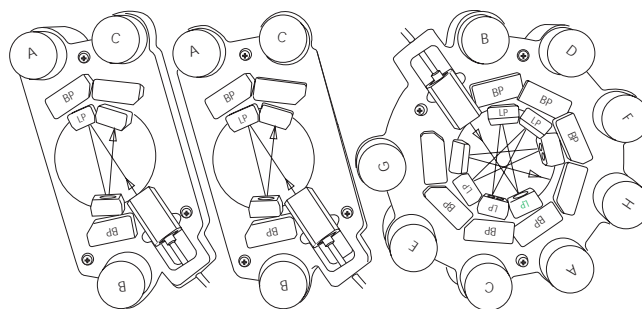


## Technical Resources

### BD LSR II and BD FACSAria Instrument Optical Layouts and Components



Default BD LSR II flow cytometer filter configuration (four-laser system)\*

DETECTOR ARRAY (LASER)	PMT (DETECTOR)	LONGPASS DICHROIC MIRROR	BANDPASS FILTER	INTENDED DYE
Octagon (488-nm blue laser)	A	735	780/60	PE-Cy7
	B	685	695/40	PerCP-Cy5.5 or PE-Cy5.5
	C	550	575/26	PE, PI
	D	505	530/30	FITC, GFP, Alexa Fluor® 488
	E	blank	488/10	SSC
	F	blank	blank	blank
	G	blank	blank	blank
	H	blank	blank	blank
Violet Trigon (405-nm violet laser)	A	505	525/50	Cyan fluorescent protein (CFP), Alexa Fluor® 430, AmCyan
	B	blank	440/40	Pacific Blue™, Marina Blue®, Alexa Fluor® 405, Cascade Blue
	C	blank	blank	blank,
UV Trigon (325-nm or 355-nm UV laser)	A	505	530/30	Indo-1 (blue)
	B	blank	405/20	DAPI, Alexa Fluor® 350, Indo-1 (violet)
	C	blank	blank	blank,
Red Trigon (633-nm red laser)	A	735	780/60	APC-Cy7
	B	blank	660/20	APC, Alexa Fluor® 647
	C	blank	blank	blank

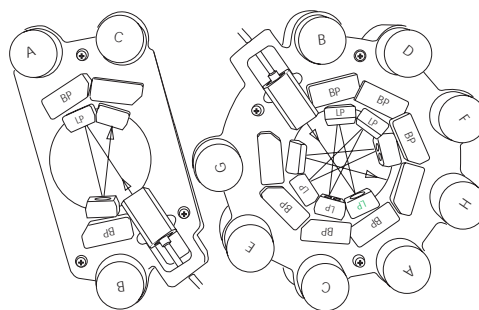
\* The BD LSR II comes with additional filters. Refer to the BD LSR II Users Guide for more information.  
blank indicates a blank optical holder should be used instead of a mirror or filter.

Default BD FACSAria flow cytometer filter configuration (three-laser system)

DETECTOR ARRAY (LASER)	PMT (DETECTOR)	LONGPASS DICHROIC MIRROR	BANDPASS FILTER	INTENDED DYE
Octagon (488-nm blue laser)	A	735	780/60	PE-Cy7
	B	655	695/40	PE-Cy5
			675/20	PerCP or PI
	C	595	610/20	PE-Texas Red®
			575/26	PE or PI
			585/42	Alternative for PE/PI when not using PE-Texas Red®
	E	502	530/30	FITC, Alexa Fluor® 488
	F		488/10	SSC
Red Trigon (633-nm red laser)	A	735	780/60	APC-Cy7
	B	blank	660/20	APC, Alexa Fluor® 647
	C	blank	blank	blank
Violet Trigon (407-nm violet laser)	A	502	530/30	Alexa Fluor® 430
	B	blank	450/40	Cascade Blue®, Pacific Blue™, Hoechst, DAPI, Alexa Fluor® 405
	C	blank	blank	blank

blank indicates a blank optical holder should be used instead of a mirror or filter.

## Technical Resources



Default BD FACSCanto flow cytometer filter configuration (two-laser system)

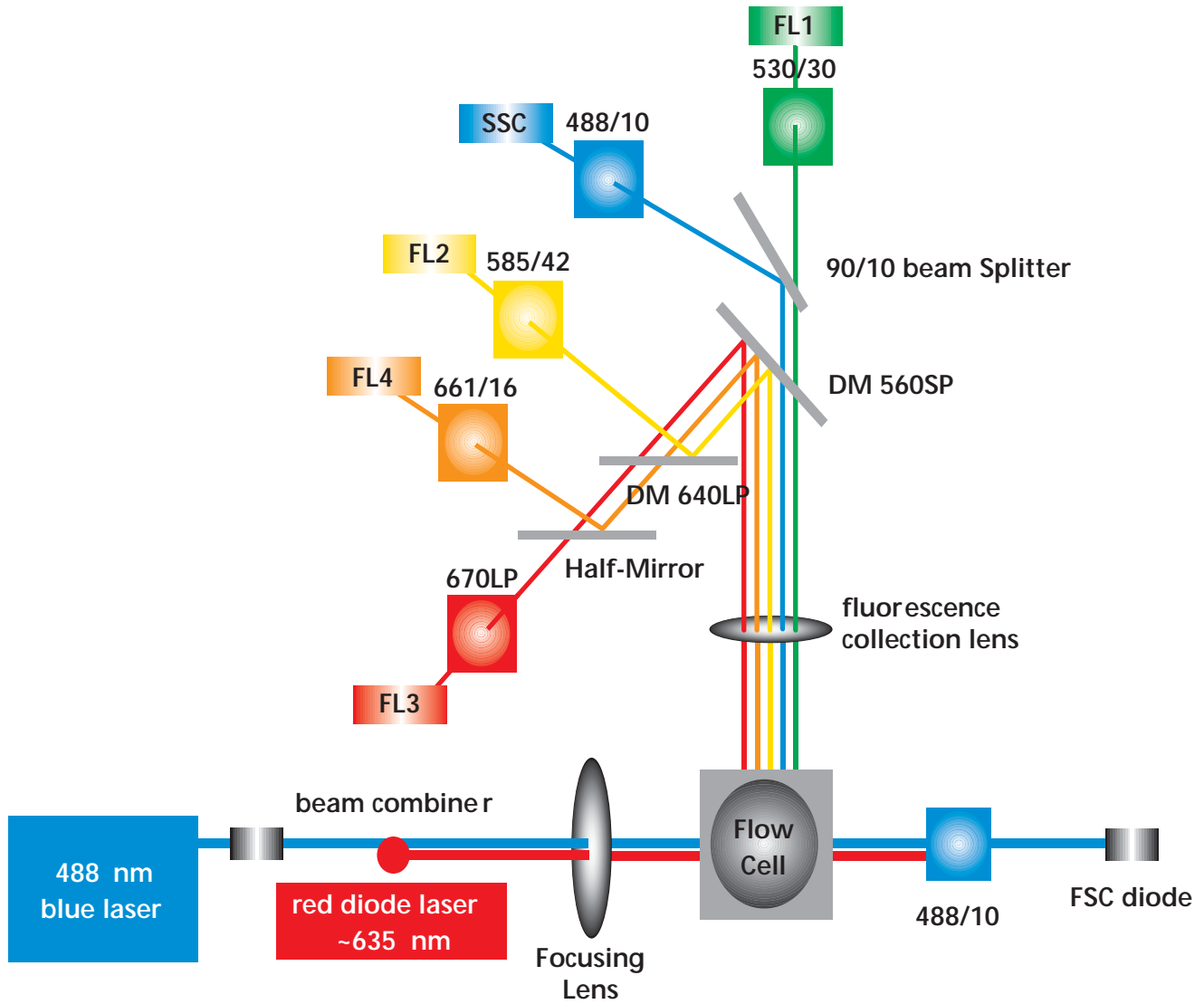
DETECTOR ARRAY (LASER)	PMT (DETECTOR)	LONGPASS DICHROIC MIRROR	BANDPASS FILTER	INTENDED DYE
Octagon (488-nm blue laser)	A	735	780/60	PE-Cy7
	B	655	670	PerCP-Cy5.5, PerCP
	C	556	585/42	PE, PI
	D	502	530/30	FITC, GFP, Alexa Fluor® 488
	E	blank	488/10	SSC
	F	blank	blank	blank
	G	blank	blank	blank
	H	blank	blank	blank
Red Trigon (633-nm red laser)	A	735	780/60	APC-Cy7
	B	blank	660/20	APC, Alexa Fluor® 647
	C	blank	blank	blank

Default BD FACSArray bioanalyzer filter configuration (two-laser system)

DETECTOR ARRAY (LASER)	PMT (DETECTOR)	BANDPASS (BP) OR LONGPASS (LP) FILTER	INTENDED DYE
532-nm green laser	Yellow	585/42 BP	PE or PI,
	Far Red	>685 LP	PE-Cy7, Per CP-Cy5.5, or PI
635-nm red laser	Red	661/16 BP	APC or Alexa Fluor® 647
	Near Infrared (NIR)	788/60 BP	APC-Cy7

# Technical Resources

## BD FACSCalibur™ Optical Layout



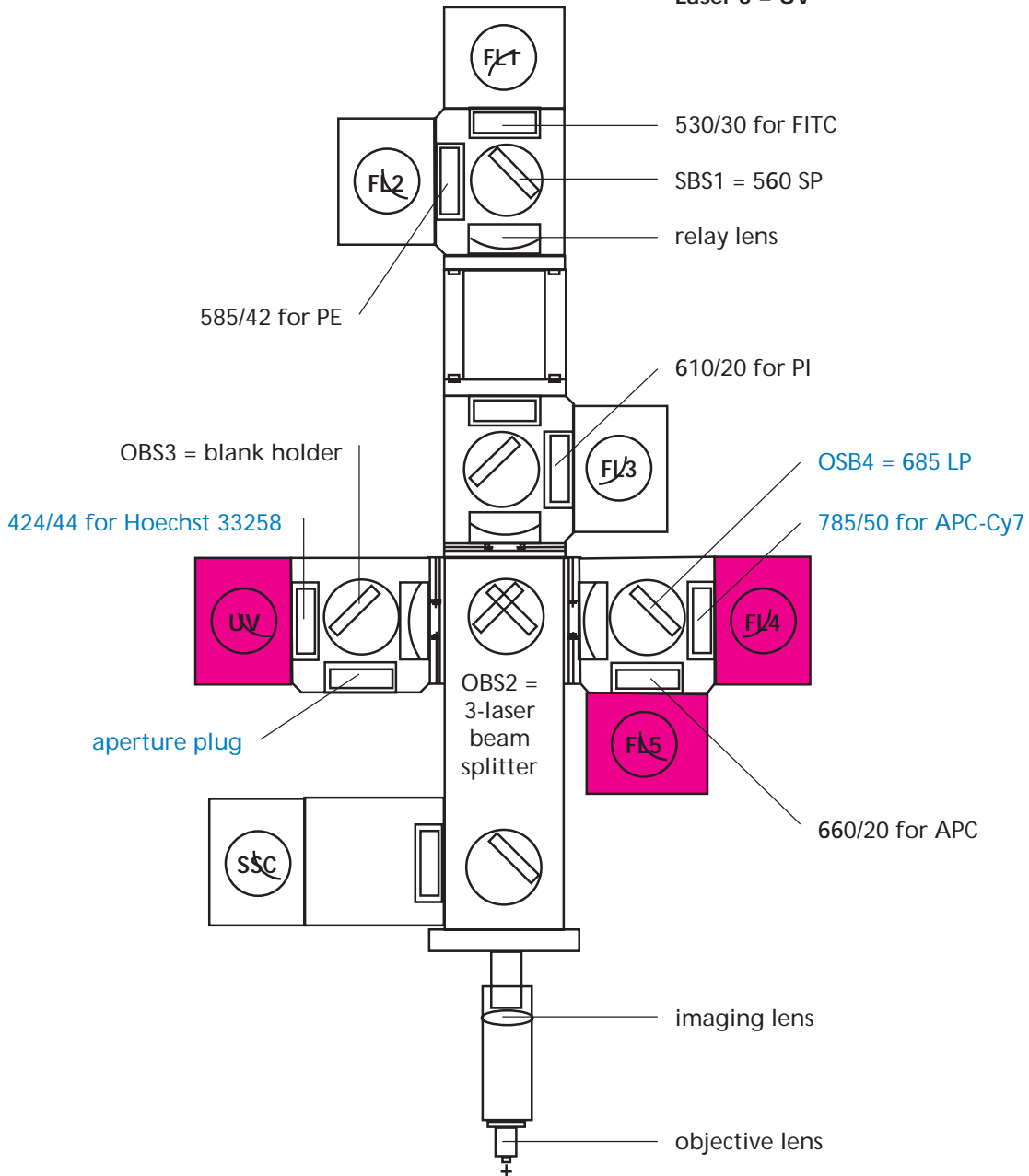


Technical Resources

BD FACSVantage™ SE Configuration (continued)

Example 2: Base Configuration + 3 **Optional Detectors\*** —  
6 Colors/3 Lasers

Laser 1 = 488 nm  
Laser 2 = 633 nm (HeNe)  
Laser 3 = UV



■ = Optional detectors

\* Other detector options and optical configurations are available. Consult with your instrument sales representative for more information.







# Technical Resources

Laser Output Table

Wavelength	BD FACSAria												BD FACSAria												BD FACSAria												BD FACSAria											
	BD LSR II				BD FACSAria				BD FACSAria				BD FACSAria				BD FACSAria				BD FACSAria				BD FACSAria				BD FACSAria				BD FACSAria															
	Lightwave Solid State UV	Kimmon HeCd	Coherent Radius 405	Coherent Sapphire 20 mW	JDS Uniphase HeNe	Point Source Violet	Uniphase Argon-Ion	Hitachi diode	JDS Uniphase 532 nm	Omnichrome HeCd	Innova 70C-Spectrum	Innova 302C Krypton	Innova 90C-A4	Innova 90C-A5	Innova 304C-UV	Innova 305C-UV	Innova 306C-UV	Enterprise IIC	Innova 70C-4	Spectra-Physics 177G	Coherent Sapphire 200 mW	Spectra-Physics HeNe																										
Multiline Visible																																																
UV (MM)	0.020W	0.008W								0.035W	2.500W								4.00W																													
Multiline UV (Ar-Kr)																																																
Multiline UV (Kr)																																																
Multiline UV (Ar)																																																
Multiline Violet																																																
Violet																																																
Violet			0.025W			0.017W																																										
Blue																																																
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Far Red																																																

## Technical Resources

### Choosing Antibody-Fluorochrome Combinations

When performing multicolor flow cytometric analysis, a major factor in the success of the analysis is choosing which antibody to use with which fluorochrome. There are often many correct combinations possible. A number of factors needs to be considered.

#### Intensity (Brightness) of the Fluorochrome/mAb Conjugate

Each fluorochrome has a unique quantum efficiency and therefore differs in its relative fluorescence intensity. The ability of a given antibody to resolve a positive from a negative result depends on which fluorochrome conjugate is used. Figure 1 shows an example of the staining pattern using the same monoclonal antibody (mAb) conjugated to ten different fluorochromes. The resulting data depends on several factors: conjugates choice, background levels, and instrument sensitivity.

Note the following.

- For a given mAb the S/N ratio of positive and negative cells can differ four- to six-fold depending on the fluorochrome used.
- The relative fluorochrome intensity depends on the instrument (not shown). This is because of differences in the laser and filter combinations used on the different instruments.

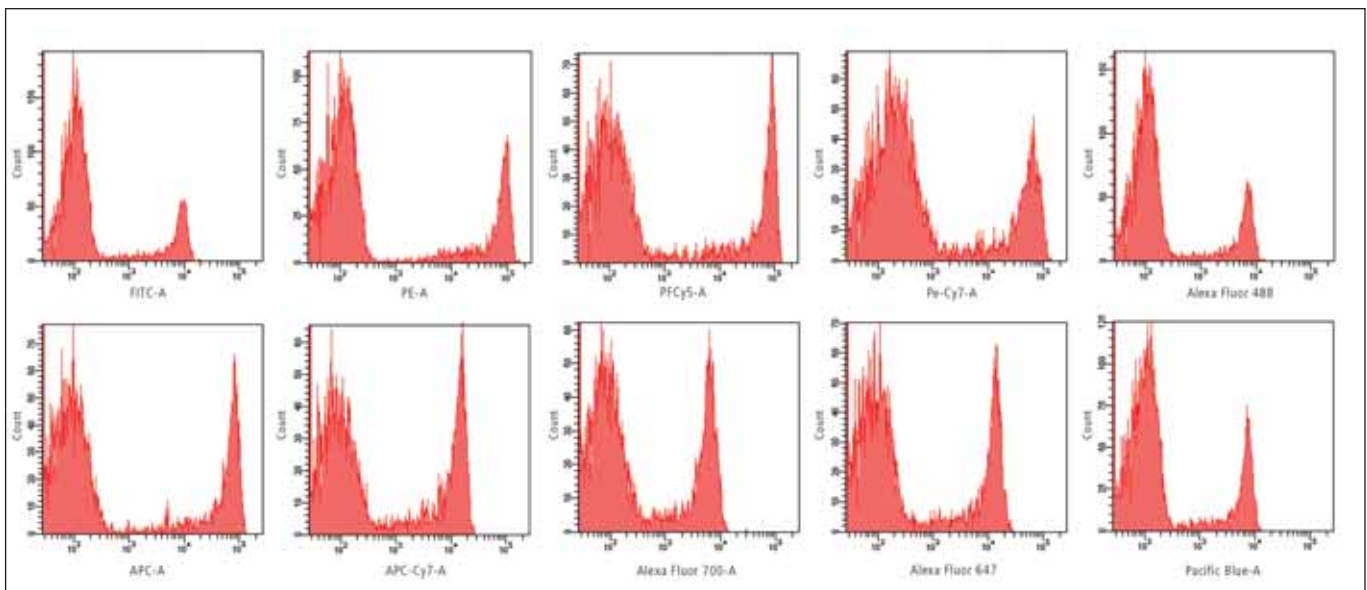
A general guideline for the relative intensities of the various fluorochromes is shown in the following table. Note that this is a general pattern when using a S/N ratio. Some differences are seen for individual mAbs.

INSTRUMENT	RELATIVE FLUOROCHROME INTENSITY (BRIGHTER → DIMMER)
BD LSR II instrument equipped with standard laser configuration	PE, PE-Cy7, PE-Cy5, APC > APC-Cy7 Alexa Fluor 647, Alexa Fluor 700 > FITC, Pacific Blue, Alexa Fluor 488

#### F/P Ratio

The number of fluorochromes present on the antibody (F/P ratio) can also affect the relative brightness. FITC and PerCP conjugates are made with several (2 to 9 depending on the antibody) fluorochromes per antibody, while APC and PE conjugates are made at approximately one fluorochrome per antibody. Tandem conjugates of Cy7 with PE and APC have multiple Cy7 molecules bound per PE or APC protein, whereas approximately one PE or APC molecule is conjugated to each antibody. In contrast, tandem conjugates of Cy5.5 with PerCP contain approximately one Cy5.5 molecule per PerCP molecule. Because of the conjugation chemistry required, IgM antibodies are conjugated only to small-molecule fluorochromes, such as FITC, Texas Red®, Cy3, and Cy5.

Figure 1. BD LSR II SE Flow Cytometer



## Technical Resources

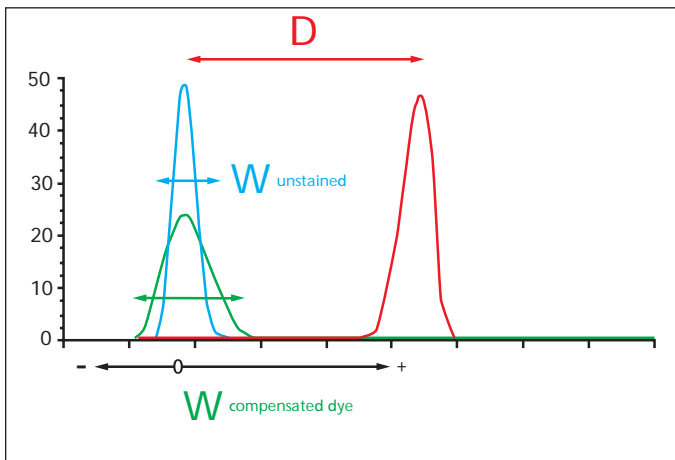
### Choosing Antibody-Fluorochrome Combinations (continued) Antigen Density

A highly expressed antigen will usually be resolved with almost any fluorophore. An antigen expressed at lower density might require the higher S/B ratio provided by a PE or APC conjugate, to separate the positive cells adequately from the unstained cells.

#### Stain Index\*

A useful metric to express the relative brightness of a positive signal is shown below by calculating the Stain Index (SI):

$$\frac{D}{W} = \frac{\text{Mean}_{(\text{pos})} - \text{Mean}_{(\text{neg})}}{2 \times \text{sd}_{(\text{neg})}} = \text{S.I.}$$



#### Definitions

D = the difference of the populations means

W = the width of negative (at 2 standard deviations)

Mean<sub>(pos)</sub> = the mean of the positive population

Mean<sub>(neg)</sub> = the mean of the negative population

Std<sub>(neg)</sub> = the standard deviation of the negative population

The algorithm normalizes the positive population signal with the degree of spread observed in the negative population or compensated cells from another dye dimension.

In certain instrument systems the median, rather than the mean, should be used.

#### Autofluorescence

Individual cell populations have characteristic levels of autofluorescence (fluorescent signals generated by the cells themselves).

While autofluorescence is observed in all fluorescence channels, it decreases dramatically at longer wavelengths (>600 nm).

For cell types that are very autofluorescent, using a dye with a longer excitation and/or emission wavelength (eg, APC) can give a better signal-to-background ratio.

For cell types that are not very autofluorescent, the improved separation seen with long-wavelength excitation is less apparent. FITC conjugates can be used.

\*Ref: Bigos M, Stovel R, Parks D. "Evaluating multicolor fluorescence data quality among different instruments and different laser powers—methods and results." *Cytometry* 59A:42 (2004).

## Technical Resources

### Non-Specific Binding

A number of antibody conjugates exhibit low-level non-specific binding that can increase the fluorescence of negative cells to levels above autofluorescence. This non-specific binding is typically caused by the following.

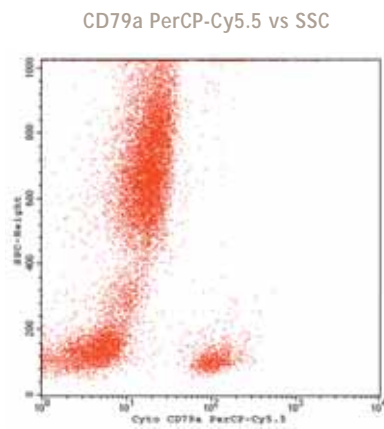
- Isotype of the mAb

Some IgG isotypes are more likely to bind to Fc receptors on some cell types.

- Fluorochrome used

Carbocyanin (Cy3, Cy5, Cy5.5, and Cy7) and Texas Red® direct conjugates and certain tandem conjugates can sometimes show a tendency to increased binding to some cell subsets. In the case of Cy5, this has been shown to be caused by a very low affinity interaction of the dye with the low affinity Fc receptors. This is also a property of PE-Cy5 tandem conjugates. See Figure 2.

*NOTE: In certain situations, such as in the identification of monocytes using our BD Quantibrite Anti-HLA-DR PE/Anti-Monocyte PerCP-Cy5.5 product, this property can be exploited by intentionally putting more carbocyanin dye on the conjugate. This helps ensure detection of all monocytes regardless of CD14 antigen expression levels.*



*Figure 2. PerCP-Cy5.5 tandem conjugates differ from PE-Cy5 tandem conjugates in that substantially less of the carbocyanin component is required to achieve more intense emission. As a result, the degree of monocyte staining of these reagents can be virtually eliminated as demonstrated in the CD79a PerCP-Cy5.5 vs. SSC plot.*

# Technical Resources

## Fluorochrome Reference Chart

### Typical Instrument Configuration

Instrument	Laser	Excitation Laser Line (nm)	Fluorescence Channel	Fluorochromes Provided by BD Biosciences			
BD FACScan™	Argon (L1)	488	FL1 Green	FITC Alexa Fluor® 488			
			FL2 Yellow	PE			
			FL3 Red FL4 Red	PE-Texas Red® PE-Cy5* PerCP PerCP-Cy5.5 PE-Cy7			
BD FACSCalibur™	Argon (L1)	488	FL1 Green	FITC Alexa Fluor® 488			
			FL2 Yellow	PE			
			FL3 Red FL4 Red	PE-Cy5* PerCP PerCP-Cy5.5 PE-Cy7 APC* Alexa Fluor® 647			
BD FACSCanto™	Argon (L1)	488	Green	FITC			
			Yellow	PE			
			Red Infra Red	PerCP PerCP-Cy5.5 PE-Cy7			
	HeNe (L2)	633	Red Infra Red	APC* APC-Cy7			
			BD FACSArray™ SE (typical setup)	Argon (L1)	488	FL1 Green	FITC Alexa Fluor® 488
						FL2 Yellow	PE
FL6 Red FL3 InfraRed	PE-Texas Red® PE-Cy5* PerCP-Cy5.5 PE-Cy7						
Krypton (L2)	407	FL4 (1) Blue	Alexa Fluor® 405 Pacific Blue®				
		FL4 (2) Red FL5 InfraRed	APC* Alexa Fluor® 647 APC-Cy7				
BD™ LSR (typical setup)	Argon (L1)	488	FL1 Green	FITC Alexa Fluor® 488			
			FL2 Yellow	PE			
			FL3 Red FL4, FL5 Blue	PE-Texas Red® PE-Cy5* PerCP PerCP-Cy5.5 PE-Cy7			
	HeCd (L2)	325	FL4, FL5 Blue				
	HeNe (L3)	633	FL6 Red	APC* Alexa Fluor® 647 APC-Cy7			
BD™ LSR II (typical setup)	Argon (L1)	488	Green	FITC Alexa Fluor® 488			
			Yellow	PE			
			Red Infra Red	PE-Texas Red® PE-Cy5* PerCP PerCP-Cy5.5 PE-Cy7			
	HeNe (L2)	633	Red Infra Red	APC* Alexa Fluor® 647 APC-Cy7			
			UV (L3)	355	Violet		
	Violet (L4)	405			Blue	Alexa Fluor® 405 Pacific Blue®	
			Green	AmCyan			
Blue	Alexa Fluor® 405 Pacific Blue®						
BD FACSaria™ (typical setup)	Argon (L1)	488	Green	FITC Alexa Fluor® 488			
			Yellow	PE			
			Red Far Red InfraRed	PE-Texas Red® PE-Cy5* PerCP PerCP-Cy5.5 PE-Cy7			
	HeNe (L2)	633	Red InfraRed	APC* Alexa Fluor® 647 APC-Cy7			
			Violet (L3)	407	Green	AmCyan	
	Blue	Alexa Fluor® 405 Pacific Blue®					
BD FACSArray™ (typical setup)	Green Diode (L1)	532	Yellow	PE			
			Far Red	PerCP-Cy5.5 PE-Cy7			
	Red Diode (L2)	635	Red Infra Red	APC* Alexa Fluor® 647 APC-Cy7			

\* APC and PE-Cy5 may be used together on instruments with cross-beam compensation.

# Technical Resources

## Fluorochrome Reference Chart

### Fluorochrome Specifications

Fluorochrome	Fluorescence Emission Color	Ex-Max (nm)	Excitation Laser Line (nm)	Em-Max (nm)	BD FACScan™	BD FACSCalibur™	BD FACSCanto™	BD FACSVantage™ SE	BD™ LSR	BD™ LSR II	BD FACSMia™	BD FACSAria™
Alexa Fluor® 405	Blue	401	360, 405, 407	421				✓		✓	✓	
Pacific Blue®	Blue	405	360, 405, 407	455				✓		✓	✓	
AmCyan	Green	457	405, 407	491				✓		✓	✓	
Alexa Fluor® 488	Green	495	488	519	✓	✓		✓	✓	✓	✓	✓
FITC	Green	494	488	519	✓	✓	✓	✓	✓	✓	✓	✓
PE	Yellow	496, 564	488, 532	578	✓	✓	✓	✓	✓	✓	✓	✓
PE-Texas Red®	Orange	496, 564	488, 532	615	✓			✓	✓	✓	✓	
Texas Red®**	Orange	595	595	615				✓				
APC*	Red	650	595, 633, 635, 647	660		✓	✓	✓	✓	✓	✓	✓
Alexa Fluor® 647	Red	650	595, 633, 635, 647	668		✓		✓	✓	✓	✓	✓
PE-Cy5*	Red	496, 564	488, 532	667	✓	✓		✓	✓	✓	✓	
PerCP	Red	482	488, 532	678	✓	✓	✓	✓	✓	✓	✓	
PerCP-Cy5.5	Far Red	482	488, 532	695	✓	✓	✓	✓	✓	✓	✓	✓
Alexa Fluor® 700***	Far Red	696	633, 635	719				✓		✓	✓	
PE-Cy7	InfraRed†	496, 564	488, 532	785	✓	✓	✓	✓	✓	✓	✓	✓
APC-Cy7	InfraRed†	650	595, 633, 635, 647	785			✓	✓	✓	✓	✓	✓

† InfraRed detection requires a Hamamatsu R3896 Photomultiplier Tube (comes with detector option).

\* APC and PE-Cy5 may be used together on instruments with cross-beam compensation.

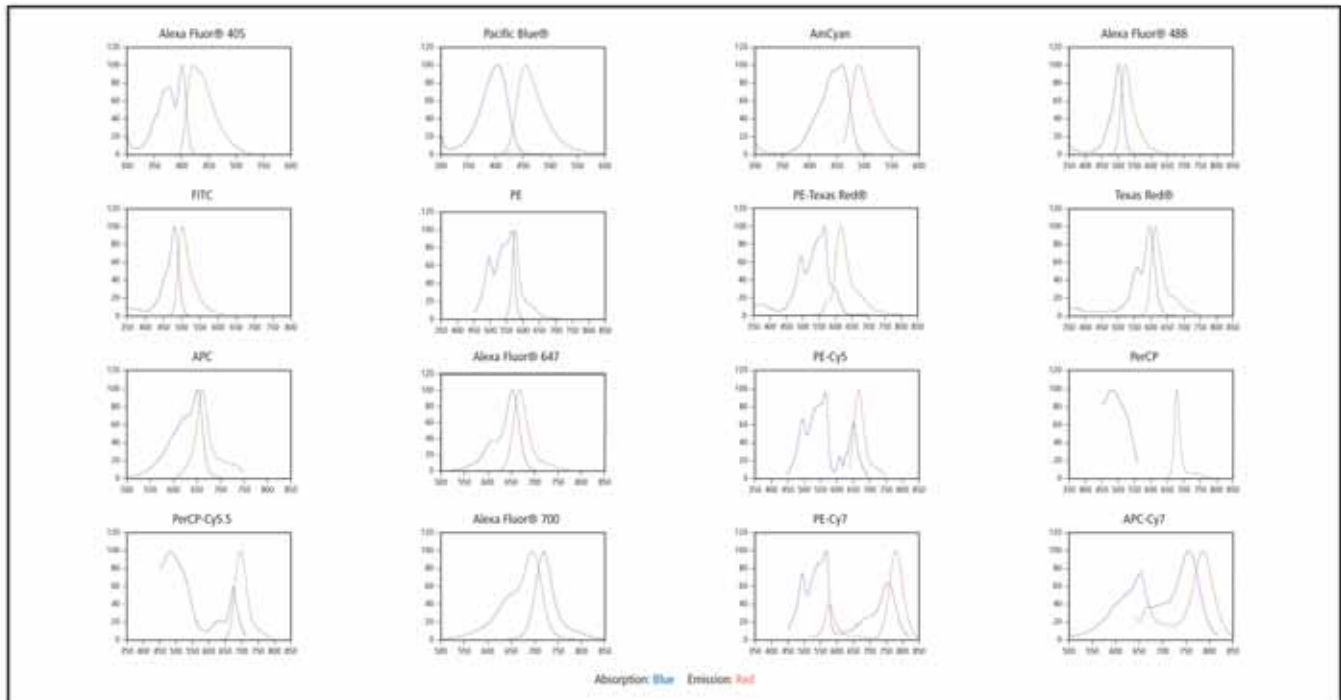
\*\* Texas Red® detection requires a dye laser for 595-600 nm excitation.

\*\*\* Alexa Fluor® 700 detection is available through an expanded optical configuration.

# Technical Resources

## Fluorochrome Reference Chart

### Absorption and Emission Spectra for BD Biosciences Fluorochromes



## Technical Resources

### Analog vs Digital Data Display

#### Notes on Digital Flow Cytometry

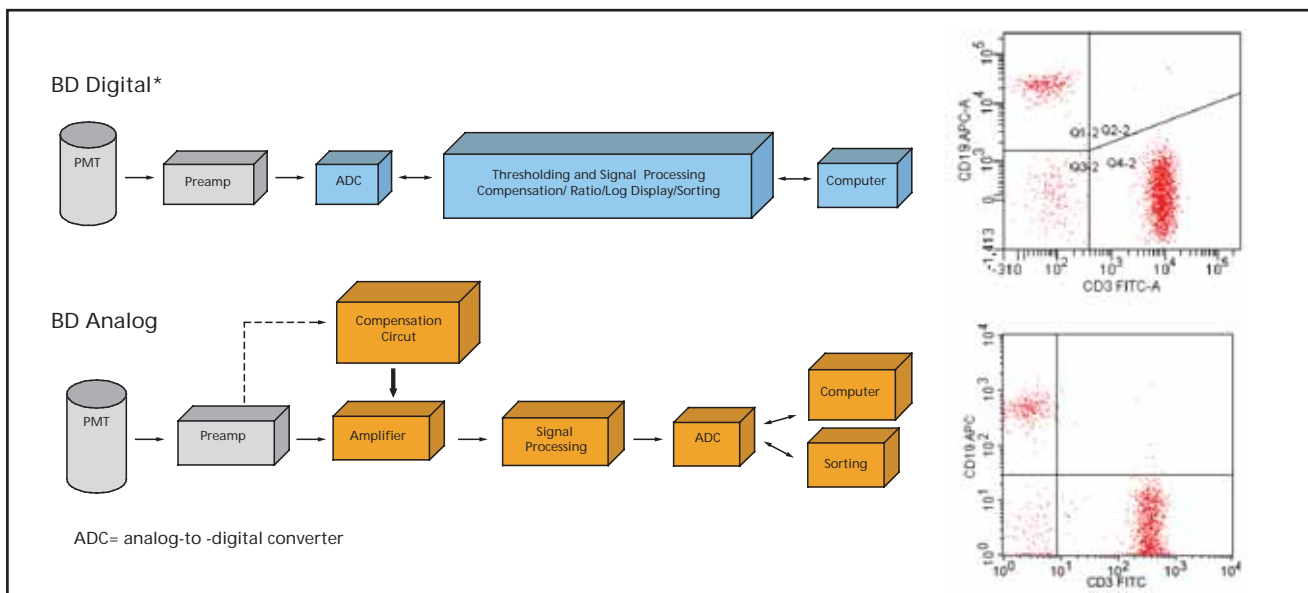
- Conversion of analog signals to digital occurs earlier in digital systems. Note the placement of the analog-to-digital converter (ADC) in the diagrams below.
- Early digitization provides more accurate data for several reasons.

Signals are digitized before processing, where electronic errors can be introduced. For example, compensation circuits and log amplifiers in analog systems introduce errors during signal processing. Digital systems avoid these errors because compensation and log visualization are computed mathematically.

Pulse area analysis for each parameter is the default and measures total fluorescence.

Matrix algebra can be used to calculate compensation real time for all parameter combinations.

- Digital systems are more versatile than analog systems because they can threshold off any laser or combination of lasers.



\* BD Digital data displayed using biexponential scaling and pivoted quadrant gates are both found in BD FACSDiva Software v4.1 or higher.