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Flow Cytometry Expands Application Range

Novel Dimensions and Capabilities Keep Technology Fresh and in Demand at Labs

by Catherine Shaffer

Flow cytometry is and always has been a multiparametric technology. Increasingly simultaneous and correlated measurements can be used to provide greater detail about the phenotype of cellular subpopulations. Clinical laboratories now routinely employ 5- or 6-color flow cytometry, while research labs are experimenting with 10- to 20-color capabilities.

Innovations such as image flow cytometry and acoustically focused flow cytometry have added new dimensions and capabilities, and in some cases flow cytometers have begun to replace other instruments like microscopes.

At the same time, in order to reach its full potential, the field of flow cytometry must overcome some significant challenges. Chief among these is the abundance of data generated from increasingly large multiparametric analyses.

At the other end of the spectrum, there are some basic problems that are still being solved, such as how to accurately calculate the sensitivity of the instrument. Potential solutions to a range of problems impeding the efficient use of flow cytometry were presented at the recent "Great Lakes International Imaging and Flow Cytometry Association" conference.

[...]

Broad Stream, Tight Spot

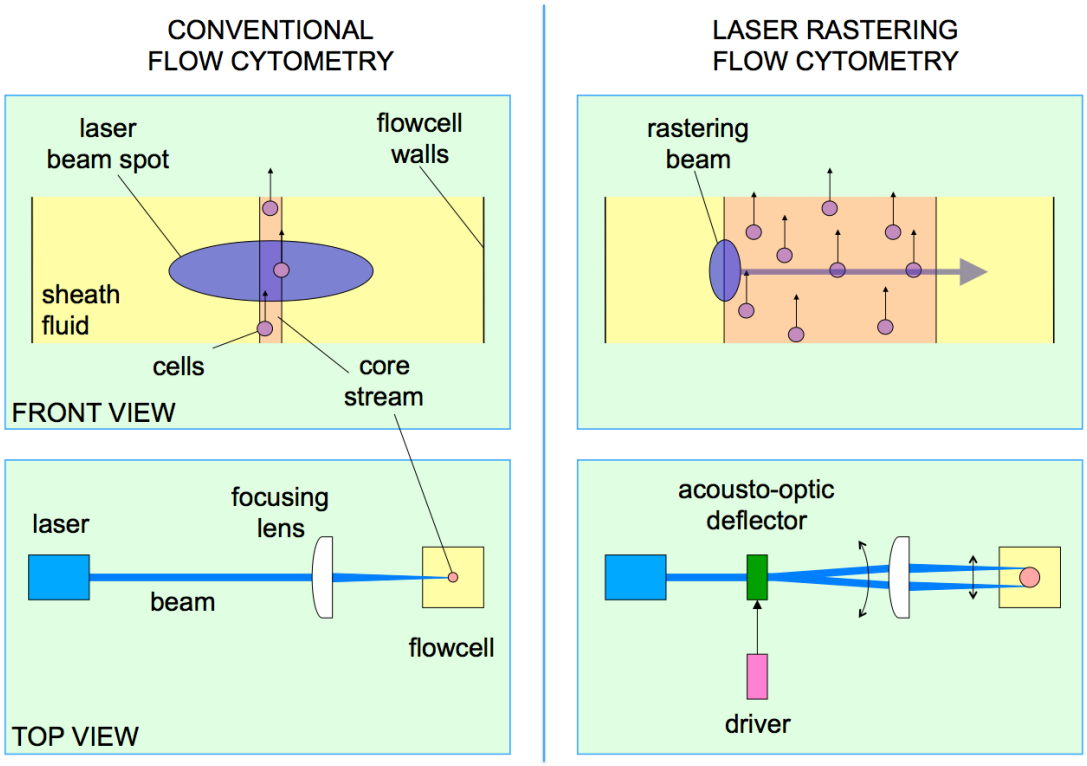
Giacomo Vacca, Ph.D., R&D program manager and research fellow at Abbott Laboratories drew interest with his presentation on laser rastering in flow cytometry. Three features distinguish laser rastering flow cytometry from conventional flow cytometry. First, instead of narrowing the stream of fluid to a single line of cells, the laser-rastering system uses a wide stream with many cells coming through at the same time to increase throughput.

Second, instead of a broad, elongated spot, it uses a tight laser spot in both dimensions. And lastly, instead of a fixed laser beam that waits for cells to flow past, the laser beam is scanned, or rastered, across the core stream as the cells go by, and interacts with each cell multiple times.

Dr. Vacca and his colleagues have been building and perfecting laser rastering flow cytometry at Abbott Hematology for several years with the goal of creating a better hematology analyzer. "We can measure the same things you can in a flow cytometer or hematology analyzer, but at a much greater rate," he said.

The large amount of complex data does, however, require some “heavy-duty signal processing.” The system can process more than 300,000 cells per second, claims Dr. Vacca, whereas a high-end hematology analyzer can handle only about 10,000 to 20,000 cells per second. One advantage of this higher rate might be to scan for rare events.

One common problem in using a flow cytometer to analyze blood cells is called the coincidence problem. This is when two cells are in the beam at the same time and cannot be resolved. Surprisingly, Dr. Vacca’s laser-rastered system does not have a greater degree of coincidence than a conventional flow cytometer because the wider stream is compensated for by the tighter laser spot.



Over the last several years, researchers at Abbott Laboratories have been building and perfecting laser rastering flow cytometry. Three features distinguish laser rastering from conventional flow cytometry: instead of a single line of cells, a wide stream is used; instead of a broad elongated spot, a tighter laser spot in both dimensions is utilized; and, finally, the laser beam is scanned or rastered across the core stream as the cells go by.