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ClearScope®: Image large and small intact brains using light sheet theta microscopy

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Introduction

The recent advancements in tissue clearing techniques and Light Sheet Microscopy (LSM) hold the promise of novel insights into brain-wide exploration of connectivity and phenotypic profiling of behavior and pathological states. Whole-organ clearing used in conjunction with this imaging modality is a proven method for biological structure quantification and morphometric analysis. LSM provides a unique combination of speed, resolution, tissue depth penetration, and low phototoxicity compared to confocal and multiphoton microscopy. However, limitations of the standard LSM prevent its users from imaging large intact tissue specimen.

To improve on existing LSM technologies, we collaborated with the Tomer laboratory at Columbia University to develop ClearScope – an angled, dual light-sheet microscope that produces high-resolution images of large, intact specimens while minimizing photo-damage and out of focus features. Utilizing revolutionary Light Sheet Theta Microscopy (LSTM), ClearScope applies two light sheets, oblique to the specimen and detector axes, to image tissue specimens of extraordinary sizes at high resolution with no lateral limitations. It is commercially available for research laboratories using tissue clearing techniques that need a robust multi-channel imaging solution. ClearScope's unique hardware configuration and intuitive software make it an industry-leading solution for cleared tissue imaging.

Light Sheet Theta Microscopy

At its core, LSTM technology is the use of dual illumination pathways oblique to the specimen and detection pathway. This provides ClearScope with the ability to image thicker tissue specimens over a large lateral area (XY) at higher optical resolutions while maintaining fast imaging speed, high imaging quality, and low photo-bleaching.

+ Stage range of motion is the only limit to specimen lateral dimensions.
+ The oblique light sheet arrangement allows for utilization of high numerical aperture (NA) and long working distance (WD) objectives.
+ Two intersecting light sheets form an ultra-thin light sheet (2µm) that optimizes axial image resolution and quality, produces uniform illumination, and reduces out of focus features.

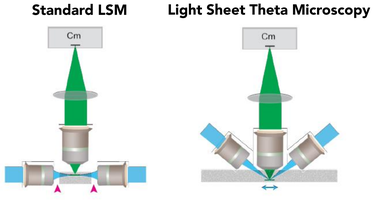

Figure 1. Graphic depiction of standard light sheet illumination pathway in comparison to light sheet theta microscopy. Figure reprinted from Migliori, B., Datta, M.S., Dupre, C. *et al.* Light sheet theta microscopy for rapid high-resolution imaging of large biological samples. *BMC Biol* **16,** 57 (2018). https://doi.org/10.1186/s12915-018-0521-8

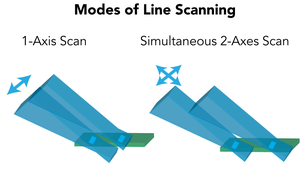

Figure 2. Demonstrates the out of focus light introduced when imaging with single axis scanning. Simultaneous dual axis scanning uniformly illuminates the sample at the thinnest section of the light sheet. Figure reprinted from Migliori, B., Datta, M.S., Dupre, C. *et al.* Light sheet theta microscopy for rapid high-resolution imaging of large biological samples. *BMC Biol* **16,** 57 (2018). https://doi.org/10.1186/s12915-018-0521-8

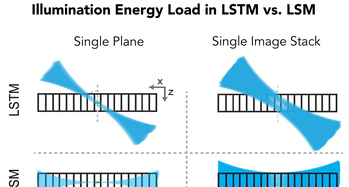

Figure 3. A comparison of sample exposure to light using LSTM verses LSM in single plane and single stack acquires. Figures reprinted from Migliori, B., Datta, M.S., Dupre, C. et al. Light sheet theta microscopy for rapid high-resolution imaging of large biological samples. *BMC Biol* **16,** 57 (2018). https://doi.org/10.1186/s12915-018-0521-8

+ Simultaneous 2-axis scanning focuses the light sheet using an electronically tunable lens (ETL) in conjunction with the galvanometer scanner ensuring that only the thinnest part of the light sheet intersects the detection plane.
+ Dual illumination pathways eliminate shadow effects providing uniform illumination across the field of view.
+ Oblique illumination mitigates redundant illumination of the scanned areas.
+ Synchronized scanning of the light sheet with exposure of the rolling shutter optimizes axial image resolution and improves signal to noise ratio.

ClearScope: The Complete Solution

+ **Advanced and Basic User Modes**
  Intuitive software interface allows easy design and execution of sophisticated imaging workflows.
+ **Multi-TB Stitching**
  Seamlessly stitch multi-TB image volumes within the ClearScope application.
+ **Image Data Analysis**
  Complete with all the software needed to render large 3D image volumes and obtain quantifiable imaging data pertaining to fluorescence, neurons, vessels, and subcellular structures like dendritic spines.
+ **Imaging Flexibility**
  Nearly all clearing techniques are compatible with ClearScope via its Intelligent Refractive Index Compensation (IRIC). Modular hardware design allows for scanning of biologic structures at a variety of objective lens magnifications (4x-25x) and up to 7 laser wavelengths customizable to researcher needs.
+ **Big Data Solutions**
  Optimized data storage systems with fast transfer rates take the stress out of working with multi-TB image datasets.

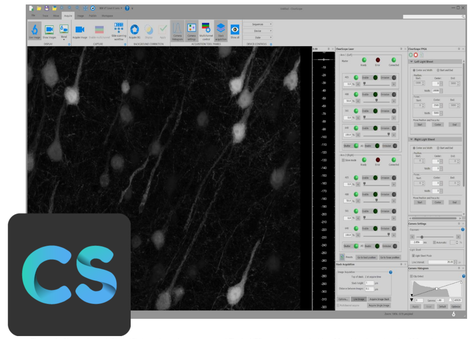

Figure 4. Clarity cleared (RI=1.45) tissue from the neocortex of Thy1-eYFP mouse imaged with a 20x objective using the 488nm laser (max. intensity projection image).

Figure 5. ClearScope light sheet theta microscopy imaging system with Hamamatsu Fusion camera, two oblique light sheets, one detection objective, and 4"x3" motorized XY stage system. Monitors show clear scope software and example image data.

Acquired Images

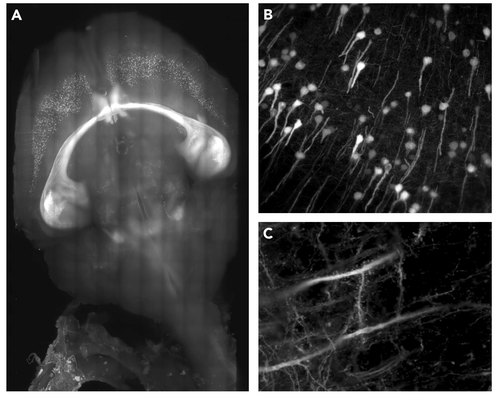

Figure 6. Images taken with ClearScope. **A.** Brain and spinal cord of Thy1-eYFP mouse imaged with a 10X objective (max. intensity projection image). Total imaging time was 9 hours. **B.** Clarity cleared (RI= 1.45) tissue from the neocortex of Thy1-eYFP mouse imaged in three dimensions with a 20X objective using the 488nm laser (max. intensity projection image). **C.** Dendritic spines from a clarity cleared (RI= 1.45) tissue sample of Thy1-eYFP mouse brain imaged with a 20X objective using the 488nm laser (max. intensity projection image).

Specifications and Capabilities

Laser Channels
405, 488, 515, 532, 561, 638 and 660 nm

Light Sheet
+ Number of light sheets: 2
+ Illumination: Bi-directional, oblique angle
+ Light sheet thickness: 2 – 6 µm

Detector Objectives
+ Magnification 4X – 25X
+ Numerical Aperture 0.28 – 1.0
+ Working Distance 8 – 12 mm

Specimen Chambers
+ Customizable chambers can accommodate specimen of many sizes from whole drosophila brains to whole human brains.
+ Isolate tissue and immersion media from the Refractive Index matching solution in the tub
+ Refractive index compatibility range: 1.33-1.56
+ Magnetic coupling holds chambers in place while allowing for quick swapping

Figure 7. A. Specimen chamber containing cleared mouse brain. Note that this specimen has been in the chamber for 6+ months with no signs of leaking. B. Specimen chamber holder with specimen. C. Slide holder with fluorescent bead slide. D. Immersion medium chamber with mouse brain specimen in place as for imaging on the ClearScope system.

Stage Range of Motion
The stage range of motion is the only limitation on the lateral dimensions of tissue specimens.
+ 114mm x 75mm with standard XY stage
+ 150mm x 100mm with extended travel XY stage

ClearScope components are upgradable and customizable to suit the imaging needs of most research investigations.

Future Directions and Advancements

ClearScope is compatible with most cleared tissue imaging objectives from the leading lens manufacturers and will continue to be updated as new lenses come to market. Currently, we are working to support a multi- position objective changer. We expect to release fully automated magnification switching synchronized with acquisition presets later this year.

Engineers and scientists at MBF Bioscience are working with research labs in academia and industry to develop cutting edge image processing solutions that dramatically speed up multi-TB image stitching, viewing, and analysis. This is a major bottleneck in big data imaging.

Acknowledgements and References

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+ Conflict of Interest: Content reflects use of products made by commercial employer of author.
+ Migliori B, Datta MS, Dupre C, Apak MC, Asano S, Gao R, Boyden ES, Hermanson O, Yuste R, Tomer R. Light sheet theta microscopy for rapid high-resolution imaging of large biological samples. BMC Biology. 2018; 16-57.