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

Check it out! Have a look at *quanti*Flash® at APE's booth

Konrad von Volkmann[1], Kristen Feher[2], Jan Popien[1], Toralf Kaiser[2]

[1] APE Angewandte Physik & Elektronik GmbH, Plauener Str. 163-165, 13053 Berlin, Germany
[2] Deutsches Rheuma-Forschungszentrum, Charitéplatz 1, 10117 Berlin, Germany

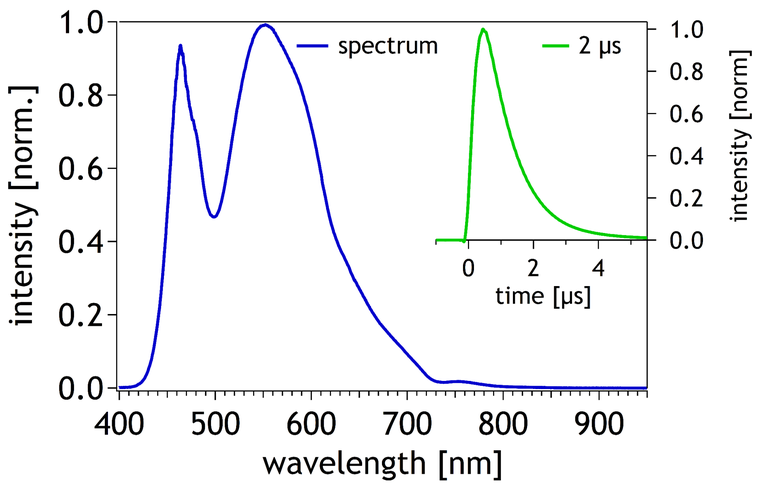
Motivation

Traditionally, flow cytometers are characterized (sensitivity, linearity, long time stability etc.) by fluorescent microspheres. As shown by others*) the coefficient of variation (CV) of a stable light source can be used for scale calibration in numbers of estimated photoelectrons. This allows the quantitative comparison of flow cytometers in terms of light detection efficiency. Due to the intrinsic CV (2-4%) of microspheres they are not suitable for such calibration. Here we show a comparison of the detection efficiency of 3 flow cytometers (FACSAria™) using *quanti*Flash®.
*quanti*Flash ® is an ultra stable (CV < 0.1%) easy to use LED pulse generator made for cytometer characterization.

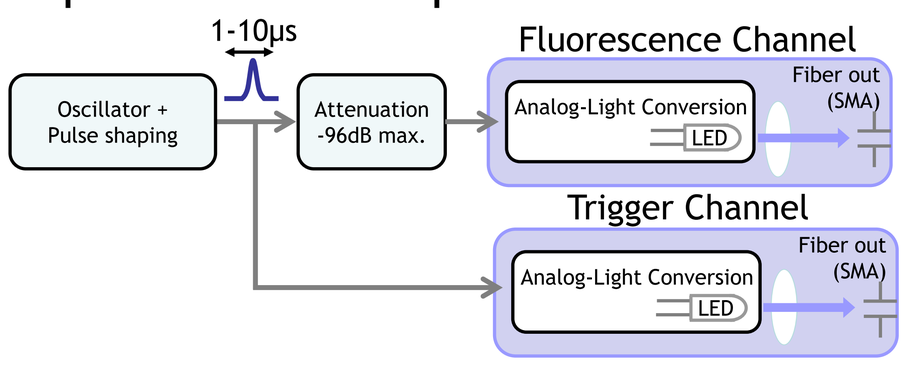
*) H. B. Steen, „Noise, Sensitivity, and Resolution of Flow Cytometers“, *Cytometry*, Bd. 13, Bd. 8, S. 822-830 (1992)
M. J. McCutcheon und R. G. Miller, „Fluorescence intensity resolution in flow systems.“, *J Histochem Cytochem*, Bd. 27, Nr. 1, S. 246-249, (1979)

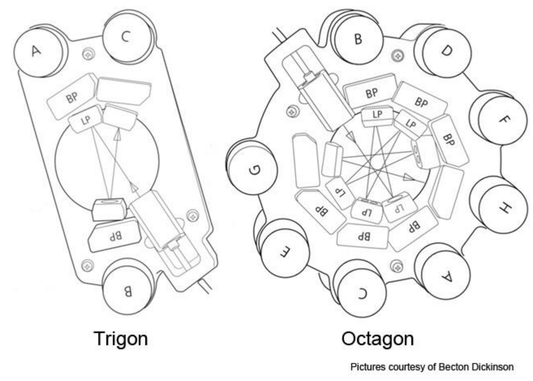
*quanti*Flash®

Specs	V2 digital
Pulse width	1-10 µs, variable
Repetition rate	0.5-10 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



Experimental setup



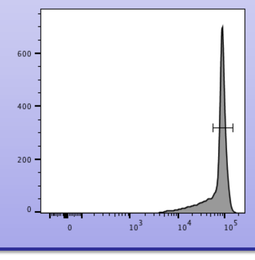


Pictures courtesy of Becton Dickinson

Scale calibration in ABS

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

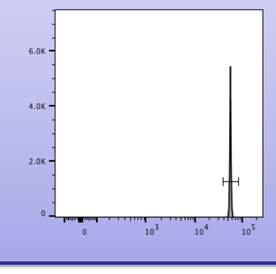
Quantum™ Simply Cellular® Microspheres



Scale calibration in statistical photoelectron estimate (Spe)

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

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



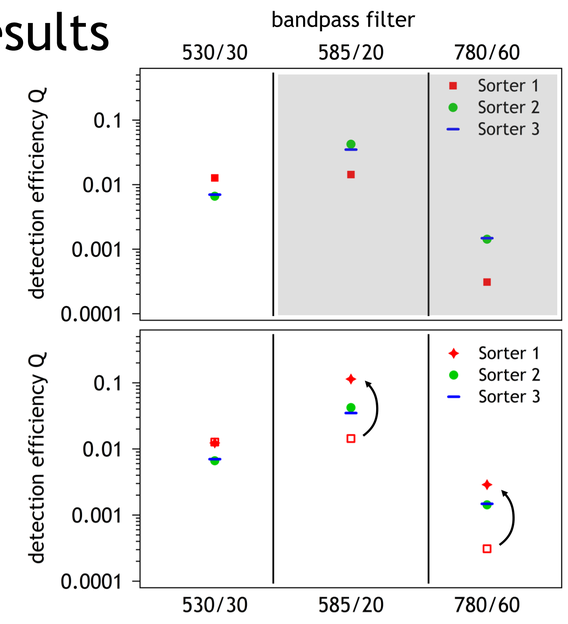
Calculation of Q *)

Q = Spe / ABC

1/Q gives the required number of ABC to detect one Spe

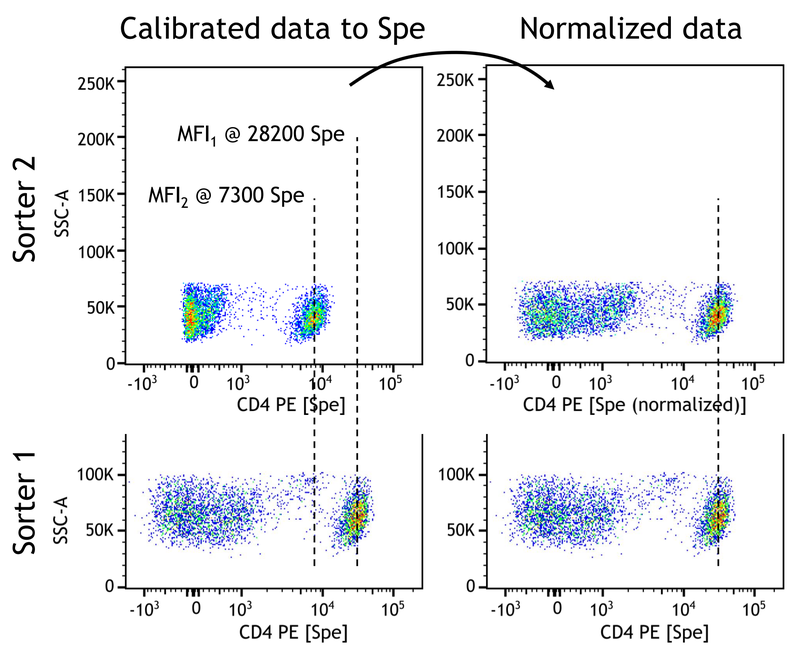
*) **high Q = high detection efficiency**

Results



- comparable Spe values of all sorters
  ➡ detection & PMTs working well
- Significant lower Q of sorter 1 in channels 585/20 and 780/60
  ➡ laser alignment ? Yes, indeed!

Sorter 1: **Q** value is about **2-3 times higher**



1. Reference sample of single stained CD4-PE human PBMCs measured on two different sorters
2. Scale calibration to Spe using *quanit*Flash®
3. Normalization factor ist given by

$$MFI_1 / MFI_2$$

Conclusions

*quanti*Flash® allows the quantitative characterization of flow cytometers defined as Q. Thus, it is possible to predict the optical detection efficiency of a certain channel. This information is useful for optimal panel design. Moreover, *quanti*Flash® allows the comparison of flow cytometers regardless of the manufacturer design which is very useful in multicenter studies or even long-term experiments.