Permanently aligned multi-line lasers: A simplified solution for optical integration in biomedical instrumentation and fluorescence microscopes

Melissa Haahr, Elizabeth Illy, Håkan Karlsson, Magnus Rådmark, Gunnar Elgcrona
Cobolt AB, a part of HÜBNER Photonics, Vretenvägen 13, Solna, Sweden, 17145

ABSTRACT

The integration of multi-color laser excitation into biomedical instrumentation is associated with several challenges which must be overcome to meet the desired performance requirements of the instrument. Multi-color lasers are needed in fluorescence-analysis based applications such as flow cytometry, DNA sequencing, and various types of fluorescence microscopes such as scanning confocal microscopes, TIRF, Light-sheet, SIM, STORM and STED techniques. In many cases, these techniques require capability for excitation of multiple fluorophores and therefore access to several laser lines within the instrument.

The advantages of lasers over other light-sources, such as LEDs, for these techniques are high-brightness and wavelength precision. Unfortunately, the inclusion of lasers also introduces complexity in the design. Laser combiners including individual lasers have been integrated with the intention of simplifying the design, as an alternative to traditional multi-line gas lasers. This solution, however, is still susceptible to misalignment over time, and can increase the size and cost of the instrument.

A compact, permanently aligned, multi-line laser simplifies the integration of multiple laser wavelengths by eliminating the need for in-field alignment and service, reducing manufacturing cost, and allowing for more compact designs.

In addition to overcoming the initial design challenges of integrating lasers into bio-instrumentation, a multi-line laser is also an easy-to-upgrade field replacement for previous generations of technology, such as Argon Ion gas lasers.

Here we demonstrate how a compact and robust permanently aligned multi-line solid-state laser can be achieved using novel techniques for optical assembly and miniaturization. We also show how the integration of such a multi-line laser can deliver the required optical performance while simplifying the design and enabling commercialization of a new bioimaging technology, and exemplify the integration of this solution as a drop-in replacement for an Argon Ion lasers in existing microscope set-ups.

Keywords: Fluorescence microscopy, flow cytometry, multi-line laser, lasers for microscopy, biomedical instrumentation, light-sheet microscopy, argon ion replacement, Cobolt Skyra

1. INTRODUCTION

Fluorescence microscopy instrumentation relies on illumination sources to excite fluorophores. Common illumination sources are LEDs, super-continuum white-light sources, or single-wavelength lasers. Lasers are primarily used for high-resolution and high-throughput imaging techniques and each wavelength excites a different set of fluorophores. In order to efficiently excite multiple fluorophores, it is necessary to use multiple single-wavelength lasers in one instrument or experiment.

Along with the advantage of activating more fluorophores, comes the challenge of integrating each of the individual wavelengths required. Typically, there is a need to use between two and eight different lasers. Often this is solved with a laser-combiner, which includes separate lasers and beam-combining optics. Unfortunately, laser combiners can be a large and bulky solution, and difficult to keep aligned[1]. In addition, fiber coupling or alignment to a detection target add further complexity to the optical system.

A simplified solution for integrating multiple laser wavelengths into a fluorescence microscope is to use a multi-line laser, with permanent and stable fixation of all beam alignment optics included in the same package. It is now possible to deliver up to four laser colors from one compact and permanently aligned laser package, with co-linear beam output or stacked beams, and an option for direct fiber coupling. The introduction of multi-line lasers to fluorescence instrumentation provides a reliable, easy-to-use, and service-free solution to the challenges of including all the desired wavelengths with reliable, stable performance.

Here we will present how the Cobolt Skyra™ multi-line laser was engineered to improve the reliability and design of bio-instrumentation. We will discuss how a reliable, permanently-aligned, and easy-to-use multi-line laser can simplify the integration of lasers into fluorescence microscopes, accelerate the commercialization of new technology, and offer customized solutions within flow cytometry. We will also comment on how a multi-line laser can assist with replacement of previous technologies, such as Argon Ion lasers, in microscopy.

2. TECHNOLOGY

The development and design of the Cobolt Skyra™ multi-line laser was motivated by the technical, practical, and user-specific requirements of fluorescence microscopy applications for both research and instrument manufacturing. Further development followed to specifically address the requirements of flow cytometry, and the need for in-field replacement of Argon-Ion lasers in existing instrumentation.

The availability of such a compact, easy-to-use, and reliable high-performance multi-line laser has obvious benefit in the design of bio-instrumentation. However, the development and manufacture of such a solution is a significant challenge. The simplicity of the Cobolt Skyra™ multi-line laser package externally contrasts with the intricacy and complexity of the optical platform inside. The design is enabled by patent-pending alignment techniques and utilizing Cobolt's proprietary HTCure™ technology.

The HTCure™ technology is based on careful thermo-mechanical matching and high-temperature fixation of miniaturised optics. The lasers are built on a single, temperature-controlled platform for stable operation and protection from thermomechanical mis-alignment. All the optical elements, including components for beam combining, beam-shaping and alignment, are precision-mounted and the entire package is exposed to high-temperature baking and hermetically sealed. This offers high-precision in beam position, and strong fixation of optical components. The temperature-stabilized and compact package (meaning short beam paths) provide stable beam-pointing and robustness in varying environmental conditions.

Cobolt's HTCure™ technology was an integral part of developing a compact and reliable multi-line laser source for fluorescence microscopy techniques. It eliminates the need to align lasers in the field, by maintaining alignment under various ambient operating conditions, and keeping the laser lines focused into a fiber delivery system. Similarly, the stable beam alignment and beam shaping capabilities are beneficial to applications in flow cytometry for uniform exposure of a cell stream and alignment to detection optics. In addition, the control electronics of the multi-line laser are integrated directly into the laser head, for a simple, clean, and easily-integrated solution (Figure 1).

Figure 1: Cobolt Skyra™ multi-line laser with integrated electronics Free space dimensions: 70 x 144 x 38mm. Fiber coupled dimensions: 70 x 144 x 48 mm.

3. PERFORMANCE

The Cobolt Skyra™ can be fiber coupled with single-mode, polarization-maintaining fiber directly on the laser head. The fixed collinear beam alignment of the multi-line laser package is demonstrated by measuring the power stability through the fiber at each wavelength in changing environmental conditions (Figure 2). The data in Figure 2, below, is measured as a percent change in power per wavelength through the SM/PM fiber across a temperature range of 20°C to 50°C.

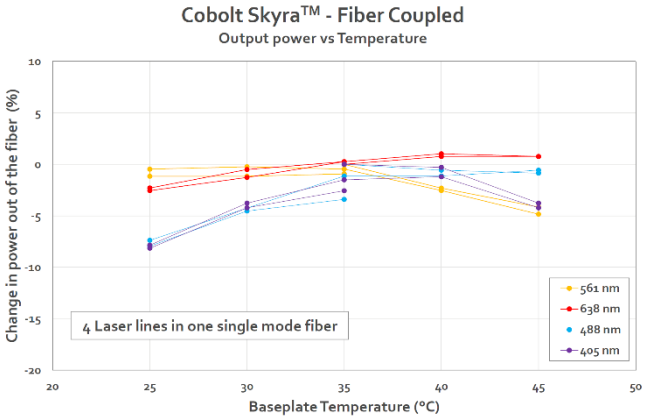


Figure 2 : Typical power stability out of the SM/PM fiber at each wavelength across the temperature range 20-50ºC.

The modulation of each wavelength is independent from the others, which is essential for use in fluorescence microscopy when exciting different fluorophores in various sequences. The external modulation controls are compatible with digital and analog inputs, as well as software commands via USB. Fast and deep digital modulation up to 5MHz is possible (Figure 3) and up to 500kHz in analog modulation, both are accessible through SMA connection on the back of the laser head. Programming the laser wavelengths for on/off operation or with different modulation sequences, often with only one USB connection, greatly simplifies the equipment needed to integrate and operate the laser. Furthermore, the software compatibility allows for remote control as well as remote servicing of the laser, further reducing the cost of ownership.

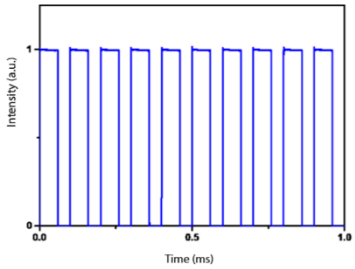
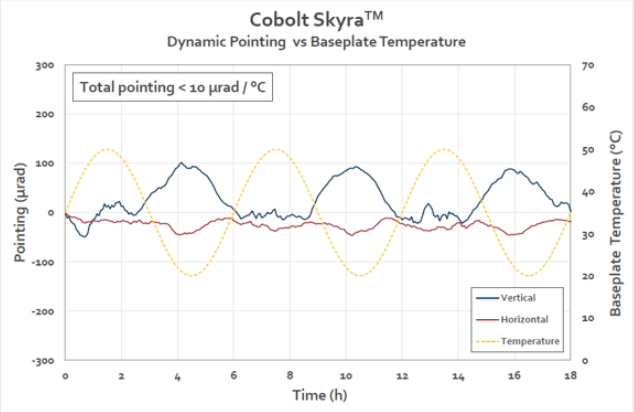


Figure 3: Typical pulse train with sub-microsecond pulses of Cobolt 473nm diode laser with direct digital modulation.

Numerous wavelength combinations are available, with or without direct fiber coupling. By including both direct-diode and diode pumped solid state laser technology on the multi-line laser platform, a wide range of wavelengths are available. The Cobolt Skyra™ can include up to four wavelengths, within the range of 405 nm to 660 nm with beam position overlap <50um at the exit and pointing stability <10urad/ºC over a temperature range of 20ºC to 50ºC (Figure 4). The output beams of the Cobolt Skyra™ can be collinear free-space or coupled into single mode fibers for convenient launching into microscope set ups, or tailored to form stacked light sheets at a precisely defined location in front of the laser for direct alignment to, for example, a flow cell in a cytometer.



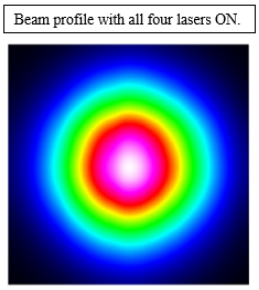


Figure 4a (left): Vertical and horizontal beam pointing (urad) over 18 hours continuous laser operation and temperature cycling between 20°C to 50°C.
Figure 4b (right): Beam profile of typical 4-line Cobolt Skyra™ demonstrating Gaussian beam overlap.

4. APPLICATIONS IN FLOW CYTOMETRY

Recent developments on the platform have also allowed for custom beam shaping and beam positions. These capabilities are possible through utilizing the HTCure™ technology and specialized optical design. For example, it is now possible to build top-hat profiles or elliptical beams within the Skyra™ platform. Such capabilities allow for complete customization of the multi-line laser for specific manufacturing needs. The flexibility of the HTCure™ technique, and the stability of the Cobolt Skyra™ enable the generation of top hat beam shapes on each individual wavelength using a single optical element (Figure 5). The platform and design also allow for generating stacked beams for applications requiring beam separation.

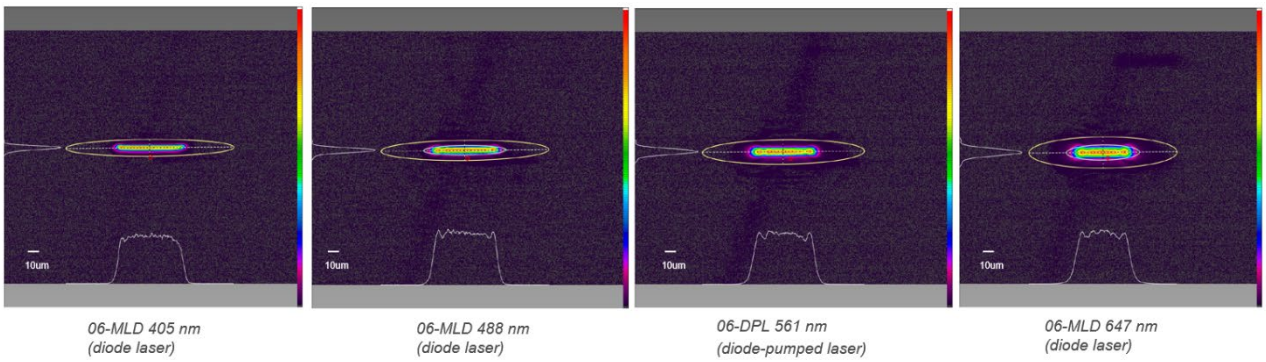


Figure 5: Example of top-hat profiles of four wavelengths (405nm, 488nm, 561nm, and 647nm) achieved with Cobolt Skyra™ and a single optical element.

Notable development has been made on the Cobolt Skyra™ platform specifically for integration into flow cytometry instrumentation. In addition to the requirement of multiple available colors within a flow cytometer, the alignment of the system and beam parameters of the lasers is of utmost importance. Lasers must be aligned to detectors, after passing through a flow cell of sample stream. This places strict requirements on laser pointing stability and beam position, to achieve precision alignment to targets further on in the optical path, as well as tight beam parameters to ensure even laser illumination within the cell stream of the flow cell[2]. A generic schematic of spatially separated light sheets showing laser lines passing through a flow cell is illustrated in Figure 6.

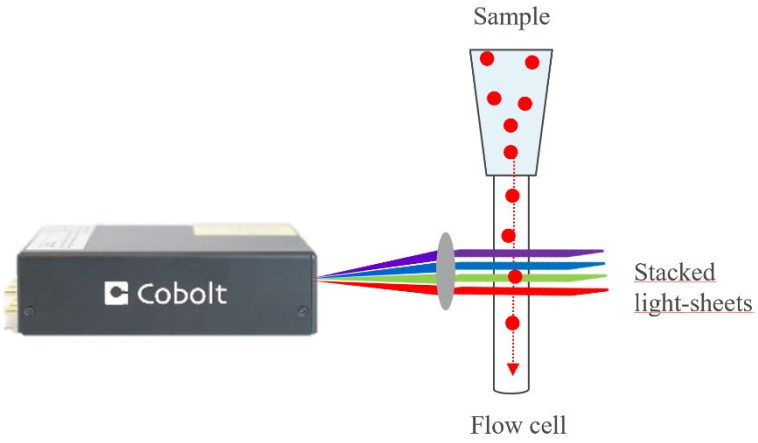


Figure 6: Schematic of stacked beams with spacial separation passing through a flow cell. Not drawn to scale.

Traditional optical paths within flow cytometers can be complex and subject to misalignment and require maintenance in the field. Utilizing the HTCure™ manufacturing technique and expertise in miniaturized optical design, it is now possible to incorporate more of the optical path inside of the Skyra™ platform solution. Thus, integration of the lasers and optics within the instrument becomes simplified and customized. It minimizes the need for additional optics for beam shaping and beam steering external of the laser platform and provides easier replacement and installation. An integrated solution can also reduce the time and cost of maintenance during the instrument life cycle.

5. APPLICATIONS IN FLUORESCENCE MICROSCOPY

An interesting technique for use of multi-line lasers is in the field of cancer diagnostics and the progression towards increasing fluorescence instrumentation in clinical settings. The barrier of developing such suitable instrumentation and achieving clinical certification is high, but a critical step is creating advanced instrumentation that also has the capability to be commercialized and accessible. The first challenge is to develop the technique, but it is often followed by a second challenge of making that new technology dependable and user-friendly.

A team from Dr. Jonathan Liu's laboratory at the University of Washington has recently developed a cutting-edge open-top light-sheet microscope for fast, non-destructive, slide-free, 3D pathology[3]. The technique rapidly images 3D biological samples, without slicing the tissue-sample as in traditional pathology techniques. A unique application for this technology is applied to prostate needle-core biopsies and cancer diagnosis. Furthermore, Dr. Liu and his team have continued to drive their technology towards commercialization[4]. The use of an easy to control, compact, and permanently aligned multi-line laser assisted in the simplifying of the optical assembly in their innovative instrument design[4].

In academia, some of the earliest users of the Cobolt Skyra™ have utilized the technology to equip laboratories with a powerful tool for multiple types of microscopy techniques. One such laboratory is that of Prof. Dr. Markus Sauer at the Department of Biotechnology and Biophysics at Julius-Maximilian-University of Würzburg. Researchers in Prof. Dr. Markus Sauer's lab are focusing on single molecule sensitive fluorescence spectroscopy and imaging techniques, including super-resolution microscopy and its applications in biomedical sciences. The Cobolt Skyra™ laser has, for example, been used in a single-molecule localization microscopy (SMLM) setup to gain new insights into the organization of proteins within a cell. The system provides images with spatial resolution nearing the molecular level, from which quantitative biological data can be extracted (Figure 7). The Cobolt Skyra™ was an economical, high-performing, and easy-to-use solution in their instrumentation, helping to move research along at a faster pace with consistent results.[5]

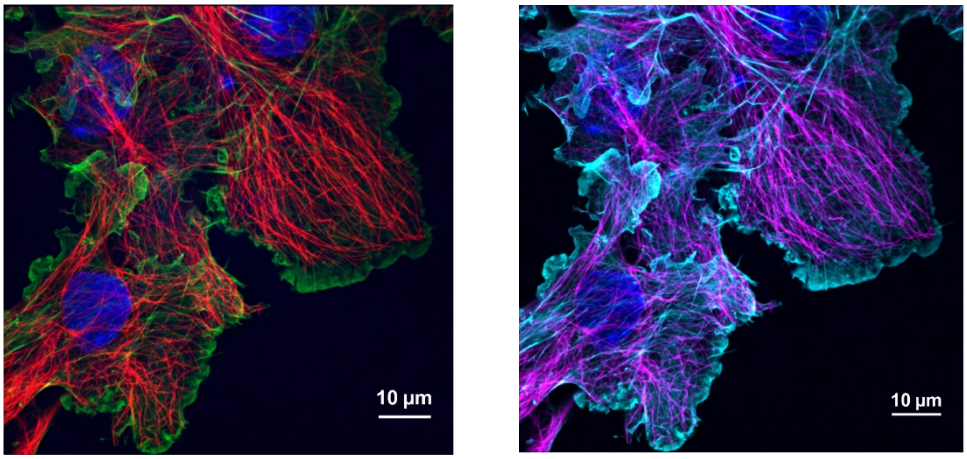


Figure 7. An example of an images taken in a single-molecule localization microscopy (SMLM) setup from Department of Biotechnology & Biophysics at Julius-Maximilian-University of Würzburg.The 3-color image shows african green monkey kidney cell (COS7) with nucleus (blue), microtubules (red/magenta) and the actin sceleton (green/cyan) staining. Recording time 4s per channel at 2048x2048px field of view.[5]

Another application for multi-line lasers is as in-field replacement of previous generations of technology. In particular, the replacement of Argon-Ion gas lasers which have been utilized with earlier models of commercial microscope systems. The Argon-Ion lasers are large, difficult and expensive to maintain, and in some cases no longer supported by the original manufacturer. As much of the fluorescence-based microscopy instrumentation has transitioned from gas lasers to compact and reliable solid state laser sources[1], it is a further advantage to replace Argon Ion lasers directly with a multi-line solid state laser with permanent alignment of all of the necessary wavelengths (for example: 457nm, 488nm, and 515nm) and direct fiber coupling. Due to the availability of wavelengths and stable fiber coupling, it is possible to make a direct drop-in replacement with the Cobolt Skyra™ (Figure 8). Cost of ownership over time, and general ease-of-use, are also advantages to transitioning from gas lasers to solid state laser sources as maintenance-free alternatives.

Figure 8: Fiber-coupled Skyra™ multi-line laser including 457nm, 488nm, and 515nm.
Fiber coupled configuration dimensions: 70 x 144 x 48 mm.

6. CONCLUSION

Fluorescence imaging is a key technique in both biomedical research and clinical diagnosis. Fluorescence microscopes for high-resolution and high-throughput multi-fluorophore imaging typically rely on the use of several individual laser sources at different wavelengths, within the same instrument. Traditionally these lasers have been coupled into the microscopes through laser combiners, which have added bulk, cost, and alignment complexity. The new solution to improve instrument simplicity and design can be accomplished instead with a multi-line laser.

In this work, we have shown how the design of the Cobolt Skyra™ multi-line laser was specifically developed to address the common challenges of optics and laser integration within multi-color fluorescence instrumentation. Through the use of the HTCure™ manufacturing process, we produced quality laser performance in a reliable, simple, and cost-effective package for both commercial systems and laboratory-built instrumentation. The HTCure™ technology has been further applied to address the specific needs of flow cytometry with custom beam outputs and controls. The simple and reliable multi-line laser solution can assist in instrument design, commercialization, and in-field performance over time.

The very precise, stable and compact beam alignment enabled by the HTCure™ technology allows for direct integration of the laser source into the instrument without the need for further alignment of individual lasers or optics for beam shaping or combining. In this way, the Cobolt Skyra™ concept can transform the way laser-based multi-color bioinstrumentation is designed and manufactured. It enables smaller and more cost-efficient instruments which are easier to manufacture and maintain. This supports the strive for bringing more advanced laser-based instrumentation into research and clinical settings for improved medical diagnostics and further development of new analytical techniques.

We have demonstrated how multi-line lasers can simplify the use of a research laboratory fluorescence microscopy set-up, facilitate commercialization of a new fluorescence microscopy technology, be customized for specific beam requirements to address flow cytometry demands, and be considered for drop-in replacement of Argon-Ion gas lasers in the field.

REFERENCES

[1] E.Illy and H. Karlsson, *BioOptics World*, Highly stable multi-line lasers for next-generation imaging. Oct 2018.
[2] M. Wallace, *Laser Focus World,* Lasers for Biosciences: Lasers for flow cytometry are small, solid, and reliable, Jan 2019
[3] Glaser, A. K. et al. Light-sheet microscopy for slide-free non-destructive pathology of large clinical specimens. Nat. Biomed. Eng. 1, 0084 (2017).
[4] Glaser, A., Reder, N., Liu, J., Buckley, S., True, L. https://lightspeedmicro.com/ January 2019.
[5] M. Sauer and M. Heilemann, *Chem. Rev.*, 117, 7478−7509 (2017); doi:10.1021/acs.chemrev.6b00667.