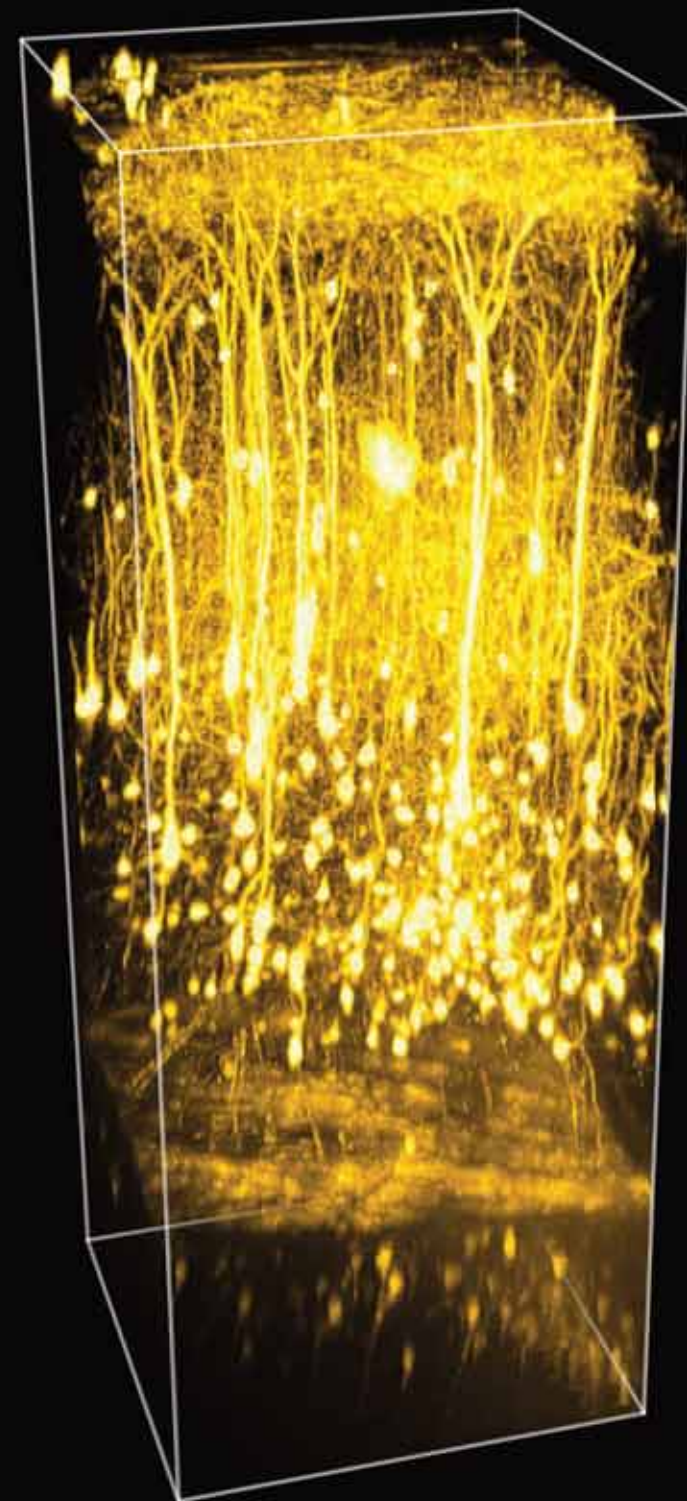




# A1MP<sup>+</sup>

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Multiphoton confocal microscope



In combination with NI-E

The A1 MP+/A1R MP+ multiphoton confocal microscopes provide faster and sharper imaging from deeper within living organisms, extending the boundaries of traditional research techniques in biological sciences.

- Ultrahigh-speed imaging up to 420 frames per second (fps) (512 x 32 pixels) with multiphoton imaging using A1R MP+ high efficiency optics and resonant scanner.
- Deep specimen imaging with high-sensitive non-descanned detectors (NDD) located close to the back aperture of the objective lens. Newly developed ultrasensitive gallium arsenide phosphide (GaAsP) NDD allows much deeper *in vivo* imaging of mouse brain over 1.2 mm.
- Auto laser alignment function quickly corrects the IR laser beam shift caused after changing the multiphoton excitation wavelength.
- The IR laser is coupled to the microscope using a compact Incident Optical Unit that contains an acousto-optic modulator and features auto-alignment functions.
- Compatible with both upright and inverted microscopes. Provides optimum multiphoton imaging configurations for brain research, other neuroscience applications and *in vivo* imaging of living specimens.

**Amazingly deep — A1 MP+/A1R MP+ sharply visualize ultra-deep dynamics within living organisms.**



In combination with TI-E



In combination with FN1



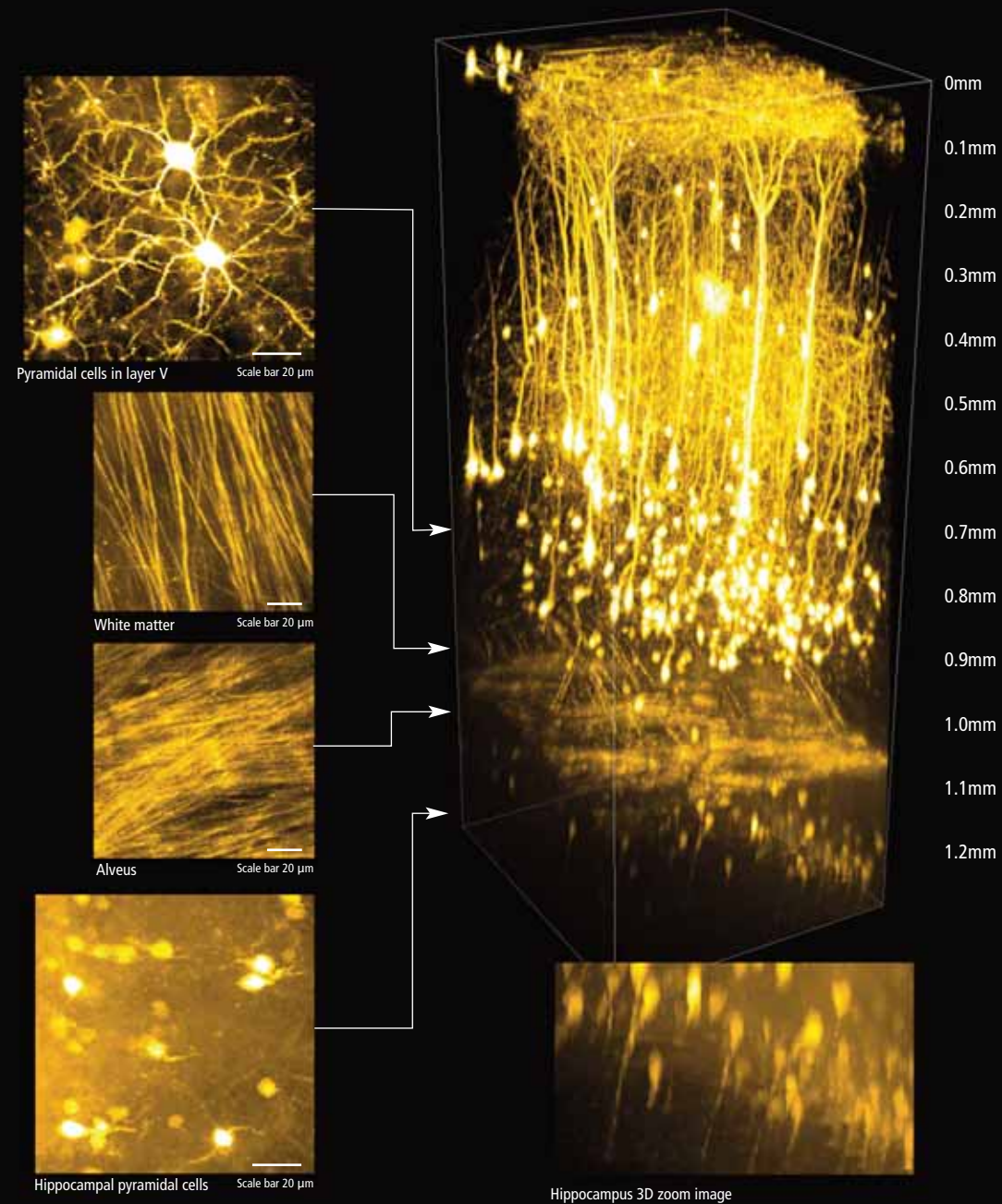
## Ultra-deep imaging with the new GaAsP NDD

The new ultrasensitive GaAsP NDD allows clear *in vivo* imaging in deeper areas than ever before and is powerful enough to analyze the mechanisms, such as brain neurons, of living specimens.

GaAsP

### Deep brain imaging in *in vivo* mouse

*In vivo* imaging of an anesthetized YFP-H mouse (4-week-old) via open skull method. Visualization of the entire layer V pyramidal neurons and the deeper hippocampal neurons. Deep imaging achieved for 3-dimensional imaging of hippocampal dendrites over 1.1 mm into the brain.



Captured with episcopic GaAsP NDD and CFI75 Apochromat 25xW MP objective lens (NA 1.10, WD 2.0 mm)

Photographed with the cooperation of:  
Dr. Ryosuke Kawakami, Dr. Terumasa Hibi, Dr. Tomomi Nemoto, Research Institute for Electronic Science, Hokkaido University

## Fast multiphoton imaging, powerful enough for *in vivo* imaging

The Nikon resonant scanner is capable of high-speed 420-fps imaging, the world's fastest for a multiphoton microscope using point scanning technology. Unique to this design is a resonant scan mirror capable of imaging full fields of view at much higher speeds than traditional galvano scanners. Nikon's optical pixel clock system, which monitors the position of the resonant mirror in real time, adjusts the pixel clock to ensure more stable, geometrically correct and more evenly illuminated imaging even at high speeds. This enables the successful visualization of *in vivo* rapid changes, such as reactions in living organisms, dynamics and cell interactions.

### Visualization of intravital microcirculation

Blood cells in blood vessels within a living organism were excited by a femtosecond pulsed IR laser with the A1R MP++ ultrahigh-speed resonant scanner, and their movements were simultaneously captured in three successive fluorescence images at 30 fps (30 msec), with three separate color channels.

The arrowhead indicates the tracking movement of the white blood cell nucleus.

Three fluorescent probes are simultaneously excited and imaged—nucleus (blue), endothelium (green), and plasma (red).

The long-wavelength ultrafast laser in combination with the ultrahigh-speed resonant scanner effectively reduces photodamage and makes time resolved multiphoton imaging of biomolecules possible.

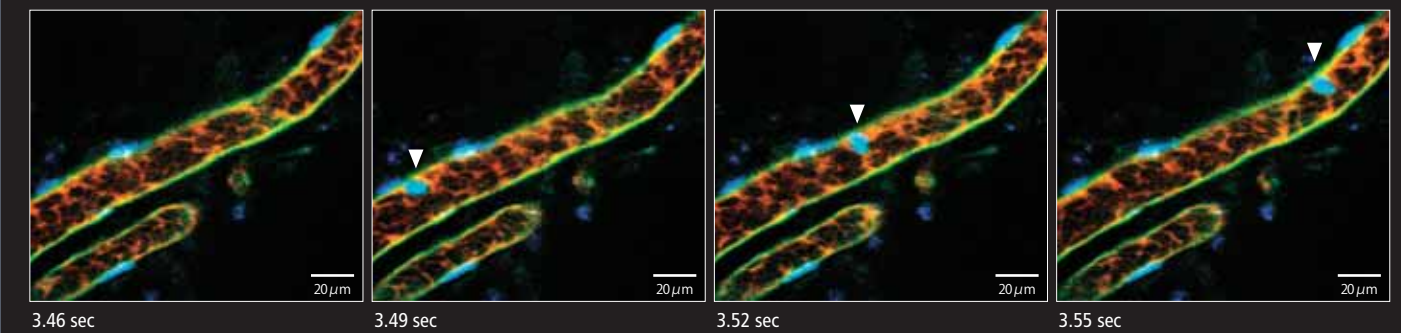


Image resolution: 512 x 512 pixels, Image acquisition speed: 30 fps, Objective: water immersion objective 60x

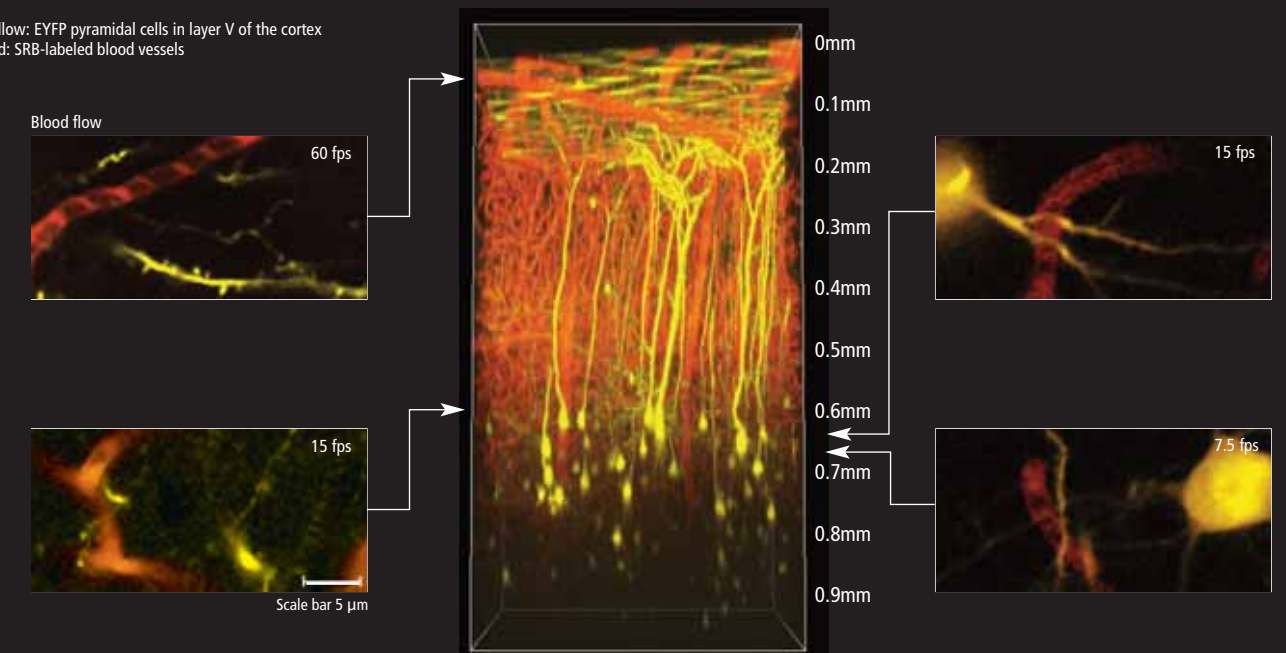
Photographed with the cooperation of: Dr. Satoshi Nishimura, Department of Cardiovascular Medicine, the University of Tokyo, TSBMI, the University of Tokyo, PRESTO, Japan Science and Technology Agency

### Mouse brain *in vivo* high-speed imaging

The cerebral cortex of an anesthetized YFP-H mouse (4-week-old) was studied with the open skull method. SRB (Sulforhodamine B) was injected into the tail vein. Using resonant scanning with episcopic GaAsP NDD, blood flow can be imaged at various deep Z positions.

GaAsP

Yellow: EYFP pyramidal cells in layer V of the cortex  
Red: SRB-labeled blood vessels



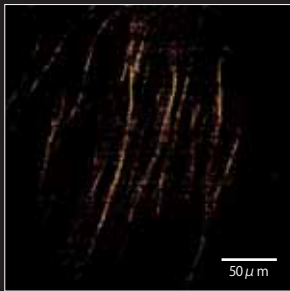
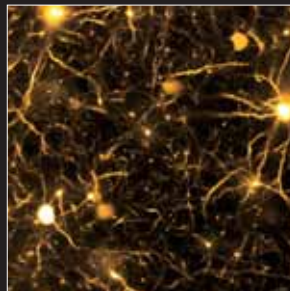
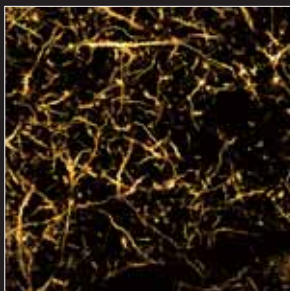
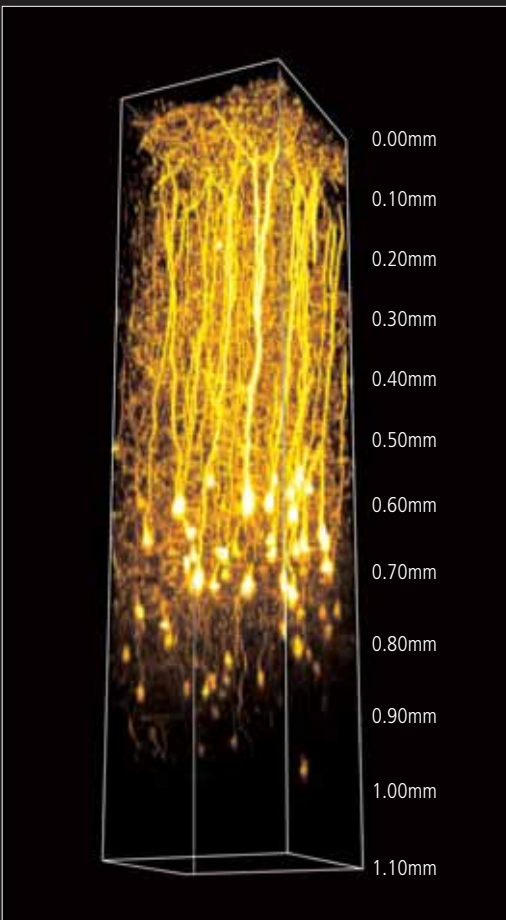
Photographed with the cooperation of:

Dr. Ryosuke Kawakami, Dr. Terumasa Hibi, Dr. Tomomi Nemoto, Research Institute for Electronic Science, Hokkaido University



## Deep imaging of living specimens with highly efficient standard NDD

### *In vivo* image of deep areas of cerebral cortex of a mouse



The cerebral cortex of an H-line 5-week-old mouse was studied with the open skull method. The entire shape of dendrites of pyramidal cells in layer V expressing EYFP were visualized from the bottom layer into a superficial layer. In addition, the fluorescence signal of white matter in deeper areas was also studied.

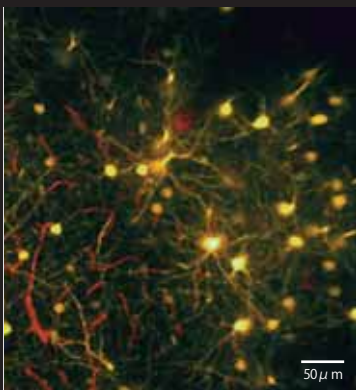
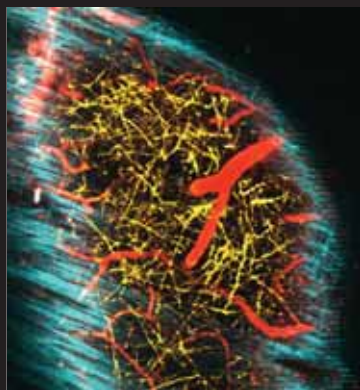
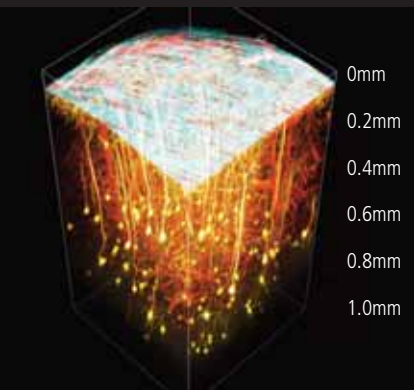
Left) 3D reconstruction image  
Right) Z-stack images  
Top: dendrites located in superficial layers in the layer V pyramidal cells  
25 μm from the surface  
Middle: basal dendrites in the layer V pyramidal cells  
625 μm from the surface  
Bottom: fluorescence from white matter

Excitation wavelength: 930 nm  
Objective: CFI75 Apochromat 25xW MP (NA 1.10 WD 2.0)

Photographed with the cooperation of:  
Dr. Tomomi Nemoto, Research Institute for Electronic Science, Hokkaido University  
Dr. Shigenori Nonaka, National Institute for Basic Biology  
Dr. Takeshi Imamura, Graduate School of Medicine, Ehime University

### Mouse cerebral cortex multi-color imaging

Simultaneous acquisition of three channels in anesthetized YFP-H mouse using IR excitation of 950 nm and imaging Second Harmonic Generation (SHG) and two fluorescence emissions.



Cyan: SHG signal of dura mater  
Yellow: EYFP pyramidal neurons in layer V of the cortex  
Red: SRB-labeled blood vessels

Photographed with the cooperation of:  
Dr. Ryosuke Kawakami, Dr. Terumasa Hibi, Dr. Tomomi Nemoto, Research Institute for Electronic Science, Hokkaido University

## Channel unmixing

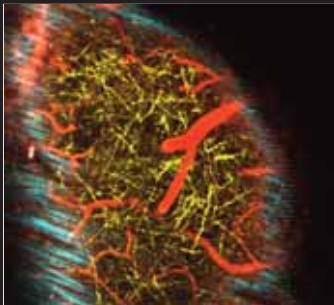
With multiphoton excitation, fluorophores have a considerably broader profile of the absorption spectra than with single photon excitation. Therefore simultaneous excitation of multiple fluorophores with single excitation wavelength is possible. Additionally, the wavelength of a pulsed laser for multiphoton excitation can be changed and the user can select a suitable and well-balanced wavelength for the excitation of multiple fluorophores. A1 MP+/A1R MP+ NDD and channel unmixing technology enables the user to clearly isolate multiple fluorophores and obtain information on the minute structure of a specimen deep within a living organism.

### Unmixing with three-color simultaneous excitation

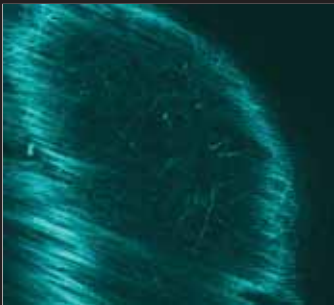
Simultaneous imaging of three colors in anesthetized YFP-H mouse with IR excitation of 950 nm

The upper four images are acquired original data and the lower four images are unmixed images by utilizing the unmixing function. Blood vessels and neurons are clearly separated.

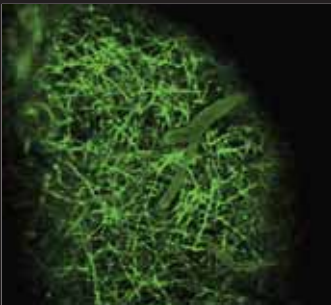
#### Acquired



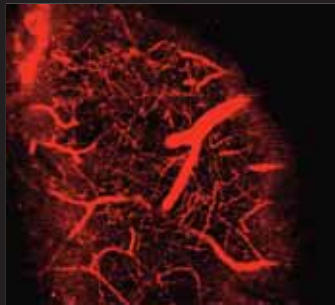
All channels merged



Dura mater

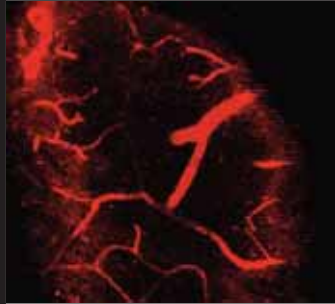
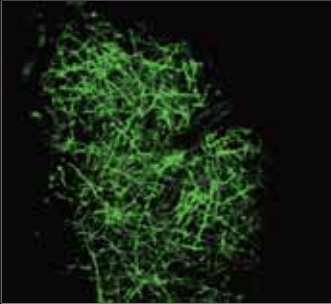
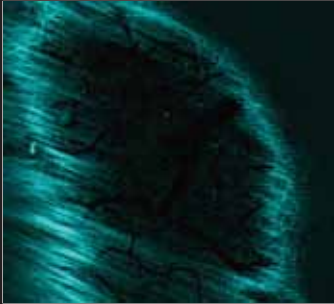
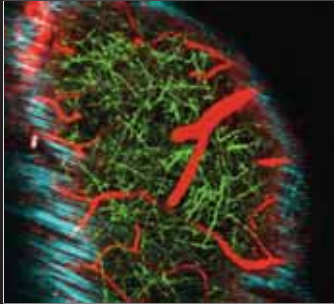


Pyramidal neuron



Blood vessels

#### Unmixed



Cyan: SHG signal of dura mater  
Yellow: EYFP pyramidal neurons in layer V of the cortex  
Red: SRB-labeled blood vessels

Photographed with the cooperation of:  
Dr. Ryosuke Kawakami, Dr. Terumasa Hibi, Dr. Tomomi Nemoto, Research Institute for Electronic Science, Hokkaido University

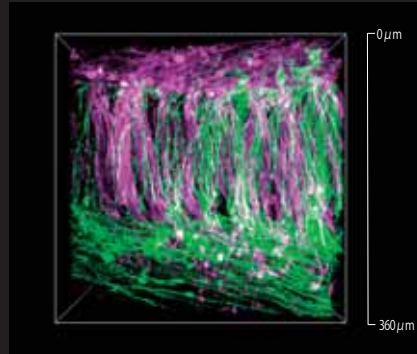
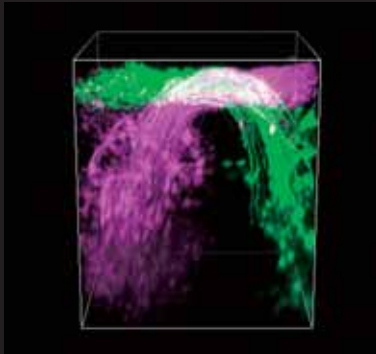
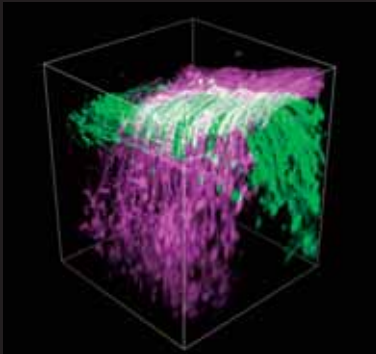
### Unmixing with two-color simultaneous excitation

Spinal cord primordia (neural tube) of a 12.5-day-old rat embryo

The entire embryo was cultured for approximately 44 hours after transfection of the right and left nerve cells with eGFP and YFP (Venus) by electroporation. A cross-sectional slice of spinal cord was embedded in gel and simultaneous excitation of eGFP and YFP was conducted using pulsed IR laser (930 nm).

The image is captured with NDD and processed by the unmixing function.

Observation of interneuron and its commissural axon is clearly achieved.

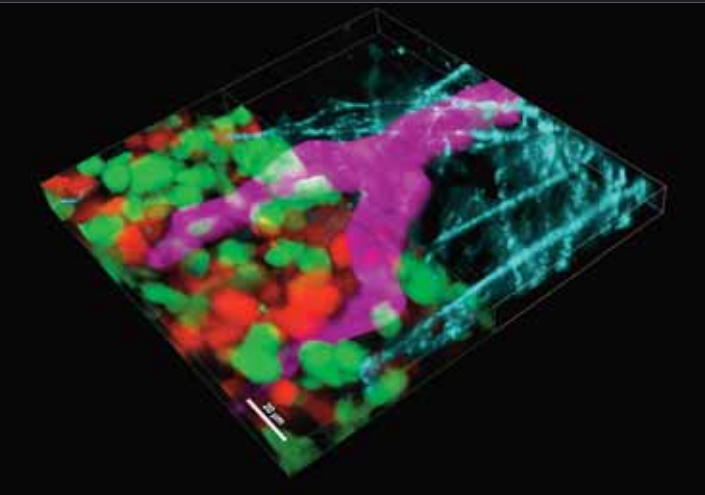


Photographed with the cooperation of:  
Dr. Noriko Osumi, Dr. Masanori Takahashi, Division of Developmental Neuroscience, United Center for Advanced Research and Translational Medicine (ART), Tohoku University Graduate School of Medicine



Four-color imaging of human colon cancer cells in *in vivo*

Three-dimensional volume rendering of implanted subcutaneous tumor of HCT116 expressing Fucci. The cell cycle of tumor cells and the environment (collagen fiber and vessels) are visualized. Upper right, only collagen fiber and vessels are shown.



Red: Fucci mkO2/cancer cell  
Green: Fucci mAG/cancer cell  
Cyan: SHG/collagen fiber  
Purple: Qtracker655/neovascular vessels

Objective: CFI Plan Fluor 20xA MI  
Excitation Wavelength: 940 nm

Photographed with the cooperation of Dr. Yoshinori Kagawa and Dr. Masaru Ishii, Immunology Frontier Research Center, Osaka University

Image width: 156.61  $\mu\text{m}$ , height: 156.61  $\mu\text{m}$ , depth: 22.50  $\mu\text{m}$

Dynamic *in vivo* imaging of granulocytes in live adipose tissues

The epididymal adipose tissue of a LysM-EGFP mouse was observed using intravital multiphoton microscopy. Granulocytes patrolling around adipocytes were visualized. Time-lapse images show the movement of the granulocytes. (arrowhead : granulocyte-A, arrow : granulocyte-B)

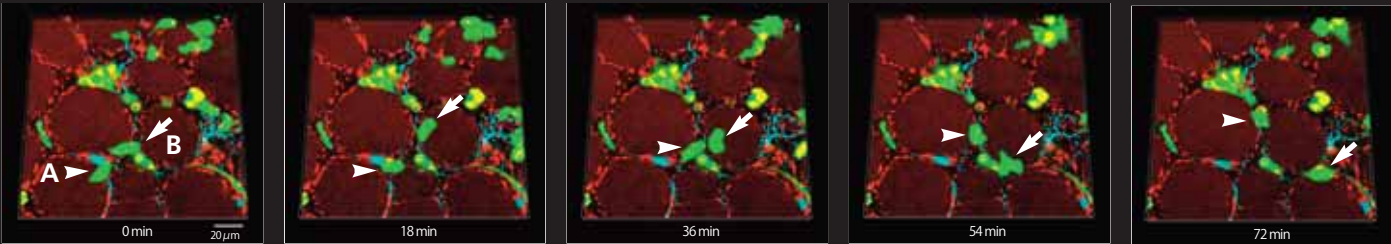


Image width: 159.10  $\mu\text{m}$ , height: 159.10  $\mu\text{m}$ , depth: 8.00  $\mu\text{m}$

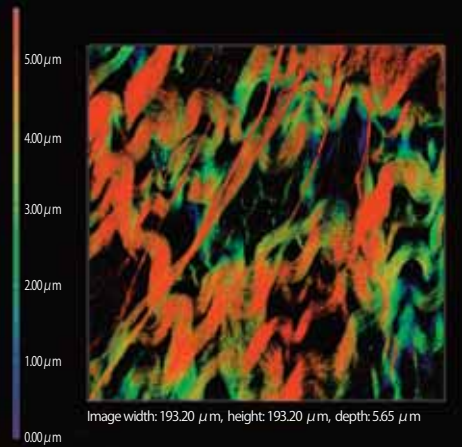
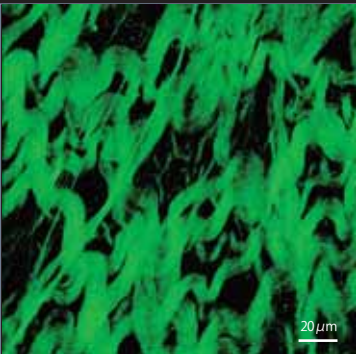
Red: BODIPY /fat droplet  
Green: EGFP /granulocyte  
Cyan: Hoechst /nucleus and SHG/collagen fiber

Objective: CFI Apochromat LWD 40x WI  $\lambda\text{S}$   
Excitation Wavelength: 920 nm

Photographed with the cooperation of Junichi Kikuta, Shoko Yasuda and Dr. Masaru Ishii, Laboratory of Cellular Dynamics, Immunology Frontier Research Center, Osaka University

Knitted stitch structure of colon wall muscle by SHG imaging

NOD/SCID mouse colon wall was observed toward mucosal membrane from serosal membrane side. Knitted stitch structure of colon wall muscle fibers was clearly visualized using SHG. Left, maximum intensity projection calculated from Z stack. Right, three-dimensional volume rendering using depth-code pseudo color.



SHG of collagen fiber

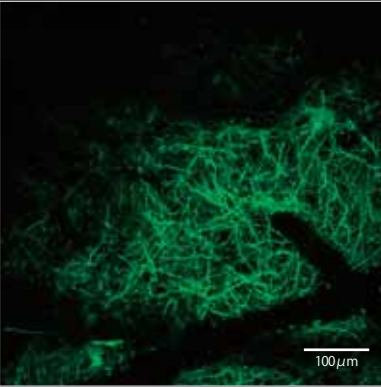
Objective: CFI Plan Fluor 20xA MI  
Excitation Wavelength: 840 nm

Photographed with the cooperation of Dr. Yoshinori Kagawa and Dr. Masaru Ishii, Immunology Frontier Research Center, Osaka University

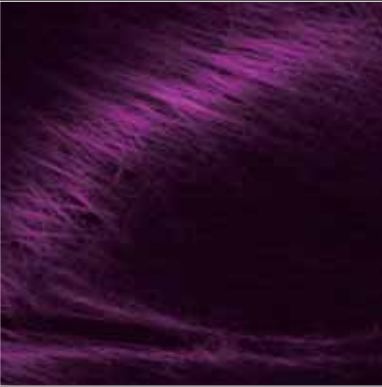
Image width: 193.20  $\mu\text{m}$ , height: 193.20  $\mu\text{m}$ , depth: 5.65  $\mu\text{m}$

SHG image of the brain surface of a mouse

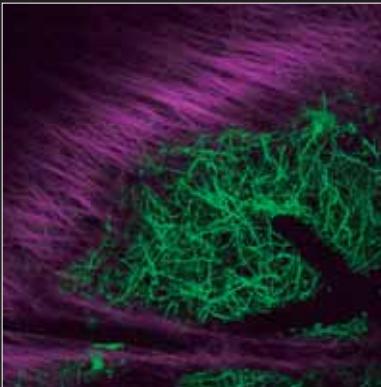
The neocortex of an H-line 5-week-old mouse was studied with the open skull method. The SHG signals from dura mater and EYFP fluorescence signals were simultaneously acquired using the NDD.



EYFP fluorescent image



SHG image of the dura mater



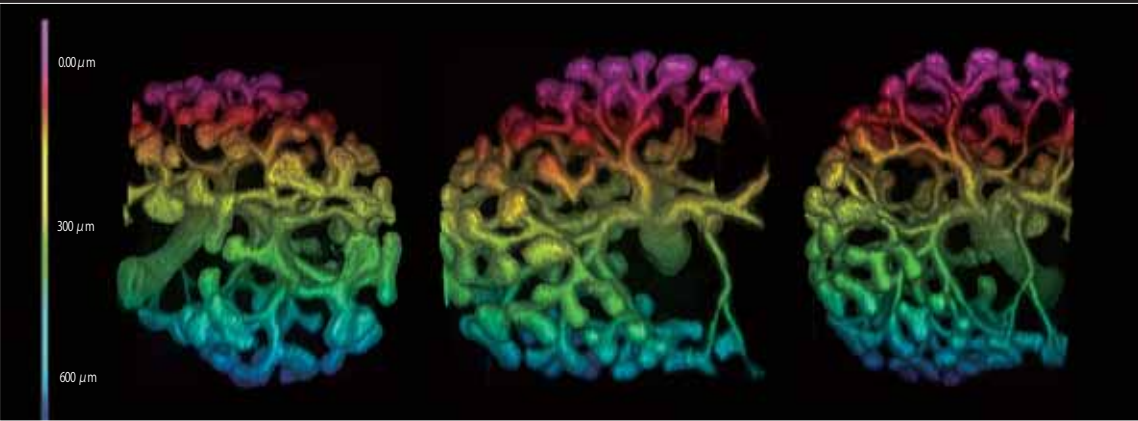
overlay image

Excitation wavelength: 950 nm  
Objective: CFI75 Apochromat 25xW MP (NA 1.10 WD 2.0)

Photographed with the cooperation of:  
Dr. Takeshi Imamura, Graduate School of Medicine, Ehime University  
Dr. Yusuke Oshima, Dr. Shigenori Nonaka, National Institute for Basic Biology  
Dr. Terumasa Hibi, Dr. Ryoshuke Kawakami, Dr. Tomomi Nemoto, Research Institute for Electronic Science, Hokkaido University

3D volume rendering images

Three-dimensional volume renderings of a kidney labeled with Hoxb7/myrVenus marker (Chi et al, 2009 Genesis), using depth-code pseudocolor volume rendering to reference Z depths (pseudocolored by depth - 1  $\mu\text{m}$  step for 550  $\mu\text{m}$ ).

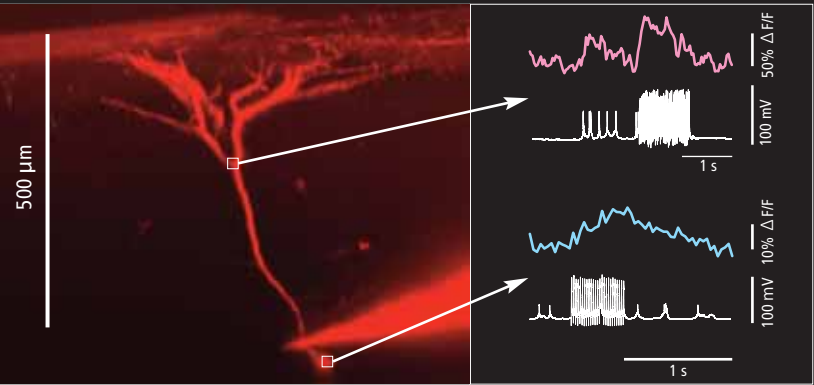


Objective: CFI Apochromat 25xW MP, Scan zoom: 1x, Z step size: 1  $\mu\text{m}$ , IR excitation wavelength: 930 nm  
Image resolution: 1024x1024 pixels, Image volume: 460  $\mu\text{m}$  (length) x 460  $\mu\text{m}$  (width) x 600  $\mu\text{m}$  (height)  
Photographed with the cooperation of Dr. Frank Costantini and Dr. Liza Pon, Columbia University Medical Center, New York

Ca<sup>2+</sup> signals from the layer V pyramidal neuron

*Left*, Two-photon image of Alexa 594 fluorescence. Lateral-medial (x axis) and dorsal-ventral (y axis) projections were calculated from 3D stacks. The soma was located at > 500  $\mu\text{m}$  from the surface.  
*Right*, Fluorescent change evoked by action potentials. The soma and dendrites were loaded with Oregon Green 488 BAPTA-1 using a patch pipette. The duration of current pulses was 500 ms or 1 s.

Photographed with the cooperation of Dr. Satoshi Manita and Dr. Masanori Murayama, Brain Science Institute (BSI), Riken





A1 MP+/A1R MP+ achieve the most advanced multiphoton imaging

Standard NDD

The fluorescence emissions from deep within a specimen are highly scattered in multiphoton excitation, and therefore the conventional detector using a pinhole cannot provide bright fluorescent images. The episcopic NDD in the A1 MP+/A1R MP+ is located close to the back aperture of the objective to detect the maximum amount of scattered emission signals from deep within living specimens. The use of this four-channel detector in combination with special spectral mirrors, together with Nikon's unmixing algorithm, eliminates cross talk between fluorescent probes with highly overlapping emission spectra. Background auto-fluorescence is also eliminated, enabling high-contrast image capture from deep within the specimen.

Using diasopic NDD\* together with episcopic NDD, brighter images can be acquired by detecting fluorescence signals from both reflected and transmitted.

\*Compatible with Ni-E focusing nosepiece microscope

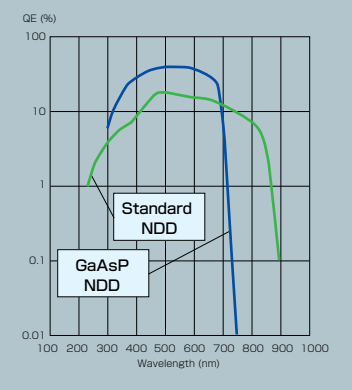
4-channel episcopic NDD

4-channel diasopic NDD

Super High-sensitive GaAsP NDD

The newly developed GaAsP NDD\* has approximately twice the sensitivity of a standard NDD and allows clear imaging of deeper areas of living specimens than ever before. Its ability to acquire bright images enables faster imaging and higher quality Z-stack imaging. Its high sensitivity allows acquisition of fluorescent signals with less laser power, resulting in less photo damage to living specimens.

\* Compatible with FN1 fixed stage microscope



Nikon's high-NA objectives are ideal for multiphoton imaging

High-NA objectives have been developed that highly correct chromatic aberrations over a wide wavelength range, from ultraviolet to infrared. Transmission is increased through the use of Nikon's exclusive Nano Crystal Coat technology. In particular, the CFI Apochromat 25xW MP objective lens provides an industry leading highest numerical aperture of 1.10 while still maintaining a 2.0 mm working distance. It also has a collar that corrects chromatic aberrations depending on the depth of the specimen and a 33° manipulator pipette access angle, making it ideal for deep multiphoton imaging and physiology research applications.

Nano Crystal Coat is a Nikon exclusive lens coating technology using an ultralow refractive index nanoparticle thin film originally developed for the semiconductor fabrications industry. The Nano Crystal Coat particle structure dramatically reduces stray reflections and boosts transmission over a wide wavelength range, producing images with higher signal-to-noise (S/N) ratios.

Objectives

CFI75 Apochromat 25xW MP	NA 1.10	WD 2.0	Nano Crystal Coat
CFI Apochromat LWD 40xWI λ S	NA 1.15	WD 0.6	Nano Crystal Coat
CFI Apochromat 40xWI λ S	NA 1.25	WD 0.18	Nano Crystal Coat
CFI Plan Apochromat IR 60xWI	NA 1.27	WD 0.17	Nano Crystal Coat

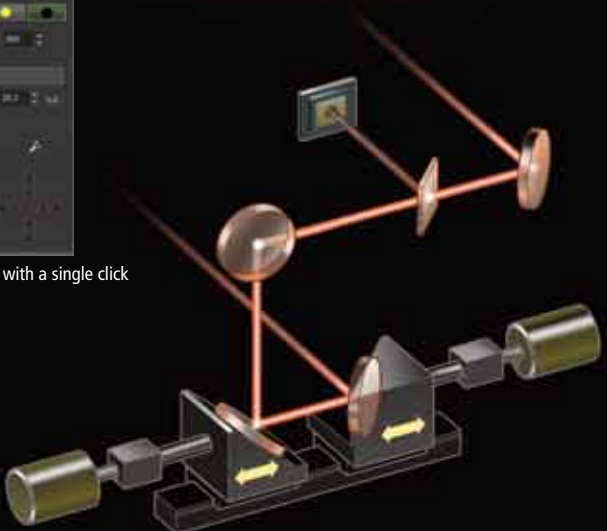
Auto laser alignment when changing multiphoton excitation wavelength

When the multiphoton laser wavelength or group velocity dispersion pre-compensation is changed, the multiphoton laser beam positional pointing at the objective back aperture may also change, resulting in uneven intensity across the image, or a slight misalignment between the IR and visible laser light paths.

Verifying the IR laser beam pointing and setting the alignment has traditionally been difficult. Nikon's A1 MP+ series' auto laser alignment function, housed in the Incident Optical Unit for the multiphoton excitation light path, automatically maximizes IR laser alignments with a single click in NIS-Elements C.



Auto laser alignment with a single click



# Two types of scanning head enable high-speed, high-quality imaging

A1 MP+ is equipped with a galvano (non-resonant) scanner for high-resolution imaging. A1R MP+ is a hybrid scanning head that incorporates both galvano and ultrahigh-speed resonant scanners. A1R MP+ allows imaging and photoactivation at ultrafast speeds necessary for revealing cell dynamics and interaction.

## High-resolution imaging A1 MP+ A1R MP+

The A1 MP+/A1R MP+ galvano scanner enables high-resolution imaging of up to 4096 x 4096 pixels. In addition, with the newly developed scanner driving and sampling systems, plus Nikon's unique image correction technology, high-speed acquisition of 10 fps (512 x 512 pixels) is also possible.

1D scanning	5,200 lps (lines per second)
2D scanning	130 fps (512 x 32 pixels)
Full frame scanning	10 fps (512 x 512 pixels)



## Ultrafast imaging A1R MP+

A1R MP+ is a hybrid scanning head equipped with both a galvano scanner and a resonant scanner with an ultrahigh resonance frequency of 7.8 kHz. It allows ultrafast imaging and photoactivation at 420 fps (512 x 32 pixels), the world's fastest image acquisition.

1D scanning	15,600 lps
2D scanning	420 fps (512 x 32 pixels)
Full frame scanning	30 fps (512 x 512 pixels)



### Stable, ultrafast imaging

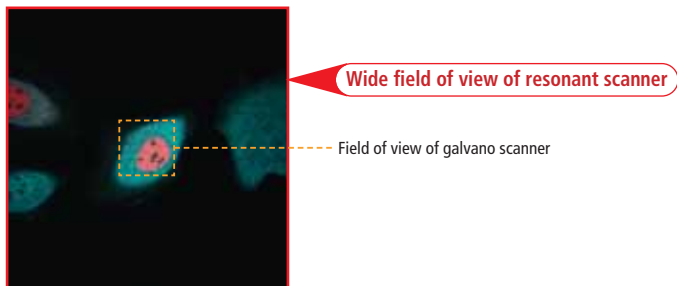
The Nikon original optical clock generation method is used for high-speed imaging with a resonant scanner. Stable clock pulses are generated optically, offering images that have neither flicker nor distortion even at the highest speed.

### High-speed data transfer with fiber-optic communication

High-speed data transfer with fiber-optic communication  
The fiber-optic communication data transfer system can transfer data at a maximum of 4 Gbps. This allows the transfer of five channels of image data (512 x 512 pixels, 16 bit) at 30 fps.

### Wide field of view

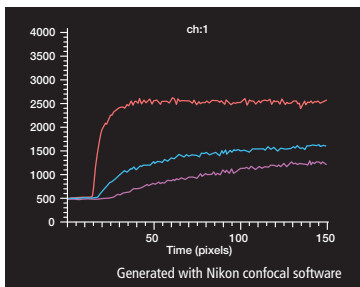
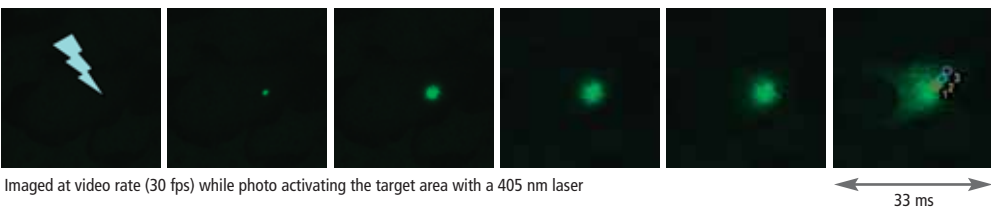
Resonant scanners do not suffer from overheating of the motor during high-speed image acquisition. Therefore, it is not necessary to reduce the field of view of the scanned image in order to avoid overheating, thus enabling a wide field of view.



## Simultaneous photoactivation and imaging

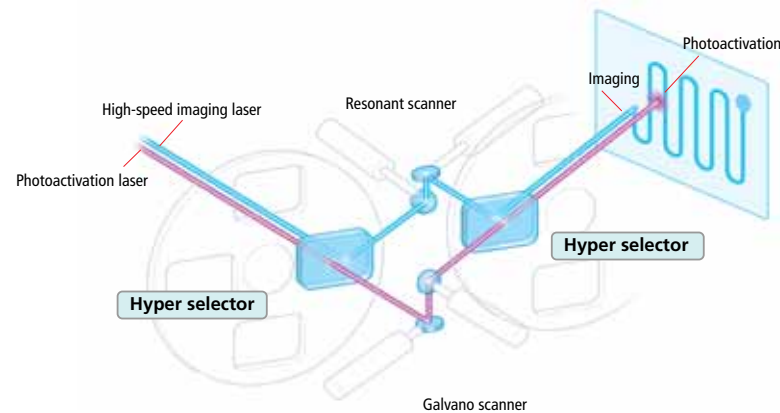
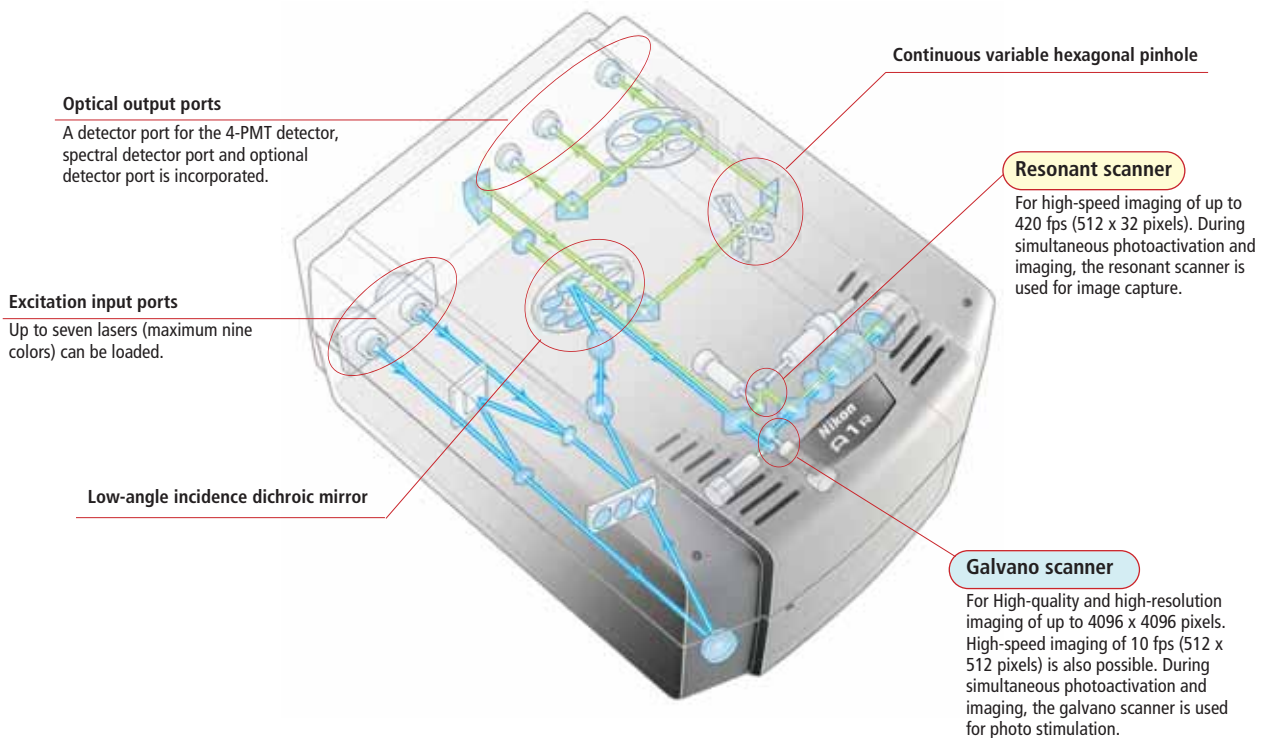
Simultaneous photoactivation and fluorescence imaging is conducted using galvano and resonant scanners. Because the resonant scanner can capture images at 30 fps, image acquisition of high-speed biological processes after photoactivation is possible.

### High-speed imaging of photoactivation



Points within the cell and changes of fluorescence intensity (From the point closer to the activated point: red, blue and purple)

## Optical path in the A1R MP+ scanning head



### What is a hybrid scanner?

This mechanism allows flexible switching or simultaneous use of two scanners (resonant and galvano) with the use of a hyper selector.

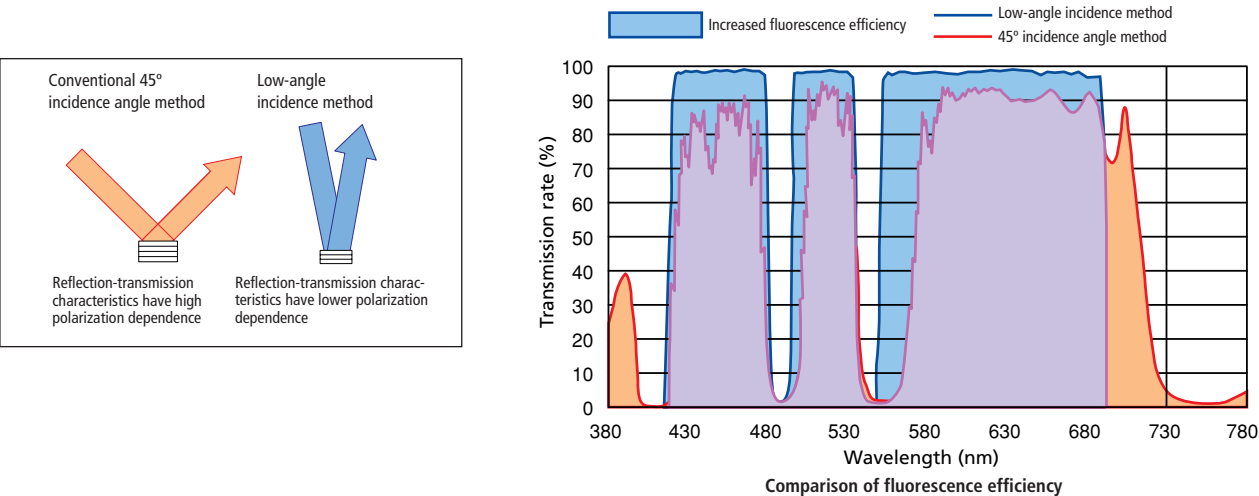


# Key Nikon innovations for improving image quality

The best image quality is achieved by an increased light sensitivity resulting from comprehensive technological innovations in electronics, optics and software.

## Low-angle incidence dichroic mirror creates a 30% increase in fluorescence efficiency

With the A1 MP+ series, the industry's first low-angle incidence method is utilized on the dichroic mirrors and a 30% increase of fluorescence efficiency is realized.



## Brighter images with continuous variable hexagonal pinhole

Instead of a continuous variable square pinhole, the industry's first hexagonal pinhole is employed. Higher brightness, equivalent to that of an ideal circular pinhole is achieved while maintaining the confocality.

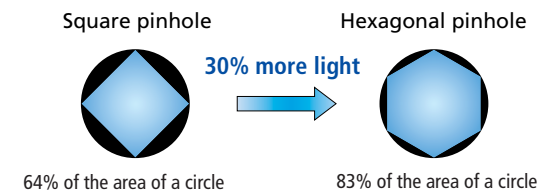
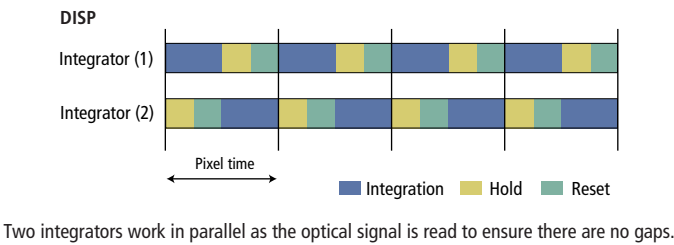


Image of a zebrafish labeled with four probes (captured with galvano scanner)  
Nucleus (blue): Hoechst33342, Pupil (green): GFP, Nerve (yellow): Alexa555,  
Muscle (red): Alexa647  
Photographed with the cooperation of: Dr. Kazuki Horikawa and Prof.  
Takeharu Nagai, Research Institute for Electronic Science, Hokkaido University

## DISP improves electrical efficiency

Nikon's original Dual Integration Signal Processing (DISP) technology has been implemented in the image processing circuitry to improve electrical efficiency, preventing signal loss while the digitizer processes pixel data and resets. The signal is monitored for the entire pixel time resulting in an extremely high S/N ratio.



# Enhanced spectral detector

Nikon's original spectral performance is even further enhanced in the A1 MP+ series, allowing high-speed spectral acquisition with a single scan. In addition, advanced functions, including real-time unmixing, are incorporated.

### DEES system

High diffraction efficiency is achieved by matching the polarization direction of light entering a grating to the polarizing light beam S.

### Optical fiber

The wavelength resolution is independent of pinhole diameter.

### Multiple gratings

Wavelength resolution can be varied between 2.5/6/10 nm with three gratings. Each position is precisely controlled for high wavelength reproducibility.

### 32-channel detector

A precisely corrected 32-PMT array detector is used. A three-mobile-shielding mechanism allows simultaneous excitation by up to four lasers.

## High-quality spectral data acquisition

### Diffraction Efficiency Enhancement System (DEES)

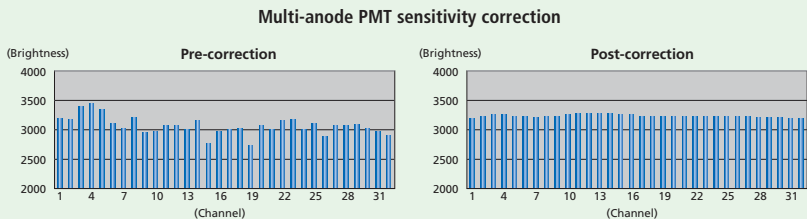
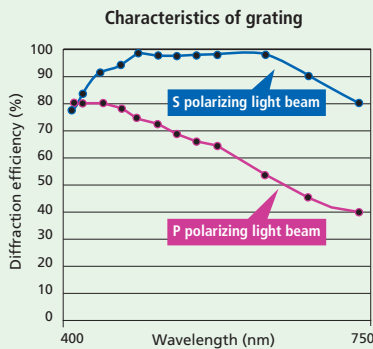
With the DEES, non-polarized fluorescence light emitted by the specimen is separated into two polarizing light beams P and S by a polarizing beam splitter. P is then converted by a polarization rotator into S, which has higher diffraction efficiency than P, achieving vastly increased overall diffraction efficiency.

### High-efficiency fluorescence transmission technology

The ends of the fluorescence fibers and detector surfaces use a proprietary anti-reflective coating to reduce signal loss to a minimum, achieving high optical transmission.

### Accurate, reliable spectral data: three correction techniques

Three correction techniques allow for the acquisition of accurate spectra: inter-channel sensitivity correction, which adjusts offset and sensitivity of each channel; spectral sensitivity correction, which adjusts diffraction grating spectral efficiency and detector spectral sensitivity; and correction of spectral transmission of optical devices in scanning heads and microscopes.





# Intuitive, easy-to-use software for multiphoton imaging

## NIS-Elements C Acquisition and Analysis software

### Simple operations common with Nikon confocal microscopes

- All necessary operations for image capture are displayed in one window.
- Lasers and detectors for visible laser excitation can be switched simply by selecting fluorescent probe to be used.
- One-touch switching of high speed resonant scanner and high-resolution galvano (non-resonant) scanner
- Simultaneous photoactivation with high speed imaging is possible with visible laser excitation.



### Functions for high quality multiphoton imaging

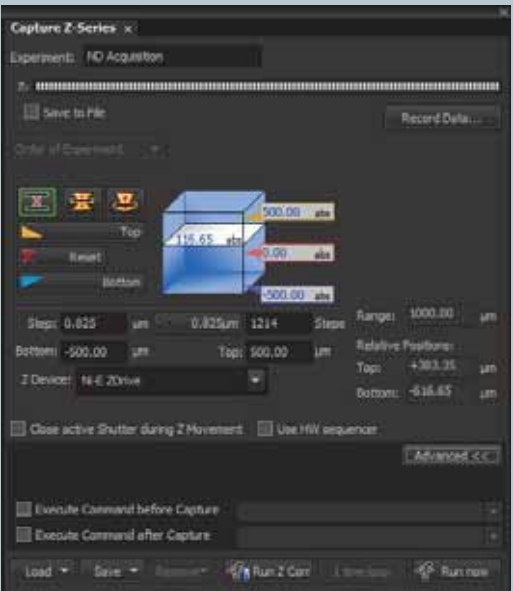
#### Auto laser alignment function

The IR laser alignment can be quickly optimized with a single click when changing the multiphoton excitation wavelength



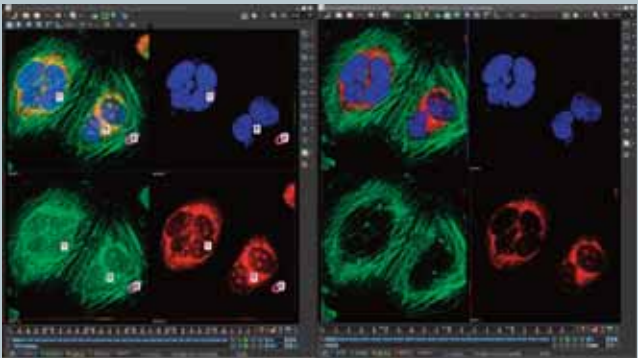
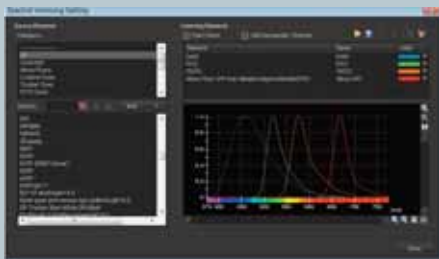
#### Z-intensity control function

Users can define the laser power and PMT gain to use at different depths in a Z series using the Z intensity control function, so that even when imaging dense and thick specimens, the intensity of the emission is maintained throughout the specimen.

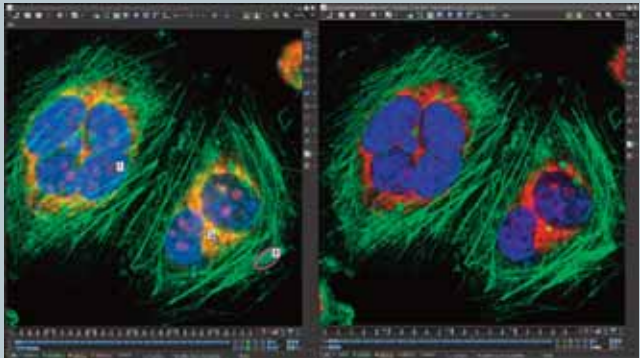


### Channel unmixing function

Nikon's channel unmixing allows you to obtain emissions from multiple NDD PMTs simultaneously, using one IR excitation wavelength, and unmix overlapping emission spectra.



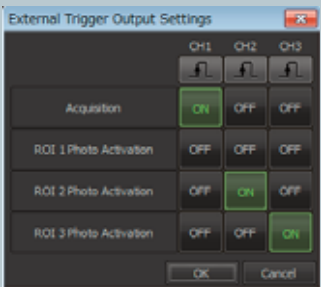
Three color simultaneous fluorescent imaging with 850 nm pulsed IR excitation (left: before unmixing, right: after unmixing)



Channel unmixing reduces crosstalk (left: before unmixing, right: after unmixing)

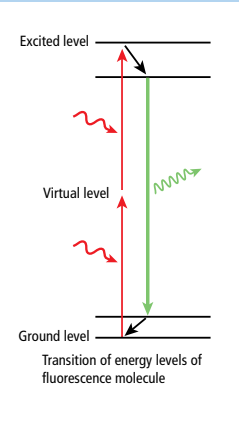
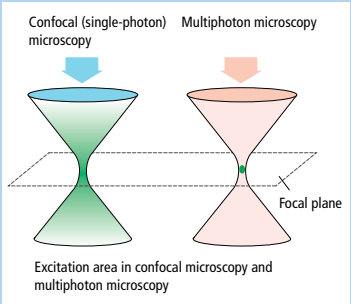
### External trigger function

A1 MP+/A1R MP+ and NIS Elements C support triggering applications. This is effective for synchronizing frame and scanning times with electrophysiology recordings, or to externally trigger the confocal to scan.

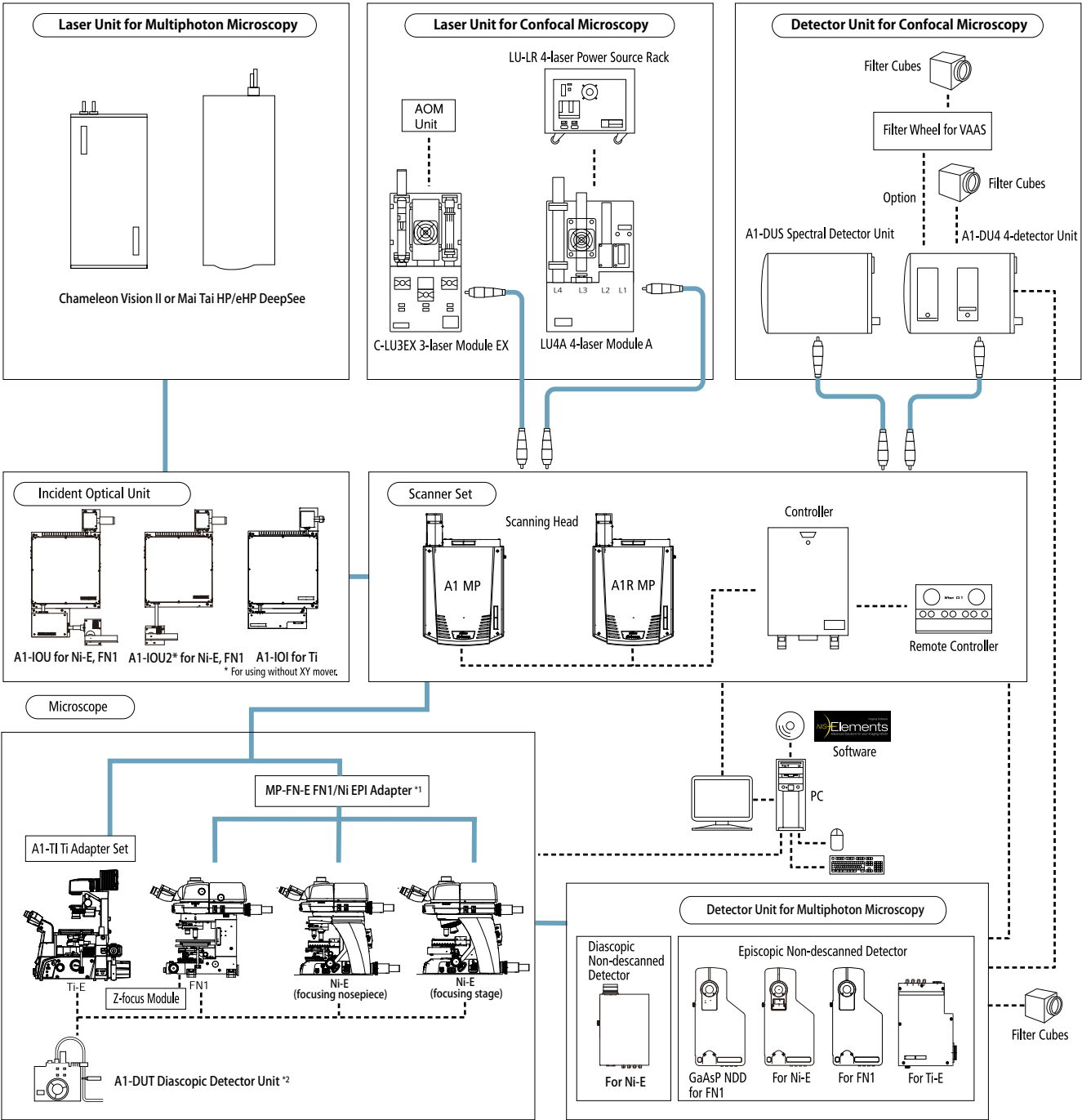


### Principle of multiphoton excitation

When two photons are absorbed simultaneously by a single fluorescent molecule (two-photon excitation), the excitation efficiency is proportional to the square of the excitation light intensity. In order to achieve multiphoton excitation, a pulsed beam with high photon density or flux is used. Because the laser beam is delivered in very short (femtosecond) pulses and is converged on a focal point through an objective lens, the probability of simultaneous absorption of two photons becomes high enough to be useful for imaging. In two-photon excitation, the excitation efficiency decreases inversely with the fourth power of the distance from the center of the focal volume. As a result, only fluorescence molecules located within the diffraction-limited focal volume of the objective lens are excited and can emit fluorescence. This principle allows the use of non-descanned detectors (NDD's), where an emission pinhole is not necessary to achieve confocal results. There is less absorption and scattering of near infrared light than visible wavelengths through a specimen so the excitation beam can easily penetrate deep into thick tissue. Because two photon excitation is highly confined to only the diffraction-limited focal volume of the objective lens, the need for a confocal pinhole aperture to block the emitted fluorescence from out of focus plane from reaching the detector is eliminated. Photo damage to a specimen can be minimized, and maximum fluorescence detection is made possible, creating conditions suitable for *in vivo* imaging of living tissue. The combination of the group velocity dispersion pre-compensation "pre-chirping" system incorporated in the multiphoton laser and the use of the non-descanned detector (NDD) allows fluorescence imaging deeper into a specimen than is possible with standard confocal technique.



System diagram



\*1 When attaching a diascopic detector to the Ni-E, use the MP-FN1/Ni DIA/EPI Adapter.  
\*2 Dedicated adapter is required depending on microscope model.

Femtosecond pulsed lasers

When pulsed light of very short duration, typically about 100 femtoseconds, passes through microscope optics (e.g. objective), the pulse is spread out in time on its way to the specimen because of group velocity dispersion, (the variation by wavelength in velocity of the speed of light through glass substrates), causing a reduction of peak power. To prevent the reduction of peak pulse power, Nikon has equipped the femtosecond pulsed lasers for multiphoton microscopy with built-in group velocity dispersion precompensation that restores the original pulse width at the specimen. The parameters of the precompensation have been optimized for Nikon's optical system. This enables bright fluorescence imaging of areas deep within a specimen with minimum laser power.



Specifications

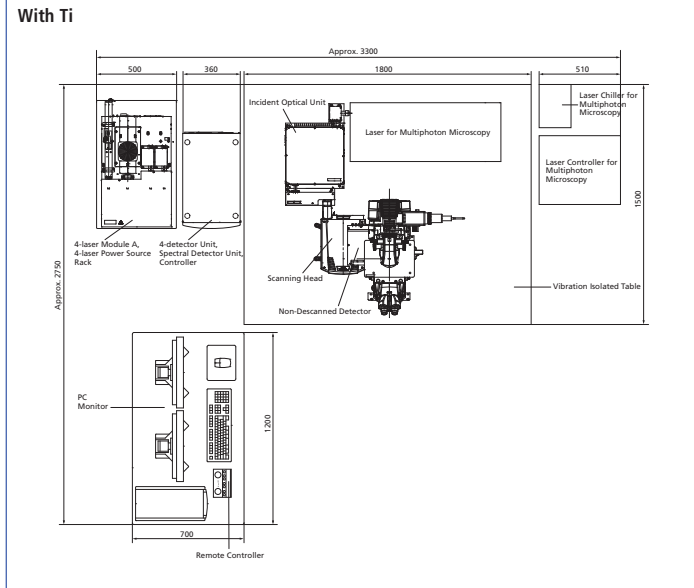
		A1 MP+	A1R MP+
Input/output port		3 laser input ports 4 signal output ports for 4-PMT detector, spectral detector, VAAS (optional), and third-party detector (FCS/FCCS/FLIM)	
Laser for multiphoton microscopy	Compatible laser	Mai Tai HP/eHP DeepSee (Newport Corp.) Chameleon Vision II (Coherent Inc.)	
	Modulation	Method: AOM (Acousto-Optic Modulator) device Control: power control, return mask, ROI exposure control	
	Incident optics	700-1000 nm, auto alignment	
Laser for confocal microscopy (option)	Compatible laser	405 nm, 440/445 nm, 488 nm, 561/594 nm, 638/640nm, Ar laser (457 nm, 488 nm, 514 nm), HeNe laser (543 nm)	
	Modulation	Method: AOTF (Acousto-Optic Tunable Filter) or AOM (Acousto-Optic Modulator) device Control: power control for each wavelength, return mask, ROI exposure control	
	Laser unit	Standard: LU4A 4-laser module A or C-LU3EX 3-laser module EX Optional: C-LU3EX 3-laser module EX (when 4-laser module is chosen as standard laser unit)	
NDD for multiphoton microscopy	Wavelength	400-650 nm	
	Detector	4 PMT	
	Filter cube	Filter cubes commonly used for a microscope Recommended filter sets for multiphoton: 492SP, 525/50, 575/25, 629/53, DM458, DM495, DM511, DM560, DM593	
	Detector type	Episcopic NDD (for Ni-E/FN1/Ti-E) Diascopic NDD (for Ni-E) Episcopic GaAsP NDD (for FN1)	
Standard fluorescence detector (option)	Wavelength	400-750 nm (400-650 nm for multiphoton observation)	
	Detector	4 PMT	
	Filter cube	6 filter cubes commonly used for a microscope mountable on each of three filter wheels Recommended wavelengths for multiphoton/confocal observation: 450/50, 482/35, 515/30, 525/50, 540/30, 550/49, 585/65, 595/50, 700/75	
Diascopic detector (option)	Wavelength	440-645 nm	
	Detector	PMT	
FOV		Square inscribed in a ø18 mm circle	
Image bit depth		4096 gray intensity levels (12 bit)	
Scanning head	Standard image acquisition	Scanner: galvano scanner x2 Pixel size: max. 4096 x 4096 pixels Scanning speed: Standard mode: 2 fps (512 x 512 pixels, bi-direction), 24 fps (512 x 32 pixels, bi-direction) Fast mode: 10fps (512 x 512 pixels, bi-direction), 130 fps (512 x 32 pixels bi-direction)*1 Zoom: 1-1000x continuously variable Scanning mode: X-Y, X-T, X-Z, XY rotation, Free line	
		—	
	High-speed image acquisition	Scanner: resonant scanner (X-axis, resonance frequency 7.8 kHz), galvano scanner (Y-axis) Pixel size: max. 512 x 512 pixels Scanning speed: 30 fps (512 x 512 pixels) to 420 fps (512 x 32 pixels), 15,600 lines/sec (line speed) Zoom: 7 steps (1x, 1.5x, 2x, 3x, 4x, 6x, 8x) Scanning mode: X-Y, X-T, X-Z Acquisition method: Standard image acquisition, High-speed image acquisition, Simultaneous photoactivation and image acquisition	
Spectral detector (with galvano scanner) (option)	Dichroic mirror	Low-angle incidence method Position: 8 Standard filter: 405/488, 405/488/561, 405/488/561/638, 400-457/514/IR, 405/488/543/638, BS20/80, IR, 405/488/561/IR	
	Pinhole	12-256 µm variable (1st image plane)	
	Wavelength detection range	400 nm-750 nm (400 nm-650 nm with multiphoton microscopy)	
Compatible microscopes	Number of channels	32 channels	
	Spectral image acquisition speed	4 fps (256 x 256 pixels), 1000 lps	
	Wavelength resolution	80 nm (2.5 nm), 192 nm (6 nm), 320 nm (10 nm) Wavelength range variable in 0.25 nm steps	
	Unmixing	High-speed unmixing, Precision unmixing	
Z step		Ti-E: 0.025 µm, FN1 stepping motor: 0.05 µm Ni-E: 0.025 µm	
Option		Motorized XY stage (for Ti-E/Ni-E), High-speed Z stage (for Ti-E), High-speed piezo objective-positioning system (for FN1/Ni-E), VAAS	
Software	Display/image generation	2D analysis, 3D volume rendering/orthogonal, 4D analysis, spectral unmixing	
	Image format	JP2, JPG, TIFF, BMP, GIF, PNG, ND2, JFF, JTF, AVI, ICS/IDS	
	Application	FRAP, FLIP, FRET, photo activation, three-dimensional time-lapse imaging, multipoint time-lapse imaging, colocalization	
	OS	Microsoft Windows®7 Professional 64 bits SP1 (Japanese version/English version)	
	CPU	Intel Xeon X5672 (3.20 GHz/8 MB/1333 MHz/Quad Core) or higher	
	Memory	12 GB (2 GB x 3 + additional 2 GB x3)	
Control computer	Hard disk	300 GB SAS (15,000 rpm) x2, RAID 0 configuration	
	Data transfer	Dedicated data transfer I/F	
	Network interface	10/100/1000 Gigabit Ethernet	
	Monitor	1600 x 1200 or higher resolution, dual monitor configuration recommended	
Vibration isolated table		1800 (W) x 1500 (D) mm recommended, or 1500 (W) x 1500 (D) mm	

\*1 Fast mode is compatible with zoom 8-1000x and scanning modes X-Y and X-T. It is not compatible with Rotation, Free line, CROP, ROI, Spectral imaging, Stimulation, CLEM and FLIM.



# Layout

Unit: mm



## Operation conditions

- Temperature: 20 °C to 25 °C ( $\pm 1$  °C), with 24-hour air conditioning
- Humidity: 75 % (RH) or less, with no condensation
- Completely dark room or light shield for microscope

## Power source

Multiphoton system	Multiphoton system (scanner set, laser unit)	120 VAC	6.7 A
	Computer unit	220 VAC	3.6 A
Laser	Ar laser (457 nm, 488 nm, 514 nm)	120 VAC	12.2 A
		220 VAC	6.6 A
	Except Ar laser (457 nm, 488 nm, 514 nm)	120 VAC	12.5 A
		220 VAC	6.8 A
		120 VAC	2.5 A
		220 VAC	1.4 A
Microscope	Inverted microscope Ti-E with HUB-A and epi-fluorescence illuminator	120 VAC	19.2 A
		220 VAC	10.5 A
		120 VAC	4.4 A
		220 VAC	2.4 A

Specifications and equipment are subject to change without any notice or obligation on the part of the manufacturer. December 2011 ©2010-11 NIKON CORPORATION

	<b>WARNING</b>	TO ENSURE CORRECT USAGE, READ THE CORRESPONDING MANUALS CAREFULLY BEFORE USING YOUR EQUIPMENT.
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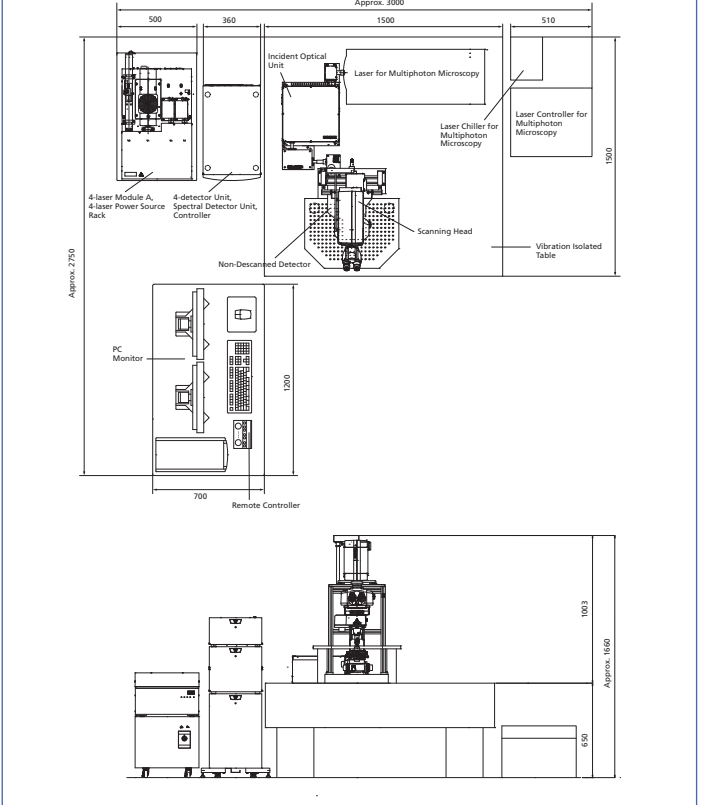
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## With FN1



## Dimensions and weight

Scanning head	276 (W) x 163 (H) x 364 (D) mm	Approx. 10 kg
Incident optical unit (A1-IOU)	363 (W) x 186 (H) x 676 (D) mm	Approx. 16 kg
Controller	360 (W) x 580 (H) x 600 (D) mm	Approx. 40 kg
4-detector unit	360 (W) x 199 (H) x 593.5 (D) mm	Approx. 16 kg (approx. 22 kg with VAAS)
Spectral detector unit	360 (W) x 323 (H) x 595 (D) mm	Approx. 26 kg
Episcopic NDD (for Ti-E)	206 (W) x 60 (H) x 262 (D) mm	Approx. 5 kg
Episcopic NDD (for FN1, Ni-E)	216 (W) x 112 (H) x 425 (D) mm	Approx. 10 kg
Diascopic NDD (for Ni-E)	301 (W) x 66 (H) x 185 (D) mm	Approx. 10 kg
4-laser module	438 (W) x 301 (H) x 690 (D) mm	Approx. 43 kg (without laser)
4-laser power source rack	438 (W) x 400 (H) x 800 (D) mm	Approx. 20 kg (without laser power source)
3-laser module EX	365 (W) x 133 (H) x 702 (D) mm	Approx. 22 kg (without laser)

Dimensions exclude projections.



The AOTF incorporated into the 4-laser unit and the AOM optionally incorporated into the 3-laser unit are classified as controlled products (including provisions applicable to controlled technology) under foreign exchange and trade control laws. You must obtain government permission and complete all required procedures before exporting this system.



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