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Automated cytometer characterization using calibration particles in combination with an LED pulsed light source

Toralf Kaiser[1], Ana Teichmüller[1], Sjoerd Visser[2], Jenny Kirsch[1], Kerstin Heinrich[1], Konrad v. Volkmann[2], Erik Mul[3], Simon Tol[3], Claudia Giesecke-Thiel[4]

[1] DRFZ, Berlin, Germany, [2] APE GmbH, Berlin, Germany, [3] Sanquin Center, Amsterdam, Netherlands, [4] MPI for Molecular Genetics, Berlin, Germany

correspondence APE quantiFlash: konrad_vonvolkmann@ape-berlin.de **scientific correspondence: kaiser@drfz.de**

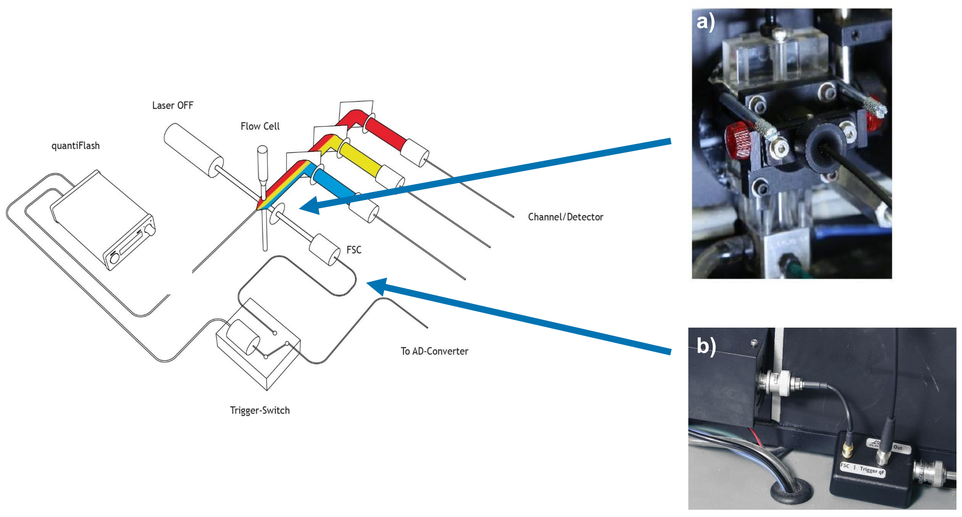
1. Introduction

Quality control and performance tracking of a flow cytometer is a prerequisite for measurement assurance. State of the art bead-based approaches can only evaluate flow cytometer performance in total as sum of all components. This complicates determination of single component performance and the origin of potential issues. Here, we present an LED pulser based method for quality control which in combination with bead measurements allows dissection of illumination characteristics and performance versus detector performance. We present a semi-automated data acquisition method that enables full characterization of the cytometer detection performance in less than half an hour.

3. Setup & Measurement

Four different steps were performed on three flow cytometers (2 BD FACS Symphony and one FACSAriaFusion) from three different institutes in Berlin (Germany) and Amsterdam (Netherlands):

1. Connection of LED pulser to a) flow cell and b) FSC trigger cable



2. quantiFlash measurements for detector characterization

- Intensity ramp-up of LED pulses in steps of 2dB from -96dB to 0dB (for details see quantiFlash specifications), collecting 10,000 events per step, exported as *.fcs file. Repeatedly performed on PMT voltages from 300V to 900V in steps of Δ50V for each detector. Lasers were switched OFF.

- Laser background (LIB) determination: Collection of 10,000 trigger events (LED pulser intensity OFF) for PMT voltages from 300V to 900V in steps of Δ50V with lasers switched ON, saved as *.fcs file

3. Calibration particle measurements

- Measurement of peak 2 Beads (Spherotec) again for PMT voltages from 300V to 900V in steps of 50V. Collecting 10,000 events for each voltage and saved as *.fcs file.

4. Data processing

Data processing was performed using a Phyton script provided by APE (online download from quantiFlash website).

- definition of a reference signal S_{100pe} (100 photoelectrons at 600V)
- calculation of scale calibration factor K for each detector
- output of SNR (based on S_{100pe} to LIB) and DNR curves
- output of all relevant statistical values

Conclusion

- The measurement of LED pulses and calibration particles allows an independent characterization of illumination- (laser) and detector performance, the latter can be determined in an automated fashion
- The SNR and DNR values of the detectors differed by a maximum of 12dB
- Based on the measurement of beads, a difference of up to 20dB could be determined between the comparison instruments. This difference is apparently due to different laser power & alignment

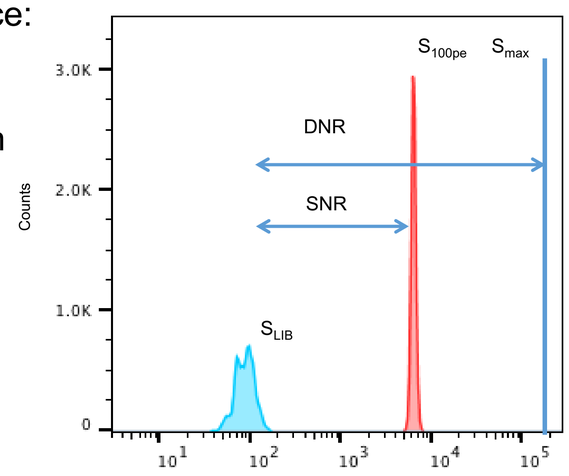
2. Theory[1]:

quantiFlash measurement:

- characterization of detection performance: SNR and DNR
- visualization of signal intensities **S** on a calibrated decibel scale using calibration factor **K**:

$$\mathbf{SNR_{dB}} = 20 \times \log_{10}(S_{100pe}/S_{LIB})$$

$$\mathbf{DNR_{dB}} = 20 \times \log_{10}(S_{max}/S_{LIB})$$



$$\mathbf{K} = \frac{\mathrm{N_{pe\_qF}}}{\mathrm{Mean}_{qF}} \quad \text{with} \quad \mathbf{N}_{pe} = \frac{1}{CV_{qF}{}^2}$$

calibration particles:

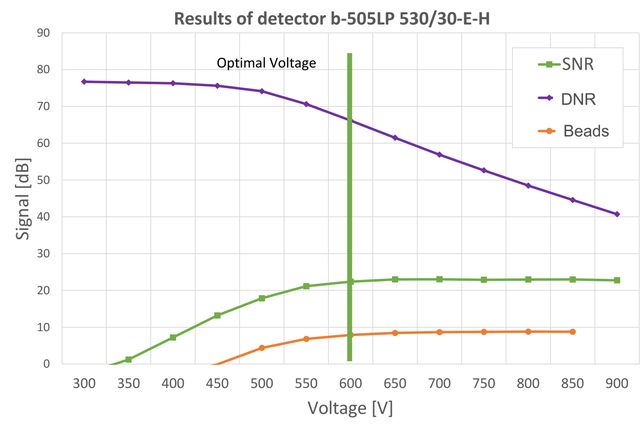
- characterization of the instrument as a entire unit (signal processing, optics, laser, detectors)
- visualization of bead intensities in relation to LIB on calibrated decibel scale

$$\mathbf{Beads_{dB}} = 20 \times \log_{10}(S_{beads}/S_{LIB})$$

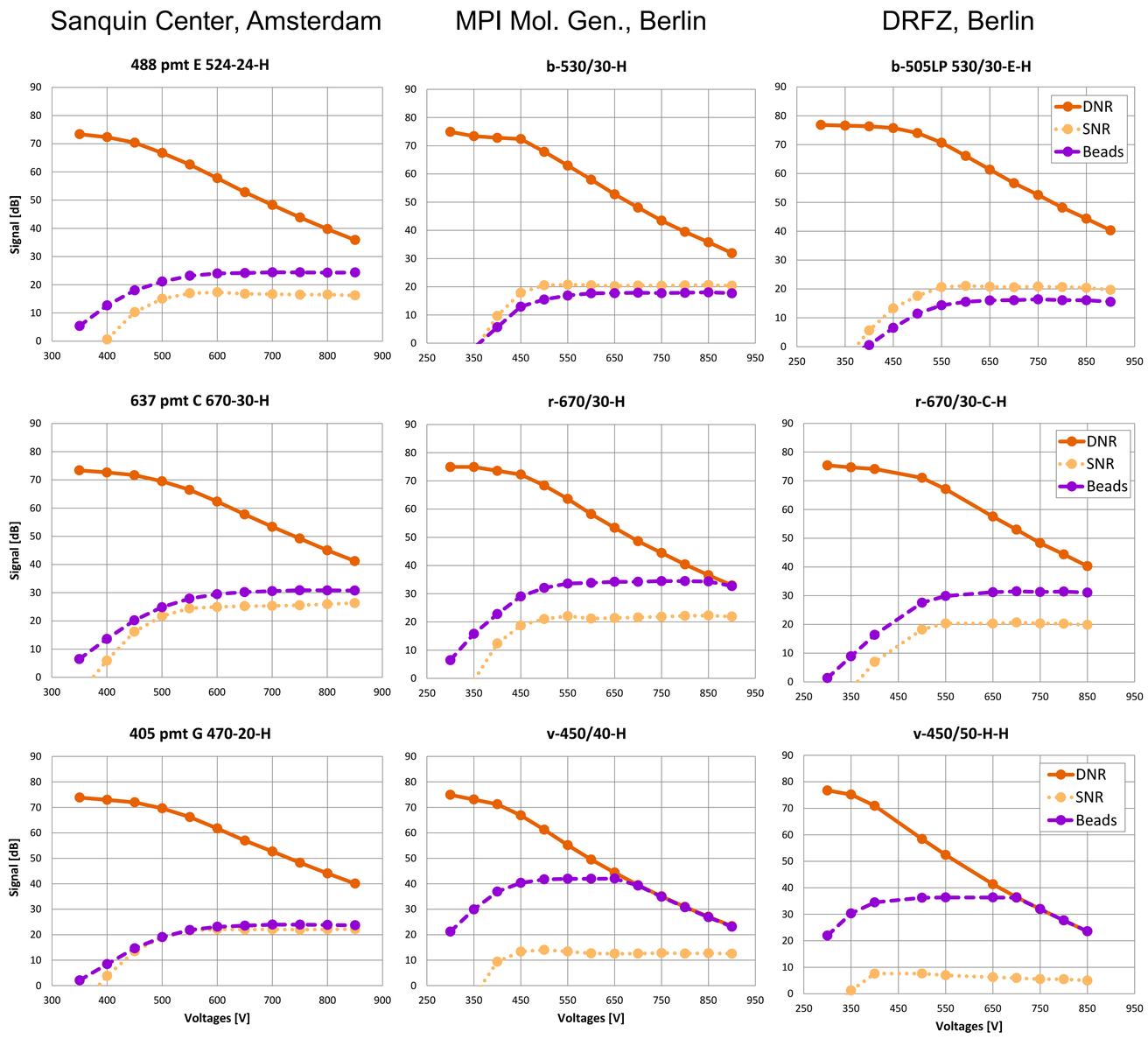
4. Results:

Finding the optimal PMT voltage with quantiFlash

The optimum PMT voltage in this example is at 600V. A further increase of the voltage will reduce the DNR and not increase the SNR.



Instrument comparison:



[1]Further details on method can be found at:
Giesecke, C., Feher, K., von Volkmann, K., Kirsch, J., Radbruch, A., & Kaiser, T. (2017). Determination of background, signal-to-noise, and dynamic range of a flow cytometer: A novel practical method for instrument characterization and standardization. *Cytometry Part A*, *91*(11), 1104-1114.

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