



Automation of High-Throughput Flow Cytometry with the ZE5 Cell Analyzer

Integration of flow cytometers into automated workcells increases throughput, enables 24/7 operation, and provides consistent results. With hardware designed specifically to manage high-throughput sample handling and analysis, the ZE5 Cell Analyzer is robust and automation friendly. Moreover, the ZE5 Cell Analyzer user interface (Everest Software) and application program interface (API) are platform-agnostic and work with most scheduling software. Past successful integrations have been with automation products from Biosero, Inc., Peak Analysis & Automation (PAA), Hamilton Company, HighRes Biosolutions, Retisoft, Inc., Beckman Coulter, Inc., Propel Labs, PerkinElmer, Inc., and Sunset Resources Group LLC. This allows for flexibility and an intuitive user interface that takes you from simple walk-up operation to complex workcell setups. Bio-Rad works closely with each partner and their customers to deliver automated, high-throughput flow cytometry for almost any need.

What Can Be Accomplished through Automated High-Throughput Flow Cytometry

Historically, limited color detection, large sample volumes, and manual loading were the reality for flow cytometers. Modern flow

cytometers have dramatically increased sampling capacity while decreasing the amount of sample needed for analysis. Today's high-parameter cytometers detect dozens of colors/markers simultaneously, which saves precious samples and speeds up processing time. Now an entire plate of 384 samples can be analyzed in less than 60 minutes.

When throughput and reproducibility need to be taken to the next level, automation is the answer. A reliable robotic arm can load and unload the flow cytometer continuously, reducing downtime while maximizing the productivity of a busy laboratory. A core lab that recently integrated the ZE5 Cell Analyzer into an automated workcell sees no time wasted between experiments. Scientists load all of their plates onto a plate hotel in the morning, and the robotic arm loads a new plate automatically right after each experiment ends.

Sample preparation can be integrated into a workcell with liquid handlers and timed incubation, which generates reliable, consistent data. Instruments capable of 24/7 operation can dramatically increase assay throughput, enabling scale-up to meet the needs of high-priority projects.

Features and Applications of the ZE5 Cell Analyzer

The ZE5 Cell Analyzer was built for high-throughput analysis with a focus on speed, flexibility, high-parameter analysis, and automation. The integrated sample loader is able to accommodate tube racks, 96-well plates, or 384-well plates. Screening is fast, with the ability to analyze up to 100,000 events per second. In high-throughput mode, a 96-well plate can be analyzed in less than 15 minutes and a 384-well plate in less than 60 minutes. In addition to its speed, the ZE5 Cell Analyzer can accommodate up to five lasers and 30 detectors, giving it the ability to simultaneously detect 27 fluorescence parameters. Consideration for sample integrity is also built in, with temperature control and vortexing available to keep cells viable and uniform during sampling. The ZE5 Cell Analyzer features flexible capabilities for use in a wide range of applications that can be improved with high-throughput automation.



The ZE5 Cell Analyzer integrated into the Biosero Automated Plate Handling System.

Range of Applications for High-Throughput Flow Cytometry

- Antibody screening
- Immunophenotyping and immunoassays
- Phenotypic drug discovery
- Functional assays
- Target screening
- Receptor binding assays
- Protein-protein interactions
- Toxicity assays
- Hybridoma screening

The Automated ZE5 Cell Analyzer Dramatically Increases Throughput of Protein Interaction Screening

User: Nonprofit research institute

Read-out: FRET assay for protein self-assembly and interaction

Old setup: Imaging flow system — 8 hr to analyze 96-well plate with ~20,000 gated cells

New setup: ZE5 Cell Analyzer integrated into a turnkey automation workcell — just minutes to analyze 96-well plate; up to 20 samples per minute; 100-fold throughput increase

The Automated ZE5 Cell Analyzer and Barcode Multiplexing Enable Large-Scale Drug Screening

User: Leading pharma company

Read-out: Receptor binding assay

Setup: ZE5 Cell Analyzer integrated into custom automation workcell; 24/7 operation

Application: Combined automated high-throughput flow cytometry and multiplexed barcoding assays enable large-scale screening; up to 16 cell lines in a single sample; >30,000 assays completed per day

Considerations for Automating Flow Cytometry

When deciding which steps in a process to automate, identify the steps of the workflow that are slowing time to results, such as cost, space, equipment, or staffing. Consider partial or total automation of the workflow, keeping the final goals in mind. Carefully map out steps to be automated, considering each critical detail, including timing, temperature, mixing, and reagent volumes.

Staff/Training	Equipment
Even for complex workcells, intuitive scheduling software can make an automated system user friendly, requiring little training. However, training is still vital to success for an automated flow cytometry lab, so choosing an integration company, such as Biosero, PAA, HighRes Biosolutions, or Retisoft, that provides ample training for its users is key. Some partners offer three levels of training to enable lab staff to create their own protocols, execute layout changes, install new instruments, and develop advanced scripts.	Instruments with agnostic APIs, such as the ZE5 Cell Analyzer, are able to easily connect with any scheduling software. The typical workcell might include the ZE5 Cell Analyzer, a robotic arm, and plate hotels. Larger workcells might also include incubators and liquid handlers to store and prepare samples and assays. It is not unusual to start with a smaller workcell and then add components as needs change over time.
Space	Costs
The flexibility of modern robotics provides space efficiency, allowing workcells to be built in compact spaces. The ZE5 Cell Analyzer has internal, hot-swappable fluidics tanks to minimize the space needed. Larger external tanks with run times up to 24 hours are available as well. If steps performed with stationary equipment located in various rooms of a lab need to be automated, robots are able to travel from room to room, fitting into any assay workflow.	Automation saves time, increases productivity, reduces waste, and cuts downtime. When setting a budget for automation and integration, consider how those savings or increased revenue over many years compares to the initial cost of purchasing and integrating the components. It is also vital to consider the ongoing cost of service and maintenance.

Three Ways to High-Throughput Flow Cytometry

The ZE5 Cell Analyzer was designed with automation in mind, from simple plate loading systems to complex custom workcells. The ZE5 Cell Analyzer's software-agnostic API is the key to making integration with any existing or new automation system simple and straightforward. Whether using an internal automation team, choosing an off-the-shelf system, or working with an external integration service provider, the ZE5 Cell Analyzer's API allows for instrument control, communication with other instruments, and specified data storage and analysis.

Existing Workcell Integrating the ZE5 Cell Analyzer

For labs that have an existing automated flow cytometry workcell but want to upgrade the flow cytometer for higher throughput, more parameters, or faster analysis, the ZE5 Cell Analyzer allows for easy integration. An internal automation team or a service provider can easily write new drivers to enable the scheduling software to control the instrument. From there, the ZE5 Cell Analyzer instantly becomes part of the workcell and replaces the outdated flow cytometer.

Off-the-Shelf Automation System Integrating the ZE5 Cell Analyzer

A relatively easy way to get up and running with automated flow cytometry is to purchase a new off-the-shelf system like the one from Biosero. The advantage of off-the-shelf systems is that all of the components, including the ZE5 Cell Analyzer, are pretested and optimized to work together, making setup quick and easy. However, these systems can be limited to the components that have already been integrated, so it is important to know up front that the needs of the lab match the features and capabilities of the system.



The intuitive user interface of Green Button Go Scheduling Software.

interface with the ZE5 Cell Analyzer, virtual simulations can be run to ensure the workflow setup will meet productivity expectations. Biosero's Green Button Go Software, for example, coordinates all the work, and the ZE5 Cell Analyzer can be run with little to no operator intervention. The ZE5 Cell Analyzer offers automatic probe washing, probe crash detection, sample temperature control, and large-capacity fluidics tanks, all desirable features in high-throughput analysis. These features ensure that the workcell is efficiently producing data and not wasting precious resources.

Optimize High-Throughput Workflow and Data Analysis

Moving to a high-throughput process requires adjusting the manual workflow and data analysis to accommodate a multitude of samples. To get the desired throughput and to generate actionable results, it is critical to optimize the workflow and create automated scripts to speed up data analysis.

High-Throughput Workflow Optimization

For complex protocols, lab scientists and software engineers need to work closely together to develop workflows that will achieve the expected or desired results.

High-Throughput Data Analysis

High-throughput data require high-throughput data analysis. The ZE5 Cell Analyzer's Everest Software provides versatile and customizable data visualization, analysis, and export functions that meet basic analysis needs, and every ZE5 Cell Analyzer purchase includes De Novo's FCS Express Software for advanced needs. Custom scripts can be generated to analyze data in a user-friendly format. Customization will depend on the assay and desired result layouts. All automation partners have software that can direct data generated from the ZE5 Cell Analyzer to any location desired by the user, such as the cloud or a network server. Data from each sample analyzed on the ZE5 Cell Analyzer are immediately available for analysis, giving users the ability to view results in real time for rapid decision making.



Automation Solution from Retisoft integrated with a ZE5 Cell Analyzer.

Custom Workcell Integrating the ZE5 Cell Analyzer

Because of the agnostic API, the ZE5 Cell Analyzer can easily integrate into any custom workcell. Simple workcells can initially be built and new components added as needs change. Custom workcells require the use of scheduling software that integrates each piece of equipment in the workcell and provides the user interface. Ease of use, system support, and software flexibility vary greatly among scheduling software providers and should be carefully considered.

Liquid handling stations, centrifuges, incubators, refrigerators, plate hotels, and more can be integrated with the right software and API. Through the ZE5 Cell Analyzer's API, integrators are able to customize their scheduling software to control the instrument.

All Bio-Rad partners offer close support and expertise during a workcell design process, delivering optimized systems even for complex assays. Once a workflow is created in a scheduling software

Continued Support and Maintenance

Ongoing customer support is a commitment taken seriously by both Bio-Rad and all of its automation partners throughout the installation, integration, and ongoing use of their automated flow cytometry products.

Bio-Rad ZE5 Cell Analyzer Support and Built-In Maintenance Features

- User events hosted by Bio-Rad for additional training and information
- Open line of communication between Bio-Rad and any automation service provider
- Engineers available to help with integration when needed
- Built-in unclog process to clean the sample line and probe when a clog is detected
- Automatic cleaning of the sample path at shutdown to prevent contamination
- Automatic probe crash detection to prevent probe damage

Automation and Integration Products from Our Partners

Biosero Automation Software

- Global support network for international customers to provide onsite and call-in customer support
- Courses available for basic, advanced, and developer-level customers
- First year of support included with custom automation with additional plans or hourly service available
- Unique error handling to eliminate run abandonment and restart; Green Button Go Software's Pause mode stops the system if an instrument fails and resumes seamlessly without any loss of data

PAA Workcell Products

- Global support network for international customers to provide onsite and call-in customer support
- First year of support included with custom automation with additional plans or hourly service available
- Range of automation solutions for 1, 2, or many instruments integrated with room temperature or incubated storage

Retisoft Lab Automation Solutions

- Flexible, reconfigurable, and functional designs providing consistent sample handling for reproducible results
- Powered by Genera, a true dynamic scheduler. Genera's open architecture allows easy customization of your workflow
- Seamless integration with other processes such as upstream liquid handling or downstream data handling into your laboratory information management system (LIMS)
- 100% backed up by the service and support team. Includes 1 year full technical support

HighRes Biosolutions Laboratory Automation Systems

- Customized integration of complete automation workflows tailored to your science
- Flexible automation through the use of the MicroDock and MicroCart technology to evolve and scale your automation over time as your science changes
- Easy method creation, simulation, and execution in the dynamic scheduler, Cellario Software
- Robust, open API architecture for seamless integration with other data systems, such as LIMS and downstream analysis

Visit bio-rad.com/HTFlow for more information on high-throughput flow cytometry and to talk with a specialist.

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