



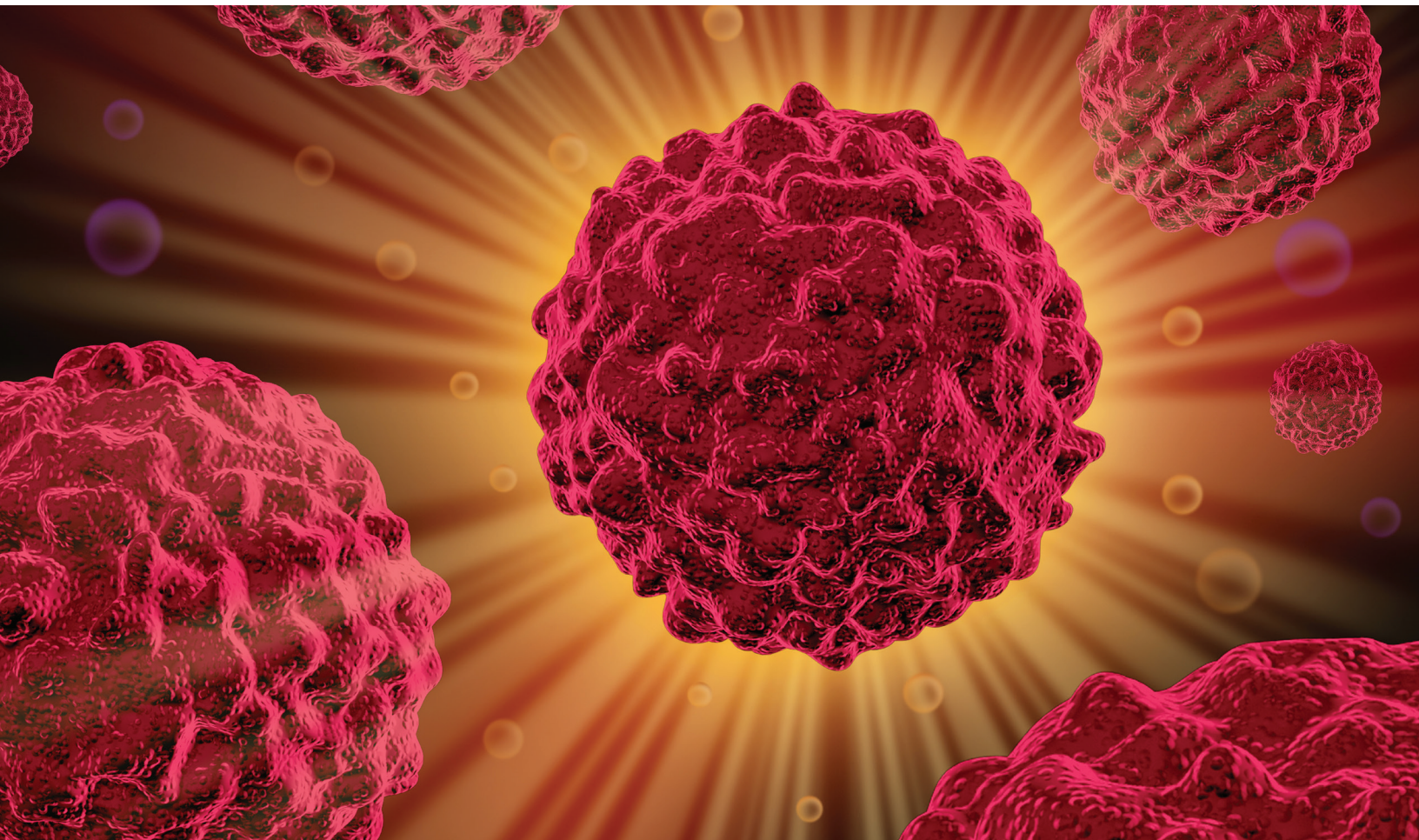
BD FACSCelesta™ Flow Cytometer

Data Book



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Introduction



All data samples in this paper were collected and analyzed on the BD FACSCelesta™ flow cytometer. With the ability to detect and analyze up to 14 parameters in a bench-top footprint, the BD FACSCelesta makes sophisticated flow cytometry accessible, which enables deeper scientific discoveries.

For more information regarding the BD FACSCelesta system, please see the appendix.

Immunology

Enabling deep immunophenotyping

Among the methodologies that contribute to immunology research, multicolor flow cytometry is preeminent. Immunophenotyping was one of the first applications of flow cytometry, and for over 40 years, BD Biosciences has actively supported groundbreaking immunological research.

The BD FACSCelesta is an ideal bench-top platform for core immunophenotyping studies and is configured for rapid detection and analysis of up to 14 parameters simultaneously on an individual cell level. With areas of research rapidly expanding and new protein markers continuously being discovered, the BD FACSCelesta allows researchers to quickly reveal extensive biologic information from their sample.

Figure 1 shows how the full capability of the 2-laser BD FACSCelesta BV configuration can be maximized to take advantage of all 10 colors. This 10 color T-cell analysis demonstrates the sensitivity and resolution of the 2-laser system to detect rare subpopulations. After normal human whole blood was washed and lysed, BD Horizon Brilliant™ and traditional reagents were used to identify rare T-cell and Treg subpopulations.

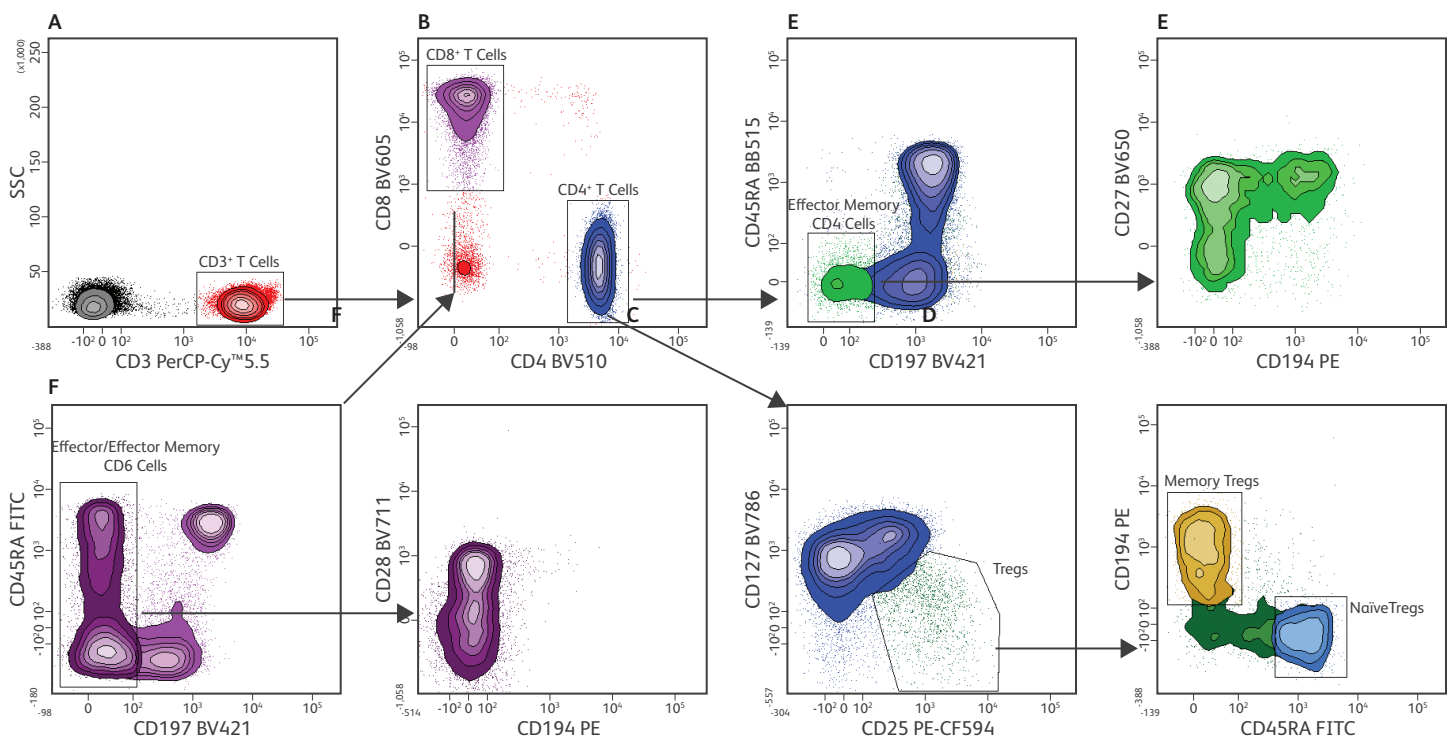


Figure 1. Ten-color T-cell analysis on the BD FACSCelesta BV configuration.

This T-cell panel demonstrates the sensitivity and resolution of the BD FACSCelesta flow cytometer, even in detecting rare subpopulations using a 2-laser system. After normal human whole blood was washed and lysed, BD Horizon Brilliant™ and traditional dyes were used to identify rare T-cell and Treg subpopulations. **A.** Cells were gated to select the CD3⁺ T cells. **B.** CD3⁺ lymphocytes were gated to show the CD4⁺ helper T cells and CD8⁺ cytotoxic T cells. **C.** Gated on the CD4⁺ T cells, surface markers were used to identify CD25⁺CD127⁺ Tregs. **D.** Gated on Tregs, surface markers were used to identify memory and naive Treg subsets (CD194, CD45RA). **E.** CD4⁺ helper T cells were analyzed for memory T-cell subsets using CD45RA and CD197. Memory subsets were further defined using CD27 and CD194 surface markers. **F.** CD8⁺ cytotoxic T cells were analyzed for memory T-cell subsets using CD45RA and CD197. Memory subsets were further defined using CD28 and CD194 surface markers.

Enabling deep immunophenotyping

Figure 2 shows how the full capability of the 3-laser BD FACSCelesta BVUV configuration can be maximized to take advantage of all 12 colors. This T-cell panel demonstrates the sensitivity and resolution of the 3-laser

system to detect rare subpopulations. After normal human whole blood was washed and lysed, BD Horizon Brilliant dyes were used to stain 9 of the 12 markers, allowing easy resolution of rare T-cell and Treg subpopulations.

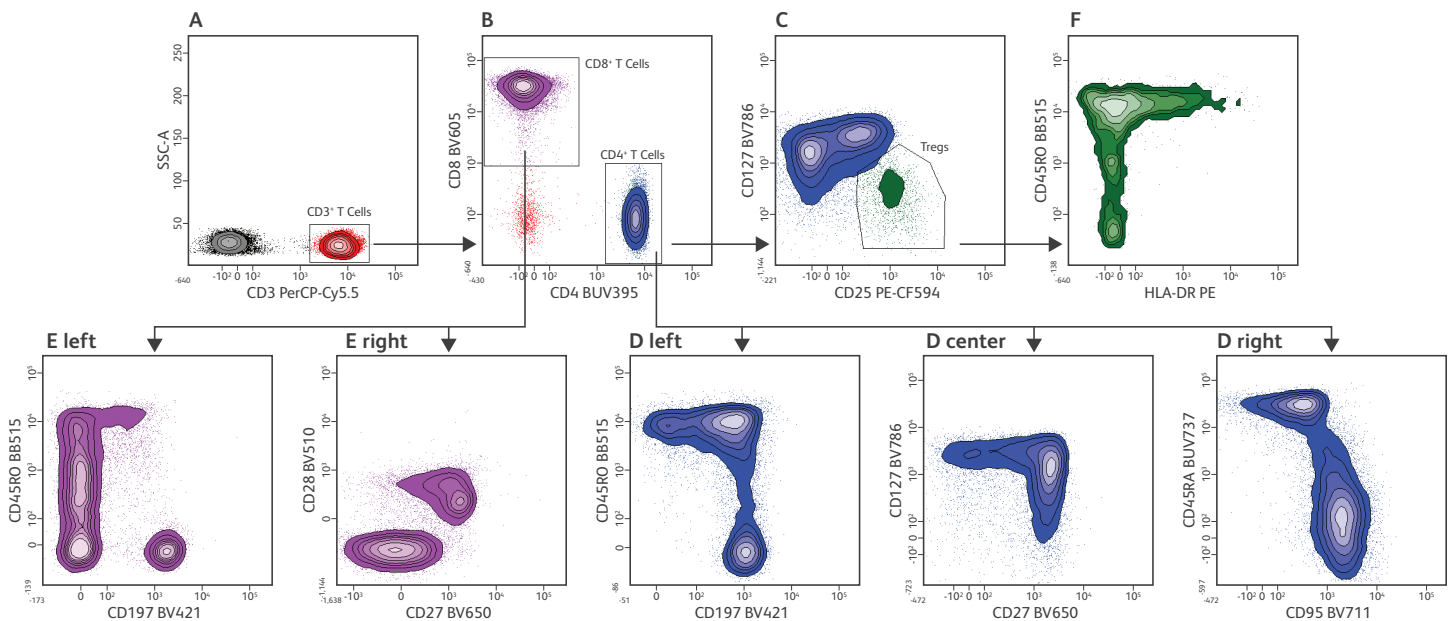


Figure 2. Twelve-color T-cell analysis on the BD FACSCelesta BVUV configuration

This T-cell panel demonstrates the sensitivity and resolution of the BD FACSCelesta, even in detecting rare subpopulations. After normal human whole blood was stained, lysed, and washed. BD Horizon Brilliant dyes were used to stain nine of the 12 markers, allowing easy resolution of rare T-cell and Treg subpopulations. **A.** Cells were gated to select the CD3⁺ T cells. **B.** CD3⁺ lymphocytes were gated to show the CD4⁺ helper T cells and CD8⁺ cytotoxic T cells. **C.** Gated on the CD4⁺ T cells, surface markers were used to identify CD25⁺CD127⁻ Tregs. (The absence of CD127 is a proxy for the presence of the classic intracellular Treg marker FoxP3.) **D.** CD4⁺ helper T cells were analyzed for memory T-cell subsets using CD45RO, CD197 and CD27. Additional surface markers were used to distinguish CD127, CD45RA and CD95 expression levels.

E. CD8⁺ cytotoxic T cells were analyzed for memory T-cell subsets using CD45RO, CD197, CD27 and CD28. **F.** HLA-DR and CD45RO expression levels were plotted for the Treg population.

Enabling deep immunophenotyping

Figure 3 shows a 10-color T-cell analysis on the 3-laser BD FACSCelesta BVR configuration. After normal human whole blood was washed and lysed, BD Horizon Brilliant and traditional fluorochromes were used to identify rare T-cell and Treg subpopulations.

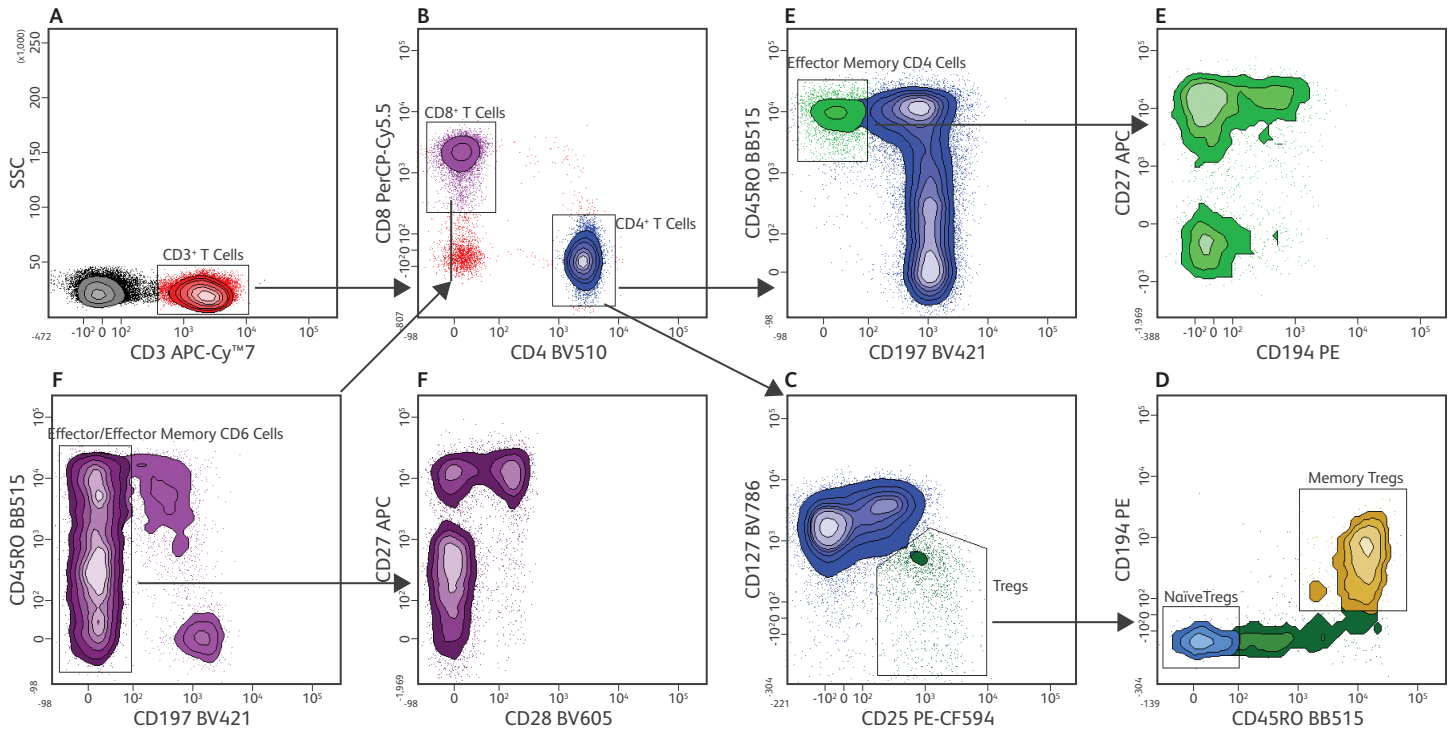


Figure 3. Ten-color T-cell analysis on the BD FACSCelesta BVR configuration

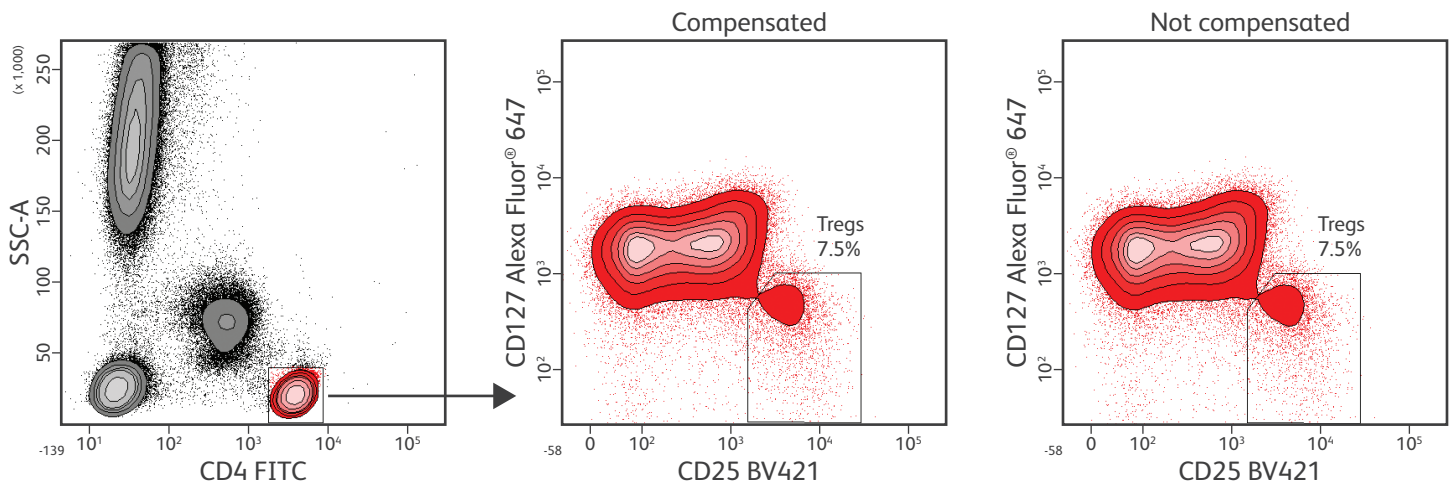
This T-cell panel demonstrates the sensitivity and resolution of the BD FACSCelesta system, even in detecting rare subpopulations. After normal human whole blood was washed and lysed, BD Horizon Brilliant and traditional dyes were used to identify rare T-cell and Treg subpopulations. **A.** Cells were gated to select the CD3⁺ T cells. **B.** CD3⁺ lymphocytes were gated to show the CD4⁺ helper T cells and CD8⁺ cytotoxic T cells. **C.** Gated on the CD4⁺ T cells, surface markers were used to identify CD25⁺CD127⁺ Tregs. **D.** Gated on Tregs, surface markers were used to identify memory and naïve Treg subsets (CD194, CD45RA). **E.** CD4⁺ helper T cells were analyzed for memory T-cell subsets using CD45RO and CD197 (left) and effector memory subsets were further defined with surface markers for CD27 and CD194 (right). **F.** CD8⁺ cytotoxic T cells were analyzed for effector and memory subsets using CD45RO and CD197 (left) and effector/effector memory subsets were further defined using CD27 and CD28 surface markers. Similar analysis was completed for CD8⁺ cytotoxic T cells (right).

Minimal spectral overlap panels to enhance data quality

With up to three lasers and twelve fluorescence parameters, the BD FACSCelesta™ flow cytometer is an easy-to-use 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.

Although fluorescence compensation is routinely used to offset increased background, it does not remove the effects of increased spread. As an example, Figure 4 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 4. Three-color minimal spectral overlap human Treg panel on the BD FACSCelesta BVR configuration

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.

Ability to resolve rare and dim cell populations

The biology of rare cell types, such as dendritic cells (DCs) and regulatory T cells (Tregs), can be difficult to study because it's not easy to isolate component subsets for investigation, and their markers often overlap with other cell types. Flow cytometry is well suited for this analysis since, by assessing the expression of multiple intracellular and cell surface markers, it can rapidly analyze thousands of individual cells to provide both phenotypic and functional data in the same assay. Combining innovations in instrumentation with optimization for 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. By enabling both deep and broad immunophenotyping, it allows you to design more complex biological panels and address important questions in biology. Even when cell subsets of interest comprise less than 0.1% of a heterogeneous sample, "deep immunophenotyping" on the BD FACSCelesta system can resolve them clearly.

Figure 5 (left side) shows a standard immunophenotyping panel for mouse dendritic cells on the BD FACSCelesta Blue/Violet/Red (BVR) laser configuration. Two blue channels (FITC/ BD Horizon Brilliant™ Blue 515 or BB515 and PE), two violet channels (BD Horizon Brilliant™ Violet dyes, BV421 and BV605), and two red channels (APC and APC-R700) were used to discriminate myeloid, lymphoid and plasmacytoid (mDC, IDC and pDC) subsets, based on their expression of I-A/I-E (MHC-II), CD11b, CD11c, CD8 α and B220. On the right, three additional discriminatory markers were added for a deeper immunophenotypic characterization of the three DC subsets. Signal Regulatory Protein Alpha (Sirp α /CD172a) was used to characterize CD11c⁺ CD11b⁺ CD8 α ⁻ mDCs, while Clec12A (CD371) was used to characterize CD8 α ⁺ IDCs and pDCs. The expression of CD4 was also analyzed in all three subsets of DCs. The BD FACSCelesta, with its multiple violet channels and optimization for BD Horizon Brilliant dyes, is an ideal instrument to develop and analyze sophisticated panels showing deeper insights.

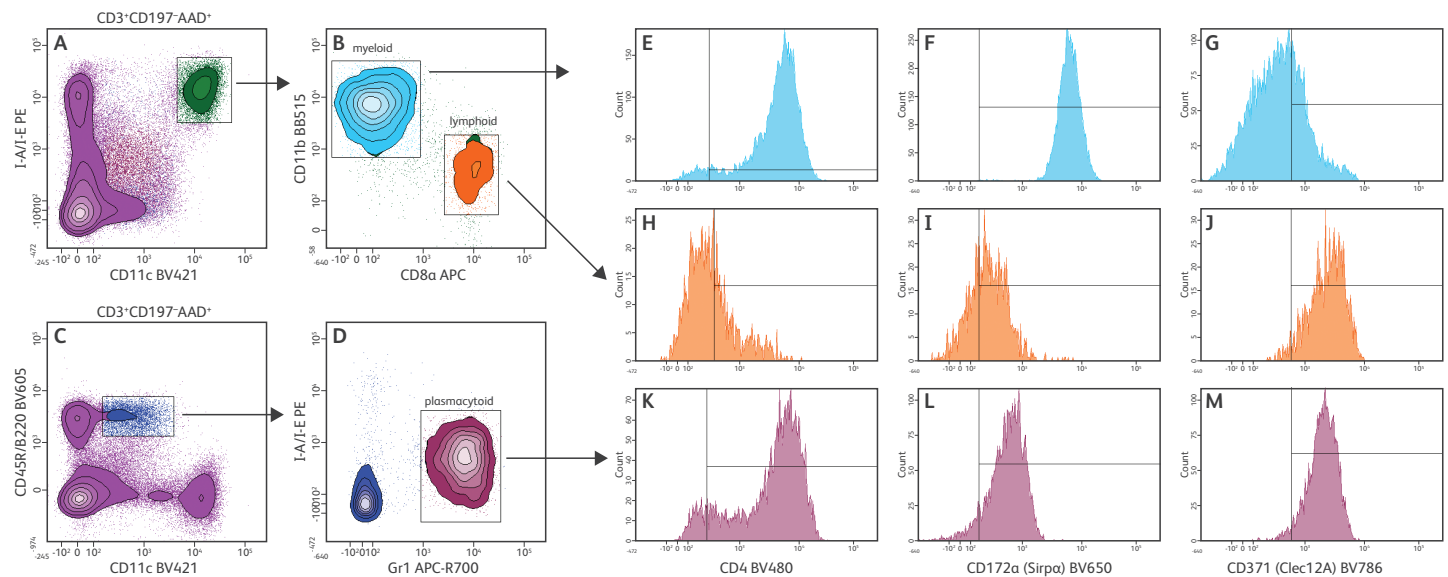


Figure 5. Adding drop-ins: 10-color mouse DC panel on the BD FACSCelesta system

Ten-color immunophenotypic characterization of the three main subsets of mouse DCs (myeloid, lymphoid and plasmacytoid) in the mouse spleen. BALB/c mouse spleen was enzymatically digested, stained with a cocktail of antibodies (including BD OptiBuild™ versions of Sirp α BV650 and Clec12A BV786) and analyzed on a BD FACSCelesta Blue/Violet/Red (BVR) laser configuration. Cells were initially gated on CD3, CD19 and 7-AAD negative cells (not shown). **A-D.** Gating strategy: I-A/I-E^{high}CD11c^{high} conventional DCs were further discriminated into CD11b⁺ cDCs and CD8⁺ cDCs. I-A/I-E^{low}CD11c^{low}B220⁺Gr1⁺ cells were recognized as pDCs. **Results:** Differential expression of the additional markers CD4, CD172a (Sirp α) and CD371 (Clec12A) was further analyzed within the mDC (**E, F, G**), IDC (**H, I, J**) and pDC (**K, L, M**) subsets, respectively. Gates were drawn based on fluorescence minus one (FMO) controls.

Ability to resolve rare and dim cell populations

In Figure 6, the functional responses of human dendritic cell subsets to different stimuli were assessed using the BD FACSCelesta Blue/Violet/Ultraviolet (BVUV) laser configuration. The cells were stimulated with lipopolysaccharide (LPS, a TLR4 ligand), R837 (a TLR7 ligand), or R848 (a TLR7/8 ligand), and analyzed for production of TNF- α and IFN- α using intracellular cytokine staining (ICS). Figure 2A shows the identification of mDCs and pDCs. Cells were gated using light scatter, HLA-DR expression and a lineage-negative cocktail. HLA-DR was assessed using the UV laser to minimize spillover, both into and back from other fluorescence channels. Minimal spillover is key in detecting

rare cell populations. ICS (Figures 6B and 6C) showed that each dendritic subset exhibited a unique cytokine response depending on the stimulus (activating ligand) used. As expected, pDCs (blue), which express TLR7, secreted TNF- α when stimulated with R837 and R848, both of which activate that receptor. Also, as the predominant cell type that produces type I interferons, pDCs expressed IFN- α , but only when stimulated with R848. mDCs (pink), which express TLR4 and TLR8, produced TNF- α when stimulated with LPS and R848, ligands specific to those receptors, but not with the TLR7 ligand R837.

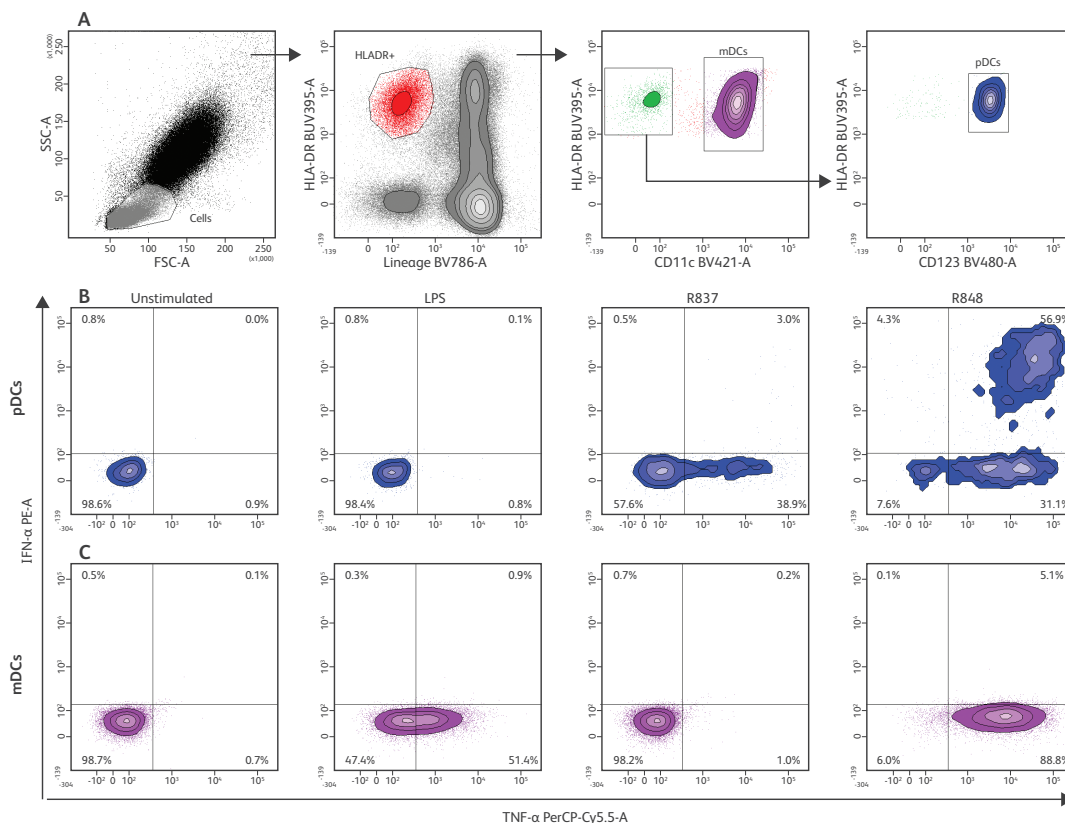


Figure 6. Intracellular cytokine response in TLR ligand-activated human dendritic cell subsets

Six-color immunophenotypic and functional characterization of the two main subsets of human DCs (plasmacytoid and myeloid). Peripheral blood from a normal human donor was treated with lipopolysaccharide (LPS, a TLR4 ligand), Imiquimod (R837, a TLR7 ligand), or Resiquimod (R848, a TLR7/8 ligand) or left unstimulated for four hours in the presence of BD GolgiPlug™ protein transport inhibitor. Samples were stained with a cocktail of antibodies for detection of surface markers, lysed, fixed and permeabilized using BD Cytofix/Cytoperm™ buffers prior to staining with intracellular antibodies for detection of cytokines TNF- α and IFN- α . Cells were analyzed on the BD FACSCelesta Blue/Violet/UV (BVUV) laser configuration. **Results: A.** Cells were first gated based on light scatter, and then on HLA-DR expression and absence of lineage markers (HLA-DR⁺CD3⁻CD56⁻CD14⁻). DC subsets were then identified as myeloid (HLA-DR⁺CD11c⁺) or plasmacytoid (HLA-DR⁺CD11c⁻), with further confirmation as CD123⁺. **B-C.** Plots show the differential expression of TNF- α and IFN- α (x- and y-axis respectively) within the pDC (blue) and mDC (pink) subsets in unstimulated cells (left column) as well as cells stimulated with LPS, R837 and R848. Unstimulated cells expressed neither cytokine, while mDCs expressed TNF- α when stimulated with LPS or R848, and pDCs expressed TNF- α when stimulated with R837 or R848. Only pDCs stimulated with R848 expressed IFN- α .

High sensitivity to resolve populations with low antigen density

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.

Figure 7 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 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.

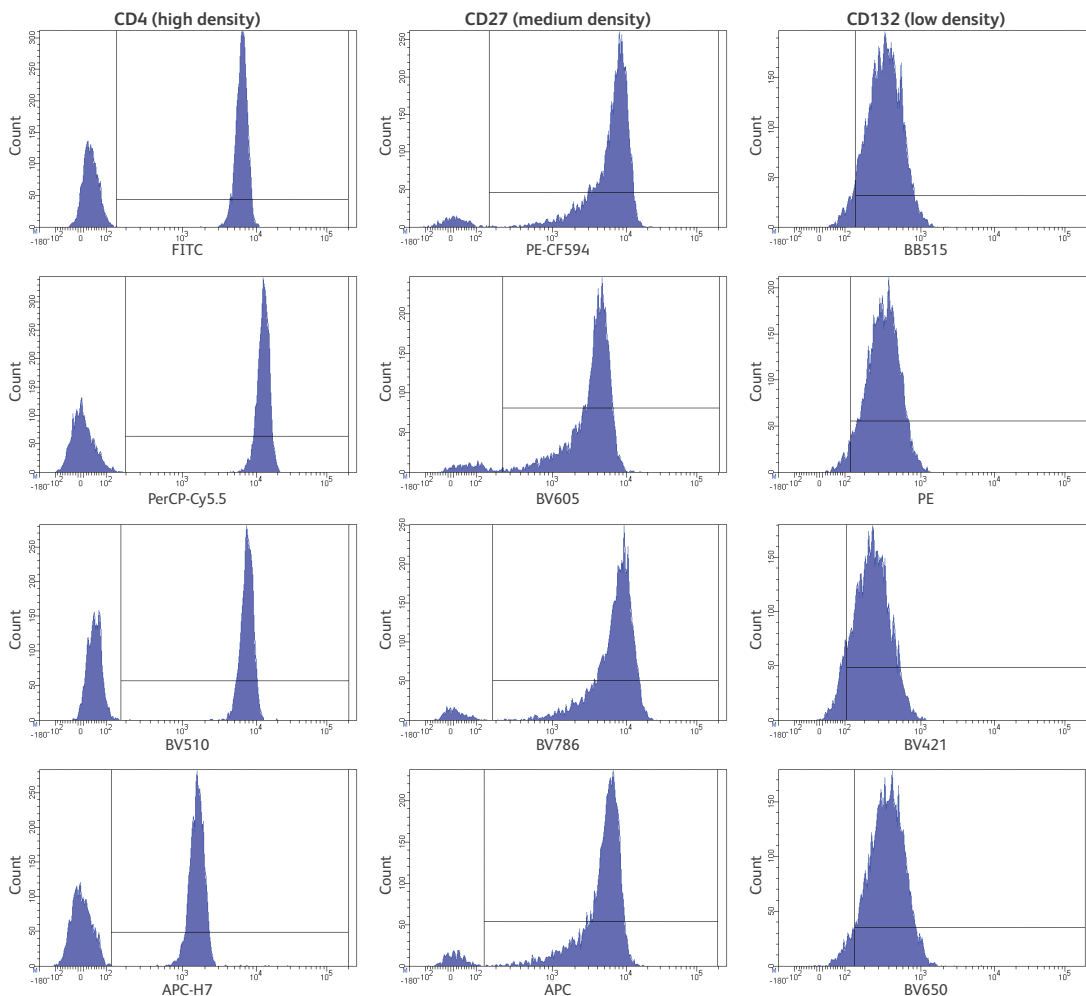


Figure 7. Resolution of T-cell markers on the BD FACSCelesta BVR configuration

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.

High sensitivity to resolve populations with low antigen density

Figure 8 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.

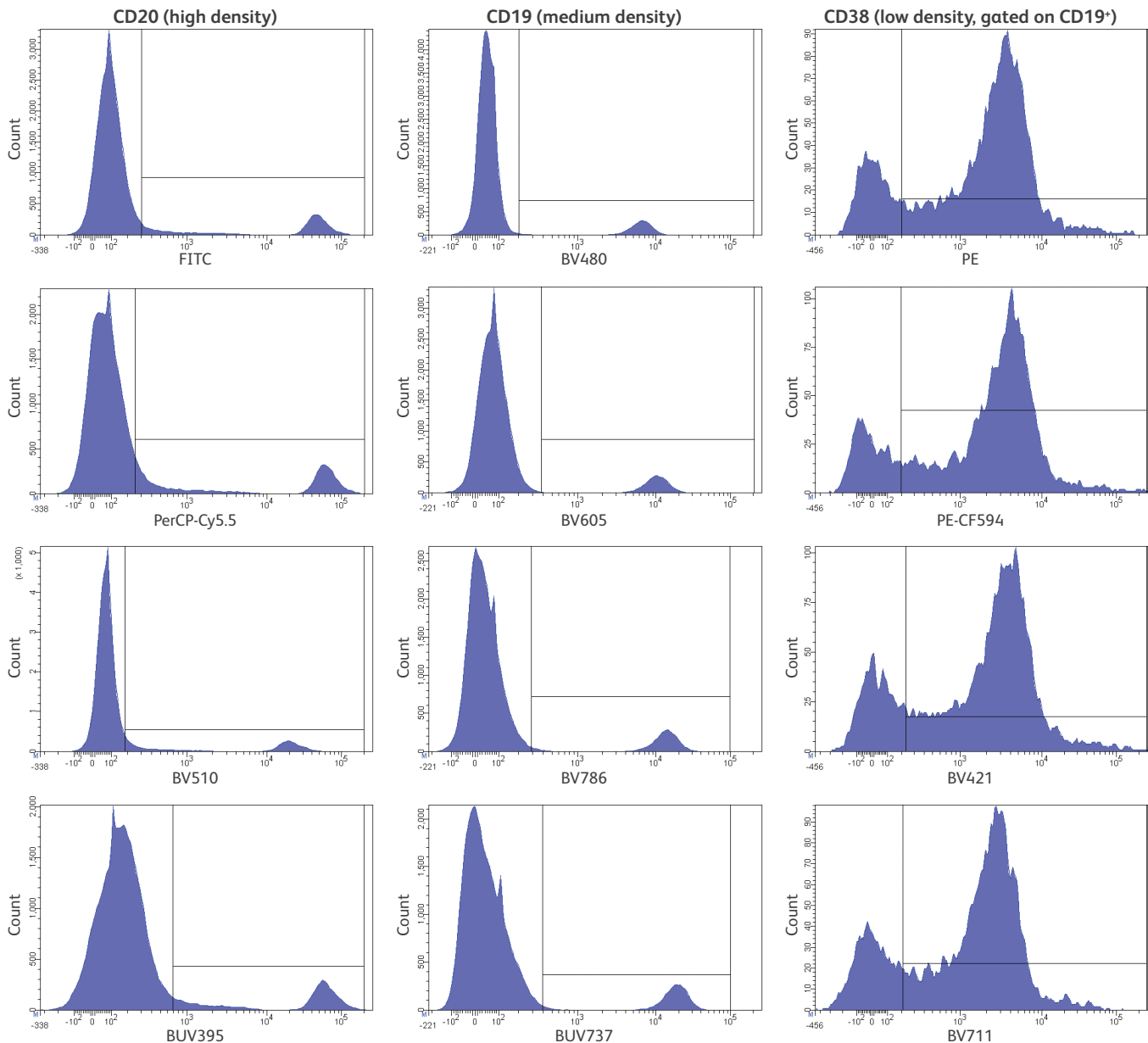


Figure 8. Resolution of B-cell markers on the BD FACSCelesta BVUV configuration

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.

Cell Biology

A flow solution for fluorescent protein analysis

Fluorescent proteins (FPs) are used as reporters in many kinds of experiments, from cell and molecular biology to stem cell and cancer research to microbiology. Flow cytometry, specifically designed to detect and measure fluorescence, is an excellent methodology for research using FPs. The BD FACSCelesta flow cytometer is well suited for FP analysis, while leaving additional channels available for immunophenotyping or cell function analysis. Some popular FPs, such as green and yellow fluorescent proteins (GFP and YFP), are excited by a blue (488-nm) laser, which is standard in most flow cytometers. However, RFP and its variants, such as mCherry, are only weakly excited by blue and other standard lasers. When the red FP signal is dim, it can be difficult to detect and resolve the FP+ cells. A yellow-green (561-nm) laser excites these FPs much more strongly, which

results in a brighter emission signal. This laser has become essential for many applications using red FPs, including the fruit series of fluorescent proteins (such as Living Colors[®] mCherry, mPlum, and DsRed dyes). The BD FACSCelesta Blue/Violet/Yellow-Green (BVYG) configuration is ideal for this research, since it can enable research using all of these RFP variants, as well as GFP and YFP.

Figure 9 shows human embryonic kidney cells transfected with GFP or mCherry, excited by the blue or yellow-green laser of the BVYG configuration, respectively. In each case, separation between the positive and negative peaks is more than two orders of magnitude. For GFP, an intermediate peak is also resolved clearly. The development of transgenic mouse models in which key genes are tagged with a fluorescent protein has opened up new approaches to research.

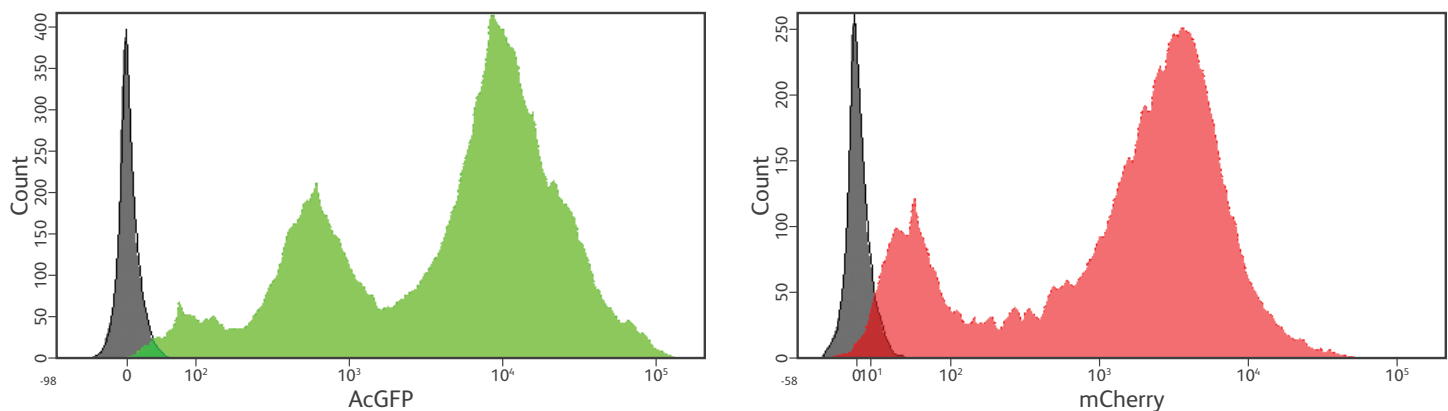


Figure 9. Expression of fluorescent proteins in transfected human embryonic kidney cells

HEK-293 cells were transfected over 24 hours with AcGFP (left) or mCherry (right), fixed with BD Cytotfix™ Fixation Buffer (Cat. No. 554655), and cryopreserved for one week. After thawing, cells were washed and then analyzed with the BD FACSCelesta BVYG configuration. Transfected cells (green or red) were compared to the wild type (black) for expression of AcGFP (excited by the blue laser) or mCherry (excited by the yellow-green laser), respectively.

A flow solution for fluorescent protein analysis

Figure 10 shows an experiment using a mouse model in which a reporter expressing an RFP (specifically, monomeric red fluorescent protein or mRFP) has been knocked into the endogenous locus for FoxP3, the transcription factor that is the master regulator for regulatory T cells (Tregs). As an intracellular marker, FoxP3 detection requires fixation and permeabilization of cells, and no stable and reliable surface marker correlate has yet been found. The development of this mouse model has streamlined and facilitated Treg research, since Tregs can be definitively identified without fixation or permeabilization. Not only does this simplify and streamline the experimental protocols, but the cells also remain viable for use in downstream applications.

In Figure 10, after gating on lymphocytes and then on T cells, CD4⁺ CD25⁺ Tregs were identified. Ninety percent expressed FoxP3, as indicated by mRFP fluorescence. The clear resolution of this population, without the need to fix or permeabilize the cells, attests to the value of this transgenic mouse model. As a low-density antigen, CD25 often stains dimly, so CD25 expression is not always easy to detect. That's where the violet laser facilitates discovery. In this experiment, CD25 was paired with BD Horizon Brilliant™ Violet 421, one of a family of advanced polymer fluorochromes for the violet laser. The brightness of BV421, and the violet laser that is

standard in all configurations of the BD FACSCelesta, make it easier to detect and resolve populations that express low-density antigens. With the BD FACSCelesta BVYG configuration, you don't have to give up the violet laser to get the yellow-green laser, which you can dedicate to detecting the tagged cells from your transgenic mice or transfected cell line. That leaves the blue and violet laser channels open for other purposes. For example, you can use them to immunophenotype the cells, allowing you to determine which subpopulations are expressing the FP. You can also use them for measures of cell function, such as apoptosis, viability, cell cycle, DNA damage and/or proliferation. As an added bonus, the yellow-green laser also maximizes resolution of other fluorochromes conventionally excited by the blue laser, including phycoerythrin (PE) and PE tandem dyes such as PE-CF594 and PE-Cy7. Combining innovations in instrumentation with optimization for bright new reagents and BD's unparalleled service and support, the BD FACSCelesta flow cytometer is designed to help you extract a deeper level of biological information from your cell types of interest. By enhancing FP detection while simultaneously enabling immunophenotyping and cell function analysis, the BD FACSCelesta BVYG configuration helps you gain new understanding and insights about your transgenic model or transfected cells.

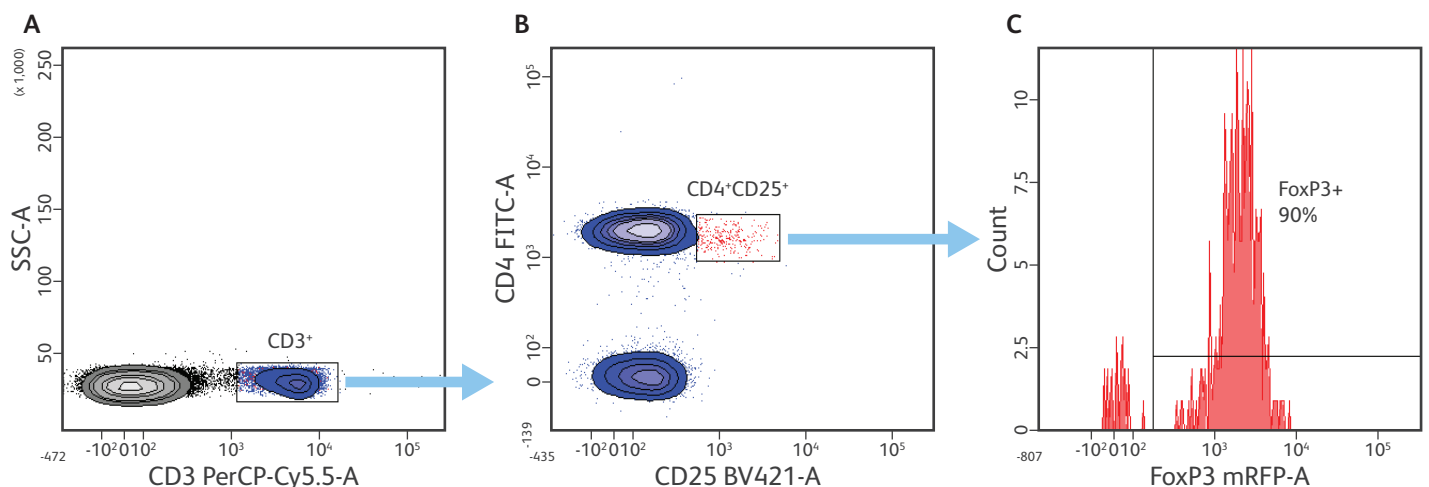


Figure 10. Treg analysis of FoxP3 transgenic mouse splenocytes

Splenocytes from FoxP3-IRES-mRFP mice (Jackson Laboratories) were isolated and stained with a cocktail of surface-marker antibodies, including CD3 PerCP-Cy5.5, CD4 FITC and CD25 BV421. Lymphocytes were first gated based on light-scatter properties (not shown), followed by gating of CD3⁺ T cells (A). From the gated T cells, CD4⁺CD25⁺ Tregs were identified (B) and analyzed for the expression of the signature transcription factor FoxP3, which was expressed by 90% of the cells (C). The yellow-green laser optimally excites mRFP, resulting in clear resolution of FoxP3.

Enhanced cell cycle analysis

The BD FACSCelesta flow cytometer provides a flexible tool for cell biologists to complement traditional Western blot analysis with rich cell cycle data and immunophenotyping. While Western blot analysis analyzes populations in bulk, multiparameter flow cytometry enables the quantitative analysis of populations at the single-cell level. It is ideal for applications that require quantitation of subsets within a heterogeneous population, such as cell cycle analysis in which different cells may be at different points of the cell cycle.

Figure 11 shows an example in which flow cytometry provides information on cyclin B1 expression on a cell-by-cell basis across the cell cycle. Here, cell cycle was measured using the DNA dye DAPI and the thymidine analog ethynyl-deoxyuridine (EdU), which is incorporated into newly synthesized DNA during the S phase. Results allow segmentation of cells in the G₀/G₁ phase, S phase and G₂/M

phase. While Western blot would provide the average cyclin B1 expression level of the entire population—resulting in a single, average value—flow cytometry allows the dissection of heterogeneous cyclin B1 expression within different cell cycle compartments. The results show that cyclin B1 expression increases over the course of the cell cycle. In this experiment, the BD FACSCelesta Blue/Violet/Red (BVR) configuration was used. The three separate lasers facilitate the design of 3-color panels that minimize spectral overlap. This simplifies multicolor panel design and workflow, since compensation can be omitted without altering the biological relevance of the data. In addition, panels composed of fluorochromes with low spectral overlap are not subject to data spread due to spillover, increasing data quality and resolution. Such panels can be used to simplify the design and analysis of many types of experiments.

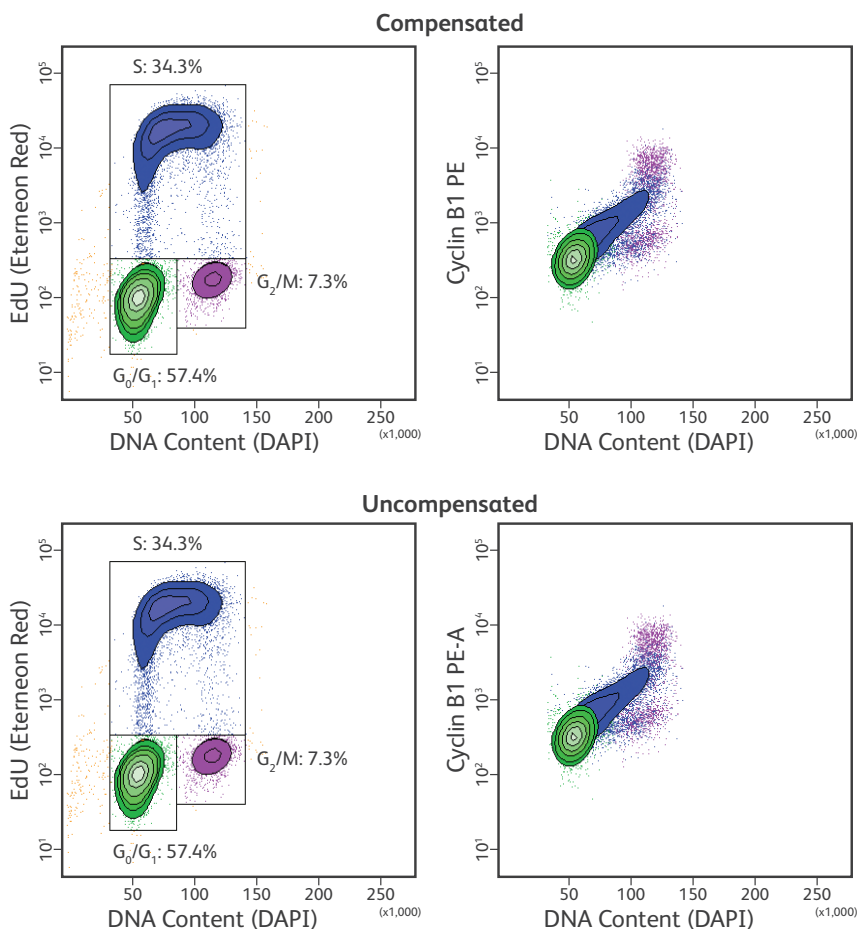


Figure 11. Minimal spectral overlap panel for cell cycle analysis on the BD FACSCelesta

MCF-7 (ATCC® HTB-22™) cells were pulsed with EdU for one hour, and then fixed, permeabilized, and stained according to the recommended assay procedure for the BD Pharmingen™ 647 EdU Click Proliferation Kit. After two washes to remove the click reaction cocktail, cells were stained with BD Pharmingen™ PE Mouse Anti-Human Cyclin B1. After another washing, cells were stained with 1 µg/mL BD Pharmingen™ DAPI Solution and acquired and analyzed on the BD FACSCelesta BVR configuration. Results: Cells were gated based on light scatter, followed by doublet discrimination (not shown). The EdU data (left plots) allowed identification of cells in different cell cycle compartments: G₀/G₁-phase cells are EdU⁻ with 2N DNA content (green), S-phase cells are EdU⁺ (blue), and G₂/M-phase cells are EdU⁻ with 4N DNA content (purple). Cyclin B1 (right plots) increased as cells progressed through the cell cycle. Data with (top) or without (bottom) compensation showed similar staining patterns and equal numbers of cells in each cell cycle compartment, confirming that compensation was unnecessary for this experiment.

Detailed apoptosis analysis

Apoptosis (programmed cell death) is characterized by a number of morphological and biochemical cellular changes. Studies have revealed distinct pathways (intrinsic and extrinsic) leading up to cell death. Apoptosis can be experimentally induced in vitro to understand mechanisms regulating cell death, as well as to test the effect of drugs on cell viability. Annexin V, the most common flow cytometry assay to study apoptosis, binds to the phosphatidylserine (PS) that has been translocated to the outer layer of the cell membrane of apoptotic cells. A cell-impermeable dye such as propidium iodide (PI) or 7-AAD often is added to discriminate dead from apoptotic cells.

Figure 12 shows an experiment in which Jurkat cells, an acute human T-cell leukemia cell line, were treated with Camptothecin, a topoisomerase I inhibitor, to induce apoptosis and cell death in Jurkats. The samples were stained with Annexin V and PI to assess cell viability and then analyzed. Cells treated with just DMSO were used as a control. When comparing the untreated (upper right plot) with the Camptothecin-treated (lower right plot), we can see the treated cells show an increase in the number of Annexin V+ (apoptotic) and Annexin V+/PI+ (dead) cells, indicating that camptothecin treatment did induce apoptosis and cell death.

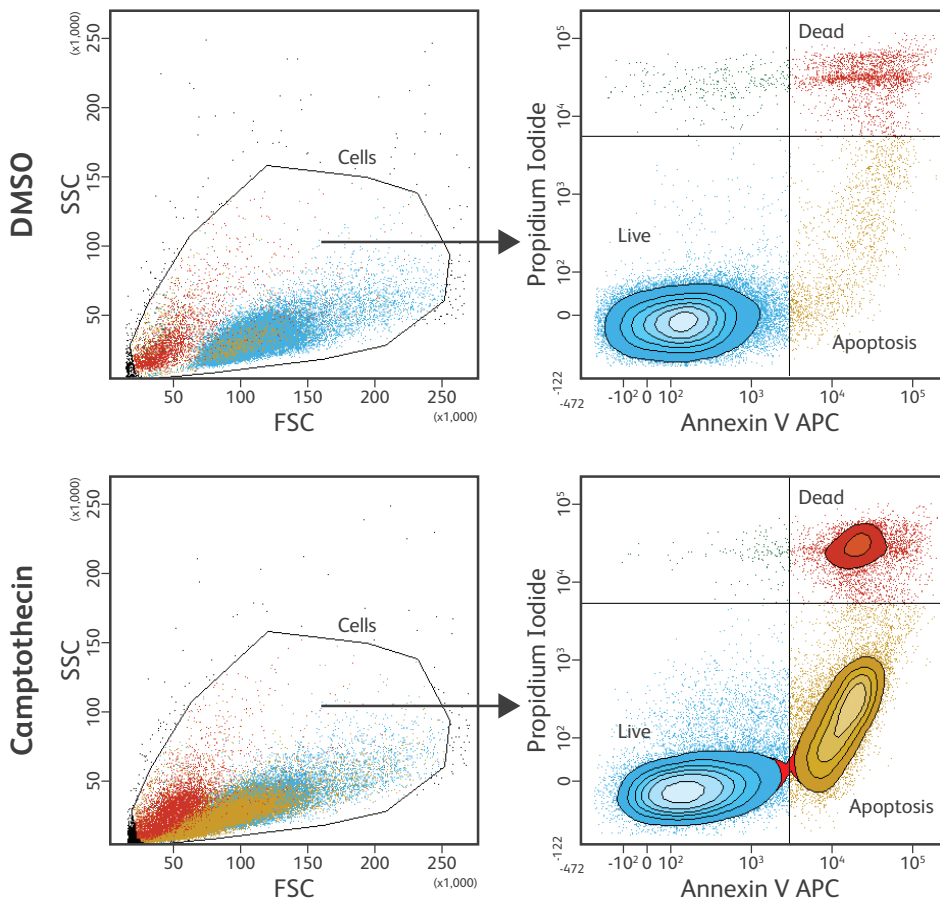


Figure 12. Two-color flow cytometric analysis of apoptosis and viability in Jurkat cells

Jurkat cells were treated with 0.025% DMSO vehicle (top plots) or 5 μ M camptothecin (bottom plots) for 4 hours, harvested from culture, washed, and resuspended in Annexin V Binding Buffer. Cells were then incubated with BD Pharmingen™ APC Annexin V (Cat. No. 550475) and BD Pharmingen™ Propidium Iodide Staining Solution (Cat. No. 556463) (PI) for 15 minutes at room temperature protected from light, and then analyzed by flow cytometry on a BD FACSCelesta system. Debris was excluded based on the light-scatter properties of Jurkat cells (left plots). DMSO vehicle-treated cells were primarily Annexin V⁻PI⁻, indicating that most cells were live. Camptothecin-treated cells show an increase in the number of Annexin V⁺ (apoptotic) and Annexin V⁺PI⁺ (dead) cells, indicating that camptothecin treatment induced apoptosis and cell death.

Appendix: Overview of the BD FACSCelesta

This appendix summarizes the features and capabilities of the BD FACSCelesta system. For more information, including options please visit our website at bdbiosciences.com.

The BD FACSCelesta

Combining proven legacy BD components and pairing it with innovative new technology, the BD FACSCelesta offers performance, efficiency and accessibility at an affordable price. The 14 parameter, bench-top analyzer enables researchers of all skill levels to perform sophisticated, multicolor cellular analysis and confidently drive their experiment from hypothesis to results. Equipped with state-of-the-art electronics that deliver high signal to low noise you are able to resolve rare and dim cell populations. Available in four instrument configurations, each configuration has blue (488-nm) and violet (405-nm) lasers which can be paired with a red (640-nm), ultraviolet (355-nm) or yellow-green (561-nm) laser.

The Complete Solution

The BD FACSCelesta system brings together innovative instrument, reagent and software technologies in a single system, backed by high level of field and application support.

Reagents: Optimized for use with bright, narrow-spectrum BD Horizon Brilliant dyes, the BD FACSCelesta is designed to reduce the need for compensation as well as increase fluorescent intensity.

Software: The BD FACSDiva™ software, already used by many researchers worldwide, simplifies setup, operation, panel design and compensation. It requires minimal training for new and experienced users alike.

Service: The BD FACSCelesta system is backed by BD's world-class service and support organization. Hands-on training, field application support, highly trained field technicians, remote assistance and an included a full-year warranty limits downtime of your instrument and provides the training and support necessary to excel.

Options

High Throughput: The BD™ High Throughput Sampler (HTS) supports reliable, multi-sample applications and is compatible with 96-well U, V and flat-bottom plates as well as 384-well microtiter plates. In high-throughput mode, the HTS can process a 96-well plate in fewer than 15 minutes with less than 1% carryover.

BD FACSFlow™ Supply System: The optional BD FACSFlow Supply System (FFSS) fluidics cart increases capacity and ease of use while maintaining a stable fluidics pressure. It includes automated sheath and waste fluid control system that reduces daily maintenance by incorporating two 20-L containers (Cubitainers®).

Additional information

1. BD Biosciences. Designing minimal spectral overlap panels to enhance data quality: Increasing resolution and ease-of-use with the BD FACSCelesta™ flow cytometer. More information at: http://static.bdbiosciences.com/documents/BD-FACSCelesta-Compensation-DS.pdf?_ga=2.127497421.1995174449.1499277913-1876293953.1497977228

2. BD Biosciences. Immunophenotyping and functional analysis of rare cell populations: Analyze dendritic cell markers and functional properties with the BD FACSCelesta™ flow cytometer. More information at: http://static.bdbiosciences.com/documents/BD-FACSCelesta-Rare-Cell-DS.pdf?_ga=2.26220158.215067626.1499700972-1876293953.1497977228

3. BD Biosciences. Combining instrument sensitivity with bright dyes to resolve low-density antigens: Taking advantage of the sensitivity of the BD FACSCelesta™ flow cytometer. More information at: http://static.bdbiosciences.com/documents/BD-FACSCelesta-Sensitivity-Antigen-DS.pdf?_ga=2.27228670.215067626.1499700972-1876293953.1497977228

4. BD Biosciences. A flow cytometry solution for fluorescent protein analysis and beyond: Maximizing resolution with the BD FACSCelesta™ BVYG configuration. More information at: http://static.bdbiosciences.com/documents/BD-FACSCelesta-Fluorescent-Protein-Analysis-BVYG-DS.pdf?_ga=2.27228670.215067626.1499700972-1876293953.1497977228

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