OLYMPUS®

Your Vision, Our Future

Multiphoton Laser Scanning Microscope

FVMPE-RS

FLUOVIEW

NEW

Advanced multiphoton microscopy with extended IR range at high speed

INNOVATIVE, PRECISE, HIGH-SPEED, AND DEEPLY FOCUSED

High speed

Multicolour

Extended IR range

Laser light stimulation

Deep observation

The Olympus FVMPE-RS satisfies a myriad of performance needs for deep observation.

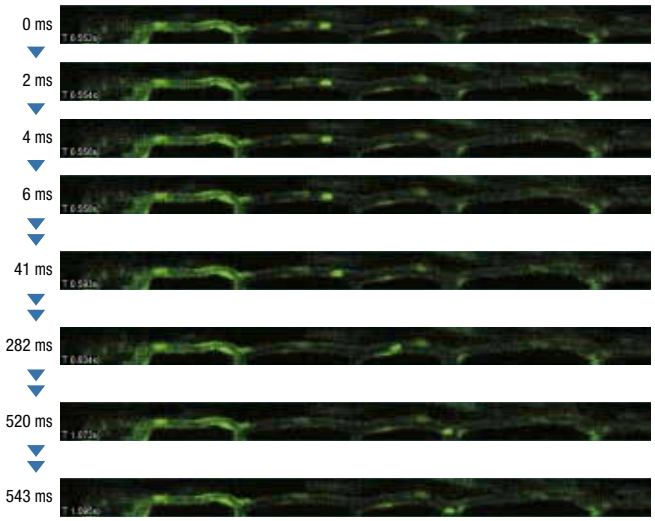
It delivers high-speed millisecond imaging essential for the capture of rapid *in vivo* responses, and offers ideal spot excitation with intense energy up to 1300 nm – even at deep sites. It also offers high S/N imaging for the efficient detection of scattered fluorescence photons, simultaneous dual-wavelength excitation at deep sites, visible or multiphoton laser light stimulation, and synchronisation with patch clamp data.

Put simply, the Olympus FLUOVIEW FVMPE-RS combines high-speed, deep observation capability with multicolour imaging and powerful laser light stimulation for the researcher who refuses to compromise.

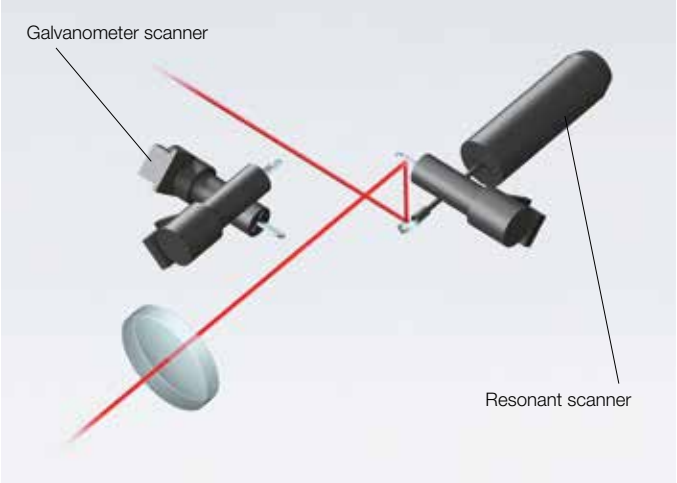
HIGH-SPEED SCANNING CAPTURES *IN VIVO* RESPONSES WITH 438 fps

A high-speed scanner providing unique 438 fps at 512 × 32 scan performance

The scanner unit combines a newly developed high-speed resonant scanner with a conventional galvanometer scanner to provide high-speed and high-definition imaging in a single system. High-speed imaging delivers 30 fps at 512 × 512 at full field of view (FN 18), while clip scans optimise return time to achieve an unmatched 438 fps at 512 × 32 pixels – making it possible to capture rapid calcium channel reactions and membrane potential-sensitive dyes in action.



Images show data acquired with 438 fps frame rate. Fast-moving fluorescently labelled cells are captured without distortion in blood vessel of zebra fish.

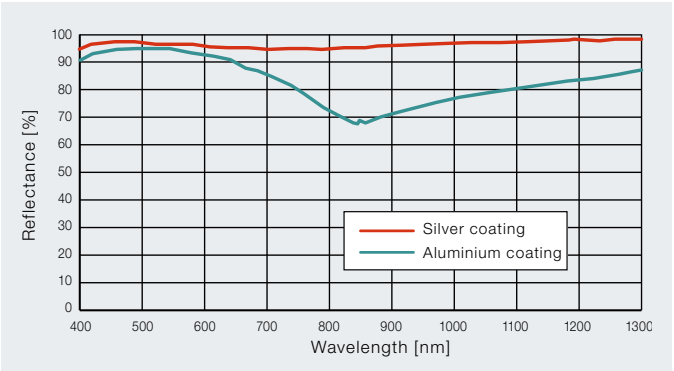


High-speed imaging

A proprietary silver coating improves excitation efficiency by 50%*

Silver-coated scanner mirrors achieve extremely high reflectance characteristics across a broad wavelength range from visible to near infrared. Total reflectance for the XY scanner is enhanced by more than 25% in the near-infrared range compared to conventional aluminium-coated mirrors, and this increased reflectance provides more than a 50% improvement when converted to multiphoton excitation efficiency. The result is a highly effective apparatus that delivers the superior power needed for deep *in vivo* probing.

*Compared to standard aluminium coating



A cooled, high-sensitivity GaAsP detector acquires high S/N images

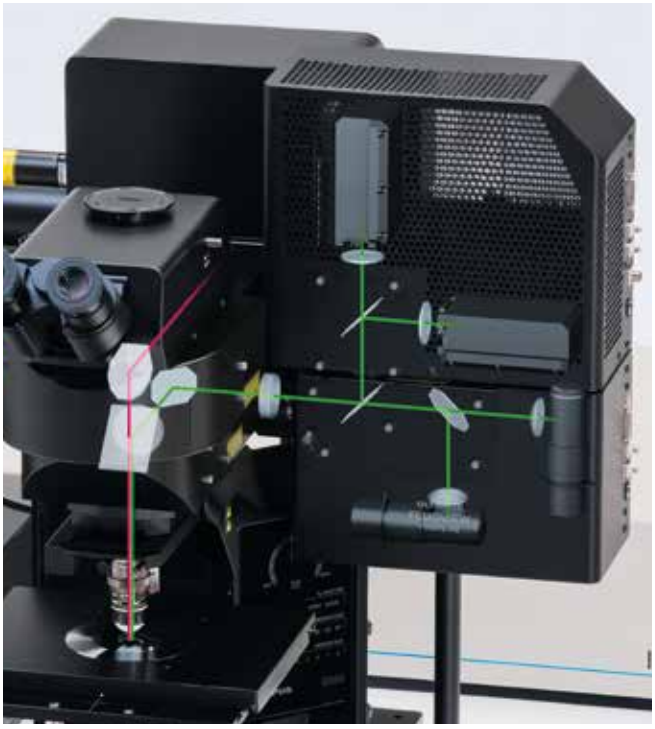
High S/N imaging can be acquired even under faint fluorescence through the use of gallium arsenide phosphide (GaAsP) in the photomultiplier tube (PMT) – delivering greater quantum efficiency than multi-alkali PMT along with Peltier cooling that improves S/N even further.

Image with multi-alkali detector

Image captured with GaAsP detector

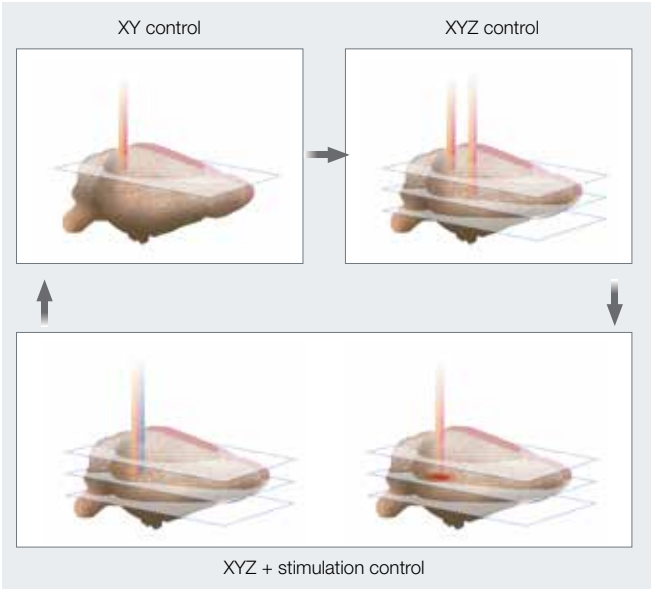
Arc-dVenus transgenic mouse (8 weeks old), coronal brain block, hippocampal dentate gyrus
Projection image of 300–400 µm depth (5 µm steps)

Image data courtesy of:
Dr Norio Takata, Dr Hajime Hirase
Laboratory for Neuron-Glia Circuitry, RIKEN BSI
Dr Shun Yamaguchi
Gifu University Graduate School of Medicine



Microsecond precision and hardware sequencer control

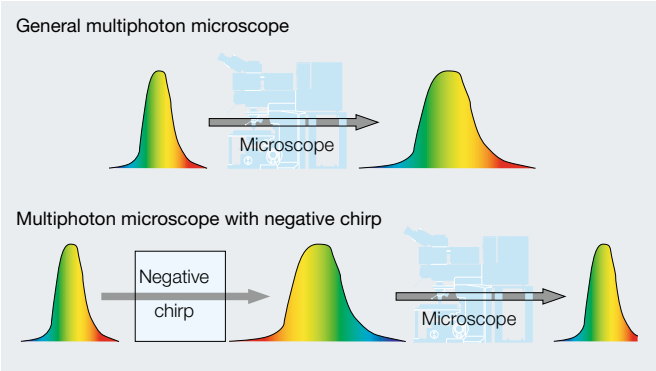
Microsecond repeatability precision provides the power needed for precise control of triggering and point of stimulation. The optional sequencer manager enables extra-long-term (two-week) procedures for complicated observational testing that requires switching between different imaging tasks. Even in extra-extended lab work cycles, repeatability retains millisecond precision. Microsecond repeatability precision is critical for many applications requiring high speed. This is particularly true for electrophysiology and optogenetic stimulation, where microsecond timing can mean the difference between observing synchronous and asynchronous stimulus responses.
For extra-long (two-week) acquisitions with complicated experimental procedures that require switching between different imaging tasks, the optional sequencer manager can still maintain millisecond precision, ensuring data integrity in the most demanding *in vivo* and *in vitro* experiments.



OPTIMISED FOR DEEP OBSERVATION

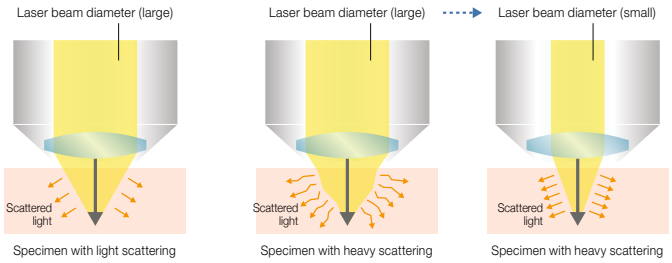
Laser with negative chirp improves excitation efficiency at the focal plane

A laser beam with optimally adjusted pulse widths can be delivered to the focal plane, thanks to the application of negative dispersion that perfectly corresponds to the magnitude of the pulse-width dispersion generated during transmission through the microscope optics. The result is brighter images without needing to increase laser power, sample heating, photobleaching or photo-toxicity.



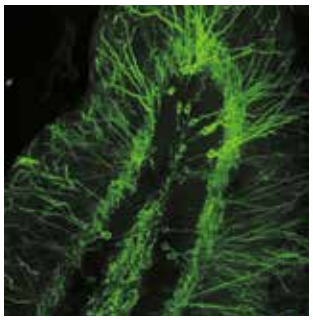
Deep Focus Mode elevates light-condensation performance for specimens with heavy scattering

A newly developed Deep Focus Mode responsively adjusts the laser beam diameter in accordance with laser scattering conditions across specimens. For *in vivo* specimens with heavy laser scattering, more excitation photons reach deep sites with the Deep Focus Mode and brighter high-resolution images are produced.



Both images are maximum intensity projection from 23 slices, Deep Focus Mode provides brighter image

Normal image



Deep Focus Mode image

Image data courtesy of:
Urs Ziegler and Jose Maria Mateos,
Center for Microscopy and Image Analysis, University Zurich
Mouse line L15 kindly provided by Pico Caroni, FMI, Basel

Depth-brightness compensation keeps brightness consistent from the surface to deep levels

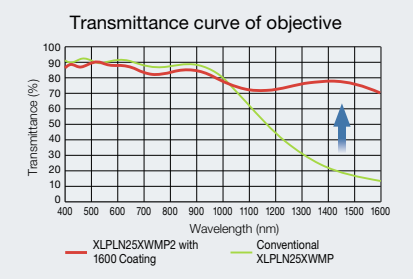
When observing thick specimens, images can often get darker as the focal point goes deeper. But with depth-brightness compensation, detector sensitivity and laser power are constantly retuned to keep brightness at a consistent level.

Image without compensation

Image with compensation

Optics with new outperforming IR coating

An innovative IR coating (1600 coating) for the 25× dedicated multiphoton objective range and scanner optics further refines deep observation quality. This coating with improved long wavelength transmittance enables excitation without a decrease in laser power, even at deep sites. Since transmittance at 405 nm also remains high, the feature is suited to uncaging applications that employ a 405 nm laser.



Detection light path redesigned for more efficient fluorescence capture

The non-descanned detection light path has been positioned close to the specimen. The signal collecting optics have been enlarged to increase the detection efficiency of scattered fluorescence.

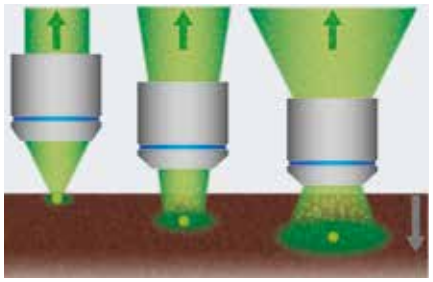
Deep observation of *in vivo* and fixed transparent specimens through dedicated multiphoton objectives with a maximum depth of 8 mm

The XLPLN25XWMP2 water immersion objective with a working distance of 2 mm delivers a high resolution and a wide field of view for the deep observation of live specimens. Two other objectives in the same family with working distances of 4 mm and 8 mm deliver maximum performance with fixed transparent specimens for high-definition observation at deep levels. All of these objectives feature correction collars that allow them to correct spherical aberration generated by the difference in refractive index between the immersion solution and the specimen – forming optimal light-condensed spots without energy density loss, even during observations deep within the specimen. Furthermore, each objective features a wide-field design that permits the efficient acquisition of scattered fluorescence photons for bright observations.

XLPLN25XWMP2
W.D. 2 mm

XLPLN25XSVMP2
W.D. 4 mm

XLSLPLN25XSVMP2
W.D. 8 mm



•Wide field of view

Despite efficient excitation, fluorescence light is scattered deep within the specimen. These wide-field objectives can collect scattered fluorescence photons to generate brighter images.

Dedicated multiphoton objectives	NA	W.D. (mm)	Immersion index
XLPLN25XWMP2	1.05	2	1.33
XLPLN25XSVMP2	1.0	4	1.33–1.40
XLSLPLN25XSVMP2	0.95	8	1.33–1.40

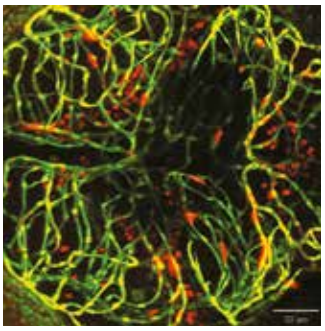
HIGH-PRECISION LASER BEAM CONTROL UP TO 1300 nm FOR FLEXIBLE DUAL-LINE MULTIPHOTON IMAGING

Multi-wavelength excitation and multiphoton imaging

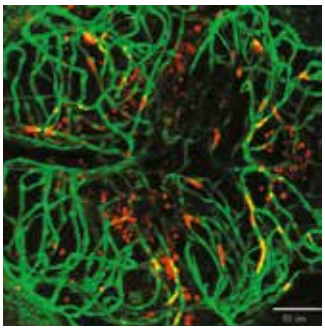
Multichannel multiphoton excitation imaging is accomplished with a dual-wavelength IR pulsed laser or two independent IR pulsed lasers – enabling simultaneous excitation of chromophores with different wavelengths. Thanks to the flexible and precise IR introduction optics, both lines are accurately merged. Simultaneous excitation provides perfected registration and balanced images for different chromophores. Optimal excitation wavelengths for individual chromophores may also reduce auto-fluorescence by avoiding the use of excitation at around 800 nm.

InSight DeepSee supports simultaneous two-laser-line excitation and extended NIR multiphoton imaging

The InSight DeepSee pulsed IR laser systems ideally support multi-photon imaging with excitation from 680–1300 nm. The Dual Line version of the InSight DeepSee system offers two laser beam outputs: main output with a tunable line from 680–1300 nm and the second output at 1040 nm. Higher laser power beyond 1000 nm provides a host of new multiphoton imaging capabilities, covering a variety of dyes and fluorescence proteins and third-harmonic generation imaging without UV damage.



Crosstalk occurred after simultaneous excitation of GFP and DS-RED with a single-wavelength IR pulsed laser.

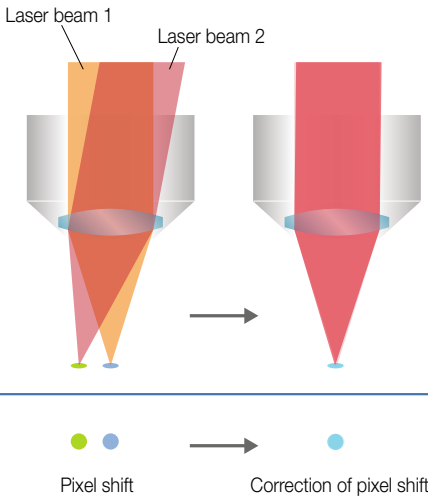


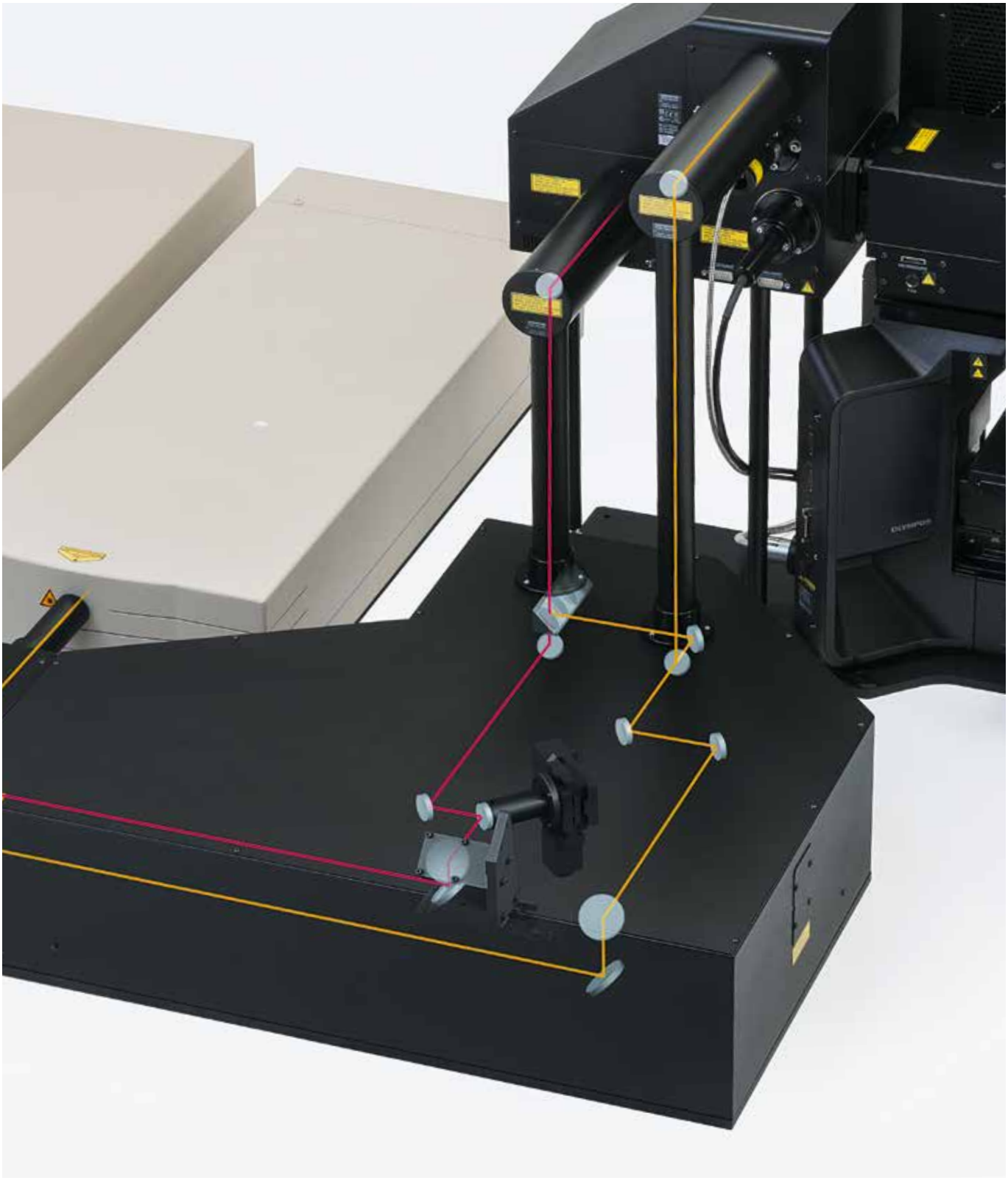
Two fluorescence proteins are clearly separated after individual excitation of GFP and DS-RED with a dual-wavelength IR pulsed laser.

Image data courtesy of:
Director Naoki Mochizuki
Department of Cell Biology, National Cerebral and Cardiovascular Center

Auto-correct for laser beam misalignment and pixel shift with Quadralign 4-axis auto-alignment

Multicolour multiphoton laser acquisition provides optimised excitation of different fluorophores, reducing channel crosstalk and photobleaching due to the need to choose a suboptimal middle wavelength for excitation. To ensure proper co-localisation of fluorescent signals, the Quadralign 4-axis auto-alignment is incorporated into two horizontal and two angular axes per laser line, and single-click compensation is also enabled for laser beam position as well as incident laser angle – a common cause of pixel shift. Saving time and effort, this auto-alignment mechanism tunes the optical axes of the lasers to the laser wavelength used during multicolour excitation. Software-based fine-tuning is also available.





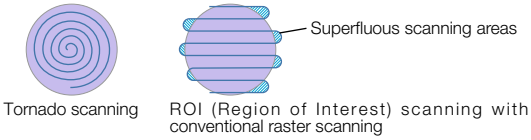
TOOLS FOR ADVANCED APPLICATIONS

Light-stimulation SIM scanner from the visible to IR range

A laser light stimulation scanner can be installed separately to form a unique Triple Scanner system. This enables optogenetics laser light stimulation of Channel Rhodopsin (ChR2) and Halorodopsin (NpHR) with simultaneous real-time imaging of neural cell activity with a visible or IR-range laser.

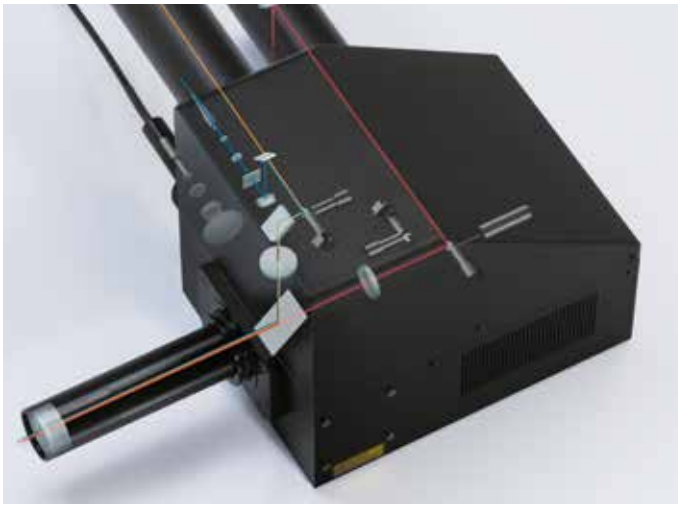
•Wide choice of scan modes

The FVMPE-RS comes with AOM as standard and provides fine position and time control of imaging and light stimulation. Using Olympus' own tornado scanning allows rapid bleaching and laser light stimulation of desired fields in experiments.



Tornado scanning

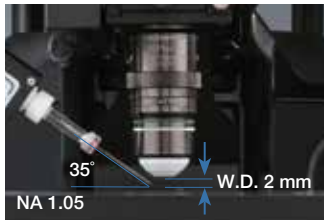
ROI (Region of Interest) scanning with conventional raster scanning

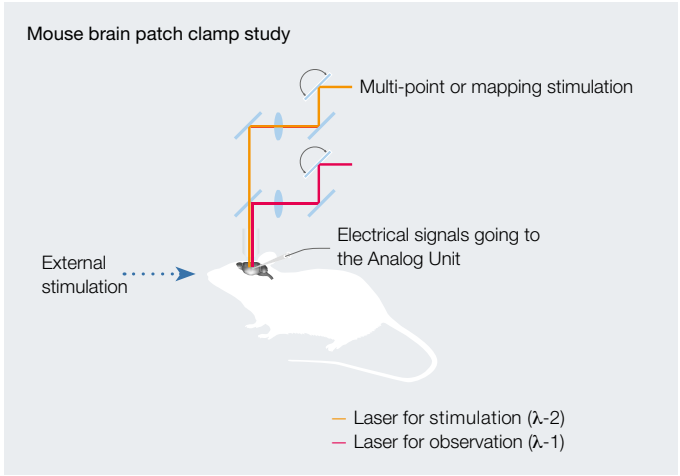


SIM scanner add-on forms a unique Triple Scanner unit

Analog Unit synchronises electro-physiological data and laser light stimulation

Electro-physiological experiments are enabled through analogue inputs and TTL I/O support. The Analog Unit converts voltage to images that can be treated in the same manner as fluorescent images – enabling light-stimulated electrical signals measured with patch clamps to be synchronised with image capture and displayed as a pseudo-colour intensity overlay.



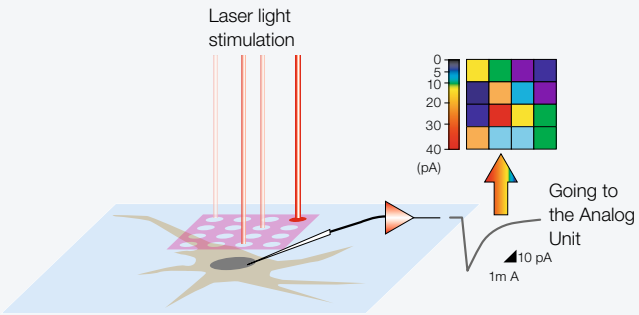


*Multicolour point by point laser control supported using SIM scanner with multiple lasers for mixed ChR2 and NpHR experiments.

3D-mapping stimulation creates reaction maps based on multiple coordinates

Highly targeted laser light stimulation is achieved by dividing the observation domain into a grid and laser irradiating each specific area in a software-controlled sequence while eliminating adjacent areas from stimulation. The Z-position setting is available to enable stimulation at a depth different from that of the imaging layer. Changes in intensity during stimulation can also be mapped onto the image and reaction maps can be created for multiple coordinates.

A typical combination of laser light stimulation and an Analog Unit



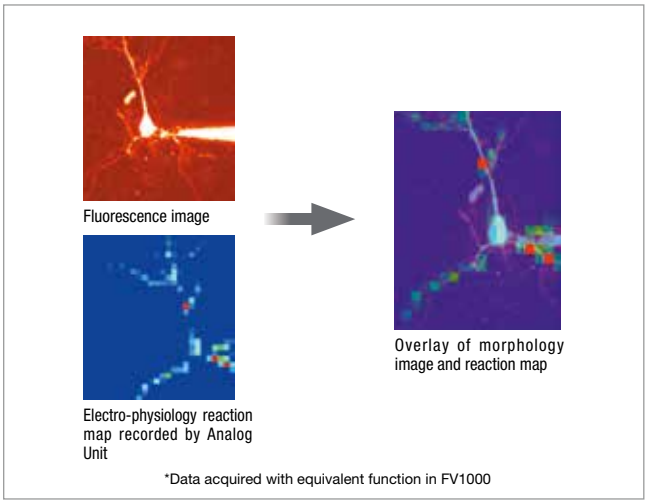
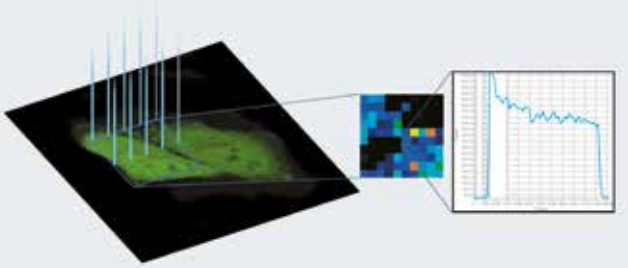


Image data courtesy of:
Haruo Kasai
Center for Disease Biology and Integrative Medicine, Faculty of Medicine,
The University of Tokyo

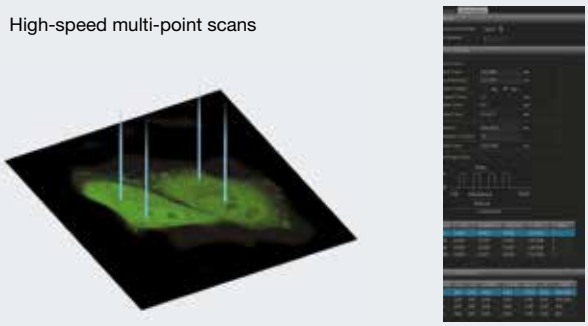
Select between continuous and pulsed multi-point stimulation

The user can designate the number of points on an image for light stimulation. Stimulation timing, duration and interval can be defined in the magnitude of µs and the user can program the experiment with continuous or pulsed stimulation. The same software also provides features that allow extended multiple points surrounding one single point to cover a small area.

Mapping scans

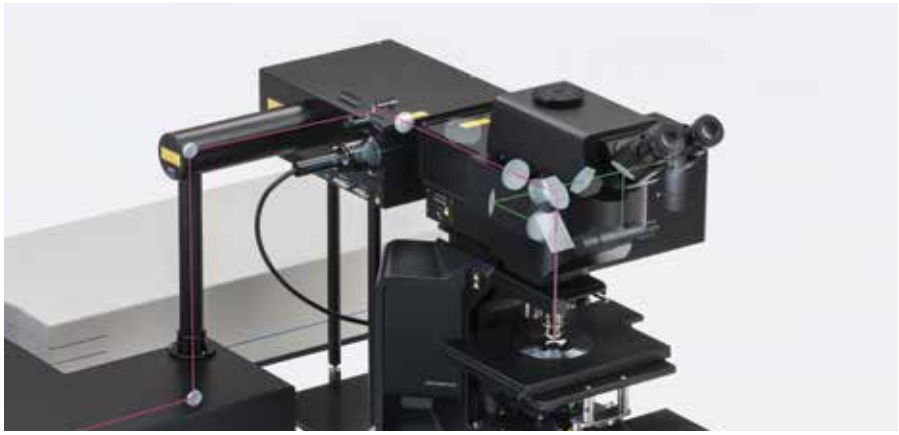


High-speed multi-point scans

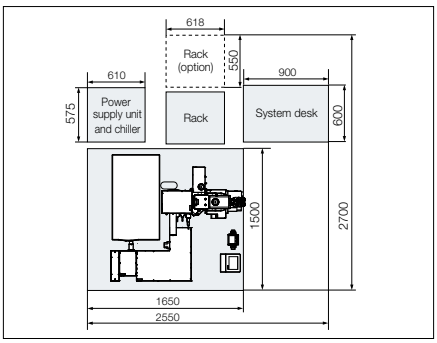


A VARIED LINE-UP OFFERING HIGH FLEXIBILITY AND INNOVATIVE IR LASER BEAM CONTROL

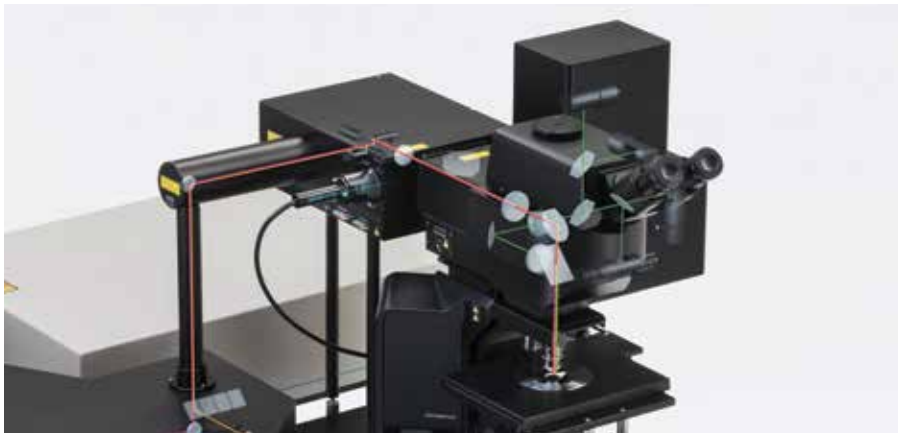
Single-laser system



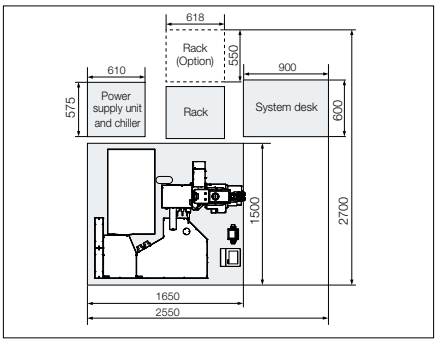
This streamlined system uses a single multi-photon IR laser for imaging. A SIM scanner for visible laser light stimulation is optional.



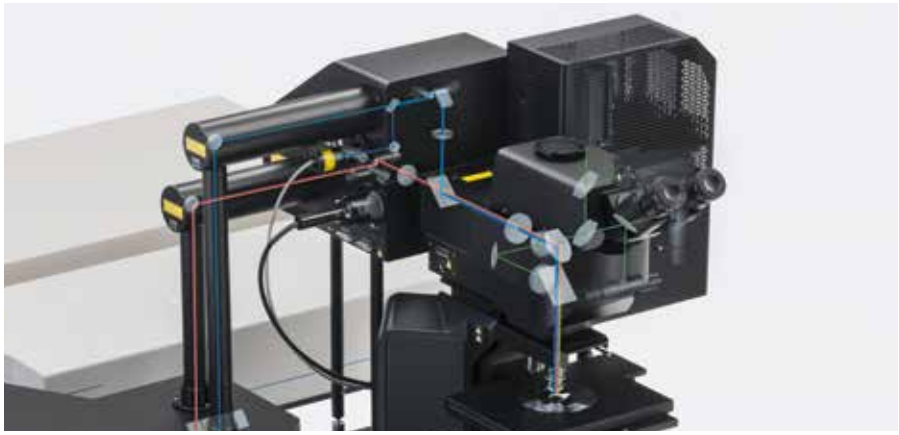
Dual-line system



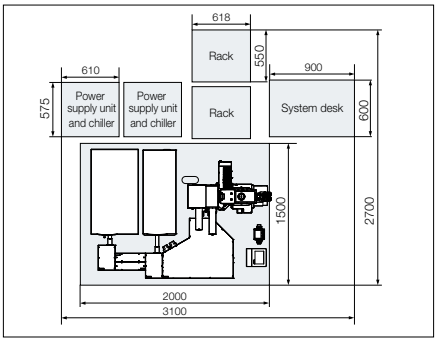
Employing the InSight DeepSee Dual laser, this system supplies dual wavelengths for multiphoton multicolour imaging. A SIM scanner for simultaneous laser light stimulation is also optional.



Twin-laser system (with SIM scanner)



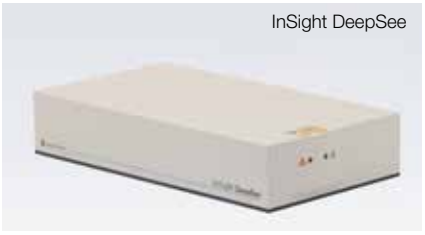
This system employs two multiphoton IR lasers for imaging. In addition to multiphoton multicolour imaging, simultaneous laser light stimulation is also supported in combination with an optional SIM scanner.



Lasers adapted for a range of multiphoton configurations

InSight DeepSee enables dual-wavelength simultaneous imaging for deep observation – with a high peak power with short 120 fs pulse widths, a continuously variable broadband range from 680 nm to 1300 nm, and a fixed wavelength of 1040 nm. A broad selection of dedicated models is available to make the most of multiphoton performance, including the MaiTai HP/eHP DeepSee-OL (Spectra-Physics).

InSight DeepSee



MaiTai HP/eHP DeepSee-OL

Chameleon Vision I/II-OL

Manufacturer	Model	Wavelength covered
Spectra-Physics	MaiTai HP DeepSee-OL	690 nm–1040 nm
	MaiTai eHP DeepSee-OL	690 nm–1040 nm
	InSight DS-OL	680 nm–1300 nm
	InSight DS Dual-OL	680 nm–1300 nm 1040 nm (fixed)

Manufacturer	Model	Wavelength covered
COHERENT	Chameleon Vision I Olympus	690 nm–1040 nm
	Chameleon Vision II Olympus	680 nm–1080 nm
	Chameleon Vision S Olympus	690 nm–1050 nm

*The Chemeleon series of lasers is not available from Olympus in some regions.

Visible beam combiner for laser light stimulation

The beam combiner allows solid-state laser combinations for laser light stimulation at wavelengths of 405 nm, 458 nm and 588 nm.

Multi-alkali PMT 2CH detector

Basic configuration of multi-alkali PMT 2CH.

Light-guide illumination source U-HGLGPS

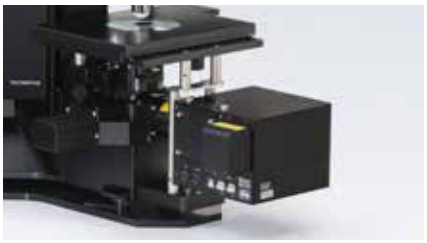
This light-guide illumination source is equipped with a liquid light guide that minimises the impact of vibration and lamp heat on the microscope and specimens alike. Employing a high-pressure mercury bulb, the light source offers a durable average lifetime of 2000 hours.

Multi-alkali PMT 2CH + 2CH detector

Multi-alkali PMT 2CH and optional addition of multi-alkali PMT 2CH.

Transmitted non-descanned light detector

A high-NA condenser and transmitted non-descanned light detector for multiphoton imaging detect fluorescence emitted from the focal plane and light scattered within the specimen.



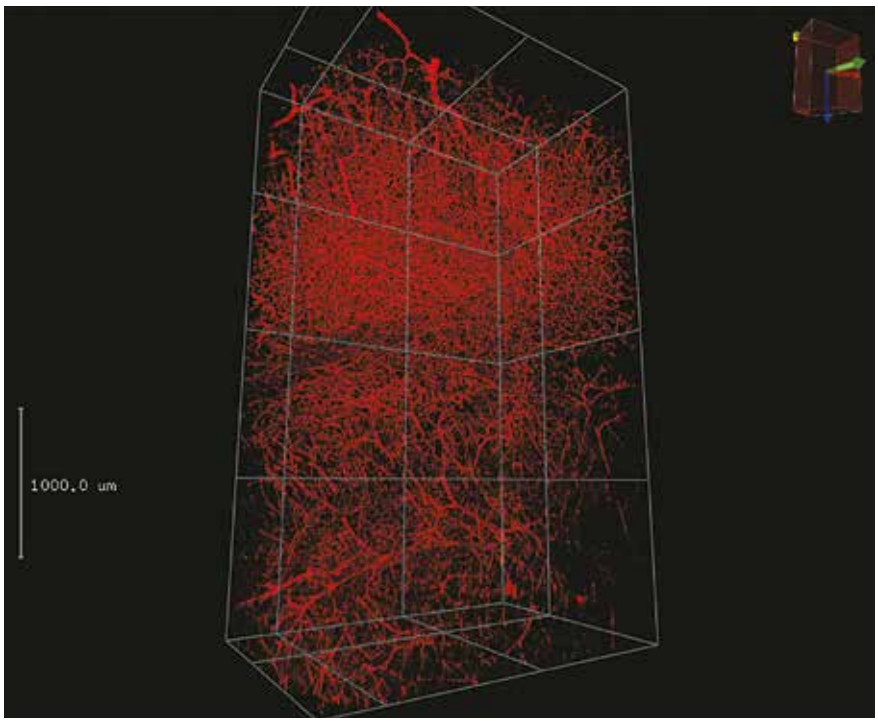
Multi-alkali PMT 2CH + cooled GaAsP PMT detector

Multi-alkali PMT 2CH and optional cooled GaAsP PMT 2CH in combination.

STREAMLINED SOFTWARE FOR MULTIPHOTON IMAGING

Software architecture supports massive data needs

Smooth 3D-rendered display is possible for massive Z-stack data comprising high-definition images captured from the sample's surface to deep sites. Key frame registration is also available, making it easy to create animated views of 3D images that zoom and transition to different camera angles.

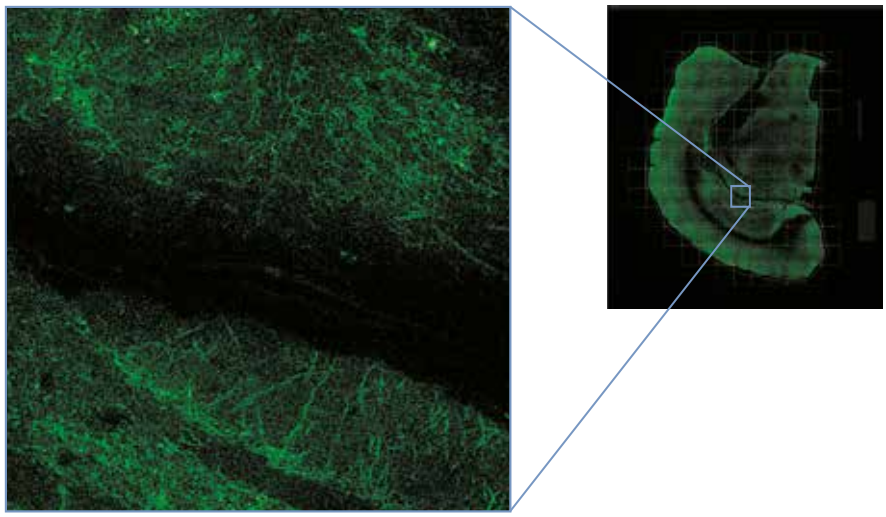


4 mm 3D stack on blood vessel label with Texas Red in mouse brain

Image data courtesy of:
Hiroshi Hama, Rie Ito, Atsushi Miyawaki
Laboratory for Cell Function Dynamics, RIKEN Brain Science Institute

Tiling significantly extends the imaging range

The tiling function scans multiple adjacent views and stitches them together to build a large image beyond the physical field of view. Use of a motorised stage supports tiling for an even wider field of view, while the mapping feature makes it easy to locate a specific cellular position within the resultant large image.

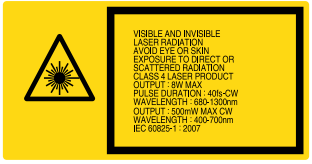


Using the map function with motorised stage, finding target field of view is easy

Image data courtesy of:
Urs Ziegler and Jose Maria Mateos
Center for Microscopy and Image Analysis, University Zurich
Mouse line L15 kindly provided by Pico Caroni, FMI, Basel

FLUOVIEW FVMPE-RS specifications

		Single-laser system	Dual-line system	Twin-laser system
Laser unit	Qualified IR pulsed lasers with negative chirp for multiphoton excitation	Mode-locked Ti: sapphire laser [femtosecond laser (equipped with a group velocity compensation)], laser power unit, water-cooled circulating chiller · Spectra-Physics products : MaiTai HP DS-OL: 690 nm–1040 nm MaiTai eHP DS-OL: 690 nm–1040 nm InSight DS-OL: 680 nm–1300 nm InSight DS Dual-OL: 680 nm–1300 nm + 1040 nm · Coherent products: Chameleon Vision I Olympus: 690 nm–1040 nm Chameleon Vision II Olympus: 680 nm–1080 nm Chameleon Vision S Olympus: 690 nm–1050 nm		
	Main IR pulsed laser	MaiTai HP DS-OL MaiTai eHP DS-OL InSight DS-OL Chameleon Vision I Olympus Chameleon Vision II Olympus Chameleon Vision S Olympus	InSight DS Dual-OL	MaiTai HP DS-OL MaiTai eHP DS-OL InSight DS-OL Chameleon Vision I Olympus Chameleon Vision II Olympus Chameleon Vision S Olympus
	Additional IR line/laser: Use as second imaging line/ laser or for simultaneous stimulation (optional SIM scanner)		1040 nm fixed line from InSight DS Dual-OL	MaiTai HP DS-OL MaiTai eHP DS-OL Chameleon Vision I Olympus Chameleon Vision II Olympus Chameleon Vision S Olympus
	Automatic introduction optic	Introduction optic with AOM attenuation (0%–100%, 0.1% increment). Including fully automated beam expander, XY shifter and two axes angle alignment. (4-axes Quadralign auto-alignment optic) Direct coupling to laser port of scanning unit.	Introduction optic with 2 sets of AOM attenuation (0%–100%, 0.1% increment). Including 2 sets of fully automated beam expanders, XY shifter and two axes angle alignment. (4-axes Quadralign auto-alignment optic) Direct coupling to laser port of scanning unit.	
	IR laser combining optic		Motorised light path switcher, with DM900, DM1000R, DM1100 to combine two IR wavelengths for imaging.	
	Optional visible light laser for stimulation	405 nm/50 mW, 458 nm/20 mW, 588 nm/20 mW laser source with AOTF attenuation. 0%–100%, 0.1% increment, < 2 µs rising time.		
Scanning unit	Scanning method	Light deflection via 2 silver-coated galvanometer scanning mirrors, or silver-coated resonant scanning mirror.		
	Scanning speed	Galvanometer scanner (normal imaging): 512 × 512 with 1.1 s–264 s. Pixel time: 2 µs–1000 µs. Resonant scanner (high-speed imaging): 30 fps at 512 × 512, 438 fps at 512 × 32.		
	Scanning mode	XY, XYZ, XYT, XYZT, free line, XZ, XT, XZT, PointT		
	Galvanometer scanner (normal imaging)	Galvanometer ROI scanning: rectangle clip, ellipse, polygon, free area, line, free line and point Zoom: 1.0×–50.0× with 0.01x increment, support 0°–360° rotation and pan Scanning field number: 18 Image size: 64 × 64 to 4096 × 4096		
	Resonant scanner (high-speed imaging)	Resonant ROI scanning: rectangle clip, line Zoom: 1.0× — 8.0× with 0.01× increment Scanning field number: 18 Image size: 512 × 512		
	Optical coating	IR support optic with 1600 coating.		
	Non-descanned MPE imaging detectors	Reflected detection: 2- or 4-channel configuration: 2-PMT configuration, 4-PMT configuration or 2 PMTs + 2 cooled GaAsP-PMTs Transmitted detection: 2 PMTs, unit with high-NA condenser		
	Transmitted-light detector	Module with integrated external transmitted-light photomultiplier detector and 100 W halogen bulb, motorised switching, fibre adaptation to microscope frame		
	Z-drive	Integrated motorised focus module of the microscope, minimum increment 0.01 µm Optional: highly rigid piezo nosepiece.		
	Control unit	CPU: Intel Xeon 4-core 3.6 GHz, memory: 12 GB, storage: 1 TB HDD x2, 240 GB SSD, graphic card: NVIDIA Quadro 600 1 GB. OS: Microsoft Windows 7 Professional 64 bit, display: 30 inch Hardware sequencer for highly precise timing repeatability control		
	Optional simultaneous stimulation scanner	Highly synchronised simultaneous stimulation scanner, including a set of galvanometer scanner, VIS and IR laser port. ROI scanning: rectangle clip, ellipse, polygon, tornado, free area, line, free line and point.		
	Optional analogue and digital in out box	4-channel analogue signal input, 6-channel digital TTL trigger input, 5-channel digital TTL trigger output. Scanner timing output.		
Operation environment		Room temperature: 20–25°C, humidity: 75% or less at 25°C, requires continuous (24-hour) power supply		
Size of anti-vibration table		1500 mm × 1650 mm	1500 mm × 1650 mm	1500 mm × 2000 mm
Software	Basic features	Dark room matching GUI design. User-arrangeable layout. Acquisition parameter reload features. Hard disk recording capability, adjust laser power and HV with Z-stack acquisition. Z-stack with alpha blending, maximum-intensity projection, iso-surface rendering.		
	IR laser controlling	Fully integrated IR laser wavelength control and Deep Focus Mode		
	Optional motorised-stage software	XY motorised-stage control, map image acquisition for easy target locating. Tiling acquisition and software image stitching. Define multiple areas for time-lapse imaging.		
	Optional mapping and multiple-point stimulation software	Multiple-point stimulation and data acquisition software. Mapping multiple-point stimulation to generate reaction map. Filtering feature to select points. Multiple-point stimulation. Single or repeat stimulation. Independent stimulation wavelength selection for each point.		
	Optional sequencer manager	Advanced programmable software to define multiple imaging/stimulation tasks and execute by hardware sequencer. Minimum gap 100 ms delay between tasks.		



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