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.

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.

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.

**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.

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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
Optics with new outperforming IR coating
An innovative IR coating (1600 coating) for the 25x 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.

<table>
<thead>
<tr>
<th>Dedicated multiphoton objectives</th>
<th>NA</th>
<th>W.D. (mm)</th>
<th>Immersion index</th>
</tr>
</thead>
<tbody>
<tr>
<td>XLPLN25XWMP2</td>
<td>1.05</td>
<td>2</td>
<td>1.33</td>
</tr>
<tr>
<td>XLPLN25XSVM2</td>
<td>1.0</td>
<td>4</td>
<td>1.33–1.40</td>
</tr>
<tr>
<td>XLPLN25XSVM2</td>
<td>0.95</td>
<td>8</td>
<td>1.33–1.40</td>
</tr>
</tbody>
</table>

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.
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 multiphoton 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.

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.

**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.
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.

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.
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).

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Model</th>
<th>Wavelength covered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectra-Physics</td>
<td>MaiTai HP DeepSee-OL</td>
<td>690 nm–1040 nm</td>
</tr>
<tr>
<td></td>
<td>MaiTai eHP DeepSee-OL</td>
<td>690 nm–1040 nm</td>
</tr>
<tr>
<td></td>
<td>InSight DS-OL</td>
<td>680 nm–1300 nm</td>
</tr>
<tr>
<td></td>
<td>InSight DS Dual-OL</td>
<td>680 nm–1300 nm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1040 nm (fixed)</td>
</tr>
<tr>
<td>COHERENT</td>
<td>Chameleon Vision I Olympus</td>
<td>690 nm–1040 nm</td>
</tr>
<tr>
<td></td>
<td>Chameleon Vision II Olympus</td>
<td>680 nm–1080 nm</td>
</tr>
<tr>
<td></td>
<td>Chameleon Vision S Olympus</td>
<td>690 nm–1050 nm</td>
</tr>
</tbody>
</table>

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.

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.

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 detector
Basic configuration of multi-alkali PMT 2CH.

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

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.

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.

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

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
Software

- **Basic features**
  - Dark room matching GUI design. User-arrangeable layout.
  - Parameter control, includes adjustable laser power and HV with Z-stack acquisition.
  - Z-stack with alpha blending, maximum intensity projection, isosurface 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.

- **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.

- **Optional sequencer manager**
  - Advanced programmable software to define multiple imaging/stimulation tasks and execute by hardware sequence.

Hardware

- **Optical laser sources**
  - Multiple-wavelengths for imaging.

- **Laser operating systems**
  - Coherent products: Chameleon Vision I, Chameleon Vision II, Chameleon Vision III, MaiTai HP, MaiTai eHP, MaiTai HP Dual-OL, InSight DS-OL, InSight DS Dual-OL.

- **Laser configurations**
  - Main IR laser
    - MaTa HP DS-OL
    - MaiTa eHP DS-OL
    - InSight DS-OL
    - Chameleon Vision I Olympus
    - Chameleon Vision II Olympus
    - Chameleon Vision S Olympus
  - Additional IR line/laser
    - 1040 nm fixed line from InSight DS Dual-OL
  - 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**
  - Resonant scanner (high-speed imaging): 512 × 512 with 1.1 s –264 s. Pixel time: 2 µs –1000 µs.
  - Resonant scanner (normal imaging): 512 × 512 with 0.01 s –2000 ms. Pixel time: 2 µs –1000 µs.

- **Optical coating**
  - IR support optic with 1600 coating.

- **Software**
  - Advanced programmable software to define multiple imaging/stimulation tasks and execute by hardware sequence.
  - Minimum gap: 100 ms delay between tasks.

- **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 × 2000 mm

- **Software**
  - Dark room matching GUI design. User-arrangeable layout.
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