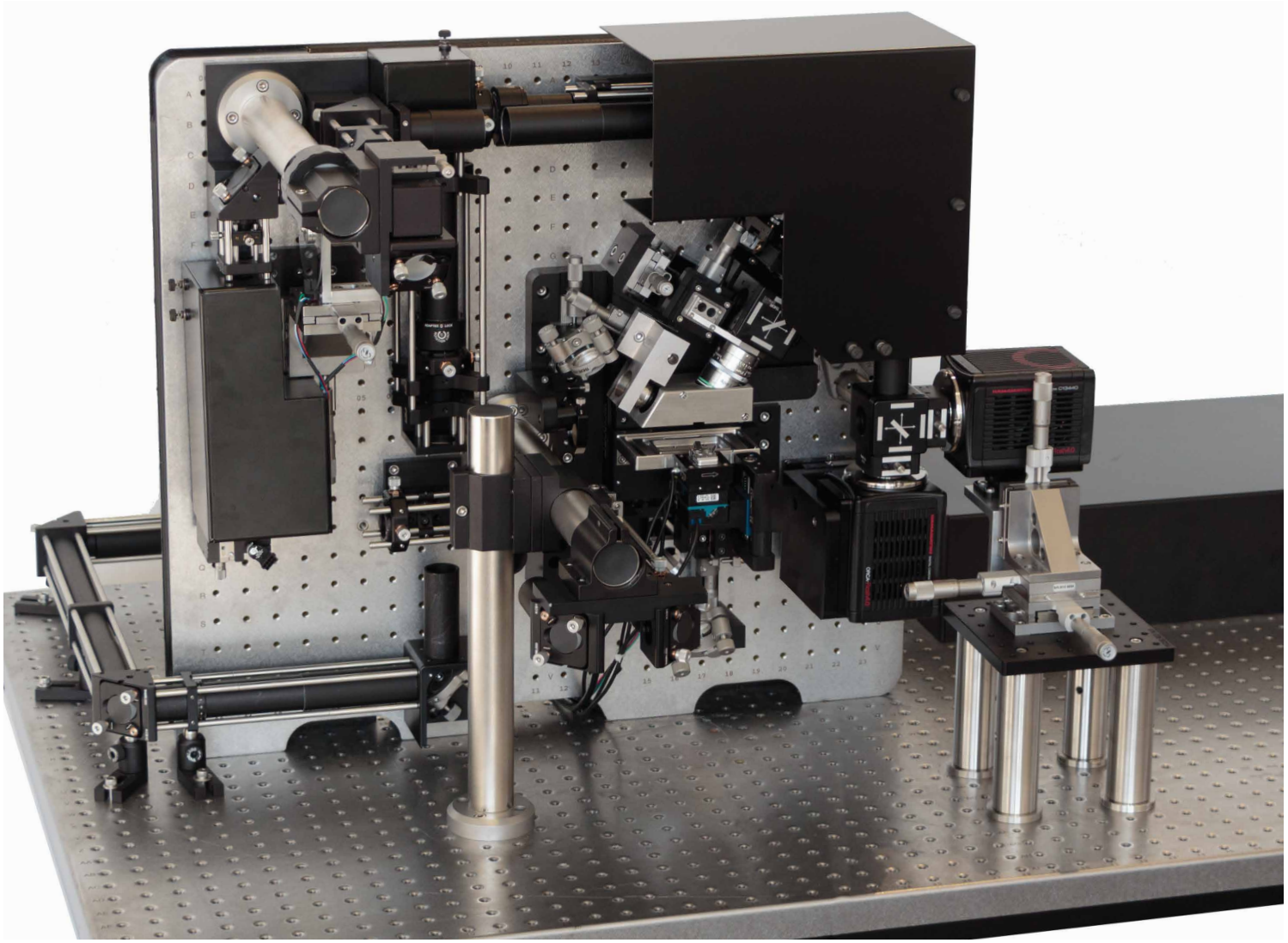
LATTICE LIGHTSHEET



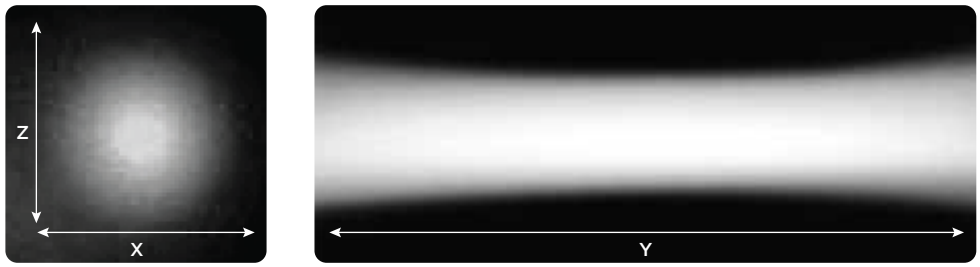
High-resolution, low light-dose lightsheet microscope

First developed by Nobel Laureate Dr. Eric Betzig, the 3i Lattice LightSheet microscope is capable of imaging biological systems spanning four orders of magnitude in space and time. The system generates an optical lattice to create an ultra-thin light sheet to image biological samples over long periods of time and with very fine resolution. This allows for 4D living cell imaging, where experiments limited to seconds or minutes on other imaging platforms can be extended to hours or even days. The combination of high spatiotemporal resolution, imaging speed and sensitivity make Lattice LightSheet the ultimate imaging tool for a new era of living cell microscopy.

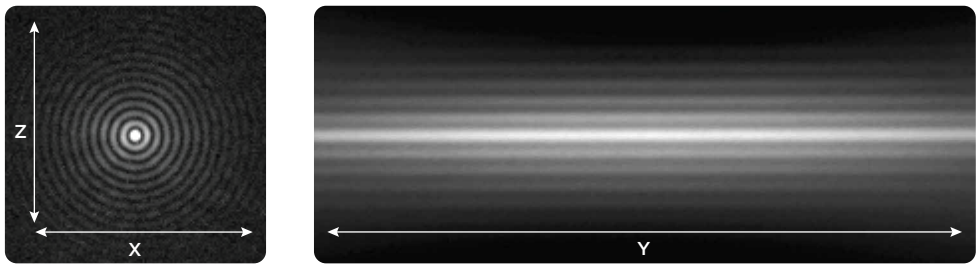
Ideal Lightsheet for Live Cell Imaging

Lightsheet imaging offers fast fluorescence imaging with decreased light dose at the sample plane compared to confocal imaging. Conventional selective plane illumination microscopy (SPIM) methods use a Gaussian beam where light sheet thickness is proportional to light sheet length, leading to a beam that is typically too short or thick for ideal sub-cellular imaging. The innovative Lattice LightSheet combines the flexibility of a Gaussian light sheet with the thin optical sectioning of a Bessel beam to achieve the best optical sectioning of any light sheet microscope.

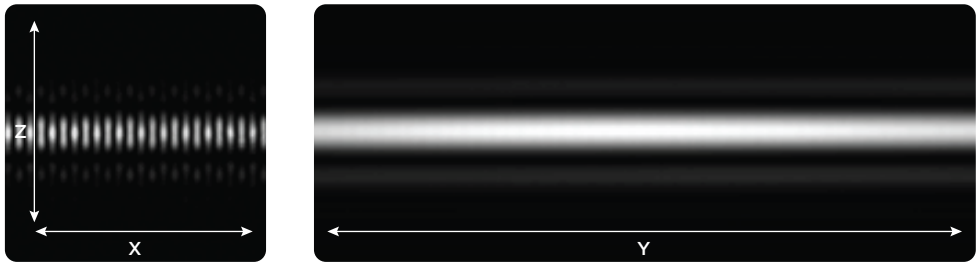
Gaussian



Bessel



Lattice

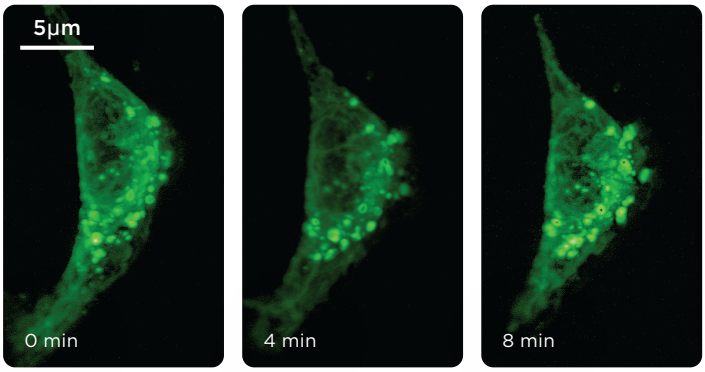


MAKING A LATTICE LIGHTSHEET

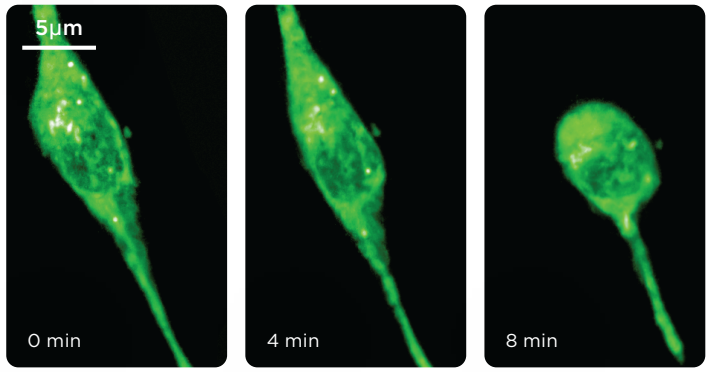
1. Cylindrical lenses stretch and collimate the beam to form a sheet projected onto a spatial light modulator (SLM)
2. SLM generates an optical lattice of Bessel beams
3. Annular mask acts as a zero order filter, removing artifacts and lengthening the sheet
4. Galvos dither the sheet in X and sweep in Z

LOW PHOTOTOXICITY FOR PROLONGED LIVING CELL IMAGING

Lattice LightSheet



Spinning Disk Confocal



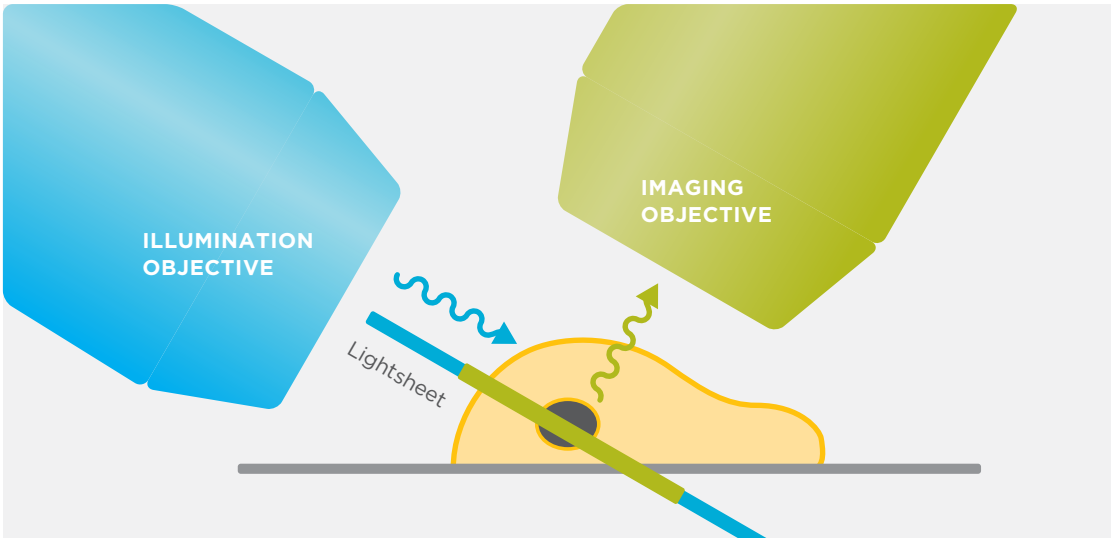
Mouse embryonic fibroblasts expressing SiR-Actin (642 nm) imaged with 25 ms exposures. After 8 minutes, the cells died on the spinning disk confocal but the Lattice LightSheet cells remained viable.

Optimal Lightsheet Illumination

ILLUMINATION: Custom-made 0.71NA long working distance water immersion objective for lightsheet illumination, mechanically and optically matched to the imaging objective.

IMAGING: High resolution 1.1NA water immersion objective with depth of field matched to lightsheet thickness for excellent optical sectioning.

LIGHTSHEET EXCITATION EFFICIENCY REDUCES PHOTOTOXICITY



- 0.4µm lightsheet thickness
- 0.5µm objective depth of field

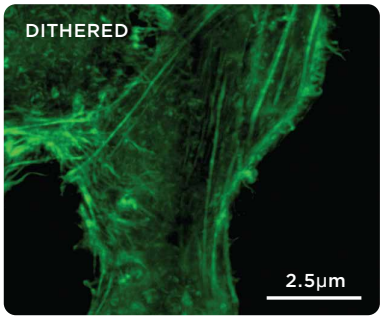
Because the light is entering the specimen along the plane of focus, the likelihood of a useful fluorescence event is far higher than in other methods that illuminate through the cell.

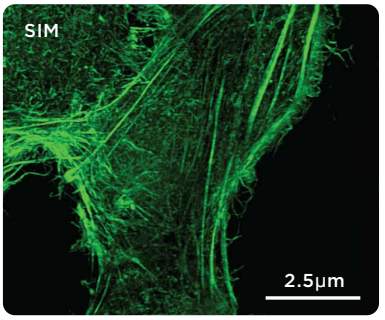
DISTINCT CAPTURE MODES

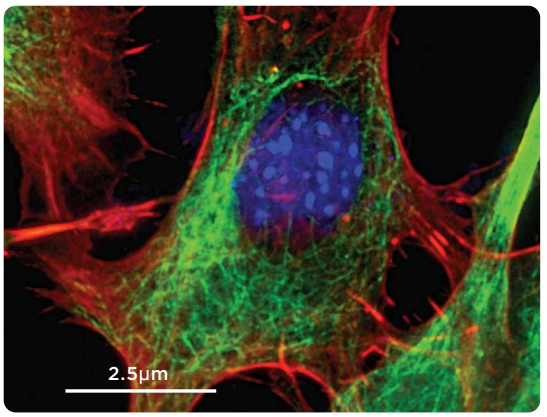
SHEET SCAN: The light sheet and objective are moved in tandem to form a 3D image.

SAMPLE SCAN: For larger imaging areas, the sample is moved while the light sheet and objective are stationary.

STRUCTURED ILLUMINATION MICROSCOPY (SIM): The light sheet is stepped along 5 discrete phase steps on the X axis in either sheet scan or sample scan mode. Five raw images are collected that are reconstructed to produce an image that is beyond the diffraction limit of the detection objective.



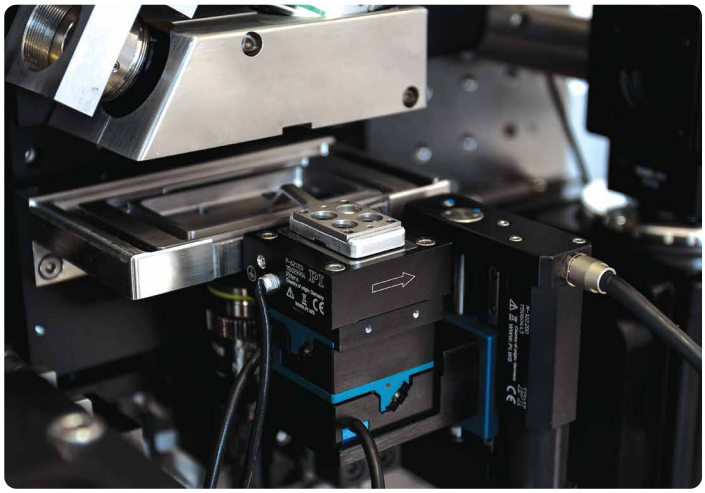




Mouse embryonic fibroblasts expressing SiR-Actin (642nm, red) labeled with Tubulin Tracker (488nm, green) and NucBlue (405nm, blue). 3 minutes into a 10 minute live capture with 25ms exposures for all channels.

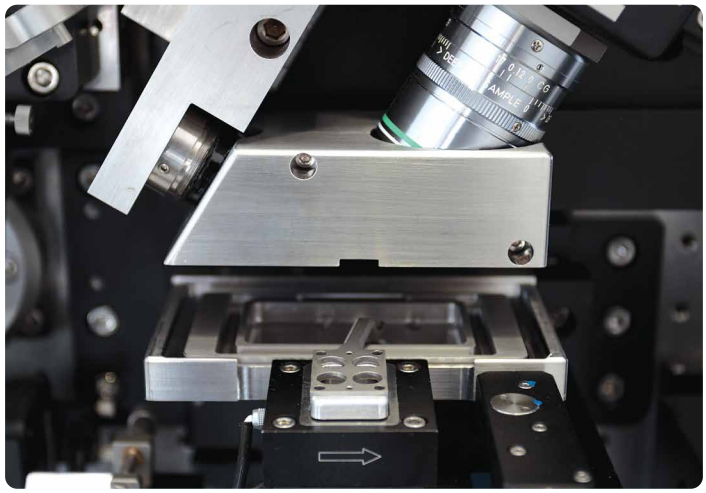
Lattice Lightsheet V2 Updates

MOTORIZED SAMPLE CHAMBER



- Objectives remain fixed and aligned
- Rapid and repeatable sample changing
- Expanded specimen access when loading
- Load and home positions for 2-move sample change
- Removable inner chamber for easy cleaning

SOLID STATE HEATING SYSTEM



- Accurate and stable temperature setting for the objectives and sample holder
- No water, tubing, heaters or pumps
- Active feedback for improved thermal stability

MOTORIZED ANNULAR MASK

- Fast, automated changing of annuli
- Easy testing of various mask positions and patterns on a single biological sample
- No alignment required after change

LED BRIGHTFIELD & EPI-FLUORESCENCE ILLUMINATION

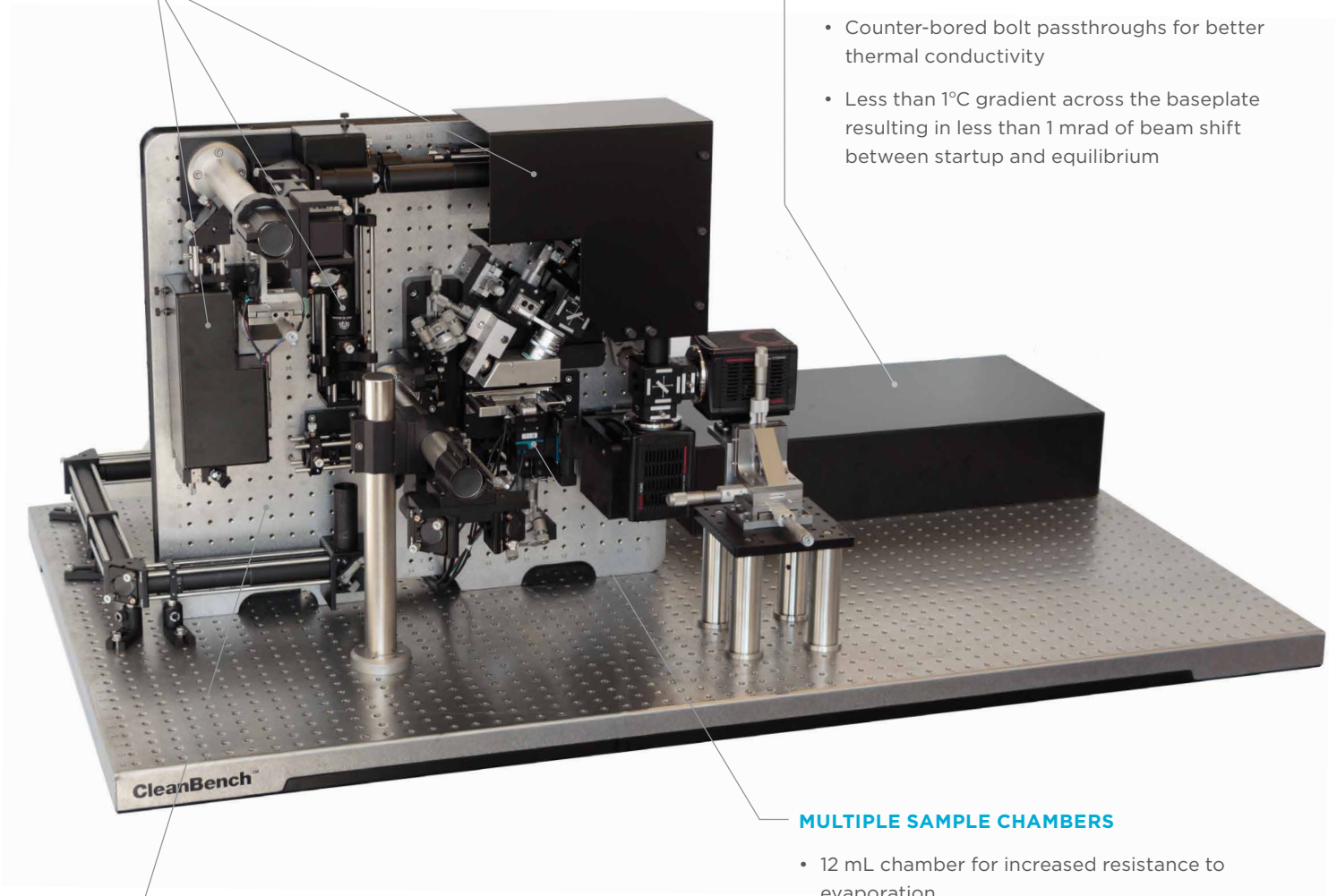
- Proper epi-fluorescence illumination via LEDs, mirrors and filters behind the vertical board
- Significant reduction in light dose compared to laser epi-excitation
- Multi-band dichroic replaces 90/10 beamsplitter
- Multi-channel LED illuminator to match the imaging lasers
- White brightfield LED illumination through excitation objective

IMPROVED OPTICAL MOUNTS AND BEAM SHIELDING

- Cylindrical lens assembly redesigned to replace large, glued elliptical mirror at 37° angle with a pair of mirrors in adjustable 90° mounts
- Precise alignment with improved optical stability
- Covers prevent incidental contact with galvo assembly, SLM and emission path
- Beam shielding increases laser safety and keeps dust from disturbing the optical path

LASER LAUNCH SOLID BASEPLATE

- Solid aluminum baseplate with no anodization and a clear alodine treatment to prevent oxidation
- Large heatsink increasing thermal stability
- Counter-bored bolt passthroughs for better thermal conductivity
- Less than 1°C gradient across the baseplate resulting in less than 1 mrad of beam shift between startup and equilibrium



MULTIPLE SAMPLE CHAMBERS

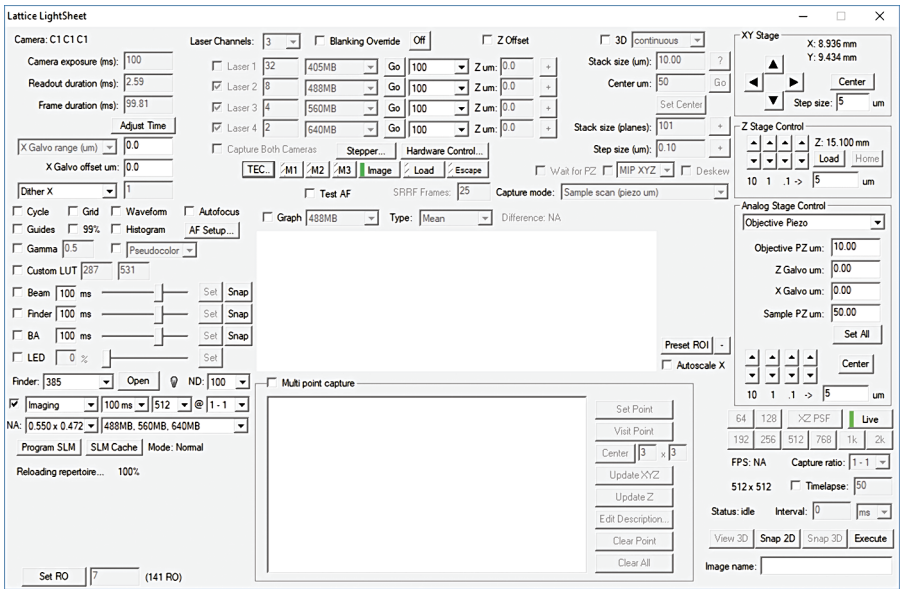
- 12 mL chamber for increased resistance to evaporation
- 3 mL chamber for reduced cost of expensive media and reagents
- Two ports for active perfusion of media
- Slots into lower assembly for easy exchange and cleaning

NEW VERTICAL MOUNTING BOARD

- Front and back plates mechanically secured with metal rivets
- Breadboard extended down to contact air table for increased stability
- Holes for cable management and access to rear-mounted epi-fluorescence light path

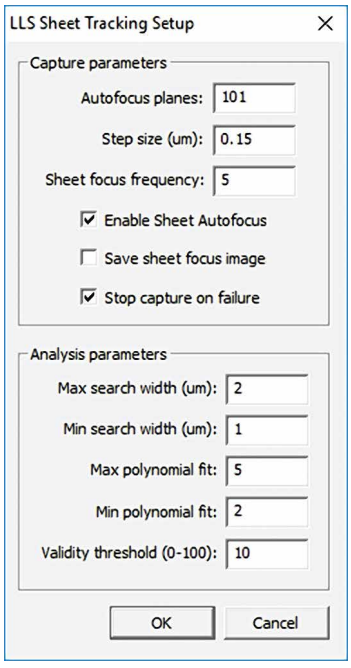
SlideBook

SINGLE CONTROL PANEL



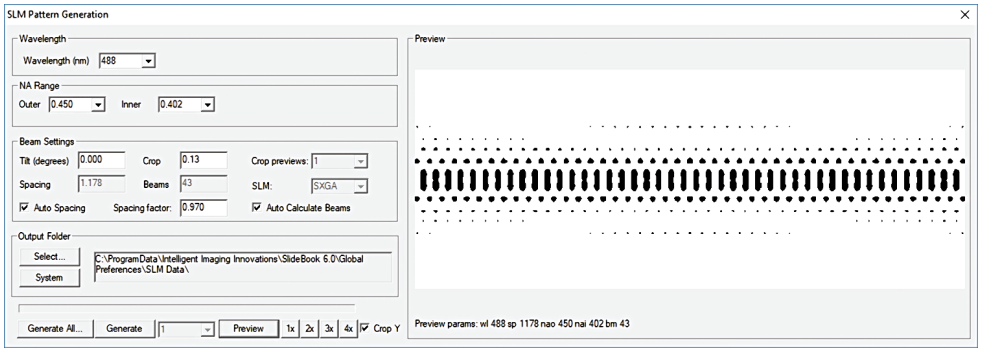
- Streamlined control module for image loading, sample finding, and image acquisition
- Tools for daily alignment adjustment
- SLM presets for rapid switching between exposures, wavelengths, and ROIs
- Integrated SIM capture and processing

LIGHTSHEET TRACKING



- SlideBook monitors lightsheet position during capture
- Dynamic adjustment of objective piezo maintains a focused image on the camera

INTEGRATED SLM PATTERN GENERATION

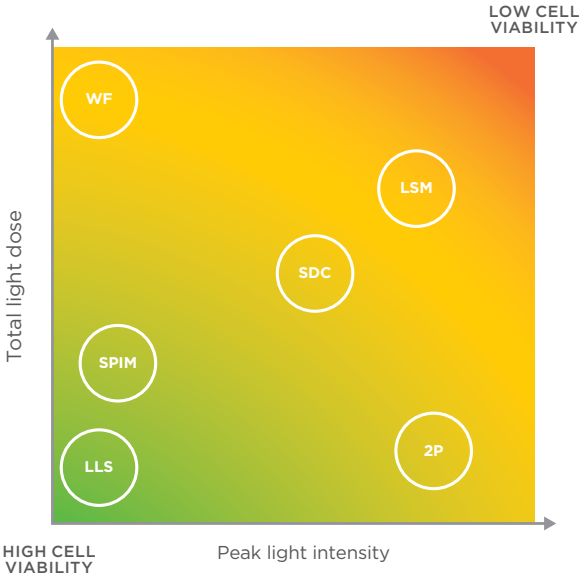


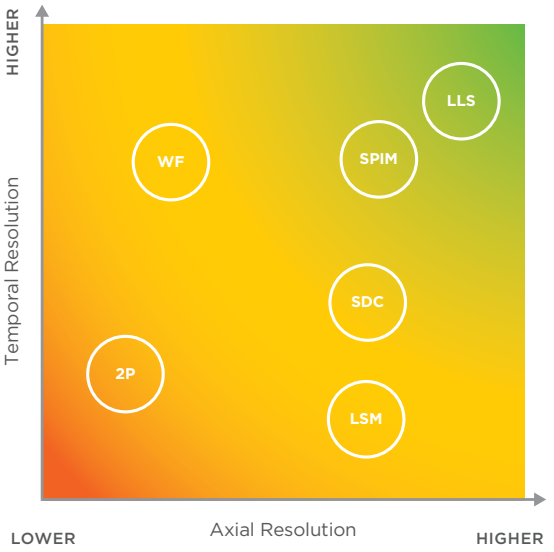
- SLM pattern generator for complete adjustment of pattern parameters
- Pattern defaults for quick starting points
- Pattern generation tied to specific position on motorized annular mask for rapid annulus changes

Microvolution

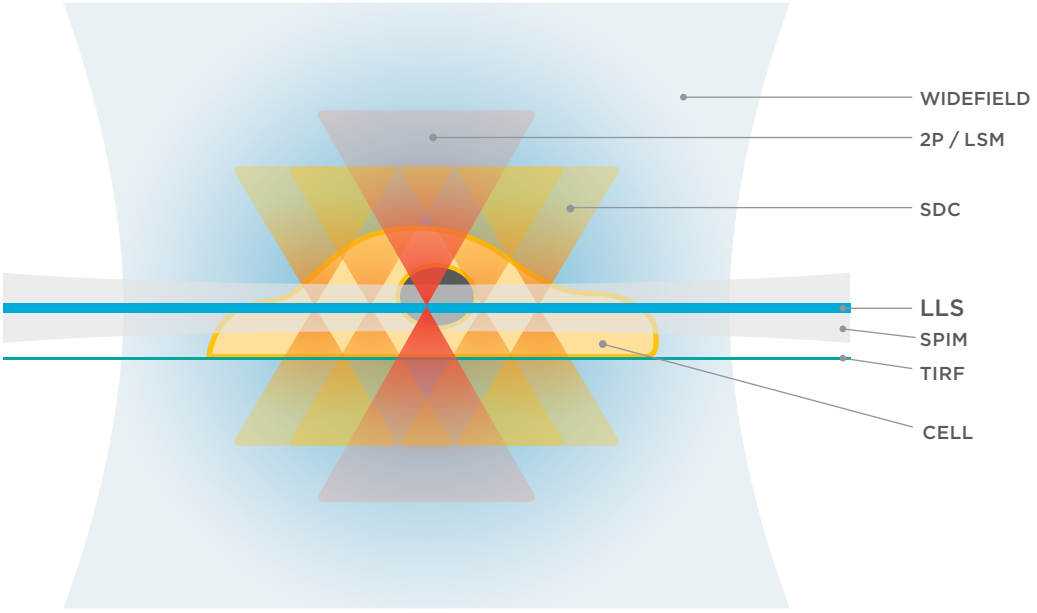
Microvolution GPU based deconvolution package integration for fast, post-acquisition processing

Cell Viability





LIGHT DOSE / PHOTOTOXICITY



Specifications

LIGHT SHEET THICKNESS: 0.4µm at 50µm length

DETECTION OPTICS: 1.1NA water objective, 2.0mm WD, 62.5x total magnification

ILLUMINATION OPTICS: 0.71NA water objective, 3.7mm WD

LASER OPTIONS: 405 350mW, 445 100mW, 488 500mW, 515 150mW, 560 500mW, 592 500mW, 642 500mW

STANDARD CAMERA: Hamamatsu ORCA-Flash 4.0 v3 sCMOS

CAMERA OPTIONS: Single sCMOS, Dual sCMOS direct 1x projection, Dual EMCCD relayed 2.5x projection

SAMPLE CHAMBER: Medical grade stainless steel with TEC temperature control and perfusion capabilities

SPECIMEN MOUNTING: Standard, horizontally-oriented 5mm round coverslip

ACQUISITION COMPUTER: Dual 10-Core Xeon 2.4GHz processors, 128GB RAM, NVIDIA Quadro P4000 8GB workstation graphics card, 1TB OS SSD and 6TB SSD array, 10GbE (copper) Adapter

ANALYSIS COMPUTER: Dual 10-Core Xeon 2.4GHz processors, 256GB RAM, NVIDIA Quadro P6000 24GB workstation graphics card, 1TB OS SSD and 6TB SSD array, 10GbE (copper) Adapter

STORAGE SOLUTIONS: DDN® unified storage systems for direct full-speed acquisition and analysis starting at 300TB. DDN systems utilize a BioScaler GPFS file system and are easily expandable to multiple petabytes

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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