3i Intelligent Imaging Innovations

Nouveau Phasor

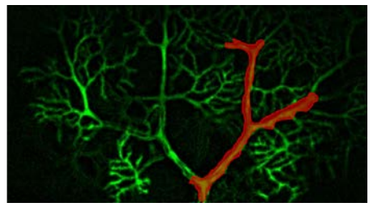
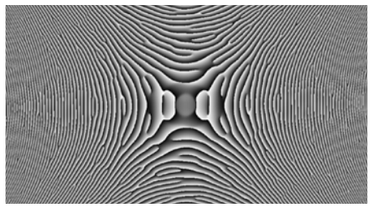
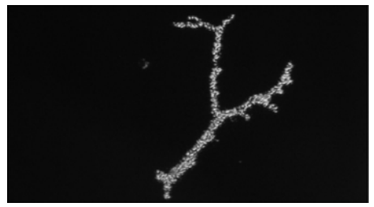
All Optical Electrophysiology

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.

Nouveau Phasor | All Optical Electrophysiology

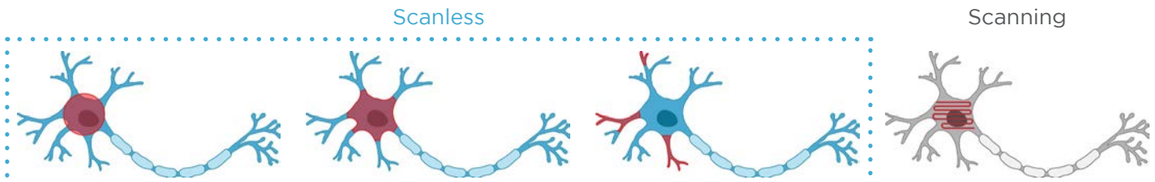
Computer-Generated Holography

Nouveau Phasor is based on a computer-generated holography (CGH) technique that uses a spatial light modulator (SLM) to modify the phase of an incoming laser beam. It is a highly efficient, phase-only modulation technique that uses all of the power of the incoming laser to generate a holographic pattern at the objective's focus. Using an iterative Fourier transform, it is possible to apply phase patterns onto the SLM to generate any hand-drawn or computationally outlined pattern in 3D.

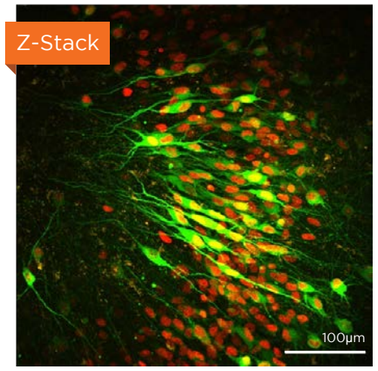
Scanless Photostimulation

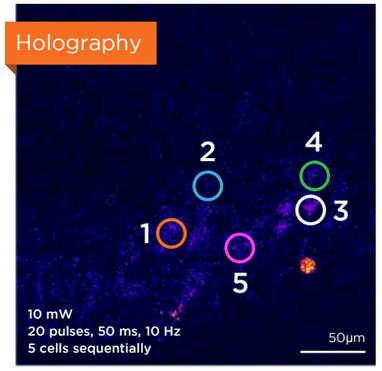
Scanning photostimulation techniques are generally limited to slower opsins and studies at non-physiological rates. Scanless techniques like holography enable photostimulation across all opsin channels simultaneously so that the cell response speed is only dependent on the cell and the opsin kinetics. As a result Nouveau Phasor enables the use of fast opsins (e.g., Chronos, ChETA, vf-Chrimson, ChroME) allowing for studies at physiological scale with millisecond temporal resolution and sub-millisecond jitter.

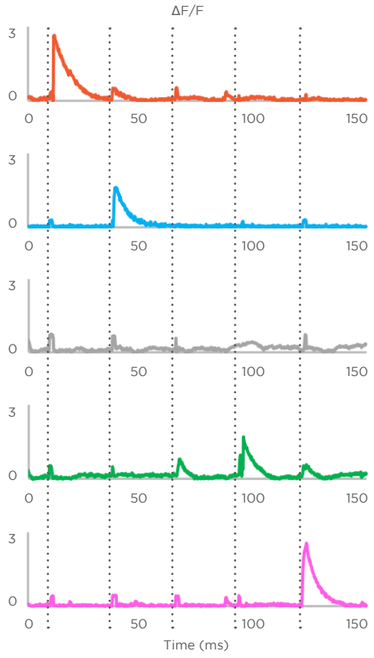


Nouveau Phasor is the latest generation of 3i holographic products that have supplied labs for over ten years. Nouveau Phasor combines four different modalities that can be chosen by a single software click.

- 2-Photon Computer Generated Holography (2P-CGH)
- 2D Temporal Focusing (2D-TF)
- 3D Temporal Focusing (3D-TF) also known as Multiplexed Temporally Focused Computer Generated Holography (MTF-CGH)
- 3D Scanless Holographic Optogenetics with Temporal Focusing (3D-SHOT)







Easy

Intuitive software with seamless switching between modes

Flexible

Supports most research-grade microscope frames

Fast

High-speed spatial light modulators

Modular

Upgrade hardware anytime for evolving experimental paradigms

Optimized

Intuitive holographic pattern generation software including uniform distribution of power in XYZ, optical aberration correction, and user-defined power assignment, developed in collaboration with user labs

Stable

Stable imaging paths remain parfocal over time without realignment

User-Defined Imaging Platform

User-defined MATLAB and Python code can interactively control complex experiments directly in SlideBook

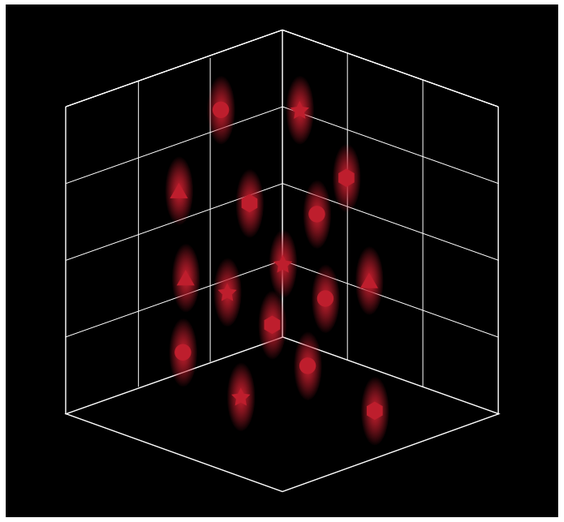
Nouveau Phasor

Stimulation Modalities

	2P Holography	2D-TF	3D-TF	3D-SHOT
Power throughput	●●●	●●	●●	●●
3D spatial resolution	●	●●	●●●	●●
Hand drawn ROIs	Yes	Yes	Yes	No
Compatible with any laser	Yes	Yes	Yes	Yes
Compatible with fast SLMs	Yes	Yes	Yes	Yes
Sample	Cell cultures, Plants	Brain slices, *Drosophila*, Zebrafish	*Drosophila*, Zebrafish, *in vivo* rodents, non-human mammals	*Drosophila*, Zebrafish, *in vivo* rodents, non-human mammals

Scan for a video walkthrough of Nouveau Phasor.

2P Holography | Maximum Power



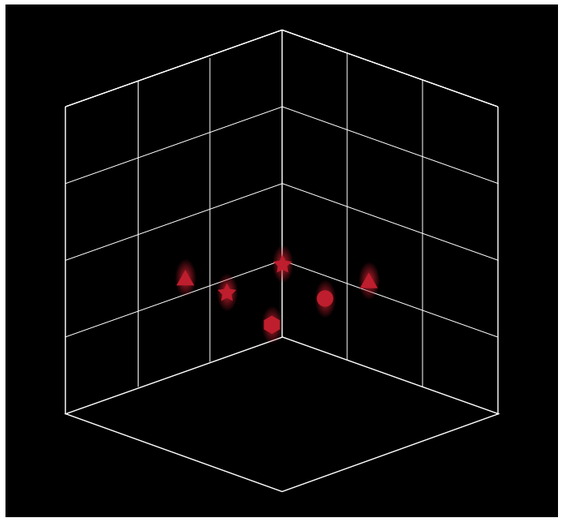
3D Holographic Stimulation

The SLM is exclusively used to modulate the phase of an incoming wavefront from a femtosecond IR laser using the incoming laser's full power for stimulation patterns.

Proprietary software based on iterative Fourier transform algorithms (IFTA) simultaneously generate multiple hand-drawn regions of interest at any position in sample space.

SlideBook's exclusively licensed libraries generate holographic patterns at multiple Z positions with corrections for power discrepancies inherent to the use of SLMs and each pattern can be assigned a specific power density.

2D-TF | Cell Cultures and Thin Samples



2D Temporal Focusing

A grating is added to the optical path for temporal spreading of the femtosecond beam outside the focal plane. This minimizes the probability of 2-photon absorption above and below the plane of focus.

Temporal Focusing thus offers the possibility to generate axially-confined holographic patterns at the plane of focus.

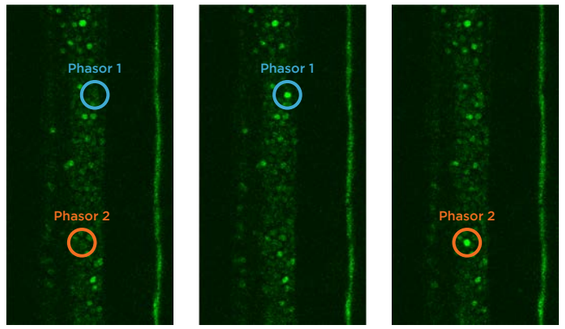
Optimal Samples

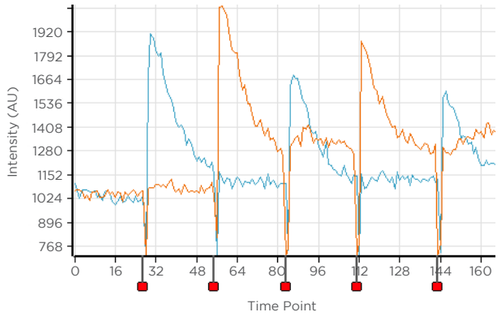
Areas of Study

Locomotion

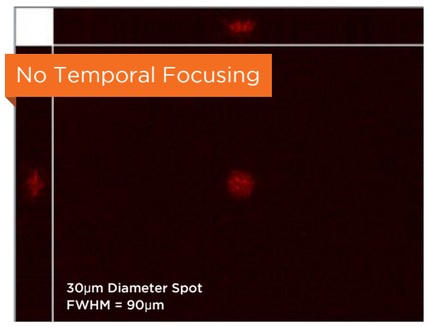
Neuronal Cell Activity

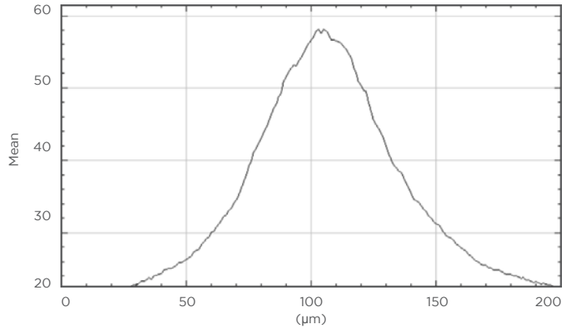
Visual Systems

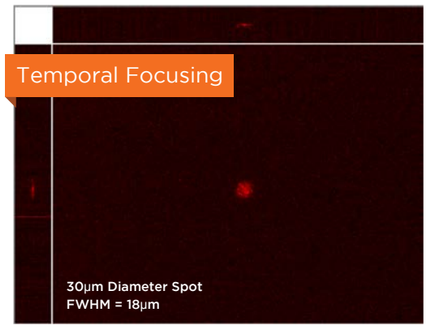


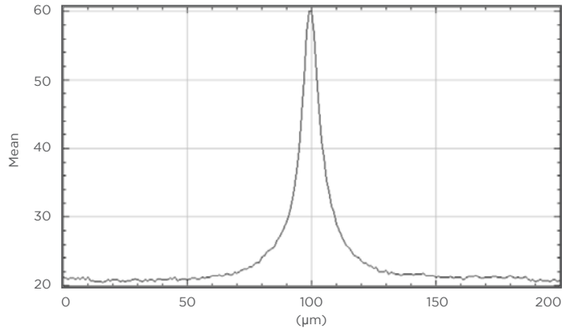


All-optical electrophysiology data derived from transgenic zebrafish larvae expressing GCaMP6f in spinal neurons co-expressing the opsin Chrimson; neurons were selectively activated using Phasor 2P photostimulation. 3-Panel montage shows Phasor-elicited calcium increases (left) and graphed results (right).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

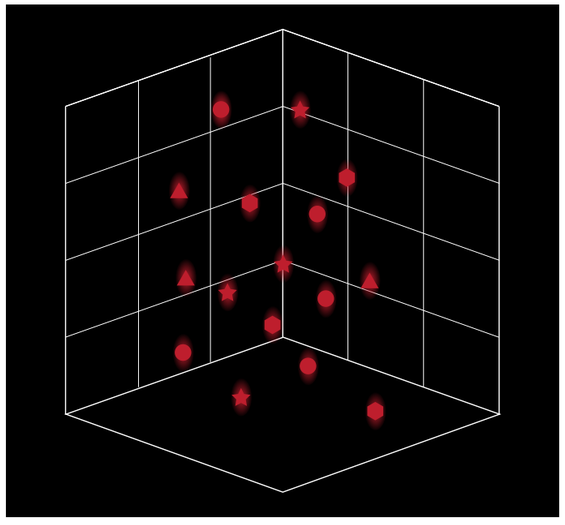








3D-TF & 3D-SHOT | Flexible Stimulation of Thick Samples

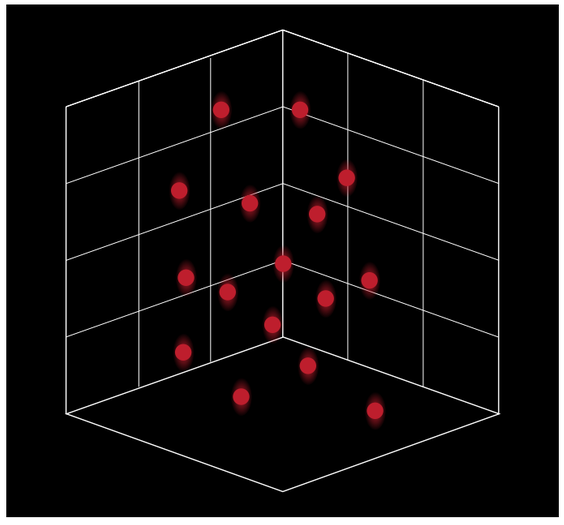


3D Temporal Focusing

A second SLM is added to the optical path.

The first SLM generates the desired patterns, the grating confines the axial extent of the patterns and the second SLM multiplexes the patterns in 3D.

This configuration enables simultaneously-generated axially-confined holographic patterns in 3D.



3D-SHOT

A grating and SLM are used to generate axially-confined Gaussian patterns in sample space. The grating axially confines the Gaussian activation pattern and the SLM multiplexes the pattern at multiple positions in 3D.

A motorized beam expander changes the size of the Gaussian activation pattern.

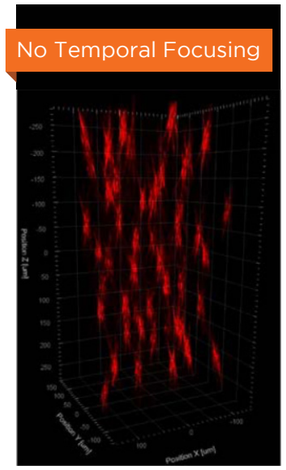
Optimal Samples

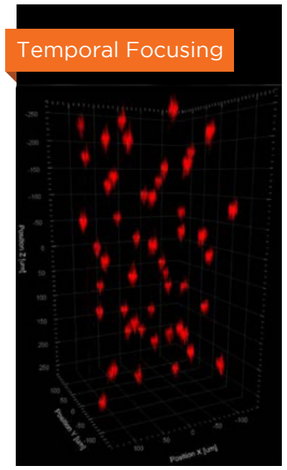
Areas of Study

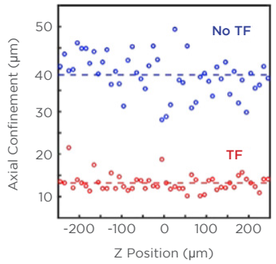
Food & Drug Dependency

Learning & Memory

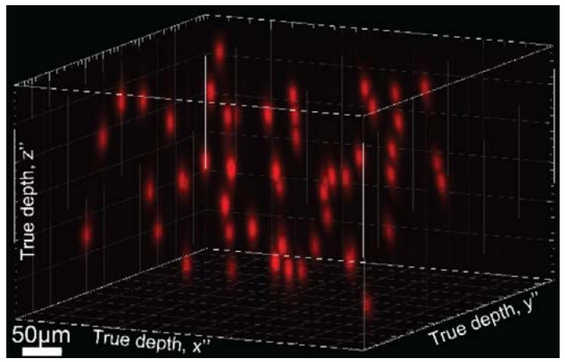
Optogenetics Therapy

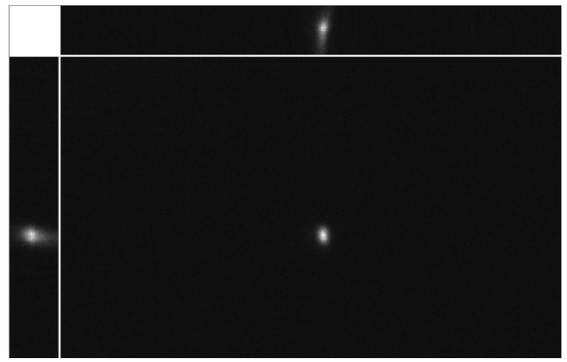






50 Holographic circular spots of 15 µm diameter at 50 different axial positions. Average FWHMz 11µm with 40x 0.8NA. Courtesy of Nicolò Accanto, Institut de la Vision.





50 randomly distributed targets in a 250µm x 250µm x 500µm volume. FWHMz is ~20µm for a 25µm diameter Gaussian pattern. Courtesy of Nicolas Pégard, University of North Carolina

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

Power Calibration

Uniform distribution of power density in 3D
Geometry calibration of Nouveau Phasor to most research-grade microscope frames

NVIDIA CUDA GPU Acceleration

Fast hologram computation for physiological timescales

Stimulation Region of Interests

Hand-drawn or computed stimulation regions at any position in 3D with user-defined power density value

Phasor Streaming

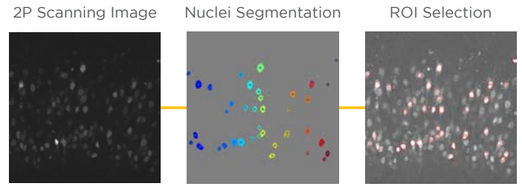
High temporal resolution imaging and simulation sequence recordings

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.

MATLAB Interface

Imaging pipeline for user-defined experimental designs



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.

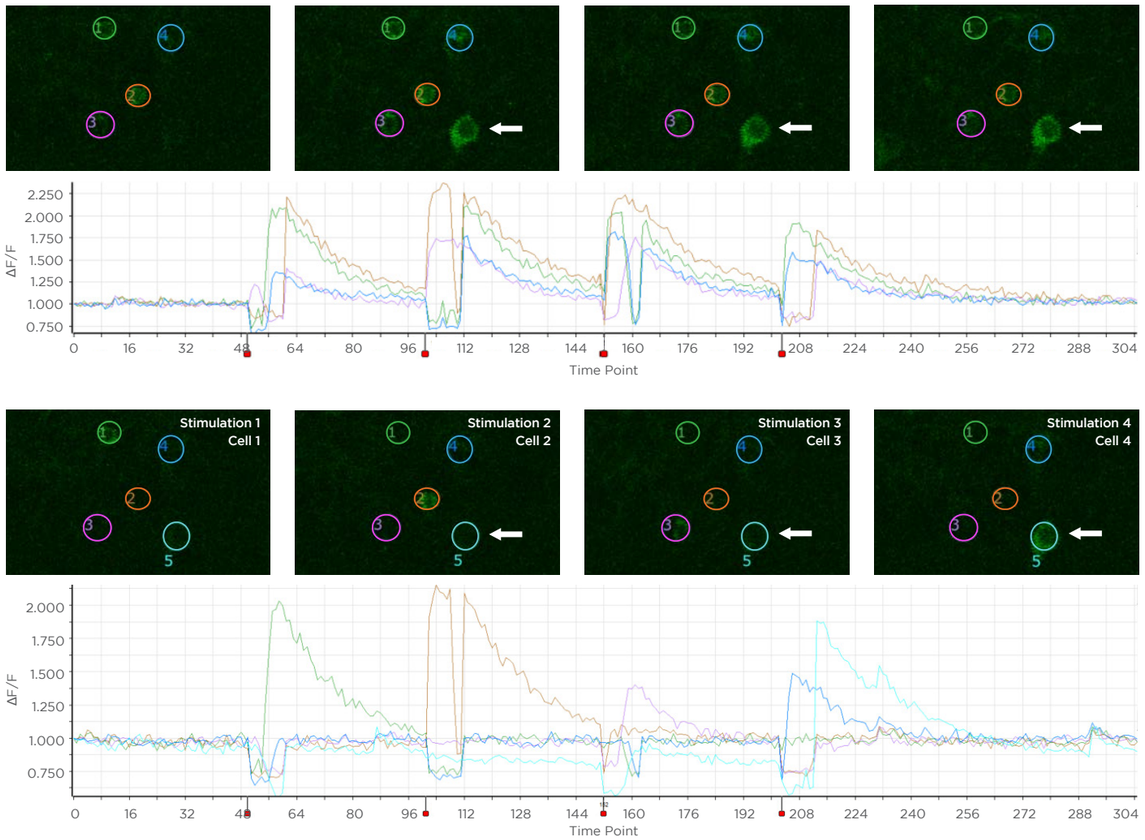
Aivia

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

Dell

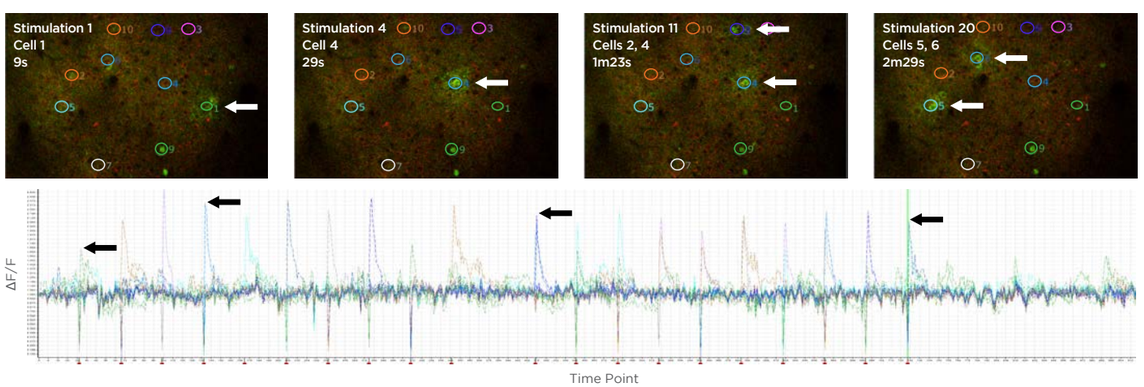
The latest high-power computer workstations control all microscope hardware and enable high-speed processing, segmentation and volume rendering of terabyte (TB) datasets.

Cell Connectivity



Organotypic neurons expressing GCaMP7s and ChroME. Top: 4 cells are simultaneously stimulated at each event. All 4 cells show an increase in calcium activity. One cell responds to the activation without being stimulated (white arrow). Bottom: Sequential activation of each cell shows specific connectivity between cell 4 and the cell shown with the white arrow.

Awake Animal Behavior



3 minute recording of mouse brain calcium activity in vivo via cranial window expressing ChRmine and GCaMP6m. 20 stimulation events were performed for single-cell activation and pair-of-cells activation. All cells had an increase in calcium activity upon stimulation.

Scientific Spotlight

Locomotion

Chopek JW, Zhang Y, Brownstone RM. Intrinsic brainstem circuits comprised of Chx10-expressing neurons contribute to reticulospinal output in mice. *J Neurophysiol.* 2021 Dec 1;126(6):1978-1990. doi: 10.1152/jn.00322.2021. Epub 2021 Oct 20. PMID: 34669520; PMCID: PMC8715053.

Jia X, Wyart C. Holographic Optogenetic Activation of Neurons Eliciting Locomotion in Head-Embedded Larval Zebrafish. *Methods Mol Biol.* 2024;2707:125-140. doi: 10.1007/978-1-0716-3401-1_8. PMID: 37668909.

Learning & Memory

Eschbach C, Fushiki A, Winding M, Schneider-Mizell CM, Shao M, Arruda R, Eichler K, Valdes-Aleman J, Ohyama T, Thum AS, Gerber B, Fetter RD, Truman JW, Litwin-Kumar A, Cardona A, Zlatic M. Recurrent architecture for adaptive regulation of learning in the insect brain. *Nat Neurosci.* 2020 Apr;23(4):544-555. doi: 10.1038/s41593-020-0607-9. Epub 2020 Mar 23. PMID: 32203499; PMCID: PMC7145459.

Neuronal Cell Activity

Berlin S, Carroll EC, Newman ZL, Okada HO, Quinn CM, Kallman B, Rockwell NC, Martin SS, Lagarias JC, Isacoff EY. Photoactivatable genetically encoded calcium indicators for targeted neuronal imaging. *Nat Methods.* 2015 Sep;12(9):852-8. doi: 10.1038/nmeth.3480. Epub 2015 Jul 13. PMID: 26167640; PMCID: PMC4597790.

Carroll EC, Berlin S, Levitz J, Kienzler MA, Yuan Z, Madsen D, Larsen DS, Isacoff EY. Two-photon brightness of azobenzene photoswitches designed for glutamate receptor optogenetics. *Proc Natl Acad Sci U S A.* 2015 Feb 17;112(7):E776-85. doi: 10.1073/pnas.1416942112. Epub 2015 Feb 4. PMID: 25653339; PMCID: PMC4343171.

Fontaine AK, Futia GL, Rajendran PS, Littich SF, Mizoguchi N, Shivkumar K, Ardell JL, Restrepo D, Caldwell JH, Gibson EA, Weir RFF. Optical vagus nerve modulation of heart and respiration via heart-injected retrograde AAV. *Sci Rep.* 2021 Feb 11;11(1):3664. doi: 10.1038/s41598-021-83280-3. PMID: 33574459; PMCID: PMC7878800.

Futia GL , Fontaine AK, Littich S, McCullough S, Restrepo D, Weir RF, Caldwell JH, Gibson AH. In vivo holographic photo-stimulation and two photon GCaMP6 imaging of vagus nerve axons using a GRIN lens integrated nerve cuff. *Proc. SPIE*. 2019 Feb 22;Optogenetics and Optical Manipulation 2019, 108660K. doi: 10.1117/12.2521830.

Jain S, Lin Y, Kurmangaliyev YZ, Valdes-Aleman J, LoCascio SA, Mirshahidi P, Parrington B, Zipursky SL. A global timing mechanism regulates cell-type-specific wiring programmes. *Nature.* 2022 Mar;603(7899):112-118. doi: 10.1038/s41586-022-04418-5. Epub 2022 Feb 23. PMID: 35197627.

Peng Z, Zhang N, Wei W, Huang CS, Cetina Y, Otis TS, Houser CR. A reorganized GABAergic circuit in a model of epilepsy: evidence from optogenetic labeling and stimulation of somatostatin interneurons. *J Neurosci.* 2013 Sep 4;33(36):14392-405. doi: 10.1523/JNEUROSCI.2045-13.2013. PMID: 24005292; PMCID: PMC3761049.

Sales EC, Heckman EL, Warren TL, Doe CQ. Regulation of subcellular dendritic synapse specificity by axon guidance cues. *Elife.* 2019 Apr 23;8:e43478. doi: 10.7554/eLife.43478. PMID: 31012844; PMCID: PMC6499537.

Tanese D, Weng JY, Zampini V, De Sars V, Canepari M, Rozsa B, Emiliani V, Zecevic D. Imaging membrane potential changes from dendritic spines using computer-generated holography. *Neurophotonics.* 2017 Jul;4(3):031211. doi: 10.1117/1.NPh.4.3.031211. Epub 2017 May 12. PMID: 28523281; PMCID: PMC5428833.

Valdes-Aleman J, Fetter RD, Sales EC, Heckman EL, Venkatasubramanian L, Doe CQ, Landgraf M, Cardona A, Zlatic M. Comparative Connectomics Reveals How Partner Identity, Location, and Activity Specify Synaptic Connectivity in Drosophila. *Neuron.* 2021 Jan 6;109(1):105-122.e7. doi: 10.1016/j.neuron.2020.10.004. Epub 2020 Oct 28. PMID: 33120017; PMCID: PMC7837116.

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

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