Automating the Personal Flow Cytometer

White Paper

Contents

Abstract

Flow cytometry offers the ability to analyze multiple proteins in heterogeneous samples comprising thousands of cells, rapidly and accurately, with single-cell resolution. This powerful methodology is now used in a broad range of applications. However, flow cytometry's capacity to collect and process an abundance of data can also increase the effort needed to prepare, collect, and analyze samples.

Automation can improve productivity, increase the quality and reproducibility of data, boost throughput, and enable the generation of data that would not otherwise be obtainable. This white paper explores the value of automating flow cytometry and illustrates the kinds of data an automated system can provide.

Sample data was collected and analyzed on the BD Accuri™ C6 personal flow cytometer, which offers its own automated sampling accessory as well as an open communications interface with which it can be integrated with third-party automation options. This paper profiles automated flow cytometry systems based on the BD Accuri C6 that can monitor bioreactors, fermenters, and other cell culture applications online in real time, or perform high-throughput screening for drug discovery, gene mutagenesis, genotoxicity testing, and other applications.

BD

Introduction

Flow cytometry brings significant benefit to life science research: the ability to analyze multiple proteins in heterogeneous samples comprising thousands of cells, rapidly and accurately, with single-cell resolution. This powerful methodology is now used in a broad range of applications, from immunophenotyping and cell counting to gene expression studies, DNA and cell cycle analysis, characterization of stem cells and their derivatives, marine and environmental research, biofuel development, and bioprocess monitoring. The introduction of personal flow cytometers like the BD Accuri C6 has brought this essential tool within reach of more researchers, laboratories, and institutions than ever before.

The ability of flow cytometry to collect and analyze more data can accelerate the process of discovery. However, an abundance of samples can also mean an exponential increase in the effort needed to prepare, collect, and analyze them.

Automation can improve productivity by handling many manual steps automatically, allowing researchers to process the proliferation of samples. In addition, automation can dramatically reduce the variability inherent in manual sample preparation, thus increasing the quality and reproducibility of data. Automation can boost throughput, making screening applications such as drug discovery and gene mutagenesis feasible. Automated flow cytometry can even enable the collection of data that would not otherwise be obtainable, such as continuous, multiparametric monitoring of cultures in a bioreactor.

This white paper explores the value of automating flow cytometry and illustrates the kinds of data an automated system can provide. All of the data in the examples in this paper was collected on a BD Accuri C6 flow cytometer, which offers its own automated sampling accessory as well as an open communications interface with which it can be paired or integrated with automation options from partners and independent vendors.

The BD Accuri C6 as an automation platform

An open system offers simplified operation and maintenance

The BD Accuri C6 (Figure 1) used to generate the data in this white paper offers performance, simplicity, and affordability. Light, rugged, and small enough to fit on a benchtop, it connects to standard electrical supplies and can easily be placed in a laminar-flow hood or transported into the field without the need for re-alignment.

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.

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.

BD Accuri C6 software offers an open platform that makes it ideal for use in an automated system. It allows the flow cytometer to take commands for all basic functions directly from a master application through a seamless software interface. The cytometer software can communicate individually with other instrumentation software or be integrated into a turnkey system.

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 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 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 and continuous sampling

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.

The non-pressurized pumps and open fluidics system allow the use of open sample containers, such as microcentrifuge tubes, or direct connections to culture systems. This allows the convenient addition of test compounds to the cell suspension without interruption during sampling, or continuous sampling from a bioreactor or other culture system as described under “Automated, real-time monitoring and control of cell cultures” later in this paper. Samples need merely to be made available at the SIP in the correct quantity at the right time. The BD Accuri C6 does the rest, gently pulling the fluid into the SIP, just as if used manually.

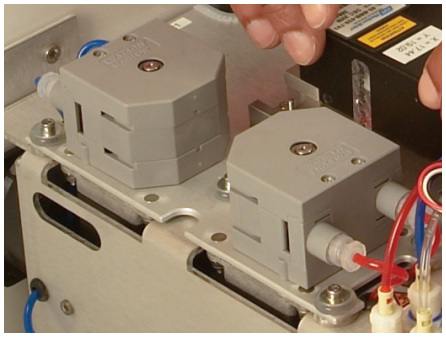


Figure 2. The BD Accuri C6 peristaltic pump system.

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.

Finally, the peristaltic pumps enable independent regulation of both the sheath and sample flow rates. Users (or automation software) 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.

Automated data collection

Researchers can run large experiments that would otherwise be unfeasible by using an automated sampling accessory such as the BD CSampler™ (Figure 3), which adds a first level of automation to the BD Accuri C6 system. This simple, reliable, easy-to-use sample processing option offers walkaway convenience and can significantly increase throughput (Table 1).

Table 1. One research lab's estimated time to count 30 samples using various methods.

Method	Time
BD Accuri C6 (with BD CSampler)	22.5–37.5 min
BD Accuri C6 (manual run)	60 min
Particle Counter	150–262.5 min
Hemocytometer	390 min

Estimates for single samples courtesy of James Barker and Rose Ann Cattolico, PhD, University of Washington, Seattle, WA.

Figure 3. The BD CSampler accessory.

An automated sampling 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.

With the BD CSampler, samples can be prepared in 96-well plates and analyzed directly on the flow cytometer. This saves time and reduces cost, waste, and potential for operator error. This option is compatible with both 48- and 96-well plates and 24-tube racks. Open access to the BD CSampler arm permits robotics-guided plate loading. The BD CSampler adds minimal footprint to the system—about 3 ft^2 (0.28 m^2) for the pair—keeping the benchtop free for other uses.

Automated, real-time monitoring and control of cell cultures

The rapid, multiparametric analysis offered by flow cytometry provides significant benefits for bioprocess monitoring,[1] biofuel development,[2] fermentation, and other cell culturing applications. However, sample preparation for flow cytometric assays is often elaborate, complex, and time consuming. Without a means to automate the wash, fixation, stain, dilution, and incubation steps, it can be difficult to use flow cytometry in these applications.

BD Biosciences has worked with MSP Corporation to interconnect the BD Accuri C6 with the MSP M5000 FlowCytoPrep™ sample preparation system (Figure 4). The FlowCytoPrep micro-reactor platform enables user-initiated or fully automated sterile bioreactor sampling, sample preparation, and sample injection into the BD Accuri C6 flow cytometer. Together, the two instruments can perform rapid online measurements of single-cell properties, which is not feasible by classical methods.[3] The seamless interface between the two instruments results in a turnkey system for real-time, online monitoring of bioreactors and other cell culture applications.

Figure 4. The MSP M5000 FlowCytoPrep sample preparation system connected to the BD Accuri C6.

The seamless interface between the two instruments results in a turnkey system for real-time, online monitoring of bioreactors and other cell culture applications.

The FlowCytoPrep consists of a micro-reactor, fluidics system, control hardware, operational software, and flow cytometric connection kits. Samples are automatically introduced into and prepared in the micro-reactor, a miniature (150 µL) continuous stirred-tank reactor (CSTR) configured with special ports, and mixing and bidirectional temperature control. Figure 5 shows a schematic of sample preparation in the micro-reactor that includes fixation, two stains (with incubation), four washes, and injection into the BD Accuri C6. Sample concentration can also be adjusted if needed.

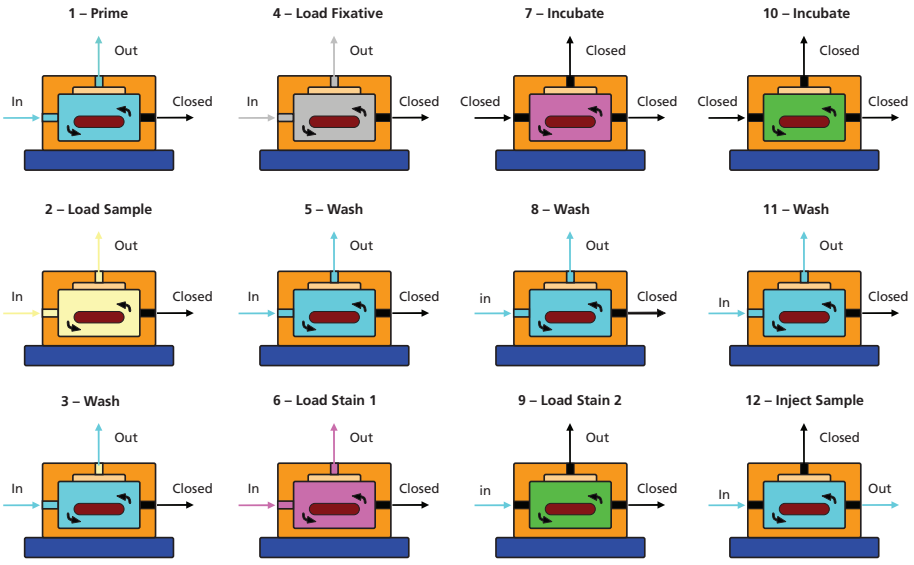


Figure 5. Twelve FlowCytoPrep micro-reactor steps in a typical monitoring study.

Samples are automatically prepared in the FlowCytoPrep micro-reactor, a miniature (150 µL) continuous stirred-tank reactor configured with special ports, and mixing and bidirectional temperature control.

Unlike yield-affecting filtration methods, in which cells are pushed against a membrane and then dislodged, the micro-reactor performs fluid exchanges slowly enough to keep the cells in suspension while the supernatant is replaced. Demonstrated yields are >90% per operation, vs typical 60%–80% yields from a single manual spin-down cycle.

After switching on the BD Accuri C6 and starting BD Accuri C6 software, the click of a button enables remote control. From that point on, the FlowCytoPrep software coordinates seamlessly with the BD Accuri C6 through its open communications interface. Users select protocols from the FlowCytoPrep console. The FlowCytoPrep automatically samples from the bioreactor or fermenter, prepares the samples, and transports them to the BD Accuri C6 SIP. A precisely machined tee connector (Figure 6) ensures smooth fluid flow.

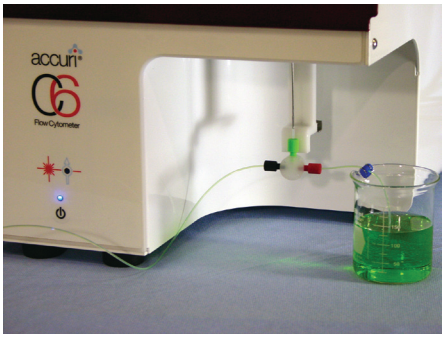
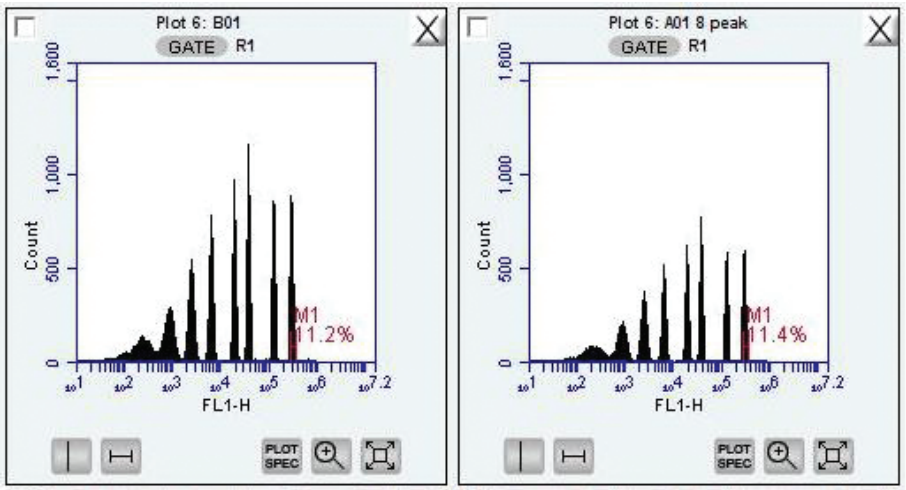


Figure 6. The tee connection from the FlowCytoPrep to the BD Accuri C6 SIP.

The cytometer takes the amount of sample it needs and discards the rest into a waste reservoir.

The FlowCytoPrep software triggers standard BD Accuri C6 functions, telling it to load sample, cleaning solution, sheath fluid, or other fluid as appropriate, and controlling the sample flow rate. The cytometer uses reagents from the BD Accuri C6 fluidics tray just as during manual use. The cytometer takes the amount of sample it needs and discards the rest into a waste reservoir. The non-pressurized fluidics of the BD Accuri C6 allow this simple interface—from the flow cytometer's point of view, it's just like sampling from an open tube, and the peaks generated from manual and automated sampling are similar (Figure 7). No positive pressure is generated, eliminating any risk of pressure buildup in the fluidics line.



Plot 6: B01 Gated on R1	Count	Volume (µL)	% of All	Mean FL1-H	CV FL1-H	Median FL1-H
This Plot	15,119	45.7	65.67%	61,501.93	168.05%	7,048.0
M1 (281,39...	1,693	45.7	7.35%	303,267.21	2.91%	304,100.0

Plot 6: A01 Gated on R1	Count	Volume (µL)	% of All	Mean FL1-H	CV FL1-H	Median FL1-H
This Plot	10,202	47.2	72.06%	60,307.05	167.09%	6,956.0
M1 (281,39...	1,166	47.2	8.24%	303,592.32	3.03%	304,693.0

Figure 7. Comparison of 8-peak calibration beads between manual and automatic sample injection.

Spherotech™ 8-Peak Validation Beads (BD Cat. No. 653144) were analyzed on the BD Accuri C6. ***Left Plot, Top Table:*** *Sample loaded manually into the BD Accuri C6.* ***Right Plot, Bottom Table:*** *Sample injected automatically into the BD Accuri C6 from the FlowCytoPrep. Mean and median peak values and CVs are similar. (Peak amplitudes differ because different numbers of beads were collected.)*

Once data is collected, the BD Accuri C6 software exports the FCS file directly to the FlowCytoPrep software for automated analysis, such as standard operation performance on data sets. Based on that information, the FlowCytoPrep software then sends decision-making information via an I/O module to the bioreactor, fermenter, or Laboratory Information Management System (LIMS), replacing manual operations.

Because two-week continuous operation is common for bioprocess monitoring applications, cleaning is also programmed into the FlowCytoPrep, enabling compliance and eliminating material accumulation and carryover. The system can incorporate more specific assay-defined cleaning protocols, if desired. The only daily task is to inspect the BD Accuri C6 and MSP FlowCytoPrep fluidics levels and refill or empty the containers as needed. Even daily QC can be automated by setting up the flow cytometry QC beads as a FlowCytoPrep reagent.

The combination of the BD Accuri C6 with MSP M5000 FlowCytoPrep results in a turnkey system that can operate 24/7 over long periods with minimal operator intervention. It is ideal for bioprocess monitoring, cell line development, and media optimization. The system can be shut down manually when necessary.

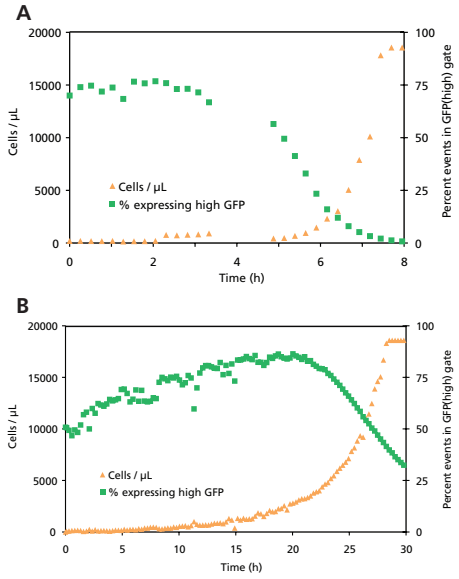


Figure 8. Batch growth and GFP fluorescence of *E. coli* BL21 pRSET-GFP in LB and M9 media.

A 5-mL starter culture of E. coli BL21 cells was grown at 37°C in LB-Amp (1.0% tryptone; 0.5% yeast extract; 1.0% sodium chloride; pH 7; 100 µg/mL ampicillin). Cells were inoculated into a bioreactor as described below and acquired and analyzed on the MSP FlowCytoPrep and BD Accuri C6. Samples were collected for 1.5 minutes at the Medium flow rate (35 µL/min), with an acquisition threshold of FSC-H = 11,000 to exclude debris. GFP fluorescence was detected in FL1 (533/30) using the standard emission filter.

A. *The cultured E. coli cells were inoculated into 500 mL of LB-Amp at approximately 200 cells/µL in a 2-L bioreactor. The culture was grown at 37°C and stirred at 250 RPM while samples were analyzed over time. Cell concentrations increased rapidly toward the end of culturing, while the percentage of GFP⁺ events decreased.*

B. *The cultured E. coli cells were transferred from LB to M9 minimal medium (5 g/L glucose) and grown overnight before similarly inoculating the bioreactor and analyzing samples over time. Cell concentrations increased gradually over time, while the percentage of GFP⁺ events increased, reached a plateau, and then decreased.*

Applications in bioprocessing, biofuels, and bacterial cultures

Researchers at the Biotechnology Institute of the University of Minnesota use the BD Accuri C6/MSP M5000 FlowCytoPrep system in their work on improving monitoring and control of bioprocesses, biofuel development, and cultures of yeast and bacteria. All of these applications use automated flow cytometry to obtain time-series culture samples, which are then used to adjust the culture environment. Sample publication topics to date include process control of CHO cell cultures,[4] effect of growth medium on cell cycle distribution,[5] development of a cytostat to control cell environment and cell growth,[6] and evolution of stress-resistant yeast strains.[7]

For example, researchers at the Institute used the BD Accuri C6/FlowCytoPrep system to assess the kinetics of batch growth and GFP expression of *E. coli* BL21 cells cultured in either nutrient-rich lysogeny broth (LB) or M9 minimal medium. GFP was expressed from the pRSET-GFP expression plasmid, which was propagated in the cells using the ampicillin selectable marker. The MSP FlowCytoPrep acquired and delivered the samples, automatically executing acquisition and cleaning commands on the BD Accuri C6.

Samples were analyzed for exponential growth and GFP expression every 15 minutes following inoculation. Monitoring GFP expression over time allows discrimination between producing and non-producing cells in a fermentation. For example, one can distinguish whether reduced overall production is due to reduced production in every cell of a homogeneous population, or to a subset of low- or non-producers.

As shown in Figure 8A, the concentration of cells (▲) grown in LB expanded from 192 cells/µL at inoculation to 18,557 cells/µL over 8 hours of monitoring. (Beyond this point, cell concentrations in undiluted samples fall outside the linear range of the BD Accuri C6.) Concurrently, GFP expression (■) decreased over time.

In M9 minimal medium (Figure 8B), growth (▲) was much slower than in LB, taking nearly 30 hours to expand from approximately 150 cells/µL to 18,000 cells/µL. However, the GFP⁺ expression phenotype (■) was retained during the early exponential phase (10–20 hours post inoculation); the percentage of GFP⁺ cells increased transiently, only to decrease rapidly later on.[8]

The Institute also uses the BD Accuri C6/FlowCytoPrep system in its effort to improve the process of biofuel production. They focus on isolating and characterizing yeast strains that are resistant to the main inhibitors in existing industrial processes, such as the accumulation of toxic substances like ethanol and acetate. The researchers use a cytostat cultivation method[9] to maintain cultures in very low cell concentrations—100 cells/µL—in a precisely defined environment to evolve high-resistance strains through accelerated natural selection. Specific environments provide the ability to isolate various clones of interest. Glucose, minerals, and other nutrients are kept constant, allowing optimal cell growth and rapid selection of the most robust and fastest-growing cells under industrially challenging conditions.

As with the *E. coli* cultures, the automated system samples the yeast cultures every 15 minutes. The cell counts are used to automatically dilute and maintain the low cell-density culture in the nutrient-rich environment. To track the response to treatments such as ethanol or acetate stress, the samples are stained automatically for viability in the FlowCytoPrep micro-reactor and analyzed on the BD Accuri C6.

The researchers find the automated system a vast improvement over past methods, in which time-consuming serial dilutions were performed at discrete time points in an attempt to maintain cell density. In the old system, cells grown overnight used up the glucose in the medium, transitioned into different metabolic states, and grew more slowly due to nutrient depletion stress. Serial dilution experiments could take up to three weeks to complete, and 15-minute sampling cycles were unrealistic due to time constraints.

The Institute's process advances would not have been possible without the use of automated flow cytometry for time-series analysis. The time-series data is the key to process automation of culture expansion, detailed monitoring of cell cycle and growth, precise control of the cell environment using the cytostat, and rapid evolution of technologically useful strains.

Automated high-throughput screening

The multiparametric, single-cell resolution of flow cytometry can add significant value to biologic drug discovery, development of therapeutic antibodies and vaccines, genotoxicity testing, and other screening applications. However, throughput often does not match the standards set by plate readers and image-based systems. Two instruments from IntelliCyt Corporation—the HTFC® (High-Throughput Flow Cytometry) Screening System* and the iQue™ Screener—integrate a BD Accuri C6 into a high-throughput, flow cytometric screening system. The iQue system, for example, can analyze a 96-well plate in less than 3 minutes or a 384-well plate in less than 12 minutes.

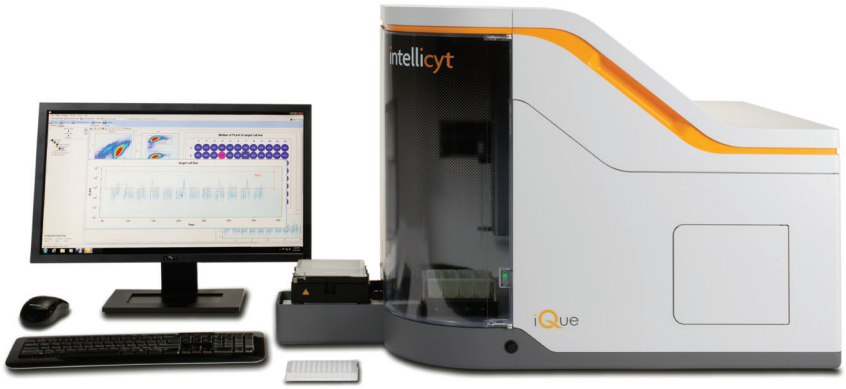


Figure 9. The IntelliCyt iQue Screener (pictured) and HTFC Screening System integrate a BD Accuri C6 to provide high-throughput, flow cytometric screening.

These versatile systems are ideal for cell- and bead-based screening, saving time over plate readers and image-based systems while providing a deeper understanding of data. For example, cell lines in which the cells float in suspension, such as Jurkat cells, cannot be analyzed by imaging systems. However, they can be screened on the HTFC and iQue systems, expanding the breadth of research.

Flow cytometry's ability to multiplex means that researchers can label samples with multiple probes to obtain correlated data for several biomarkers, or even mix cell lines from different species by tagging them with different probes. Up to six measurements can be taken on each cell as it passes through the BD Accuri C6 laser beams.

*As of July 1, 2013, IntelliCyt has replaced the HTFC Screening System with the next-generation iQue Screener.

In the sampler unit, a shaker stand keeps cells in suspension, and an external, low-pulsation, programmable peristaltic pump moves samples from the wells of a microtiter plate, gently pushing the fluid into the BD Accuri C6 SIP. Samples from adjacent wells are separated by an air gap. The resulting meniscus reduces well-to-well carryover so that discrete samples are 98% contained. A rinse station is available if the application requires absolutely no carryover. The user decides which factor—speed or purity—is most important for the application. The non-pressurized fluidics of the BD Accuri C6 eliminate any variability inherent in pressurized systems, increasing the reliability of sample collection.

ForeCyt® screening software, which controls the integrated systems, was designed specifically for large-run cell- and bead-based applications. It receives real-time cytometric information from the BD Accuri C6, providing a seamless, robust interface. Researchers can create assays to meet each experiment's individual criteria. ForeCyt software controls flow speed, core size, rinse cycles, and the number of cells sampled by communicating directly with the BD Accuri C6.

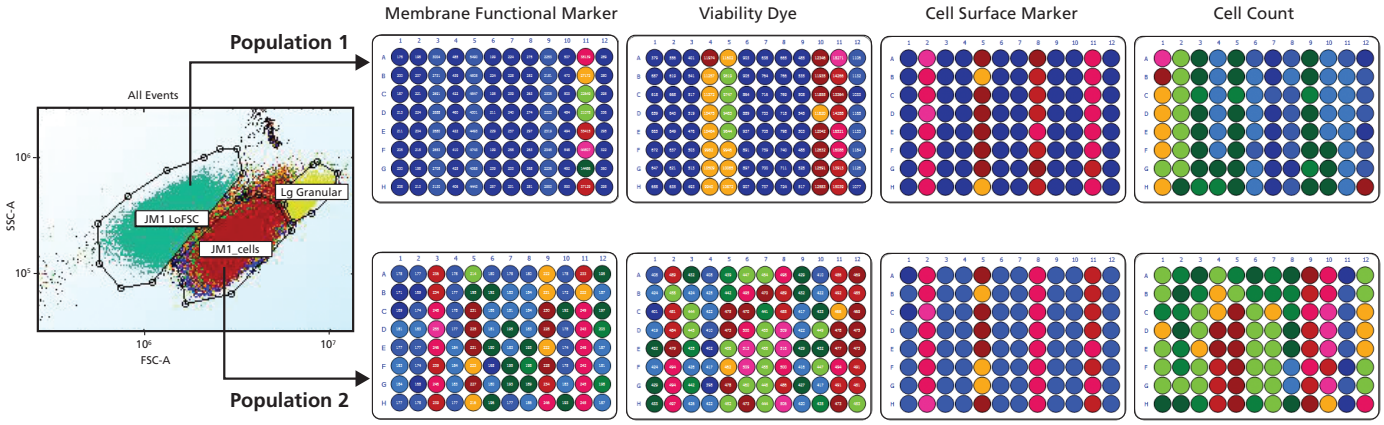


Figure 10. ForeCyt data analysis can generate heatmaps for each subpopulation and each measured parameter.

If desired, IntelliCyt Data Manager (iDM®) informatics software can be used to manage the files and keep the plates in a specific order. For simplicity, iDM puts all the information into one file (rather than one file per well), and sends the files to a data management system or LIMS.

The HTFC and iQue systems inherit the dynamic range and ease of use of the BD Accuri C6, making them particularly attractive to researchers who are new to flow cytometry. If a customer already owns a BD Accuri C6, the flow cytometer can be retrofitted to the HyperCyt® Autosampler, which extends the cytometer's capabilities by reducing sample volume requirements and increasing throughput. The combination enables researchers to process multiple samples or take time-course measurements that were previously impractical.

Applications in site-directed mutagenesis

Gene mutagenesis is a powerful tool for mapping antibody epitopes, functional domains, and optimizing the stability and expression of proteins. However, traditional site-directed mutagenesis methods are laborious and require prior knowledge of important domains in the protein of interest.

In contrast, Shotgun Mutagenesis technology, developed by Integral Molecular, enables rapid and comprehensive analysis of every amino acid in the protein. Using Shotgun Mutagenesis and the HTFC Screening System, Integral Molecular creates alanine-scan libraries in which each cell represents a unique alanine substitution, and all mutants together constitute substitutions for every possible position in a given protein. The workflow, shown in Figure 11, creates a mutation library containing hundreds to thousands of alanine scan mutations, which are then screened. The approach can be used to map epitopes and functional domains of even complex proteins such as G protein-coupled receptors (GPCRs), ion channels, and viral envelopes. The ability to map epitopes efficiently, accurately, and on structurally complex targets is an important advancement for the discovery and development of antibody therapeutics.[10]

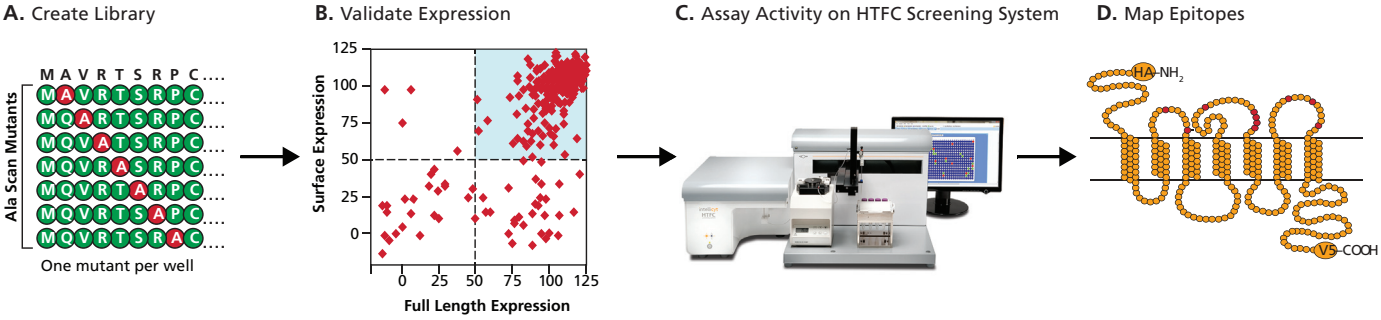


Figure 11. Shotgun Mutagenesis epitope mapping workflow.

A. Shotgun Mutagenesis is used to create custom libraries of hundreds to thousands of alanine-scan mutations containing single substitutions in every possible position. B. Each mutant is validated for full-length and surface expression. C. Mutants are expressed in human cells and then assayed for binding using the HTFC Screening System. Binding results are analyzed and displayed for each individual mutant. D. The collection of critical residues is mapped to the protein structure, highlighting important regions of interest.

Shotgun Mutagenesis begins with the creation of a customized plasmid library for a target gene, each clone in the library bearing a unique amino acid mutation. Clones are individually arrayed in microplates, where they are expressed within living mammalian cells—the best environment to obtain proper folding and post-translational modification. Mutants are simultaneously validated for expression and tested for antibody binding or other functional activities. Because each clone is sequenced at the time of library creation, amino acids critical for the epitope or functional region are readily identified by a loss of reactivity. Critical residues can be mapped onto the protein structure to visualize epitope and functional motifs.

Figure 12 highlights the advantages of HTFC Screening System assays over traditional horseradish peroxidase (HRP)–based assays. Data generated with the HTFC Screening System is higher quality and better correlated than results obtained by HRP immunoluminescence.

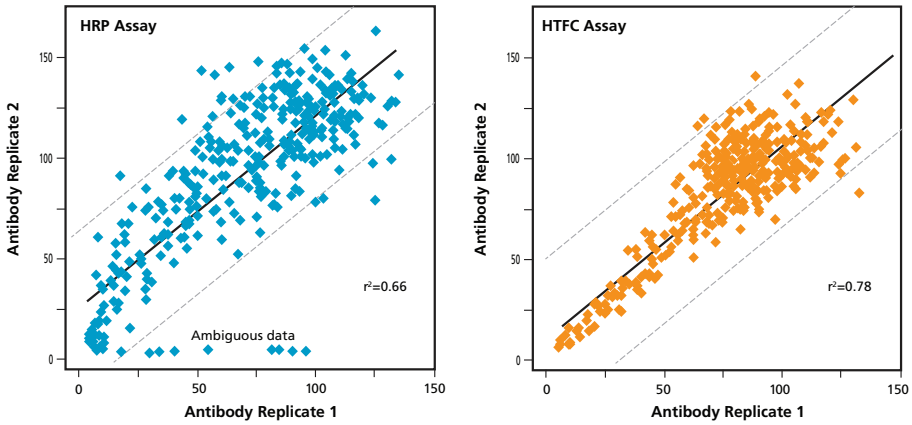


Figure 12. HTFC assays are less variable and more reproducible than traditional assays.

An anti-CXCR2 antibody (MAB331, R&D Systems) was tested for binding against an alanine-scan mutant panel of CXCR2 using ***(A)*** *an HRP immunoluminescence assay and* ***(B)*** *the HTFC Screening System. For the HTFC assays, each mutant was first incubated with a primary antibody, and then with a fluorescently labeled secondary antibody conjugated to Alexa Fluor® 488. Data points represent the percent antibody binding of mutated CXCR2 normalized to wild-type protein for two replicates. Points that lie between the dashed lines are replicates that differ less than ±40% from the linear regression, indicating a reproducible result.*

Results: *The HRP assay showed significant variability between replicates (r^2 = 0.66), with a substantial subset of replicates providing ambiguous data (both strong binding and no binding in one replicate pair, lying near the x-axis). In contrast, the HTFC assay showed less variability (r^2 = 0.78) and no ambiguous replicate pairs, generating a highly reproducible data set and reducing the need for repeat experiments.*

Figure 13 illustrates the epitope mapping process using the CXCR2 antibody library and the HTFC Screening System. For this particular anti-CXCR2 antibody, the four critical antibody binding sites were clustered tightly at a single extracellular domain.

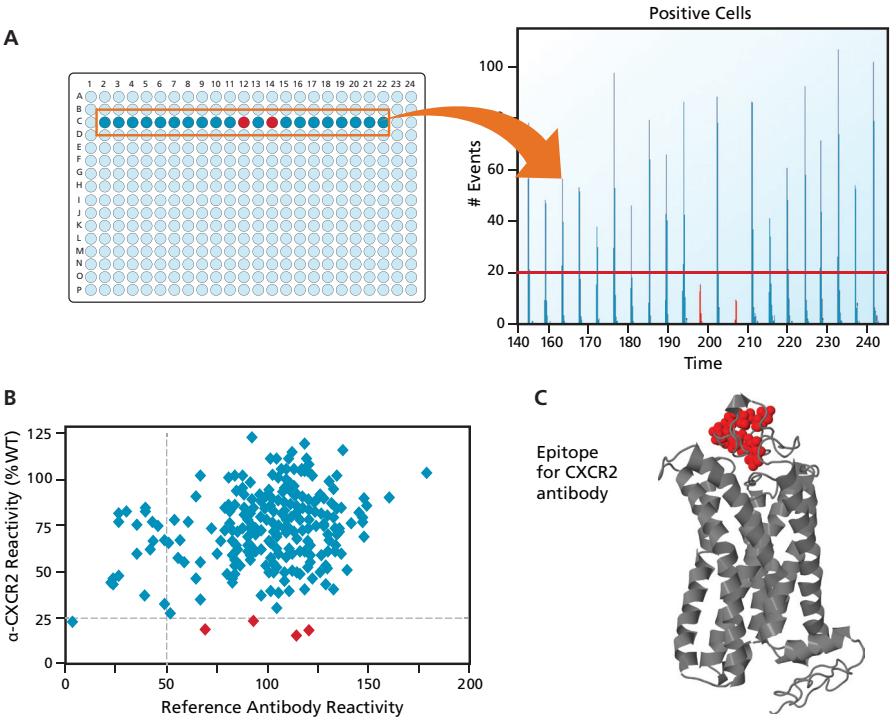


Figure 13. GPCR epitope mapping using the CXCR2 library and the HTFC Screening System.

A. *Every amino acid of CXCR2 was individually replaced by alanine. In total, 357 mutants were created, sequenced, arrayed into a 384-well plate (left), and validated for expression. Screened samples were analyzed using the HTFC Screening System and peaks representing the number of antibody reactive cells in each well were displayed as a function of the time they were sampled (right). Loss of fluorescent signal (two peaks colored red) provides quick visual identification of potentially weak antibody binding reactions.* ***B.*** *After final analysis, mutations at four positions (red data points) showed substantially reduced reactivity compared to a reference polyclonal antibody.* ***C.*** *When mapped to a theoretical 3-dimensional structure of CXCR2, these four positions clustered tightly at a single extracellular domain, highlighting the epitope for the anti-CXCR2 antibody tested in this study.*

Summary and conclusions

Flow cytometry can accelerate discovery by collecting and processing an abundance of data, but the assays often require substantial effort to prepare, collect, and analyze samples. Automation can improve productivity, enhance the quality and reproducibility of data, speed throughput, and enable the generation of data that would not otherwise be obtainable.

This white paper has explored the value of automating flow cytometry by examining three automation platforms that include the BD Accuri C6 personal flow cytometer. At a basic level, the BD CSampler accessory offers automated sample processing with walkaway convenience. Interconnecting the BD Accuri C6 with the MSP M5000 FlowCytoPrep sample preparation system results in a turnkey system for real-time online monitoring of bioreactors, fermenters, and other cell culture applications. Finally, by integrating a BD Accuri C6 into a high-throughput system, the IntelliCyt HTFC Screening System and iQue Screener provide rapid and powerful screening tools for drug discovery, gene mutagenesis, genotoxicity testing, and other applications.

Powerful, compact, cost-effective, and easy to use, the BD Accuri C6 is an excellent choice for automation applications. A non-pressurized fluidics system allows for straightforward connections between the cytometer's SIP and other instruments. An open software platform allows the instrument to respond to commands for all basic functions through a seamless software interface. Whether paired with the BD CSampler, an independent sample preparation accessory, or integrated into a turnkey system, an automated BD Accuri C6 system can save time and effort and enhance research and discovery.

If your application requires even faster throughput or a higher level of automation, BD Biosciences offers a full portfolio of flow cytometry instruments and automation accessories to suit a broad range of needs. Contact your BD instrument specialist or see the instruments and sample preparation options at bdbiosciences.com/instruments.

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