

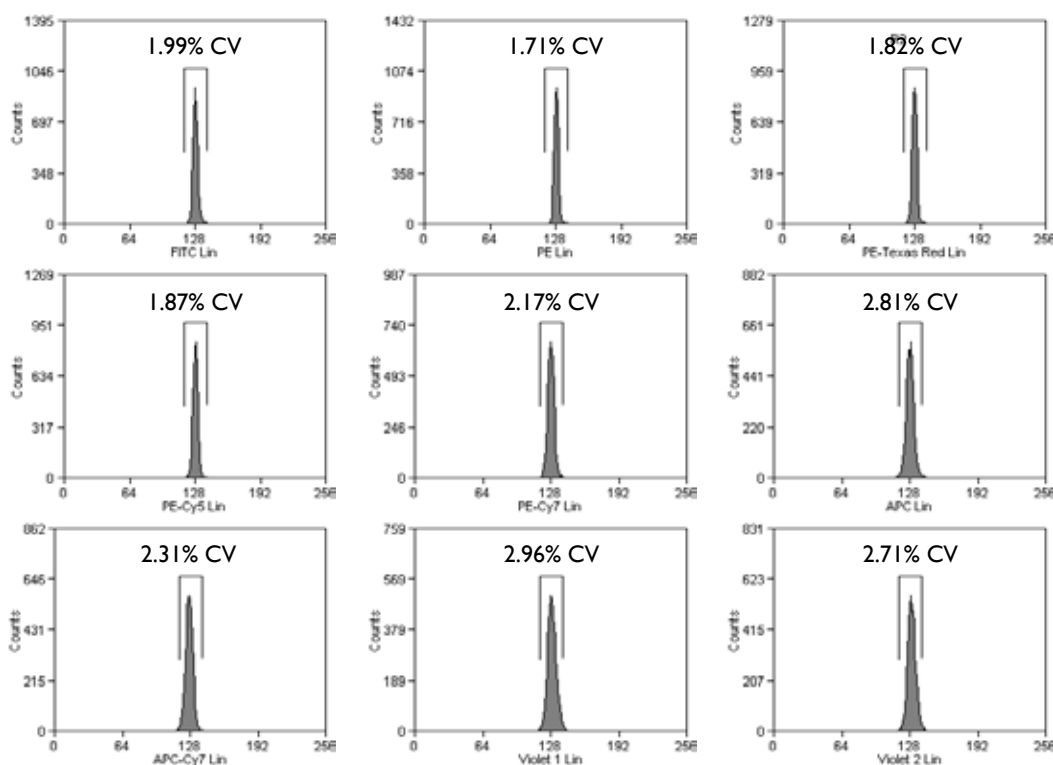
SPHERO™ Ultra Rainbow Fluorescent Particles

- 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, μm	Catalog No.	Unit
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 42 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 43 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.

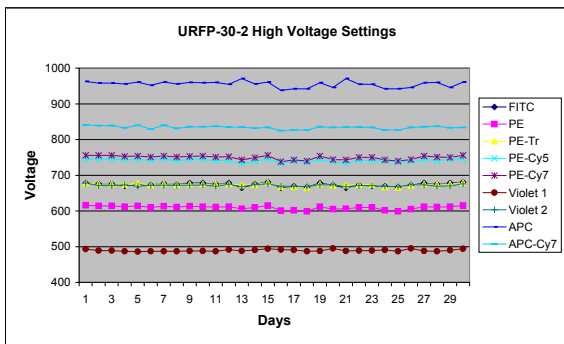


Figure 44 Levy Jennings Graphs for the %CV of the URFP-30-2 at the 128 Channel Number on a Beckman Coulter CyAn™ ADP.

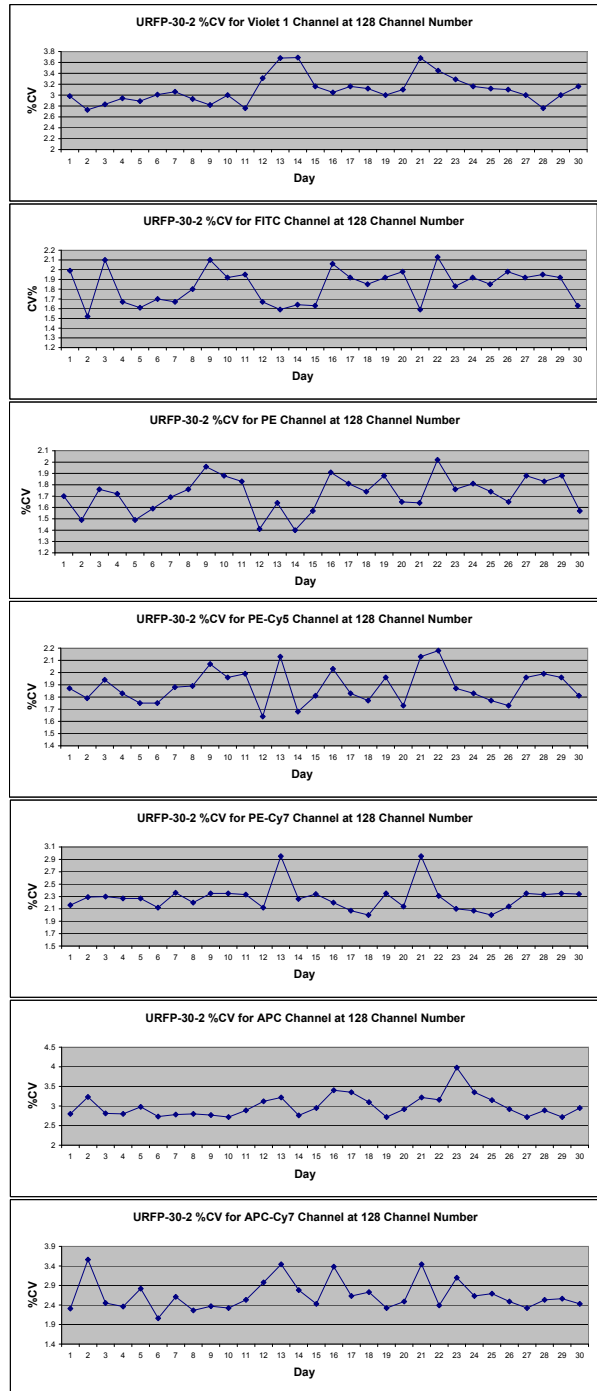
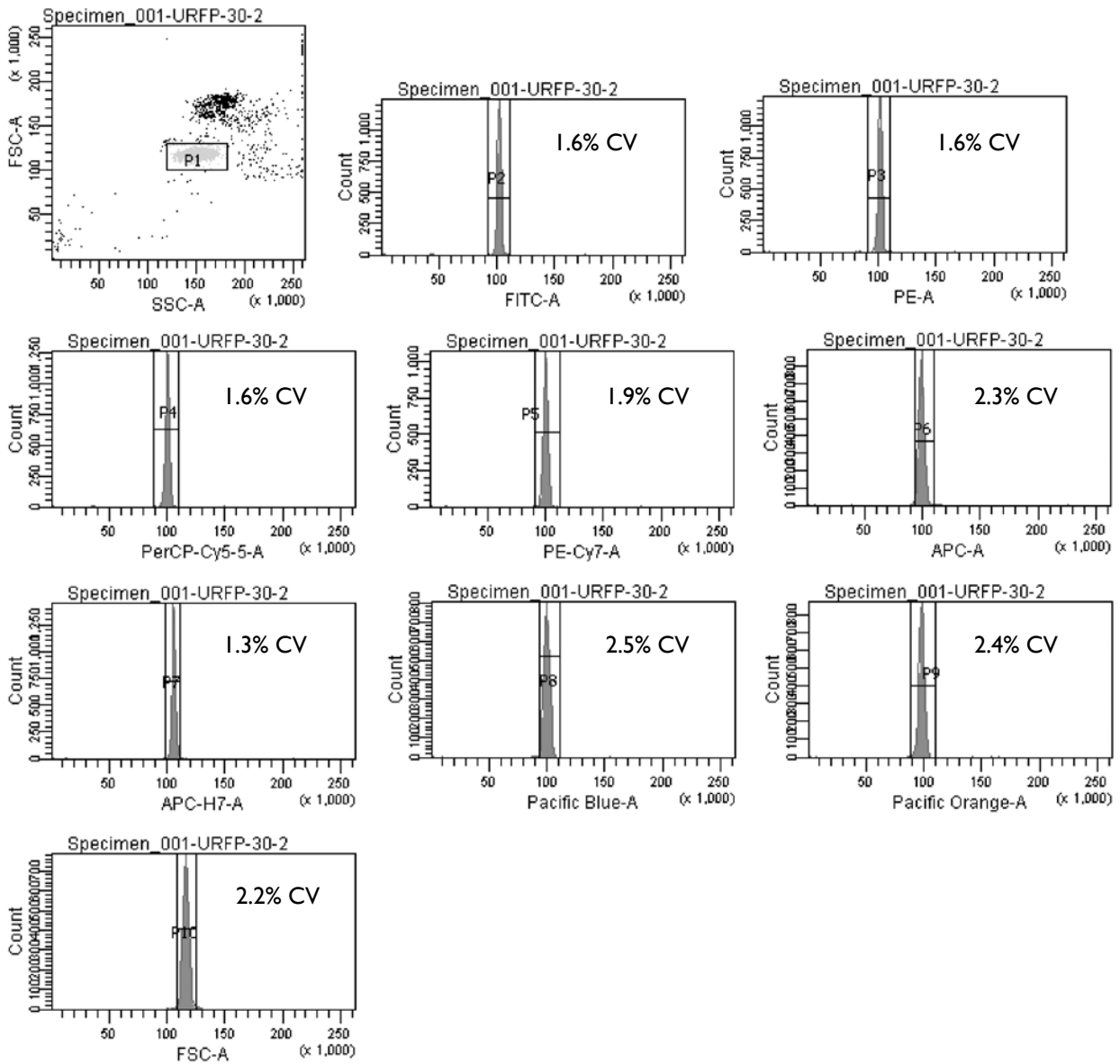


Figure 45 Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-30-2, Lot No. AA02) on a BD Canto™ II.



* Data provided by Laura Marszalek, Northwestern Memorial Hospital.

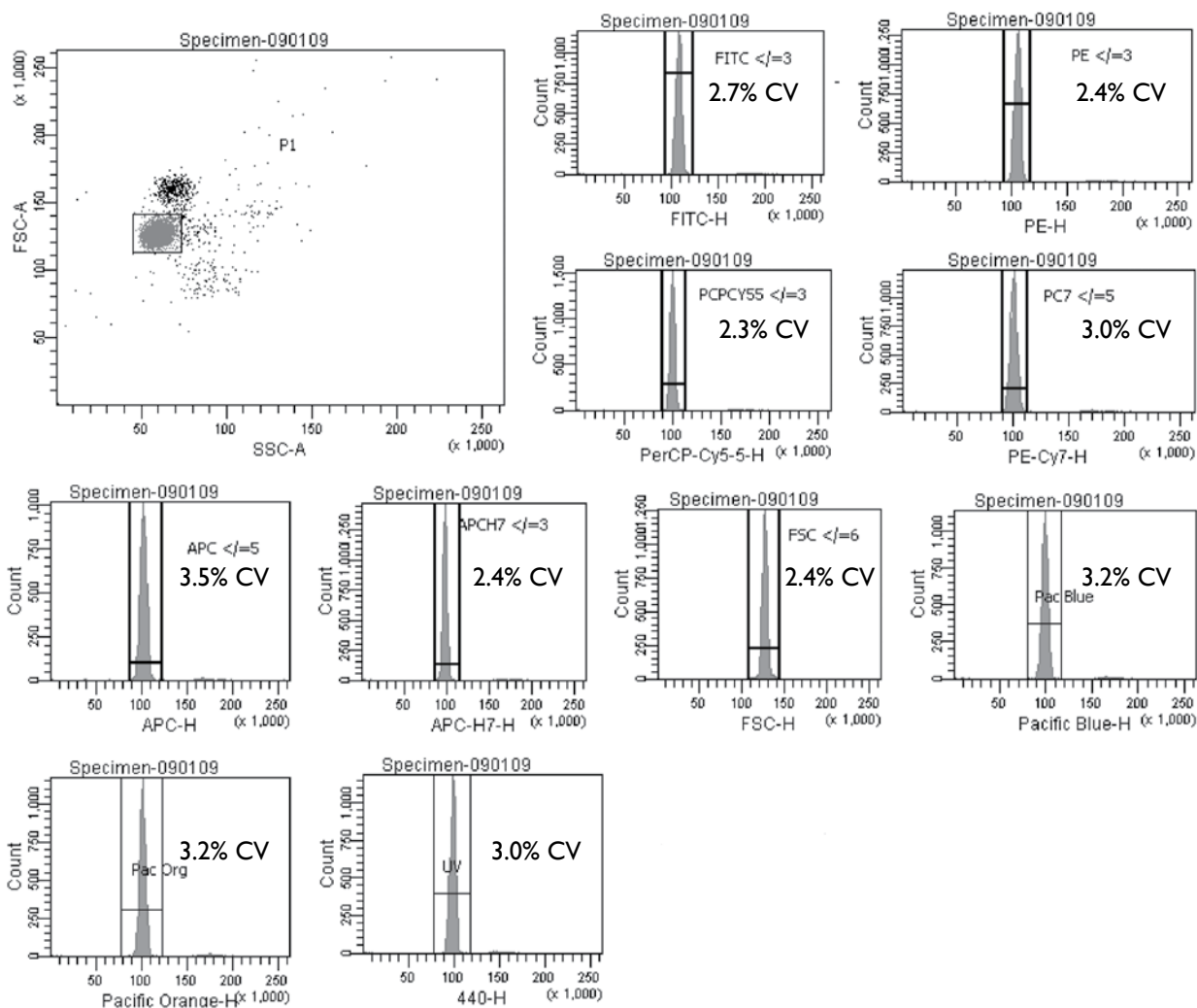
To see more information on Spherotech beads for flow cytometry calibration and standardization go to:

www.Spherotech.com/tech.htm

Spherotech, Inc.

27845 Irma Lee Circle, Unit 101, Lake Forest, IL 60045

Figure 46 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

Tel.: 800-368-0822 or 847-680-8922; Fax: 847-680-8927; E-Mail: service@spherotech.com

Visit us on the web at <http://www.Spherotech.com>

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27845 Irma Lee Circle, Unit 101, Lake Forest, IL 60045

Figure 47 Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-38-2, Lot No. Z02) on a Beckman Coulter Cyan™ ADP.

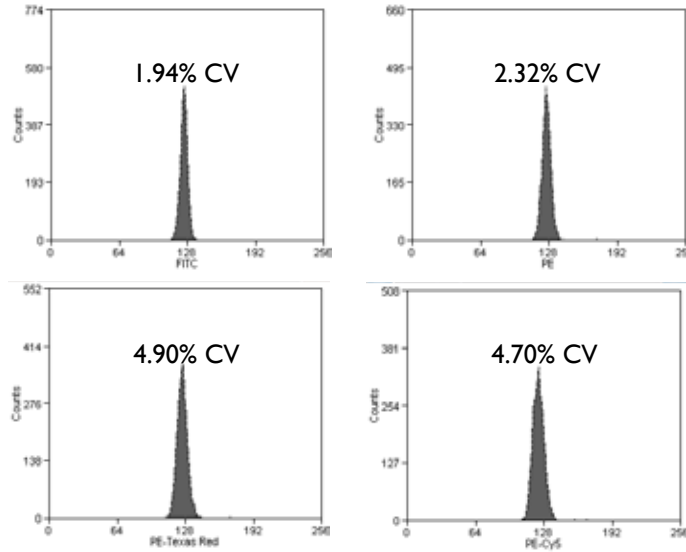


Figure 48 Histograms of the Ultra Rainbow Fluorescent Particles (Cat. No. URFP-100-2, Lot No. Z02) on a Beckman Coulter Cyan™ ADP.

