Fig 1: Dr. Nobuko Uchida, Cell Biology for Transplantation Group, SyStemix, Inc., Palo Alto, CA.

Fig 2: Flow cytometry by David Houck and Laurie Gilmour, BD; FISH and photomicrography courtesy of Nga Bui, BD.

Fig 3, 5, 8, 12, 13, 15, 16, 17: Flow cytometry courtesy of Dr. Matthias Haury, Institut Pasteur, Flow Cytometry Laboratory, Department of Immunology, Paris, France.

Device Master File (FDA): BD-MF7149. BD FACSVantage is constructed under ISO 9001, UL, and is CE certified for the low voltage EMC directive.

Laser safety practices for a Class IV device should be observed during servicing or laser alignment procedures.


* For Research Use Only

Phycocyanin (PE) and Allophycocyanin (APC): US Patent No. 4,520,110; European Patent No. 76,695; Canadian Patent No. 1,179,942

Attractors: US Patent No. 5,627,040; 5,739,000; and 5,795,727

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delay calculations directly from the video monitor, assuring fast, reproducible, and accurate startup for each of your sorting experiments. The MasterSort controller automatically provides six sort modes after automated drop-delay calculations have been made and stored in the instrument. Each mode is specifically designed to optimize for purity, recovery, or count accuracy to meet your experimental objectives.

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BD Biosciences offers a wide range of software enhancements for the BD FACSVantage SE flow cytometry system that lets you stretch the limits of your experiment and gain the advantage in state-of-the-art research.

Superior Service
To help you gain the most from your BD FACSVantage SE flow cytometry system, BD Biosciences provides you with all the support services you’ll need, including system installation and comprehensive training, as well as applications support, expert field services, and comprehensive maintenance programs, all to ensure that your BD FACSVantage SE flow cytometer performs to its full potential. With just a click of the mouse, BD Customer Support can be in your lab remotely to help resolve your instrument or software questions. The FACSConnect remote diagnostics feature allows you to dial directly into BD from the FACStation and get comprehensive technical support.

Remote diagnostics available only in North America.

Gain the Research Advantage Today
Call us today to arrange for a complete demonstration, so you can see firsthand the power and flexibility of the most advanced flow cytometer available today. BD FACSVantage SE—another first from BD Biosciences, gives you the research advantage with the most powerful vehicle on the road to discovery.

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BD FACSVantage SE gained even more speed and power with new features and options that seamlessly integrate with its standard multicolor fluorescence, multi-laser excitation, and data management capabilities. Features such as non-rectangular sort regions are standard on the instrument. New options include a sixth fluorescence detector, third laser excitation spot, and CloneCyt™ Plus with faster well-to-well access. We've added a new dimension to automated single-cell deposition with IndexSort, which is standard with CloneCyt Plus. For the first time, specific information regarding single cells sorted into a 96-well tray can be recorded and linked to clonal expansion—a very exciting and enabling technology!

The proven AutoSort computerized drop-delay setup feature is available, so you don't have to bother with complex calculations and can set up your sorting experiments faster and with higher precision than ever before. And you can expand your range of applications to include large-particle analysis and sorting with the innovative MacroSort™ Plus or high-speed sorting with the TurboSort Plus options.

That's not all you'll find in the BD FACSVantage SE flow cytometer. Extensive analytical tools ensure sensitivity and speed, as well as simple-to-use, time-saving formats that let you focus on the challenges of the experiment, not the technology of the equipment. The sleek, ergonomic design allows you more functionality in less space, and BD FACSVantage SE's versatile architecture is fully modular and can be upgraded for specialized applications.

As the cornerstone of our research instrument line, BD FACSVantage SE carries BD Biosciences' reputation for providing the best service, support, and training in the industry. All of this enables you to be first in the race to find answers.
Gain the Research Advantage with BD FACSVantage SE

BD revolutionized cell sorting in 1992 with the introduction of the FACSVantage flow cytometer for biomedical research. Today, the new Sort Enhanced (SE) edition makes BD FACSVantage™ SE the most powerful and flexible vehicle on the road to discovery.

BD FACSVantage SE gains even more speed and power with new features and options that seamlessly integrate with its standard multicolor fluorescence, multi-laser excitation, and data management capabilities. Features such as non-rectangular sort regions are standard on the instrument. New options include a sixth fluorescence detector, third laser excitation spot, and CloneCyt™ Plus with faster well-to-well access. We’ve added a new dimension to automated single cell deposition with IndexSort, which is standard with CloneCyt Plus. For the first time, specific information regarding single cells sorted into a 96-well tray can be recorded and linked to clonal expansion—a very exciting and enabling technology!

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Another industry first from BD is the MacroSORT Plus option that allows you to sort cells larger than previously possible. Potential applications include sorting pancreatic islets, plant protoplasts, or megakaryocytes.

With MacroSORT Plus fluid-coupled, piezo-driven plunger assembly, efficient and stable drop formation is attained with nozzle diameters of up to 400 µm. BD FACSVantage SE automates your experiment with the CloneCyt Plus option. CloneCyt Plus precisely deposits a predefined number of cells onto microscope slides and filters, in individual wells of 96- or 24-well microtiter plates, or even a user-defined collection device, all with the highest purity and variability in the industry. The CloneCyt software works transparently with CellQuest, so users need only set the sort deposition requirements once to achieve total automation. The system does the rest!

CloneCyt Plus is also the only system to provide IndexSort—a powerful verification tool that links the information from a deposited cell in a 96-well tray to precise cell phenotype measurements. Ongoing studies in research utilize this feature to ensure that the sorted cells have the same phenotype after cultured under defined conditions, then correlated to subsequent colony morphology. You can now determine the best cell marker combination to accurately define and characterize subpopulations of cells with a new level of confidence!

The core technology that enables and simplifies advanced cell sorting is based on the AutoSort feature, which greatly simplifies instrument setup. This unique capability provides for automatic drop-
Designed for Flexibility

BD Biosciences designed the BD FACSVantage SE system with unique image collection optics that provide high sensitivity and resolution. Using up to three independent laser beam spots, you can collect as many as six fluorescence signals per cell.

The system’s innovative pulse processing technology, available as an option, allows you to measure the area, width, and ratio of detector pulses. Pulse processing can be used to detect doublets in DNA analysis and produces the ratio of two fluorescence signals for use in calcium flux measurements.

To meet your unique research requirements, a wide range of air- and water-cooled lasers is available. The newest include mixed-gas and the latest argon-ion lasers.

Automated for Greater Productivity

The BD FACSVantage SE provides software-assisted instrument setup, allowing you to spend more time analyzing your data, instead of defining software and experiment parameters. With automated setup, experiments are consistent and reproducible—critical requirements in core laboratories or facilities where users and applications often change.

With just a click of the mouse in CellQuest™ software, a predefined document containing a user-defined combination of acquisition and analysis tools tailored for a particular experiment can be instantaneously recalled and used. The document saves setup time and ensures consistency when running the procedure again. With seamless acquisition and analysis, CellQuest™ allows you to rapidly assess the progress of an experiment and the status of the instrument. No guessing! Just give the command and let BD FACSVantage SE do the work.

Figure 1: Sorting data

Isolation of c-kit+Thy-1.1, lo-Lin-1, Sca-1+ stem cells from C57BL/Ka Thy 1.1 murine bone marrow. Figure 1a shows the light scatter profile of the unfractionated bone marrow cells. The cells were stained with Phycocerythrin (PE) lineage cocktail and Thy-1.1 Fluorescein isothiocyanate (FITC), Sca-1 Texas Red, and c-kit Allophycocyanin (APC). Dead cells were excluded from the lymphoid sort gate using propidium iodide (PI). In this example, positively selected bone marrow cells are shown as the pre-sort data (1c, 1d, and 1e) and used to better visualize the stem cell phenotypes. The percent noted for the gate in each plot was based on the actual preselection bone marrow analysis. R2 delineates 0.3-0.5% of the Lineage lo versus Thy-1.1, R3 defines 0.1-0.2% of the Sca-1 versus c-kit, and R4 0.04-0.08% in the Thy-1.1 versus c-kit. Upon subsequent reanalysis (1f, 1g, 1h), cell purity was determined to be >98%.

Streamlined Design

From an engineering and design level, BD Biosciences provides the scientific tools that will reward you with superior results without the need to focus on technology. The BD FACSVantage SE is designed and optimized, from end to end, as a complete system that incorporates reliability, safety, and the flexibility that you have come to expect from your hardware and software. This modular flexibility allows the BD FACSVantage SE to be adapted and expanded as your research needs change and expand.

All of this technology is accessible through a familiar user interface and efficient ergonomic design. The compact ergonomic workstation of the BD FACSVantage SE provides a large, open workspace that allows clutter-free instrument operation without sacrificing precious laboratory space.

The BD FACSVantage SE open-architecture system is easily upgraded. As your needs expand, you can add multiple options to your system—from lasers to automation enhancements—protecting your technology investment.

Cell Sorting Benchmark

BD Biosciences cell sorters hold the distinction of being the industry benchmark in sort performance against which all other sorters are compared. The BD FACSVantage SE system provides the tools to collect the cells of interest under the best conditions.

Figure 2: Sorting for subsequent in situ hybridization

Figure 2a illustrates the region used to sort the lymphocyte fraction of normal, male lyzed whole blood for subsequent in situ hybridization using a density plot on CellQuest software. Cells were sorted, then spun onto glass slides, and hybridized with a probe to detect the Y chromosome. The probe is biotinylated with a streptavidin FITC second step. The nuclei are counter-stained with propidium iodide. The fluorescence photomicrograph in figure 2b illustrates the hybridized lymphocytes sorted from region 1.

Cell Phenotype Percent

- Helper T Cells: 28.7%
  - CD3+CD8–CD4+CD19– Violet
- Suppressor/Cytotoxic T Cells: 13.6%
  - CD3+CD8+CD4– Red
- B Cells: 19.9%
  - CD3–CD8–CD4–CD19+ blue
- T Cells: 11.3%
  - CD3+CD8–CD4+CD19– yellow
- NK Cells: 16.5%
  - CD3–CD8+CD4+CD19+ green

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Figure 3: Six-color fluorescence

Six-color fluorescence analysis of human peripheral blood using PAINT-A-GATE™ software. The cells were stained with CD3 FITC, CD16-CD56 PE, CD4 PE, Texas Red Tandem conjugate, CD45 PerCP-Cy5.5, CD20 APC, and CD68 Biotin/SAV APC-Cy7. The BD FACSVantage SE was set up with dual laser excitation using an air-cooled argon-ion laser, and a helium-neon laser. The cells colored gray on the plots represent the non-staining cells present within the sample. The following lymphocyte subsets were identified:

- **Helper T Cells**: 28.7% CD3+CD4+CD8- (Violet)
- **Suppressor/Cytotoxic T Cells**: 13.6% CD3+CD8+CD4- (Red)
- **B Cells**: 19.5% CD20+CD19+CD14- (Blue)
- **T Cells**: 11.3% CD3+CD16+CD56+CD8- (Yellow)
- **NK Cells**: 16.5% CD3-CD16+CD56+ (Green)

Figure 4: Four-color immunofluorescence

Peripheral blood cells were stained with CD2 FITC, CD3 RED613, CD4 APC, and CD8 PE in a longitudinal series of four-color immunofluorescence experiments to monitor changes in T-cell subsets. Using BD Attractors software on the FACStation, subsets were automatically classified using a customized Attractor Set based upon dynamic gates. These adjust to relative changes in the subpopulation and furnish quantitative values for reports for each subsequent file. BD Attractors also provides batch processing for walk-away automation, consistency, and increased productivity.

Figure A: Phenotype Percent

- Helper T Cells: 28.7% CD3+CD4+CD8-
- Suppressor/Cytotoxic T Cells: 13.6% CD3+CD8+CD4-
- B Cells: 19.5% CD20+CD19+CD14-
- T Cells: 11.3% CD3+CD16+CD56+CD8-
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**Versatile BD FACSVantage SE—Applications & Experiments**

The versatility of the BD FACSVantage SE flow cytometer allows you to explore many new research applications not possible with conventional cell sorting systems. These applications include genetics, kinetics, and multicolor analysis. And with BD’s wide range of state-of-the-art lasers, advanced optics, signal processing technologies, and enhanced automation, you have the potential to conduct experiments from molecular biology to large particle analysis like islets of Langerhans, sperm, or plant cells.

**Multicolor Analysis**

Now it’s easy to explore new subsets with BD FACSVantage’s SE’s multicolor analysis capabilities. Choose as many as three lasers for up to six-color fluorescence measurement. The unique optical design and spatial beam separation of the BD FACSVantage SE system mean you need minimal electronic compensation and few optical components. The results are enhanced signal discrimination and sensitivity (<200 Molecules of Equivalent Soluble Fluorescin [MESF]—well below the limiting autofluorescence of most biological cells*).

BD Biosciences software programs like CellQuest, PAINT-A-GATE™, and Attractors, also provide easy-to-use interfaces for the simplest or the most complex multicolor analysis. The patented BD Attractors software can rapidly and automatically analyze series of data files containing complex mixtures of cell populations to discover new cell subsets.

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**Figure 5: Calcium flux with indo-1 and 3 surface markers**

Peripheral blood mononuclear cells were prepared and stained based on the method of Rabinovitch, et al. Cells were surface stained with CD4 FITC, CD8 PE, and CD20 PerCP monoclonal antibodies. For this experiment, baseline unstimulated measurements were followed by the addition of a CD3 stimulus. Figures 5a and 5b display CD4+, CD8+, and CD20+ gated lymphocytes. Analysis was gated on the FITC-labeled CD4+ lymphocytes (Figure 5c), PE-labeled CD8+ lymphocytes (Figure 5d), and PerCP-labeled CD20+ lymphocytes (Figure 5e). For these three plots, the indo-1 violet/green fluorescence ratio is shown as a function of elapsed time. The arrows indicate the point where the CD3 stimulus was added.

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**Figure 6: Single-cell sorting and PCR analysis**

Tracking the fate of antigen-specific helper T cells in vivo has been difficult due to their low frequencies in normal animals. Using five-color flow cytometry, these antigen-responsive CD4 subsets responding to pigeon cytochrome c (PCC) were characterized by excluding CD8, B220, and Mac-1 (Figure 6a, 6b) and sorted based upon the expression of the T-cell receptor and modulation of CD44 and CD62L (Figure 6c, 6d, 6e). Single cells were sorted using CloneCyt Plus into an oligo (dT)-primed cDNA reaction mix and incubated. The Vol.1V3 cDNA was amplified using an RT-PCR nested strategy then directly sequenced. The distinctive CDR3 loops of PCC-specific helper T cells are shown in the PCR results (Figure 6f).

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**Figure 7: Chromosome analysis**

Figure 7a shows a Hoechst 33258 versus Chromomycin A3 flow karyotype as a dot-plot distribution for a karyotypically normal human male. Measurements were made in pulse area using the pulse processing option. Figure 7b demonstrates the “zoom” feature of the CellQuest software. An exploded view of a portion of the display aids in the identification of minor subpopulations for more accurate analysis window definition.

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**Figure 8: DNA analysis**

Figure 8a is a dot plot of FL2 signals electronically processed to provide pulse width and pulse area measurements. Pulse processing is a powerful tool used in the discrimination of doublets for optimal DNA analysis. Figure 8b is a histogram displaying DNA content analysis of a fine needle aspirate of a lung tumor, stained with the BD CycleTEST™ Reagent Kit, for research use only.

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**Figure 9: Analysis of Green Fluorescent Protein (GFP)**

Figure 9 shows a graph of wild-type GFP expression after infection with an adenovirus vector; the wild-type GFP is expressed at reduced levels due to the presence of an in-frame stop codon in the inserts. The insertions are indicated by solid bars. The red line shows the expression of a construct containing a premature stop codon, while the green line shows the expression of a construct containing a wild-type GFP sequence. The horizontal axis represents the number of days after infection, and the vertical axis represents the relative fluorescence intensity. The data were collected using BD FACSVantage SE flow cytometry, and the process of discrimination is not compromised. BD’s dye-specific chromosome filter set optimizes and maximizes signal collection. The innovations designed into the new BD FACSVantage SE flow cytometer provide you with all the resolution you need to succeed in genetics research, now and in the future.
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Kinetic Research

Time is the most important parameter for the measurement of dynamic cellular events in intact cells under physiologic or near-physiologic conditions. This parameter is recorded for each event by BD FACSVantage SE’s software. The kinetic research opportunities possible with the BD FACSVantage SE system include studying of such processes as the rates of influx or efflux of molecules across the cell membrane; binding of ligands to intracellular calcium, pH, and membrane potential in response to various stimuli.

Sample heterogeneity can be easily unmasked by combining multicolor, multiparameter analysis with the powerful logical gating routines offered by CellQuest software. And realistic determinations are as easy as a click of the mouse with the systems optimal pulse processing hardware.

BD FACSVantage SE offers speed, high sensitivity, and extraordinary measurement precision for monitoring critical time-dependent biological events.

Figure 6: Single-cell sorting and PCR analysis

Tracking the rate of antigen-specific helper T cell in vivo has been difficult due to their low frequencies in normal animals. Using five-color flow cytometry, these antigen-responsive CD4 subsets responding to pigeon cytomegalovirus (PCMV) were characterized by excluding CD8, B220, and Mac-1 (Figure 6a, 6b) and sorted based upon the expression of the T cell receptor and modulation of CD44 and CD62L (Figure 6c, 6d, 6e). Single cells were sorted using CloneCyt Plus into an oligo (dT)-primed cDNA reaction mix and incubated. The Volv1323 cDNA was amplified using an RT-PCR nested strategy and then directly sequenced. The distinctive CDR3 loops of PCC-specific helper T cells are shown in the PCR results (Figure 6f).

Figure 7: Karyotypically normal male human chromosomes

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Figure 9: Calcium flux with indo-1 and 3 surface markers

Red outline overlay indicates the background control.

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* Using Spherotech Rainbow RCP 30-SA particles; >5000 MESF using the Flow Cytometry Standards Corporation Quantum particle.
Advanced Cell Sorting

BD pioneered flow cytometry instrumentation more than two decades ago. We continue to provide pioneering enhancements in cell sorting that can make a significant difference in your research, along with advanced features that increase the speed, accuracy, functionality, and reliability of cell sorting.

The new TurboSort Plus option analyzes and sorts cells at rates of 25,000 events per second using multiple laser beam excitation. Precise fluid control and core stream adjustments are maintained using a new integrated fluidic subsystem. High throughput rates coupled with high purity and recovery eliminate the dilemma of sorting rare events or samples with large cell numbers. TurboSort Plus easily cuts processing time to a fraction of what a standard instrument requires while maintaining high purity, recovery, and viability.

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Figure 10: Sorting performance

Figure 10 illustrates the purity and recovery determination for a 50/50 mix if FITC- and PE-labeled CaliBRITE™ beads are sorted at the threshold rates shown. Percent purity is determined by reanalysis, and percent yield is the number of particles sorted determined by an automated cell counter as a fraction of the sort count total determined by the BD FACSVantage SE.

Figure 11: Index Sorting

Figure 11 illustrates the use of index sorting to provide verification of the quality of a single-cell sort using CloneCyt Plus.
delay calculations directly from the video monitor, assuring fast, reproducible, and accurate start-up for each of your sorting experiments. The M-assort Software controller automatically provides six sort modes after automated drop-delay calculations have been made and stored in the instrument. Each mode is specifically designed to optimize for purity, recovery, or count accuracy to meet your experimental objectives.

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BD Biosciences offers a wide range of software enhancements for the BD FACSVantage SE flow cytometry system that lets you stretch the limits of your experiment and gain the advantage in state-of-the-art research.

**Superior Service**

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