The many faces of Cytometry
26th Annual Conference of the German Society for Cytometry
October 5 – 7, 2016, Berlin

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Deutsches Rheuma-Forschungszentrum
Ein Institut der Leibniz-Gemeinschaft
A·P·E

Quantitative comparison study of flow cytometers using a novel ultra-stable calibration light source

Konrad von Volkmann, APE GmbH, Berlin

Oral Presentation, Emerging Technologies Session, 26th Annual Conference of the German Society for Cytometry.

Introduction

Need for comparison between cytometers

- allow reproducible measurements
- at different times on the same instrument
- on different instruments (same site or at distinct sites)

T. Kalina *et al.*: EuroFlow standardization …, Leukemia **26**, 1986–2010 (2012)

- System check
- Performance monitoring
- Automated data analysis
- others

Calibration standards

Standard ways today:

- Comparison is based on the measurements of latex microspheres
- Quality control, instrument characterization (Q & B), instrument setup …

- Standardized „cells“, reproducible and stable

Limitations of today's standards

- Mainly relative calibration, absolute reference is demanding (variable gain, light collection, alignment)
- Variation in size due to the manufacturing process
  intrinsic CV > 2%
- Variations from lot to lot
- Degradation process, aging: beads get lumpy

Alternative idea

Cytometry 13:822–830 (1992)

Noise, Sensitivity, and Resolution of Flow Cytometers[1]

Harald B. Steen

Department of Biophysics, Institute for Cancer Research, The Norwegian Radium Hospital, 0310 Oslo, Norway

Received for publication January 8, 1992; accepted April 20, 1992

Cells passing through the laser focus = flashes of light

Detection of light by a PMT

- detection process is governed by Poissonian statistics
- intrinsic broadening of the detected mean value

$$CV = \frac{1}{\sqrt{N_{photo\ electrons}}} \quad \Longleftrightarrow \quad N_{photo\ electrons} = \frac{1}{CV^2}$$

$N_{photo\ electrons}$ = Spe (statistical photoelectron estimate)

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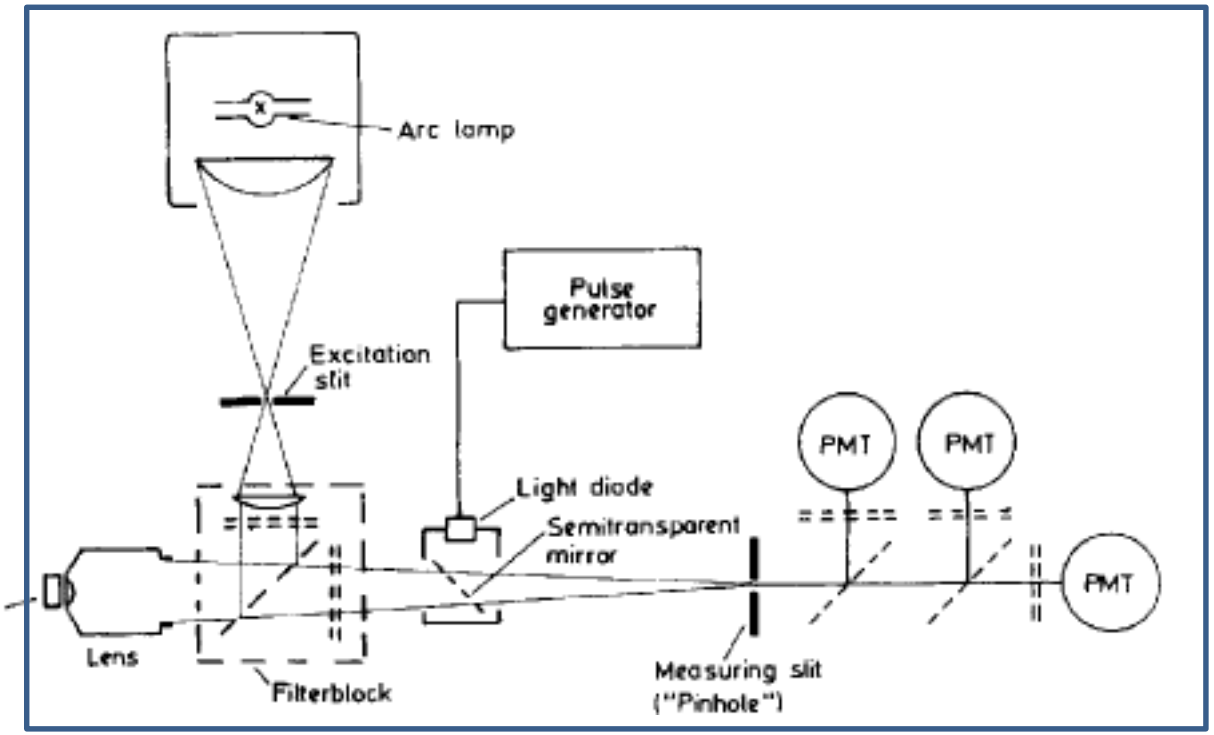

www.quantiFlash.de

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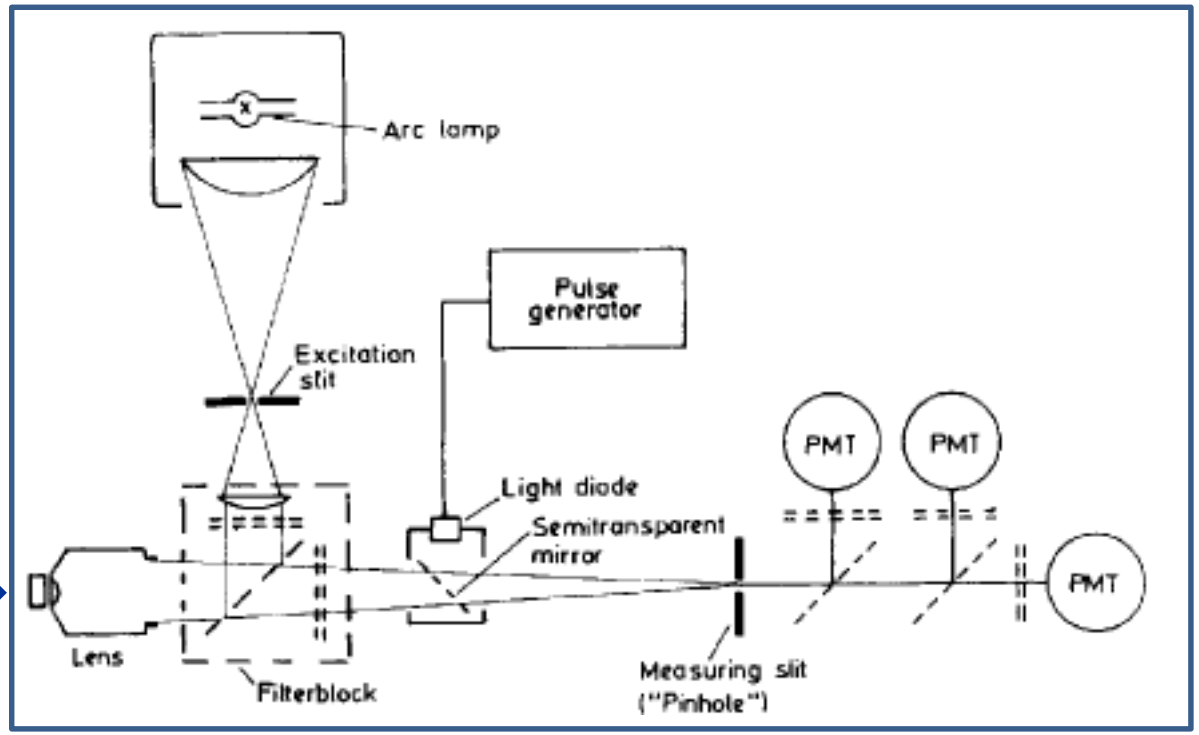

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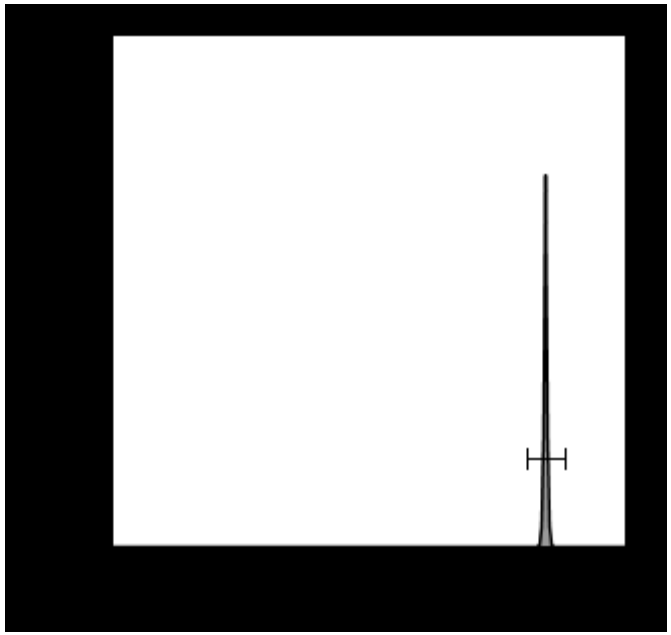
Scale calibration in Spe (Statistical Photoelectron Unit)

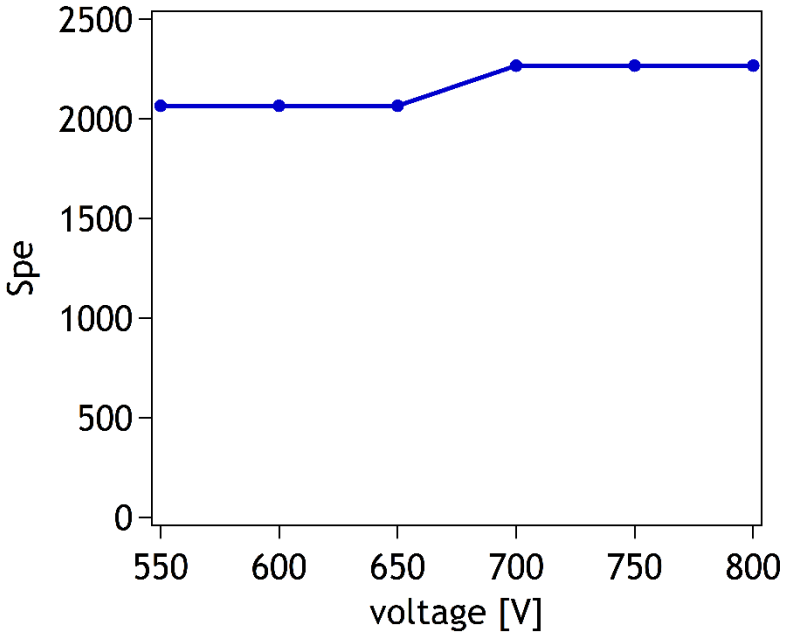
Why Spe?
- Absolute reference
- Independent of gain
- Independent of filters used
- Independent of staining and biology

*quanti*Flash® LED pulse **-> Spe/Ch**

$Spe = (100/rCV_{pulse})^2$



Spe @ constant light intensity



*quanti*Flash®: calibration tool for cytometers

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Scale calibration in Spe

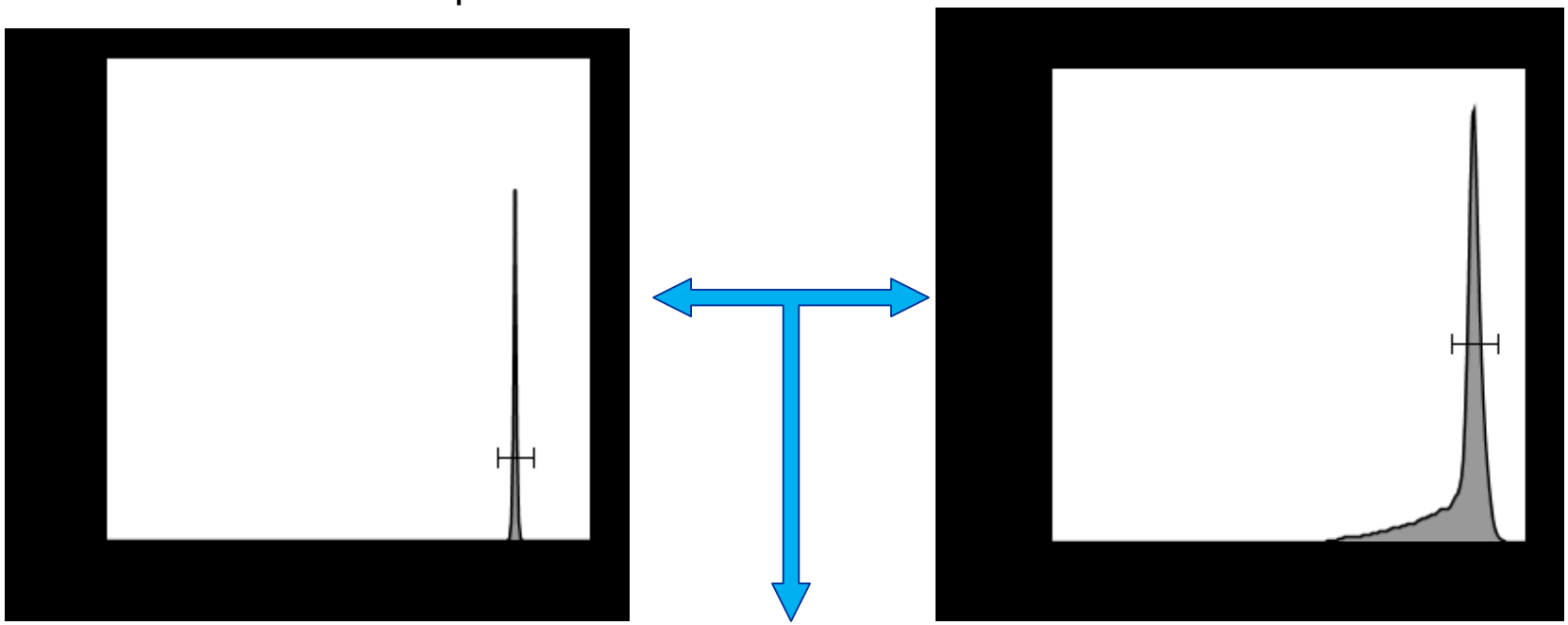
*quanti*Flash LED pulse **-> Spe/Ch**

Spe = $(100/rCV_{pulse})^2$

Scale calibration in ABC

measuring of beads with a calibrated fluorescent dye value -> ABC/Ch

Quantum™ Simply Cellular® Microspheres



Calculation of Q

Q = Spe / ABC (or MESF)

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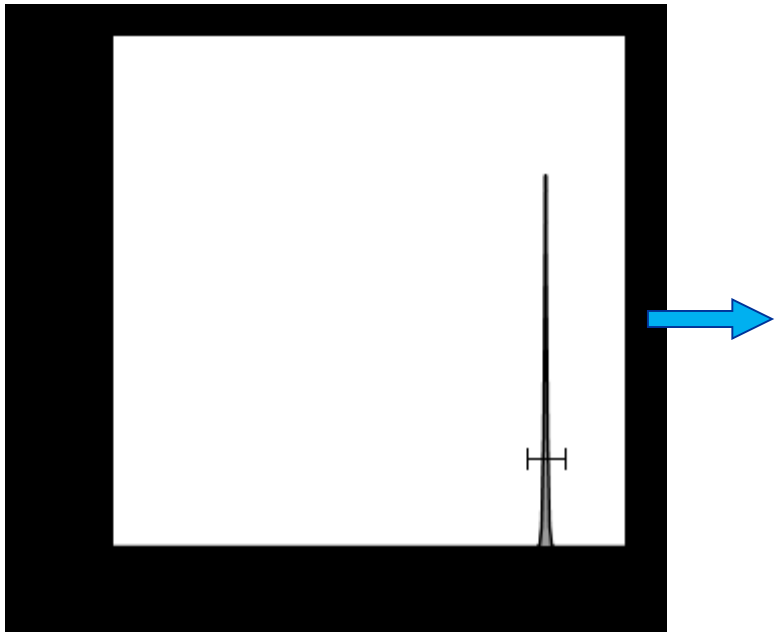
Scale calibration in Spe

*quanti*Flash LED pulse **-> Spe/Ch**

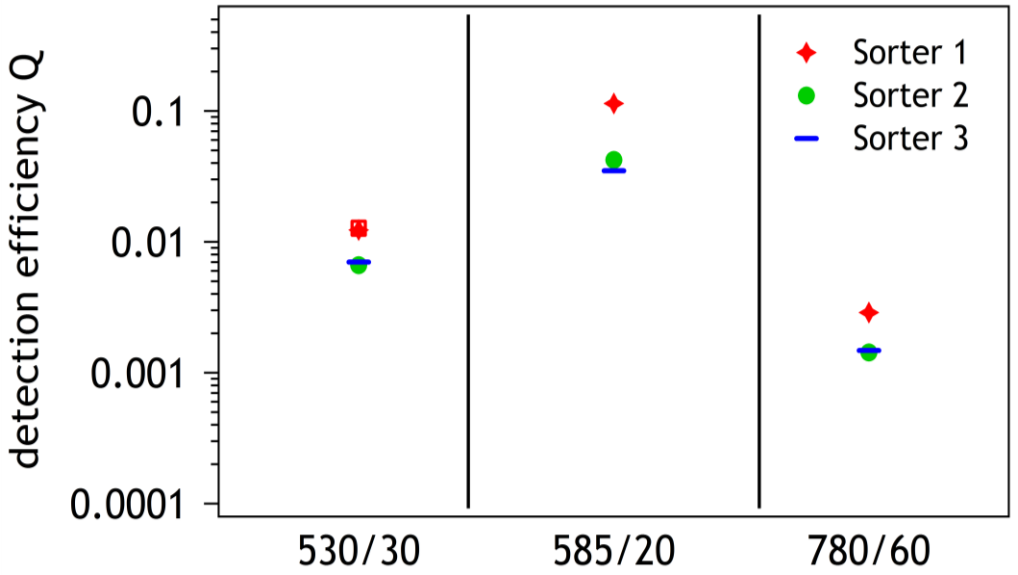
$$Spe = (100/rCV_{pulse})^2$$



Sorter 1: FACSAria II SORP
lasers: 488, 561, …

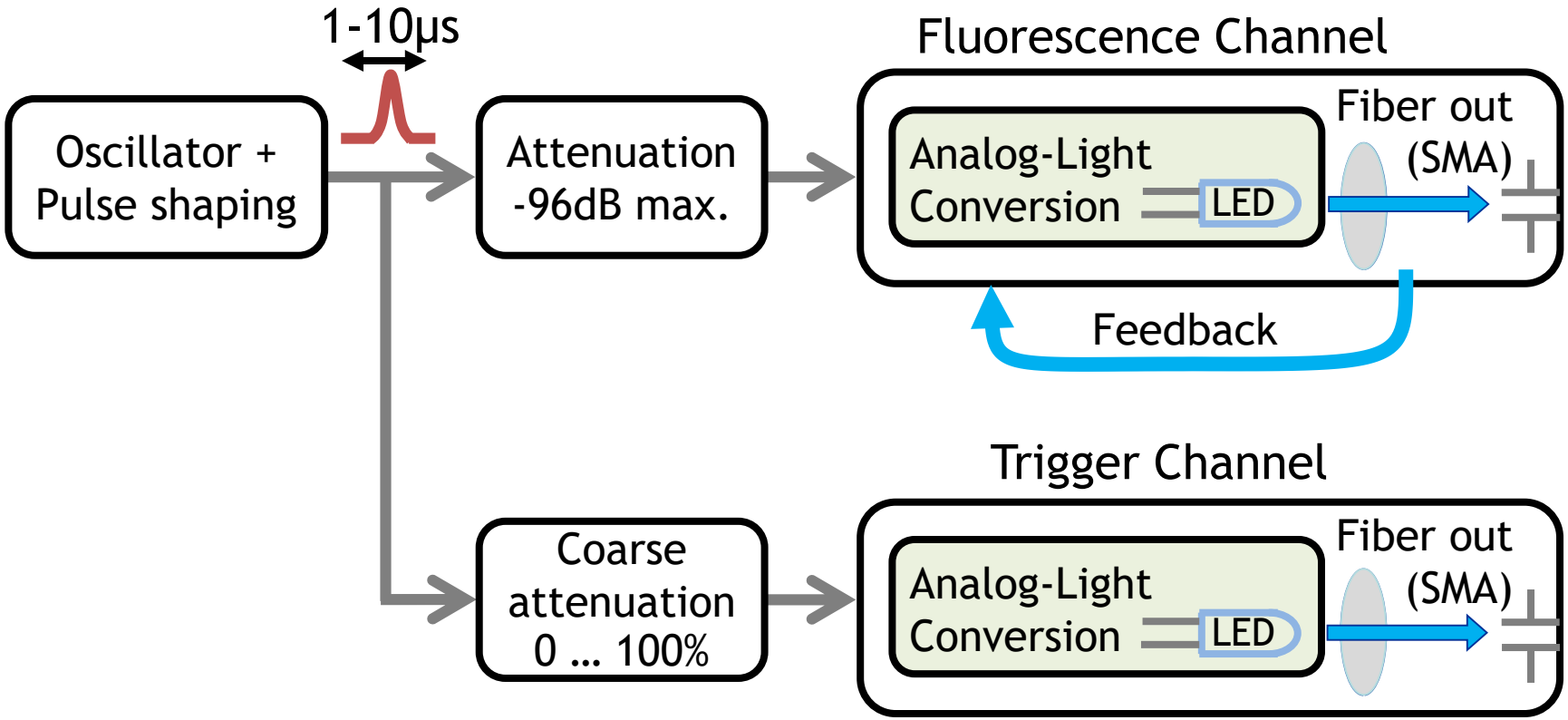
Sorter 2,3: FACSAria I
lasers: 488, …

bandpass filter



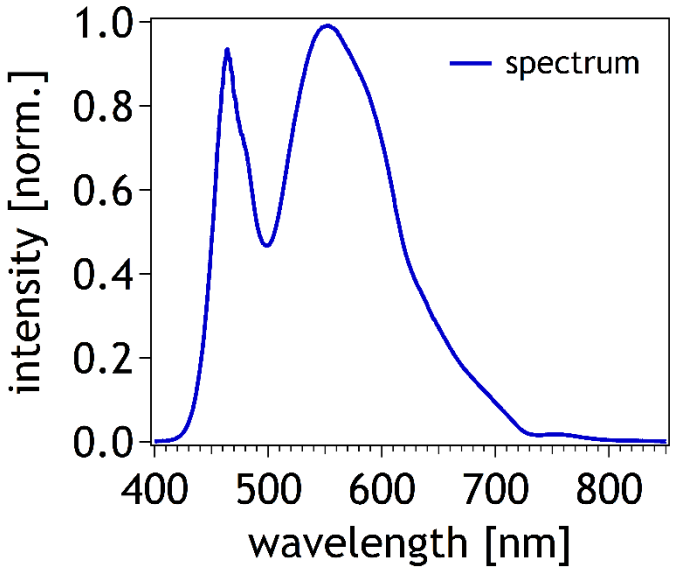
In the PE Channel 585/20 and the PE-Cy7 Channel 780/60 the improved excitation efficiency leads to a 2-3 times higher detection efficiency.

*quanti*Flash® : schematic setup



Specifications of *quanti*Flash®

Pulse width	1-10 µs, variable
Repetition rate	0.5-20 kHz, variable
Pulse shape	variable
Pulse amplitude	0 … -96 dB
Pulse amplitude precision	CV < 0.1%
Fiber coupled	f-SMA termination
Power supply	rechargeable, USB powered



*quanti*Flash® Software

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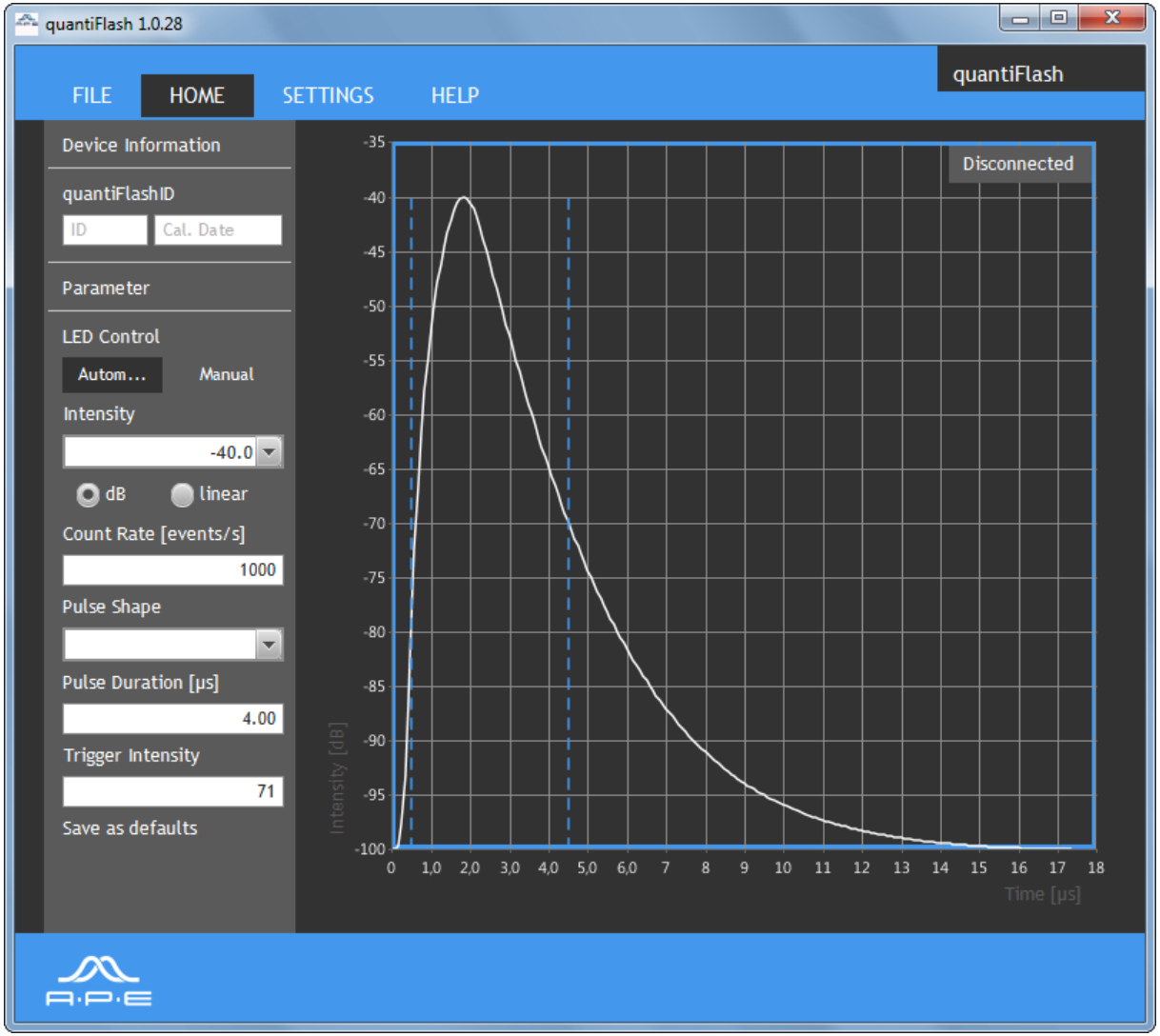
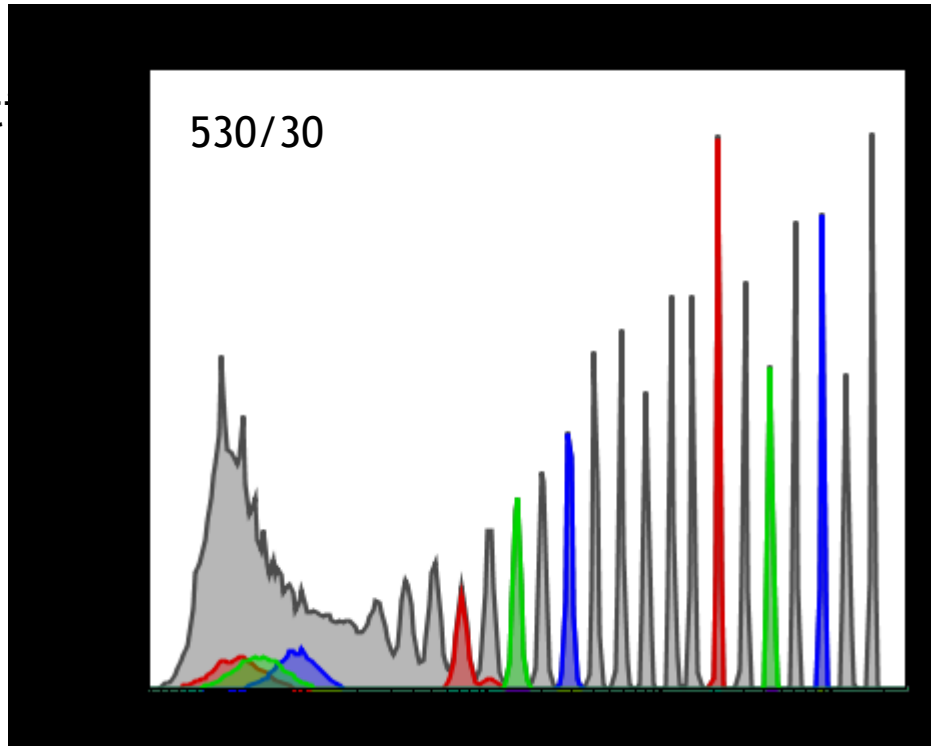
Graphical user interface for comfortable setup

- save/load parameter sets
- API for scripting (based on TCP/IP)
- easy to use software and firmware upgrades („one-click“)



Application examples using *quanti*Flash®

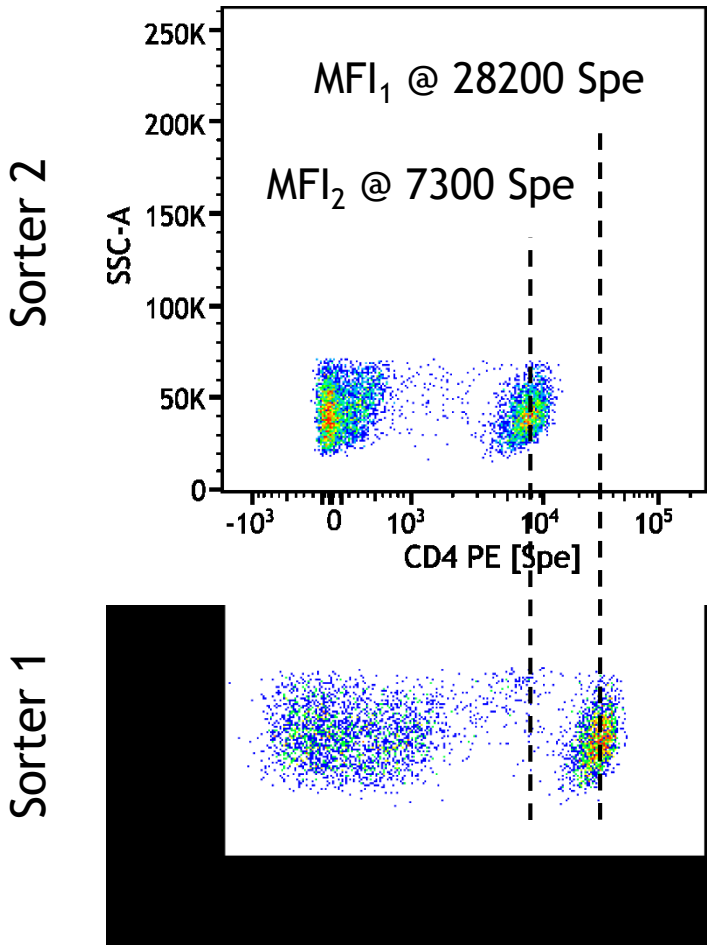
- Scale calibration (channels → Spe)
- Simulating multi-peak beads
- High-quality characterization measurement
- Optimized PMT voltages
- Check of detectors independent of laser alignment and sample preparat
- Check of linearity of detectors
- Study effects of pulse shape / pulse duration (check doublet discrimination)
- Gerneral alignment tool
- …



Simulated 30-peak beads (3 dB intensity steps) measured on FACSAria I, T. Kaiser / DRFZ

Scale calibration – part II

Calibrated data to Spe



1. Reference sample of single stained CD4-PE human PBMCs measured on two different sorters
2. Scale calibration to Spe using *quanti*Flash®

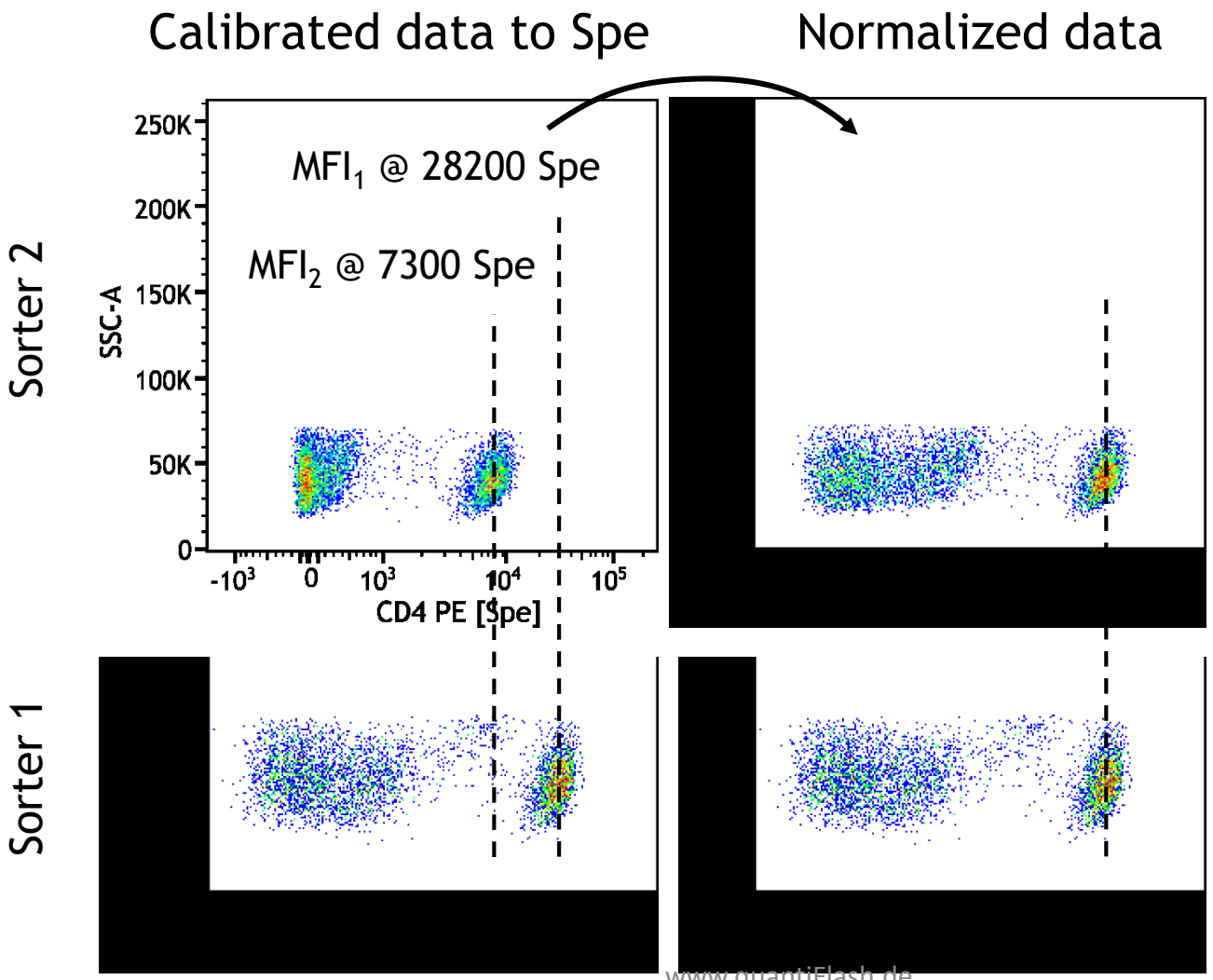
Sensitivity of sorter 1 is about 4× higher

Resolution is the relevant measure depends on sensitivity **AND** background

→ Stain index

SI_1= 22.3, SI_2 = 17.5

Scale calibration – part II



Normalization factor

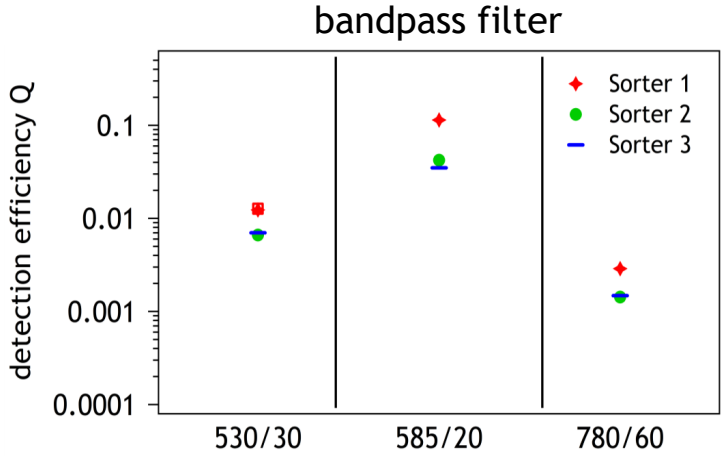
$$\frac{MFI_1}{MFI_2}$$

Summary

- Calibration is important for reproducible measurements and results
- *quanti*Flash®: a new tool for calibration
- Calibration of intensity scales leads to a meaningful basis for comparision

Calculation of Q

Q = Spe / ABC (or MESF)



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Wake Forest University School of Medicine, USA

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Backup Slides

Q & B measurement (by Jim Wood)

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Sample Name V570A
Date 08-Dec-2015

Version 1.11 12/04/2015

	Mean	SD	SD^2	Fluorescence (F) Spe	All Photonic (F + B) Spe	ERF	Q
1	52696	333	110889.00	32470.09	32540.42	68070.96	4.77E-01
2	22639	200	40000.00	13949.64	14019.97	29244.32	4.77E-01
3	9636	121	14641.00	5937.49	6007.82	12447.47	4.77E-01
4	3911	86.8	7534.24	2409.87	2480.20	5052.10	4.77E-01
5	1217	47.7	2275.29	749.89	820.22	1572.08	4.77E-01
6	120	19.4	376.36	73.94	144.27	155.01	4.77E-01
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
Marker Beads	46448			28620.21	28690.54	60000.00	4.77E-01

CLEAR

PASTE

CALCULATE

Intrinsic CV	0.28%
Spe per Channel	6.16E-01
Background Intensity Units	1.14E+02
Background Spe	7.03E+01
Background ERF	239.29
Goodness of Fit	1.000
Q	0.4770