A screening flow cytometer without limits.

Break bottlenecks with the ZE5 Cell Analyzer

You've waited long enough for a screening flow cytometer built for automated, high-parameter screening assays. The ZE5 Cell Analyzer is the only screening instrument with the ideal combination of high sample throughput, smart sample handling, and superior speed, resolution, and sensitivity to meet your drug discovery needs. Its application programming interface (API) facilitates integration into any workcell, so you can power through screening campaigns faster and with even larger panels than ever before.

Explore robotics-ready flow cytometry at bio-rad.com/HTFlow

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Cell proliferation is a complex biological process that involves DNA synthesis, growth, and cell division to expand a population of cells. As a core component of normal tissue development and homeostasis, cell growth is monitored to examine the effects of external or internal stimuli on cellular fitness. For example, developmental biologists monitor cell growth to identify stem cells, cancer researchers analyze cell proliferation to determine a cell's ability to divide uncontrollably, and immunologists assess B and T cell division to determine the extent of an immune response. Cell proliferation assays are also inherent components of the drug discovery pipeline to test a therapeutic candidate's effects on cellular fitness and cell division as part of a toxicity screen, to identify molecules that slow tumor growth, or to assess T cell clonal expansion following activation.

Monitoring Cell Growth

Various cell proliferation assays accommodate in vivo or in vitro analyses at a single or multiple time point(s), in real time, or as an endpoint measurement. Some assays quantify cell cycle–associated proteins to determine the percentage of cells in the various stages of cell growth. Others use cell-permeable, fluorescent dyes, such as CytoTrack Dyes, for live-cell analysis. Because these dyes are diluted when a cell divides, individual generations are identified based on fluorescence intensity. In general, assay choice depends on sample type, experimental goals, and available resources.

One of the most accurate and reliable cell proliferation assays involves monitoring DNA replication with nucleotide-mimicking synthetic compounds, such as the thymidine analog bromodeoxyuridine (BrdU), that are incorporated into nascent DNA. Because nucleotides are highly conserved molecules, BrdU efficiently labels dividing cells in a variety of organisms ranging from plants to mammals. In addition, the compound is well suited for both in vitro and in vivo experiments, providing researchers with the flexibility to adjust the assay to best fit their needs. To label proliferating cells, BrdU is added to the cell culture medium or directly injected into a model organism of choice and incubated for a set time depending on the specimen's cell proliferation rate. Next, specific antibodies are used to label incorporated BrdU, followed by immunohistochemistry, ELISAs, or flow cytometry to detect and quantify newly emerged cells.

Gearing up with Flow Cytometry

A flow cytometer's multiparametric nature allows researchers to simultaneously interrogate cells for the presence of multiple specific markers or antigens. For BrdU-based experiments, cell division rates among specific subsets of the total population can be determined. How many fluorophores a flow cytometer can detect depends on its configuration: the more lasers and detectors, the higher the instrument's multiparametric ability. Powerful flow cytometers, such as Bio-Rad's ZE5 Cell Analyzer, can characterize many antigens and markers simultaneously.

Successful flow cytometry–based BrdU assays require planning and optimization. First, DNA-labeling dyes should be titrated to the highest level that does not affect cell function or viability. Given its short half-life at 4°C, freshly prepared BrdU should be used for all experiments. Next, it is important to select a sensitive, specific anti-BrdU antibody that works for the intended assay. Bio-Rad offers a range of PrecisionAb Monoclonal Anti-BrdU Antibodies that are extensively validated for use in a variety of model organisms and applications, including flow cytometry, immunofluorescence, and immunohistochemistry. Testing several antibodies and selecting the best antibody for the intended assay saves time and ensures data accuracy. Antibody-based BrdU detection protocols include a DNA denaturation step that enables antibodies to bind DNA. Because such denaturation steps may alter protein structure, acid concentrations, temperature, incubation time, and washes should be tested for the conditions that provide optimal detection of all antigens. Finally, it is important to include appropriate positive and negative controls to properly interpret results. Incorporating these tips will facilitate flow cytometry–based cell proliferation assay optimization and ensure robust data generation.

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Scientists most commonly use flow cytometry to identify cells and quantify the types of cells present in a heterogeneous mixture such as blood. For these immunophenotyping experiments, samples are incubated with fluorescently-labeled antibodies that recognize cell type–specific proteins and detect their presence or absence. Ideal cell markers uniquely label a cell type, but these kinds of proteins are difficult to identify because most have broader expression patterns. Therefore, several markers are typically analyzed at once to identify a cell type of interest from a heterogeneous population. In addition, flow cytometers that are equipped with cell sorting technology allow isolation and further characterization of a cell population.

**Laser Focus on Cancer**

Immunophenotyping is indispensable for translational and preclinical studies to develop cancer therapies, including immunotherapy and hematopoietic stem cell (HSC) transfer. For example, flow cytometry enables the analysis of immunotherapy resistance mechanisms such as antigen escape — the reduced expression of antigens recognized by chimeric antigen receptor (CAR) T cells on the surface of target cells. Patients with refractory multiple myeloma (MM), a blood cancer that originates in plasma cells, respond well to immunotherapy with B cell maturation antigen (BCMA)-targeting CAR T cells, but typically develop resistance over time.

To study antigen escape mechanisms in vitro, Martin Kampmann’s team at the University of California, San Francisco performed a CRISPR screen to alter gene expression in MM. The researchers next cultured these cells with BCMA-targeting CAR T cells and used flow cytometry to isolate MM cells with altered BCMA expression, revealing several molecular immunotherapy resistance mechanisms that could be targeted pharmacologically.

When targeting cancer cells, it may be safer and more effective to direct CAR T cells to an antigen not expressed by healthy cells. Here, flow cytometry is used to isolate cancer cells, profile them, and develop strategies to bypass CAR T cell toxicity. CD33-targeting CAR T cell therapy is a promising acute myeloid leukemia (AML) treatment. Unfortunately, HSCs also express CD33, so the treatment targets both cancer and stem cells. To overcome this issue, Siddhartha Mukherjee and colleagues at Columbia University Irving Medical Center in New York deleted CD33 in wildtype HSCs and showed that these cells still properly engraft to repopulate a functional hematopoietic system in mice.

Culturing CD33-ablated HSCs with AML cells and CAR T cells specifically cleared leukemic cells without suppressing blood cell production, revealing the potential of preceding targeted immunotherapy with a genetically engineered HSC transfer to improve treatment safety.

HSC transplantation is another effective treatment for blood cancers. The HSC isolation process typically yields few HSCs, because they are retained in the bone marrow through a mechanism that involves CXC chemokine receptor 4 (CXCR4). To optimize it, Ziwei Huang’s team at Tsinghua University in Beijing, China, developed a small molecule CXCR4 inhibitor and used a ZE5 Cell Analyzer to show that this molecule rapidly and potently mobilizes HSCs into the blood of both mice and monkeys.

**The More (Parameters), the Merrier**

Whether to increase analysis speed or decrease sample requirements, scientists strive to optimize methods and protocols so that they can analyze more variables with less input material. While the first flow cytometer analyzed one parameter per cell, you can now routinely analyze ten fluorescent parameters at once. Bio-Rad’s ZE5 Cell Analyzer further increases a flow cytometer’s multiparametric potential. Equipped with five lasers and up to 30 detectors, this instrument simultaneously detects 27 fluorescent parameters at up to 100,000 events per second.

Multiparametric experiments require diligent experimental design because of the need for antibody-fluorophore combinations with minimal spectral overlap and optimal population resolution. To facilitate multicolor panel design, Bio-Rad designed StarBright Dyes, fluorescent nanoparticles with narrow excitation and emission windows that minimize signal spillover into other channels. In addition, StarBright Dyes are exceptionally bright, which improves low abundance antigen detection and rare cell identification. Finally, these dyes are compatible with virtually any buffer, staining protocol, and flow cytometer, which greatly facilitates multiparametric flow cytometry experimental design. When StarBright Dyes are combined with a ZE5 Cell Analyzer, complex cell populations can be identified and profiled at an exceptional speed and depth.

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AUTOMATING FLOW CYTOMETRY FOR HIGH-THROUGHPUT SCREENS

High-throughput (HT), cell-based screens have transformed how scientists identify genetic and pharmacological factors that modify disease pathways. This is especially true for drug discovery programs, where compound libraries are routinely screened for their effects on a cellular process. Because such compound libraries contain tens to hundreds of thousands of candidate molecules, effective candidate selection and safety studies require automated, HT technologies.

Flow cytometry’s ability to analyze multiple parameters at once makes it a desirable technology to integrate into HT drug discovery workflows. Until recently, flow cytometers were unable to handle large numbers of samples, restricting the instrument’s use in pharmaceutical lead characterization.1 To expand the technology’s ease of use and applicability, flow cytometers were developed with automated plate-based sampling and data analysis technologies.2 HT flow cytometers are now integral parts of drug discovery workflows, including target identification and validation, lead and candidate selection, and safety studies.

Sampling Like a Pro
Compared to traditional flow cytometers, where scientists manually change and load sample tubes, HT flow cytometers use a sample handler to deliver a constant stream of samples taken directly from multiwell plates and separated by small air bubbles.1,2 To prevent carryover of cells, HT flow cytometers should wash aspiration probes and tubing between wells. Depending on the technology, such washes can substantially lengthen a plate’s processing time. One way to minimize time lost between samples is to integrate a washing station in the sampling head. The ZE5 Cell Analyzer contains a flying collar wash station that surrounds the aspiration probe and cleans it in place, avoiding travel between the multiwell plate and washing station. In HT mode, the ZE5 Cell Analyzer’s sampling pump draws small amounts of air and wash fluid between samples to separate them and simultaneously clean the sampling lines to maximize processing speed and minimize clog formation.3,4,5 The incorporation of a vortexer into the instrument’s sampling station further reduces clogs because it resuspends cells prior to aspiration.4

Sample integrity is another important factor to consider for HT flow cytometers. Incubation settings vary depending on the nature of the screen and cell type, and suboptimal conditions will affect data integrity. Combined with an incubator and automation-ready platform and software, samples can be stored and resuspended prior to analysis with the ZE5 Cell Analyzer.4,5

A Need for Automation
To analyze the effects of hundreds of thousands of compounds on cell function, HT flow cytometers need to automate as many elements of the screening process as possible. For example, HT ZE5 Cell Analyzer workstations contain robotic arms that travel between the sampling station and a plate hotel to automatically exchange multiwell plates. Liquid handlers and centrifuges can also be added to workstations for automated sample preparation, further reducing downtime between experiments. Combined with ZE5 Cell Analyzer’s automation-ready software and a barcode scanner that confirms plate identity prior to analysis, scientists can schedule and run analyses around the clock. Finally, the instrument contains onboard quality control (QC) calibration beads that are automatically vortexed and analyzed, with QC results plotted daily to monitor trends and allow timely intervention when issues arise.3,4

The Future Is (Star)Bright
The most informative screens simultaneously analyze numerous parameters to profile manipulated cells. Such multiparametric experiments require an array of bright, specific antibodies in diverse colors to distinguish between antigens. To facilitate multicolor panel design, Bio-Rad designed StarBright Dyes, exceptionally bright dyes with narrow excitation and emission profiles that reduce spillover between channels to improve data resolution.6 As they work in virtually any buffer and staining protocol, these dyes easily fit into existing multicolor assays. Staining solutions that contain StarBright Dyes are also stable; they can be premixed up to 28 days in advance without any loss of performance.7 Next, researchers need HT flow cytometers that can detect a wide range of colors. The ZE5 Cell Analyzer can detect 27 fluorescence parameters at once, including up to 7 colors of UV-excitable dyes.1 In addition, the instrument’s high-speed flow cell and fast electronics enable stream velocities of 8 m/sec to scan a 384-well plate in less than 60 minutes.4,5 Therefore, multiparametric, HT flow cytometry screens can be expanded and accelerated with StarBright Dyes and a ZE5 Cell Analyzer.

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Accelerate High-Throughput Screens with Flow Cytometry

High-throughput screens that aim to identify biomarkers, therapeutic targets, or hits from extensive libraries are powerful components of the drug discovery workflow. Early flow cytometers lacked the speed and processing power to efficiently analyze thousands of samples. Several technological advances significantly increased these instruments’ throughput, and flow cytometers are now incorporated in almost every stage of the drug development process.

The ZE5 Cell Analyzer Meets Every High-Throughput Need

- Automation-ready software
- High-speed flow cell
- Superior optics
- Sample integrity protection
- Flying collar washing station
- Automated QC

Automating the Entire Process

The ZE5 Cell Analyzer is automated with a robotic arm that moves multiwell plates between a plate hotel, lid removal station, barcode scanner (to confirm plate identity), and the flow cytometer. In addition, liquid handlers and centrifuges can be added to workstations for automated sample preparation prior to analysis.

Expand Fluorescent Horizons with StarBright Dyes

- Narrow excitation and emission profiles minimize spillover
- Exceptional brightness helps detect low abundance antigens
- Premixing ability without loss of performance for up to 28 days
- Compatibility with all instruments, staining protocols, and buffers
- Lot-to-lot reproducibility ensures data quality
References

Article 1: Streamlining Cell Proliferation Assays


Article 2: Flowing Towards Therapeutic Success


Article 3: Automating Flow Cytometry for High-Throughput Screens


StarBright Dyes for flow cytometry

StarBright Dyes are unique fluorescent nanoparticles conjugated to Bio-Rad’s highly validated immunology antibodies. Developed specifically for flow cytometry, StarBright Dyes have narrow excitation and emission spectra and give you exceptional brightness without the need for a special buffer. StarBright Dyes are currently available for use with violet, blue, and ultraviolet lasers.

Explore StarBright Dyes at bio-rad-antibodies.com/StarBrightDyes

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