Flow Cytometry for Immunology Research

New multiwavelength engines support medical applications.

Ingo Waldeck and Matthias Schulze

The fight against the Covid-19 pandemic highlights the need for and benefits of personalized medicine. Thus, there is a growing demand for more detailed analysis of disease pathology as well as patients' individual immune system characteristics and hence predicted disease progression and optimum treatment. Consequently, clinical labs and research facilities try to increase the performance of multiparameter flow cytometry as a key tool to analyze the cellular components of the immune system. In response, Coherent is supporting faster instrument development and is increasing the number of parameters in two ways – integrated multiwavelength OEM subsystems and new laser wavelengths in the ultraviolet. In both cases, the output beam parameters are optimized for common flow cytometry platforms.

Personalized medicine refers to an optimized medical treatment matching the genetic/proteomic profile of both the patient and the disease strain. It is already widely used in oncology. Here, many new drugs are highly effective, but usually only for tumors with particular genetic characteristics and mutations. The ongoing pandemic has provided further impetus for personalized treatments since the disease shows a wide and continuous spectrum of symptoms in addition to a broad set of long-term complications.

Researchers and clinicians have already identified several correlators for an enhanced risk of disease severity, including age and obesity. But the genetics of the immune system also play an important role. A twenty-something patient may die and a sixty-something patient may be asymptomatic. Therefore, the genetic markers and immune system characteristics responsible for a serious course of disease are explored. Knowing these factors enables the treatments to be reserved and deployed for those most in need.

There are two parts to successfully address a disease like cancer or Covid-19 with a personalized medicine approach. At first, extensive research identifies the markers correlating with each disease outcome. The second part will be the targeted clinical analysis (pre-screening) of patients, presumably focusing on high-risk age groups and pre-existing complications. In both efforts, the frontline tools will be DNA sequencing and flow cytometry. An excellent example are repeatedly performed highly detailed blood analyses during the entire disease progression of a hospitalized patient with non-severe Covid-19 [1].

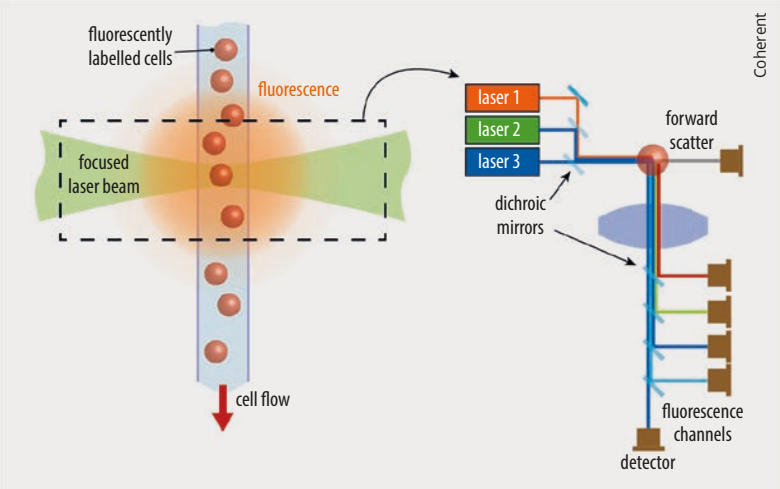

Fig. 1 The use of several lasers and fluorescence detection channels enables a single instrument to analyze for multiple parameters simultaneously.

Flow cytometry background

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. The cells are forced to pass one-by-one through an interaction zone, often using a flow conformation called hydrodynamic focusing. In this zone, the cells are sequentially irradiated by one or more focused laser beams (**Fig. 1**). The resultant scatter and Stokes-shifted fluorescence is collected by optics and separated by cut-off and band-pass filters into discrete wavelength bands. Afterwards, an avalanche photodiode (APD) determines the light intensity in each band quantitatively. For example, during the analysis of blood cells in research and clinical laboratories, the cells are treated with fluorescent labels (fluorochromes) that are bonded to antibodies targeted at specific antigens on the outer cell membranes. For every cell passing the interaction zone, the analysis of the relative intensities in the wavelength bands allows counting the population as a function of different parameters, typically using multivariate parameter analysis. In addition, the scattering angles determine the cell shape.

In oncology, immunology and drug discovery, flow cytometry can also sort cells, i.e. selectively collect a specific cell type and discard all other types. In a sorting instrument, the flow passes to the interaction zone. The cells are individually held in tiny droplets picking up a small static charge from the nozzle. Electrostatic plate electrodes create a field which deflects the charged droplets into a collection tube according to the cell type.

New UV laser wavelengths

Covid-19 is but one example of the need to maximize the number of parameters in order to obtain more detailed information in research applications. One solution is to include more excitation wavelengths in the instruments in combination with new fluorochromes matching these wavelengths. However, smart plug-and-play laser wavelengths such as the OBIS series from Coherent already cover the entire visible spectrum. Both instrument builders and laser manufacturers have recognized that a significant increase in the number of parameters relies on an extended wavelength bandwidth – into the near IR and especially into the ultraviolet.

Research instruments typically require a few tens of milliwatts at each excitation wavelength. For a

Fig. 2 Two new UV laser wavelengths (349 nm and 360 nm) are now available as part of the OBIS family of compact lasers.

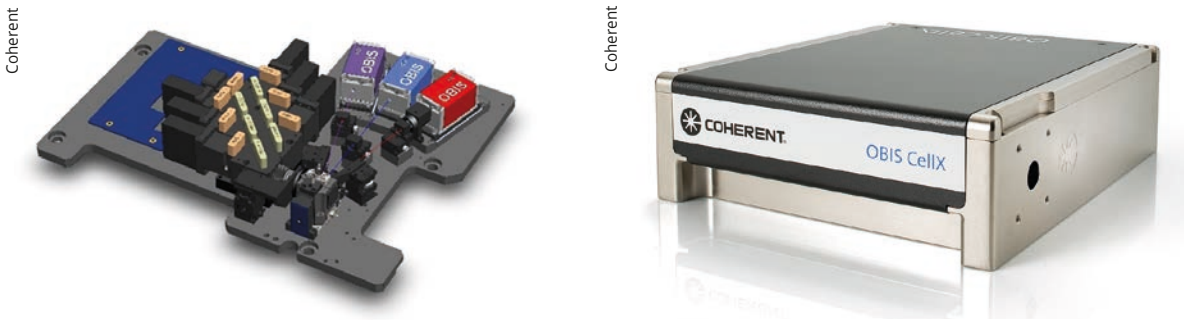

Fig. 3 The use of several lasers in a single instrument involves numerous adjustable optomechanical components which must all be specified and aligned by the instrument builder (left; source ACEA Biosciences). A self-contained multiwavelength light engine with independent adjustment of each wavelength can replace all these standalone lasers and optomechanics (right).

multi-laser flow cytometry instrument, a compact laser package with low power and thermal budgets – high efficiency – is highly desirable. Unfortunately, most UV lasers rely on frequency tripling to 355 nm with a limited efficiency.

Coherent recently introduced two additional laser wavelengths to their family of compact plug-and-play lasers. These OBIS XT lasers feature wavelengths of 349 nm and 360 nm, respectively, with a choice of 20, 60, or 100 mW of output (**Fig. 2**). They are diode-pumped solid-state (DPSS) lasers based on frequency-doubled praseodymium (Pr) technology. Previous attempts to commercialize this technology have had limited success because Pr presents some unique laser challenges, particularly with respect to reliability. However, Coherent engineers have developed proprietary solutions which overcome these limitations. Moreover, these UV lasers are electrically efficient with a low thermal load and, thus, can be produced in a compact OBIS-style laser package. This package supports simple integration as discrete lasers or alternatively in turnkey multiwavelength OEM light engines. In addition, the lifetime and reliability are similar to existing OBIS laser wavelengths based on OPSLs and diode lasers with the same low noise characteristics. They provide the same electronic interface like other OBIS lasers and have the same output beam characteristics: the standard 0.7 mm TEM_{00} circular beam used in flow cytometry as well as identical specifications for beam pointing and beam circularity.

These new 349 nm and 360 nm lasers serve as direct replacements for 355 nm lasers. They excite a similar set of fluorochromes coming in a smaller case with lower heat generation, lower power consumption and lower output noise. In addition to these advantages, some early

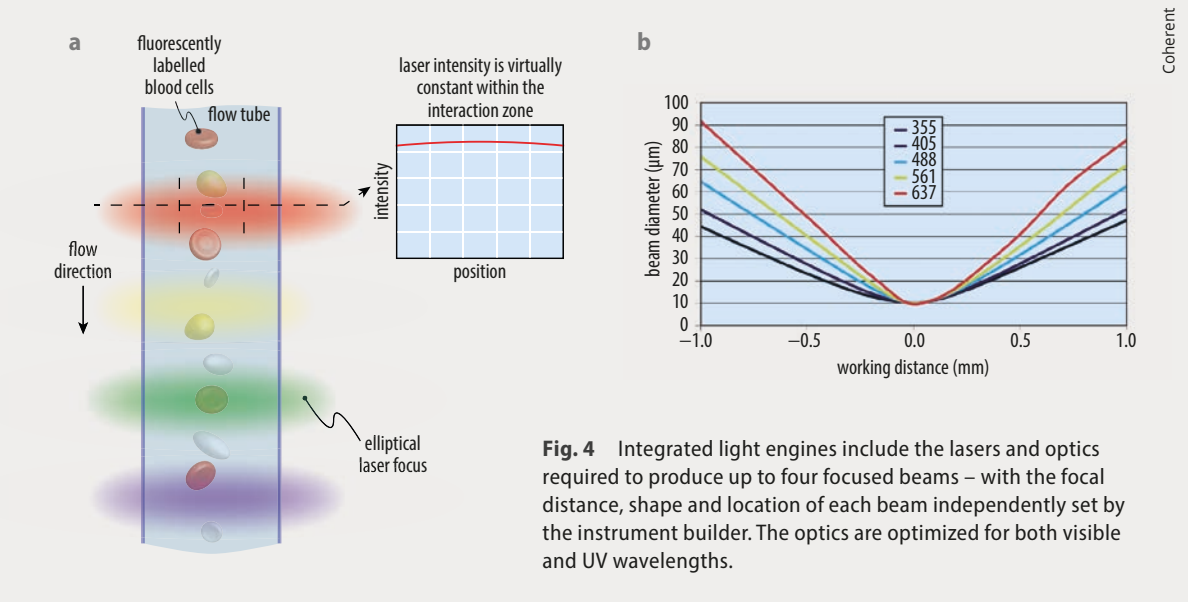

Fig. 4 Integrated light engines include the lasers and optics required to produce up to four focused beams – with the focal distance, shape and location of each beam independently set by the instrument builder. The optics are optimized for both visible and UV wavelengths.

adopters favour 349 nm because it may also excite short wavelength fluorochromes more efficiently than 355 nm. Other users indicate an interest in the 360 nm wavelength based on the better transmission through BK7 glass than 355 nm.

New multiwavelength engines

From the clinical point of view, personalized medicine is creating a demand for multiparameter flow cytometry instruments with high throughput and market sustainable costs. Coherent provides completely integrated multiwavelength light engines called the CellX series incorporating multiple OBIS lasers (**Fig. 3**). All the lasers, electronics, beam shaping and focusing optics are housed in a single module to streamline the development of multiparameter instruments. These off-the-shelf, standard engines are currently supplied with the four wavelengths most commonly used in such instruments: 405, 488, 561, and 637 nm. All the optics are already compatible with the new UV laser wavelengths. Instruments typically use the laser beams as a series of elliptical foci (**Fig. 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 cross the interrogation points.

These integrated light engines are designed to offer highly flexible output and support different instrument designs. Removing the cover allows a simple, precise, independent adjustment of each of the four beams and enables the separation of the staggered beam spots (**Fig. 4**) to be varied from zero (co-aligned) to ±250 µm. The *x* and *y* ellipse dimensions can independently be adjusted for each wavelength. Thus, the shape, size and position of each of the four focused laser beams can 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. Firstly, by outsourcing the beam conditioning and laser integration, the instrument builder reduces development costs and shortens the time to market while also minimizing performance risk. In addition, this integration offers cost reduction by consolidating hardware and electronics, e.g. by using a single laser controller board, a common power supply and a single I/O connector. Moreover, outsourcing the photonics technology allows the instrument builders to focus on fluorochrome chemistry and other key differentiators, such as novel data analysis and other features.

Summary

Lasers and flow cytometry have closely and synergistically co-developed. In the form of multiparameter instruments flow cytometry has a history spanning decades. Currently, the field shows remarkable dynamics in both immunological research and clinical applications, driven to a large part by the push for personalized medicine. Laser manufacturers support this trend with new ultraviolet wavelengths and streamlined multiwavelength products designed to improve the performance of instruments, accelerate their development, and contain their costs.

[1] *I. Thevarajan, T. H. O. Nguyen, M. Koutsakos* et al., Nat. Med. **26**, 453 (2020)

Authors

Dr. Matthias Schulze and **Ingo Waldeck**, Coherent Inc; e-mail: ingo.waldeck@coherent.com, Web: www.coherent.com;