Spherotech, Inc.
27845 Irma Lee Circle, Lake Forest, Illinois 60045

SPHERO™ Technical Note STN-17 Rev A 070915

DETERMINATION OF A FLOW CYTOMETER'S SENSITIVITY USING DETECTION EFFICIENCY (Q) AND THE BACKGROUND LIGHT LEVEL (B)

Introduction

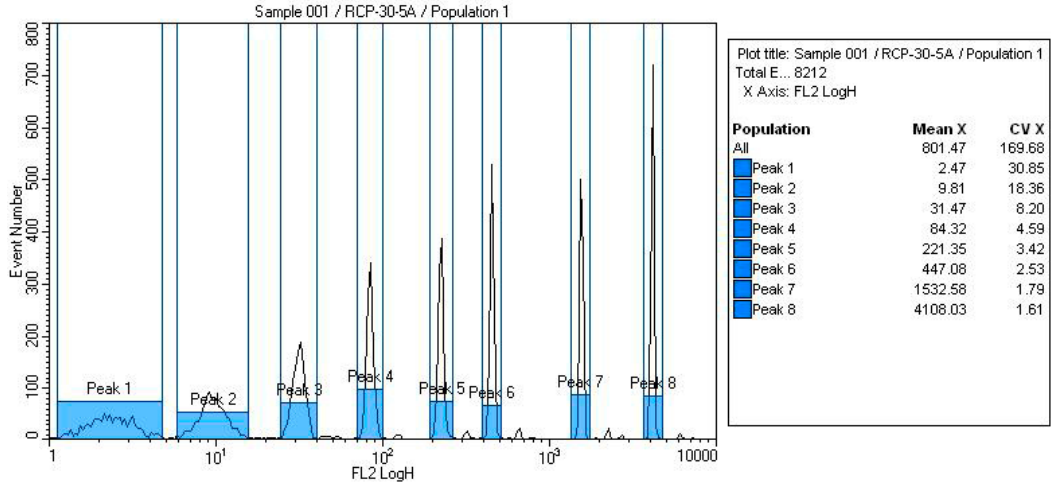
In order to obtain accurate and scientifically sound flow cytometric data it is critical to use standardization techniques and a robust and reliable instrument. The standardization protocol should address specific operational parameters to determine the performance of the instrument at any point in time. The flow cytometer's optic, fluidic and electronics design should be simplistic while still being sensitive and effective.

Experimental Procedure

One major parameter to be included into the standardization protocol is a test for sensitivity. Sensitivity is an important parameter since it defines the ability to detect particles above background. However, sensitivity determination should also include resolution, the ability to resolve dim particles. This can be measured using Spherotech Rainbow Calibration Particles with eight intensities, Cat. No. RCP-30-5A (Rainbow Calibration Particles, 8 peaks, 1E7/mL, 3.0-3.4 µm, 5 mL). Since each intensity of the RCP-30-5A has been calibrated to the molecules of equivalent fluorophores (MEF) it can be used to quantitate sensitivity. **Figure 1** is the histogram of the RCP-30-5A on Stratedigm's S1000 cutting-edge flow cytometer in the PE channel. See http://stratedigm.com/instrumentation for more information on Stratedigm flow cytometers.

A flow cytometer's sensitivity can be described as the detection efficiency (Q) and the background light level (B). The detection efficiency is how well light is collected in the cytometer, while the background light level shows how much noise is created by the instrument in the background. In order to determine the efficiency (Q) and the background light level (B) the MEF values of blank beads and another dim population must be known. These values can be obtained using the Spherotech PMT QC Template. The PMT QC template can be used to determine the linear equation for the 8 peak Rainbow Calibration Beads. As a result, the MEF values for the Dim Bead 1 (Blank) and Dim Bead 2 can be determined based on the Calculated MEF extrapolated from the 8 peak Rainbow Calibration Beads calibration curve after its 256 Relative Channel Number has been determined. See SpheroTECH Note # 8 and 14 for more information on obtaining 256 Relative Channel Numbers, creating calibration curves and determining the MEF of unknowns with the RCP-30-5A, http://www.spherotech.com/tech.htm. **Figure 2** shows the PMT QC Template data entry table and calibration graph for the histogram above.

Figure 1. Stratedigm's S1000 histogram of the RCP-30-5A in the PE channel



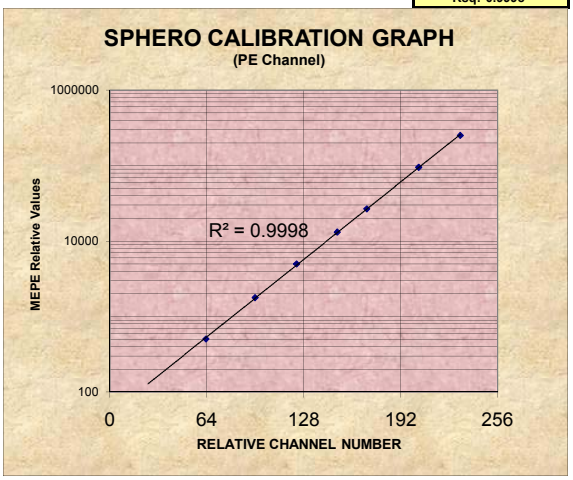
In addition, **Figure 3** shows the calculations of detection efficiency (Q) and the background light level (B) for Stratedigm's S1000 based on the PMT QC template and histogram. Once Q and B values are obtained a Levey-Jennings plot of the data can be used to monitor the performance over time. As a result, the system performance can be verified and validated at any given point in history.

Figure 2. Spherotech PMT QC Template for the determination of the Dim Bead 1 and Dim Bead 2 MEF values

PMT LINEARITY QC RECORD

PEAK #	CH #	MEPE	MEPE LOG	CALC.	RESIDUAL	CALC. MEPE
1	25.13			2.109		128
2	63.47	505	2.703	2.723	7.30%	529
3	95.87	1777	3.250	3.243	2.20%	1748
4	123.26	4974	3.697	3.682	4.08%	4805
5	150.09	13118	4.118	4.112	1.50%	12934
6	169.62	26757	4.427	4.425	0.56%	26604
7	203.87	94930	4.977	4.974	0.71%	94161
8	231.27	250470	5.399	5.413	2.67%	258936
			Ave Residual		2.72%	

Slope: 0.0160
Intercept: 1.7058
Rsq: 0.9998



a. Highlighted fields are obtained from the RCP-30-5A histogram and PMT QC Template

Microsphere	Linear MFI	%CV	Assigned MEF
RCP-30-5a Peak 8	231.27	1.61	250470
Dim Bead 1 (Blank)	25.13	30.85	n/a
Dim Bead 2	62.40	18.36	n/a

b. Enter MEF value of dim beads from PMT QC Template Calculated MEF

Microsphere	MEF
Dim Bead 1 (Blank)	128.0
Dim Bead 2	529.0

c. Determine SD^2 of dim beads from CV:
$SD^2 = (MEF)^2 \times (CV^2 \text{ of dim bead} - CV^2 \text{ of Peak 8})$

Microsphere	CV	CV2	MEF	MEF^2	SD^2
Brightest Bead	0.01610	0.000259	n/a	n/a	n/a
Dim Bead 1 (Blank)	0.30850	0.095172	129.0	16384.00	1555.06
Dim Bead 2	0.18360	0.033709	529.0	279841.00	9360.61

d. Calculate Detection Efficiency (Q) :
$Q = 1 / (CV^2 \times MEF_{Blank})$

Q
0.082

e. Calculate Background (B) :
$B = (SD^2_{Blank} / SD^2_{Mid}) \times MEF_{Blank}$

B
21.26

Summary

By using the Spherotech 8 peak Rainbow Calibration Particles (Cat. No. RCP-30-5A) and the Stratedigm's S1000 robust and reliable data is obtained when a standardization protocol is successfully adopted and implemented. The standardization protocol should include the statistical analyses of sensitivity measurements to promote innovative, scientifically sound experimental results.