OLYMPUS®

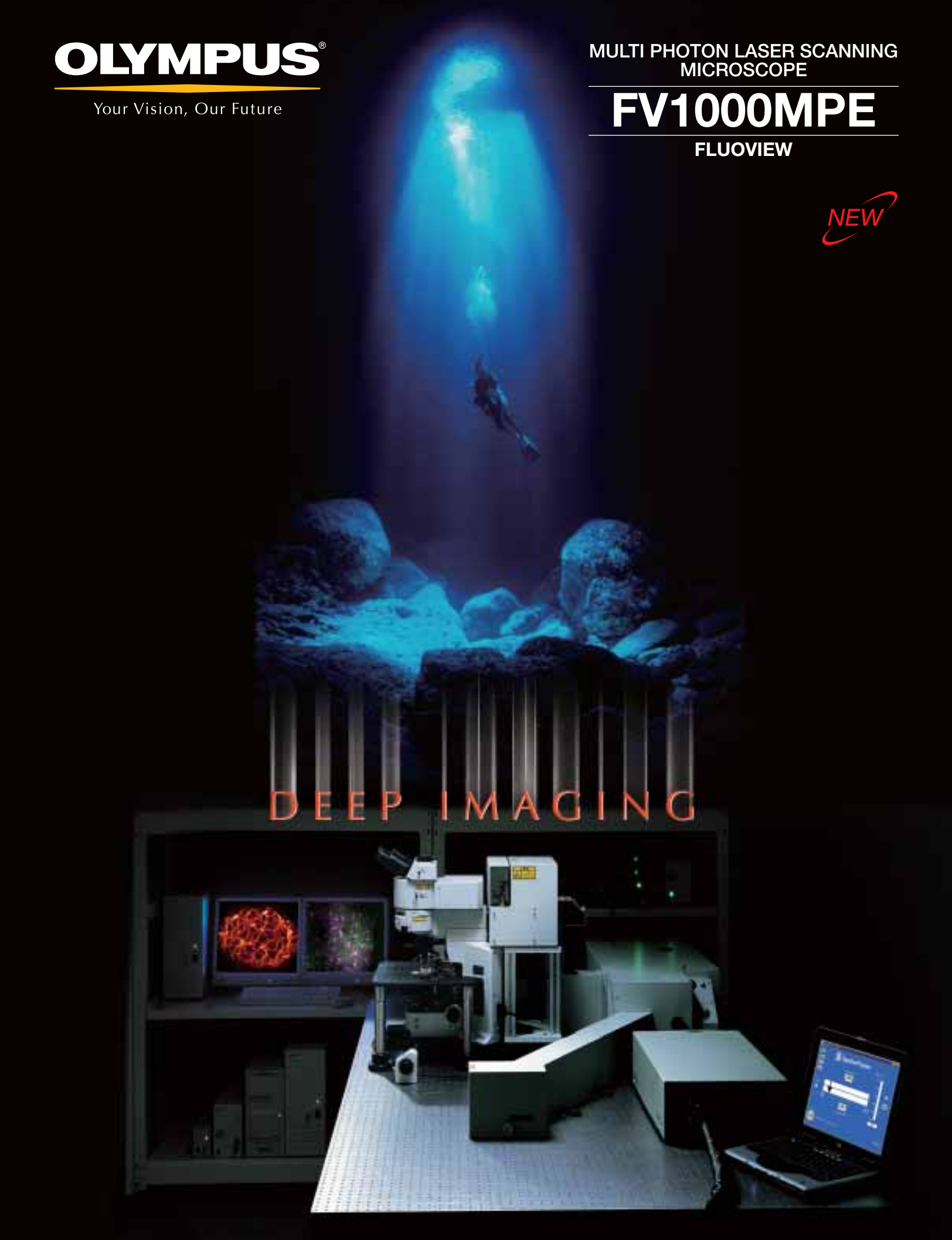
Your Vision, Our Future

MULTI PHOTON LASER SCANNING MICROSCOPE

FV1000MPE

FLUOVIEW

NEW



Multi photon laser scanning microscope for deep imaging

The Olympus Fluoview FV1000MPE, a multi photon laser scanning microscope, allows deeper imaging of living or thickly sliced specimens than conventional confocal laser microscopes can provide. Scientists can delve deeper into living tissue to study dynamic processes, with much less damage to specimens.

Optimal for *in vivo* observation deep within tissue

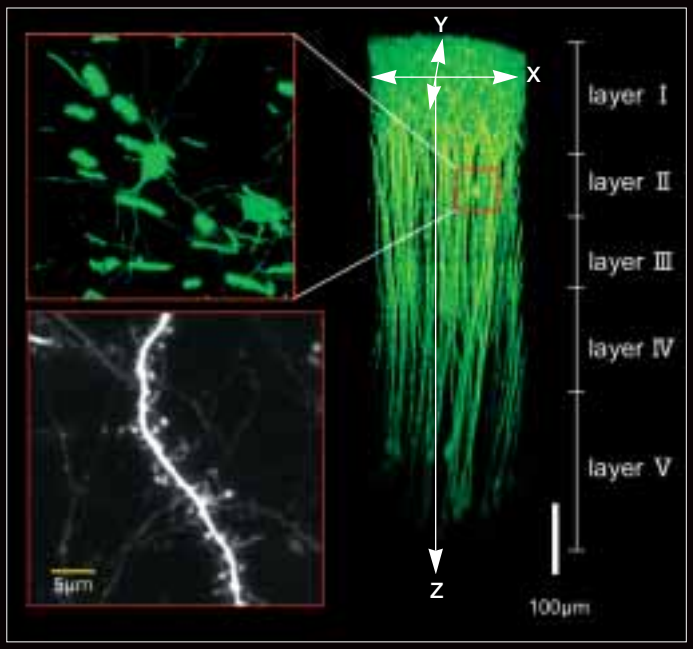
Because it uses a near-infrared laser with superior tissue penetration, the FV1000MPE allows observation deep within living specimens.

Minimizes damage to specimens

The FV1000MPE uses a near-infrared laser, for greatly reduced phototoxicity. Photodamage to specimens and fluorochromes is minimized, making long-range and time-lapse studies possible.

Reduces photobleaching

With multi photon excitation, the laser excites only the fluorescent molecules located at the focal plane. Fluorescent molecules above and below the focal plane are not stimulated by the excitation light, so there is minimal photobleaching.

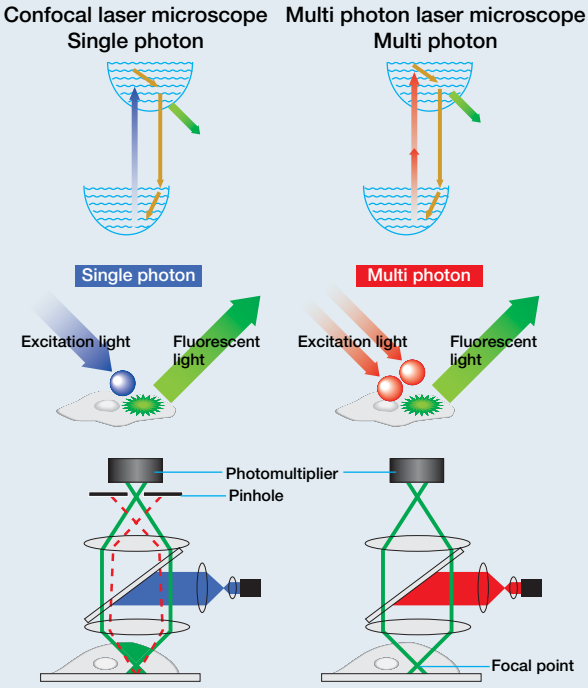


3-dimensionally constructed images of neurons expressing EYFP in the cerebral neocortex of a mouse under anesthesia.
Cross-sectional images down to 0.7 mm from the surface can be observed after attachment of a special adapter to the specimen.
Objective: LUMPlanFL 60xW/IR

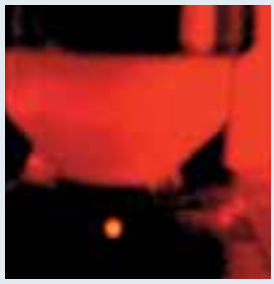
Hiroaki Waki, Tomomi Nemoto, and Junichi Nabekura
National Institute for Physiological Sciences,
National Institutes of Natural Sciences, Japan

Multi photon excitation

A laser radiates high-density light at wavelengths up to several times the emission wavelength, causing only those fluorescent molecules located exactly at the focal point to become excited. Confocal-type optical sectioning can be achieved without the use of a pinhole, since light is not emitted from areas outside the focal plane.

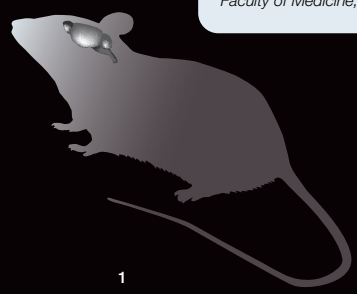


Single photon image



Multi photon image

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



More efficient fluorescence

Streamlined optical system offers enhanced multi photon performance

The FV1000MPE offers aberration correction over a wide range from the visible spectrum to the near-infrared range, thanks to its integrated optical design featuring a dedicated IR laser port, pupil projection lens and objectives. Because every optical element was designed with multi photon imaging in mind, the optical system functions efficiently, and allows better optical penetration when using near-infrared lasers.
Olympus provides a full line of water immersion objectives designed for near-infrared imaging, including the five most often-requested objectives.

	Numerical aperture	Working distance (mm)
XLUMPlanFI 20 x W	0.95	2.0
LUMPlanFI 40 x W/IR	0.80	3.30
LUMPlanFI 60 x W/IR	0.90	2.00
LUMFL60 x W	1.1	1.5
UPlanSApo60 x W	1.2	0.28

Efficient fluorescence acquisition via non-descanned detector system

With regular confocal systems, returning fluorescence emissions traveling from deep within a specimen can become dispersed through the specimen and degrade acquisition efficiency. But the FV1000MPE provides more efficient detection with minimal loss using an external two-channel photomultiplier detection system placed close to the specimen. The system provides effective fluorescence detection without the need for a pinhole for de-scanning, because fluorescence excitation occurs only in the confocal plane. Dichromatic mirrors are used in the light path to separate the light going to the two photomultiplier tubes; the mirrors and filters can be changed easily according to the specimen's fluorescence emission characteristics.

Use of a femtosecond pulsed IR laser

In multi photon excitation, a pulsed laser is used to increase photon density in the focal plane. Fluorescent molecules can be efficiently detected even at low emission levels by using a femtosecond pulsed laser. Either a Mai Tai® (Spectra-Physics, a unit of Newport Corporation) or Chameleon™ (Coherent, Inc.) laser with PC-controlled wavelength adjustment can be adapted.


Mai Tai

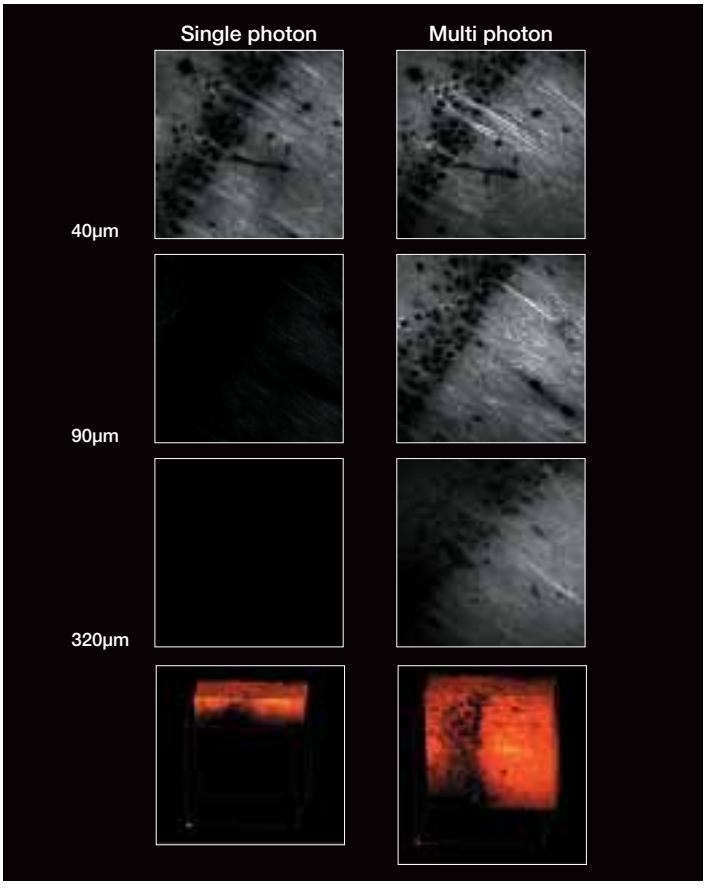

Chameleon

Optics adapted following lasers

Manufacturer	Model	Wavelengths covered
Spectra-Physics	Mai Tai XF-1	710 — 920 nm
	Mai Tai XF-950	710 — 950 nm
	Mai Tai BB	710 — 990 nm
	Mai Tai HP	690 — 1020 nm
COHERENT	Chameleon 210	720 — 950 nm
	Chameleon XR	705 — 980 nm
	Chameleon Ultra II	680 — 1080 nm

Optimal for *in vivo* observation up to several hundred µm deep

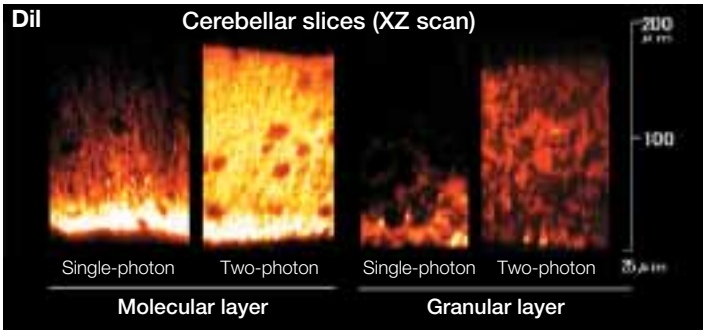
The FV1000MPE's streamlined light path involving fewer optical elements permits the microscope to operate with far less laser attenuation. The heightened rate of penetration allows deeper observation of in vivo and thickly sliced specimens.



XYZ images of mouse brain sections expressing GFP, comparing single-photon 488 nm excitation and two-photon 910 nm excitation. With single photon excitation, tissue can be observed only to a depth of about 90 µm, but with two photons, observation to a depth of about 320 µm is possible.

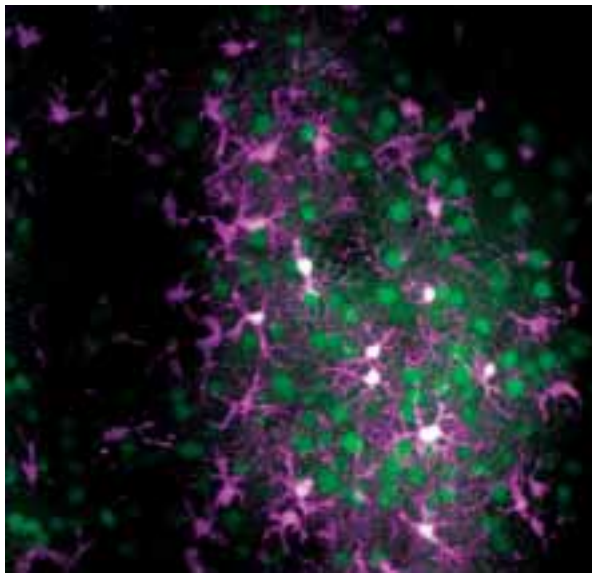
Items displayed in color are vertical cross sections of 3-dimensionally constructed images.

Specimens provided by:
Kimihiko Kameyama, Tomoyo Ochiishi, Kazuyuki Kiyosue, Tatsuhiko Ebihara
Molecular Neurobiology Group, Neuroscience Research Institute,
National Institute of Advanced Industrial Science and Technology, Japan



Vertical cross sections of the mouse cerebellum molecular layer (L) and granular layer (R) marked with DiI and obtained by XZ scans using single-photon 488 nm and two-photon 720 nm excitation. Multiphoton excitation's superiority to confocal observation is apparent in deep observation of brain slices.

Yasuyuki Hayakawa, Haruo Kasai
Center for Disease Biology and Integrative Medicine, Faculty of Medicine, University of Tokyo



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.

Norio Takata, Hajime Hirase
Neuronal Circuit Mechanisms Research Group, Riken Brain Science Institute, Japan

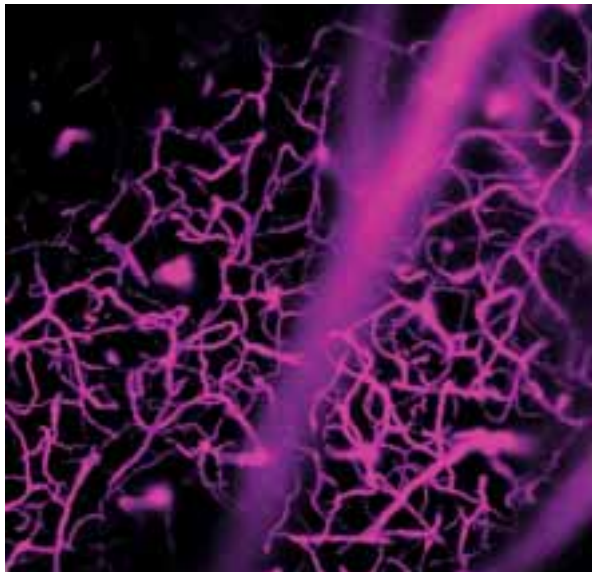
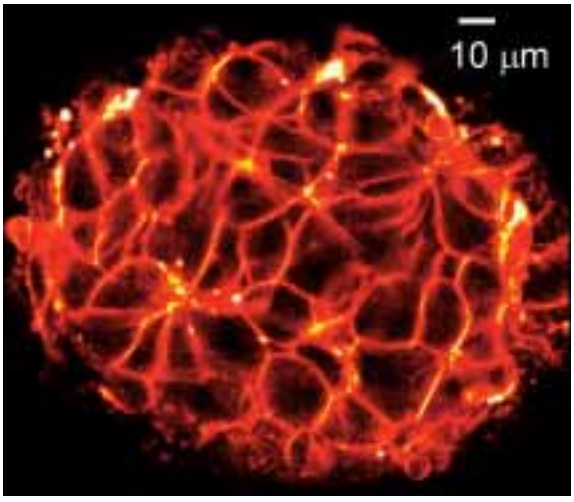
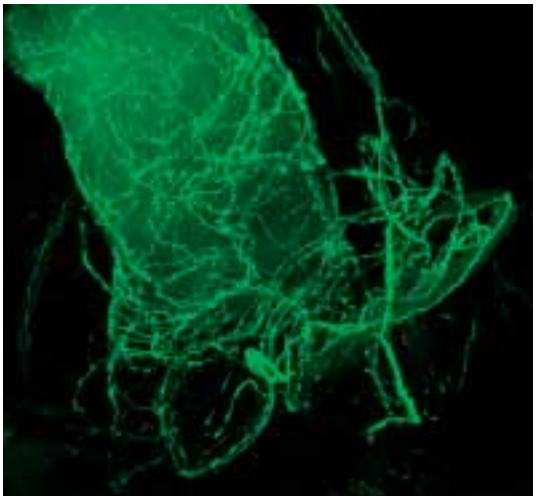


Image of cerebral blood flow of a rat under anesthesia.
Cerebral blood flow FITC-dextran 50-350 µm Z-stack image.
Hajime Hirase
Neuronal Circuit Mechanisms Research Group, Riken Brain Science Institute, Japan



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

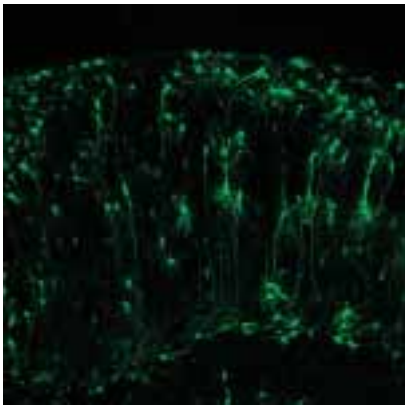
Noriko Takahashi, Haruo Kasai
Center for Disease Biology and Integrative Medicine, Faculty of Medicine, University of Tokyo



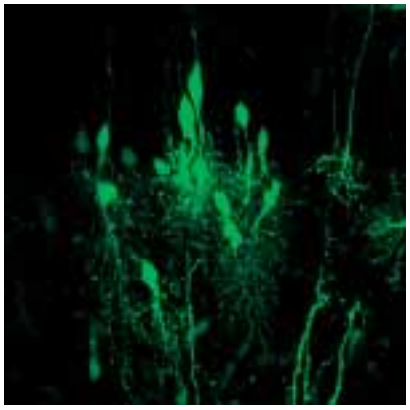
3-dimensionally constructed image of cGMP-containing cells marked with CY3 running along the antenna nerve of the silkworm.

200 µm projection image.

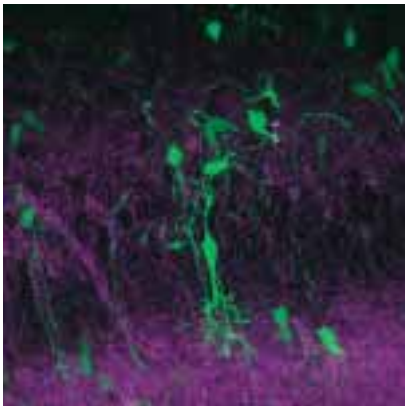
Hitoshi Aonuma
Research Institute for Electronic Science, Hokkaido University, Japan



Cells distributed in the CA1 region of the hippocampus
(Mouse embryo with the GFP gene introduced in utero)

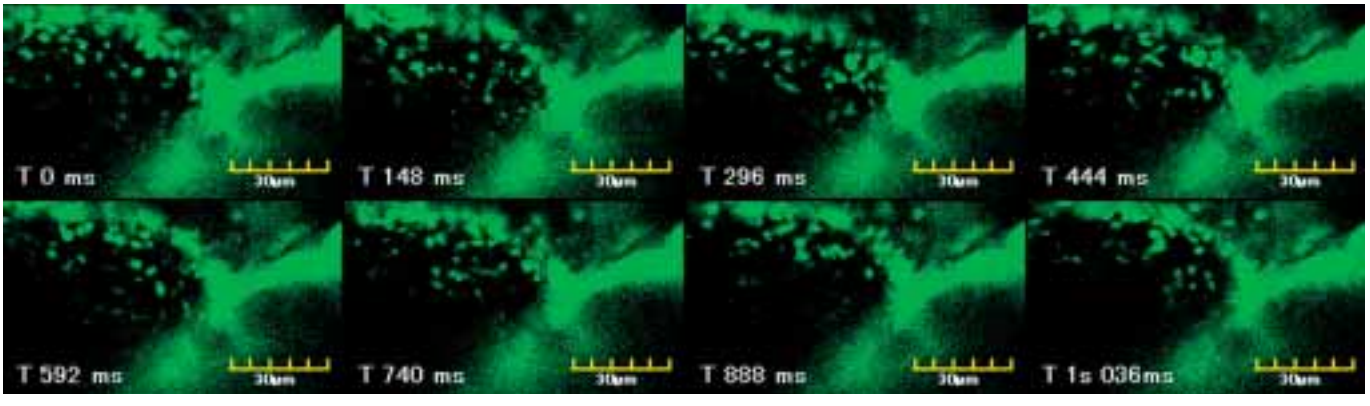


Cell body and projections of a neural progenitor cell
(Mouse embryo with the GFP gene introduced in utero)



Cell body and projections of a neural progenitor cell
(Mouse embryo with the GFP gene introduced in utero)
Neural progenitor cell: GFP (green)
Actin fibers: Phalloidin-Alexa 594 (red)

Akira Mizoguchi
Department of Neural Regeneration and Cell Communication , Faculty of Medicine, Mie University



Visualization of erythrocytes following the blood flow in the brain and vascular endothelial cells in a GFP-actin transgenic mouse
XYT (time interval 148 ms)

Akira Mizoguchi
Department of Neural Regeneration and Cell Communication, Faculty of Medicine, Mie University

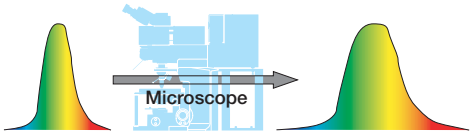
Scanning specific areas using optimal pulse width

**The FV1000MPE can handle applications using multiple points or regions of interest through high-speed toggling ON/OFF of the laser with an acousto-optic modulator (AOM).
In addition, the system's negative chirp enables highly efficient multi photon excitation.**

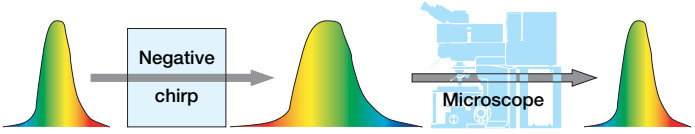
What is negative chirp?

In microscopes, dispersion through the optics causes delays in laser pulse components, which in turn can create problems with multi photon imaging. By narrowing the pulse width of the laser (also called creating a negative chirp), the beam emerging from the objective is adjusted so that the optimal width of the pulse is nearly restored. This negative chirp results in ultra-short pulses of light for multi photon excitation, and helps reduce specimen damage.

General multi photon laser microscope

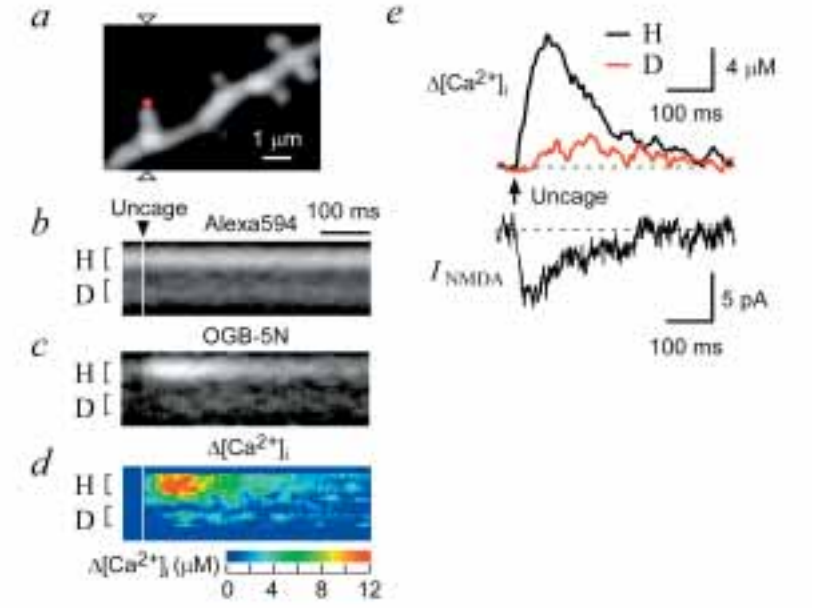


Multi photon laser microscope with negative chirp



Multi photon simultaneous imaging and laser stimulation

Laser light stimulation can be adjusted as desired without the user being limited by imaging settings. The FV1000's SIM scanner for laser light stimulation, which is independent of the scanner for observation, is available. A multi photon laser provides simultaneous excitation of the same focal plane as that used in imaging.



Calcium signal of a single dendritic spine examined by two-photon uncaging and fluorescence

a) Stacked fluorescent image of dendritic spines in the hippocampus (excitation of 830 nm). Whole-cell recording was performed, and Alexa 594 and the calcium indicator OGB-5N were injected. At the head of the single spine (red), two-photon uncaging of caged glutamate was performed and glutamate was administered (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 toward the dendritic trunk.

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

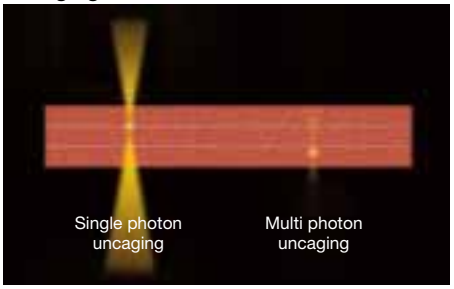
d) Calcium concentration determined from the fluorescence emission ratios of OGB-5N and Alexa 594.

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

Uncaging area

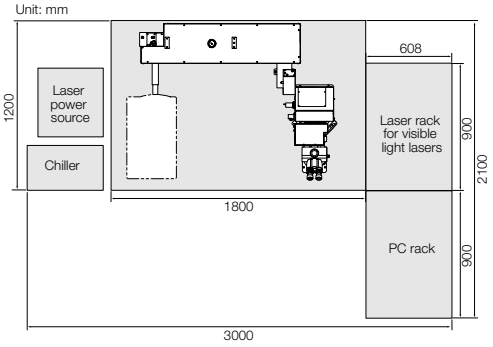


Three types of optics are available for multi photon laser integration.

Basic system

Optimized for *in vivo* imaging, this system allows deeper sections of specimens to be viewed using the multi photon laser. The optics are designed to have the beam enter the pupil of the objective efficiently. Precise adjustment of the light intensity is possible through use of a highly durable λ/2 Plate & Glan-Thompson prism. The system features a streamlined, compact design to save valuable laboratory space.

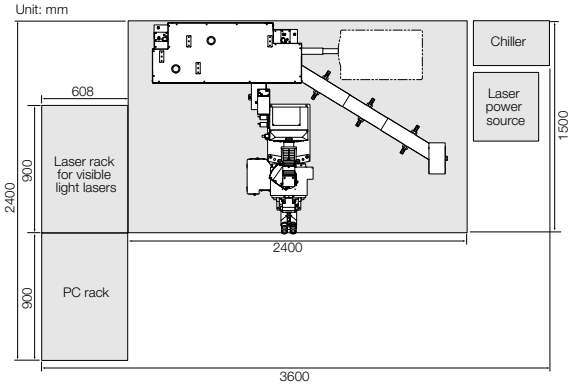
Fixed stage upright microscope with motorized focusing BX61WI system layout



AOM system

The AOM module allows high-speed ON/OFF powering of the laser. Local laser radiation of regions of interest is possible, so the FV1000MPE can be used in experiments requiring laser light stimulation, such as photoconversion. Negative chirp comes standard, so efficient multi photon excitation can be achieved with relatively weak excitation light.

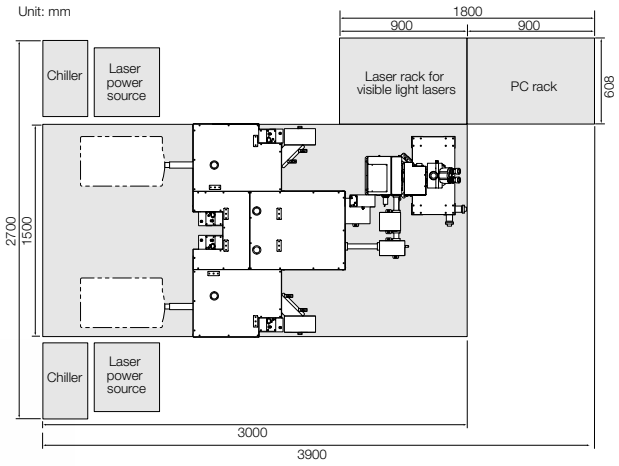
Motorized inverted system microscope BX61WI/IX81 system layout

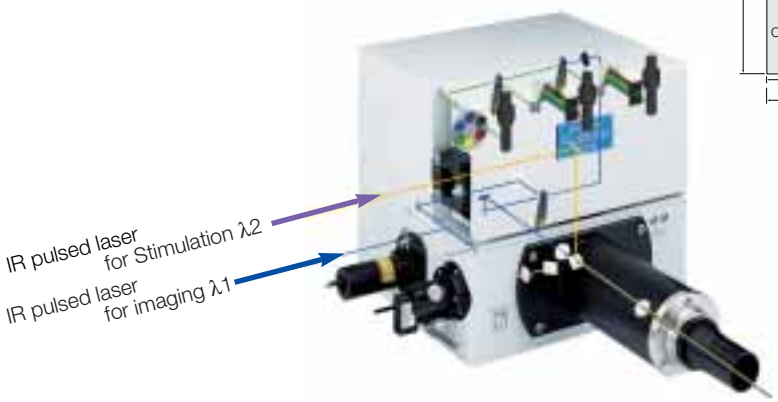


MPE-SIM system

Multi photon lasers have been integrated in the imaging unit as well as in the scanner for laser light stimulation. With multi photon excitation, excitation occurs only in the focal plane, so there is minimal bleaching. Thus, laser light stimulation of a specific site can be performed in a localized manner, in three dimensions. Additionally, this set of optics is equipped with two AOM modules, and exact laser light stimulation of the targeted area may be achieved in 2 dimensions. (Use of the single photon laser for laser light stimulation by attaching it to the dedicated scanner for laser light stimulation is not possible while imaging with the multi photon laser.)

Fixed stage upright microscope with motorized focusing BX61WI with SIM scanner system layout





FV1000MPE specifications

		Basic system	AOM system	MPE-SIM system
Single photon laser unit	Visible light laser	• LD laser: 405nm: 50mW, 440nm: 25mW, 473nm: 15mW, 559nm: 15mW, 635mW, 20mW, Multi Ar laser (457nm, 488nm, 515nm, Total 30mW), HeNe(G) laser (543nm, 1mW) • Modulation: Continuously adjustable via an AOTF (0.1 — 100% in 0.1% increments) • Operating mode: Allows laser turn-off during the retrace period, adjustment of laser power for a given region, and selection of the laser and selection of the laser wavelength		
	AOTF laser combiner	• Visible light laser platform with implemented AOTF system, ultra-fast intensity with individual laser lines, additional shutter control • Connected to scanner via single-mode fiber • Equipped with laser feedback mechanism to limit changes in laser light intensity over time		
Multi photon laser unit	Multi photon near-infrared pulsed laser	• For the adjustable wavelengths of a mode-locked Ti:sapphire laser (femtosecond), refer to additional lasers that can be used on page 6: Mai Tai (Spectra-Physics) or Chameleon (COHERENT) • Can be directly connected to a scanner via Olympus' optics integrating a multi photon near-infrared pulsed laser • Multi photon near-infrared pulsed laser power unit, water-cooled circulating chiller		
	Optics integrated	• Integrates a multi photon near-infrared pulsed laser in the scanning unit • Laser safety measures implemented via a protective cover for laser safety		
	Shutter/attenuator	λ/2 Plate & Glan-Thompson prism	AOM	AOM x 2
Scanning unit	Basic configuration	• Detector: 3 channels for fluorescence detection (photomultipliers), 1 channel for transmitted light detection (photomultiplier) • Laser ports: visible-light laser port (integrated by fiber), multi photon near-infrared pulsed laser port (directly integrated) • Dichromatic mirrors for excitation, dichromatic mirrors for multi photon excitation, dichromatic mirrors for fluorescence, emission filter, infrared cut filter: using a high-performance filter • A filter or spectral type of fluorescence detector can be selected Spectral type: Channels 1 and 2 provided with independent grating and slit Selectable wavelength range: 1 — 100 nm, wavelength resolution: 2 nm, wavelength switching speed: 100 nm/ms		
	Scanning method	• Light deflection via 2 galvanometer scanning mirrors		
	Scanning modes	• Pixel size: 64 x 64 — 4096 x 4096 pixels • Scanning speed: (pixel time): 2 µs — 5ms • High-speed scanning mode: 16 frames/sec (256 x 256) • Dimensions: Time, Z, (wavelength) (or any combination thereof) • Line scan: straight line (includes rotation), free line, point XY scan		
	Photo detection method	Analog integration		
	Pinhole	Single motorized pinhole, adjustable in 0.5 µm increments		
	Field Number	18		
	Zoom size	1 — 50x (adjustable in 0.1x increments)		
External detector		• External detector: photomultiplier (2 channels)		
SIM scanner unit		For single photon laser (optional)	For single photon laser (optional)	For multi photon laser
Vibration isolation table size		• Large vibration isolation table for a multi photon near-infrared pulsed laser		
		1,800(W) x 1,200 (D) mm	2,400(W) x 1,500 (D) mm	3,000 (W) x 1,500 (D) mm
Microscopes	Motorized microscopes	BX61WI	BX61WI/ IX81	BX61WI
System control		• OS: Windows XP Professional (English version) • CPU: Pentium IV 3.2GHz or larger • Memory: 2.0GB or larger • Hard disk: 120GB or larger • Dedicated I/F board: built-in PC • Graphics board: Open GL-compliant • Recording media: Equipped with DVD dual drive • Monitor: two SXGA 1280 x 1024 19-inch monitors		
Software		FV10-ASW		
Required installation environment		Room temperature: 20°C — 25°C ± 1°C, humidity: 60% or less, dust level: Class 10000, requires continuous (24-hour) power supply		
Power source environment		AC100-120/220-240V 60VA For details, refer to supplied environmental specifications		

*The cover page shows an FV1000MPE BX61WI system

The use of lasers with SUB-PICOSECOND pulses for two-photon microscopy is protected by US Pat No. USP5034613, JP Pat No. JP2848952B2, EU Pat No. EP500717B2, EU Pat No. EP807814B1. This technology are under a license from Carl Zeiss MicroImaging GmbH and Cornell Research Foundation Inc.

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OLYMPUS CORPORATION Micro-Imaging System Division

IISO14001 Certification
OLYMPUS CORPORATION Ina-Plant

ISO9001/ISO14001 Certification
Olympus Optical Technology Philippines Inc.

OLYMPUS CORPORATION has obtained ISO9001/ISO14001

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OLYMPUS CORPORATION
Shinjuku Monolith, 3-1, Nishi Shinjuku 2-chome, Shinjuku-ku, Tokyo, Japan
OLYMPUS LIFE AND MATERIAL SCIENCE EUROPA GMBH
Postfach 10 49 08, 20034, Hamburg, Germany
OLYMPUS AMERICA INC.
3500 Corporate Parkway, Center Valley, Pennsylvania 18034-0610, U.S.A.

www.olympus.com