

BD Influx™



A high-speed cell sorter that
adapts to your way of thinking

The BD Influx cell sorter adapts to your way of thinking

The BD Influx™ cell sorter is a flexible flow cytometry platform that easily adapts to a researcher's application or environmental requirements. A modular architecture and a powerful combination of detection capabilities, hands-on controls, and high performance allow researchers to configure the BD Influx system to their site and application needs.

The optical system offers up to ten lasers to adapt to application requirements. Each laser can be customized with detectors and filters best suited to your research requirements. A variety of exchangeable detector options are also offered. To meet needed speed and accuracy, the BD Influx system can handle a throughput rate of up to 200,000 events per second.

A unique fluidics system protects cells and addresses contamination. The nozzle assembly is designed to produce high droplet frequencies at relatively low pressures, enabling high-speed sorting while maintaining cell viability and functionality. To simplify drop-delay determination, patented BD FACS™ Accudrop technology allows researchers to quickly and easily see the best drop-delay value.

The BD Influx supports up to six-way sorting to maximize efficiency, and plate sorting to accommodate isolation of single cells. To support aseptic sorting, disposable fluidics allow researchers to replace a sample line or the complete fluidics path, from sheath tank to nozzle tip.

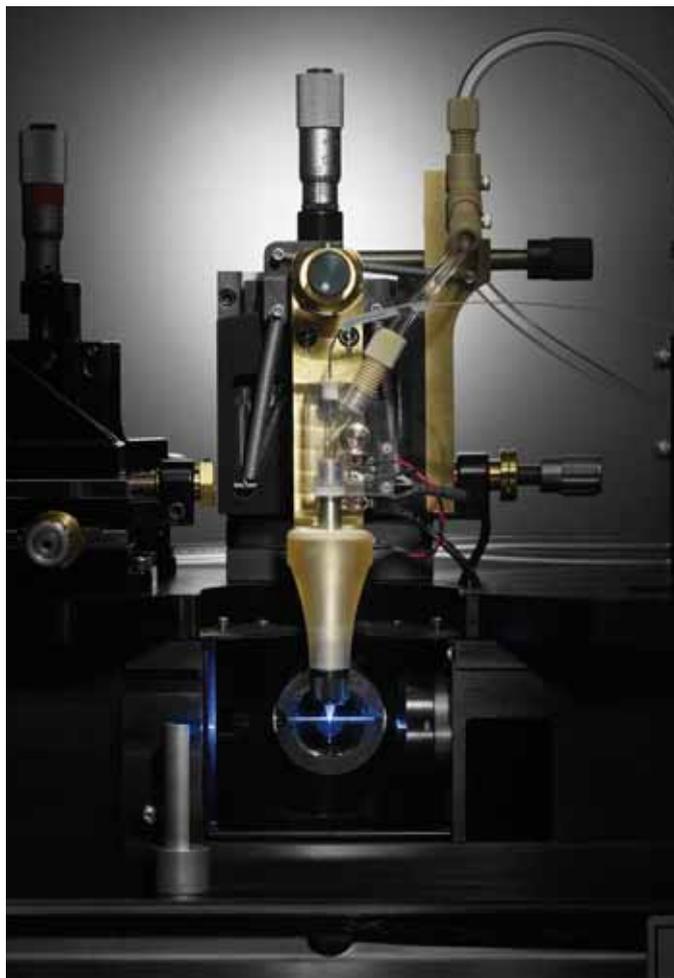
To protect operators and samples, a Class II Type A2 biosafety cabinet specifically designed for the BD Influx is available as an option.

BD FACS™ Software sorter software gives researchers comprehensive control of the sorter from configuration and compensation setup to acquisition, sorting, and analysis. Unlike traditional software, BD FACS Software supports sorting by capturing all information about an event and making it available to researchers on demand for later analysis. To visualize experiment data, rich output formats including histograms, density, and contour plots, are available to support analysis.

As part of BD Biosciences ongoing commitment to bringing innovative tools to life scientists and emerging areas of cell-based research, the BD Influx system can be fully optimized for routine tasks or emerging new applications. A full array of BD technical and application support comes standard to help facilitate and streamline research.

Control cross-contamination

Innovative fluidics design



Nozzle assembly and exchangeable fluidics

The BD Influx fluidics system features a unique acoustical coupling in the nozzle assembly to reliably create droplets for sorting, while ensuring low shear stress to optimize cell viability, even at high pressures. To support aseptic sorting, the fluidics path is easily removed and replaced with the optional disposable fluidics kit.

Nozzle assembly and nozzle sizes

The core of the BD Influx system is the nozzle assembly, which features an optimized acoustical coupling, enabling the most efficient droplet formation. This unique design minimizes noise from the droplet formation process and results in high droplet formation rates at relatively low sheath pressures.

In addition, the form of the nozzle assembly enables cells to accelerate smoothly to the laser intercept, increasing the viability and functionality of even the most fragile cells. A variety of nozzle sizes are available to allow researchers to easily adapt the system to different cell or particle types.

Sample station and fluidics console

A sample station and fluidics console control sample flow, sample line backflush, boost, and differential pressure. Bubble detectors prevent air bubbles from reaching the nozzle by stopping flow when the sample tube is empty.

Stainless steel tanks

Stainless steel pressurized sheath and waste tanks allow researchers to adapt cleaning procedures to match their application needs.

Exchangeable fluidics

An important concern in cell sorting is cross-contamination, either between samples or with foreign biological agents such as bacteria, viruses, and DNA. To mitigate the risks of cross-contamination, researchers can replace the sample line in a matter of minutes. The sample line extends into the nozzle tip to minimize contact between cells and non-replaceable parts. For rigorous control, the complete fluidics system, from sheath tank to nozzle tip, can be exchanged. The unique design of the BD Influx fluidics system allows for rapid exchange of all parts that potentially contact cells, addressing cross-contamination concerns.





A quick and simple alignment procedure

Intuitive alignment

The optical system that directs lasers to the interrogation point is easy to use, intuitive, and adaptable. Up to 10 laser paths can be configured. BD is constantly expanding the choice of lasers available for the BD Influx. Check the current technical specifications for the most up-to-date information.



Final focus lens assembly

The 10 laser paths and 7-pinhole optical collection system support 24 parameters simultaneously and 24 x 24 compensation. Systems with up to 5 lasers support 16 simultaneous parameters and 16 x 16 compensation.

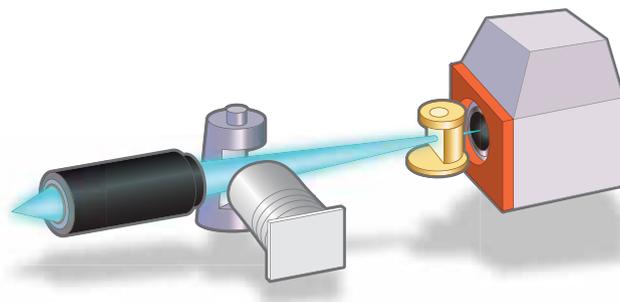
For each laser, light is shaped by individual optics, and final alignment and focusing are achieved using a dedicated lens that steers light to the stream at different angles. For the ten-laser system, the laser paths are collinear through the steering optics and focusing lens, but intersect the stream at seven spatially separated points. Fluorescence is collected through a high NA lens and focused on individual mirrored pinholes, reducing crosstalk between lasers. The modular design of the BD Influx optical system makes it easy to upgrade lasers as research requirements change. In addition, the variety of available lasers is constantly growing to keep pace with the needs of advanced research.

The patented optical design uses a special pinhole camera to image the stream and the pinholes simultaneously, simplifying optical alignment. With the help of the pinhole camera, near-optimal alignment can be achieved within seconds without using beads.

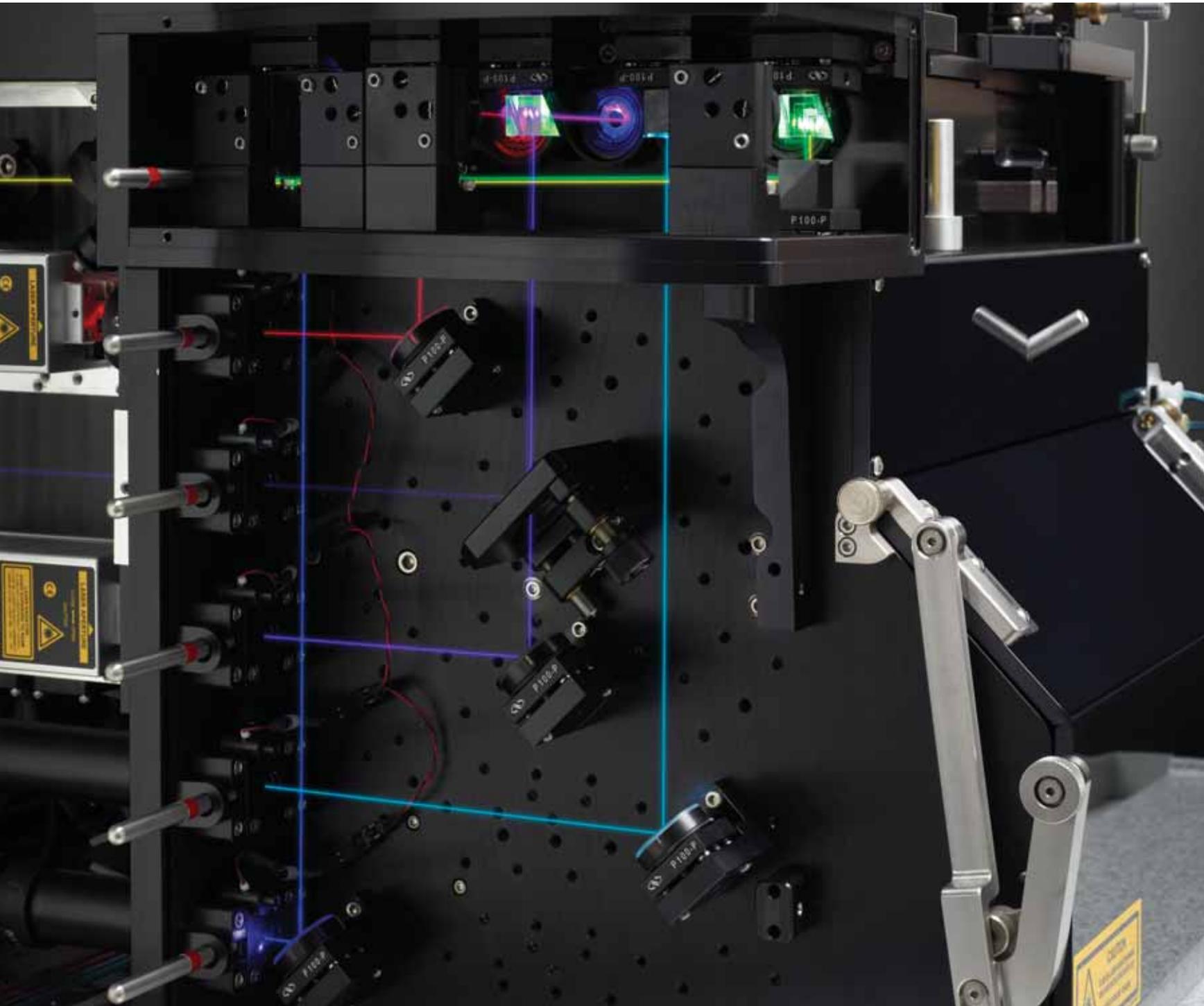


Pinhole camera view

The BD Influx system uses a pinhole camera that helps ensure that the fluorescence is in perfect alignment with the detectors. The pinhole camera lets a researcher view the stream at its optical center through the pinhole aperture's mirrored surface and subsequently align it so that the cells are in focus. When the cells are in focus for the camera, the system is optimally aligned for detecting fluorescence.



Schematic representation of collected lens, pinhole camera, and mirrored pinholes



Individual laser positioners

Independent laser beam alignment and video control of laser intercepts and pinholes allow for fast and intuitive alignment of multiple lasers.



A range of features for application-specific configurations

Adaptable

The BD Influx system can adapt to virtually any environment. A small footprint allows the instrument to fit into tight spaces, and a modular component design allows the instrument to be easily configured to specific needs. Footprint and modularity have made the BD Influx cell sorter the instrument of choice for marine biology applications. The BD Influx Mariner version includes pre-amp modifications for dimly fluorescent microorganisms and a brace to secure fluidics tanks for shipboard installation.

A range of standard detection modules is available to configure the system based on application needs.

Optional, exchangeable detector modules provide the capability to measure small particles, the polarization state of scatter, or fluorescence signals. This flexibility allows the user to adapt the system to the needs of routine or emerging applications.



Small Particle Option



Small Particle Option with polarization

Polarization

The BD Influx system can be equipped with polarization-sensitive detectors. A unique polarization design uses two detectors mounted under Brewster angles to measure changes in parallel and perpendicular light for both scatter or fluorescence. Changes in polarization can help differentiate between organisms containing highly reflective inclusions such as diatoms, or discriminate among different populations of granulocytes.

Small Particle Option

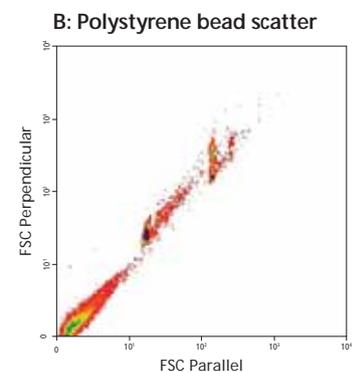
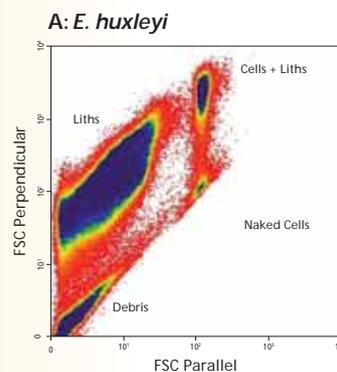
The Small Particle Option on the BD Influx system improves forward scatter detection. This option lowers the threshold on size measurements using a special detector with a high NA microscope lens, a pinhole, and a photomultiplier tube (PMT). The pinhole reduces the amount of stray light reaching the detector and prevents saturation of the PMT. Optimized sheath fluid (0.1-micron filtered) lowers the intrinsic noise level, which enables measurement of particles as small as 200 nm.

This resolution makes the Small Particle Option particularly valuable for applications in marine biology, microbiology, and environmental biology. The Small Particle Option also allows researchers to detect fluorescence in the forward direction. This capability is used in applications such as chlorophyll detection.

Unique properties revealed with polarization-sensitive detectors

A Viewed with polarization-sensitive detectors on the BD Influx, liths (scales) and lithed cells of the phytoplankton *E. huxleyi* can be discriminated using perpendicular and parallel FSC.

B Polystyrene beads serve as a negative control.



Computerized Cell Deposition Unit

For precise sorting, the mechanics and speed of the XY table in the Computerized Cell Deposition Unit (CCDU) allow sorting into microtiter plates or other custom devices such as slides or petri dishes. Single-cell cloning or sorting for single-cell PCR or single-cell imaging techniques is possible with the standard CCDU.

Make exploratory cloning more efficient with index sorting

Index sorting functionality has been completely rewritten and extended to put a very powerful analytical technique in the hands of researchers. It is now possible to review the complete flow phenotype of every cell sorted into a multiposition sort device, such as a 96-well tray. Index sort mode creates an FCS file containing all the sort deposition information and tray position information on an event-by-event basis. Each sorted event in the file is "indexed" one-by-one according to the X and Y coordinates of the sort collection device. Post-sorting results can be precisely traced back to the flow characteristics of the specific cell or combinations of cells sorted.



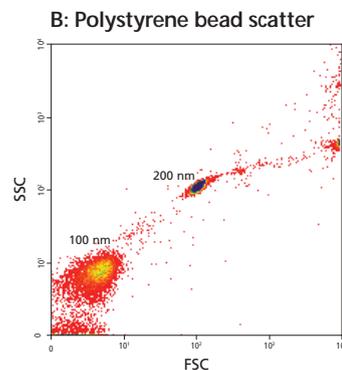
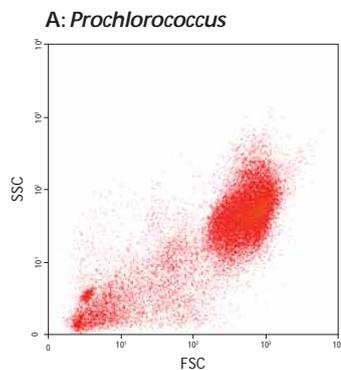
Flexible collection formats

In addition to sorting into tubes, the BD Influx supports an endless variety of slides, plates, and custom devices. Predefined trays and slides include 384-, 96-, 24- and 6-well plates, coarse and fine calibration slides, and 4 x 12 slides.

Prochlorococcus and polystyrene bead scatter using the Small Particle Option

A Small cells of the cyanobacteria *Prochlorococcus* are visible with the BD Influx with the Small Particle Option.

B The 100-nm polystyrene beads would not be visible without the Small Particle Option.

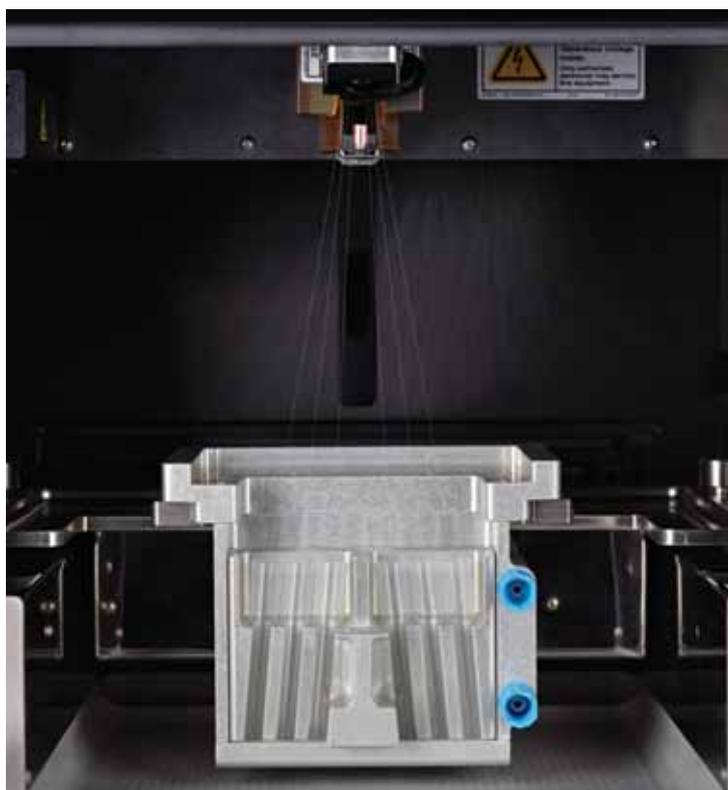


Multi-way sorting and Accudrop technology

Flexible sorting

The BD Influx system features two-, four-, and six-way sorting, as well as plate sorting. The BD Influx electronics support high-speed sorting, and BD FACS Accudrop technology helps simplify sort optimization.

In addition, new BD FACS Software sorter software facilitates sorting QC by allowing users to learn about how sort parameters impact results, to optimize research.



Six-way sorting into 5-mL tubes

Multi-way sorting

Isolating multiple populations at once saves time and makes efficient use of sample. Whether sorting one subpopulation or six, the BD Influx sorter can be quickly configured to best fit researchers' application requirements. A wide range of standard collection options supports two, four, and six-way sorting. Tube holders include sizes from microtubes, to 12 x 75-mm tubes, to 15-mL tubes.

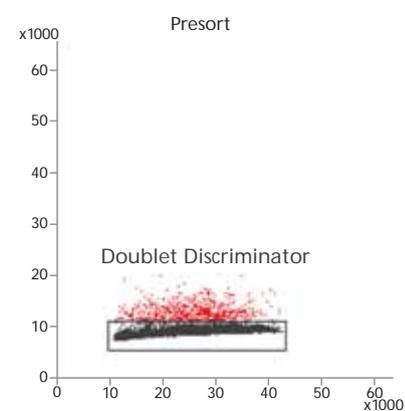
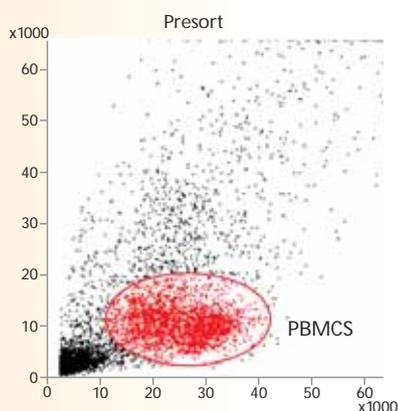
Electronics

The BD Influx system uses parallel electronics to reach a throughput rate of 200,000 events per second, independent of the number of parameters. For high sensitivity and low noise, the BD Influx system uses analog electronics in the first stage of signal amplification. The output of the analog peak and hold circuitry is digitized using 16-bit ADCs and placed on a patented parallel processing digital bus. The bus handles real-time compensation as well as other digitized signals, including area and width of up to eight additional pulses. To sort cells, researchers can select subpopulations through the creation of up to 32 sort regions. A variety of sort modes, optimized to favor purity, yield, or count accuracy, is standard. A custom mode allows researchers to optimize those settings for a specific application.

If purity is of no concern, theoretically sort speed is as fast as the throughput rate. In this case, cells are sorted independently and the target population is enriched.

Hierarchical gating strategy

Human lymphocyte subpopulations were identified with fluorochrome-conjugated antibodies excited by the 488-nm, 561-nm, 640-nm, and 405-nm lasers. A hierarchical gating strategy identified peripheral blood mononuclear cells (PBMCs) (forward scatter vs side scatter), singlets (forward scatter vs forward scatter width), lymphocytes (CD45-PerCP-CyTM5.5 vs side scatter) and lymphocyte subset populations (fluorescence plots).



SORTING

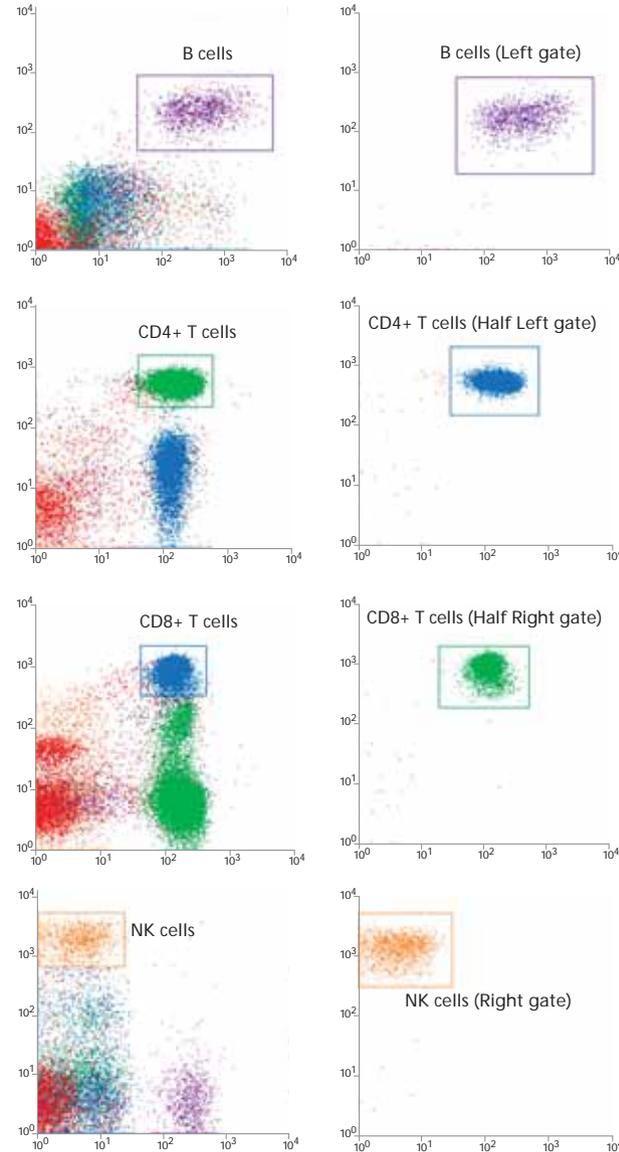
In most sorts, however, purity is a major concern, and factors such as droplet formation rate, event rate, and sample quality play a deciding role. When these factors are taken into account, the yield can be calculated by using Poisson's statistics. The BD Influx system will reach >80% of Poisson's expected yield with properly prepared samples.

Accudrop technology simplifies drop-delay determination
BD FACS Accudrop technology simplifies setup and eliminates manual calculations normally required. Accurately calculating the drop-delay determination—the point at which the drop separates from the stream—is important because it ensures that the instrument precisely places the charge on the drop containing the particle of interest.

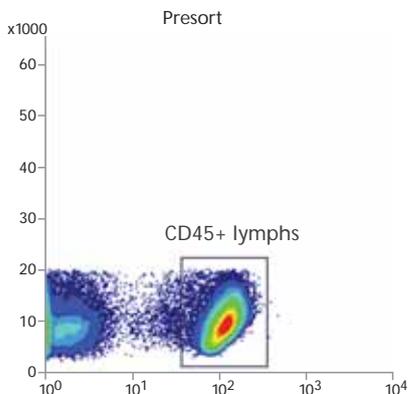
Accudrop technology assists the researcher to set the optimal drop-delay value. The drop-delay value can be adjusted while viewing BD FACS™ Accudrop beads, which are illuminated in the center and side sort streams. The researcher can identify the precise drop-delay value without a fluorescence microscope or manual calculations.

Four-way sorting

The BD Influx system was set up for a 4-way sort using an 86-µm nozzle (30 psi with a frequency of 60 kHz). Lymphocyte subset populations were sorted in purity mode at a rate of 10,000 events per second. Sort purity was 98% or greater.



Statistics: Post-sort	
Populations	% Parent
B cells (Left gate)	97.80%
NK cells (Right gate)	99.73%
CD4+ T cells (Half Left gate)	98.91%
CD8+ T cells (Half Right gate)	98.86%



Statistics: PRESORT		
Populations	% Total	% Parent
All Events	100.00%	###
PBMCs	41.11%	41.11%
Doublet Discriminator	40.47%	98.43%
CD45+ lymphs	27.36%	67.61%
B cells	1.09%	3.99%
NK cells	3.28%	12.01%
CD4+ T cells	14.17%	51.80%
CD8+ T cells	7.07%	25.84%



Real-time viewing of the sort in process is enabled by live videos of the side streams, drop breakoff point and laser intercept on the stream.

Multiple levels of control to match application and environmental needs

Custom Cabinet meets biosafety standards

A biological safety cabinet (BSC) designed specifically for the BD Influx by The Baker Company, measuring 86.2 x 53.5 x 94 in. (219.0 x 136 x 239 cm) is available as an option. Biological safety in flow cytometry is an emerging requirement for core laboratories concerned about the potential accidental exposure of operators to biological samples.

Protecting personnel, products, and the environment

BSCs are designed to protect operators from risks associated with exposure to biological agents in samples. These cabinets are among the most effective and commonly used primary containment devices in laboratories working with infectious agents. BSCs protect personnel and the environment from harmful agents and protect product (cells) from contamination.

Verified to meet biosafety standards

The Baker Company has verified that the BSC designed specifically for the BD Influx meets personnel and product protection standards for a Class II Type A2 biosafety cabinet, the National Sanitation Foundation International Standard 49. Importantly, all microbiological testing was performed with the BD Influx placed inside the work area of the BSC to validate performance in an as-used condition.

Airflow control

The BSC controls the direction, volume, and speed of airflow to direct potentially harmful particles away from the operator. Air is filtered and circulated around the work surface, and a separate airflow at the front of the cabinet creates a protective barrier for the operator. Aerosols from the BD Influx sort chamber are directly evacuated into the high efficiency particulate air (HEPA) filters of the BSC, eliminating the need for an aerosol management option. The HEPA filters remove microorganisms and airborne particulates (aerosols) from the air. Filters are placed where the air enters and exits the work area. Filters are placed where a percentage of the air is exhausted from the cabinet and where the cabinet air is recirculated. HEPA filters in the cabinet support the removal of a minimum of 99.97% of particles or equivalent with a diameter of 0.3 μm .



PROTECTION



Sorting software for a better level of control

BD FACS Software: from acquisition to sorting and analysis

BD FACS Software sorter software provides comprehensive control of the cell sorter from configuration and compensation setup to acquisition, sorting, and analysis, making it easy to customize the instrument for specific applications.

BD FACS Software fundamentally improves the way cytometer software supports cell sorting. The software captures all the information about an event such as time, position in sorted drop, position relative to other events, firmware classifier status, etc, and makes it available to researchers on demand as needed for quality control, or for post-sort analysis.

The software uses industry-standard protocols and manages hundreds of system parameters, giving researchers a higher level of control and engagement with the instrument to support advanced research applications.

To maximize reproducibility and accelerate study, researchers can save and recall previous workspaces, configurations, instrument states, and parameter settings. A multitasking capability allows researchers to sort cells from a sample, record the information in a data file, and work with a previous data file—all at the same time.

Software wizards and controls assist researchers to classify cell populations, perform compensation, monitor sorting, and analyze results.

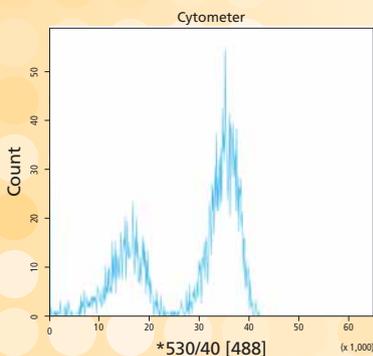
Hierarchical gating tools make it easy and intuitive to classify cell populations.

The automatic compensation wizard creates a spillover matrix based on user-selected population controls and automatically calculates a compensation matrix to align populations.

Sort controls and event counters monitor sorting, with the ability to pause, resume, reset, and stop the sort streams individually or all at once.

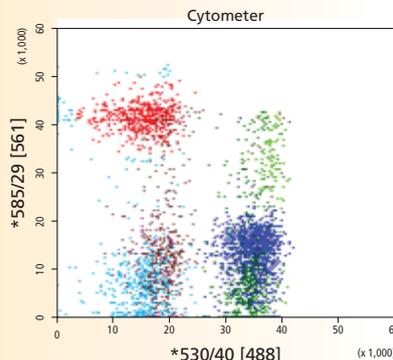
Data and sort analysis tools provide robust statistics on cell populations and sorting quality control at the individual cell level.

To support analysis, BD FACS Software lets researchers visualize data from experiments in a variety of rich output formats. Output formats include histograms, overlay histograms, and dot, density, and contour plots—all with linear, log, or biexponential (Logicle) scaling. Support for standard software protocols allows researchers to export both graphical data and text into standard productivity software (such as Microsoft® Office®) for presentation or publication. Results can also be exported and imported as FCS data files for other software applications.



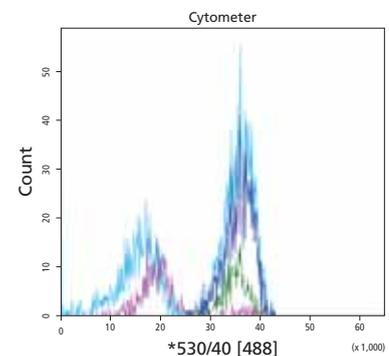
Histogram

Histograms show cell counts for a single scatter or fluorochrome variable.



Dot plot

Dot plots show two variables plotted against each other, useful for identifying subpopulations.



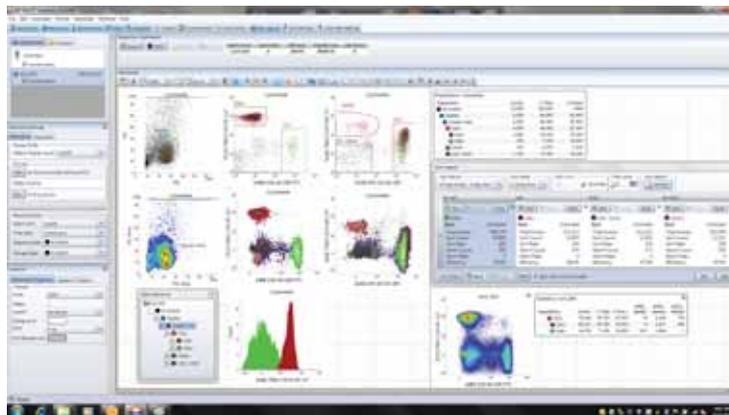
Overlay histogram

Overlay histograms show cell counts for a variable, such as fluorescence, for multiple subpopulations.

Cytometer



Acquisition, analysis, and sorting



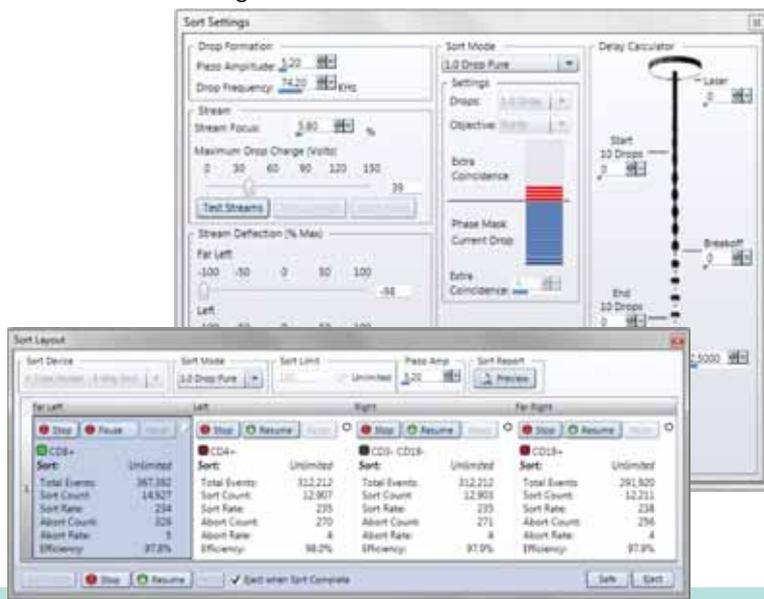
Intuitive workflow

BD FACS Software has a simple, intuitive workflow, and its familiar Microsoft® Windows® interface allows researchers to focus on their experiment rather than on commands and dialog boxes. Researchers can perform cytometer setup, compensation, data acquisition, gating, analysis, and sorting progressively—or they can choose to return to any step for instant adjustment.

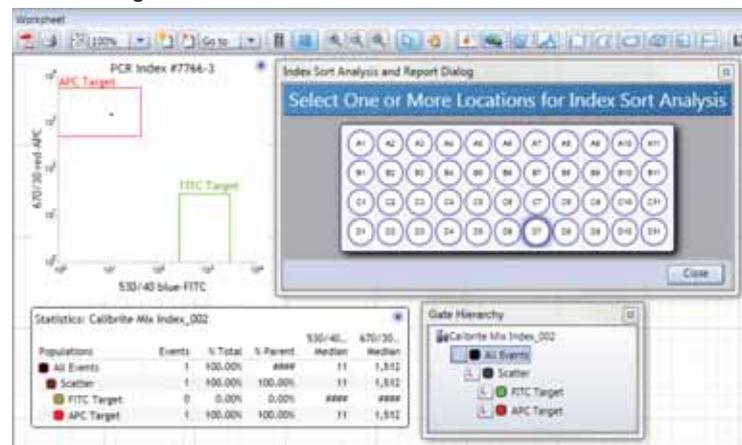
Compensation



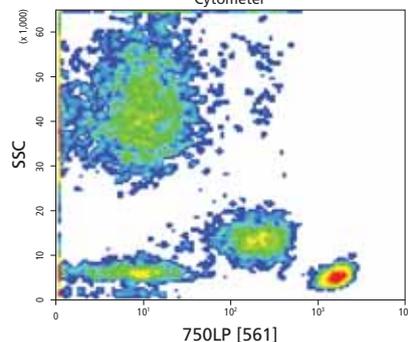
Sorting



Index sorting



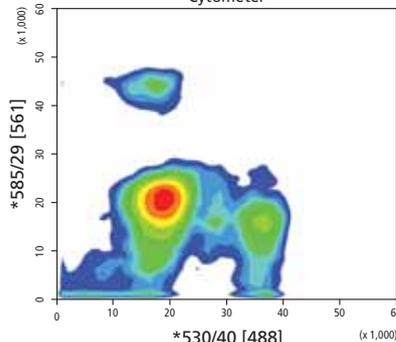
Cytometer



Density plot

Density plots show two variables plotted against each other using color to illustrate heavily concentrated events.

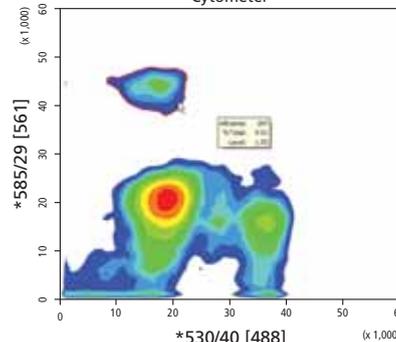
Cytometer



Contour plot

Like density plots, contour plots show two variables plotted against each other, but instead use topographic lines to show the boundaries of populations.

Cytometer



Analysis of contour plot

Employ topographic lines to define subpopulations or move the cursor over a topographic line for instantaneous subpopulation statistics.

Choices now and in the future

Special order products for unique needs

The BD special order program allows customers to configure BD flow cytometers and cell sorters to fit precise research and assay needs. Tailored to the special needs of research at the leading edge of biomedical discovery, this program offers a wide range of choices to help researchers create the ultimate customized instrument for their requirements.

Evolving capabilities

The modular design of the BD Influx system is highly flexible, and as a result, it offers a range of standard choices and options. These choices allow customers to purchase an instrument that meets their needs today and expand the system as future requirements evolve.

The open design of the BD Influx system also makes it possible to accommodate application challenges as they arise.

Multiple options

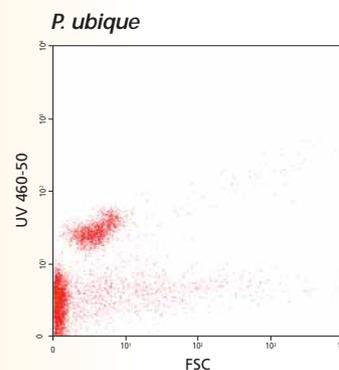
The BD Influx system can be ordered with a range of innovative options to meet research requirements. Laser options include the choice of diode and solid state lasers across a spectrum of wavelengths and power options. Currently, researchers can configure a BD Influx system with up to 10 lasers and choose specific filters from a wide selection of exchangeable detector modules to meet precise requirements. See the technical specifications and filter guide for the current offerings.

An innovation process

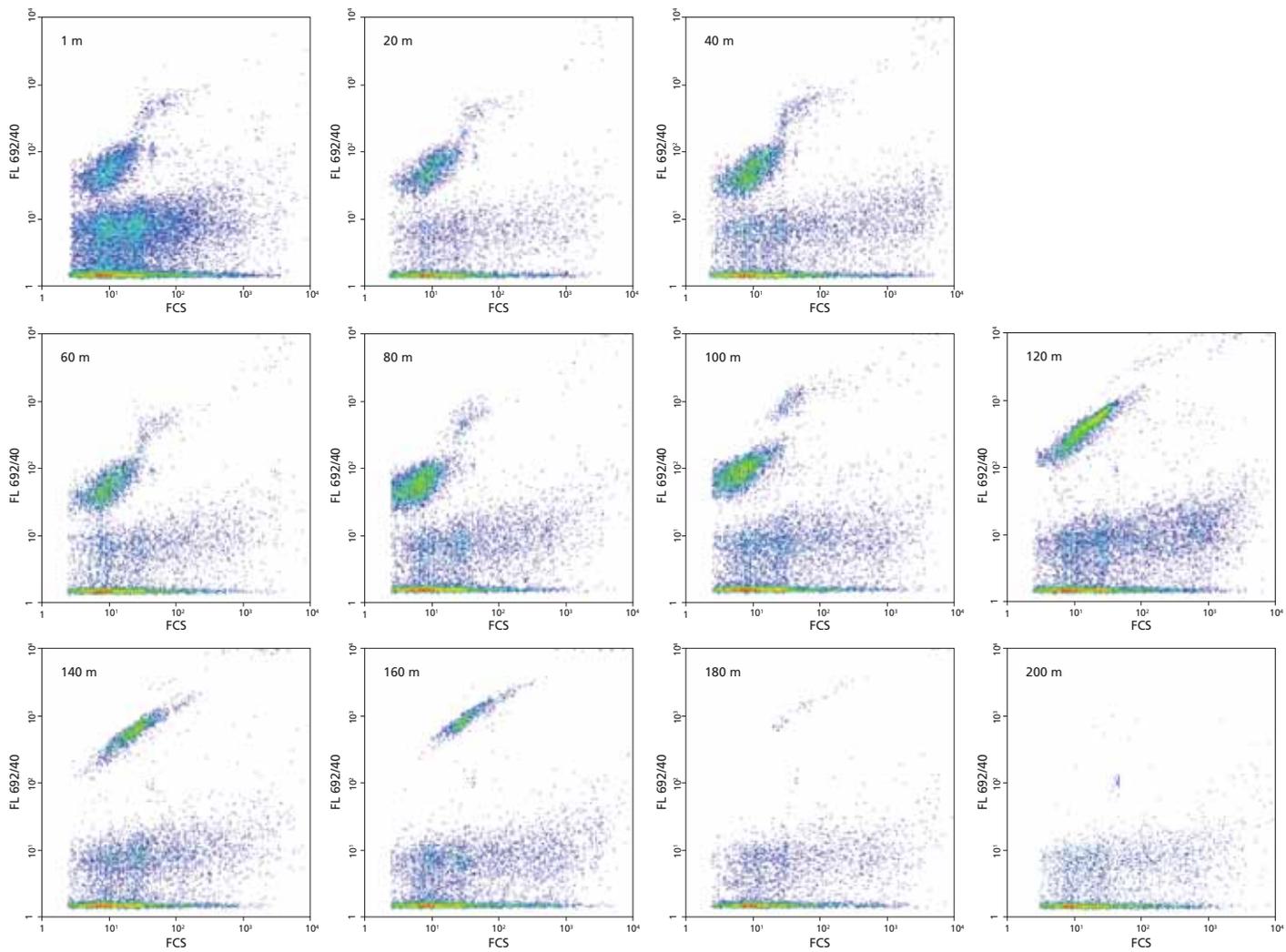
Vigorous sourcing of the latest and best laser technologies ensures that an unparalleled range of configuration choices is offered. New technologies are regularly incorporated into the product line as soon as they become available.

The ever-expanding list of available lasers demonstrates BD's ongoing commitment to perpetual innovation. This is one of the many ways BD ensures that the BD Influx cell sorter continues to support the evolving needs of leading researchers around the world.

Discrimination of small particles can be further improved by measuring fluorescence
Representative Hoechst staining of *Pelagibacter ubique*.



CONFIGURATION

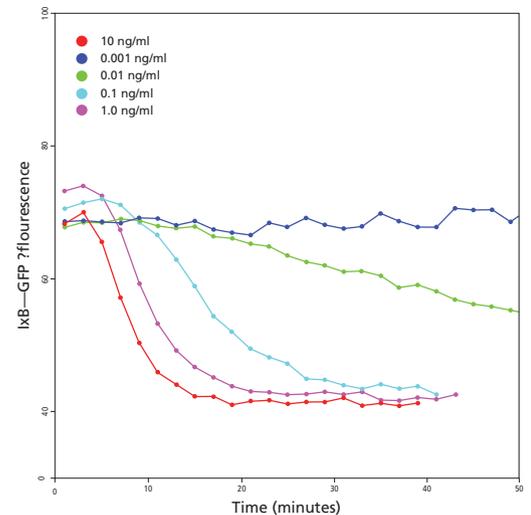
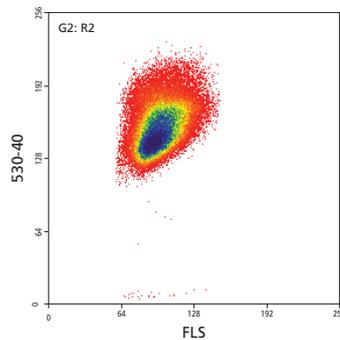


Analysis of aquatic samples

Aquatic samples contain a large variety of very small particles. Data shows forward scatter (200 mW, 488 nm) vs fluorescence (692/40) in samples taken at different depths from the Sargasso Sea. Distinction of *Prochlorococcus* and *Synechococcus* cyanobacterium clusters by flow cytometry illustrates the exceptional sensitivity of the standard BD Influx forward scatter detector.

Measurement of macrophage cell line stimulation using GFP

Murine RAW macrophage cells were transfected with a Green Fluorescent Protein (GFP) and stimulated with varying concentrations of the TLR ligand PAM2. Degradation of GFP fluorescence was measured over a 50-minute time period using a BD Influx system. The data was binned and averaged to produce the mean fluorescence for each time point. The BD Influx system is able to make many measurements over short time scales to increase the accuracy of time course studies.



Services and support

BD Biosciences instruments and reagents are backed by a world-class service and support organization with unmatched flow cytometry experience.

For over 25 years, BD has actively worked with researchers to develop tools that help improve workflow, ease of use, and performance. With in-depth knowledge and experience, BD delivers comprehensive training, application expertise, excellent technical support, and world-class field service.

Training

Held at BD training centers worldwide, BD Biosciences flow cytometry training courses combine theory and hands-on practice to provide participants with the skills and experience they need to take full advantage of the capabilities of their instruments.

Technical application support

BD technical application support specialists are available to provide field- or phone-based assistance and advice. Expert in a diverse array of topics, BD technical application specialists are well equipped to address customer needs in both instrument and application support.

Field service engineers

BD field service engineers are located across the world. When instrument installation or service is required, a BD field service engineer can be dispatched to the customer site. On-site service and maintenance agreements are available to provide long-term support.

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Office locations are available on our websites.

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