Euroflow standardisation using CST beads

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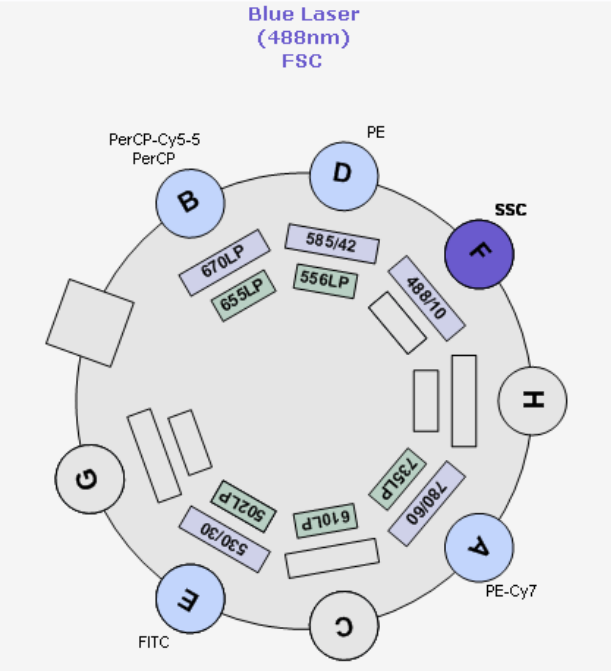
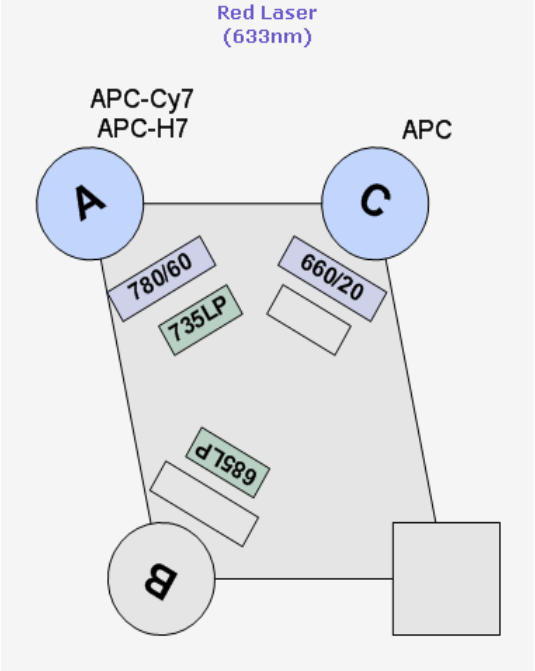
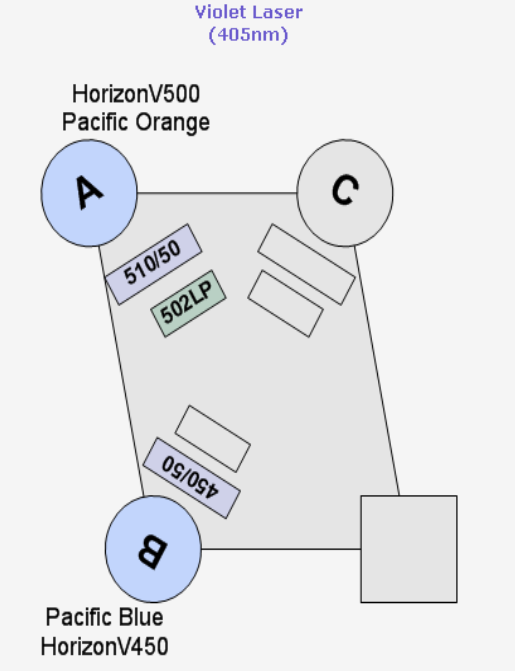
Introduction

Configuration

- Setting the configuration: EUROFLOW8
- Filter/mirror settings







Calibration

- Perform a baseline (according to manufacturer) using CS&T beads
- Define PMTv using Spherotech 8-peak rainbow beads, to place the brightest peak at predefined target values.
- Perform a baseline with the PMTv defined with rainbow beads
- Perform a baseline with the new dim bead median channel values
- Perform a performance check using CS&T beads
- Perform a control with Spherotech 8-peak rainbow beads

- Frequency of calibration: once a year

Step 1: Perform a baseline (according to manufacturer)

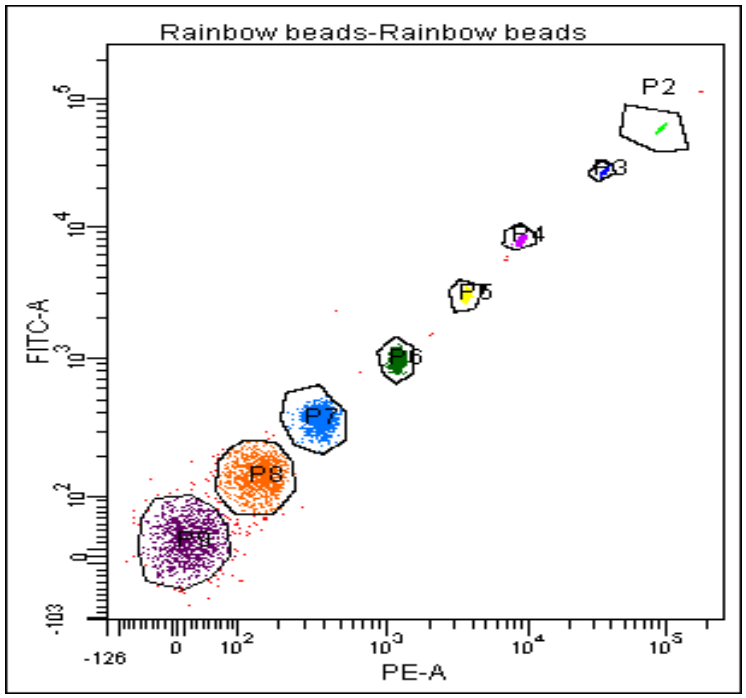
Rainbow beads

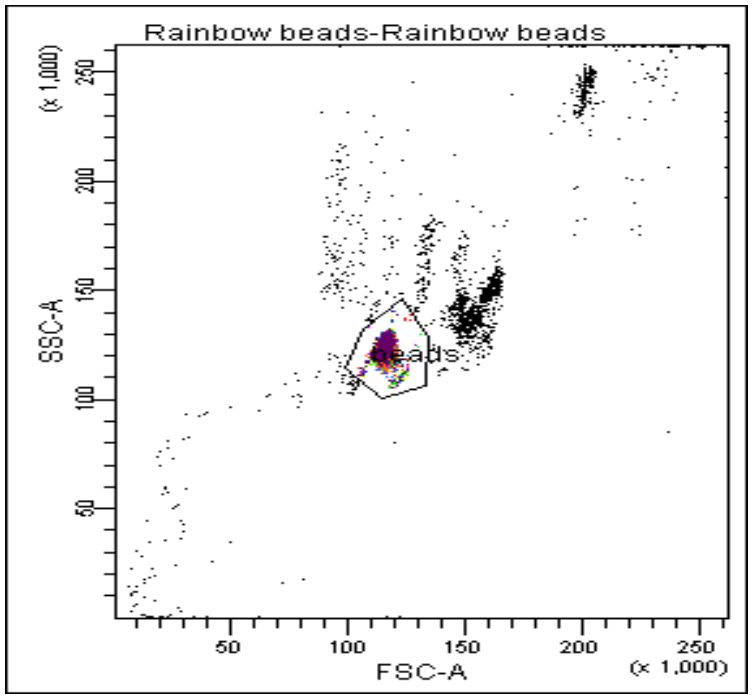
Step 2: Define PMTv according to EUROFLOW with rainbow beads

- PMTv are experimentally determined by placing the brightest peak of the rainbow beads (P2) at predefined target values.

Experiment Name:	Rainbow beads CST19102010
Specimen Name:	Rainbow beads
Tube Name:	Rainbow beads
Record Date:	Oct 21, 2010 11:08:38 AM
$OP:	Administrator
GUID:	1af16d12-568b-4edf-b30e-20275c1d8d8e

Population	FITC-A Mean	PE-A Mean	PerCP-C... Mean	PE-Cy7-A Mean	APC-A Mean	APC-Cy7-A Mean	Pacific Bl... Mean	HorizonV... Mean
P2	58,908	94,384	218,656	29,740	183,342	58,162	185,723	244,225
P3	27,369	36,891	64,660	8,557	74,952	20,849	110,696	111,552
P4	7,935	9,047	13,783	1,807	20,894	5,670	33,818	29,544
P5	2,966	3,624	5,383	711	10,421	2,916	26,520	15,821
P6	978	1,187	1,879	256	6,057	1,719	22,479	9,370
P7	363	365	603	89	2,478	650	6,793	3,052
P8	146	144	275	46	1,725	437	6,088	2,295
P9	25	12	68	15	700	155	255	209





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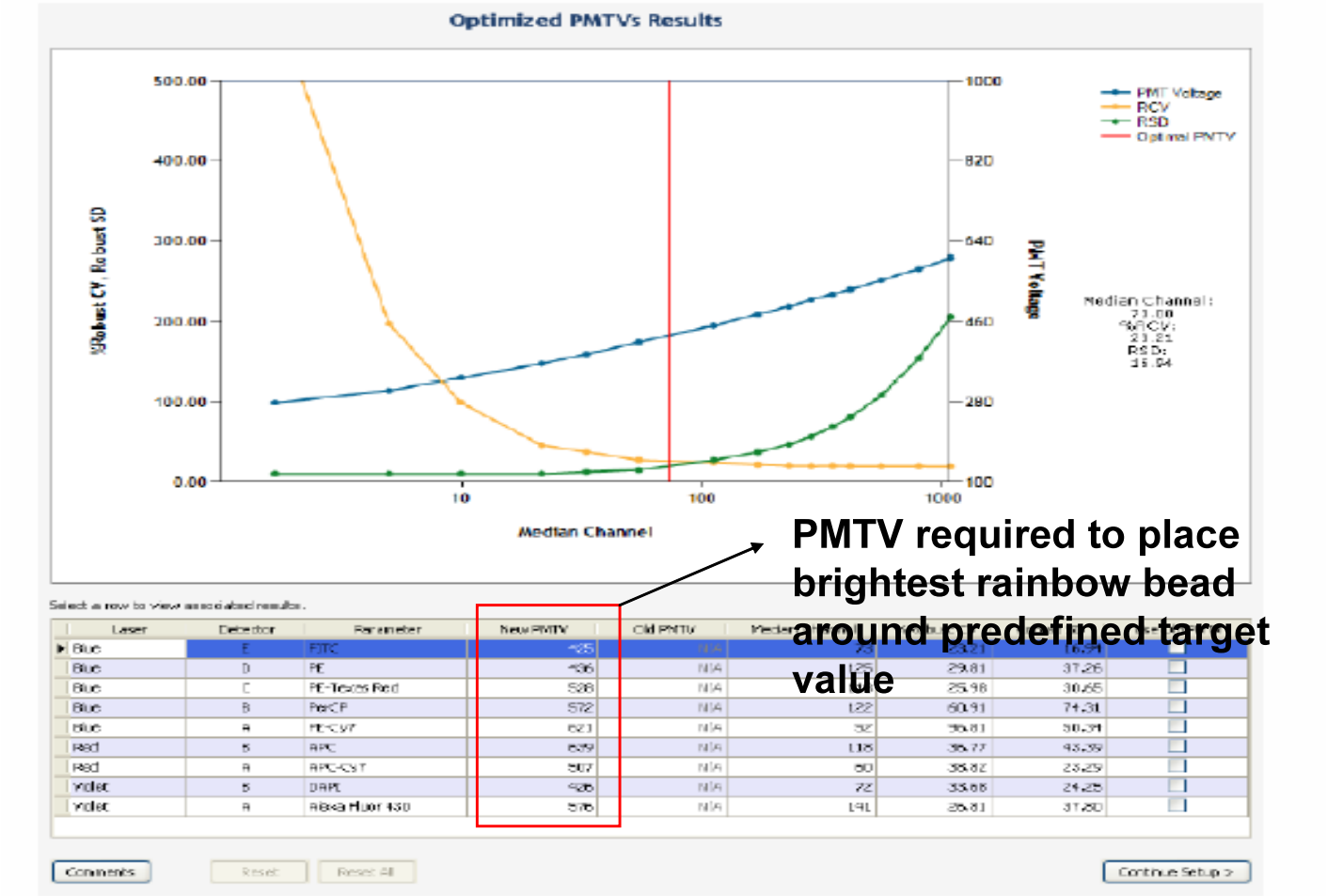
Kanaal	**Laagste MFI waarde (-10%)**	**Target MFI waarde**	**Hoogste MFI waarde (+10%)**
Pacific blue/Horizon V450	176015	195572	215129
Pacific Orange/ Horizon V500	208139	231265	254391
FITC	53617	59574	65531
PE	91710	101900	112090
PerCPcy5.5	194458	216064	237670
PEcy7	24716	27462	30208
APC	159102	176780	194458
APC-H7	50794	56437	62080

Step 3: Run a CS&T- baseline and replace the PMTv values in the “PMTv review section” by those obtained in step 2 (by moving red line)



- Continue baseline setup. Go to report and look to the dim bead median channel values.

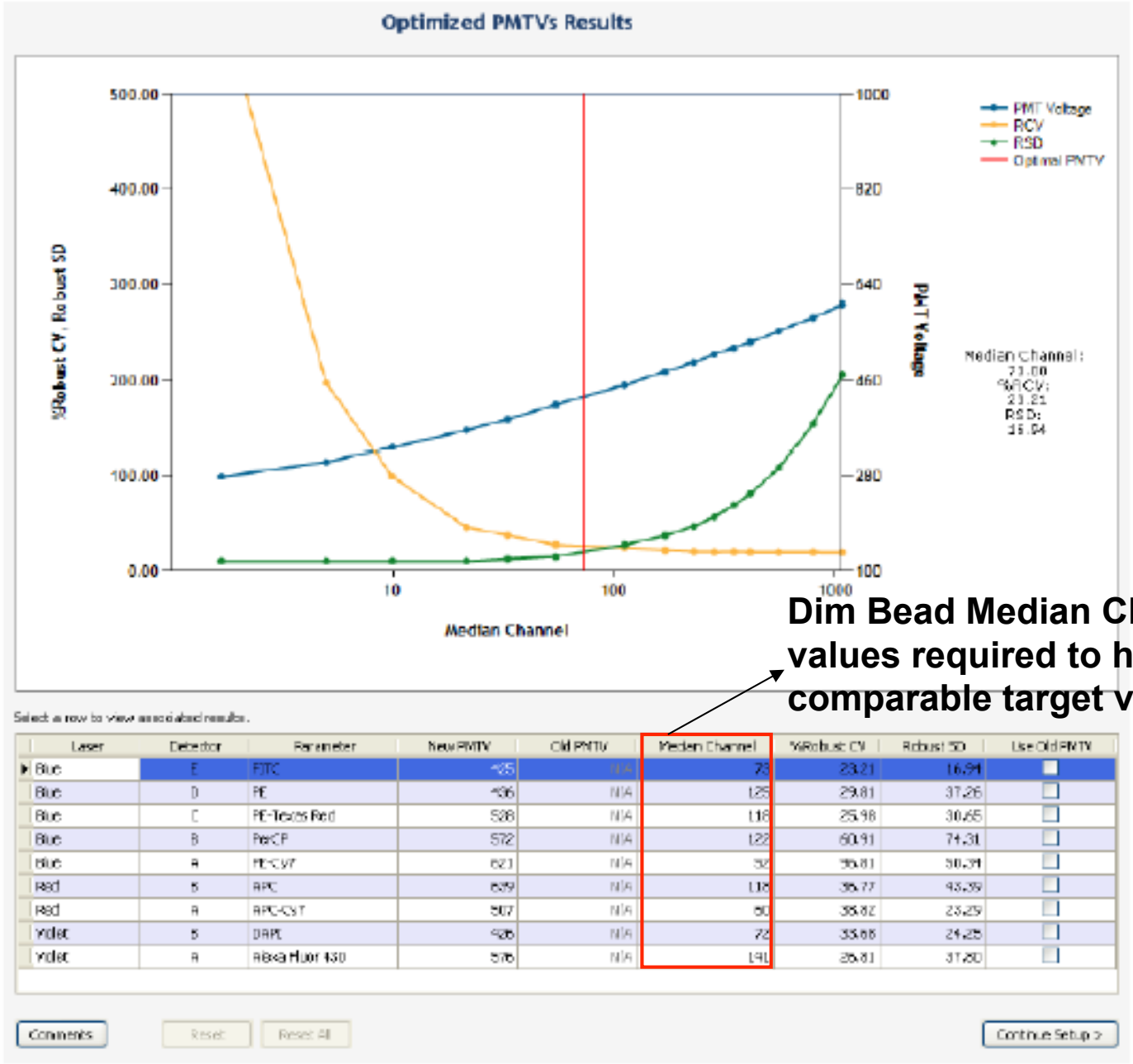
Cytometer Baseline Report

Detector Settings

Laser	Detector	Parameter	PMTV	New Target Value	Old Target Value	Bright Bead %Robust CV	Mid Bead Median Channel	Mid Bead % Robust CV	Dim Bead Median Channel	Dim Bead % Robust CV
Blue	FSC	FSC	373	125000	N/A	2.24	126809	2.09	18034	5.68
Blue	E	SSC	348	125000	N/A	5.1	123989	4.45	64420	2.73
Blue	D	FITC	640 (m)	15960	N/A	3.17	735	12.61	81	43.24
Blue	C	PE	437 (m)	13903	N/A	2.47	512	10.76	104	31.09
Blue	B	PerCP-Cy5-5	745 (m)	49419	N/A	4.86	1861	21.41	224	63.54
Blue	A	PE-Cy7	699 (m)	30078	N/A	7.42	767	37.55	45	159.24
Red	B	APC	648 (m)	46800	N/A	2.96	2389	12.03	175	42.89
Red	A	APC-H7	622 (m)	124797	N/A	3.69	4825	11.65	423	35.93
Violet	B	Pacific Blue	476 (m)	22381	N/A	3.14	1278	7.23	156	27.55
Violet	A	HorizonV500	559 (m)	72261	N/A	2.88	3239	6.53	240	23.01

- STEP 4: Perform a new baseline and put the obtained dim bead median channel values in the PMTv review section on the flowcytometer.

	Lot 54102	**Lot 75445**
V450	156	149
V500	240	204
FITC	84	86
PE	106	106
PerCP-Cy5-5	253	213
PE-Cy7	56	62
APC	157	224
APC-H7	388	341


Optimized PMTVs Results
500.00
400.00
300.00
200.00
100.00
0.00
%Robust CV, Robust SD
1000
820
640
460
280
100
PMT Voltage
10
100
1000
Median Channel
PMT Voltage
RCV
RSD
Optimal PMTV
Median Channel:
%RCV:
RSD:
Dim Bead Median Channel values required to have comparable target values
Select a row to view associated results.
Laser
Detector
Parameter
New PMTV
Old PMTV
Median Channel
%Robust CV
Robust SD
Use Old PMTV
Blue
E
FITC
425
N/A
23.21
16.94
Blue
D
PE
436
N/A
29.81
37.26
Blue
C
PE-Texas Red
528
N/A
25.98
30.65
Blue
B
PerCP
572
N/A
60.91
74.31
Blue
A
PE-Cy7
621
N/A
30.81
30.39
Red
B
APC
659
N/A
35.77
43.39
Red
A
APC-Cy7
807
N/A
35.82
23.29
Violet
B
DAPI
426
N/A
35.68
24.25
Violet
A
AmCyan
576
N/A
28.81
37.80
Comments
Reset
Reset All
Continue Setup >

- Step 5: Perform a performance check using CS&T beads

- Step 6: Perform a control with Spherotech 8 - peak rainbow beads

Compensation

- Comp beads with CD3 in FITC, PE, PerCP-Cy5.5, PE-Cy7, APC, APC-H7, and CD45 in Pacific Blue, Horizon V500

Compensation

Procedure:

- 9 tubes:

  - Unstained: 50µL BD Cell Wash + 30µl pos beads
  - FITC: 50µL BD Cell Wash + 30µl pos beads + 30µl neg beads + 20µl anti-CD3-FITC
  - PE: 50µL BD Cell Wash + 30µl pos beads + 30µl neg beads + 20µl anti-CD3-PE
  - PerCP-Cy5.5: 50µL BD Cell Wash + 30µl pos beads + 30µl neg beads + 20µl anti-CD3-PerCP-Cy5.5
  - PE-Cy7: 50µL BD Cell Wash + 30µl pos beads + 30µl neg beads + 5µl anti-CD3-PE-Cy7
  - APC: 50µL BD Cell Wash + 30µl pos beads + 30µl neg beads + 5µl anti-CD3-APC
  - APC-H7: 50µL BD Cell Wash + 30µl pos beads + 30µl neg beads + 5µl anti-CD3-APC-H7
  - Pacific Blue: 50µL BD Cell Wash + 30µl pos beads + 30µl neg beads + 5µl anti-CD45-Pacific Blue
  - HorizonV500: 50µL BD Cell Wash + 30µl pos beads + 30µl neg beads + 5µl anti-CD45-HorizonV500

- Incubate 20min. by room temperature in the dark
- Add 2mL Cellwash and centrifugate 5min. at 1500rpm
- Decant the supernatans
- Add 500µL BD Cellwash and analyze

FSC and SSC settings

- mix of 5 peripheral blood samples (healthy individuals)
- 50µL mix of PB + 2mL 1/10 BD Facs Lyse
- vortex slightly
- incubate 10min. in the dark
- centrifuge 5min. at 540g
- aspirate supernatans
- wash with 2mL BD Cellwash
- centrifuge 5min. at 540g
- aspirate supernatans
- add 250µL Cellwash
- acquire cells using a threshold: FSC: 10.000
- gate lymfocytes and adjust – fine tune FSC and SSC voltages to reach the target (see next slide)

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

- Control the FSC and SSC settings with the 5 peripheral blood samples

Thank you for your attention!