FV1200MPE specifications

| | | B system | S system | T system | M system |
|---|---|---|---|---|---|
| Laser unit | IR pulsed laser 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<br>•MaiTai BB DeepSee-OL, MaiTai HP DeepSee-OL or MaiTai eHP DeepSee-OL (Spectra-Physics products)<br>MaiTai BB DeepSee-OL: 710 nm–990 nm<br>MaiTai HP DeepSee-OL: 690 nm–1040 nm<br>MaiTai eHP DeepSee-OL: 690 nm–1040 nm (70 femtoseconds at a specimen plane)<br>•Chameleon Vision I-OL, Chameleon Vision II-OL (Coherent products)<br>Chameleon Vision I-OL: 690 nm–1040 nm<br>Chameleon Vision II-OL: 680 nm–1080 nm | | | |
| | Visible light laser AOTF laser combiner | LD laser: 405 nm: 50 mW, 440 nm: 25 mW, 473 nm 15 mW, 559 nm 15 mW, 635 nm 20 mW<br>Multi Ar laser (458 nm, 488 nm, 515 nm, Total 30 mW), HeNe (G) laser (543 nm, 1 mW)<br>Modulation: continuously adjustable via an AOTF (0.1 — 100% in 0.1% increments)<br>•Operating mode: allows laser turn-off during the retrace period<br>REX: adjustment of laser power for each region, and selection of the laser and laser wavelength<br>•Visible light laser platform with implemented AOTF system, ultra fast intensity control for individual laser lines, additional shutter control, connected to scanner via single-mode fibre<br>•Equipped with laser feedback mechanism to limit changes in laser light intensity over time | | | |
| | Single laser for visible light | LD473 laser (15 mW) depending on the type of modulation: light intensity modulation, shutter control, connected to the scanner via single-mode optical fibre | | | |
| Scanning unit | Scanning method | •Light deflection via 2 silver-coated galvanometer scanning mirrors | | | •Light deflection via 2 gold-coated galvanometer scanning mirrors |
| | Scanning modes | •Pixel size: 64 x 64 — 4096 x 4096 pixels Scanning speed: (pixel time): 2 µs — 200 µs High-speed scanning mode: 16 frames/s (256 x 256)<br>•Dimensions: time, Z, (wavelength) (or any combination thereof) •Line scan: straight line (includes rotation), free line, point XY scan | | | |
| | Zoom size | Observation position zoom with inclination width modification of galvanometer mirror: 1—50x (adjustable in 0.1x increments) | | | |
| | Confocal detector (The M scanner does not have a confocal detector) | •Detector: multi-alkali photomultiplier; 3 channels, optional cooled GaAsP-PMT 2 channels detector or 4CH detector for expansion<br>•Dichromatic mirrors for excitation, dichromatic mirrors for multiphoton excitation, dichromatic mirrors for fluorescence, emission filter<br>•Infrared cut filter: using a high-performance filter<br>•A filter or spectral type of fluorescence detector can be selected<br>Spectral type: Channels 1 and 2 provided with independent grating and slit<br>Selectable wavelength range: 1–100 nm, wavelength resolution: 2 nm, wavelength switching speed: 100 nm/ms<br>•Pinhole: single motorised pinhole, pinhole diameter: ø 50 – 800 µm (spectral type ø 50 – 300 µm), adjustable in 1 µm increments<br>•Field number: 18 | | | |
| Optics with infrared laser for multiphoton imaging | | •Integrates a multi photon near-infrared pulsed laser in the scanning unit (Laser safety measures implemented)<br>•Continuously variable output using AOM (0.1 – 100%, 0.1% increments) | | | |
| Component incorporating the laser for multiphoton imaging | | Main scanner for observation | SIM scanner for laser light stimulation, Main scanner for observation: VIS laser | Incorporating 2 independent lasers for laser light stimulation/observation | M scanner for observation |
| Detector for multiphoton imaging | Reflected light fluorescent detector | Multi-alkali photomultiplier (2 or 4 channels) or<br>Cooled GaAsP-PMT 2 channels plus multi-alkali photomultiplier, 2 channels | | | |
| | Transmitted light fluorescent detector | Photomultiplier (2 channels),<br>Fluorescence wavelength can be selected with the dedicated filter cube (replaceable) (not combinable with IX-SVL2)<br>Exclusive equipment for the BX61WI upright microscope | | | |
| Transmitted DIC unit | | •Integrated transmitted light detector and transmitted illuminator, motorised switching<br>Connected to microscope via fiber cable (IR-DIC observation using an infrared laser is not possible) | | | |
| Z-drive | | •A motorized focus module inside the microscope is used<br>•Minimum increment: 0.01 µm | | | |
| Microscope | | Upright microscopes: BX61WI, BX61 Inverted microscope: IX83 (IX83P2ZF) | | | |
| System control | | •OS: Windows 7 Professional (English version) •CPU: Intel Xeon E5-1620 (3.60 GHz) or higher •Memory: 8 GB (2GB X 4) •Hard disk: 1 TB or more for data storage<br>•Dedicated I/F board: built-in control unit •Graphics board: NVIDIA Quadro 600 •Optical drive: DVD ± R/RW Super-Multi | | | |
| Required installation environment | | Room temperature: 20–25 °C, humidity: 75% or less @ 2 °C, requires continuous (24-hour) power supply | | | |
| Vibration isolation table for microscope and laser installation, size | | 1500 mm x 1250 mm | 1500 mm x 1500 mm | 1700 mm x 1700 mm | 1500 mm x 1250 mm |


Image data on cover page provided by:
Hiroshi Hama, Hiroshi Kurokawa, Atsushi Miyawaki
Laboratory for Cell Function Dynamics RIKEN Brain Science Institute

Reference material on cover page and top of page 13:
Hiroshi Hama, Hiroshi Kurokawa, Hiroyuki Kawano, Ryoko Ando, Tomomi Shimogori, Hisayori Noda, Kiyoko Fukami, Asako Sakaue-Sawano & Atsushi Miyawaki
*Sca/e*: a chemical approach for fluorescence imaging and the reconstruction of a transparent mouse brain
Nature Neuroscience, advance online publication, 30 August 2011 (doi:10.1038/nn.2928)

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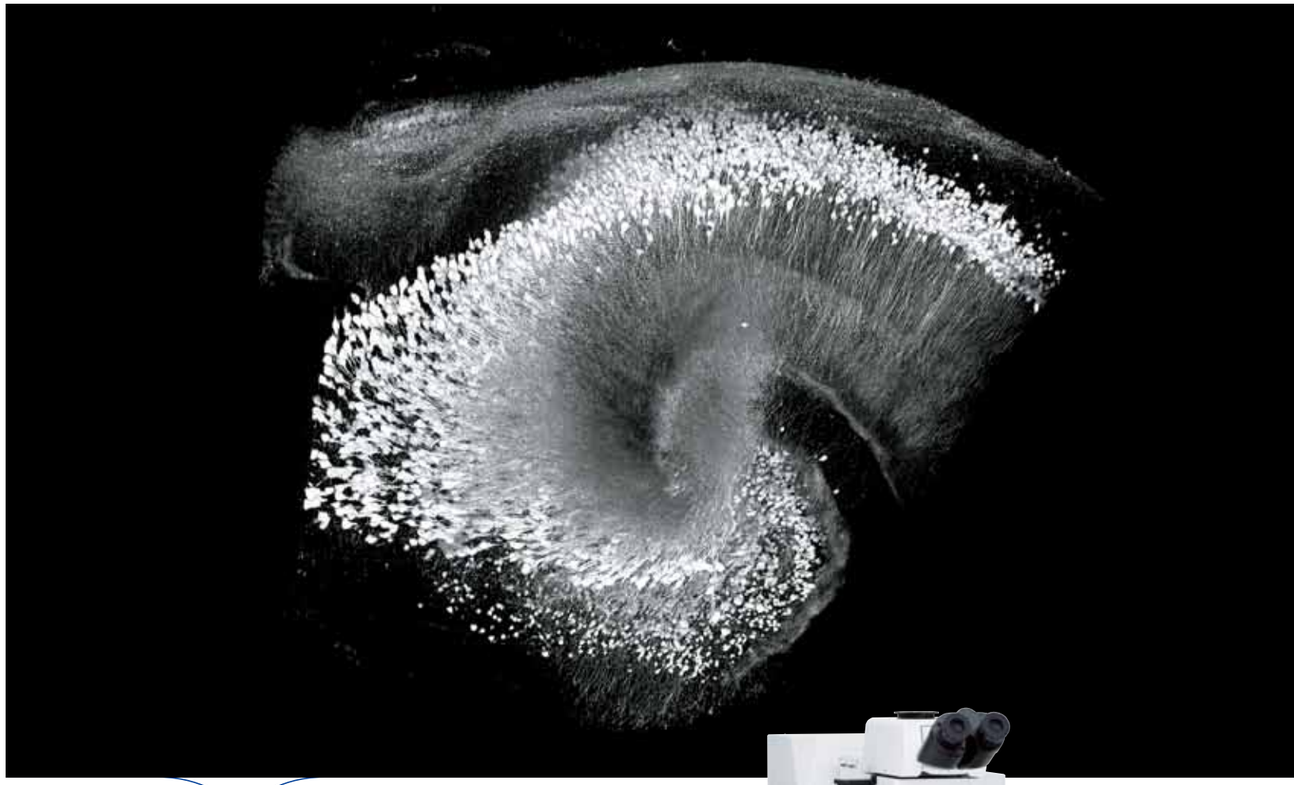
OLYMPUS
Your Vision, Our Future

Multiphoton laser scanning microscope

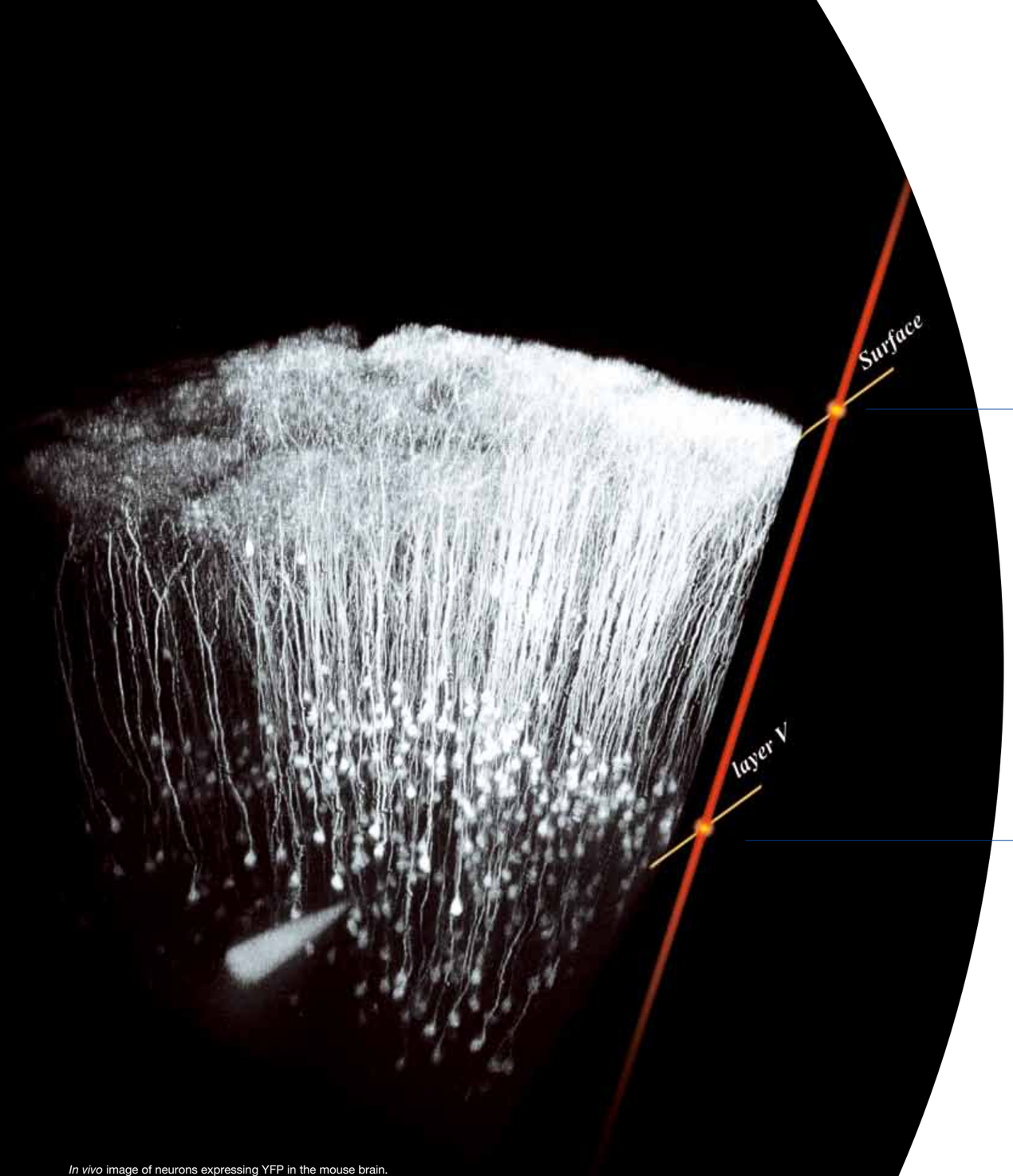
# FV1200MPE

## FLUOVIEW

*Think deep. Look deeper... with Olympus.*



UIS2
World-leading optics




*In vivo* image of neurons expressing YFP in the mouse brain.

The FV1200MPE allows observation at the depths of 0.8mm or more from the tissue surface, down to layer 5 in the mouse cerebral cortex. Images acquired *in vivo* were rendered in 3 dimensions and tilted for display.

Objective: XLPLN25XWMP

*Image data provided by:*
*Kei Eto, Hiroyuki Inada, Yusuke Takatsuru, Hiroaki Waki, Tomomi Nemoto, and Junichi Nabekura*
*National Institute for Physiological Sciences, National Institutes of Natural Sciences, Japan*



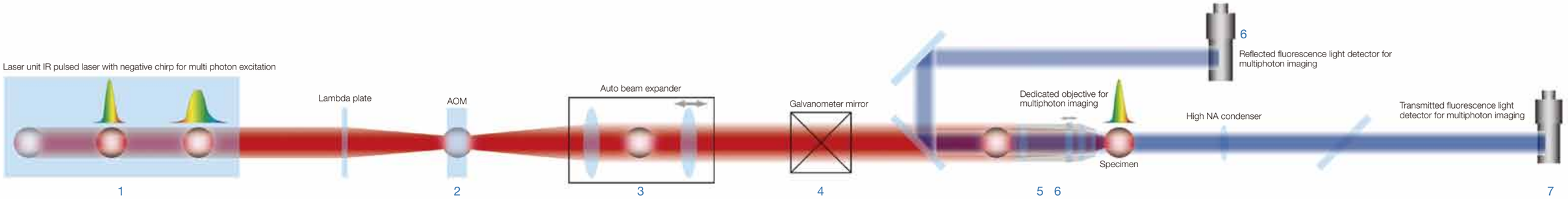
# BRIGHTER AND DEEPER IMAGING WITH BETTER RESOLUTION

The Olympus FV1200MPE multiphoton laser scanning microscope offers brighter, clearer imaging from deeper within specimens. This is thanks to its optical design, which has been optimised for efficient multiphoton excitation and signal detection.

From the deepest imaging with dedicated Olympus multiphoton objectives, to robust laser stimulation for high-speed electrophysiology, uncaging and optogenetics experiments, the FV1200MPE has been optimized for scientific discovery.

By closely adhering to optical principles while designing microscopes, Olympus opens up greater possibilities for discovery with brighter and deeper imaging with better resolution.

# THE FV1200MPE ALLOWS BRIGHT, HIGH-RESOLUTION OBSERVATION DEEP WITHIN SPECIMENS WITHOUT DAMAGING THEM




## 1 Brighter and deeper imaging with less damage

In multiphoton microscopy, fluorescence excitation efficiency is maximised by using a short pulse width in the focal plane. However, the pulse width of a femtosecond laser disperses as it passes through optics, broadening the pulse width when the beam exits from an objective.

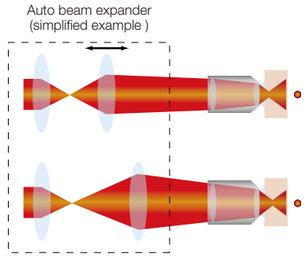
The laser beam-shaping optics establish a compensatory dispersion, the exact inverse of that produced by the microscope's optics (negative chirp), thus restoring the ideal pulse width for the specimen.

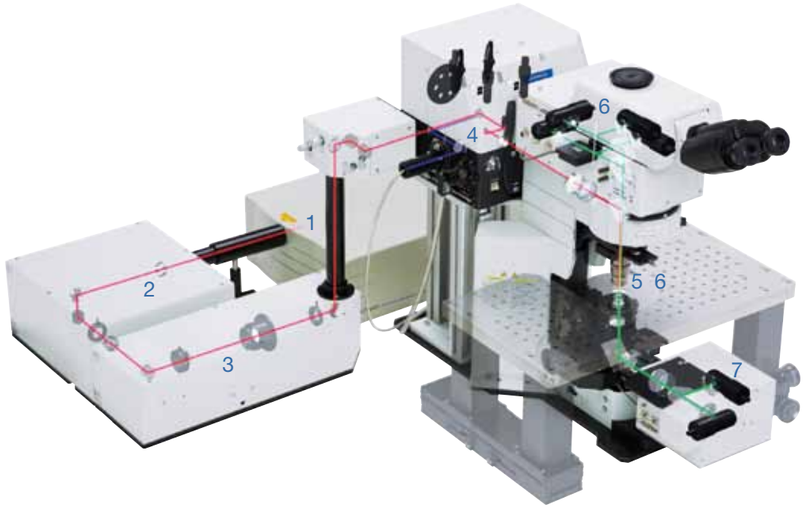
## 2 Custom light adjustment for the exiting laser beam

The FV1200MPE is equipped with an AOM to adjust laser light. The AOM allows changes in laser intensity and rapid ON/OFF switching of the laser to microsecond control. This provides laser output control to restrict irradiation to the region of interest, avoiding the surrounding areas. In thick specimens, laser intensity, and PMT voltages can be adjusted with specimen depth, allowing image capture without changes in image brightness.

## 3 Auto-adjustment of the beam in accordance with the excitation wavelength and objective

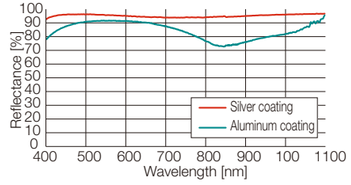
To achieve efficient multiphoton excitation, the laser beam, described by a Gaussian distribution of intensity, must fill the pupil diameter as it enters the objective.
The beam expander of the FV1200MPE automatically adjusts the beam diameter depending on the objective and excitation wavelength. This optimises laser beam characteristics for multiphoton excitation microscopy.



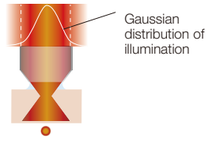

## 4 New galvanometer mirror delivers 50% greater excitation efficiency due to non-linear excitation

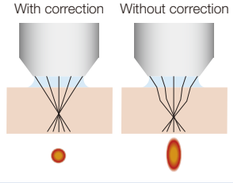
Our galvanometer mirror features an innovative silver coating that delivers outstanding reflective characteristics across a bandwidth from visible light to near infrared. The total reflectivity rate of the XY scanner is also improved—providing as much as 25% greater reflectivity in the near-infrared range compared to conventional aluminium mirrors. And where absolute power is essential, the increase in reflectivity translates into a 50% improvement in multiphoton excitation frequency compared to the FV1000MPE, making the mirror ideal for deep observation.




## 5 Correcting for light refraction in the specimen and providing deep imaging

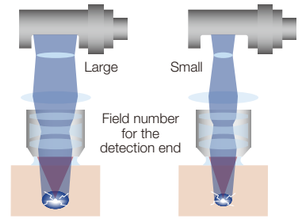
Refraction index differences within the specimen create a problem in deep imaging by disrupting the focal spot.
The FV1200MPE's dedicated objective compensates for the refractive index mismatches thanks to its correction collar, allowing the formation of an ideal focal spot deep within the specimen without loss of energy density.



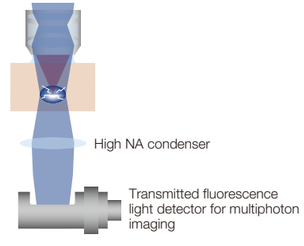

## 6 Wide field of view design to detect fluorescence with minimal loss of scattered light

In multiphoton excitation, fluorescence is emitted from the focal spot inside the specimen. Cells and tissue components scatter light such that it emerges from the surface of the specimen at some distance from the incident beam. Incorporating a wide field of view, the FV1200MPE can capture the maximum amount of fluorescent signal, including scattered light, to provide highly efficient fluorescence imaging in scattering tissue.




## 7 Even brighter in-depth observation with transmitted light detection

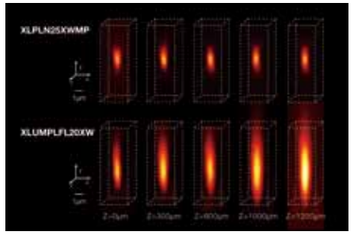
A transmitted fluorescence light detector for multi photon imaging with a dedicated high NA condenser detects transmitted fluorescence as well as transmitted laser light and forward scattered fluorescence. These additions allow extremely bright fluorescence imaging deep within a specimen and is especially effective for second harmonic generation (SHG) imaging.



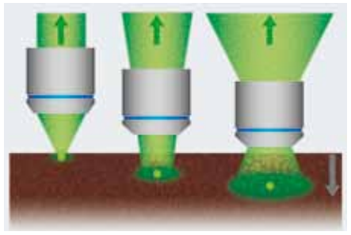
# DEDICATED OBJECTIVES AND HIGH-SENSITIVITY MULTIPHOTON FLUORESCENCE DETECTORS

### XLPLN25XWMP, dedicated water immersion objective with exceptional brightness and resolution for multiphoton imaging

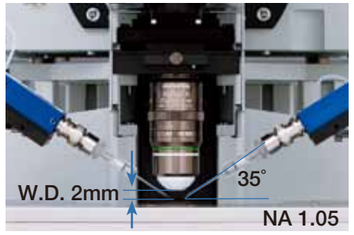
This water immersion objective with a high NA and wide field of view design has improved near-infrared transmittance to optimise multiphoton fluorescence microscopy. The correction collar minimises spherical aberrations caused by refractive index differences between the water and the specimen. This allows the formation of a tightly focused spot without reducing energy density during deep imaging. Its wide field of view design and efficient capture of scattered fluorescence allows for extremely bright, high-resolution fluorescence microscopy. In addition, it provides an approach angle of 35 degrees while maintaining a high NA, allowing easy access to execute simultaneous patch clamping and imaging.




**•Highly focused light deep within the specimen**
In this example, fluorescent microspheres 0.5µm in diameter were observed in a highly refractive medium. Axial resolution has been markedly improved compared to the conventional 20x objective.



**•Wide field of view**
Despite efficient excitation, fluorescence is scattered deep within the specimen. This widefield objective can collect scattered fluorescence to generate brighter images.



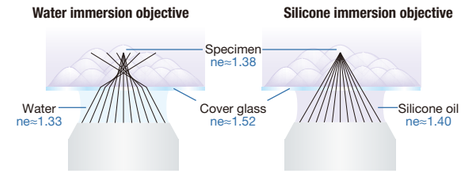

**•Sharp approach angle**
An approach angle of 35 degrees provides easy access for patch clamping. Use of this dedicated objective for multiphoton imaging allows for simultaneous imaging and patch clamp recordings.

### Silicone immersion objectives for live imaging

This immersion objective is designed exclusively for use with silicone oil, which has a refractive index even closer to live cells than that of water. The objective features a large numerical aperture and wide-ranging transmission capability from UV to IR for use in both multiphoton and single photon microscopy. Time-lapse observations become more reliable and less elaborate, because silicone oil does not dry at 37ºC and its refractive index remains constant. This objective also offers a long working distance to enable observation at deeper tissue levels and across broader fields. In a nutshell, this silicone objective offers a comprehensive solution for both macro- and deep-tissue observation in the fields of generative and regenerative science.

**Silicone immersion objective UPLSAPO30XS**
Magnification: 30x
NA: 1.05 (silicone immersion oil)
W.D.: 0.8mm
Cover glass thickness: 0.13–0.19mm
Operation temperature: 23ºC–37ºC

**The refractive index is important for deep tissue observation**




When working with a water immersion objective, the difference between the refractive index of the sample and water results in spherical aberration in deep tissue, causing resolution to deteriorate and fluorescence to become dim.

When working with a silicone immersion objective, the difference between the refractive index of the sample and the silicone oil is minimal. So it achieves brighter fluorescence images with higher resolution for deep tissue.

*Correction Collar is used to adjust for refractive index mismatch with water immersion objective XLPLN25XWMP.*

**Objectives for BX61WI**

| Model | Numerical aperture | Working distance (mm) |
|---|---|---|
| MPLN5X | 0.10 | 20.0 |
| UMPLFLN10XW | 0.30 | 3.5 |
| UMPLFLN20XW | 0.50 | 3.5 |
| LUMPLFLN40XW | 0.80 | 3.3 |
| LUMPLFLN60XW | 1.00 | 2.0 |
| LUMFLN60XW | 1.10 | 1.5 |
| XLUMPLFLN20XW*1 | 1.00 | 2.0 |
| XLPLN25XWMP*1 | 1.05 | 2.0 |
| XLPLN25XSVMP*1 | 1.00 | 4.0 |
| UPLSAPO60XW | 1.20 | 0.28 |

*1 Exclusively for BX61WI configuration.

**Objectives for IX83**

| Model | Numerical aperture | Working distance (mm) |
|---|---|---|
| UPLSAPO10X2 | 0.40 | 3.1 |
| UPLSAPO20X | 0.75 | 0.6 |
| UPLSAPO30XS | 1.05 | 0.8 |
| UPLSAPO40X2 | 0.95 | 0.18 |
| UPLFLN40XO | 1.30 | 0.2 |
| UPLSAPO40XS*2 | 1.25 | 0.3 |
| UPLSAPO60XO | 1.35 | 0.15 |
| UPLSAPO60XW | 1.20 | 0.28 |
| UPLSAPO60XS | 1.30 | 0.3 |

*2 Scheduled to be available in 2013.

### Reflected high-sensitivity GaAsP detector for upright microscopes

Achieve images with a high S/N ratio, even in cases of extremely faint fluorescence, with a detector that makes use of hand-selected gallium arsenide phosphide (GaAsP, with 45% QE). What's more, while this detector offers superior photon detection efficiency compared to conventional PMTs, noise is kept to an absolute minimum thanks to the benefits of Peltier cooling.

•Features a choice of two conventional PMT channels and two GaAsP PMT channels – enabling easy fluorescence imaging by simply switching between channels. Select a conventional PMT channel for identification of the imaging site. Switch to a GaAsP PMT channel for high-sensitivity imaging with a high S/N ratio.
•Keeps noise to an absolute minimum, with GaAsP PMT channels cooled by a Peltier element.
•Reduces degradation caused by ambient light, such as room lighting or excessive fluorescence.

Image captured with current detector

Image captured with GaAsP detector

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

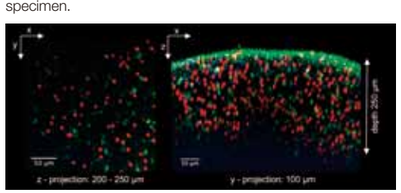
*Image data provided by:*
*Dr Norio Takata, Dr Hajime Hirase*
*Laboratory for Neuron-Glia Circuitry, RIKEN BSI*

*Dr Shun Yamaguchi*
*Gifu University Graduate School of Medicine*

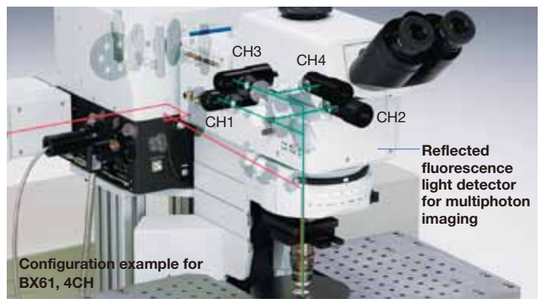
### Reflected fluorescence light detector

Fluorescent signals are not only extremely faint, but also scatter within a thick specimen, causing further decay in signal intensity. The FV1200MPE uses a detector installed at a position as close as possible to the specimen in order to maximise detection efficiency. Because multiphoton excitation is restricted to the focal plane, the emitted fluorescence does not need to pass through a confocal aperture (pinhole). This allows high-sensitivity imaging, minimising light loss due to scattering.

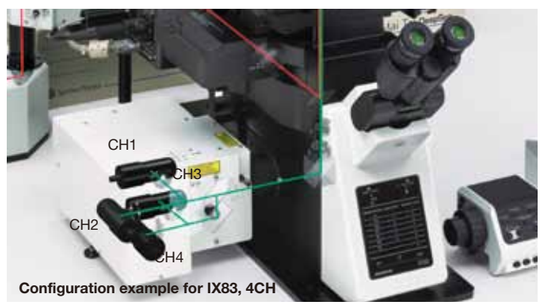
•In addition to the standard 2-channel type equipped with 2 photomultiplier tubes, a 4-channel reflected fluorescence light detector for multi photon imaging is available. All detectors are equidistant from the specimen and allow bright, high-sensitivity multicolour imaging.
•Olympus's own high-performance filter is used for wavelength separation. It can be replaced with other filters depending on the fluorescence characteristics of the specimen.




**Two-photon imaging of an explanted lymph node following transfer of B lymphocytes labeled with either SNARF (red) or CMAC (blue).**
The transferred cells and autofluorescence (green) can be observed through the collagen-rich capsular region to a depth greater than 250 µm. The left-hand panel depicts the Z-projection of an image stack at a depth of between 200 and 250 µm. The right-hand panel shows the 100 µm Y-projection of the same stack resliced along the XZ-plane. Excitation at 800 nm, objective: XLPLN 25XWMP, NA 1.05.
*Julia Eckl-Dorna, Patricia Barral, Andreas Bruckbauer, Facundo Batista*
*Cancer Research UK, London Research Institute, London, UK*




**Configuration example for BX61, 4CH**

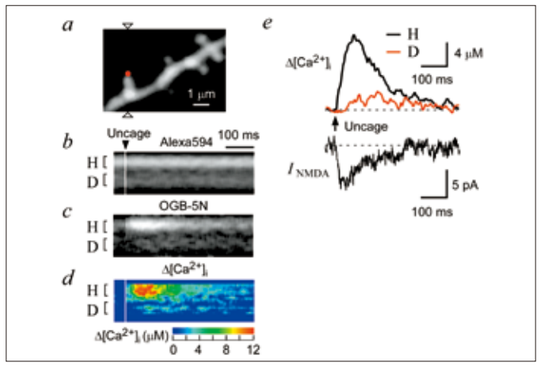



**Configuration example for IX83, 4CH**

# POWERFUL OPTIMISATIONS FOR HIGH-SPEED ELECTROPHYSIOLOGY, UNCAGING, CALCIUM IMAGING AND OPTOGENETICS

## Multiphoton simultaneous imaging and laser stimulation

Laser light stimulation can be adjusted as desired without the user being limited by imaging settings. This is due to the independent FV1200's second scanner (SIM) used for laser light stimulation (available as an option). Connected to SIM scanner, the second multiphoton laser provides simultaneous stimulation at the same focal plane that is used for imaging.




**Calcium signal of a single dendritic spine examined by multiphoton uncaging and fluorescence**

a) Stacked fluorescent image of dendritic spines in the hippocampus (excitation of 830 nm). Whole-cell recording was performed. Alexa 594 and the calcium indicator OGB-5N were injected. At the head of the single spine (red), multiphoton uncaging of caged glutamate was done and glutamate was injected (excitation of 720 nm). A line scan was performed on the line (the line linking the 2 triangles) from the head of this single spine and towards the dendritic trunk.

b), c) Simultaneous line scanning for Alexa 594 and OGB-5N.

d) Calcium concentration determined from the fluorescence emission ratios of OGB-5N and Alexa 594.

e) Changes in calcium concentration at the head of the spine (H, black), changes in calcium concentration at the dendritic trunk (D, red), current from whole-cell recorded NMDA receptors (INMDA). Calcium flow into the trunk via NMDA receptors at the head of the spine is apparent from these observations.

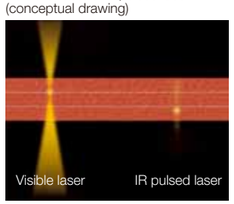
Reprinted from Noguchi et al. Neuron 46(2005)609-622.

*Jun Noguchi, Haruo Kasai*
*Center for Disease Biology and Integrative Medicine, Faculty of Medicine, University of Tokyo*

## Providing both visible light stimulation and multiphoton stimulation

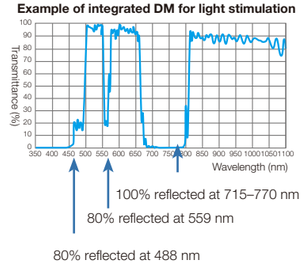
Multiple-point stimulation software (optional) allows continued stimulation, switching between IR and visible lasers in one experiment. Example: uncaging with multiphoton excitation followed by channel-rhodopsins visible light stimulation without the need to stop image acquisition.

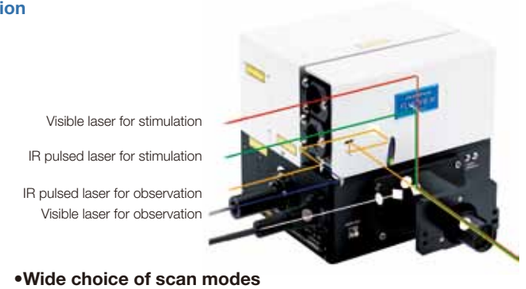
■ Comparison of stimulation with a visible laser and IR pulsed laser (conceptual drawing)




For example, with the dichromatic mirror indicated below, stimulation can be done with visible light at 488 nm and 559 nm; excitation can then be done with IR light at 92 0nm to allow observation.

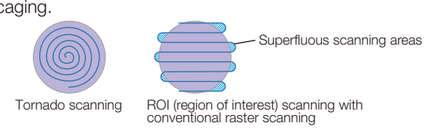
**Example of integrated DM for light stimulation**



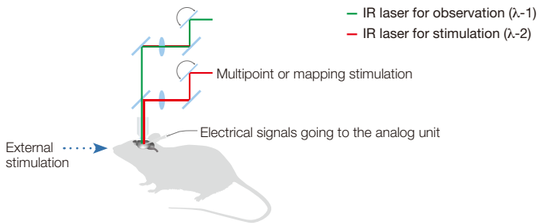

### •Wide choice of scan modes

The FV1200MPE comes with AOM as standard and enables precise position and time control of imaging and light stimulation. Using Olympus's own tornado scanning allows rapid bleaching and laser light stimulation of desired fields in experiments like those involving FRAP and uncaging.



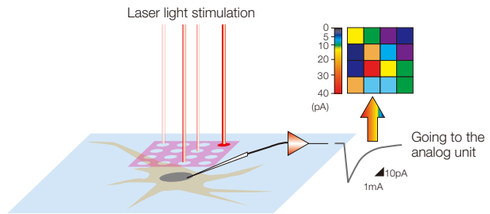

## Synchronization of laser light stimulation and patch clamp signals

The FV1200MPE's analog unit enables voltages to be converted into images and handled just like fluorescence images. For example, electrical signals measured by patch clamping during laser light stimulation can be synchronised with the image acquisition and displayed with pseudo colour.




## Laser light mapping and multipoint stimulation for electrophysiology and high-speed fluorescent measurements

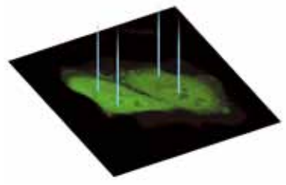
The observation field is divided into a grid and separate fields are discreetly irradiated with a laser, allowing laser light stimulation while excluding the signal influence from adjacent fields. The mapping and multipoint software enables autostimulation at multiple points (optional software).

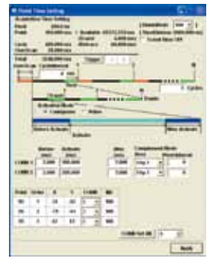


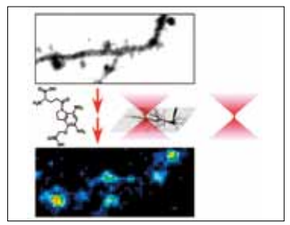

A typical combination of laser light stimulation and an analog unit

### High-speed multipoint scans

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







Functional mapping of glutamate receptors at the single spine level via multiphoton excitation of caged glutamate.

Left: stacked multiphoton fluorescence images (excitation of 830nm, Alexa594 as fluorochrome) of hippocampal CA1 pyramidal cells.

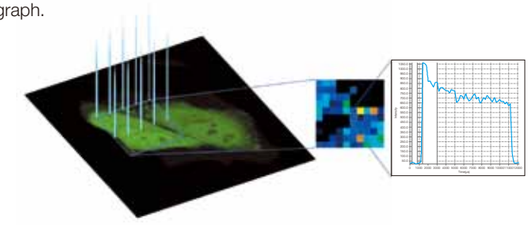
Top right: An enlargement of the mapping field.

Bottom right: Electrical signals from a glutamate receptor current, obtained with whole-cell recording. The separate points in the top right-hand figure are irradiated with the laser, captured and then mapped with colour-coding to represent the values of cell responses. At that point, caged glutamate (CDNI glutamate) is then injected into specimen slices.

*Image data provided by:*
*Masaki Matsuzaki, Haruo Kasai*
*Center for Disease Biology and Integrative Medicine, Faculty of Medicine, University of Tokyo*

### Mapping scans

Light stimulation can be applied to a rectangular region of interest. Software control of the stimulation of each point assures neighbouring points will not be excited. This allows the user to observe the reaction of a sample more accurately. Changes in intensity from those points can be processed as a mapped image or graph.



## Brightness compensation function in the Z-direction

Sample brightness typically decreases when imaging deeper into a thick specimen. Use of this function means that detector sensitivity and laser power can be adjusted while continuously acquiring an image to match the focal position, thus allowing high-sensitivity and high-precision imaging without losing information from the thick portion of the specimen.

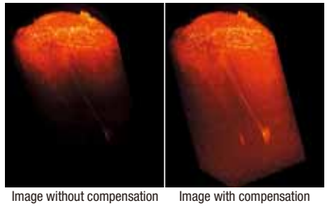


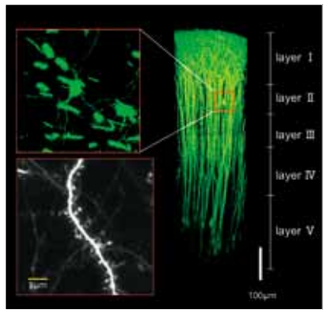
Image without compensation  Image with compensation

## The arm-height-raising kit enables small animal experiments

The arm-height-raising kit provides an additional 40 mm of clearance and is mounted between the microscope frame and the reflected light illuminator. This facilitates experiments requiring small animals.

# APPLICATION

## Mouse brain

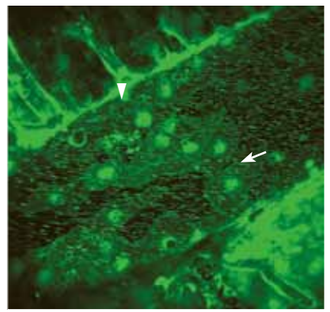



3-dimensionally constructed images of neurons expressing EYFP in the cerebral neocortex of a mouse under anesthesia.

Cross-sectional images down to 0.7 mm from the surface can be observed after a special adapter is attached to a specimen.

Objective: LUMPlanFL 60XW/IR

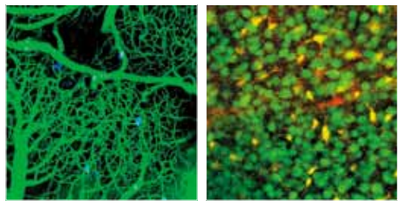
*Image data provided by:*
*Hiroaki Waki, Tomomi Nemoto, and Junichi Nabekura*
*National Institute for Physiological Sciences,*
*National Institutes of Natural Sciences, Japan*



*In vivo* observation inside the brain of a GFP-actin transgenic mouse.

One hundred and three minutes after a low concentration of lipopolysaccharide was intravenously injected into the mouse, attachments between epithelial cells detached (arrow) and a thrombus formed (triangle).

*Image data provided by:*
*Hisako Nakajima, Akira Mizoguchi*
*Neural regeneration and cell communication, Genomics and regenerative biology,*
*Mie university graduate school of medicine*

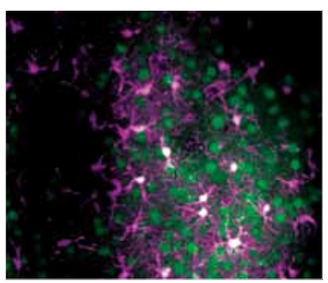


**Left:** Fluorescence angiogram in the brain of a living mouse represented by a maximum intensity projection of the imaging volume of ~600 x 600 x 600 microns. Imaging was performed on a transgenic mouse that develops senile plaques similar to those found in case of Alzheimer's disease. They are labelled with the fluorescent compound methoxy-XO4 (blue).

**Right:** A group of neurons and astrocytes loaded with the intracellular calcium reporter OGB-1 (green). Astrocytes are labeled with SR101 (red). Astrocytes that are loaded with OGB and tagged with SR101 are yellow.

*Image data provided by:*
*Brian J. Bacskai, PhD*
*Alzheimer's Disease Research Unit, Mass. General Hospital*

## Rat brain

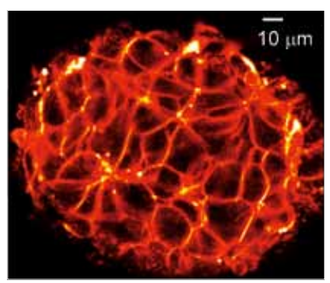


Z-stack image of neurons and glial cells in layers II and III of the cerebral cortex of a rat under anesthesia.

Magenta: glial cells (astrocytes) marked by specific fluorescence marker Sulforhodamine 101, green: neurons and glial cells, Ca-sensitive fluorescent dye Oregon Green 488 BAPTA-1 200 μm.

*Image data provided by:*
*Norio Takata, Hajime Hirase*
*Neuronal Circuit Mechanisms Research Group, Riken Brain Science Institute, Japan*

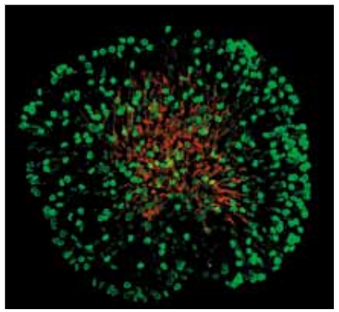
## Mouse spleen




Living pancreatic islet of Langerhans stained with FM1-43 lipid-soluble fluorescent dye. The cell membrane structure of the islet of Langerhans and growth of the membrane area accompanying insulin exocytosis of a single insulin granule can be observed.

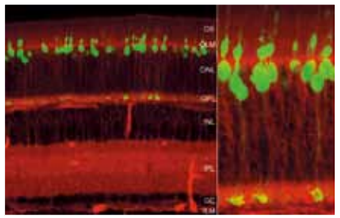
*Image data provided by:*
*Noriko Takahashi, Haruo Kasai*
*Center for Disease Biology and Integrative Medicine, Faculty of Medicine,*
*University of Tokyo*

## Mouse retina



Observation of neurogenesis in the early mouse retina

Whole-mount specimen of the mouse retina in which mitotic progenitor cells are stained with Alexa488 (green) and neurons are stained with Alexa568 (red).
Using this specimen, images were superimposed after about 120 cross-sectional images were acquired (with an XLPLN25XWMP objective and excitation wavelength of 890 nm).

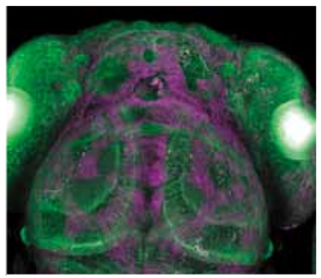


Observation of the retina in which rod photoreceptors were labelled with EGFP (green) and ubiquitous retina cells were labelled with tdTomato (red).

The specimen was fixed for a short period of time, but images were acquired under conditions for live cell imaging (low laser power) (with an XLPLN25XWMP objective and excitation wavelength of 890 nm).

*Specimens provided by:*
*Dr Branden R. Nelson, PhD at the University of Washington*

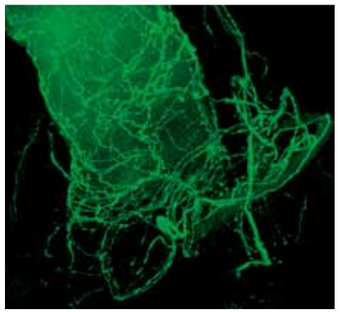
## Zebrafish



Transgenic zebrafish with cell membranes labelled with CFP. CFP is shown in green and YFP in magenta.

*Image data provided by:*
*Dr Rachel O Wong, Mr. Philip Williams,*
*Dept. Biological Structure, University of Washington*

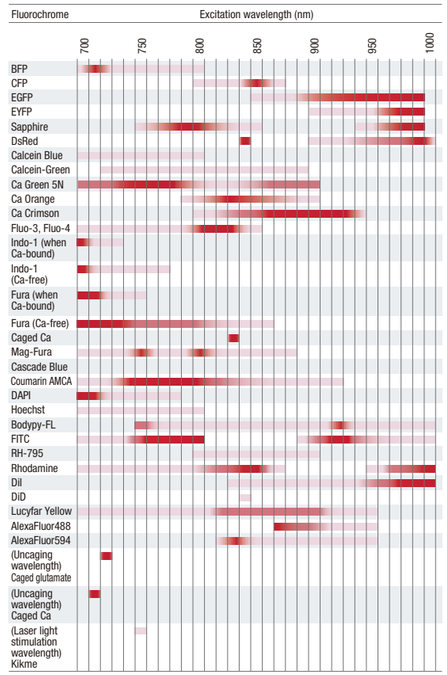
## Silkworm



3-dimensionally constructed image of cGMP-containing cells marked with CY3 and located along the antenna nerve of the silkworm.

200 μm projection image.

*Image data provided by:*
*Hitoshi Aonuma, Research Institute for Electronic Science, Hokkaido University, Japan*




References
Xu, C. and W. W. Webb, J. Opt. Soc. Am. B 13 (3), 481-491, 1996.
Xu, C., W. Zipfel, J.B. Shear, R.M. Williams and W.W. Webb, PNAS 93(20), 10763-10768, 1996
Xu, C., R.M. Williams, W.R. Zipfel and W.W. Webb, Bioimaging 4(3), 198-207, 1996
Heikal, A.A., S.T. Hess, G.S. Baird, R.Y. Tsien and W.W. Webb, PNAS97(22), 11996-12001, 2000

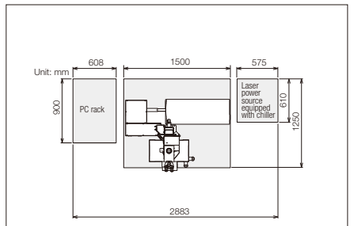
# A VARIED LINE-UP FOR LASER LIGHT STIMULATION AND IN-DEPTH OBSERVATION, FROM *IN VIVO* TO LIVE CELL IMAGING

## M System (multiphoton exclusive system)

### M scanner multiphoton exclusive system

This multi photon exclusive system is not equipped with visible light lasers. Simple optics optimised for multiphoton microscopy allow for a smaller size, simpler operation and deeper imaging within a specimen. The system uses a gold-coated galvanometer scanning mirror.

*Red: IR pulsed laser, green: fluorescent light*



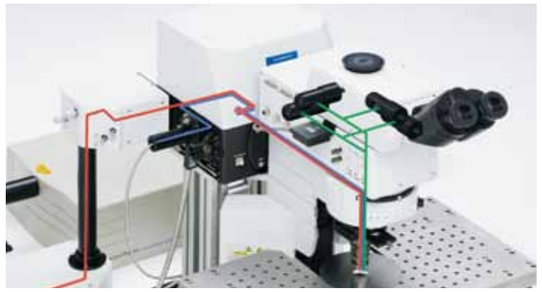

BX61/IX83 configuration

## B System (basic system)

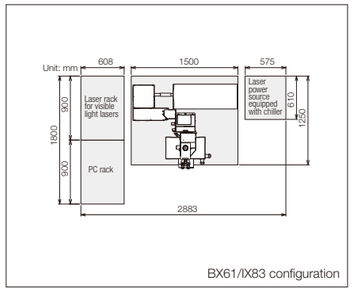
### Standard scanner multiphoton microscopy system

This system is equipped with an IR laser for multiphoton imaging and a laser for visible light, so it is designed for deep imaging using multiphoton microscopy and confocal imaging with a visible laser. The system is designed for a variety of imaging methods, including live cell and *in vivo* imaging.

** Using this system along with the double laser combiner allows multiphoton imaging and visible light stimulation.*



*Red: IR pulsed laser, blue: visible light laser, green: fluorescent light*



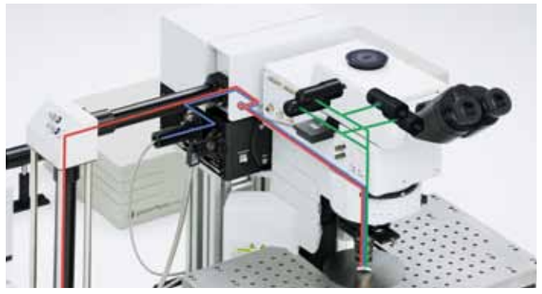

BX61/IX83 configuration

## S System (stimulation system)

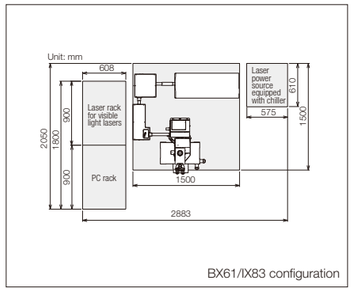
### Multiphoton laser light stimulation system

This system is equipped with an IR laser, which delivers light to the scanner for stimulation. In addition to general multiphoton microscopy, the system allows for pinpoint light stimulation by multiphoton excitation during imaging with a visible laser.

**Multiphoton microscopy does not allow some image acquisition modes such as Time Controller.*



*Red: IR pulsed laser (for stimulation/observation), Blue: visible light laser, green: fluorescent light*

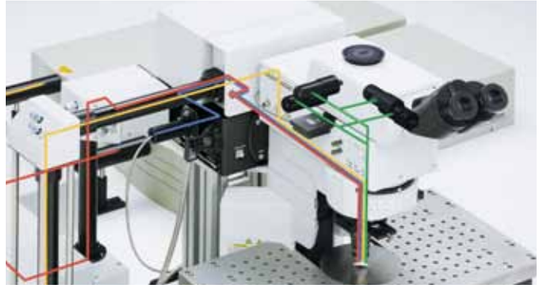



BX61/IX83 configuration

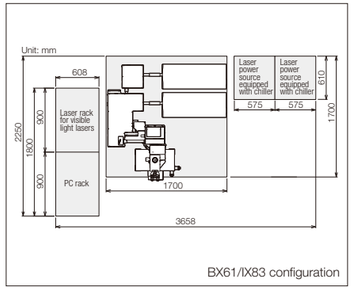
## T System (twin system)

### Multiphoton imaging plus multiphoton laser light stimulation system

This system synchronises laser light from 2 independent IR lasers for stimulation and imaging. It provides the multiphoton imaging capability of visualising deep within the tissue, while at the same time, enabling pinpoint 3D stimulation with multiphoton excitation. For example, to stimulate a single dendritic spine located deep within the tissue. The newly introduced SIM dual-port feature allows the SIM scanner to accurately stimulate with both the visible laser as well as the IR laser.



*Red: IR pulsed laser (for observation), yellow: IR pulsed laser (for stimulation), blue: visible light laser, Green: Fluorescent light*



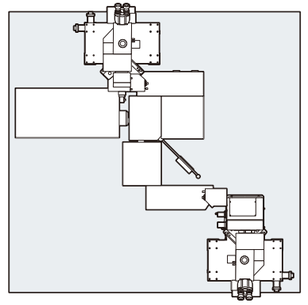

BX61/IX83 configuration

## Laser sharing system

### This system allows 2 microscopes to share a single laser

**Example of a B system basic system) sharing a laser with an M system (multiphoton exclusive system)**

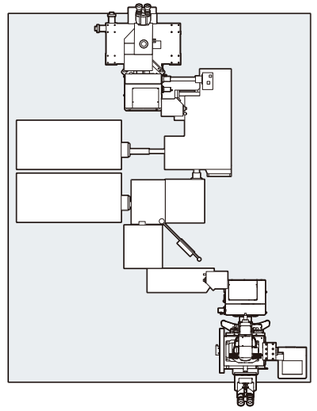
Both the B system's BX61WI and the M system's BX61WI share a single laser.



Recommended system combinations
BXM-BXB system (BX61WI-M & BX61WI-B)
IXB-BXB system (IX83-B & BX61WI-B)
BXT-BXB system (BX61WI-T & BX61WI-B)
BXT-IXB system (BX61WI-T & IX83-B)

**Example of a T system (twin system) sharing lasers with a B system (basic system)**

The BX61WI in the B system and IX83 in the T system share 2 lasers.



### Optics adapted following lasers

Both the MaiTai BB/HP/eHP DeepSee-OL lasers (from Spectra Physics, a division of Newport Corporation) and Chameleon Vision I/II-OL lasers (from Coherent, Inc.) are designed exclusively for the FV1200MPE, to provide optimal multiphoton performance.

| Manufacturer | Model | Wavelength covered |
|---|---|---|
| Spectra-Physics | MaiTai BB DeepSee-OL | 710 nm—990 nm |
| | MaiTai HP DeepSee-OL | 690 nm—1040 nm |
| | MaiTai eHP DeepSee-OL | 690 nm—1040 nm |
| COHERENT | Chameleon Vision I-OL | 690 nm—1040 nm |
| | Chameleon Vision II-OL | 680 nm—1080 nm |



### Visible lasers use for single photon confocal imaging

The multi-combiner enables combinations with all of the following diode lasers: 405 nm, 440 nm, 473 nm, 559 nm and 635 nm. The system can also be equipped with conventional Multi-line Ar laser and HeNe-G laser.

**Double output type**
The multi-combiner outputs laser light with two fibers. Light can be used for both observation and laser light stimulation.

**Single output type**
The multi-combiner with a single output fibre for visible light observation. AOTF is standard equipment.

# TAKE A REVOLUTIONARY APPROACH TO DEEP IMAGING

### Take imaging to new depths with transparent specimens and a dedicated multiphoton objective

Olympus makes it possible to perform high-precision imaging of transparent biological specimens at exceptionally deep tissue levels, with an innovative solution: comprising a dedicated 4mm working distance objective for multiphoton imaging and a groundbreaking aqueous agent that renders biological specimens transparent.

**SCALEVIEW immersion 25x objective XLPLN25XSVMP**
Specially designed to deliver optimum performance with SCALEVIEW immersion, this dedicated multiphoton objective with an ultra long working distance enables the high-precision imaging of transparent biological specimens to a depth of 4 mm.

**Optical clearing agent SCALEVIEW-A2**
SCALEVIEW-A2 revolutionises the imaging of formalin-fixed specimens. Simply render mammalian brain tissue transparent through immersion in the SCALEVIEW-A2 solution. Because SCALEVIEW-A2 eliminates light scattering, it doesn't decrease the intensity of signals emitted by fluorescent proteins in the tissue and so enables structures labelled with fluorescent proteins to be imaged in detail, from the surface to a significant depth, without the need for mechanical sectioning.

SCALEVIEW-A2 contains the fundamental components of Sca*l*eA2 developed by the RIKEN Institute. It is adjusted to achieve optimal performance with the XLPLN25XSVMP objective.

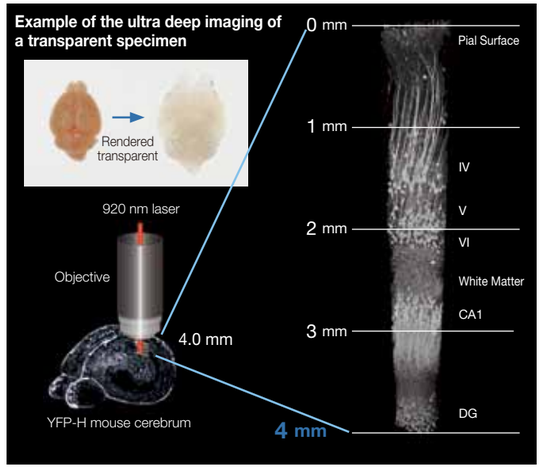
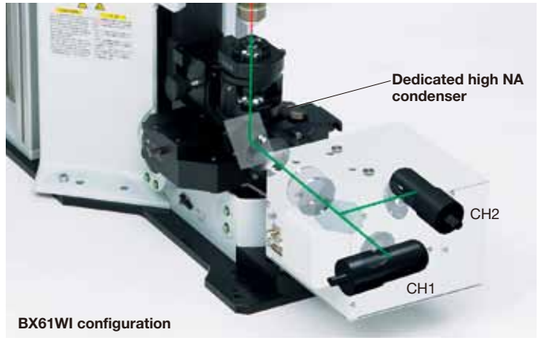



*Image data provided by: Hiroshi Hama, Hiroshi Kurokawa, Atsushi Miyawaki*
*Laboratory for Cell Function Dynamics, RIKEN Brain Science Institute*

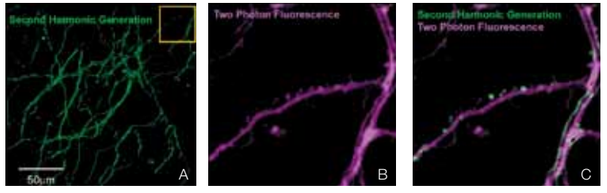
### Transmitted fluorescence light detector

A high-NA condenser and transmitted fluorescence light detector for multi photon imaging detect fluorescence emitted from the focal plane and light scattered within the specimen. With this transmitted light detector, fluorescence can be detected with a high level of efficiency, especially in deep layers of the specimen.

- The transmitted fluorescence light detector has 2 channels. These 2 channels can be used to detect fluorescence or SHG. Taking into account the reflected fluorescence light detector, FV1200MPE allows a maximum 6-channel simultaneous acquisition.
- Two types of dichromatic mirrors are available: one is a fluorescence collection type for wavelength separation in 2 channels, and another for fluorescence and SHG (475 nm).
- Two types of condensers are available: one with an oil top lens for high NA (NA 1.45) and another with a dry top lens (NA 0.8).
- Switching between transmitted light fluorescence detection and DIC observation is easy. This is optimal for patch clamping (transmitted light fluorescence detection and DIC observation cannot be performed simultaneously).




**BX61WI configuration**




Second harmonic generation imaging of neurons.

A: SHG image of neurons in dissociated culture from the mouse cerebral cortex. After FM4-64 was injected to neurons, the cells were irradiated with a femtosecond laser at 950nm and the SHG signal at 475nm was detected with a transmitted light detector.

B: Zoomed fragment (5x) of the specimen in the yellow box in image A. As it is apparent, spines protruding from dendrites can be observed with fluorescent.

C: SHG and multiphoton images have been superimposed.

*Image data provided by:*
*Mutsuo Nuriya, PhD, Masato Yasui, MD, PhD*
*Department of Pharmacology School of Medicine, Keio University*



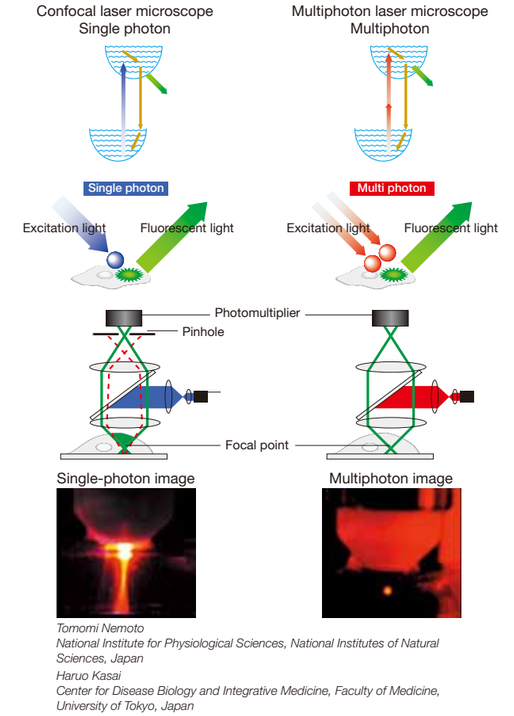


# THE MULTIPHOTON PRINCIPLE

## Multiphoton principle

### Multiphoton excitation

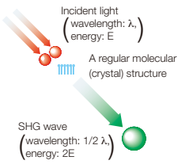
A laser radiates high-density light at wavelengths up to several times longer (approx.) than the emission wavelength, exciting the fluorescence of molecules located exactly at the focal point only. Confocal-type optical sectioning can be achieved without the use of a pinhole, since flurophores are not excited from areas outside the focal plane.



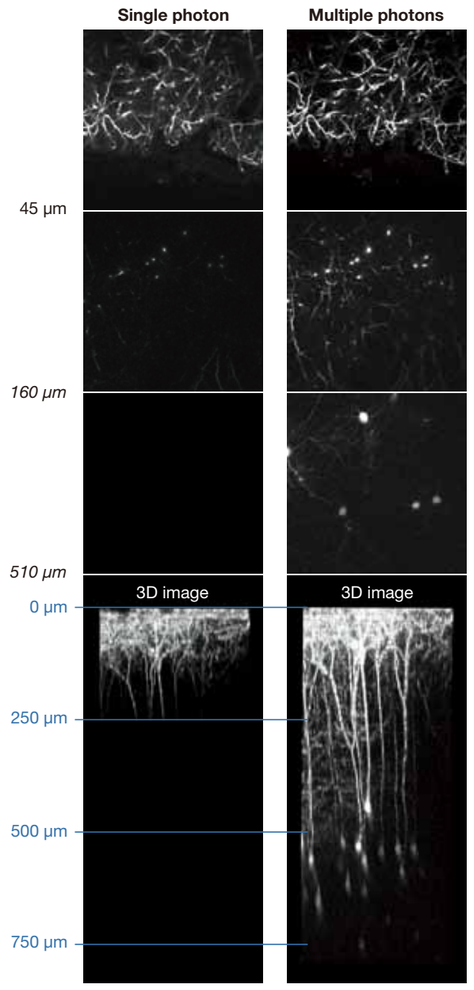

*Tomomi Nemoto*
*National Institute for Physiological Sciences, National Institutes of Natural Sciences, Japan*
*Haruo Kasai*
*Center for Disease Biology and Integrative Medicine, Faculty of Medicine, University of Tokyo, Japan*

### What is second harmonic generation (SHG)?

SHG is a secondary non-linear optical phenomenon. In SHG, the energy of 2 photons entering a specimen is combined, producing energy in the form of light. That is, the wavelength of light observed is half of the incident wavelength (the frequency is doubled). An SHG signal is not produced unless molecules in the material are noncentrosymmetric (i.e. a center of inversion symmetry is absent). The signal is linear, so a transmitted light detector is needed. In addition, SHG signal intensity is proportional to the size of the potential, so changes in membrane potential in the vicinity of lipid bilayers of cells with a regular molecular structure can also be analysed.




## Deep observation using multiphoton excitation




The cerebral cortex of M-line, a strain of transgenic mouse (GFP), was exposed and *in vivo* Z-stack imaging was performed with excitation at 488 nm for single photon excitation and with excitation at 920 nm for multiple photons. With single photon excitation only depths as far as 250 μm can be observed, but with multiplephotons depths to about 750 μm can be observed.

Images were acquired at a live cell imaging seminar (National Institute of Advanced Industrial Science and Technology, Tsukuba Research Center).

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