Multi-line lasers simplify biomedical imaging

The introduction of multi-line lasers to fluorescence instrumentation provides a compact, easy-to-use, and service-free solution for integrating up to four laser wavelengths with reliable, stable performance. In this white paper we explore the advantages of a permanently aligned multi-line laser with fully integrated electronics.

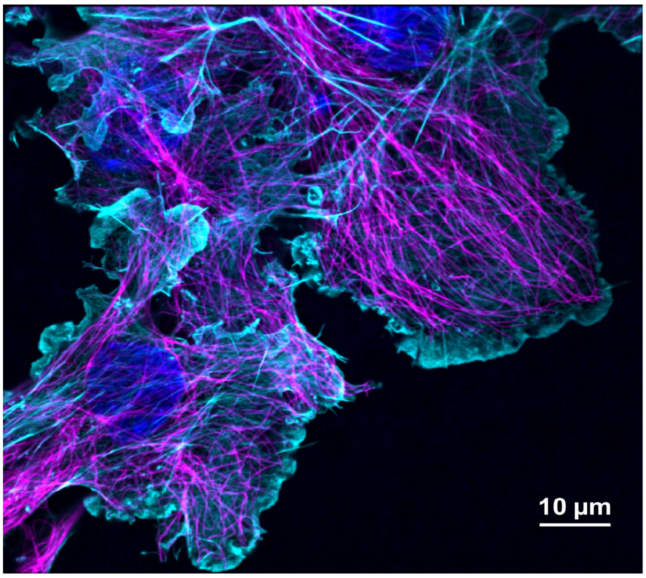
Over the last decade, the fluorescence-based life science industry has already been transitioning from bulky gas-laser sources into solid-state lasers with a smaller footprint, longer lifetime, and lower maintenance requirements. The development of compact, reliable solid-state lasers was an initial enabling technology for commercialization and expansion into new markets and applications. While some applications are able to utilize the advancements in LED and super-continuum white-light sources, the high-resolution, high-speed techniques still rely on the high-brightness and wavelength precision of lasers.

Currently, many researchers and manufacturers align and integrate individual laser sources for each desired wavelength either onto an optical bench or within the instrument. These assemblies require additional optics for each laser line, all of which need to be aligned with high precision and typically launched into a fiber delivery system. This design often requires the time and cost of installation and service by a technician from the instrument manufacturer or the time of a researcher spent aligning optics instead of collecting new data. Laser combiners and laser light engines have simplified some of these assemblies substantially. However, they do not eliminate the need for alignment (and re-alignment) over time, and can contribute the overall bulkiness of a manufactured solution.

As new techniques are developed for clinical applications, ease-of-use and the ability to commercialize the instrumentation become increasingly important. While maintaining the highest quality and performance, laser manufacturers must deliver reliable, simple, and cost-effective solutions for both commercial systems and laboratory instrumentation for fundamental research.

The use of multi-line lasers as an alternative to conventional laser combiners or laser engines solves many of these common pain-points in fluorescence microscopy applications. A "multi-line laser" is several individual laser wavelengths built into one laser platform, with permanent and stable fixation of all beam alignment optics included the same package. The Cobolt Skyra™ is a totally customizable, permanently aligned multi-line laser solution offering up to four individual wavelengths, ranging from 405 nm to 660 nm, in a single laser output.

The availability of a compact, easy-to-use, and reliable high-performance multi-line laser will assist with the commercialization of new fluorescence-based-instrumentation and further expand existing technologies into laboratories with a lower barrier of entry, for both the instrument manufacturer and end-user.

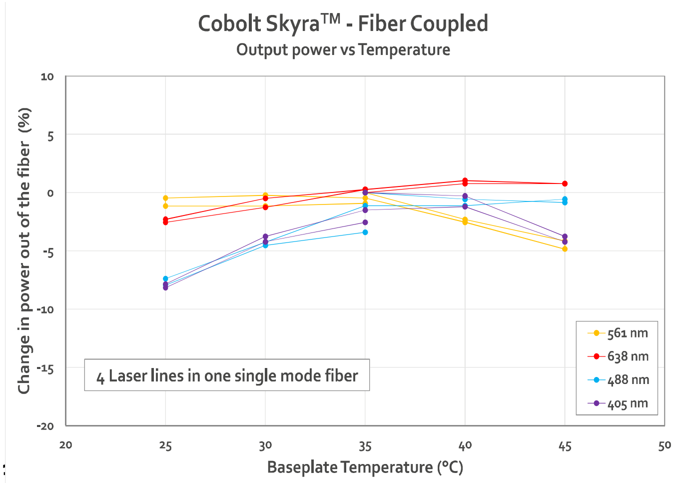


An example of an image taken in a single-molecule localization microscopy (SMLM) setup including Cobolt Skyra™ from Department of Biotechnology & Biophysics at Julius-Maximilian-University of Würzburg.

Enabling technology

The Cobolt Skyra™ multi-line laser is unique in its' design and manufacturing. It is built using patent-pending alignment techniques and utilizing Cobolt's proprietary HTCure™ technology.

The lasers are built on a single, temperature-controlled platform for stable operation and protection from thermomechanical misalignment. All the optical elements, including components for beam combining, beam-shaping, and alignment, are precision-mounted and the entire package is hermetically sealed. The temperature-stabilized and compact package provide stable beam-pointing and robustness in varying environmental conditions **(Figure 1a).**



1a

1b

Figure 1a: – typical power stability out of the SM/PM fiber at each wavelength across the temperature range 20 °C to 50 °C.

Figure 1b: – Cobolt Skyra™ multi-line laser with integrated electronics (Dimentions: 70 x 144 x 38 mm).

The Cobolt Skyra™ can be coupled with single-mode, polarization-maintaining fiber coupling directly on the laser head. The output power stability in figure 1a below is measured through the SM/PM fiber, from 20 °C to 50 °C.

Cobolt's HTCure™ technology is integral to the development of compact and reliable multi-line laser sources 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. 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 1b)**.

Different fluorescence microscopy techniques, applications, and day-to-day experiments can utilize multiple combinations of performance and capabilities in the experimental design. Most of these demands can now be met simultaneously with one standard or customized variation of a multi-line laser source. As standard on Cobolt Skyra™, the modulation and control of each wavelength is independent from each other. The controls are compatible with digital and/or analog inputs, as well as software commands via USB. Fast and deep digital modulation up to 5 MHz is possible as well as 500 kHz in analog modulation.

The Cobolt Skyra™ can include up to four wavelengths, within the range of 405 nm to 660 nm with beam position overlap <50 um at the exit and pointing stability <10 urad/°C over a temperature cycling between 20 °C and 50 °C **(Figure 2a).**

The output beams of the Cobolt Skyra™ can be collinear and coupled into single mode fibers for convenient launching into microscope set-ups. Alternatively, thanks to the flexible design and patented manufacturing technique, the output beams can be tailored to form stacked light sheets at a precisely defined location in front of the laser for direct alignment onto an external target.

In the LAB

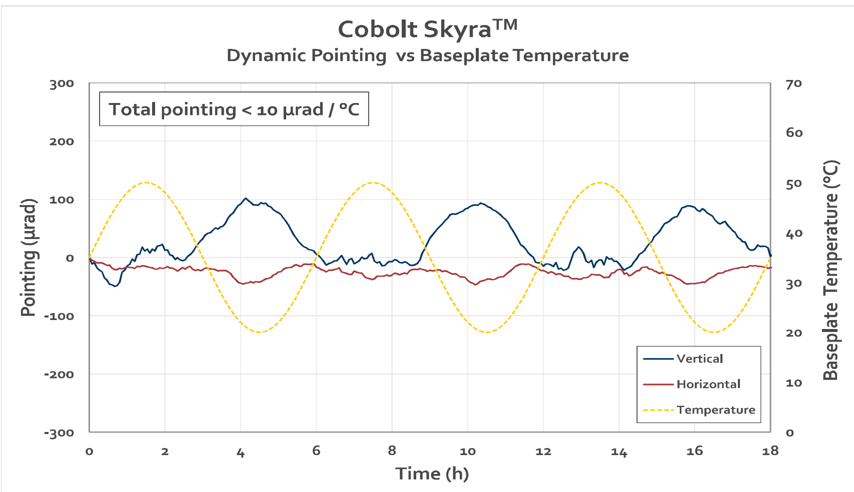
Some of the earliest users of the Cobolt Skyra™ in academia have utilized the technology to equip laboratories with a powerful tool for multiple types of microscopy techniques. One such laboratory is that of Prof. Dr. Markus Sauer at the Department of Biotechnology and Biophysics at Julius-Maximilian-University 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 set-up in their laboratory system provides images with spatial resolution nearing the molecular level, from which quantitative biological data can be extracted **(Figure 3a)**. The Cobolt Skyra™ was an economical, high-performing, and easy-to-use solution in their instrumentation, helping to move the research along at a faster pace with consistent and reliable results **(Figure 3b)** [1].

Another interesting use of multi-line lasers is in the field of cancer diagnostics and the progression towards increasing fluorescence instrumentation in clinical settings [2]. The barrier of developing suitable instrumentation and achieving clinical certification is high, but a critical step is creating advanced instrumentation that can be commercialized and accessible.

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. 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 [3]. Furthermore, Dr. Liu and his team have continued to drive their technology towards commercialization. The use of an easy to control, compact, and permanently aligned multi-line laser assisted by simplifying the optical assembly in their innovative instrument design.

2a



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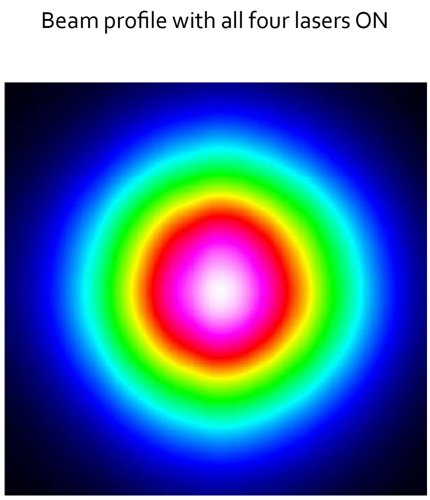
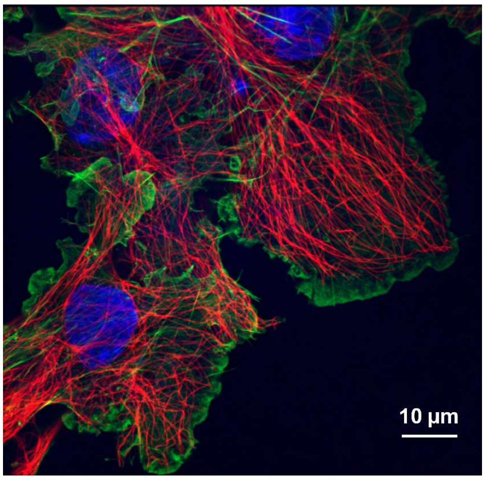


Figure 2a: Vertical and horizontal beam pointing (stability urad) over 18 hours continuous laser operation and temperature cycling between 20 °C to 50 °C.

Figure 2b: Beam profile of typical 4-line Cobolt Skyra™ demonstrating Gaussian beam overlap.

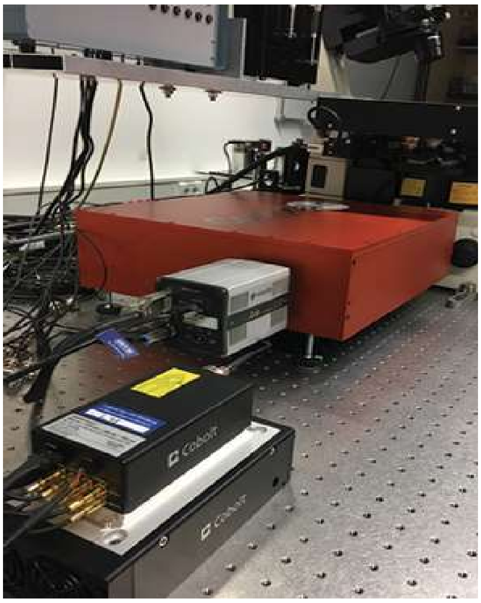


3a

Outlook

Fluorescence imaging is a key technique in both biomedical research and clinical diagnosis. Fluorescence based 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 can add bulk, cost, and alignment complexity.

Multi-line laser solutions are an attractive alternative to laser combiners to simplify fluorescence based imaging instrumentation and furthermore aid in the process of commercialization for new, cutting-edge imaging systems for clinical use. Multi-line lasers enable smaller and more cost-efficient instruments which are much easier to manufacture and maintain. This development 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.



3b

Figure 3a - An example of an image taken in a single-molecule localization microscopy (SMLM) setup from Department of Biotechnology & Biophysics at Julius-Maximilian-University of Würzburg. The 3-color image (above and on page 1) 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.

Figure 3b - Cobolt Skyra™ laser is shown in use in the Department of Biotechnology & Biophysics at Julius-Maximilian-University of Würzburg [1].

References

[1] M. Sauer and M. Heilemann, Chem. Rev, 117, 7478–7509 (2017); doi:10.1021/acs.chemrev.6b00667.
[2] Glaser, A. K. et al. Light-sheet microscopy for slide-free non-destructive pathology of large clinical specimens. Nat. Biomed. Eng. 1, 0084 (2017).
[3] Glaser, A., Reder, N., Liu, J., Buckley, S., True, L. https://lightspeedmicro.com/ January 2019.

About the company

Cobolt AB is a part of HÜBNER Photonics.
At HÜBNER Photonics, we not only make gamechanging lasers, but also rethink all kinds of other technologies including terahertz imaging and high-frequency radar. Since 2015, our portfolio has been further reinforced by the acquisition of Cobolt AB, a world leading manufacturer of high performance lasers for analytical instrumentation. Together, we unite proven corporate values with innovative ideas and top-notch technologies for the whole electromagnetic spectrum.

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Melissa is the product manager for Cobolt's lasers for Life Sciences.

Melissa holds a Master's degree in physical chemistry from the University of Oregon and Bachelor's degrees in Chemistry and Environmental Science from Alfred University.
She joined the company in 2014 with knowledge in analytical instrumentation and analysis techniques, as well as technical sales experience.