Photonic calibration, determination of background, signal-to-noise and dynamic range of a flow cytometer – a novel practical method for instrument characterization and standardization

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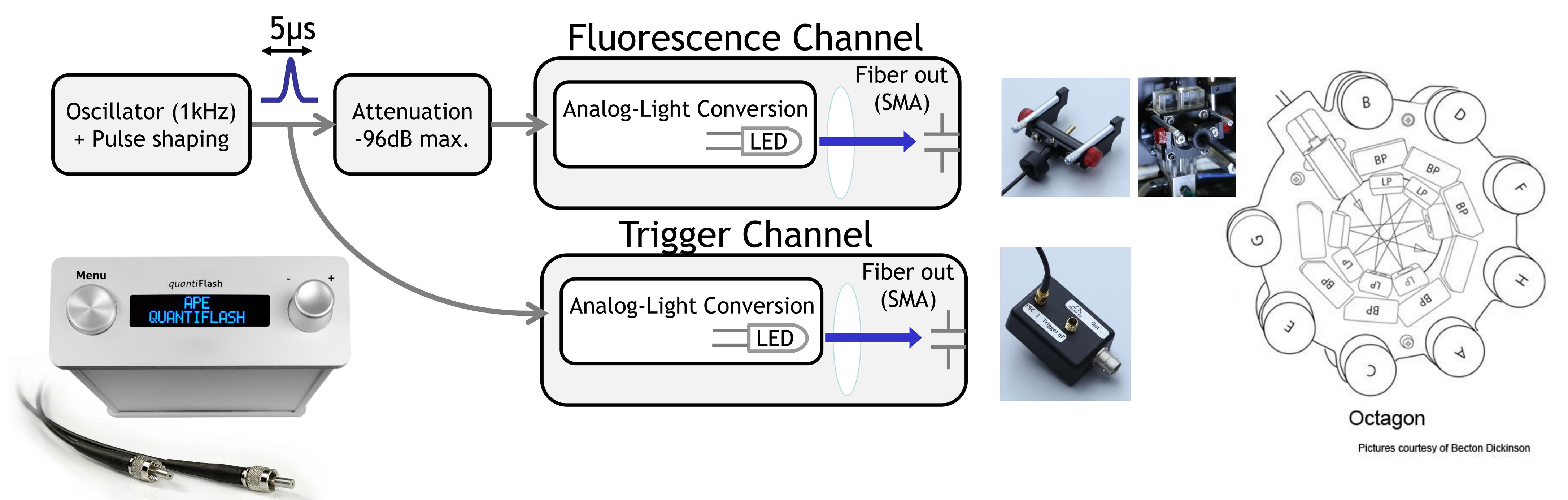
Motivation

A well-defined scale calibration in flow cytometry can improve many aspects of data acquisition such as cytometer setup, instrument comparison and sample comparison. The theory for scale calibration was proposed by Steen over two decades ago [1], but it has never been put into regular use due to the lack of a widely available precision light source. The introduction of such a light source, the quantiFlash®, gave this possibility. Here, we want to describe how this light source can be used to characterize a cytometer's PMT performance and the instrument's response over the entire PMT voltage range.

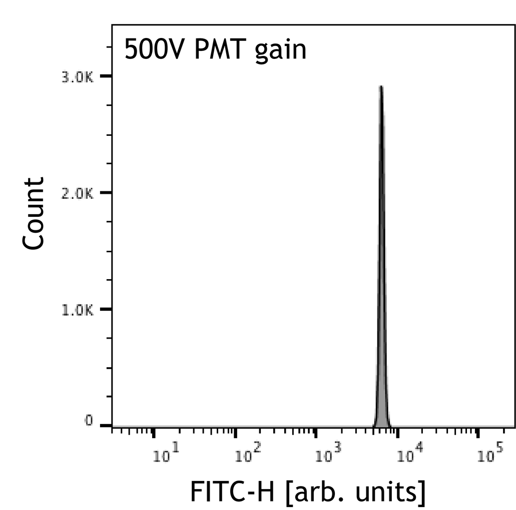
[1] H. B. Steen, „Noise, Sensitivity, and Resolution of Flow Cytometers", *Cytometry*, Bd. 13, Bd. 8, S. 822-830 (1992)

Experimental setup



Calibration steps

1. Scale calibration

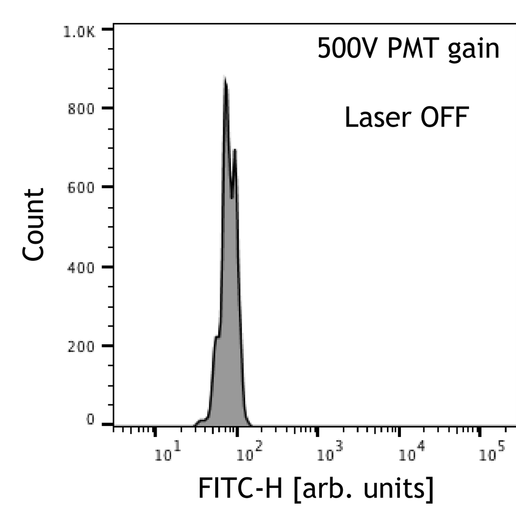


$$CV = \frac{1}{\sqrt{N_{PE}}} \Leftrightarrow N_{PE} = \frac{1}{CV^2}$$

$$K = \frac{N_{PE}}{\text{mean ch.}}$$

CV: coefficient of variation of light pulses
N_{PE}: number of generated photoelectrons
K: calibration factor

2. Measure background

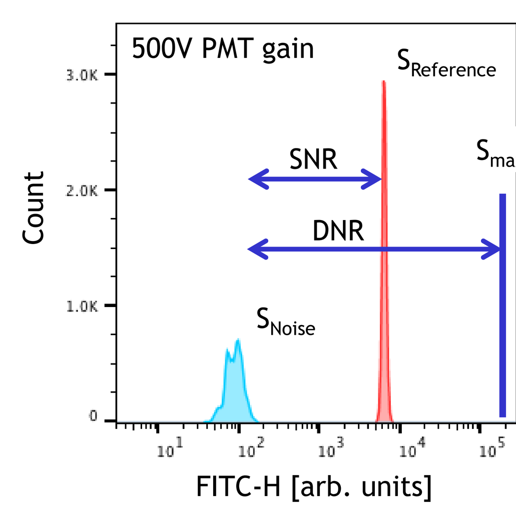


	OFF	ON
GeoMean	76	85
N_{PE} equivalent	5	6

electronic noise

electronic noise + laser induced background

3. Determine SNR and DNR



reference signal: N_{PE} = 445
noise (background): N_{PE} = 6
SNR = 20 × $\log_{10}$(445/6) = **37 dB**

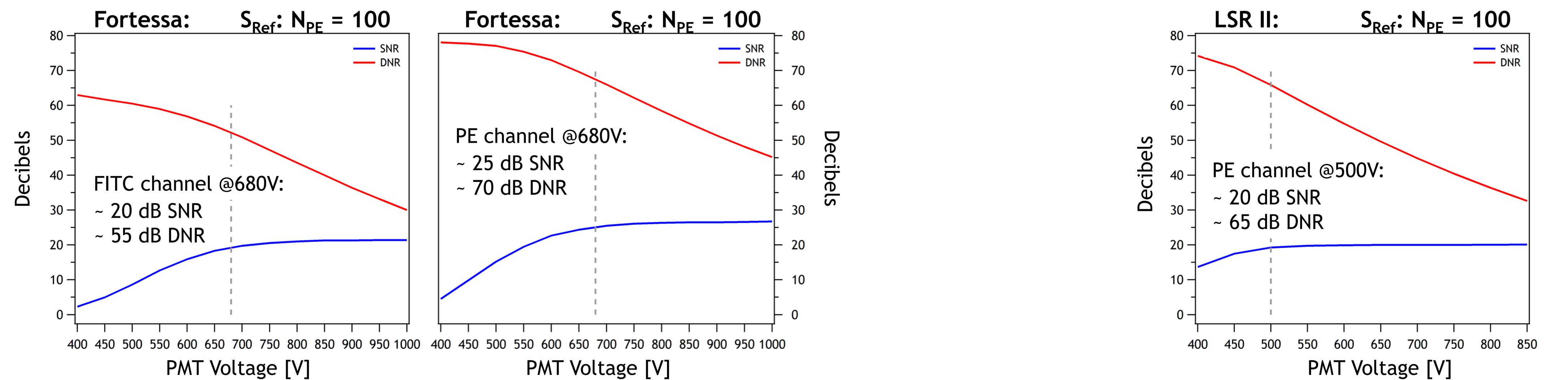
max. signal: N_{PE} = 7300
(= 0.073 × 1e5)
DNR = 20 × $\log_{10}$(7300/6) = **62 dB**

Conclusions

The *quanti*Flash® allows the quantitative characterization of flow cytometers defined by supplying a precise reference signal for comparable SNR and DNR values. Further the possibility to directly measure the background makes it easy to determine whether negative cells can be clearly separated from the background leading to improved results and cleaner sorting results.

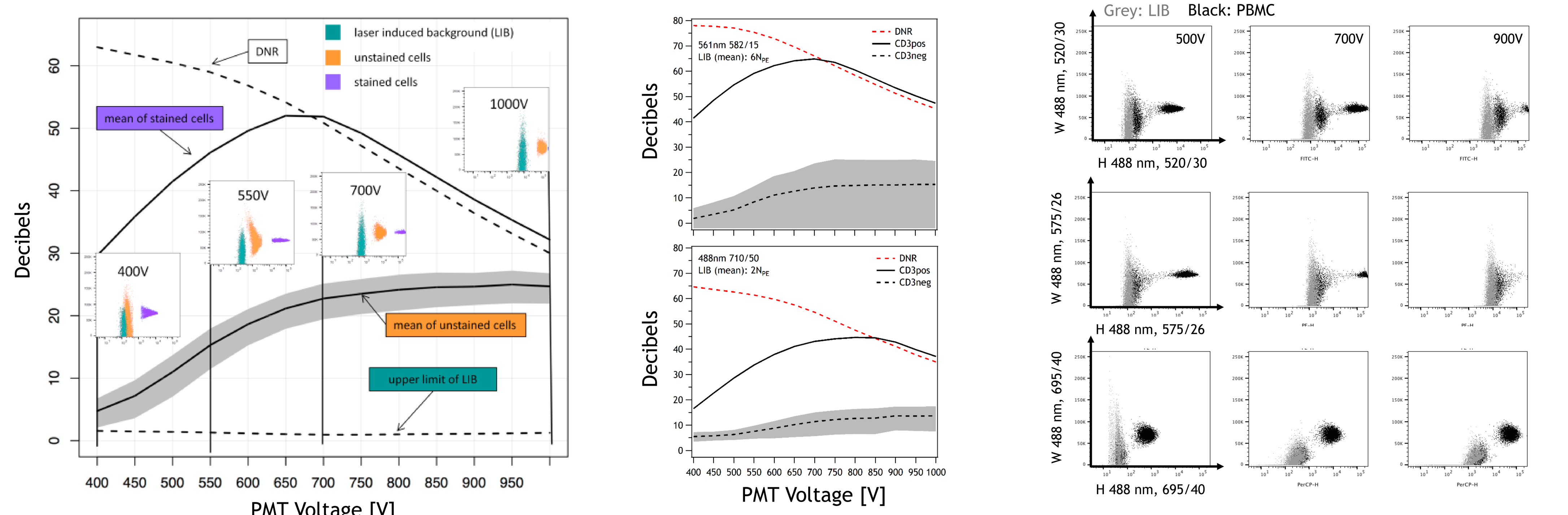
Results and applications

1. Better comparison of channels and cytometers



Measuring SNR and DNR as a function of PMT voltage clearly shows the point where best compromise for best resolution is reached while maintaining a good dynamic range. This value makes is extremely easy to compare different fluorescent channels or even flow cytometer amongst each other.

2. Choosing colors and gain voltages with even more reason



This example shows single stainings of human PBMCs with FITC, PE and PerCP linked to CD3. While both the positive and negative cells are clearly resolved on the FITC channel it is not possible to distinguish them from the background in the other two channels.

3. Transferring gate settings

Setting gates with reference to N_{PE} (calibration step 1) leads to greatly improved counting frequencies when comparing measurements from different sorters.