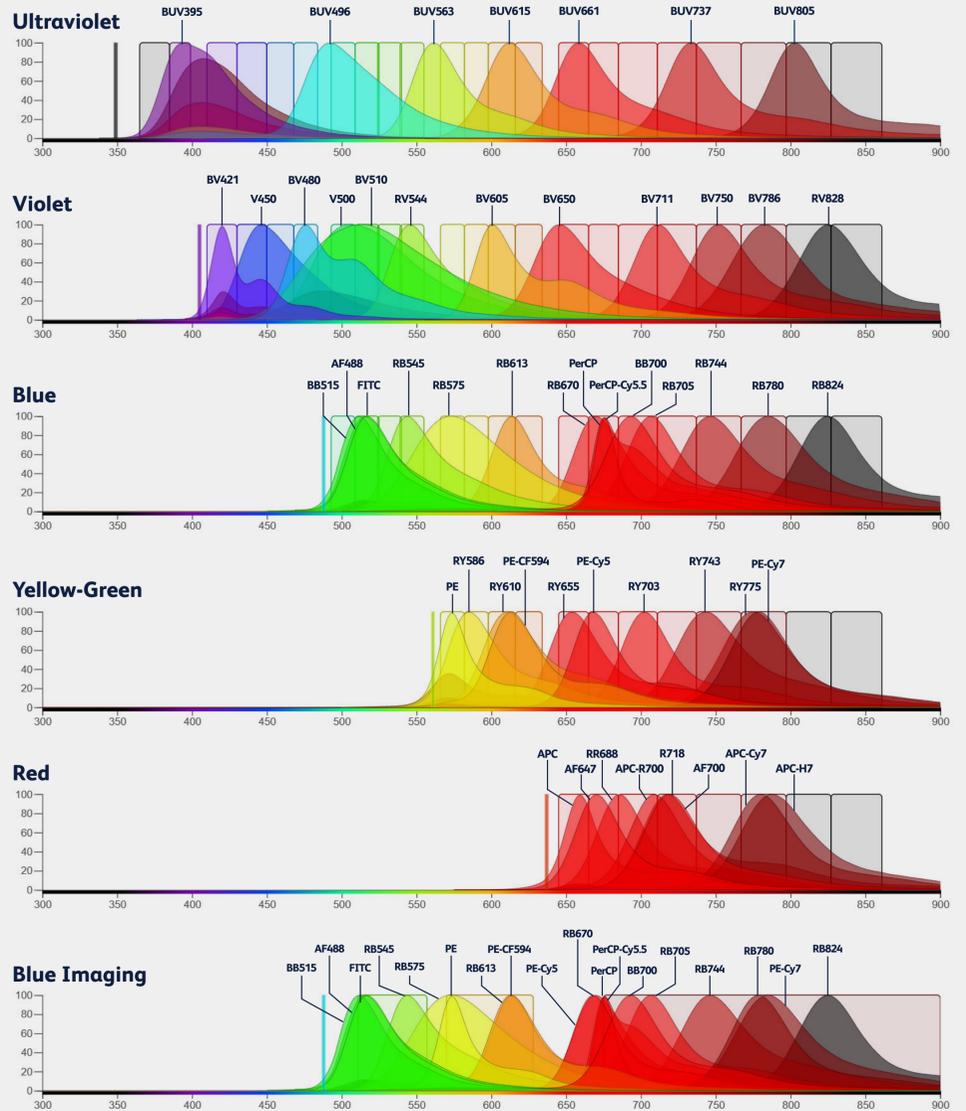


Fluorochrome Selection Guide: BD FACSDiscover™ A8 Cell Analyzer and BD FACSDiscover™ S8 Cell Sorter

Spectral and Imaging Flow Cytometry



Fluorochrome Common Name	Em (nm)	Resolution (CD4 Stain Index)	Spectral Spillover (Cross-Laser and Residual)					Imaging (Signal or Spillover)				
			UV	V	B	YG	R	IMG 1	IMG 2	IMG 3		
Ultraviolet 349 nm 30 mW	BUV395	395	●●●●○	○	○	○	○	○	○	○	○	○
	BUV496	496	●●●●○	○	○	○	○	○	○	○	○	○
	BUV563	564	●●●●○	○	○	○	○	○	○	○	○	○
	BUV615	615	●●●●○	○	○	○	○	○	○	○	○	○
	BUV661	660	●●●●○	○	○	○	○	○	○	○	○	○
	BUV737	735	●●●●○	○	○	○	○	○	○	○	○	○
	BUV805	803	●●●●○	○	○	○	○	○	○	○	○	○
Violet 405 nm 50 mW	BV421	423	●●●●○	○	○	○	○	○	○	○	○	○
	V450	450	●●●●○	○	○	○	○	○	○	○	○	○
	BV480	479	●●●●○	○	○	○	○	○	○	○	○	○
	V500	499	●●●●○	○	○	○	○	○	○	○	○	○
	BV510	512	●●●●○	○	○	○	○	○	○	○	○	○
	RV544	544	●●●●○	○	○	○	○	○	○	○	○	○
	BV605	605	●●●●○	○	○	○	○	○	○	○	○	○
	BV650	649	●●●●○	○	○	○	○	○	○	○	○	○
	BV711	713	●●●●○	○	○	○	○	○	○	○	○	○
	BV750	754	●●●●○	○	○	○	○	○	○	○	○	○
	BV786	786	●●●●○	○	○	○	○	○	○	○	○	○
	RV828	828	●●●●○	○	○	○	○	○	○	○	○	○
Blue 488 nm 100 mW	BB515	515	●●●●○	○	○	○	○	○	○	○	○	○
	AF488	517	●●●●○	○	○	○	○	○	○	○	○	○
	FITC	518	●●●●○	○	○	○	○	○	○	○	○	○
	RB545	545	●●●●○	○	○	○	○	○	○	○	○	○
	RB575	575	●●●●○	○	○	○	○	○	○	○	○	○
	RB613	613	●●●●○	○	○	○	○	○	○	○	○	○
	RB670	670	●●●●○	○	○	○	○	○	○	○	○	○
	PerCP	675	●●●●○	○	○	○	○	○	○	○	○	○
	PerCP-Cy5.5	695	●●●●○	○	○	○	○	○	○	○	○	○
	BB700	695	●●●●○	○	○	○	○	○	○	○	○	○
	RB705	708	●●●●○	○	○	○	○	○	○	○	○	○
	RB744	744	●●●●○	○	○	○	○	○	○	○	○	○
	RB780	781	●●●●○	○	○	○	○	○	○	○	○	○
	RB824	824	●●●●○	○	○	○	○	○	○	○	○	○
Yellow-Green 561 nm 50 mW	PE	576	●●●●○	○	○	○	○	○	○	○	○	○
	RY586	586	●●●●○	○	○	○	○	○	○	○	○	○
	RY610	610	●●●●○	○	○	○	○	○	○	○	○	○
	PE-CF594	615	●●●●○	○	○	○	○	○	○	○	○	○
	RY655	654	●●●●○	○	○	○	○	○	○	○	○	○
	PE-Cy5	670	●●●●○	○	○	○	○	○	○	○	○	○
	RY703	703	●●●●○	○	○	○	○	○	○	○	○	○
	RY743	743	●●●●○	○	○	○	○	○	○	○	○	○
	RY775	775	●●●●○	○	○	○	○	○	○	○	○	○
	PE-Cy7	781	●●●●○	○	○	○	○	○	○	○	○	○
Red 637 nm 100 mW	APC	660	●●●●○	○	○	○	○	○	○	○	○	○
	AF647	669	●●●●○	○	○	○	○	○	○	○	○	○
	RR688	686	●●●●○	○	○	○	○	○	○	○	○	○
	APC-R700	706	●●●●○	○	○	○	○	○	○	○	○	○
	R718	718	●●●●○	○	○	○	○	○	○	○	○	○
	AF700	719	●●●●○	○	○	○	○	○	○	○	○	○
	APC-Cy7	779	●●●●○	○	○	○	○	○	○	○	○	○
	APC-H7	782	●●●●○	○	○	○	○	○	○	○	○	○



BD FACSDiscover™ A8 Cell Analyzer and BD FACSDiscover™ S8 Cell Sorter configuration for a five-laser system with BD CellView™ Image Technology.

Fluorochrome emissions shown are primary laser-specific profiles, highlighting adjacent spillover into neighboring fluorochromes. It is best practice to select fluorochromes across the spectrum, spacing fluorochromes apart can reduce signal overlap, which may create spread and impact panel resolution. For cross-laser and residual donor spectral overlap, see the Spectral Spillover column in the fluorochrome performance chart. Fluorochrome emissions shown were generated using anti-CD4 stained human PBMCs acquired on a BD FACSDiscover™ S8 Cell Sorter and is available on BD™ Spectrum Viewer.

BD fluorochrome performance, specific to the BD FACSDiscover™ A8 Cell Analyzer and BD FACSDiscover™ S8 Cell Sorter.

Spectral Spillover: Cross-Laser: ○ 0-14.9% ○ 15-39.9% ○ 40-69.9% ○ 70-100%
Residual: ○ 0-14.9% ○ 15-39.9% ○ 40-69.9% ○ 70-100%

Imaging: Signal: ○ 0-4.9% ○ 5-29.9% ○ 30-84.9% ○ 85-100% Spillover: □ Minimal impact □ Medium impact □ High impact

Resolution is based on CD4 stain index value calculations and grouped by magnitude to create a five-level ranking.

Spectral Spillover, also known as spectral overlap, is shown for both cross-laser and residual donor. Values are calculated as a percentage of max MFI for each fluorochrome and shown in four levels based on magnitude. For adjacent overlap, see spectral profiles per laser on the BD FACSDiscover™ A8 Cell Analyzer and BD FACSDiscover™ S8 Cell Sorter configuration figure.

Imaging signal or spillover values are shown across the imaging detectors (IMG 1, IMG 2 and IMG 3). Imaging signal indicates a fluorochrome may be used in BD CellView™ Image Technology applications. Levels of signal are shown as percentage of max MFI of the primary imaging detector. Imaging spillover indicates signal from a fluorochrome used in a spectral panel has signal into the imaging detectors. It is best practice to assess the signals from the spectral panel reagents on the resolution of the imaging detector regardless of the expected impact.

All data were generated using anti-CD4 stained human PBMCs and are specific for the BD FACSDiscover™ A8 Cell Analyzer and the BD FACSDiscover™ S8 Cell Sorter.

The information above can help guide fluorochrome selection for applications with or without imaging. Following general panel design principles, it is best practice to assign bright fluorochromes to lower expression markers and use dimmer fluorochromes for high expression markers. When assigning fluorochromes, pay close attention to spectral overlap which can be from cross-laser excitation (e.g., BV711 excitation from the red laser), residual donor signal (e.g., BUV805 spillover into BUV395's emission) and signal into adjacent detectors (e.g., RB705 spillover into RB744's emission). The amount of spread resulting from spectral overlap and the impact on resolution, is dependent on fluorochrome brightness (MFI) and antigen density of the marker. Additionally, fluorochrome spillover characteristics depend on the instrument's configuration and settings. When using a bright fluorochrome with high spillover, assign it to a lower antigen density marker as this may reduce the impact of the spillover-spread. Another strategy is to assign it to a marker that is not co-expressed with markers already paired with fluorochromes that have high spectral overlap.

When designing a spectral panel that uses BD CellView™ Image Technology, fluorochrome assignment becomes critical. Signals from the spectral panel cannot be compensated out of the imaging channels. Therefore, it is best practice to select fluorochromes in the spectral panel that have little to no signal into the imaging channels being used. The resolution impact of signal into the imaging channels can be managed by assigning medium impact dyes to low antigen density markers in the spectral panel. It is essential to assess the impact of all signals from the spectral panel on the resolution of the imaging detector. For high-impact fluorochromes, an alternative strategy is to assign them to markers that are not expressed within the imaging populations.

BD CellView™ Image Technology

The BD FACSDiscover™ S8 Cell Sorter and BD FACSDiscover™ A8 Cell Analyzer leverage BD CellView™ Image Technology, a novel high-speed imaging technology that facilitates analysis and sorting through real-time integration of image and flow data.

Compatible Fluorochromes

	Fluorochrome	Brightness	Biological Examples: Image Wall	Specificity	Target Cell
IMG1	AF488	Moderate		BCMA CAR	CAR T Cells
	BB515	Bright		CD326 (Epcam)	Cell Line: HT-29
	FITC	Moderate		Her-2-Neu	Cell Line: SKBR3
	RB545	Moderate		IFN-γ	Stimulated PBMC
IMG2	PE	Moderate		CD147	Cell Line: HT-1080
	PE-CF594	Moderate		CD14	Resting PBMC
	RB613	Bright		CD25	Resting PBMC
	PE-Cy5	Bright		CD4	Cell Line: Jurkat
IMG3	RB670	Bright		γδ TCR	Resting PBMC
	BB700	Bright		CD4	Resting PBMC
	RB705	Bright		CD54	Stimulated PBMC
	RB744	Bright		CD25	Resting PBMC
	PE-Cy7	Bright		CD8	Murine PBMC
	RB780	Bright		CD13	Cell Line: HT-1080
	RB824	Moderate		CD4	Cell Line: Jurkat

BD CellView™ Image Technology compatible fluorochromes for each of the three imaging channels: IMG 1 (534/66), IMG 2 (600/60) and IMG 3 (788/225). Fluorochrome brightness designations (moderate or bright) are based on image-derived fluorescence signal/noise separation using fluorescence detected outside the region of analysis as background. Biological example images for each fluorochrome are shown using lightloss for reference (left column), fluorescence channel alone (middle column) and then lightloss/fluorescence overlays (right column). The specificity of each antibody used as well as target cell for each example are listed.

Fluorochrome brightness designations were determined using CD4 stained cells with uniform antigen density and cell size. Brightness should not be judged based on examples shown as antigen density and cell size can significantly impact the appearance of brightness. PerCP and PerCP-Cy5.5, which are compatible with BD CellView™ Image Technology, are not shown because they were determined to be too dim for typical use cases.

Image Features

Center of Mass (X) (Scatter and fluorescence) Applications: » Internalization » Cell-cell interaction » Intracellular trafficking Center of Mass (X): Distance between the left edge of the field of view (-) and relative center (centroid) (□) of signal distribution	Size (Scatter and fluorescence) Applications: » Morphology » Label-free sorting » Cell cycle Size: Pixel count (□)
Center of Mass (Y) (Scatter and fluorescence) Applications: » Internalization » Cell-cell interaction » Intracellular trafficking Center of Mass (Y): Distance between the top edge of the field of view (-) and relative center (centroid) (□) of signal distribution	Total Intensity (Scatter and fluorescence) Applications: » Morphology » Imaging FMOs Total Intensity: Additive intensity of all pixels (□)
Long Axis Moment (Scatter and fluorescence) Applications: » Cellular morphology » Cluster identification » Doublet discrimination Long Axis Moment: The longest axis across the distribution of signal (-)	Correlation (Fluorescence: Any two channels) Applications: » Nuclear translocation » Co-localization » Intracellular trafficking Correlation: Score based on the percentage of pixels from two fluorescence channels that occupy the same space (□)
Short Axis Moment (Scatter and fluorescence) Applications: » Cellular morphology » Cluster identification » Doublet discrimination Short Axis Moment: The shortest axis across the distribution of signal (-) that is perpendicular to the long axis moment	Delta Center of Mass (Fluorescence: Any two channels) Applications: » Intracellular trafficking » Cell-cell interaction Delta Center of Mass: Distance between the centroids of two fluorescence channels (-)
Eccentricity (Scatter and fluorescence) Applications: » Morphology » Cluster identification » Doublet discrimination Eccentricity: Long axis (-) / Short axis (-)	Max Intensity (Scatter and fluorescence) Applications: » Punctate fluorescence » Phagocytosis assay » Cell cycle analysis Max Intensity: Brightest pixel (□)
Radial Moment (Scatter and fluorescence) Applications: » Morphology » Cluster identification » Doublet discrimination Radial Moment: Median distance of all pixels from the centroid (-)	Diffusivity (Scatter and fluorescence) Applications: » Punctate fluorescence » Cell morphology » Phagocytosis Diffusivity: Fluorescence-A / Max Intensity (□)

BUV: BD Horizon Brilliant™ UV; BV: BD Horizon Brilliant™ Violet; V: BD Horizon Violet; RV: BD Horizon RealViolet™; BB: BD Horizon Brilliant™ Blue; RB: BD Horizon RealBlue™; RY: BD Horizon RealYellow™; RR: BD Horizon RealRed™. BD flow cytometers are Class 1 Laser Products. For Research Use Only. Not for use in diagnostic or therapeutic procedures.

