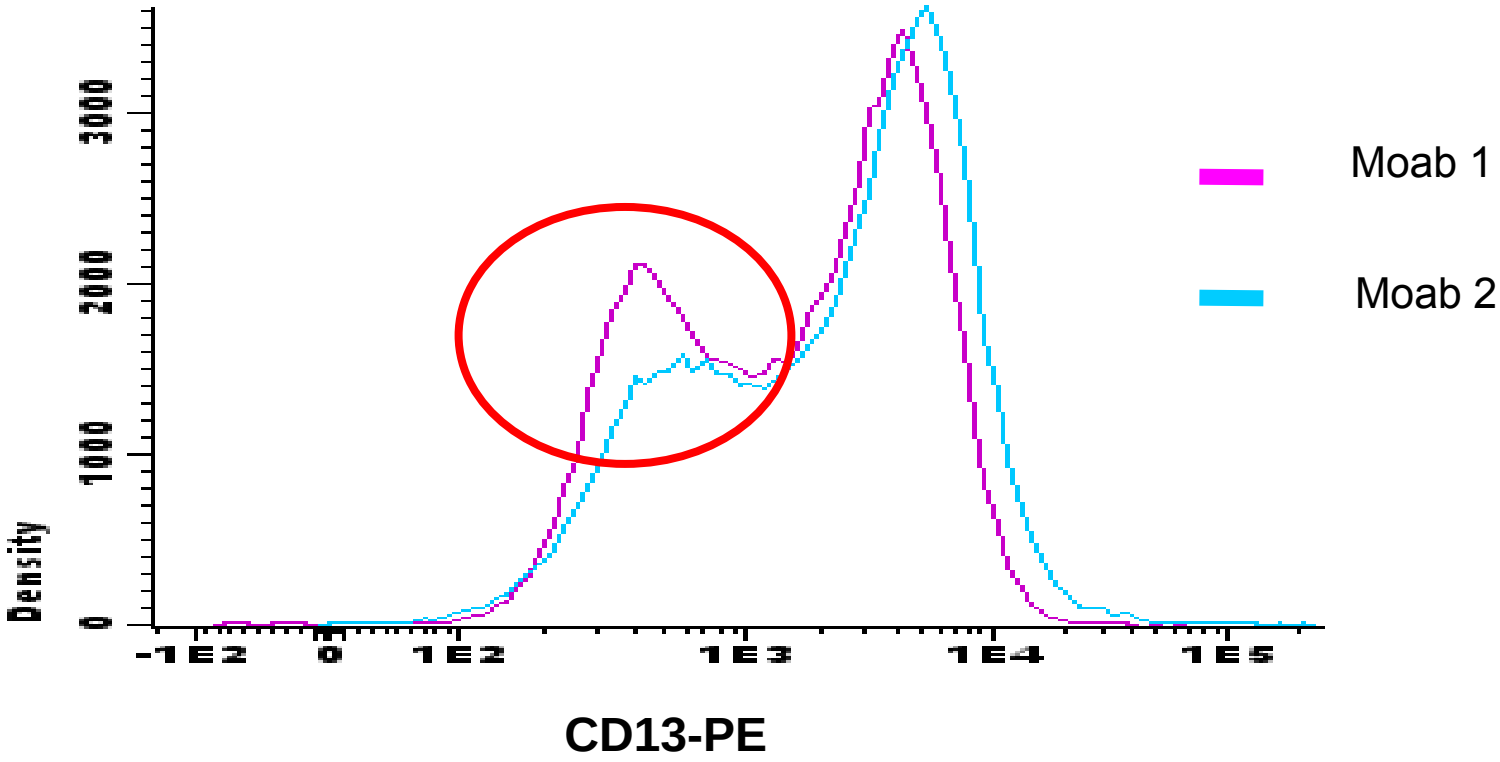
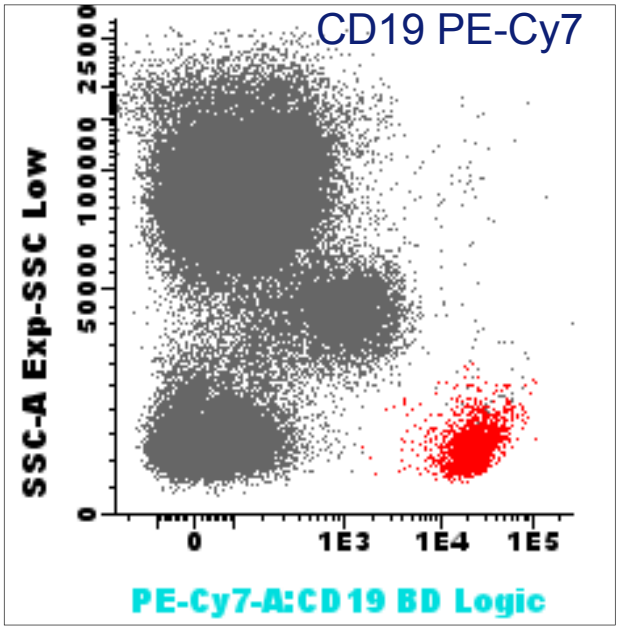
# Valkuilen

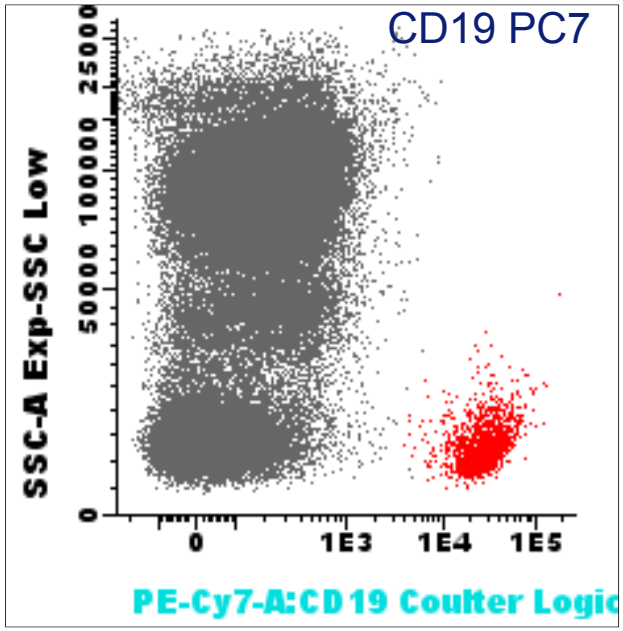
- Experimenteel
  - Instrument Setup
    - Scatter
    - Fluorescentie
  - Procedure
  - Antistoffen
    - Fluorochromen
    - Clonen
    - Tandemfluorochromen
    - Data Spread
  - Fluorescentiepatronen
- Data interpretatie

# Discussie


Density
0
1000
2000
3000
-1E2
0
1E2
1E3
1E4
1E5
CD13-PE
Moab 1
Moab 2

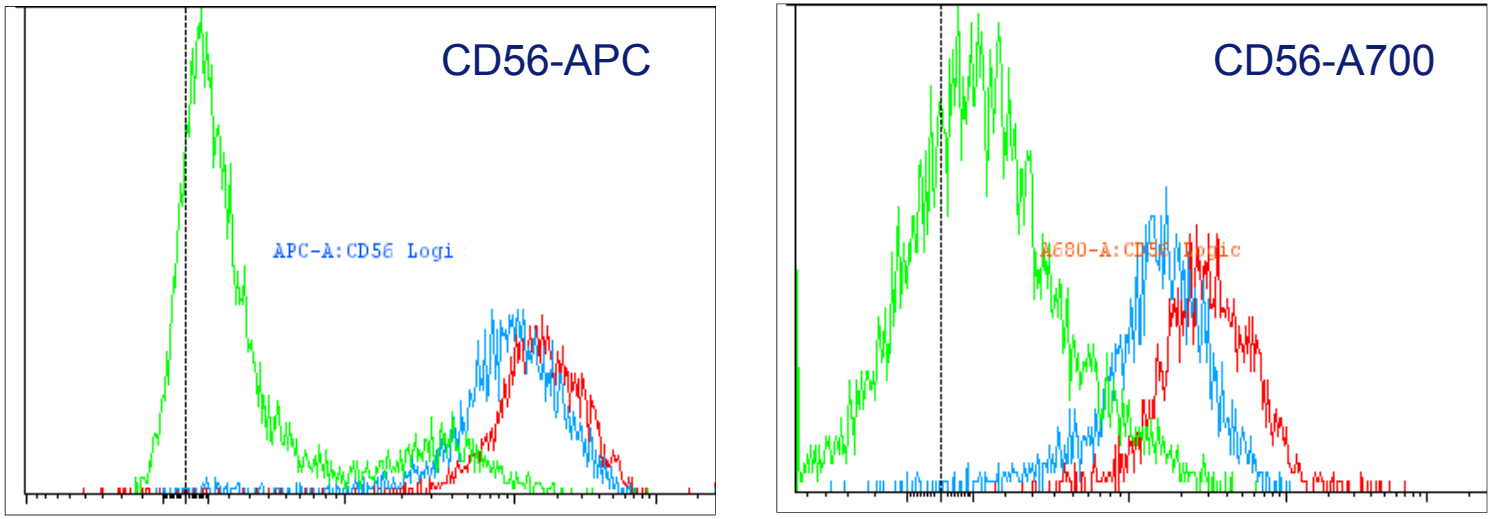
# Discussie



# Antistoffen

- Fluorochromen

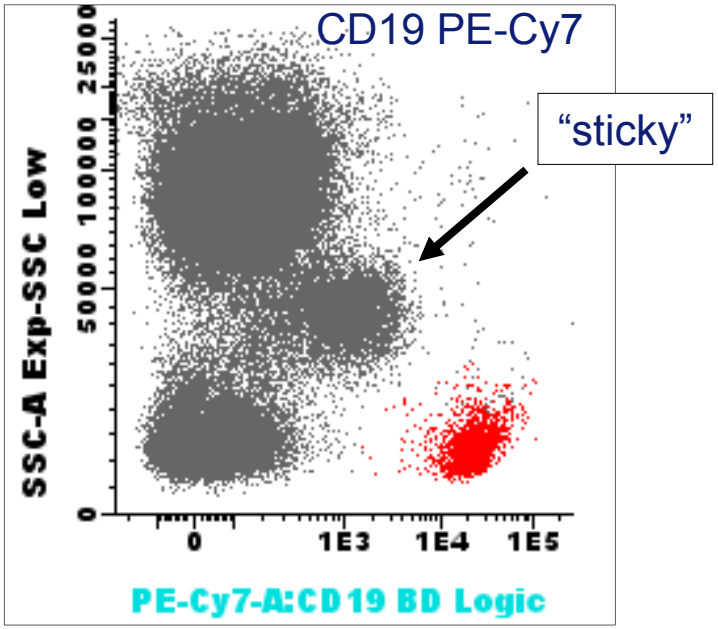


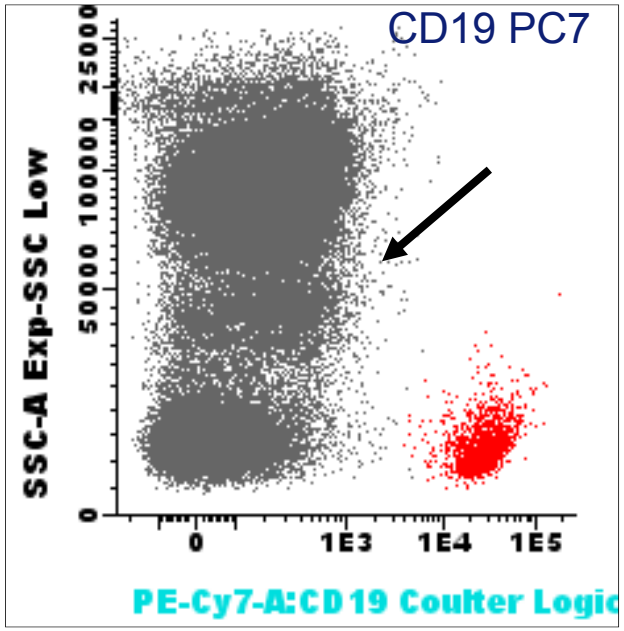

Nk cellen

• Voor expressie van CD56 op blasten is een voldoende sterke fluorescentie nodig

# Antistoffen

- Fluorochromen

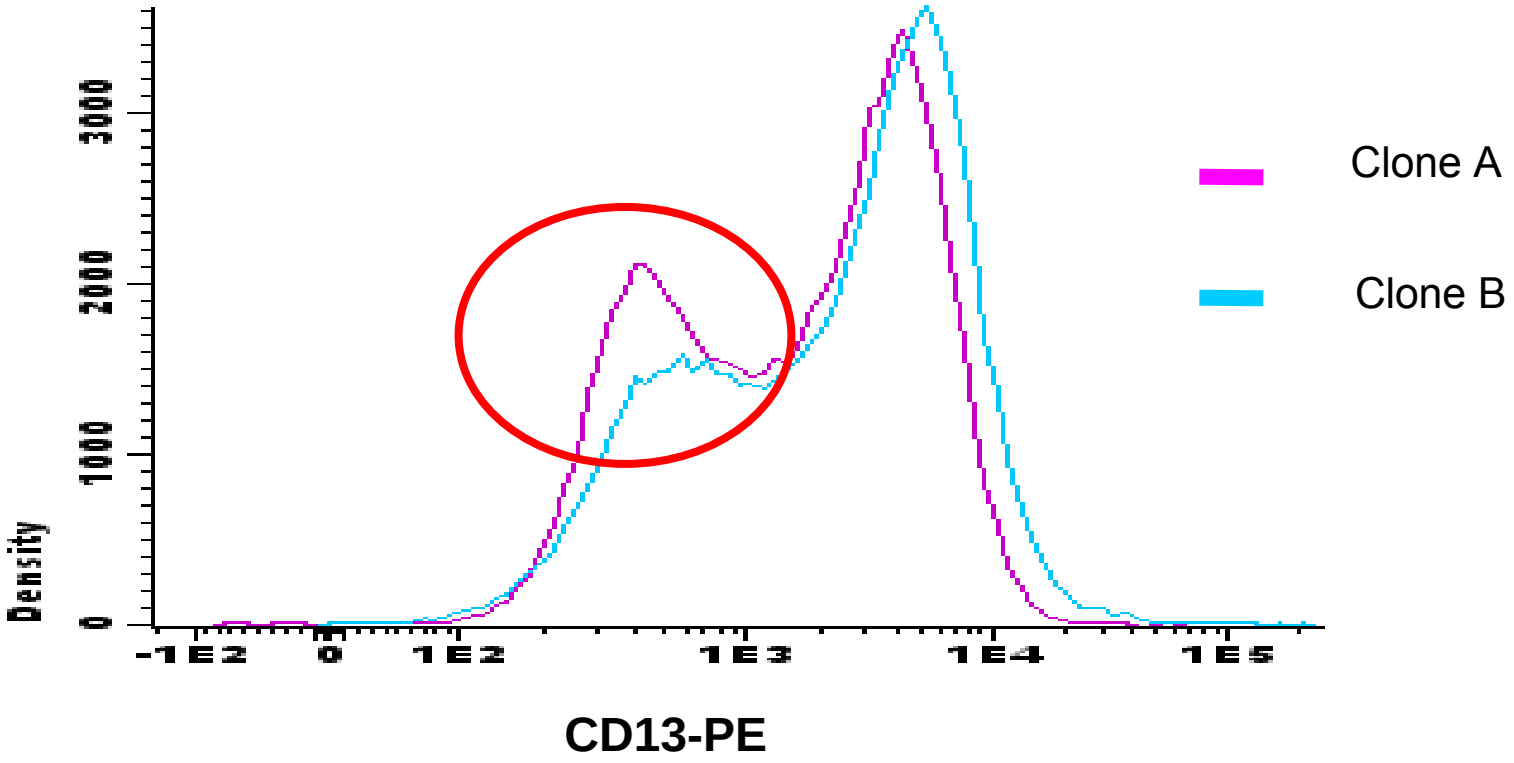



• Lage achtergrondfluorescentie vergemakkelijkt gating en interpretatie

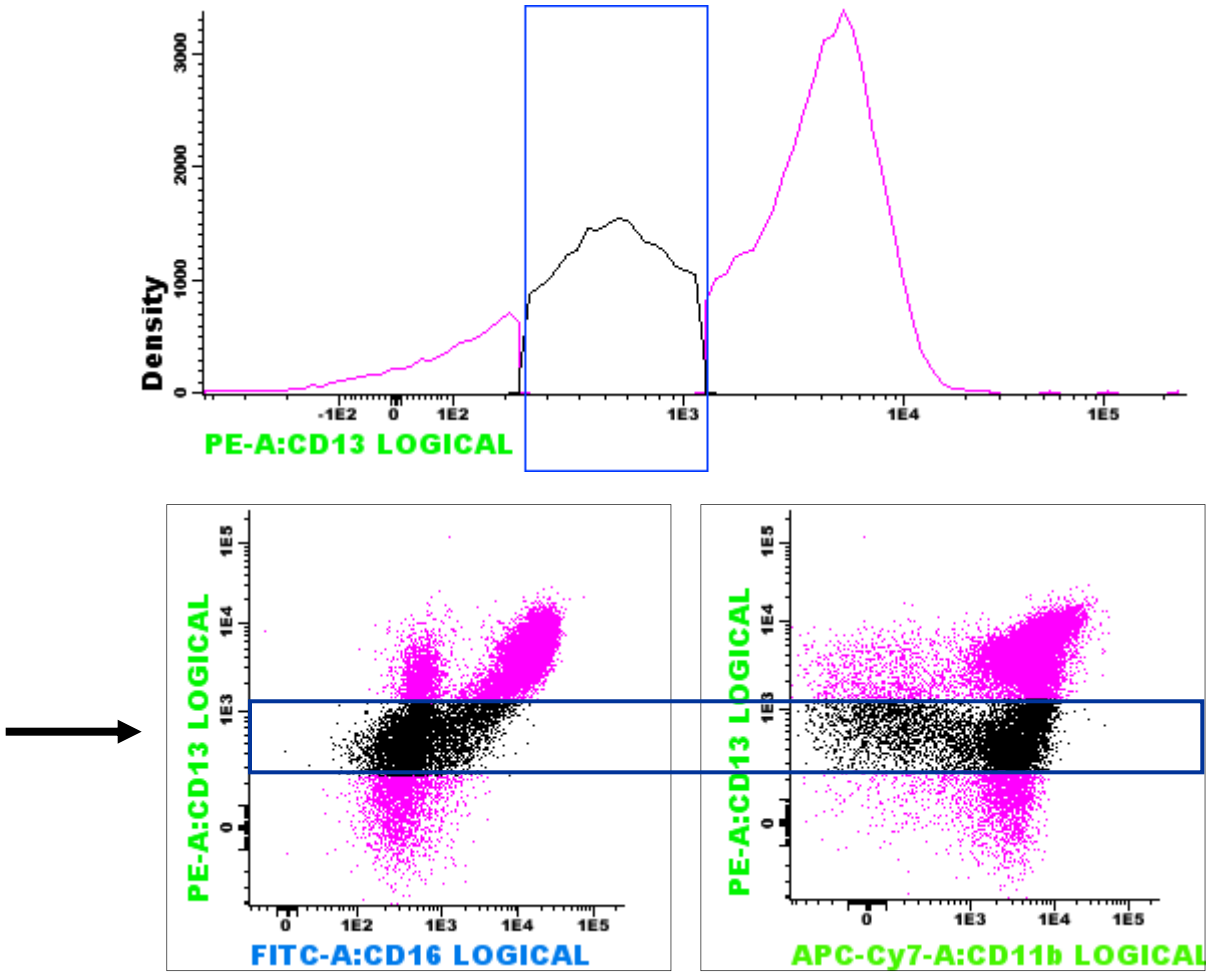
# Antistoffen

- Clonen



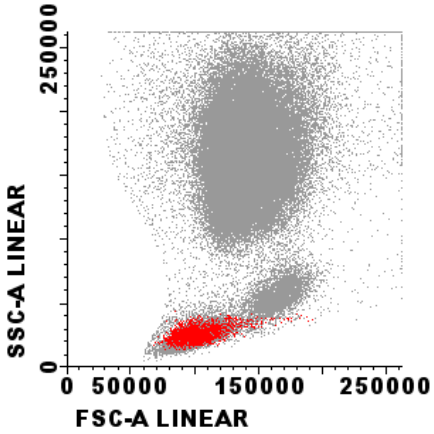
# Antistoffen

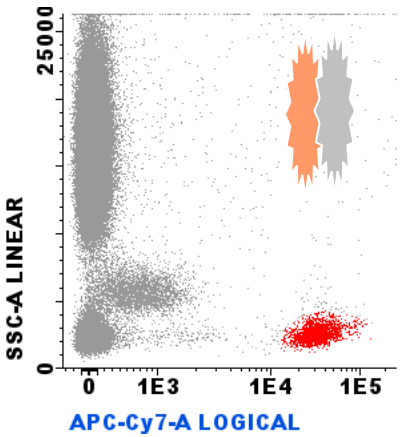
- Clonen

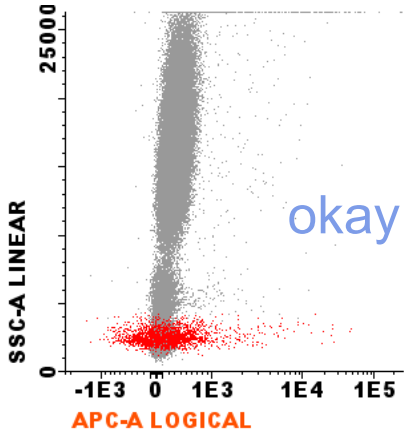


# Antistoffen

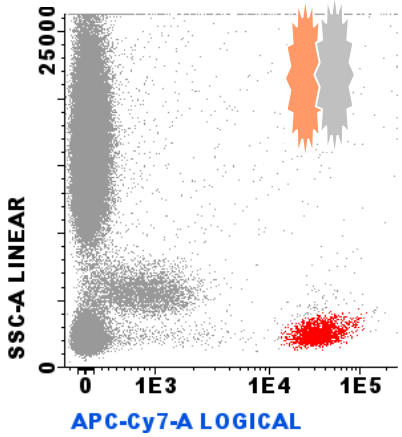
- Tandemfluorochromen

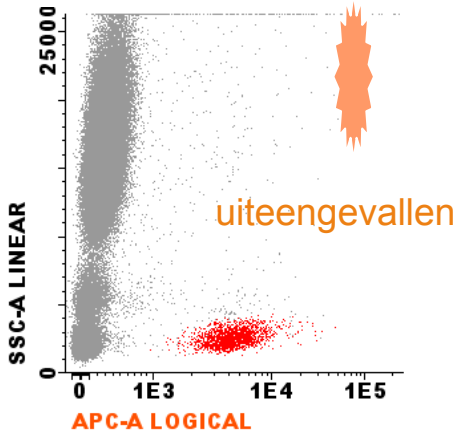



Enkelkleuring met CD20-APC-Cy7



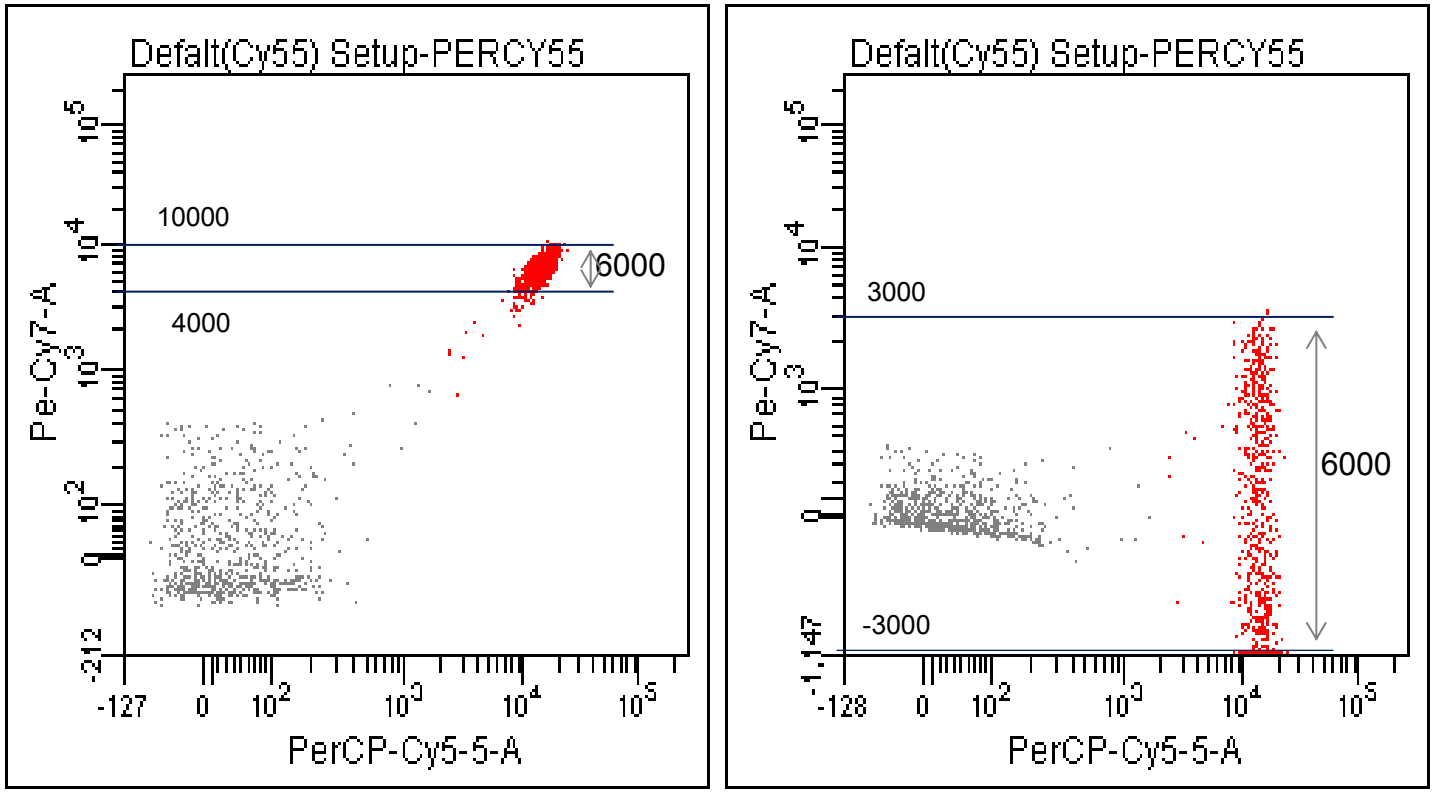




Instabiliteit tandemfluorochromen o.i.v. licht (APC-Cy7 → APC-H7

# Antistoffen

- Data Spread

Enkel kleuring met CD4-PerCP-Cy5.5

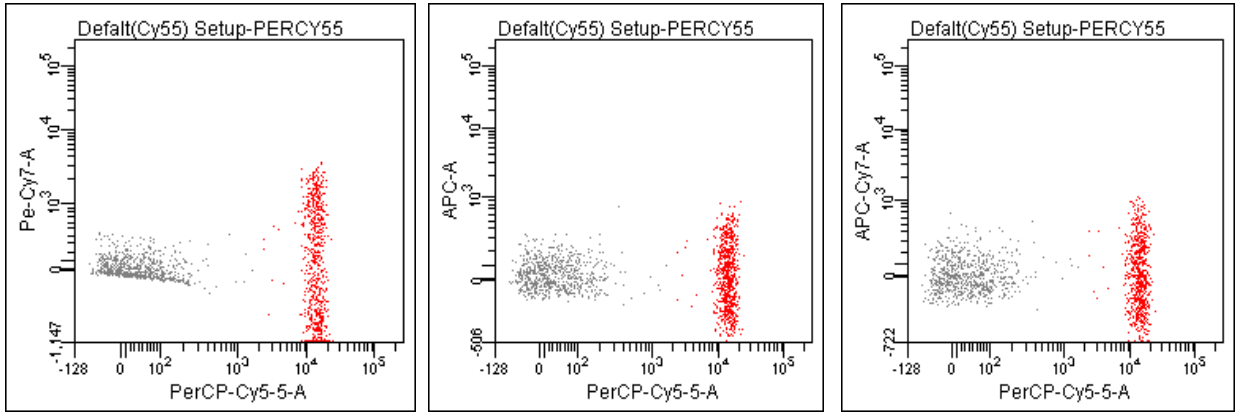



Digital data processing → data spread

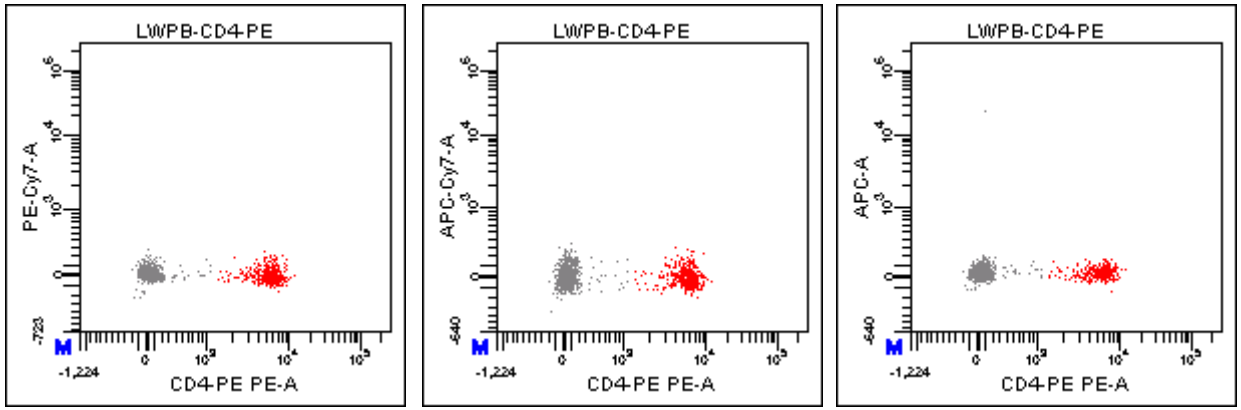
# Antistoffen

- Data Spread

CD4 PerCP-Cy5.5 “Spread”




CD4 PE “Spread”




1. Fluorochroom afhankelijk (i.h.a.: hogere λ, meer spread)
2. Verlies van gevoeligheid

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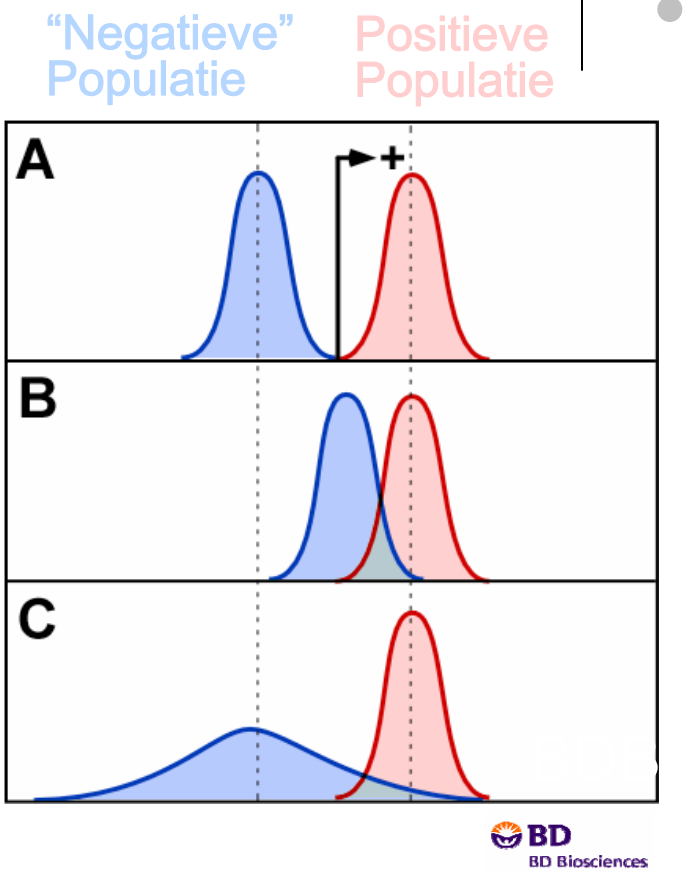
# Antistoffen

- Effect van Data Spread

Negatieve populatie heeft
een lage achtergrond
Populaties goed gescheiden

Negatieve populatie heeft
een hoge achtergrond
Populaties slecht gescheiden

Negatieve populatie heeft
een lage achtergrond
hoge CV (Spread)
Populaties slecht gescheiden




Het vermogen om populaties te onderscheiden is een funktie van zowel achtergrond als ook van "spread" van de negatieve populatie

# Valkuilen

- Experimenteel
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    - Clonen
    - Tandemfluorochromen
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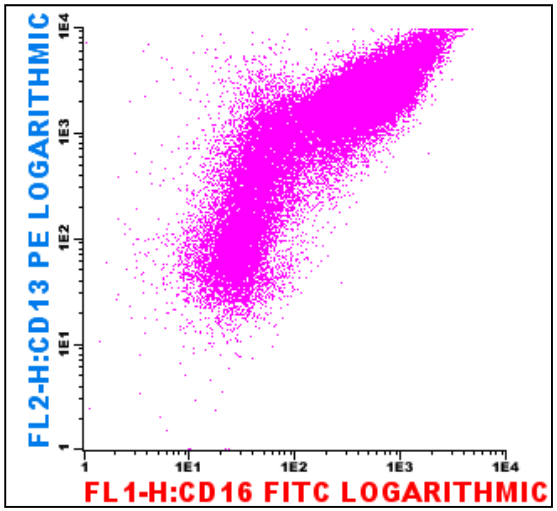
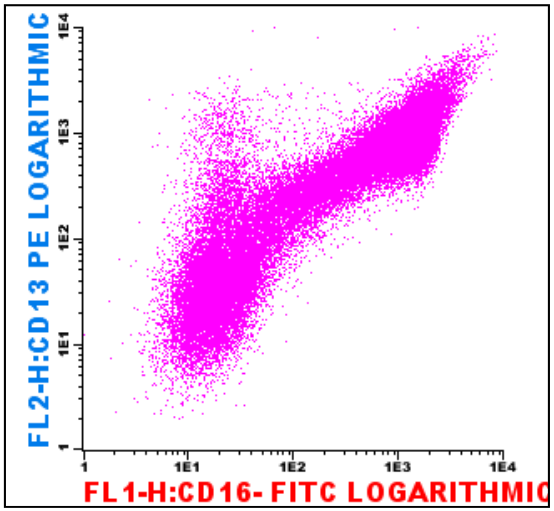
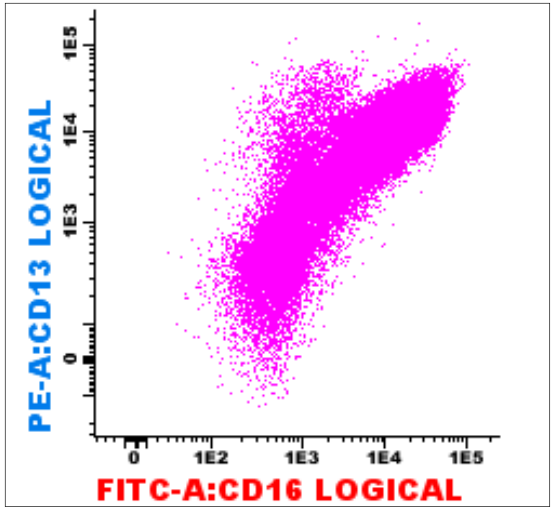
  - Fluorescentiepatronen
- Data interpretatie

# Discussie

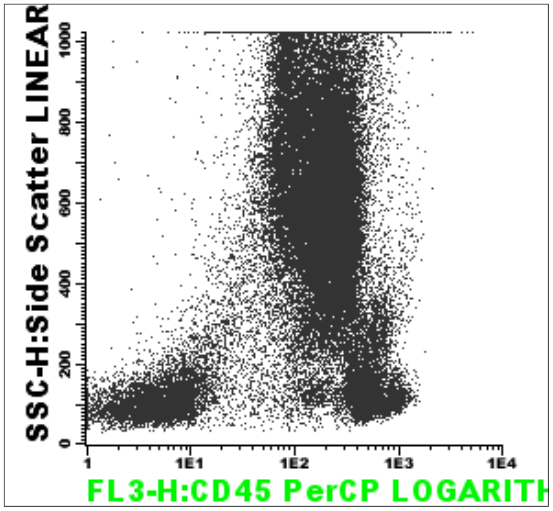


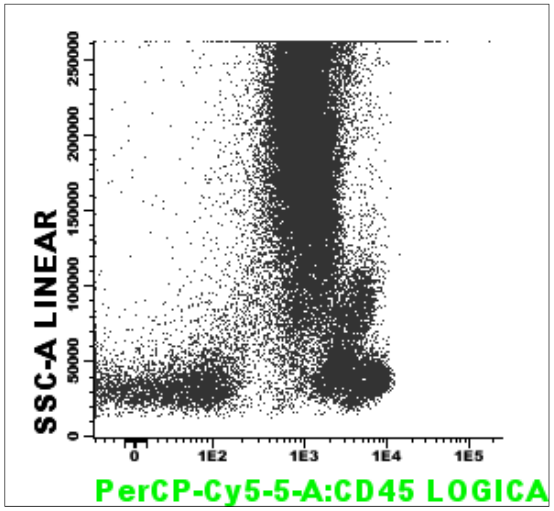



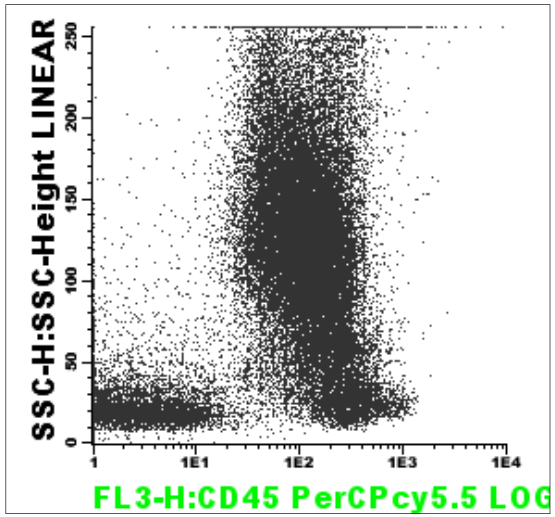



# Fluorescentiepatronen

- CD45-SSC
  - 1 Beenmerg
  - 3 Centra

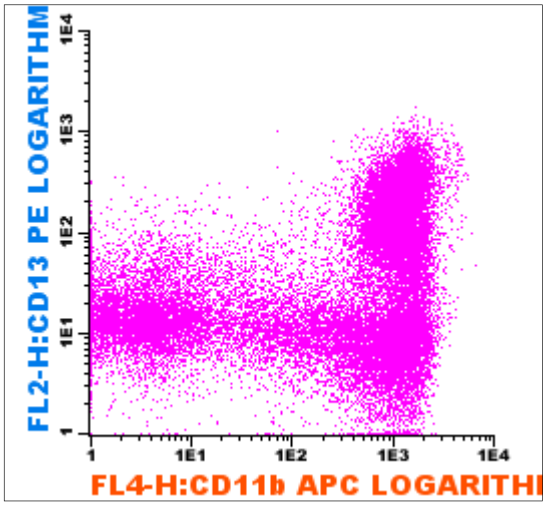


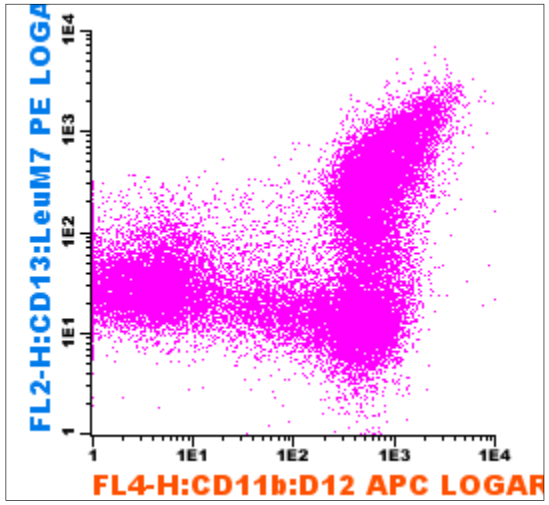



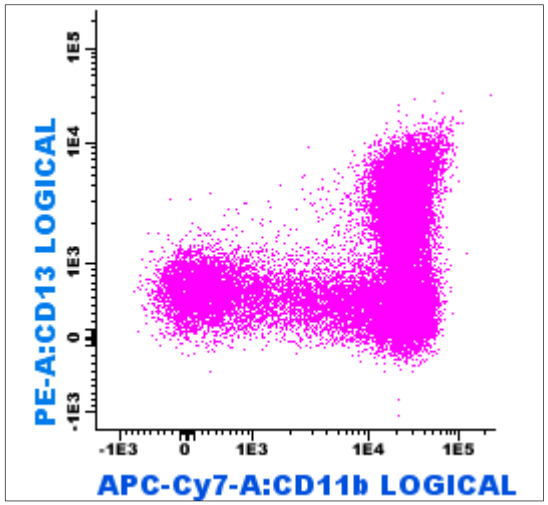



# Fluorescentiepatronen

- CD11b-CD13
  - 1 Beenmerg
  - 3 Centra

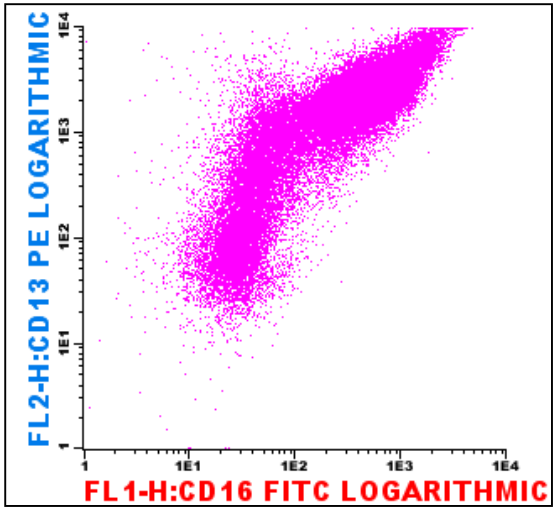


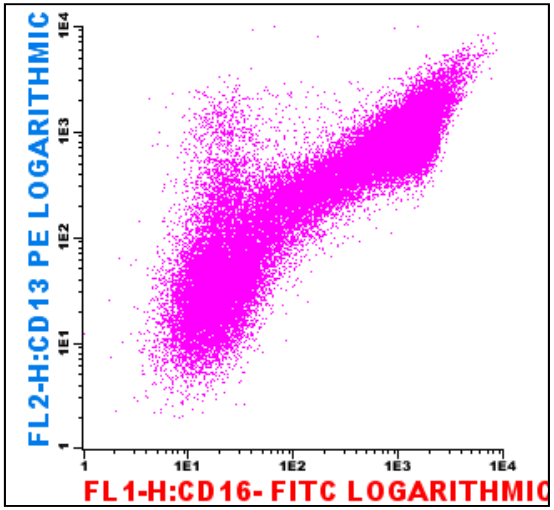



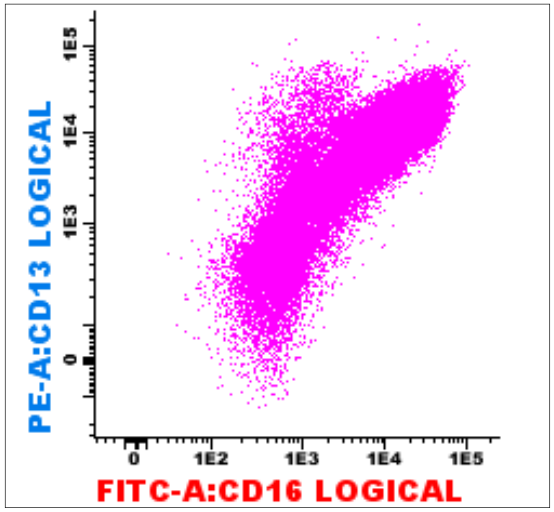



# Fluorescentiepatronen

- CD16-CD13
  - 1 Beenmerg
  - 3 Centra

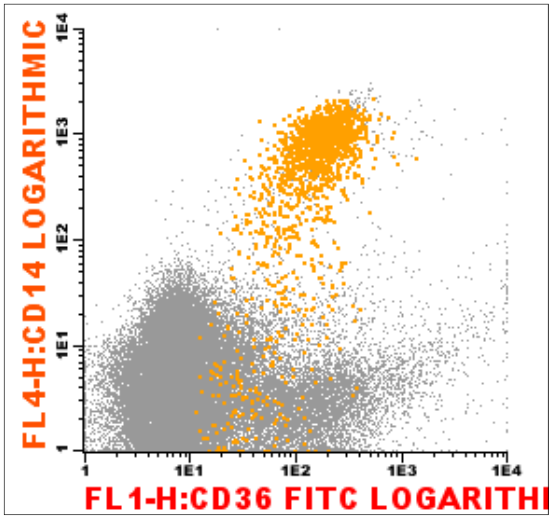


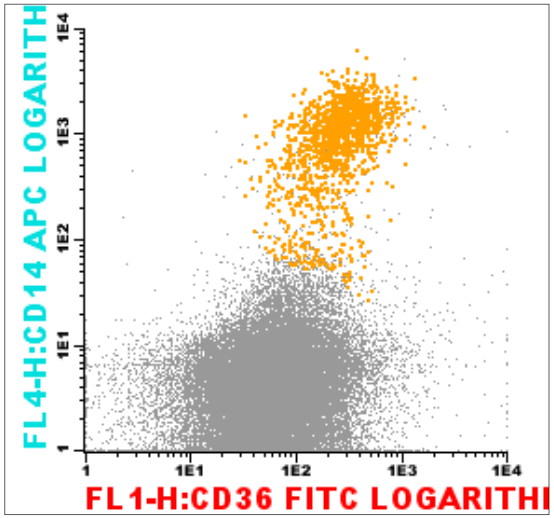



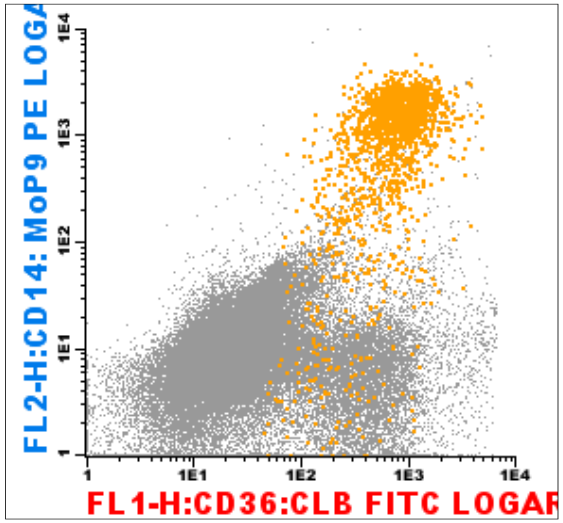



# Fluorescentiepatronen

- 36-14
  - 1 Beenmerg
  - 3 Centra

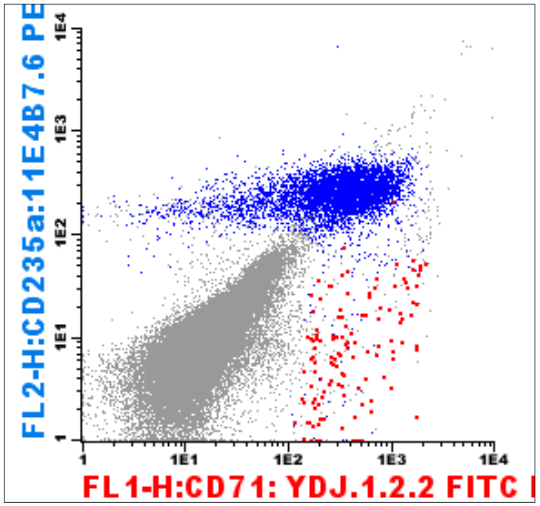


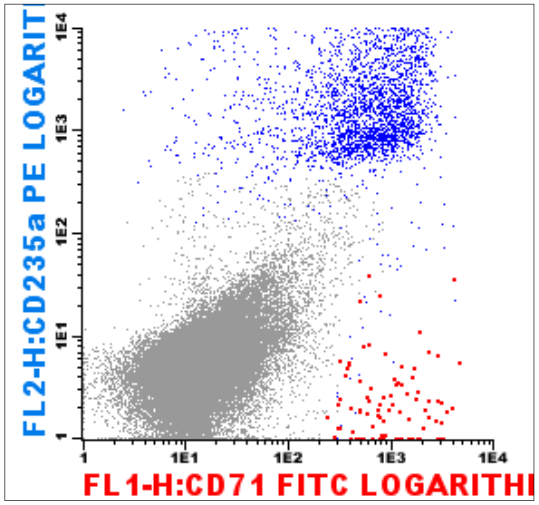



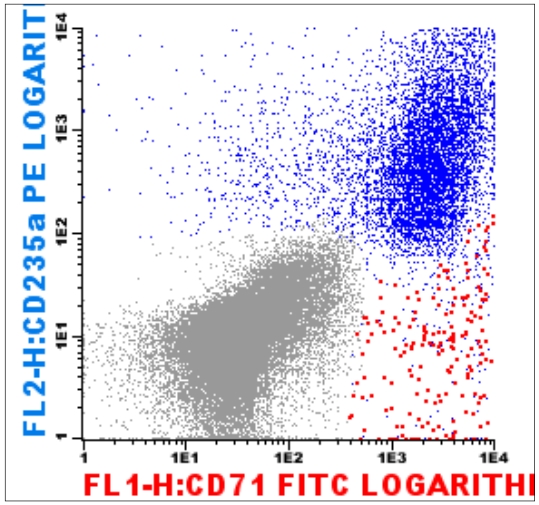



# Fluorescentiepatronen

- CD71-CD235a
  - 1 Beenmerg
  - 3 Centra



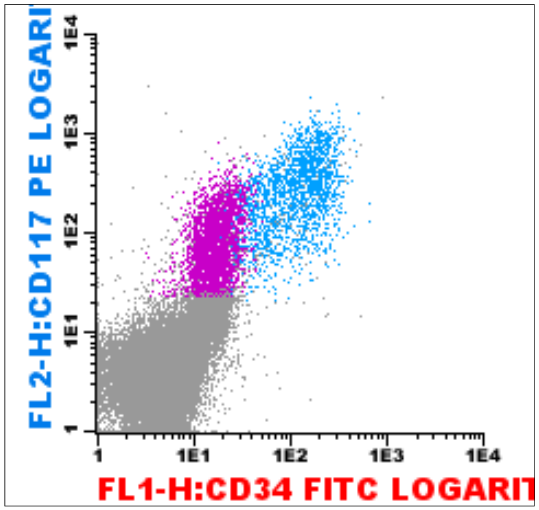



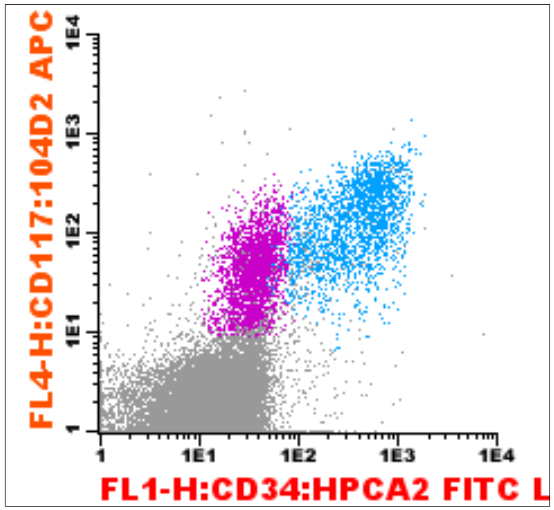



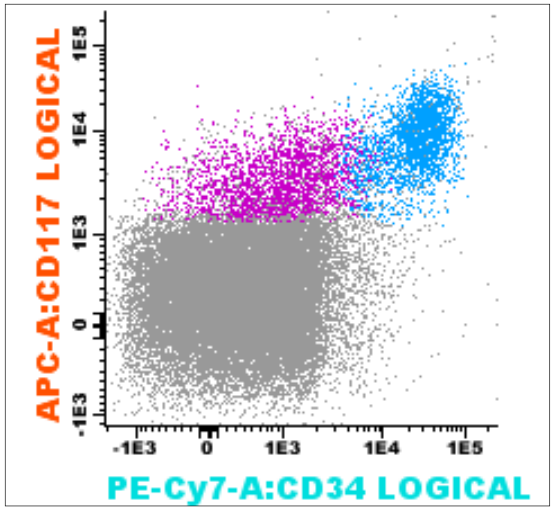

NVC
CYTOMETRIE.NL
NEDERLANDSE VERENIGING VOOR CYTOMETRIE

# Fluorescentiepatronen

- CD34-CD117
  - 1 Beenmerg
  - 3 Centra



# Conclusies

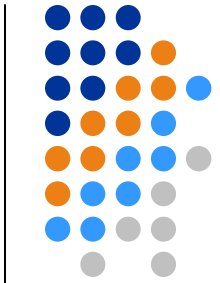
- Experimenteel
  - Instrument Setup
    - Correct m.b.t. scatter, treshold en fluorescentie
    - Continuïteit waarborgen
  - Procedure
    - Celsuspensie verkrijgen door bulklysis (zonder fixatief)
  - Antistoffen
    - Fluorochroomkeuze afhankelijk van het doel
    - Reaktiviteit verschillende clonen testen
  - Fluorescentiepatronen
    - Afhankelijk van apparatuur, setup, procedure en antistoffen

# Stelling

Vergelijkingen in de tijd binnen één centrum maar ook tussen centra:

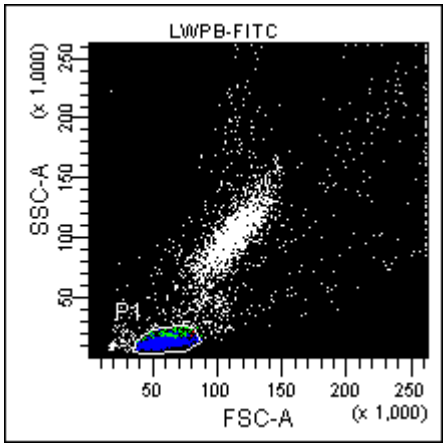
Richtlijnen voor instrument setup, procedures en antistoffen noodzakelijk.

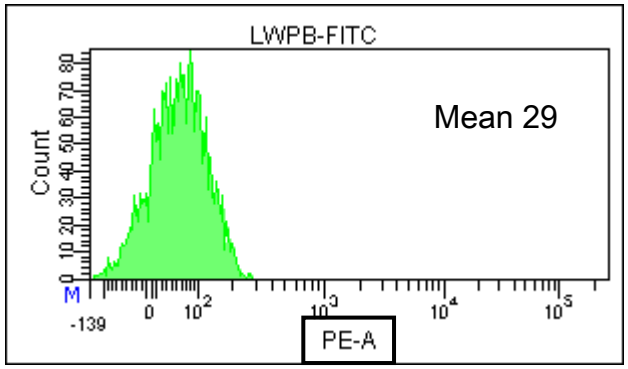
CYTOMETRIE.NL
NEDERLANDSE VERENIGING VOOR CYTOMETRIE

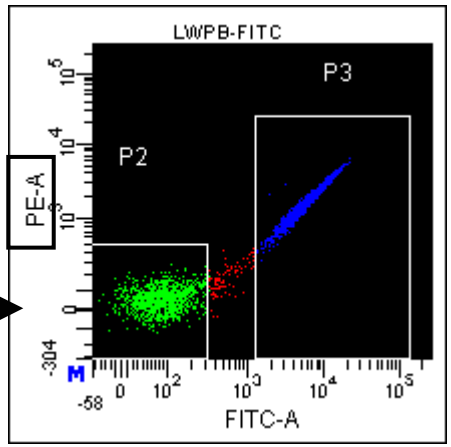


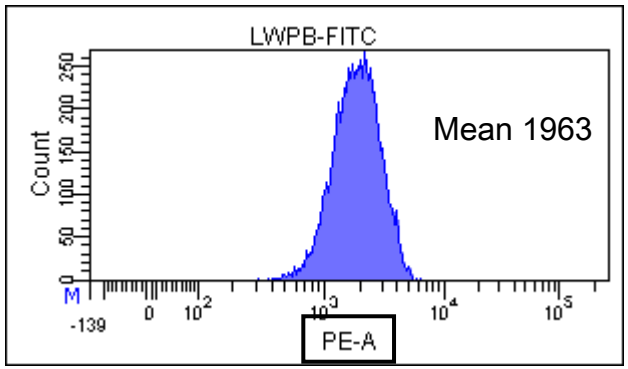
# Manueel Instellen van de flowcytometer

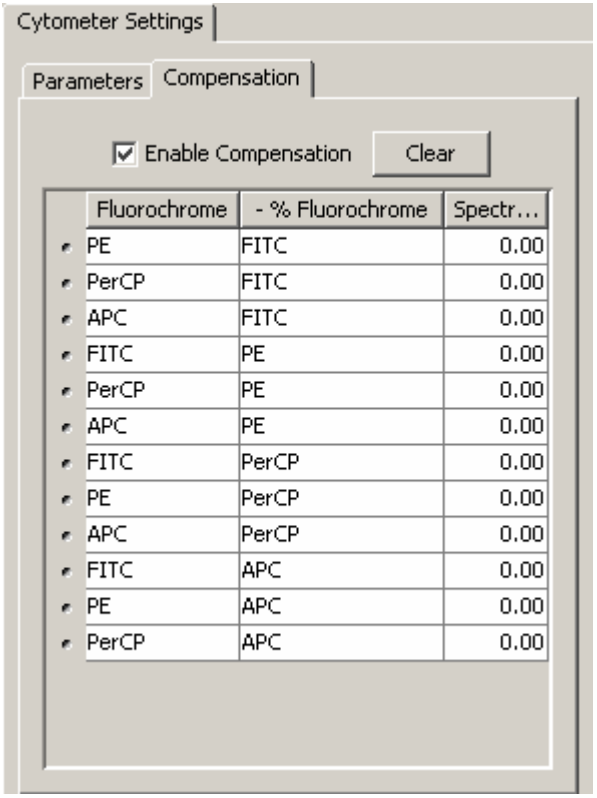
- Stel de compensatie in voor correctie van spectrale overspraak



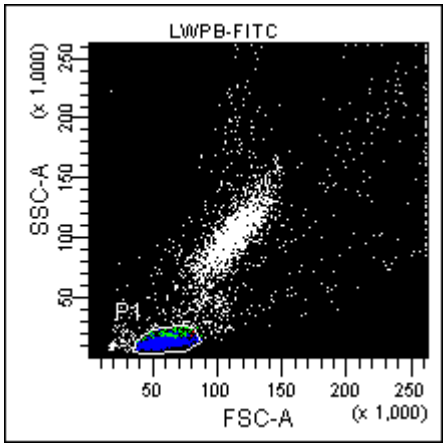



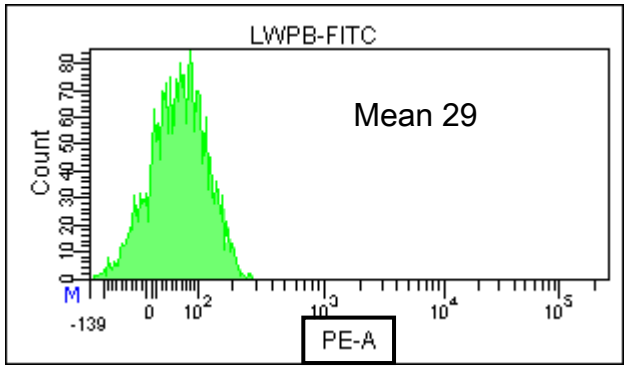



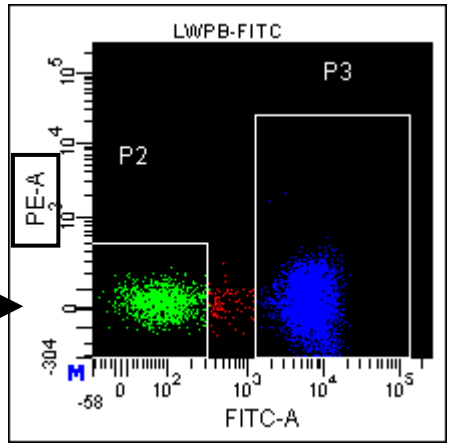

| Fluorochrome | - % Fluorochrome | Spectr... |
|---|---|---|
| PE | FITC | 0.00 |
| PerCP | FITC | 0.00 |
| APC | FITC | 0.00 |
| FITC | PE | 0.00 |
| PerCP | PE | 0.00 |
| APC | PE | 0.00 |
| FITC | PerCP | 0.00 |
| PE | PerCP | 0.00 |
| APC | PerCP | 0.00 |
| FITC | APC | 0.00 |
| PE | APC | 0.00 |
| PerCP | APC | 0.00 |

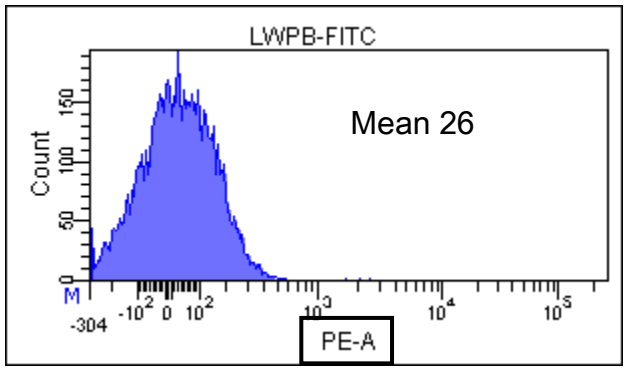
# Manueel Instellen van de flowcytometer

- Stel de compensatie in voor correctie van spectrale overspraak




Cytometer Settings

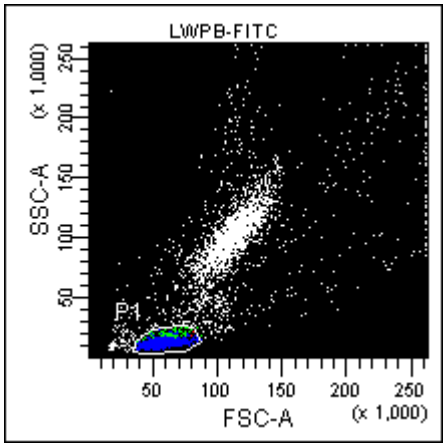
Parameters | Compensation

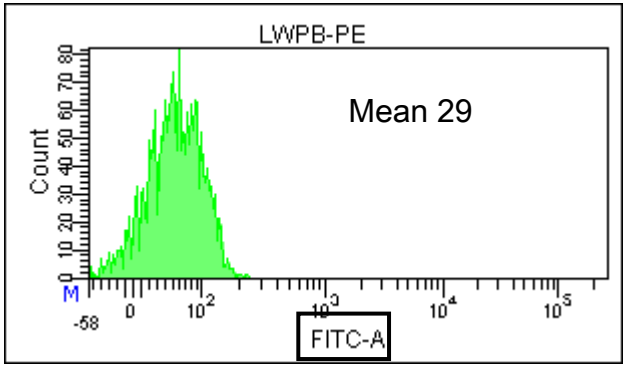
Enable Compensation | Clear

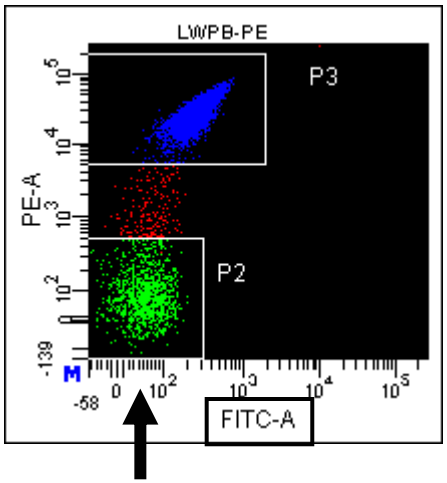
| Fluorochrome | - % Fluorochrome | Spectr... |
|---|---|---|
| PE | FITC | 27.50 |
| PerCP | FITC | 0.00 |
| APC | FITC | 0.00 |
| FITC | PE | 0.00 |
| PerCP | PE | 0.00 |
| APC | PE | 0.00 |
| FITC | PerCP | 0.00 |
| PE | PerCP | 0.00 |
| APC | PerCP | 0.00 |
| FITC | APC | 0.00 |
| PE | APC | 0.00 |
| PerCP | APC | 0.00 |

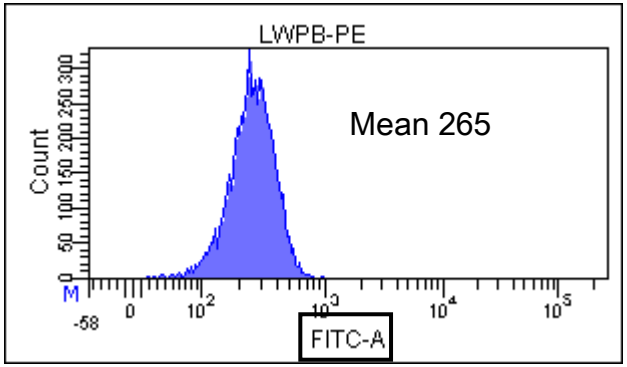
# Manueel Instellen van de flowcytometer

- Stel de compensatie in voor correctie van spectrale overspraak




Cytometer Settings

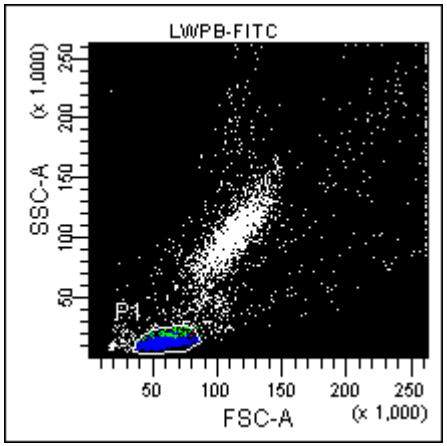
Parameters | Compensation

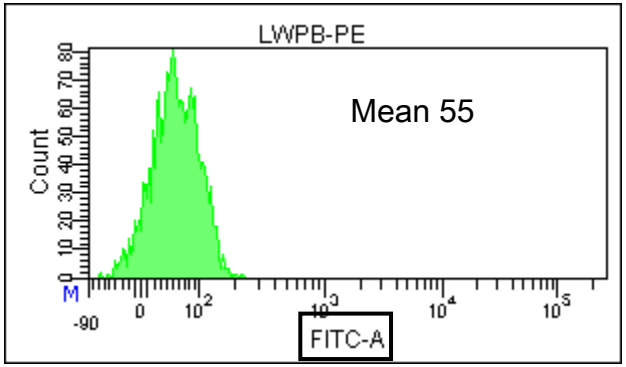
Enable Compensation | Clear

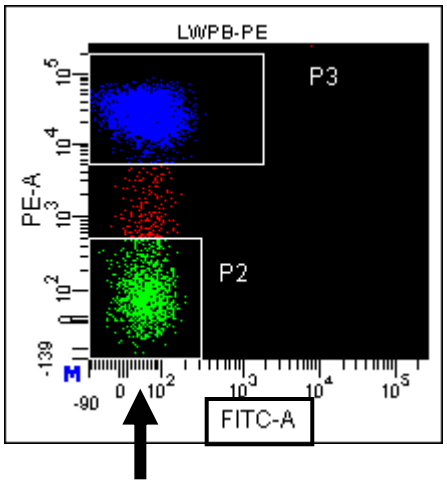
| Fluorochrome | - % Fluorochrome | Spectr... |
|---|---|---|
| PE | FITC | 0.00 |
| PerCP | FITC | 0.00 |
| APC | FITC | 0.00 |
| FITC | PE | 0.00 |
| PerCP | PE | 0.00 |
| APC | PE | 0.00 |
| FITC | PerCP | 0.00 |
| PE | PerCP | 0.00 |
| APC | PerCP | 0.00 |
| FITC | APC | 0.00 |
| PE | APC | 0.00 |
| PerCP | APC | 0.00 |

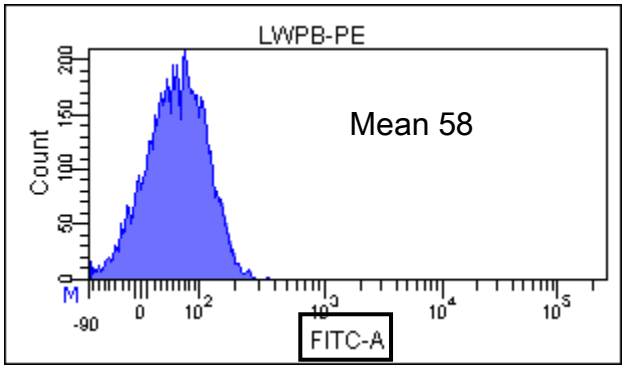
# Manueel Instellen van de flowcytometer

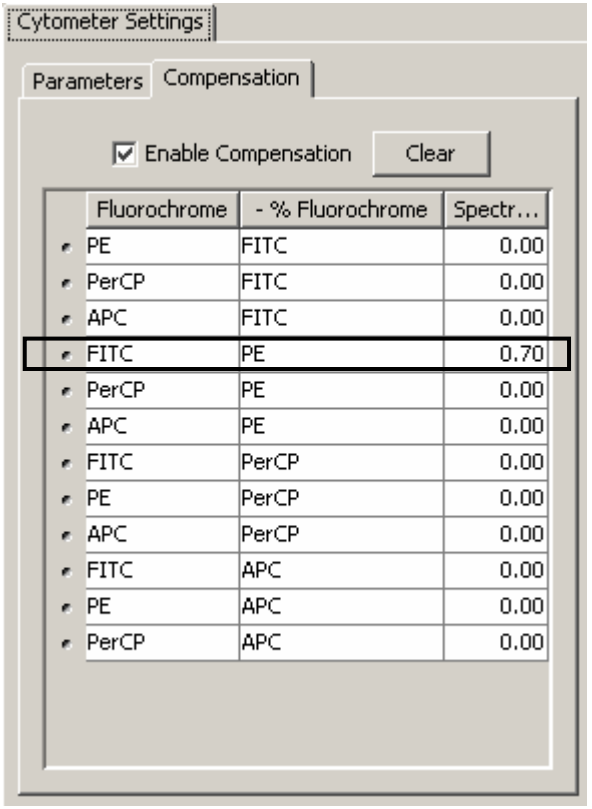
- Stel de compensatie in voor correctie van spectrale overspraak



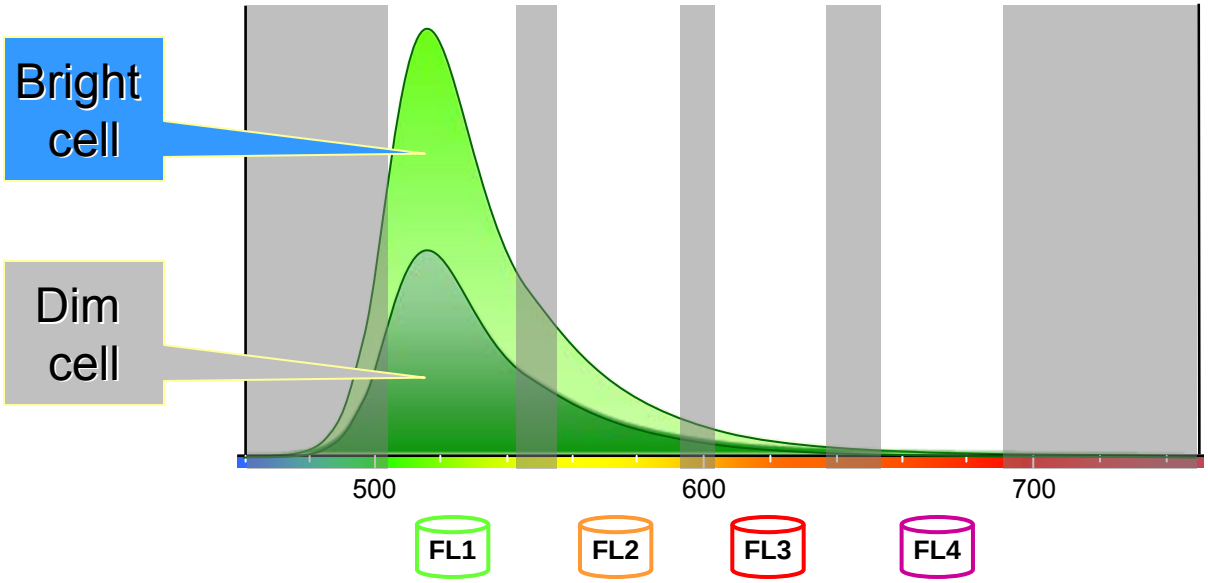



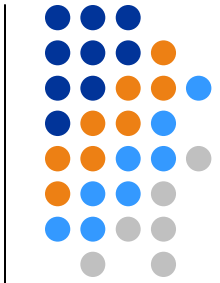

| Fluorochrome | - % Fluorochrome | Spectr... |
|---|---|---|
| PE | FITC | 0.00 |
| PerCP | FITC | 0.00 |
| APC | FITC | 0.00 |
| FITC | PE | 0.70 |
| PerCP | PE | 0.00 |
| APC | PE | 0.00 |
| FITC | PerCP | 0.00 |
| PE | PerCP | 0.00 |
| APC | PerCP | 0.00 |
| FITC | APC | 0.00 |
| PE | APC | 0.00 |
| PerCP | APC | 0.00 |

# The “Rules” of Crosstalk: Constant Proportionality




| | | | | |
|---|---|---|---|---|
| Bright cell Intensities | 500 | 60 | 5 | 0.4 |
| **Intensity ratios (%)** | **100** | **12** | **1** | **<0.1** |
| Dim cell Intensities | 250 | 30 | 2.5 | 0.2 |

No matter how the fluorescence intensity varies, the ratios of measured values stay constant.

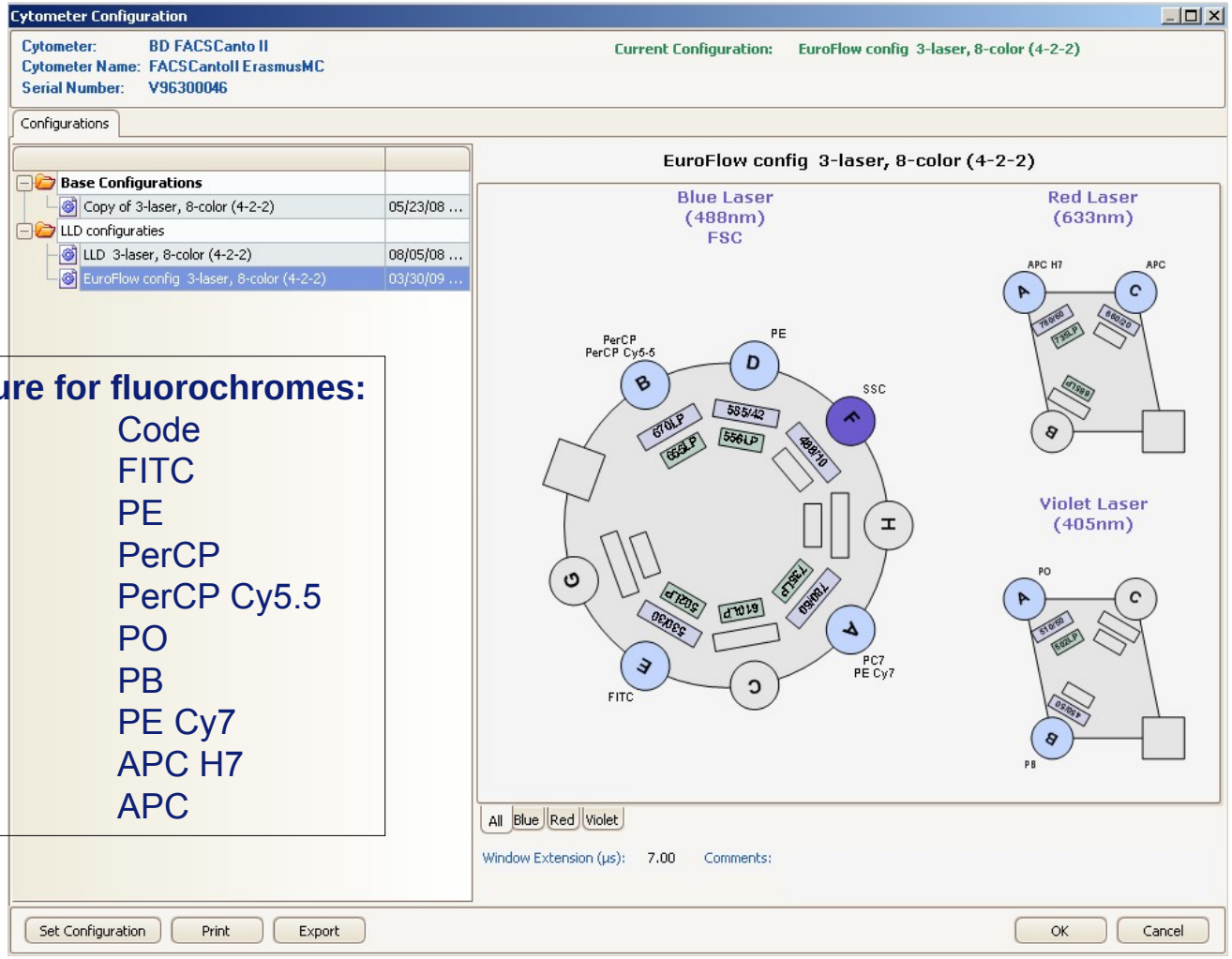


# Standardization of Instrument Setup

1. Configuration
2. Scatter settings
   - Forward scatter (FSC), Side scatter (SSC) and treshold
3. PMT settings for fluorescence
   - CST beads
   - EuroFlow approach (8-pk Rainbow beads)
4. Compensation matrix
5. Guidelines for uniform Instrument Setup
   - Synchronized experiment
6. Scatter beads
   - Synchronized experiment

# Configuration

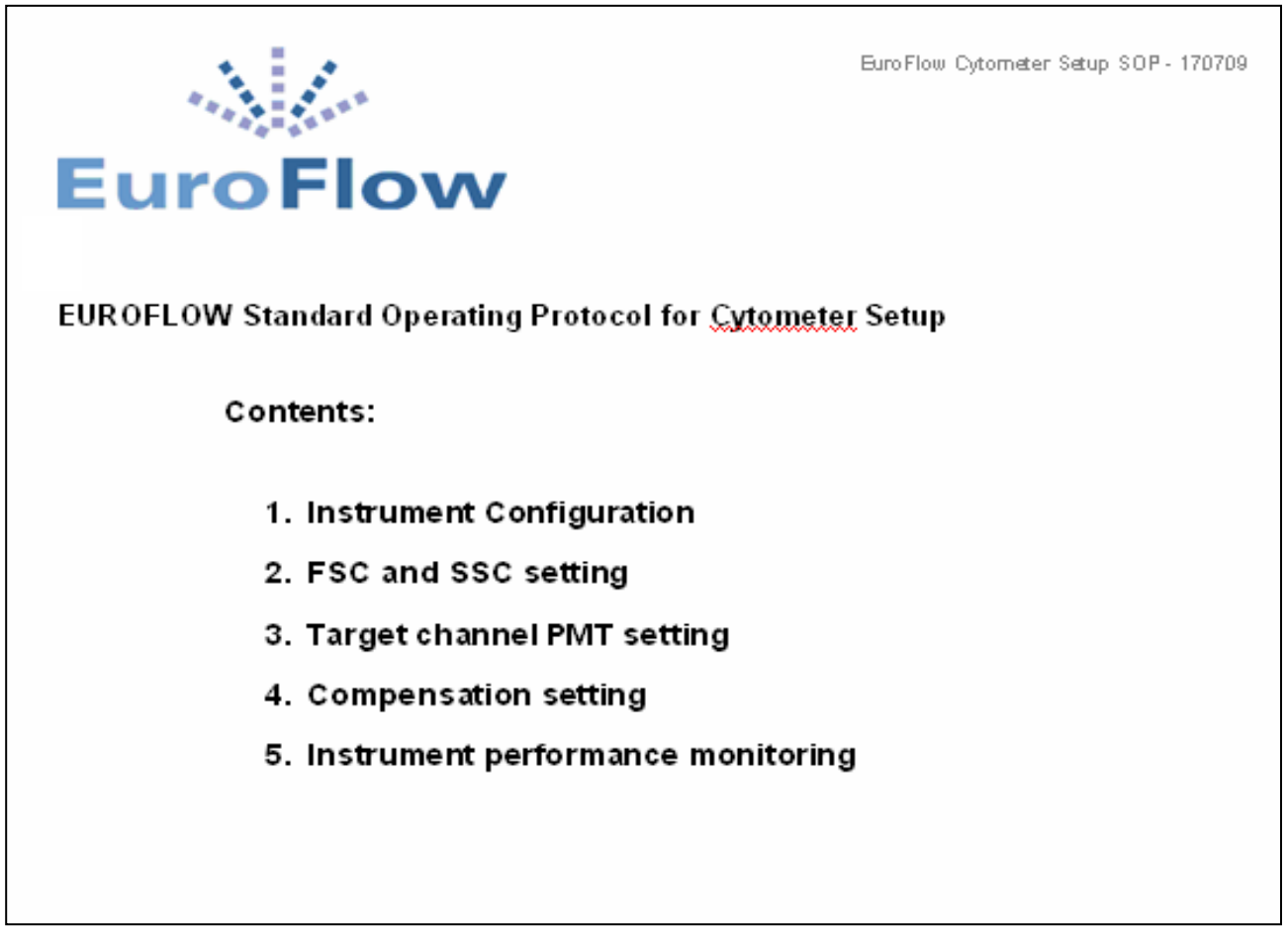
- Which fluorochromes or cell parameters will be measured at each photomultiplier tube (PMT)




**EF Nomenclature for fluorochromes:**

| Fluorochrome | Code |
|---|---|
| FITC | FITC |
| PE | PE |
| PerCP | PerCP |
| PerCP Cy5.5 | PerCP Cy5.5 |
| Pacific Orange | PO |
| Pacific Blue | PB |
| PE-Cy7 | PE Cy7 |
| APC-H7 | APC H7 |
| APC | APC |

# Uniform Instrument Setup




EuroFlow

# Synchronized Experiment

- Synchronized data acquisition in 8 different centers
  1. Instrument Setup according to EF SOP
  2. 8-peak Rainbow beads as control
  3. Staining according to EF procedure for:
     - 1 stabilized blood
     - 3 to 4 healthy donors in each center:

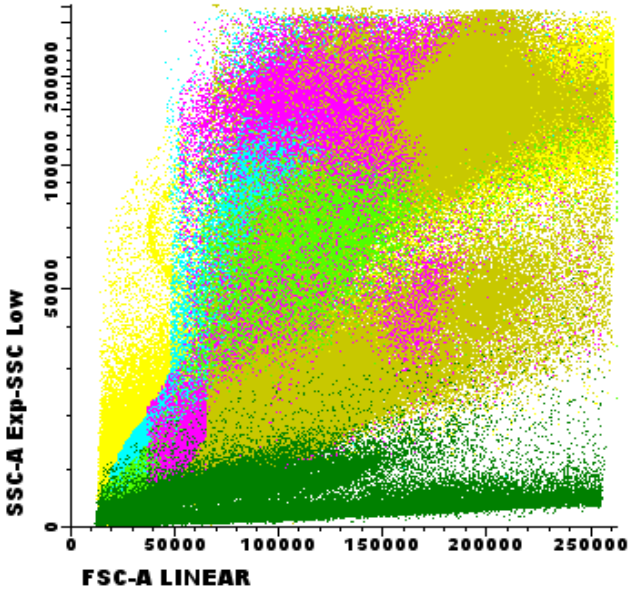
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 3.1 | Use **50ul** human peripheral blood | | | | | | | |
| 3.2 | Prepare 1 tube for Modified CLPD | | | | | | | |
| 3.3 | Add the amount of antibody to the tube: | | | | | | | |
| | | | | | | | | |
| | Pacific Blue | PacOr | FITC | PE | PerCP-Cy5.5 | PECy7 | APC | APC-H7 |
| | CD20 | CD45 | CD8 | CD27 | CD4 | CD19 | CD14 | CD3 |
| Ab amo | 10 | 3 | 10 | 10 | 10 | 5 | 3 | 3 |

EuroFlow

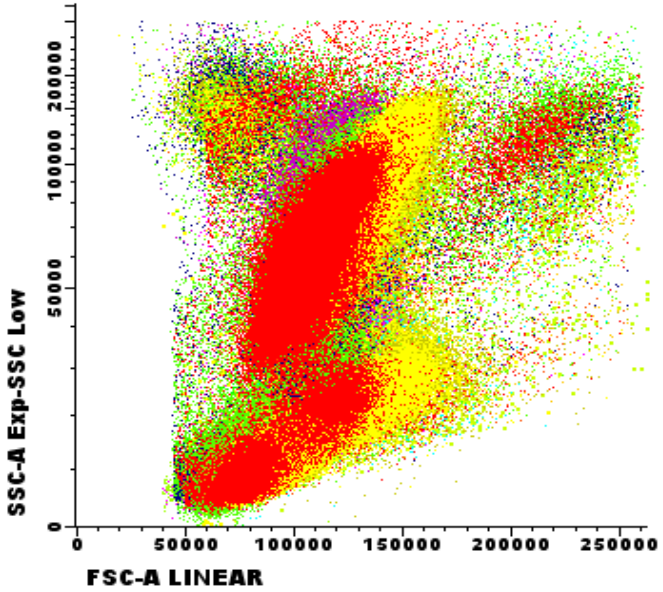
# Results of synchronized experiments

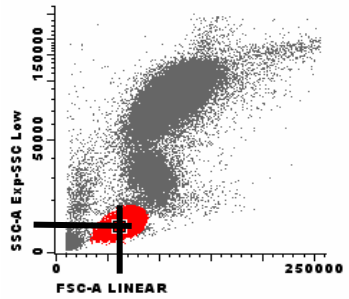
- Scatter

"Local" settings




EuroFlow settings




- Use 50ul peripheral blood of healthy donor
- Standard EF procedure using FACS Lysing solution

- Target values lymphocytes:
  FSC: 55.000 (range 50.000 - 60.000)
  SSC: 13.000 (range 11.000 - 15.000)

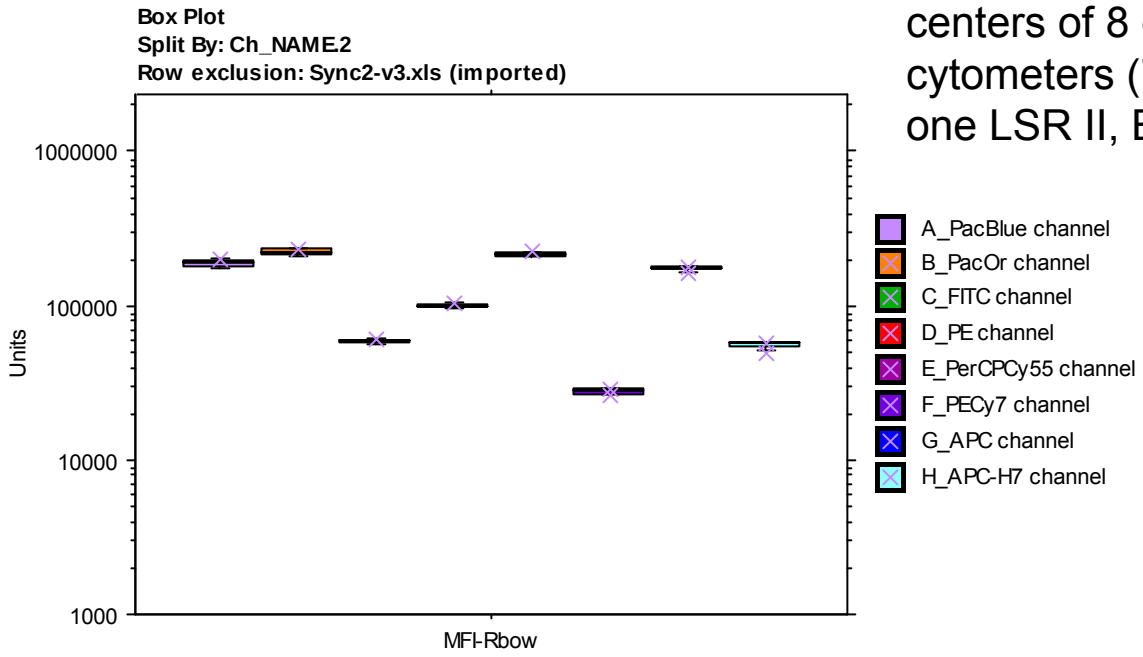
  Fixed treshold: 10,000

Remark: results of 7 different centers

EuroFlow

# Results of synchronized experiments

Data Rainbow beads in 8 different centers of 8 different flow cytometers (7x FACSCanto II and one LSR II, BD)




**Descriptive Statistics**
**Split By: Ch_NAME.2**
**Row exclusion: Sync2-v3.xls (imported)**

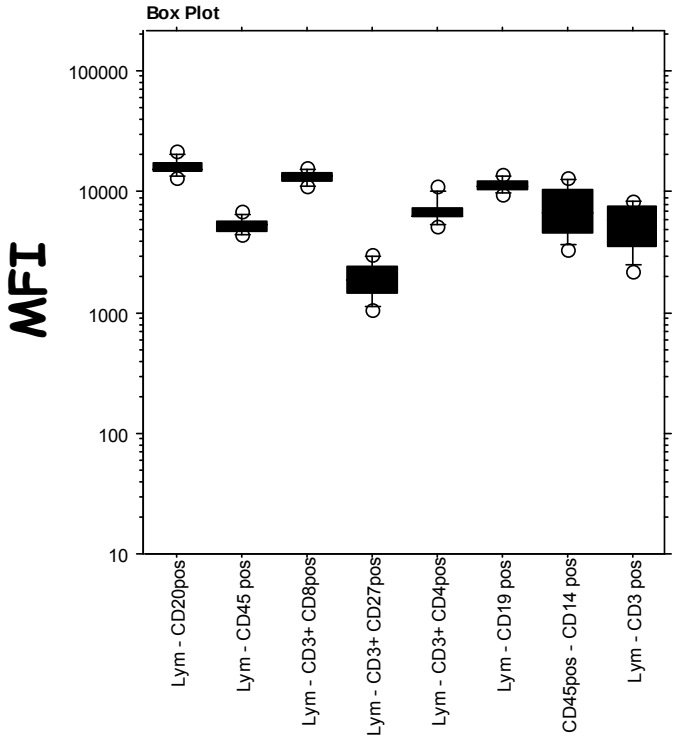
| | Mean | Std. Dev. | Coef. Var. |
|---|---|---|---|
| MFI-Rbow , Total | 130165,452 | 73835,014 | ,567 |
| MFI-Rbow , A_PacBlue channel | 186995,128 | 10463,338 | ,056 |
| MFI-Rbow , B_PacOr channel | 222018,096 | 9364,052 | ,042 |
| MFI-Rbow , C_FITC channel | 58771,019 | 1548,629 | ,026 |
| MFI-Rbow , D_PE channel | 100027,144 | 2768,766 | ,028 |
| MFI-Rbow , E_PerCPCy55 channel | 214431,445 | 7009,384 | ,033 |
| MFI-Rbow , F_PECy7 channel | 27777,652 | 948,720 | ,034 |
| MFI-Rbow , G_APC channel | 173227,432 | 4684,230 | ,027 |
| MFI-Rbow , H_APC-H7 channel | 55656,722 | 2781,647 | ,050 |

- The Coeff. of Variation is less then 5,6% between centers

EuroFlow

# Results of synchronized experiments

Paciifc Blue
Pacific Orange
FITC
PE
PerCP-Cy5.5
PC7
APC
APC-H7




**Stabilized normal PB**

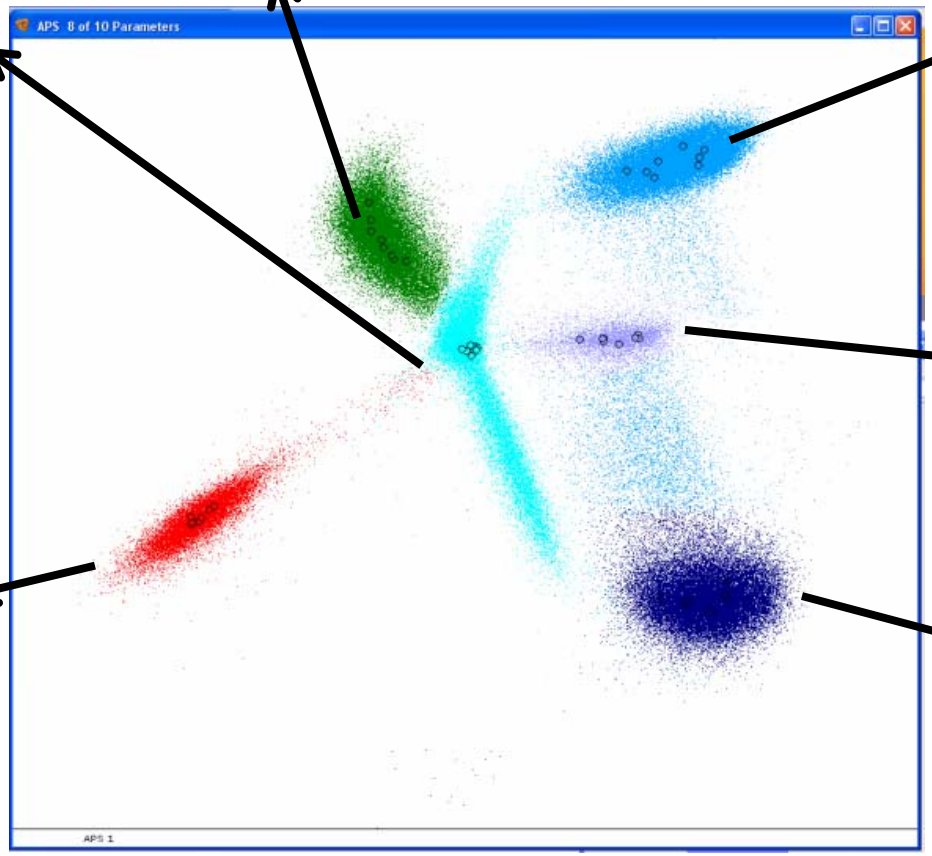
## Interlaboratory MFI CV of gated cell subsets

| Cell subset | CV |
|---|---|
| Lym - CD20pos | 15,25% |
| Lym - CD45 pos | 13,90% |
| Lym - CD19 pos | 11,13% |
| Lym - CD3 pos | 38,67% |
| CD45pos - CD14 pos | 43,77% |
| Lym - CD3+ CD4pos | 24,68% |
| Lym - CD3+ CD8pos | 11,35% |
| Lym - CD3+ CD27pos | 32,09% |

EuroFlow

# Results of synchronized experiments

APS view of 8 merged data files from different centers (n=8)




CD14+ Monocytes

CD8+ CD3- T cells

CD3+ CD4+ T-cells

CD3+ CD8- CD4- T-cells

CD19+ CD20+ B-cells

CD3+ CD8+ T cells

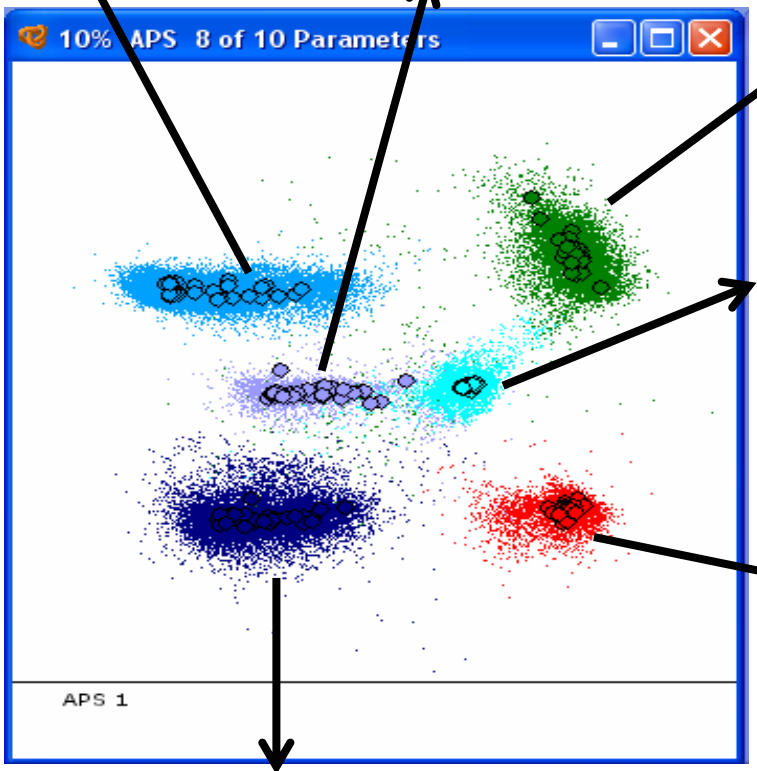
Stabilized normal PB

EuroFlow

# Results of synchronized experiments

## APS view of 30 merged data files from different centers

**Local normal donors**

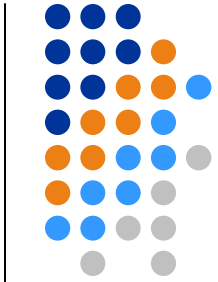



CV MFI subset

| | | |
|---|---|---|
| PacBlue channel | Lym - CD20pos | 16,90% |
| PacOr channel | Lym - CD45 pos | 15,52% |
| FITC channel | Lym - CD3+ CD8pos | 16,93% |
| PE channel | Lym - CD3+ CD27pos | 27,95% |
| PerCPCy55 chann | Lym - CD3+ CD4pos | 28,39% |
| PECy7 channel | Lym - CD19 pos | 15,40% |
| APC channel | CD45pos - CD14 pos | 22,68% |
| APC-H7 channel | Lym - CD3 pos | **48,35%** |

EuroFlow

# Conclusion



- Using a SOP for cytometer setup it is possible to generate identical data on different flow cytometers* independent of time and place
- Conditions:
  1. Universal Instrument Setup
  2. Identical sample preparation and staining protocols