BD FACSDiVa Option

The Digital Advantage for the BD FACSVantage SE
Flow Cytometry System
Proven Performance in Flow Cytometry with BD Biosciences

Since 1974, when we developed the first commercially available fluorescence activated cell sorter (FACS™) in collaboration with Stanford University, BD Biosciences has been the leader in flow cytometry technology and innovation. BD Biosciences continues to be the industry leader with the BD FACSDiVa™ option for the BD FACSVantage™ SE cell sorter, designed and developed with over 25 years of experience in flow cytometry instrumentation. Our proven performance continues to provide the advances that keep customers at the forefront of science.

BD Biosciences has proven performance in all aspects of flow cytometry. We are committed to offering complete systems to help each laboratory excel scientifically. Our reliability and performance in instrumentation, reagents, service, and support has been key to customer satisfaction. BD Biosciences offers the most advanced and complete solution in flow cytometry.
BD innovation meets the needs of the flow cytometry community with continuous advances in research instrumentation to keep pace with science. Introduced in 1998, the BD FACS Vantage SE flow cytometer has a proven performance record. To build upon the foundation of the BD FACS Vantage SE instrument, a completely new digital option has been created.
The BD FACSDiVa option includes fully digital electronics and new PC-based software. The digital electronics provide higher performance and expanded capabilities. The BD FACSDiVa option can acquire up to 10 channels with a completely configured BD FACSVantage SE instrument. The digital electronics support a maximum of 16 channels with additional optical components. The new digital electronics provide the means to utilize all standard optical pathways and acquire every fluorescent measurement possible.

New tools are available to enhance and improve all aspects of sorting. Four-way sorting capabilities increase sorting efficiency, providing faster and more efficient results. Sort setup is easier and more convenient with new enhanced sort modes. Drop delays are determined quickly and accurately within minutes using the BD AccuDrop* option, eliminating tedious manual drop delay profiles.

The BD FACSDiVa option software operates on a state-of-the-art PC providing a new high-end platform for data acquisition and analysis. The software contains data management features to improve experiment setup and organization of data files. The software organizes data stored in the database in the browser for easy batch analysis and template generation. The ability to create data acquisition and analysis templates provides consistency between experiments.

The Digital Advantage

The BD FACSDiVa option operates as an independent cytometer interface with its own unique set of digital electronics and computer workstation for acquisition and analysis. A digital/analog switch on the front panel of the BD FACSDiVa module provides the flexibility to operate in the new digital mode or traditional analog mode.

The BD FACSDiVa option digitizes signals 10 million times per second in 16,384 discrete levels. As a result, logarithmic amplifiers and analog peak-and-hold circuits are no longer required. Electronic dead-time is eliminated and more particles can be processed, improving sort yields. By eliminating the log amps, more accurate fluorescent measurements can be made, improving linearity, compensation, and quantitation of fluorescence measurements.

The new digital electronics allow all optical detectors to be utilized at once. With a fully loaded optical configuration, the BD FACSDiVa option has the ability to acquire all 10 channels simultaneously, 8 fluorescent and 2 scatter. This increases the amount of information that can be acquired from each cell. The BD FACSDiVa option electronics can support a maximum of 16 channels with an expanded, customized optical bench.

BD FACSDiVa electronics can trigger off of any parameter generated from any laser. This enhances the instrument's ability to discriminate between specific cells. Triggering criteria can be combined with and/or logic for two or more parameters. This improves the performance and speed of data acquisition by focusing on specific cell characteristics.

The ability of the BD FACSDiVa option to digitize signals at 10 million times per second in 16,384 discrete levels expands channel resolution. The area measurement is the sum of all data points measured for each cell. The area value for a typical pulse of 3 µsec is in the range of 0 to 262,144. The BD FACSDiVa option provides increased detection and channel resolution per pulse to acquire more information per cell.

* US and foreign patents filed

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Compensation is now performed in the firmware for sorting and in the software for analysis. This increases flexibility and efficiency in data acquisition and analysis. The BD FACSDiVa option performs compensation based upon the inverse matrix of the so-called spillover coefficients. A spillover coefficient is the amount of fluorescent light that leaks into other channels. The spillover matrix can be entered between any channels that are measured, with expanded capabilities for interbeam compensation. There is no limitation to the compensation combination that can be set up and acquired.

Enhanced Sort Performance

Enter a new realm of sorting performance with the added features incorporated into the BD FACSDiVa option. The ability to sort four populations simultaneously increases sorting efficiency and yield. At sort rates of 30,000 events/second, yields are significantly increased in comparison to sorting without the BD FACSDiVa option (Figure 1).

The Sort Output layout feature in the software aids in sort setup and data management. This software tool simplifies sort setup and enables collection of sort-specific information. Use the Sort Output layout to define the sort output device as a tube, plate, or slide. For each sort destination, define and save the sort regions and the number of cells to sort. Once the sort is complete, the exact number of cells sorted is saved. The Sort Output layout is a permanent record for the experiment. The BD FACSDiVa option provides easier sort setup and saving of sort results, which improves the overall performance of the BD FACSVantage SE flow cytometer.

Increased flexibility makes the BD FACSDiVa option single-tube sort array a clear advantage. This feature allows you to define a specific number of cells per population to be sorted in a destination, thus allowing you to define the exact mix of cells sorted for each experiment. The single-tube sort array enables customized experimental design.

Increased drop resolution provides more accurate investigation of each sorted drop and results in better sort purities and yields. Each drop is investigated in 32 increments. By examining the sorted drops in smaller increments, each sort decision that takes place becomes more accurate and efficient. Increased resolution provides increased sort accuracy and performance by sorting only the drops of interest.

Because the investigation of each drop is more stringent, each sort is limited to one drop unless the target particle is detected too close to the edge of a drop. The Sort Precision modes set the criteria for each sort decision. Depending on the Sort Precision mode chosen, the cell will be sorted for purity, yield, or count accuracy. The Sort Precision modes and increased drop resolution improve flexibility with high-level performance.
Figure 1 shows that as the threshold rate (events/second) increases, the BD FACSDiVa digital electronics maintain a greater yield than the analog electronics. Yield was calculated by sorting a 40% bead population on each instrument and comparing the counters.

Figure 2 shows the pre-sort plots and the four subpopulations of beads to be sorted. Figure 3 shows the post-sort plots that have been gated on scatter to eliminate debris. Table 1 shows the sort results achieved from a four-way purity sort of 20,000 events/second at 45 psi and 65 kHz.

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<th>Table 1</th>
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<td><strong>Pre- and post-sort results</strong></td>
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New Software Expands Acquisition and Analysis Tools

The BD FACSDiVa option was designed with additional benefits that are not directly related to the digital electronics. One is the creation of a new PC-based software program to acquire and analyze the FCS data files generated from the BD FACSDiVa electronics. This software brings many additional features to both data acquisition and analysis.

The BD FACSDiVa option holds data in memory after it is recorded. This feature conserves precious sample because you can pause the sample flow during acquisition and then perform compensation on the data held in memory. After compensation is adjusted, begin recording the sample and save the FCS data file. This improves efficiency and time in the overall experiment.

Compensation can also be adjusted on saved FCS data files generated from the BD FACSDiVa electronics. Post-acquisition compensation adjustments increase flexibility and demonstrate the analytical advantages of BD FACSDiVa software.
Pulse processing is built into the BD FACSDiVa electronics. Both area and height can be saved for each pulse generated from each detector. Width is generated from the threshold channel. Ratios are calculated on any two parameters with no limitations. Built-in pulse processing saves time when performing cell cycle, doublet discrimination, and calcium flux experiments.

The BD FACSDiVa electronics handles log display as it handles compensation. Both are mathematical processes that can be performed or repeated at any time. The system uses 18-bit precalculated log lookup tables. The main advantage of using log lookup tables is accuracy. Analog systems use logarithmic amplifiers that are manufactured to deviate by no more than 5% from a perfect logarithmic response in its optimal range. At the beginning and end of the response curve, even greater deviations can be observed. The digital electronics and software provide a more accurate log calculation for improved data quality. Figure 4 illustrates a six-color experiment setup for human lymphocyte subset analysis of CD3/CD16+56/CD45/CD4/CD19/CD8.

Figure 4
Example of a six-color experiment

The BD FACSDiVa option brings an entirely new experience to data acquisition and analysis. The new software operates on a PC-based Windows™ platform. This new platform is more powerful and flexible than any other workstation BD Biosciences has offered for its flow cytometry instruments. The BD FACSDiVa option workstation is compatible with many PC-based computers used in the market place, so it is easier for IT departments to support.

The workstation computer has a dual processor with 2 GB of RAM or memory. The system includes the following removable storage devices: 4-mm DAT, CD-ROM, CD-RW, 250 internal Zip™ Drive, and 3.5” floppy drive. The workstation comes equipped with an 18” LCD flat-panel monitor and speakers. Video cards are provided to operate two monitors at once. Ethernet is built in for networking capabilities on 10/100 Ethernet connections. A three-port Firewire® PCI adapter is also provided. A wireless keyboard and mouse provide freedom of movement and flexibility while performing sample optimization and sort setup.

BD Biosciences continues to meet the needs of its customers by expanding the capabilities of the BD FACSVantage SE flow cytometer with the BD FACSDiVa option. New digital electronics and acquisition software increase sorting efficiency, expand multicolor analysis, and improve data management. BD Biosciences provides the flow cytometry instrumentation to advance your scientific discoveries.