The Essential Cell Analysis Tool

BD Biosciences

White Paper

Contents

Introduction

Cell biologists today employ a wide range of cell analysis technologies to advance their research. Preeminent among these methodologies is multicolor flow cytometry, which offers the advantage of multiparametric analysis at the level of individual cells and subpopulations.

Over the past forty years, life science researchers have used flow cytometry for an expanding set of cell analysis applications. Use of this technology has further blossomed with intensified interest in proteomics, increased use of biomarkers in drug development research, and the spread of high-throughput, cell-based drug screening research. Table 1 shows a sampling of these applications.

BD Biosciences has long been a leading provider of products that support numerous cell analysis methodologies, including fluorescence microscopy, immunohistochemistry, Western blot, ELISA, ELISPOT, intracellular staining assays, and a full range of flow cytometry analyzers and sorters. This white paper examines several cell analysis applications that take advantage of the capabilities of flow cytometry, including apoptosis detection, DNA analysis, analysis of complex populations, GFP transfection studies, kinetic analysis, and cell counting. Other BD Biosciences publications cited in the references offer additional details on selected topics.

All data examples in this paper were collected and analyzed on the BD Accuri™ C6, the first personal flow cytometer (Figure 1). With a footprint, weight, and price similar to a real-time qPCR system or microplate reader, the BD Accuri C6 puts this essential cell analysis tool into the hands of more cell biologists than ever before.

Table 1. Selected cell analysis applications for flow cytometry.

Application	Sample Research Questions	Sample Reagents
T-cell immunology	• Cellular and molecular mechanisms of T-cell development • Cell plasticity between Tregs and Th17s	Anti-CD3, CD4, CD8, CD45, CD127, FoxP3, IL-17
Apoptosis	Treatment effects on apoptosis	Annexin V, JC-1
DNA/cell cycle	• Treatment effects on cell cycle • DNA content of plant tissues	Propidium iodide (PI), 7-AAD
Analysis of complex populations	• Characterization of heterogeneous stem cell cultures • RNA knockdown • Rare events	Anti-Sox2, Nanog, Oct3/4
GFP transfection	• Gene or protein expression • Transfection efficiency	GFP, YFP, mCherry
Kinetic analysis	• Effects of test compounds on intracellular calcium • Nanoparticle uptake	Fluo-4
Cell counting	• Viable cell concentrations in cultured cell lines • Platelet concentrations in whole, unlysed blood	7-AAD, Anti-CD41, CD61
Intracellular flow cytometry	• Cytokines and transcription factors • Cell signaling	Anti–IL-4, IL-17, IFNγ, Anti-Stat1, Stat5, BD Phosflow™ Perm Buffer III

Figure 1. The BD Accuri C6 flow cytometer system.

The instrument weighs just 13.6 kg (30 lb). Exterior dimensions (H x W x D) are 27.9 x 54.6 x 41.9 cm (11 x 21.5 x 16.5 in.) with fluid tanks in place.

Overview of the BD Accuri C6

Operation and maintenance

The BD Accuri C6 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™ accessory (see Figure 14, page 11), it can process multiwell plates and racks with unattended operation.

BD Accuri™ C6 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 3-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 FCS Express™, FlowJo™, or other flow cytometry analysis programs.

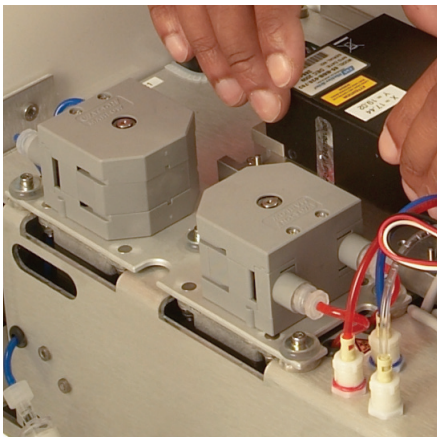

Figure 2. The BD Accuri C6 peristaltic pump system.

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.

Routine instrument cleaning and priming are 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 features two lasers, two light scatter detectors, and four fluorescence detectors. A state-of-the-art digital signal processing (DSP) system gives the BD Accuri C6 a dynamic range greater than six 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 standardized factory detector settings without the need for optimization or tuning. In rare cases where the fluorescence is off scale, such as some cell lines transfected with green fluorescent protein (GFP), 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 Lasers Module (Cat. No. 653126) 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 flow cytometer has a unique laminar-flow fluidics system driven by push-pull peristaltic pumps (Figure 2). By monitoring the pressure in the sample introduction probe (SIP), 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 accurately determine sample volume and automatically report concentrations for any identified population in a sample.

This ability to measure absolute counts and concentrations of cells or particles in samples is crucial for many cell analysis applications. Concentration measurements from the direct volume method are highly correlated with those from counting beads (see Figure 12, page 11), and are more precise than hemocytometer counts, as described under *Cell counting* later in this paper.

The non-pressurized pumps and open fluidics system allow the use of open sample containers, such as microcentrifuge tubes. This allows the convenient addition of test compounds to the cell suspension without interruption during sampling (see Figure 10, page 9), as described under *Kinetic analysis* later in this paper.

Finally, the peristaltic pumps 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.

Apoptosis detection

Several methodologies can detect apoptosis and cell proliferation. BD Biosciences carries a broad portfolio of reagents for determination and detection of these events by ELISA, immunohistochemistry, cell imaging, Western blot, and flow cytometry.

Over the years, multicolor flow cytometry has become essential in the study of apoptosis, cell cycle, and cell proliferation. Different flow cytometric methods can detect different indicators of apoptosis, including Annexin V, caspase activation, PARP cleavage, changes in mitochondrial membrane potential, and DNA fragmentation (see Table 2). The success of these methods results from the capability of flow cytometry to monitor these processes along with other cellular events, such as protein phosphorylation or cytokine secretion, within heterogeneous cell populations.

Table 2. Selected methods for detecting apoptosis using flow cytometry.

Apoptosis Indicator	Assay	Examples	Cat. No.
Plasma membrane alterations (Phosphatidylserine exposure)	Annexin V binding assays: • Single conjugates • Annexin V kits	Annexin V FITC Apoptosis Detection Kit	556570
		Annexin V PE Apoptosis Detection Kit	559763
Mitochondrial changes	JC-1 assays	BD™ MitoScreen (JC-1) Kit	551302
Caspase activation	Caspase activity assay kits and reagents	Caspase-3 Active Form PE Apoptosis Kit	550914
DNA fragmentation	TUNEL assays	APO-BrdU Apoptosis Detection Kit	556405
		APO-Direct Apoptosis Detection Kit	556381

Changes in the plasma membrane are one of the first detectable characteristics of the apoptotic process. In normal cells, phosphatidylserine (PS) molecules are confined to the inner leaflet of the plasma membrane (Figure 3). During apoptosis, these molecules externalize and can be bound to FITC- or PE-labeled Annexin V in the presence of calcium. Dead cells can be excluded with membrane-impermeant dyes such as propidium iodide (PI) or 7-aminoactinomycin D (7-AAD).

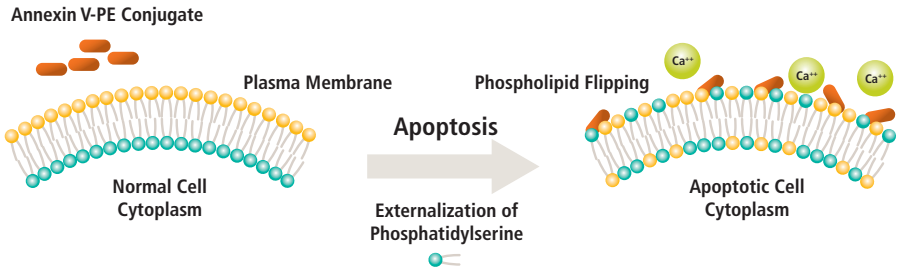

Figure 3. Changes in the plasma membrane are an early sign of apoptosis.

With the ability to detect four fluorochromes in addition to forward and side scatter, the BD Accuri C6 can perform most flow cytometric apoptosis assays.[1] Figure 4 shows sample Annexin V data.

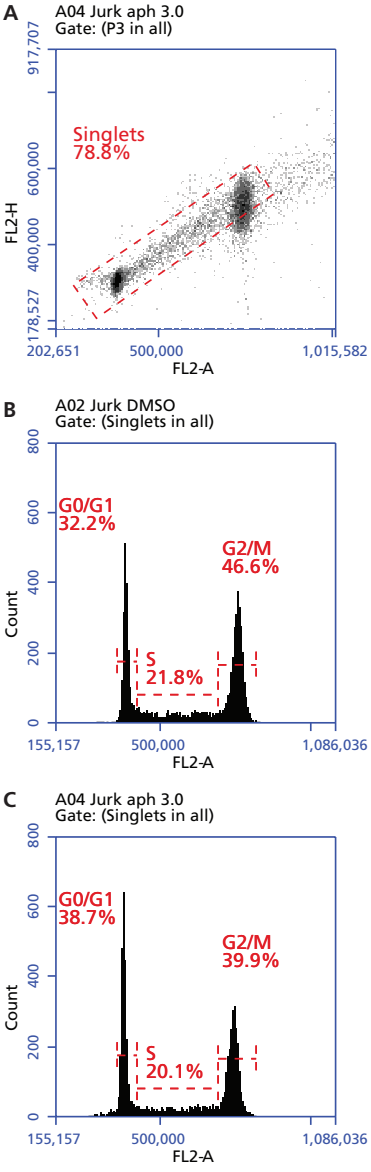

Figure 5. Cell cycle disruption after treatment with aphidocolin.

*Jurkat cells were treated with DMSO or 3 µg/mL of aphidocolin, stained with PI, acquired, and analyzed on the BD Accuri C6. **A.** Cells treated with DMSO were gated to exclude aggregates on a PI FL2-A vs PI FL2-H plot. **B, C.** PI histograms of Jurkat cells (gated on singlets, Panel A) show distinct peaks for cells at the G_0/G_1 and G_2+M cycle phases, with cells in the S phase falling in between. Treatment with aphidocolin (C), which blocks cells in the early S phase, resulted in more G_0/G_1 cells and fewer G_2+M cells.*

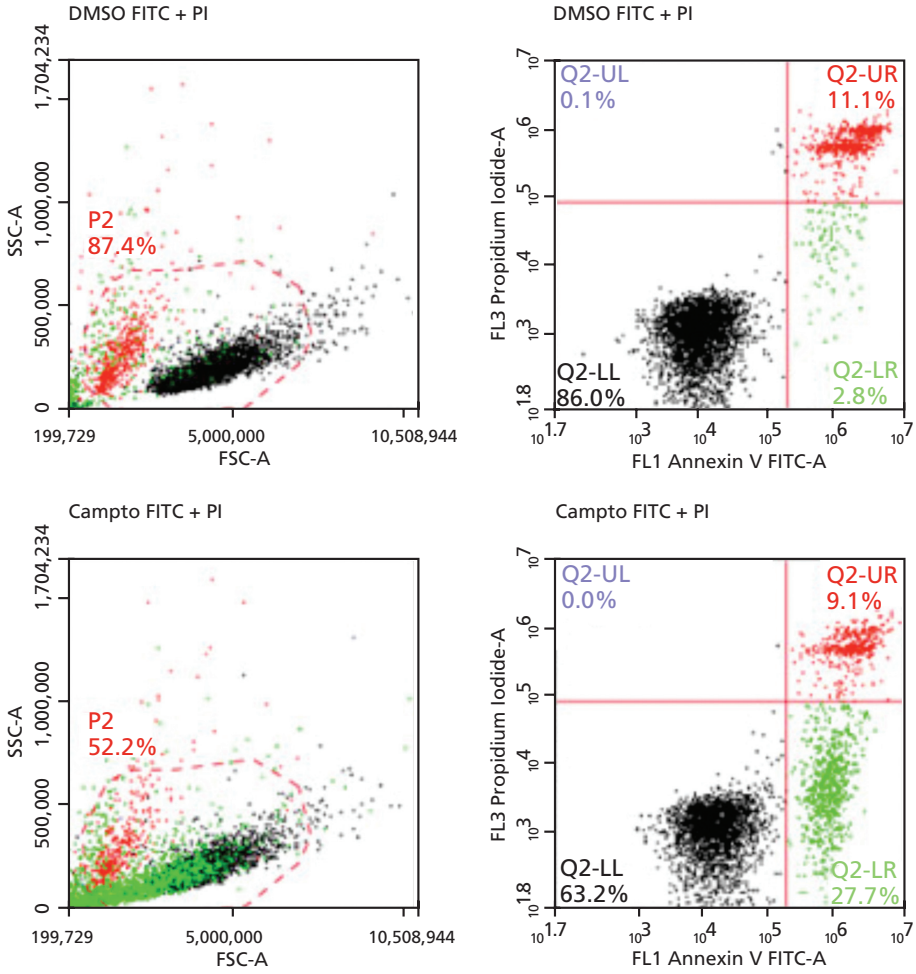

Figure 4. Flow cytometric analysis of FITC Annexin V staining.

*Jurkat cells (human T-cell leukemia; ATCC TIB-152) were treated with 6 µM of camptothecin or 0.1% DMSO (negative control) for four hours to induce apoptosis. Cells were stained with FITC Annexin V and PI according to the BD Pharmingen™ Annexin V FITC Apoptosis Detection Kit staining protocol (Cat. No. 556570). Data was acquired on a BD Accuri C6 flow cytometer using BD Accuri C6 software. **Results:** Camptothecin treatment (lower plots) resulted in an increase in early apoptotic cells (PI^-Annexin V^+, shown in green) compared to the DMSO control (upper plots). Dead cell (PI^+, red) and live cell (PI^-Annexin V^-, black) populations were easily distinguished.*

DNA and cell cycle analysis

Fluorescence intensities of DNA-intercalating dyes such as PI and 7-AAD correlate directly with DNA content and can be easily measured using flow cytometry. Because different phases of the cell cycle have their own characteristic chromosome content (2N to 4N), PI staining can differentiate cycling and aneuploid cells.

Figure 5 shows flow cytometric PI data evaluating how aphidocolin, a DNA polymerase inhibitor that disrupts the cell cycle by blocking cells in the early S phase, alters the proportion of cells in specific phases.

DNA analysis by flow cytometry can identify apoptotic cells (which contain less than 2N DNA), proliferating cells, and other subpopulations of interest. Table 3 profiles a convenient kit for performing multiparametric analysis of cell status, including proliferation, DNA damage, and apoptosis. (Sample data from an induced stem cell culture is shown in Figure 6, page 6.)

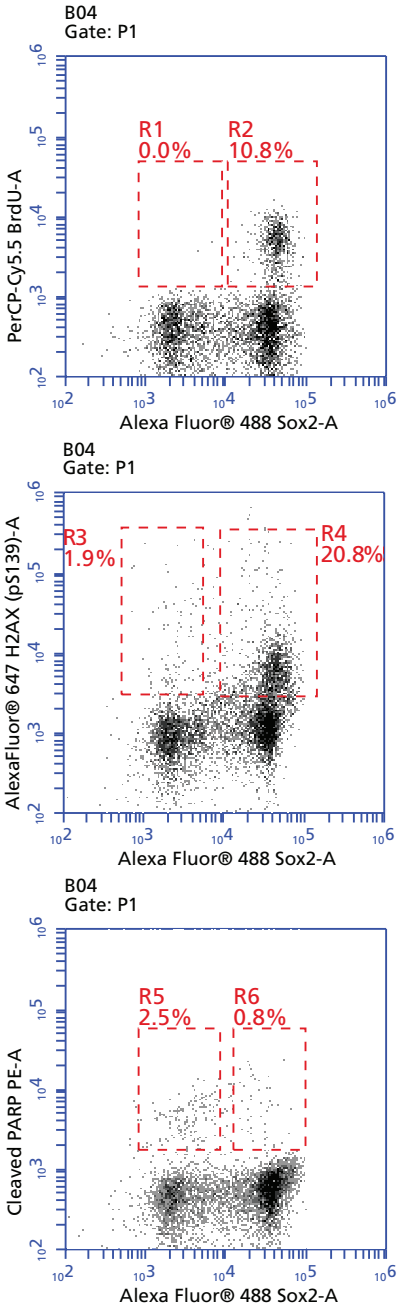

Figure 6. Cell status in a heterogeneous population of neural stem cells.

H9 hESCs were differentiated to neural ectoderm for six weeks as described in Yuan, et al.[4] Cells were dissociated and analyzed for expression of the hESC and NSC marker Sox2, as well as the cell function markers in the BD Pharmingen Apoptosis, DNA Damage, and Cell Proliferation Kit (Cat. No. 562253). Data was collected and analyzed on a BD Accuri C6, gating on the light scatter properties of H9 hESC derivatives. ***Results:*** *Sox2⁻ cells (differentiated neurons and glia) showed* ***(A)*** *reduced proliferation (BrdU⁺, R1),* ***(B)*** *reduced DNA damage (pH2AX, R3), and* ***(C)*** *slightly increased apoptosis (cleaved PARP, R5) compared to other cells in the population.*

Table 3. Contents of the BD Pharmingen™ Apoptosis, DNA Damage, and Cell Proliferation Kit (Cat. No. 562253).*

Component	Format	Laser	Purpose
Bromodeoxyuridine (BrdU)	—	—	Incorporated into newly synthesized DNA during incubation
Anti-BrdU	PerCP-Cy™5.5	Blue	Detects cell proliferation
Anti-H2AX (pS139)	Alexa Fluor® 647	Red	Detects DNA damage
Anti-Cleaved PARP (Asp214)	PE	Blue	Detects apoptosis

*Kit also contains DAPI, DNase, buffers, and a detailed protocol.

One application that demonstrates the power of flow cytometry in DNA research is determining the haploid nuclear DNA content (C-value) for higher plants. Plant C-values, which vary over an extensive range, can be difficult to determine due to autofluorescence, endoreduplication, mitotic activity, and cellular and subcellular debris. Galbraith and colleagues at the University of Arizona have exploited the broad dynamic range, sensitivity to PI, and transportability of the BD Accuri C6 to determine plant C-values quickly and accurately, both in the lab and in the field.[2]

Analysis of complex populations

A major strength of flow cytometry is its ability to study complex populations. Western blot, immunoprecipitation, 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, are hard to scale up for quantitative analysis. Flow cytometry can characterize large numbers of individual cells, allowing for the identification and quantification of different subsets of cells in a heterogeneous population.

Stem cell differentiation

One example of a complex population is a stem cell culture, which often contains pluripotent or multipotent stem cells along with adult cells in various stages of differentiation. Each type of cell has its own distinct intracellular and/or surface markers, which are enumerated in the interactive Stem Cell Explorer at **bdbiosciences.com**.[3] BD Biosciences offers an expansive portfolio of individual antibodies and convenient kits for the detection of markers of embryonic, adult, and induced pluripotent stem cells and their derivatives.

For example, induction of human embryonic stem cells (hESCs) using the serum-free embryoid body (SFEB) method produces "variable and heterogeneous" mixtures of uninduced hESCs, neural stem cells (NSCs), differentiated cells such as neurons and glia, and cells from other germ cell lineages.[4] Flow cytometric analysis using a fluorescent antibody to the transcription factor Sox2 allows the rapid identification of large numbers of both $Sox2^+$ (hESC and NSC) and Sox^- (differentiated) cells in this mixed population, and greatly simplifies further analysis.

Figure 6 compares the proliferation status (BrdU staining), DNA damage (phorphorylated H2AX staining), and apoptotic status (cleaved PARP staining) of induced stem cells in a differentiated culture. Results indicate that $Sox2^-$ (differentiated) cells have stopped proliferating and show reduced DNA damage compared to other cells in the population.

RNAi and protein expression

The analysis of multiple parameters in individual cells or subsets of cells in complex populations is also important in protein expression experiments such as RNA interference (RNAi). Variables such as transfection and knockdown efficiency can create complexity that can be missed when looking at RNA or protein levels at the population level using qPCR or Western blot. A population in which 80% of the cells are transfected with a short interfering RNA (siRNA) that results in 50% protein reduction would show the same 40% overall protein reduction as one with 50% transfection efficiency and 80% protein reduction.

Chan, et al (2005)[5] provided an elegant example of the use of flow cytometry for RNAi analysis. They knocked down Lck, a Src-family kinase that plays an early role in the T-cell receptor signaling pathway in Jurkat T cells. This resulted in two distinct subpopulations—Lck^{lo}, in which transfection was successful (Lck expression was reduced), and Lck^{hi}, in which it was not. Using additional markers, they were able to analyze these different populations and found that, when stimulated with phytohemagglutinin (PHA), Lck^{lo} and Lck^{hi} cells differed in expressing CD69, an early T-cell activation marker.

Rare events and cell subpopulations

The advantages of flow cytometry over Western blot are multiplied when conducting research on rare events or on cells that make up a small minority of a population. For example, in research on acute myeloid leukemia (AML), one hypothesis suggests that minimal residual disease (MRD) cells persist because they resist apoptosis. Using Western blot to study apoptosis-related proteins in MRD cells is difficult, however, due to their rarity in the population. Flow cytometry offers a rapid, highly sensitive method for investigating apoptosis in these samples because the MRD cells can be identified by phenotype and selected for detailed analysis.[6]

GFP transfection studies

Transfection, or introduction of foreign DNA, RNA, or proteins into cells, has become an important tool in studying gene and protein expression and function. Because of variability in transfection efficiency, a reporter gene is often co-transfected into the cells. Green fluorescent protein (GFP, cloned from the jellyfish *A. victoria*) and other derivatives are commonly used as reporter molecules.

Many researchers use microscopy to measure GFP transfection efficiency, although the process is time-consuming and expression levels cannot be readily quantified. Alternatively, Western blot can rapidly quantify protein levels across an entire population, but cannot query individual cells or determine the proportion of cells that express the protein. A flow cytometer can bridge these techniques to determine GFP fluorescence levels for individual cells while collecting data on up to 10,000 cells/second, and can screen for stable transfectants quickly and easily. In addition, the BD Accuri C6 can measure changes in gene expression over time without data loss, even in subpopulations, as described under *Kinetic Analysis* later in this paper.

Figure 7, page 8, shows sample microbiology data acquired and analyzed on the BD Accuri C6. Flow cytometry was readily able to distinguish GFP-infected vs wild-type *E. coli* in a mixed sample.

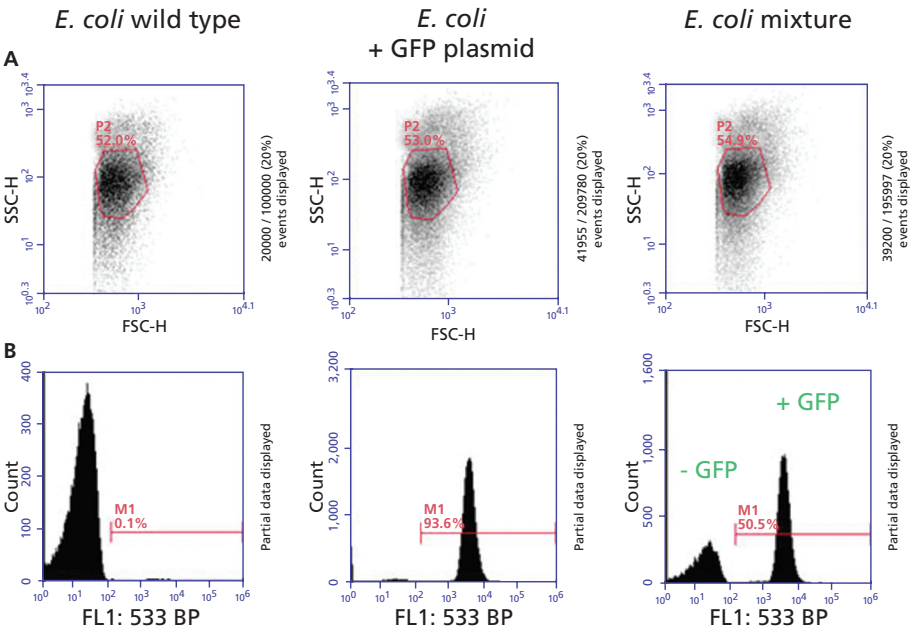

Figure 7. Detection of GFP expression in bacteria.

Samples containing wild-type (left column) or GFP-transfected (middle column) Escheria coli B, *or a mixture of the two (right column), were acquired on a BD Accuri C6.* ***A.*** *FSC vs SSC scatter plots were used to gate cells and exclude debris.* ***B.*** *FL1 histograms were used to identify transfected (peak around $10^{3.5}$) and non-transfected (peak around 10^{1}) populations. Both subpopulations were resolved clearly in the mixed population. Data courtesy of Tim F. Cooper, Dept. of Biology and Biological Chemistry, University of Houston, Houston, TX, USA.*

Table 4. Fluorescent protein detection with standard BD Accuri C6 optical filters.

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

*Includes enhanced versions

With more than six decades of dynamic range, the BD Accuri C6 can detect both bright and dim signals from GFP and other fluorescent proteins using the standard filter configuration as shown in Table 4. Optional filters can increase signal resolution and allow separation of signals that might overlap using the standard configuration, as shown in Figure 8.[7]

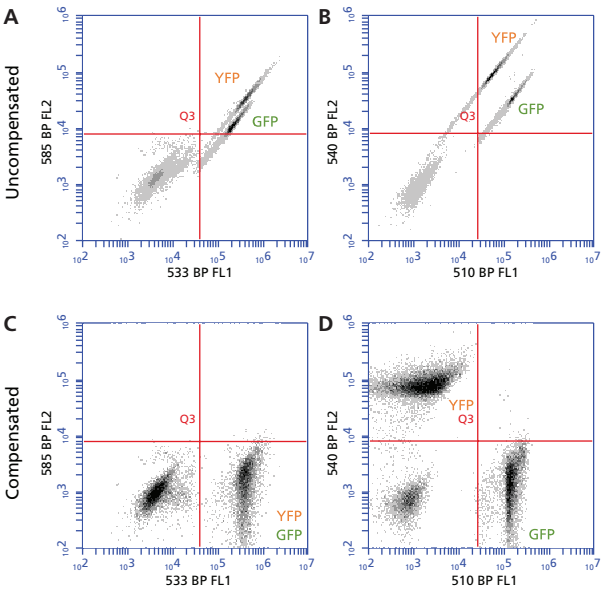

Figure 8. Detection of green and yellow fluorescent proteins using standard and optional filters.

A, C. *Either GFP or YFP signals can be detected in FL1 and FL2 with the standard BD Accuri C6 filter configuration (533/30 in FL1, 585/40 in FL2).* ***B, D.*** *To detect both GFP and YFP at once, researchers can separate the signals by using the 510/15 filter (Cat. No. 653184) in FL1 and the 540/20 filter (Cat. No. 653528) in FL2. The top and bottom graphs show uncompensated and compensated data, respectively.*

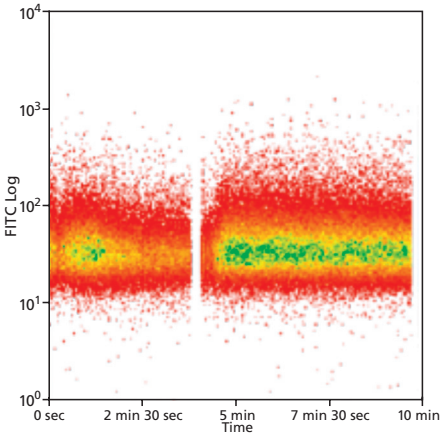

Figure 9. The stop-flow kinetic analysis method leaves data gaps that may miss cellular responses.

Cytogram obtained on a Beckman Coulter CyAn™ ADP analyzer using the stop-flow method, showing a time gap when compounds were added.

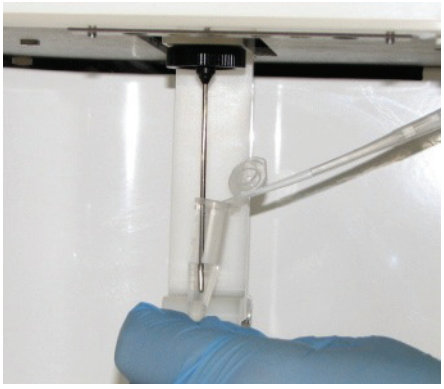

Figure 10. Continuous-flow addition of test compounds with the BD Accuri C6.

Cells or compounds can easily be added to open tubes on the BD Accuri C6 without interrupting sample analysis.

Kinetic analysis

Small-molecule agonists and other test compounds can elicit powerful and rapid cellular responses—some within seconds or faster. Such dynamic responses can be difficult to quantify with microscopy and difficult to even detect with other techniques.

Many flow cytometers use a pressurized fluidics system in which the tubes must be sealed. To add test compounds to the cell suspension, a stop-flow method is used in which sampling is paused, the sample tube opened, the agonist added, and the tube resealed. This technique leaves a gap or blind spot in data collection (Figure 9) that may fail to capture essential cellular responses.

The BD Accuri C6, in contrast, employs peristaltic pumps (Figure 2, page 3) in an open fluidics system. The use of open sample containers, such as microcentrifuge tubes, allows convenient addition of test compounds to the cell suspension without interrupting sampling (Figure 10). This "continuous-flow" method enables nonstop monitoring of thousands of cells and accurate dynamic measurement of the entire population.

Calcium flux, for example, mediates many important cellular functions. Changes to intracellular calcium ($Ca2^+$) levels occur rapidly—in some cases within nanoseconds of stimulation—and obtaining accurate data on these changes is a significant research challenge. Figure 11 illustrates gap-free measurement of Ca^{2+} levels using the continuous-flow method.

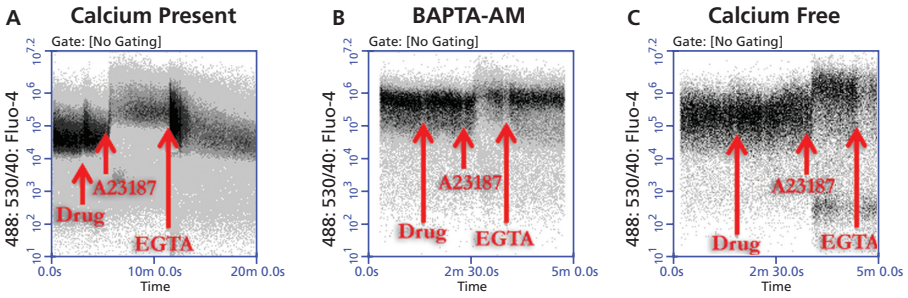

Figure 11. Continuous, gap-free recording of intracellular calcium levels on the BD Accuri C6.

An unspecified drug compound, the calcium ionophore A23187, and the extracellular calcium chelator EGTA were sequentially added to cultured SH-SY5Y neuronal cells using the continuous-flow method on the BD Accuri C6 under three experimental conditions. Cytograms show Fluo-4 fluorescence indicating Ca^{2+} levels over time, with arrows indicating addition of test compounds. ***A.*** *Test compounds were added in the presence of calcium, mediated by thapsigargin.* ***B.*** *Test compounds were added in the presence of BAPTA-AM, the intracellular calcium chelator.* ***C.*** *Test compounds were added in the absence of calcium (EGTA).* ***Results:*** *In the presence of calcium, the drug compound provoked a momentary spike in intracellular calcium, which might have been missed using the stop-flow method. As expected, A23187 caused a lasting increase in Ca^{2+} concentration, and EGTA reduced it. The presence of BAPTA-AM or the absence of calcium negated the effects of the drug and moderated the effects of A23187 and EGTA. Data courtesy of Alfonso Blanco Fernández, UCD-Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland.*

The continuous-flow method on the BD Accuri C6 can quantify dynamic changes in Ca^{2+} levels while simultaneously identifying and analyzing different subpopulations within a sample, using cell-permeable, calcium-sensitive fluorescent dyes and antibodies to cell marker proteins.[8] This method can also be applied to other research areas that measure dynamic live cell response, such as pH, reactive oxygen and nitrogen species, mitochondrial membrane potential, and nanoparticle uptake.

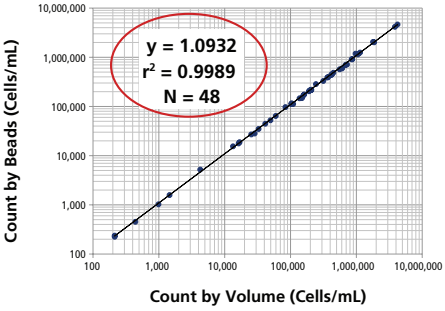

Figure 12. Comparison of cell concentration measurements based on direct volume vs counting beads.

Serial dilutions of Jurkat, 3T3, and U937 cells, and T cells, B cells, and platelet samples from four human peripheral blood donors were counted on the BD Accuri C6 by two methods. X-axis values represent direct-volume measurements, while y-axis values were calculated based on counting beads. ***Results:*** *The direct counts (x-axis values) correlated highly (r^2 = 0.9989) with counting bead counts (y-axis values).*

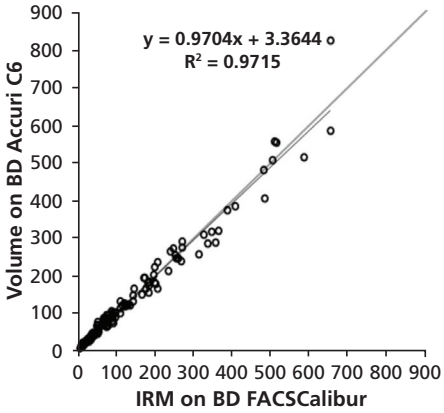

Figure 13. Comparison of direct volume platelet counts on the BD Accuri C6 vs the International Reference Method.

EDTA anti-coagulated human whole blood samples (N = 144) were acquired and analyzed, and platelet counts (x 10^9/L) calculated using the International Reference Method (IRM) on the BD FACSCalibur™ flow cytometer (x-axis) and by direct volume on the BD Accuri C6 (y-axis). ***Results:*** *The two methods on the two instruments were highly correlated (R^2 = 0.9715) for all platelet counts. Bland-Altman mean-difference analysis (not shown) indicated minimal bias between the two methods across the full range of platelet counts, and modest bias when analysis was confined to samples with low platelet counts. Data courtesy of Paul Harrison, Oxford Haemophilia Centre & Thrombosis Centre, Churchill Hospital, Oxford, UK.*

Cell counting

Quick and precise cell concentrations for a given phenotype are highly useful in drug discovery and cell biology. Historically, laboratories performed such counts by combining a light microscope and hemocytometer, using a dye such as Trypan blue to distinguish viable and dead cells. However, this method is slow and prone to error, and cannot easily identify specific subsets of cells.

Flow cytometers can quickly and accurately identify phenotypes and count cells, but their fluidics systems often cannot measure sample volume, so determining cell concentrations may require the addition of counting beads to the sample. The BD Accuri C6 peristaltic pump system (Figure 2), in contrast, allows for determination of the sample flow rate, and thus can calculate concentrations for any identified population in a sample.

A recent study explored three cell-counting applications: viable cell concentrations in cultured cell lines; immune cell concentrations in human peripheral blood; and platelet counts in whole, unlysed human blood. Direct volume measurement on the BD Accuri C6 was compared with counting beads and hemocytometer counts.[9]

The cell concentrations determined by direct volume on the BD Accuri C6 correlated highly with concentrations calculated by counting beads (Figure 12). Both methods were consistent over a broad range of concentrations and were more precise than hemocytometer counts. In experiments where cell counts are compared across different conditions, either technique is appropriate when used consistently. The direct volume method offers the added advantages of increased speed and reduced cost and complexity.

One application of direct volume cell counting is the determination of platelet concentrations, which is crucial in both basic science and clinical research laboratories. An International Reference Method (IRM), introduced in 2001, provides fast, accurate platelet counts, but requires both a flow cytometer and a hematology analyzer. A recent study validated platelet concentrations measured by direct volume on the BD Accuri C6 without using a hematology analyzer.[10] When applied to platelet concentrations spanning the normal range, the direct volume method was found to be interchangeable with the IRM (Figure 13).

A flexible system for every cell biology lab

A flow cytometer is an essential cell analysis tool for every cell biology lab. With the ability to analyze large, 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 brings this unique perspective to a broad range of applications, yet fits comfortably on a laboratory benchtop. It can process 30–40 samples per hour with manual operation, or 50–60 samples per hour with the optional, automated BD CSampler option (Figure 14). It can also accept virtually any sample tube, from 12 x 75-mm test tubes to microfuge tubes to multiwell plates. Researchers can even add reagents to samples during data acquisition to study dynamic cell processes (Figure 10, page 9).

Figure 14. The BD CSampler accessory.

An automation option allows preparation and analysis of samples in the same plate, eliminating manual transfers from plate to tubes. The BD CSampler is compatible with both regular and deep-well 96-well plates, as well as 12 x 75-mm tubes.

Versatile, compact, and affordable, the BD Accuri C6 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 while freeing up more complex instruments for more complex experiments. With its combination of performance and simplicity, the BD Accuri C6 puts this essential cell analysis tool into the hands of more cell biologists than ever before.

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