3i Intelligent Imaging Innovations

VIVO Multiphoton

Upright, Open & Inverted Imaging Systems with Advanced Photostimulation

VIVO Multiphoton | Upright

Built on a research-grade microscope platform, VIVO Multiphoton Upright is an intravital imaging system for brain slice and *in vivo* multiphoton imaging. A flexible and modular design allows for the integration of best-in-class components from platform stages to scanheads to holographic photostimulation.

TTL Sync

Millisecond timing and trigger control of multiple devices

Kaktus2 Multi-PMT Array

Detector array that can accommodate up to 4 PMTs in a combination of types

SidePort

Allows for the combination of up to 3 optical paths

Phasor

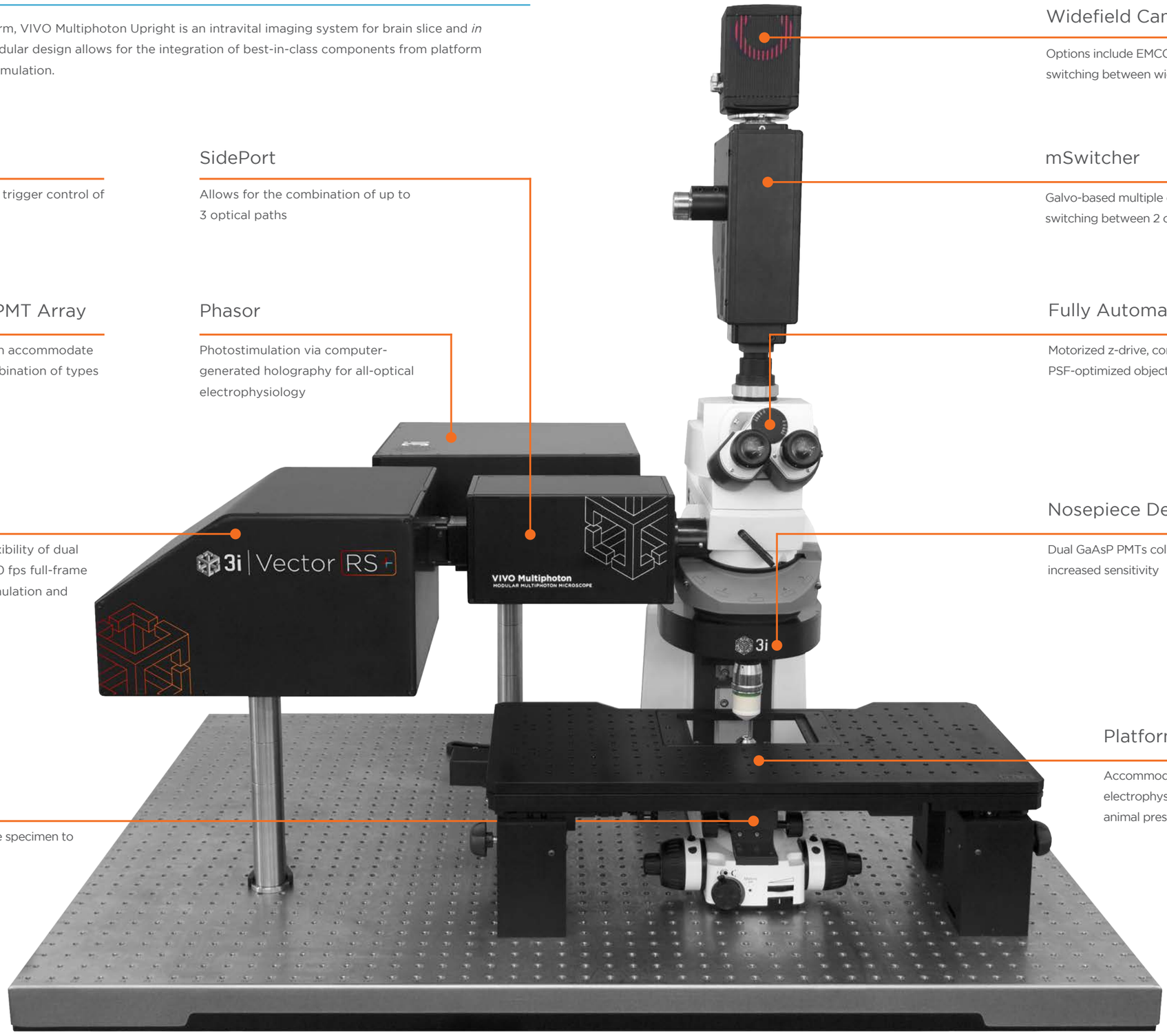
Photostimulation via computer-generated holography for all-optical electrophysiology

Vector RS+

The speed of resonant scanning with the flexibility of dual galvos allows for rapid switching between 30 fps full-frame resonant scanning and spiral/spot photostimulation and ablation using dual galvos

Substage Detector

Up to 2 GaAsP PMTs and a 1.2NA lens below the specimen to increase collection of emission signal



Widefield Camera

Options include EMCCDs and high-resolution CMOS cameras for fast switching between widefield and multiphoton imaging

mSwitcher

Galvo-based multiple detector mounting allowing for 1ms switching between 2 output paths

Fully Automated Research Microscope

Motorized z-drive, condenser, optical path selection and PSF-optimized objectives

Nosepiece Detector

Dual GaAsP PMTs collect reflected light close to the sample for increased sensitivity

Platform Stage

Accommodates multiple manipulators for electrophysiology or oversized trays for tissue or whole animal presentation

VIVO Multiphoton | Open

VIVO Multiphoton Open is based on a movable objective microscope platform to provide ultimate flexibility. The system is optimized for mammalian intravital imaging while accommodating all-optical electrophysiology with Nouveau Phasor holographic photostimulation. VIVO Multiphoton Open can be custom-configured for nearly any experimental paradigm with full control of hardware configuration, acquisition, processing and analysis in SlideBook.

Widefield Camera

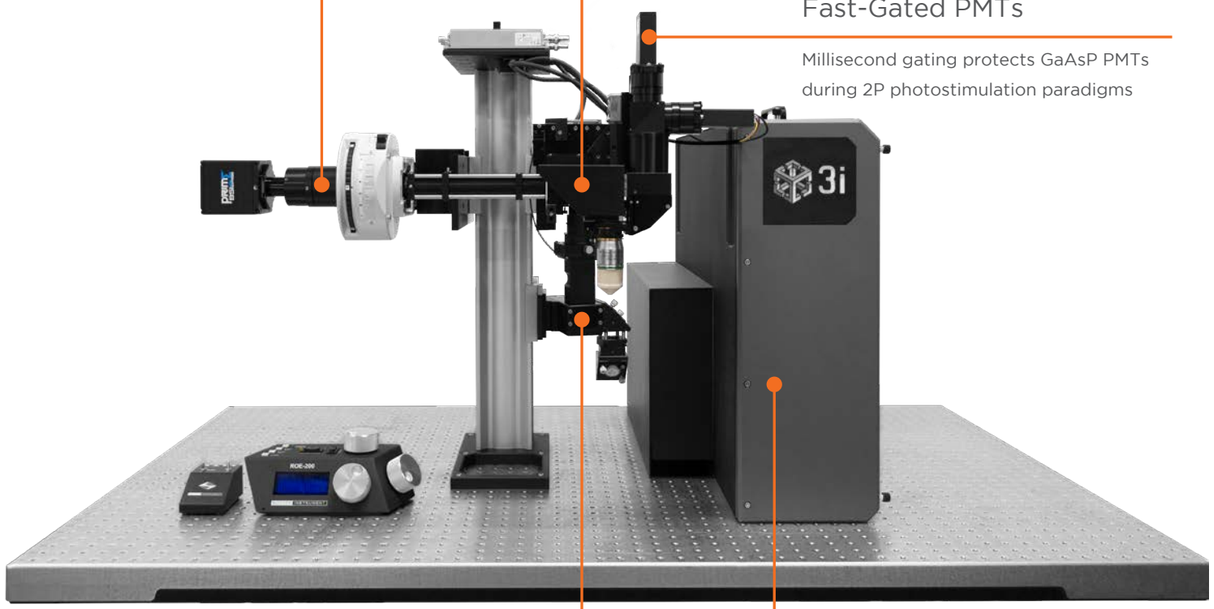
EMCCD and sCMOS cameras for locating labeled neurons or a specific brain area for investigation

Movable Objective Microscope (MOM)

22mm of objective travel in the x, y, and z axes and motorized path selection

Fast-Gated PMTs

Millisecond gating protects GaAsP PMTs during 2P photostimulation paradigms



RS+ Scanhead

3-galvo design allows for high-speed resonant scanning and flexible dual-galvo scanning in one system

Nouveau Phasor

2P photostimulation via computer-generated holography for all-optical electrophysiology

VIVO Multiphoton | Inverted

VIVO Multiphoton Inverted brings 2-photon imaging capabilities to the Marianas live-cell research platform. At the heart of the system is a research-grade inverted microscope customizable with an array of stages, cameras, sample autofocus, light sources, photomanipulation devices and environmental control.

Motorized XY Stage

Transmitted IR Detection

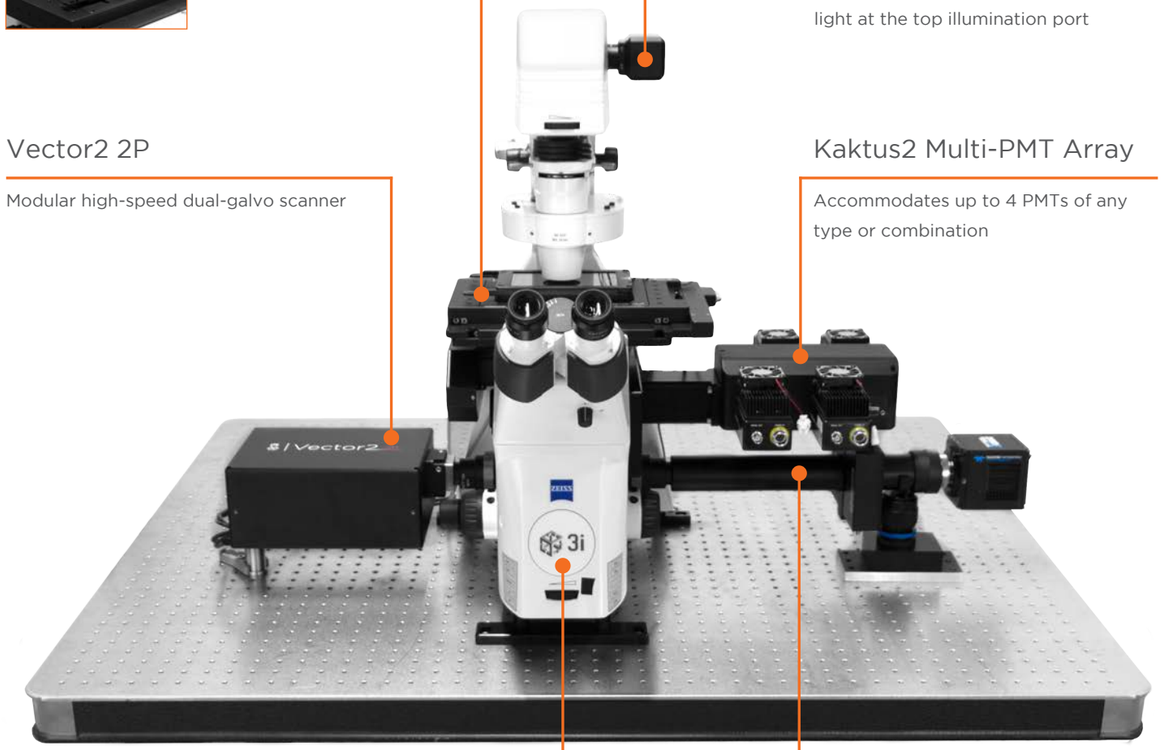
Bialkalai PMT to collect transmitted IR light at the top illumination port

Vector2 2P

Modular high-speed dual-galvo scanner

Kaktus2 Multi-PMT Array

Accommodates up to 4 PMTs of any type or combination



Fully Automated Research Microscope

Motorized objective and path selection with autofocus (Definite Focus 3) and PSF-optimized objectives

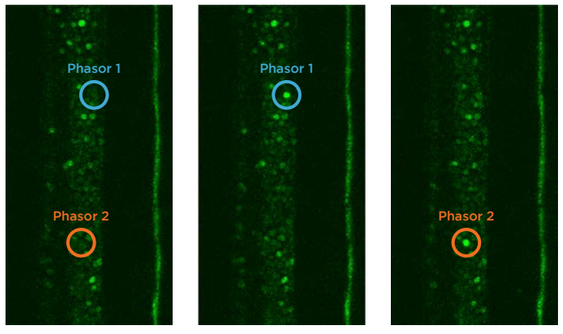
Widefield Camera

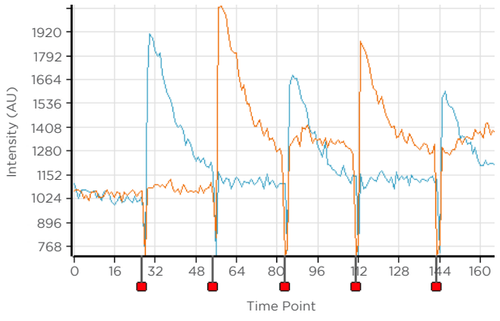
EMCCD and sCMOS cameras for epifluorescence imaging

Nouveau Phasor | All Optical Electrophysiology

Nouveau Phasor is designed for 3D 2-photon stimulation of neurons *in vivo*. Four distinct modalities enable flexible optogenetics studies involving organisms from *C. elegans* and *Drosophila* to zebrafish, mice and larger mammalian species. Nouveau Phasor is able to simultaneously illuminate multiple regions in a 3D volume with the use of SLM-based computer-generated holography (CGH).

Holographic patterns have an axial extent that increases linearly with their lateral extent. This makes it difficult to avoid stimulation above and below the location of interest. The addition of 2D or 3D Temporal Focusing (TF) allows stimulation to be confined along the plane of interest. In 2D-TF the confinement will be in the objective's plane of focus, while with 3D-TF the confinement can be in any plane in the 3D imaging volume. This allows for selective stimulation of only the cellular components of interest regardless of their spatial location. Nouveau Phasor offers exclusive, patented TF performance in multiple modalities.



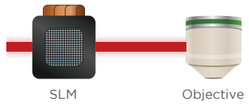


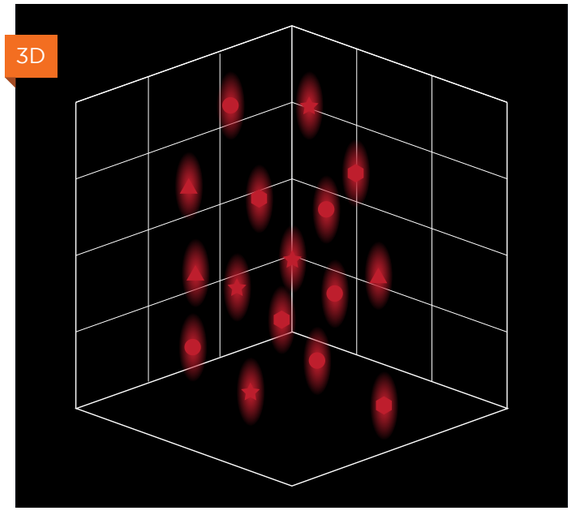
All-optical electrophysiology data derived from transgenic zebrafish larvae expressing GCaMP6f in spinal neurons co-expressing the opsin Chrimson; neurons were selectively activated using Phasor 2P photostimulation. 3-Panel montage shows Phasor-elicited calcium increases (left) and graphed results (right). Specimen provided by Dr. David Lyons, University of Edinburgh.

Model Organisms

3D Holographic Stimulation

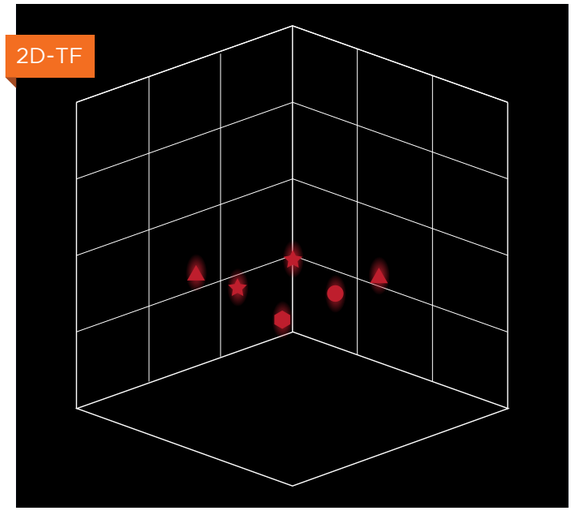
3D flexible region photostimulation via CGH





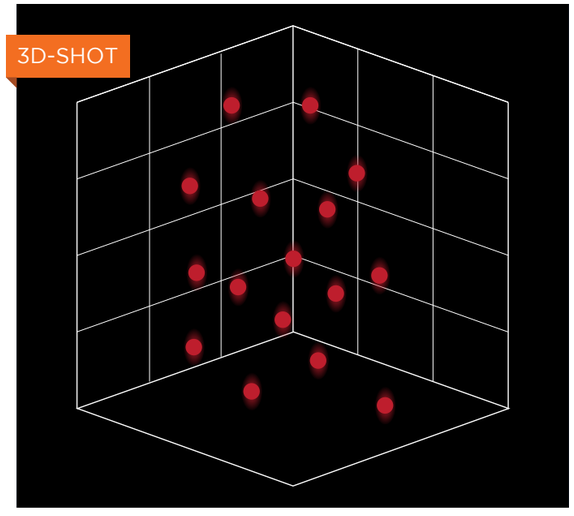
2D Temporal Focusing

3D flexible region photostimulation via CGH with axial confinement in the plane of focus



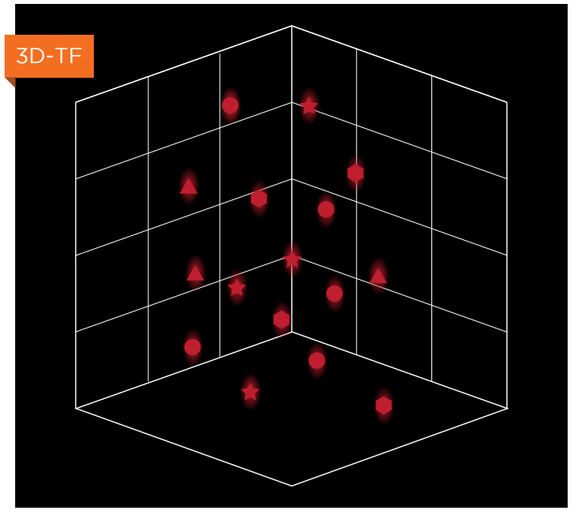
3D-SHOT

3D uniform region photostimulation with axial confinement in the imaging volume



3D Temporal Focusing

3D flexible region photostimulation via CGH with axial confinement at any axial position in the imaging volume



Kaktus2 Multi-PMT Array

The Kaktus2 Multi-PMT Array is for experiments where two PMTs are insufficient for signal collection across multiple channels. It is standard for VIVO Multiphoton Inverted systems. It can be mounted in conjunction with mSwitcher to alternate between PMT and high-speed camera detection.

Kaktus2 can accommodate any combination of up to 4 PMTs. Available PMTs include multialkali for high dynamic range, compact GaAsP for high sensitivity, peltier-cooled GaAsP for low light applications, and fast-gated GaAsP for use with photostimulation. Emission signal separation is accomplished by exchangeable drop-in cassettes containing customizable dichroic mirrors and emission filters.

Multialkali

High dynamic range

Peltier-Cooled GaAsP

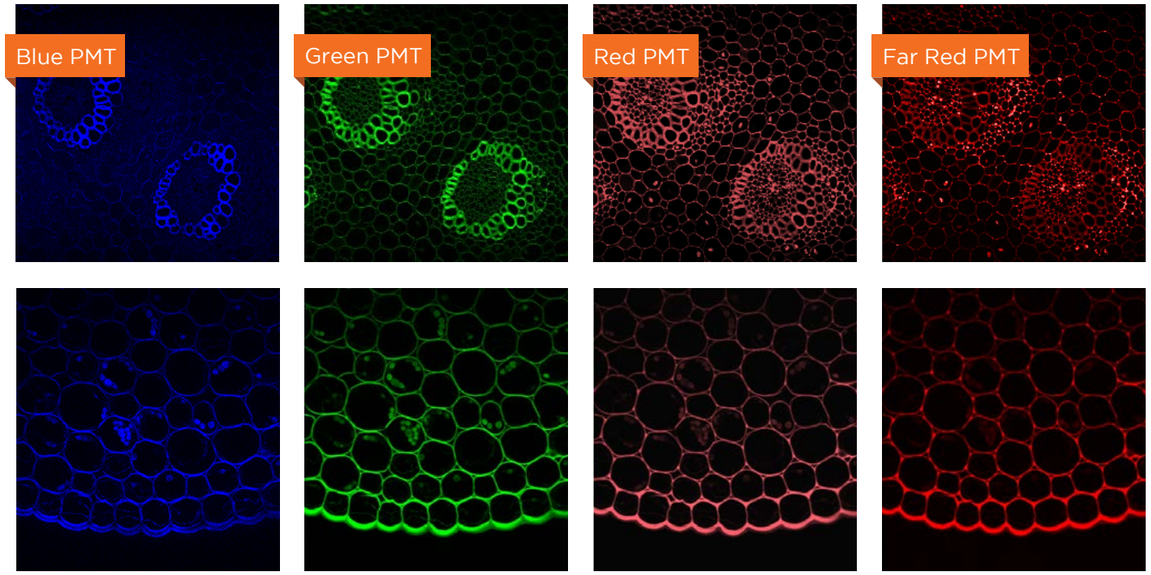
High sensitivity and low dark current with active cooling

Fast-Gated GaAsP

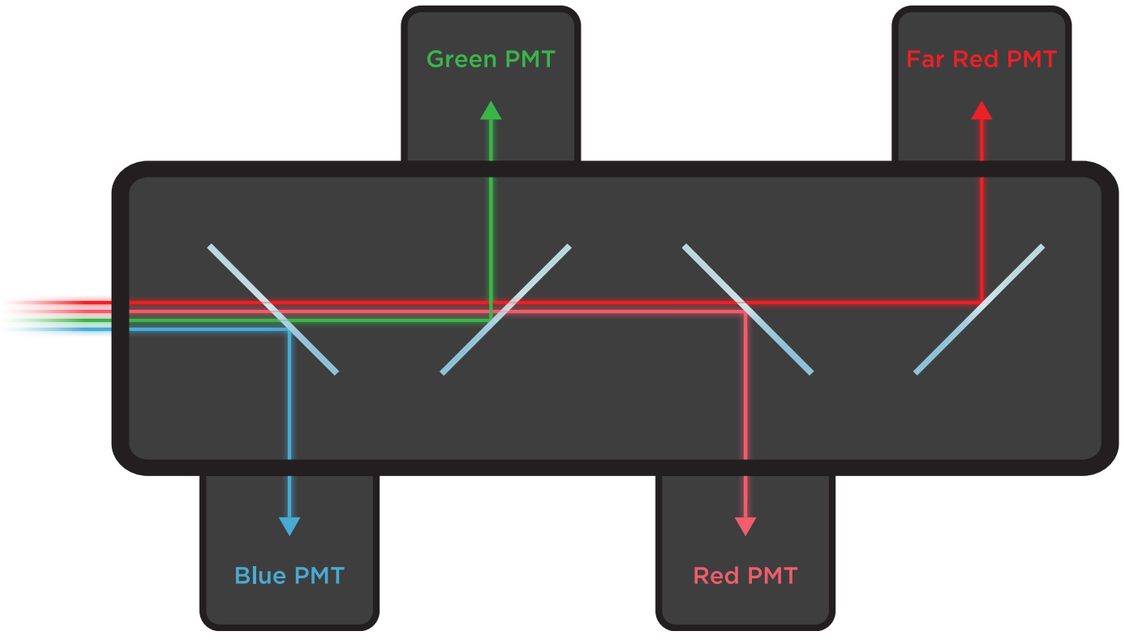
PMT of choice when paired with photostimulation at the millisecond timescale

Compact GaAsP

Highly sensitivity with a small footprint



Convallaria slide imaged with excitation at 920nm showing emission split between four channels.



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.

NVIDIA CUDA GPU Acceleration

GPU acceleration of computationally-intensive operations such as deconvolution

Multiwell and Montage

Streamlined multiwell interface
Montaging with a variety of methods

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

Multiphoton System Capture Console

Consoles are a single easy-to-use window featuring all frequent controls and status displays. The VIVO Multiphoton scanning console also features an intuitive tool for adjusting laser power delivery at different depths with dynamic signal feedback.

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.

Microvolution

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

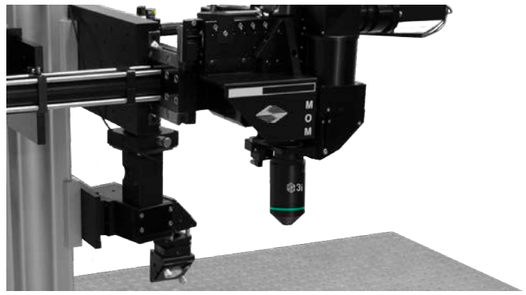
Aivia

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

Dell

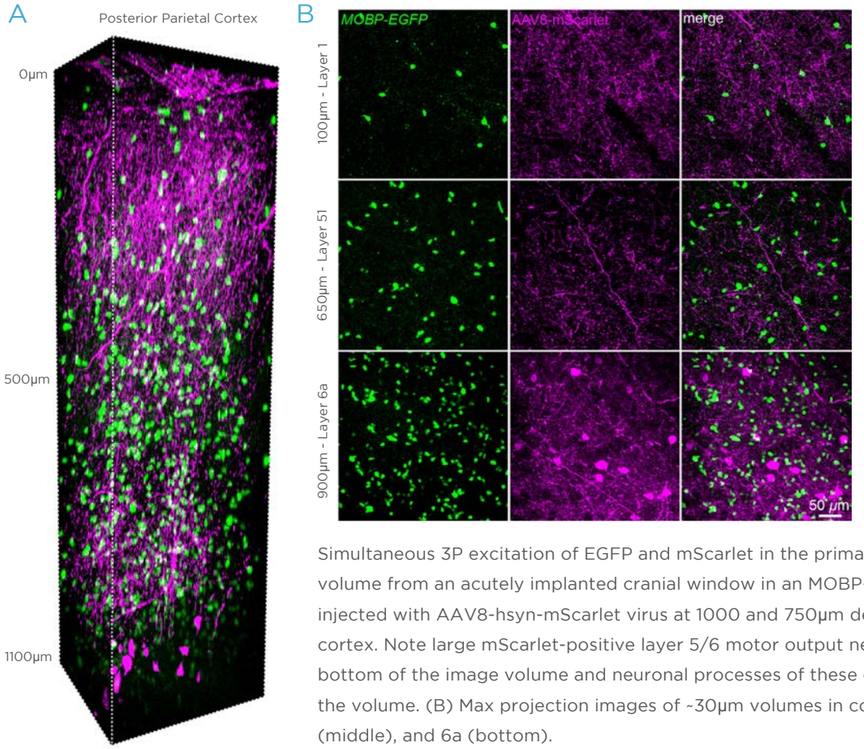
The latest high-power computer workstations control all microscope hardware and enable high-speed processing, segmentation and volume rendering of terabyte (TB) datasets.

3-Photon | Deep Imaging



Three-photon microscopy (3P) uses longer wavelength NIR light with lower scattering than two-photon microscopy (2P). This allows 3P microscopy to achieve deeper imaging with better signal to noise than traditional 2P microscopy. Whereas 2P has proven to be exceptionally useful to depths of 100µm to 500µm, 3P has increasingly been shown to produce useful data to 1mm and beyond. 3i offers 3P microscopy systems with objectives, adaptive optics, and software to achieve the transmission, pulse compression and point spread function (PSF) integrity needed for successful imaging.

Dual Color Imaging with 3P Excitation



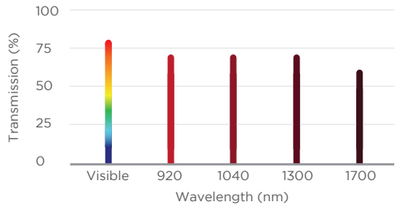
Simultaneous 3P excitation of EGFP and mScarlet in the primary motor cortex. (A) 3D image volume from an acutely implanted cranial window in an MOBP-EGFP mouse at P65 that was injected with AAV8-hsyn-mScarlet virus at 1000 and 750µm depths in the primary motor cortex. Note large mScarlet-positive layer 5/6 motor output neurons are labeled at the bottom of the image volume and neuronal processes of these cells are labeled throughout the volume. (B) Max projection images of ~30µm volumes in cortical layers 1 (top), 5 (middle), and 6a (bottom).

Michael A. Thornton,[1] Gregory L. Futia,1 Michael E. Stockton,[1] Barish N. Ozbay,[2] Karl Kilborn,[2] Diego Restrepo,[1] Emily A. Gibson,[1] Ethan G. Hughes[1] Characterization of red fluorescent reporters for dual-color in vivo three-photon microscopy. Neurophotonics, 9(3), 031912 (2022). https://doi.org/10.1117/1.NPh.9.3.031912

[1]Univ. of Colorado Anschutz Medical Campus (United States)
[2]Intelligent Imaging Innovations, Inc. (United States)

DeepScan 3P Objective

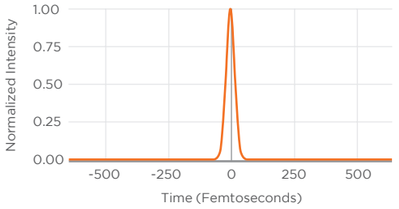
The DeepScan 3P objective is a high NA long working distance water dipping lens specifically designed for >70% transmission in the 1300nm window and >60% in the 1700nm window. Coupled with the remote focus capability of M-Shaper, it can rapidly capture 3D volumes to depths of well beyond 1mm.



2.0mm Working Distance
0.95NA, f=10mm
Water Dipping

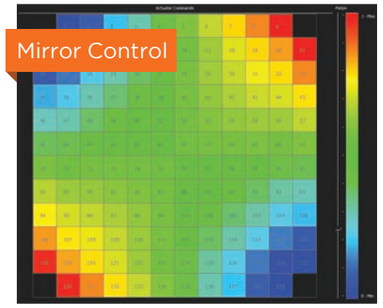
Pulse Compression

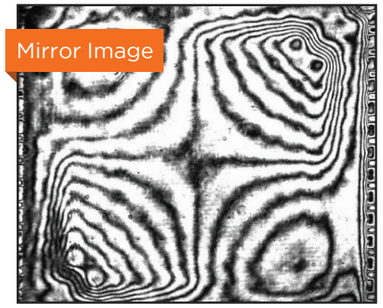
Pulse compression is critical to successful three-photon imaging, even more so than in two-photon imaging. 3i engineers design customized beam paths with highly-dispersive mirrors and NIR-optimized optics to deliver sub-50fs pulses to the specimen.



M-Shaper Adaptive Optics

When imaging deep in tissue, PSF integrity is compromised by changing optical conditions through the specimen. M-Shaper uses adaptive optics to dynamically adjust to changing specimen conditions in order to maintain a near-optimal PSF. Additionally M-Shaper allows for remote focus, enabling high-speed capture of 3D data deep in the specimen without moving the objective.





Powered by SlideBook

SlideBook

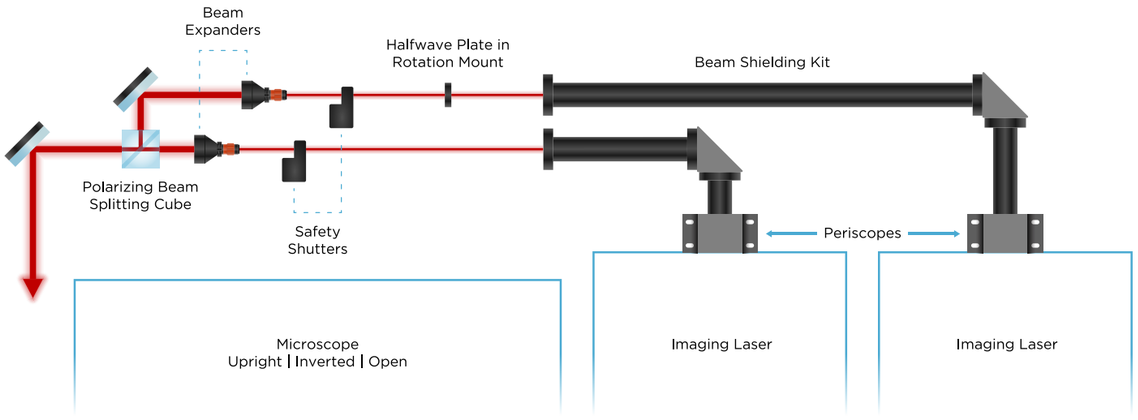
Synchronization (TTL) module provides sophisticated electronic control of carefully timed devices such as lasers, detectors, shutters, fine motion devices, electrodes, patch clamp recording devices, perfusion systems and more.

Pulse-synchronized boxcar acquisition and digitization provides signal selection and background rejection based on anticipated fluorescence lifetimes.

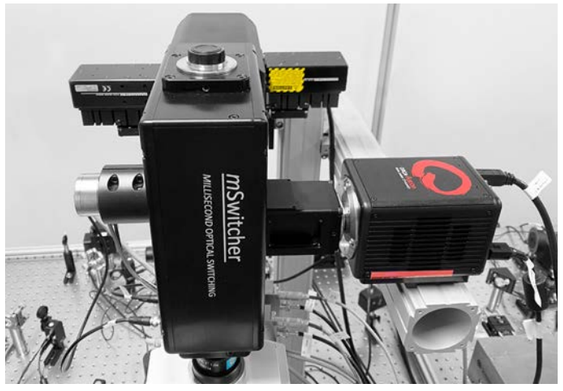
ECG-based cardiac gating utilizes an electrocardiograph to reduce specimen-induced motion artifacts by selectively capturing data only during periods with the least anticipated movement.

Beam Path

3i configures a customized beam path for each multiphoton system. This ensures the multiphoton excitation beam from the laser head is delivered to the sample with maximum efficiency and minimal power loss. Periscope-based height adjustment accommodates the various laser output heights of imaging and photostimulation lasers. Beam paths can be designed to incorporate a single imaging laser, two imaging lasers (combined with either a polarizing beam splitting cube or dichroic), one imaging laser and one photostimulation laser, or other configurations required to achieve specific scientific objectives.



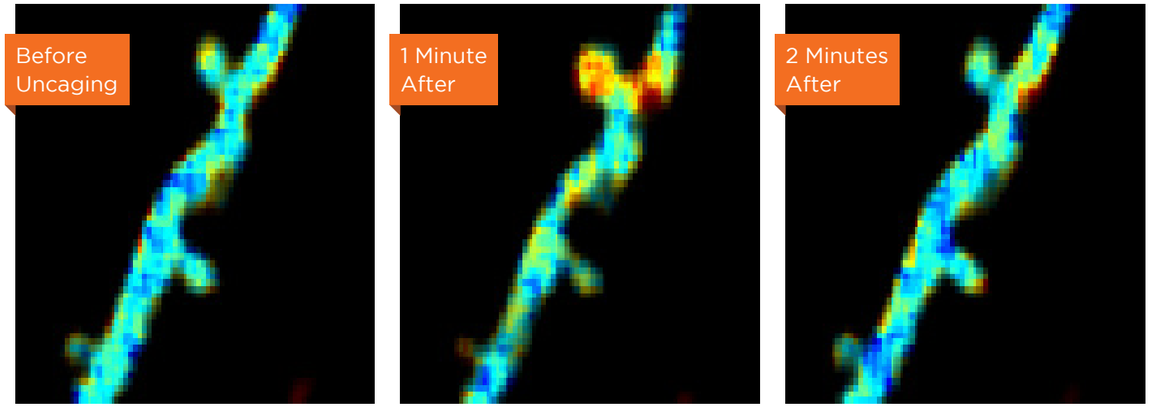
mSwitcher



mSwitcher allows for millisecond switching between a single input and multiple outputs using a high-speed galvo port switcher. This enables the fluorescence emission signal to be projected onto multiple detectors, including cameras, single PMTs, or the Kaktus2 Multi-PMT Array.

Multi-Channel TCSPC FLIM

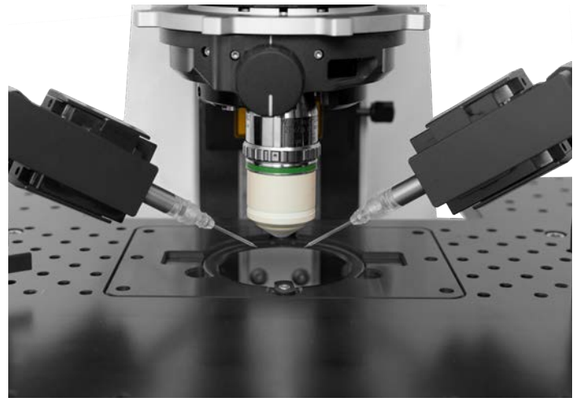
VIVO Multiphoton systems are able to perform 2-photon time-correlated single photon counting fluorescence lifetime experiments. SlideBook software controls Becker & Hickl TCSPC boards, GaAsP PMTs or hybrid detectors, and an electronic signal switch to direct PMT signals to either standard A/D conversion for imaging or single photon counting for 2pFLIM. The Becker & Hickl hardware, combined with SlideBook software, provides an effective yet easy to manage tool for conducting FLIM experiments fully integrated with the rest of the system.



PM-ER interaction in dendritic spines imaged with 2pFLIM, MBL Neurobiology, 2015.

Patch Clamp Electrophysiology

Traditional 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 Multiphoton systems including existing electrophysiology hardware.



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

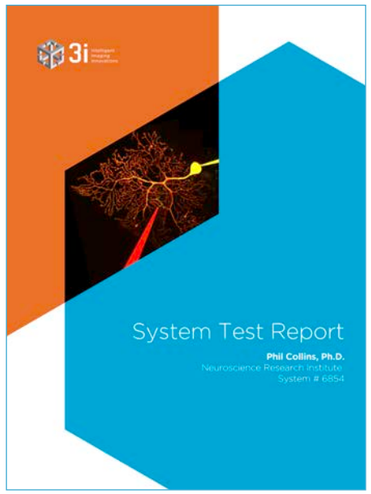
When a technically advanced experiment requires specific system performance to succeed in the lab, a custom test plan assures the system meets that mark prior to delivery.

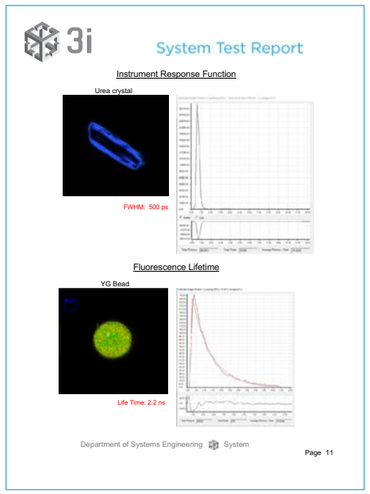
System Integration | Synchronization of Dozens of Instruments

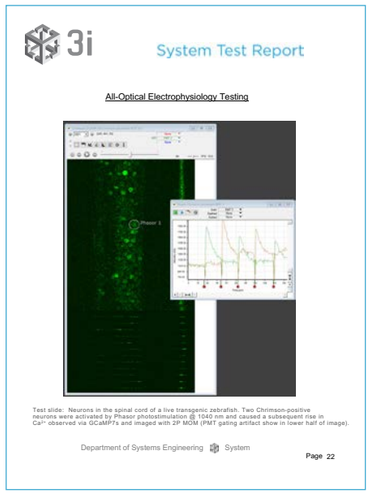
Systems Engineering combines institutional knowledge and scientific consultation to ensure that the instruments in each system are configured for experimental success in the lab.

System Test Report | Guaranteed Performance

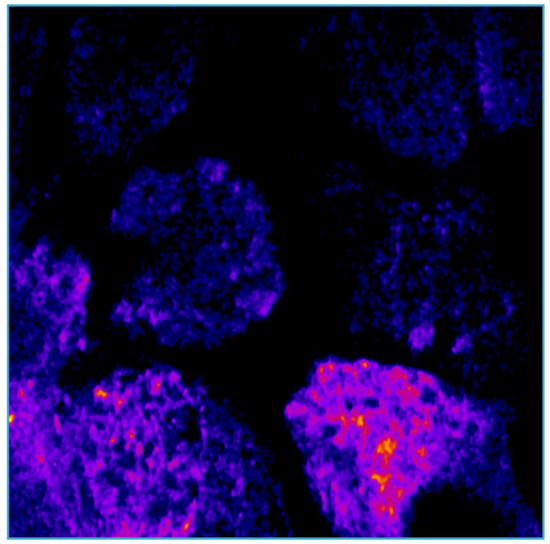
Performance metrics and results of the custom test plan are documented in a System Test Report delivered with each system.



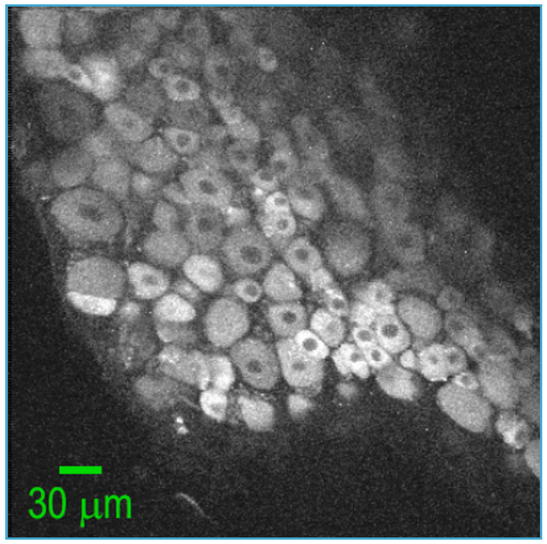




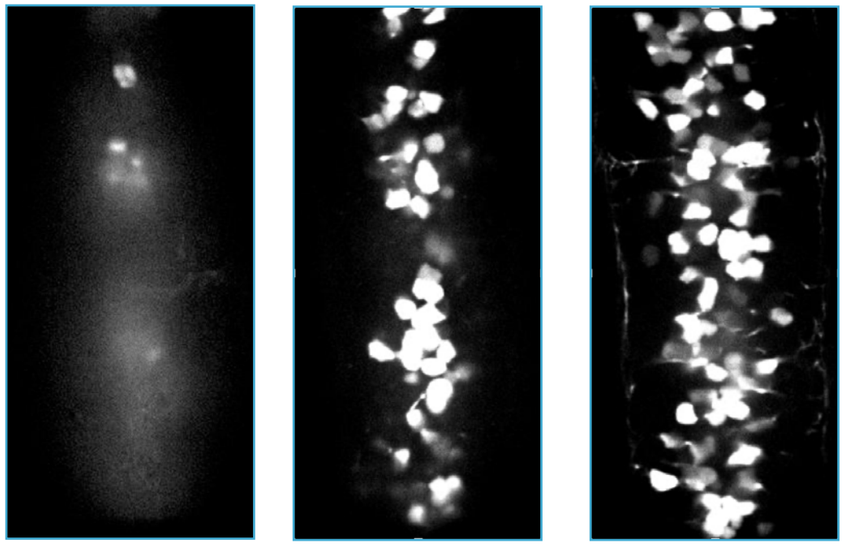
Application Data



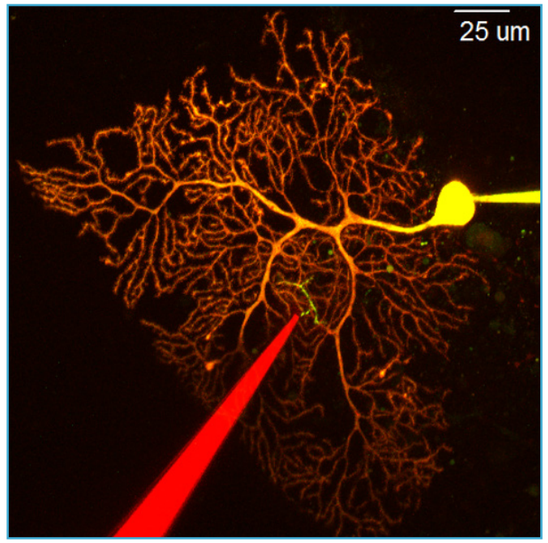
MOUSE
Calcium imaging of odor responses in mice expressing GCaMP6s in mature olfactory sensory neurons. Courtesy of Claire Cheetham, Carnegie Mellon University.



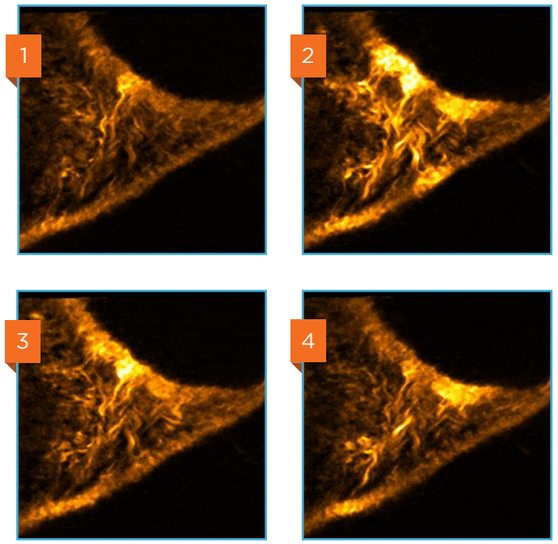
MOUSE
Two photon imaging of GCaMP6f responses in a subset of neurons from an intact mouse dorsal root ganglion. Courtesy of Petri Takkala, Prescott lab, University of Toronto.



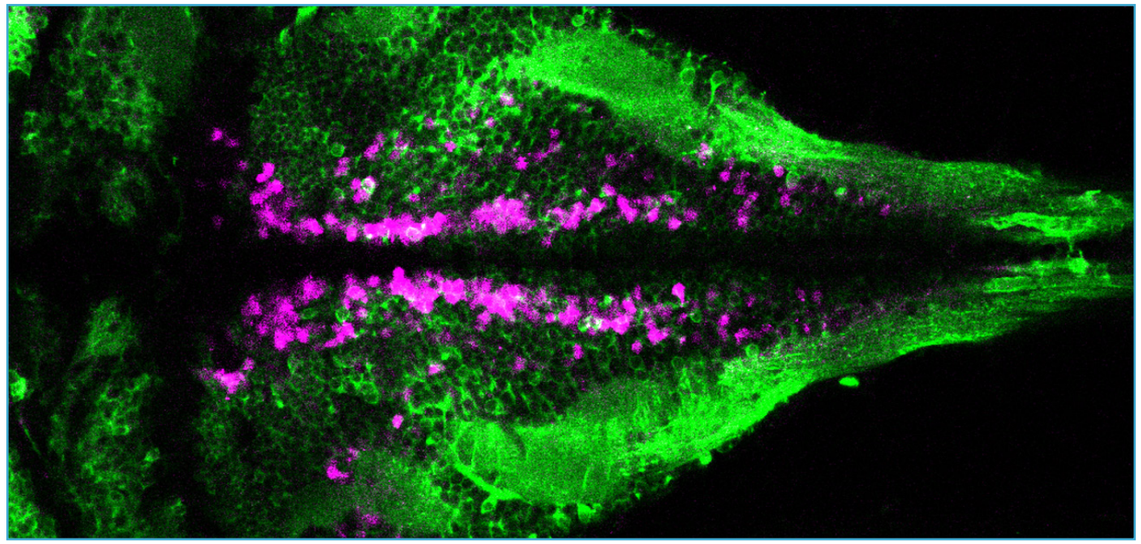
ZEBRAFISH
Z stack of GFP-expressing GABAergic neurons in the zebrafish spinal cord. Courtesy of Jenna Sternberg, Wyart lab, Institut du Cerveau et de la Moelle Épinière, Paris.



MOUSE
Two photon calcium imaging of a Purkinje neuron filled with Oregon Green BAPTA-1. Courtesy of Meera Pratap, Otis lab, University of California Los Angeles.



DROSOPHILA
T4T5 neurons in the *Drosophila* visual system responding to various directions of visual motion. Courtesy of Dr. Ben Hardcastle, Frye Lab, University of California Los Angeles.



ZEBRAFISH
A z-stack 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 Épinière, Paris.

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.

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