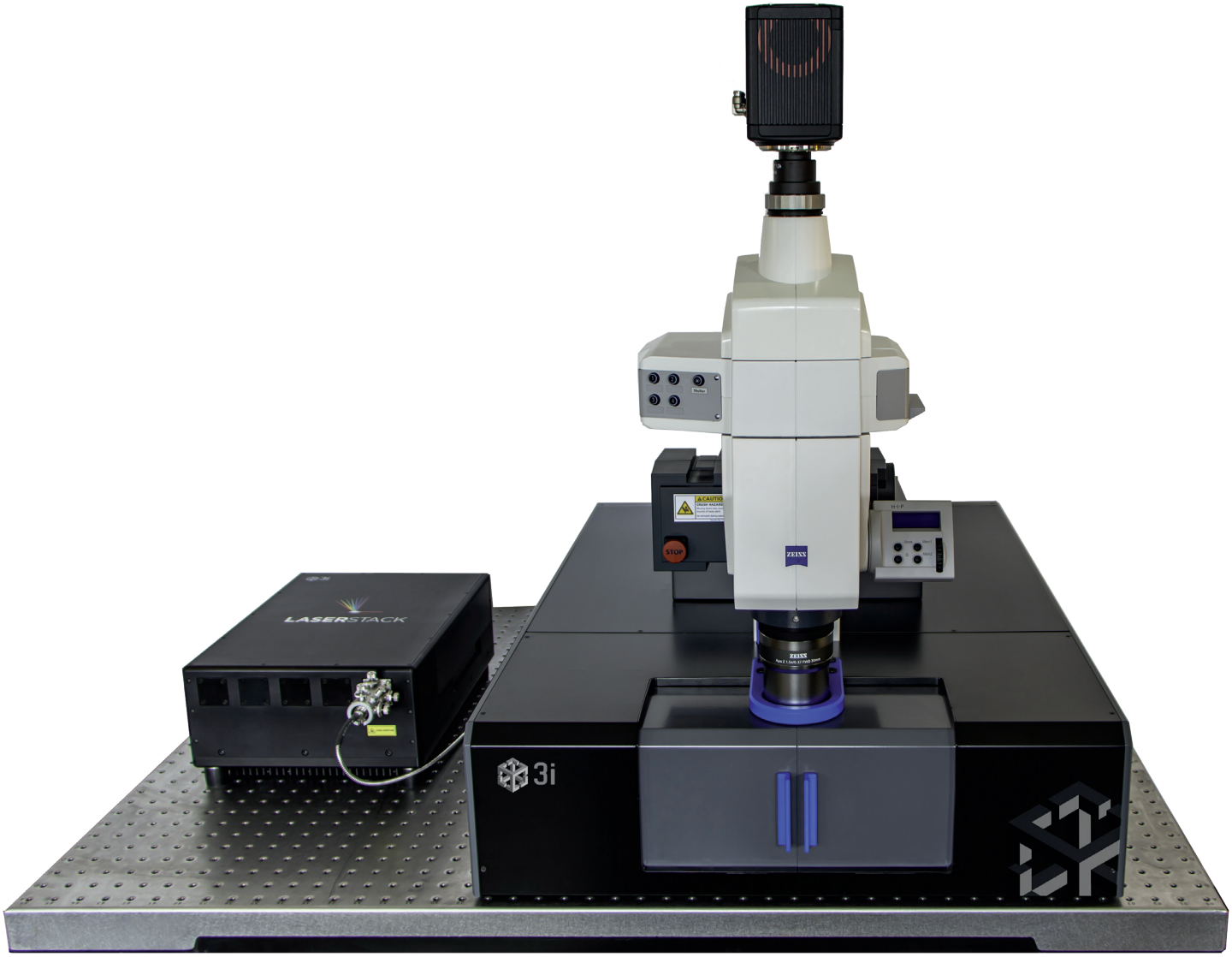
CLEARED TISSUE LIGHTSHEET



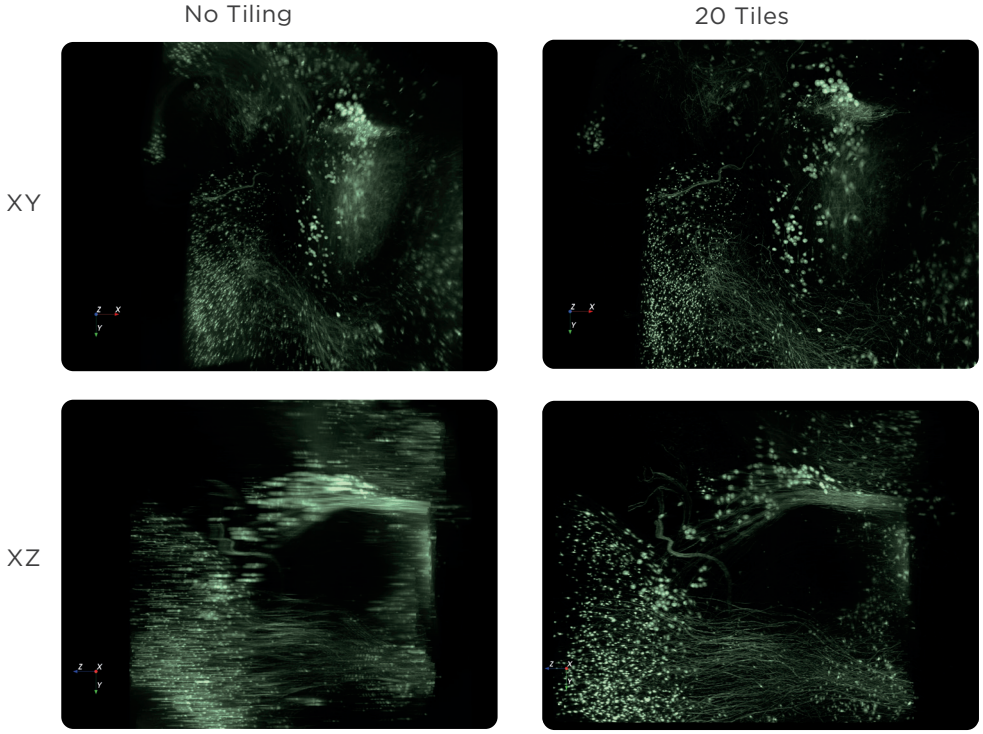
High Speed High Resolution Imaging of Cleared Tissue and Whole Organs

Cleared Tissue LightSheet (CTLS) is a large field light sheet microscope designed to image whole organs at high speed. CTLS creates a focused sheet with a narrow waist for better optical sectioning, then uses a spatial light modulator (SLM) to rapidly shift the waist of the sheet along the axis of propagation. A dual excitation setup allows imaging from the right and left sides of the specimen for optimal light sheet projection throughout. Piezoelectric stages move the specimen in x, y, and z with sub-micron resolution. The result is clear: a 1 cm^3 volume can be imaged at up to 1µm x 1µm x 3µm (XYZ) resolution, and a cleared mouse brain can be imaged in as little as 1.5 hours.

The Difference is Clear

Light sheet microscopy is a powerful technique for imaging large specimens by taking full advantage of emerging tissue clearing methods. The chemistry behind these techniques has advanced to where we can easily penetrate 1, 5 even 10mm into a specimen with a focused sheet of light. In combination with a macro zoom microscope using high NA large field of view lenses, Cleared Tissue LightSheet can image large field sizes with high resolution in short periods of time.

CTLS acquisition is extremely flexible, from ultrafast capture with a 20µm light sheet (left) to high-resolution capture with a 3µm light sheet shifted 20 times and the resulting 20 sections of best focus tiled to one best-focus image (right). Images compare lateral (XY) and axial (XZ) views of the ventral Tegmental Nuclear (VTN) group of the mouse cleared with PEGASOS (Jing et al. (2018). Tissue clearing of both hard and soft tissue organs with the PEGASOS method. *Cell Research*). Sample courtesy of Dr. Hu Zhao (Texas A&M University).



How Tiling Works

The optical sectioning ability of a light sheet is dependent on the thickness of the waist of the focused beam used to create the sheet. The thickness of the waist is directly proportional to the beam length. A thinner waist is generally required for better optical sectioning, but the thinner the waist the shorter the usable length of the beam. Imaging large cleared specimens with a light sheet requires a beam with a long waist matched to the large field of view. The long waist has a correspondingly high thickness, and the result is often poor optical sectioning.

To dramatically improve on this limitation, CTLS uses a spatial light modulator to create a sharply focused beam with a thin waist much shorter than the detector's field of view. The beam waist is tiled along the axis of propagation and the camera is synchronized to capture one image per tile. The optimal region of each capture is selected and stitched together forming a continuously optimized image. The resulting data has excellent axial resolution compared to a non-translated focused beam.

Benefits of CTLS

- **Excellent resolution** from the macro objectives
- **Excellent optical sectioning** from the thin waist of the tiled light sheet
- **Low photobleaching** via light sheet as compared to confocal
- **Ultrafast acquisition** from large-format macro objectives, large-format sCMOS camera, and digital focus via SLM eliminating the need to move optical elements
- **Fully automated hardware control, image acquisition and data stitching** via SlideBook software

Features

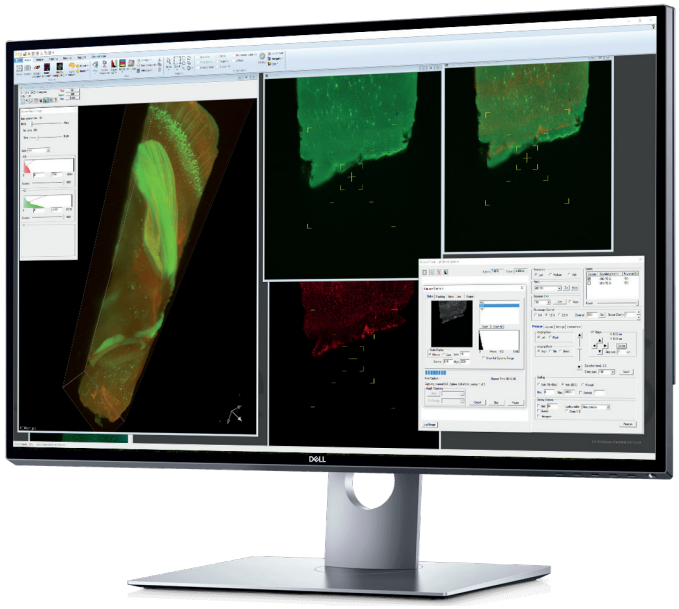
DUAL LEFT AND RIGHT ILLUMINATION	Allows for optimal sheet penetration across wide specimens and avoidance of opaque structures that may be present on one side but not the other.
HIGH NA / LARGE FIELD OF VIEW / LONG WORKING DISTANCE OBJECTIVES	Macro lenses at 1x/0.25NA and 1.5x/0.37NA can image through any cleared organ with excellent resolution.
PIEZO STAGES DRIVING MULTIPLE SAMPLE CHAMBERS	Sub-micron resolution stages allow precise positioning of the specimen in sample chambers sized to fit the biology.
OPTICAL ZOOM PRESCAN	CTLS includes a motorized optical zoom to automatically zoom out and create a 2D map of the entire specimen. This map serves as virtual eyepieces allowing inspection of the entire specimen at higher magnification and identification of regions of interest for zoomed-in high resolution imaging in 3D.
LASERSTACK LASER COMBINER	Fiber-coupled laser combiner allows up to six lasers covering the entire visible spectrum at multiple power levels.
GPU-OPTIMIZED SLIDEBOOK SOFTWARE	SlideBook directs all hardware synchronization and data capture, creating 3D datasets at over 1TB ready for analysis and rendering.
LARGE DATA SOLUTIONS	Available DDN® unified storage systems allow direct acquisition and analysis without time-consuming file transfers for 200TB to over 1PB.

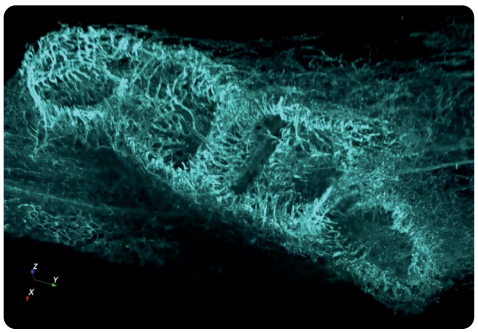
Advanced Features

AXIAL CHROMATIC ABERRATION CORRECTION	Leveraging the SLM, SlideBook creates patterns specific to each laser wavelength to correct for axial chromatic aberration.
AUTOMATIC REDUCTION OF SHADOWING & STRIPING EFFECTS	Light sheets can be subject to distortion as they encounter objects that have not been cleared, resulting in shadows or striping in the image plane. SlideBook uses the SLM to create a patterned light sheet that interrogates the specimen from 3 different angles to mitigate (and in some instances completely eliminate) these artifacts.

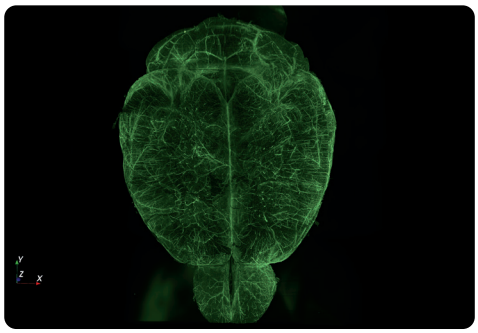
SlideBook

SlideBook allows scientists to focus on investigation rather than instrumentation, controlling every aspect of CTLS from hardware configuration to image acquisition, data reconstruction, image processing and 3D visualization. With over 20 years of active development in collaboration with researchers worldwide, SlideBook is intuitive yet powerful with features from visual imaging ROI selection to automated SLM pattern generation and axial chromatic aberration correction. SlideBook SLD files can be accessed via any application supporting Bio-Formats OME, allowing seamless collaboration in any workflow.

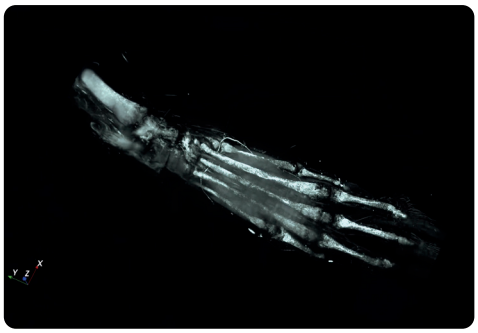




Mouse mandible cleared with PEGASOS showing vasculature and dentin in the molar teeth reported by endogenous fluorescence cdh5cre. Sample courtesy of Dr. Dian Jing and Dr. Yating Li, Sichuan University, Department of Orthodontics.



Smooth muscle cells in the arteries, veins and capillaries of mouse brain cleared with PEGASOS reported by NG2BacDsRed. Sample courtesy of Dr. Woo-Ping Ge, University of Texas Southwestern Medical Center.



Mouse hind paw labelled with Thy-1 YFP and cleared with PEGASOS. Specimen courtesy of Dr. Wenjing Luo, Texas A&M Health Sciences Center.

Specifications

RESOLUTION	1 µm x 1 µm x 3 µm
OBJECTIVES	1x/0.25NA, 1.5x/0.37NA
IMAGING SPEED	< 1 min/mm^3
SAMPLE TRAVEL RANGE	25mm x 25mm x 25mm (XYZ)
SPECIMEN CHAMBERS	Multiple glass specimen chambers optimized for 1x/0.25NA and 1.5x/0.37NA objectives, with specimen holder. Custom-shaped specimen holders available upon request.
COMPATIBLE CLEARING METHODS	Organic and aqueous clearing solutions
CAMERA	2048x2048 16-bit sCMOS
LASER LINES	LaserStack compact modular laser launch with 488nm and 561nm lasers standard. Up to 4 additional wavelengths upon request.
XYZ TRANSLATION STAGE	Piezoelectric with sub-micron resolution in X, Y and Z
SOFTWARE	SlideBook™ software for acquisition and GPU-accelerated analysis

3i Intelligent Imaging Innovations

BUILT BY SCIENTISTS FOR SCIENTISTS. Intelligent Imaging Innovations (3i) designs and manufactures cutting edge live cell and intravital microscopy imaging platforms driven by 64-bit SlideBook software. 3i was established in 1995 by a group of scientists whose wide range of research activities includes cell biology, immunology, neuroscience and computer science. Our collective aim is to provide advanced multi-dimensional microscopy platforms that are intuitive to use, modular in design, and meet the evolving needs of investigators in the biological research community.

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