Removing Bottlenecks from Flow Cytometry

High-throughput flow cytometry is changing the drug discovery landscape, but has until now been limited by reduced flexibility and lagging analytical capabilities

Technological advances over the last two decades have turned flow cytometry into a powerful high-throughput tool for a broad range of large-scale applications requiring rapid multi-parameter analysis. These advances enable high-throughput phenotypic screening and structure-activity relationship screening (SAR) in areas like drug discovery, therapeutics development, and biomarker discovery. As a result, flow cytometry is gaining widespread use in high-throughput niches across the pharmaceutical and biotech industries and academia. Analytical pipelines for the enormous datasets have, to date, relied on custom ad hoc solutions, however. Truly high-throughput capabilities are now possible off-the-shelf through advanced complimentary instrument and software solutions.

Flow cytometry as a high-throughput screening solution

Flow cytometry has only recently become a viable method for high-throughput screening. While it has

always been ideal for multiparametric analysis of cells based on their physical characteristics and surface protein expression, flow cytometry has traditionally been too slow in processing large sample numbers to gain widespread use in high-volume applications.

Drug screening efforts have traditionally relied primarily on time-consuming or computationally demanding techniques like ELISA-type assays and high-content imaging cytometry. Though relatively straightforward, ELISA-type assays typically test for a single parameter at a time and may require testing out of context, which could affect results. “For instance, you've got a protein that would normally be expressed on the surface of the cell—if you take it off the surface of the cell and put it onto a plate, then potentially you're changing the conformation of that protein,” explains Richard Cuthbert, PhD, flow cytometry product manager at Bio-Rad. Like flow cytometry, high-content imaging cytometry is multiparametric, but it requires considerable processing power for analyzing and storing large image data files, and capturing rare events is difficult.

The demand for high-throughput flow cytometry solutions offering rapid multiparameter analysis is not surprising, given the context. Improvements to sample processing speed enable modern instruments to catalogue 100,000 events per second, satisfying volume requirements even for rare event capture. However, developing truly high-throughput capabilities requires the systematic removal of process bottlenecks from sample loading through data analysis. Thus far, analysis bottlenecks have been addressed at the user-level by those with the resources to create custom solutions. New attention towards removing the last of the bottlenecks associated with high-throughput flow cytometry has produced accessible, comprehensive commercial solutions. Here we review the key recent innovations that ensure reliable, high-throughput, automated workflows.

The sample bottleneck

One of the earliest, and most important, innovations in high-throughput flow cytometry allowed for more rapid cycling through samples. Traditional flow cytometers need to fill the sample line up to the interrogation point with material from a single sample, which is time consuming. The introduction of air spaces in the line without destabilizing flow allows samples to be queued while remaining recognizably distinct. This not only speeds up sample loading and data acquisition, it supports assay miniaturization by reducing sample volume requirements.

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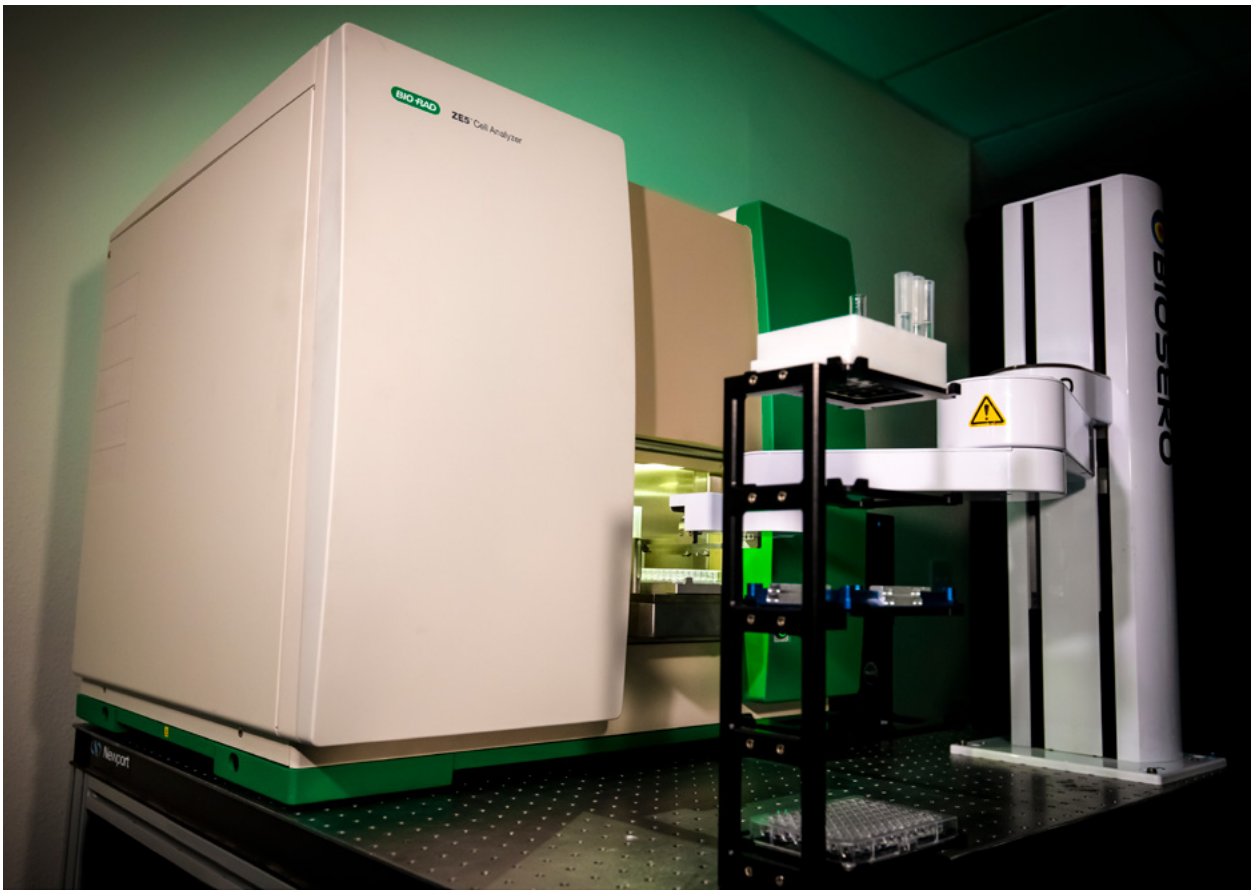
One drawback associated with this advancement has been extensive sample carryover, detrimentally affecting accuracy in well-parsing and ramping up contamination concerns. Bio-Rad’s high-throughput ZE5 Cell Analyzer solved this with an integrated wash station that travels with the probe, cleaning it thoroughly between each sample.

Instrument blocking has formed another major concern, according to Richard, who reports further innovation in sample pumps along with increased pressure and speed in flow cells within the ZE5 Cell Analyzer to mitigate this problem.

The plate bottleneck

As plate processing becomes faster, the limiting factor becomes prepping and feeding plates into the cytometer. The ideal solution is the automation of sample preparation and plate loading using robotic workcells, particularly when they are combined with upstream instruments. This integration adds complexity to the operating requirements to ensure compatibility with a range of automation platforms from multiple manufacturers. According to Richard, Bio-Rad has achieved compatibility in their ZE5 Cell Analyzer five-laser high-throughput flow cytometer by developing a “device-agnostic application programming interface (API) and working with several



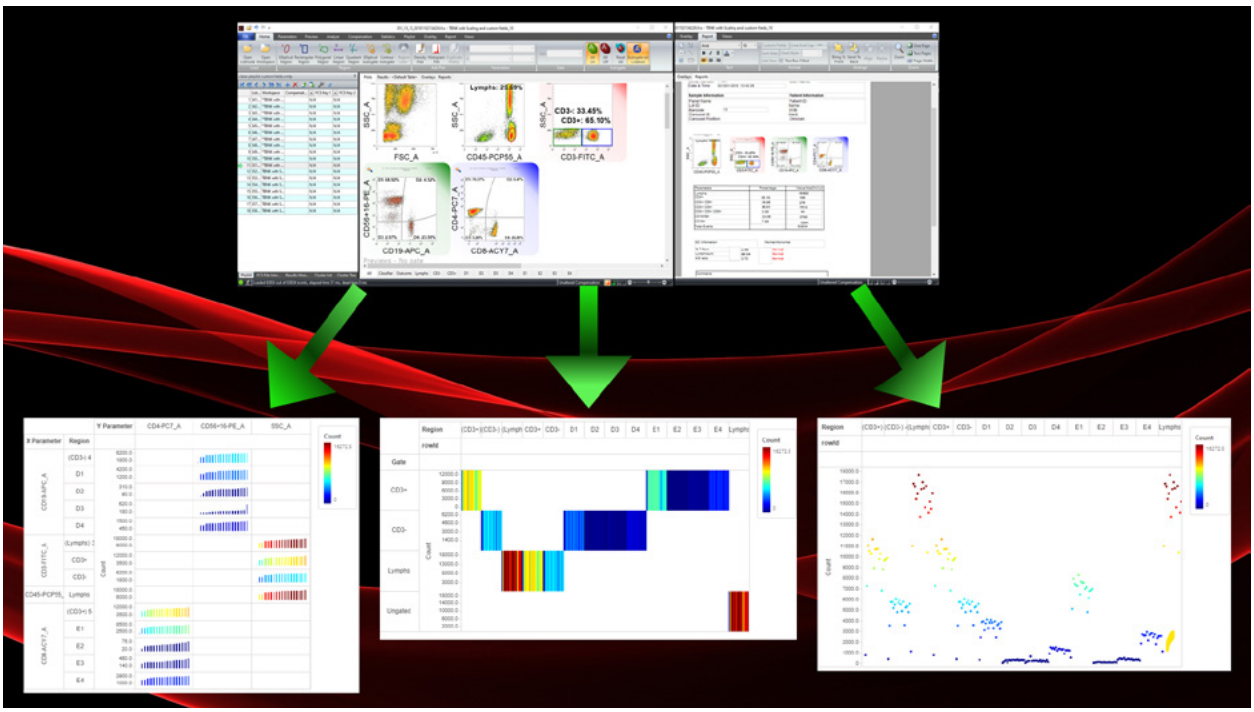
automation partners to integrate the ZE5 Cell Analyzer into new and existing robotic workcells." LIMS can be integrated with instruments as well as other laboratory and enterprise applications and systems.

One drawback thus far during the evolution of high-throughput flow cytometry has been reduced flexibility and multiplexing capabilities as instruments are primarily focused on expanding throughput. While that may be acceptable in terms of screening functionality, it limits the instruments' relevance to the full drug discovery and rare event analysis workflows. Improving multiplexing capabilities and sample formats, as demonstrated with the ZE5 Cell Analyzer's 27 available fluorescence parameters and ability to handle 5 ml tubes, increases instrument flexibility across all workflows.

The data bottleneck

High-throughput flow cytometry rapidly generates enormous quantities of data, leading to the next bottleneck—data processing and analysis. Standard flow cytometry analysis software packages do not have the power, automation, or scalability to handle the computational demands of high-throughput data.

Custom industrial solutions have been individually developed by some organizations, such as large pharmaceutical companies, though these have largely been under the purview of in-house statisticians or computer programmers running a basic set of analyses. Increasing complexity in fluorescence parameters at high-throughput levels, however, has reintroduced the need for flow cytometrist involvement early in the analytical process.



To find an ideal, all-in-one, easy-to-use solution for rapid, automated analysis, Bio-Rad worked with Applied Cytometry. With a long history of flow cytometry data analysis development, Applied Cytometry has followed technological changes closely.

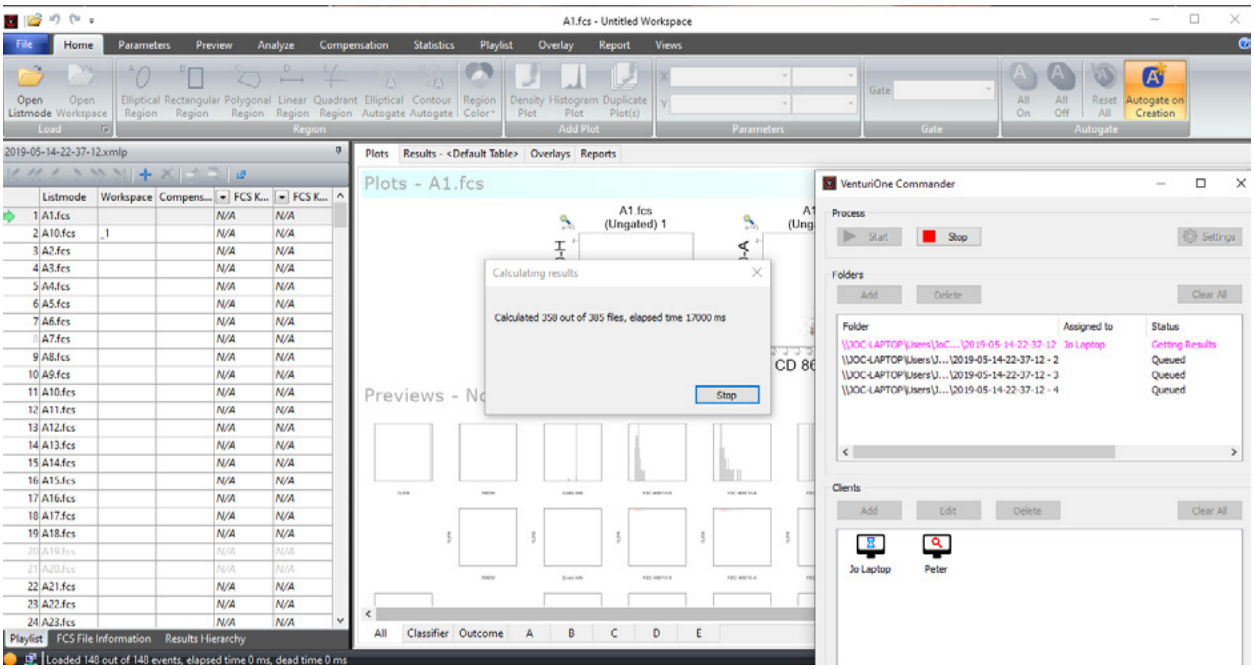
Peter Nobes, director of product development at Applied Cytometry, describes a major pivot in their software starting a decade ago with the introduction of 64-bit processors, “we took the opportunity to look at where we thought the market was going, both flow cytometry-wise and computer-wise. People were moving from four or five colors to ten colors or more. We took a bet and redesigned our entire software.” The newly developed software, VenturiOne, was 64-bit compatible and built to utilize full memory capacity, banking on a future need to process broader, longer data files with increasing parameters and events at a faster rate. As Peter explains, “however many colors a machine has, people always want another five, and however long a data file is, people looking for rare events will always want another million—or 10 million—events.”

Designed to be used by flow cytometrists over statisticians or programmers, VenturiOne allows users to set up a workspace with as much or as little complexity as the experiment requires, complete with the requisite plots and easy gate-setting, or auto-gating, which can then be applied across multiple plates.

The automation bottleneck

Though their initial aim was to process a minimum of 400 files at a time for a rapid, one-shot analysis of a 384-well plate, it was working with Bio-Rad and a client in the drug discovery field that drove Applied Cytometry to design software enabling high-throughput screening by analyzing thousands of files at once. The client also wanted a software solution that provided finer control over the output for flow cytometrists compared to their existing statistician workflows.

Like with robotic plate feeders, the answer was automation. Applied Cytometry built a new scheduling software, Commander, to feed multiple plates of data from the instrument to the core flow cytometry data analysis software, VenturiOne. This provides full scalability while maintaining the core analytical capabilities and interface. To enable rapid analysis of hundreds of plates, Commander uses parallel computing principles to run the analyses across multiple installations of VenturiOne on PCs across the lab or servers within the intranet. It shuffles a plate’s



worth of data to each computer at a time, repeating as space becomes available.

Working with the drug development client's data, Applied Cytometry found that each sample took less than 1 second to analyze. Ten thousand files, the equivalent of 26 384-well plates, can be analyzed in 2 hours using a single PC. Spreading the workload across multiple PCs reduces the amount of processing time essentially by the number of PCs used, e.g. 10 PCs could analyze 10,000 files in 15 minutes. This system, which allows the import of whole data folders, not only rolls out experimental results quickly and efficiently, but it can reanalyze years' worth of accumulated data on entire compounds in minutes when new insight is gained, for example, changing gates.

The massive initial analysis efforts are moved from the realm of programmers and statisticians into that of flow cytometrists, while making it just as quick and easy to run 100 or 200 plates as one or two. Richard agrees, "if you can run standard flow cytometry analysis software, then you can use this tool." The requested statistics are delivered in CSV format for each plate, which in turn may be fed into a corporate analysis tool or delivered to statisticians as needed.

Modern advancements in throughput have greatly expanded the applications for flow cytometry, turning it into a powerful screening or profiling tool. The multiparameter, information-rich analysis allows, for example, large-scale compound toxicity investigations greatly speeding drug discovery efforts, while the new analytical pipelines support rapid reanalysis of years of data stores based on new information and adjusted parameters.

Twenty years in, full scalability of high-throughput flow cytometry workflows has been achieved.