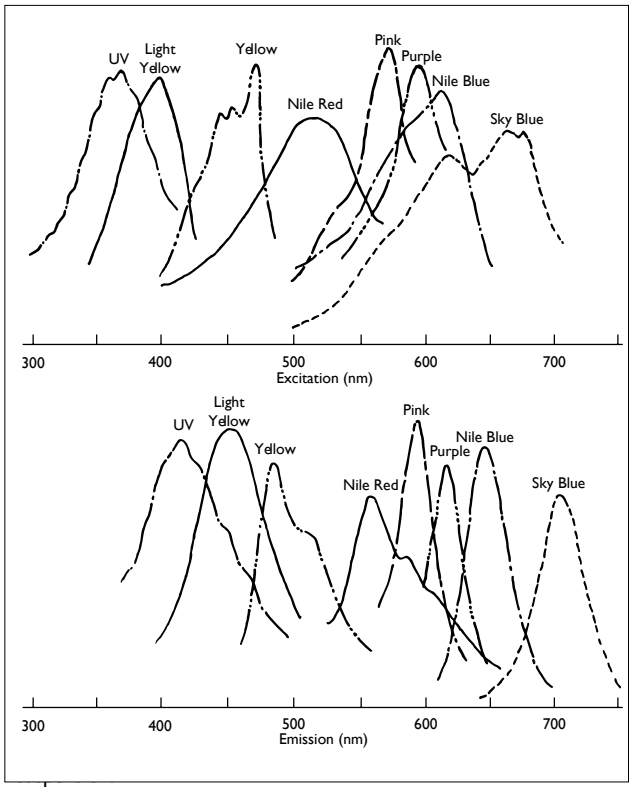
SPHERO™ Fluorescent Particles

- Beneficial to bioimaging and biosensing applications
- Uniform and stable fluorescence
- Available with functional groups for covalent binding

The SPHERO™ fluorescent microparticles are prepared by either staining polystyrene particles with a fluorophore solution or by polymerizing a fluorophore in styrene in the presence of polystyrene core particles. As a result, a wide variety of fluorescent particles can be prepared ranging in size, type of fluorophore, fluorescence intensity and surface functional groups. The fluorophores chosen for use in the preparation of SPHERO™ fluorescent particles are water insoluble and therefore are very stable. These fluorophores, once incorporated into the particles, do not leach and their color and fluorescence remains stable for long periods of time under proper storage conditions.

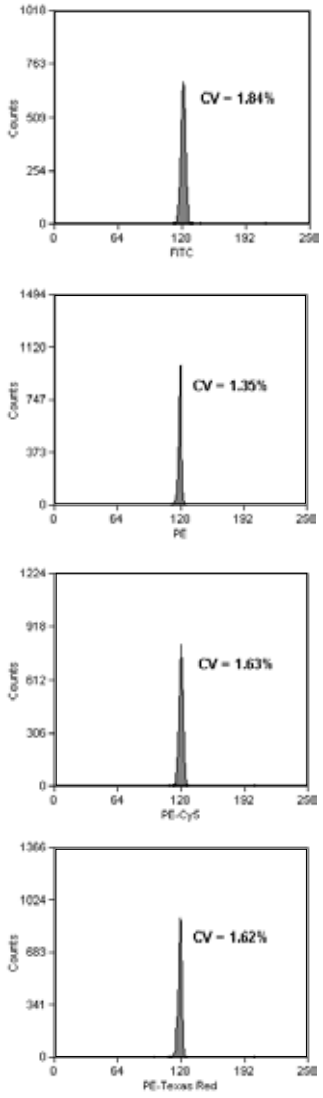
The excitation and emission spectra of some of the fluorophores used in the SPHERO™ fluorescent particles are shown in Figure 19.

Figure 20 Excitation & Emission Spectra of the fluorophores used in SPHERO™ fluorescent particles.



The SPHERO™ fluorescent particles are available in single or multiple fluorophores of various sizes and fluorescence intensities with very small coefficient of variation in both size and fluorescence. They can be used for latex agglutination, fluorescence microscopy, confocal fluorescence microscopy. Many of these particles can be used for flow cytometry. The flow cytometer histograms of 2.9 µm Nile Red Particles (Catalog # FP-3056-2) at four channels are shown in Figure 20. More flow cytometry data for SPHERO™ fluorescent particles is shown on page 15.

Figure 21 Flow cytometry histograms of 2.9 µm, Nile Red Particles (Cat. No. FP-3056-2)



The flow cytometry data of some of the fluorescent particles are shown in **Table I**.

Table I

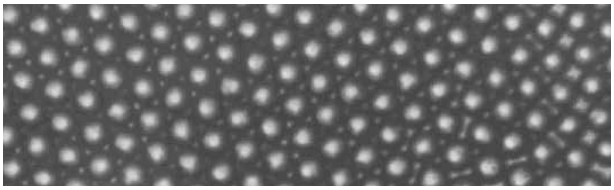
Fluorophores	Cat. No.	Size (µm)	%CV*								
			Violet 1	Violet 2	FL	PE	TR	PE-Cy5	PE-Cy7	APC	APC-Cy7
SPHERO™ Fluorescent Particles, 1.7-2.2 µm											
UV, Low Intensity	FL-2040-2	1.81	6.34	14.51							
UV, High Intensity	FH-2040-2	2.14	3.65								
Light Yellow, Low Intensity	FL-2045-2	2.10	3.89	4.40							
Light Yellow, High Intensity	FH-2045-2	2.16	3.26	3.25							
Yellow, Low Intensity	FL-2052-2	1.80	4.04	4.08	4.53	5.60					
Yellow, High Intensity	FH-2052-2	1.84			2.30	3.50					
Nile Red, Low Intensity	FL-2056-2	2.07		8.88	4.22	3.89	4.34	4.9	9.67		
Nile Red, High Intensity	FH-2056-2	2.27			2.80	2.64	2.55	3.00	5.10		
Pink	FP-2058-2	1.80				2.86	3.55	7.92			
Purple, Low Intensity	FL-2062-2	1.93					10.33				
Purple, Mid-level Intensity	FP-2062-2	2.0					7.13	13.0	10.99		
Purple, High Intensity	FH-2062-2	1.80					5.33	8.02	9.55		
Sky Blue	FP-2070-2	2.07								10.18	11.23
SPHERO™ Fluorescent Particles, 2.5-4.5 µm											
Yellow	FP-4052-2	4.10	3.42	3.47	2.65	2.83	5.15				
Nile Red	FP-3056-2	2.88	3.88	2.70	2.49	2.09	2.10	2.53	5.28		
Nile Blue	FP-3065-2	3.00	8.62	8.10	6.23	6.24	6.59	7.77	13.89	4.99	6.99
Blue	FP-3068-2	3.30								2.78	4.83
SPHERO™ Multiple Fluorophore Particles, 1.7-2.2 µm											
UV/LY	FP-2042-2	2.00									
PR/Y, Low Intensity	FL-2060-2	2.20	2.68	2.66	3.32	3.44	5.02				
PR/Y, Mid-level Intensity	FP-2060-2	2.20	2.88	2.87	2.48	3.35	3.87	6.18			
PR/Y, High Intensity	FH-2060-2	2.02	3.75	3.69	3.50	4.00	2.79	3.86	12.65		
SPHERO™ Multiple Fluorophore Particles, 2.5-5.0 µm											
UV/LY	FP-3042-2	3.20	4.22	4.55							
PK/Y	FP-3055-2	3.00									
PR/Y	FP-4060-2	4.00	3.30	4.05	3.94	4.00	5.56	12.86			
SPHERO™ Fluorescent Particles, 0.7-0.9 µm											
Yellow	FP-0852-2	0.85			5.76	8.14					
Pink	FP-0858-2	0.91				8.22	9.65				
Purple	FP-0862-2	0.84					6.77				

*Data for particles with sizes above 1.0 micron obtained using a Dako Cyan ADP with the following Excitation and Emission wavelength: Violet 1: Ex 405nm, Em. 450/50nm; Violet 2: Ex. 405nm, Em. 530/40nm; FITC: Ex. 488nm, Em. 530/40nm; PE: Ex. 488nm, Em. 575/25nm; TR: Ex 488nm, Em. 613/20nm; PE-Cy5: Ex 488nm, Em. 680/30nm ; PE-Cy7: Ex 488nm, Em. >750nm ; APC: Ex. 633nm, Em. 665/20nm; APC-Cy7: 633nm, Em.> 750 nm

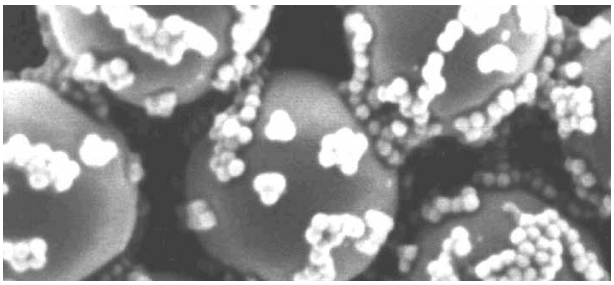
**Data for particles with sizes below 1.0 micron obtained using a Stratedigm S1400 with the following Excitation and Emission wavelength: FITC: Ex. 488nm, Em. 530/30nm; PE: Ex. 488nm, Em. 545/60nm; TR: Ex 488nm, Em. 615/30nm

Figure 22 Microscope photos (a) 10 µm Nile Red beads (40x), (b) 0.4 µm Avidin fluorescent beads binding to the surface of 6.0 µm Biotin polystyrene beads (5000x).

(a)



(b)



SPHERO™ Fluorescent Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Yellow	0.04-0.09	1.0	FP-00552-2	2 mL
Nile Red	0.04-0.06	1.0	FP-00556-2	2 mL
Pink	0.04-0.09	1.0	FP-00558-2	2 mL
Purple	0.04-0.06	1.0	FP-00562-2	2 mL
Sky Blue	0.04-0.09	0.25	FP-00570-2	2 mL
Light Yellow	0.1-0.3	1.0	FP-0245-2	2 mL
Yellow	0.1-0.3	1.0	FP-0252-2	2 mL
Nile Red	0.1-0.3	1.0	FP-0256-2	2 mL
Purple	0.1-0.3	1.0	FP-0262-2	2 mL
Sky Blue	0.1-0.3	0.25	FP-0270-2	2 mL
Light Yellow	0.4-0.6	1.0	FP-0545-2	2 mL
Yellow	0.4-0.6	1.0	FP-0552-2	2 mL
Nile Red	0.4-0.6	1.0	FP-0556-2	2 mL
Pink	0.4-0.6	1.0	FP-0558-2	2 mL
Purple	0.4-0.6	1.0	FP-0562-2	2 mL
Sky Blue	0.4-0.6	1.0	FP-0570-2	2 mL
Light Yellow	0.7-0.9	1.0	FP-0845-2	2 mL
Yellow	0.7-0.9	1.0	FP-0852-2	2 mL
Nile Red	0.7-0.9	1.0	FP-0856-2	2 mL
Pink	0.7-0.9	1.0	FP-0858-2	2 mL
Purple	0.7-0.9	1.0	FP-0862-2	2 mL
Blue	0.7-0.9	1.0	FP-0868-2	2 mL
Sky Blue	0.7-0.9	1.0	FP-0870-2	2 mL
Jade Green	0.7-0.9	1.0	FP-0878-2	2 mL
Yellow	1.0-1.9	1.0	FP-1552-2	2 mL
Light Yellow, Medium Intensity	1.7-2.2	1.0	FP-2045-2	2 mL
Nile Red	1.7-2.2	1.0	FP-2056-2	2 mL
Pink, Medium Intensity,	1.7-2.2	1.0	FP-2058-2	2 mL
Purple , Medium Intensity	1.7-2.4	1.0	FP-2062-2	2 mL
Nile Blue	1.7-2.2	1.0	FP-2065-2	2 mL
Blue	1.7-2.2	1.0	FP-2068-2	2 mL
Sky Blue	1.7-2.2	1.0	FP-2070-2	2 mL
Yellow	2.5-4.5	1.0	FP-3052-2	2 mL
Nile Red	2.5-4.5	1.0	FP-3056-2	2 mL
Pink	2.5-3.4	1.0	FP-3058-2	2 mL
Nile Blue	2.5-4.5	1.0	FP-3065-2	2 mL

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Blue	2.5-4.5	1.0	FP-3068-2	2 mL
Ocean Blue	2.5-4.5	1.0	FP-3069-2	2 mL
Sky Blue	2.5-4.5	1.0	FP-3070-2	2 mL
Yellow	2.5-4.5	1.0	FP-4052-2	2 mL
Nile Red	2.5-4.5	1.0	FP-4056-2	2 mL
Pink	2.5-4.5	1.0	FP-4058-2	2 mL
Purple	2.5-4.5	1.0	FP-4062-2	2 mL
Sky Blue	2.5-4.5	0.2	FP-4070-2	2 mL
Nile Red	5.0-7.9	1.0	FP-5056-10	10 mL
Yellow	5.0-7.9	1.0	FP-6052-2	2 mL
Nile Red	5.0-7.9	1.0	FP-6056-2	2 mL
Pink	6.0-8.0	1.0	FP-6058-2	2 mL
Ocean Blue	5.0-7.9	0.2	FP-6069-2	2 mL
Yellow	7.0-7.9	1.0	FP-7052-2	2 mL
Sky Blue	7.0-7.9	0.25	FP-7070-2	2 mL
UV	10.0-14.0	1.0	FP-10040-2	2 mL
Light Yellow	10.0-14.0	1.0	FP-10045-2	2 mL
Yellow	10.0-14.0	1.0	FP-10052-2	2 mL
Nile Red	10.0-14.0	1.0	FP-10056-10	10 mL
Nile Red	10.0-14.0	1.0	FP-10056-2	2 mL
Purple	10.0-14.0	1.0	FP-10062-2	2 mL
CyBlue	10.0-14.0	10^7/mL	FP-10066-2	2 mL
PAK Blue	10.0-14.0	10^7/mL	FP-10067-2	2 mL
Sky Blue	10.0-14.0	0.2	FP-10070-2	2 mL
UV	15.0-19.0	1.0	FP-15040-2	2 mL
Light Yellow	15.0-19.0	1.0	FP-15045-2	2 mL
Yellow	15.0-19.0	1.0	FP-15052-2	2 mL
Nile Red	15.0-19.0	1.0	FP-15056-2	2 mL
Purple	15.0-19.0	1.0	FP-15062-2	2 mL
CyBlue	15.0-19.0	10^7/mL	FP-15066-2	
PAK Blue	15.0-19.0	1.0	FP-15067-2	2 mL
Sky Blue	15.0-19.0	0.2	FP-15070-2	2 mL
Yellow	18.0-24.9	1.0	FP-20052-5	5 mL
Nile Red	18.0-24.9	1.0	FP-20056-5	5 mL
Yellow	25.0-35.0	1.0	FP-30052-5	5 mL
Nile Red	25.0-35.0	1.0	FP-30056-5	5 mL
Purple	25.0-35.0	1.0	FP-30062-5	5 mL

SPHERO™ Low Intensity Fluorescent Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Light Yellow	0.7-0.9	1.0	FL-0845-2	2 mL
Yellow	0.7-0.9	1.0	FL-0852-2	2 mL
UV	1.7-2.2	1.0	FL-2040-2	2 mL
Light Yellow	1.7-2.2	1.0	FL-2045-2	2 mL
Yellow	1.7-2.2	1.0	FL-2052-2	2 mL
Nile Red	1.7-2.2	1.0	FL-2056-2	2 mL
Purple	1.7-2.2	1.0	FL-2062-2	2 mL
Blue	1.7-2.2	1.0	FL-2068-2	2 mL
Blue	2.5-4.5	1.0	FL-3068-2	2 mL
Sky Blue	3.6-4.5	1.0	FL-4070-2	2 mL
Nile Red	5.0-7.9	1.0	FL-6056-2	2 mL
Blue	6.0-8.0	1.0	FL-6068-2	2 mL
Nile Red	10.0-14.0	1.0	FL-10056-2	2 mL

SPHERO™ Fluorescent PMMA

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Yellow	38.0-44.0	1.0	FPMA-40052-5	5 mL
Nile Red	38.0-44.0	1.0	FPMA-40056-5	5 mL
Purple	38.0-44.0	1.0	FPMA-40062-5	5 mL
Nile Red	45.0-52.0	1.0	FPMA-50056-5	5 mL
Purple	53.0-62.0	1.0	FPMA-60062-5	5 mL

SPHERO™ Multiple Fluorophore Fluorescent Particles

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
Multiple Fluorophore	0.1-0.3	0.2	FP-0257-2	2 mL
Multiple Fluorophore	0.4-0.6	0.2	FP-0557-2	2 mL
Multiple Fluorophore	0.7-0.9	0.2	FP-0857-2	2 mL
Multiple Fluorophore	1.7-2.2	0.2	FP-2057-2	2 mL
Multiple Fluorophore	2.5-5.0	0.2	FP-3057-2	2 mL
Multiple Fluorophore	6.0-7.9	1.0	FP-6057-2	2 mL
UV / Light Yellow	0.7-0.9	1.0	FP-0842-2	2 mL
UV / Light Yellow	1.7-2.2	1.0	FP-2042-2	2 mL
UV / Purple / Yellow / Pink / Nile Blue	1.7-2.2	1.0	FP-2054-2	2 mL
Purple / Yellow	1.7-2.2	1.0	FP-2060-2	2 mL
UV / Light Yellow	2.5-5.0	1.0	FP-3042-2	2 mL
Pink / Yellow	2.5-5.0	1.0	FP-3055-2	2 mL
Nile Red / Blue	2.5-4.5	1.0	FP-3066-2	2 mL
Purple / Yellow	2.5-5.0	1.0	FP-4060-2	2 mL
Nile Red / Blue	4.6-5.9	1.0	FP-5066-2	2 mL
Purple / Yellow, High Intensity	1.7-2.2	1.0	FH-2060-2	2 mL
Purple / Yellow, Low Intensity	1.7-2.2	1.0	FL-2060-2	2 mL

SPHERO™ High Intensity Fluorescent Polystyrene

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
UV	1.7-2.2	1.0	FH-2040-2	2 mL
Light Yellow	1.7-2.2	1.0	FH-2045-2	2 mL
Yellow	1.7-2.2	1.0	FH-2052-2	2 mL
Nile Red	1.7-2.2	1.0	FH-2056-2	2 mL
Purple	1.7-2.2	1.0	FH-2062-2	2 mL
Sky Blue	1.7-2.2	0.2	FH-2070-2	2 mL
Nile Red	2.5-4.5	1.0	FH-3056-2	2 mL
Nile Red	5.0-7.9	1.0	FH-5056-2	2 mL
UV	10.0-14.0	1.0	FH-10040-2	2 mL
Yellow	10.0-14.0	1.0	FH-10052-2	2 mL
Nile Red	10.0-14.0	1.0	FH-10056-10	10 mL
Nile Red	10.0-14.0	1.0	FH-10056-2	2 mL
Purple	10.0-14.0	1.0	FH-10062-2	2 mL
Purple	15.0-19.0	1.0	FH-15062-2	2 mL

SPHERO™ FITC Polystyrene Particles

- Surfaced labeled with FITC
- Used as calibration particles for flow cytometry
- Also used to cross calibrate different flow cytometers for data normalization.

Particle Type and Surface	Size, µm	% w/v	Catalog No.	Unit
FITC	0.7-0.9	0.1	FICP-08-2	2 mL
FITC	2.0-2.9	0.1	FICP-20-2	2 mL
FITC	3.0-3.9	0.1	FICP-30-2	2 mL
FITC	5.0-5.9	0.1	FICP-50-2	2 mL
FITC	7.0-7.9	0.1	FICP-70-2	2 mL
FITC	8.0-8.9	0.1	FICP-80-2	2 mL

Figure 23 Flow cytometry histograms for Cat. No. FH-2052-2 lot AC01 (Fluorescent Particles, Yellow, High Intensity, 1% w/v, 1.84 µm, 2 mL).

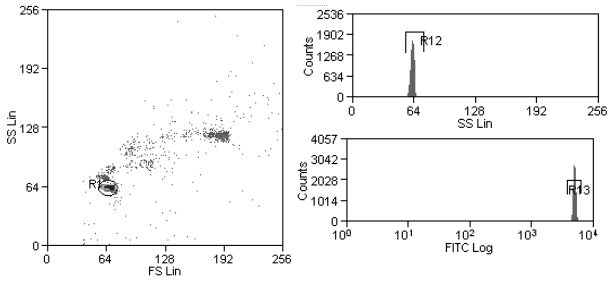


Figure 24 Histogram of SPHERO™ Cat. No. FICP-30-2 (FITC Polystyrene Particles, 0.1% w/v, 3.72 µm , 2 mL) from a BD Bioscience LSRFortessa™ X-20

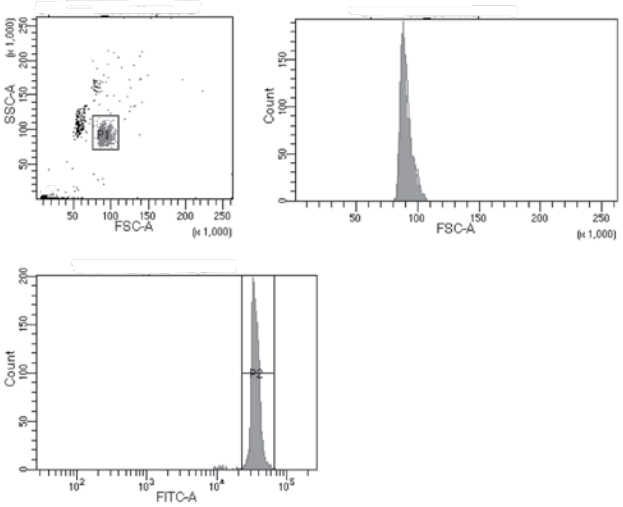


Figure 25 Histogram of SPHERO™ Cat. No. FP-10045-2 (Fluorescent Particles, Light Yellow, 1% w/v, 10.8 µm, 2 mL) from a BD Bioscience LSRFortessa™ X-20

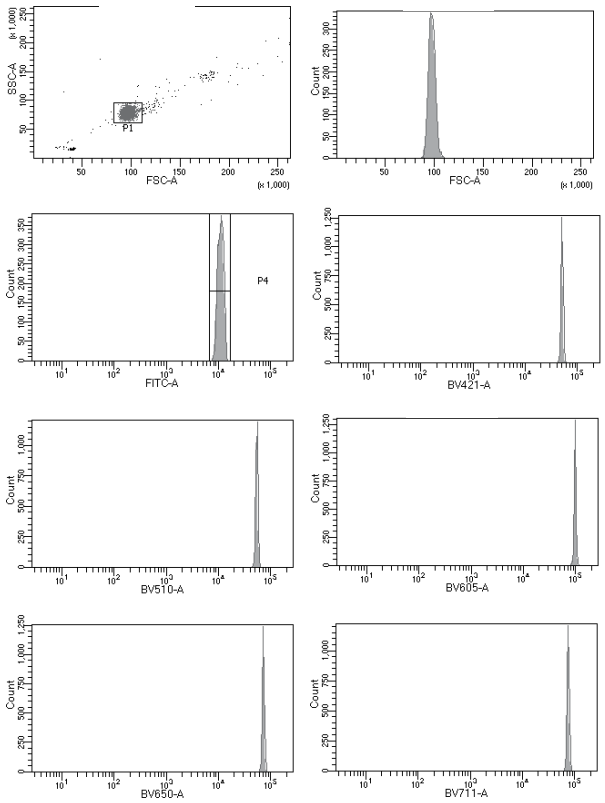


Figure 26 Histogram of SPHERO™ Cat. No. FICP-20-2 (FITC Polystyrene Particles, 0.1% w/v, 1.87 µm , 2 mL) from a BD Bioscience LSRFortessa™ X-20

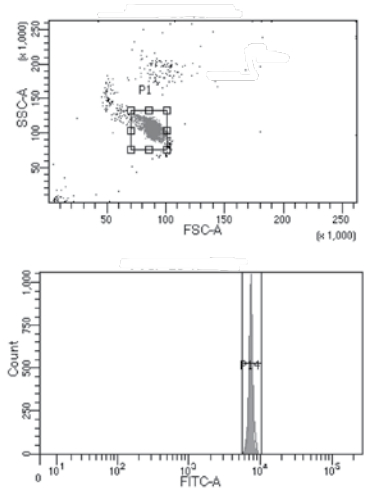


Figure 27 Histogram of SPHERO™ Cat. No. FP-10040-2 (Fluorescent Particles, UV, 1% w/v, 10.2 µm from a BD Bioscience LSRFortessa™ X-20