

# Ultima 2Pplus

● All-Optical Multiphoton Workstation

Innovation with Integrity

Fluorescence Microscopy

*See the Biology of Life More Clearly*

# Next-Generation Multiphoton Imaging for In Vivo Studies

The Ultima 2Pplus All-Optical Multiphoton System delivers the best commercially available combination of flexibility, resolution, imaging depth and speed, allowing researchers to perform simultaneous imaging, photostimulation, and electrophysiology protocols with greater efficiency and effectivity.

From the introduction of the first commercial system to allow simultaneous imaging and uncaging in 2003, to the latest system, Ultima multiphoton microscopes have grown into the systems of choice for cutting-edge research around the world. Built upon this industry-leading platform and Prairie View software, the 2Pplus microscope incorporates over a decade of laboratory experience and close collaboration with leading neuroscientists around the world, resulting in a host of unique capabilities and features found only on Ultima systems.

## Ultima 2Pplus Uniquely Provides

- Best-in-class field of view for multiphoton imaging
- High-efficiency light collection and detection
- Simultaneous imaging and photoactivation

“The neuroscience community has long desired microscopes that support both 3D, high-speed photoactivation and full-field, deep imaging. Bruker’s novel **Ultima 2Pplus** promises a single-tool solution, providing a new all-optical resource for quantitative investigation.”

– Dr. Adam Packer, Oxford University



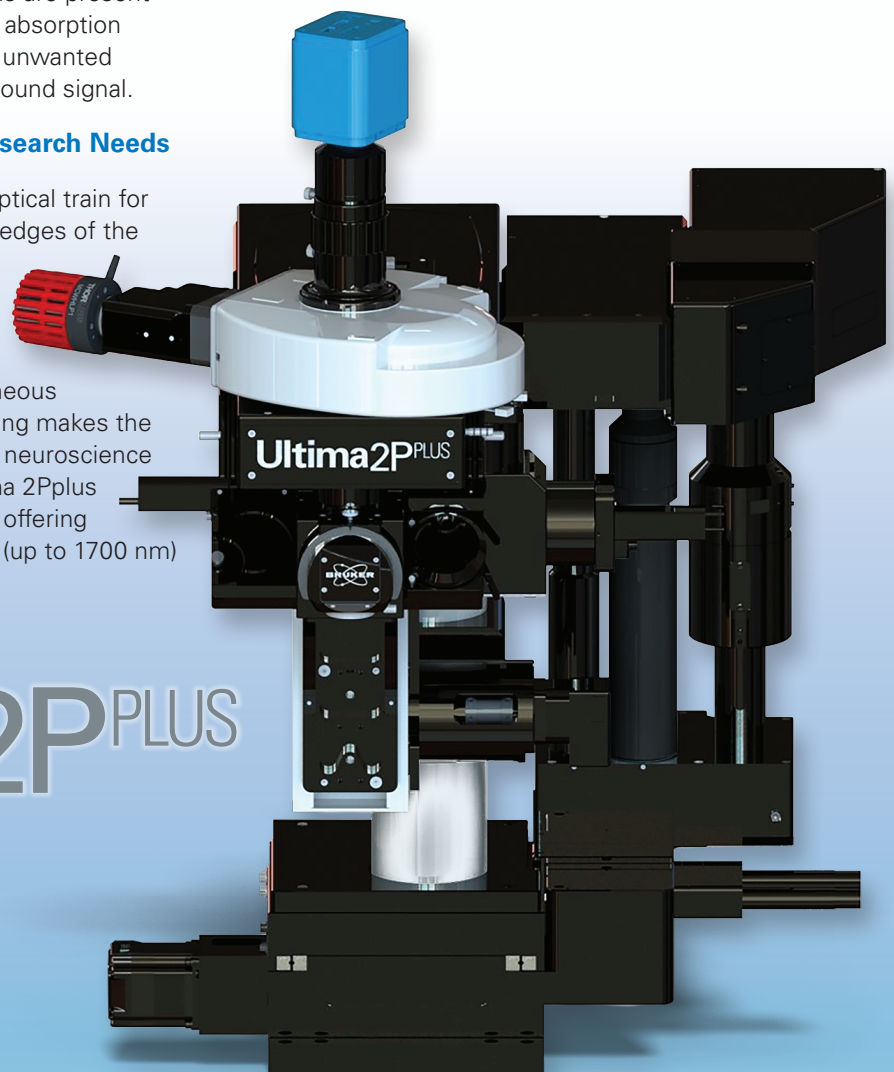
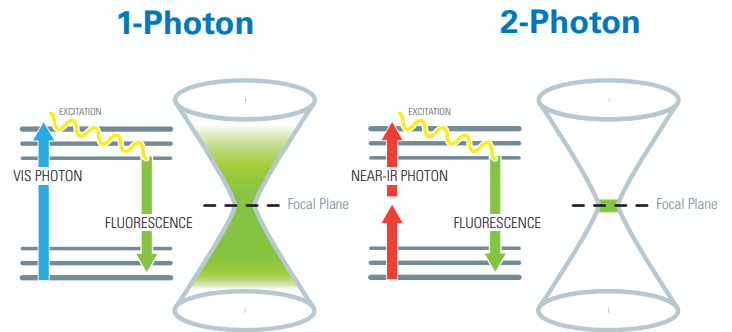
# ● Take the Next Step

## How Multiphoton Imaging Works

In confocal microscopy, a single photon of visible light is absorbed by a fluorophore, causing it to emit a fluorescent photon. By contrast, in two-photon microscopy, two photons are absorbed simultaneously by the fluorescent molecule. The two photons used to excite the molecule are of half the energy (twice the wavelength) of its single-photon counterpart to induce fluorescence. The two-photon effect, in comparison to single-photon excitation, requires higher powered, pulsed lasers that deliver the energy to the molecule almost simultaneously. These ultrashort laser pulses are focused to a single point within the sample, where it is the only spot that has sufficient photon flux to cause fluorescence. In other parts of the specimen, where photons are present at much lower densities, two-photon absorption generally does not occur, minimizing unwanted fluorescence and out-of-focus background signal.

## Technology for Your Evolving Research Needs

Ultima 2Pplus utilizes an optimized optical train for exceptional performance to the very edges of the wide field. The extended clearance stage is ideal for large-animal imaging. And the optically corrected, decoupled electrically tunable focusing module for simultaneous holographic stimulation and 3D imaging makes the system uniquely suited for advanced neuroscience inquiry into awake animals. The Ultima 2Pplus also anticipates future techniques by offering longer wavelength 3-photon imaging (up to 1700 nm) for looking deep into living tissue.



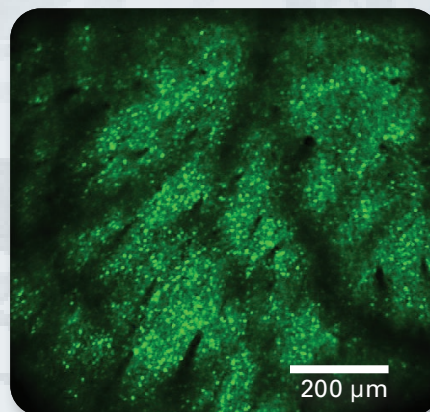
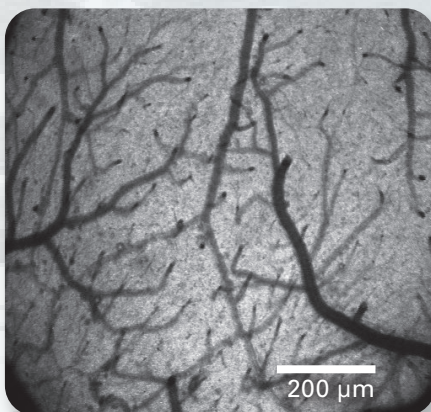
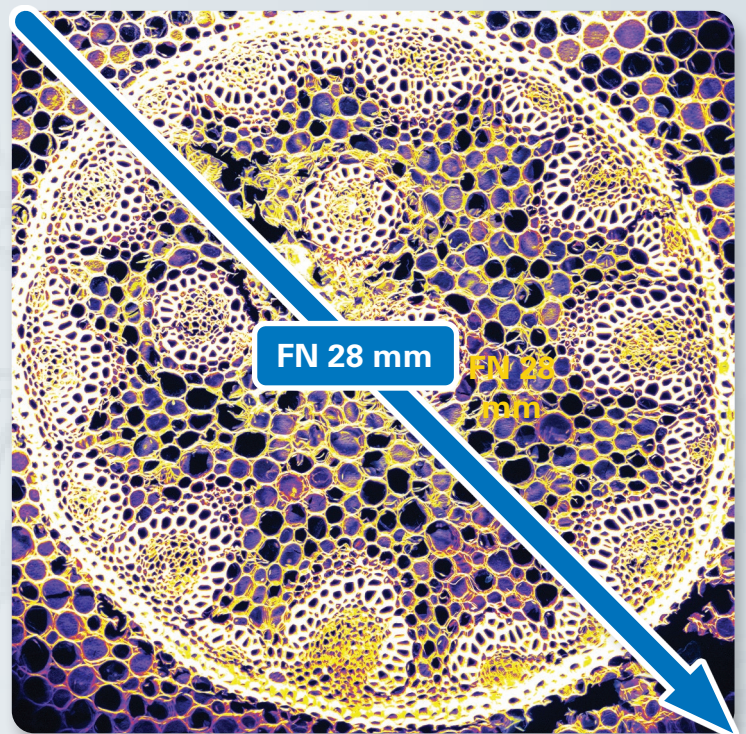
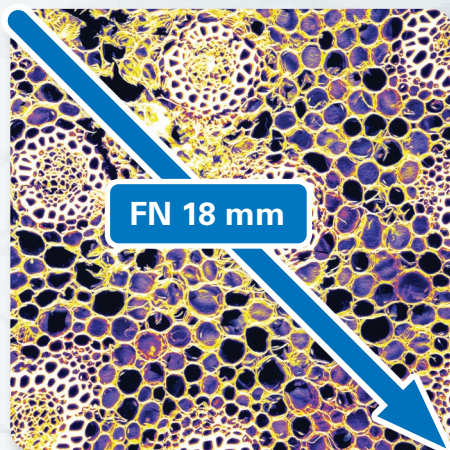
# Ultima 2P<sup>PLUS</sup>

## • See More, Learn More

### Largest Field of View and Superior Field Uniformity

The Ultima 2Pplus field of view is over 50% larger to facilitate modern optogenetics experiments, which require simultaneous imaging and photostimulation of distant regions in a sample. A new, custom optical design preserves spatial and temporal resolution throughout the entire field, which is essential for researchers to be able to continue their work without changing experimental designs.

Field of view on Ultima 2Pplus is FN 28 mm in comparison to industry standard of FN 18 mm.



(Left) Exemplary two-photon imaging of cortical surface through a cranial glass window on Ultima 2Pplus. (Right) GCaMP6s expression in layer 5A neurons of mouse visual cortex at a depth of ~400 μm below the pia. Both acquired with Nikon 16x 0.8 NA objective at 920 nm. Scale 200 μm.

Data courtesy of Dustin Herrmann, Mehmet Fisek, Michael Häusser's lab, UCL, London.



# ● Look Deeper

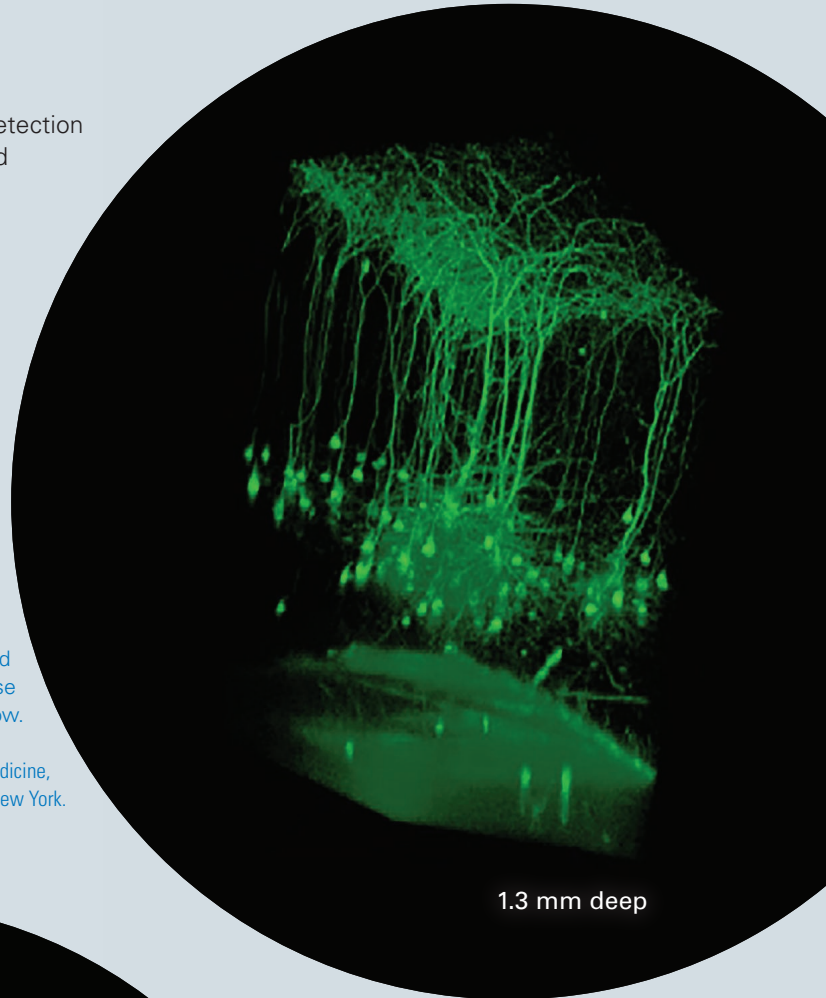
## High-Sensitivity Deep Imaging

The Ultima 2Pplus light collection path ensures detection of almost all photons scattered from dim, thick and opaque biological samples. The superior collection efficiency is provided with 2-inch or larger optics throughout the emission path up to the sensitive close-proximity detectors.

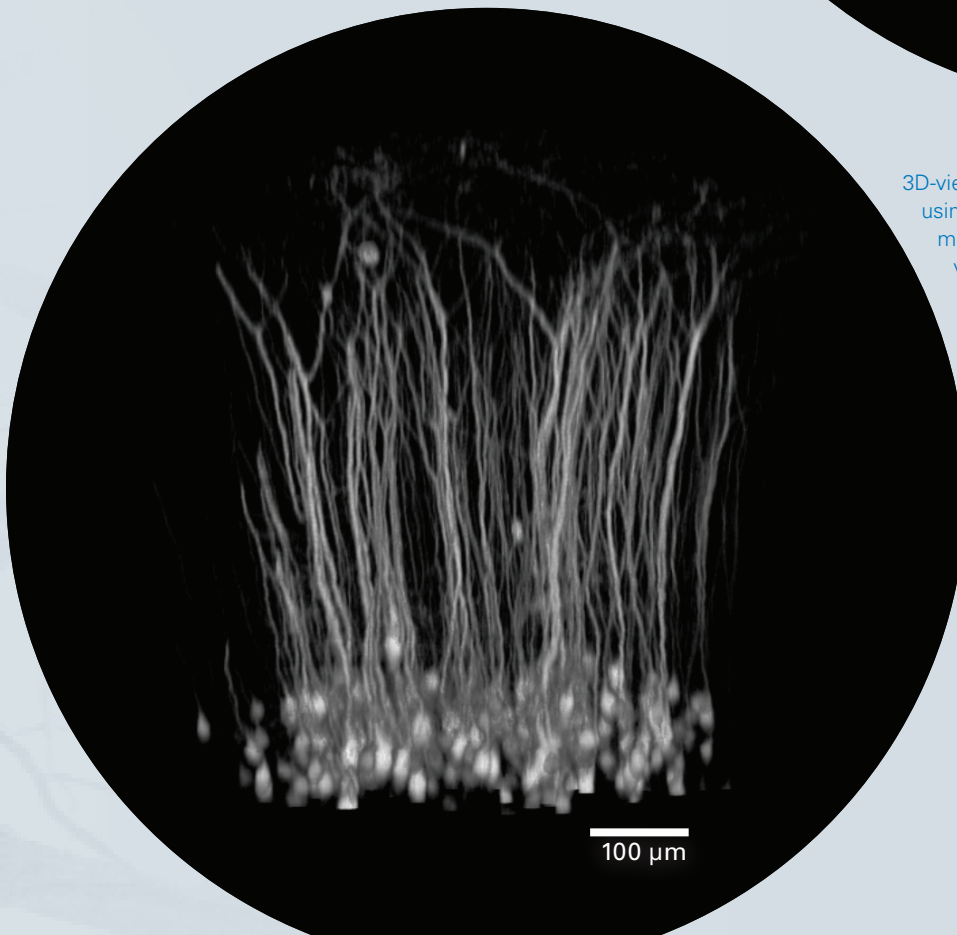
As two-photon microscopy has become well adopted, emerging three-photon microscopy offers the potential to image even deeper into samples. Since further infrared wavelengths are scattered even less, three-photon excitation has the promise to be able to image several millimeters into the brain. Ultima 2Pplus already uniquely incorporates this capability to take your research to new levels of potential.

3D-view of pyramidal cells in layer 5. Data collected in one-month-old transgenic Thy1-YFP-H line mouse through a glass-covered cranial window.

Data courtesy of Guang Yang, School of Medicine, New York University, New York.



1.3 mm deep



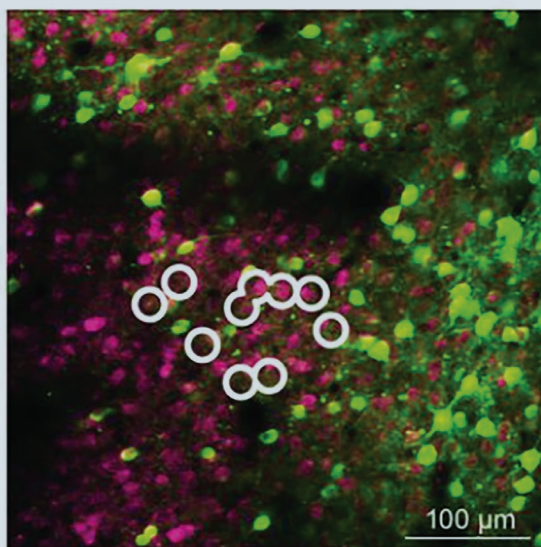
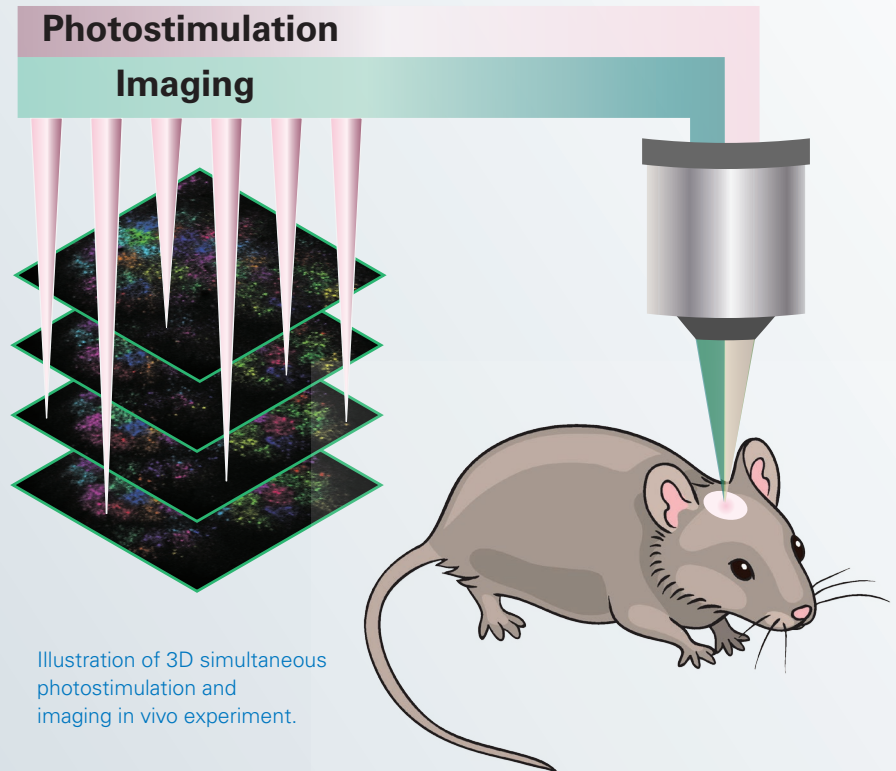
3D-view of volumetric stack recorded using the electrically tunable focusing module. Layer 5B neurons in mouse visual cortex in vivo, labelled with tdTomato. Scale bar 100  $\mu\text{m}$ .

Data courtesy of Lisa Bauer, Dustin Herrmann, Mehmet Fisek, Michael Häusser's lab, UCL, London.

# ● Acquire Even More Data

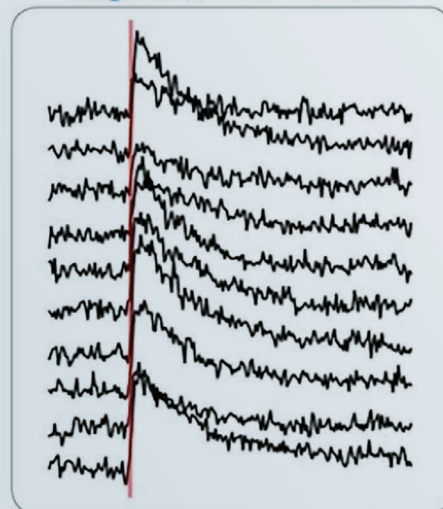
## Perfected Photostimulation and Imaging

The Ultima 2Pplus empowers sophisticated optogenetics experiments with Bruker's Neuralight 3D™ Spatial Light Modulator option. Large populations of targets can be stimulated simultaneously in multiple focal planes with computer-generated holograms. During 3D holographic experiments, targets at different depths are imaged with a custom-designed, Two-photon imaging electrically tunable focusing module that allows for independent Z-positioning of the imaging plane relative to photostimulation.



■ GCaMP6s  
■ C1V1-2A-mCherry

## Targeted 2P stimulation



Optical stimulation of circled cells with Neuralight 3D. 3D hologram of points was created which was then spirally scanned over neuronal cell bodies.

Data Courtesy of Adam Packer, Lloyd Russell, Henry Dalglish, Michael Häusser's lab, UCL, London.



# ● Expand Your Capabilities

## Unmatched Options for Extensibility

The true power of the Ultima systems lies in their ability to be custom configured. An array of modules is available to extend the systems to meet your particular research needs:

**Photostimulation Path Module** provides the capability for simultaneous photostimulation or uncaging with imaging.

**LED Module** supports full-field optogenetics applications.

**Resonant Scanner Module** enables fast data acquisition with 30 Hz at 512x512 resolution.

**Rotating Nosepiece Module** provides off-axis imaging to access specimen areas of interest not accessible through the vertical axis. Ensures a proper angle to minimize scatter in tissue with highly oriented cells. Motorized option is controlled via remote manual controller and in the software.

**Dual Wavelength Imaging Module** enables routing of two lasers into the imaging path of the microscope, providing the capability to use multiple probes without laser tuning during imaging. Scanning can be performed simultaneously or in a line-interlaced mode.

**Moving Scope Stage Module** allows for controlled movement of the scope around a sample.

**Moving Sample Stage Module** includes a low-profile, motorized stage with a specimen platform. Easily inserted or removed, the Bridge Stage converts the Ultima into a slice rig.

**Objective Z-Piezo Stage Module** supports fast volumetric acquisition. This module is fully controlled in the software. It can be synchronized with resonant scanner data acquisition.

**Remote ETL Module** features an optically corrected electro-tunable lens with 450  $\mu\text{m}$  working range.

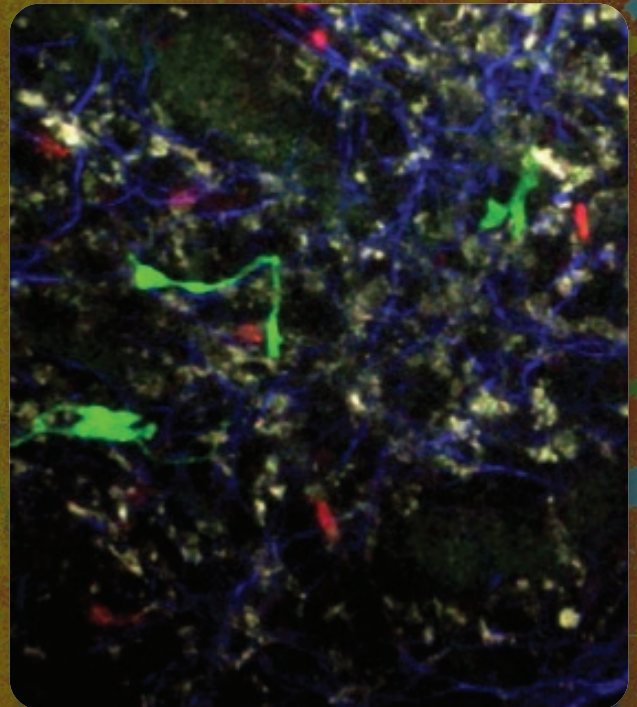
**FLIM Module** supports Fluorescence Lifetime and Phosphorescence Lifetime applications. The module provides additional electronics for fluorescence detection and software for data acquisition.

**Substage Detectors Module** supports in vitro applications. It improves detection of fluorescent signal in dim samples. This module can be used to collect Second Harmonic Generation (SHG) forward signal.

**Dot Gradient Contrast Module** improves contrast of images of unstained samples (i.e., brain slices) during electrophysiological experiments.



Rotating nosepiece.



Dual wavelength imaging in lymph node explant.



## Ultima 2Pplus Specifications

Scanning Method	Matched pair of 6 mm Cambridge galvanometers with raster and spiral scanning capabilities
Field of View	~1.375 mm x 1.375 mm with 16x objective ( $\leq 28$ mm FN)
Scan Speed	Raster scan: 1.65 fps at 512 x 512, >12 fps at 64; Spiral scan: 6 fps at 512 x 512, ~30 fps at 64 x 64
Scan Customization	User-definable straight, freehand and circular (infinite) linescan with included software; User-definable pixels/line and lines/scan from 1 to 2048; $\leq 120\times$ scan zoom; 360° of scan rotation; Point scan
Uncaging Option	Second set of matched 3 mm or 6 mm Cambridge galvanometers in same scan head provide high-precision visible or multiphoton laser sample photomanipulation
High-Speed Imaging Option	8 kHz resonant galvanometer; ~30 fps at 512 x 512, >1300 fps at 512 x 8 region of interest

### DETECTORS

Reflected Non-Descanned	1 to 4 hand-picked Hamamatsu Multi-Alkali PMTs; Upgradeable to high-sensitivity Hamamatsu GaAsP PMTs
Transmitted Non-Descanned	1 or 2 hand-picked Hamamatsu Multi-Alkali PMTs; Upgradeable to high-sensitivity Hamamatsu GaAsP PMTs
Dot	Single Hamamatsu PMT for DIC-like image collection
Transmitted	Single Hamamatsu PMT for transmitted light image collection
Camera	Standard C-mount camera port built into scan head

### OPTICAL INPUTS


Multiphoton Laser	Optimized for multiphoton laser input from 690 to 1700 nm
Epifluorescence	High-Powered LED in epifluorescence turret
Visible Laser	Fibered laser inputs for visible laser introduction
Ultima Laser Rating	Class 1 (Contains Class 3b and Class 4 lasers) with light box
Helios Laser Rating	Class 3b
LED	Full-field photoactivation with LED module

### MOTOR CONTROL

X,Y Stage	Variable height with ~15 cm X and ~7.5 cm Y movement and ~0.3 $\mu\text{m}$ step size
X,Y Microscope Base	Fine and coarse-movement platform for scope with ~35 mm travel and 0.1 $\mu\text{m}$ step size
Z-Focus	Range of 30 mm with ~0.2 $\mu\text{m}$ step size
Z-Piezo	Travel range $\leq 1000$ $\mu\text{m}$ with 0.05 $\mu\text{m}$ step size
Orbital Nosepiece	Motorized control of objective angle and rotation

### SOFTWARE

Prairie View	Turnkey intuitive and customizable operation for imaging
Z-Series	Easy creation of depth stacks with user-customizable slice number, step size and laser power
T-Series	Easy creation of complex series involving Z-Series and triggered images
Stage Montage	Atlas Imaging simplifies setup and optimizes acquisition of 2D and 3D stage montages
Peripherals Integration	Wavelength and power control available for multiphoton and visible laser launches
Photoactivation	User-defined points and regions with synchronized laser modulation
Regions of Interest	User-defined regions for faster scanning capabilities
Brightness	Intensity mapping and plotting for user-defined regions over time
Voltage Inputs/Outputs	Signal inputs and outputs for electrophysiological experiments, stimulus control, and synchronization with external devices


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