

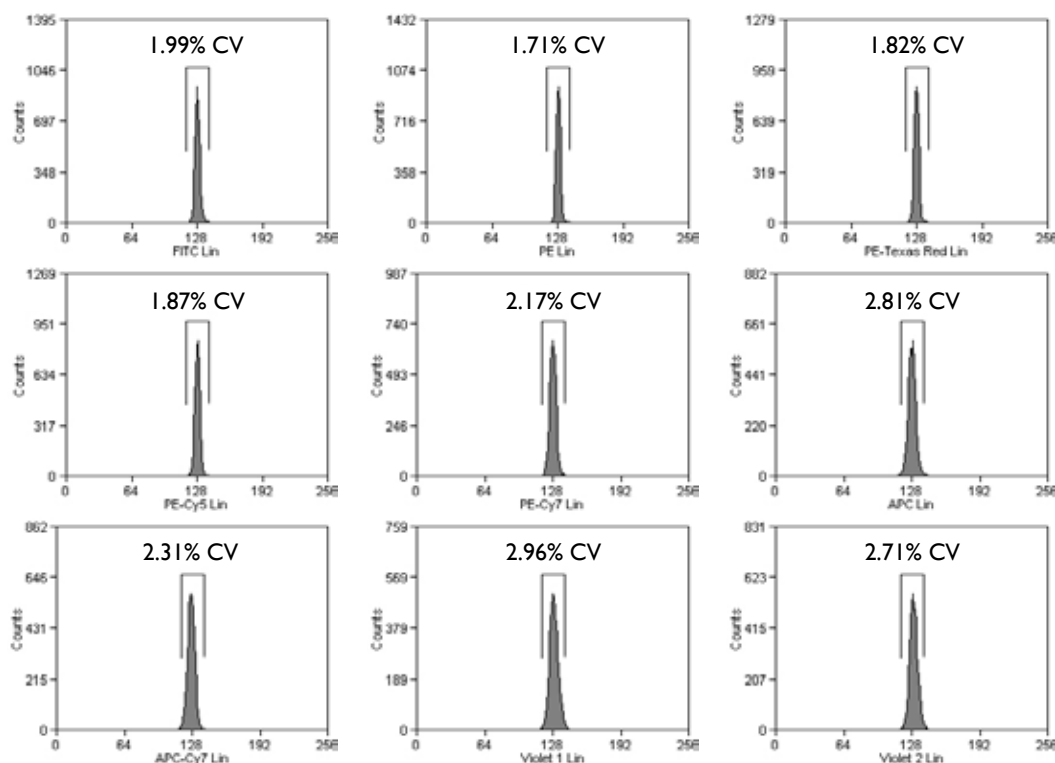
## SPHERO™ Ultra Rainbow Fluorescent Particles

- Consists of a single peak for optical alignment of any flow cytometer in all channels from UV to Far Red
- Determines if the flow cell is clean and without fluidic blockage
- Measures the coefficients of variation (CVs), peak channels, and histogram distribution to determine the functionality of flow cytometers.

New flow cytometers, with fluorescent channels from the UV to Far Red, and corresponding fluorescent conjugates are now available. As a result, we have developed the Ultra Rainbow Fluorescent Particles with enhanced UV and Far Red fluorescence intensity. The Ultra Rainbow Fluorescent Particles contain a single peak and are designed for checking the optical alignment of any flow cytometer in all channels.

Particle Type and Surface	Size, $\mu\text{m}$	Catalog No.	Unit
Ultra Rainbow Fluorescent, $10^{10}/\text{mL}$	0.1-0.3	URFP-02-5	5 mL
Ultra Rainbow Fluorescent, $10^7/\text{mL}$	0.4-0.6	URFP-05-5	5 mL
Ultra Rainbow Fluorescent, $10^7/\text{mL}$	1.0-1.4	URFP-10-5	5 mL
Ultra Rainbow Fluorescent, $10^7/\text{mL}$	3.0-3.4	URFP-30-2	2 mL
Ultra Rainbow Fluorescent, $10^7/\text{mL}$	3.0-3.4	URFP-30-20	20 mL
Ultra Rainbow Fluorescent, $10^6/\text{mL}$ , Ready-to-Use	3.0-3.4	URFP01-30-2K	2x15mL
Ultra Rainbow Fluorescent, $10^6/\text{mL}$ , Ready-to-Use	3.0-3.4	URFP01-30-10K	10x3mL
Ultra Rainbow Fluorescent, $10^7/\text{mL}$	3.5-3.9	URFP-38-2	2 mL
Ultra Rainbow Fluorescent, Mid-Range Intensity, $10^7/\text{mL}$	3.5-3.9	URFP-38-5A	5 mL
Ultra Rainbow Fluorescent, $10^7/\text{mL}$	8.1-12.0	URFP-100-2	2 mL
Ultra Rainbow Fluorescent, $5 \times 10^6/\text{mL}$	13.0-17.9	URFP-150-2	2 mL
Ultra Rainbow Fluorescent, 1% w/v	18.0-24.9	URFP-200-5	5 mL
Ultra Rainbow Fluorescent, 1% w/v	25.0-35.0	URFP-300-5	5 mL

**Figure 52** Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-30-2, Lot No. AA02) on a Beckman Coulter Cyan™ ADP. NOTE: %CV is dependent on the flow rate, concentration, and the instrument used to evaluate the Ultra Rainbow Fluorescent Particles.



**Spherotech Cat. No. URFP-30-2**  
**Instructions for use:**

**A. Preparation of Particles**

1. Vortex the particles vigorously
2. Add 2 to 4 drops of particles to 1mL of sheath fluid or DI water. The inclusion of a small amount of detergent (~0.01%) in the dilution buffer will increase the percentage of the singlet population.

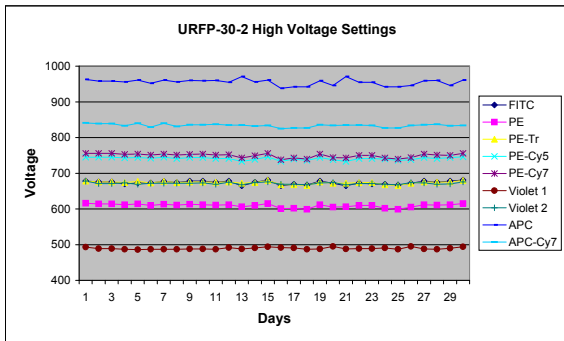
**B. Daily Alignment**

To determine the optical alignment of the system perform the following:

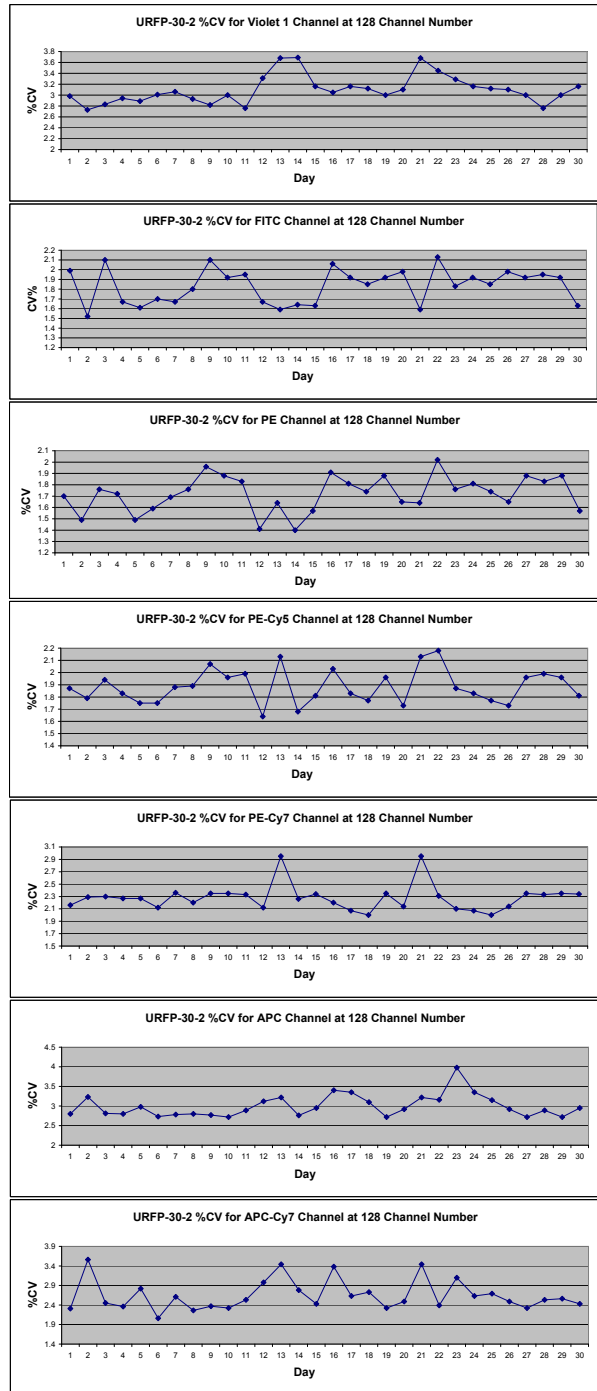
1. Set a live gate for the singlet population on the FSC vs SSC histogram to exclude aggregates
2. Adjust the Gain and High voltage so that the mean channel number of the peak is in a predetermined position on each histogram of interest. The histograms on the previous page can be used as a guide.
3. Collect 5000 events inside the gate
4. Record the % CV and High Voltage for all fluorescence channels of interest.
5. Use a computer program such as Excel to generate the Levy Jennings graphs.

NOTE: If the values on any parameter exceed those of the day-to-day average or preset values, which are determined by at least one months worth of data, additional calibration or alignment procedures should be performed according to the instrument operation manual.

**Figure 53** Levy Jennings Graph for the Voltage Setting used to place the URFP-30-2 at the 128 Channel Number on a Beckman Coulter CyAn™ ADP.

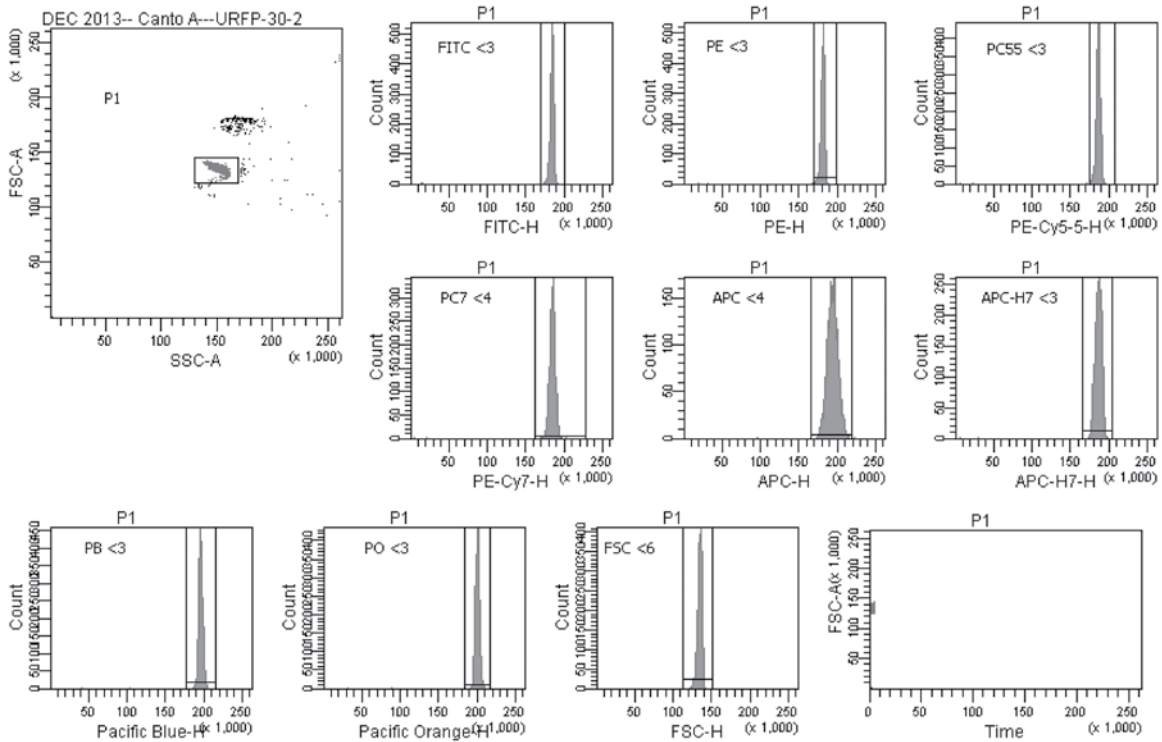


**Figure 54** Levy Jennings Graphs for the %CV of the URFP-30-2 at the 128 Channel Number on a Beckman Coulter CyAn™ ADP.



Flow Cytometry Particles-Alignment

**Figure 55** Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-30-2, Lot No. AE02) on a BD Canto™ II.

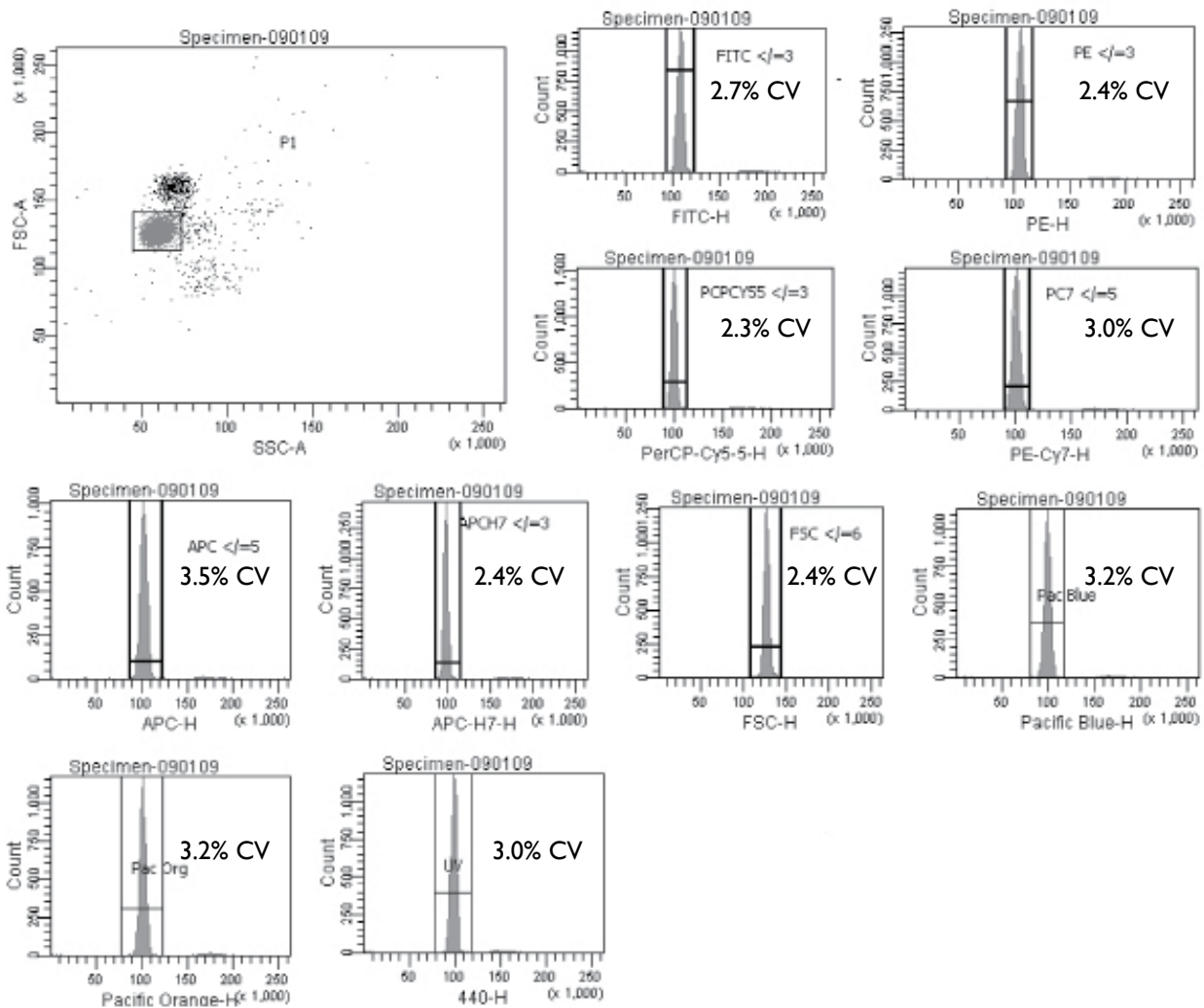


Population	FITC-H Mean	FITC-H %CV	Population	Pacific Blue-H Mean	Pacific Blue-H %CV	
<input checked="" type="checkbox"/> FITC <3	184,254	1.3	<input checked="" type="checkbox"/> PB <3	196,026	1.3	
Population	PE-H Mean	PE-H %CV	Population	Pacific Orange-H Mean	Pacific Orange-H %CV	
<input checked="" type="checkbox"/> PE <3	181,156	1.3	<input checked="" type="checkbox"/> PO <3	200,938	1.3	
Population	PE-Cy5-5-H Mean	PE-Cy5-5-H %CV	Population	FSC-H Mean	FSC-H %CV	
<input checked="" type="checkbox"/> PC55 <3	186,116	1.4	<input checked="" type="checkbox"/> FSC <6	134,238	2.1	
Population	PE-Cy7-H Mean	PE-Cy7-H %CV	Tube: URFP-30-2			
<input checked="" type="checkbox"/> PC7 <4	184,672	1.9	Population	#Events	%Parent	%Total
Population	APC-H Mean	APC-H %CV	<input checked="" type="checkbox"/> All Events	3,000	###	100.0
<input checked="" type="checkbox"/> APC <4	194,079	3.5	<input checked="" type="checkbox"/> P1	2,729	91.0	91.0
Population	APC-H7-H Mean	APC-H7-H %CV	<input checked="" type="checkbox"/> FITC <3	2,727	99.9	90.9
<input checked="" type="checkbox"/> APC-H7 <3	186,429	2.1	<input checked="" type="checkbox"/> PE <3	2,725	99.9	90.8
			<input checked="" type="checkbox"/> PC7 <4	2,727	99.9	90.9
			<input checked="" type="checkbox"/> APC <4	2,727	99.9	90.9
			<input checked="" type="checkbox"/> APC-H7 <3	2,727	99.9	90.9
			<input checked="" type="checkbox"/> FSC <6	2,729	100.0	91.0
			<input checked="" type="checkbox"/> PC55 <3	2,726	99.9	90.9
			<input checked="" type="checkbox"/> PB <3	2,727	99.9	90.9
			<input checked="" type="checkbox"/> PO <3	2,727	99.9	90.9

\* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

To see more information on Spherotech beads for flow cytometry calibration and standardization go to: [www.Spherotech.com/tech.htm](http://www.Spherotech.com/tech.htm)

**Figure 56** Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-30-2, Lot No. AA02) on a BD LSR™ II.



\* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

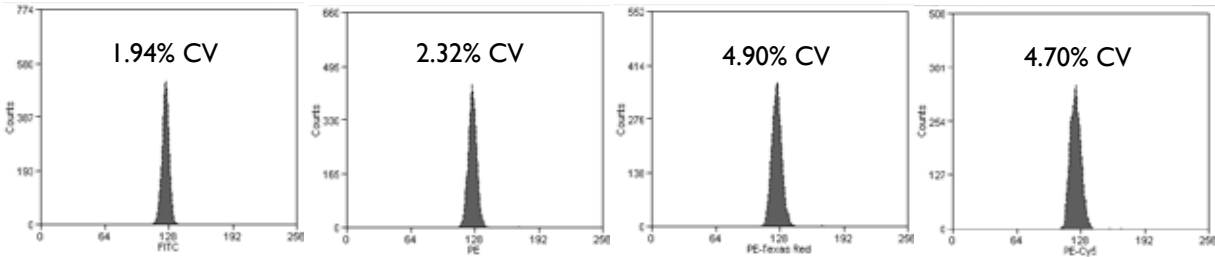
## SPHERO™ Ultra Rainbow Fluorescent Particles

One Bead Aligns all Channels

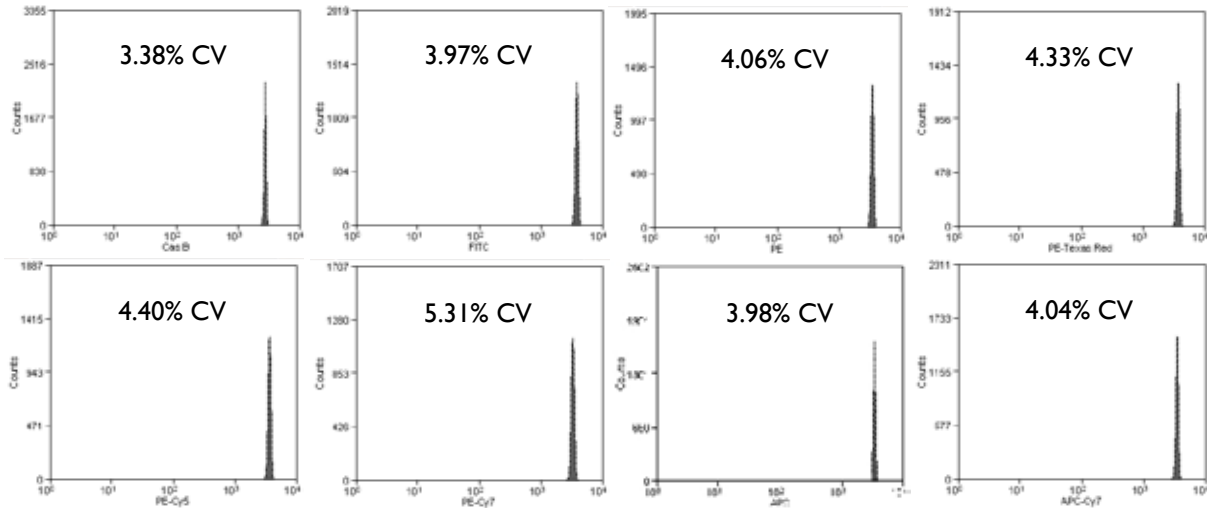
- Aids in the alignment and optimization of all fluorescent and scatter parameters
- Determines if the flow cell is clean and without fluidic blockage
- Measures the coefficients of variation (CVs), peak channels, and histogram distributions to determine the functionality of the flow cytometer

[www.spherotech.com](http://www.spherotech.com)

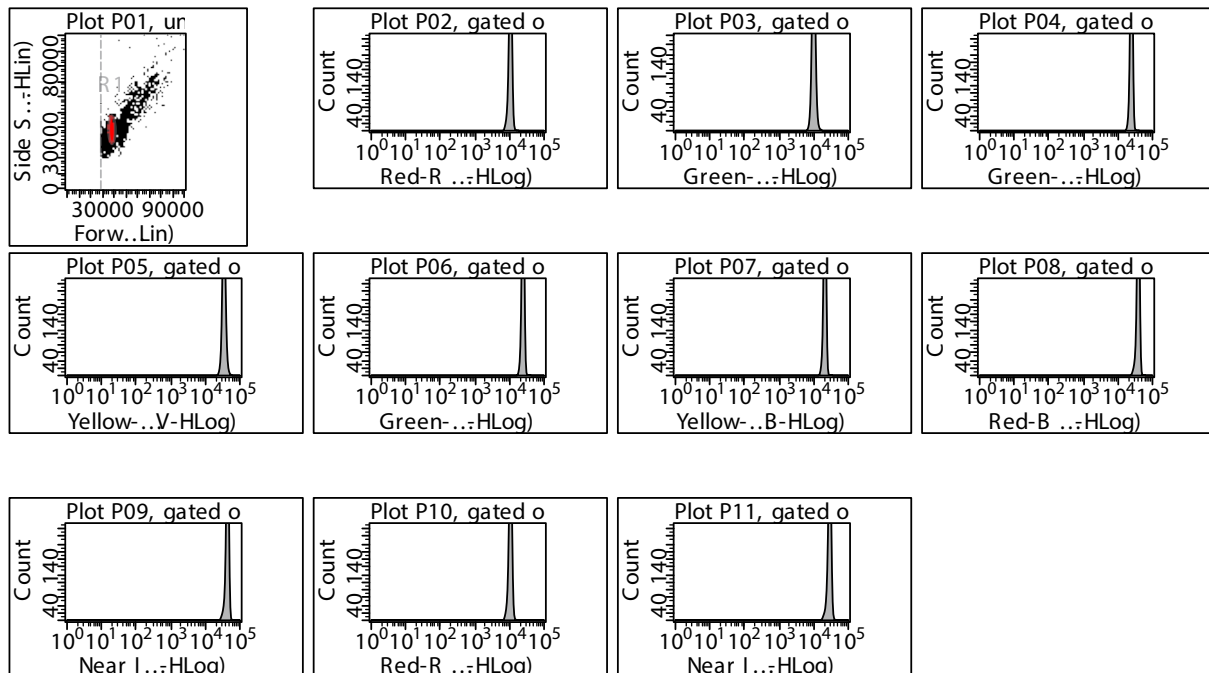
**Figure 57** Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-38-2, Lot No.AB01) on a Beckman Coulter Cyan™ ADP.



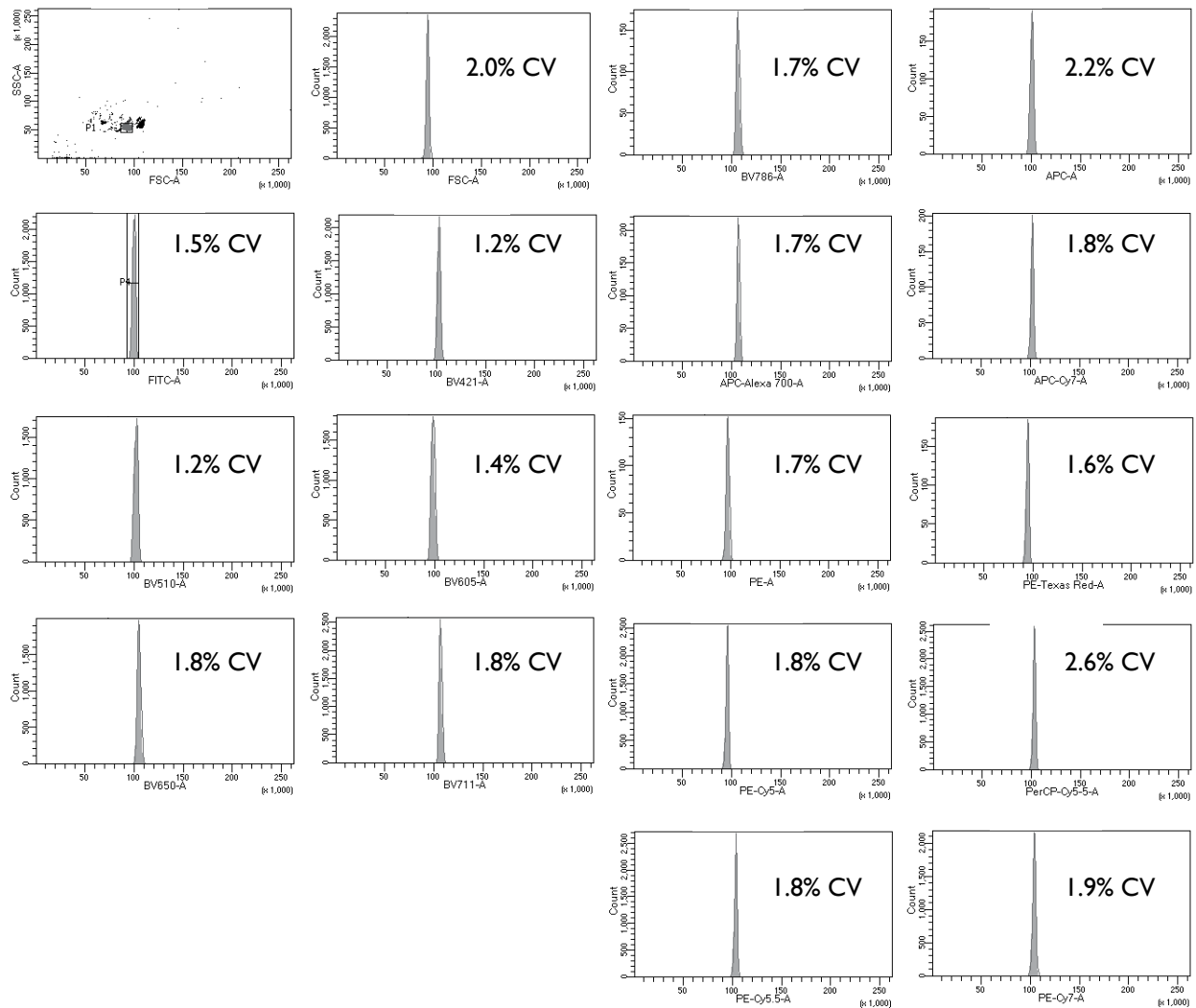
**Figure 58** Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-100-2, Lot No.AF01) on a Beckman Coulter Cyan™ ADP.



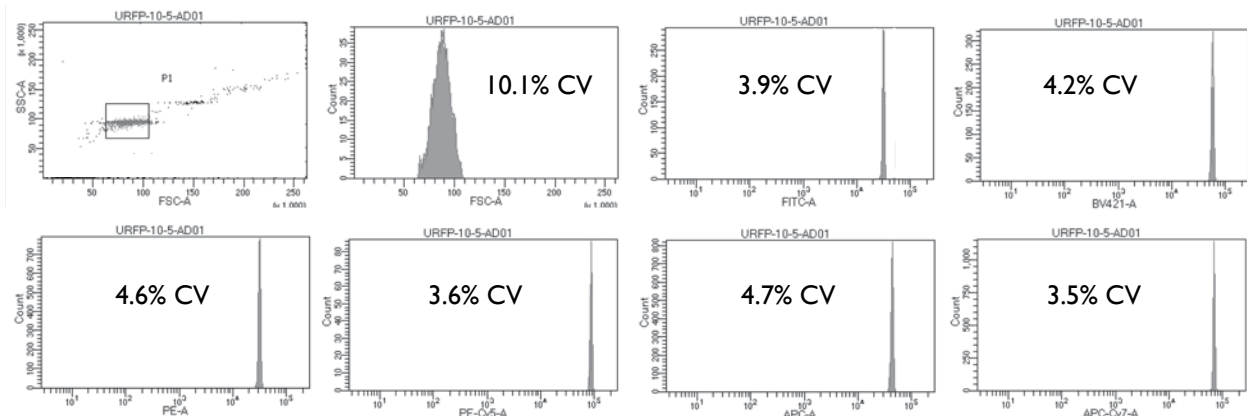
**Figure 59** Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-30-2, Lot No.AG03) on a EMD Millipore Guava easyCyte™ I2.



**Figure 60** Histograms of the Ultra Rainbow Fluorescent Particles (Catalog No. URFP-30-2, Lot No. AG03) on a BD Bioscience LSRFortessa™ X-20 are shown below.



**Figure 61** Histograms of the Ultra Rainbow Fluorescent Particles (Catalog No. URFP-10-5, Lot No. AG03, 1.06 micron) on a BD Bioscience LSRFortessa™ X-20 are shown below.



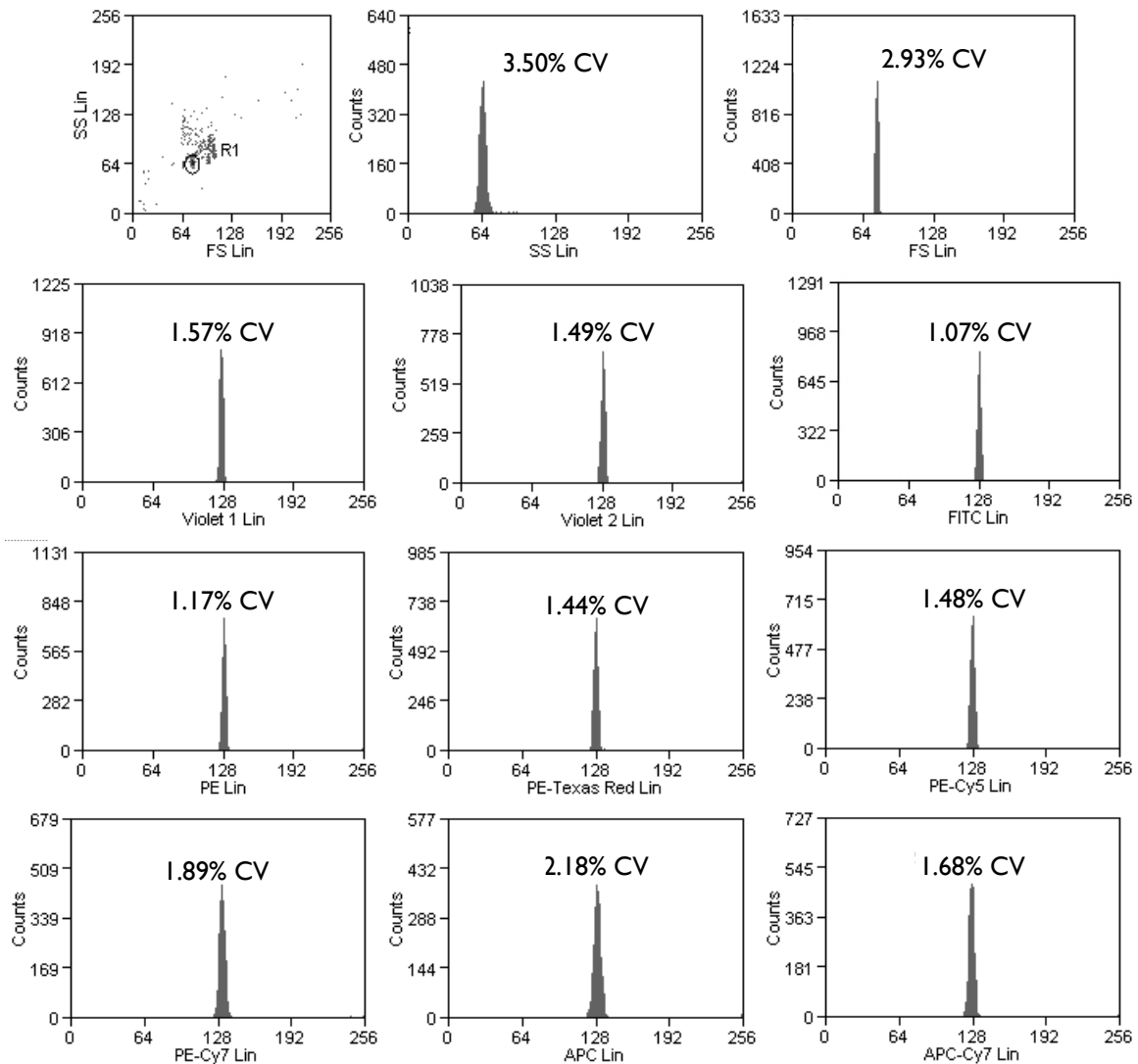
Flow Cytometry Particles - Alignment

## SPHERO™ Ultra Rainbow Fluorescent Particles for Beckman Coulter Cytometers

- Consists of a single peak for performance verification of Beckman Coulter flow cytometers
- Determines cleanliness of the flow cell and fluidics

Particle Type and Surface	Size, $\mu\text{m}$	Catalog No.	Unit
Calibration Beads for MoFlo™ Astrios™, $10^7/\text{mL}$	3.0-3.4	B27034	2 mL
Calibration Beads for MoFlo™, MoFlo™ XDP, and CyAn™ ADP, $10^7/\text{mL}$	3.0-3.4	B28479	2 mL
AccuCount Ultra Rainbow Fluorescent Particles (Calibration Beads for Aquios CL™, $10^6/\text{mL}$ )	3.8 (+/-0.3)	ACURFP-38-5	5 mL
AccuCount Ultra Rainbow Fluorescent Particles (Calibration Beads for Aquios CL™, $10^6/\text{mL}$ )	3.8 (+/-0.3)	ACURFP-38-15	15 mL

**Figure 62** Histograms of the Calibration Beads for MoFlo™, MoFlo™ XDP, and CyAn™ ADP (Cat. No. B28479, Lot No. BAE01) on a Beckman Coulter Cyan™ ADP.

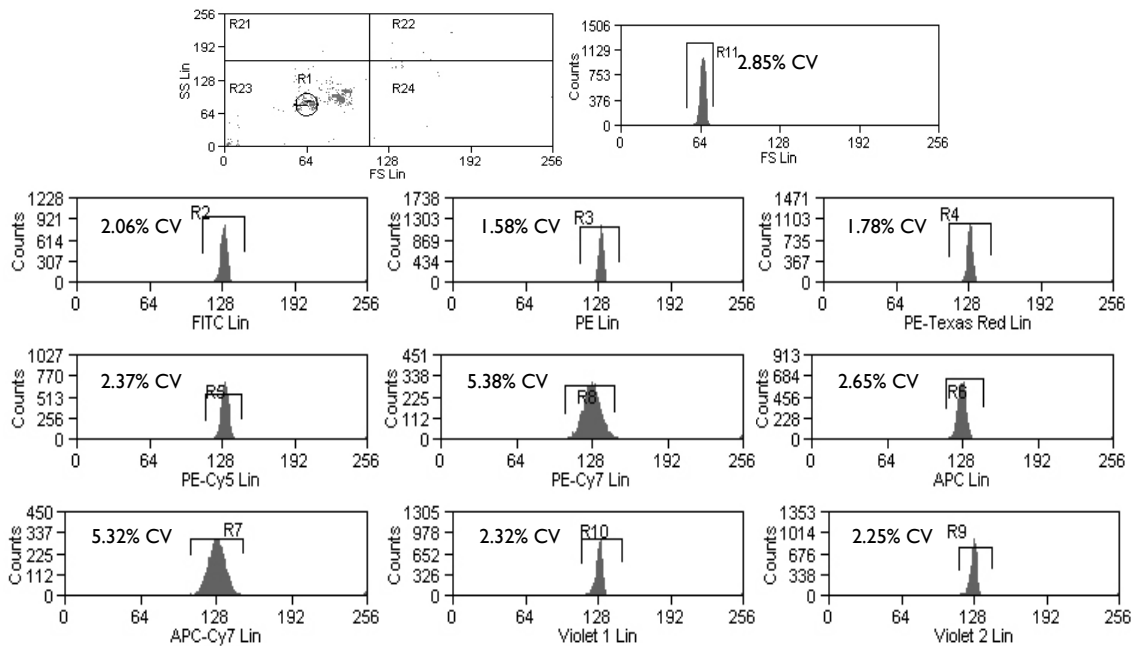


## SPHERO™ Rainbow Fluorescent Particles

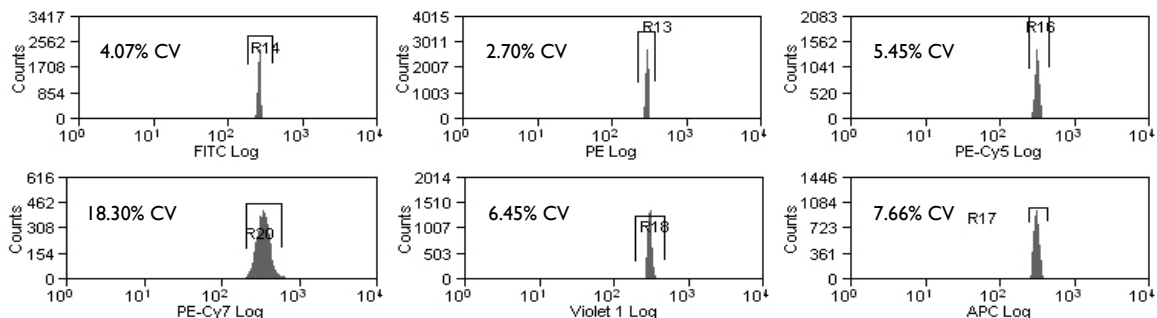
The Rainbow Fluorescent Particles are similar to Rainbow Calibration Particles except that these represent uniform size particles with a single intensity. The Rainbow Fluorescent Particles are usually the brightest peak of the corresponding Rainbow Calibration Particles with the exception of RFP-50-5, RFP-70-2, RFP-100-2 and RFP-30-5A. The RFP-30-5A has the fluorescence intensity similar to stained cells in all channels. Since these particles contain a single peak with very small fluorescence and size CV, they are very useful in the alignment of the optical system of the flow cytometer in all channels.

Particle Type and Surface	Size, $\mu\text{m}$	Catalog No.	Unit
Rainbow Fluorescent, $10^7/\text{mL}$ (Intensity similar to brightest peak in RCP-20-5)	1.8-2.2	RFP-20-5	5 mL
Rainbow Fluorescent, $10^7/\text{mL}$ (Intensity similar to brightest peak in RCP-30-5)	3.0-3.4	RFP-30-5	5 mL
Rainbow Fluorescent, $10^7/\text{mL}$ (Intensity similar to mid range FLI fluorescence in RCP-30-5)	3.0-3.4	RFP-30-5A	5 mL
Rainbow Fluorescent, $10^7/\text{mL}$ (Intensity similar to brightest peak in RCP-35-5)	3.5-4.0	RFP-35-5	5 mL
Rainbow Fluorescent, $10^7/\text{mL}$	5.0-5.9	RFP-50-5	5 mL
Rainbow Fluorescent, $10^7/\text{mL}$ (Intensity similar to brightest peak in RCP-60-5)	6.0-6.4	RFP-60-5	5 mL
Rainbow Fluorescent, $10^7/\text{mL}$	6.5-8.0	RFP-70-5	5 mL
Rainbow Fluorescent, $10^7/\text{mL}$	8.1-12.0	RFP-100-2	2 mL

**Figure 63** Histograms of the Rainbow Fluorescent Particles (Cat. No. RFP-30-5, Lot No. AF02).

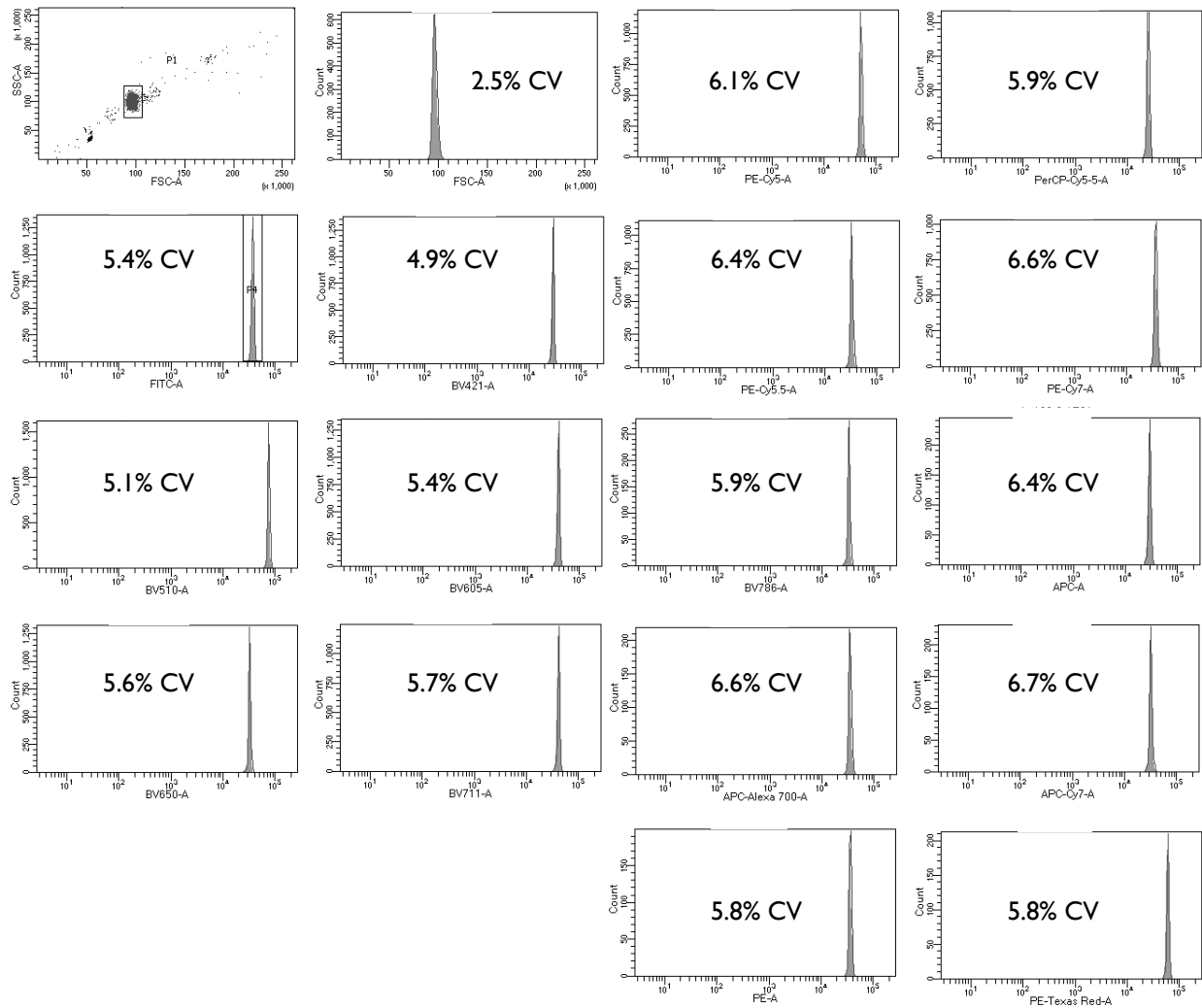


**Figure 64** Histograms of the Rainbow Fluorescent Particles, Mid-Range (Cat. No. RFP-30-5A, Lot No. AF02).

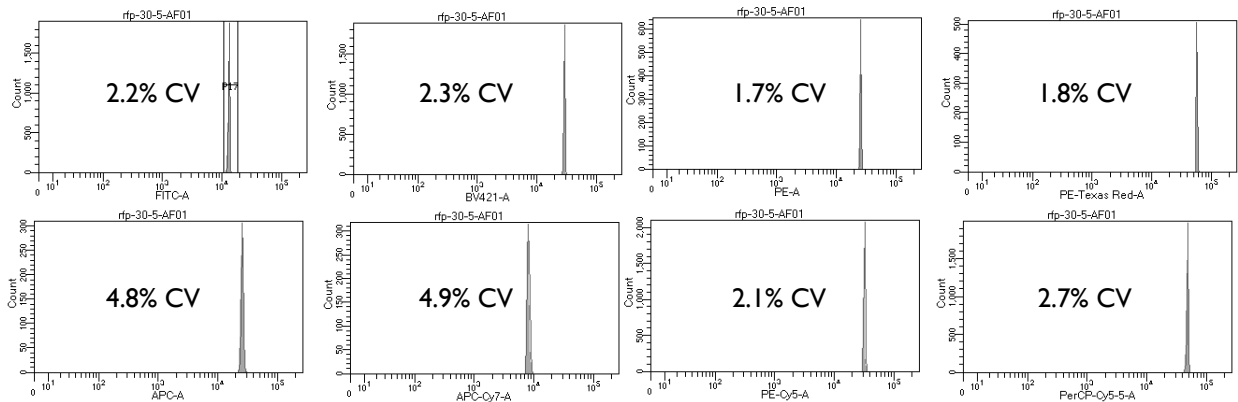




**Figure 65** Histograms showing fluorescence intensities of Rainbow Fluorescent Particles (Catalog No. RFP-100-2, Lot No. AE01) on a BD Bioscience LSRFortessa™ X-20 are shown below.



**Figure 66** Histograms showing fluorescence intensities of Rainbow Fluorescent Particles (Catalog No. RFP-30-2, Lot No. AF02) on a BD Bioscience LSRFortessa™ X-20 are shown below.



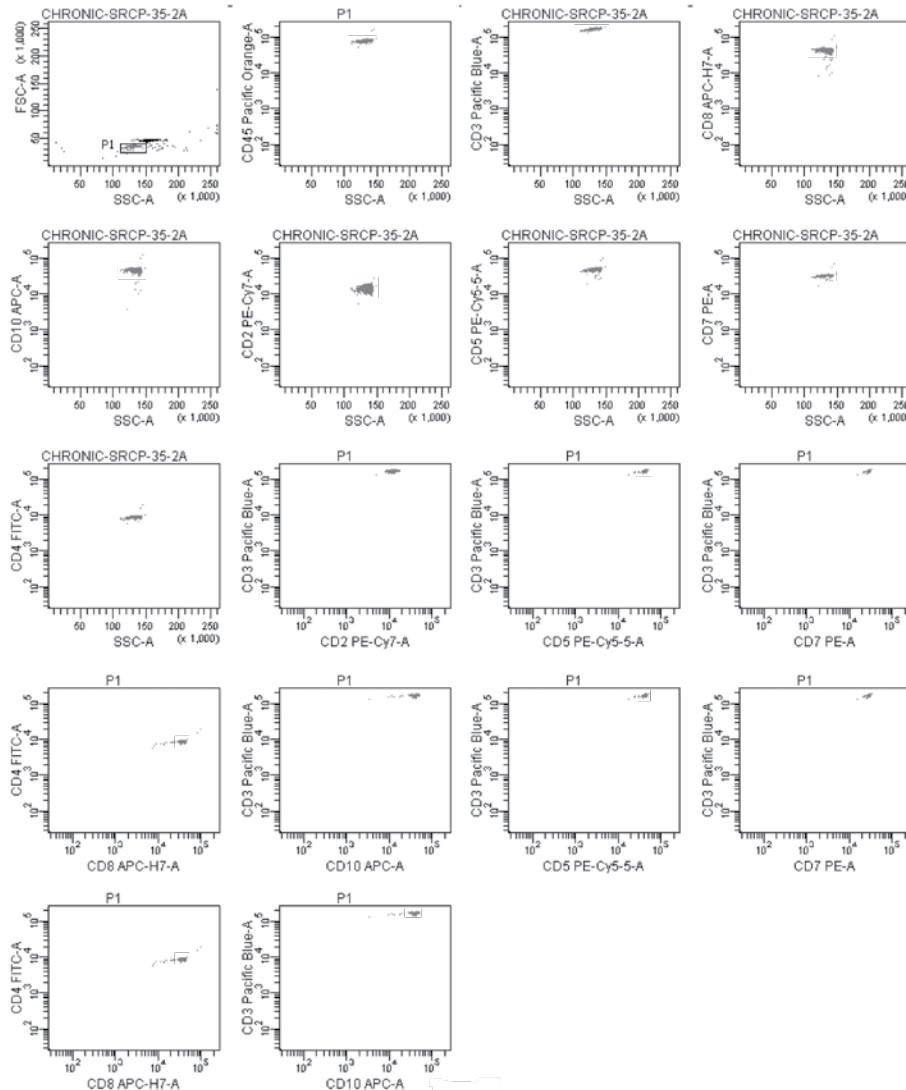
## SPHERO™ Supra Rainbow Midrange Fluorescent Particles

- Consists of a single peak which has an intensity similar to real samples
- Contains a single peak with very low fluorescence and size CV
- Fluorescent in UV, FITC, PE, PE-TR, PE-Cy5, PE-Cy7, APC, APC-Cy7, and IR
- Measures the coefficients of variation (CVs), and target channels using experimental setting.

Particle Type and Surface	Size, $\mu\text{m}$	Catalog No.	Unit
Supra Rainbow Midrange Fluorescent, $10^7/\text{mL}$	3.0-3.59	SRCP-35-2A	2 mL

The Supra Rainbow Midrange Fluorescent Particles (SRCPs) have an intensity close to that of cellular samples with excellent CVs. As a result, once the optimal voltages for a particular experiment are determined, the setting can be captured as target channels based on the mean fluorescence intensity of the SRCPs. This allows creation of Levy-Jennings plots of the voltages to get the beads to a specific target channel number. The target channel numbers are more robust to instrument changes than the voltages themselves. As a result, changes in the instrument are easier to detect.

**Figure 67** Histograms of the Supra Rainbow Midrange Fluorescent Particles (Cat. No. SRCP-35-2A) using the setting of cellular samples.

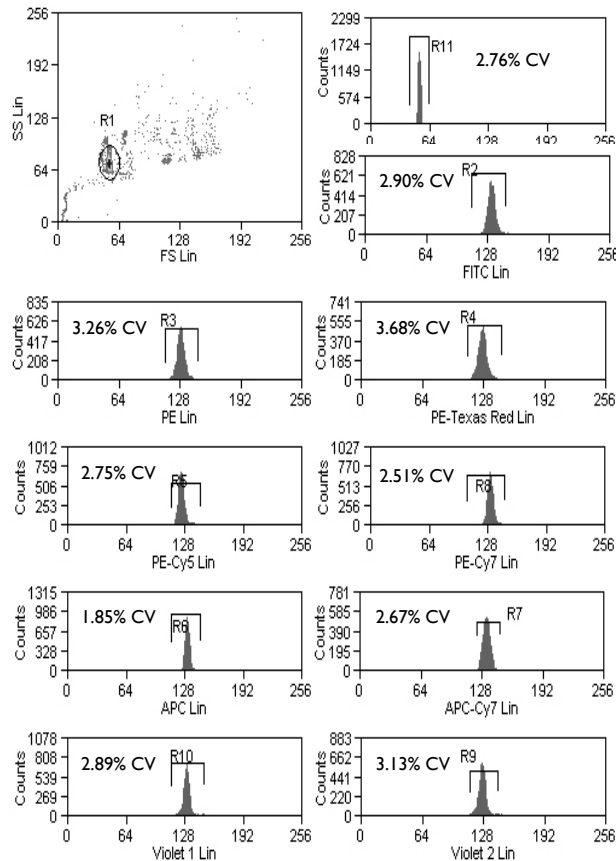


## SPHERO™ Rainbow Alignment Particles

- Consists of a prediluted, single peak for optical alignment of any flow cytometer in any channel
- Contains a single peak with very low fluorescence and size CV
- Fluorescent in UV, FITC, PE, PE-TR, PE-Cy5, PE-Cy7, APC, and APC-Cy7 channels
- Measures the coefficients of variation (CVs), peak channels, and histogram distribution of flow cytometers.

Particle Type and Surface	Size, $\mu\text{m}$	Catalog No.	Unit
Rainbow Fluorescent Alignment Particles, $1.2 \times 10^6/\text{mL}$	3.5-3.9	RAP-38-5	5 mL

**Figure 68** Histograms of the Rainbow Alignment Particles (Cat. No. RAP-38-5, Lot No. AD01) on a Beckman Coulter Cyan™ ADP.

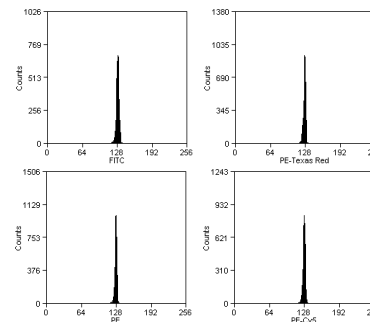


## SPHERO™ Fluorescent Alignment Particles

- Consists of a single peak for optical alignment of any flow cytometer
- Contains a single peak with very low fluorescence and size CV
- Fluorescent in FITC, PE, PE-TR, and PE-Cy5 channels
- Measures the coefficients of variation (CVs), peak channels, and histogram distribution of flow cytometers.

Particle Type and Surface	Size, $\mu\text{m}$	Catalog No.	Unit
Fluorescent Alignment Particles, $10^8/\text{mL}$	3.0-3.4	FAP-3056-5	5 mL

**Figure 69** Histograms of the Fluorescent Alignment Particles (Cat. No. FAP-3056-5, Lot No. AG01) on a Beckman Coulter Cyan™ ADP.



## SPHERO™ Rainbow Calibration and Rainbow QC Kits

The Rainbow Calibration Kit and Rainbow QC Kit are designed to simplify the routine calibration of flow cytometers.

### SPHERO™ Rainbow QC Kit (Cat. No. RCK-3K)

Consists of one vial each of the following particles:

- RCP-30-5A-1 (Peak 1), 3.0-3.4 $\mu\text{m}$ ,  $10^7/\text{mL}$ , 5mL
- RCP-30-5A-4 (Peak 4), 3.0-3.4 $\mu\text{m}$ ,  $10^7/\text{mL}$ , 5mL
- RCP-30-5A-8 (Peak 8), 3.0-3.4 $\mu\text{m}$ ,  $10^7/\text{mL}$ , 5mL

### SPHERO™ Rainbow Calibration Kit (Cat. No. RQC-4K)

Consists of one vial each of the following particles:

- BCP-60-2, 6.0-6.4 $\mu\text{m}$ ,  $10^7/\text{mL}$ , 2mL
- RFP-60-2, 6.0-6.4 $\mu\text{m}$ ,  $10^7/\text{mL}$ , 2mL
- RCP-30-2L (Peaks 1-4), 3.0-3.4 $\mu\text{m}$ ,  $10^7/\text{mL}$ , 2mL
- RCP-30-2H (Peaks 5-8), 3.0-3.4 $\mu\text{m}$ ,  $10^7/\text{mL}$ , 2mL

Particle Type and Surface	Catalog No.	Unit
Rainbow Calibration Kit	RCK-3K	1 Kit
Rainbow QC Kit	RQC-4K	1 Kit