Multiphoton Microscopy Systems (Page 1 of 6)

Multiphoton Microscopy offers several advantages over other laser scanning techniques, particularly the ability to image deeper into a sample. The modular design of Thorlabs’ Multiphoton Microscopy Systems is flexible enough to suit both researchers looking to bring standard turnkey multiphoton technology into their laboratories as well as those desiring to build customized multiphoton systems from scratch. Three systems, which include both 2-channel and 4-channel variations, are detailed below.

The diverse product portfolio of Thorlabs gives us the unique ability to provide all the necessary pieces to create a turnkey multimodal imaging workstation. This includes the complete multiphoton imaging system, beam conditioner, physiology stage, ultrafast laser source, dispersion compensation, epi-fluorescence illuminator, fluorescence filters and filter cubes, anti-vibration tables, and beam diagnostics equipment.

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
- Two-Channel, Four-Channel, and Four-Channel-Ready Systems Available

Two-Channel Multiphoton System
Thorlabs’ MPM200-2 Two-Channel Multiphoton 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. The two high-sensitivity, non-descanned detectors maximize signal collection efficiency to image deeper and with less damage. An easy-to-change filter cube allows the user to select which wavelengths are directed to each detector.

Maximum resolution is ensured by using high-numerical-aperture objective lenses. The dedicated multiphoton optical path allows high NA objectives from a variety of manufacturers to be used with the system. In particular, low magnification, high-NA water dipping physiology objectives are well supported. The included ThorImageLS™ acquisition software controls all the necessary functions for capturing three-dimensional data. Thorlabs aims to offer the most versatile multiphoton microscope system on the market. We encourage customers to contact us at ImagingSales@thorlabs.com to discuss alternative upgrades or modifications to our systems.

Multiphoton System Accessories
Thorlabs offers a range of accessories for use with our multiphoton imaging systems, including physiology stages, a beam conditioner, and a dispersion compensation unit. Please see pages 1667 – 1677 for details.

Label-Free Imaging
Multiphoton systems can also be used for label-free imaging of biological tissues with an ordered structure. Some samples, like collagen-based samples, are naturally suited for two-photon microscopy because they can absorb two photons from the multiphoton system’s excitation laser and spontaneously re-emit a photon with double the frequency (Second Harmonic Generation, SHG). Due to this natural phenomenon, labeling dyes do not need to be used when imaging these samples.

This technique is used in a growing number of applications involving cellular membranes and intact tissue imaging, as well as material science applications. SHG created by myelin and collagen yields excellent extracellular structure determination without the need for an externally applied fluorophore. SHG microscopy has been used extensively in studies of the cornea and lamina cribrosa, structures that consist mostly of collagen.
Four-Channel Multiphoton System

Thorlabs’ Four-Channel Multiphoton System, an extension of our MPM200-2 Two-Channel Multiphoton System presented on the previous two pages, includes an additional transmitted light detection module (TLDM). This system provides a total of four detection channels, making it ideal for monitoring fluorescence resulting from multiphoton excitation as well as photons attributed to second and third harmonic generation.*

The TLDM allows the MPM200-4 to acquire four fluorescence channels simultaneously by using the sub-stage condenser lens as an opposing objective. If the same filter cube is placed in the back scattered detector as the TLDM, the forward propagating signal can be summed with the backscattered signal, in software, effectively increasing the signal-to-noise ratio of the resultant image. Alternatively, the backscattered detectors can detect two fluorescence signals, and the forward detectors can collect the signals that arise from second and third harmonic generation.

When not needed, the TLDM can slide forward and allows sample observation using the white-light wide-field illuminator.

Included with MPM200-4

- Nikon FN1 Microscope
- Four High-Sensitivity GaAsP PMTs
- Transmitted Light Detection Module (See Next Page for Details)
- Beam Delivery Periscope
- Z-Focus Motor
- Four-Channel Electronics and Dual Quad-Core (64-Bit) Computer with 24” Monitor
- ThorImageLS™ Acquisition Software (See Page 1666 for Details)
- Installation Included

*Third harmonic generation requires a laser excitation wavelength greater than 1.2 µm, which is well supported by the broad excitation wavelength range of the MPM200 series of multiphoton systems.

Deep Tissue Imaging

Imaging depth is primarily limited by the scattering of both excitation laser light into the sample and subsequent signal emitted from the sample. In multiphoton microscopy, longer excitation wavelengths from a pulsed laser are used, which leads to less scatter and deeper penetration into the specimen. Both the excitation laser and emission signal are spatially limited to the focal plane, and hence, the emitted signal is only from this focal plane. Positioning the GaAsP PMTs directly behind the objective ensures that more photons reach the detector.

Mouse Embryo Section.
Sample Courtesy of Dr. Rieko Ajima, National Cancer Institute, Center for Cancer Research.
Multiphoton Microscopy Systems (Page 3 of 6)

Four-Channel-Ready Multiphoton System

Thorlabs recognizes that some customers may wish to have the option to upgrade their multiphoton system at a later time. To enable this level of flexibility, we offer our MPM200-2 Two-Channel Multiphoton System in a format that is ready for upgrade to a four-channel system at a future time. The MPM200-4R Four-Channel-Ready Multiphoton System provides all the same imaging capabilities as our MPM200-2 but includes the appropriate acquisition software and hardware to accommodate two additional channels of detection.

Included with MPM200-4R

- All Standard Features of the MPM200-2
- ThorImageLS™ Acquisition Software (See Page 1666 for Details)
- Dual Quad-Core Computer with 64-Bit Operating System and 24” Monitor
- Upgraded 4-Channel PCIExpress Digitizer
- Installation Included

In combination with the MPM-TLDM (see below for details), the MPM200-4R is converted from the two-channel MPM200-2 to our MPM200-4 Four-Channel System. The MPM200-4R includes four-channel-ready high-performance data acquisition and is controlled by our ThorImageLS™ software. This system is compatible with all of our MPM200 Series Accessories detailed on pages 1667 – 1677.

Transmitted Light Detection Module Upgrade for MPM200-4R

The Transmitted Light Detection Module (TLDM) is a modular upgrade for Thorlabs’ MPM200-4R Four-Channel-Ready Multiphoton System. This module converts the two-channel MPM200-4R into our four-channel MPM200-4. By adding two extra detection channels, the TLDM enables collection of additional information; the two existing channels of the MPM200-4R measure backscattered signal while the two additional channels from the TLDM measure forward scattering signal.

The two additional detection channels consist of two high-sensitivity GaAsP PMTs with full field-of-view collection optics similar to the backscattered detection module. The sub-stage condenser lens acts as an opposing objective (0.78 NA) 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. An easy access filter cube, shown in the image below, allows the user to appropriately select which signal is going to each detector.

This Transmitted Light Detection Module (MPM-TLDM) mounts directly onto the Nikon FN1 base and conveniently slides forward when white-light wide-field visualization is required. The MPM-TLDM interfaces with the existing ThorImageLS software and data acquisition electronics that are built into the MPM200-4R Multiphoton System.

Features

- Provides Two-Channel Forward Propagating Signal Collection
- Converts MPM200-4R into a Four-Channel Detection System
- Includes Two High-Sensitivity GaAsP PMTs and Easy Access Filter Cube
- Compatible with Existing Software and Control Electronics in the MPM200-4R System
- Mounts Directly onto Nikon FN1 Base

The TLDM provides easy access and exchange of filter sets for forward signal detection.
MPM200 Optical Path

The MPM200 Series of multiphoton systems is specially designed to operate in the near-infrared wavelength range from 680 – 1400 nm. Through optimization in this region, these systems are well-suited for use with Ti:Sapphire excitation laser sources. A diagram of the optical beam path in a typical MPM200 series system is shown below. The excitation light (red) is directed through a beam periscope to the multiphoton scanning system. High-speed XY scanning is achieved using a galvo-resonant scanner pair. The scanning beam passes through a customized scan lens and tube lens system that has been designed for aberration correction and antireflection in the NIR. Careful attention was also paid to minimize Group Delay Dispersion of the excitation path as much as possible. The multiphoton emission signal from the sample (green) is coupled back through the imaging microscope objective and redirected to the PMT detector module in a non-descanned detection scheme. By minimizing the emission beam path from the sample to the detector, the detection efficiency is greatly improved. Furthermore, the PMTs are placed immediately “behind” the objective to minimize light loss in the microscope body itself. An NIR blocking filter placed ahead of the secondary dichroic mirror prevents any scattered excitation laser light from reaching the detector.

The fluorescence filter cube consists of the secondary dichroic and two emission filters placed in front of each PMT. This cube is easily accessed through the front panel and changed by the user to suit experimental conditions.

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**Multiphoton Physiology Objectives**

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. Please visit our website, www.thorlabs.com, for additional information on each objective.

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**MPM200 Cross-Section**

The light path of the MPM-200 is optically separate from the wide-field light path of the FN1 microscope. This allows the multiphoton scanning and detection optics to be specifically designed to perform multiphoton imaging without compromise. By keeping the existing optical path of the FN1 intact, the traditional brightfield and epi-fluorescence capabilities of the microscope remain unaffected, further enhancing the experimental flexibility of the MPM-200 series of multiphoton imaging systems.

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**Have you seen our...**

Fluorescence Imaging Filters and Sets

- Ø25 mm Excitation and Emission Filters
- 25.2 mm x 35.6 mm Dichroic Beamsplitter
- Available Fluorescence Filter Sets
  - YFP  •  CY3.5  •  TXRED
  - CFP  •  TRITC  •  WGF
  - GFP  •  FITC  •  BFP

*See pages 1718 - 1719*
## Multiphoton Microscopy Systems (Page 6 of 6)

### Specifications

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<tr>
<th>Microscope</th>
<th>Stand</th>
<th>Upright Nikon FN1</th>
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<tr>
<td>Recommended Objectives</td>
<td>Nikon CFI LWD 16XW, 0.80 NA, WD = 3.0 mm; Nikon CFI Apo 25XW, 1.10 NA, WD = 2.0 mm; Nikon CFI Apo NIR 40XW, 0.80 NA, WD = 3.5 mm; Nikon CFI Apo NIR 60XW, 1.0 NA, WD = 2.8 mm; Nikon CFI Apo Lambda S LWD 40XW, 1.15 NA, WD = 0.61 mm; Nikon CFI Apo Lambda S 40XW, 1.25 NA, WD = 0.18 mm; Nikon CFI Plan Apochromat 60XW, 1.20 NA, WD = 0.27 mm; Olympus XLUMPLFLN 20XW, 1.0 NA, WD = 2.0 mm;</td>
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<td>Z-Drive</td>
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<td>XY Stage (Optional)</td>
<td>FN1 XY Rectangular Stage (Manual); XY Physiology Stage (Manual or Motorized, See Page 1673)</td>
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### Excitation

| Beam Conditioner | Variable Beam Expander (1X - 4X); Motorized Beam Attenuation (λ/2 Wave Plate and Polarizer); |
| Dispersion Pre-Compensation | .6300 fs² |
| Wavelength Range | 680 – 1400 nm |
| Objective Pupil Diameter | 20 mm (Max) |
| Field of View | 16 mm Diagonal Square (Max) at the Intermediate Plane |
| Scan | X: 7.8 kHz Resonant Scanner |
| Scanner Y: Galvanometric Scan Mirror |
| Scan Speed | 30 fps @ 512 x 512 Pixels |
| Scan Zoom | 1X to 8X (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 (NDD) Detectors | Two High-Sensitivity GaAsP PMTs Positioned Directly Behind the Objective |
| PMT Sensitivity Wavelength Range | 300 – 720 nm |
| Filter Cube | Single, User-Changeable |

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### Dimensions

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

### Item List

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