Comparison study of flow cytometers combining novel ultra-bright calibration particles with state-of-the-art characterization methods

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Introduction

Good flow cytometer setup is fundamental to empower good experimental results. In simple words: for maximum resolution of the populations of interest the primary goal is that the negative populations should be above noise and positive populations should be onscale. In engineering sciences specific performance metrics are assigned to these requirements, namely signal-to-noise (SNR) and dynamic range (DNR).

There are many factors that influence a flow cytometer's sensitivity. One of the prerequisites for the best sensitivity of a cytometer is the selection of the optimal PMT voltage. Beads are well established to determine the optimal voltage, e.g. by measuring their fluorescence at different PMT voltages. However, such a measurement is a result of the instrument performance and the excitation/emission properties of the beads. Therefore, the voltages determined may not be applicable to cells with different autofluorescent properties. Using a pulsed precision LED light source instead, the *quanti*Flash® (APE GmbH), offers an independent possibility to deeply characterize, calibrate the detectors (PMTs or APDs) and choose PMT voltages wisely.

Combining this with well-established bead-based methods give a whole new perspective on the instrument's performance and characteristics. Such knowledge allows to discriminate biological background signals from technical ones which can help with experiment design.

Furthermore, SNR and DNR are stand-alone metrics that allow a direct qualitative comparison of different instruments.

Literature

[1] H. B. Steen, *Cytometry*, **13**, 822–830 (1992)

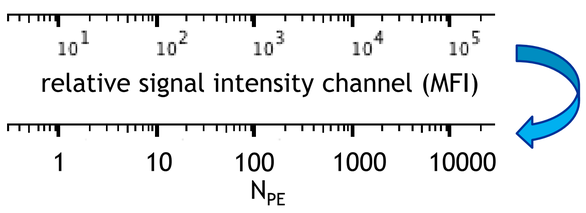
[2] C. Giesecke, *et al. Cytometry Part A, **91**, 1104-1114*

Basics and basic applications

1. Scale calibration

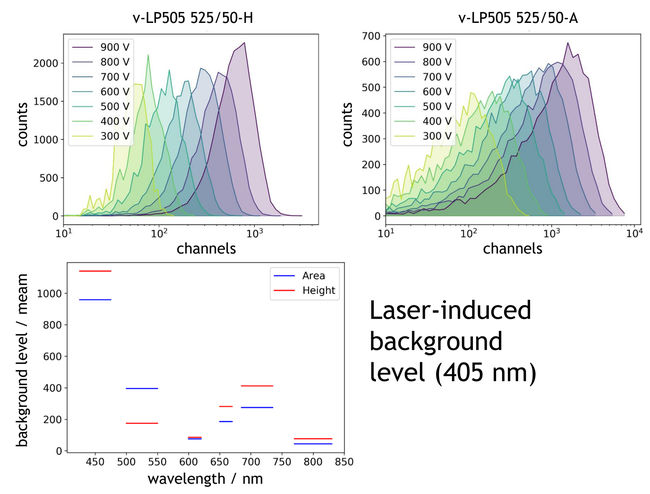
$$CV = \frac{1}{\sqrt{N_{PE}}} \quad \Leftrightarrow \quad N_{PE} = \frac{1}{CV^2}$$

N_{PE}: number of generated photoelectrons



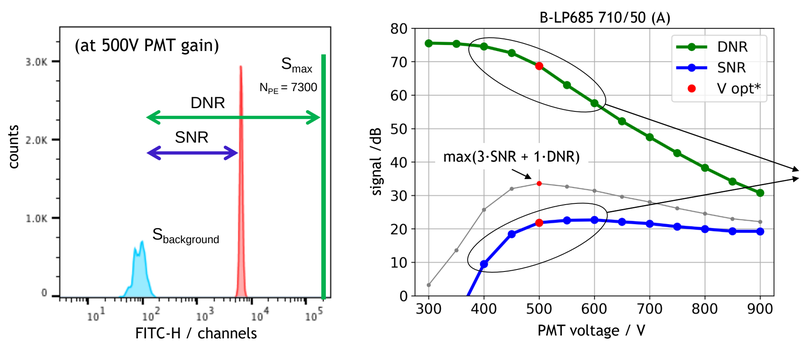
2. Background measurement

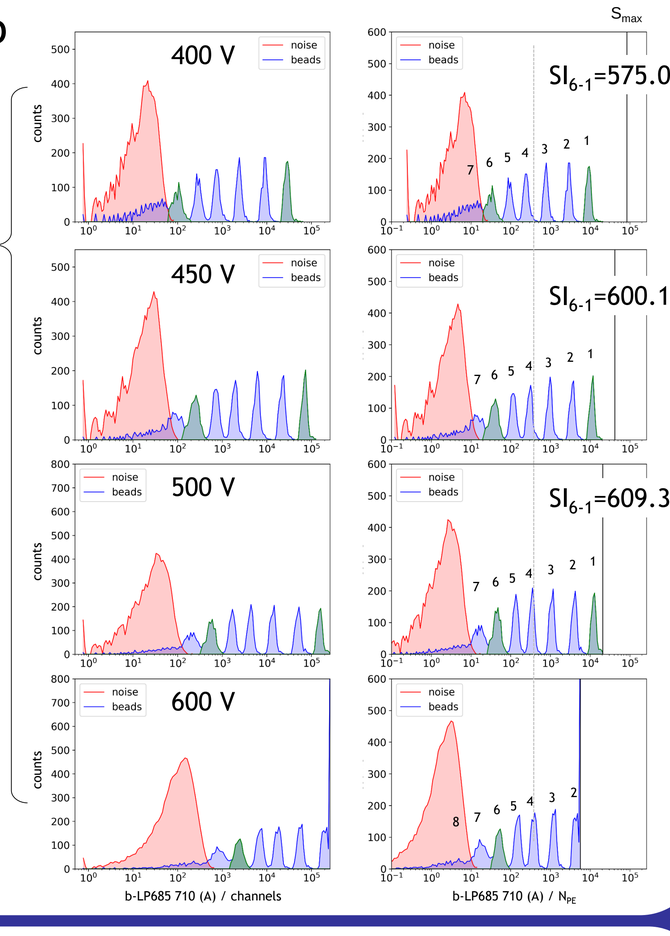
Using 2nd independent output of quantiFlash



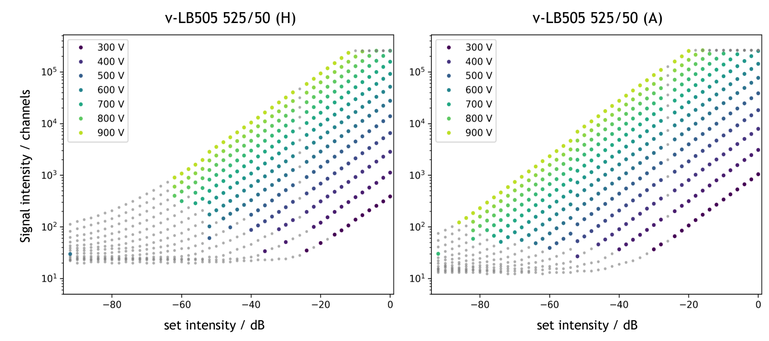
Laser-induced background level (405 nm)

3. Reference signal, SNR, DNR, PMT setup





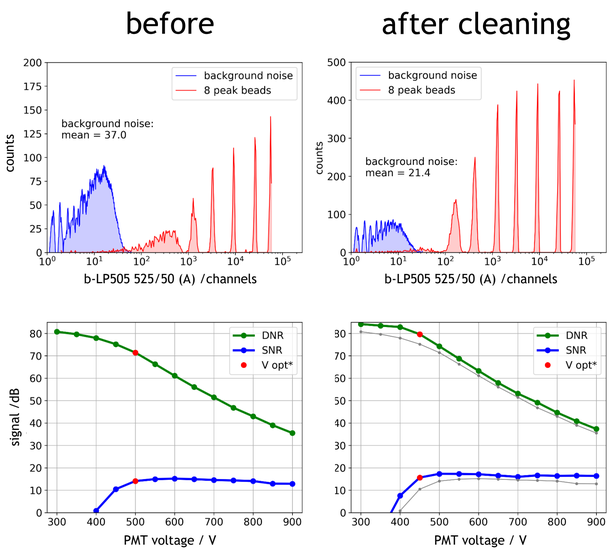
4. Linearity test, detector performance



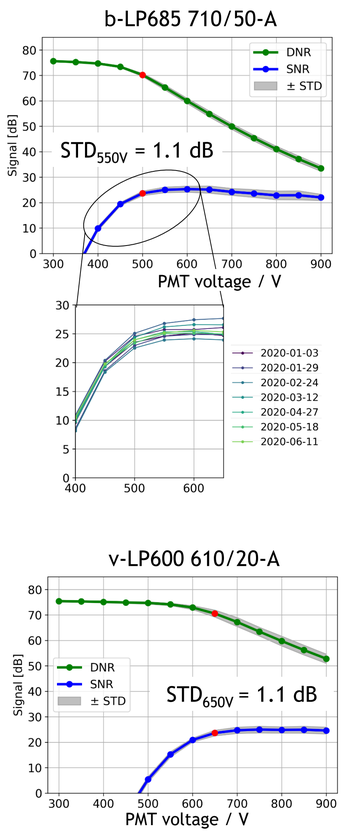
Advanced applications

1. Maintenance: dirty PMT connection

- Low resolution of dim population
- Reduced DNR of ~ -6 dB (= factor 2)
- Higher optimal voltages needed (~50 V)

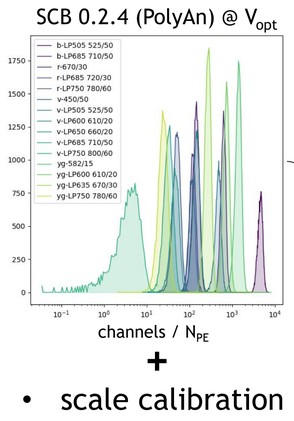


2. Long-term stability



3. Comparison of calibration particle by Spherotec and PolyAn

The novel Spectrum Calibration Beads (SCB 0.2.4) by PolyAn have an extended emission spectrum, especially in the IR. Comparison with Peak-2-Beads by Spherotec



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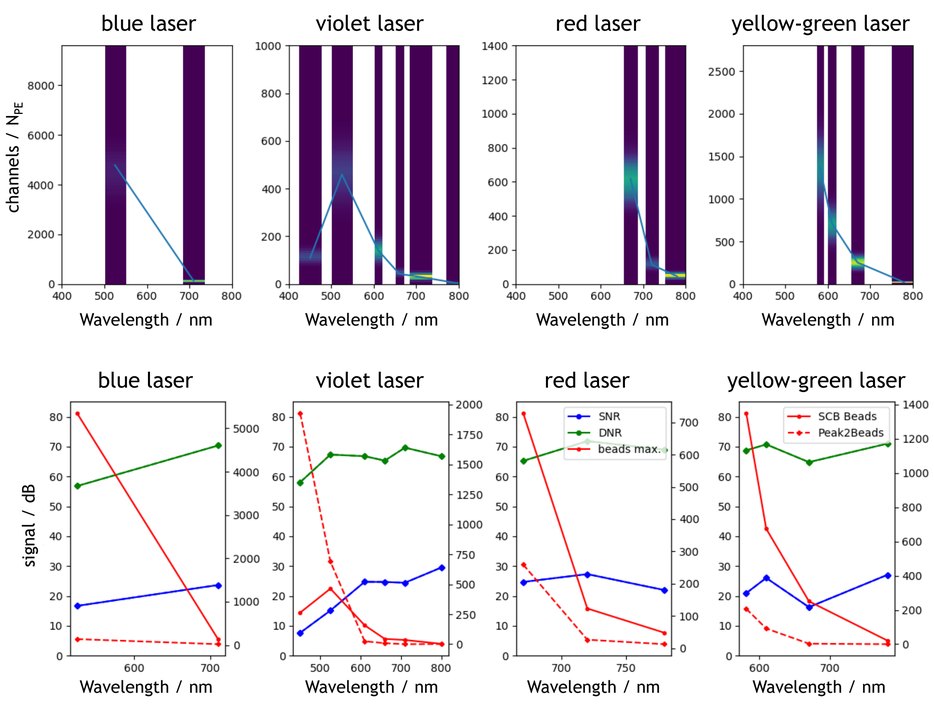
- scale calibration with quantiFlash
- determination of SNR / DNR
- determination of optimal PMT voltage



Summary

Applying the characterization methods of Giesecke *et al.* yields very stable results for the PMTs. Based on these findings we propose new way determine optimal PMT voltages by a weighted sum of SNR + DNR. Combining these results with the established bead-based methods provide a deeper understanding to setup and choose the cytometer parameters. The excellent stability of PMTs proof once again the validity of fixed application settings. If working in the linear range of the detectors, variations due to laser fluctuations or alignment should be compensated by mathematically and not by changing PMT voltages.

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