56 Sparta Avenue • Newton, New Jersey 07860
(973) 300-3000 Sales • (973) 300-3600 Fax
www.thorlabs.com

THORLABS

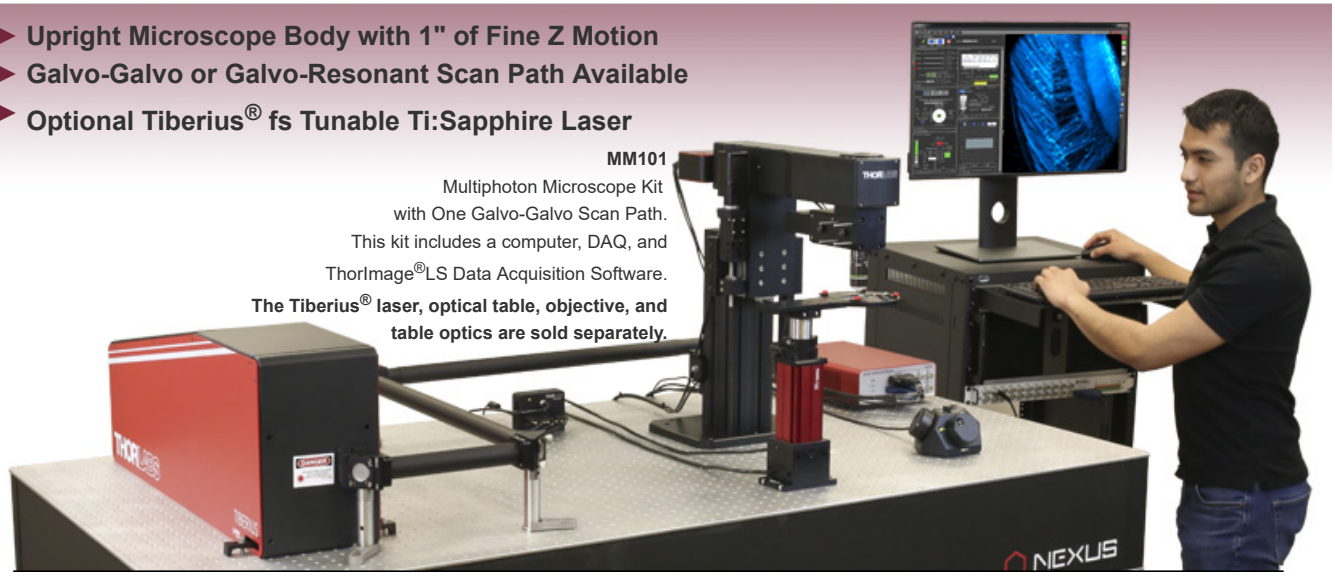
MM201 - October 4, 2022

Item # MM201 was discontinued on October 4, 2022. For informational purposes, this is a copy of the website content at that time and is valid only for the stated product.

DIY MULTIPHOTON MICROSCOPE KITS

- Upright Microscope Body with 1" of Fine Z Motion
- Galvo-Galvo or Galvo-Resonant Scan Path Available
- Optional Tiberius® fs Tunable Ti:Sapphire Laser

MM101
Multiphoton Microscope Kit
with One Galvo-Galvo Scan Path.
This kit includes a computer, DAQ, and
ThorImage®LS Data Acquisition Software.
The Tiberius® laser, optical table, objective, and table optics are sold separately.



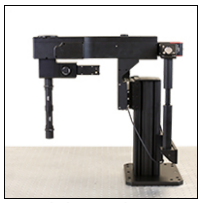
OVERVIEW

Imaging Capabilities

- Scan Path Wavelength Range: 450 - 1100 nm
- Field of View (FOV): 20 mm Diagonal Square (Max) at the Intermediate Image Plane
- 8° Collection Optics in Epi Direction (for Ø20 mm Entrance Pupil)

Microscope Kit Features

- Multiphoton Scan Path with Galvo-Galvo or Galvo-Resonant Scanner
- One GaAsP Photomultiplier Tube (PMT)
  - Upgradeable to Two PMTS
- Upright Microscope Body with Fine Z-Axis Motion
- Single-Objective Holder
- Rigid Stand Slide Holder with XY Motorized Translation Stage
- Computer with DAQ Card Included
- ThorImage®LS Data Acquisition Software with Lifetime Support
- Tiberius® fs Tunable Ti:Sapphire Laser Available Upon Request (Contact ImagingSales@thorlabs.com)



Click to Enlarge
A laser alignment tool is included with the Multiphoton Microscope Kits. The MM101 kit is shown with the alignment tool attached to the objective nosepiece.

Sam Tesfai
General Manager,
Thorlabs Imaging Systems

Feedback?
Questions?
Need a Quote?

Contact Me

Scan Path Options	
Galvo-Galvo	• User-Defined ROI Shapes and Photostimulation Patterns: Squares, Rectangles, Circles, Ellipses, Lines, and Polylines • Consistent Dwell Times Across Field of View • Up to 2 fps at 512 x 512 Pixels, Single Channel
Galvo-Resonant	• High-Speed Imaging • Image 30 fps at 512 x 512 Pixels

Thorlabs' Modular Do-It-Yourself (DIY) Multiphoton Microscope Kits are user-configurable kits that provide the backbone for constructing a multiphoton microscope; see the *Shipping List* tab for details on all included items. These kits are designed for DIY microscopists who are interested in building and aligning their own custom microscopes. For detailed step-by-step assembly instructions, please refer to the manual, which can be found by clicking on the red "Docs" icon ( ) below. To request installation support, please click on the Contact Me button in the green box to the right.

We offer kits with either a galvo-galvo scanner for user-defined scan geometries, or a galvo-resonant scanner for high-speed imaging. The galvo-galvo scanner supports user-drawn scan geometries (lines, polylines, squares, and rectangles), custom photoactivation patterns (circles, ellipses, polygons, and points), and consistent pixel dwell times for better signal integration and image uniformity. Alternatively, the 8 kHz galvo-resonant scanner is ideal for high-speed imaging applications with a maximum frame rate of 400 fps, while still utilizing the entire field of view.

Each microscope kit has one GaAsP PMT with high quantum efficiency in the visible spectrum, providing elevated sensitivity to incoming illumination compared to photocathodes composed of alkali metals. The kit can be easily upgraded by adding a second PMT to the included dual-path epi-detection module with 8° collimation optics. To aid users in aligning the microscope, a laser alignment tool is provided; see the image to the upper right. For details on how to use this tool, please refer to the manual.

These kits allow the users to build a customized multiphoton microscope by adding their own laser and M32 x 0.75 objective. RMS-, M25 x 0.75-, or SM1-threaded objectives can also be used with the included thread adapters. Other objectives can be incorporated using our microscope objective threading adapters. A 95 mm dovetail on the microscope body and female D1N dovetail on the top panel can be used to add additional components, such as a camera tube and scientific camera for widefield imaging. For more information on how to integrate additional modules into your kit, please contact ImagingSales@thorlabs.com or call (703) 651-1700.

Every microscope kit includes a PC with a National Instruments (NI) PCIe-6363 Series DAQ card (see the *Specs* tab for details) and ThorImage®LS data acquisition software. ThorImageLS was developed in conjunction with our laser scanning microscopy systems to provide an intuitive program for acquiring and analyzing images. This open-source software package enables synchronization of external hardware and events, multi-dimensional data acquisition and display, region-of-interest scanning, and multi-user operation. All images are saved in the standard TIFF image format so that they can be viewed using software packages such as ImageJ/Fiji. See the *ThorImageLS* tab for additional information on software features. Upon the purchase of a multiphoton microscope kit, Thorlabs provides lifetime support for the ThorImageLS package.

Optional Tiberius® fs Tunable Femtosecond Ti:Sapphire Laser

With the Multiphoton Microscope Kits, there is an option to include the Tiberius® fs Tunable Femtosecond Ti:Sapphire Laser. This two-photon tunable laser offers an average power of more than 2.3 W at 800 nm and a wavelength that is tunable from 720 nm to 1060 nm allowing the user to target specific compounds for two-photon fluorescence imaging and photostimulation / uncaging. More details on this laser are available at its full web presentation. If you are interested in this option, please contact ImagingSales@thorlabs.com or call (703) 651-1700.

SPECS

Specifications for Thorlabs' DIY Multiphoton Microscope Kits are provided here. If you are interested in a system with different specifications or additional modules than those listed below, contact ImagingSales@thorlabs.com or call our applications engineers at (703) 651-1700.

Item #	MM101	MM201
Highlighted System Specifications		
FOV	20 mm Diagonal Square (Max) at the Intermediate Image Plane	
Imaging Speed	Up to 2 fps at 512 x 512 Pixels, Single Channel	30 fps at 512 x 512 Pixels
Scanning Resolution	Bi-Directional: Up to 2048 x 2048 Pixels Uni-Directional: Up to 4096 x 4096 Pixels	Bi-Directional: Up to 2048 x 2048 Pixels Uni-Directional: Up to 4096 x 4096 Pixels
Microscope Components		
Microscope Body	95 mm Dovetail Rail with Z-Axis Motion	
Scanner	LSKGG4 4 mm Galvo-Galvo Scanner	LSK-GR08 8 kHz Galvo-Resonant Scanner
Scan Path Wavelength Range	450 - 1100 nm	
Detection	1 GaAsP PMT, Upgradeable to 2 PMTs	
Collection Optics Module	8°	
Objective Holder	Single	
Sample Stage	MP150-RCH2 Rigid Stand Slide Holder[a] with PLS-XY Motorized XY Translation Stage	
Laser (Optional)	Tiberius® Tunable fs Ti:Sapphire Laser	
Data Acquisition		
Type	National Instruments PCIe-6363 Series DAQ Card	4-Channel, 14 Bit PCI Digitizer
Analog Output[b]	4 Channels Resolution: 16 Bits Voltage Range: ±10 V Accuracy: 1.89 mV Update Rate: 2.86 MS/s	
Analog Input[b]	1 Channel Resolution: 16 Bits Voltage Range: ±10 V Accuracy: 1.66 mV	
Digital I/O[b]	48 Bidirectional Channels	
Clock Rate	10 MHz (Max)	
Frame In/Out Triggering	TTL	
Line Trigger Out	TTL	
Counter/Timers[b]	4	
Computer and Software		
Computer	PC with DAQ	
Software	ThorImage®LS with Lifetime Support	

a. This previous-generation item is not available for individual purchase.
b. All digital channels and one counter are available for use by the user. All other channels and counters are either actively used by the multiphoton system or reserved to support future upgrades.

SHIPPING LIST

MM101 Multiphoton Microscope Kit with Galvo-Galvo Scan Path

Item # MM101 consists of:

- 95 mm Rail Microscope Body
- LSKGG4 Galvo-Galvo Scanner and Controller
- Primary Scan Path Housing
- Protected Silver Mirror
- SL50-CLS2 Scan Lens
- Laser Scanning Tube Lens
- Manual Primary Dichroic Block
- M32RMSS, M32M25S, M32SM1S Thread Adapters
- Epi-Detection Module, 8° Collection (Upgradeable to 2 Channels)
- GaAsP PMT
- MDFM-MF2 Filter Cube with GFP Filter
- 3-Axis Motorized Stage Control Module
- MP150-RCH2 Rigid Stand Slide Holder[a] with PLS-XY Motorized XY Translation Stage
- NI PCIe-6363 Multifunction Data Acquisition Board
- NI Breakout Box with NI BNC-2090A and NI Cables
- Computer with 24" Monitor, Keyboard, and Mouse
- Computer Rack
- 20 Port USB and 7 Port USB Hubs
- Alignment Tool
- SMA to BNC Cable, 10 ft
- Hardware Kit with All Components Required for Mounting to an Optical Table
  - Ten 1/4"-20 and Twelve M6 Socket Head Cap Screws
  - Four 1/4" (M6) Washers
  - 2 mm, 2.5 mm, 3 mm, 4 mm, 5 mm, and 3/16" Hex L-Keys
  - Four 2 mm (5/64") Hex Key Thumbscrews

MM201 Multiphoton Microscope Kit with Galvo-Resonant Scan Path

Item # MM201 consists of:

- 95 mm Rail Microscope Body
- LSK-GR08 8 kHz Galvo-Resonant Scanner and Controller
- Primary Scan Path Housing
- Protected Silver Mirror
- SL50-CLS2 Scan Lens
- Laser Scanning Tube Lens
- Manual Primary Dichroic Block
- M32RMSS, M32M25S, M32SM1S Thread Adapters
- Epi-Detection Module, 8° Collection (Upgradeable to 2 Channels)
- GaAsP PMT
- MDFM-MF2 Filter Cube with GFP Filter
- 3-Axis Motorized Stage Control Module
- MP150-RCH2 Rigid Stand Slide Holder[a] with PLS-XY Motorized XY Translation Stage
- 4-Channel, 14 bit PCI Digitizer
- NI PCIe-6363 Multifunction Data Acquisition Board
- NI Breakout Box with NI BNC-2090A and NI Cables
- Computer with 24" Monitor, Keyboard, and Mouse
- Computer Rack
- 20 Port USB and 7 Port USB Hubs
- Alignment Tool
- SMA to BNC Cable, 10 ft
- SMA to SMB Cable, 10 ft
- 2 SMB to BNC Cables, 10 ft
- Hardware Kit with All Components Required for Mounting to an Optical Table
  - Ten 1/4"-20 and Twelve M6 Socket Head Cap Screws
  - Four 1/4" (M6) Washers
  - 2 mm, 2.5 mm, 3 mm, 4 mm, 5 mm, and 3/16" Hex L-Keys
  - Four 2 mm (5/64") Hex Key Thumbscrews

a. This previous-generation item is not available for individual purchase.

These Multiphoton Microscope Kits can be controlled using the same software as our Bergamo® II Series Multiphoton Microscope, Confocal Imaging Systems, and Hyperspectral Imaging System.

ThorImage®LS Software

ThorImageLS is an open-source image acquisition program that controls Thorlabs' microscopes, as well as supplementary external hardware. From prepared-slice multiphoton Z-stacks to simultaneous *in vivo* photoactivation and imaging, ThorImageLS provides an integrated, modular workspace tailored to the individual needs of the scientist. Its workflow-oriented interface supports single image, Z-stacks, time series, and image streaming acquisition, visualization, and analysis. See the video to the lower right for a real-time view of data acquisition and analysis with ThorImageLS.

ThorImageLS is included with a Thorlabs microscope purchase and open source, allowing full customization of software features and performance. ThorImageLS also includes Thorlabs' customer support and regular software updates to continually meet the imaging demands of the scientific community.

For additional details, see the full web presentation.

OPEN SOURCE

The full source code for ThorImage®LS is available for owners of a Bergamo®, Cerna®, Veneto® or confocal microscope. Click here to receive your copy.

Advanced Software Functionality

- Multi-Column Customizable Workspace
- Image Acquisition Synced with Hardware Inputs and Timing Events
- Live Image Correction and ROI Analysis
- Independent Galvo-Galvo and Galvo-Resonant Scan Areas and Geometries
- Tiling for High-Resolution Large-Area Imaging
- Independent Primary and Secondary Z-Axis Control for Fast Deep-Tissue Scans
- Automated Image Capture with Scripts
  - Compatible with ImageJ Macros
- Multi-User Settings Saved for Shared Workstations
- Individual Colors for Detection Channels Enable Simple Visual Analysis

Seamless Integration with Experiments

- Simultaneous Multi-Point Photoactivation and Imaging with Spatial Light Modulator
- Fast Z Volume Acquisition with PFM450E or Third-Party Objective Scanners
- Electrophysiology Signaling
- Wavelength Switching with Tiberius® Laser or Coherent Chameleon Lasers
- Pockels Cell ROI Masking
- Power Ramped with Depth to Minimize Damage and Maximize Signal-to-Noise

New Functionality: Version 4.0 (Click to Expand for More Details)

Please contact ImagingTechSupport@thorlabs.com to obtain the latest ThorImageLS version compatible with your microscope. Because ThorImageLS 4.0 adds significant new features over 3.x, 2.x and 1.x versions, it may not be compatible with older microscopes. We continue to support older software versions for customers with older hardware. See the full web presentation for functionality of previous versions.

Contact Us

New Hardware Support

- Added Support for Windows® 10 OS
- Added Support for CS895MU and CS505MU Monochrome Cameras (Requires ThorCAM 3.2)
  - Allows for Hot Pixel Correction
- Added Support for CSN210 Motorized Dual-Objective Nosepiece
  - Allows for Improved Objective Setup and Control
- Added Support for Secondary Three Channel Controller
- Added Support for Second LED of the DC2200 LED Driver
- Added Support for New Version of Thorlabs' Tiberius® Femtosecond Ti:Sapphire Laser (Up to 1060 nm)
- Added Support for Second Channel for GGNI (Allows for Sequential Imaging with 2 Channels)
- Added Support for Controlling Up to 6 Digital Shutters (ThorShutterDig)
- Added Support for Resonant-Galvo-Galvo Scan-Head (Galvo-Resonant or Gavlo-Galvo Scan Modes Only)
- Added Support for Coherent® Discovery with AOM Support (Requires Coherent® Discovery GUI

New Features

- Added Ability to Save Experiment Data in Multi-Page TIFF Format (OME TIFF)
- Added Rapid Image Update for Galvo-Galvo Scanner
  - Updates Image Every 16 Scan Lines During Acquisition
- Added Galvo-Galvo External Trigger Sync (Minimum 1 MHz) (GGNI Not Supported)
- Added Improved Galvo-Galvo and Galvo-Resonant Triggering Times
- Added Ability to Read Resonant Frequency Probe
- Added Configurable Trigger Output (Signal Generator) Based on Time or Other Digital Events
- Added Auto Update for Histograms
- Added Dedicated Bleach Shutter Control for Galvo-Galvo and GGNI
- Added Stimulation Epoch Control
- Added Additional Stimulation Features (Pre Idle, Post Idle) and Control Lines (Active, Cycle Output, Epoch)
- Added SLM Multiple Epoch Control (Random Epoch)
- Added Ability to Invert Z Control's Plus and Minus Buttons (Supports Both Primary and Secondary Z

Version 1.8.3 and 3rd Party Virtual Serial Port Software)

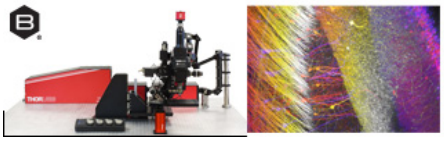
User Interface (UI) Improvements

- Renamed “Bleaching” to “Stimulation”
- Added Scale Bar in Image
- Added Help Menu Features
    - Allows User to Check for Updates
    - Allows User to View Log File for Trouble-Shooting
- Added Shortcuts to Hardware Settings and Application Settings in Hardware Connections Window and Edit Under Settings Menu
- Changed Capture Preview of Image to Show Averaged if Cumulative Mode is Used
- Added Control Digital Switches within Script
- Updated Digital Switch Configuration to be Saved in Experiment Settings and Viewable in Experiment Settings Browser
- Updated Extend Filing Numbering Index Out to 6 Digits

Controllers)

- Added Ability to Display X and Y Positions in Microns or Millimeters
- Added BCM-PA Slider Step Size
    - Allows for Setting Slide Step Size When Using Slider Plus and Minus Buttons for Power Adjustment.
- Added Auto Saving of Changed Fine Alignment Values
- Added Ability to Save Image Location and Zoom Level When Switching Image Modalities
- Added ThorSync Changes
    - Stack Panel Option
    - Virtual Channel

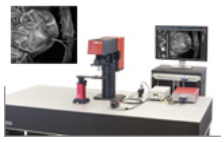
Supported Imaging Platforms



Bergamo® II Multiphoton Microscopes

DIY Multiphoton Microscope Kits

Veneto® Inverted Microscopes



Confocal Imaging Systems

Hyperspectral Imaging System

Laser Scanning Microscopy Tutorial

Laser scanning microscopy (LSM) is an indispensable imaging tool in the biological sciences. In this tutorial, we will be discussing confocal fluorescence imaging, multiphoton excitation fluorescence imaging, and second and third harmonic generation imaging techniques. We will limit our discussions to point scanning of biological samples with a focus on the technology behind the imaging tools offered by Thorlabs.

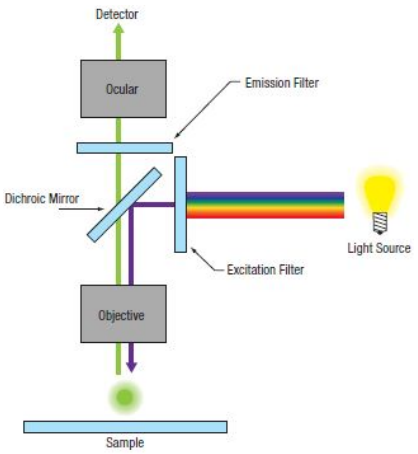
Introduction

The goal of any microscope is to generate high-contrast, high-resolution images. In much the same way that a telescope allows scientists to discern the finest details of the universe, a microscope allows us to observe biological functioning at the nanometer scale. Modern laser scanning microscopes are capable of generating multidimensional data (X, Y, Z, τ, λ), leading to a plethora of high-resolution imaging capabilities that further the understanding of underlying biological processes.

In conventional widefield microscopy (Figure 1, below left), high-quality images can only be obtained when using thin specimens (on the order of one to two cell layers thick). However, many applications require imaging of thick samples, where volume datasets or selection of data from within a specific focal plane is desired. Conventional widefield microscopes are unable to address these needs.

LSM, in particular confocal LSM and multiphoton LSM, allows for the visualization of thin planes from within a thick bulk sample, a technique known as optical sectioning. In confocal LSM, signals generated by the sample outside of the optical focus are physically blocked by an aperture, preventing their detection. Multiphoton LSM, as we will discuss later, does not generate any appreciable signal outside of the focal plane. By combining optical sectioning with incremented changes in focus (Figure 2, below right), laser scanning microscopy techniques can recreate 3D representations of thick specimen.

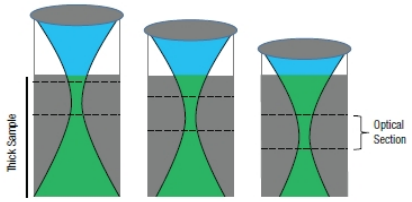
Figure 1 Widefield Epi-Fluorescence



Click to Enlarge

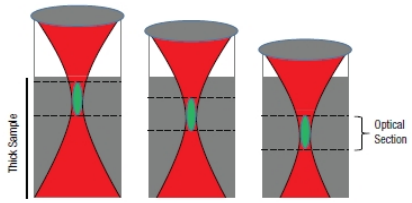
Figure 2 Optical Sections (Visualization of Thin Planes within a Bulk Sample)

Optical Sectioning in Confocal Microscopy



Click to Enlarge

Optical Sectioning in Multiphoton Microscopy



Click to Enlarge

Signal generated by the sample is shown in green. Optical sections are formed by discretely measuring the signal generated within a specific focal plane. In confocal LSM, out-of-focus light is rejected through the use of a pinhole aperture, thereby leading to higher resolution. In multiphoton LSM, signal is only generated in the focal volume. Signal collected at each optical section can be reconstructed to create a 3D image.

Contrast Mechanisms in LSM

Biological samples typically do not have very good contrast, which leads to difficulty in observing the boundaries between adjacent structures. A common method for improving contrast in laser scanning microscopes is through the use of fluorescence.

In fluorescence, a light-emitting molecule is used to distinguish the constituent of interest from the background or neighboring structure. This molecule can already exist within the specimen (endogenous or auto-fluorescence), be applied externally and attached to the constituent (chemically or through antibody binding), or transfected (fluorescent proteins) into the cell.

In order for the molecule to emit light (fluoresce) it must first absorb light (a photon) with the appropriate amount of energy to promote the molecule from the ground state to the excited state, as seen in Figure 3A below. Light is emitted when the molecule returns back down to the ground state. The amount of fluorescence is proportional to the intensity (I) of the incident laser, and so confocal LSM is often referred to as a linear imaging technique. Natural losses within this relaxation process require that the emitted photon have lower energy—that is, a longer wavelength—than the absorbed photon.

Multiphoton excitation (Figure 3B, below) of the molecule occurs when two (or more) photons, whose sum energy satisfies the transition energy, arrive simultaneously. Consequently, the two arriving photons will be of lower energy than the emitted fluorescence photon.

There are also multiphoton contrast mechanisms, such as harmonic generation and sum frequency generation, that use non-absorptive processes. Under conditions in which harmonic generation is allowed, the incident photons are simultaneously annihilated and a new photon of the summed energy is created, as illustrated in Figure 3C below.

Further constituent discrimination can be obtained by observing the physical order of the harmonic generation. In the case of second harmonic generation (SHG), signal is only generated in constituents that are highly ordered and lacking inversion symmetry. Third harmonic generation (THG) is observed at boundary interfaces where there is a refractive index change. Two-photon excitation and SHG are nonlinear processes and the signal generated is dependent on the square of the intensity (I^2).

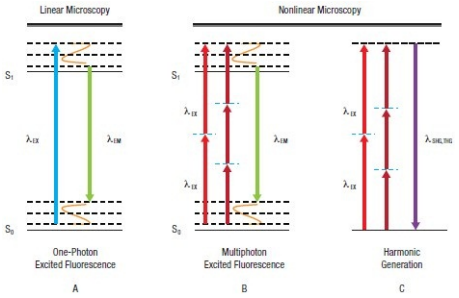
The nonlinear nature of signal generation in multiphoton microscopy means that high photon densities are required to observe SHG and THG. In order to accomplish this while maintaining relatively low average power on the sample, mode-locked femtosecond pulsed lasers, particularly Ti:Sapphire lasers, have become the standard.

Another consideration to be made in nonlinear microscopy is the excitation wavelength for a particular fluorophore. One might think that the ideal excitation wavelength is twice that of the one-photon absorption peak. However, for most fluorophores, the excited state selection rules are different for one- and two-photon absorption.

This leads to two-photon absorption spectra that are quite different from their one-photon counterparts. Two-photon absorption spectra are often significantly broader (can be >100 nm) and do not follow smooth semi-Gaussian curves. The broad two-photon absorption spectrum of many fluorophores facilitates excitation of several fluorescent molecules with a single laser, allowing the observation of several constituents of interest simultaneously.

All of the fluorophores being excited do not have to have the same excitation peak, but should overlap each other and have a common excitation range. Multiple fluorophore excitation is typically accomplished by choosing a compromising wavelength that excites all fluorophores with acceptable levels of efficiency.

Figure 3 Signal Generation in Laser Scanning Microscopy



Click to Enlarge

Absorptive Process (A, B):

The absorption of one or more excitation photons (λ_{EX}) promotes the molecule from the ground state (S_0) to the excited state (S_1). Fluorescence (λ_{EM}) is emitted when the molecule returns to the ground state.

Non-Absorptive Process (C):

The excitation photons (λ_{EX}) simultaneously convert into a single photon ($\lambda_{SHG,THG}$) of the sum energy and half (for SHG) or one-third (for THG) the wavelength.

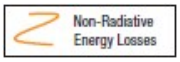


Image Formation

In a point-scanning LSM, the single-plane image is created by a point illumination source imaged to a diffraction-limited spot at the sample, which is then imaged to a point detector. Two-dimensional *en face* images are created by scanning the diffraction-limited spot across the specimen, point by point, to form a line, then line by line in a raster fashion.

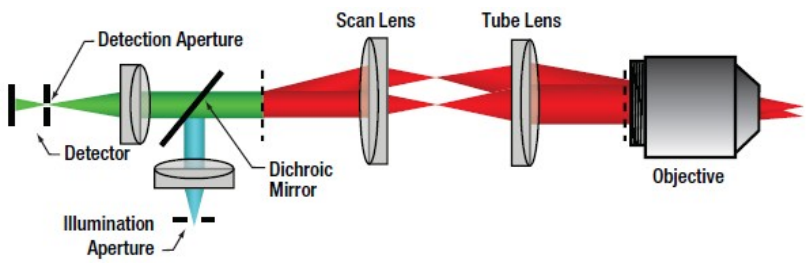
The illuminated volume emits a signal which is imaged to a single-element detector. The most common single-element detector used is a photomultiplier tube (PMT), although in certain cases, avalanche photodiodes (APDs) can be used. CCD cameras are not typically used in point-scanning microscopes, though are the detector of choice in multifocal (i.e. spinning disk confocal) applications.

The signal from the detector is then passed to a computer which constructs a two-dimensional image as an array of intensities for each spot scanned across the sample. Because no true image is formed, LSM is referred to as a digital imaging technique. A clear advantage of single-point scanning and single-point detection is that the displayed image resolution, optical resolution, and scan field can be set to match a particular experimental requirement and are not predefined by the imaging optics of the system.

Confocal LSM

In confocal LSM, point illumination, typically from a single mode, optical-fiber-coupled CW laser, is the critical feature that allows optical sectioning. The light emitted from the core of the single mode optical fiber is

Figure 4 Confocal Optical Path



collimated and
used as the illumination beam for scanning. The scan system is then imaged to the back aperture of the objective lens which focuses the scanned beam to a diffraction-limited spot on the sample. The signal generated by the focused illumination beam is collected back through the objective and passed through the scan system.

After the scan system, the signal is separated from the illumination beam by a dichroic mirror and brought to a focus. The confocal pinhole is located at this focus. In this configuration, signals that are generated above or below the focal plane are blocked from passing through the pinhole, creating the optically sectioned image (Figure 2, above). The detector is placed after the confocal pinhole, as illustrated in Figure 4 to the right. It can be inferred that the size of the pinhole has direct consequences on the imaging capabilities (particularly, contrast, resolution and optical section thickness) of the confocal microscope.

The lateral resolution of a confocal microscope is determined by the ability of the system to create a diffraction-limited spot at the sample. Forming a diffraction-limited spot depends on the quality of the laser beam as well as that of the scan optics and objective lens.

The beam quality is typically ensured by using a single mode optical fiber to deliver the excitation laser light as a Gaussian point source, which is then collimated and focused into a diffraction-limited beam. In an aberration-free imaging system, obtained by using the highest quality optical elements, the size of this focus spot, assuming uniform illumination, is a function of excitation wavelength (λ_{EX}) and numerical aperture (NA) of the objective lens, as seen in Equation 1.

$$\text{Spot Size} = \frac{1.22\lambda_{EX}}{\text{NA}}$$

Equation 1 Spot Size

In actuality, the beam isn't focused to a true point, but rather to a bullseye-like shape. The spot size is the distance between the zeros of the Airy disk (diameter across the middle of the first ring around the center of the bullseye) and is termed one Airy Unit (AU). This will become important again later when we discuss pinhole sizes.

The lateral resolution of the imaging system is defined as the minimum distance between two points for them to be observed as two distinct entities. In confocal (and multiphoton) LSM, it is common and experimentally convenient to define the lateral resolution according to the full width at half maximum (FWHM) of the individual points that are observed.

Using the FWHM definition, in confocal LSM, the lateral resolution ($R_{lateral,confocal}$) is:

$$R_{\text{lateral,confocal}} = \frac{0.51\lambda_{EX}}{\text{NA}}$$

Equation 2 Lateral Resolution, Confocal LSM

and the axial resolution ($R_{axial,confocal}$) is:

$$R_{\text{axial,confocal}} = \frac{0.88\lambda_{EX}}{n - \sqrt{n^2 - (\text{NA})^2}}$$

Equation 3 Axial Resolution, Confocal LSM

where *n* is the refractive index of the immersion medium.

It is interesting to note that in a confocal microscope, the lateral resolution is solely determined by the excitation wavelength. This is in contrast to widefield microscopy, where lateral resolution is determined only by emission wavelength.

To determine the appropriate size of the confocal pinhole, we multiply the excitation spot size by the total magnification of the microscope:

$$D_{\text{pinhole}} = M_{\text{objective}} \times M_{\text{scan head}} \times \text{Spot Size}$$

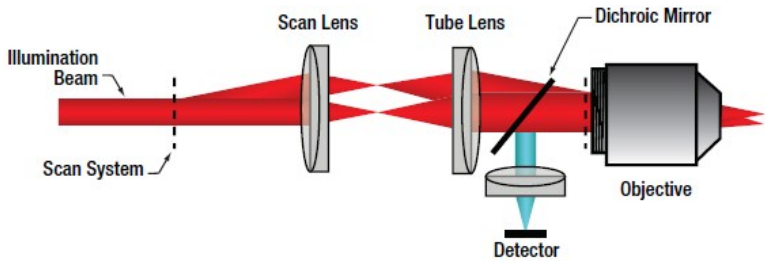
Equation 4 Pinhole Diameter

As an example, the appropriate size pinhole for a 60X objective with NA = 1.0 for λ_{EX} = 488 nm ($M_{scan\ head}$ = 1.07 for the Thorlabs Confocal Scan Head) would be 38.2 µm and is termed a pinhole of 1 AU diameter. If we used the same objective parameters but changed the magnification to 40X, the appropriate pinhole size would be 25.5 µm and would also be termed a pinhole of 1 AU diameter. Therefore, defining a pinhole diameter in terms of AU is a means of normalizing pinhole diameter, even though one would have to change the pinhole selection for the two different objectives.

Theoretically, the total resolution of a confocal microscope is a function of the excitation illumination spot size and the detection pinhole size. This means that the resolution of the optical system can be improved by reducing the size of the pinhole. Practically speaking, as we restrict the pinhole diameter, we improve resolution and confocality, but we also reduce the amount of signal reaching the detector. A pinhole of 1 AU is a good balance between signal strength, resolution, and confocality.

Multiphoton LSM

Figure 5 Multiphoton Optical Path



In multiphoton LSM, a short pulsed free-space laser supplies the collimated illumination beam that passes through the scanning system and is focused by the objective. The very low probability of a multiphoton absorption event occurring, due to the I^2 dependence of the signal on incident power, ensures signal is confined to the focal plane of the objective lens. Therefore, very little signal is generated from the regions above and below the focal plane. This effective elimination of out-of-focus signal provides inherent optical sectioning capabilities (Figure 2, above) without the need for a confocal pinhole. As a result of this configuration, the collected signal does not have to go back through the scanning system, allowing the detector to be placed as close to the objective as possible to maximize collection efficiency, as illustrated in Figure 5 to the right. A detector that collects signal before it travels back through the scan system is referred to as a non-descanned detector.

Again using the FWHM defintion, in multiphoton LSM, the lateral resolution ($R_{lateral,multiphoton}$) is:

$$R_{\text{lateral,multiphoton,NA}>0.7} = \frac{0.383\lambda_{EX}}{(\text{NA})^{0.91}}$$

Equation 5 Lateral Resolution, Multiphoton LSM

and the axial resolution ($R_{axial,multiphoton}$) is:

$$R_{\text{axial,multiphoton,NA}>0.7} = \frac{0.626\lambda_{EX}}{n - \sqrt{n^2 - (\text{NA})^2}}$$

Equation 6 Axial Resolution, Multiphoton LSM

These equations assume an objective NA > 0.7, which is true of virtually all multiphoton objectives.

The longer wavelength used for multiphoton excitation would lead one to believe (from Equation 5) that the resolution in multiphoton LSM, compared to confocal LSM, would be reduced roughly by a factor of two. For an ideal point object (i.e. a sub-resolution size fluorescent bead) the I^2 signal dependence reduces the effective focal volume, more than offsetting the 2X increase in the focused illumination spot size.

We should note that the lateral and axial resolutions display a dependence on intensity. As laser power is increased, there is a corresponding increase in the probability of signal being generated within the diffraction-limited focal volume. In practice, the lateral resolution in a multiphoton microscope is limited by how tightly the illumination beam can be focused and is well approximated by Equation 5 at moderate intensities. Axial resolution will continue to degrade as excitation power is increased.

Image Display

Although we are not directly rendering an image, it is still important to consider the size of the image field, the number of pixels in which we are displaying our image (capture resolution) on the screen, and the lateral resolution of the imaging system. We use the lateral resolution because we are rendering an *en face* image. In order to faithfully display the finest features the optical system is capable of resolving, we must appropriately match resolution (capture and lateral) with the scan field. Our capture resolution must, therefore, appropriately sample the optical resolution.

In LSM, we typically rely on Nyquist sampling rules, which state that the pixel size should be the lateral resolution divided by 2.3. This means that if we take our 60X objective from earlier, the lateral resolution is 249 nm (Equation 2) and the pixel size in the displayed image should be 108 nm. Therefore, for a 1024 x 1024 pixel capture resolution, the scan field on the specimen would be ~111 µm x 111 µm. It should be noted that the 40X objective from our previous example would yield the exact same scan field (both objectives have the same NA) in the sample. The only difference between the two images is the angle at which we tilt our scanners to acquire the image.

It may not always be necessary to render images with such high resolution. We can always make the trade-off of image resolution, scan field, and capture resolution to create a balance of signal, sample longevity, and resolution in our images.

Considerations in Live Cell Imaging

One of LSM's greatest attributes is its ability to image living cells and tissues. Unfortunately, some of the by-products of fluorescence can be cytotoxic. As such, there is a delicate balancing act between generating high-quality images and keeping cells alive.

One important consideration is fluorophore saturation. Saturation occurs when increasing the laser power does not provide the expected concurrent increase in the fluorescence signal. This can occur when as few as 10% of the fluorophores are in the excited state.

The reason behind saturation is the amount of time a fluorophore requires to relax back down to the ground state once excited. While the fluorescence pathways are relatively fast (hundreds of ps to a few ns), this represents only one relaxation mechanism. Triplet state conversion and nonradiative decay require significantly longer relaxation times. Furthermore, re-exciting a fluorophore before it has relaxed back down to the ground state can lead to irreversible bleaching of the fluorophore. Cells have their own intrinsic mechanisms for dealing with the cytotoxicity associated with fluorescence, so long as excitation occurs slowly.

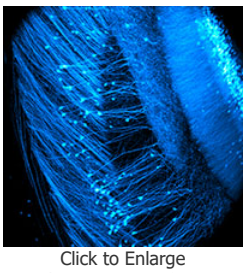
One method to reduce photobleaching and the associated cytotoxicity is through fast scanning. While reducing the amount of time the laser spends on a single point in the image will proportionally decrease the amount of detected signal, it also reduces some of the bleaching mechanisms by allowing the fluorophore to completely relax back to the ground state before the laser is scanned back to that point. If the utmost in speed is not a critical issue, one can average several lines or complete frames and build up the signal lost from the shorter integration time.

The longer excitation wavelength and non-descanned detection ability of multiphoton LSM give the ability to image deeper within biological tissues. Longer wavelengths are less susceptible to scattering by the sample because of the inverse fourth power dependence (I^{-4}) of scattering on wavelength. Typical penetration depths for multiphoton LSM are 250 - 500 µm, although imaging as deep as 1 mm has been reported in the literature, compared to ~100 µm for confocal LSM.

Thorlabs recognizes that each imaging application has unique requirements.
If you have any feedback, questions, or need a quotation, please contact
ImagingSales@thorlabs.com or call (703) 651-1700.

Multiphoton Microscope Kit with Galvo-Galvo Scanner

- Galvo-Galvo Scanner for User-Defined Scan Geometries
- Small Footprint with Large Throat Depth
- 1/4" (M6) Slots to Mount to an Optical Table (Not Included)
- Customize with User Supplied:
  - Laser
  - Objective
  - Scientific Camera



Click to Enlarge
Two-Photon Image of Neurons Expressing Thy1-YFP in a Cleared Region of the Hippocampus. (Courtesy of the 2017 Imaging Structure and Function in the Nervous System Course at Cold Spring Harbor Laboratory, Cold Spring Harbor, NY)

Thorlabs' MM101 Multiphoton Microscope Kit includes a galvo-galvo scanner, AR-coated scan lens for the visible and NIR wavelength ranges, epi-detection module, and a single GaAsP PMT. A single-objective holder accepts objectives with M32 x 0.75 threads. Brass thread adapters are included for use with RMS-, M25 x 0.75-, or SM1-threaded components. The sample stage provides 1" of motorized XY translation to adjust the sample position. The MM101 kit also includes a computer with DAQ and the ThorImage®LS data acquisition software.

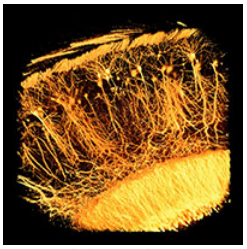
This microscope kit allows the users to build their own microscope and customize with their own illumination source, objective, and scientific camera. The 95 mm dovetail on the microscope body and female D1N dovetail on top of the scan path allow the user to add additional components, such as a camera tube and scientific camera for widefield imaging. We offer an option to include the Tiberius® fs Tunable Femtosecond Ti:Sapphire Laser with the kit; please contact ImagingSales@thorlabs.com or call (703) 651-1700 to request this laser.

The microscope body features a large 12.41" (315.2 mm) throat depth. To achieve this large free space beneath and around the objective, the microscope is not designed to be free-standing and must be bolted to an optical table. For this purpose, 1/4"-20 and M6 cap screws are included to provide compatibility with both metric and imperial optical tables.

Part Number	Description	Price	Availability
MM101	**DIY Multiphoton Microscope Kit, Galvo-Galvo Scanner**	**$84,525.99**	**Lead Time**

Multiphoton Microscope Kit with Galvo-Resonant Scanner

- Galvo-Resonant Scanner for High-Speed Imaging
- Small Footprint with Large Throat Depth
- 1/4" (M6) Slots to Mount to an Optical Table (Not Included)
- Customize with User Supplied:
  - Laser
  - Objective
  - Scientific Camera



Click to Enlarge
Two-Photon Image of Neurons Expressing Thy1-YFP in a Cleared Region of the Dentate Gyrus. (Courtesy of the 2017 Imaging Structure and Function in the Nervous System Course at Cold Spring Harbor Laboratory, Cold Spring Harbor, NY)

Thorlabs' MM201 Multiphoton Microscope Kit includes a galvo-resonant scanner, AR-coated scan lens for the visible and NIR wavelength ranges, epi-detection module, and a single GaAsP PMT. A single-objective holder accepts objectives with M32 x 0.75 threads. Brass thread adapters are included for use with RMS-, M25 x 0.75-, or SM1-threaded components. The sample stage provides 1" of motorized XY translation to adjust the sample position. The MM201 kit also includes a computer with DAQ and the ThorImage®LS data acquisition software.

This microscope kit allows the users to build their own microscope and customize with their own illumination source, objective, and scientific camera. The 95 mm dovetail on the microscope body and female D1N dovetail on top of the scan path allow the user to add additional components, such as a camera tube and scientific camera for widefield imaging. We offer an option to include the Tiberius® fs Tunable Femtosecond Ti:Sapphire Laser with the kit; please contact ImagingSales@thorlabs.com or call (703) 651-1700 to request this laser.

The microscope body features a large 12.41" (315.2 mm) throat depth. To achieve this large free space beneath and around the objective, the microscope is not designed to be free-standing and must be bolted to an optical table. For this purpose, 1/4"-20 and M6 cap screws are included to provide compatibility with both metric and imperial optical tables.

For the MM201 DIY Galvo-Resonant Microscope Kit, the typical lead time is approximately 3 weeks from when the order is placed to when the kit is shipped out of Thorlabs' Newton, NJ Warehouse.

Part Number	Description	Price	Availability
MM201	**DIY Multiphoton Microscope Kit, Galvo-Resonant Scanner**	**$120,553.45**	**Lead Time**

Optical Table, Objective and Routing Optics Not Included