Fluorescence microscopy instrumentation simplified using novel multi-line lasers

Melissa Haahr, Elizabeth Illy, Håkan Karlsson, Magnus Rådmark
Cobolt AB Vretenvägen 13, Solna, Sweden, 17145

Cobolt
a HÜBNER Group company

SPIE.

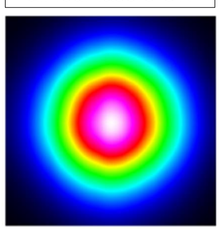
INTRODUCTION

Conventional fluorescence-based bio-instrumentation equipment typically uses multiple individual lasers combined through optical elements into one beam or an optical fiber. The systems can become bulky, costly to manufacture, and challenging to keep aligned. An extremely compact, permanently aligned, and service-free multi-line laser device can reduce the size and cost of these systems for fluorescence-based research. Removing the complexity of integrating individual lasers with a multi-line solution makes the techniques more cost-efficient, user-friendly, and accessible for all levels of researchers. Here we demonstrate how multi-line lasers are integrated into fluorescence-based instrumentation to simplify experiments and assist in commercialization of new technology. Integrated electronics, software interfacing, and individual control of each laser-line allow for full flexibility to tailor the laser for the exact experimental needs.

Functionality

- **Permanently aligned**
- **Integrated electronics**
- **Compact and easy-to-use**

Beam Overlap-
all four lasers ON



Cobolt Skyra™
144mm x 70mm x 38mm

Customized Solution

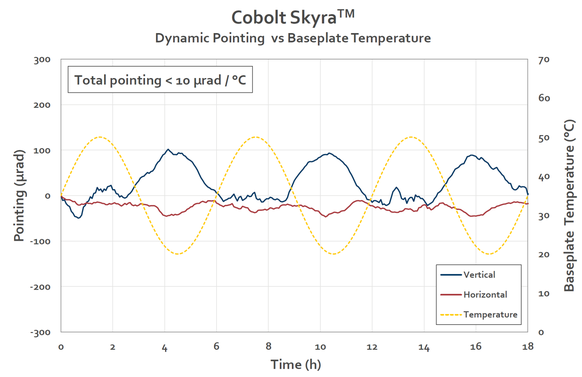
- **Up to 4 wavelengths**
- **Digital and Analog Modulation**
- **Individual software control**

ENABLING TECHNOLOGY

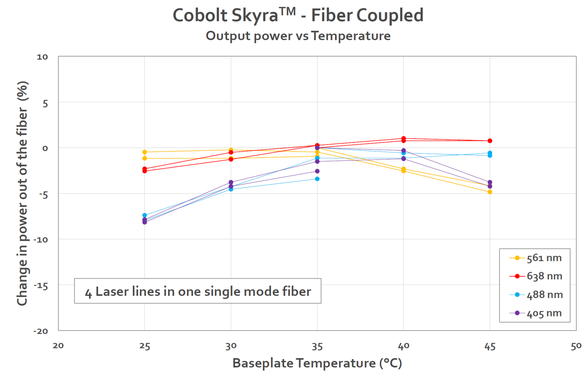
The Cobolt Skyra™ multi-line laser is unique in its' design and manufacturing. It is built using patent-pending alignment techniques and utilizing Cobolt's proprietary HTCure™ technology, based on careful thermo-mechanical matching and high-temperature fixation of miniaturized optics. The lasers are 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.

PERFORMANCE

The temperature-stabilized and compact package (meaning short beam paths) provide stable beam-pointing and robustness in varying environmental conditions. Below, dynamic beam pointing is measured over 18hours and temperature fluctuations between 20°C and 50°C.



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



APPLICATIONS

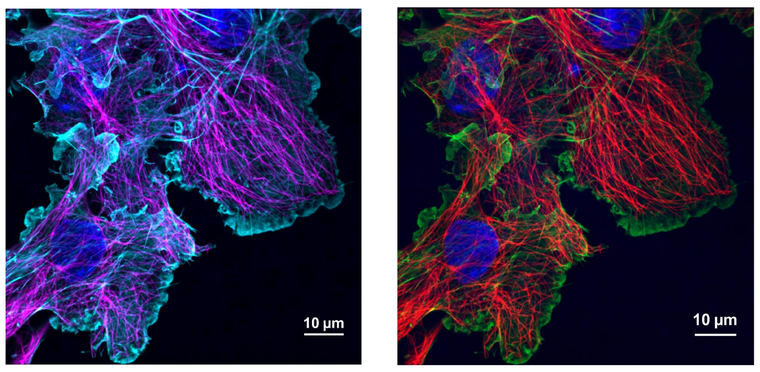
1. Instrument Design and Commercialization

- Multi-line lasers support progression towards increasing fluorescence instrumentation in clinical settings.
- An easy to control, compact, and permanently aligned multi-line laser simplify the optical assembly in innovative instrument design.
- Cobolt Skyra™ can assist in bringing new technology from benchtop to reliable, commercially viable solutions.

2. Laboratory and Research

- Cobolt Skyra™ is a powerful tool for multiple types of microscopy techniques and cross-disciplinary laboratories.
- Versitile solution with capability to support many unique experimental requirements.
- Cobolt Skyra™ is an economical, high-performance, and easy-to-use solution, helping to move laboratory research along at a faster pace with consistent results.

In one example, a fiber-coupled Cobolt Skyra™ with 405nm, 488nm, 561nm, and 638nm lasers is used by researchers from the group of Prof. Dr. Markus Sauer at Julius-Maximilian-University of Würzburg for single molecule localization microscopy (SMLM) 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.



Images courtesy of Dr. Andreas Kurz. The 3-color images show african green monkey kidney cell (COS7) with nucleus (blue), microtubules (red/magenta) and the actin skeleton (green/cyan) staining. Recording time 4s per channel at 2048x2048px field of view. Cobolt Skyra™ laser.

CONCLUSION

- **Multi-line lasers are an attractive solution to simplify the optical assemblies in fluorescence imaging instrumentation.**
- **One such multi-line laser source is the Cobolt Skyra™, which is integrated into one single hermetically sealed package using HTCure™ laser manufacturing technology.**
- **The very precise, stable and compact beam alignment allows for direct integration of 4 wavelengths into the instrument without the need for further alignment of individual lasers or optics for beam shaping or combining.**
- **The Cobolt Skyra™ concept can transform the way laser-based multi-color bioinstrumentation is designed and manufactured.**

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.