Supporting information of "Quantification of light scattering detection efficiency and background in flow cytometry"

Customized FACSCanto

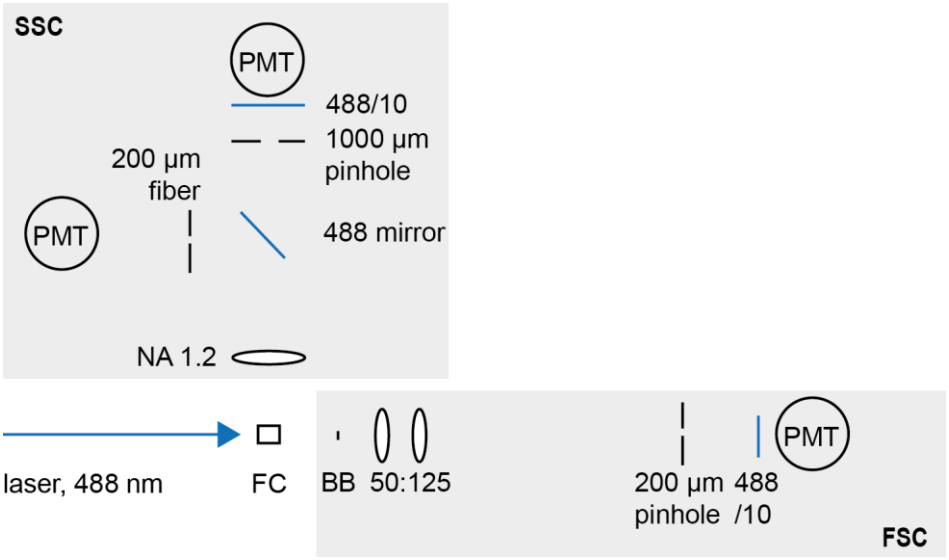

Figure S1: Schematic representation of the detection optics of the customized FACSCanto. 488/10: bandpass filter, 488 mirror: NFD01-488 (Semrock), BB: blocker bar, FC: flow cell, NA: numerical aperture, PD: photodiode, PMT: photomultiplier tube.

Results for the height parameter

The exact same method as described in the manuscript for the area parameter has been applied to the height parameter of the data. The only difference is that for the height parameter, the background signals were measured at each illumination power by measuring PBS while triggering using simulated trigger pulses (quantiFlash, APE Angewandt Physik & Elektronik GmbH, Berlin, Germany), which was not possible for the area parameter due to a difference in trigger pulse widths and thereby integration time for the LED pulser and the beads.

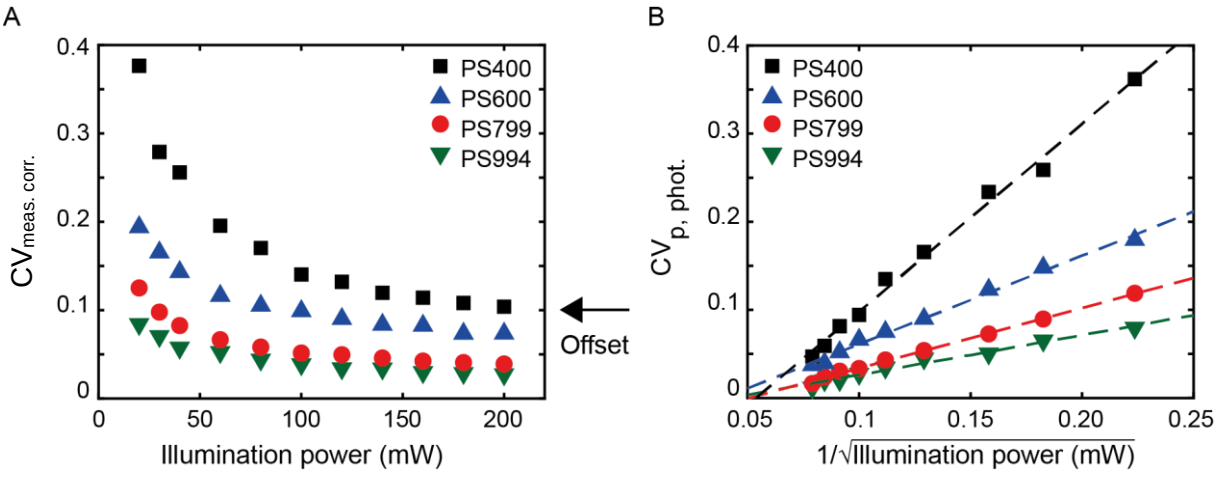

Figure S2: Presence of Poisson statistics in the height parameter of light scatter. A) Background corrected robust coefficient of variation ($CV_{meas.\ corr.}$) on side scatter versus illumination power for polystyrene (PS) beads of different diameters. $CV_{meas.\ corr.}$ decreases with increasing illumination power, because detection of photons can be described by Poisson statistics. The remaining offset in $CV_{meas.\ corr.}$ is due to intrinsic and illumination variations. B) CV due to Poisson statistics ($CV_{p,\ phot.}$) versus $1/\sqrt{illumination\ power}$ for different bead diameters (symbols). A linear relation can be fitted through all bead data (dashed lines, $R^2>0.98$), confirming the presence of Poisson statistics in the signals.

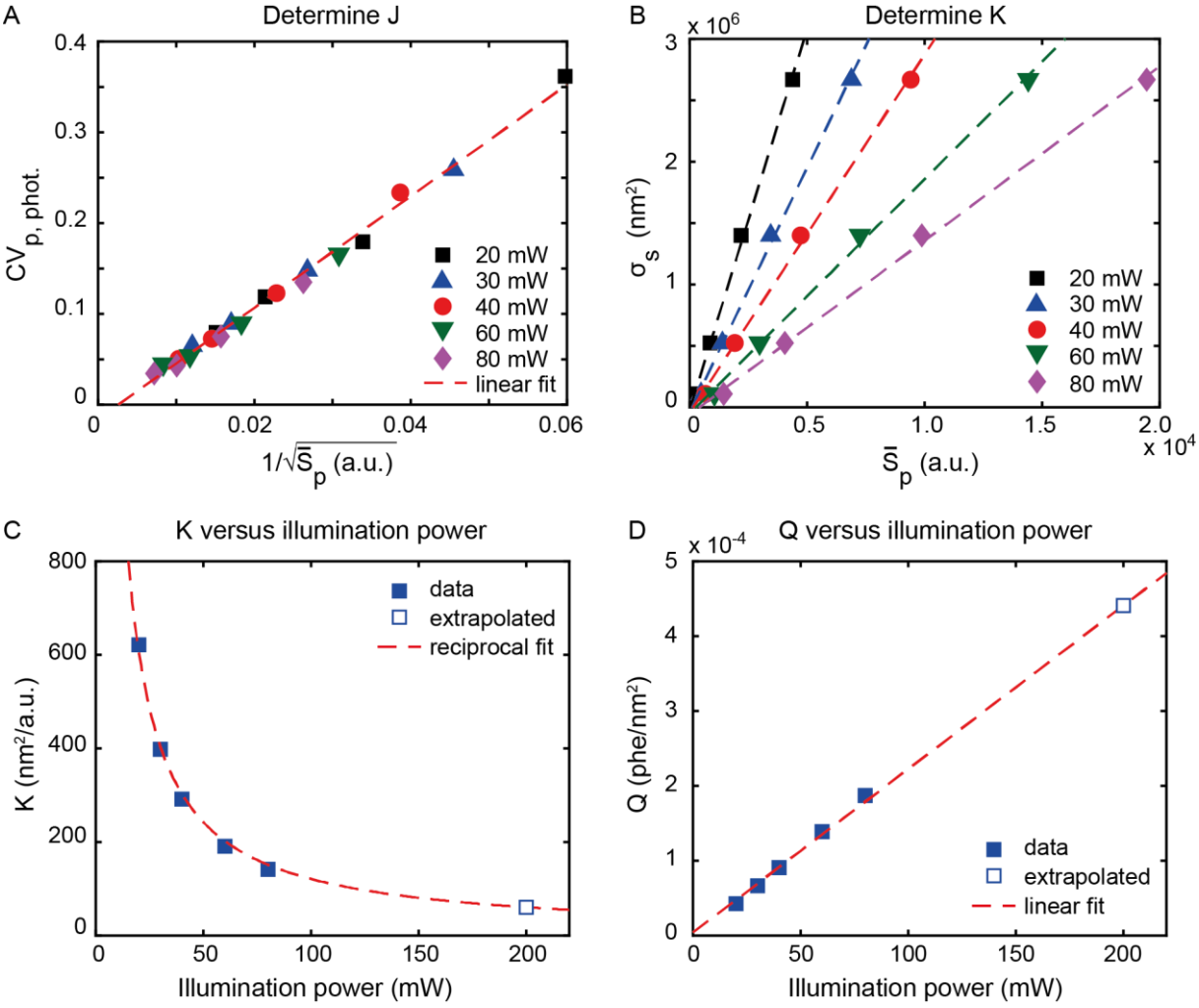

Figure S3: Deriving Q and B for the height parameter. A) Measured robust coefficient of variation due to Poisson statistics ($CV_{p,phot.}$) versus $1/\sqrt{\bar{S}_p}$ (symbols), with $\bar{S}_p$ the background corrected median light scatter signal of 400, 600, 799, 994 nm polystyrene beads at different illumination powers. All data can be described by the linear fit (dashed line, R2=0.993) with slope J = 6.1 $\sqrt{a.u.}$. B) Scattering cross section (σs) versus $\bar{S}_p$ (symbols) of 400, 600, 799, 994 nm polystyrene beads at different illumination powers. The slope of the linear fits (dashed lines, R2>0.998) represents the factor K per illumination power. C) K versus illumination power. The data (symbols) can be described by a reciprocal relation (dashed line, R2=0.996), which was used to extrapolate K at 200 mW. D) Q versus illumination power (symbols). As expected, Q increases linearly (dashed line, $R^2=0.999$) with illumination power.

Table S1: *Q*, *B* and *R* at different illumination powers for the height parameter

Illumination power (mW)	*Q* ($1/nm^2$)	*B* (nm^2)*	*R*	
			(nm^2)*	PS bead (nm)
20	0.000043	$1.90 \cdot 10^4$	$1.23 \cdot 10^5$	412
30	0.000067	$1.48 \cdot 10^4$	$8.19 \cdot 10^4$	373
40	0.000091	$1.27 \cdot 10^4$	$6.20 \cdot 10^4$	349
60	0.00014	$1.14 \cdot 10^4$	$4.38 \cdot 10^4$	321
80	0.00019	$1.04 \cdot 10^4$	$3.43 \cdot 10^4$	303
200**	0.00044_	$9.20 \cdot 10^3$	$1.83 \cdot 10^4$	263

* Total scattering cross section

** Values at 200 mW are calculated using extrapolation of K

PS: polystyrene

Counter intuitively, Table S1 shows that background level *B* decreased with increasing illumination power, which can possibly be attributed to the baseline restorer being more accurate at subtracting high background signals (= at high illumination powers) than low background signals. This phenomenon has been described before [1, 2], and results in a *B* that decreases with illumination power.

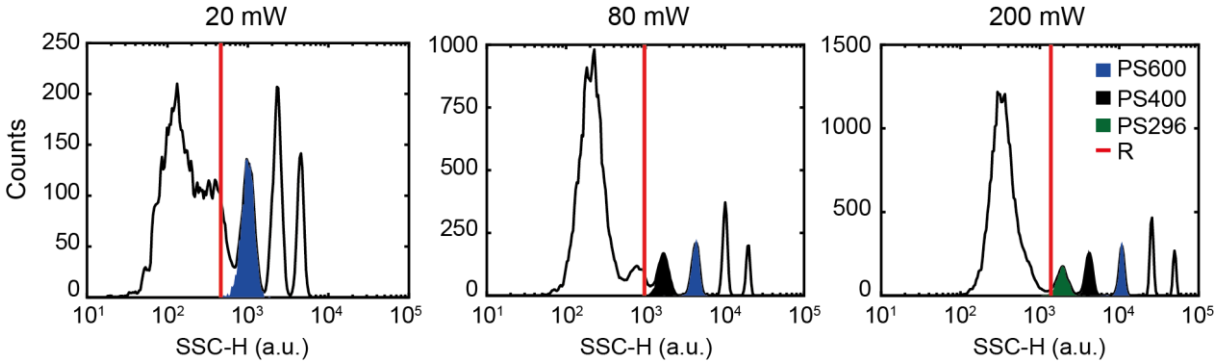

Figure S4: Side scatter histogram of the height parameter (H) of a mixture of polystyrene (PS) beads at 20, 80 and 200 mW as measured by the flow cytometer. The red solid line indicates R as calculated from Q and B and shown in Table S1. Bead populations can be clearly discriminated from the noise for signals exceeding R.

Spectrum of the LED pulser

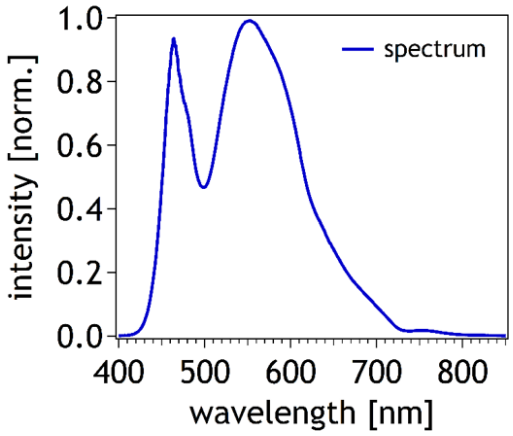

Figure S5: Output intensity versus wavelength of the LED pulser as provided by the supplier.

Standard deviation of the background noise

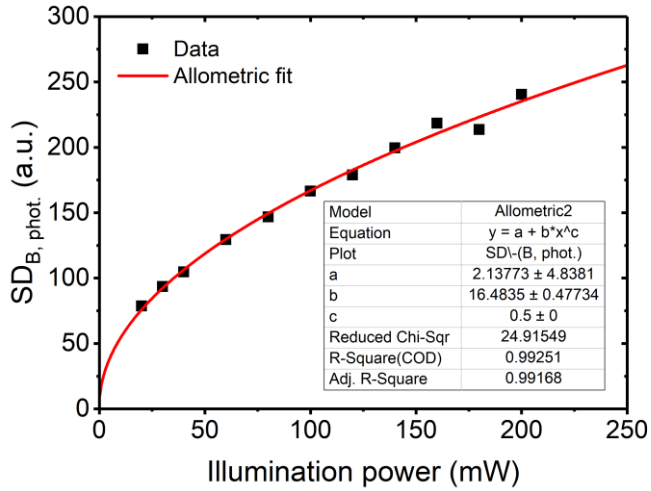

Figure S6: Measured standard deviation of the background noise $SD_{B,\,phot.}$ versus illumination power fitted by an allometric function. Parameter c was fixed at 0.5 to model a square root function. Because the offset (parameter a) at 0 mW is small compared to $SD_{B,\,phot.}$, electronic noise was negligible throughout our experiments.

References

1. Steen HB. Noise, Sensitivity, and Resolution of Flow Cytometers. *Cytometry*. 1992; **13**: 822-30.
2. Wood JC. Fundamental flow cytometer properties governing sensitivity and resolution. *Cytometry*. 1998; **33**: 260-6.