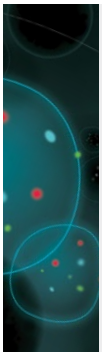


Faster, Better, and More of It: What's Next for Flow Cytometry–Based Screening?

By Richard J Cuthbert

In recent years, the use of flow cytometry in drug discovery has become widely accepted. It offers an unparalleled combination of speed, sensitivity, and multiparameter analysis, making it an essential tool for antibody screening and phenotypic drug discovery. The emergence of flow cytometry as the foremost technology for screening has been an incremental process. Early flow cytometers were large and difficult to operate, yet with comparatively few parameters. Despite significant improvement over the years, one fundamental limitation persisted — speed.

Early innovations like the addition of plate loaders or tube carousels attempted to address the low throughput rate associated with manual sample handling. However, flow cytometry paled in comparison to other technologies, such as the enzyme-linked immunosorbent assay (ELISA), where results could be rapidly collected from microtiter plates. The fundamental problem was that flow cytometers typically relied on each sample being drawn through a section of capillary tubing before being focused into a stream of particles and presented one by one to a single set of detection optics.

The breakthrough came with the idea that, rather than drawing a single sample at a time and then clearing the line, multiple samples could be drawn one after another into the sample line. In order to differentiate one sample from the next, an air bubble is introduced between them. This approach has key advantages, as it facilitates a reduction in the sample volume and a corresponding increase in the concentration, thus increasing the number of samples that can be processed in a given time. It also allows the miniaturization of assays, meaning a significant reduction in running costs.

The Power of Flow Cytometry

With this innovation, the power of flow cytometry could finally be realized in a format amenable to high-throughput screening. Rather than being limited to a single parameter, as is the case with ELISA, researchers could apply multiple parameters to their assays and save huge amounts of time, effectively performing two or more assays in the same well. Another great advantage is that assays can be performed using live cells, which means that targets normally expressed on the surface of cells are not subjected to any conformational change and binding epitopes are preserved.

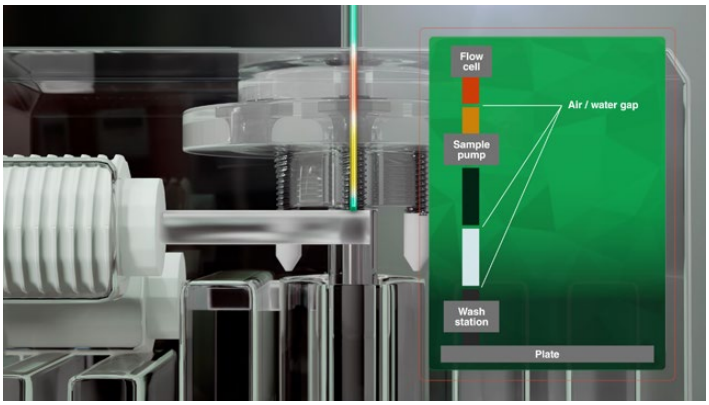
Compared to technologies such as imaging cytometry, what was once the Achilles' heel of flow cytometry has become one of its greatest strengths. The fact that particles are presented to the detection optics one by one means that data collection is at the single-particle or cell level and is not subject to resolution limitations. Because of this, flow cytometry does not rely on population averaging, so unwanted events can be easily discounted from analysis, unlike in imaging cytometry.

The ZE5 Cell Analyzer with a simple robotic workcell.

Today, some features are generally accepted as essential for a screening flow cytometer. These include compatibility with high-density microtiter plates and low sample volumes, suitability for automation, and above all, short acquisition times. However, just because an instrument delivers on these points does not mean that all challenges are met. Excessive sample carryover, downtime caused by instrument blockages, labor-intensive file separation, and lack of flexibility can slow down projects. Limitations on the number of excitation wavelengths and available detection channels can also reduce productivity. These are among the unresolved issues that limit the capability of some screening instruments.

The Future of Flow Cytometry–Based Screening

The ZE5 Cell Analyzer from Bio-Rad Laboratories, Inc. has been designed to break through screening limits. It has a plate loader with built-in temperature regulation (4–37°C), performs orbital shaking calibrated to plate type, and is compatible with a wide range of input sample types. This makes it the most capable plate loader available today. As you would expect, it uses air and water gaps to separate samples in the line so that it is able to move through them incredibly quickly. Unlike other systems, it combines this ability with industry-leading detection electronics, enabling collection of up to 100,000 events/second with no data loss. This is the difference between a system that can power through detection of rare events in a screening assay and one that simply cannot.



Samples are drawn into the sample line and separated from each other by air and water gaps, facilitating rapid sampling.

When it comes to robotics, integration could not be simpler. The ZE5 Cell Analyzer has an application programming interface (API) that is designed to be broadly compatible. It is vendor agnostic, laboratory information management system (LIMS) friendly, and can be seamlessly integrated into new or existing workcells — from a simple robot arm and plate hotel to complex multi-instrument platforms.

So the ZE5 Cell Analyzer truly delivers on the essentials. But what about those previously unmet challenges? If a system uses a probe that moves between samples, it's a potential source of carryover. Recognizing this, the ZE5 Cell Analyzer includes a cleaning station that quickly washes the probe both inside and out after each and every sample. This means that, even at top speed, carryover is minimized to less than 0.5%.

Blockage has always been an issue for flow cytometry and can lead to downtime. One of the stand-out characteristics of the ZE5 Cell Analyzer is the quality of its fluidic system. This alone significantly reduces the risk of blockage. Additionally, it has a reversible sample pump, allowing any problems that do arise to be addressed quickly and easily.

To save further time, there is no need to separate individual samples from one big file. The ZE5 Cell Analyzer separates FCS files for each individual well on the fly so the data are ready to go by the time the run is complete. Unlike other systems that compromise data resolution, especially at low signal strength, data quality remains excellent across the full dynamic range.

In terms of flexibility, the ZE5 Cell Analyzer is second to none. With up to 27 fluorescence channels, it can handle much more complex assays and do more in every single well. With five lasers, problematic compensation is avoided, simplifying experiments and preserving data quality.

The Bio-Rad ZE5 Cell Analyzer represents the next step in drug screening. With fast data acquisition and processing, flexible sample handling, superior data quality, and a wide variety of parameters and lasers, it makes flow cytometry more powerful than ever before.

Visit **bio-rad.com/CellAnalysis** for more information.

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