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# Imaging Software

ThorImage<sup>®</sup>LS is an intuitive, powerful program with diverse functionality and a customizable interface, providing flexible control of Thorlabs' imaging systems.



# ThorImage<sup>®</sup>LS Capabilities

ThorImageLS is a comprehensive software tool for managing Thorlabs' Bergamo<sup>®</sup> multiphoton microscopes, Veneto<sup>®</sup> inverted microscopes, Cerna<sup>®</sup> epi + DIC rigs, and confocal microscopes, as well as supplementary products, including the Tiberius<sup>®</sup> laser, scientific cameras, and sample scanning stages. Use one integrated software suite for every step of your imaging experiment.

#### Deep Physiological Scans

Use Built-In Controls for Scanning Large 3D Volumes:

- For deep, high-speed 3D imaging, coordinate between long-travel stepper control and high-resolution piezo motion to traverse large z ranges at user-defined step sizes.
- Compensate for depth-dependent scatter through tissue by ramping laser power as a function of depth, resulting in an even signal response throughout the scan.

#### High-Speed In Vivo Acquisition

Easily Perform In Vivo Imaging via Image Streaming, Time Series, and Z-Stack Acquisition:

- Software Supports Both Full-Frame and Fast-Frame-Rate Laser Scanning Image Acquisition
- Data Immediately Stored to the Hard Drive
- Experiment Sizes Constrained Only by the Size of Available Hard Drive Space
- High-Speed Volumetric Imaging Coordinates Fast Z Piezo Travel with Data Collection
- Store, Visualize, and Analyze Images Simultaneously with Acquisition Frame Rates up to 400 fps





Widefield Image with Fast Frame Rate Images (42 fps) of In Vivo Zebrafish Embryo Heart



1.2 mm In Vivo Deep Brain 3D Image Stack<sup>a</sup>



Cell Layers in a Mouse Retina<sup>b</sup>

#### Multi-Modal Imaging

Utilize One Software Package for Multiple Imaging Modalities to Capture Feature-Rich Samples:

- Supported Imaging Modalities:
  - Laser Scanning (Resonant-Galvo-Galvo, Galvo-Galvo, and Galvo-Resonant):
     Fluorescence, Dodt, Second Harmonic Generation, and Third Harmonic
     Generation
  - Widefield: Fluorescence, Brightfield, DIC, Dodt, Darkfield, and Phase Contrast
- Software Workflow Customizable for Each Modality
- Simultaneously Obtain Laser-Scanned Images Co-Registered With Widefield Images
- Acquire External Camera Data Synced with Microscopy Image Data to Correlate Image Features with Specimen Behavior

#### Photostimulation

For Optogenetics Research Requiring Patterned Light Control, Choose from Multiple Techniques:



Simultaneous Photostimulation of 100 Cells Co-Expressing GCaMP6f (Green) and C1V1 (Red)^  $\,$ 

- Spatial Light Modulator (SLM) Holography
  - 2D or 3D Photostimulation
  - Combined SLM and Galvo-Galvo Control Enable Users to Spread Laser Activation Over Whole Cells to Ensure More Reliable Stimulation
     Simultaneously Activate Hundreds of Cells
  - Classify Groups of Cells to Activate Independently
  - Synchronize with High-Speed Imaging
- Random Epoch Control
- Galvo Region of Interest (ROI)
  - Serially Stimulate Thousands of Cells
  - Choose from a Variety of ROI Types and Scan Patterns
  - Tightly Synchronize with Imaging to Maximize PMT Lifetime
- Full Field
  - Compatible with LEDs and Laser Configurations
  - Configurable Stimulation Timing
    - Pre- or Post-Imaging Frames
    - Inter-Frame Stimulation







Stitched Confocal Fluorescence Image of Rat Retina Stained with DAPI, Alexa Fluor® 555 and Alexa Fluor®  $633^{\rm d}$ 

#### Large-Area Mosaic Tiling

Capture Large Tiled Images to View Entire Samples at High Resolution:

- Selectable and Configurable Tiles
- Overlap Tiles in X and Y to Aid Analysis and Post-Processing in Fiji/ImageJ
- Multiplex with Time Series and Z Stacks
- Enable Tilt Adjustment to Correct for Samples Not Orthogonal to the Objective

#### Image Credits

#### **Cover Page:**

Top Left: Neurons captured using the Bergamo® II Multiphoton Microscope.

Top Right: 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.

Middle Left: Neurofilaments (green), glial filaments (red), and DAPI nucleic stain (blue) from hippocampus.

Middle Right: 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.

Bottom Left: Confocal mosaic image of GFP and NC82 antibodies in a fly brain. Sample courtesy of Dr. Berry Dickson, Janelia Research Campus, HHMI, Ashburn, VA.

Bottom Right: Confocal Z-stack maximum intensity projection of a mosquito eye.

#### From the Brochure Contents:

- a. Courtesy of Dr. Hajime Hirase and Katsuya Ozawa, RIKEN Brain Science Institute, Wako, Japan.
- b. Sample courtesy of Dr. Robert Fariss, Biological Imaging Core, National Institutes of Health, Bethesda, MD.
- c. Courtesy of Lloyd Russell, Dr. Adam Packer, and Prof. Michael Häusser, University College London, United Kingdom.
- d. Courtesy of Dr. Jennifer Kielczewski, National Eye Institute, National Institutes of Health, Bethesda, MD.

# **Explore the Workspace**

#### Automated Image Capture

Program routine image acquisitions:

- Create Command Sequences Using a Drag-and-Drop Interface
- Run Fiji/ImageJ Macros
- Execute MATLAB® Scripts

#### Multi-Modality Support

Capture a data set using multiple imaging modalities without jumping between applications.

- Quickly Switch Modalities to
   Reduce Wait Times Over a Single Run
- Customize Capture Layouts and
  Settings for Each Modality

#### Ease of Use

Intuitive user interface design provides easy access to all necessary hardware control elements.

#### Customizable Workspace

Visualize only the settings relevant to each aspect of your individual experiment.

- Customizable Multi-Column Layout
   Save by User
  - Save by Imaging Modality
- User-Defined Keyboard Shortcuts
- Multi-User Settings Saved for Shared Workstations

#### Signal Generator

Custom programmable TTL output trigger for triggering external stimulation equipment based on:

- Time
- Bergamo Generated Triggers
- Custom Input Trigger



Screenshot of the Capture Setup tab during an SLM holographic photostimulation experiment using our Bergamo® Multiphoton Microscope.

#### Coordinated Imaging and Experimentation

Use one software package to control and synchronize experimental processes with captured data.

Applications

- Photoactivation

- Software Features
- ThorSync™
- Laser-Power-Ramped Z Stacks
- Third-Party Script CompatibilityDirectly Manipulate
- Open-Source Software Code



#### Intuitive Visualization

Once acquired, easily explore multi-dimensional datasets.

- Simultaneously Scan Through 2D Slices in the X-Y, X-Z, and Y-Z Planes
- Render and Rotate 3D Volumes to View the Complete Sample and Identify Non-Orthogonal Features

#### Real-Time ROI Analysis

Select an ROI to perform real-time calculations simultaneously with image acquisition.

- Multiple ROI Shape Options
- Intensity as a Function of Time
- Mean, Minimum, and Maximum Intensity
- Standard Deviations
- Arithmetic Operations
   ΔF/F
- Ratiometric Imaging

SLM Multiple Epoch Control

Execute SLM Patterns in pre-defined order.

# **Integrated Acquisition**

To provide a flexible user experience, our team has designed ThorImageLS to easily communicate with external hardware and third-party software. The application code is also freely available for complete software customization.

#### ThorSync™

Integrate ThorImageLS with ThorSync, our supplemental software program designed to record and generate digital/ analog signals for timing-critical applications. This enables synchronization of data acquisition with external equipment.

- Create Output Signals to Trigger Image Acquisition, Experiment Stimulation, and Auxiliary Equipment
- Create or Respond to Signals from Behavioral Devices (e.g. Virtual Reality Environments)
- Customize Display Settings to Improve Signal Interpretation
- Stack Panels for Individual Signal Display
- Measure Time and Y-Axis Values Using Location Indicators
- Rollover Statistics for All Signals
- Digital and Analog Data Saved in an Open HDF5 Scientific Format that is Easily Imported into Analysis Packages
- Support for Spectral Analysis
- Virtual Channel Support
- Custom Control of TTL Lines



From the Review tab, export acquired data into Fiji or MATLAB®.





Top: ThorSync recording of tethered Drosophila flight to study rapid steering maneuvers around looming stimuli. The tachometer to monitor wingbeat frequency (AI 0) is timed alongside the visual patterns presented to the Drosophila (AI 2: ai2, frame number; AI 3: AuxPhidgAO1, pattern type), left and right wing stroke amplitude (AI 6: LW and AI 7: RW), and image acquisition (AI 1: AuxPhidgAO0).

Bottom: GCaMP6f fluorescence expression ( $\Delta F/F)$  in an active descending neuron during the experiment.

#### Third-Party Integration

Export data or run third-party scripts directly using ThorImageLS, which standardizes and streamlines automated acquisition and analysis processes.

- Launch and Immediately Open Files in Fiji/ImageJ or MATLAB<sup>®</sup> with a Quick Connect Button in the ThorImageLS Viewer
- Create ThorImageLS Scripts that Execute ImageJ and MATLAB<sup>®</sup> (m-files<sup>®</sup>) Scripts Either Synchronously or Asynchronously
- Simplified Hardware Integration Through Third-Party Scripts
- Open SDKs Enable Users to Code Their Own Device
   Plugins

#### Open Source

ThorlmageLS is provided with every Thorlabs microscope purchase. Full customization of software features and performance is made possible with open-source availability. Contact us at ImagingTechSupport@thorlabs.com for ThorImageLS source code and technical support.



### Specifications

Microscopes	<ul> <li>Bergamo<sup>®</sup> (Upright &amp; Rotating)</li> <li>Confocal</li> </ul>	♦ Veneto <sup>®</sup> (Inverted)
Imaging Modalities	Laser Scanning	Widefield
	◆ Two Photon	<ul> <li>Epi-Illumination:</li> </ul>
	♦ Three Photon	– Reflected Light
	♦ Confocal	- Fluorescence
	◆ DIC	◆ Trans-Illumination:
	♦ Dodt	- Hyperspectral with KURIOS®
	◆ Fluorescence	– DIC
		- Dodt
		– Darkfield & Brightfield
Applications	Photostimulation	
	– 2D or 3D Multi-Point Spatial Light Modulation	
	- Sequential Multi-Point (Galvo-Galvo)	
	- Full Field	
	- Protein Uncaging	
	Rapid Excitation Wavelength Switching with Tiberius®	
	Electrophysiology Signaling     A Fast Daga Timus Comparing	
	Fast Deep-Tissue Scanning	
Computer	Microsoff® Windows® 10 (64 Bit)     Microsoft® Constitute ® Constitute Processor, 14 CB DAM	
	<ul> <li>Minimum Specifications: Intel<sup>®</sup> Core i7 3.2</li> </ul>	2 GHz Processor, 16 GB RAM
Features <sup>a</sup>		
Acquisition	Mosaic Large-Area Imaging	
	<ul> <li>High-Speed ROI Masking to Eliminate Photodamage Outside Resonant Imaging Areas</li> </ul>	
	<ul> <li>Fast Z-Stack/Volume Acquisition</li> </ul>	
	<ul> <li>Multi-Modal Imaging Compatibility:</li> </ul>	
	- Resonant-Galvo-Galvo	– Galvo-Resonant
	- Galvo-Galvo	<ul> <li>Thorlabs' and Some Third-Party Scientific Camera</li> </ul>
	<ul> <li>Data Acquisition Synced with Hardware Inputs and Timing Events</li> </ul>	
	<ul> <li>Intuitive Scripting Tool for Customized Image Acquisition</li> </ul>	
	◆ Real-Time ROI Analysis and Image Correction	
	◆ Customizable Scan Area Modes	
	◆ Acquire in 4D, 3D, 2D, Line, Polyline and Point	
	◆ User-Defined Laser Power Ramping in Z	
	<ul> <li>Unlimited Data Size Acquisition (Capture to Hard Drive Capacity)</li> </ul>	
Settings	♦ Multi-Column Customizable Workspace	
	<ul> <li>Multi-User Settings Saved for Shared Workstations</li> </ul>	
Export	Non-Proprietary File Formats	
	- TIFF	
	– OME TIFF	
	- RAW	
	– XML	
	◆ Interface with MATLAB® Scripts & Fiji/ImageJ Macros	
Support	Lifetime Software Technical Support	
	<ul> <li>Open-Source Access to Full Application Code via Cloud-Based Git Servers</li> </ul>	
	<ul> <li>Multiple Feature Updates per Calendar Year</li> </ul>	

a. For a complete list of software features and user interface upgrades, visit our website at www.thorlabs.com.

## Worldwide Support



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