

OPSLs for quick flow cytometry

Optically pumped semiconductor lasers fulfill requirements of virtually all cytometry tasks

● Applications for flow cytometry continue to diversify and evolve. That is because cytometry is both a widely used clinical tool for fast blood cell analysis as well as a powerful research tool. As novel therapeutic modalities (e.g. stem cell treatment) are developed, new cytometry applications often blur the traditional distinction between clinical and research measurements. Yet a single laser technology – the optically pumped semiconductor laser (OPSL) – is proving optimum in virtually all cases, whether the application needs high power, multiple wavelengths, low cost, small packaging or a low thermal or electrical budget. From routine counting of CD4 immune receptor cells to stem cell sorting, this article will show how and why OPSL is meeting the evolving needs of flow cytometry.

The purpose of flow cytometry is to use a laser to interrogate a population of living cells in order to count or sort specific cell types. Most commonly, a whole blood sample is mixed with one or more fluorescent antibodies that bind to specific cell types. The prepared specimen are forced to flow in single file through a tightly focused laser beam, with electronics rapidly collecting and graphically displaying any detected fluorescence and other valuable discriminating parameters such as scatter (see Figure 1). Should there be interest in further analysis or a culture of a specific cell population, a cell sorter may be employed. In this type of instrument, cells of interest are electrostatically deflected into a collection vessel, or onto a microscope slide based on their fluorescence or scattered light signature.

Stem cell sorting

Stem cells have long held the promise of revolutionizing the treatment of many classes of medical conditions. Better outcomes and none of the side-effects of drug-based treatments such as chemotherapy are ex-

pected. Potential treatments include conditions such as spinal cord injuries (paralysis), leukemia and other malignancies, as well as genetically-based disorders like Parkinson's disease. Indeed, rarely a week goes by without stories in the general press about a stem cell-based advance in one or more of these areas. The growing importance of stem cells is reflected in the fact that every flow cytometer manufacturer has already developed or is actively developing instruments optimized for the special needs of sorting stem cells. In turn, laser manufacturers are supporting them with lasers with the appropriate specifications.

Speed is by far the biggest challenge in stem cell sorting. Depending on the type of sample, the targets may represent only 0.01 % of the total population – a true "needle in a haystack." Depending on the type of cell and treatment, at least several hundred, but typically several thousand cells, are required to start a culture for eventual implantation. Currently, total sort times for such an amount of cells can reach up to 5 to 6 hours. The overarching goal is to significantly speed up this process. Although slow, stem cell sorting is conceptually simple. Only one cell type is being targeted and fluorescent probes are sufficiently specific so that only a single wavelength is needed. The preferred laser wavelength is 488 nm, which excites standard fluorescence markers.

Several approaches to higher speed sorting have been launched or are under development. These include novel cell handling techniques as well as parallel processing by splitting the laser beam to simultaneously sort at multiple nozzles. The one common requirement is a need for higher laser power. Reflecting this, instruments based on 200 mW were considered state of the art for speed in 2009, but by 2011, instruments using 488 nm lasers as high as 2 W are expected. At the same time, cytometer manufacturers want to keep all the laser advantages that they already have: e.g., small footprint, high efficiency and high reliability.

OPSLs are ideally positioned to meet this demand for increased power because

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of their unique power scalability. The key elements of an OPSL are shown in Figure 2. Power can be scaled in various ways. Up to a few hundred milliwatts or even a few watts, the simplest approach is to use a gain chip with a larger diameter and to pump this with a single laser diode, albeit at higher power. In most lasers, expanding the mode volume in this way would require a corresponding cavity length increase, and hence a larger laser head, in order to maintain the TEM00 output beam profile which is a key requirement for high-performance flow cytometry. However, the use of telescopic optics enables mode volume and cavity length to be uncoupled. For example, a 2 W laser head such as the Coherent Genesis measures only 281 mm x 156 mm x 85 mm. Powers higher than 10 W can be achieved by pumping the gain chip with multiple diodes and incorporating two or more gain chips in a folded zigzag cavity arrangement.

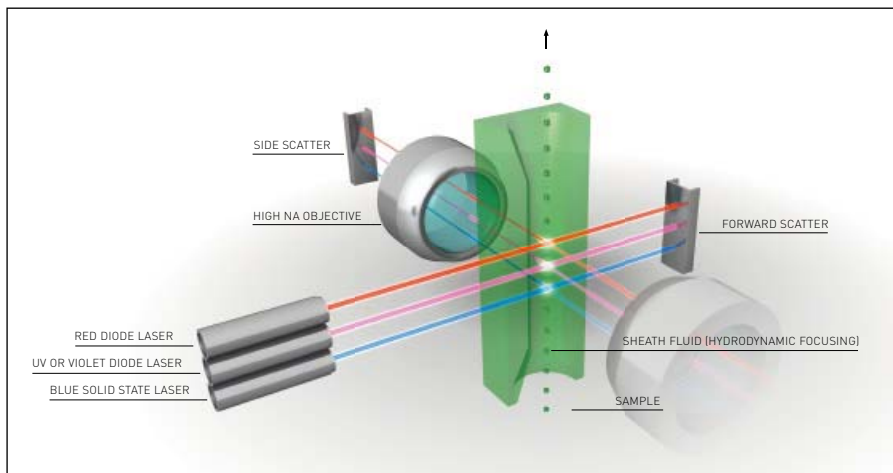


FIG. 1: In flow cytometry cells are counted according to an optical signal related to their size, shape or fluorescence label. (Courtesy: Partec Essential Healthcare)

Hematology

Flow cytometry has long been used for clinical blood analysis, most notably for complete blood count (CBC) measurements that simultaneously count multiple cell types. Here, simplification of the instruments, a smaller footprint, and lower costs has in many cases enabled it to be performed at the doctor's office rather than in an external lab. However, the crucial counting of CD4 immune cells in HIV patients is still the domain of specialized labs. The absolute number of CD4 cells and the ratio of that number to other white cell types require the use of two laser wavelengths, typically 488 nm and a red one (e. g., 640 nm). Usually the red is provided by a laser diode and the blue by an OPSSL or a diode pumped solid-state (DPSS) laser. Blue laser diodes do not have the required combination of long lifetime, stable output mode and low cost

needed for clinical flow cytometers. While this type of test is a critical element in treating HIV patients in developed countries, in the third world it is often still too expensive for widespread use.

Indeed, for this application lowering the costs is a key driver, together with the minimization of the size of the laser and the increase of laser efficiency to reduce thermal load. Consequently, OPSSLs have become the preferred laser choice as they excel in all these areas.

In an OPSSL, all the active and passive components are completely solid state, delivering the advantages of small size, high efficiency, consistent volume fabrication, and long-term reliability. Moreover, because the gain chip is a thin (approx. 10 microns) semiconductor chip rather than a long laser crystal, optical pumping is a two-dimensional problem. This constitutes a benefit when compared to the typical three-dimensional problem in pumping a laser crystal where the laser mode volume needs to be matched along the length of the crystal. In an OPSSL, the diode output simply has to be re-imaged with a circular profile onto the surface of this chip. These relaxed geometric tolerances for pumping mean lower component costs and also enable the entire laser cavity to be very compact. Simplification of assembly and alignment as well as the possibility of semi-robotic automated assembly can be achieved by mounting all components to a single block, thus ensuring high unit-to-unit consistency and volume cost savings.

For an instrument maker such as a flow cytometry manufacturer, the true cost of ownership includes other factors beyond the initial cost of the laser. These are e. g., reliability and lifetime, power consumption (i.e. efficiency), and cost of integration and installation. Again, OPSSLs offer advantages

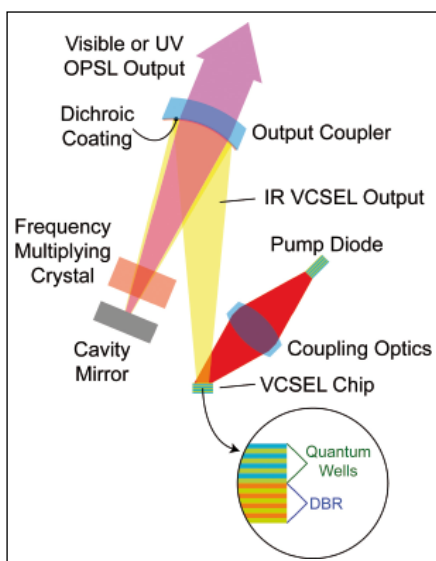


FIG. 2: This figure schematically illustrates the main components in an OPSSL (not to scale).

in these areas. For example, because they utilize solid state technology, OPSSLs are inherently much more efficient at converting electrical power to optical output, compared to traditional ion lasers and solid state lasers based on optical gain crystals such as Nd:YAG and Nd:YVO4. In an OPSSL the gain conversion medium is a monolithic III-V semiconductor chip, containing layers of tertiary quantum wells (InGaAs) alternated between binary (GaAs) layers (see Figure 2). These binary layers are optimized to efficiently absorb pump radiation, with an absorption coefficient orders of magnitude higher than in a typical laser crystal. In fact, the entire chip is less than 10 microns thick yet can absorb the pump radiation more completely than a laser crystal several millimeter in length. The end result is a typical wallplug efficiency for OPSSL lasers exceeding 2 %.

Another factor beyond OPSSL efficiency also contributes to lowering thermal management requirements. In an OPSSL, the pump diode wavelength does not have to be tightly controlled. Because at wavelengths shorter than their bandgap, the GaAs layers have a broad and truly continuous absorption profile, such that changes in the pump wavelength have no effect on efficiency or performance. In contrast, in DPSS lasers, based on Nd:YVO4 or Nd:YAG, the absorption peak in the laser crystal is only 1 to 2 nm FWHM, so even minor shifts in the pump diode wavelength can significantly affect laser performance. The output wavelength of the pump diodes strongly depends on their operating temperature, which must be closed-loop controlled using a thermoelectric cooler. In some DPSS lasers, operating this cooler represents a sizeable proportion of the total power budget, energy that is solely converted into heat. Therefore, in OPSSL systems, the pump diode temperature does not have to be separately and actively controlled. It is sufficient to just maintain the overall laser head within its safe thermal operating range via monitoring and control of its baseplate or heatsink.

Research instruments

The third application segment for flow cytometry is basic and applied research. Many of the instruments are configured in a modular format to accept multiple laser wavelengths – see Figure 3. Thus, multiple cell types can be counted and sorted. Also, it provides the ability to sort cell populations where multiple fluorophores are required to distinguish subtleties in their outer proteins. OPSSLs are well suited for this segment for



FIG. 3: Research instruments such as this MoFlo XDP from Beckman Coulter combine high speed and high sorting reliability. Notice the open architecture of the instrument which enables configuration modularity to be optimized for diverse sorting applications, including multiple excitation lasers. (Source: Beckman Coulter)



FIG. 4: The wavelength scalability of OPSL technology enables lasers to be built for nearly any visible wavelength.

THE COMPANY

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Since its foundation as a laser manufacturer in 1966, COHERENT Inc. has become the technology and market leader in a number of areas. The company, which is headquartered in California, has R&D and manufacturing facilities around the world – in Europe these are in Germany and Great Britain. A global service and sales network supports customers in industry, medicine and science.

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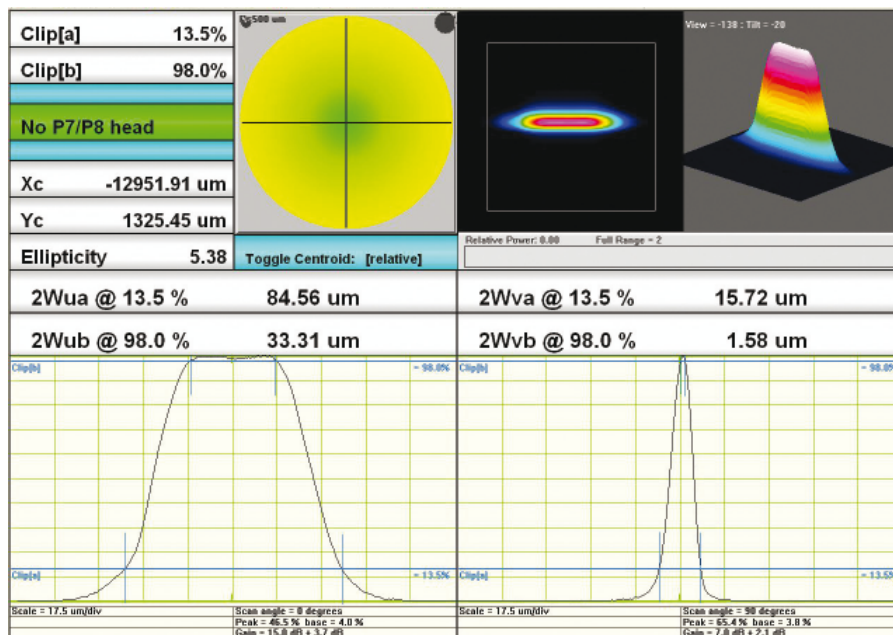


FIG. 5: Increasingly, laser manufacturers are offering a higher level of integration including optics to produce specific beam shapes – in this case a top hat line profile.

two reasons. First, the technology is wavelength scalable, allowing OPSLs to be built for nearly any visible wavelength, provided there is a volume demand – see Figure 4. For example, the first “new” wavelength developed for flow cytometry was 560 nm. The main purpose of this wavelength is optimized excitation of DsRed proteins, commonly attached to reporter genes. DsRed has been previously used in flow cytometry using the “nearest fit” 568 nm output line of the krypton ion laser. Conversely, while laser diodes are now available at many visible wavelengths, they do not have the beam quality and often not the necessary power.

Small size is another advantage of OPSLs for multiple wavelength applications. With their low thermal load, several lasers can be closely packed into a small volume, simplifying construction and operation of multi-wavelength cytometers.

Future trends

Looking to the future, what can we expect with respect to these applications for CW visible lasers? First, we anticipate a next generation featuring even greater ease of installation and operation. OPSLs will continue on their road to success as a plug and play device. Scalability in power and wavelength will further expand the OPSL based solutions portfolio to support new application demands in flow cytometry.

Reduced costs can be expected as OPSP technology has not yet fully leveraged its design fundamental cost advantages.

Another anticipated trend is vertical integration from laser manufacturers – the development of photonic engines which include the special beam shaping requirements for flow cytometry. Specifically, full optimization of flow cytometry needs a beam shaped to match the geometry of the fluid stream in the instrument, rather than a simple circular beam. Traditionally this has been done by instrument builders using a combination of cylindrical and spherical lenses. Increasingly this function will be integrated by laser manufacturers enabling faster time to market for new instruments as well as lower overall instrument costs – see Figure 5.

Conclusion

Flow cytometry is both a diverse and dynamic field. As with other key life science applications, the needs of cytometry have helped define a new generation of CW visible lasers and are now defining a future generation of lasers and integrated sub-systems. OPSP technology has proven to be a particularly effective and successful match for these applications because it offers flexibility in terms of wavelength and power scalability, together with inherent efficiency.