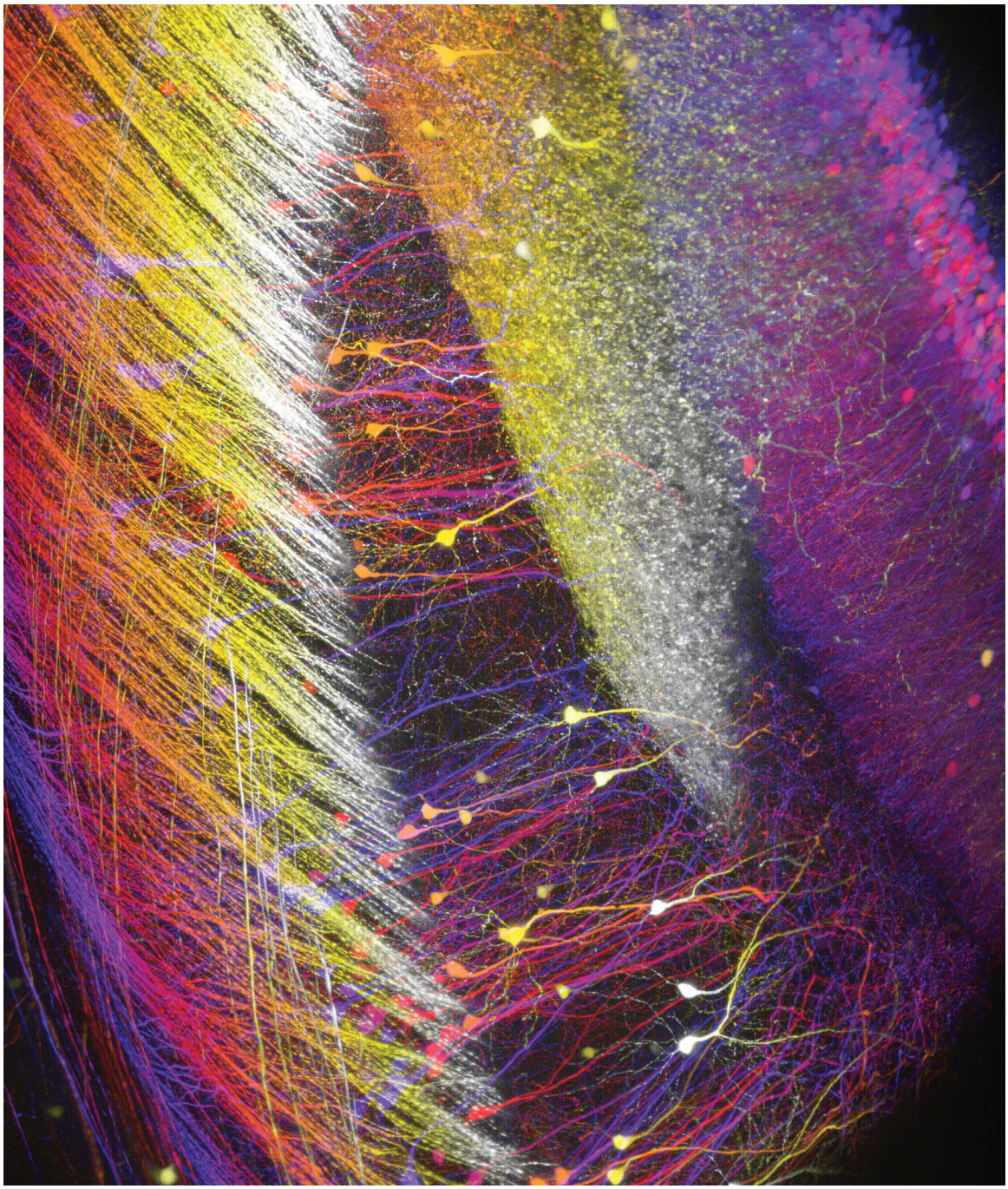
Multiphoton Microscopes

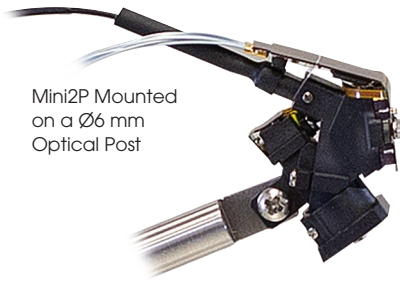


Multiphoton Microscopes: From Mini to Meso

Multiphoton microscopes are ideally suited for applications requiring deep tissue observation. Thorlabs' selection of two- and three-photon microscopes are designed with application in mind and include systems for imaging neuronal activity in freely-moving specimens at the miniscale, simultaneous capture of multiple brain regions at the mesoscale, and everything in between.

THORLABS

Mini2P Miniature Two Photon Microscope



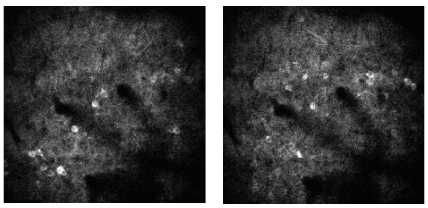
Mini2P Mounted on a Ø6 mm Optical Post

Thorlabs' smallest multiphoton system, the Mini2P is a head-mounted two-photon microscope designed for imaging GCaMP6 (GFP) in freely-moving animals. The lightweight design allows for stable calcium imaging without impediment to the sample. The Mini2P allows for hundreds of neurons to be monitored in a single FOV, while visualization of up to a thousand cells is achievable with multiplane imaging controlled through the built-in microtunable lens.

A near-infrared, 920 nm femtosecond pulsed laser provides ideal excitation for two-photon imaging of GFP. Fiber coupling with a hollow-core photonic crystal fiber creates a stable system without complicated alignment procedures. Laser scanning is performed by a 2D microelectromechanical system (MEMS) device that is available for either fast scanning or large FOV applications. Signal detection is achieved with an on-board, dual-color silicon photomultiplier (SiPM) detector, eliminating the need for a fiber collection bundle that could impede movement.

Application Highlight: Synchronized Behavioral and 2P Imaging

Two-photon imaging from the Mini2P can be synchronized with an external IR camera allowing the user to monitor animal behavior and corresponding neuronal activity. The images to the right show two time points of spontaneous neuronal activity in a freely-moving mouse's somatosensory cortex.



Objective Options

Magnifications	3X	3X	7X
Collection NA	0.50	0.45	0.70
Working Distance	0.582 mm (Air)	0 - 0.7 mm (Air/Water) 1.5 - 2 mm (Glass)	0.24 mm (Water) 0.17 mm (Glass)
Use Case	GRIN Lens	Prism	Window
Focus Range	240 µm	240 µm	~40 µm

Key Features

- Interchangeable Objectives for Application-Specific Customization
- Fast Multiplane Imaging with a Microtunable Lens* (<0.4 ms Response Time)
- Laser-Alignment-Free Operation
- Two MEMS Laser Scanning Options
  - Fast Scanning: 0.17 mm^2 FOV, 40 Hz (256 x 256 Pixels)
  - Large FOV: 0.25 mm^2 FOV, 15 Hz (256 x 256 Pixels)
- On-Board SiPM Detection

* Manufactured by poLight

Mini2P Specifications[a]

Scanner	Large FOV	Fast Scanning
Imaging Speed	15 Hz (256 x 256 Pixels)	40 Hz (256 x 256 Pixels)
Detector	Silicon Photomultiplier (SiPM)	
Laser	920 nm	
Pulse Width	125 fs	

a. This item is still in development and specifications are subject to change.

Mini2P Accessories

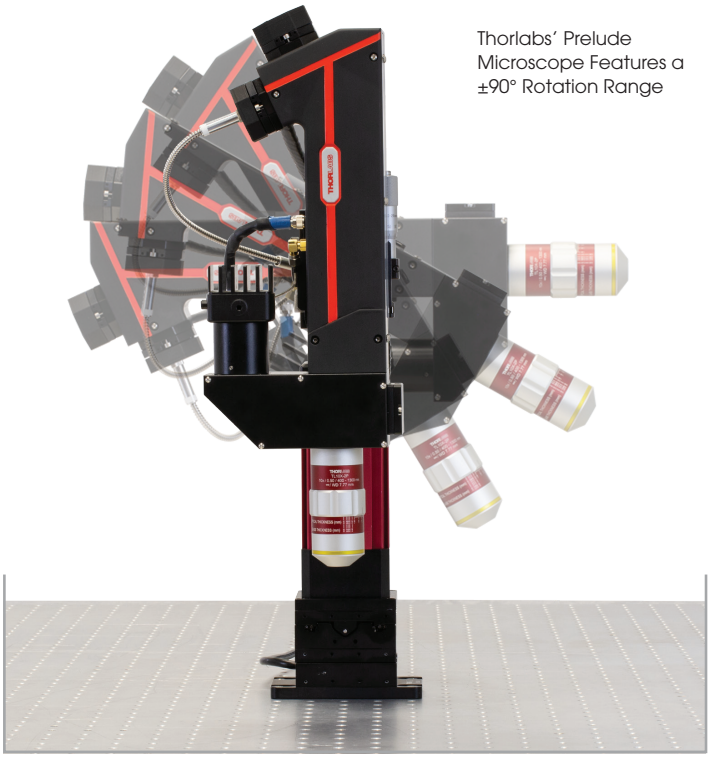
An array of accessories are available to support imaging with the Mini2P. Interchangeable objectives facilitate use with cranial windows, prisms, and GRIN lenses for imaging tissue at different depths and orientations. We also offer the Mini2P as a complete portable system that includes a rolling cart with rack-mounted computer and laser, breadboard mounting, and a camera as well as micromanipulators for alignment.

A Behavioral Imaging Environment seen at Neuroscience 2024 is also available by special request. This light-secure enclosure features a built-in IR LED and IR-sensitive camera allowing behavioral imaging which can be synchronized to Mini2P imaging. A counterbalanced arm in the enclosure relieves weight of the Mini2P while also keeping wires organized, allowing for minimal specimen impediment.

PRELUDE® Functional Imaging Microscope

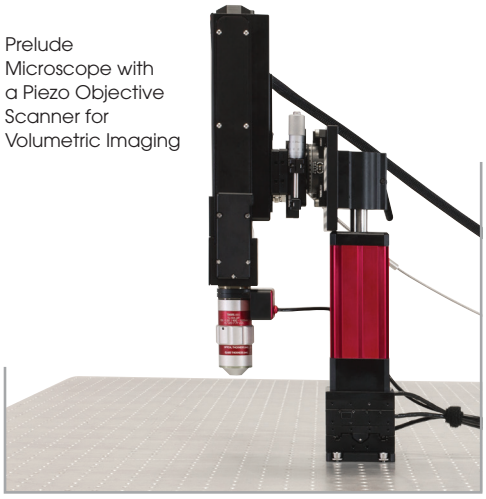
Key Features

- Laser-Alignment-Free Operation
- Integrated Optics and Filters for Two-Photon Fluorescence Imaging of GFP
- Fine Z-Focus Options
  - Vibrationless Remote Focus
  - High-Speed Piezo Objective Scanner
- Hypersensitive Silicon Photomultiplier (SiPM) Detector
- Fiber-Coupled fs Laser Source with Dispersion Compensation for Improved Contrast



Thorlabs' Prelude Microscope Features a ±90° Rotation Range

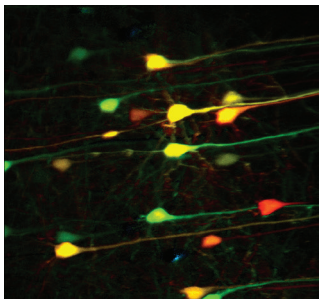
For samples with difficult positioning requirements, the Prelude® is a fully-integrated microscope ideal for two-photon neuronal imaging of GCaMP6 (GFP). The Prelude includes a motorized post with XYZ translation and can be manually rotated through a ±90° range. The fiber-coupled 920 nm laser, built-in MEMS scanner, and integrated optics lends to a compact design, offering simple set-up and a high-degree of maneuverability.



Prelude Microscope with a Piezo Objective Scanner for Volumetric Imaging

Application Highlight: Volumetric Imaging

We offer two fine focus options with the Prelude designed to provide fast volumetric imaging. Our remote focus unit utilizes liquid crystal lenses to switch between 16 discrete focal planes; this fast and vibrationless technology makes it ideal for *in vivo* applications. We also offer a high-speed piezo objective scanner, ideal for precisely setting the focal position of the microscope as well as high-speed, high-resolution Z scanning.



GFP-labelled mouse brain section. This maximum intensity Z-stack was taken with the Prelude and is color-coded by depth.

Prelude Specifications

Laser		920 nm, 125 fs Pulse Width
Scanner		4.7 kHz Resonant-MEMS Scanning
Scan Speed		>12 fps at 512 x 512 Pixels
Detector		PDA45 Silicon Photomultiplier (SiPM)
Collection Optics		7° Non-Descanned Fluorescence Collection Angle for Ø20 mm Entrance Pupil
Translation	**X, Y, and Z**	1" of Motorized Travel
	Rotation	±90° of Manually Operated Rotation
Fine Z Focus	**Piezo Objective Scanner**	Open Loop: 600 µm ± 10% Travel Range; 1 nm Resolution Closed Loop: 450 µm Travel Range; 3 nm Resolution
	Vibrationless Remote Focus 16 Discrete Steps	600 µm Total Travel for a 10X Objective 341 µm Total Travel for a 15X Objective 300 µm Total Travel for a 16X Objective

Veneto® Inverted Microscopes

Veneto® Inverted Mutiphoton Microscope

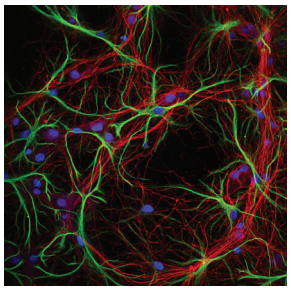
Key Features

- Supports Widefield, Brightfield, Darkfield, Phase, Confocal, and/or Multiphoton Imaging
- Built-In Motorized Focusing Module Allowing for Users to Image Multiple Z-Stacks Easily
- Trans-Illumination Module Tilts to Allow Large Samples Underneath
- Light Path Selector Allows Users to Switch Between Up to 3 Light Paths
- Up to 7 Customizable Optical Pathways

Designed to meet the needs of labs working in cell biology and other life science applications, our Veneto® microscopes feature an inverted design making them a versatile research tool that can accommodate a range of applications, such as fluorescence, *in vivo*, *ex vivo*, 3D, high-resolution, high-speed (video-rate), and live tissue imaging. Available as a turnkey solution for multiphoton, confocal, and widefield imaging, this system is also highly customizable, offering accessory ports, easily accessible light paths, and built-in breadboards allowing users to create custom optical pathways.

Application Highlight: Confocal Imaging

Our Veneto microscopes allow users to image in multiple modalities with one system. Our confocal configuration offers up to four user-selectable laser lines, high-speed galvo-resonant or galvo-galvo scanners, and four high-sensitivity PMT detectors.



Multi-Channel Confocal Image of Neurofilaments (Green), Glial Cells (Red), and DAPI Nucleic Stain (Blue) from Hippocampus Cells

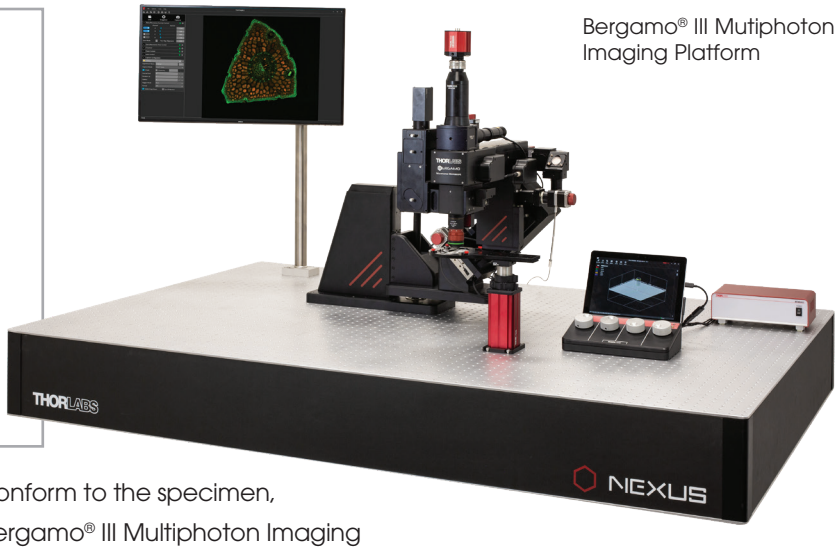
Veneto Specifications

Laser Scanning	**Scan Path Wavelength Range**	450 - 1100 nm, 680 - 1300 nm, or 800 - 1800 nm
	Scan Paths	Resonant-Galvo-Galvo Scanner, Galvo-Resonant Scanners, or Galvo-Galvo Scanners; Single or Dual Scan Paths
	8 kHz Resonant-Galvo-Galvo or Galvo-Resonant Scan Speed	2 fps at 4096 x 4096 Pixels 30 fps at 512 x 512 Pixels 400 fps at 512 x 32 Pixels
	12 kHz Resonant-Galvo-Galvo or Galvo-Resonant Scan Speed	4.4 fps at 2048 x 2048 Pixels 45 fps at 512 x 512 Pixels 600 fps at 512 x 32 Pixels
	Field of View	20 mm Diagonal Square (Max) at the Intermediate Image Plane (12 mm Diagonal Square (Max) for 12 kHz Scanner)
	Scan Zoom	1X to 16X (Continuously Variable)
	Scan Resolution	Up to 2048 x 2048 Pixels (Bi-Directional) (Up to 1168 x 1168 Pixels for 12 kHz Scanners) Up to 4096 x 4096 Pixels (Unidirectional) (Up to 2336 x 2336 Pixels for 12 kHz Scanners)
Motorized XY Scanning Stage		110 mm x 75 mm (4.3" x 2.95") Travel Range 250 mm/s Max Velocity
Objective Turret		Five Manual Positions with M25 x 0.75
Focusing Unit		15 mm of Travel Stepper Motor: 15 mm Travel Range, 50 nm Resolution Piezo: 100 µm Travel Range, 50 nm Resolution
Reflected Light Illumination Turret		Six Motorized Positions for 32 mm x 44 mm Dichroics and Ø32 mm Excitation and Emission Filters

BERGAMO® III Series Microscopes

Key Features

- Microscope Bodies with Up to Five Axes of Motion
- Fiber Coupled 2P Lasers for Alignment-Free Imaging
- Available with Single or Dual Scan Paths
- Scan Optics Optimized for Photoactivation, 2P, and 3P Imaging
- Up to Four Simultaneous Detection Channels



Bergamo® III Mutiphoton Imaging Platform

Following the principle that the microscope should conform to the specimen, rather than the other way around, we created the Bergamo® III Multiphoton Imaging Platform, a completely modular system that adapts to a wide range of experimental setups.

Completely Modular Platform

Microscope Bodies: Designed to be brought to your sample, our Bergamo III microscopes are available in three body configurations. Our rotating Bergamo III microscope bodies include five axes of motion, providing near-total freedom to study *in vivo* systems. Our upright bodies feature an industry-leading throat depth and offer either one or three axes of motion control.

Scan Optics: Bergamo III microscopes accommodate the most recent lasers, fluorophores, and techniques. Laser wavelength ranges can be selected based on the user's needs and include choices for photoactivation, 2P, and 3P applications. For 2P lasers, we offer the option for fiber-coupled laser delivery, allowing for highly-stable, alignment-free operation, while also maximizing space for the sample.

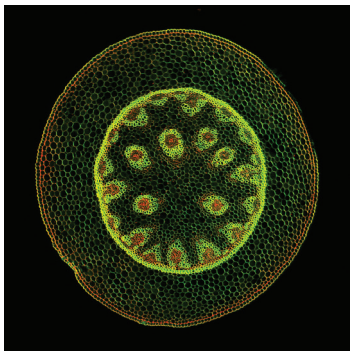
Signal Detection: We employ high-sensitivity GaAsP PMTs in our multiphoton systems, which can offer high quantum efficiency, aiding in imaging weakly fluorescent or highly photosensitive samples.

Key Techniques

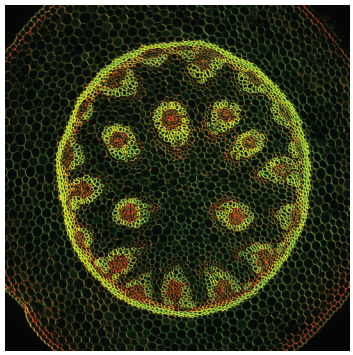
- Two- and Three-Photon Imaging
- 2D or 3D Photoactivation/Uncaging
- Simultaneous Multi-Channel Detection
- Rapid Volumetric Imaging Using Bessel Beams
- Fast Functional Imaging
- Multi-Target Photoactivation
- Confocal Imaging
- Dodt Gradient Contrast
- Spatial Light Modulation
- Deep Brain Imaging

Application Highlight: Large FOV Imaging

Our Bergamo III microscopes can be configured with a Field Number (FN) of 40, allowing users to image multiple regions of interest within a single FOV. A secondary path with a spatial light modulator can also be configured to manipulate a smaller portion of the field. The two images below were taken with a Bergamo III microscope configured for large FOV imaging.



FOV: 2.83 mm x 2.83 mm
Taken with a Thorlabs TL10X-2P 10X Objective

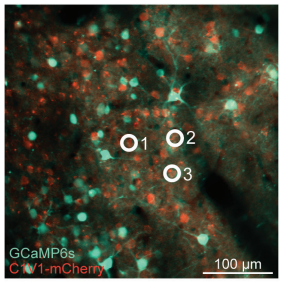


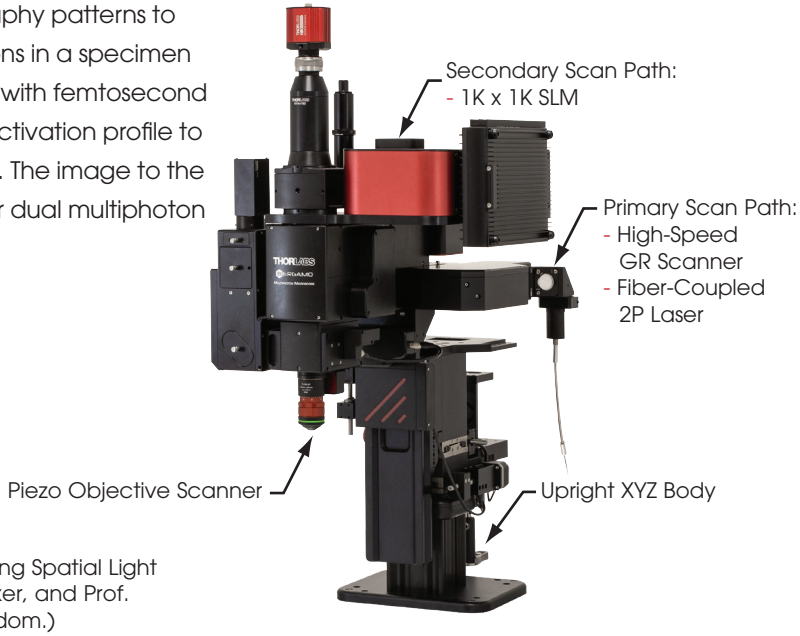
FOV: 1.89 mm x 1.89 mm
Taken with a Thorlabs TL15X-2P 15X Objective

BERGAMO® III Series Microscopes

Application Highlight: Spatial Light Modulation

Thorlabs' Spatial Light Modulator (SLM) uses holography patterns to enable 2D or 3D photoactivation of multiple locations in a specimen simultaneously. Designed for two-photon excitation with femtosecond pulses, the SLM manipulates the phase across the activation profile to generate hundreds of user-determined focal points. The image to the right shows a Bergamo III microscope configured for dual multiphoton imaging and spatial light modulation.





Left: Photoactivation and Ca^{2+} Imaging of Three Cells Using Spatial Light Modulation (Courtesy of Dr. Lloyd Russell, Dr. Adam Packer, and Prof. Michael Häusser, University College London, United Kingdom.)

Bergamo III Specifications

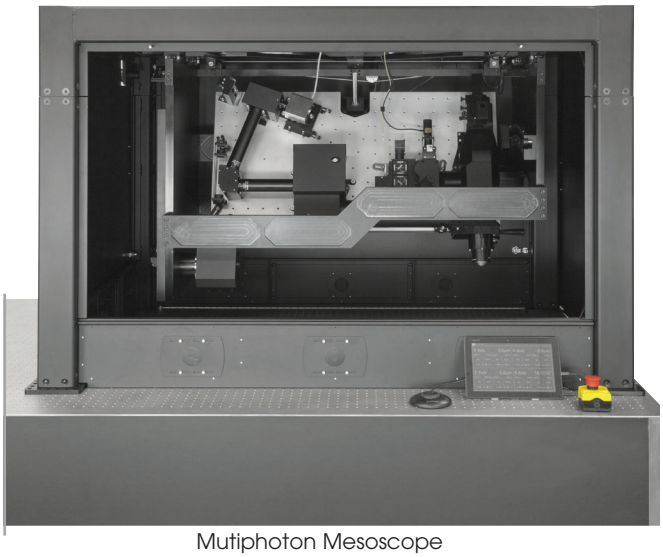
Multiphoton Excitation	**Wavelength Range**	450 - 1100 nm, 680 - 1300 nm, or 800 - 1800 nm
	Scan Paths	Resonant-Galvo-Galvo Scanner, Galvo-Resonant Scanners, Galvo-Galvo Scanners, or Spatial Light Modulator; Single or Dual Scan Paths
	Resonant-Galvo-Galvo and Galvo-Resonant Scan Speeds	8 kHz: 2 fps (4096 x 4096 Pixels); 30 fps (512 x 512 Pixels); 400 fps (512 x 32 Pixels) 12 kHz: 4.4 fps (2048 x 2048 Pixels); 45 fps (512 x 512 Pixels); 600 fps (512 x 32 Pixels)
	Galvo-Galvo Scan Speeds	3 fps at 512 x 512 Pixels; 48 fps at 512 x 32 Pixels; 70 fps at 32 x 32 Pixels Pixel Dwell Time: 0.4 - 20 µs
	Galvo-Galvo Scan Geometries	Imaging: Line, Polyline, Square, or Rectangle Non-Imaging: Circle, Ellipse, Polygon, or Point
	Field of View	FN40 Equivalent to 40 mm Diagonal Square (Max) at the Intermediate Image Plane >2.8 mm x 2.8 mm at the Sample Plane with a 10X Objective
	Scan Zoom	1X - 16X (Continuously Variable)
	Scan Resolution	Bi-Directional: 2048 x 2048 Pixels (Max) (1168 x 1168 Pixels (Max) for 12 kHz Scanner) Unidirectional: 4096 x 4096 Pixels (Max) (2336 x 2336 Pixels (Max) for 12 kHz Scanner)
Multiphoton Signal Detection	**Epi Detection**	Up to Four Ultrasensitive GaAsP PMTs, Cooled or Non-Cooled
	Forward-Direction Detection	Two Highly Sensitive Forward Fluorescence Detection Channels
Translation	**X and Y**	2" (50.8 mm); 0.5 µm Encoder Resolution
	Z	1" (25.4 mm); 0.1 µm Encoder Resolution
	Microscope Base (Rotating Bodies)	5" (127 mm); 1 µm Encoder Resolution
	Rotation (Rotating Bodies)	Up to 90°; 0.1° Encoder Resolution
Fine Z Focus	**Piezo Objective Scanner**	Open Loop: 600 µm ± 10% Travel Range; 1 nm Resolution Closed Loop: 450 µm Travel Range; 3 nm Resolution
	Vibrationless Remote Focus	16 Discrete Steps ~400 µm for a 10X Objective ~160 µm for a 16X Objective

Multiphoton Mesoscope

Key Features

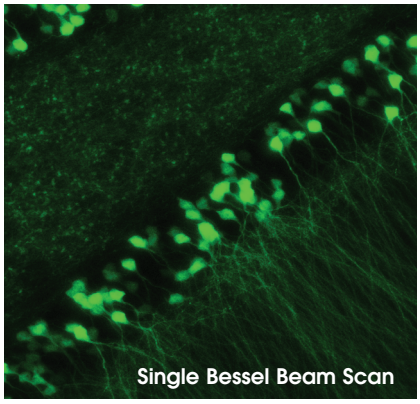
- Subcellular Functional Imaging Over a 5 mm x 5 mm FOV
- ±20° Rotation Around Sample and Fine XYZ Motion
- Configurable Scans Over Entire FOV or Multiple Non-Contiguous Regions
- Translatable FOV While Specimen Remains Fixed

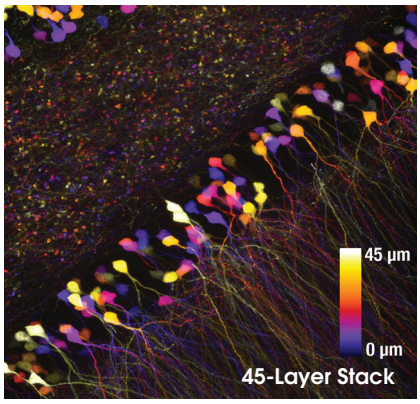
Our largest two-photon system, the Mesoscope offers subcellular resolution over an exceptionally large 5 mm x 5 mm FOV, allowing for simultaneous imaging of multiple brain regions at near video frame rates. The Mesoscope features motion control systems that permit the Mesoscope body to move while the specimen remains fixed.



Mutiphoton Mesoscope

Application Highlight: Bessel Beam Imaging





Thorlabs offers a Bessel beam module for our multiphoton Mesoscope. A single Bessel scan (left) captures the same structural information obtained from a Gaussian volume scan created by stacking 45 optical sections (right), reducing the total scan time by a factor of 45. (Sample Courtesy of Dr. Qinrong Zhang and Matthew Jacobs; the Ji Lab, Department of Physics, University of California, Berkeley.)

Mesoscope Specifications

Scan Path Wavelength Range		900 - 1070 nm
Field of View		5 mm x 5 mm
Objective	**Excitation NA[a]**	0.6
	Collection NA[a]	1.0
	Working Distance (Minimum)[b]	2.7 mm or 5.0 mm
Lateral Scan Unit		12 kHz Resonant Scanner + Virtually Conjugated Galvo Scanner Set
Scan Speed		Field of View is Divided into 608-µm-Wide Vertical Stripes Time per Scan Line is 42 µs Scan Speed = 42 µs × Number of Stripes × Number of Scan Lines
Epi-Detection		Two Ultrasensitive GaAsP PMTs
Objective Rotation		-20° to +20° Around Objective Focus; 0.1° Encoder Resolution
X Translation		2" (50.8 mm); 0.5 µm Encoder Resolution
Y Translation		6" (152.4 mm); 0.5 µm Encoder Resolution
Z Translation	**Stepper Motor**	2" (50.8 mm); 0.1 µm Encoder Resolution
	Remote Focusing Mirror	1 mm Travel Range

a. These NAs are valid over the entire scan path wavelength range.
b. The Mesoscope's remote focusing mirror can be used to translate the focal plane over a 1 mm range without movement of the objective or the specimen, allowing the specimen to be placed farther from the edge of the objective than its working distance.

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