Benchtop Flow Cytometers Support Expanding Base of Applications

A new generation of compact instruments incorporating plug & play, fiber-coupled, smart laser modules supports fast growing and diverse applications including oncology, stem cell research, bio fuels, and epidemiology, plus traditional applications such as immunology.

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Applications for flow cytometry continue to grow and diversify, supported by a new generation of benchtop instruments that combine ease of use, modular flexibility and economy, while still delivering performance that rivals traditional large platform instruments. Around the size of a kitchen microwave, the latest instruments incorporate compact, fiber-coupled, smart lasers having plug & play functionality. This enables a single instrument platform to be configured for a range of different application needs, and even allows "hot swapping" of lasers in the field. This article shows how the combination of perfect flat top beam output and a novel flow technique called acoustic focusing now eliminates the traditional flow cytometry trade-off between speed and sensitivity in the latest instruments.

Benchtop Instruments – a Growing Market Segment

As recently as just 10 years ago, the vast majority of flow cytometry applications were in the areas of immunology and hematology: counting various types of blood cells, such as CD4 (T helper cells), that is key in monitoring HIV infections, for example.Both research and clinical testing needs were well-served by large platform instruments, which typically incorporated multiple lasers and over a dozen detection channels. (These detection channels are mostly different fluorescence wavelength windows, as well as a few channels that monitor scattering at different angles.)

Several factors have contributed to the development of benchtop instruments as alternatives to these large, costly machines. First is the overarching trend of product miniaturization, in this case made possible through the availability of smaller electronic and photonic components with increased functionality. At the same time, several life science applications have begun to access flow cytometry's unique ability to simultaneously count multiple cell types.These emerging

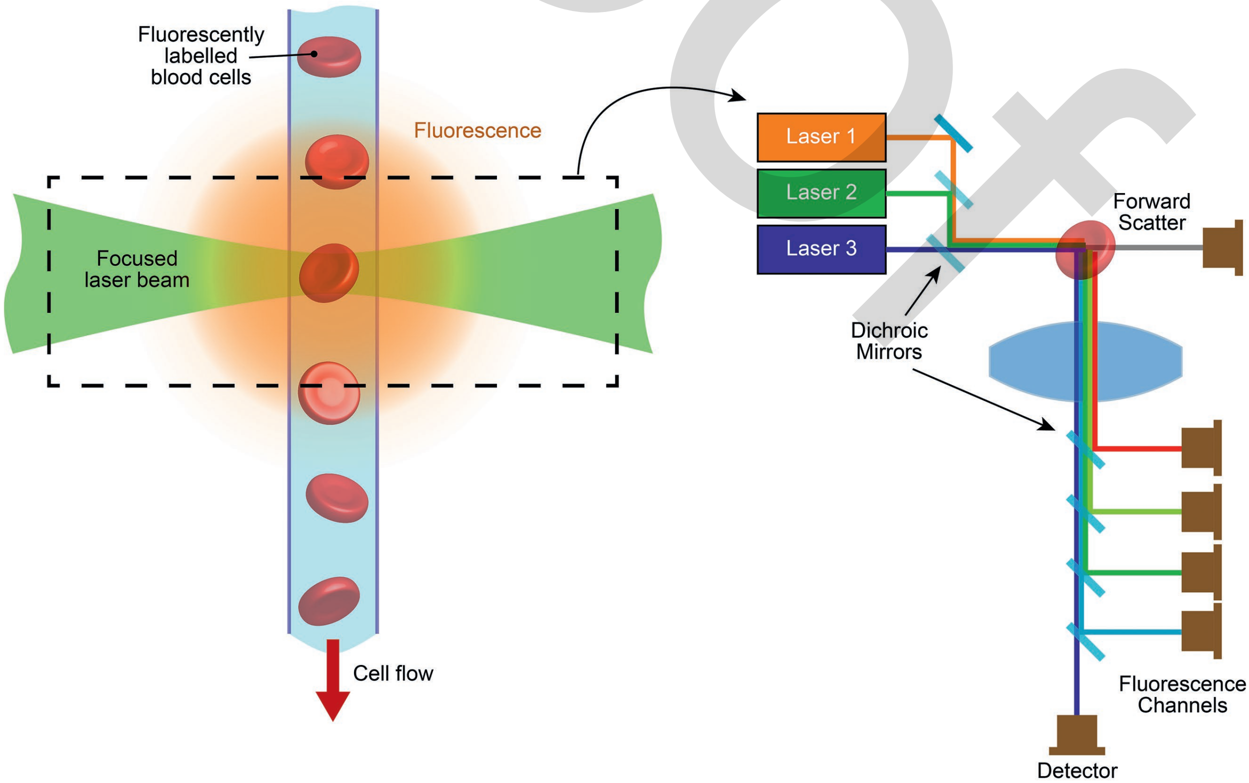

Figure 1: Flow cytometry uses focused laser light to count cells according to fluorescent labels (fluorochromes) that bind to target proteins and other cell surface markers

Image: Coherent

applications have themselves been enabled by an ever expanding portfolio of dyes, probes and fluorescent protein labels. Examples include bio fuel research, epidemiology (to quickly identify and characterize responsible pathogens), oncology (to find rare cells in the blood that cause metastasis for example), stem cell research (encompassing all kinds of research and potential disease treatments), and pharma (to support faster screening and high throughput discovery). Many of these new applications would be limited in scope and size if the only available cell counting tools were large frame, legacy instruments.

To support the widest possible market of applications, the new benchtop instruments must provide a combination of state of the art performance (speed and sensitivity) and flexibility (applications specific feature sets). They must also deliver maximum economy and value in the form of superior reliability and easy to use software.

Plug & Play Lasers Deliver Modularity & Economy

Instrument modularity is key to providing both optimized performance and economy for these diverse applications, where a single instrument platform can be factory customized in terms of the number and wavelengths of the laser sources, and the number of detection channels. For example, the Attune NxT is a next generation benchtop instrument from ThermoFisher that features a single common platform with a modular architecture that can support between six and 16 different detection channels, and from one to four lasers, typically at 405 nm, 488 nm, 561 nm and 637 nm (**figure 2**). It also features an autosampler that optionally supports the micro-well plates widely used in pharma (96- and 384-well), plus the flow cytometry tubes used in most other applications.

The flexible and compact modular architecture is enabled in part by the use of smart, plug & play, Coherent OBIS laser modules. These simplify the addition or interchange of laser wavelengths and also support smaller instrument platforms. OBIS products utilize either optically pumped semiconductor laser (OPSL) or direct diode technologies, which both feature a physically small cavity. This is combined with highly integrated and miniaturized control and interface electronics (within the laser head itself), together with micro-optics, to yield a very compact, conductively cooled laser head. Importantly for flow cytometer manufacturers and other instrument OEMs, automated assembly of these lasers means that every unit, regardless of wavelength, generates a beam with identical opto-mechanical parameters, including beam location, beam diameter, beam shape (energy profile), and beam pointing.

Maximizing Speed, Sensitivity and Reliability

The newest benchtop flow cytometers also set new standards for speed, sensitivity and reliability thanks to a combination of the unique acoustic assisted hydrodynamic focusing technology and innovative use of a novel beam delivery module. Legacy flow cytometry solutions have a well-known trade-off between speed and sensitivity due to limitations with hydrodynamic focusing. In traditional hydrodynamic focusing, increasing the sample rate results in widening of the sample core stream, resulting in increased signal variation and compromised data quality (hydrodynamic focusing figure).This negatively impacts the cost of data, and can lower the sampling rate beyond the practical limit for some applications, even with the usual sample resuspension. In oncology for example, when counting rare cells with populations much lower than 1 per million, it could take many hours to obtain statistically meaningful data. In addition, with a stream diameter of a few microns, the laser spot has to be focused to a similar diameter to maximize the sensitivity. But this sensitivity comes at a price. Specifically, with a typical Gaussian laser beam profile, even micron shifts in the focused beam position can affect the overall detection efficiency. So these high sensitivity setups were typically less robust, requiring a higher level of maintenance. Why not use a wider core stream? The cells can move laterally in and out of the central detection zone in such a stream, thereby limiting sensitivity (**figure 3**).

This speed/sensitivity tradeoff is now avoided in two ways. First, the use of acoustic assisted hydrodynamic focusing (as used in the Attune NxT) which uses ultrasonic waves (over 2 MHz, similar to those used in ultrasound medical imaging), in combination with hydrodynamic forces, to position cells into a single, focused line along the central axis of a capillary. Acoustic focusing is largely independent of the sample input rate, enabling cells to be tightly focused at the point of laser interrogation, regardless of the sample-to-sheath ratio. This, in turn, allows the collection of more photons for high-precision analysis and allows cells

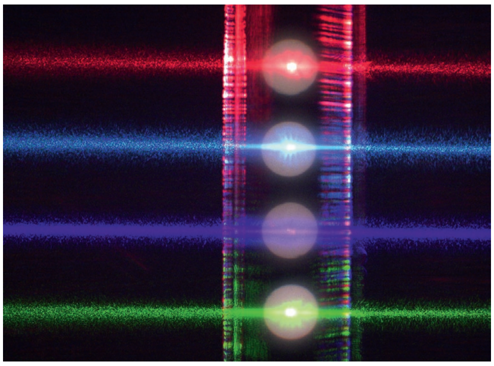

Figure 2: (or frontis photo) In the latest benchtop instruments, the flow cell is intersected by up to four different lasers

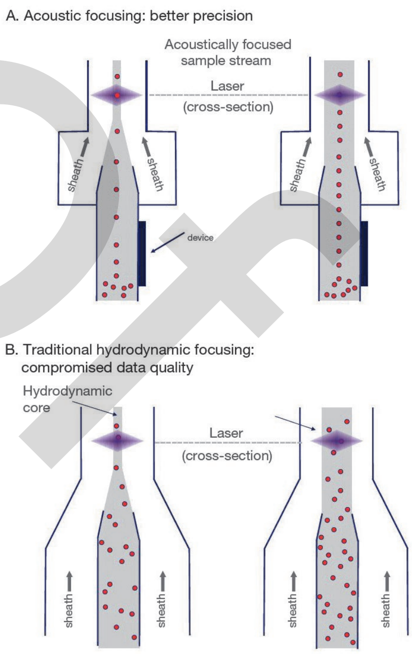

Figure 3: (A) Acoustic focusing enables tighter alignment of the target cells even when the flow rate is increased producing a wider core stream (rightside image). (B) With traditional hydrodynamic focusing, the wider streams necessary for faster sampling (rightside image) lead to increased lateral uncertainty in the position of target cells and an associated reduction in signal to noise

Images: ThermoFisher Scientific

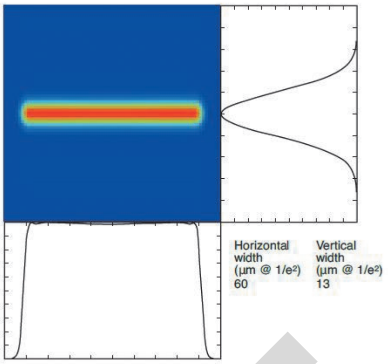

Figure 4: Speed, sensitivity and reliability are enhanced by the use of novel and efficient beam delivery modules that produce an extended top hat focused profile lateral to the flow direction

to remain in tight alignment even at higher sample rates, resulting in less signal variation and improved data quality. With this new speed advantage, assays around rare event detection and analysis of whole blood can now be utilized in flow cytometry.

At the same time, each fiber-pigtailed laser is coupled into the interaction zone using a Coherent beam focusing module that incorporates a novel aspheric optic to create an extended "top hat" beam intensity profile in the axis lateral to the core stream (**figure 4**). The result is that the detection efficiency is now relatively insensitive to the inevitable tiny shifts in instrument alignment. This yields increased reliability, a reduced need for field service, lower cost of ownership and overall lower cost of data.

Overall economy is also further enhanced by the thermo-mechanical stability of the OBIS FP modules. Specifically, many older model instruments require a 30 minute warm-up time to achieve stable alignment. But, the combination of the top hat beam profile and Coherent's permanent fiber welding technology enables a feature called simmer-mode, with instant on/off power on demand, that reduces laser aging by up to a factor of ten. Plus, the modules can be digitally modulated, eliminating the cost and complexity of external modulators.

Conclusion

In summary, a new generation of benchtop instruments represents a paradigm shift in flow cytometry, greatly expanding potential applications. These state of the art instruments economically support applications diversity through a modular architecture, enabled by plug & play, smart laser modules.

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