Life Science Applications of Personal Flow Cytometry

Introduction

Life scientists today employ a wide range of cell analysis technologies to advance their research. Among them, multicolor flow cytometry offers the advantage of multiparametric analysis at the level of individual cells and subpopulations. Over the past 40 years, life science researchers have used flow cytometry for an expanding set of applications. Currently, use of this technology has further blossomed with intensified interest in proteomics, CRISPR-Cas gene editing, targeted cancer therapies, and environmental research.

Flow cytometry allows for a comprehensive analysis of the cell. As Figure 1 shows, we can look at the cell surface to analyze expression of lineage and activation markers as well as cytokine and chemokine receptors. We can also detect intracellular molecules, such as cytokines or chemokines, signaling proteins or transcription factors, and fluorescent proteins within the cytoplasm or the nucleus. In addition, flow cytometry can provide information about cellular processes such as apoptosis, viability, and proliferation.

These insights have made flow cytometry invaluable in a broad range of life science disciplines, including immunology, cell biology, cancer research, stem cell research, molecular biology, and microbiology. The advent of BD Accuri™ flow cytometers has brought flow cytometry within reach of individual laboratories as well as core facilities, resulting in a rapid increase in scientific publications, as shown in Figure 2.

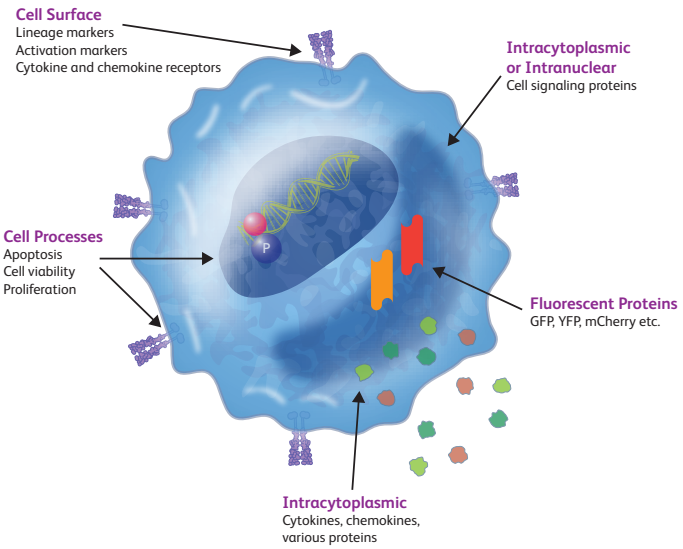


Figure 1. Analyzing a cell by flow cytometry

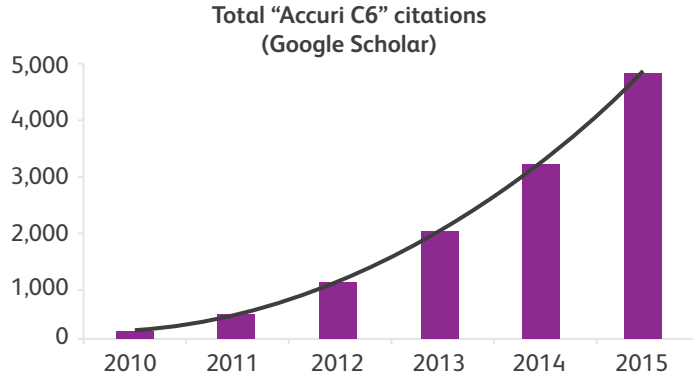


Figure 2. Google Scholar citations using BD Accuri flow cytometers, by year

BD

This white paper surveys several flow cytometry applications that can be used in various life science disciplines. A representative sampling is shown in Table 1.

Discipline	Sample Applications	Data Example
Immunology	Cytokine analysis	Figure 5
	Immunophenotyping	Figures 7–8
	T-cell activation and suppression	
Cell and Cancer Biology	Apoptosis	Figures 10–11
	Fluorescent protein detection	Figure 12
	Gene expression and regulation	Figures 13–14
	Cell signaling and calcium flux	
	Cancer stem cell immunophenotyping	
	Cell migration	
Stem Cell Research	hESC immunophenotyping	Figure 16
	MSC characterization	
Microbiology	Microbial counting and viability	Figure 18
	Environmental research	Figure 19

Table 1. Selected life science applications of flow cytometry

All data examples in this paper (unless noted otherwise) were collected and analyzed on the BD Accuri™ C6 Plus personal flow cytometer system (Figure 3).[1] With multiparametric analysis, volumetric cell counting, kits and templates, and increased sensitivity, the BD Accuri C6 Plus is a compact, affordable, easy-to-use, transportable tool that can increase discovery right in the lab or in the field.

Figure 3. The BD Accuri C6 Plus flow cytometer system
The instrument weighs just 13.6 kg (30 lb). Exterior dimensions (H x W x D) are 27.9 x 37.5 x 41.9 cm (11 x 14.75 x 16.5 in.), not including fluid tanks or the BD CSampler™ Plus automation accessory.

For more information about the BD Accuri C6 Plus, see the Appendix at the end of this white paper.

Immunology

Among the methodologies that contribute to immunology research, multicolor flow cytometry is preeminent. Immunophenotyping was one of the first applications of flow cytometry, and for over 20 years, BD Biosciences has actively supported groundbreaking immunological research with flow cytometry systems and reagents. Using panels of directly conjugated fluorescent antibodies to recognize specific cell surface and intracellular epitopes, multicolor flow cytometric analysis allows researchers to interrogate specific target protein levels expressed by individual cells in various phases of development and differentiation.

The BD Accuri C6 Plus is an ideal benchtop platform for core immunophenotyping studies and is configured for rapid 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. Cell concentrations can be calculated directly from software statistics tables without adding counting beads. BD kits and free, downloadable software templates (see sidebar, page 11) are available to streamline many common immunophenotyping panels.

For walkaway convenience, the optional BD CSampler Plus accessory (see sidebar this page) offers easy-to-use automation. The BD CSampler Plus adds minimal footprint to the BD Accuri C6 Plus—about three feet square for the pair.

Sample flexibility with optional walkaway sample loading

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

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

Figure 4. The BD CSampler Plus automatic sampling accessory

Cytokine analysis

Cytokines and growth factors are primary means of communication between cells, driving cellular differentiation that develops and supports the adaptive immune system. Different cytokines induce different cellular responses. Measurement of cytokines and growth factors can provide valuable information about immune responses and cell functionality. However, running multiple conventional ELISA assays of single analytes can consume substantial time, labor, budget, and sample material.

The BD Accuri C6 Plus offers two complementary methods for analyzing cytokine expression. Bead-based flow cytometric immunoassays allow simultaneous quantification of multiple secreted cytokines within a small volume of plasma, serum, or tissue culture supernatants. This approach assesses the total concentration of a given analyte without identifying which subset of cells secreted it.

Intracellular cytokine staining (ICS) is an alternative approach enabling researchers to interrogate distinct subpopulations of a heterogeneous sample, at the single-cell level, for expression of the cytokine of interest. Cells are treated with a protein transport inhibitor to prevent secretion and to trap cytokines within each cell. The sample can then be stained with a combination of antibodies against surface markers and cytokines for analysis of cytokine expression within distinct subpopulations of cells.

When used together, these two complementary approaches can provide a more comprehensive understanding of cytokine kinetics.[2]

Figure 5 shows a bead-based assay using the BD™ Cytometric Bead Array (CBA) method, which contains a cocktail of beads bound with specific capture antibodies that differ slightly in fluorescence intensity. The beads are mixed with samples along with PE-labeled detection antibodies and run on a flow cytometer. There are two available BD CBA formats: kits and flex sets. BD™ CBA kits provide preconfigured panels of three to seven analytes for ultimate ease of use. This experiment used the BD™ CBA Human Th1/Th2/Th7 Cytokine Kit, which can quantitate IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, and IL-17A simultaneously. Other kits include panels of human cytokines, chemokines, and anaphylatoxins; mouse cytokines and immunoglobulins; and non-human primate cytokines.

On the BD Accuri C6 Plus, the beads are excited by the red laser and detected in FL4, while the PE reporter is excited by the blue laser and detected in FL2. A BD Accuri™ C6 Plus software template simplifies setup and acquisition.

While BD CBA kits pre-package popular bead-based assays, BD™ CBA flex sets enable analysis of up to 30 analytes of your choice. Available specificities include soluble protein assays for detection of human, mouse, or rat cytokines, chemokines, and growth factors; human immunoglobulins; cell signaling assays; and enhanced sensitivity assays that can detect protein concentrations of 1.0 pg/mL or lower. The beads contain two dyes excited by the red laser that create 30 unique bead populations, while a PE reporter quantifies the analytes. Flex sets are completely configurable, and the workflow essentially allows researchers to build their own multiplex kit.

Flex sets require the optional Selectable Laser Module (see sidebar) to reconfigure the BD Accuri C6 Plus so that two detectors (instead of the usual one) detect signal from the red laser. Again, a software template simplifies setup and acquisition, and data analysis is performed in FCAP Array™ software.

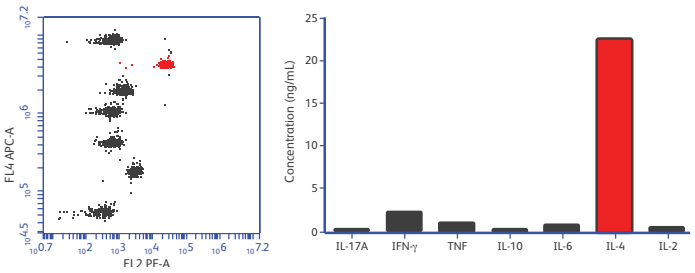


Figure 5. Analysis of cytokine expression with BD CBA kits
BD CBA assays can quantify multiple cytokines simultaneously using minimal sample. In this experiment, seven cytokines were quantified in culture supernatant of stimulated peripheral blood mononuclear cells using the BD CBA Human Th1/Th2/Th17 Cytokine Kit (Cat. No. 560484). 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.

Selectable Laser Module increases fluorochrome options

In the predefined, standard configuration of the BD Accuri C6 Plus flow cytometer, detectors FL1, FL2, and FL3 read fluorescence emissions from the blue laser, while detector FL4 reads emissions from the red laser (3-blue/1-red). By installing the optional Selectable Laser Module (Cat. No. 653126), researchers can operate the system in 2-blue/2-red and 4-blue configurations, which significantly expands the fluorochrome combinations that can be analyzed. The module includes 780/60, 610/20, and 630/30 bandpass optical filters, which optimize detection in the new configurations.

Selectable Laser Configuration	Detector Position	Laser Excitation Wavelength
3-Blue/1-Red (standard configuration)	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

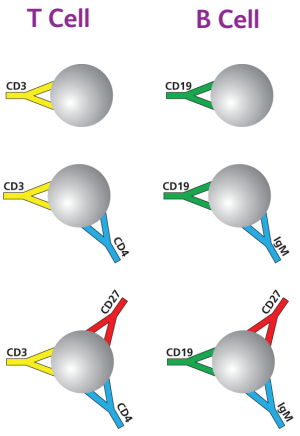
Figure 6. Selectable Laser alternate configurations
The optional Selectable Laser Module allows two or four fluorescence channels to be assigned to the blue laser, vs three in the standard configuration. The remaining channels (if any) are assigned to the red laser.

Advanced immunophenotyping: sentinel approach

In conventional multicolor flow cytometry, each marker to be analyzed is defined by signal in one fluorescence detector. With two lasers and four fluorescence detectors, the BD Accuri C6 Plus can run panels of up to four colors, including a host of common assays such as basic immunophenotyping, apoptosis, cell viability, green fluorescent protein (GFP) analysis, and much more.

The sentinel approach allows the detection of additional markers using the same four colors. Sentinel cell types, such as B and T cells, can be defined based on the expression of unique markers combined with unique fluorochromes. Markers that are unique to one sentinel cell type can be paired with the same fluorochrome as another unique marker on a different sentinel cell type. Figure 7 shows marker and fluorochrome guidelines for the sentinel approach.

Figure 8 shows an immunophenotyping analysis of T and B cells. The sentinel markers were CD3 (exclusive to T cells) and CD19 (exclusive to B cells), which were assigned to the PerCP-Cy™5.5 and APC channels respectively. The FITC and PE channels were then used to further characterize both types of cells—measuring CD8 and CD4 expression for T cells, and Ig lambda and kappa light chains for B cells. A conventional approach on the BD Accuri C6 Plus would have required two distinct panels to characterize both T and B cells (Figures 8A and 8B). With the sentinel approach, both lineages were simultaneously analyzed using a single panel, thus improving the workflow and reducing time, sample, and reagent requirements.



Sentinel markers are placed on unique fluorochromes because as they define the sentinel cell types (such as B cells and T cells) within a heterogeneous sample.

Markers that are unique to one sentinel cell type can be used with the same fluorochrome as another unique marker on a different sentinel cell type.

Markers that are expressed on more than one sentinel cell type are placed on **unique fluorochromes.**

Figure 7. Sentinel approach guidelines for analyzing more markers

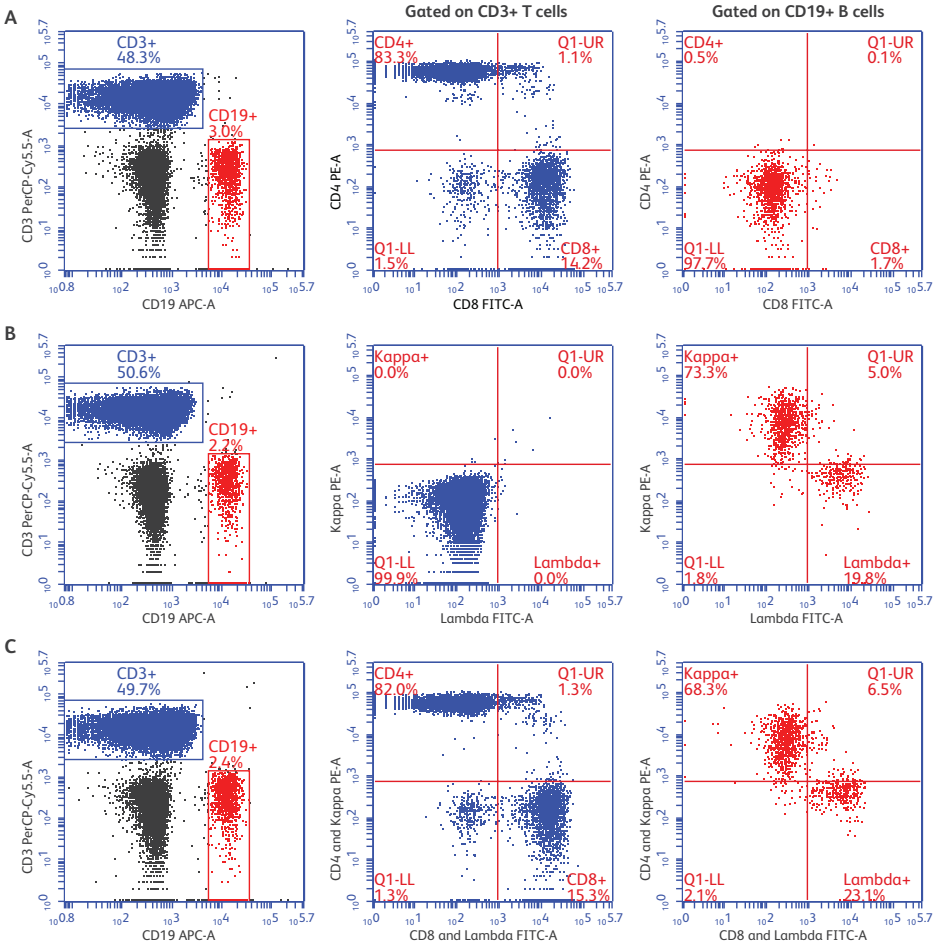


Figure 8. Sentinel analysis of T and B cells
Lysed whole blood was stained with two unique fluorochromes for the sentinel markers CD3 (PerCP-Cy5.5) and CD19 (APC), defining T and B sentinel cell types respectively. Two other fluorochromes (FITC and PE) were used as described for markers that are unique to one sentinel cell type: CD4 and CD8 within T cells and immunoglobulin lambda and kappa light chains (Ig lambda and Ig kappa) within B cells. **A.** Cells were stained with CD3 PerCP-Cy5.5, CD19 APC, CD4 PE, and CD8 FITC. Analysis confirmed that CD4 and CD8 were expressed exclusively by T cells (gated on $CD3^+$, middle plot) and not by B cells (gated on $CD19^+$, right plot). **B.** Similarly, when cells were stained with CD3 PerCP-Cy5.5, CD19 APC, Ig lambda FITC, and Ig kappa PE, only $CD19^+$ B cells expressed Ig lambda and kappa. These panels (A and B) confirmed that CD4/CD8 and Ig lambda/kappa were uniquely expressed in two sentinel cell types, T and B cells respectively, and the same fluorochromes could then be used doubly for these markers. **C.** Cells were stained with CD3 PerCP-Cy5.5, CD19 APC, CD4 PE, Ig kappa PE, CD8 FITC, and Ig lambda FITC. Both T-cell subsets ($CD4^+$ and $CD8^+$, middle) and B-cell subsets (Ig $lambda^+$ and Ig $kappa^+$, right) were clearly distinguished in a single panel using four fluorochromes paired with six markers.

Cell and cancer biology

Useful flow cytometry applications in the fields of cell and cancer biology range from cell function analysis to molecular biology. Adding functional dyes to the multiparameter analysis capability of flow cytometry allows detailed study of the mechanisms underlying changes in cell behavior. Flow cytometry can also detect fluorescent proteins and analyze protein phosphorylation for cell signaling studies.

With its ease of use and small footprint, the BD Accuri C6 Plus personal flow cytometer brings this versatility and convenience to individual laboratories. Its two lasers and four fluorescence detectors are compatible with a broad range of BD Biosciences reagents and other functional dyes, providing the flexibility to design multiplexed assays for a more comprehensive analysis of cell biology. Using flow cytometry, researchers can study multiple cellular processes such as apoptosis, cell signaling, DNA content and damage, cell cycle and ploidy, proliferation, viability, and calcium flux, often with a choice of functional indicators (Figure 9). An assortment of BD Biosciences kits and free, downloadable software templates simplify data collection and analysis.

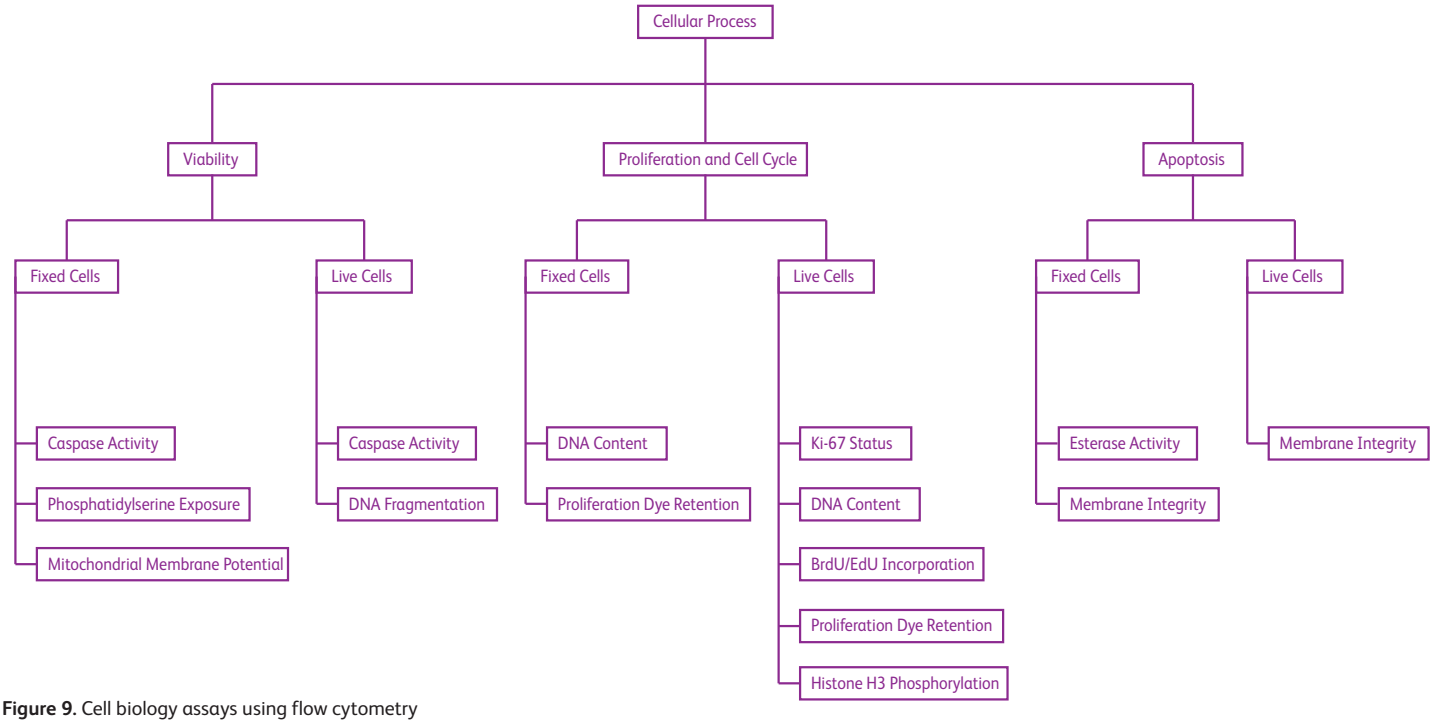


Figure 9. Cell biology assays using flow cytometry

Apoptosis

Apoptosis (programmed cell death) is characterized by a number of morphological and biochemical cellular changes. Studies have revealed distinct pathways (intrinsic and extrinsic) leading up to cell death. Apoptosis can be experimentally induced in vitro to understand mechanisms regulating cell death, as well as to test the effect of drugs on cell viability.

Annexin V, the most common flow cytometry assay to study apoptosis, binds to the phosphatydilserine (PS) that has been translocated to the outer layer of the cell membrane of apoptotic cells. A cell-impermeable dye such as propidium iodide (PI) or 7-AAD often is added to discriminate dead from apoptotic cells. Although PS exposure is a hallmark of apoptosis, other cellular events occur at different stages of apoptosis such as mitochondrial membrane depolarization, activation of caspases, and DNA fragmentation (Figure 10). The BD Accuri C6 Plus can simultaneously analyze up to four distinct cellular processes, thus providing a more comprehensive understanding of the kinetics and mechanisms of action of a tested compound.

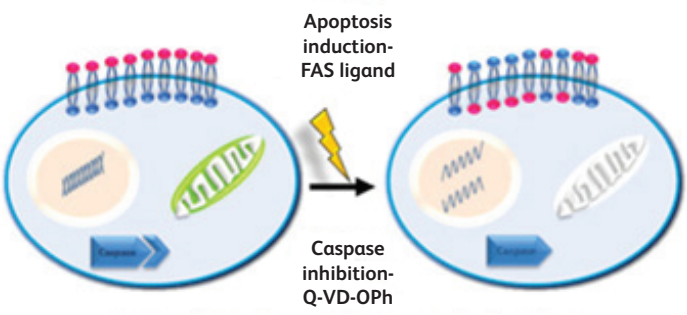


Figure 10. Apoptosis time course mechanism

Figure 11 shows an experiment in which FAS ligand was used to induce apoptosis in Jurkat cells. Some cells were treated with the caspase inhibitor Q-VD-OPh. The samples were stained with Annexin V and PI to assess cell viability and analyzed after 3 hours, 6 hours, and 24 hours. The results show that cells treated only with FAS ligand (FasL, middle column) progressed over time from the Live gate (lower left quadrant, Annexin V^-PI^-) to the Apoptotic gate (lower right, Annexin V^+PI^-) and finally to the Dead gate (upper right, Annexin V^+PI^+). However, cells treated with both FAS ligand and Q-VD-OPh (right column) remained viable, confirming that FasL induces apoptosis via a caspase-mediated mechanism.

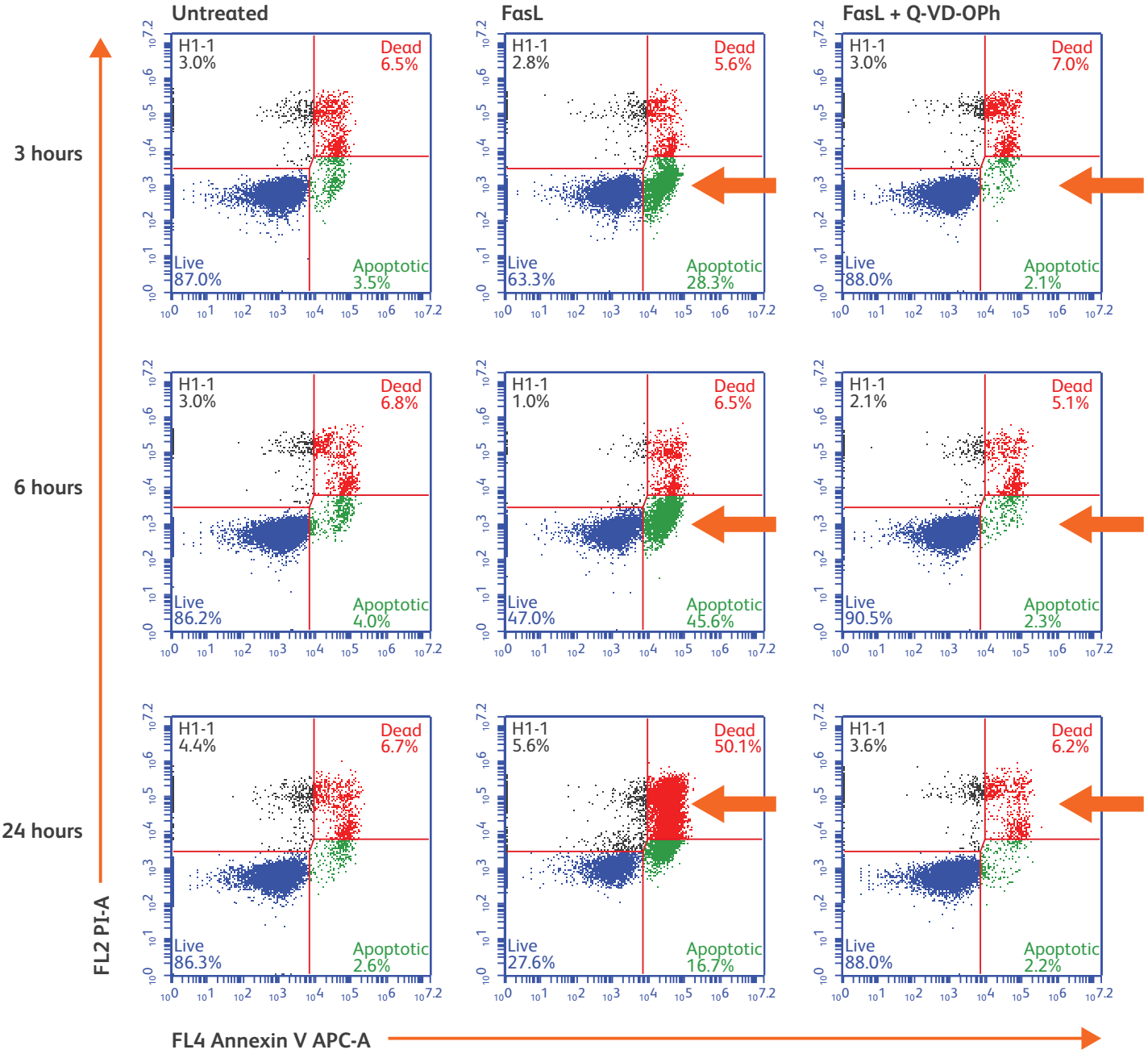


Figure 11. Time course of apoptosis in Jurkat cells

Jurkat (ATCC® TIB-52) cells were treated with 100 ng/mL of FAS ligand (Cat. No. 556375) to induce apoptosis, or with FAS ligand and BD Pharmingen™ Q-VD-OPh General Caspase Inhibitor (20 µM), or were left untreated. Cell viability was analyzed at 3, 6, and 24 hours. Cells were stained with BD Pharmingen™ Propidium Iodide Staining Solution (Cat. No. 556463) and BD Pharmingen™ APC Annexin V (Cat. No. 550474) and analyzed on the BD Accuri C6 Plus. **Results:** Over time, cells treated with FAS ligand (middle column) progressed from live to apoptotic to dead. The majority of cells treated with both FAS ligand and Q-VD-OPh (right column) remained viable, comparable to untreated cells (left column).

Gene expression and regulation

Flow cytometry is a powerful and invaluable tool for cell biologists studying gene expression and regulation. With its ability to assess protein expression at the single-cell level, flow cytometry can be employed to obtain a quantitative measure of gene expression in experimental systems, such as gene knock-out and knock-in and post-transcriptional siRNA and miRNA gene silencing.[3] This data can complement and extend the findings of more conventional methodologies such as immunoblotting and cell imaging in assessing gene modulation. Importantly, flow cytometric protocols for assessing gene modulation are often easier and faster than conventional cell analysis methodologies.

The BD Accuri C6 Plus can detect signals from many fluorescent proteins (see sidebar, below), which are widely used in cell biology as reporters to monitor gene expression. For example, when using CRISPR-Cas technology to edit a gene tagged with a fluorescent reporter, flow cytometry can rapidly quantify the percentage of cells in which the gene has been successfully knocked out by assessing the reduction in reporter expression.[4]

The quantitative measurement of fluorescent proteins is also relevant to other molecular biology applications, such as monitoring transfection efficiency. Uptake of plasmids or vectors by cells in culture is critical for successful downstream molecular biology applications. Conventionally, researchers optimize transfection in an empirical process in which different amounts of transfection reagent, foreign DNA, and varying ratios between the two need to be tested. Optimization of transfection conditions can consequently become a tedious and lengthy process that consumes precious (and expensive) reagents.

Figures 13 and 14 demonstrate the ease and speed of optimizing a transfection protocol using the BD Accuri C6 Plus. A GFP expression vector was transfected into HeLa cells using different amounts of the DNA vector and transfection reagent. Both cell imaging and flow cytometry were used to assess transfection efficiency.

The results from cell imaging (Figure 13) indicated by visual estimate that using 1.50 µL of the transfection reagent and 1.00 µg of DNA vector provided the highest transfection efficiency. Traditional fluorescence microscopy for GFP$^+$ cells relies on analysis of a few representative fields of view, as opposed to a more global assessment of all the cells in culture.

Flow cytometry-based analysis on the BD Accuri C6 Plus (Figure 14), however, demonstrated that using 1.50 µL of transfection reagent with 0.25 µg of DNA vector was optimal for transfection, resulting in 40.7% transfection efficiency. The results provided a quantitative assessment for the number of cells expressing GFP in each experimental condition tested. In addition, flow cytometry allowed detection of a gradient of GFP-expressing cells stretching from dim to bright, corresponding to cell subsets containing different copy numbers of the GFP expression vector.

Fluorescent protein detection with standard and optional filters

With more than seven decades of dynamic range, the BD Accuri C6 Plus can detect both bright and dim signals from GFP and other fluorescent proteins using the standard filter configuration as shown in Table 2. Optional filters can increase signal resolution and allow separation of signals that might overlap using the standard configuration, as shown in Figure 12.[5]

Detector	Filter	FPs Detected
FL1	533/30	GFP*, YFP*, mCitrine, YPet
FL2	585/40	mOrange, dTomato, DsRed
FL3	670 LP	RFP, mCherry

Table 2. Fluorescent protein detection with standard BD Accuri optical filters
*Includes enhanced versions

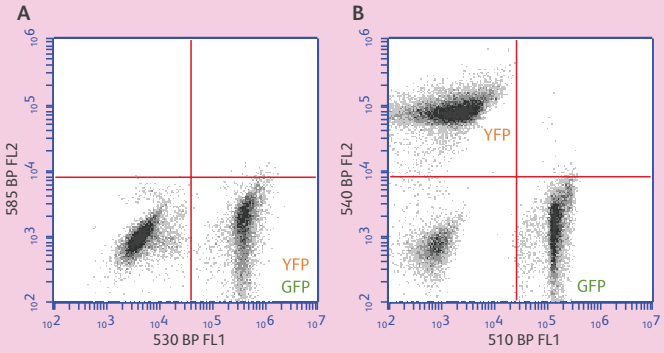


Figure 12. Detection of green and yellow fluorescent proteins using standard and optional filters
A. Both GFP and YFP signals are detected in the same FL1 channel when using the standard BD Accuri filter configuration (533/30 in FL1, 585/40 in FL2). **B.** To detect GFP and YFP individually in the same sample, use the optional 510/15 filter (Cat. No. 653184) in FL1 and the 540/20 filter (Cat. No. 653528) in FL2. Data was generated on the BD Accuri™ C6 flow cytometer.

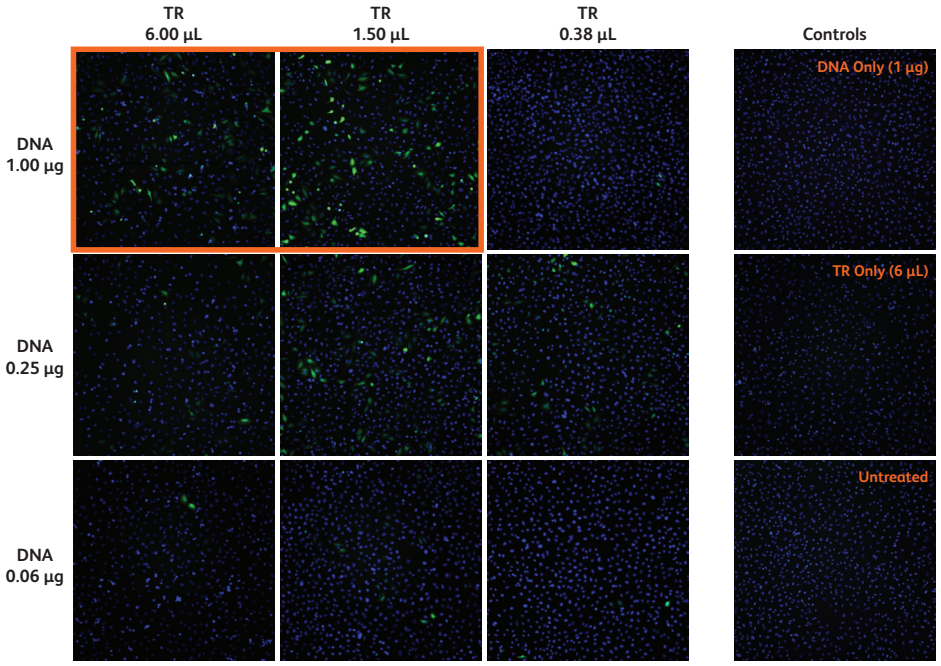


Figure 13. Monitoring transfection efficiency by imaging
HeLa cells were plated at 2 x 10^5 cells/well in 6-well plates in antibiotic-free medium. The next day, the indicated amounts of X-tremeGENE™ 9 DNA Transfection Reagent (TR, Roche) and DNA vector pAcGFP-N1 (DNA, Clontech) were mixed and then added into the culture according to the manufacturer's recommendations. **Results:** After 24 hours, cells were stained with 5 µg/mL of BD Pharmingen™ Hoechst 33342 Solution (pseudo-colored blue) and then imaged using a Molecular Devices ImageXpress® Micro XLS. Expression of GFP⁺ cells (pseudo-colored green) varied, with higher expression observed by visual estimate using between 6.00 and 1.50 µL of transfection reagent in combination with 1.00 µg of DNA vector.

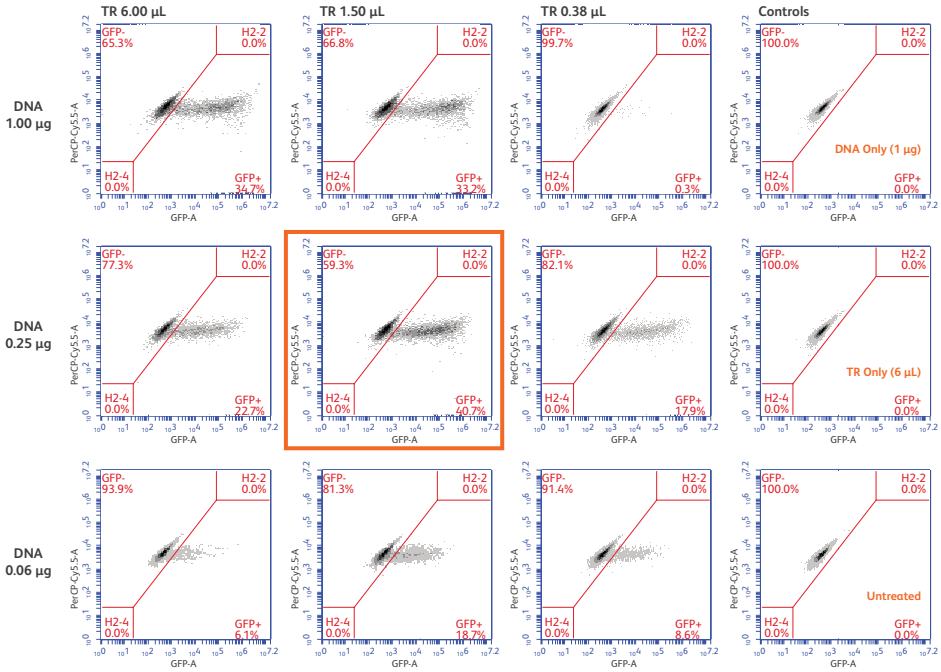


Figure 14. Monitoring transfection efficiency by flow cytometry
After fluorescent imaging, cells were harvested into a single-cell suspension in BD Pharmingen™ Stain Buffer (FBS) and then analyzed by flow cytometry using a BD Accuri C6 Plus. All data was gated based on the light scatter properties of single HeLa cells. The PerCP-Cy5.5 channel was used to distinguish dim GFP-positive cells from autofluorescence. The combination of 0.25 µg of vector and 1.50 µL of transfection reagent per well provided the highest transfection efficiency (40.7%) as assessed by percentage of cells expressing GFP.

Cell signaling and calcium flux

Cell survival, growth, and differentiation are tightly regulated through phosphorylation and dephosphorylation of key proteins in signaling cascades. Using BD Phosflow™ technology on the BD Accuri C6 Plus, researchers can study protein phosphorylation in near-native conditions, reducing the potential for artifacts. Compared to Western blot, protein phosphorylation data provided by flow cytometry demonstrates equivalent sensitivity and specificity, yet the protocol is simpler, shorter, and less time-consuming, and can provide additional insights that supplement conventional methods.[6]

Calcium also plays a key role in cell signaling. Changes in intracellular calcium levels can occur rapidly after stimulation and regulate many important cellular functions. Because the BD Accuri C6 Plus employs non-pressurized peristaltic pumps in an open fluidics system, open tubes can be used to add test compounds to the cell suspension without interrupting sampling (Figure 15). This "continuous-flow" method enables nonstop monitoring of thousands of cells and accurate, gap-free dynamic Ca^{2+} measurement of the entire population. The technique also is useful in many kinds of kinetic studies, such as nanoparticle uptake, apoptosis and viability, and platelet activation.[7]

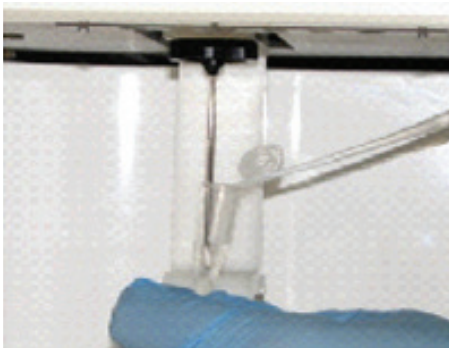


Figure 15. Adding test compounds during sampling
The open fluidics system allows convenient addition of test compounds to the cell suspension without interruption during sampling. This "continuous-flow" method enables nonstop monitoring of thousands of cells to assess calcium flux and other kinetic applications.

Stem cell research

Monitoring the potency or differentiation of target cells is a primary challenge of stem cell research due to the high degree of cell heterogeneity as well as variability of protocol efficiency. When optimizing a differentiation protocol, for example, it is crucial to quantify the percentage of differentiated cells based on the expression of lineage-specific markers. It is also equally important to determine the presence of contaminants or unwanted cells (those that are undifferentiated, partially differentiated, or differentiated into other lineages), which may affect downstream applications. Western blot and PCR-based techniques rely on lysing the entire sample, and their results apply to the population as a whole. This makes it difficult to compare specific subsets of cells within the population that might behave differently. Other techniques that examine individual cells, such as microscopy, require high-throughput systems to scale up for quantitative analysis.

BD Biosciences offers a complete solution for fast and quantitative analysis of stem cell cultures by flow cytometry. BD Stemflow kits have been designed and optimized for different stem cell applications, such as monitoring of pluripotency and reprogramming efficiency, immunophenotyping of bone marrow-derived mesenchymal stromal cells, identification of distinct neural lineage cells, and assessment of differentiation into definitive and pancreatic endoderm. Several BD Stemflow™ kits are matched to free downloadable BD Accuri™ C6 Plus software templates, as shown in the sidebar on page 11, to streamline the analysis of stem cell cultures.

For example, the BD Stemflow™ Human and Mouse Pluripotent Stem Cell Analysis Kit contains conjugated antibodies for the assessment of surface and intracellular pluripotency (SSEA-4 and OCT3/4) and differentiation (SSEA-1) markers (Figure 16). The kit also contains compensation controls and optimized fixation/permeabilization reagents.

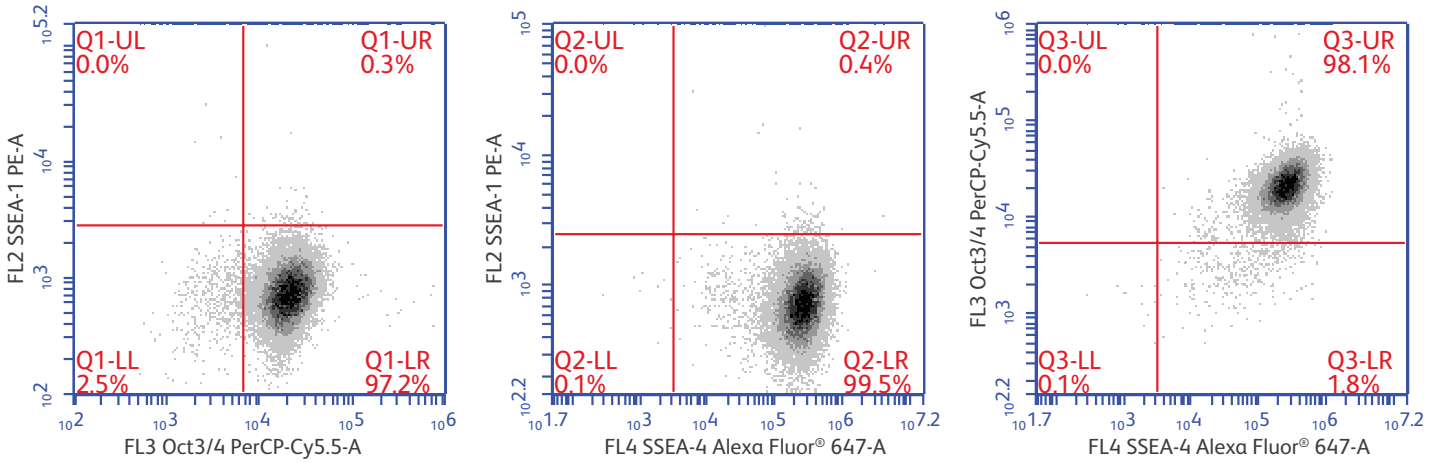


Figure 16. Assessing pluripotent stem cell phenotypes on the BD Accuri C6 Plus
The BD Accuri C6 Plus was used to measure the expression of stem cell pluripotency (SSEA-4) and differentiation (SSEA-1) markers on the cell surface, and pluripotency markers within the cells (Oct3/4). H9 human embryonic stem cells (WiCell) were stained with the BD Stemflow Human and Mouse Pluripotent Stem Cell Analysis Kit (Cat. No. 560477) and acquired using the kit template.

The BD Accuri C6 Plus is also well suited to research on non-embryonic, adult stem cells. For example, researchers who study, isolate, and culture bone marrow-derived mesenchymal stromal cells (MSCs) can use the BD Stemflow™ Human MSC Analysis Kit and template to run a complete ISCT-recommended phenotypic analysis in a single panel.

Beside the assessment of known markers for the characterization of stem cells of interest, flow cytometry is useful in exploratory studies to define novel surface marker signatures. Run the BD Lyoplate™ Human or Mouse Cell Surface Marker Screening Panel on the BD Accuri C6 Plus and the optional BD CSampler Plus as an automated, convenient solution for 96-well plate analysis.[8] This screening panel has been used in different areas of stem cell research, as well as immunology and cancer biology, to discover profiles of cells of interest through an unbiased screening of up to 242 cell surface markers. For example, this approach allowed the definition of specific surface marker signatures for neural stem cells, neurons, and glia.[9]

Free software templates speed acquisition and analysis

Free templates are available from bdbiosciences.com/accuri for many BD kits for popular applications such as stem cell pluripotency and differentiation, immunophenotyping, apoptosis, cell cycle, microbial counting, and intracellular cytokines. Figure 17 shows how the template used in the pluripotency experiment (Figure 16) appears when it is first opened, and again when data has been collected into its pre-gated, pre-labeled plots. Once the template is loaded, you can modify the preset gates and other settings to fit the cells being analyzed.

If there is a particular assay that is run often on the BD Accuri C6 Plus, save time by creating a software template and saving it for future use. A template contains a predefined workspace for quick and easy setup and analysis. All markers, regions, gates, parameter names, sample names, and compensation settings can be predefined.

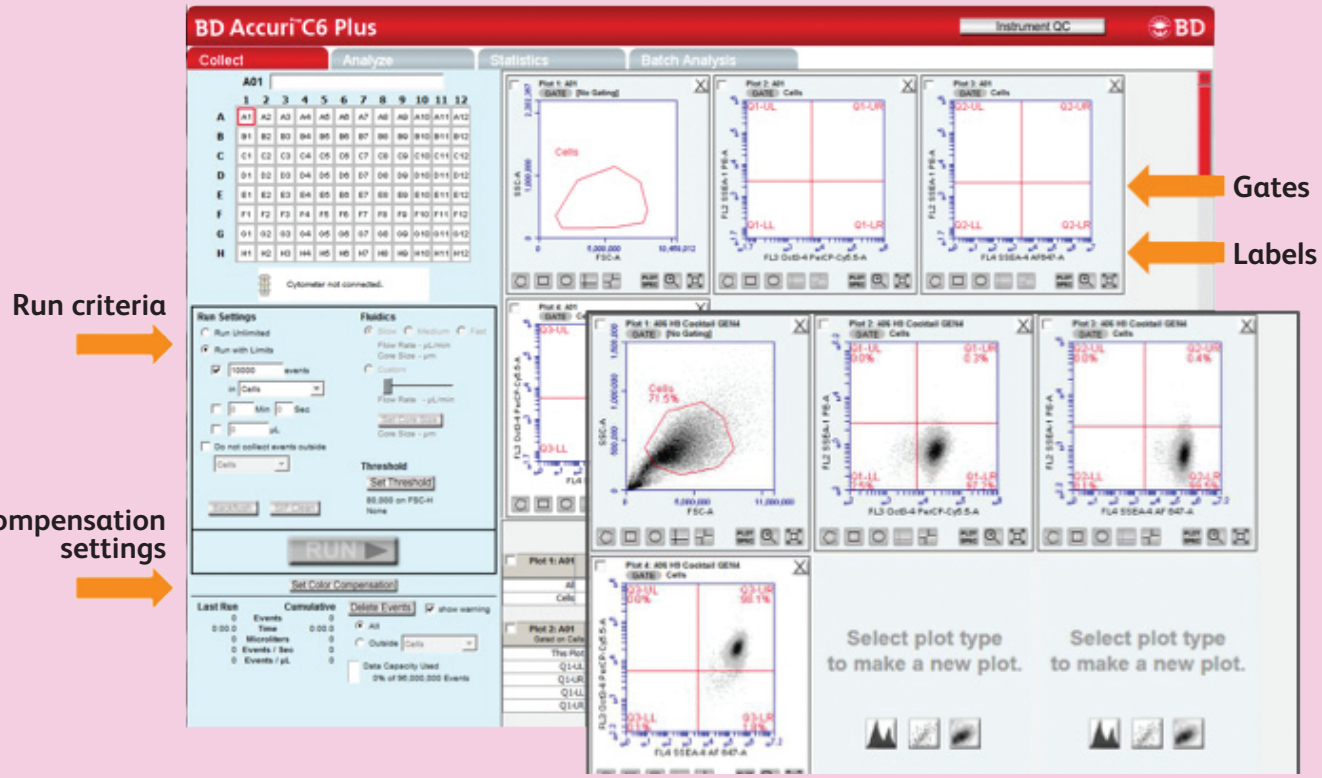


Figure 17. Free software templates speed acquisition and analysis
Free downloadable software templates, such as this one for the BD Stemflow Human and Mouse Pluripotent Stem Cell Analysis Kit (Cat. No. 560477) include gates, labels, run criteria, compensation settings, and other workspace elements. The overlay (bottom right) shows how acquired data appears in the template, ready for analysis.

Microbiology

In the diverse field of microbiology research, flow cytometry is a versatile and powerful technique for analyzing microorganisms, including bacteria and yeast. Light scatter data can reveal basic information about microbes' size, shape, and surface features, while fluorescent stains can assess their cell viability, metabolic activity, and concentration. In some cases, this information might be enough to identify specific microorganisms in a heterogeneous sample; in others, additional techniques such as PNA Flow-FISH can be added.

The BD Accuri C6 Plus can provide 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. With its adjustable core diameter and broad dynamic range, it can acquire and analyze microbes of varying size and autofluorescence. The ability to set thresholds and triggers based on both size and fluorescence allows identification of microbes of small size.

Microbial counting and viability

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. Volumetric counts on the BD Accuri C6 Plus correlate highly with values obtained using counting beads. An open, non-pressurized fluidics system also enables continuous, real-time analysis.

SYBR® Green is a cell-permeable DNA dye often used as a total cell stain to tag bacteria in environmental samples such as drinking water. Staining results in a DNA:dye complex that, when excited by the 488-nm blue laser of the BD Accuri C6 Plus, emits green light that can be measured in the FL1 channel. This approach, called fluorescence triggering, provides increased separation of SYBR® Green$^+$ bacteria from background, compared to the use of light scatter to identify bacteria based on size.

Although SYBR® Green staining allows efficient analysis of the total bacterial cell concentration, it does not differentiate between live and dead cells. However, when the cell-impermeable DNA dye PI is added to the staining protocol, it can discriminate SYBR® Green$^+$PI$^-$ viable bacteria (with intact membranes) from SYBR® Green$^+$PI$^+$ damaged or dead bacteria (with disrupted membranes). Figure 18 shows co-staining of an *E. coli* culture with SYBR® Green and PI. When treated with increasing concentrations of ethanol, damaged bacterial cells shift from the Live to the Dead cell gate on the FL1 vs FL3 plot. The line graph shows nearly identical results when the cells are counted directly by volumetric count on the BD Accuri C6 Plus, compared with the use of counting beads.

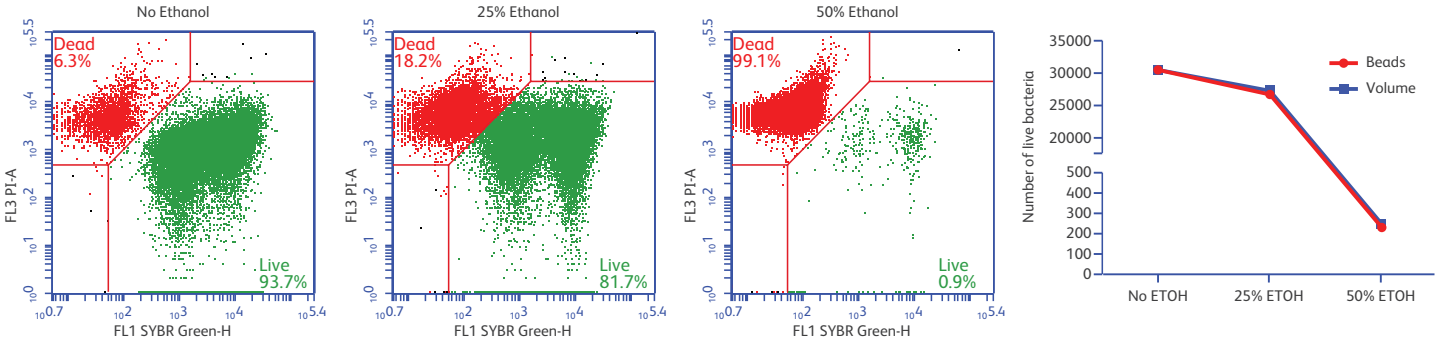


Figure 18. Direct volumetric counting of viable bacteria

SYBR® Green and 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.

Environmental research

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.

For example, researchers analyzing aquatic samples on the BD Accuri C6 Plus can identify the broad categories of phytoplankton using their characteristic fluorescence profiles, reflecting their relative production of chlorophyll, phycocyanin, and phycoerithrin (PE), detected in FL3, FL4, and FL2 respectively.

These naturally produced fluorophores allow researchers to use flow cytometry to directly detect, discriminate, analyze by morphology, and enumerate these organisms using fluorescence-detection methods on BD Accuri C6 Plus, without the addition of extraneous dyes or probes. Figure 19 shows the analysis of three aquatic samples—coastal, bay, and pond water—each with its own autofluorescence profile of microorganisms.

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)

Table 3. 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 fluorescence signals are detected.

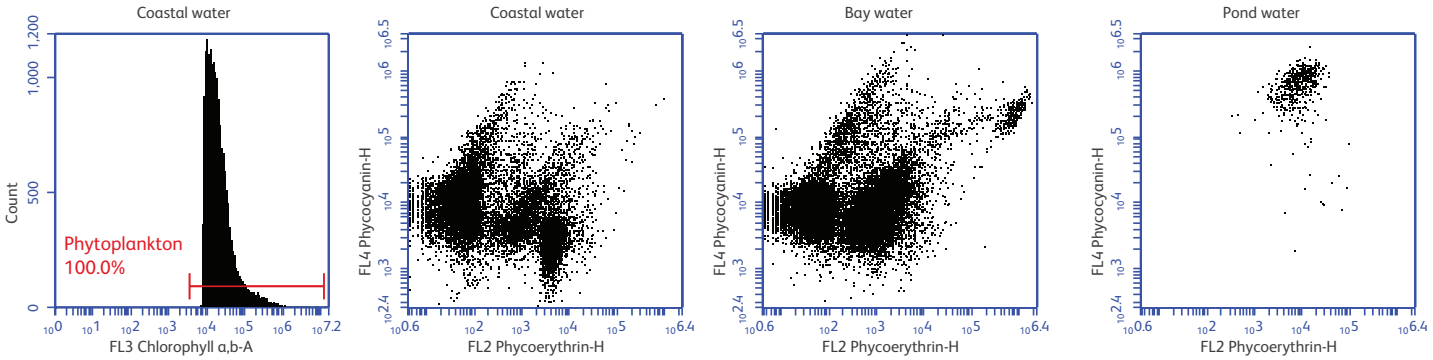


Figure 19. Algae analysis on the BD Accuri C6 Plus
In three environmental water samples, autotrophic phytoplankton were identified based on detection of chlorophyll *a* and *b*, and characterized as blue-green algae (cyanobacteria) or red algae based on phycoerythrin and phycocyanin fluorescence. Discrimination of phytoplankton from background noise was achieved by triggering on the fluorescence signal detected 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.

A flexible system for every life science lab

A flow cytometer is an essential cell analysis tool for every life science lab. With the ability to analyze complex populations at the single-cell level, multiparametric flow cytometry provides a perspective on cell process and function that other methodologies cannot.

As this white paper shows, the BD Accuri C6 Plus brings this unique perspective to a broad range of applications, yet fits comfortably on a laboratory benchtop. It can also accept virtually any sample tube, from 12 x 75-mm test tubes to microcentrifuge tubes to multiwell plates. Researchers can even add reagents to samples during data acquisition to study dynamic cell processes (Figure 15).

Versatile, compact, and affordable, the BD Accuri C6 Plus brings the power of flow cytometry within reach for individual research labs and small institutions. It is also gaining popularity in core facilities at larger institutions because it is intuitive enough for new users and frees up more complex instruments for more complex experiments.[10] With its combination of performance and simplicity, the BD Accuri C6 Plus puts this essential cell analysis tool into the hands of more life science researchers than ever before.

Appendix: Overview of the BD Accuri C6 Plus

This appendix summarizes the features and capabilities of the BD Accuri C6 Plus personal flow cytometer. For more information, including options and free software templates, see the sidebars throughout this white paper.

Operation and maintenance

The BD Accuri C6 Plus offers performance, simplicity, and affordability. Light, rugged, and small enough to fit on a benchtop, it connects to standard electrical circuitry and can easily be transported into the field without the need for re-alignment. With the optional BD CSampler Plus accessory (see sidebar, page 3), it can process multiwell plates and racks with unattended operation.

BD Accuri C6 Plus software makes operation intuitive for novice and proficient users alike. Most new users become fluent with the software in less than 30 minutes, assisted only by a three-page pictorial *Quick Start Guide*. Data files can be analyzed within the program, aided by special tools such as Zoom and VirtualGain™, or exported in FCS 3.0 format into other flow cytometry analysis programs such as FCS Express™ and FlowJo™.

Instrument QC automates daily validation using BD™ Cytometer Setup and Tracking (CS&T) beads to ensure that the instrument meets performance specifications. The software automatically generates Levey-Jennings plots that allow you to monitor instrument performance over time. Each time Instrument QC is performed, the software also updates compensation settings for selected fluorochromes.

Routine instrument cleaning and priming are also automated. Maintenance is easy, even for novice users, and requires no tools. The recommended sheath fluid is 0.22-µm filtered, deionized (DI) water. These attributes effectively expand flow cytometry beyond the core facility and onto the laboratory bench or into the field.

Optical and analytical modalities resolve bright and dim signals

The fully digital BD Accuri C6 Plus features two lasers, two light scatter detectors, and four sensitive fluorescence detectors with optical filters optimized for the detection of many popular fluorochromes, as well as the advanced polymer dye BD Horizon Brilliant™ Blue 515. A state-of-the-art digital signal processing system gives the BD Accuri C6 Plus a dynamic range greater than seven full decades. This means that it can finely resolve both faint and bright signals at once and analyze a wide span of biological variation in a single run—from dim, micron-sized platelets through large, >30-micron highly fluorescent cell lines.

The instrument detects this broad dynamic range using standard factory detector settings without the need for optimization or tuning. In rare cases when the fluorescence is off scale, such as some cell lines transfected with GFP, optional attenuation filters can easily be inserted to bring the signals back on scale while maintaining operation of the detectors within their optimal linear range.

If additional flexibility is needed, the optical configuration can easily be modified. The Selectable Laser Module (see sidebar, page 4) allows reassignment of the standard laser/detector associations, and optional filters can modify the effective detector characteristics.

High-performance fluidics offer cell counting capabilities

The BD Accuri C6 Plus flow cytometer has a unique laminar-flow fluidics system driven by push-pull peristaltic pumps (Figure 20). By monitoring the pressure in the sample introduction probe, a microprocessor can determine the sample flow rate. This arrangement combines the advantages of hydrodynamically focused cell sampling (high data acquisition rates, good light scatter and fluorescence resolution) with the ability to determine sample volume and automatically report concentrations for any identified population in a sample.

The ability to measure absolute counts and concentrations of cells or particles in samples is crucial for many life science applications. Concentration measurements from the direct volume method are highly correlated with those from counting beads (see Figure 18) or hemocytometer counts.

The non-pressurized pumps and open fluidics system allow the use of open sample containers, such as Eppendorf tubes. Test compounds can be added conveniently to the cell suspension without interruption during sampling (see Figure 15).

The peristaltic pumps also enable independent regulation of both the sheath and sample flow rates. Users can quickly optimize the sample core diameter (adjustable from 5 to 40 µm) based on the anticipated size range of cells or particles within the sample. The design also allows for easy removal of clogs (such as cell clumps) from the flow cell.

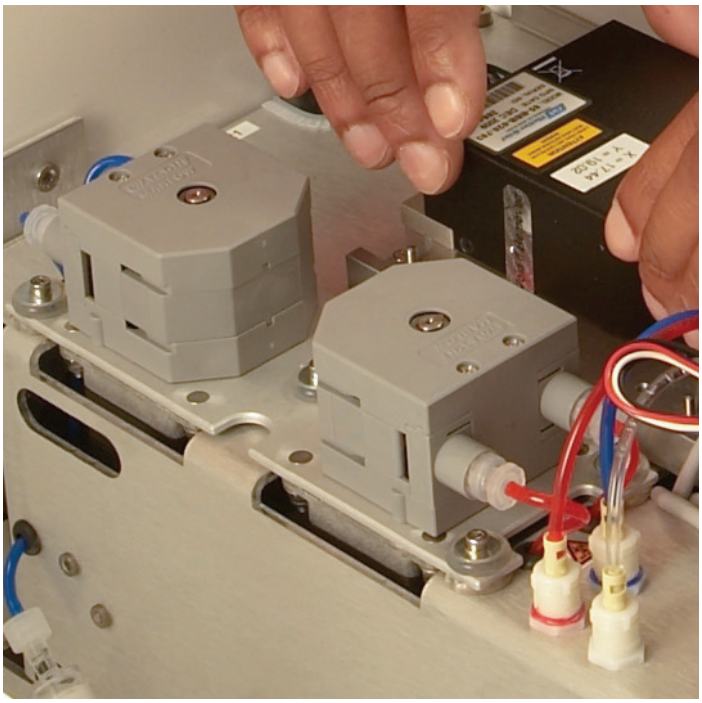


Figure 20. The BD Accuri C6 Plus peristaltic pump system
The unique laminar-flow fluidics system, driven by peristaltic pumps, combines the advantages of hydrodynamically focused cell sampling with the ability to report absolute cell counts for any identified population in a sample.

References

1. All reagents and kits are compatible with both the BD Accuri C6 and the BD Accuri C6 Plus flow cytometer systems. Data was generated on the BD Accuri C6 Plus. Information about BD reagent kits, BD Accuri™ C6 and BD Accuri C6 Plus software templates, and BD CSampler™ and BD CSampler Plus automation options is available at **bdbiosciences.com/accuri**.
2. BD Biosciences. Analysis of cytokine expression in cancer cell lines using flow cytometry. Product information sheet, 2015. Available at **bdbiosciences.com/accuri** under Resources.
3. BD Biosciences. Monitoring protein expression and transfection efficiency using flow cytometry. Product information sheet, 2016. Available at **bdbiosciences.com/accuri** under Resources.
4. Beisel C, Corselli M. Flow cytometry applications in CRISPR-Cas systems and molecular biology. Webinar, September 2016. Available at **bdbiosciences.com/accuri** under Resources.
5. BD Biosciences. Optical filter guidelines for fluorescent protein analysis with the BD Accuri™ C6 flow cytometer. Technical bulletin, June 2012. Available at **bdbiosciences.com/accuri** under Resources.
6. BD Biosciences. Protein phosphorylation analysis using flow cytometry. Product information sheet, 2015. Available at **bdbiosciences.com/accuri** under Resources.
7. Vines A, McBean GJ, Blanco-Fernández A. Continuous measurement of intracellular calcium on the BD Accuri™ C6 flow cytometer. White paper, January 2012. Available at **bdbiosciences.com/accuri** under Resources.
8. BD Biosciences. Immunophenotyping cancer cells using flow cytometry. Product information sheet, 2014. Available at **bdbiosciences.com/accuri** under Resources.
9. Yuan SH, Martin J, Elia J, et al. Cell-surface marker signatures for the isolation of neural stem cells, glia and neurons derived from human pluripotent stem cells. *PLoS ONE*. 6(3): e17540. doi:10.1371/journal.pone.0017540.
10. BD Biosciences. The personal flow cytometer in the flow cytometry core facility. White paper, April 2012. Available at **bdbiosciences.com/accuri** under Resources.

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