Combining instrument sensitivity with bright dyes to resolve low-density antigens

Taking advantage of the sensitivity of the BD FACSCelesta™ flow cytometer

Features

- Sensitive optics
- Optimized to use very bright, tight-spectrum, advanced polymer dyes
- Resolves antigens expressed in biological systems at low, medium and high levels

The sensitive optics of the BD FACSCelesta™ flow cytometer, combined with novel bright BD Horizon Brilliant™ dyes to which its configurations are optimized, allow you to resolve populations of cells with a broad range of receptor density, from very low to very high. This gives you the flexibility to design multicolor panels that resolve multiple cell populations of interest, even if their antigen expression levels differ widely.

BD

The key to successful panel design is to understand both the biology of your cell populations and the fluorescence intensity of available fluorochromes. For the best resolution of a population of interest, as a rule of thumb, pair low-density antigens with bright to very bright fluorochromes, medium-density antigens with moderate to bright fluorochromes, and high-density antigens with dim fluorochromes (Figure 1).

You can find the relative brightness of different fluorochromes on the Fluorochrome/Laser Reference Poster available on the Multicolor Tools page at bdbiosciences.com.

T-cell antigen density

CD4	CD27	CD132
FITC	PE-CF594	BB515
PerCP-Cy™5.5	BV605	PE
BV510	BV786	BV421
APC-H7	APC	BV650

B-cell antigen density

CD20	CD19	CD38
FITC	BV480	PE
PerCP-Cy5.5	BV605	PE-CF594
BV510	BV786	BV421
BUV395	BUV737	BV711

Figure 1. Antigen-fluorochrome pairings for T-cell and B-cell discrimination

Antigens expressed by T-cells (left) or B-cells (right) were chosen based on the level of expression per cells, ranking high (CD4, CD20), intermediate (CD27, CD19) or low (CD132, CD38). Four fluorochrome conjugates were chosen for each antigen based on fluorescence intensity, with the dimmer fluorochromes paired with high-density antigens, and the brighter fluorochromes with the low-density antigens. BD FACSCelesta BVR and BVUV configurations were used for T-cell and B-cell analysis, respectively.

Figure 2 shows an experiment on the BD FACSCelesta Blue/Violet/Red (BVR) configuration to detect three T-cell markers: CD4 (a high-density marker, with about 40,000 receptors per cell), CD27 (medium density, about 3,000 receptors) and CD132 (very low density, about 400 receptors). Each antigen was paired with four appropriate fluorochromes and analyzed on the BD FACSCelesta Blue/Violet/Red (BVR) system. The data shows that instrument sensitivity, combined with proper fluorochrome choice, results in clear resolution of all the antigens of interest, regardless of their expression levels.

For example, BV421, BV650, BB515 and PE—all very bright fluorochromes—were chosen to enable the resolution of the low-density antigen CD132. The histograms (gated on $CD3^+$ lymphocytes) all show clear separations from the negative control. The clear resolution of CD132, with only 400 receptors per cell, attests to the sensitivity of the BD FACSCelesta instrument.

Figure 3 shows a similar experiment on the BD FACSCelesta Blue/Violet/Ultraviolet (BVUV) configuration to detect three B-cell markers: CD20, CD19 and CD38 (in decreasing order of antigen density). Again, each antigen was paired with four appropriate fluorochromes for clear resolution of all the markers tested. For expression of CD38 (right column), distinct subsets of B cells were distinguished, expressing either low, intermediate, or high levels of CD38—very fine discrimination for this low-density marker.

With up to 3 lasers and 12 fluorescence parameters, the BD FACSCelesta is a flexible tool for running multicolor panels that can detect and resolve antigens expressed in biological systems at a broad range of levels, from very low to very high. 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.

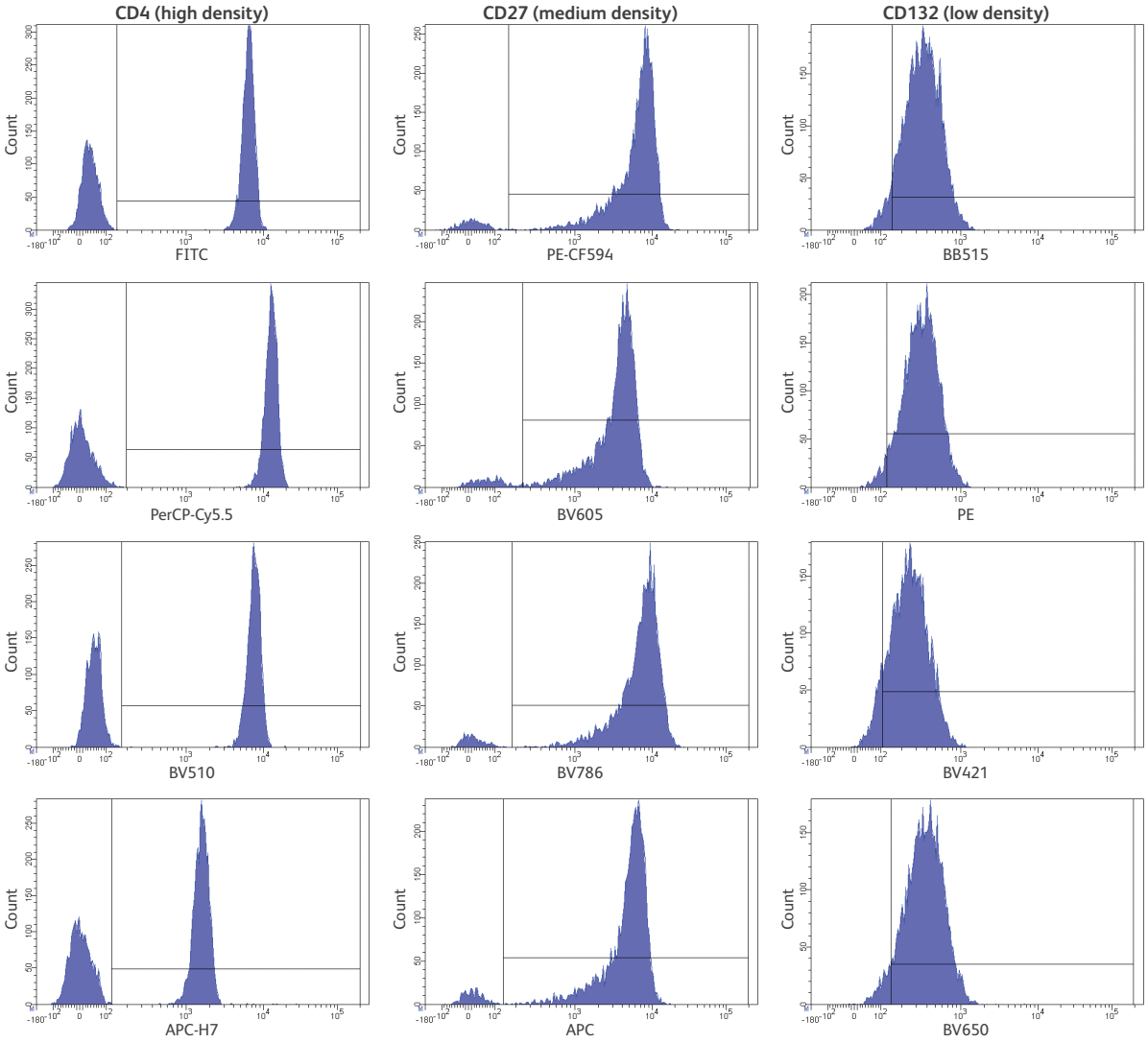

Figure 2. Resolution of T-cell markers on the BD FACSCelesta BVR system

Human whole blood was stained, lysed and fixed prior to analysis on the BD FACSCelesta BVR configuration. Four panel-appropriate fluorochromes (see Figure 1) were paired with each of three markers varying in antigen density. **Results:** T cells were first identified based on light scatter properties of lymphocytes and CD3 expression (not shown). Cells expressing high-, medium- and low-antigen density markers (CD4, CD27 and CD132), when paired with each fluorochrome, were clearly resolved from negative or unstained cells. Gates were drawn based on CD3 single-stained controls.

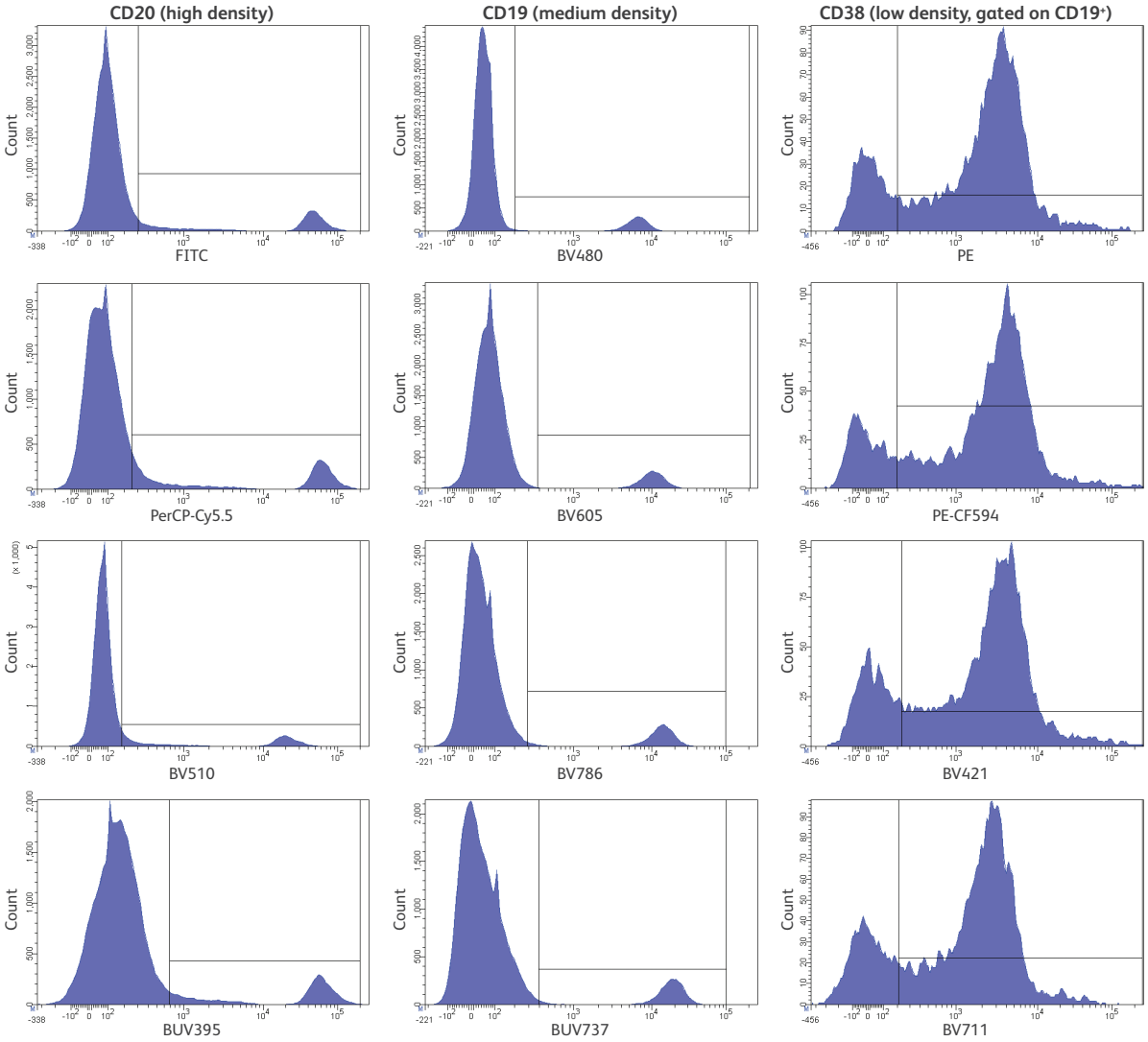

Figure 3. Resolution of B-cell markers on the BD FACSCelesta BVUV system

Peripheral blood mononuclear cells were isolated and stained prior to analysis on the BD FACSCelesta BVUV configuration. Four panel-appropriate fluorochromes (see Figure 1) were paired with each of three markers varying in antigen density. **Results:** Lymphocytes were identified based on light scatter properties. For the analysis of CD38, B cells were further defined based on CD19 expression (not shown). Cells expressing high-, medium- and low-antigen density markers (CD20, CD19 and CD38) were clearly resolved from negative cells. Within the CD19$^+$ cell population, distinct subsets of cells expressing variable levels of CD38, from low to high, were discriminated (right column). Gates were drawn based on unstained (for CD20 and CD19) or CD19 single-stained (for CD38) cells.

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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23-18286-00

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