

FACSVantage™ SE

Flow Cytometry System
from Becton Dickinson

FACSVantage SE

BECTON
DICKINSON

The Most Powerful Vehicle
on the Road to Discovery

Advancing Science Using Flow Cytometry

Since 1974, when we developed the first commercially available fluorescence activated cell sorter (FACS®) in collaboration with Stanford University, Becton Dickinson Immunocytometry Systems (BDIS) has been the leader in flow cytometry technology and innovation.

Today is no exception. Becton Dickinson systems continue to set the standard in the science of flow cytometry; and our customers expect nothing less. We're committed to offering systems with state-of-the-art advances in optics, fluidics, signal processing technology, monoclonal reagent chemistry, and customer service to provide the best possible integrated cell analysis and sorting systems in the industry.



Gain the Research Advantage with FACS*Vantage*[™] SE

Becton Dickinson revolutionized cell sorting in 1992 with the introduction of the FACS*Vantage* flow cytometer for biomedical research. Today, the new Sort Enhanced (SE) edition accelerates past the limitations of other instruments and makes FACS*Vantage* SE the most powerful and flexible vehicle on the road to discovery.

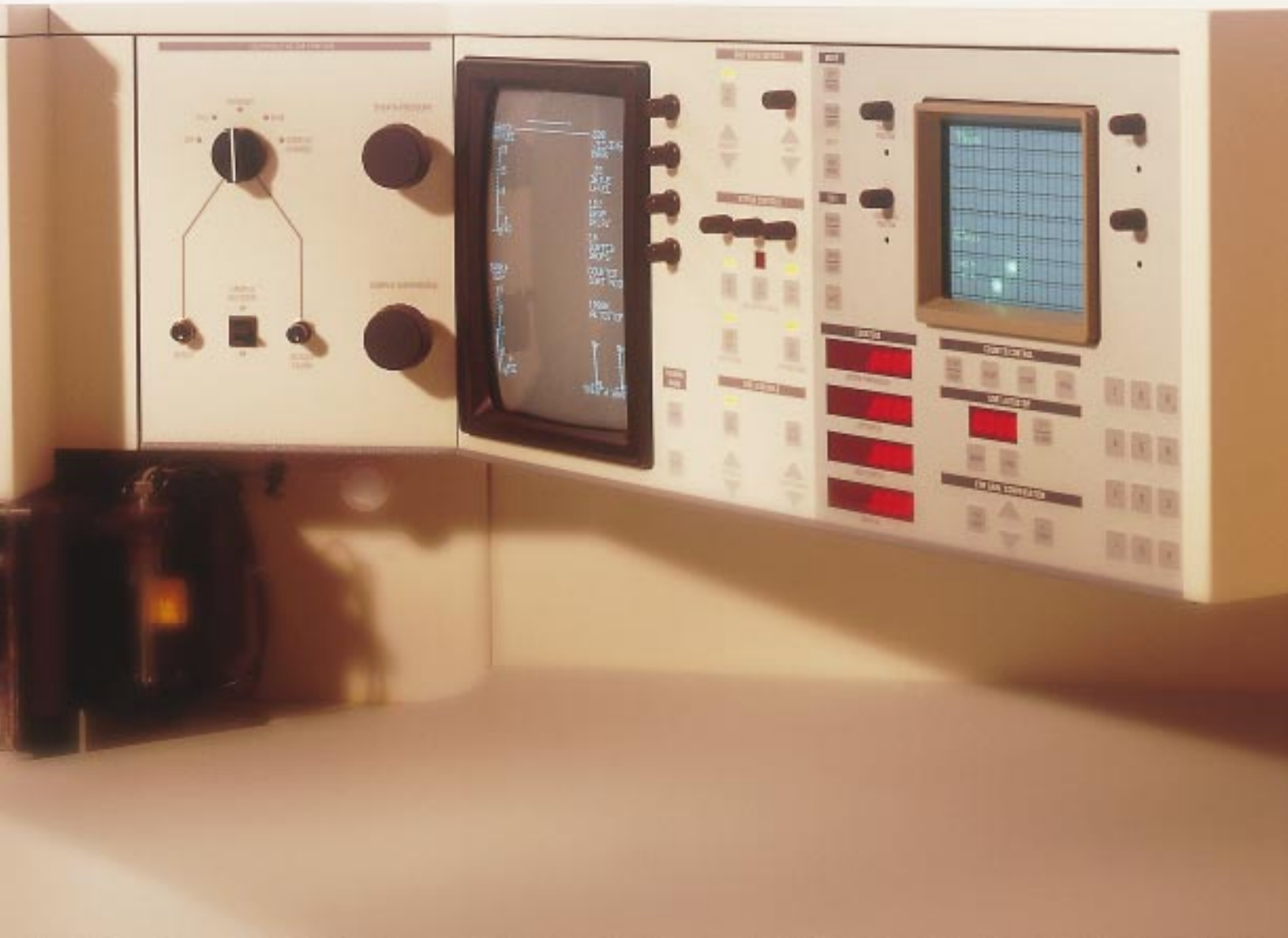
FACS*Vantage* SE gains even more speed and power with new features and options that seamlessly integrate with its standard multicolor fluorescence, multi-laser excitation, and data management capabilities. Features such as non-rectangular sort regions are now standard on the instrument. New options include a

sixth fluorescence detector, third laser excitation spot, and CLONECYT[™] Plus with faster well-to-well access. A new dimension to automated single cell deposition called IndexSort[™] 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 option.

That's not all you'll find in the FACS*Vantage* 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 FACS*Vantage* SE's versatile architecture is fully modular and can be upgraded for specialized applications for tomorrow's requirements.

As the cornerstone of our research instrument line, FACS*Vantage* SE carries Becton Dickinson's 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.







Designed for Flexibility

Becton Dickinson designed the FACS Vantage 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 FACS Vantage 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™, 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 FACS Vantage SE do the work.

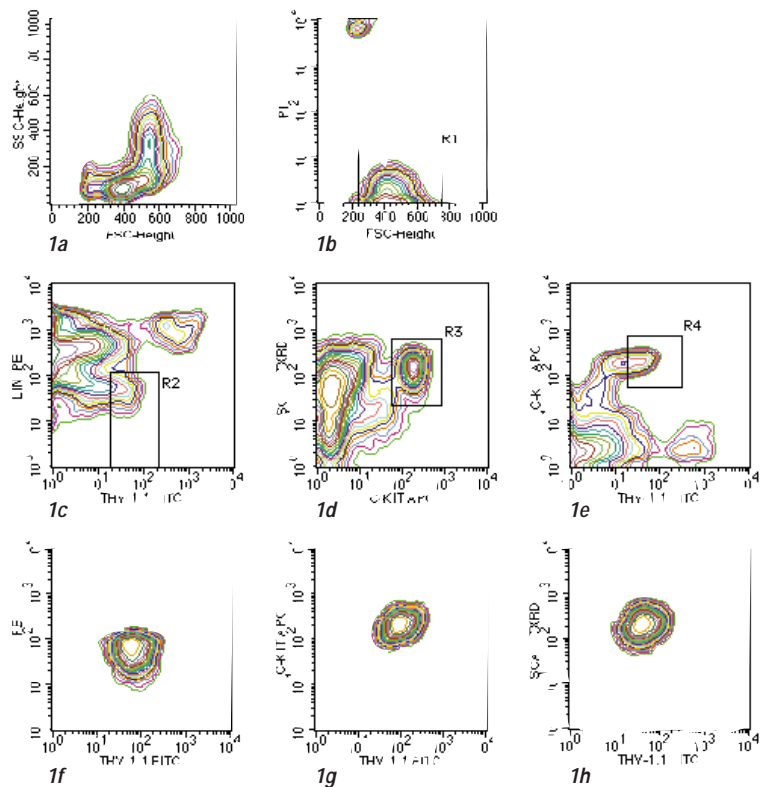


Figure 1: Sorting data

Figure 1 illustrates the isolation of *c-kit*⁺*Thy-1.1*^{lo}*Lin*^{lo}*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 a Phycoerythrin (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 (1b). 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 phenotype. The percent noted for the gates 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%.

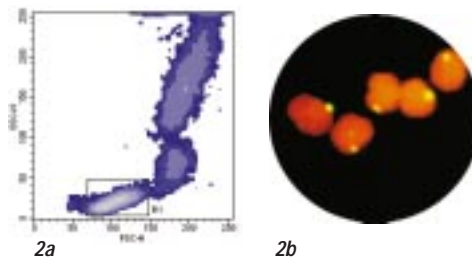


Figure 2: Sorting for subsequent in situ hybridization

Figure 2a illustrates the region used to sort the lymphocyte fraction of normal, male lysed 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. Fluorescent photomicrograph 2b illustrates the hybridized lymphocytes sorted from region 1.

Streamlined Design

On an engineering and design level, Becton Dickinson understands that a flow cytometer is far more than an aggregation of parts. It also has to be functional to meet the changing requirements in today's research lab. FACS Vantage SE is a complex interaction of many subsystems designed with the other in mind and built so they work in unison. By doing this, we assure functional and reproducible operation over many levels of experimental needs, without sacrificing the performance and precision you expect.

A compact ergonomic workstation adds more work space in a small footprint. The functional design optimizes every inch of the work area. FACS Vantage SE includes a large sample area for efficiency, aerosol management, safety, and optimum convenience, and a built-in storage area to keep critical accessories well protected and easily accessible.

The FACS Vantage 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

Becton Dickinson cell sorters hold the distinction of being the industry benchmark in sort performance against which all other sorters are compared. FACS Vantage SE meets this performance challenge by offering unprecedented sorting versatility, ease-of-use, multiple laser excitation, non-rectangular sort gates, and high speeds of up to 7,000 sorted events per second on a standard system, and 25,000 sorted events per second using the TurboSort Plus option. Whether you wish to sort bacteria, chromosomes, functional hematopoietic progenitors, or 150- μ m islets of Langerhans, the FACS Vantage SE system provides the tools to collect the cells of interest under the best conditions.

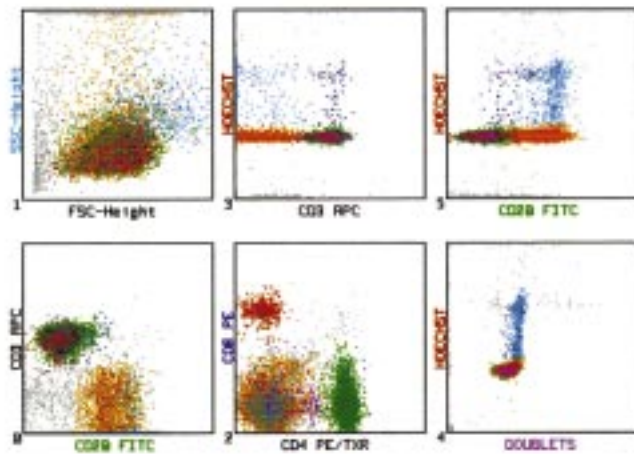


Figure 3: Five-color fluorescence

Figure 3 illustrates five-color fluorescence analysis of human tonsil tissue utilizing PAINT-A-GATE^{PLUS}™ software. The cells were stained with CD20 FITC, CD8 PE, CD4 biotin/RED613 Tandem conjugate, CD3 APC, and Hoechts 33342. The FACS Vantage SE was set up with triple laser excitation using an air-cooled argon-ion laser, a helium-neon laser, and a helium-cadmium laser. The cells colored gray on the plots represent the non-nucleated and/or non-singlet cells present within the sample. The following subsets were identified:

Phenotype	Cell Cycle Phase		% Cycling
	G ₀ /G ₁	S/G ₂ +M	
Helper T Cells CD3 ⁺ CD8 ⁻ CD4 ⁺ CD20 ⁻	22.5% Green	0.5% Blue	2%
Suppressor/Cytotoxic T Cells CD3 ⁺ CD8 ⁺ CD4 ⁻ CD20 ⁻	3.6% Red	0.1% White	3%
B Cells CD3 ⁻ CD8 ⁻ CD4 ⁻ CD20 ⁺	57% Yellow	5.2% Cyan	8%

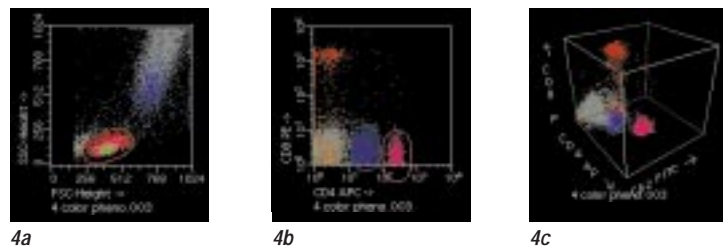


Figure 4: Four-color immunofluorescence

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 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. Attractors also provides batch processing for walk-away automation, consistency, and increased productivity.

Versatile FACSVantage SE—Applications & Experiments

The versatility of the 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 BDIS'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 FACSVantage 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 FACSVantage SE system mean you need minimal electronic compensation and few optical components. The results are enhanced signal discrimination and sensitivity (<100 Molecules of Equivalent Soluble Fluorescein [MESF]—well below the limiting autofluorescence of most biological cells*).

Becton Dickinson software programs like CELLQuest, PAINT-A-GATE^{PRO}, and AttractorsTM, also provide easy-to-use interfaces for the simplest or the most complex multicolor analysis. The patented Attractors software can rapidly and automatically analyze series of data files containing complex mixtures of cell populations to discover new cell subsets.

* Using Spherotech Rainbow RCP 30-5A particles; <1000 MESF using the Flow Cytometry Standards Corporation Quantum particles.

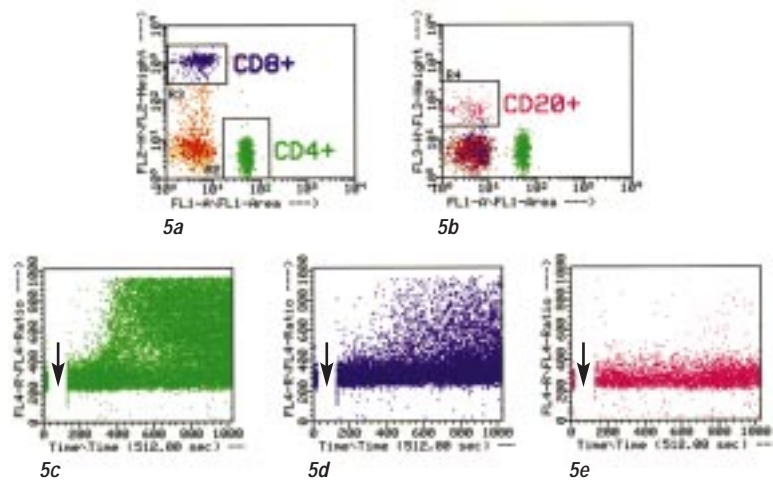


Figure 5: Calcium flux with indo-1 and 3 surface markers
Peripheral blood mononuclear cells were prepared and stained based on the method of Rabinovitch, et al.¹ Cells were surface stained with CD4 FITC, CD8 PE, and CD20 PerCP monoclonal antibodies. For this experiment, baseline unstimulated measurements were followed by the addition of a CD3 stimulus.

Fig 5a, 5b: display CD4⁺, CD8⁺, and CD20⁺ gated lymphocytes.

Analysis was gated on the FITC-labeled CD4⁺ lymphocytes (Fig 5c), PE-labeled CD8⁺ lymphocytes (Fig 5d), and PerCP-labeled CD20⁺ lymphocytes (Fig 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.

1. Rabinovitch PS, June CH, Grossman A, Ledbetter JA. Heterogeneity among T cells in intracellular free calcium responses after mitogen stimulation with PHA or Anti-CD3, simultaneous use of indo-1 and immunofluorescence in flow cytometry. *J Immunol.* 1986;137(3):952-961.

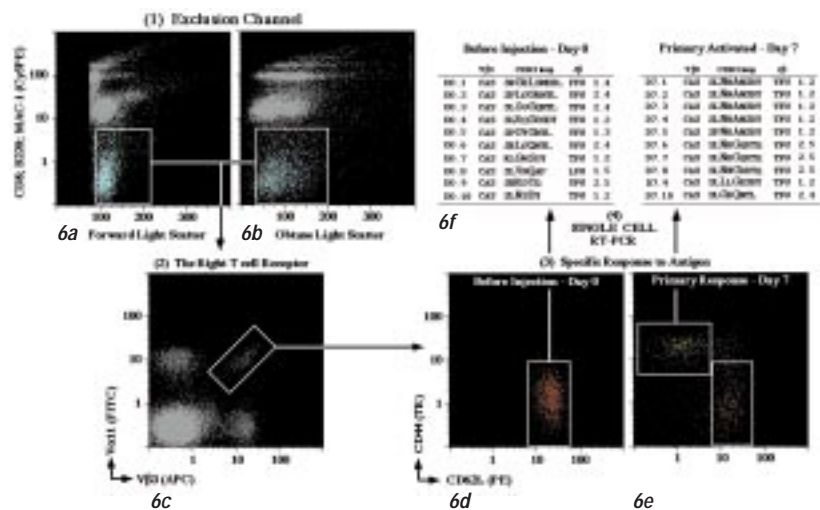


Figure 6: Single-cell sorting and PCR analysis
Tracking the fate of antigen-specific helper T cells *in vivo* has been difficult due to their low frequencies in normal animals. Using five-color flow cytometry, these antigen-responsive CD4 subsets responding to pigeon cytochrome c (PCC) were characterized by excluding CD8, B220, and Mac-1 (Fig 6a, 6b) and sorted based upon the expression of the T-cell receptor and modulation of CD44 and CD62L (Fig 6c, 6d, 6e). Single cells were sorted using CLONECYT into an oligo d(T) primed cDNA reaction mix and incubated. The V α 11V β 3 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 (Fig 6f).

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 FACS Vantage SE's software. The kinetic research opportunities possible with the FACS Vantage 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 ratiometric determinations are also as easy as a click of the mouse with the system's optional pulse processing hardware.

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

High-Performance Genetic Research

High-resolution chromosome analysis can be accomplished readily with the FACS Vantage SE system. Crucial requirements of the assay consist of exacting measurement precision and highly efficient fluorescence collection optics, as well as uniform illumination of the chromosomes. The highly stable fluidics and optical alignment provided by FACS Vantage SE meet these challenges easily.

Dual-beam flow cytometry of chromosomes improves discrimination by spreading the peaks over two dimensions. With the spatial beam separation provided by the FACS Vantage SE system, the process of discrimination is not compromised. BDIS's dye-specific chromosome filter set optimizes and maximizes signal collection. The innovations designed into the new FACS Vantage SE flow cytometer provide you with all the resolution you need to succeed in genetics research, now and in the future.

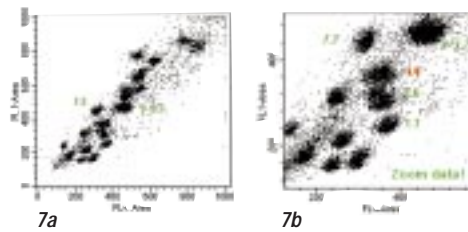


Figure 7: Karyotypically normal male human chromosomes

Fig 7a: This distribution, displayed as a dot plot, shows a Hoechts 33258 versus Chromomycin A3 flow karyotype for a karyotypically normal human male. Measurements were made in pulse area using the pulse processing option.

Fig 7b: This plot displays 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.

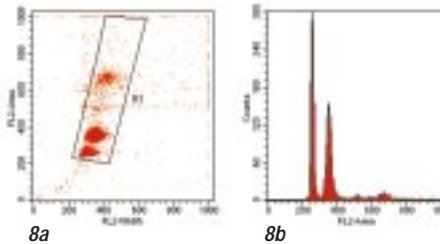


Figure 8: DNA analysis

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 BDIS's CycleTEST™ Reagent Kit, for research use only.

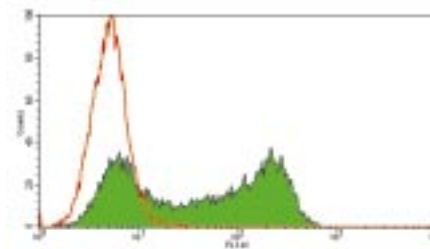


Figure 9: Analysis of Green Fluorescent Protein (GFP)

HeLa cells infected with attenuated Mengo virus vM16 containing GFP fused to viral L-peptide at 8 hours post-infection. Histogram shows 59% of the cells expressing the peptide and indicates various levels of expression due to non-synchronous expression. Red outline overlay indicates the background control.



The CLONECYT Plus option can be used with a wide variety of collection devices.

Advanced Cell Sorting

Becton Dickinson 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 tedium of sorting rare event 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.

For additional precision in the identification of cells for high speed sorting and multiparameter analysis, FACSVantage SE provides non-rectangular sort gates as a standard feature. Up to 16 cell populations can be identified using gates of various sizes and shapes, grouped in logical combinations and overlaps with no performance loss of sort or analysis rates.

Another industry first from BDIS 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



Carefully selected optical components are available for unsurpassed signal separation and detection.

plunger assembly, efficient and stable drop formation is attained with nozzle diameters of up to 400 μm !

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 versatility 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 a sorted cell with a specific phenotype is sorted, 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!

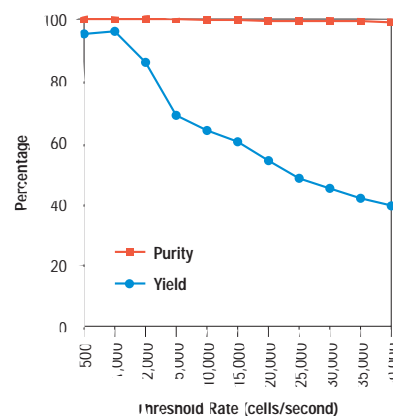


Figure 10: Sorting performance
 Figure 10 illustrates the purity and recovery determination for a 50/50 mix if FITC- and PE-labeled CaliBRITE™ Beads are sorted at the threshold rates shown. Percent purity is determined by reanalysis, and percent yield is the number of particles sorted determined by an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

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

Complete Data Management

FACSVantage SE data management provides everything you need to manage and acquire your data, including real-time statistics, up to 16 logical gates, as many as nine real-time acquisition windows, ellipsoidal or polygonal regions, and color gating tools—all in a true windowing environment with a simple-to-use, mouse-driven graphical interface.

Flexible Hardware

The FACSVantage SE comes with the powerful, industry-leading FACStation™ system for data acquisition, instrument control, and data analysis. The FACStation is based on the PowerPC RISC processor architecture for the fastest clock speed and performance available. The system comes equipped with hardware expandability, network connectivity, and processor upgradeability. They are also available with a range of options for monitors, storage devices, printers, and networking, allowing you to build a system that specifically meets your needs.

Simple Software

Perhaps the most valuable benefit to FACSVantage SE data management is CELLQuest software, which allows data to be easily handled and organized into any desired format. Logical combinations of data windows, regions, and color dot displays with real-time statistical analysis, allow you to identify more information

faster. Collect up to eight parameters with time as a ninth list-mode parameter and display the data from a selection of histograms, overlays, dot plots, contour plots, density plots, and isometric displays. Save the arrangement that works for you as an experiment document and reuse it each time you perform the experiment or analysis. The batch analysis capability also allows you to process entire series of data files while you attend to other matters. This saves time and gets to the answers you need quickly and reproducibly!

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

Superior Service

To help you gain the most from your FACSVantage SE flow cytometry system, Becton Dickinson provides you with all the support services you'll need, including

system installation and comprehensive training, as well as applications support, expert field service, and comprehensive maintenance programs, all to ensure that your FACSVantage SE flow cytometer performs to its full potential. With just a click of the mouse, BDIS Customer Support can be in your lab remotely to help resolve your instrument or software questions. The FACSCoast™ remote diagnostics feature* allows you to dial directly into BDIS from the FACStation and get comprehensive technical support.

* Remote Diagnostics available in North America.

Gain the Research Advantage Today

Call us today to arrange for a complete demonstration, so you can see first-hand the power and flexibility of the most advanced flow cytometer available today. FACSVantage SE—another first from Becton Dickinson, gives you the research advantage with the most powerful vehicle on the road to discovery.





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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, BDIS; FISH and photomicrography courtesy of Nga Bui, BDIS.

Fig 3, 5, 10: David Houck, BDIS.

Fig 4, 7: Janet Horta, BDIS.

Fig 6: Data from Dr. Michael McHeyser-Williams, Department of Immunology, Duke University Medical Center.

Fig 8: Pulse H versus Pulse W for doublet discrimination is US Patent #4,021,117 by Wolfgang and Hildegard Gohde.

Fig 9: Dr. Matthias Haury, Institut Pasteur, Flow Cytometry Laboratory, Department of Immunology, Paris, France.

Device Master File (FDA): BD-MF7149.

FACSVantage is constructed under ISO 9001, UL, and is CE certified for the low voltage EMC directive.

FACSVantage SE is a Class I laser device (IEC 825-1) under normal operating conditions.

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

Visit the BDIS website at <http://www.bdfacs.com>.

Attractors: US Patent #5,627,040.

FACS is a registered trademark of Becton Dickinson and Company. FACSVantage, FACStation, FACSCollect, Attractors, CELLQuest, CLONECYT, PAINT-A-GATE^{PLUS}, PAINT-A-GATE^{PRO}, AutoSort, IndexSort, MacroSort, TurboSort, CaliBRITE, and CycleTEST are trademarks of Becton Dickinson and Company.

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