3i Intelligent Imaging Innovations

# Marianas LightSheet

## Versatile Multiview Light Sheet Microscopy System for Imaging Model Organisms


Image courtesy of Courtney Lancaster, Pichaud lab, LMCB.

# Marianas LightSheet

Marianas LightSheet (MLS) combines the low phototoxicity and large sample space of dual inverted selective plane illumination microscopy (diSPIM) with the power and flexibility of a research-grade inverted microscope system. MLS supports a wide variety of specimen types and sizes as well as experimental designs including photoablation, photomanipulation, and computer-generated holography.  Add spinning disk confocal, TIRF and FLIM to transform the system into a versatile multimodal imaging system all controlled with SlideBook software.

## TTL Sync

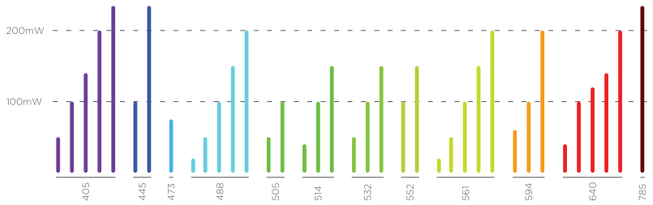
Millisecond timing and trigger
Control of multiple devices

## Scan-Optimized X,Y Stage

High-precision movement of sample during long-term experiments

## LaserStack

Modular laser combiner
Up to eight lasers




## Fiber Switcher

Up to four fiber outputs
Millisecond path switching

## Additional Light Path

Add cameras, photomanipulation devices, spinning disk confocal and more

## Light Sheet Scanners

High-speed MEMs mirror or cylindrical lens scanners

## Environmental Control

Stage top and cage incubators
Temperature, gas and humidity

## Motorized SPIM Z Drive

High precision multi-position and vertical montage capture

## sCMOS Cameras

95% QE low read noise
Large field-of-view options

## Objective Piezos

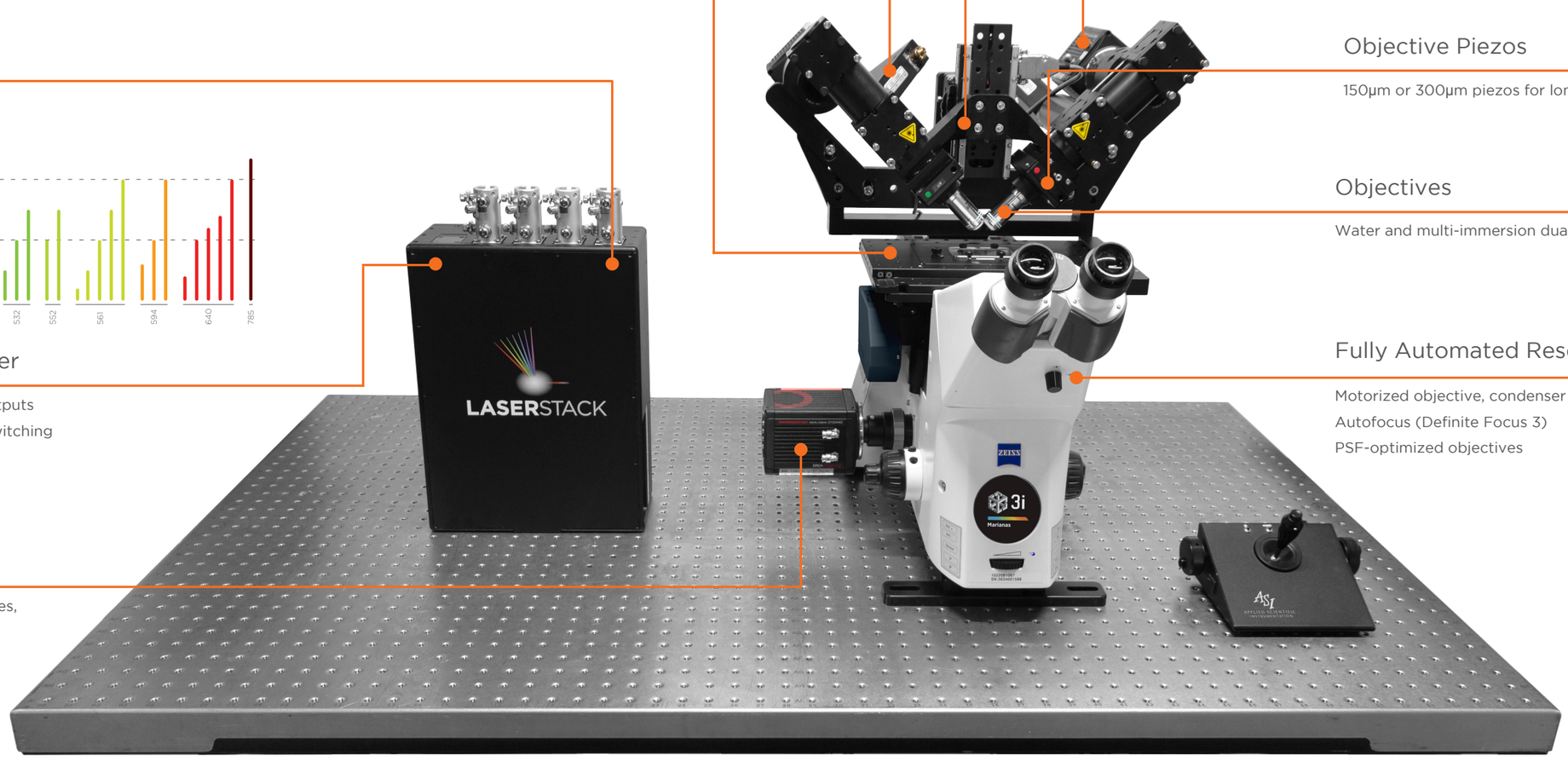
150µm or 300µm piezos for long-term high-speed 3D sampling

## Objectives

Water and multi-immersion dual-view optimized objective pairs

## Fully Automated Research Microscope

Motorized objective, condenser and path selection
Autofocus (Definite Focus 3)
PSF-optimized objectives




## Spinning Disk Confocal

Live cell 3D confocal imaging
Super-resolution dual microlens disk


## Ablate!

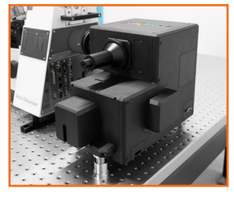
Laser ablation system
355nm and 532nm

## Vector2

Epi mounted or camera port mounted photomanipulation
Modular high-speed X,Y scanner

## Vector3

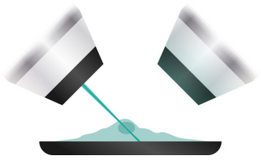
Epi-mounted TIRF and photomanipulation
Spinning X,Y TIRF with expansive FN22 FOV
Liquid light guide input for LED light source

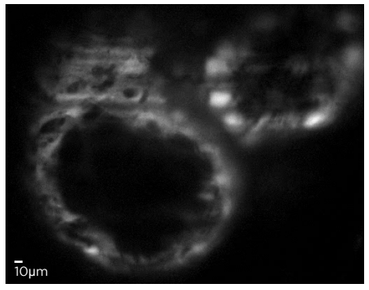


# Scanning Modes

## Fixed

Ultra-fast single plane time-lapse capture with stationary light sheet.

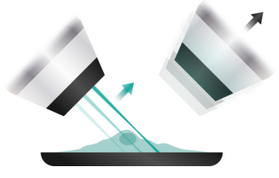


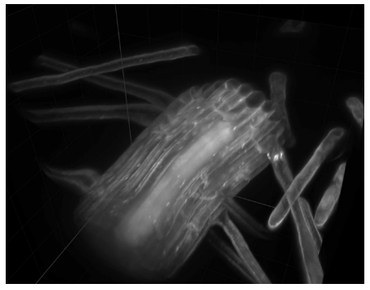



**ZEBRAFISH HEART**
Zebrafish heartbeat (GFP-labeled Cardiomyocytes). Courtesy of Dr. Jamie Nichols, University of Colorado.

## Piezo

Fast 3D capture up to 3 volumes/sec of smaller specimens with synchronized sheet and objective movement.

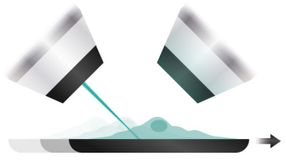


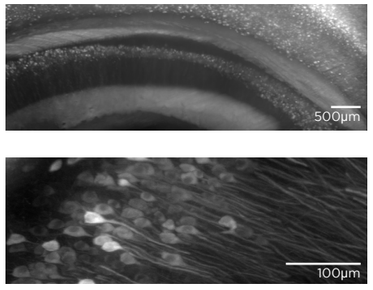


**ARABIDOPSIS ROOT**
Piezo slice scan capture of an Arabidopsis root (YFP-labeled membrane). Courtesy of Dr. David Ehrhardt, Carnegie Institution for Science.

## Stage

Large 3D capture up to 1 volume/sec of specimens translated through a stationary light sheet.

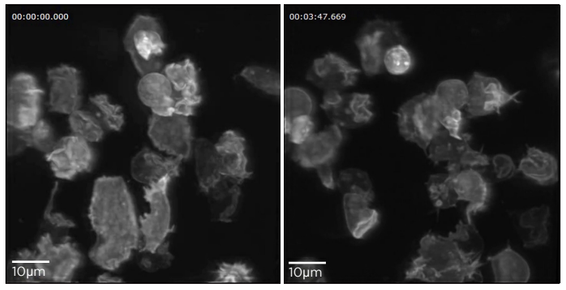





**CLEARED BRAIN SLICE**
Stage scan captures of a cleared mouse brain (GCaMP-GFP) with a pair of 10x (top) and 40x (bottom) objectives. Courtesy of Dr. Rob Campbell, University College London.

## Dual-View Piezo

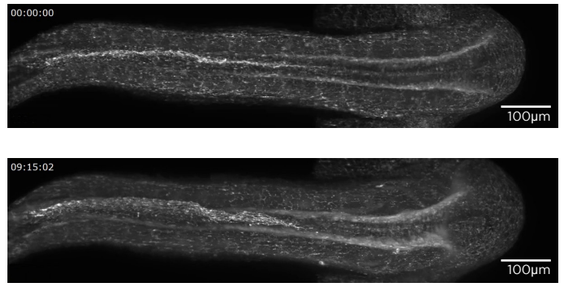
Alternating Piezo captures combined with SlideBook Multiview Reconstruction for isotropic resolution.




**DICTYOSTELIUM**
Dual-view piezo slice scan timelapse of Dictyostelium cells (membrane-rhodamin).

## Dual-View Stage

Alternating Stage captures combined with SlideBook Multiview Reconstruction for isotropic resolution.




**ZEBRAFISH**
Dual-view stage scan timelapse (GFP-actin). Courtesy of Elric Esposito, Spencer Shorte UtechS Photonic BioImaging and Nicolas Dray, Laure Bally-Cuif Zebrafish Neurogenetics lab, Institut Pasteur.

## Objective Pairs

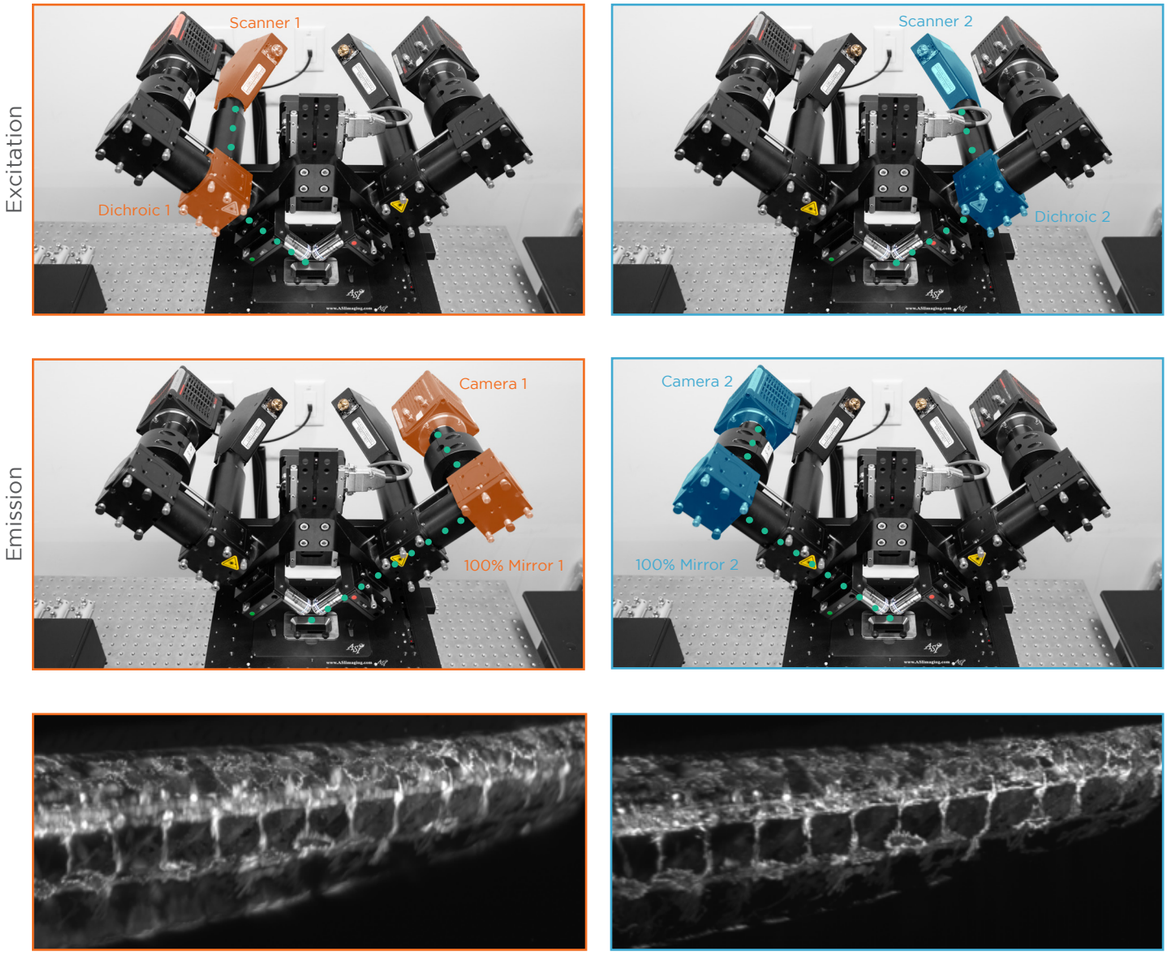
| Magnification | Numerical Aperture | Refractive Index | Maximum Field of View | Recommended Use |
|---|---|---|---|---|
| **Water Immersion** | | | | |
| 10x | 0.3 | 1.33-1.4 | 1490µm | Largest field of view |
| 20x | 0.5 | 1.33-1.4 | 620µm | Balanced field of view and resolution |
| 40x | 0.8 | 1.33-1.4 | 370µm | Highest resolution |
| **Multi-Immersion** | | | | |
| 10x | 0.3 | 1.33-1.56 | 1470µm | Largest field of view |
| 16x | 0.4 | 1.33-1.56 | 850µm | Higher resolution large field |
| 24x | 0.7 | 1.33-1.56 | 590µm | Highest resolution |

## Flexible Sample Mounting



# Dual Inverted Selective Plane Microscopy

Dual inverted selective plane microscopy (diSPIM) uses dual camera and scanner objective pairs positioned at 45° to the specimen plane to alternate capture and excitation between Path 1 and Path 2. The flexible system geometry allows for single- and dual-sided imaging with conventional sample mounting. SlideBook seamlessly controls all hardware modalities, deskewing, stitching/montaging, Multiview Reconstruction and joint deconvolution resulting in isotropic sub-cellular resolution across a wide range of samples. Marianas LightSheet integrates diSPIM with a research-grade inverted microscope enabling complex multi-modal light sheet experiments with photoablation, photostimulation and computer-generated holography.

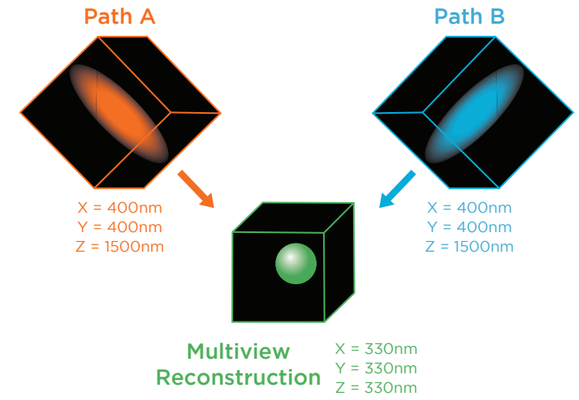


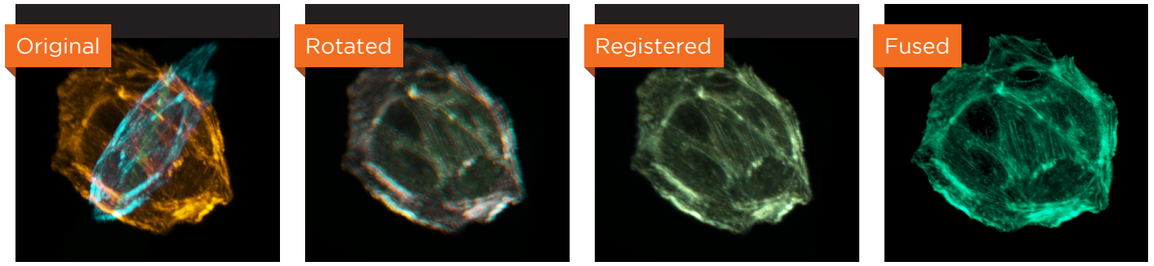

**ZEBRAFISH**
Dual-view stage scan capture of a Zebrafish (GFP-Actin). Path 1 (left) and path 2 (right). Courtesy of Dr. Cody Smith, University of Notre Dame.

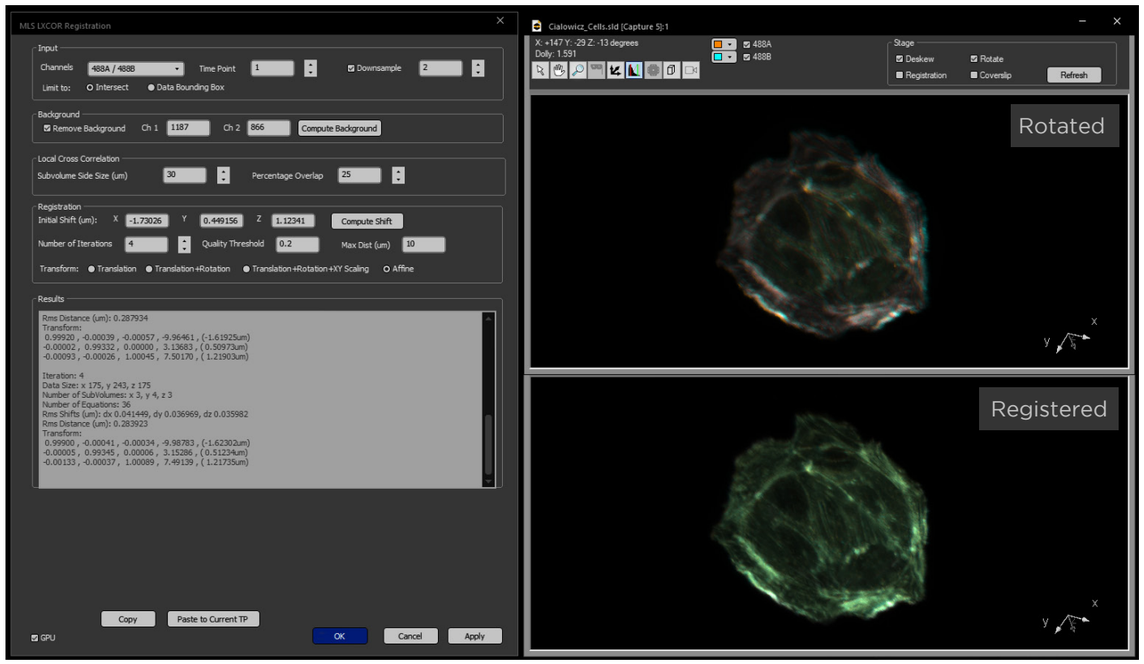


# SlideBook Multiview Reconstruction

SlideBook for MLS supports several methods to deskew, rotate, register and deconvolve diSPIM images. Dual-view capture registration is performed either through Point of Interest Detection or 3i's proprietary Local Cross-Correlation and Registration algorithms. Results are instantly displayed in 3D for quick adjustment to achieve an ideal fit before joint deconvolution produces a fused image. Large datasets that would otherwise require high-powered computer resources can be automatically split into a computer's available RAM and computed in parallel utilizing GPU-optimized processes.



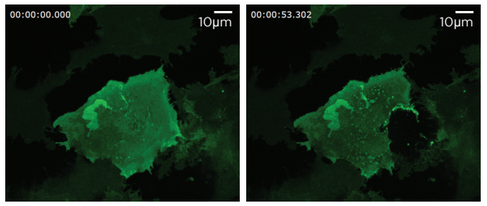



# Photomanipulation

## Ablate! Laser Ablation System

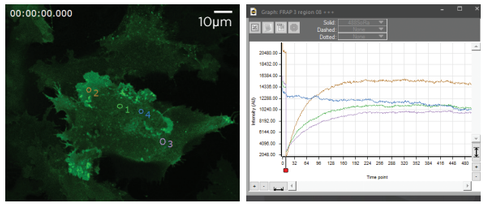
- 355nm or 532nm pulsed laser
- Fixed point or galvo-scanned variable region of interest
- 2D or 3D regions
- Diffraction limited spot


**PHOTOABLATION**
Mammalian cell with membrane labeled in green. Before (left) and after ablation (right).

## Vector2 Scanning Photomanipulation

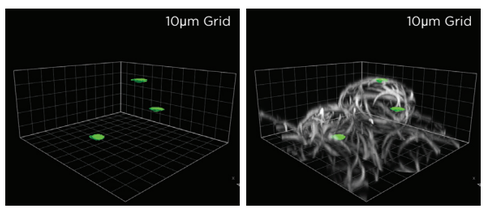
- Photoactivation/FRAP
- Galvo-scanned variable region of interest
- 2D or 3D regions
- Diffraction limited spot


**PHOTOBLEACHING**
Mammalian cell with membrane labeled in green before bleaching and FRAP curve in SlideBook.

## Phasor Holographic Photomanipulation

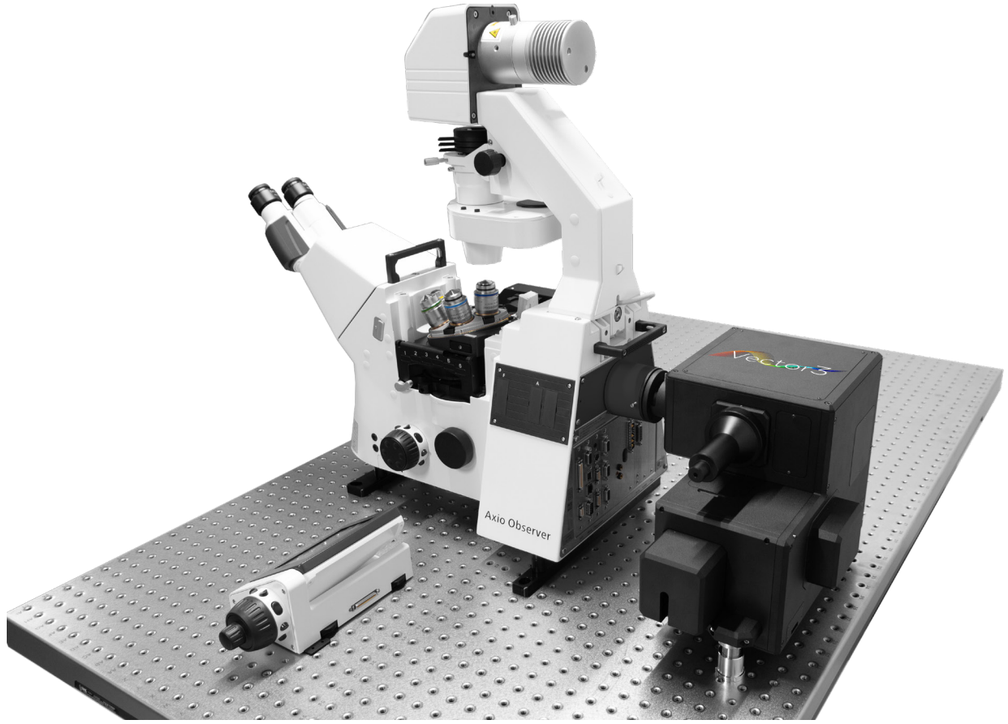
- Spatial light modulator-generated holography for optogenetics stimulation/FRAP/voltage imaging
- Simultaneous 3D stimulation of multiple, separate regions
- Visible and multiphoton stimulation without scanning


**3D PHOTOSTIMULATION**
3D illumination pattern (left) applied to a 3D specimen (right) to stimulate multiple regions simultaneously.

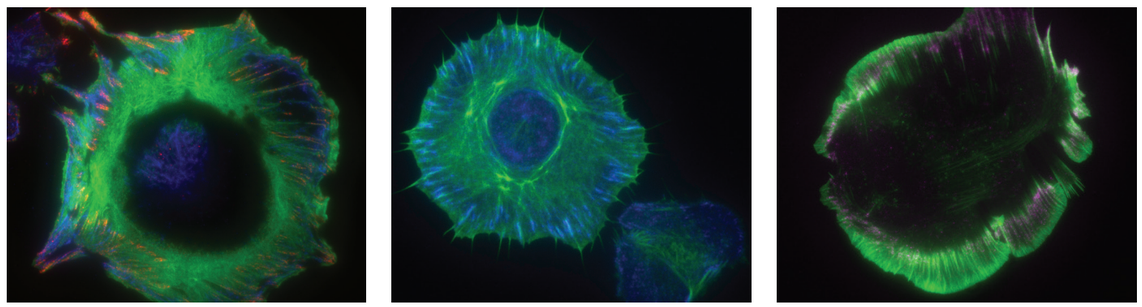


# Total Internal Reflection Fluorescence




## Vector3 Epi-Mounted TIRF and Photomanipulation

Vector3 is a motorized spinning X,Y TIRF system with built-in photomanipulation and widefield fluorescence capabilities. Vector3 offers an expansive TIRF field-of-view (FN22) designed for modern sCMOS cameras. Spinning the TIRF beam via galvo mirrors results in smooth, evenly illuminated images across the field. Vector3 is capable of galvo-scanned photomanipulation of diffraction-limited spots via user-drawn ROIs for easy FRAP and photoconversion experiments. Incorporation into the epi path of a microscope allows the use of any existing microscope cameras – for example, cameras attached to a CSU-W1 spinning disk – keeping the alignment identical between different imaging modalities and enabling powerful, multimodal imaging experiments. Integrated collimating optics are compatible with most LED illuminators (via a liquid light guide) for widefield illumination.



Data courtesy of Dr. Chris Bakal and Oliver Inge at the Institute of Cancer Research in London.

# Spinning Disk Confocal

Yokogawa spinning disk confocals utilize a dual Nipkow disk with microlenses for the best optical sectioning and minimal pinhole crosstalk. This proven technology is the best solution for intravital imaging where optical sectioning and speed are both critically important.


## CSU-X1

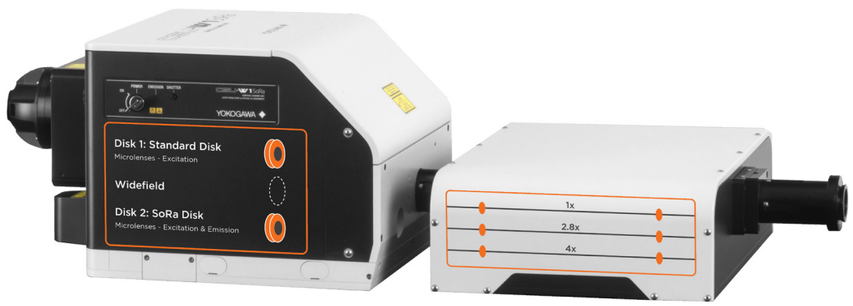
- Highest speed imaging at up to 2000fps
- Field of view 7mm x 10mm
- 50µm pinhole disk with microlenses
- Manual and motorized versions

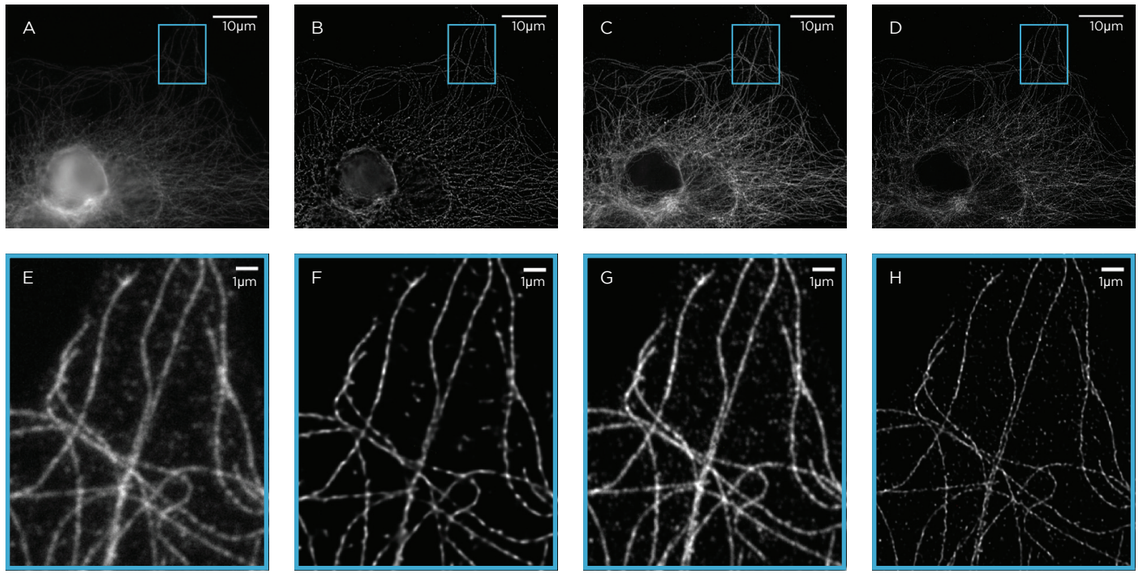

## CSU-W1

- High speed imaging up to 200fps
- Wide field of view 16mm x 17mm
- 25µm and 50µm pinhole disks for lower and higher magnification objectives
- Motorization including disk exchange, variable aperture, camera port selection and camera port magnification
- Options for split-view imaging, NIR imaging, illumination field flattening and super-resolution imaging

## CSU-W1 SoRa

CSU-W1 SoRa is an easy-to-use super-resolution microscopy solution utilizing a dual Nipkow disk assembly with microlenses on both the illuminating and pinhole disks. SoRa images have a 1.4x resolution improvement and deconvolved SoRa images have a 2x resolution improvement compared to standard spinning disk data. With a maximum speed of 200fps, low phototoxicity and no limitation on dyes or fluors, SoRa is ideal for super-resolution intravital imaging. SoRa is also available as an upgrade to existing CSU-W1 systems.

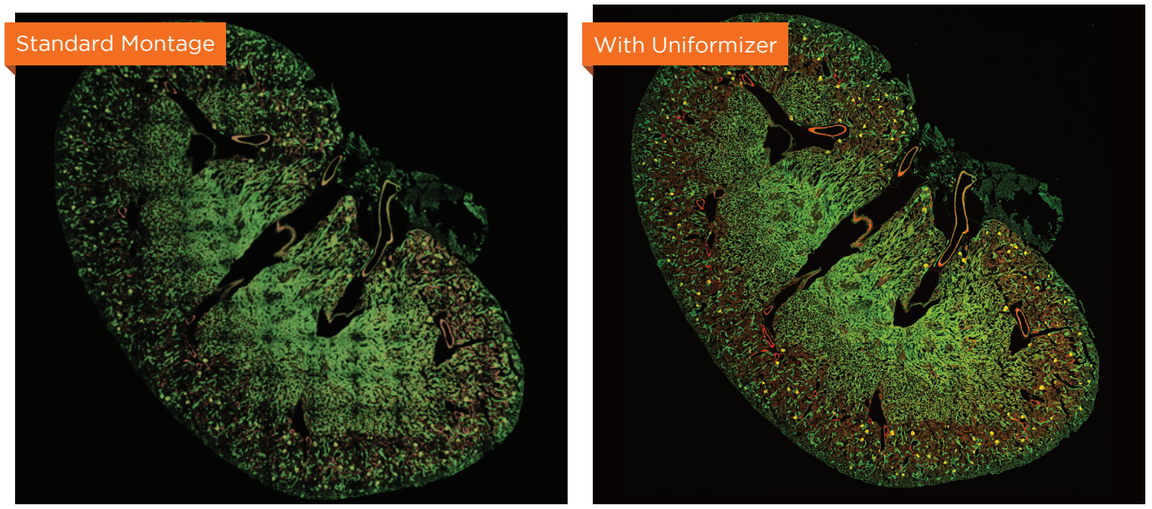



Imaging of microtubules in fixed bovine pulmonary artery endothelial cells. Azuma, T. and Kei, T. (2015) Super-resolution spinning-disk confocal microscopy using optical photon reassignment. Opt Express. Jun 1;23(11):15003-11. doi: 10.1364/OE.23.015003.

## Uniformizer | Illumination Field Uniformity

For exceptionally even illumination across the entire field, Uniformizer conditions the gaussian beam from the illumination fiber optic to distribute light evenly across the field. Using a set of microlens arrays, Uniformizer flattens the field to as little as 1% variance and boosts overall intensity up to 50%.

# SlideBook

SlideBook software supports research microscopy through the entire experimental process. By managing everything from instrument control to image processing and data analysis, SlideBook allows scientists to focus on investigation rather than instrumentation. SlideBook controls hundreds of instruments in and around the microscope from dozens of manufacturers enabling researchers to integrate their preferred components and upgrade to the latest devices once available.

## User-Selectable App Appearance

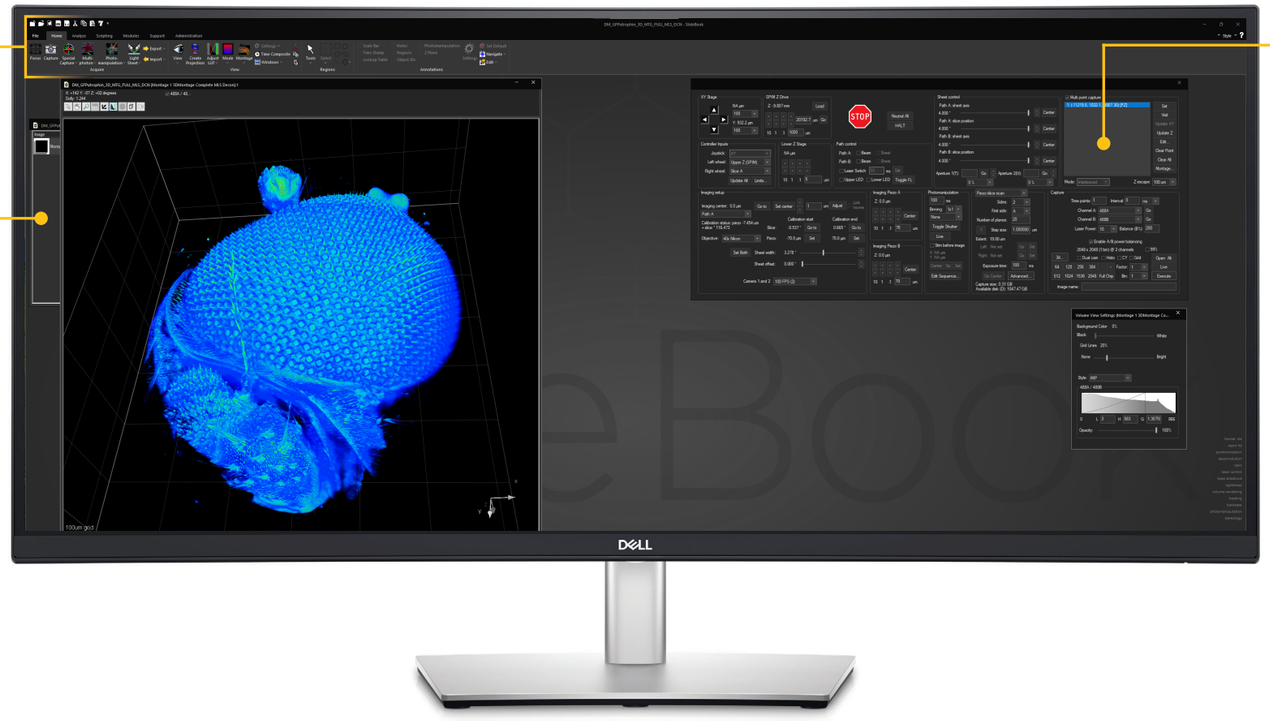
Select a color scheme from dozens of options
Switch on-the-fly from dark to light themes

## SlideBook Open File Format

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

## Multiview Reconstruction

SlideBook can use a number of methods to deskew, rotate, register and deconvolve images captured with MLS. Data is visualized, deskewed and rotated with a single click. Initial shifts and registration results can be instantly checked in 3D and adjusted before joint deconvolution to a fused image.



## Volume Rendering

3D and 4D visualization tools rotate and deskew MLS data on-the-fly and support a user specified bounding box and storyboard interface where multiple perspectives can be assembled into a single movie.

## NVIDIA CUDA GPU Acceleration

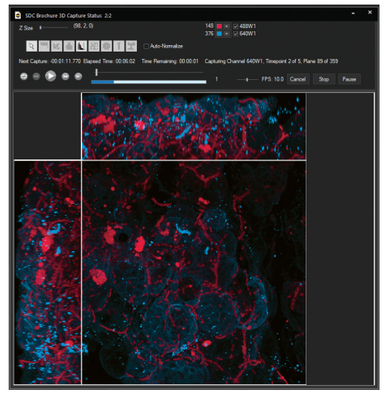
GPU acceleration of computationally-intensive operations such as deconvolution

## Multi-Position and Montage

Capture single timepoints or time-lapses at multiple positions with different experimental setups and throughout the range of capture modes

## 3D Capture Status

Volumetric projection during 4D capture supported across all instruments



## 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.

### syGlass

syGlass enables 3D and 4D visualization and analysis of SlideBook data in a virtual reality environment.

### Aivia

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

### 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

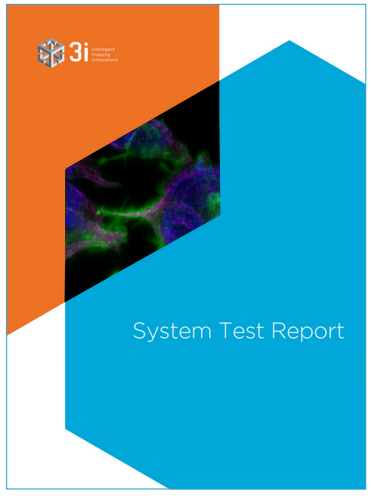
When a technically advanced experiment requires specific system performance to succeed in the lab, a custom test plan assures the system meets that mark prior to delivery.

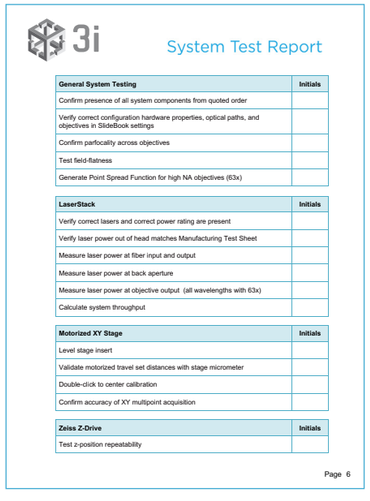
## System Integration | Synchronization of Dozens of Instruments

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.





3i System Test Report

| General System Testing | Initials |
|---|---|
| Confirm presence of all system components from quoted order | |
| Verify correct configuration hardware properties, optical paths, and objectives in SlideBook settings | |
| Confirm parfocality across objectives | |
| Test field-flatness | |
| Generate Point Spread Function for high NA objectives (63x) | |

| LaserStack | Initials |
|---|---|
| Verify correct lasers and correct power rating are present | |
| Verify laser power out of head matches Manufacturing Test Sheet | |
| Measure laser power at fiber input and output | |
| Measure laser power at back aperture | |
| Measure laser power at objective output (all wavelengths with 63x) | |
| Calculate system throughput | |

| Motorized XY Stage | Initials |
|---|---|
| Level stage insert | |
| Validate motorized travel set distances with stage micrometer | |
| Double-click to center calibration | |
| Confirm accuracy of XY multipoint acquisition | |

| Zeiss Z-Drive | Initials |
|---|---|
| Test z-position repeatability | |

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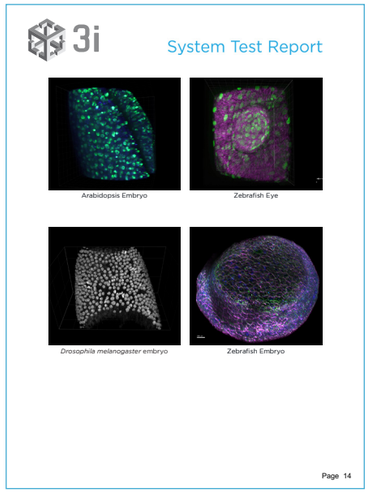
3i System Test Report


Arabidopsis Embryo


Zebrafish Eye


*Drosophila melanogaster* embryo


Zebrafish Embryo

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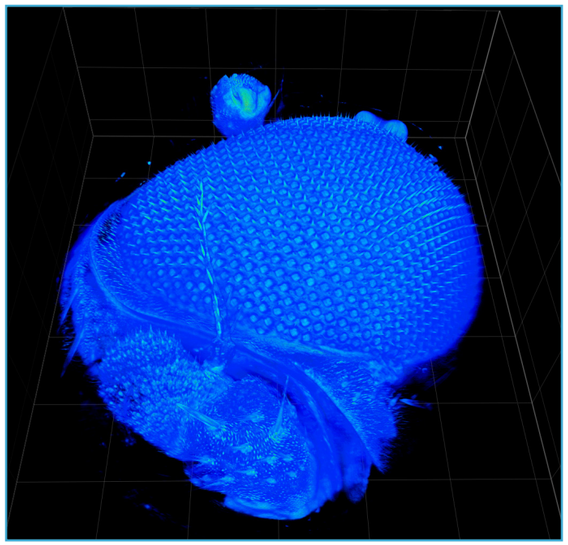
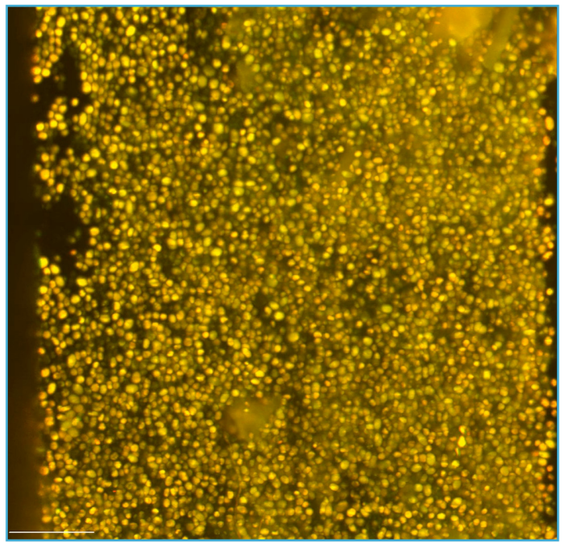
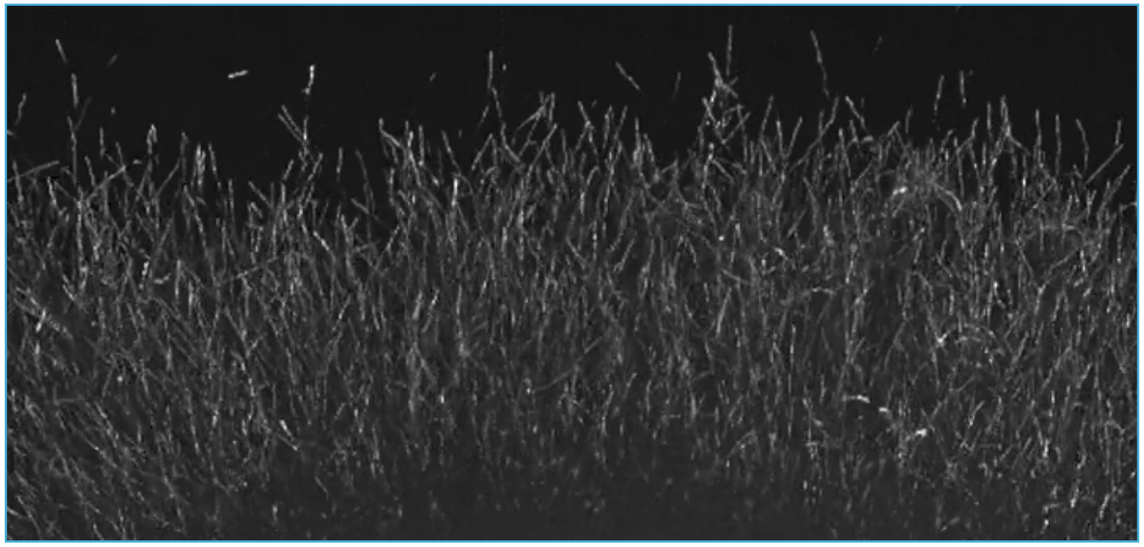
# Application Data



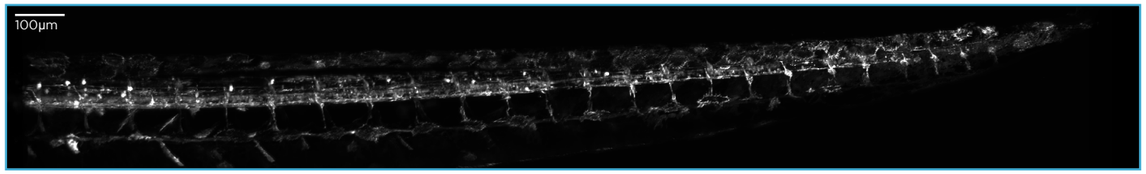
**DROSOPHILA EYE**
Stage scan Y-montage of a Drosophila pupal retina showing utrophin labeled F-actin in pseudocolour. Courtesy of Courtney Lancaster, Pichaud lab, University College London Laboratory for Molecular Cell Biology (LMCB).

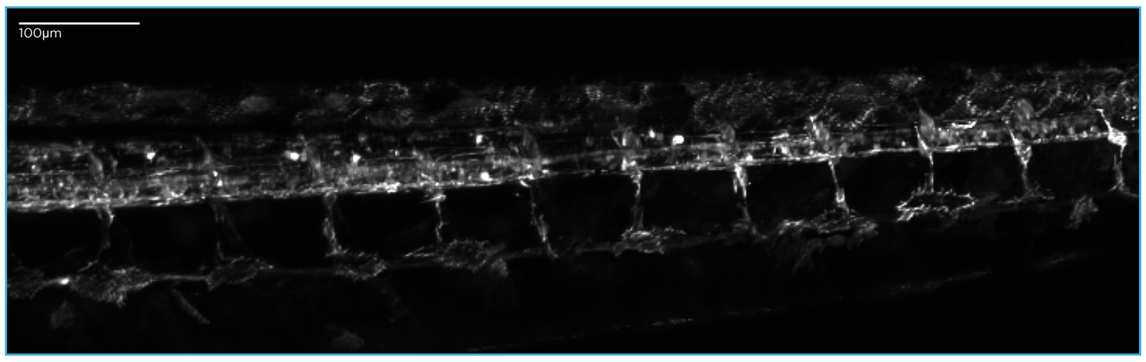


**CYANOBACTERIA**
Maximum intensity projection (MIP) of a stage scan timelapse capture of cyanobacteria biofilm autofluorescence excited with 488nm and 561nm after 48 hours. Courtesy of the Institute of Cancer Research, London.

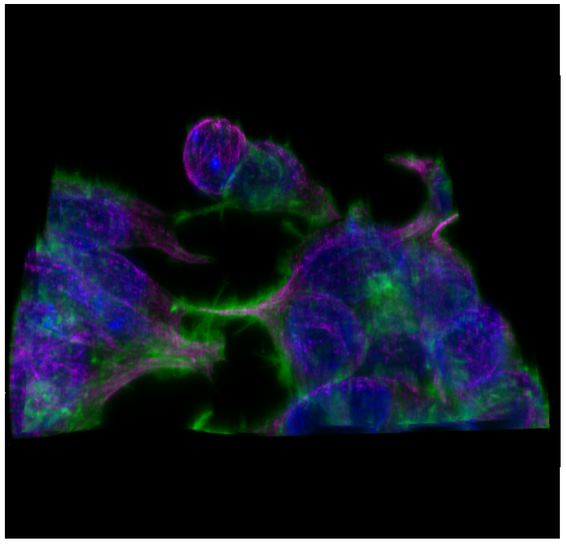


**BIOFILM**
Dual-view stage scan of Candida albicans growth (GFP-Mitochondria). Courtesy of Dr. D Pentland, collaboration Gourley-& Laissue-Labs, Universities of Kent & Essex.

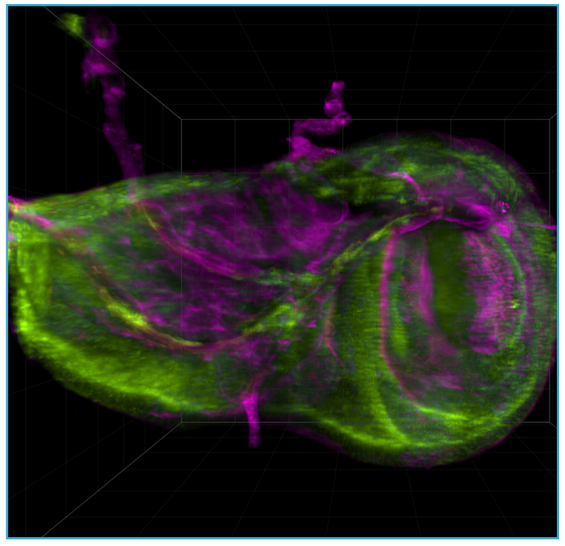



**ZEBRAFISH TAIL**
Dual-view stage scan capture of a Zebrafish (GFP-Actin). Courtesy of Dr. Cody Smith, University of Notre Dame.

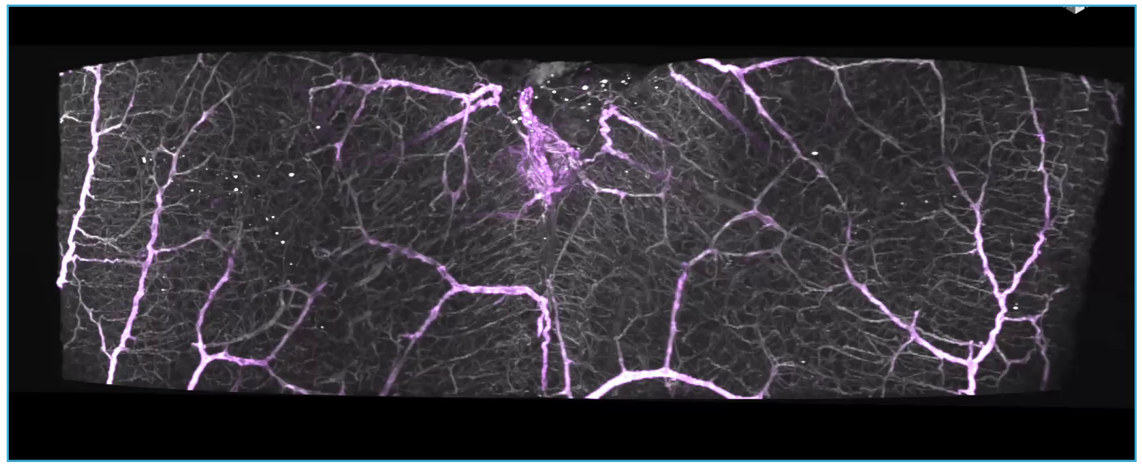


**HUMAN EMBRYONIC KIDNEY (HEK) CELLS**
Dual-view piezo scan capture of HEK cells with labeled tubulin (green), actin (magenta) and nuclei (blue). Courtesy of Dr. Deirdre Kavanagh, University of Birmingham.

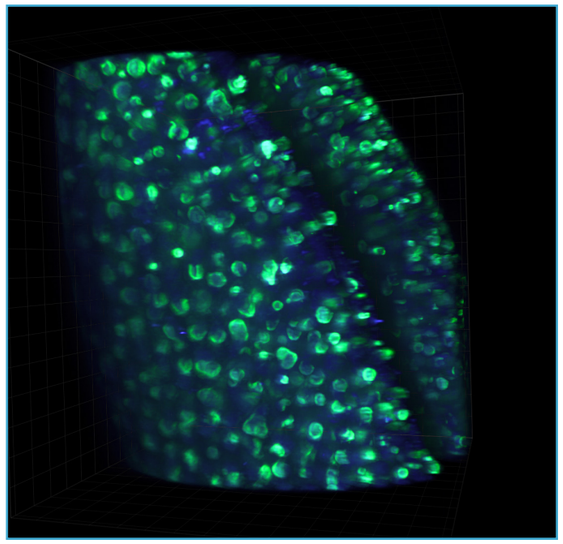


**DROSOPHILA**
Dual-view stage scan capture of a Drosophila wing disc showing Viking (magenta) and membrane (green). Courtesy of Dr. Yanlan Mao and Dr. Rob Tedley, University College London Laboratory for Molecular Cell Biology (LMCB).

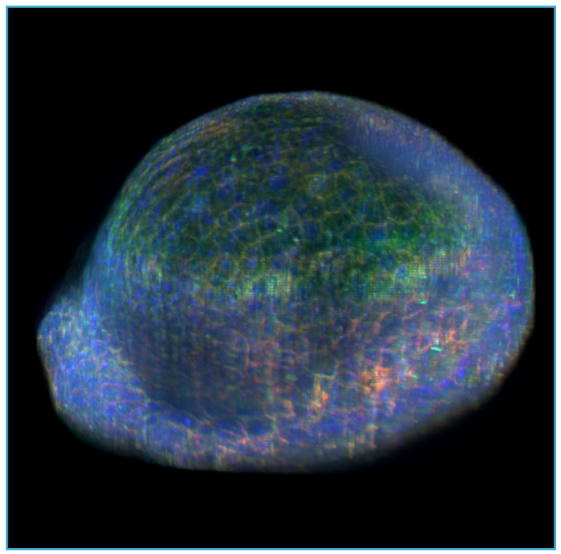
# Application Data



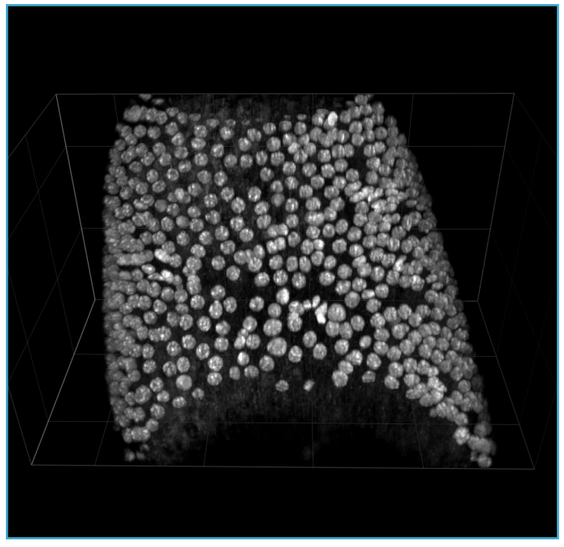
**MOUSE BRAIN**
Stage scan capture of iDISCO-cleared embryonic mouse brain with antibody stainings for smooth muscle cells (white) and endothelial cells (magenta). Courtesy of Tijana Perovice, Gerhardt lab, Max-Delbrück Center for Molecular Medicine.



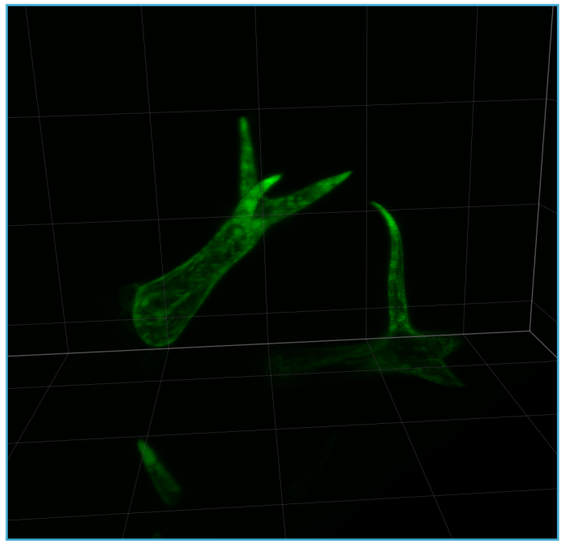
**ARABIDOPSIS EMBRYO**
Dual-view piezo scan capture of an Arabidopsis embryo showing autofluorescence excited with 405nm (blue) and 488nm (green). Courtesy of University of Warwick.



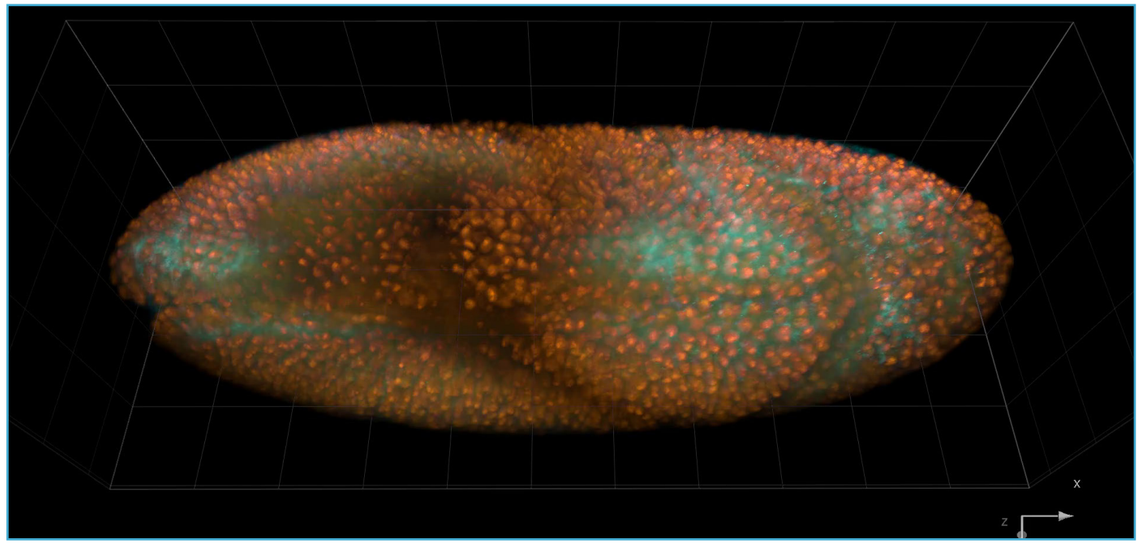
**ZEBRAFISH EMBRYO**
Stage scan capture of a zebrafish embryo 20 hours post-fertilization with labeled actin, tubulin and nuclei.



**DROSOPHILA EMBRYO**
Piezo scan timelapse of synchronised cell division of Drosophila melanogaster embryo (~ 4hours old, H2B-labeled chromatin).



**ARABIDOPSIS TRICHOMES**
Piezo scan timelapse of Arabidopsis trichomes (GFP labeled actin).




**DROSOPHILA EMBRYO**
Stage scan capture of Drosophila embryo with labeled nuclei (orange) and corpora allata cells (blue). Courtesy of Dr. Lucas Dent, the Institute of Cancer Research, London.

# 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 | | |  |

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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.

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