

New UV Wavelengths and Integrated Laser Engines Benefit Flow Cytometry

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Both clinical labs and research facilities are increasingly using multi-parameter flow cytometry, creating a demand for new (ultraviolet) lasers and integrated light engines.

Flow Cytometry Background and Trends

Flow cytometry is a widely used method to analyze (count) and/or sort cells, sperm and other bio-entities according to one or more distinct parameters. This entails forcing the cells to pass in single file through an interaction zone often using a flow conformation called hydrodynamic focusing. In this zone, the cells are then sequentially irradiated by one or more focused laser beams. The resultant scatter and (Stokes shifted) fluorescence is collected by optics and separated by cut-off and bandpass filters into discrete wavelength bands. Light in each band is then quantitatively measured using a photodetector such as an avalanche photodiode (APD). For example, in blood counting applications in clinical laboratories, the cells are treated with fluorescent labels (fluorochromes) that are bonded to antibodies targeted at specific antigens on the outer membrane of the cells. For every cell that passes through the interaction zone, analysis of the relative intensities in the wavelength bands can be used to count the population as a function of different parameters typically using multivariate parameter analysis. In addition, scatter at key angles can be measured to determine information about cell shape.

For some research applications such as oncology, immunology and drug discovery, flow cytometry can be used to sort

cells, i.e., to selectively collect a target type of cell while discarding all the others. In a sorting instrument the flow passes through a nozzle prior to the interaction zone that ensures the cells are individually held in tiny droplets which pick up a small static charge from the nozzle. Electrostatic plate electrodes are then activated to create a field that deflects the charged droplets into a collection tube, according to the cell type registered by the instrument's computer.

Although flow cytometry has a history spanning decades, it is currently a remarkably dynamic field. In research applications, a key trend is increasing the number of sorting parameters. In the clinical field, instrument builders are responding to a growing market demand for benchtop economical instruments driven by increasingly personalized medicine in the west and the demand for better health care supported by growing affluence in Asia. These trends are supported by laser manufacturers by the development of new wavelengths, particularly in the ultraviolet, and off the shelf multi-wavelength laser engines that simplify the design and manufacture of next generation cytometry instruments.

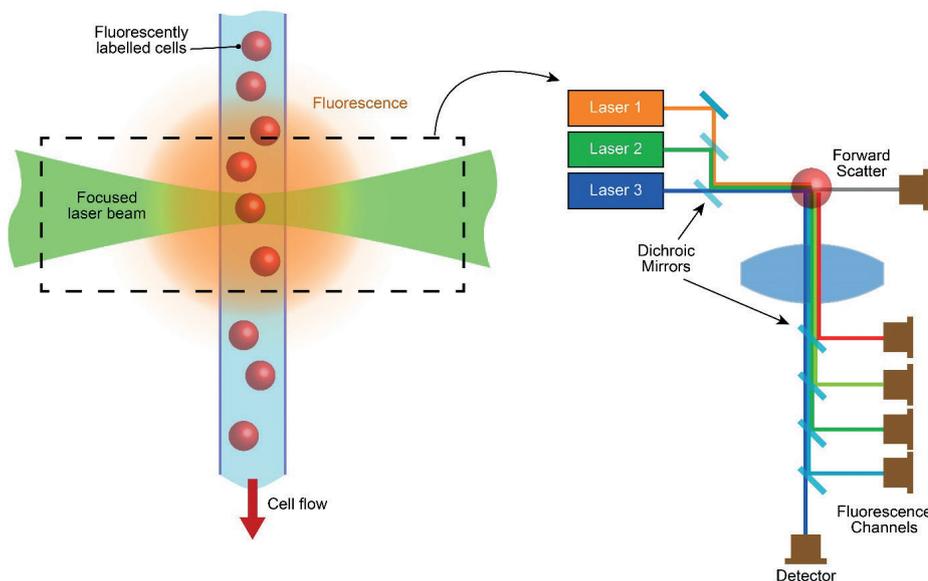
New UV Wavelengths Expand Multi-Parameter Capabilities

In both counting and sorting applications, researchers are looking to increase the

informational content and level of detail by increasing the number of different parameters. Specifically, if the number of lasers wavelengths is increased, together with additional molecular or antigen-specific probes that are optimally excited at those wavelengths, then the number of parameters that can be analyzed increases geometrically, because the fluorescence is subjected to multivariate analysis as a function of both excitation and fluorescence wavelength band. In this way, a "multi-color" flow cytometer with 5 laser wavelengths and several wavelength-specific detectors can readily analyze 25 or more different parameters simultaneously, potentially elucidating even more sub-populations from a mix.

However, the entire visible spectrum is now well covered with smart plug and play laser wavelengths such as the OBIS series from Coherent. As a result, both instrument builders and laser manufacturers have identified that the only way to significantly increase the number of parameters is to extend the wavelength bandwidth – into the near-IR and more importantly into the ultraviolet.

In response to the need for new ultraviolet capability, Coherent recently introduced two additional OBIS laser wavelengths. These OBIS XT lasers feature 349 nm and 360 nm wavelengths with a choice of 20, 60, or 100 mW of output. They are diode-pumped solid-state (DPSS) lasers based on frequency-doubled praseodymium (Pr) technology. Previous attempts by the laser industry to commercialize this technology met with limited success, because Pr presents some unique laser challenges, particularly in the area of reliability. However, Coherent engineers have developed proprietary solutions that now overcome these limitations. Moreover, these UV lasers are electrically efficient with a correspondingly low thermal load, and can thus be produced in a compact OBIS-style laser package that supports simple integration as discrete lasers, or alternatively in turnkey multi-wave-



◀ Fig. 1: The use of several lasers and fluorescence detection channels enables a single instrument run to analyze for multiple simultaneous parameters.

length OEM light engines – see figure 2. Just as important, the lifetime and reliability is similar to existing OBIS laser wavelengths based on OPSLs and diode lasers, with the same industry-leading low noise characteristics. And like other OBIS lasers, they provide the same electronic interface and have the same output beam characteristics: the standard 0.7 mm TEM00 circular beam long used in flow cytometry, as well as identical specifications for beam pointing, beam circularity, etc.

In addition to flow cytometry, these new compact UV lasers are also expected to make their mark on other applications such as confocal microscopy and semiconductor inspection.

Integrated Light Engines Simplify New Instruments

Another key trend in lasers for flow cytometry continues to be the increasing use of multi-wavelength light engines in benchtop clinical instruments. An example is the CellX from Coherent that integrates multiple OBIS lasers – see figure 3. Here all the lasers, electronics and beam shaping and focusing optics are housed in a single module to streamline the development of multi-parameter instruments. These off-the-shelf, standard engines are currently supplied with the four wavelengths most commonly used in multi-parameter instruments: 405, 488, 561, and 637 nm. (Importantly, all the optics are already compatible with the new UV laser wavelengths, in anticipation of future market demand.) Instruments typically use these as a series of elliptical foci – see figure 3 – where the short axis maximizes the instrument’s time resolution and the broad lateral axis minimizes sensitivity to changes in cell positions as the cells traverse the interrogation points.



Fig. 2: Two new UV laser wavelengths are now available as part of the OBIS family of compact lasers.

These light engines are designed to offer highly flexible output to support different instrument designs. Precise independent adjustment of each of the four beams enables the separation between the staggered beam spots (see figure 3) to be varied from zero (i.e., co-aligned) to $\pm 250 \mu\text{m}$. The x and y ellipse dimensions can also be independently adjusted for each wavelength. Thus, the shape, size and position of each of the four focused laser beams can be adjusted to exactly match the geometry of a specific instrument. In addition, each of the four lasers is independently addressable and controlled through a standard USB connection.

There are several advantages to this new type of module. First, by outsourcing the beam conditioning and laser integration, the instrument builder cuts development costs and shortens the time to market while also minimizing performance risk. And second, this integration provides cost-reduction through consolidation of hardware and electronics – for instance by using a single laser controller board, common power and single I/O connector.

Moreover, outsourcing the photonics technology allows the instrument builders to focus on fluorochemie chemistry and other key differentiators, such as novel data analysis and other features.

Summary

Flow cytometry is a dynamic field, and the demands of both research and clinical application continue to evolve. Laser manufacturers are supporting these changes with new solutions to meet the needs of the next generation of instruments that will support important future developments in personalized medicine and tailored immune cell therapies.

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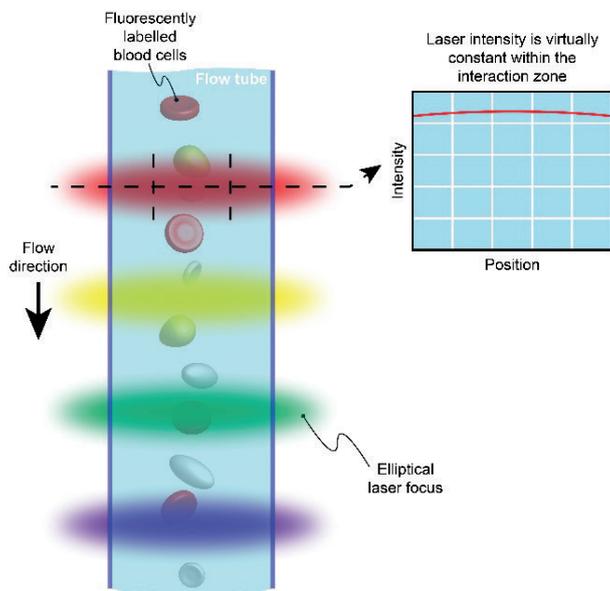


Fig. 3: Integrated light engines include the lasers and optics required to produce up to 4 focused beams, with the focal distance, shape and location of each beam independently set by the instrument builder. The optics are optimized for both visible and UV wavelengths.

