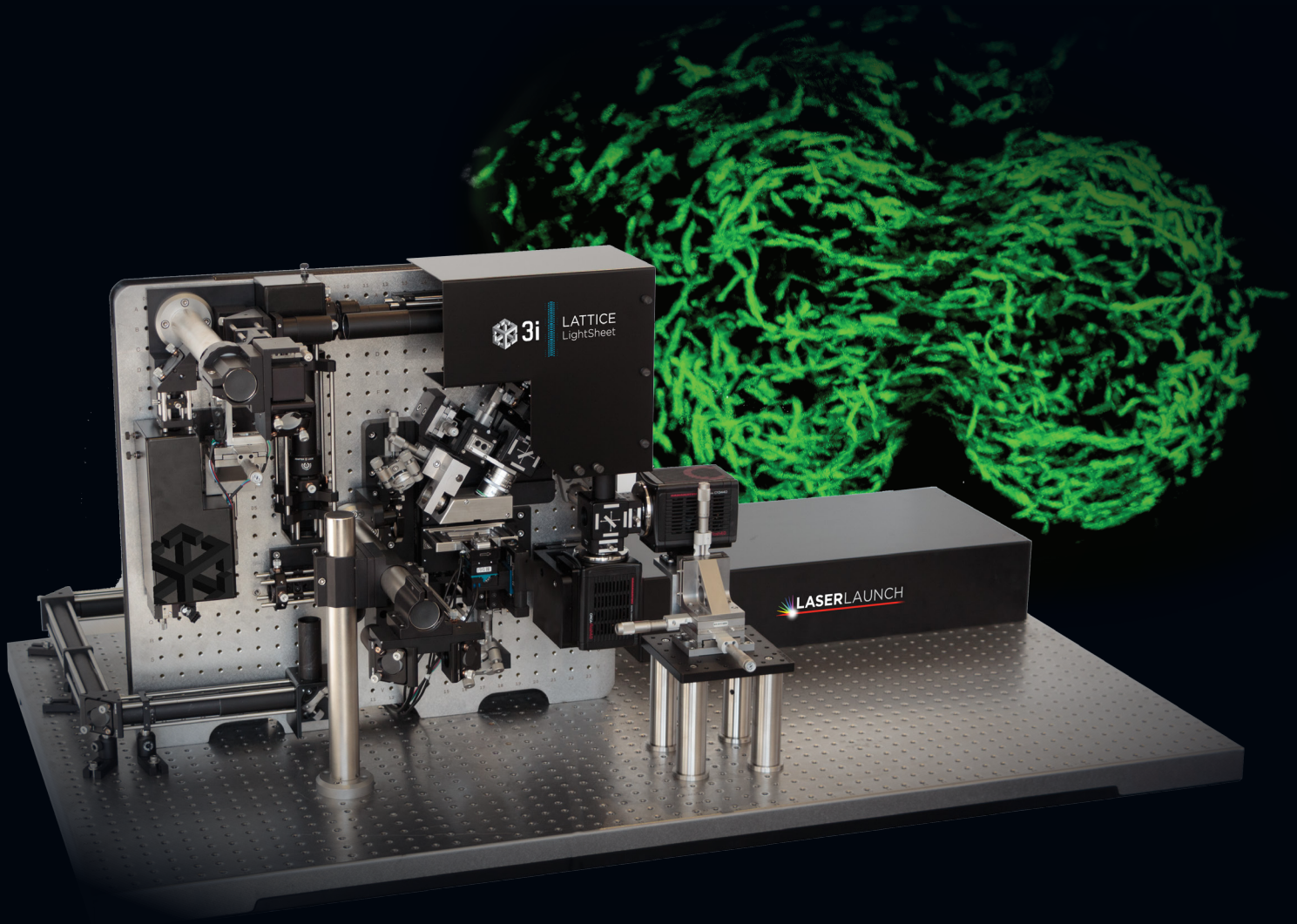
3i Intelligent Imaging Innovations

Lattice LightSheet

A Microscope for High-Resolution, Fast and Gentle 3D Live Cell Imaging

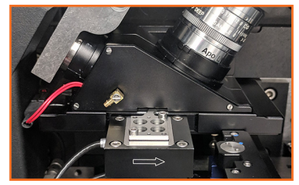


Lattice LightSheet Microscopy System

Lattice LightSheet (LLS) is a cutting-edge light sheet microscope built for cell biologists. 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 live 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 live cell microscopy.

Environmental Control

Sample chamber and objective heating
Digital temperature, gas, and humidity control



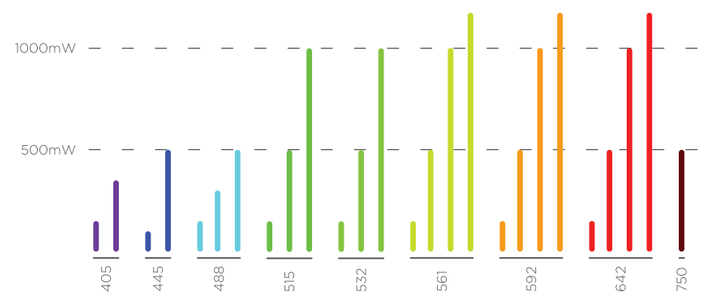
Digital Light Sheet Formation

Beam structuring via spatial light modulator (SLM)
Square, Hexagonal, Bessel, and other patterns available

Upright Microscope Configuration

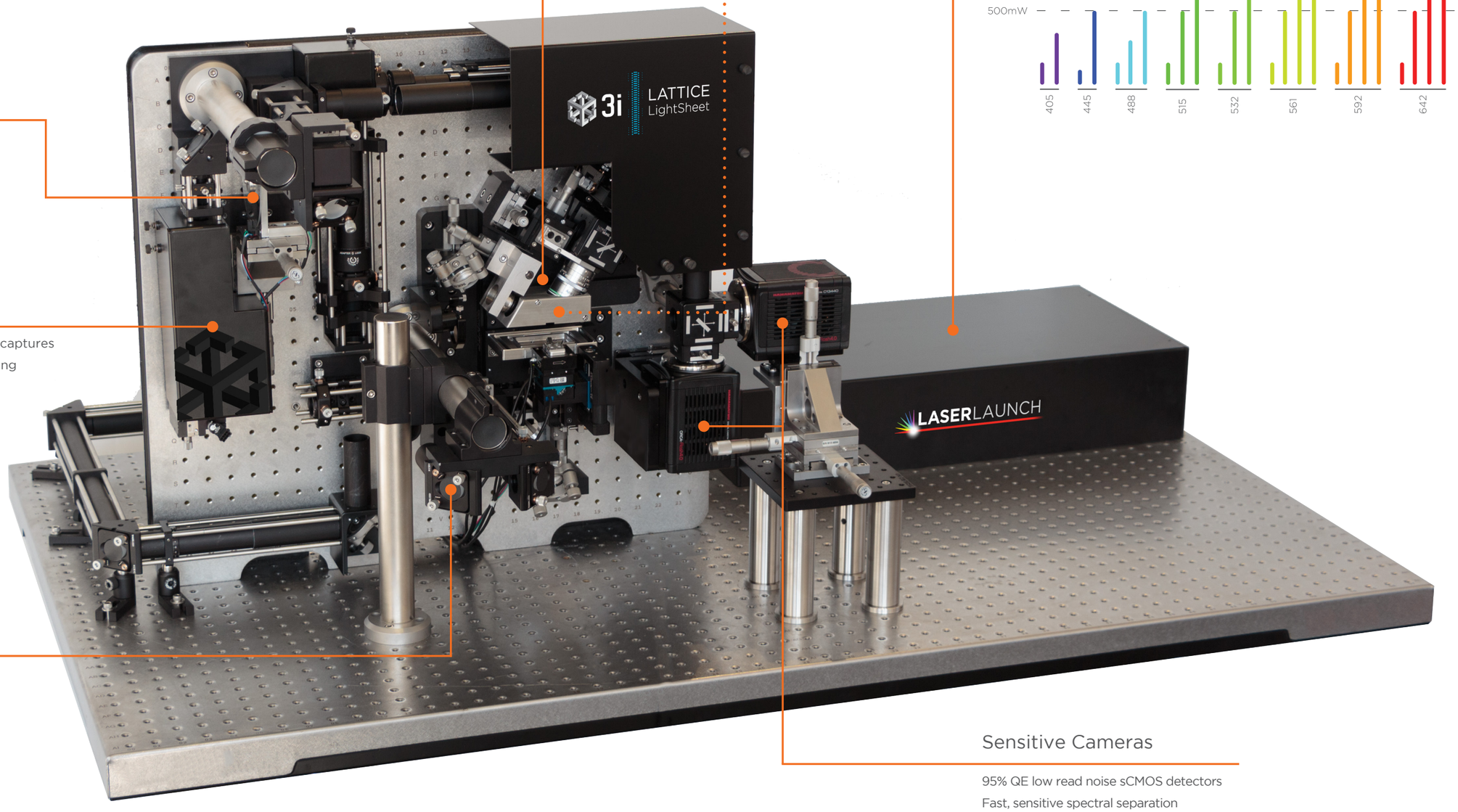
Standard glass sample mounting for cells and tissue
Custom sample mounting on other materials
Highest resolution, water-dipping objective pair
Ideal for live cells, organoids, tissue, and embryos

LaserLaunch Modular Laser Combiner



Motorized Light Sheet Selection

Adjustable light sheet length and thickness for a variety of samples, without realignment



Beam Steering

Fast X and Z galvanometers for rapid volumetric captures
Structured illumination for super-resolution imaging

Widefield Imaging Path

Inverted widefield path for easy sample finding
Brightfield LED & epi-fluorescence LED options

Sensitive Cameras

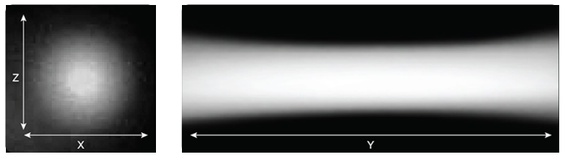
95% QE low read noise sCMOS detectors
Fast, sensitive spectral separation
Up to 2 cameras

Interfering Bessel Beams | Evolving the Gaussian

Light sheet microscopy involves illuminating a specimen orthogonal to the plane of detection. The beam is then quickly scanned or passed through a cylindrical lens to create a sheet of light. This Gaussian light sheet offers inherent optical sectioning and more chances for the excitation light to create fluorescent signal as it travels through the sample.

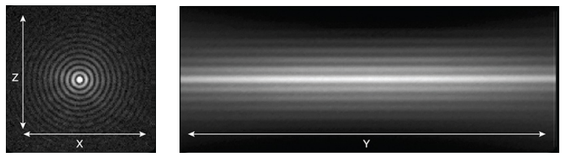
Using more exotic beam shaping, specifically Bessel and Multi-Bessel (Lattice) light sheets, the axial resolution and optical sectioning of the system can be drastically improved with minimal excitation power. The 3i Lattice LightSheet makes implementing these improved light sheets easy for the user, allowing scientists to focus on scientific exploration. The thin lattice light sheets are ideal for cell biologists pushing their experiments faster and longer than previously possible.

Gaussian



Gaussian beams are simple to create but are very short when thin (length is proportional to thickness).

Bessel



Bessel beams have great axial resolution and are uniformly thin but have a large amount of out of focus light, leading to a higher light dose on the sample.

Lattice

Interfering Bessel beams create an optical lattice that is still uniformly thin with great axial resolution but without the large amount of out of focus light. Lattice beams can also be used for structured illumination.

Specialized Objective Pair

The Lattice LightSheet's two objectives are specifically selected and designed to complement the ultra-thin light sheet and offer high-resolution imaging. Using a light sheet as thin as 400nm further improves the axial resolution.

Illumination

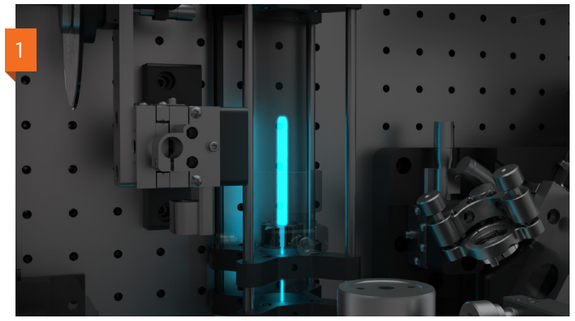
Custom 0.71NA long working distance water immersion objective for lightsheet illumination, mechanically and optically matched to the imaging objective.

Imaging

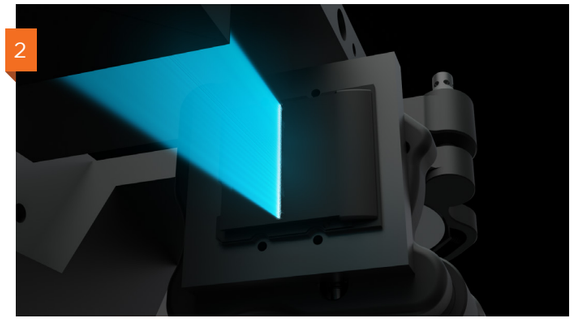
High-resolution 1.1NA water immersion objective with depth of field matched to lightsheet thickness for excellent optical sectioning.

Imaging Through a Lattice LightSheet

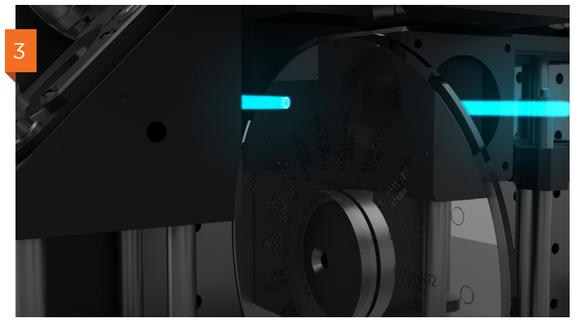
The Lattice LightSheet is a system designed to push the spatial and temporal resolution limits in live cell imaging. 3i has evolved the original design from Dr. Eric Betzig with the goal of making the system more user friendly.



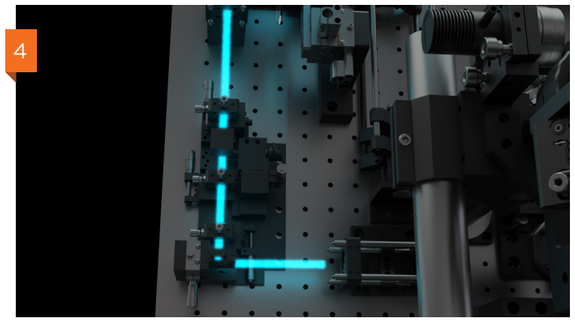
Cylindrical lenses stretch and collimate the beam to form a sheet projected onto a spatial light modulator (SLM).



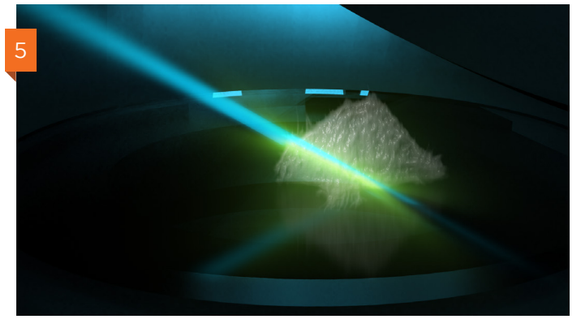
The SLM generates an optical lattice of Bessel beams.



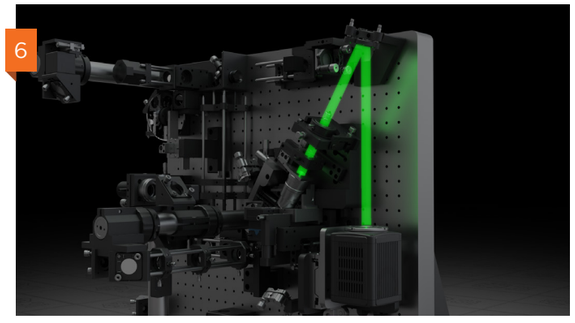
The annular mask acts as a zero-order filter, removing artifacts and lengthening the sheet.



Galvos dither the sheet in X and sweep the sheet in Z.



A lattice sheet is formed over the sample space.



Emitted fluorescence is detected on a high QE, low read-noise, sCMOS camera.

Chen BC, Legant WR, Wang K, et al. Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution. Science. 2014;346(6208):1257998. doi:10.1126/science.1257998

Intracellular to Extracellular to Multicellular Imaging

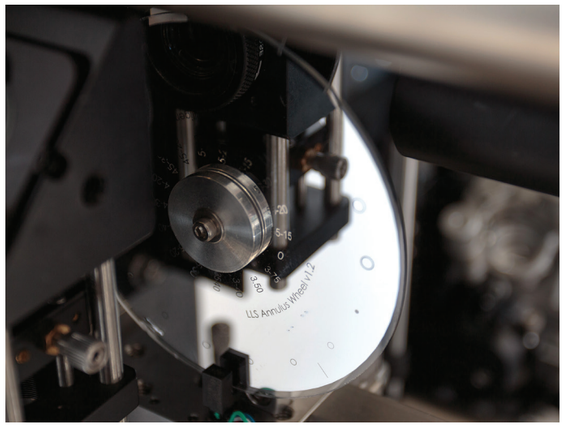
With 17 different light sheet lengths and thicknesses, it is easy to find the ideal Lattice for a given sample.

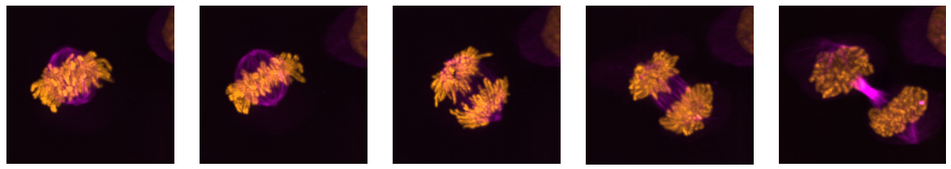
15-20µm sheets for intracellular events

30-40µm sheets for small organoids and cell-cell interactions

50-75µm sheets for large organoids and portions of embryos or larger organisms

Using the “Mask” mode allows for changing of the light sheet while the sample remains in focus, allowing users to evaluate different light sheet parameters in real time. SIM is possible with any of the sheets, opening the door for fast super-resolution imaging of an assortment of sample types.



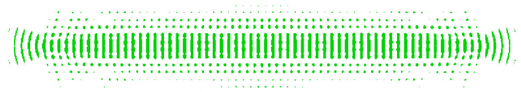


High-resolution imaging of mitotic cells over time.

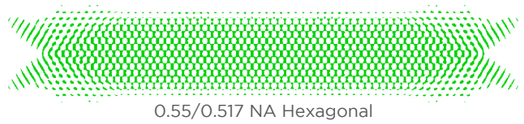
A key feature of the Lattice LightSheet is the spatial light modulator (SLM), which generates interfering multi-Bessel patterns.

For most imaging, the traditional “square” lattice pattern offers a nice blend of low light dose, optical sectioning and high axial resolution. Innovations into new patterns is an ongoing topic and has created other designs know as “hexagonal” and “hex-rect” patterns. These designs allow for fine tuning of axial resolution, SIM performance and deconvolution.

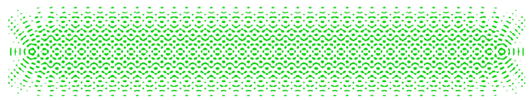
These non-traditional patterns can be easily used on the Lattice LightSheet by updating the pattern file loaded to the SLM. Since the patterns can be quickly exchanged, users can evaluate their qualitative performance in real time.



0.55/0.517 NA Square

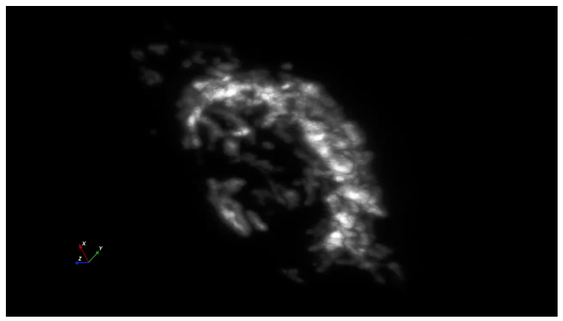


0.55/0.517 NA Hexagonal

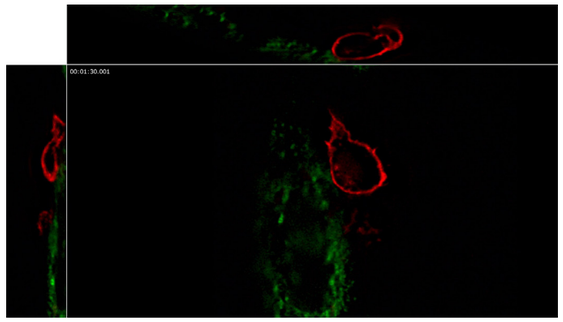


0.55/0.517 NA Hex-Rect

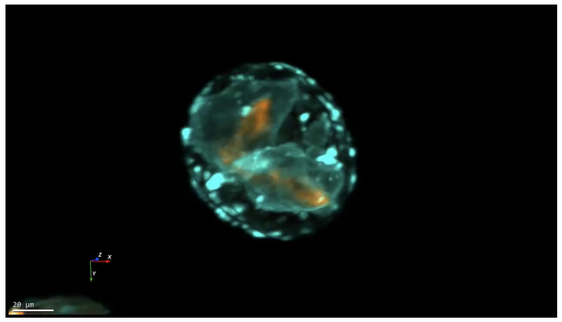
Model Organisms



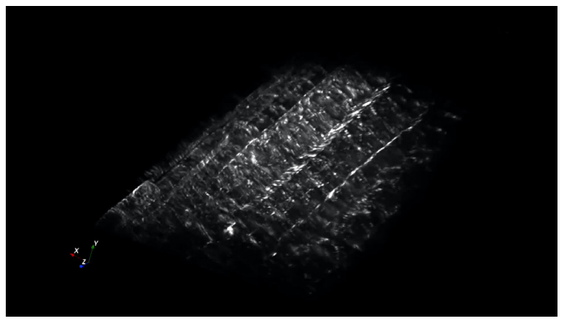
MITOCHONDRIA
Mitochondrial fusion and fission is a rapid cellular process that is also indicative of cellular damage from phototoxicity. The gentle nature of the Lattice LightSheet slows for unprecedented, volumetric imaging of this dynamic process without photodamage. Mitotracker Red in RPE1 cell.



IMMUNOLOGY
Visualizing cell-cell interactions is commonly displayed using a volume rendering. Here a slice/ortho view across X, Y, and Z allows for detailed examination of each cell’s membrane due to the high axial resolution offered by the Lattice LightSheet. Jurkat cell (red) interacting with HeLa cell (green).



MULTICELLULAR
Medium-sized samples are easy to accommodate via longer light sheets, all controlled digitally. Drosophila primary neurons with Jupiter (orange) and CD8 (blue).



PLANT BIOLOGY
Large samples, even with light scattering cell walls, can still be visualized with the Lattice LightSheet. Taking advantage of the longer 75µm long sheet, a portion of a root tip expressing GFP tubulin is imaged in high resolution.

Super-Resolution Imaging

Standard Sheet Scan

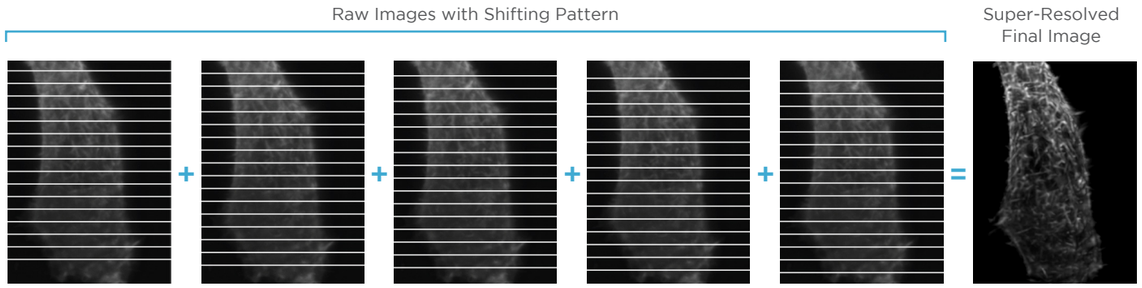
The light sheet is rapidly dithered along the X axis and one image is captured per Z plane.

Deconvolution

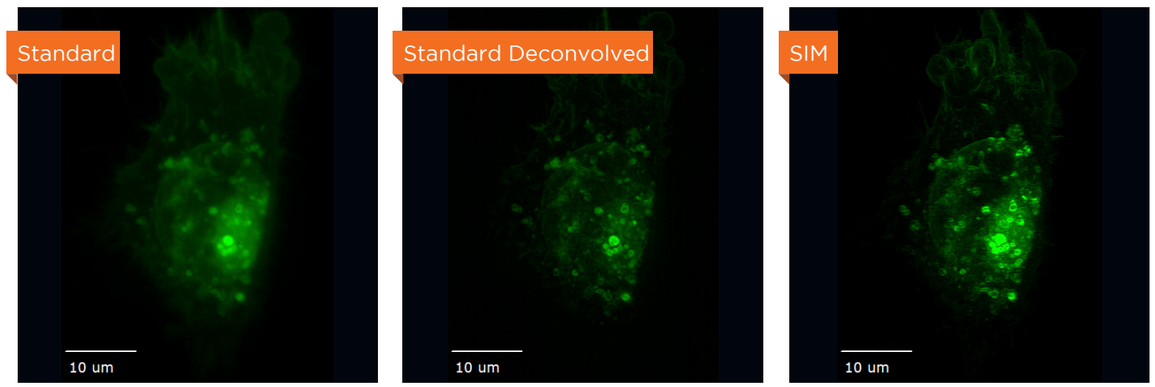
Quantitative image restoration (after standard sheet scan) via constrained iterative deconvolution in SlideBook.

Structured Illumination Microscopy (SIM)

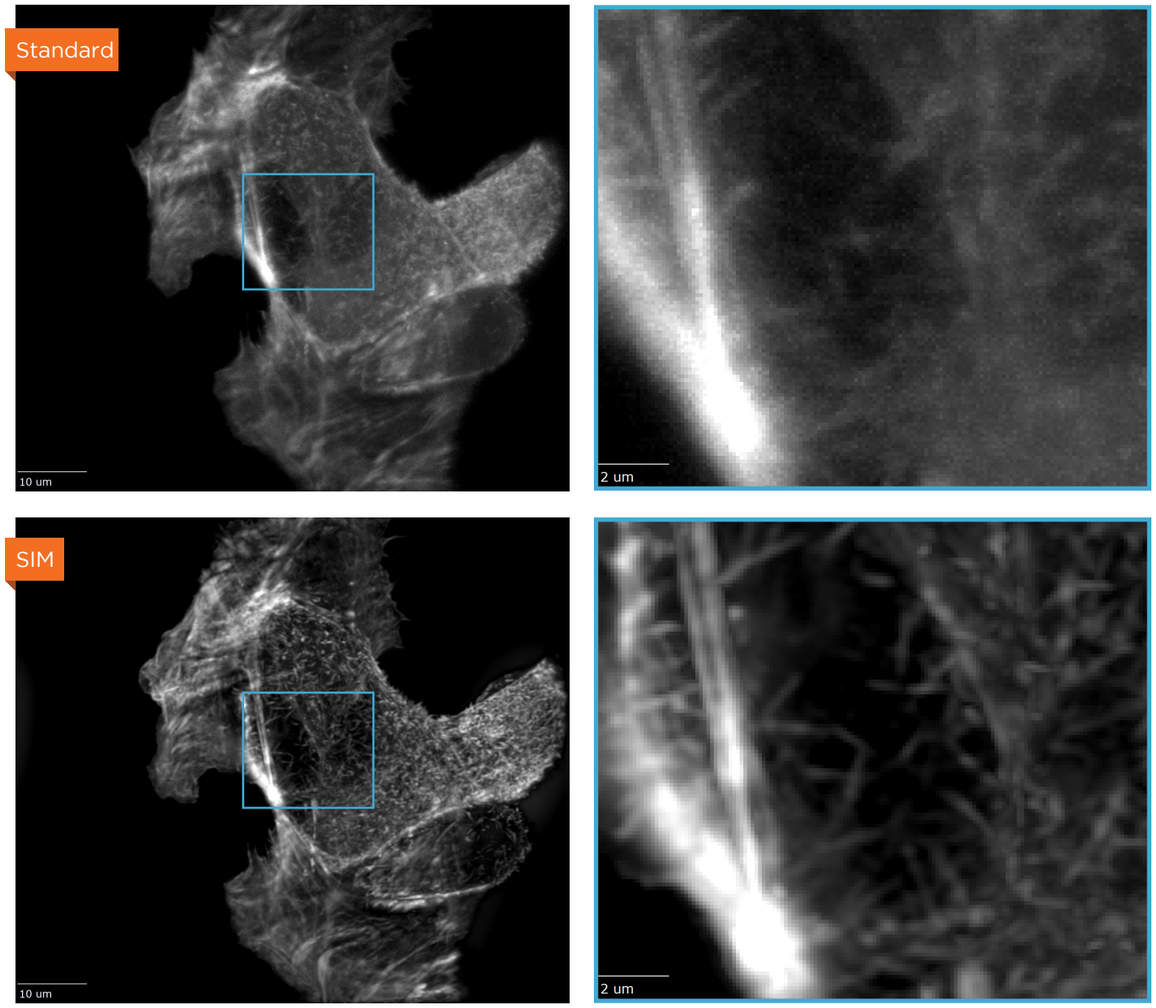
The light sheet is moved in five discrete phase steps along the X axis. Five raw images are collected that are reconstructed to produce an image that is beyond the diffraction limit of the detection objective by a factor of ~1.4x.



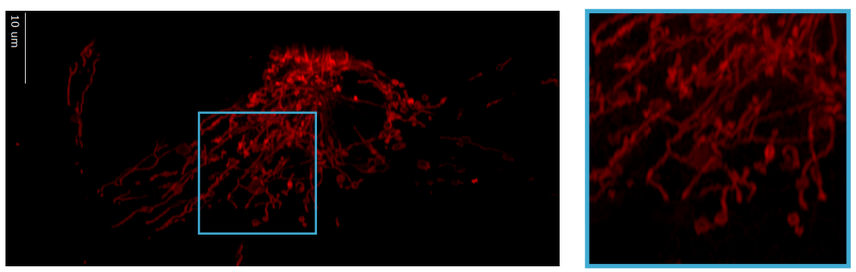
SlideBook acquires images with a stepped pattern. The step size is determined by the spacing of each pattern (wavelength and annular mask position). This is repeated throughout the entire Z-stack to acquire a full volume. SlideBook easily processes the raw SIM data into a super-resolved final image.



Mammalian cells expressing GFP-tagged lysosomes.



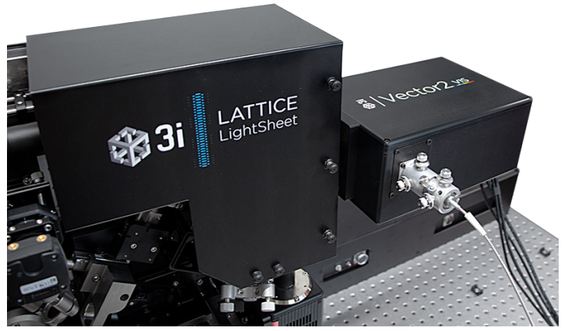
Standard (top) and SIM (bottom) image of mammalian cells expressing LifeAct GFP. Subset images on the right to highlight fine actin structures.

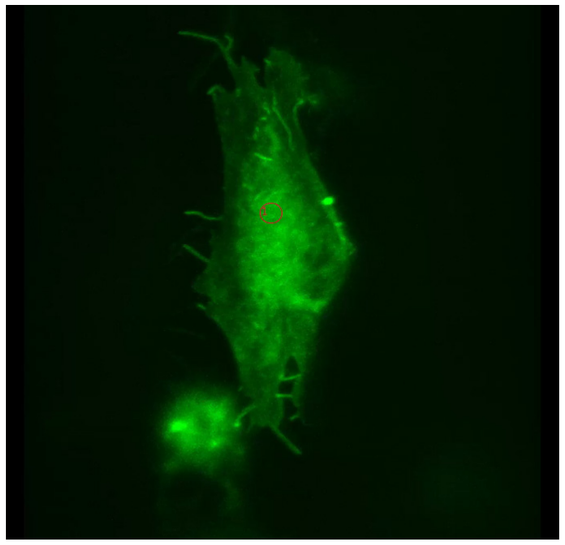


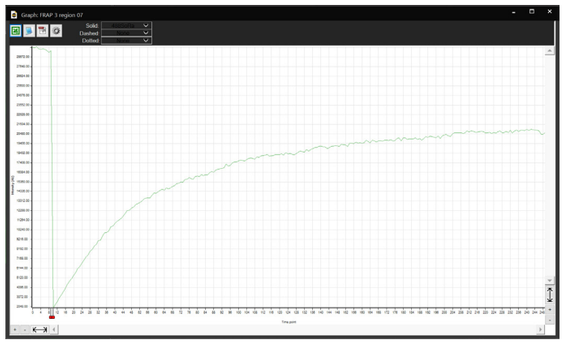
SIM image of mammalian cells stained with MitoTracker Red. Subset images on the right to highlight fine mitochondrial structures.

Photomanipulation

Photomanipulation is a powerful tool for studying membrane dynamics, receptor and vesicle movement and photo conversion of specific molecules. Addition of the Vector2 X,Y galvo scanner makes complicated bleaching, FRAP, uncaging and photoconversion experiments easy. Compared to a spinning disk or point scanning confocal, photomanipulation experiments on a lattice can be done more rapidly and with less inherent bleaching. Vector2 can be added to new or existing Lattice LightSheet systems.







PHOTOBLEACHING
Mammalian cell with membrane labeled in green before bleaching (left) with representative FRAP curve in SlideBook (above).

Single Molecule Localization Microscopy

Cell biologists and biophysicists are increasingly interested in tracking single molecules of interest instead of looking at groups of proteins as a unit. Single molecule localization microscopy (SMLM) offers researchers the ability to observe individual fluorophores on a single molecule of interest and observe how each behaves. Typically, techniques like TIRF have been required for SMLM but Lattice LightSheet offers the following capabilities to increase overall signal-to-noise and facilitate SMLM imaging in 3D:

High-powered lasers (up to 2W)

The most sensitive sCMOS cameras with low read noise and 95% QE

100+ fps acquisition for rapid single plane imaging or acquiring volumes per second

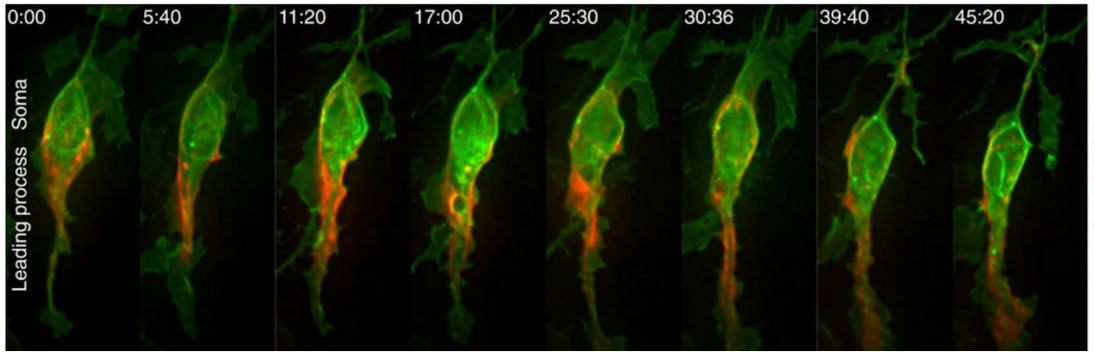
Photoswitching and photoconversion through Vector2

Scientific Spotlight

Lattice LightSheet users around the world have published dozens of papers in high-impact journals across a broad spectrum of scientific disciplines. Below are a selection of articles that use LLS data. Follow the QR code for the complete list of 3i LLS publications.

Neuroscience

Trivedi N, Stabley DR, Cain B, et al. Drebrin-mediated microtubule-actomyosin coupling steers cerebellar granule neuron nucleokinesis and migration pathway selection. Nat Commun. 2017;8:14484. doi:10.1038/ncomms14484



Cell Biology

Auckland P, Roscioli E, Coker HLE, McAinsh AD. CENP-F stabilizes kinetochore-microtubule attachments and limits dynein stripping of corona cargoes. J Cell Biol. 2020;219(5). doi:10.1083/jcb.201905018

Condon ND, Heddleston JM, Chew TL, et al. Macropinosome formation by tent pole ruffling in macrophages. J Cell Biol. 2018;217(11):3873-3885. doi:10.1083/jcb.201804137

Endocytosis

Willy NM, Colombo F, Huber S, et al. CALM supports clathrin-coated vesicle completion upon membrane tension increase. Proc Natl Acad Sci U S A. 2021;118(25):e2010438118. doi:10.1073/pnas.2010438118

Zebrafish and Leukocytes

Manley HR, Potter DL, Heddleston JM, Chew TL, Keightley MC, Lieschke GJ. Frontline Science: Dynamic cellular and subcellular features of migrating leukocytes revealed by in vivo lattice lightsheet microscopy. J Leukoc Biol. Published online April 23, 2020. doi:10.1002/JLB.3HI0120-589R

Data Science

Rosenberg J, Cao G, Borja-Prieto F, Huang J. Lattice Light-Sheet Microscopy Multi-dimensional Analyses (LaMDA) of T-Cell Receptor Dynamics Predict T-Cell Signaling States. cels. 2020;10(5):433-444.e5. doi:10.1016/j.cels.2020.04.006

Data Visualization

Valades-Cruz CA, Leconte L, Fouche G, et al. Challenges of intracellular visualization using virtual and augmented reality. Frontiers in Bioinformatics. 2022;2. Accessed October 17, 2022.Receptor Dynamics Predict T-Cell Signaling States. cels. 2020;10(5):433-444.e5. doi:10.1016/j.cels.2020.04.006

SlideBook

SlideBook manages every step in Lattice LightSheet imaging. A comprehensive control module guides users through light sheet and beam selection, the collection of 3D and 4D captures, image processing, volume rendering and finally movie making with story-board support. SlideBook is GPU optimized and readily handles the creation and processing of 3D/4D datasets over 1TB, making them ready for analysis and rendering. SlideBook SLD and SLDY files can be accessed via applications supporting BioFormats OME and Python, allowing seamless collaboration in any workflow.

User-Selectable App Appearance

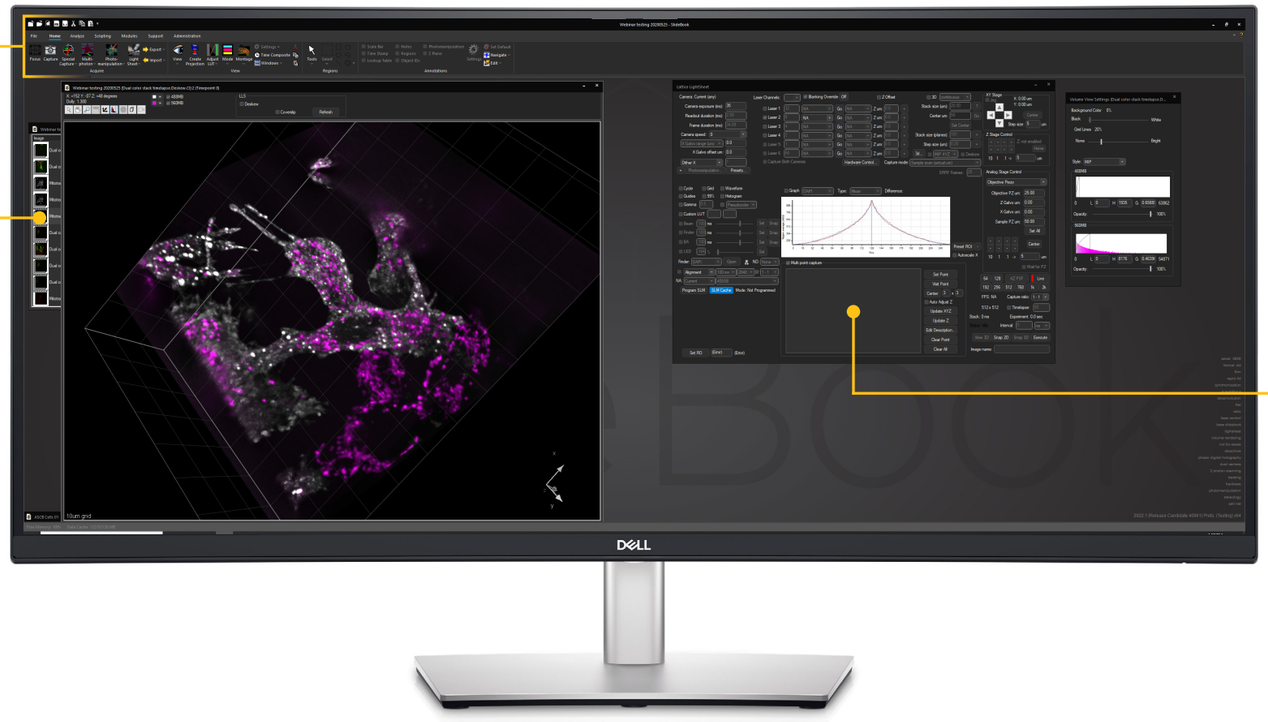
Select a color scheme from dozens of options
Switch on-the-fly from dark to light themes

SlideBook Open File Format

Directory-based open file format for big data and high performance computing applications

Volume Rendering

3D and 4D volume view visualization tools support a user-specified bounding box and a storyboard interface where multiple perspectives can be assembled into a single movie



NVIDIA CUDA GPU Acceleration

GPU acceleration of computationally-intensive operations such as deconvolution

Image Processing

Deskewing and rotation of data are built into SlideBook's workflow

System Capture Console

The LLS user interface is a single, easy-to-use window featuring all frequent controls and alignment tools from laser selection, system calibration, and dithered/SIM capture mode

Multiposition Capture

Intuitive controls for selecting multiple XYZ positions for automated, sequential capture, ideal for overnight experiments of dozens of locations

Capabilities

Capture

Control hundreds of devices including microscopes, stages, lasers, wheels, piezos, scanners, shutters and much more.

View

Visualize data through any numbers of portals, from single images to z-stacks, time lapse, color channels and 4D views.

Analyze

Analyze images and extract statistical data via a wide variety of algorithms while maintaining original data integrity.

Scripting

Macro scripting for capture and analysis enhances the flexibility and power available to users.

Communicate

Present and export data easily as 16-bit TIFFs, 3D movies, graphs or spreadsheets. Data is directly portable to MATLAB® and Excel and adheres to Open Microscopy Environment (OME) standards.

Partners

MATLAB

Through hierarchical and conditional capture, user-supplied MATLAB programs can control experimental workflows.

Microvolution

Microvolution® software delivers nearly instantaneous deconvolution by combining intelligent software programming with the power of a GPU.

Aivia

Aivia is an innovative and complete 2D-to-5D image visualization, analysis and interpretation platform.

Dell

The latest high-power computer workstations control all microscope hardware and enable high-speed processing, segmentation and volume rendering of terabyte (TB) datasets.

Systems Engineering

3i's Systems Engineering department designs, builds and extensively tests every customer system. From spinning disk confocal to multiphoton to lightsheet and photomanipulation, 3i has delivered over a thousand custom, cutting-edge microscopy systems to help answer some of the most complex scientific questions.

Application Knowledge | Scientific Consulting

A team of PhD scientists meet with each client to document and better understand the scientific context of the user group to ensure that the capabilities of the delivered system match the underlying research goals.

Performance Criteria | Targeted to Experiments

Understanding key experiments and imaging paradigms allows Systems Engineering to apply targeted testing criteria to every system.

Customized Hardware | Novel Light Creation

No matter how complex or customized a light path may be for imaging or photostimulation, our engineers ensure that light is manipulated and directed to where it is needed, when it is needed.

Custom Test Plan | Assure Experiment Success

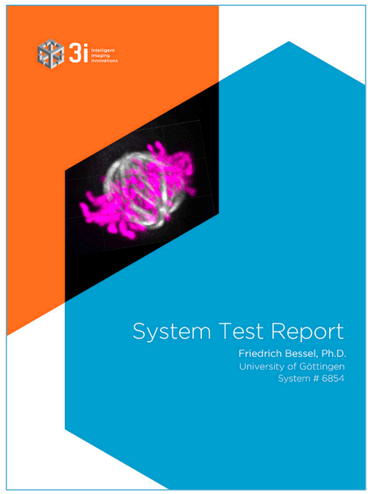
When a technically advanced experiment requires specific system performance to succeed in the lab, a custom test plan assures the system meets that mark prior to delivery.

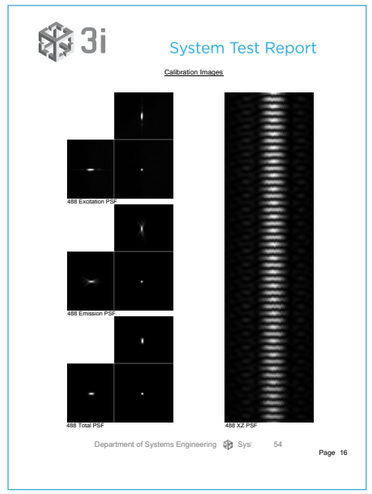
System Integration | Synchronization of Dozens of Instruments

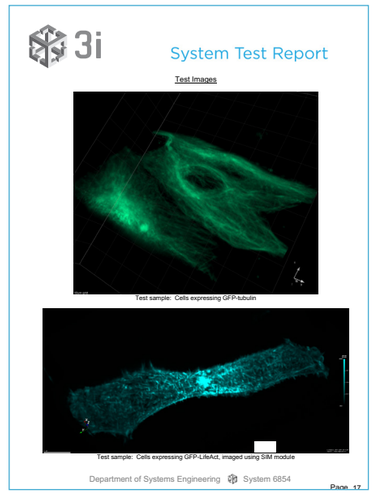
Systems Engineering combines institutional knowledge and scientific consultation to ensure that the instruments in each system are configured for experimental success in the lab.

System Test Report | Guaranteed Performance

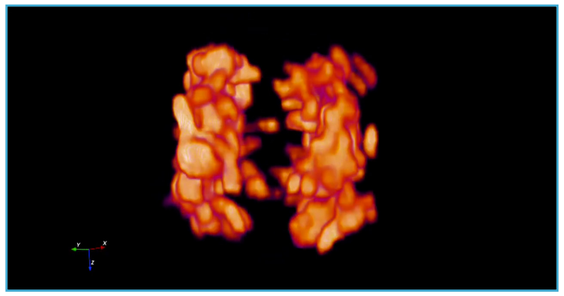
Performance metrics and results of the custom test plan are documented in a System Test Report delivered with each system.



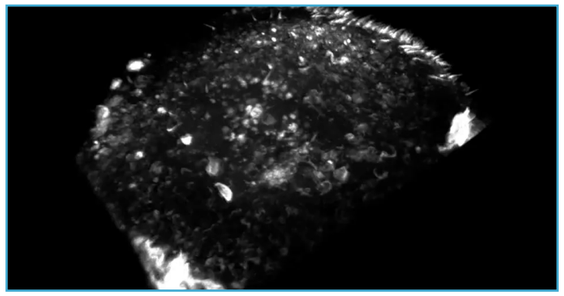




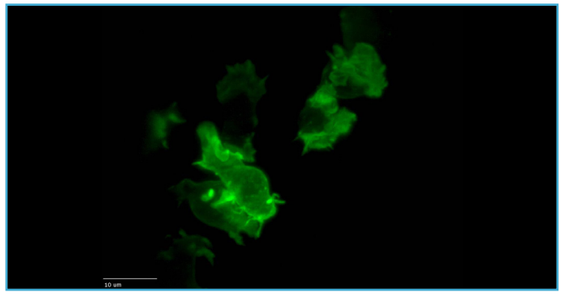
Application Data



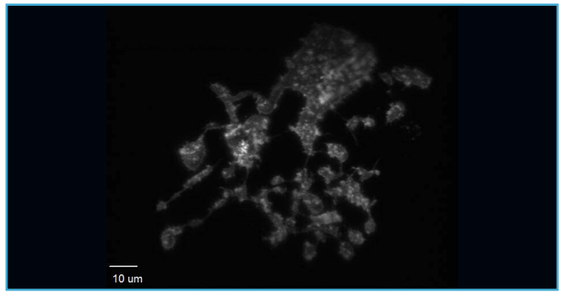
MITOSIS
The near isotropic resolution of the Lattice LightSheet allows for incredible videos of mitotic cells in 4 dimensions. Mammalian cells expressing H2B-RFP. Courtesy of Dr. Andrew McAnish, University of Warwick.



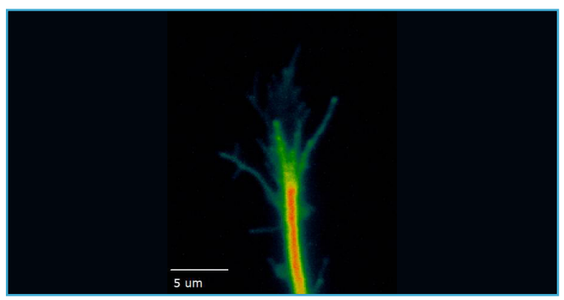
MEMBRANE DYNAMICS
Actin ruffles on a large macrophage imaged at 1 volume/second to capture rapid membrane dynamics over a large cell. Macrophage with LifeAct GFP. Courtesy of Dr. James Springfield, University of Queensland.



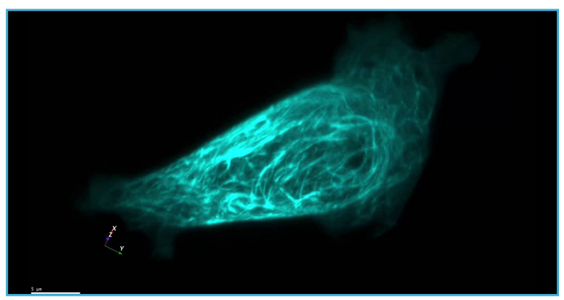
MODEL ORGANISM
Dictyostelium are a “slime mold” that tend to run away from excitation light before they begin to photobleach. The Lattice LightSheet is gentle and fast enough to image dictyostelium at over a volume/second without the cells migrating away. This allows for unprecedented imaging of this model organism. Dictyostelium expressing LifeActGFP. Courtesy of Dr. Till Bretschneider, University of Warwick.



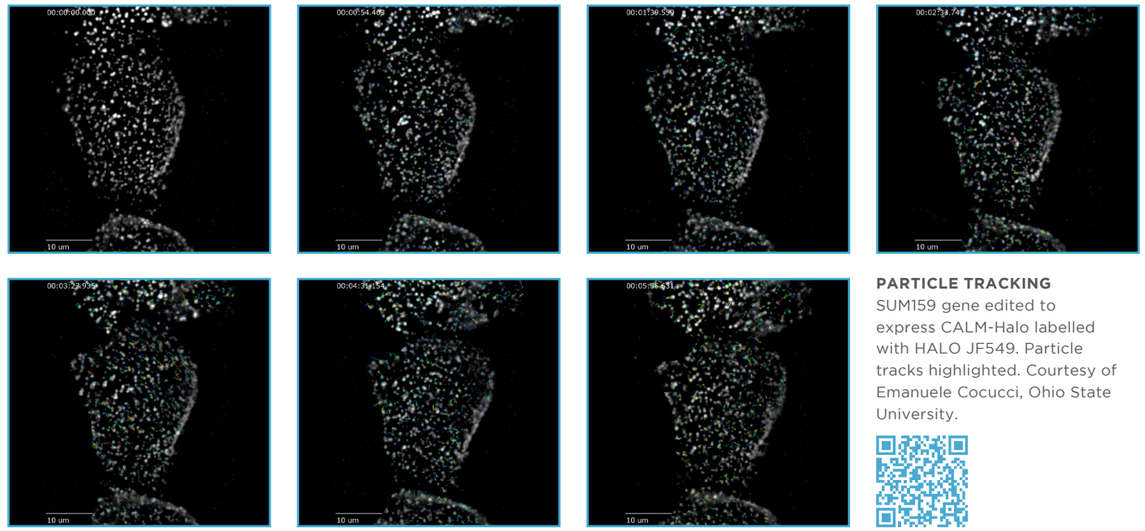
HEMATOLOGY
Platelet generation is typically a difficult process to capture using fluorescent microscopy – the megakaryocytes won’t produce platelets if they are excited with too much laser light. Here, the Lattice LightSheet is gentle enough to image this unique developmental process at high resolution. Courtesy of Dr. Watson, University of Birmingham.



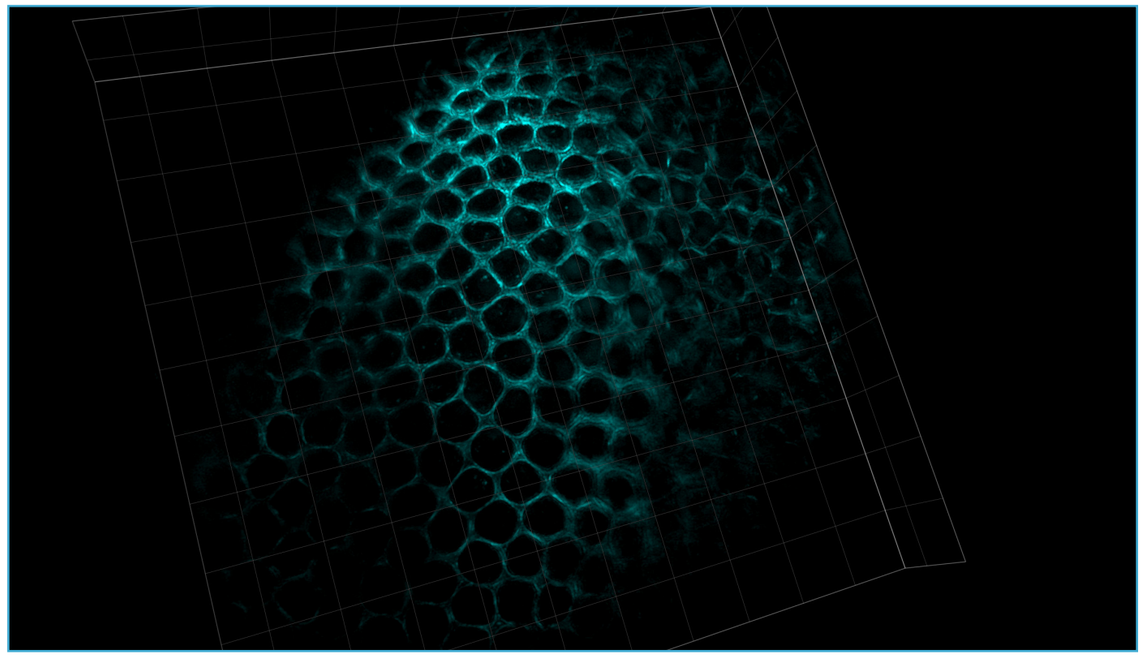
NEUROSCIENCE
Imaging the process of neuronal axon lengthening requires a gentle excitation source and rapid volumetric imaging. Sample was imaged at >1 volume/second to capture rapid microtubule dynamics in the growth cone of a primary rat neuron. Courtesy of Dr. Jeff Moore and Dr. Jayne Aiken at University of Colorado Anschutz Medical Campus.



CYTOSKELETON
Microtubules are the ever-changing cytoskeleton present in the cell. Here, fast volumetric imaging of the microtubule network displays distinct differences in the cytoskeletal dynamics throughout the cell. MCF-7 cells transfected with GFP-EMTB to visualize microtubule dynamics. Courtesy of Dr. Gordon L. Hager and David A. Garcia at the National Cancer Institute.

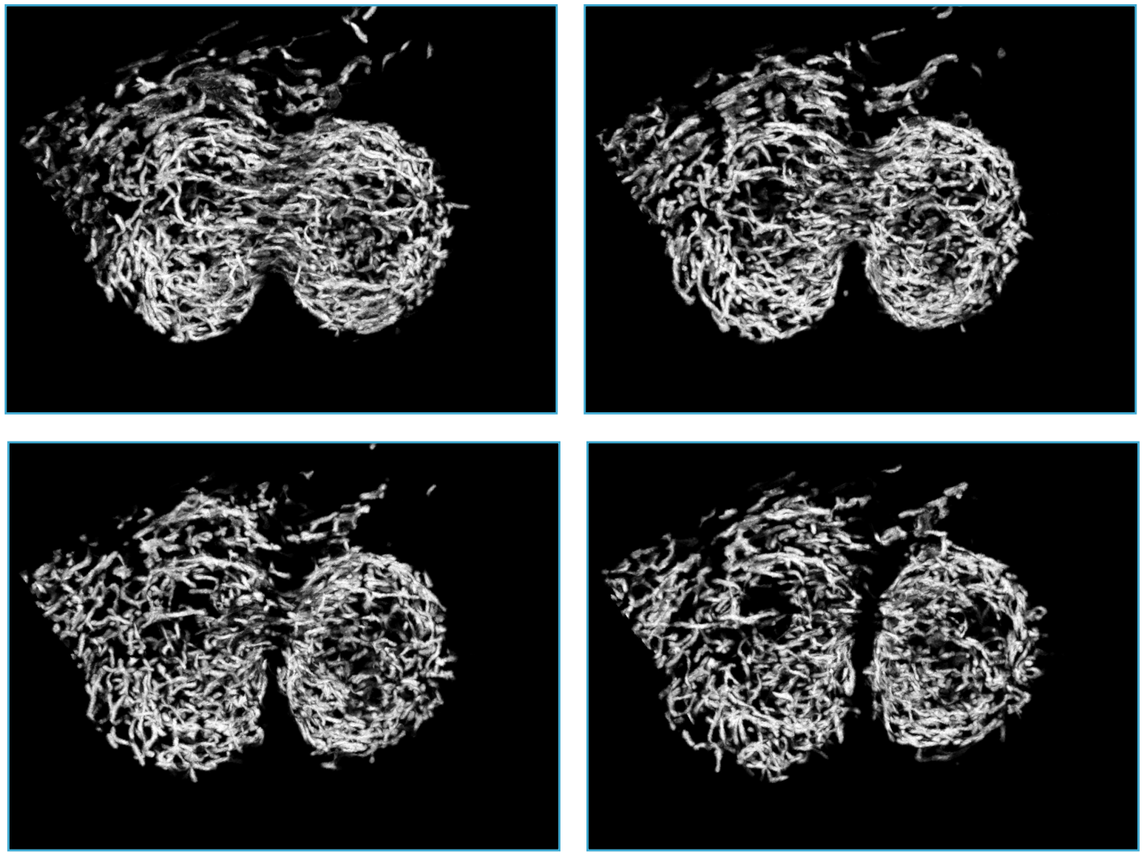


PARTICLE TRACKING
SUM159 gene edited to express CALM-Halo labelled with HALO JF549. Particle tracks highlighted. Courtesy of Emanuele Cocucci, Ohio State University.

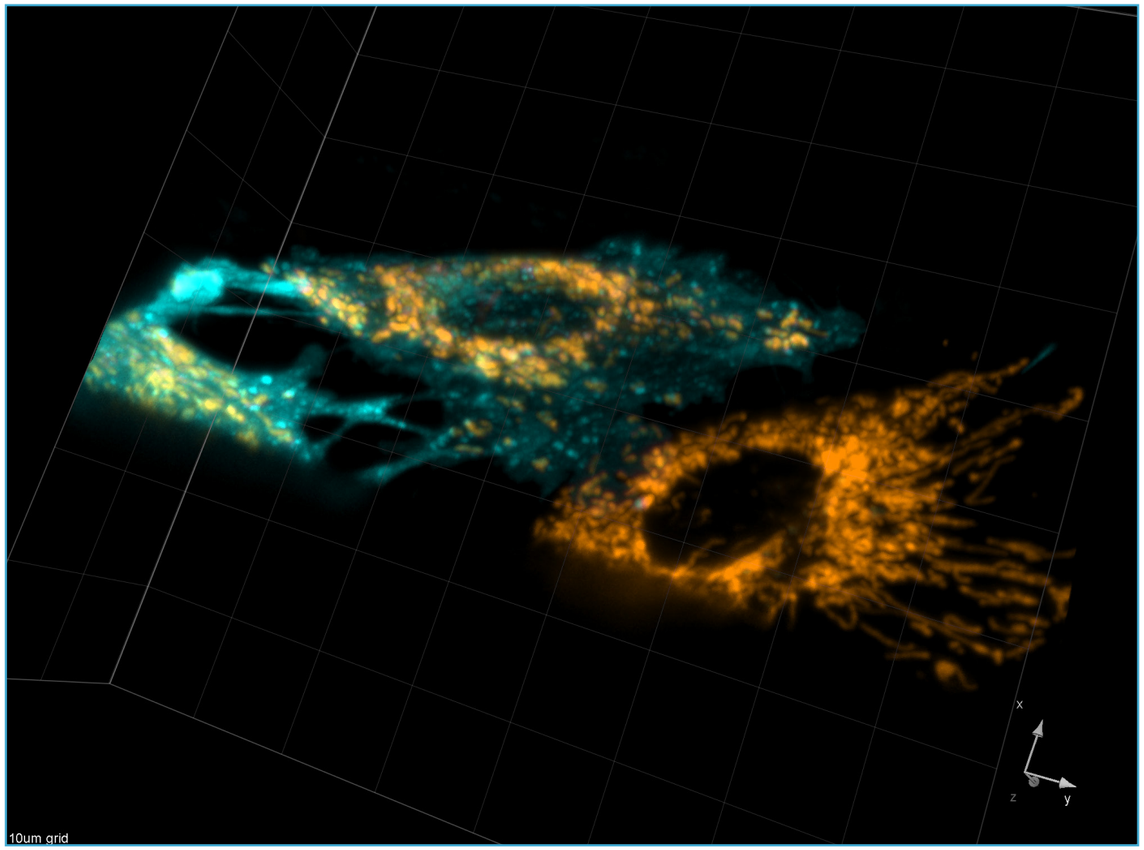


EMBRYO IMAGING
Volume rendering of GFP actin in *drosophila* embryo. Imaged with Lattice LightSheet SIM.

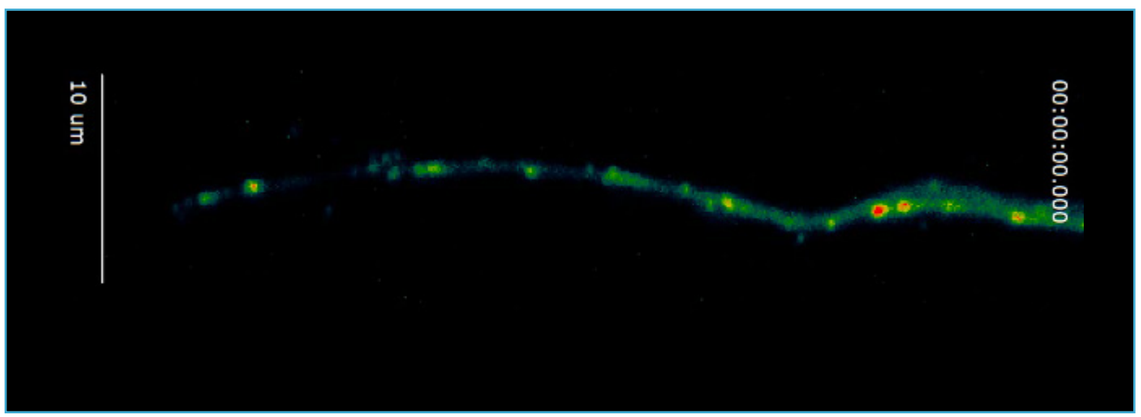
Application Data



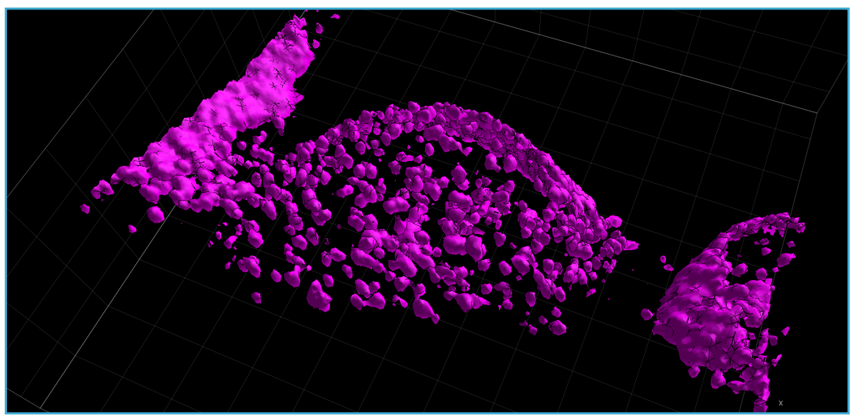
MITOCHONDRIAL FISSION
Mitochondria in dividing cells. Courtesy of Senthil Arumugam, Monash University.



SCIENCE
Volume rendering of EGFR-GFP and MitoTracker Red in mammalian cells.



NEUROSCIENCE
EB3 in primary rat neurons. Courtesy of Dr. Jeff Moore and Dr. Jayne Aiken at University of Colorado Anschutz Medical Campus.



ENDOCYTOSIS
SUM159 gene edited to express CALM-Halo labelled with HALO JF549. Particle tracks highlighted. Courtesy of Emanuele Cocucci, Ohio State University.

Support and Maintenance

A variety of software and equipment support levels help keep systems running well for years. A Software Support Agreement allows labs to run the latest version of SlideBook with new acquisition and analysis features. It includes direct access to 3i staff via email, phone and video chat. A System Maintenance Agreement adds an annual preventative maintenance visit, 3i service visits and 3i coordination of any repairs, although repair and replacement parts are not included. A System Extended Warranty adds full coverage for repairs and replacement parts. Additionally, 3i application scientists may provide in-person and webinar-based application training.

	Software Maintenance	System Maintenance	System Warranty
Phone, Email and Video Chat Support			
SlideBook Software Releases			
Service Visits and Annual PM Visit			
Repairs Coordinated by 3i			
Application Training | In-Person or Online			
Full Warranty Coverage of all System Hardware			

BUILT BY SCIENTISTS FOR SCIENTISTS

3i designs and manufactures technologies for living cell, live cell, and intravital fluorescence microscopy including superresolution, computer-generated holography, spinning disk confocal, multi-photon and lightsheet. SlideBook software manages everything from instrument control to image capture, processing and data analysis. 3i was established in 1995 by a group of cell biologists, neuroscientists, and computer scientists 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.

3i Intelligent Imaging Innovations

SlideBook

USA
Intelligent Imaging Innovations, Inc.
3575 Ringsby Court, Suite 102
Denver, CO 80216 USA
+1 303 607 9429

UK
Intelligent Imaging Innovations, Ltd
17 Westbourne Studios
242 Acklam Rd, London, UK W10 5JJ
+44 2037 440031

DE
Intelligent Imaging Innovations, GmbH
Königsallee 9-21
D-37081 Göttingen, Germany
+49 551 508 39 266

FR
Intelligent Imaging Innovations, SAS
17 rue Moreau
75012 Paris, France

info@intelligent-imaging.com
sales@intelligent-imaging.com
www.intelligent-imaging.com