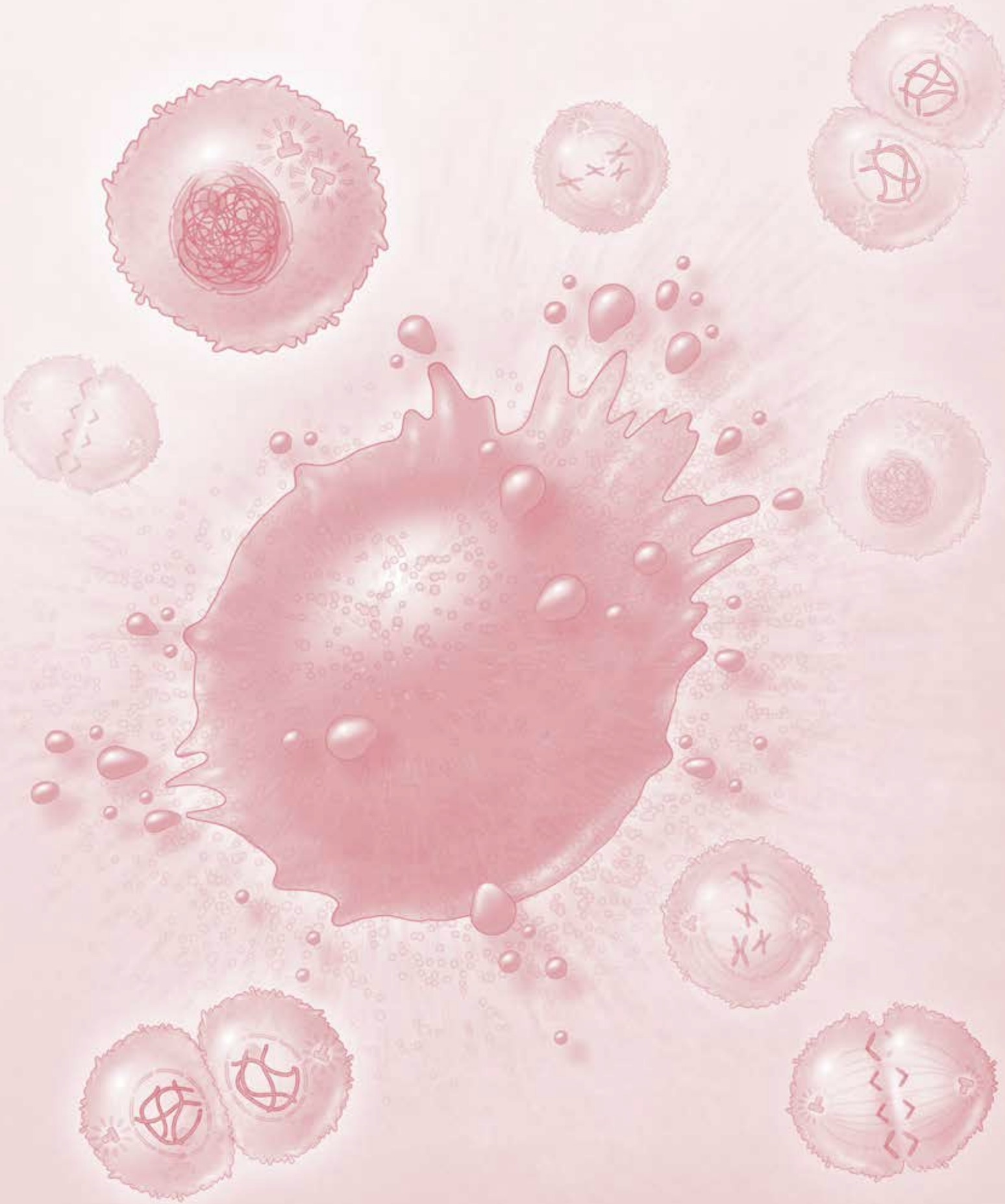
BD Accuri™ C6 Plus

FLOW CYTOMETER

Flow cytometry within reach.™



Flow cytometry within reach

Easy to use, simple to maintain, and affordable, the BD Accuri™ C6 Plus personal flow cytometer brings cell analysis within reach for new applications and new flow cytometry users.

The analytical power and versatility of today's laser-based flow cytometry systems have unlocked the mysteries of cell biology and empowered entirely new fields of research. As a result, flow cytometry has become a staple of modern laboratories around the world. Innovations in ease of use reflected in the BD Accuri C6 Plus cytometer make these powerful capabilities more accessible to a new generation of flow cytometry users.

The compact footprint, portable weight, and rugged design of the BD Accuri C6 Plus also make it a valuable personal use tool for experienced researchers who want a cytometer to be easily available when and where they need it—in the lab or in the field. The instrument is small enough to fit easily on a laboratory benchtop and can be placed in a laminar flow hood if biohazard containment is required.

Many BD Accuri C6 Plus cytometer users can begin collecting and analyzing data with the help of a quick start guide. Numerous processes are automated, such as daily QC, and the intuitive software interface guides the user through workflows. A wide dynamic range of over 7 decades ensures that all data is available all of the time. Information obtained from the BD Accuri C6 Plus can be reanalyzed at any time if gating or compensation changes are required, or to accommodate new research.

BD Accuri flow cytometers are the world's most widely cited personal flow cytometers. They are used daily in a wide range of applications, from immunology and cell biology to cancer research, aquatic research, bioprocessing, biofuels development, and synthetic biology. Their operational simplicity makes them accessible not only to technical experts and trained researchers, but also to new users such as graduate and undergraduate students.

Count on BD for quality plus reliability

Simplicity and Sensitivity on your Benchtop

Highlights

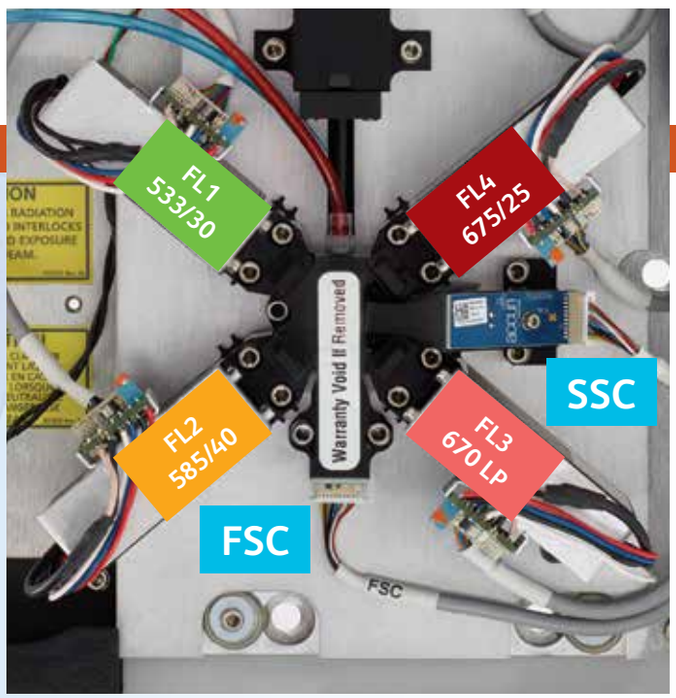
- 7.2-decade dynamic range (16 million channels of digital data)
- Highly sensitive: MESF <75 (FITC) and <50 (PE)[1]
- Non-pressurized peristaltic pump system
- Event rates up to 10,000 events per second
- Supports sample collection from multiple sources, including 12 x 75-mm (or smaller) sample tubes
- Uses laboratory-grade water as sheath fluid
- Dimensions (H x W x D): 11 x 14.75 x 16.5 in (27.9 x 37.5 x 41.9 cm)
- Weight: 30 lb (13.6 kg)

[1] MESF (Molecules of Equivalent Soluble Fluorochrome) values determined using Thermo Scientific Cyto-Cal™ Multifluor Plus Violet Beads.

The BD Accuri C6 Plus is equipped with a blue and red laser, two light scatter detectors, and four fluorescence detectors with optical filters optimized for the detection of many popular fluorochromes, including FITC, PE, PerCP-Cy™5.5, and APC, as well as newer polymer dyes such as BD Horizon Brilliant™ Blue 515. The BD Accuri C6 Plus can also analyze many variants of fluorescent proteins, such as GFP, YFP, and mCherry. Its adaptability and suitability for experiments up to four colors have allowed researchers to publish numerous scientific articles citing BD Accuri flow cytometers.

During manufacture, laser and optical alignments are set and locked down. With no need to adjust detector voltage, the system is easier to use. Data is digitally collected over a wide dynamic range and is fully available to users as needed, eliminating the risk of data loss due to incorrect settings. Software functions such as Zoom and VirtualGain™ allow visualization of data at any scale, so that users can precisely set gates and regions.

Despite its compact size, the optical system demonstrates extraordinary fluorescence sensitivity. Intensive testing ensures that the optical and fluidic design can withstand rugged conditions. Provided the system is anchored, it can run samples even if the benchtop is in motion, such as aboard a ship.



BD Accuri C6 Plus optical bench
A compact optical design, fixed alignment, and pre-optimized detector settings make the system easier to use.

OPTICS AND FLUIDICS

A unique, non-pressurized, peristaltic pump system drives the fluidics. The system accurately monitors the sample volume pulled per run, and can calculate absolute counts or sample concentration per µL without the use of counting beads. These absolute counts are more precise and far less tedious than manual counts.

The system supports a wide array of sample tubes. Fluidics are cleaned automatically on instrument shutdown, and laboratory-grade water is used as sheath fluid, reducing operating costs.

For walkaway convenience, the optional BD CSampler™ Plus accessory offers reliable and easy-to-use automation. The BD CSampler Plus adds minimal footprint to the BD Accuri C6 Plus.

Excitation Wavelength	Fluorochromes/Dyes	Standard Filter	Optional Filter
488 nm	FITC, BB515, Alexa Fluor® 488, CFSE	533/30	–
	GFP	533/30	510/15
	JC-1, Fluo-4 AM, SYBR® Green	533/30	–
	YFP	533/30	540/20
	PE, JC-1, BD™ MitoStatus TMRE	585/40	–
	OFP	585/40	565/20
	Propidium Iodide	585/40, 670 LP	610/20
	BD Horizon™ PE-CF594	670 LP	610/20
	RFP, mCherry, dsRed	670 LP	610/20
	PerCP, PE-Cy™5	670 LP	–
	PerCP-Cy™5.5	670 LP	–
	7-AAD	670 LP	–
	PE-Cy™7	670 LP	780/60
640 nm	APC, BD™ MitoStatus Red, Alexa Fluor® 647	675/25	–
	APC-H7, APC-Cy7	670 LP	780/60

BD Accuri C6 Plus fluorochrome support
All fluorochromes in the table can be detected by the BD Accuri C6 Plus equipped with standard filters. Optional filters can be used to increase resolution or to separate fluorochromes with overlapping signals, like GFP and YFP.

BD CSampler Plus automatic sampling accessory
The optional accessory offers reliable, easy-to-use automation while adding minimal footprint.
The BD CSampler Plus supports:

- 48-well plates
- 96-well plates
- 96-deep-well plates
- 24-tube rack (included) for standard 12 x 75-mm tubes

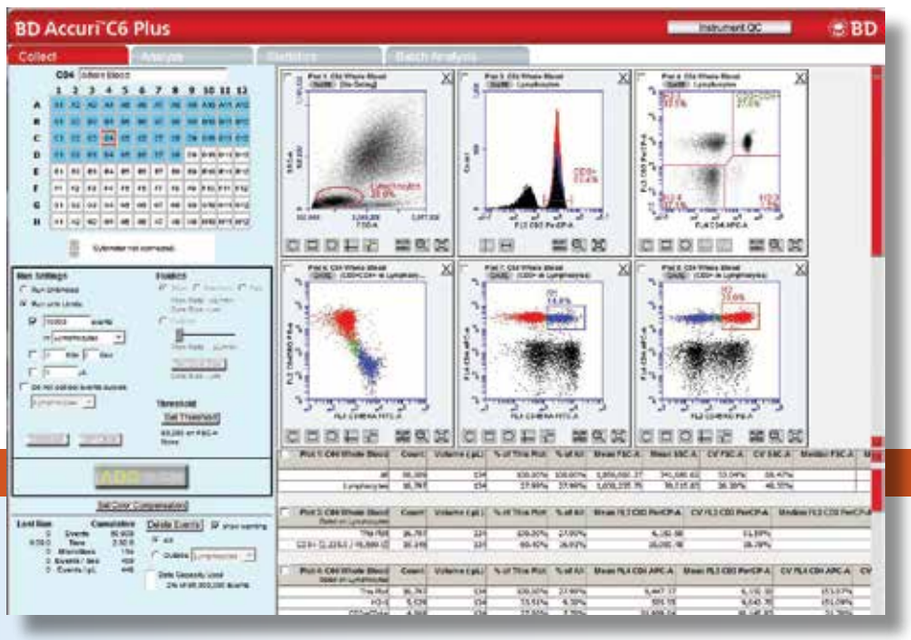
Intuitive software—master in minutes

Easy-to-Use Software with Automated QC and Application Templates

BD Accuri C6 Plus software features an intuitive user interface. Even new flow cytometry users find it so easy to learn and use that most can collect and analyze data in less than an hour. Software options and instrument controls are clearly visible from the software's tabbed interface.

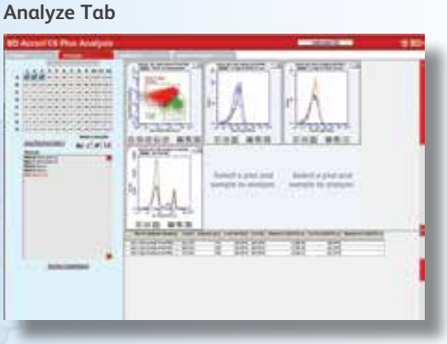
Data is acquired from the Collect tab. All tasks and settings are laid out simply on one screen for quick access and operation. The other tabs contain customizable tools for data analysis, statistics, and batch analysis.

Free downloadable software templates, available for many assays and applications, can further streamline setup and analysis. BD Accuri C6 Plus software files can be exported in FCS 3.1 format for seamless data import into flow cytometry analysis programs such as FCS Express™ software and FlowJo™ software.



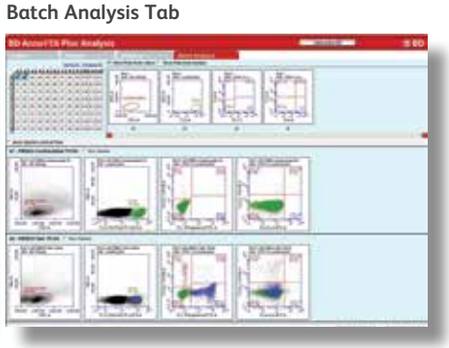
The Collect tab contains all acquisition tasks and settings for quick access and operation

Analyze Tab

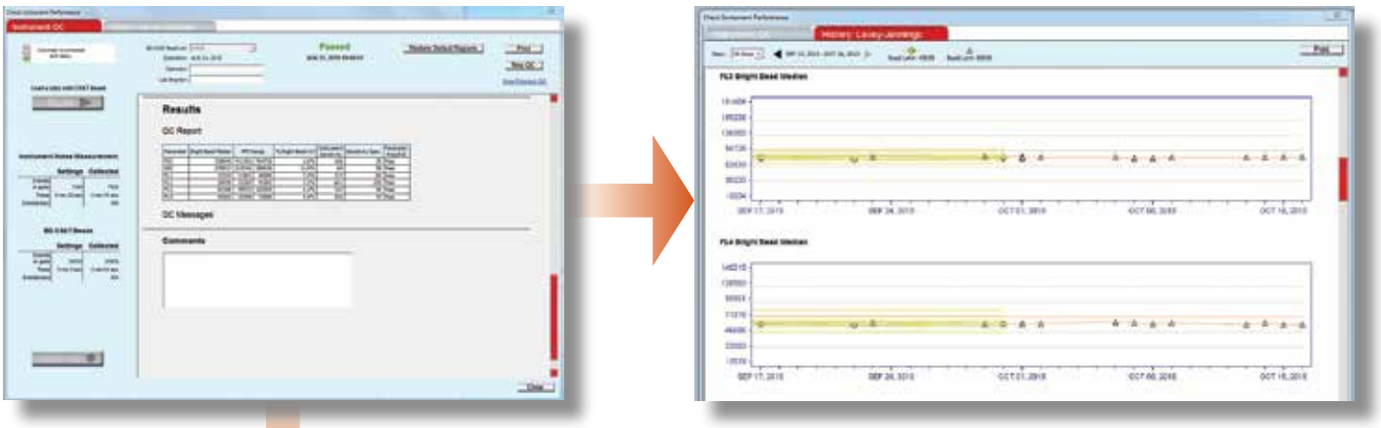


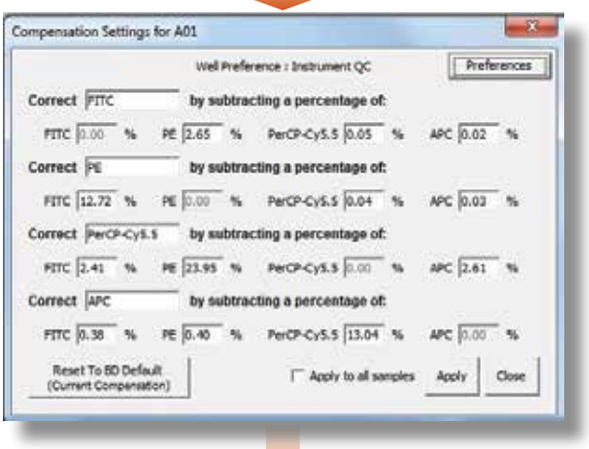
Statistics Tab

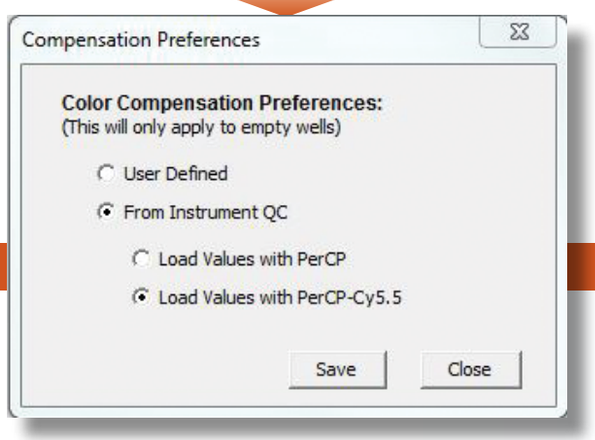
Batch Analysis Tab



SOFTWARE







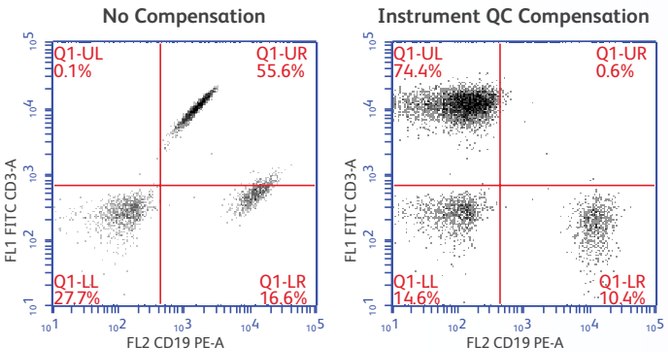
Automated instrument QC

The new *Instrument QC* feature automates the daily validation of the BD Accuri C6 Plus using BD™ Cytometer Setup and Tracking (CS&T) beads to ensure that the instrument meets performance specifications. Instrument QC results are instantly displayed in the software and saved in PDF format. The software automatically generates Levey-Jennings plots that allow you to monitor instrument performance over time.

Instrument QC Compensation

After each time Instrument QC is performed, the software also updates compensation settings for FITC, PE, APC, and PerCP or PerCP-Cy™5.5. *Instrument QC Compensation Settings* can be selected from the Compensation Preferences window as an easy and convenient starting point for proper compensation controls.

Alternatively, you can select *User Defined Compensation Settings* to calculate compensation manually, which is also necessary for other fluorochromes or dyes.



Compensation settings remove artifacts of fluorescence spillover
Whole blood was analyzed for the expression of CD3 and CD19, two markers expressed differentially on lymphocytes and B cells, respectively. Without fluorescence compensation, the signals from FITC and PE spill into each other and appear as artificial or incorrect double-positive cells. After applying Instrument QC compensation, the spillover effect is removed, and single positive $CD3^+$ lymphocytes and $CD19^+$ B cells can be readily distinguished.

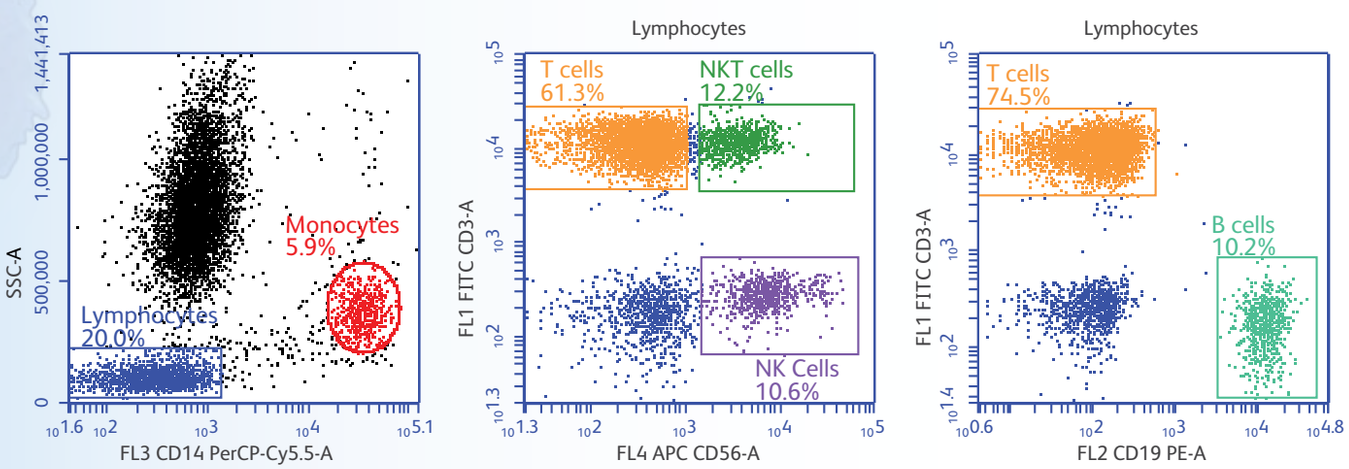
Immunophenotyping and Cytokine Analysis

Immunophenotyping was one of the first applications of flow cytometry, and for over 20 years, BD Life Sciences has actively supported groundbreaking immunological research with flow cytometry systems and reagents. The BD Accuri C6 Plus is an ideal benchtop platform for core immunophenotyping studies and is configured for rapid and accurate analysis of up to six parameters at the single-cell level. A broad dynamic range of detection makes it easy to analyze cells as varied in size as platelets and eosinophils in the same data file.

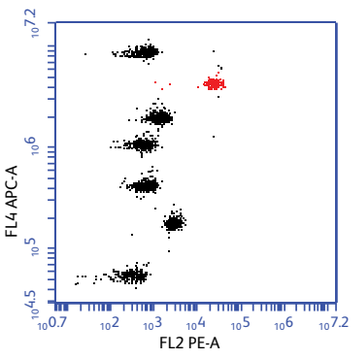
Multiparametric analysis is crucial for simultaneously detecting distinct cell types within a blood sample, based on cell size and expression of surface and intracellular markers. For example, you can perform intracellular cytokine analysis on the BD Accuri C6 Plus to define the specific source of an analyte of interest within a heterogeneous sample. You can also use BD™ Cytometric Bead Array (CBA) option for quantitative and simultaneous measurement of up to 30 secreted cytokines from a small volume of supernatant.

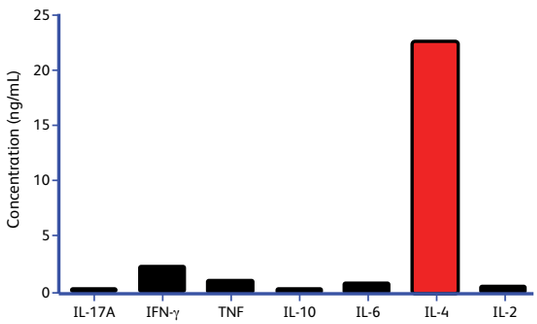
Four-color whole blood immunophenotyping panel on the BD Accuri C6 Plus

Whole blood was stained with fluorescent antibodies to CD3, CD56, CD14, and CD19 and acquired and analyzed on a BD Accuri C6 Plus. Side scatter and CD14 expression were used to discriminate lymphocyte and monocyte populations. Within the lymphocyte gate, percentages of T cells, NKT cells, NK cells, and B cells were quantified based on expression of CD3, CD56, and CD19. In all, five blood cell populations were identified in a single tube using four colors.



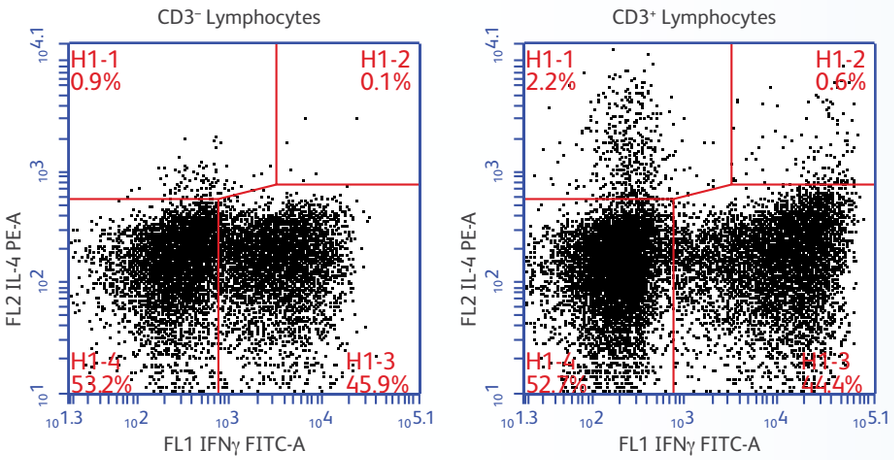
The optional BD CSampler™ Plus accessory offers reliable and easy-to-use automation to facilitate screenings. This option adds minimal footprint to the BD Accuri C6 Plus, about three feet square for the pair.





Analysis of cytokine expression with BD CBA kits

BD CBA assays can quantify multiple cytokines simultaneously using minimal sample. In this experiment, seven cytokines were quantified in culture supernatant of stimulated PBMCs using the BD™ CBA Human Th1/Th2/Th17 Cytokine Kit. Capture beads for each cytokine were identified in FL4 and cytokine levels were measured based on bead signal intensities in FL2. Cytokine concentrations were calculated using standard curves.



Analysis of cytokine expression using intracellular flow cytometry

PBMCs were stimulated with PMA + Ionomycin in the presence of BD GolgiStop™ protein transport inhibitor. The cells were fixed, permeabilized, and stained using the BD FastImmune™ Anti-Human IFN-γ FITC/IL-4 PE. Cells were acquired and analyzed on a BD Accuri C6 Plus using the kit template. IFN-γ was expressed by almost half of both CD3⁺ and CD3⁻ cells, while IL-4 was predominantly expressed by CD3⁺ T cells.

Faster, simpler protocols with continuous sampling capability

Rapid Analysis of Multiple Cellular Processes

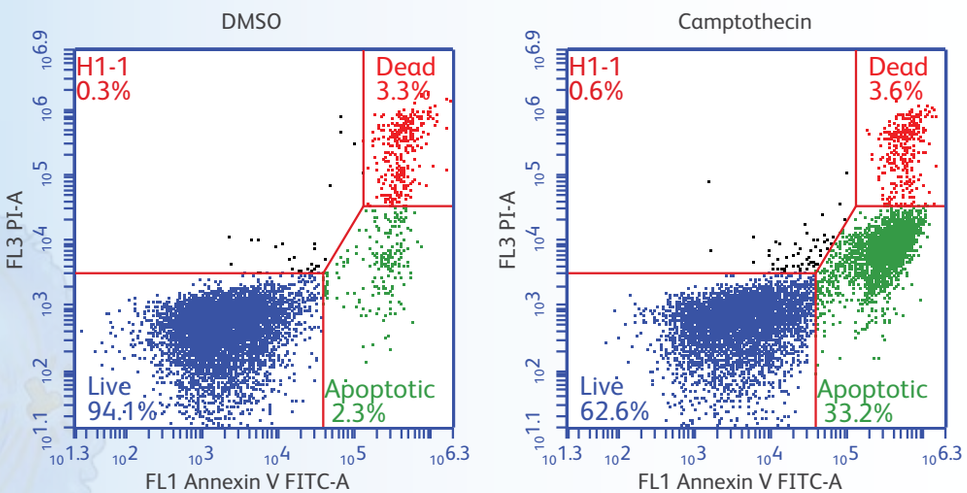
A personal flow cytometer in the lab provides many advantages for cell and cancer biology studies. When cells are ready for analysis or rare tumor samples arrive, it's crucial to have a flow cytometer at hand, ready to go.

With the BD Accuri C6 Plus, you can analyze a variety of cellular processes:

- Apoptosis
- Cell signaling
- DNA content
- DNA damage
- Cell cycle
- Proliferation
- Viability

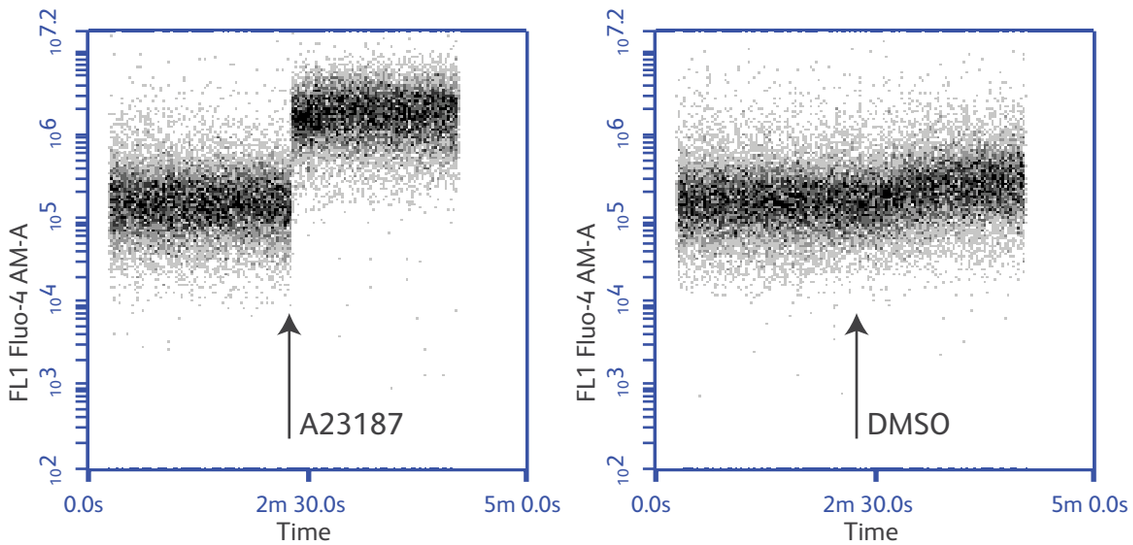
The four fluorescence detectors are compatible with a broad range of BD Life Sciences reagents and other functional dyes, providing the flexibility to design multiplexed assays for a more comprehensive analysis of cell biology.

Cell survival, growth, and differentiation are tightly regulated through phosphorylation of key proteins in signaling cascades. BD Phosflow™ reagents utilize phospho-specific antibodies that target specific amino acid phosphorylation sites in proteins. This can provide a simple and fast solution for phosphoprotein analysis that complements Western blot.



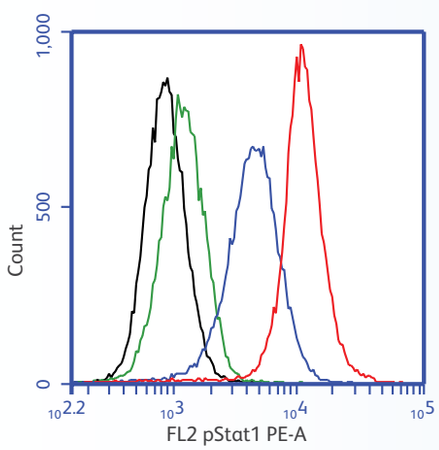
Apoptosis detection on the BD Accuri C6 Plus
Annexin V and propidium iodide (PI) were used to determine the percentage of Jurkat cells entering apoptosis (green) after treatment with camptothecin (right) compared to cells treated with DMSO (left, control). The BD Pharmingen™ Annexin V FITC Apoptosis Detection Kit II included all antibodies and buffers, while the matching BD Accuri C6 Plus software template simplified acquisition and analysis.

Because the BD Accuri C6 Plus employs non-pressurized peristaltic pumps in an open fluidics system, you can use open tubes to add test compounds to the cell suspension without interrupting sampling. This "continuous-flow" method enables nonstop monitoring of thousands of cells to facilitate accurate, gap-free kinetic analysis of cellular responses that occur within seconds. Kinetic applications include calcium flux, nanoparticle uptake, viability, and platelet activation.



Continuous sampling on the BD Accuri C6 Plus
Easily add external agents without interrupting data acquisition. Jurkat cells were pre-loaded with the calcium indicator BD Pharmingen™ Fluo-4 AM. Calcium levels increased immediately after treatment with the calcium ionophore A23187 (left plot), but not after treatment with DMSO control (right).

Cell signaling analysis using BD Phosflow reagents
The BD Accuri C6 Plus can examine post-translational modifications of cell signaling proteins. U-937 cells treated with IFN-γ showed increased Stat1 (pY701) phosphorylation in a dose-dependent manner. The level of phosphorylation can easily be quantified as a function of median fluorescence intensity (MFI).



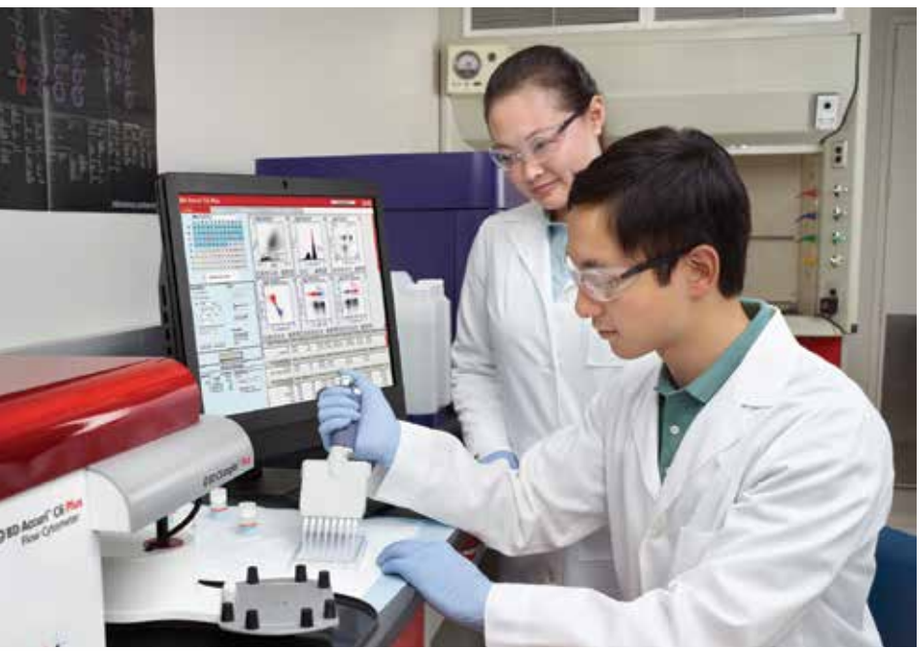
Sample	PE MFI	Fold Change
Untreated	911	1
IFNγ 1 pg/mL	1,277	1.4
IFNγ 10 pg/mL	4,935	5.4
IFNγ 100 pg/mL	12,294	13.5

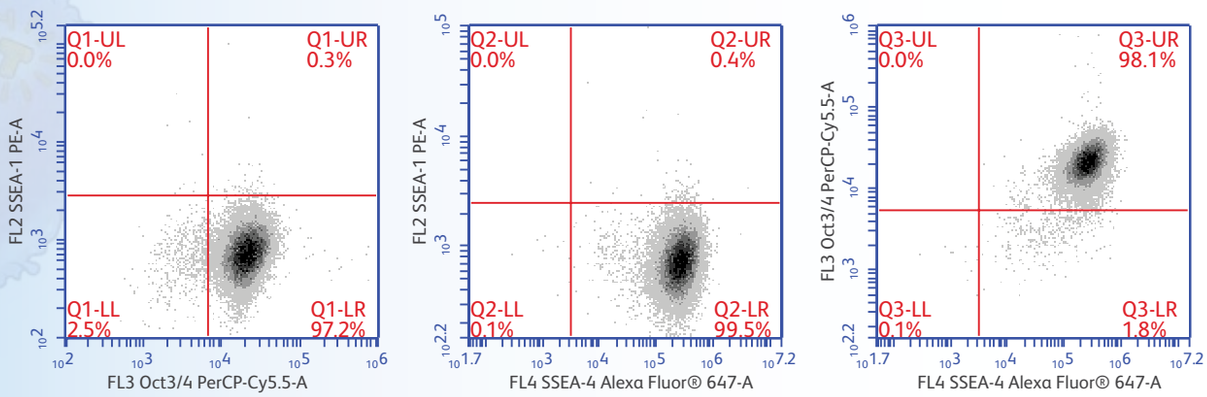
Kits and templates simplify setup and analysis

Characterizing Stem Cells and Their Derivatives

A primary challenge of stem cell research is to detect target cells within inherently heterogeneous cell cultures. The BD Accuri C6 Plus is ideal for this kind of research because of its ability to analyze the expression of multiple surface and/or intracellular markers at the single-cell level, quickly and simply.

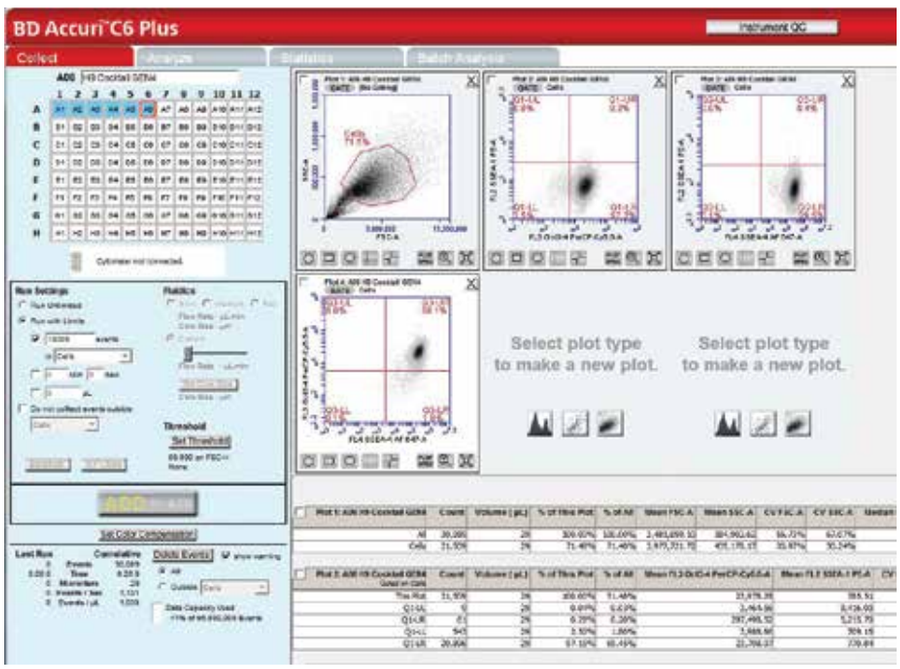
Using the BD Accuri C6 Plus, researchers can analyze stem cell cultures rapidly and accurately. BD Stemflow™ kits facilitate multiple aspects of stem cell research, from the assessment of pluripotent and adult stem cell phenotypes to iPSC reprogramming efficiency. For example, you can use the BD Stemflow™ Human MSC Analysis Kit to run a complete ISCT-recommended phenotypic analysis in a single panel. You can also choose from BD Life Sciences comprehensive portfolio of antibodies to characterize stem cells and their derivatives, including neuronal, pancreatic, hepatic, and cardiac cell types.



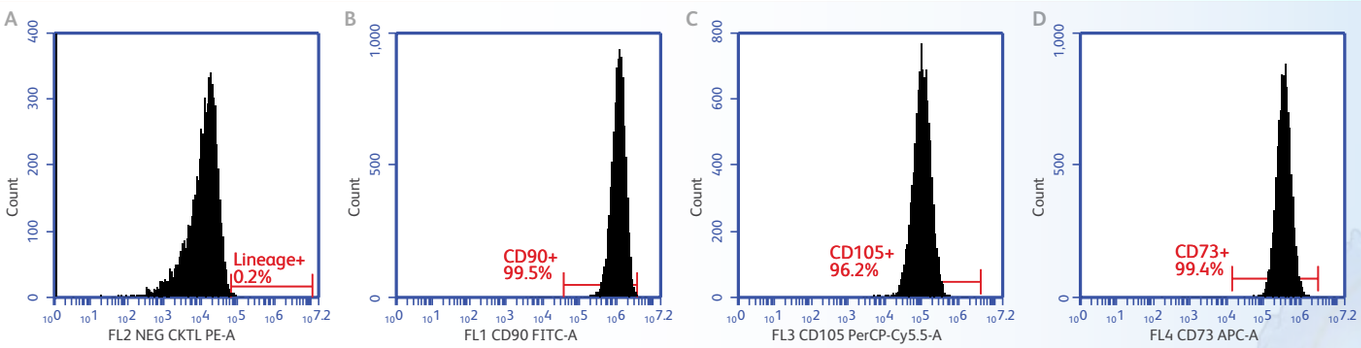


Assessing pluripotent stem cell phenotypes using surface and intracellular markers
The BD Accuri C6 Plus was used to measure the expression of stem cell pluripotency (SSEA-4) and differentiation (SSEA-1) markers on the cell surface and pluripotency markers within the cells (Oct3/4). H9 human embryonic stem cells (WiCell) were stained with the BD Stemflow™ Human and Mouse Pluripotent Stem Cell Analysis Kit and acquired using the kit template.

To further streamline setup and analysis, several BD Stemflow kits are matched to BD Accuri C6 Plus software templates. A template is a predefined workspace that includes markers, regions, gates, labels, parameter names, run criteria, and compensation settings.



Software templates simplify setup and analysis
Available for many BD Life Sciences reagent kits, BD Accuri C6 Plus software templates include predefined workspaces, markers, regions, gates, parameter names, and compensation settings for quick and easy setup and analysis. In this experiment with human embryonic stem cells, both run settings (center left) and gates (top right) were preset from the template.



Verifying the ISCT-defined MSC phenotype
Human bone marrow-derived mesenchymal stromal cells were stained using the BD Stemflow™ Human MSC Analysis Kit, acquired on a BD Accuri C6 Plus using the kit template, and analyzed for expression of MSC surface markers according to the ISCT criteria. The vast majority of analyzed cells expressed the MSC surface markers in the positive marker cocktail (CD90, CD105, and CD73, **B–D**), while very few expressed those in the negative marker cocktail (CD34, CD11b, CD19, CD45, and HLA-DR, **A**). Gates were drawn based on matched isotype control cocktails (not shown).

Microbial Analysis Across a Range of Applications

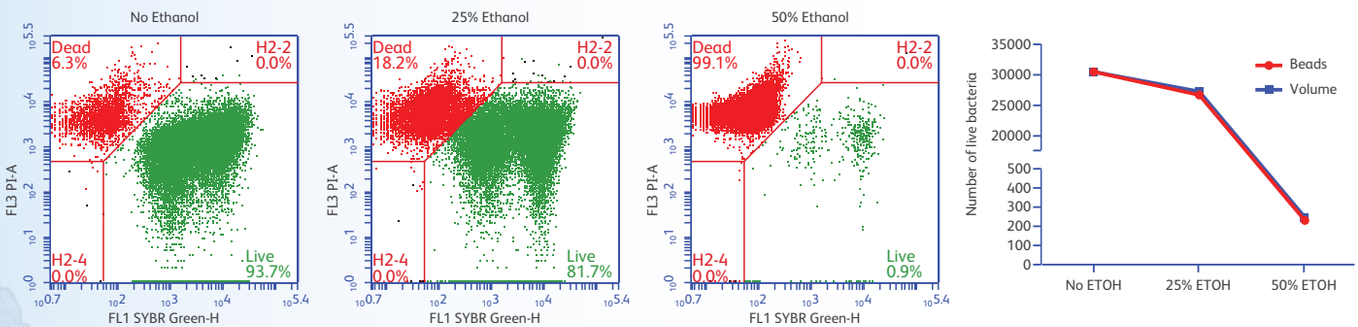
In the diverse field of microbiology research, flow cytometry is a versatile and powerful technique for analyzing microorganisms including bacteria and yeast. The BD Accuri C6 Plus can provide rich data in a range of microbiological applications, such as measurement of gene expression, monitoring bacterial and yeast fermentations, recombinant protein production in bacteria, environmental research, food processing, and monitoring drinking water.

Perhaps the most common task in microbial analysis is to identify and count microbes. The unique fluidics system of the BD Accuri C6 Plus, driven by peristaltic pumps, allows it to determine sample volume and count cells rapidly, directly, and automatically from the software, eliminating laborious plate counts. Direct counts on the BD Accuri C6 Plus correlate highly with counting beads and are more precise than manual counts.

Compact size, rugged design, and portability make the BD Accuri C6 Plus ideal for environmental research in the field. Fixed optics and capillary sheath flow fluidics enable continuous operation, even during motion and vibration. BD Accuri flow cytometers have traveled to field sites across the globe, from the peaks of the Himalaya to the forests of China, from the Great Lakes to the Gulf of Finland, and from the Arctic to the Antarctic.

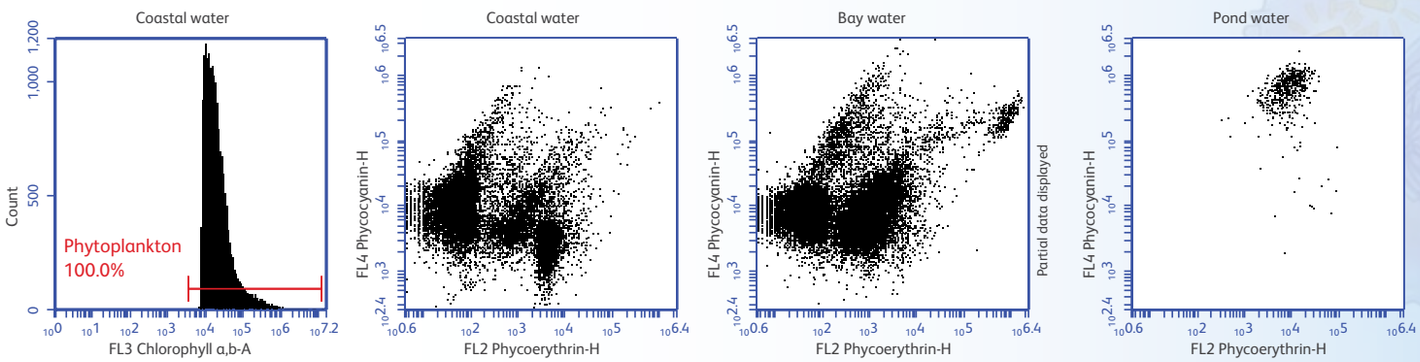
Fluorophore	Exciting Laser	Major Emission Wavelength	Detector (filter)
Chlorophyll *a,b*	488	>640 nm	FL3 (670 LP)
Phycoerythrin	488	575 nm	FL2 (585 ±20)
C-phycocyanin	640	650 nm	FL4 (675 ±12.5)
R-phycocyanin	640	646 nm	FL4 (675 ±12.5)
Allophycocyanin	640	660 nm	FL4 (675 ±12.5)

Detecting microbial autofluorescence on the BD Accuri C6 Plus
Naturally occurring fluorescent pigments in phytoplankton and the primary BD Accuri C6 Plus detectors where their fluorescent signals will be detected.



Bacterial viability on the BD Accuri C6 Plus
SYBR® Green and propidium iodide (PI) were used to discriminate live vs dead *E. coli* bacteria after treatment with varying concentrations of ethanol. Ethanol's bactericidal effect on cell viability was dose-dependent. Cell counts were similar using direct volume measurement in BD Accuri C6 Plus software compared to a normalized internal reference bead control.

MICROBIOLOGY



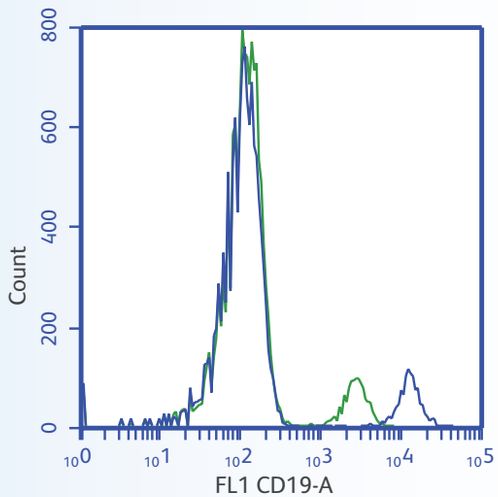
Algae analysis

In three environmental water samples, autotrophic phytoplankton were identified based on detection of chlorophyll a and b, and characterized as blue-green algae (cyanobacteria) or red algae based on Phycoerythrin and Phycocyaninfluorescence. Discrimination of phytoplankton from background noise was achieved by triggering on FL3 and by setting an appropriate threshold value. Coastal and bay water contained a variety of algae with distinct autofluorescence signatures, while chlorinated pond water contained a single dominant population.

Advanced Capabilities Expand Detection and Flexibility

Take advantage of advanced bright dyes

The BD Accuri C6 Plus is designed for compatibility with advanced polymer dyes such as BD Horizon Brilliant™ Blue 515 (BB515), which can help you detect low-density antigens and rare populations. Developed from patented technology and based on Nobel Prize-winning chemistry, BB515 is significantly brighter than FITC. Like FITC, it emits into the FL1 channel of the BD Accuri C6 Plus using the standard 533/30 filter, but has less spillover into the FL2 channel. For reagents, bright is often better, because bright fluorochromes enable researchers to identify cell populations that express low-density antigens emitting dim signals.



BD Horizon Brilliant™ Blue 515 (BB515) offers a significantly brighter alternative to FITC

Lysed whole blood was stained with anti-human CD19 antibody conjugated with FITC or BB515. Results show a brighter signal when BB515 dye (blue histogram) was used, as compared to FITC (green histogram).

Filter, laser, and configuration options provide added flexibility
The standard optical filters in the BD Accuri C6 Plus are optimized to detect common fluorochromes such as FITC/BB515, PE, PerCP, and APC. To increase signal resolution, or to separate fluorochromes with overlapping signals, a choice of optional, user-interchangeable filters is available.

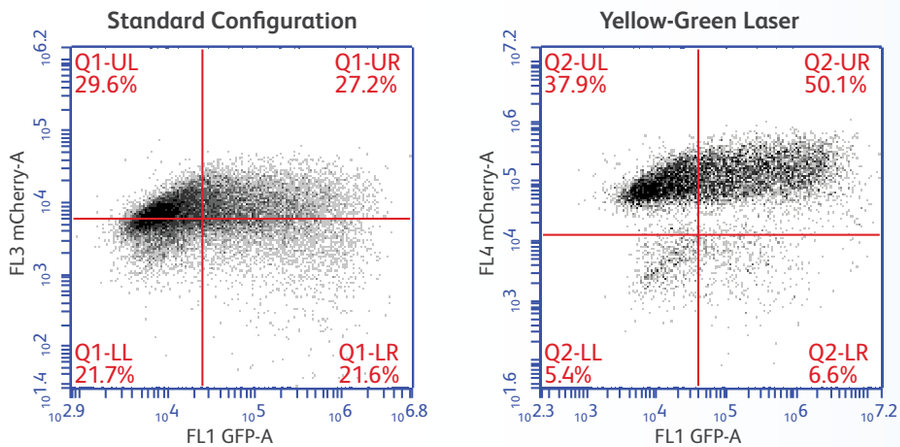
In the standard configuration of the BD Accuri C6 Plus, three detectors read fluorescence emissions from fluorochromes excited by the blue laser, while the fourth detector reads emissions from fluorochromes excited by the red laser (3-blue/1-red). By installing the optional Selectable Laser Module, you can operate the system in 2-blue/2-red and 4-blue configurations. This option significantly expands the fluorochrome combinations you can analyze.

To support the evolving needs of researchers, the BD Accuri C6 Plus can be configured with a choice of lasers. This expanded level of flexibility helps the cytometer fit a wider range of research application requirements. Your BD sales representative is your point of contact to discuss these options for your lab.

Selectable Laser Configuration	Detector Position	Laser Excitation Wavelength
3-Blue/1-Red	FL1	488 nm
	FL2	488 nm
	FL3	488 nm
	FL4	640 nm
*2-Blue/2-Red	FL1	488 nm
	FL2	488 nm
	FL3	640 nm
	FL4	640 nm
4-Blue	FL1	488 nm
	FL2	488 nm
	FL3	488 nm
	FL4	488 nm

*Required when using BD™ CBA Flex Set reagents

Selectable Laser alternate configurations
The optional Selectable Laser Module allows two or four fluorescence channels to be assigned to the blue laser, vs three in the standard configuration. The remaining channels (if any) are assigned to the red laser. Representative fluorochromes for each laser are shown in the table on page 5.



Simultaneous analysis of GFP and mCherry
CHO cells were transfected with mCherry and GFP and acquired on a standard BD Accuri C6 cytometer (left plot) or one specially equipped with a yellow-green laser (right plot).
No difference in GFP resolution was found. However, the yellow-green configuration was better able to distinguish four subpopulations of cells based on the differential expression of mCherry and GFP.

Services and Support

BD Life Sciences is fully committed to the success and satisfaction of its customers and offers a range of options for BD Accuri support.

Fast, easy installation
The BD Accuri C6 Plus cytometer can be customer installed within just an hour of taking it out of the box. A step-by-step e-learning module simplifies installation.

Preventative maintenance
Preventative maintenance procedures should be performed every two months to change the sheath filter, pump tubing, and fluidic filters, and to clean the sample introduction probe.

Free BD remote service – BD Assurity Linc™ software
Connects your BD Accuri C6 Plus with BD technical support personnel so that they can rapidly troubleshoot and answer questions. On customer authorization, technicians can securely access system diagnostic information, share your remote desktop, and in many cases make remote system adjustments, avoiding the need for a service call. This free remote service maximizes instrument uptime as well as customer productivity.

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

Training
Optional hands-on training is available on the BD Accuri C6 Plus cytometer. The training combines flow cytometry theory and practical skills to operate the BD Accuri C6 Plus. Additional e-learning modules can be accessed through the BD website or YouTube™ channel.

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