



3i Intelligent
Imaging
Innovations

VIVO SDC

Upright Intravital Spinning Disk Confocal Microscopy System

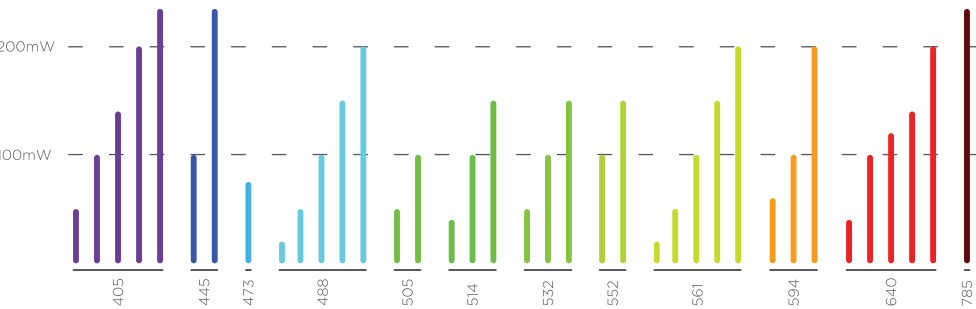


VIVO Spinning Disk Confocal

The VIVO SDC platform incorporates advanced optics, cameras, fast excitation sources, computers and proprietary electronics to achieve high speed, precision and flexibility in intravital image acquisition. The configuration of the VIVO system is designed to allow the presentation of surgically prepared animals and live tissue slices without compromising high-sensitivity imaging at fast speeds. Seamless integration of ablation lasers and photoactivation/photoconversion devices allows for complex photomanipulation experiments.

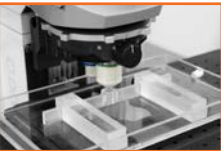
LaserStack

Modular laser combiner
Up to eight lasers



Fiber Switcher

Up to four fiber outputs
Millisecond path switching



Surgical Trays

Custom-designed surgical trays for presentation of exteriorised tissue and for stabilization of tissue presentation using cranial windows

TTL Sync

Millisecond timing and trigger
Control of multiple devices



Vector2

Modular high-speed X/Y scanner for accurate, diffraction-limited photomanipulation events



Spinning Disk Confocal

Intravital 3D confocal imaging
Super-resolution dual microlens disk

Telecorr

Focus extender for correct spinning disk confocal excitation

Phasor

Computer generated holography
1-photon and 2-photon



Fully Automated Research Microscope

Motorized z-drive (with optional fast piezo)
Long working distance water dipping objectives
Large, easily accessible sample space

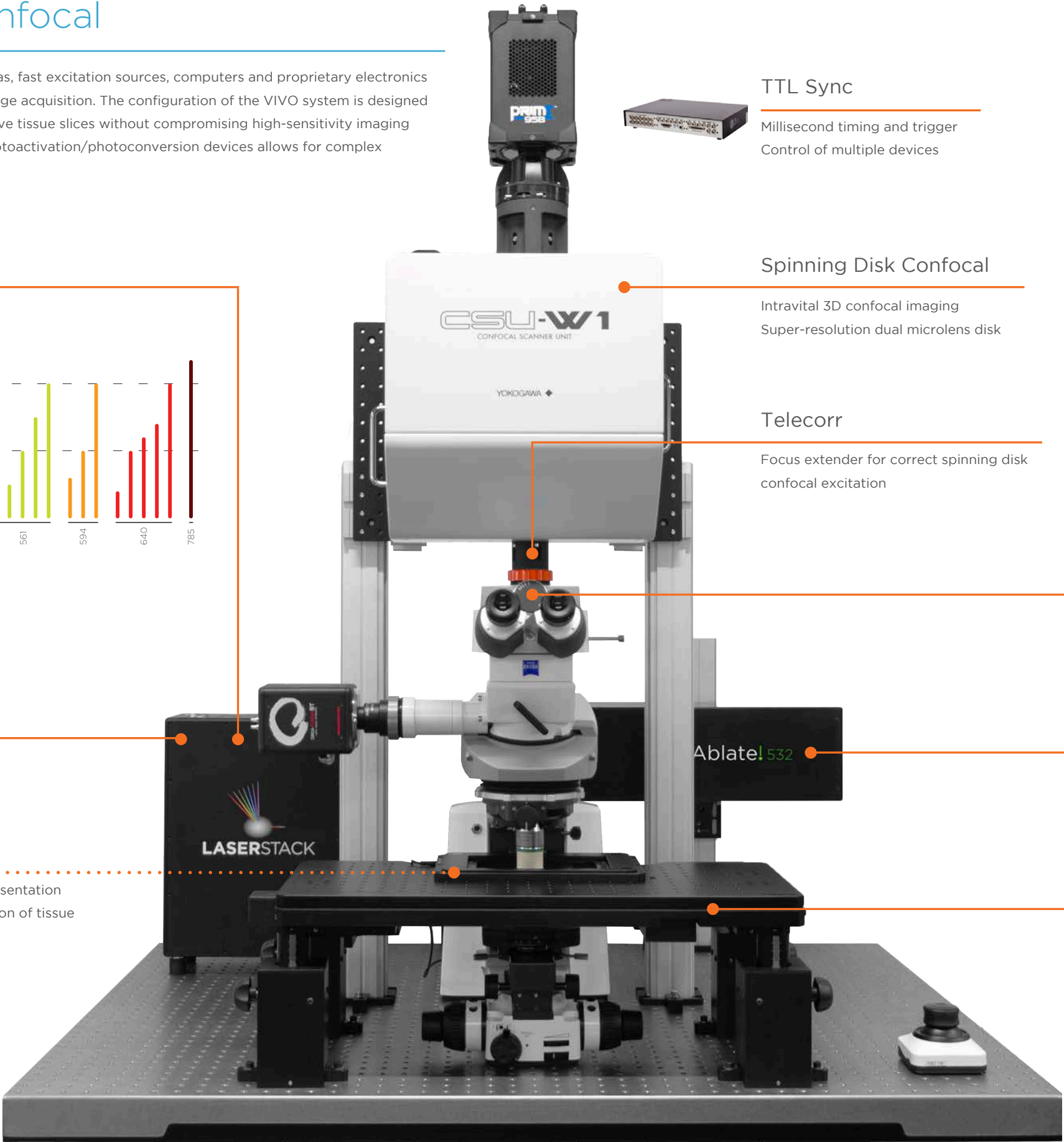
Ablate!

355nm and 532nm pulsed laser system
Fixed point or galvo-scanned for fast targeting

Ablate! 532

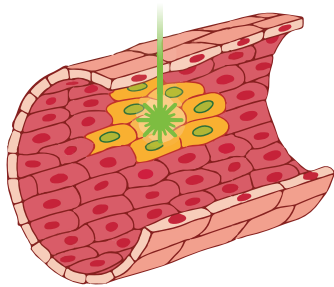
Platform Stage

Accommodates intravital imaging trays and life support systems for long-term animal imaging



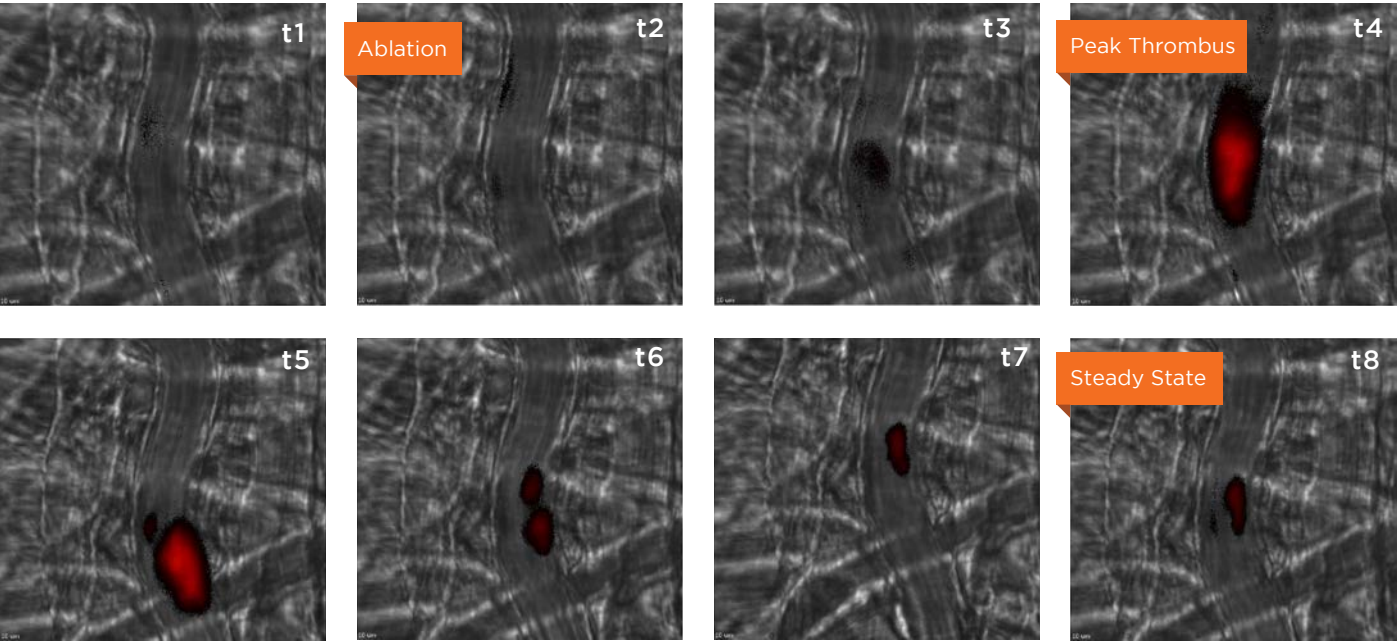
Complete Cardiovascular Imaging Platform

VIVO SDC is designed around fast imaging of the mouse vasculature for thrombosis and inflammation studies. Over the past two decades, dozens of high-impact journal articles have been published with the VIVO SDC system focusing on platelet and leukocyte biology in numerous murine vascular beds including dermis/skin-flap, mesentery, cremaster, cerebral-microvessels (via cranial window) and for imaging of larger vessels including the femoral and carotid arteries. 3i applications scientists can offer training and guidance from experimental design to publication based on extensive personal experience in animal preparation, surgical techniques, intravital imaging and data analysis.

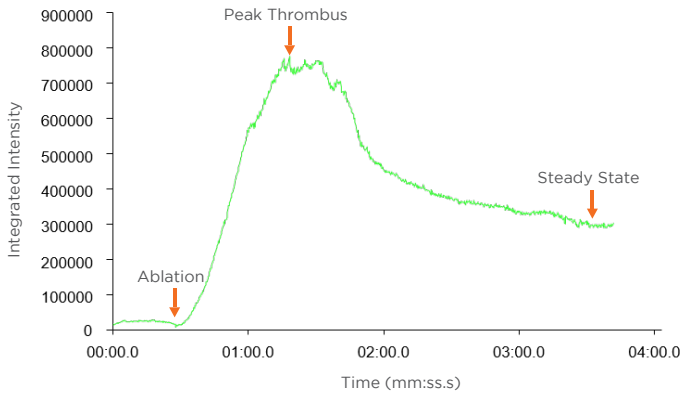


Intravital Thrombosis Imaging

The Ablate! laser system is used to trigger gentle and reproducible damage to the endothelial cells in live blood vessels to induce platelet adhesion and thrombus formation. Consistent pulses from the laser allow for tissue ablation before and after addition of an agonist or inhibitor for detailed kinetic studies of novel therapeutic targets. Power can be easily titrated from cell activation to multi-cell ablations with a diffraction-limited spot for precise targeting.



Platelet thrombosis formation is at the center of many cardiovascular diseases, including heart attacks, strokes and deep vein thrombosis (DVT). VIVO SDC equips researchers with a reproducible laser-induced thrombosis model *in vivo* by implementing a sensitive camera, fast LED brightfield & fluorescence illumination, Ablate! pulsed laser and easy-to-use SlideBook software. In this experiment the murine cremaster muscle is visualized in brightfield and platelets are fluorescently labeled in red. After the ablation event a thrombus forms and stabilizes inside of the arteriole. SlideBook offers advanced masking, segmentation and statistical analysis that makes quantifying thrombosis data easy.



Surgical Equipment & Training Resources

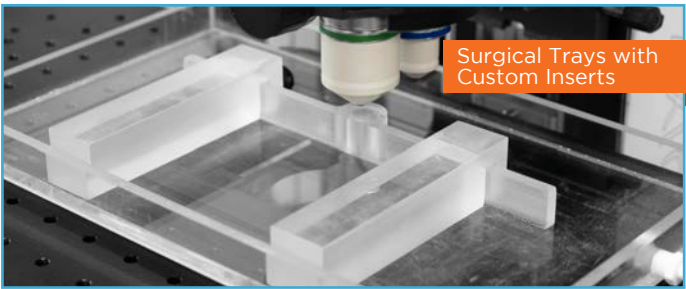
3i offers extensive equipment lists and recommendations for experimental setups. Training videos and journal articles offer researchers a quick starting point for performing advanced cardiovascular imaging experiments. Surgical trays for tissue presentation with custom inserts are available for multiple animal models.



Forceps & Scissors



Superfusion



Surgical Trays with Custom Inserts



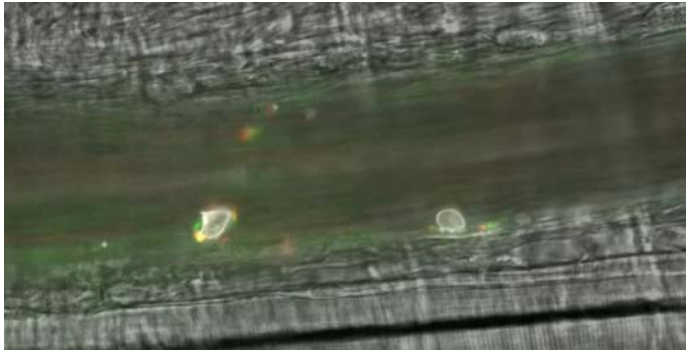
Dissection Stereomicroscopes



Thermostasis Equipment

Preclinical Research Pipeline

Intravital imaging has become essential in several fields including thrombosis & hemostasis, immunology, vascular biology, angiogenesis, biochemistry, gene therapy and cancer biology. Real-time imaging of cardiovascular events adds powerful *in vivo* results to studies of novel molecular mechanisms, accelerating drug discovery and decreasing the time to patient treatment. Intravital imaging with the VIVO SDC system is now being used to investigate and develop new therapies targeting hemophilia, leukemias and other cancers with circulating tumor cells, ischemic events, inflammation and other cardiovascular diseases.



THROMBOINFLAMMATION

The inflamed cremasteric venules of a WT mouse showing crawling neutrophils (white) capturing and releasing platelets (red) expressing CD62P (green). Courtesy of Dr. Vinatha Sreeramkumar & Dr. Andrés Hidalgo, CNIC.

Powered by SlideBook



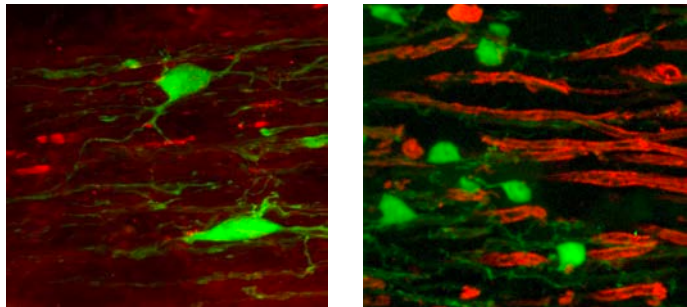
Rapid 4D imaging Accommodates high-speed confocal imaging by synchronizing the camera with a piezo Z stage.

Fast ablation targeting Click-to-fire ablation anywhere in the field of view for laser pulsing between tissue movements.

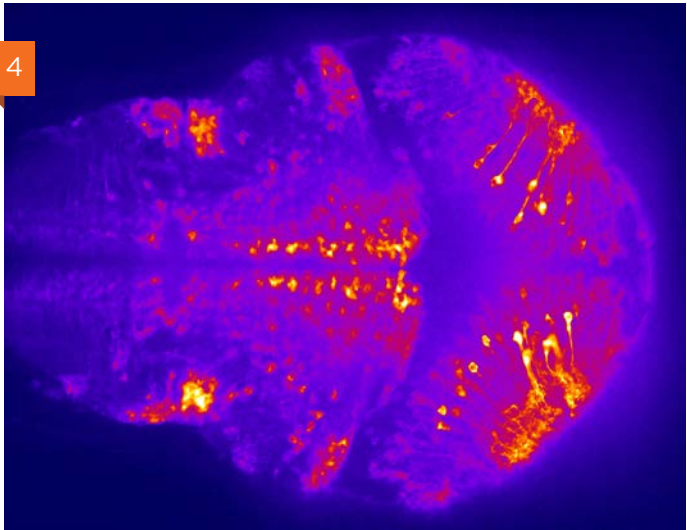
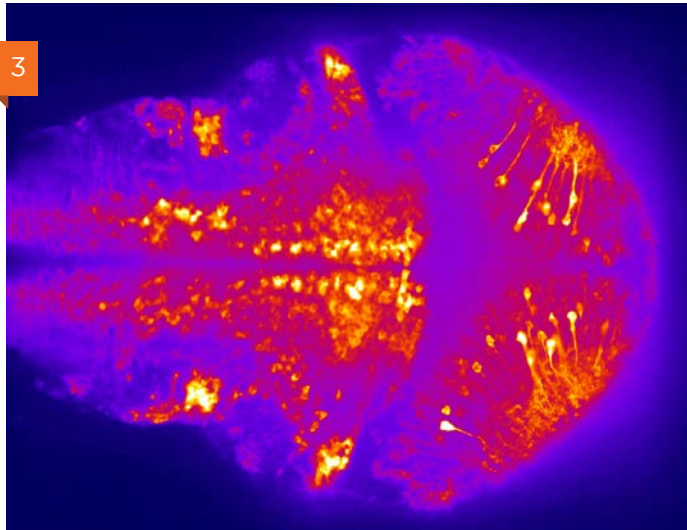
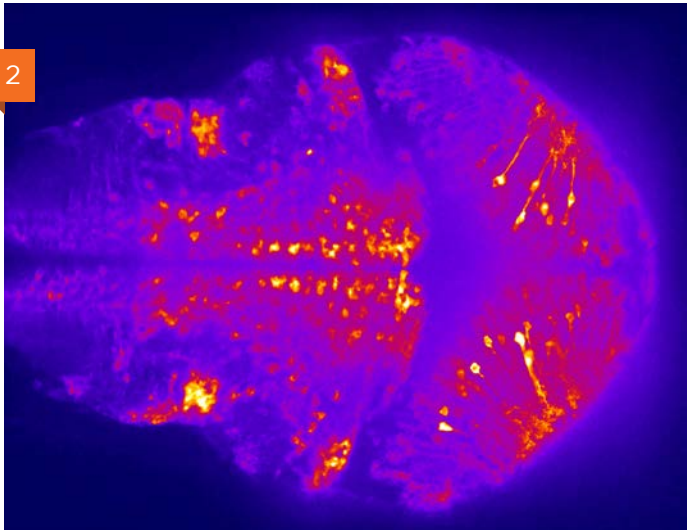
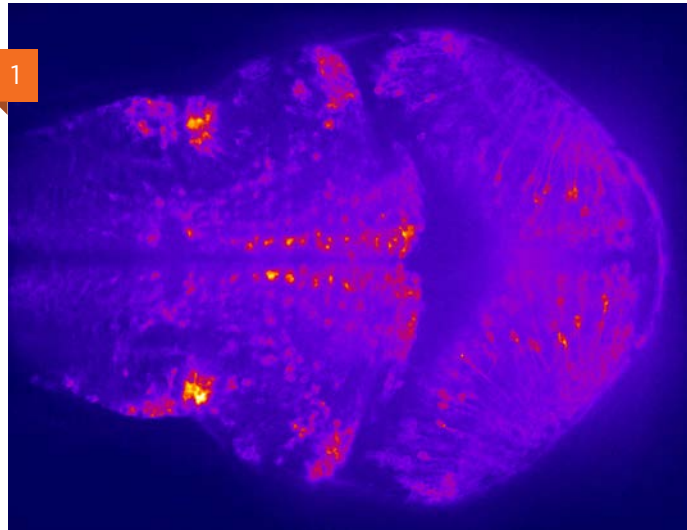
Particle tracking Acquire and automatically segment, track and quantify adherent and rolling leukocytes.

Flexible and Robust Neuroscience System

Imaging the brain and nervous system of live organisms requires a gentle, fast and high-resolution system. VIVO SDC systems are used to image structure and calcium flux in live brain tissue slices as well as zebrafish and *Drosophila* brains. A large platform stage offers room for micromanipulators for neuronal patch clamp electrophysiology, paired with fluorescence imaging modalities through software triggering and synchronization capabilities. The addition of Phasor holographic photomanipulation enables rapid optogenetic stimulation while imaging.



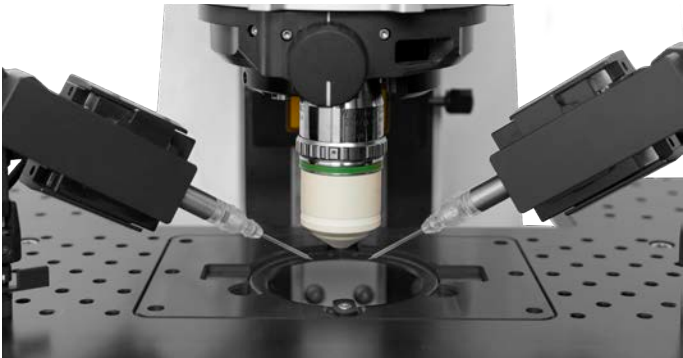
NEURONS IN SPINAL CORD TISSUE
GFP expressing neurons in spinal cord tissue. Sample courtesy Dr. Wolfram Tetzlaff, University of British Columbia.



PTZ-INDUCED SEIZURES IN THE BRAIN OF ZEBRAFISH LARVA
Calcium signals from the brain of Zebrafish larva in resting conditions (1) and after PTZ-induced seizures (2, 3, 4). The transgenic line is Tg(NeuroD:GCaMP6f). Image courtesy of Laura Desban, Andrew Prendergast and Claire Wyart.

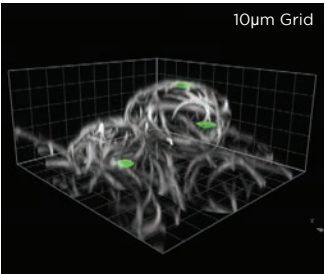
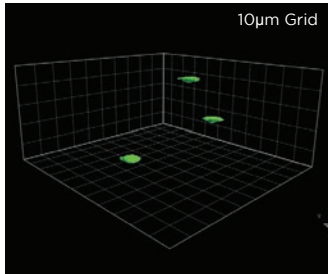
Patch Clamp Electrophysiology

Patch clamp electrophysiology may be preferred for direct measurement of current and voltage changes taking place across the membrane in neural tissue. Several combinations of stages and micromanipulators can be paired with VIVO SDC systems including existing electrophysiology equipment.



Phasor Holographic Photomanipulation

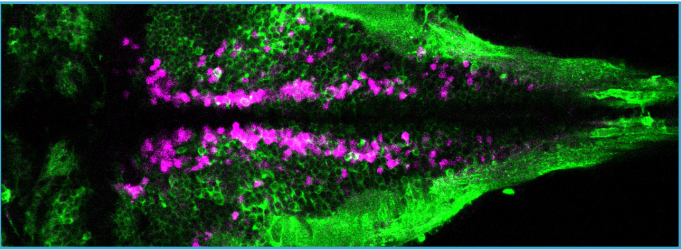
- Spatial light modulator-generated holography for optogenetics stimulation/FRAP/voltage imaging
- Simultaneous 3D stimulation of multiple, separate regions
- Visible and multiphoton stimulation without scanning



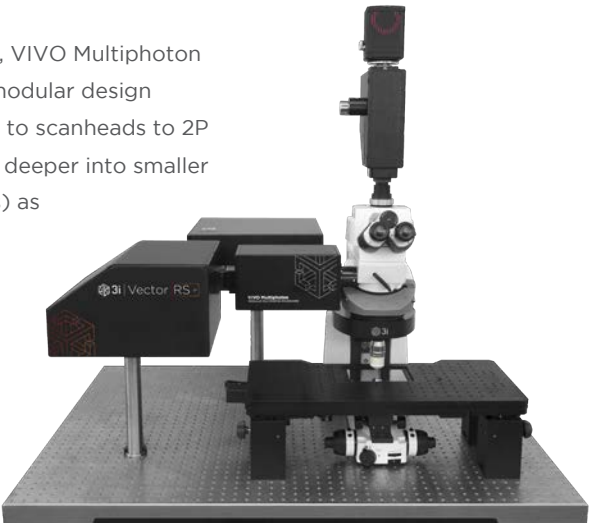
3D PHOTOSTIMULATION
3D illumination pattern (left) applied to a 3D specimen (right) to stimulate multiple regions simultaneously.

Increasing Imaging Depth with VIVO Multiphoton

An intravital imaging system for brain slice and *in vivo* multiphoton imaging, VIVO Multiphoton Upright is available as an upgrade to the VIVO SDC system. A flexible and modular design allows for the integration of best-in-class components from platform stages to scanheads to 2P holographic photomanipulation. VIVO Multiphoton is optimized for imaging deeper into smaller model systems (*Drosophila*, *C. elegans*, zebrafish, brain slices and organoids) as well as rodent animal models.



ZEBRAFISH
A maximum intensity projection of ciliated cerebrospinal fluid-contacting neurons (magenta) contacting the central canal in the spinal cord of a larval zebrafish. Cilia are labeled in green. Courtesy of Jenna Sternberg, Wyart lab, Institut du Cerveau et de la Moelle Épinrière.



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

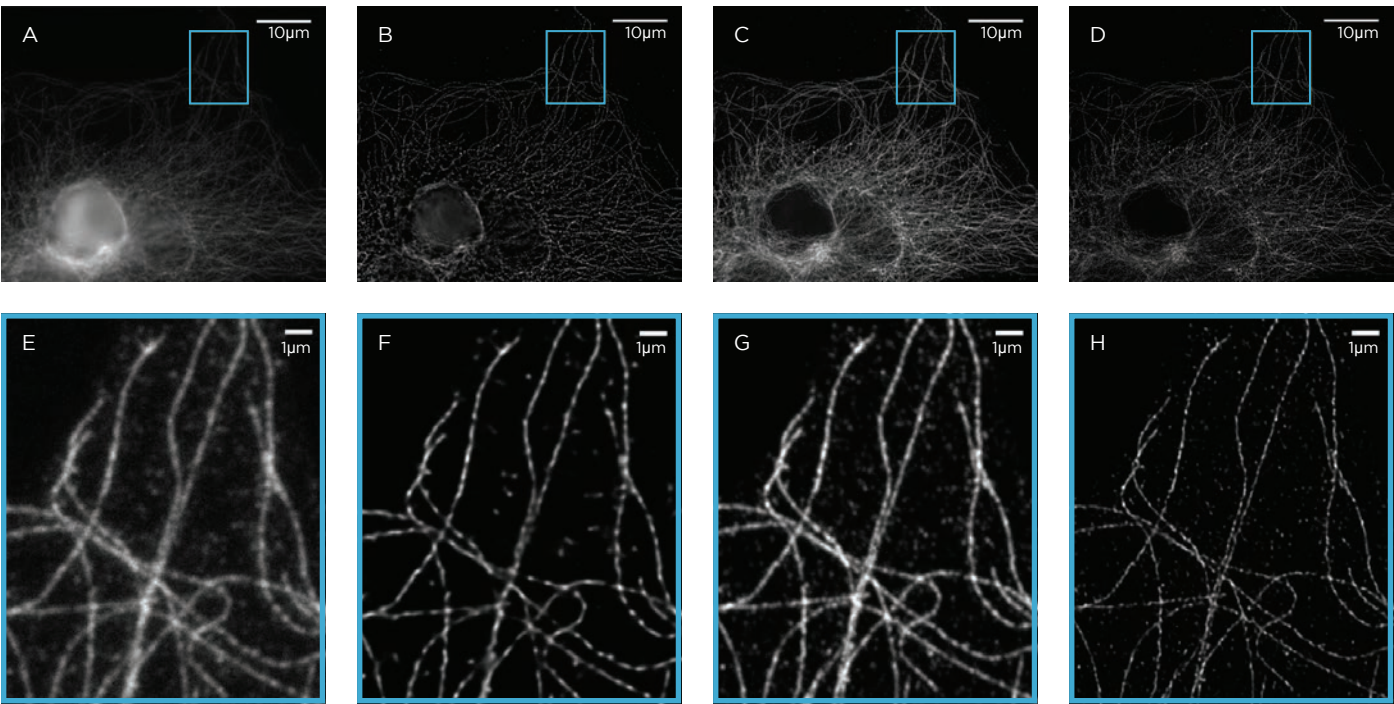
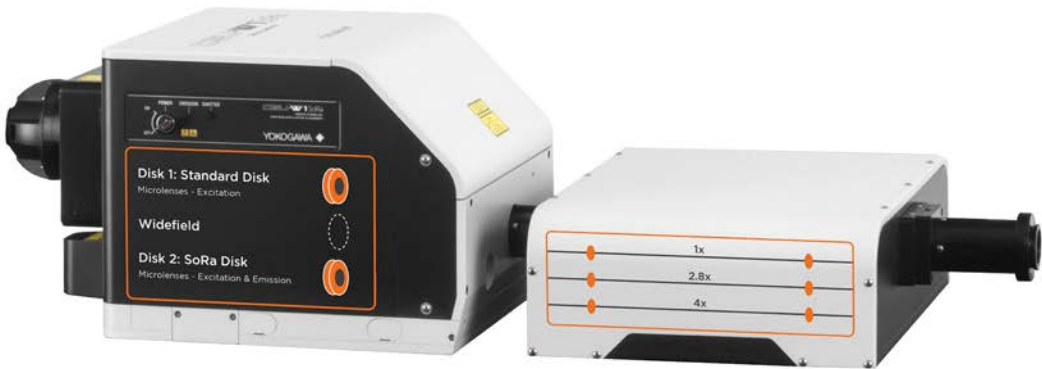


CSU-W1

- 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

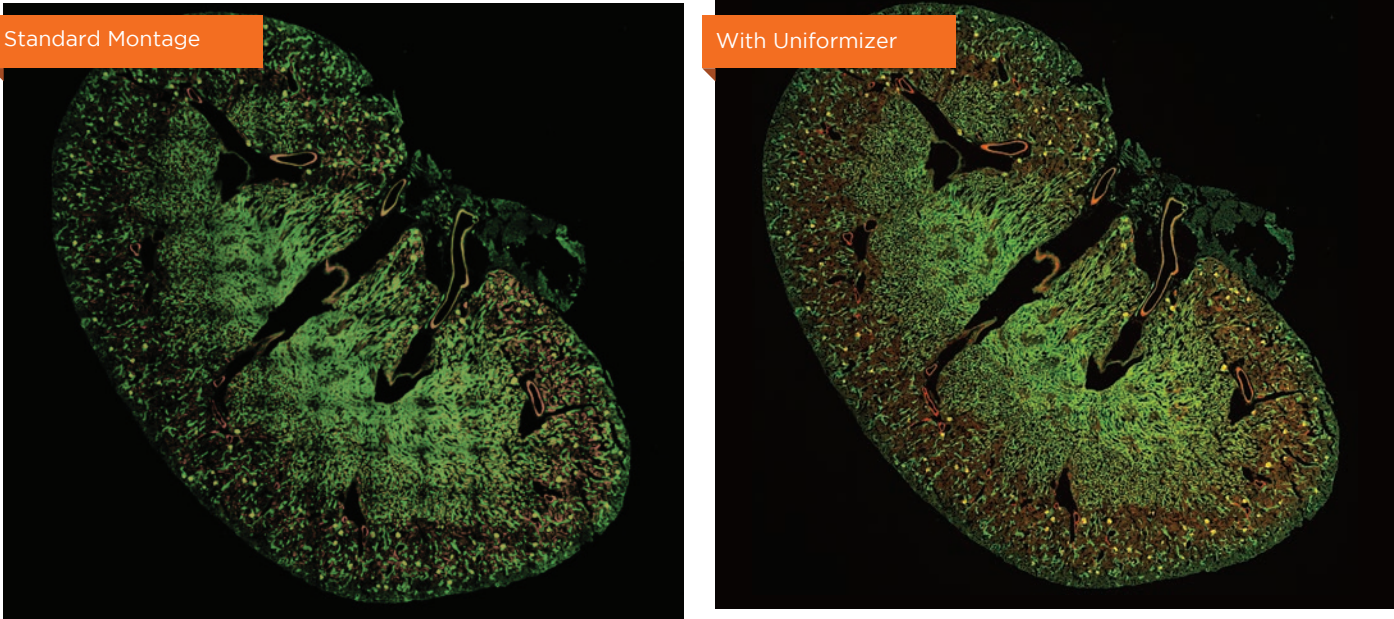
CSU-W1 SoRa is an easy-to-use super-resolution microscopy solution utilizing a dual Nipkow disk assembly with microlenses on both the illuminating and pinhole disks. SoRa images have a 1.4x resolution improvement and deconvolved SoRa images have a 2x resolution improvement compared to standard spinning 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 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

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

VAST Integration

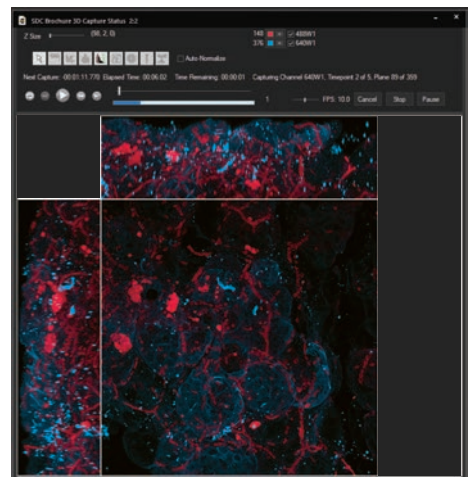
SlideBook communicates with the VAST BioImager platform for automated micron-level positioning of large specimens

Montage

Acquire and stitch 3D volumes across multiple fields of view

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.



Aivia

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



Microvolution

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

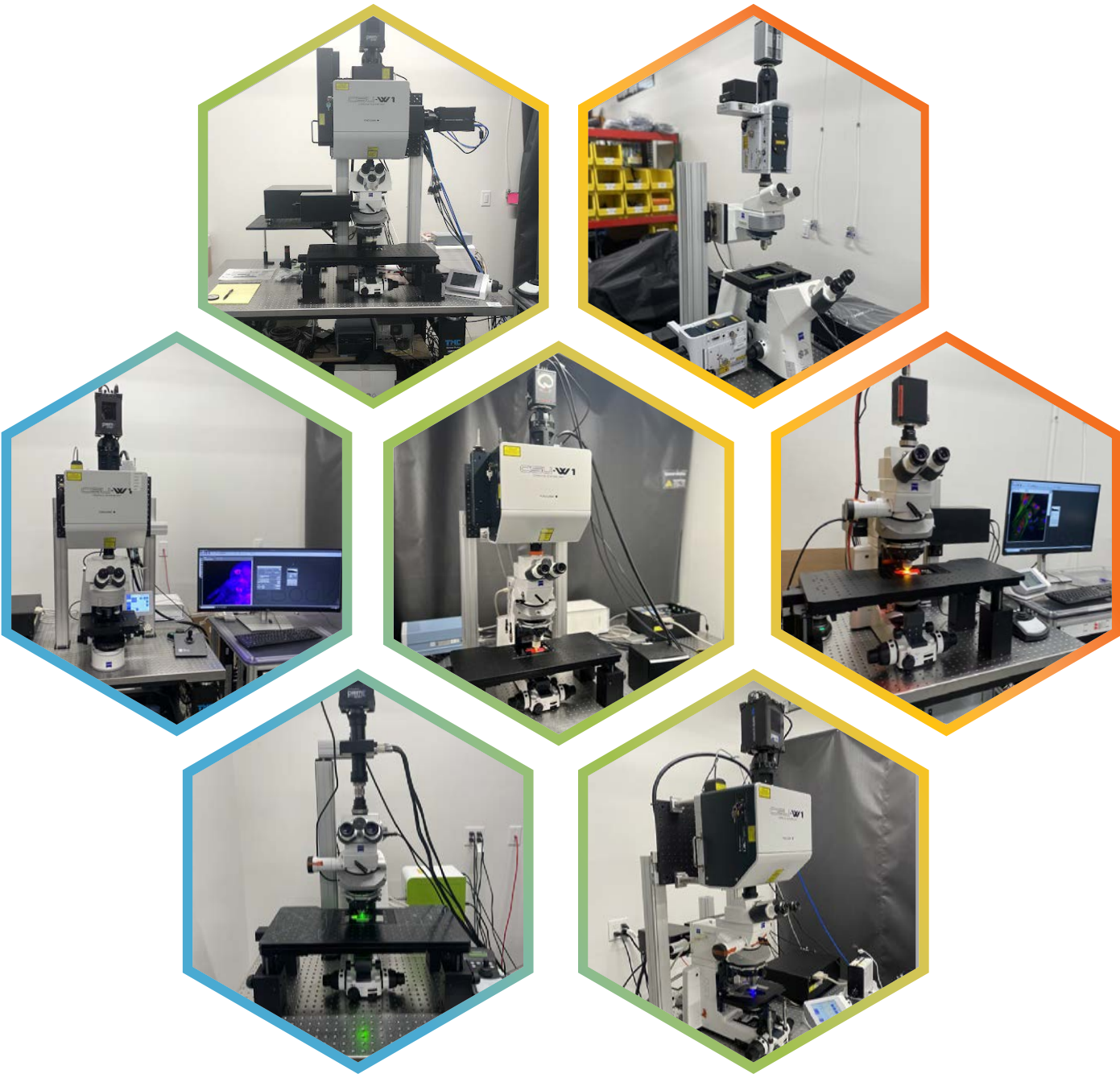


Dell

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.



System Test Report

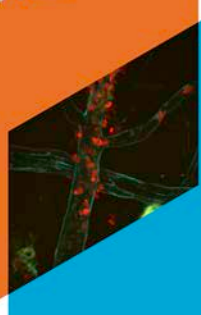
Drs. Bruce and Barbara Furie
Harvard Medical School
Division of Hemostasis and Thrombosis
Beth Israel Deaconess Medical Center

| 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 (83x) | |


| 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 | |

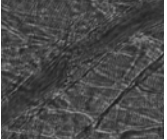
| Zeiss Z-Drive | Initials |
|-------------------------------|----------|
| Test z-position repeatability | |



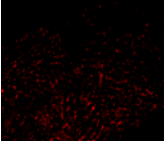
System Test Report



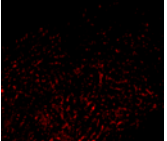
Mouse cremaster tissue



Platelet plug formation



Adherent platelets in lung microvasculature



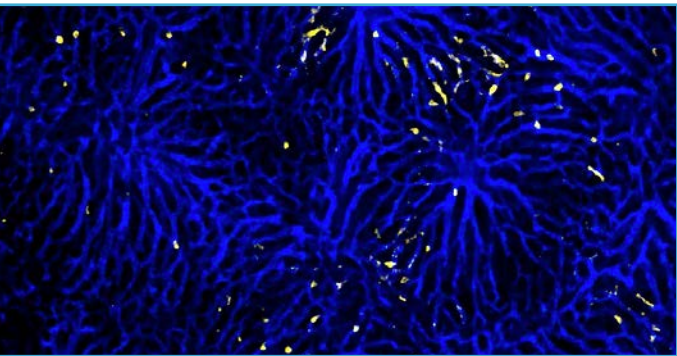
Zebrafish brain expressing GCaMP

Page 6

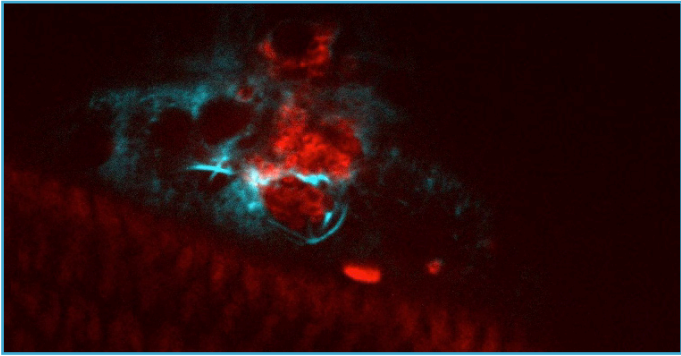
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12 Intelligent Imaging Innovations

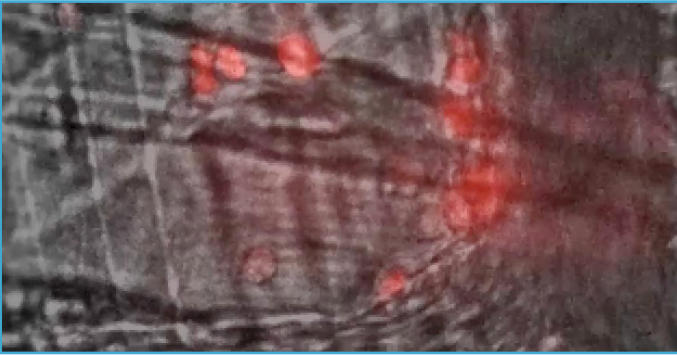
Intelligent Imaging Innovations 13



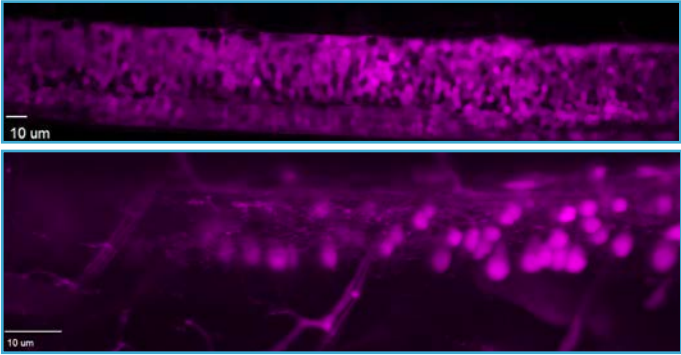
PULMONARY INFLAMMATION
Large vascular beds, as seen in the murine lung, allow for macroscale imaging of leukocyte adhesion, accumulation and transmigration in real time. Mouse lung vasculature (CD31, blue) and leukocytes (yellow). Image courtesy of Dr. Dean Kavanagh, University of Birmingham.



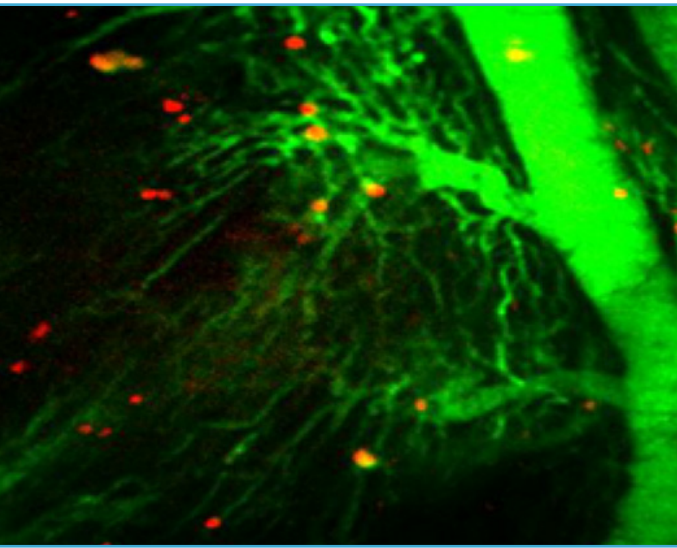
THROMBOSIS
High-speed 3D imaging is required for visualizing thrombosis formation *in vivo*. Platelets (red) and fibrin (cyan) imaged after laser-induced thrombus formation. Image courtesy Dr. Vivien Chen, University of Sydney.



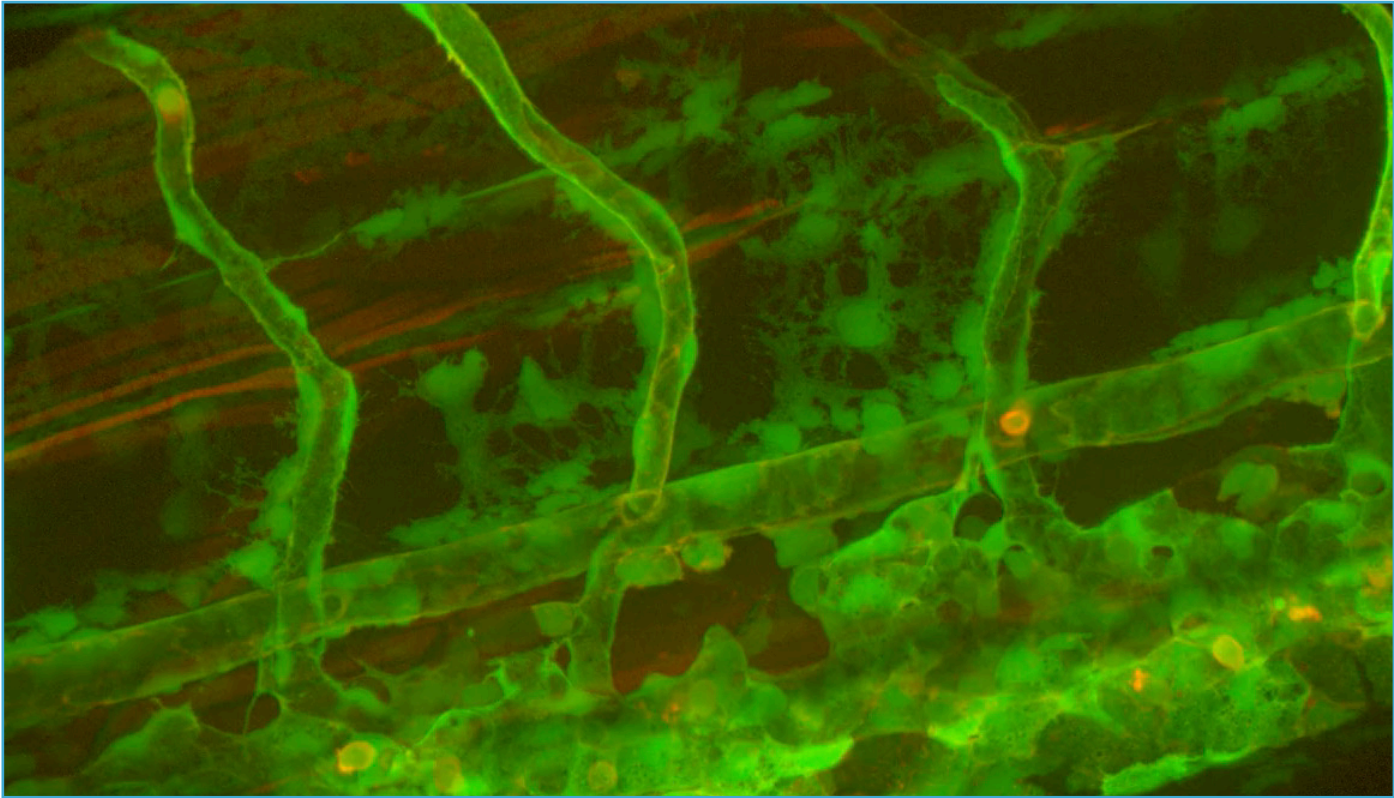
TRANSMIGRATION AND EXTRAVASATION
The inflammatory response begins with neutrophil recruitment to the site of infection and/or injury. Leukocytes (red) after leaving cremaster microvasculature following perfusion of chemoattractant.



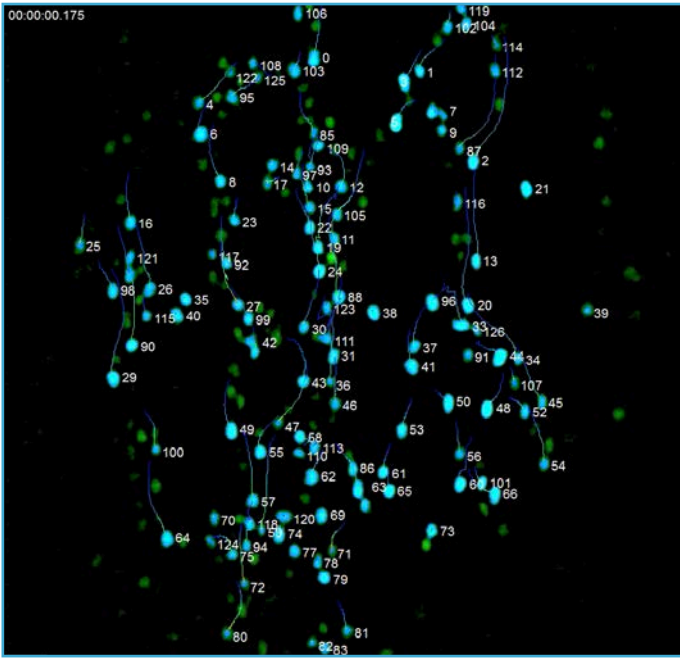
NEURONAL NETWORKS
Neurons in the spinal cord of an adult zebrafish expressing GFP.



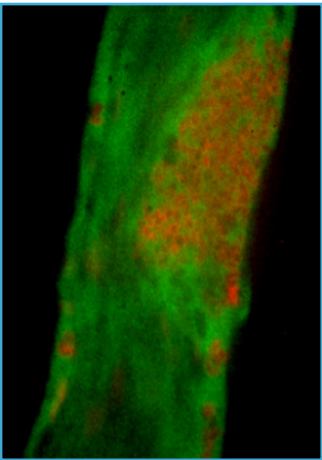
CARDIAC CIRCULATION
Murine heart vasculature visualized with FITC-BSA (green) and leukocytes with anti-Gr-1 antibodies (red). Image courtesy Dr. Dean Kavanagh, University of Birmingham.



ZEBRAFISH VASCULAR NETWORK
Small model organisms are a great substitute for mouse imaging while still offering insight into the inflammatory response. Zebrafish vascular system (green) and leukocytes (red).















CELL SEGMENTATION AND TRACKING
Neutrophil rolling and crawling inside of mouse vasculature can be easily labeled and quantified using the robust particle tracking tools in SlideBook.



THROMBOSIS
Mouse cremaster arteriole after laser-induced thrombus formation. Vasculature (green) and platelets (red).

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.



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