Designing minimal spectral overlap panels to enhance data quality

Increasing resolution and ease-of-use with the BD FACSCelesta™ flow cytometer

Features

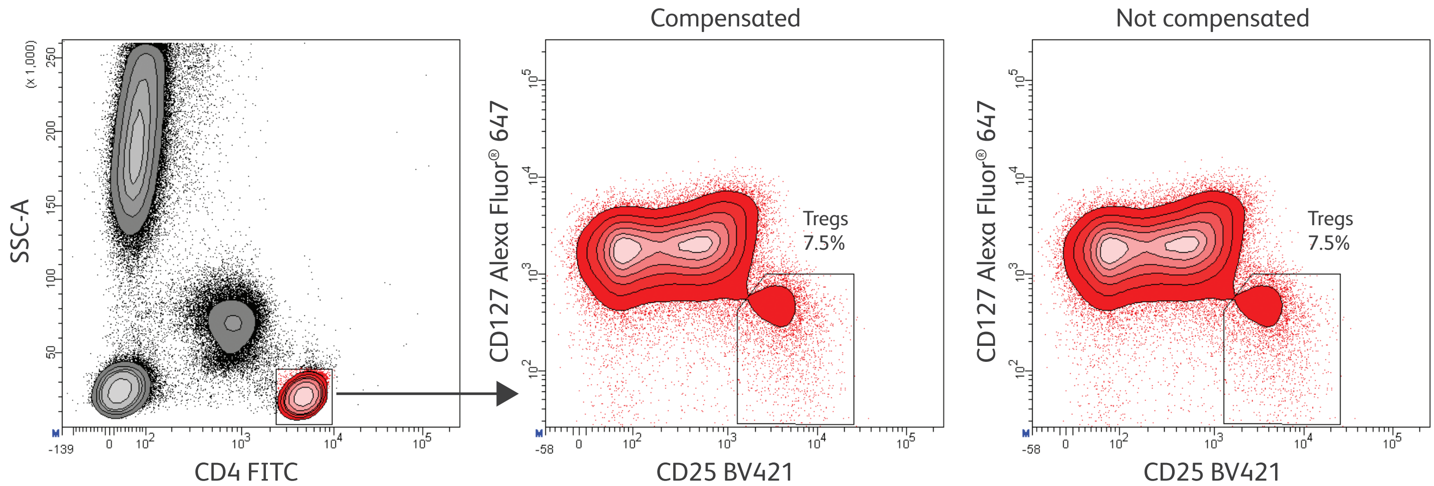
- Maximize resolution of populations of interest
- Improve visualization of populations in multicolor panels
- Enhance ease-of-use by minimizing the need for fluorescence compensation

With up to three lasers and twelve fluorescence parameters, the BD FACSCelesta™ flow cytometer is a flexible tool for designing multicolor panels with minimal spectral overlap. With configurations specifically designed to work with bright, tight-spectrum, BD Horizon Brilliant™ polymer dyes, it offers many ways to optimize panel design, increase resolution, and improve visualization of multiple populations.

Fluorescence spillover—the emission of fluorescence from one fluorochrome into the detector of another—can significantly affect the resolution of your populations of interest by increasing both the background (mean fluorescence intensity) and spread (fluorescence variability) into other detectors. Common sources of spillover to take into consideration when designing multicolor panels include adjacent-detector spillover (for example, FITC into PE); cross-laser excitation (for example, BUV737 into BV711); and residual base fluorescence (for example, BV786 into BV421). Although fluorescence compensation is routinely used to offset increased background, it does not remove the effects of increased spread.

Available in four configurations with two or three lasers, the BD FACSCelesta flow cytometer facilitates the design of multicolor panels that minimize spectral overlap. Such panels are not subject to data spread, thereby increasing data quality and resolution. This also simplifies panel design, analysis, and workflow for many types of experiments, since compensation can be omitted or minimized without altering the biological interpretation of the data.

As an example, Figure 1 shows a 3-color human regulatory T-cell (Treg) panel on the BD FACSCelesta Blue/Violet/Red (BVR) laser configuration. The three markers used to identify Tregs (CD4, CD25, and CD127) were paired with fluorochromes (FITC, BV421, and Alexa Fluor® 647) spread across the three lasers. The compensation matrix shows only nominal spectral overlap (<1%) among these three fluorochromes. When comparing compensated (middle plot) and not compensated (right plot) data, the plots are virtually identical. With each fluorochrome excited by a different laser, compensation is not required in this experiment.



Compensation

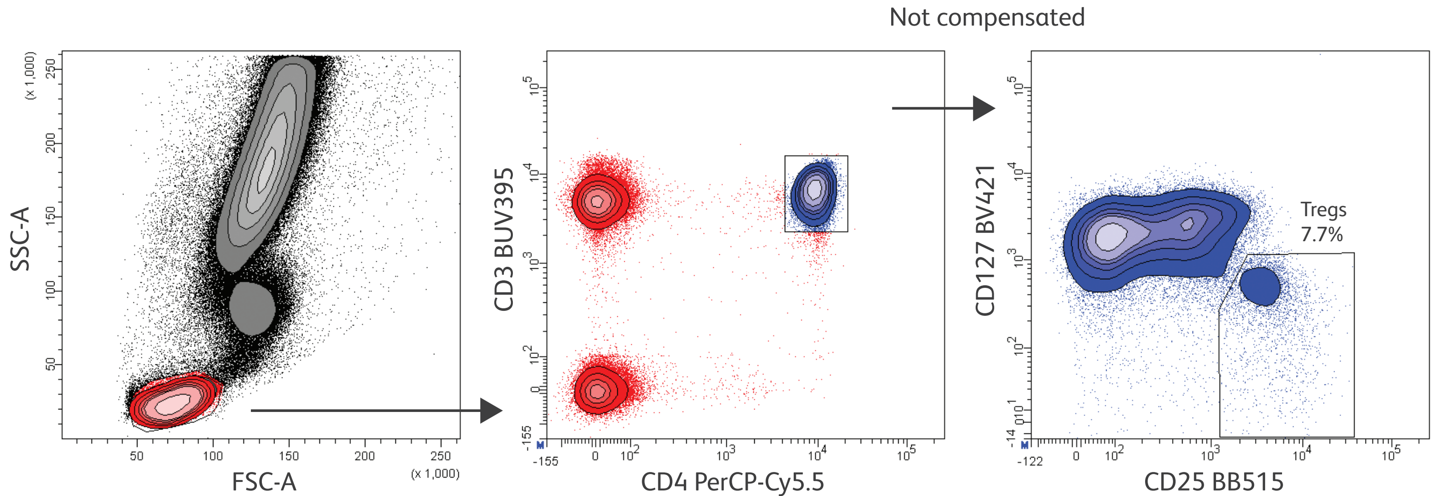
Fluorochrome	– % Fluorochrome	Spectral overlap
FITC	BV421	0.1
Alexa Fluor® 647		0.00
BV421	FITC	0.00
Alexa Fluor® 647		0.00
BV421	Alexa Fluor® 647	0.00
FITC		0.2

Figure 1. Three-color minimal spectral overlap human Treg panel on the BD FACSCelesta BVR system

Human whole blood cells were stained with fluorescent antibodies to Treg markers and acquired and analyzed on the BD FACSCelesta BVR configuration. Lymphocytes were identified based on light scatter properties and CD4 expression, measured using FITC (excited by the blue laser). Tregs were further defined based on CD25 expression using BV421 (violet laser), and CD127 expression using Alexa Fluor® 647 (red laser). Results: Tregs ($CD4^{+}CD25^{high}CD127^{low/-}$) were clearly distinguished, and comparison of compensated (middle plot) and not compensated (right plot) data showed that the plots are virtually identical. The compensation matrix shows minimal spectral overlap using these three fluorochromes together.

If more than three colors are required, you can use two fluorochromes excited by the same laser to design a minimal spectral overlap panel, as long as their emission spectra are well separated. Figure 2 shows a 4-color human Treg panel run on a BD FACSCelesta Blue/Violet/Ultraviolet (BVUV) laser configuration. This time, two markers were paired with fluorochromes excited by the blue laser with minimal overlap of emission spectra, as shown in Figure 3. Again, reviewing the compensation matrix, compensation is nominal if required at all.

By pairing innovations in instrumentation with bright new reagents, the BD FACSCelesta flow cytometer is designed to help you extract a deeper level of biological information from your cell types of interest. You can analyze more markers, design more complex and interesting experiments, and ultimately achieve greater understanding and discovery.



Compensation

Fluorochrome	– % Fluorochrome	Spectral overlap
BB515		0.2
PerCP-Cy5.5	BV421	0.00
BUV395		0.00
PerCP-Cy5.5		0.5
BV421	BB515	0.00
BUV395		0.1
BB515		0.00
BV421	PerCP-Cy5.5	0.00
BUV395		0.00
BB515		0.11
PerCP-Cy5.5	BUV395	0.00
BV421		0.32

Figure 2. Four-color minimal spectral overlap human Treg panel on the BD FACSCelesta BVUV system

Human whole blood cells were stained with fluorescent antibodies to Treg markers and acquired and analyzed on the BD FACSCelesta BVUV configuration. CD4 and CD25 expression were measured using PerCP-Cy™5.5 and BD Horizon Brilliant™ Blue 515 (BB515), respectively (both excited by the blue laser); CD127 expression using BV421 (violet laser); and CD3 expression using BUV395 (UV laser). **Results:** Tregs ($CD3^{+}CD4^{+}CD25^{high}CD127^{low/-}$) were clearly distinguished. As in Figure 1, the not compensated (shown) and compensated (not shown) data were virtually identical, and the compensation matrix shows minimal spectral overlap using these four fluorochromes together.

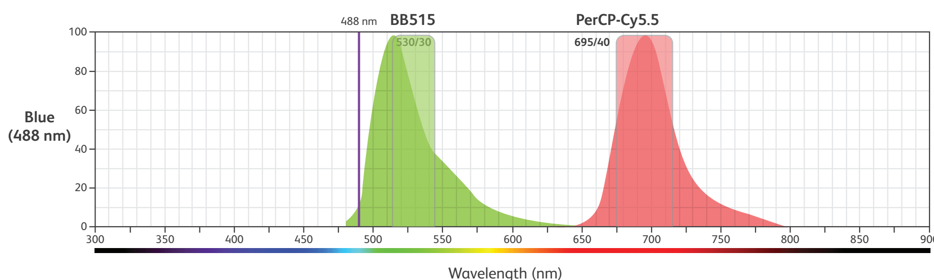


Figure 3. Analysis of emission spectra

Two fluorochromes excited by the blue laser, BB515 and PerCP-Cy™5.5, were chosen for the panel in Figure 2 based on their non-overlapping emission spectra. You can view fluorescence spectra and build multicolor panels interactively using the Fluorescence Spectrum Viewer on our **Multicolor Tools page** on bdbiosciences.com.

Ordering information	
Description	Cat. No.
BD FACSCelesta™ Flow Cytometer, BVR Configuration	660344
BD FACSCelesta™ Flow Cytometer, BVYG Configuration	660345
BD FACSCelesta™ Flow Cytometer, BVUV Configuration	660346
BD FACSCelesta™ Flow Cytometer, BV Configuration	660343

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23-19078-00

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