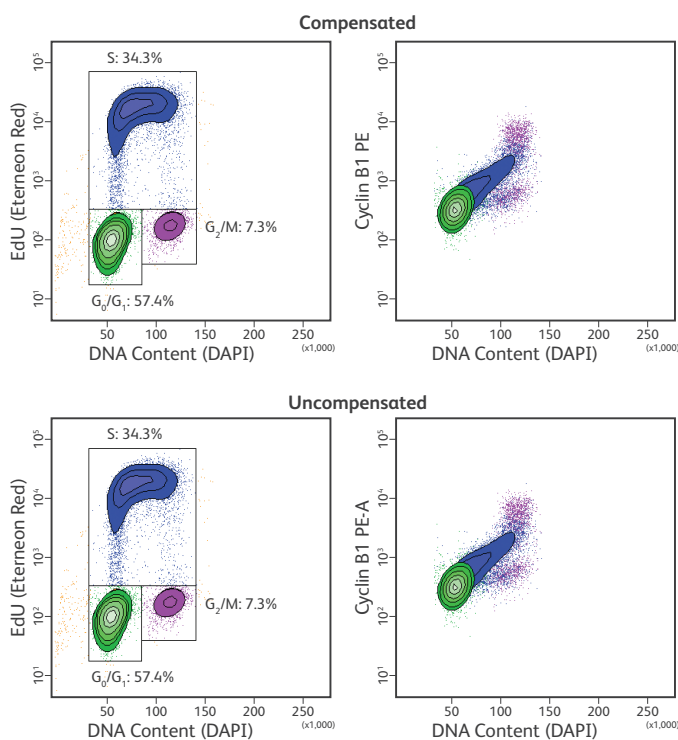# Enhanced cell cycle studies using multicolor flow cytometry
Cell biology applications on the BD FACSCelesta™ flow cytometer

Features

- Obtain enhanced cell cycle data at the single-cell level

- Reduce spectral overlap and minimize compensation by spreading fluorochromes across three lasers

- Combine multiple cell biology applications in a single tube, such as cell cycle analysis, immunophenotyping and fluorescent protein analysis

With up to three lasers and 12 fluorescence detectors, 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 1 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 G_0/G_1 phase, S phase, and G_2/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—one of four available configurations to match experimental needs—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, including cell cycle (Figure 1) and immunophenotyping (Figure 2) analyses.

In the Figure 1 panel, DNA content was measured with DAPI using the violet laser, Cyclin B1 expression using the blue laser, and EdU incorporation using the red laser. Similarly, the Figure 2 panel spreads three cell surface markers across the three lasers to interrogate breast cancer cell line immunophenotypes. With each fluorochrome excited by a different laser and cross-laser excitation accounted for, spectral overlap between channels is minimal and compensation is nominal, if required at all.

Figure 1. 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: G_0/G_1-phase cells are EdU⁻ with 2N DNA content (green), S-phase cells are EdU⁺ (blue), and G_2/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.

Enhanced cell cycle studies using multicolor flow cytometry

With up to 12 fluorescence detectors (plus two light scatter detectors) available on the BD FACSCelesta, cell cycle and immunophenotyping markers can also be multiplexed together. This can save time and sample, while also providing a deeper understanding of the cell type of interest by extracting more information from one sample. Figure 3 shows an experiment in which cell cycle and immunophenotyping analyses were combined in a single tube. In addition, the BB515/FITC channel was left open to measure GFP expression—a sixth

parameter—should researchers want to multiplex fluorescent protein expression as well.

By pairing innovations in instrumentation with bright new reagents, the BD FACSCelesta 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.
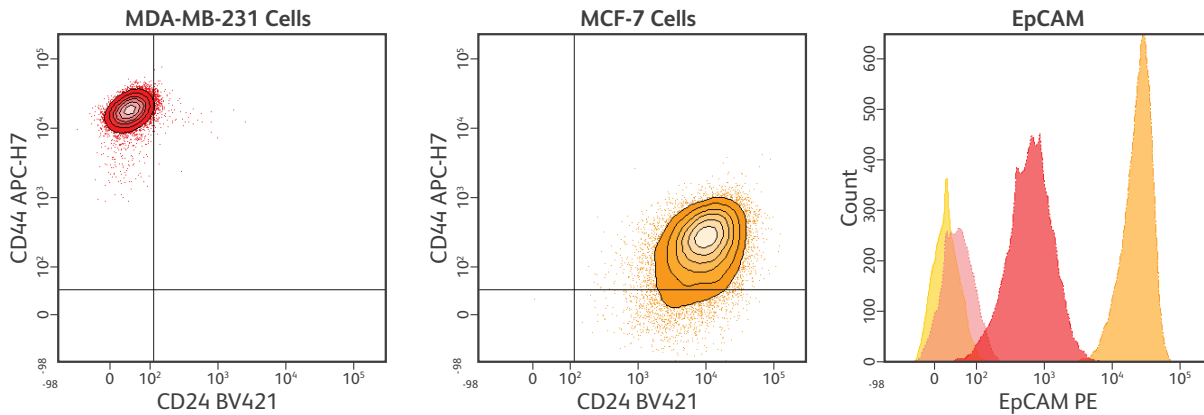
Figure 2. Minimal spectral overlap panel for immunophenotyping on the BD FACSCelesta

MDA-MB-231 (ATCC® HTB-26™) and MCF-7 (ATCC® HTB-22™) cells were stained with BD Horizon™ BV421 Mouse Anti-Human CD24, BD Pharmingen™ APC-H7 Mouse Anti-Human CD44, and Anti-EpCAM PE (Cat. No. 347211) in BD Pharmingen™ Stain Buffer (FBS), and then acquired and analyzed on the BD FACSCelesta BVR configuration. **Results:** Cells were gated based on light scatter, followed by doublet discrimination. MDA-MB-231 cells (left) expressed a cancer stem cell phenotype (CD24⁻CD44⁺), while MCF-7 cells (middle) were predominantly CD24⁺CD44⁺. Gates were drawn based on unstained controls. In the right plot, MCF-7 cells (light orange, unstained; orange, stained) expressed a higher level of EpCAM than did MDA-MB-231 cells (pink, unstained; red, stained). Staining patterns with and without compensation were similar, only uncompensated data are shown.
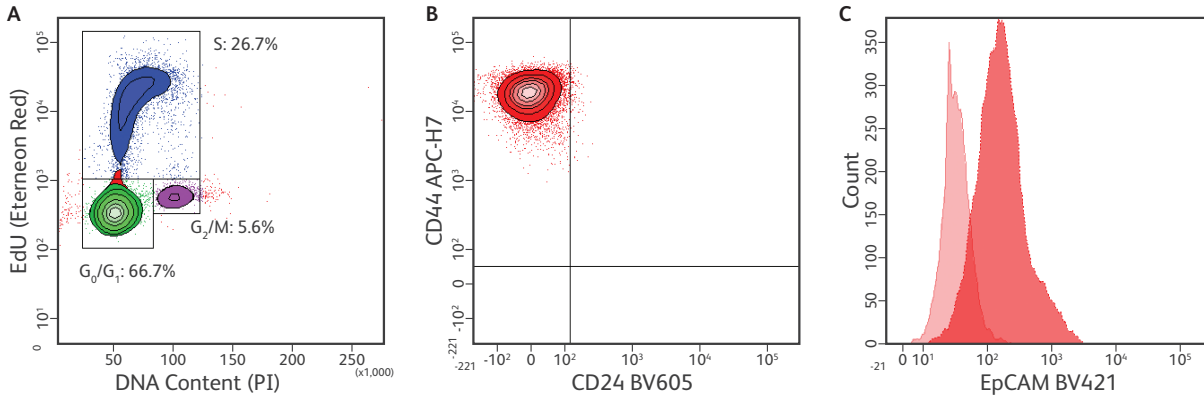
Figure 3. Multiplexed cell cycle and immunophenotyping analysis on the BD FACSCelesta

MDA-MB-231 (ATCC® HTB-26) cells were pulsed with EdU for one hour and then stained with BD Horizon™ BV605 Mouse Anti-Human CD24, BD Pharmingen™ APC-H7 Mouse Anti-Human CD44, and BD Horizon™ BV421 Mouse Anti-Human CD326 (EpCAM) in BD Pharmingen™ Stain Buffer (FBS) and BD Horizon™ Brilliant Stain Buffer. The cells were then fixed, permeabilized, and stained according to the recommended assay procedure for the BD Pharmingen™ 647 EdU Click Proliferation Kit. After washing, cells were then stained with BD Pharmingen™ Propidium Iodide Staining Solution and acquired and analyzed on the BD FACSCelesta BVR configuration. **Results:** Cells were gated based on light scatter, followed by doublet discrimination. **A.** Cell cycle analysis showed clear differentiation of G_0/G_1 (green), S (blue), and G_2/M-phase (purple) cells as in Figure 1. **B, C.** MDA-MB-231 cells expressed a cancer stem cell phenotype (CD24⁻CD44⁺) and a low level of EpCAM (pink, unstained; red, stained). The gate was drawn based on FMO controls. The BB515/FITC channel was intentionally left open to facilitate GFP drop-in for fluorescent protein analysis.

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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