

# Multi-Line Lasers Help Simplify Light Sheet Microscopes Targeting Clinical Applications

HÜBNER Photonics



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## Introduction

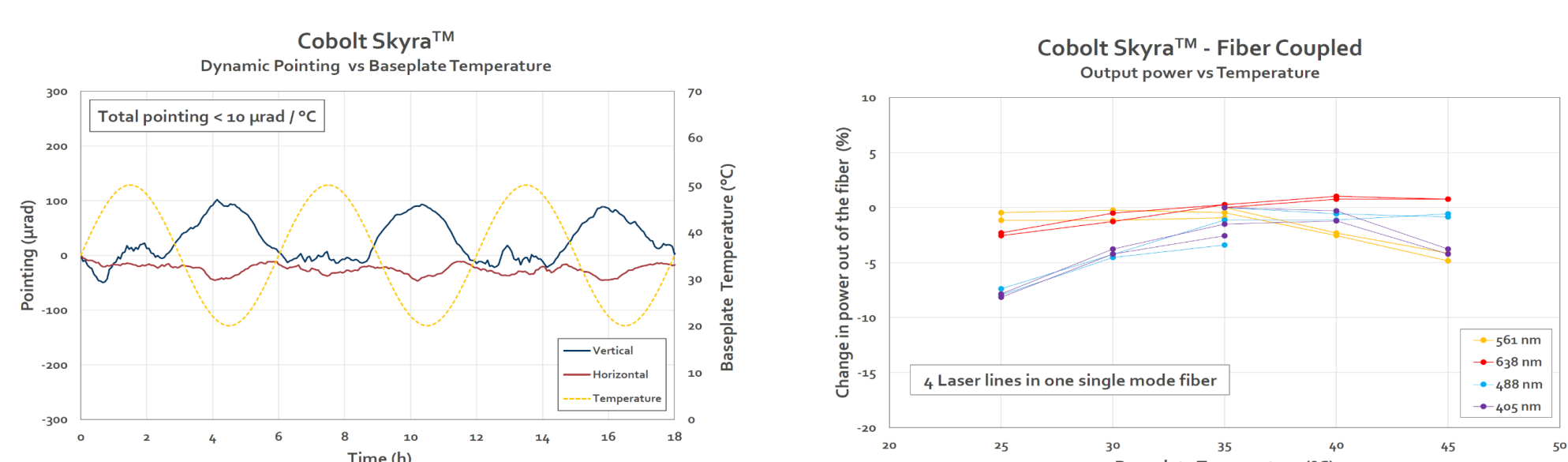
Light sheet microscopy is a fluorescence imaging method that has demonstrated strong capability to provide new ways of analyzing human specimens in the field of pathology and offering digital alternatives to the decades old and well-established field of histopathology [1]. **Light sheet microscopy** brings the combination of gentle sample illumination, high speed 3D imaging as well as high spatial resolution and molecular specificity.

However, despite increased availability of highly efficient tissue clearing techniques such as DISCO, CUBIC and SHIELD in recent years, poor access to adequate, easy-to-use and cost-efficient light-sheet fluorescence microscopy still remains a major obstacle for biomedical end-users.

In this work, we demonstrate how the concept of **multi-color light sheet microscopy** can be further improved in terms of **simplicity, cost-efficiency and spectral flexibility** through the combination of open-sourced descSPIM (desktop equipped Selective Plane Illumination Microscopy) [2] with permanently-aligned **multi-line laser sources** and new diode-pumped lasers in the **orange**. The descSPIM offers the benefits of both efficient sample evaluation in routine experimentation, with volume imaging of optically cleared samples in minutes, as well as advanced capabilities, including multi-view whole-brain imaging.

## Cobolt Skyra: Permanently aligned multi-line laser for compact multi-color illumination

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**. All the optical elements (lasers, beam combining, beam-shaping and alignment) are precision-mounted on a single platform and the entire package is baked and hermetically sealed.



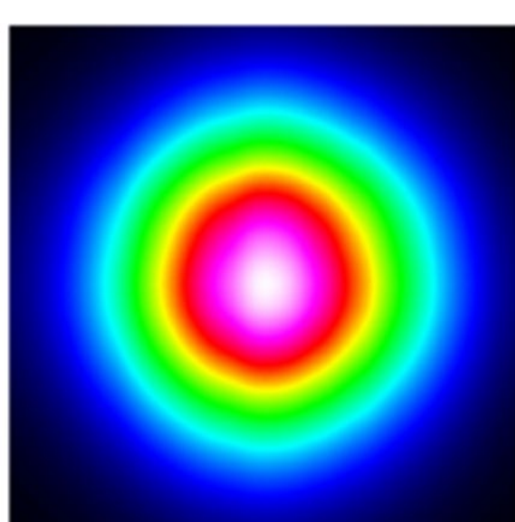
Pointing stability in temperature cycles of 20°C to 50°C over 18 hrs continuous operation

Power stability through single-mode fiber coupling over 25°C to 45°C



Cobolt Skyra™  
144mm x 70mm x 38mm

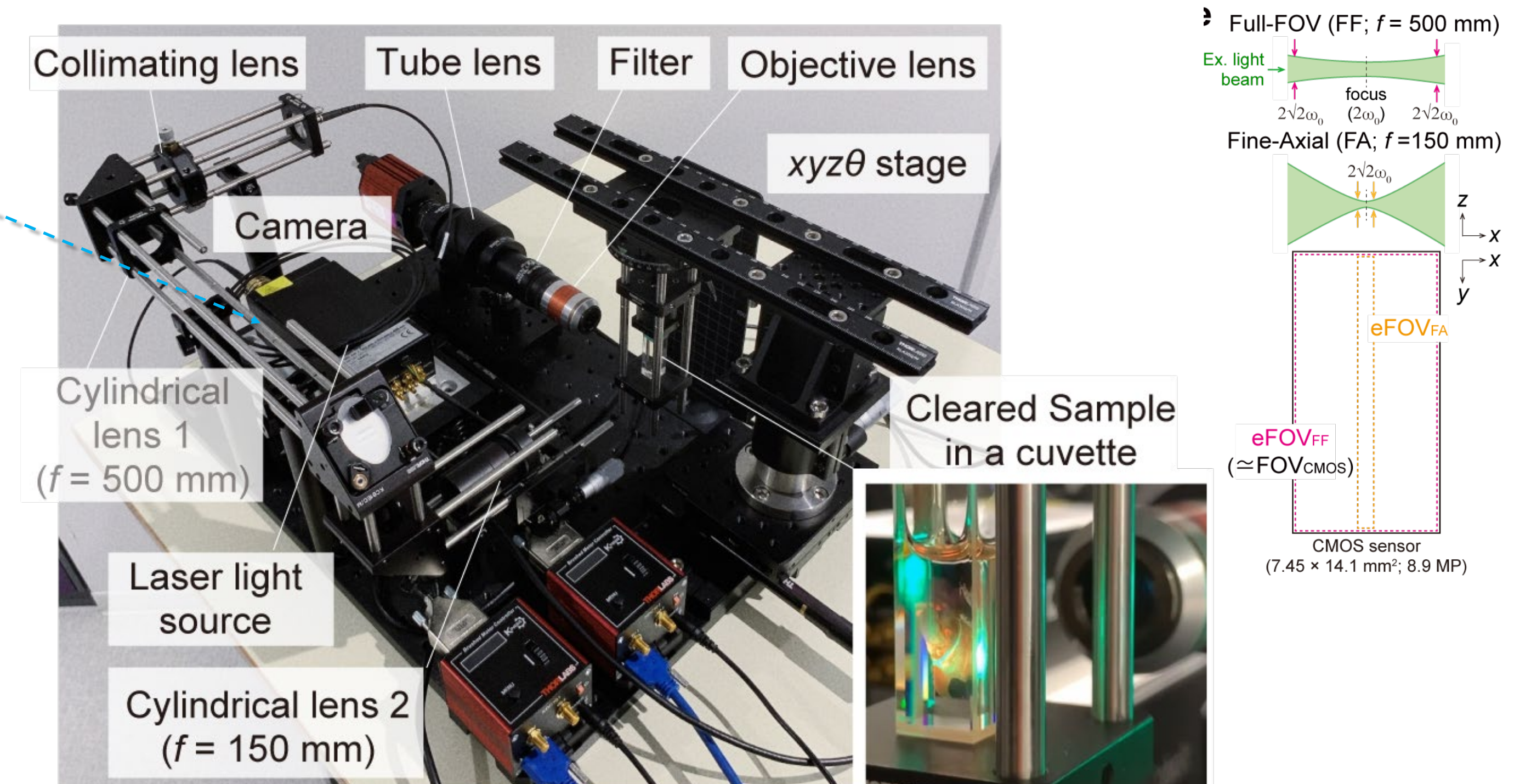
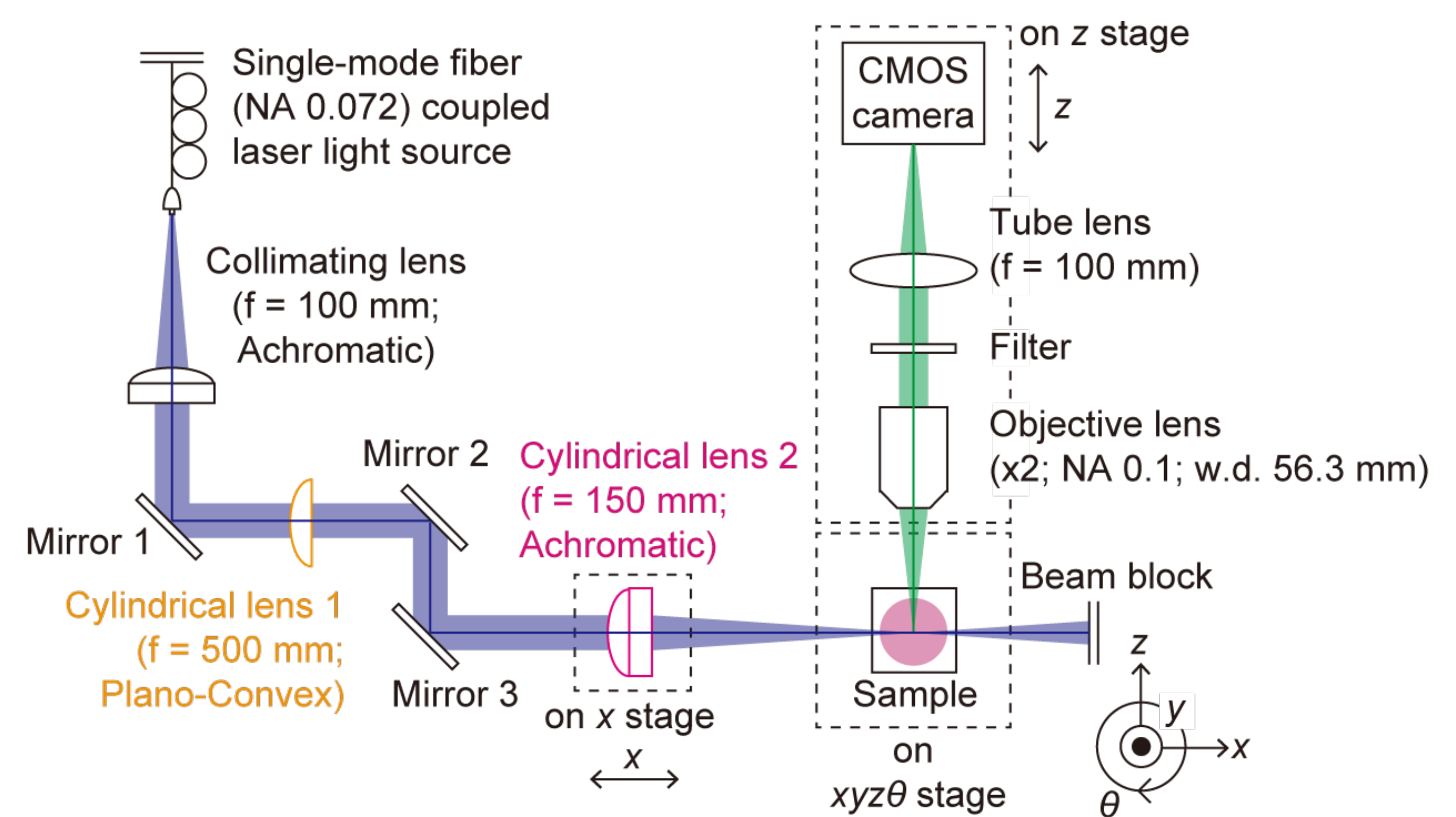
- HTCure™ technology:**
- High-temperature curing of miniature optics
  - Thermo-mechanically stable
  - Hermetically sealed packages for ultimate robustness
  - Verified insensitivity to challenging environmental conditions



Beam overlap with four laser lines ON

- Up to 4 wavelengths
- Integrated electronics
- Digital and Analog Modulation up to 5 MHz
- Free space or SM/PM fiber coupled output
- Proven reliability & lifetime, >15 000 hrs

## descSPIM set-up: Affordable and easy-to-build light sheet microscope<sup>[2]</sup>



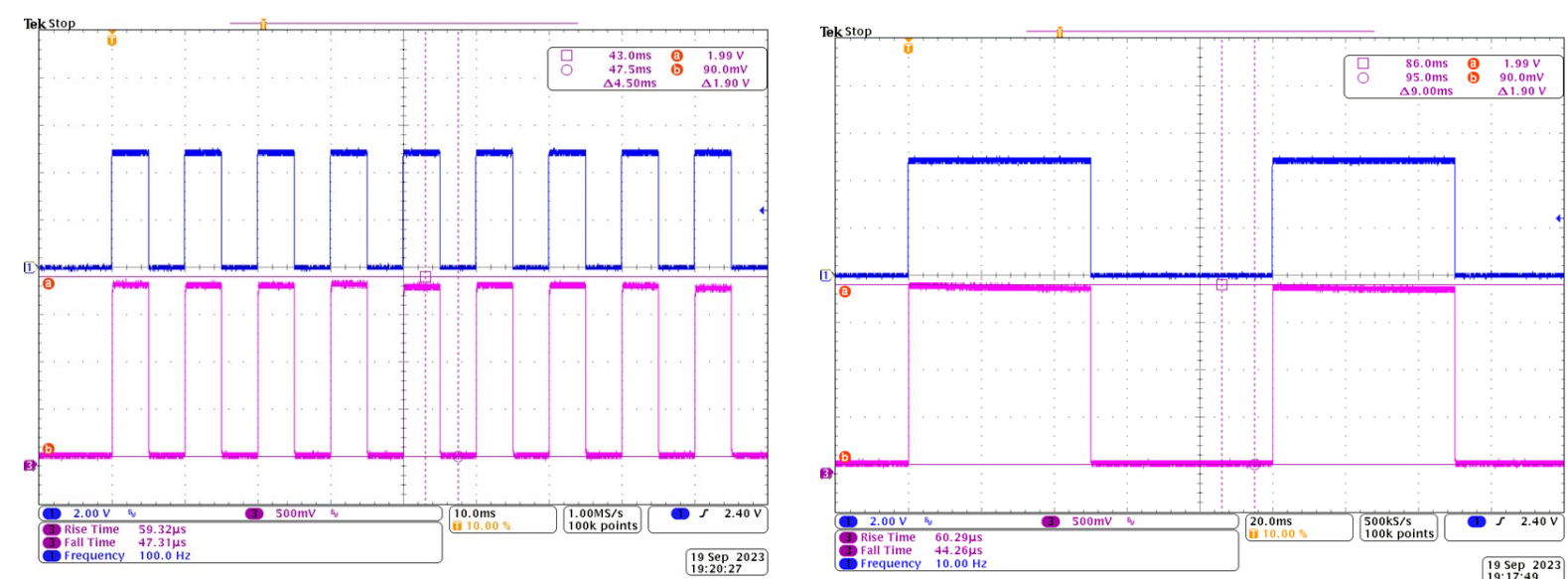
- Routine 3D imaging of cleared samples in minutes
- Open Source part list [3]
- Allows for a personalized LSFM in biomedical labs
- Compatible with custom add-ons

## New compact orange laser for increased spectral flexibility

As a further enhancement of the system flexibility, we also demonstrate a new compact and directly modulated laser line in the orange (594 nm), specifically suitable for excitation of **mCherry** and other **red fluorescent proteins**. The new diode-pumped laser is integrated into the Cobolt 06-01 Series and features up to **100 mW** output power in a perfect TEM00 beam from a compact package including all electronics.



Cobolt 06-DPL 594 nm

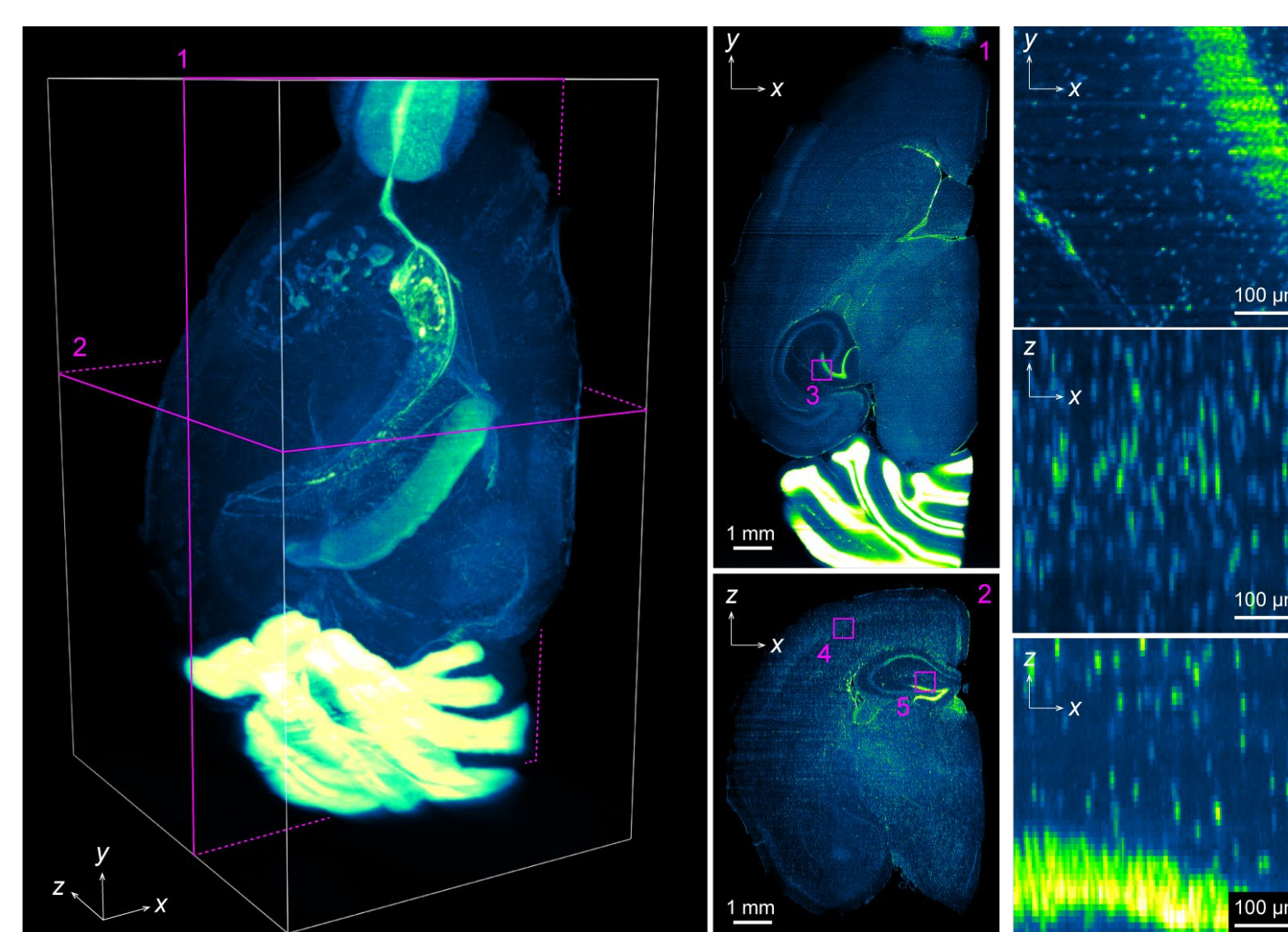


Digital intensity modulation 0-250 mW at 100 Hz and 10 Hz, including the first pulse performance

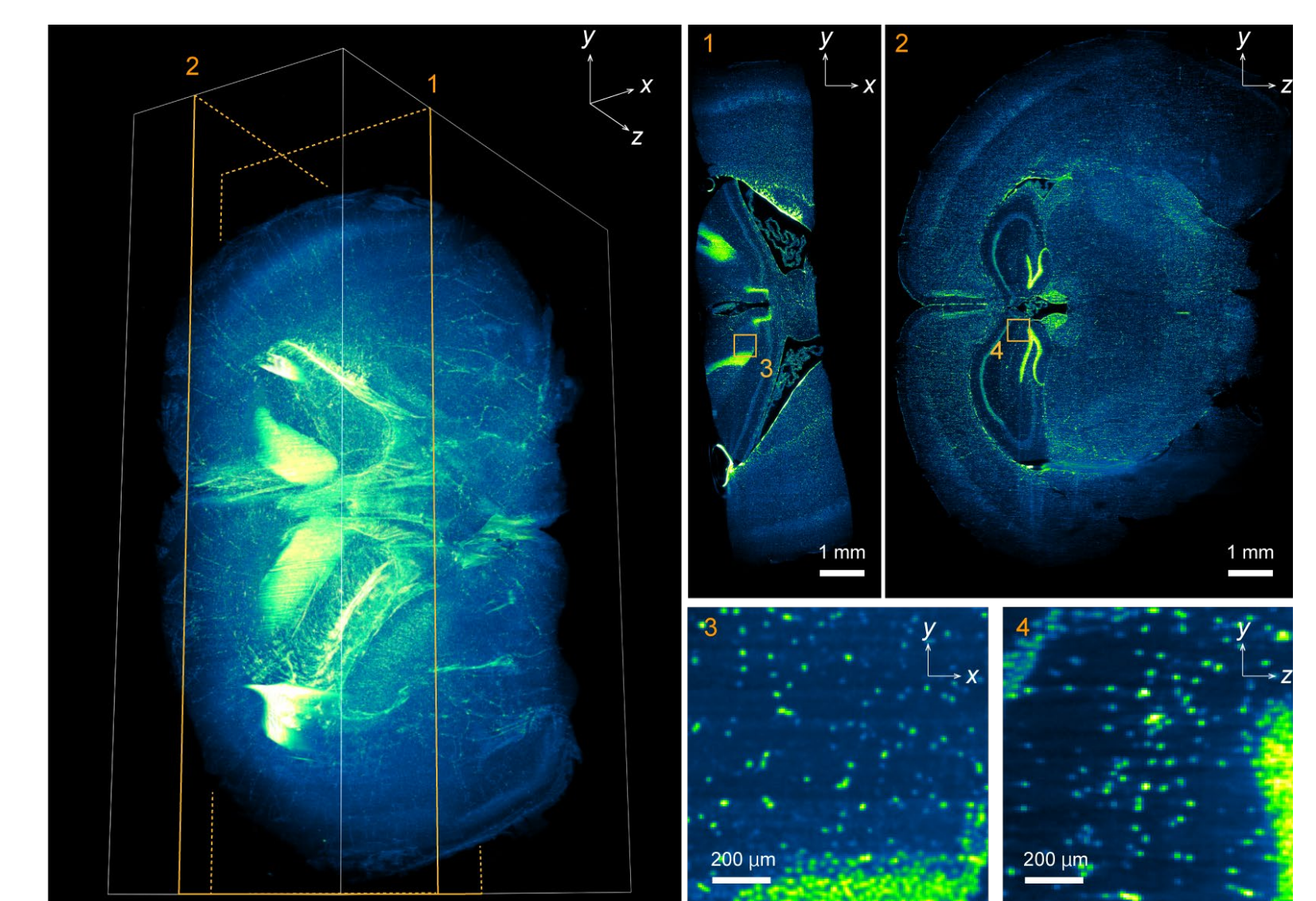
- 594 nm up to 100 mW
- Versatile intensity modulation and power control at up to 50 kHz
- True dark-state and full duty-cycle freedom

## 3D mouse brain imaging using descSPIM and multi-line laser illumination<sup>[2]</sup>

Laser excitation source: Cobolt Skyra multi-line laser at 488 nm/50 mW, 515 nm/50 mW, 561 nm/50 mW, and 647 nm/50 mW coupled into a single-mode fiber (Schäfter+Kirchhoff, Hamburg, Germany)



FF-mode imaging of a PI-stained mouse brain hemisphere. Voxel size:  $3.45 \times 3.45 \times 10 \mu\text{m}^3$   
Imaging speed: 180 sec. per 900 slices



FA mode imaging of a PI-stained, 2 mm-thick mouse brain section. Voxel size:  $3.45 \times 3.45 \times 5 \mu\text{m}^3$   
Imaging speed: 372 sec. per 1860 slices per stack

## References

- [1] Glaser, A. K. et al. Light-sheet microscopy for slide-free non-destructive pathology of large clinical specimens. *Nat. Biomed. Eng.* 1, 0084 (2017).
- [2] Otomo K et al. Biorxiv descSPIM: Affordable and Easy-to-Build Light-Sheet Microscopy for Tissue Clearing Technique Users.
- [3] <https://github.com/dbsb-juntendo/descSPIM>

## Affiliations

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## SUMMARY

- Multi-line lasers with direct modulation capability simplify the integration of multi-color illumination, eliminating the need for additional optics for alignment
- descSPIM lowers the barrier of entry for end-users of light sheet microscopy and together with the multi-line laser option, offers wide spectral coverage ultimately facilitating transition into clinical settings
- A compact orange laser with versatile direct modulation capability increases spectral flexibility



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