THORLABS

Imaging Systems

Multiphoton Microscopy Systems

Image Fast Image Deep

Multiphoton Microscope

Multiphoton microscopy is the method of choice for imaging thick, highly light scattering, living biological samples. The MPM200 series of Multiphoton Microscopes represents an ideal platform for a variety of imaging needs. These systems excel at imaging samples as small as tissue sections and as large as whole animals.

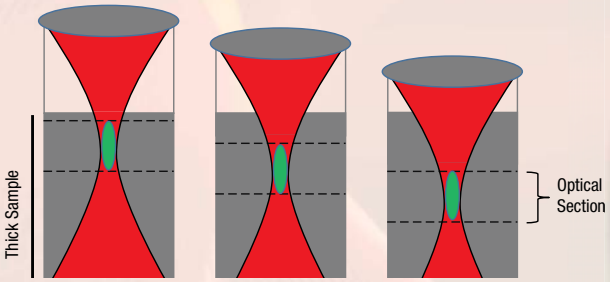
Thorlabs' MPM200 Multiphoton Microscopes are capable of providing multimodal solutions from widefield transmitted light microscopy, to epi-fluorescence imaging, to sophisticated multiphoton techniques, allowing the highest levels of detail to be extracted from data. Thorlabs offers a complete product line ranging from objectives to anti-vibration work stations, allowing you to create the ideal system to meet your imaging needs and budget.

Features

- Broadband Excitation Path: 680 – 1400 nm
- High Speed: 30 Frames per Second (at 512 x 512 Pixel Resolution)
- Full Field of View, Non-Descanned Detectors
- Supports Low Magnification/High NA Physiology Objectives
- Two-Channel, Four-Channel, and Four-Channel-Ready Systems Available
- Intuitive Image Acquisition Software

MPM200-4 Four-Channel Multiphoton System shown on a PHYS24M Physiology Stage with MPM-BCU Beam Conditioner and COMP6300 Dispersion Pre-Compensation Units, all placed on a 5' x 6' SDA150180 ScienceDesk™.

Optical Sectioning in Multiphoton Microscopy

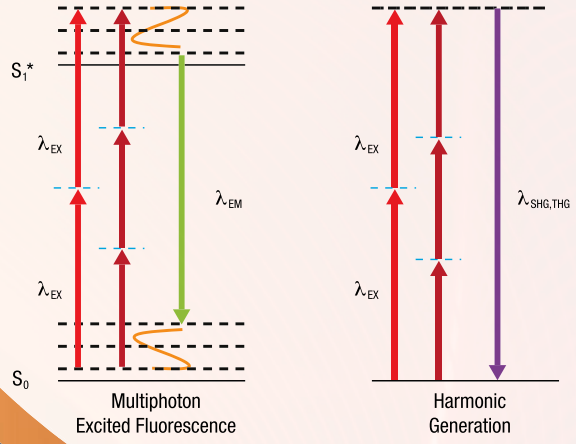


Two-photon excitation and second harmonic generation (SHG) are nonlinear processes where the signal generated is dependent on the square of the intensity (I^2), whereas for three-photon excitation and third harmonic generation (THG), signal is dependent on the cube of the laser intensity (I^3). The nonlinear nature of signal generation requires the high photon densities provided by focusing femtosecond pulses to a diffraction-limited spot. This dictates that the signal generated is mostly confined to the focal plane of the objective. Therefore, optically thin images from within thick samples are obtained. Three-dimensional reconstrctions are made by stepping the objective focus deeper into the sample.

Theory of Multiphoton Excitation

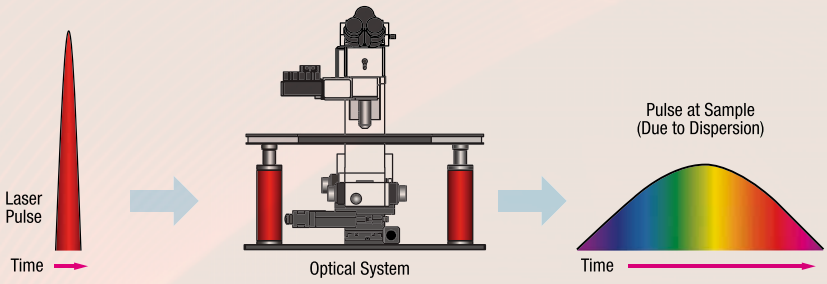
Multiphoton excitation occurs when two or more photons whose sum energy satisfies the transition energy arrive at a fluorophore simultaneously. Multiphoton techniques are also capable of contrast mechanisms using non-absorptive processes. Under conditions in which second and third harmonic generation (SHG and THG, respectively) are allowed, the incident photons are simultaneously annihilated and a new photon of the summed energy is created.

Multiphoton Microscopy



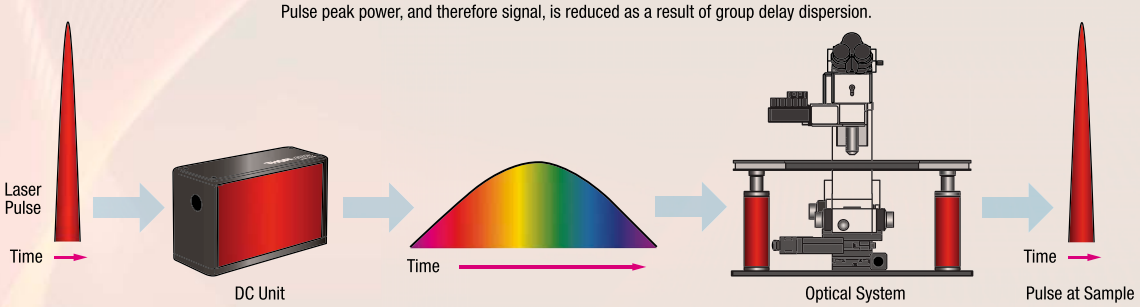
Compensation of GDD

Ultra-fast laser pulses experience group delay dispersion (GDD) as they propagate through components of an optical system.



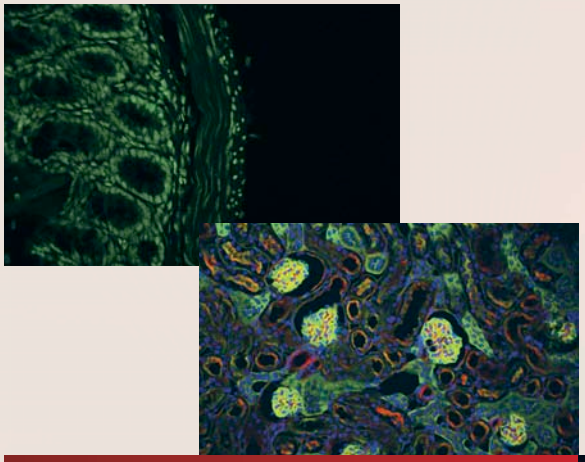
Without Dispersion Compensation

Pulse broadening results in the phase of longer wavelengths being advanced ahead of shorter wavelengths. Pulse peak power, and therefore signal, is reduced as a result of group delay dispersion.



With Dispersion Compensation

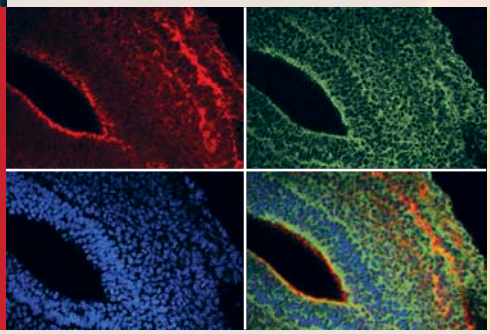
A dispersion compensating (DC) unit negates GDD by advancing the phase of shorter wavelengths relative to the longer wavelengths. As the pulse propagates through the optical system of the microscope, negative pulse broadening of the DC unit is cancelled by the positive pulse broadening of the microscope. The optical system then recompresses the pulse to reconstruct the original pulse from the laser at the sample.

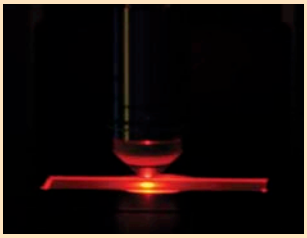


Deep Tissue Imaging

Imaging depth in multiphoton microscopy is primarily limited by the scattering of both the excitation laser light and emission signal by the sample. The extended excitation range of the MPM200 allows the use of laser sources at wavelengths longer than 1 µm. Longer wavelength photons are scattered less frequently by the sample, and thus, more photons are able to reach fluorophores deep within the sample, which improves the maximum imaging depth. In addition, the MPM200 utilizes a full field of view, non-descanned detection scheme with GaAsP PMTs positioned directly behind the objective so that both unscattered and scattered signal photons that pass through the objective are detected.

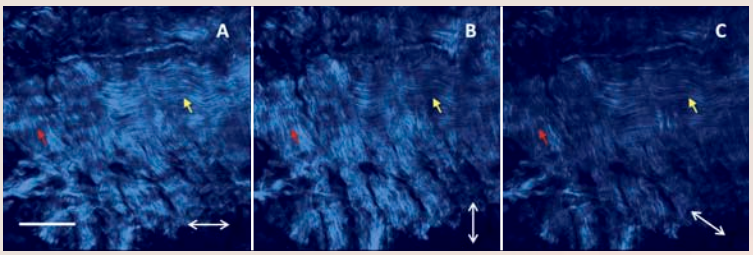
Multiphoton microscopy has become the method of choice for imaging thick, highly light-scattering living tissue…



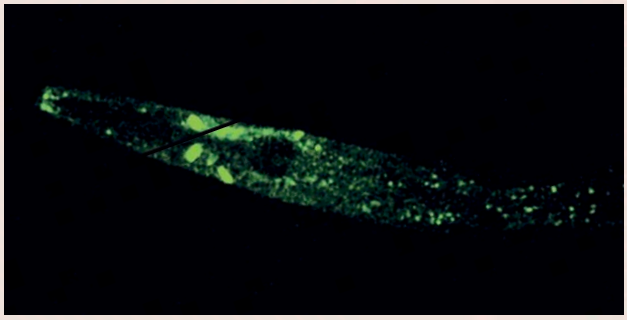


Label-Free Imaging

Through two- and three-photon excited fluorescence of endogenous fluorophores, sub-celluar structures can be observed without the use of externally applied fluorescent dyes. For example, two-photon excitation fluorescence images of NAD(P)H, retinol, or flavins can provide functional chemo-biological information or be used as structural markers. Second harmonic generation requires highly ordered structures that lack inversion symmetry. The most common constituent of biological tissue that satisfies these conditions is collagen, which can provide information on the extracellular matrix. Third harmonic generation can be observed at boundary interfaces and is useful in observing lipid bodies.



The image above shows second harmonic generation from collagen in chicken leg tendon. Although each image was taken at the same focal plane, the incident polarization was rotated as indicated by the double-ended arrow. As the laser polarization is rotated, greater signal strength can be seen from collagen fibrils that are better oriented with the incident polarization. The red arrows indicate the change in signal for vertically oriented fibers. The yellow arrows indicate the change in signal for longitudinally oriented fibers. Scale bar = 100 µm.

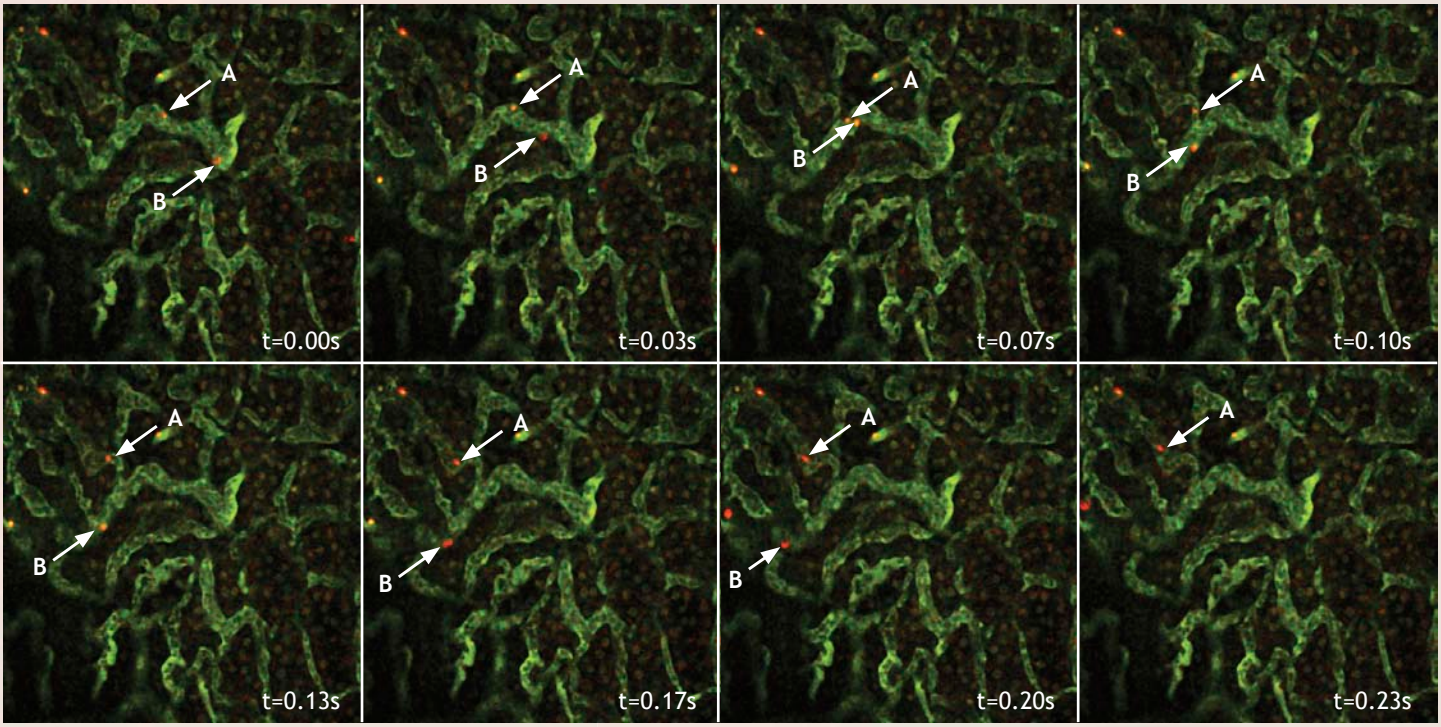


Multiphoton imaging of GFP fluorescence in dopamine receptors in C. Elegens. Olympus 20X 1.0 NA W. Sample provided by William Ryu, University of Toronto.

Live Cell Imaging

Multiphoton microscopy is well suited for imaging live cells over long periods of time. To reduce photobleaching during live cell imaging, high-speed scanning is advantageous. Thorlabs' multiphoton system achieves these high speeds by employing a galvo-resonant scanner pair.

While also minimizing photobleaching, the use of NIR two-photon excitation in multiphoton microscopy provides the additional benefits of facilitating deep penetration into the sample and lowering phototoxicity compared to single-photon excitation techniques. As a result, the multiphoton microscopy technique is well suited to imaging fluorescent proteins that can be expressed throughout the entire smaple and not just cells near the surface (see photo above).

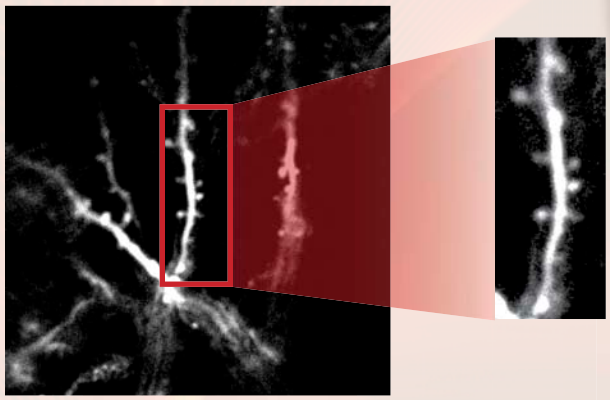


Real-Time Imaging of Dynamic Biological Processes:

Real-time *in vivo* perfusion monitoring of renal blood flow. Images from both channels were acquired simultaneously at 30 fps (512 x 512 pixels) using an Olympus 20X 1.0 NA objective (Thorlabs N20X-PFH). Blood was perfused with a mixture of FITC-Dextran (blood plasma, green) and 1 µm red fluorescent microspheres. Two individual microspheres (A & B) are tracked through the vasculature. High temporal resolution between subsequent frames allows for more accurate velocity measurements of individual particles to be obtained. Red blood cells are also observed as dark objects within the green blood plasma, providing an extra level of blood flow analysis. Images were collected during the IUPUI O'Brien Center Workshop on Applied Microscopy in Kidney Research. Preparation courtesy of Dr. Simon Rhodes.

High-Resolution Imaging

High-resolution imaging of structures deep within tissue is a cornerstone of multiphoton microscopy. Dendritic spines represent a challenging structure to image owing to their sub-micron size. The image below is a blown up view of dendritic spines from neurons in the mouse visual cortex expressing td-Tomato.



Dendritic Spine images collected with a Nikon 60X 1.0 NA (Thorlabs N60X-NIR) objective and the laser tuned to 1040 nm. Courtesy of Dr. Tobias Rose, Max Planck Institute for Neurobiology, Martinsreid Germany.

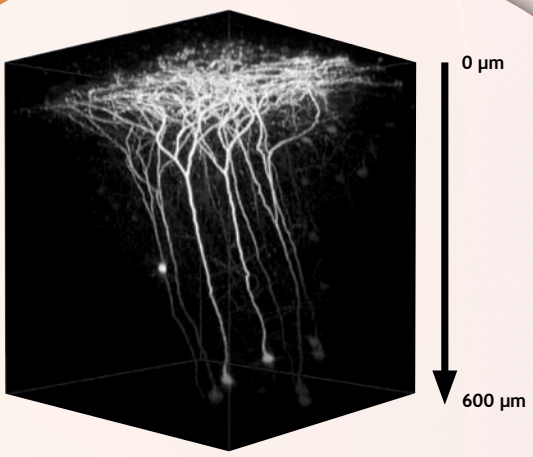


Image was collected with an Olympus 20X 1.0 NA (Thorlabs N20X-PFH) objective and the laser tuned to 1040 nm.

In Vivo Imaging

Deep tissue imaging is possible with multiphoton microscopy. The image above represents a 600 µm deep Z-Stack of neurons in the visual cortex of a mouse expressing td-Tomato. The length of the neurons can be traced from the dendrites through the cell body. Image courtesy of Dr. Tobias Rose, Max Planck Institute for Neurobiology, Martinsreid Germany.

MPM200-2 Two-Channel Multiphoton System

This system is well suited for a variety of biomedical imaging applications. The fast scanning of the MPM200 series allows for more data to be collected in less time, maintaining specimen viability over the course of the experiment. Two high-sensitivity, non-descanned detectors maximize signal detection efficiency to image deeper and with less photodamage. The dedicated multiphoton optical path allows a wide range of excitation sources and high NA objectives from a variety of manufacturers to be used with the system.

MPM200-2

MPM200-4

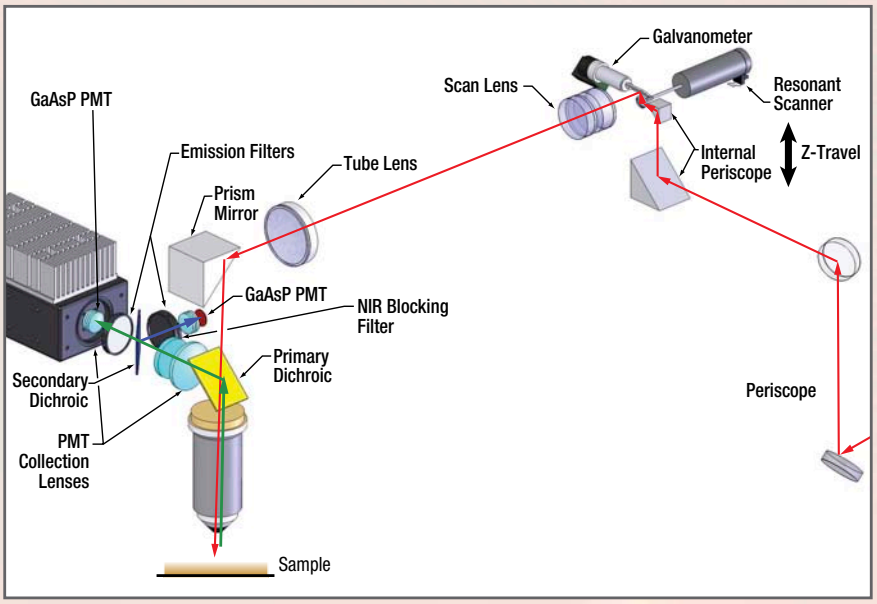
MPM200-4 Four-Channel Multiphoton System

The MPM200-4 system enhances the imaging capabilitiesof the MPM200-2 Multiphoton System by adding two PMTs that are located beneath the condenser in a Transmitted Light Detection Module (TLDM). The additional detection channels consist of two high-sensitivity GaAsP PMTs with full field-of-view collection optics. The sub-stage condenser lens acts as an opposing objective to efficiently collect the forward propagating signal. In addition to collecting fluorescence signals, the TLDM can be used for collecting second and third harmonic signals. A powerful 64 bit PC workstation* provides the computing power necessary to handle large quantities of high-speed multichannel data.

*Optional on the MPM200-2 as the MPM200-4R Four-Channel-Ready System

MPM200 Optical Path

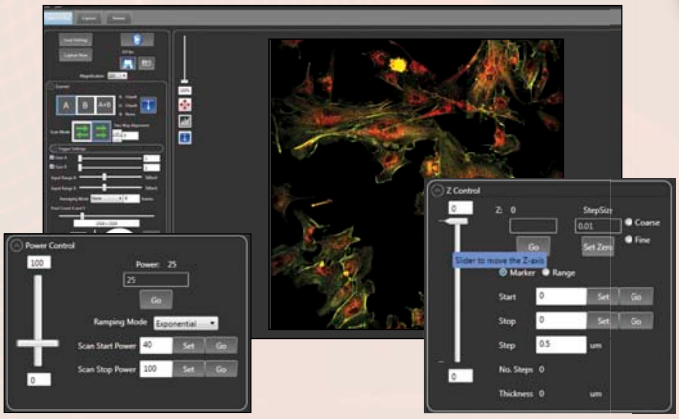
The MPM200 Series is specially designed to operate in the near-infrared wavelength range from 680 to 1400 nm, and hence, these systems are well suited for use with several types of laser excitation sources. The excitation light (red) is directed through a beam periscope to the scanning system. High-speed XY scanning is achieved using a galvo-resonant scanner pair. The scanning beam passes through dedicated scan and tube lenses. The emitted signal from the sample (green) is collected back through the objective and redirected to the non-descanned PMT detector module. The PMTs are placed immediately behind the objective to minimize light loss in the microscope body. The full field-of-view design allows light that has been scattered while exiting the sample but collected by the objective to reach the PMTs. An NIR blocking filter placed ahead of the secondary dichroic mirror prevents any scattered excitation laser light from reaching the PMTs.



The fluorescence filter cube consists of the secondary dichroic and two emission filters placed in front of each PMT. The entire optical system moves in unison with the objective to ensure the back aperture remains in the optimal location at all times.

Capture Setup

- Flexible Framework of Peripheral Control
  - Multiphoton Scan Head Control
  - Z-Stepper Motor Control
  - Photomultiplier Gain Control
  - Excitation Laser Control (Power & Wavelength)
  - Beam Size Control
- Real-Time Background and Flat Field Correction
- User Control of Detection Channels
- Flexible Image Size and Location Adjustment
- Save and Recall Experimental Settings in XML Format
- User-Selectable Color Assignments for Detection Channels

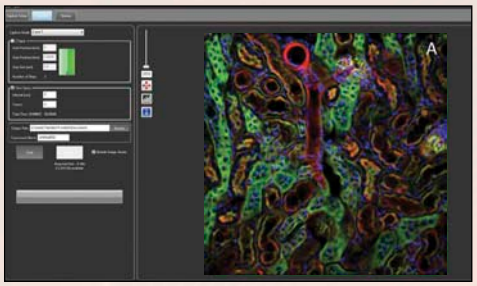


ThorImageLS™ Software

The easy-to-use ThorImageLS graphical interface enables users to quickly gather and review their data. The software coordinates the peripheral control and image acquisition for optimal data collection. Captured experiments can then be played back and converted into movies for publishing and sharing.

Software Development Kit Available
C++ and LabVIEW Libraries
Contact
ImagingSales@thorlabs.com
for more details.

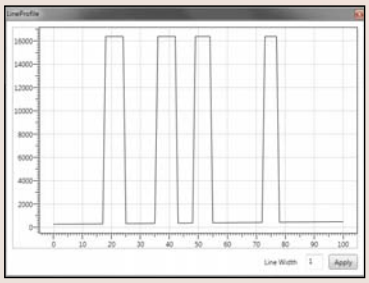
Capture

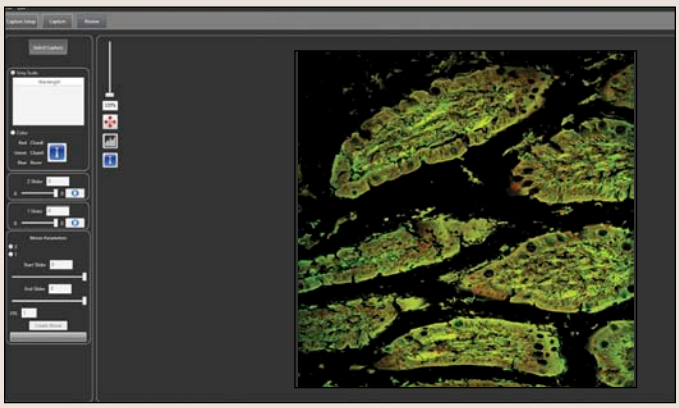


- Selectable Color Channels
  - Up to Four Simultaneous Channels
- Real-Time Capture
  - 30 Frames per Second (512 x 512 Pixels)
  - Streaming to Disk
- Z Volume Capture
- User-Defined Time Lapse
- Up to Five-Dimensional Data Collection
  - X, Y, Z, Time, and Color
- Hardware Triggering for Experiment Initiation
- Image Formats: JPEG, TIFF, and AVI Movies

Review

- Experiment Playback
- Image Histograms
- Region of Interest (ROI) Measurements
- Line Profile Measurements
- AVI Movie Creator
  - Compresses Image Sequences into an Easy-to-Share and Publish Format





Accessories

COMP6300

Disperion Compensation Unit

- Maintains Laser Pulse Width at the Sample
- Based on Chirped Mirror Technology
- Easy Drop-In Integration that Doesn't Require Tuning
- Collinear Input/Output

Beam Conditioner Unit

- Automated Control of Laser Power and Beam Diameter
- Fully Integrated into the ThorImageLS™ Software
- Automated Power Ramp Function for Creating Z-Stacks
- Stable Optical Alignment

MPM-BCU

Objectives for Multiphoton Microscopy

N20X-PFH

N16XLWD-PF

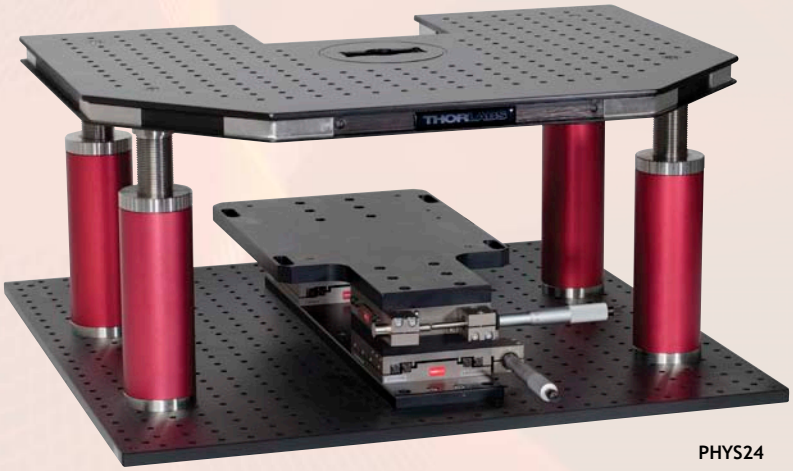
Thorlabs now offers a selection of physiology objectives especially suited for multiphoton imaging. These water immersion objectives have a high numerical aperture (NA) as well as a long working distance (WD). Additionally, they are designed to have a wide transmission and color correction range.

Item #	M	NA	WD	DESCRIPTION
N16XLWD-PF	16X	0.80	3.00 mm	Nikon Plan Fluor LWD 16XW
N20X-PFH	20X	1.00	2.00 mm	Olympus XLUMPLFLN 20XW
N40XLWD-NIR	40X	1.15	0.61 mm	Nikon Apo LWD Lambda S 40XW
N40X-NIR	40X	0.80	3.50 mm	Nikon NIR Apo 40XW
N60X-NIR	60X	1.00	2.80 mm	Nikon NIR Apo 60XW

Transmitted Light Detection Module

- Provides Two-Channel Forward Propagating Signal Collection
- Includes Two High-Sensitivity GaAsP PMTs and Easy Access Filter Cube
- Mounts Directly onto Nikon FN1 Base
- Signal can be Summed with the Back Scattered Detectors or Used Independently for Up to Four Channels of Detection

MPM-TLDM



PHYS24

Physiology Stage

- Adjustable-Height U-Shaped Breadboard Provides 270° Access to Samples
- Includes Microscope Translator
  - Manual Translation
  - Motorized Translation
  - Encoded Motorized Translation
- U-Shaped UltraLight™ Series Breadboard with Sealed Holes to Contain Spills
- Accommodates Many Types of Sample Chambers

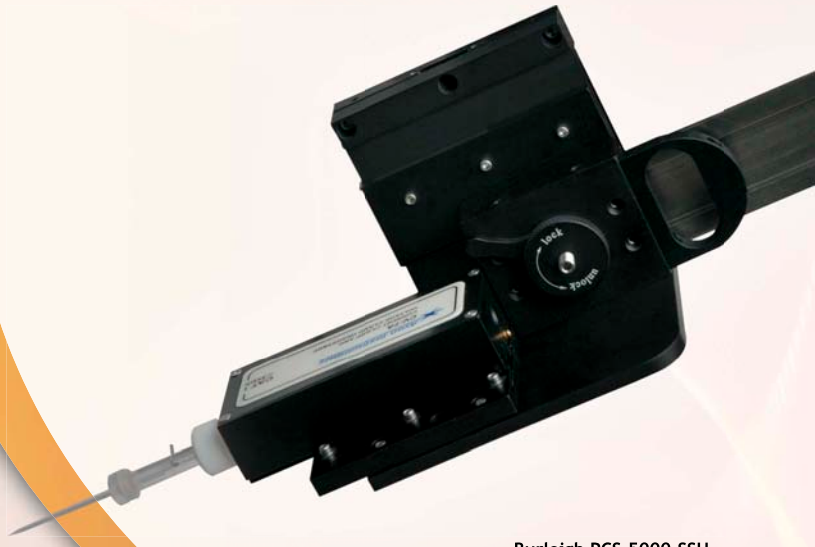
Burleigh® Micromanipulators

- Predictable Motion with Piezoelectric Technology
- Ultra-Precise Positioning Without Drift
- Superior Stability

Burleigh PCS-6000

Steep/Shallow Headstage Adapter

- Allows Patch Clamp Headstage and Pipette to be Oriented at Steep or Shallow Angles
- Simplifies and Speeds Up Pipette Exchange



Burleigh PCS-5000-SSH

Accessories Cont.

Nikon Epi-Fluorescence Illuminator

- Mounts on Top of Multiphoton Scan Head
- Does Not Interfere with Multiphoton Imaging
- Six-Position Filter Turret
- Compatible with Thorlabs' HPLS200 High-Power Light Source

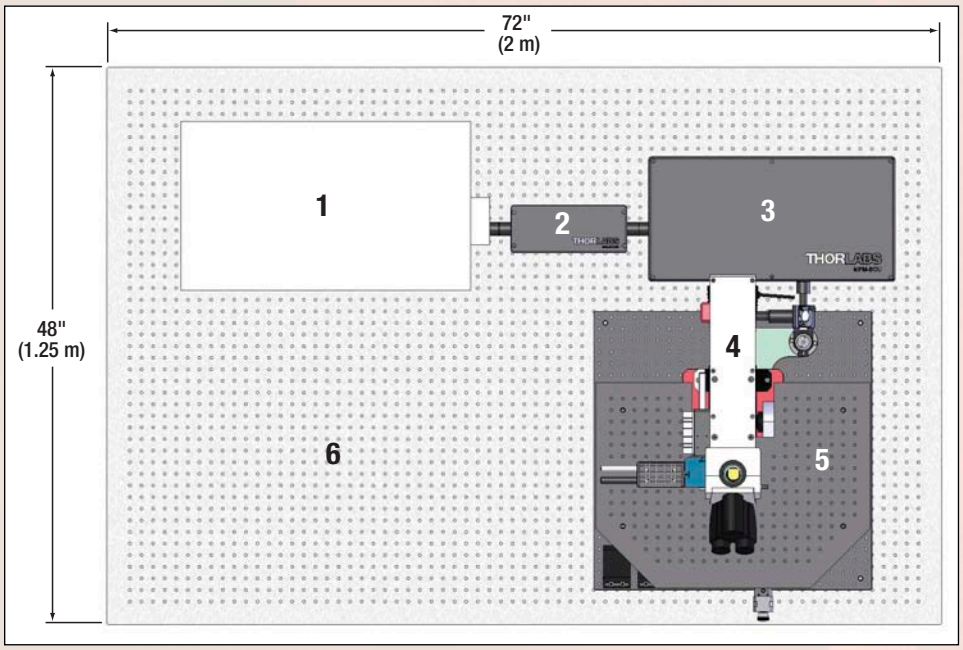
Fluorescence Light Source

- True White Light Output: 400 nm - 700 nm
- Lifetime Five Times Longer than Conventional Xenon Lamps (>10,000 Hours)
- USB or Front Panel Control
- Integrated Liquid Light Guide with Ø3 mm or Ø5 mm Core
- Easy Microscope Integration Using Available Collimation Packages

HPLS200

MPM 4' x 6' Table Schematic

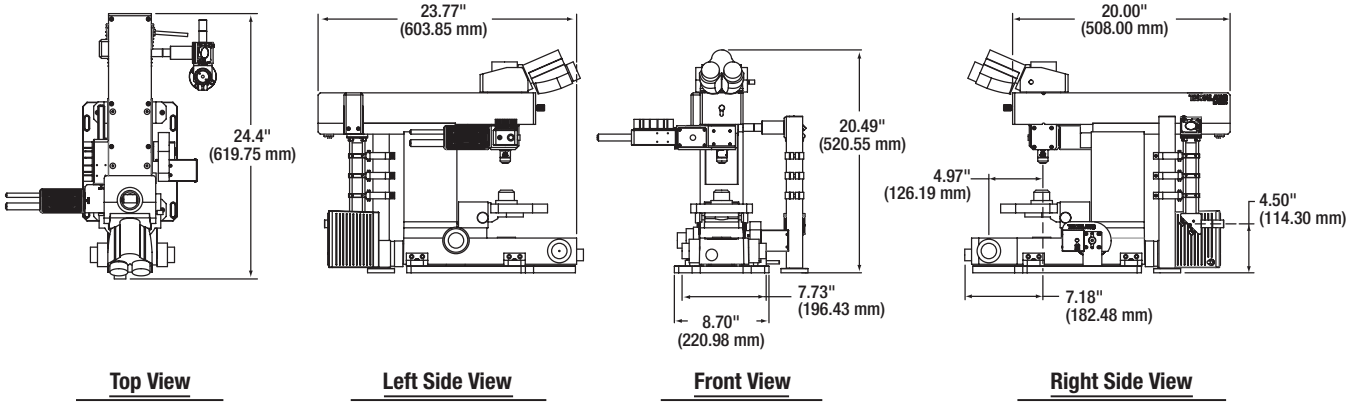
1. Ti:Sapphire Laser
2. COMP6300 Dispersion Compensating Unit
3. MPM-BCU Beam Conditioner Unit
4. MPM200 Multiphoton System
5. PHYS24M Motorized Physiology Stage
6. Thorlabs Ultra Series Optical Table



Specifications

Microscope	
Stand	Upright Nikon FN1
Recommended Objectives	Nikon LWD 16XW, 0.80 NA,WD = 3.0 mm; Nikon Apo 25XW, 1.10 NA,WD = 2.0 mm; Nikon NIR Apo 40XW, 0.80 NA, WD = 3.5 mm; Nikon NIR Apo 60XW, 1.0 NA, WD = 2.8 mm; Nikon Apo Lambda S LWD 40XW, 1.15 NA,WD = 0.61 mm; Nikon Apo Lambda S 40XW, 1.25 NA,WD = 0.18 mm; Nikon Plan Apochromat 60XW, 1.20 NA,WD = 0.27 mm; Olympus XLUMPLFLN 20XW, 1.0 NA,WD = 2.0 mm
Z-Drive	Minimum Step Size: 0.1 µm
XY Stage (Optional)	FN1 XY Rectangular Stage (Manual); XY Physiology Stage (Manual or Motorized)
Excitation	
Beam Conditioner	Variable Beam Expander (1X - 4X); Motorized Beam Attenuation (λ/2 Wave Plate and Polarizer);
Dispersion Pre-Compensation	-6300 fs^2
Wavelength Range	680 – 1400 nm
Objective Pupil Diameter	20 mm (Max)
Field of View	16 mm Diagonal Square (Max) at the Intermediate Plane 700 µm x 700 µm with Nikon 16X Objective at Sample
Scanner	X: 7.8 kHz Resonant Scanner Y: Galvanometric Scan Mirror
Scan Speed	30 fps @ 512 x 512 Pixels
Scan Zoom	1X to ~16X (Approximate)
Scan Resolution	Up to 2048 x 2048 Bi-Directional Acquisition Up to 4096 x 4096 Uni-Directional Acquisition
Scan Mode	Point XY Scan
Primary Dichroic	680 – 1600 nm Longpass
Detection	
Non-Descanned Detectors (NDD)	Two High-Sensitivity GaAsP PMTs Positioned Directly Behind the Objective or Condensor
PMT Sensitivity Wavelength Range	300 – 720 nm
Filter Cube	Single, User-Changeable

MPM200 Multiphoton Microscope Drawings



Top View **Left Side View** **Front View** **Right Side View**

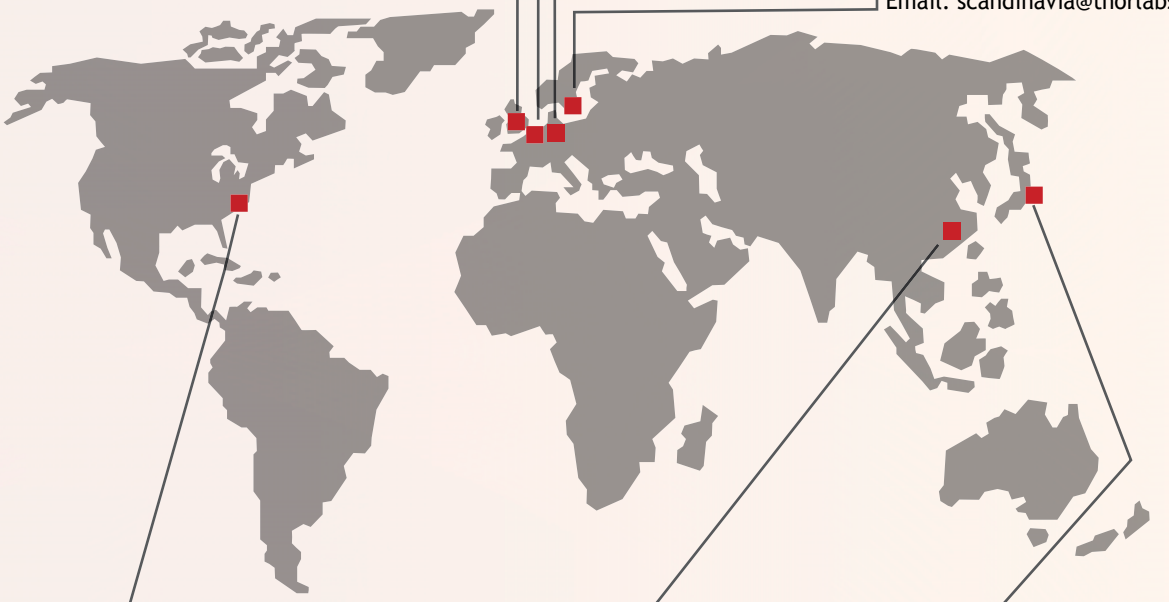
Operations and Facilities

Thorlabs SAS France
Phone : +33 (0) 970 444 844
Fax: +33 (0) 811 381 748
Email : sales.fr@thorlabs.com

Thorlabs GmbH Europe
Phone: +49 (0) 8131-5956-0
Fax: +49 (0) 8131-5956-99
Email: europe@thorlabs.com

Thorlabs Ltd. UK & Ireland
Phone: +44 (0)1353-654440
Fax: +44 (0)1353-654444
Email: sales.uk@thorlabs.com

Thorlabs Sweden AB Scandinavia
Phone: +46-31-733-30-00
Fax: +46-31-703-40-45
Email: scandinavia@thorlabs.com



Thorlabs Japan, Inc.
Phone: +81-3-5979-8889
Fax: +81-3-5979-7285
www.thorlabs.jp
Email: sales@thorlabs.jp

Thorlabs, Inc. The Americas
Phone: 1-973-300-3000
Fax: 1-973-300-3600
Email: sales@thorlabs.com

Thorlabs China
Phone: +86 (0)21-32513486
Fax: +86 (0)21-32513480
www.thorlabs.com
Email: chinasales@thorlabs.com

Contact Sales at ImagingSales@thorlabs.com to Schedule a Demo or Receive a Quotation