Making Flow Cytometry Personal

Technical Bulletin

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Since its introduction in the 1970s, flow cytometry has become an indispensable tool in a wide range of life sciences applications. The analytical power and versatility of today's laser-based flow cytometers have unlocked the mysteries of cell biology and empowered entirely new fields of research. Flow cytometry has become a staple of modern laboratories around the world.

The BD Accuri™ C6 flow cytometer offers both performance and simplicity. Its compact fit and affordable price bring flow cytometry within reach of a new generation of users. Several design innovations and technical advances—in fluidics, optics, electronics, and software—are key to making the BD Accuri C6 suitable for a wider range of researchers.

Figure 1. The BD Accuri C6 flow cytometer system.

Introduction

Over almost four decades, flow cytometry has become an essential tool for studying the cell cycle, apoptosis, cellular and molecular profiles, acellular molecular detection, cell proliferation, transfection, cell viability, and immunology. Flow cytometry has also supported recent research advances in proteomics, high throughput cell-based drug screening, and the use of biomarkers in drug development.

In flow cytometry, particles or cells are suspended in a liquid stream and flow through a beam of laser light. Optical detectors collect scattered laser light and fluorescence, and electronics measure and digitize these light emissions for analysis on a computer. The light-scatter data provide basic information about the particles or cells, such as size, shape, and surface features. To explore a wide range of other cellular properties, cells are identified with fluorescent tags, such as dye-conjugated antibodies.

The BD Accuri C6 flow cytometer, introduced in 2006, is the first *personal* flow cytometer. It is compact, affordable, simple to operate, easy to maintain, and compatible with standard research laboratory protocols. It detects forward and side scatter as well as up to four fluorescent colors. Designed from the ground up, the system's innovations in fluidics, optics, electronics, and software make it powerful, unique, and easy to use, even for those new to flow cytometry.

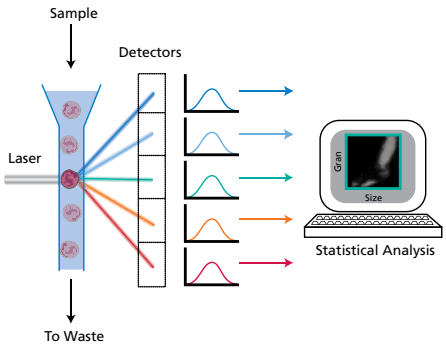

Figure 2. How flow cytometry works.

Figure 3. The BD Accuri C6 cytometer.

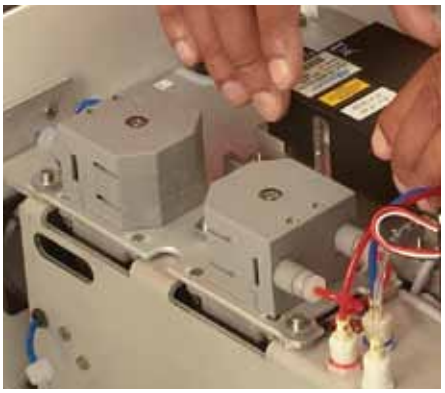

Figure 4. The BD Accuri C6 peristaltic pump system.

Patented pulse dampeners and pressure sensors allow inexpensive peristaltic pumps to provide a non-pressurized, pulse-free, push/pull system with dynamic feedback.

Innovation 1: Reliable, high-performance fluidics

The fluidics in a flow cytometer are designed to move cells or particles efficiently through the flow cell for detection, focusing the fluid stream so that the light can interrogate each cell individually.

The fluidics design of the BD Accuri C6 flow cytometer uses peristaltic pumps to drive the fluid flow. The pumps are compact, inexpensive, and need minimal maintenance. To produce a smooth flow from an inherently pulsating pump, the design team combined new pulse dampeners with a sophisticated, microprocessor-controlled, dynamic feedback system. The resulting flow is sensitive and user-adjustable. With this innovation, the BD Accuri C6 fluidics system starts automatically at power-on and allows precise control of both sheath and sample fluid velocity within extremely tight tolerances.

The BD Accuri C6 fluidics system employs two peristaltic pumps. One pushes sheath fluid into the flow cell while the other pulls the combined sample and sheath fluid from the flow cell to the waste tank. The differential between the two pump pressures creates suction, which draws up the sample. The push/pull design allows for independent adjustment of both the sheath and sample flow speeds, enabling precise control of the sample core diameter. If desired, users can quickly fine-tune the fluidics for each individual sample.

Because the direct-drive pumps can meter the sample fluid uptake, the BD Accuri C6 can automatically calculate the number of events detected per µL for each run, providing both cell counts and concentration. The design also means that clogs can easily be pushed out of the flow cell with a brief burst of sheath fluid. Most clogs (such as cell clumps) occur in the Sample Injection Probe (SIP) rather than the interior fluidics lines.

The BD Accuri C6 can accept various types of sample tubes, including 12 x 75-mm tubes and capless microcentrifuge tubes. Because the fluidics system is non-pressurized, the BD Accuri C6 can aspirate a sample from a cracked tube.

Figure 5. The BD Accuri C6 fluidics tanks.

The instrument includes four non-pressurized fluid tanks: sheath, waste, cleaner, and decontamination solutions. Sensors automatically alert users when a tank needs to be filled or emptied. Sample is drawn through the SIP into the flow cell, where the sample fluid is focused using the hydrodynamic force applied by the sheath flow.

To reduce maintenance, the BD Accuri C6 is programmed to decontaminate the fluidics system automatically. At power off, the instrument flushes the fluidics lines with decontamination and cleaning solutions. When next powered on, it flushes and primes the fluidics lines with sheath fluid in readiness for the first sample.

In summary, the high-performance BD Accuri C6 fluidics system:

1. Automatically starts up with the press of a single button.
2. Self-cleans and alerts when fluids need attention.
3. Is compatible with a variety of sample-tube configurations.
4. Meters sample fluid uptake and automatically calculates cell counts and concentrations per µL.
5. Allows the user to independently adjust the speed of the sample uptake.
6. Allows the user to adjust the core stream diameter for a range of cell sizes.
7. Minimizes the number of components, reducing footprint and cost.

Figure 6. The BD Accuri C6 optics system.
Clustered in a "pie" design around the flow cell, the photomultiplier tubes maximize light collection and reduce alignment issues.

Innovation 2: Economical, robust optics

The optics system in a flow cytometer focuses laser light on the stream within the flow cell and detects light scatter and fluorescence.

The BD Accuri C6 flow cytometer optics system is a model of simplicity. Several innovations allowed the design team to shorten and stabilize the light path, reducing cost and service requirements.

The instrument employs two solid-state lasers, emitting wavelengths of 488 and 640 nm. Multi-section focusing and gross-collection lenses increase signal strength without using mirrors. Interference filters are easy to access and swap. The fixed-alignment optic components are resistant to jarring and bumping, reducing alignment issues and service calls. This makes the instrument transportable; it can be moved from one site to another or used in the field without realignment.

The BD Accuri C6 optics system detects light directly. The laser beams, arranged co-linearly, hit the flow cell at the same point, but the red laser is modulated, allowing separation of signals from each beam. Photomultiplier tube (PMT) modules are clustered in a pie-shaped design around the flow cell to maximize light collection and reduce alignment issues. These detectors, focused on a single spot, sample data only when the specified laser combination excites the sample. Thus, all four fluorescence detectors can read from either laser. The fully digital system employs a 24-bit analog-to-digital converter (ADC) to convert the PMT signals.

In summary, the reliable BD Accuri C6 optics system:

1. Is robust and withstands jostling, making the instrument easy to transport.
2. Allows the user to swap out interference filters easily.
3. Requires no user alignment.
4. Minimizes the number of components, reducing footprint and cost.

Innovation 3: Sophisticated, simplified electronics

Electronics are the nerve system of a flow cytometer, managing communications between subsystems. For example, digital signal processing (DSP) and analog-to-digital conversion (ADC) subsystems determine an instrument's dynamic range, from faint to bright.

By using state-of-the-art 24-bit ADC chips and advanced electronic filtering, the BD Accuri C6 flow cytometer can simultaneously collect 16 million channels of digital data, displayed as seven decades. This means that it can finely resolve both faint and bright signals at once—especially useful for certain biological experiments that return signals spanning more than five decades. This allows the BD Accuri C6 to analyze a wide variety of samples, from dim, micron-sized platelets through large, >30-micron, highly fluorescent cell lines. In rare cases when the fluorescence is off scale, such as some cell lines transfected with green fluorescent protein, easily inserted attenuation filters bring the signals back on scale while maintaining operation of the detectors within their linear range.

The BD Accuri C6 detects this broad dynamic range using standard factory detector settings. Users do not need to set PMT voltage and amplifier gains to focus on the high or low end. Data can be collected first and analyzed later, without concern over lost data due to improper PMT settings. New users can collect data using a simple, preconfigured template, and the data can be analyzed later with more expert help. The data can even be re-analyzed at any time if gating or compensation errors are discovered, or in light of new research findings.

Software tools aid researchers in analyzing such a broad spectrum of data. For example, a Zoom tool allows researchers to focus (and adjust gates) on very small areas of data display. The VirtualGain™ function, which can realign the data distribution of any parameter to a chosen channel, mimics manual gain adjustment and is useful for comparing DNA distributions.

To handle communications between the cytometer and computer, the BD Accuri C6 supports plug-and-play connections using USB ports. This electronics industry standard helps to reduce complexity, cost, and size.

In summary, the sophisticated BD Accuri C6 electronics system:

1. Provides seven full decades of dynamic range.
2. Eliminates the need to adjust voltage and amplifier gain settings.
3. Provides software tools to help analyze the broad spectrum of data.
4. Supports USB plug-and-play on a standard PC or laptop.
5. Minimizes the number of components, reducing footprint and cost.

Innovation 4: Intuitive software

BD Accuri™ C6 software was designed with speed of learning and ease of use in mind, based on hundreds of hours observing researchers using flow cytometers.

For example, tabbed panels facilitate navigation. Data is acquired from the Collect tab, where users can create and manage density and dot plots, histograms, and statistics. On the Analyze tab, users can create color histogram overlays, print multiple plots, compare samples, and use the Zoom tool to magnify areas of data. Finally, on the Statistics tab, data is displayed in a master table, and statistics can easily be calculated, copied, and pasted into spreadsheets to facilitate reporting.

On each tab, users will find tools and utilities where they expect them to be. On the Collect tab, for example, each plot contains its own gating and display tools.

As a result, BD Accuri C6 software is intuitive to use. New users often become comfortable with BD Accuri C6 software in less than 30 minutes, assisted only by a 3-page pictorial *Quick Start Guide*. The software can be used on most standard PCs and laptops, and data plots and files can be exported in FCS 3.0 format for seamless import into flow cytometry analysis programs including FCS Express and FlowJo™. Plots can also be dragged and dropped into Microsoft® Office applications such as PowerPoint®.

In summary, the intuitive BD Accuri C6 software:

1. Can be used on any standard PC or laptop with 2 GB of RAM.
2. Is intuitive to both novice and proficient users, even without training classes.
3. Produces sophisticated graphics such as density plots and color histogram overlays.
4. Uses the FCS 3.0 file format for ready compatibility with other software.
5. Allows users to drag and drop plot images into Microsoft Office applications and to copy and paste statistics to spreadsheet programs.

Figure 7. The BD Accuri C6 software user interface.

Collect, Analyze, and Statistics tabs make it easy to navigate between tasks. Plots (upper right panel) include their own gating and display tools.

A truly personal flow cytometer

The innovations in fluidics, optics, electronics, and software discussed in this technical bulletin have opened up new opportunities to use flow cytometry. Accessible to novice and expert users alike, the BD Accuri C6 flow cytometer fits comfortably in any life sciences lab.

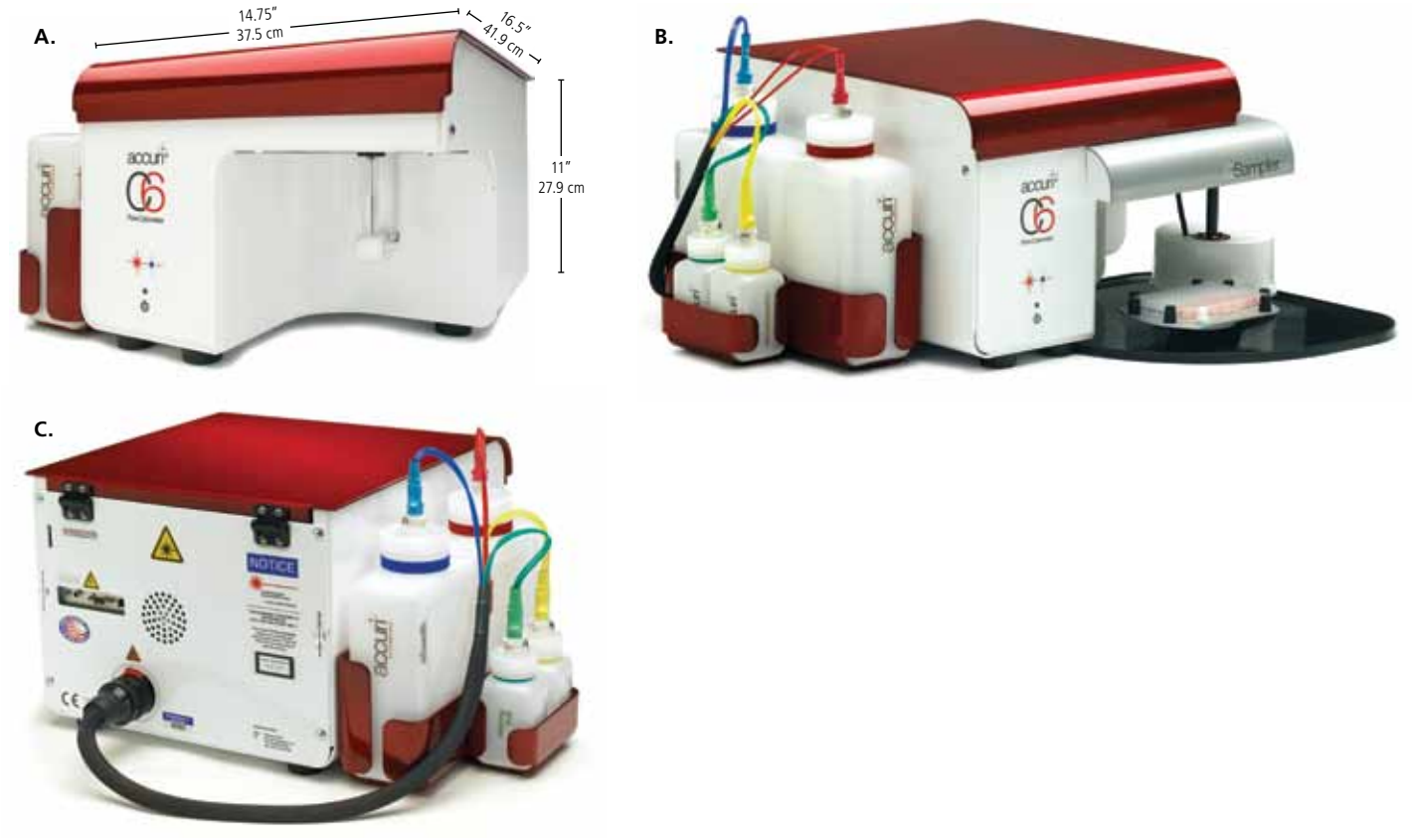

Figure 8. Three views of the BD Accuri C6 cytometer.

A. Instrument dimensions. B. The instrument with the BD CSampler™ accessory. The added automation 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. C. Reverse view, showing the BD Accuri C6 cytometer's effortless connection to fluid tanks.

The BD Accuri C6 expands the power of flow cytometry not only to a wider range of life scientists, but also to scientific disciplines that have not historically used it, such as environmental science, microbiology, and marine biology. The first personal flow cytometer gives scientists an essential tool to accelerate their research goals. The BD Accuri C6 flow cytometer truly brings *flow cytometry within reach*™.

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