



A1R MP

Multiphoton confocal microscope

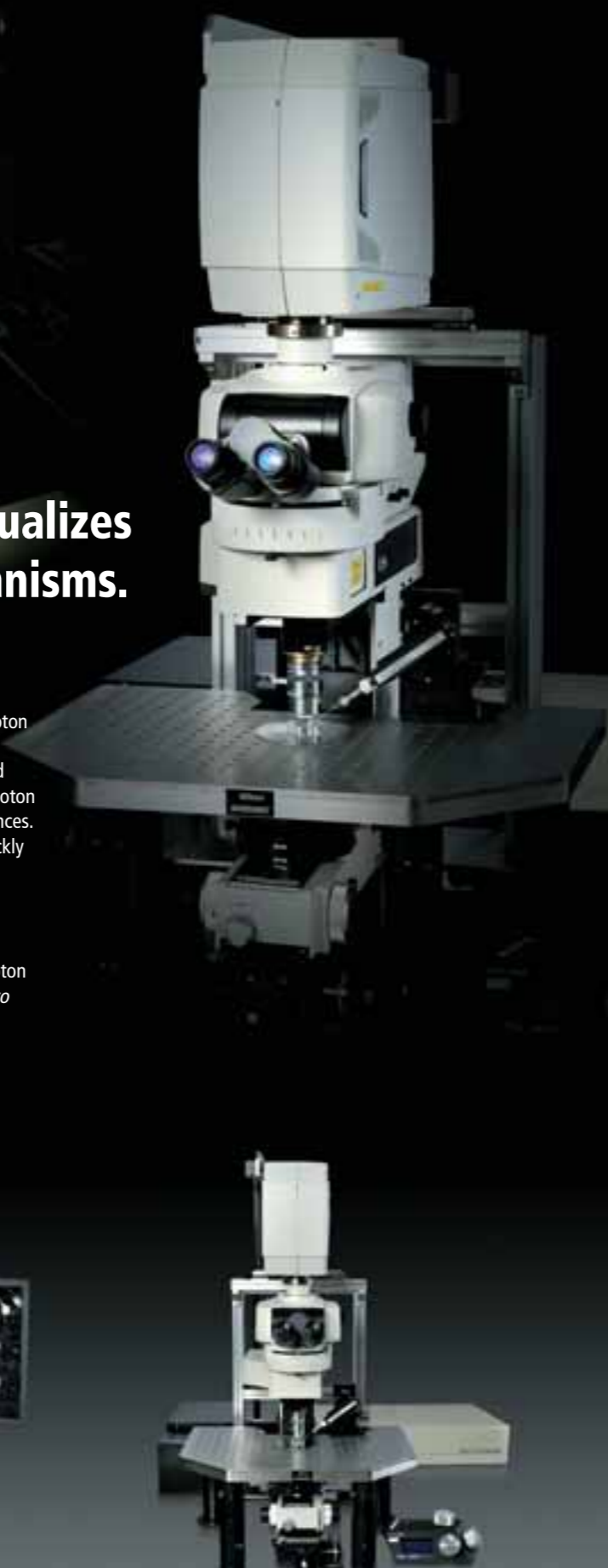


Fast, deep imaging — A1R MP visualizes dynamics deep within living organisms.

The A1R MP high-speed multiphoton confocal microscope introduces a new level of imaging potential that far exceeds that of conventional confocal microscopes.

- High-speed imaging up to 420 frames per second (fps) (512x32 pixels) with multiphoton imaging using high efficiency optics and resonant scanner.
- Deep specimen imaging with high-sensitivity non-descanned detectors (NDD) located close to the objective back aperture, and a new objective series designed for multiphoton imaging, with advanced chromatic aberration correction and very long working distances.
- Automatic alignment of the IR laser beam with an auto laser alignment function quickly corrects deviations from the ideal optical alignment while changing the multiphoton excitation wavelength.
- The IR laser is coupled to the microscope using a compact Incident Optical Unit that contains an acousto-optical 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.

A1R MP provides faster and sharper imaging from deeper within living organisms, extending the boundaries of traditional research techniques in biological sciences.



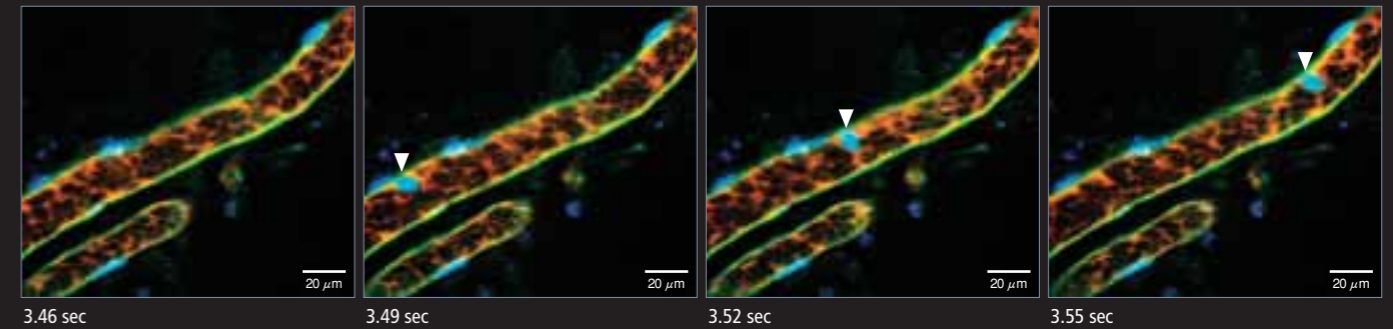
In combination with Inverted Microscope TI-E

In combination with Upright Microscope FN1

A1R MP boosts multiphoton imaging

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

The A1R MP is capable of high-speed 420-fps imaging, the world's fastest for a multiphoton microscope using point scanning technology. This enables the successful visualization of *in vivo* rapid changes, such as reactions in living organisms, dynamics and cell interactions.

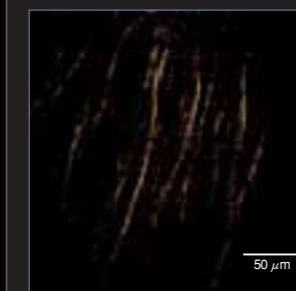
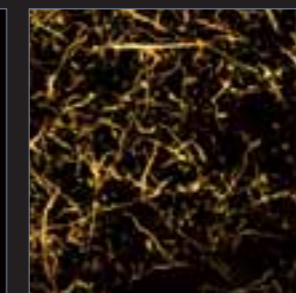
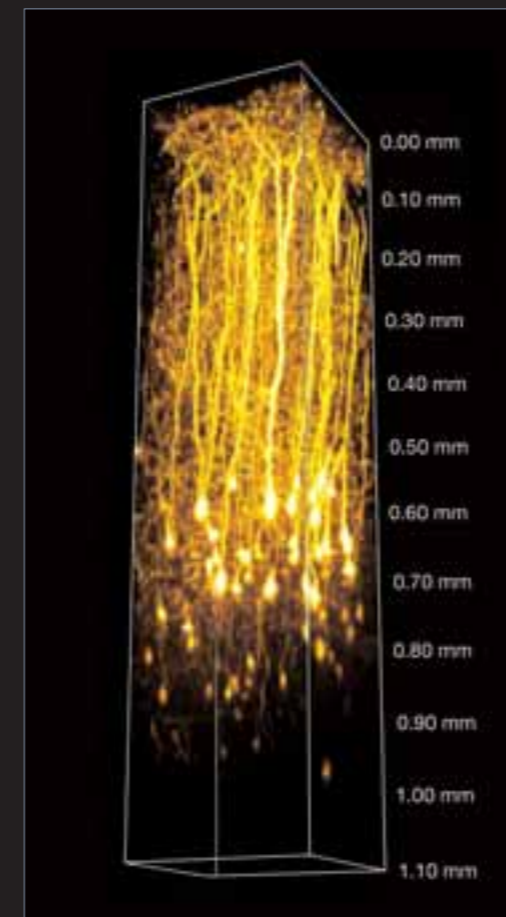


Visualization of intravital microcirculation
Blood cells in blood vessels within a living organism were excited by a femtosecond pulsed IR laser with the A1R MP's ultrahigh-speed resonant scanner, and their movements were simultaneously captured in three successive fluorescence images at 30 fps (30 msec), 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

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: CF175 Apo 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

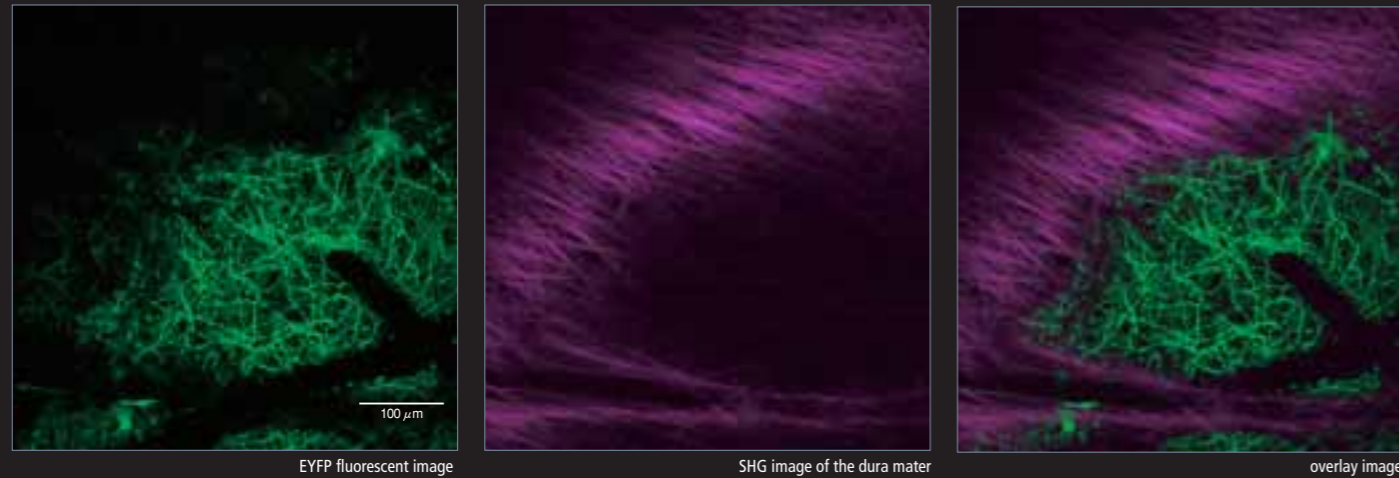
Dr. Takeshi Imamura, Graduate School of Medicine, Ehime University

50 μm

Deep imaging with highly efficient optics dedicated to multiphoton imaging

The SHG (Second Harmonic Generation) 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.

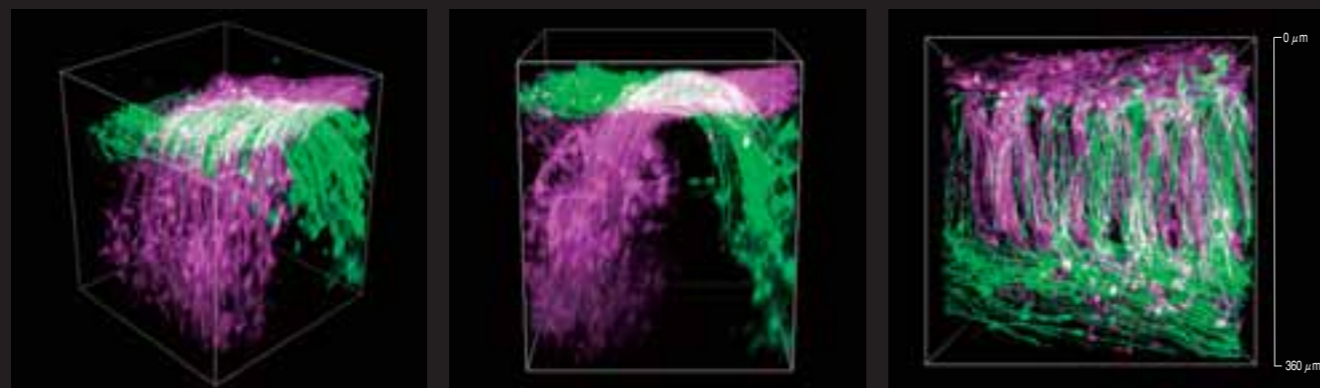


Excitation wavelength: 950 nm
Objective: CFI75 Apo 25xW MP (NA 1.10 WD 2.0)

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

Unmixed image with two color simultaneous excitation

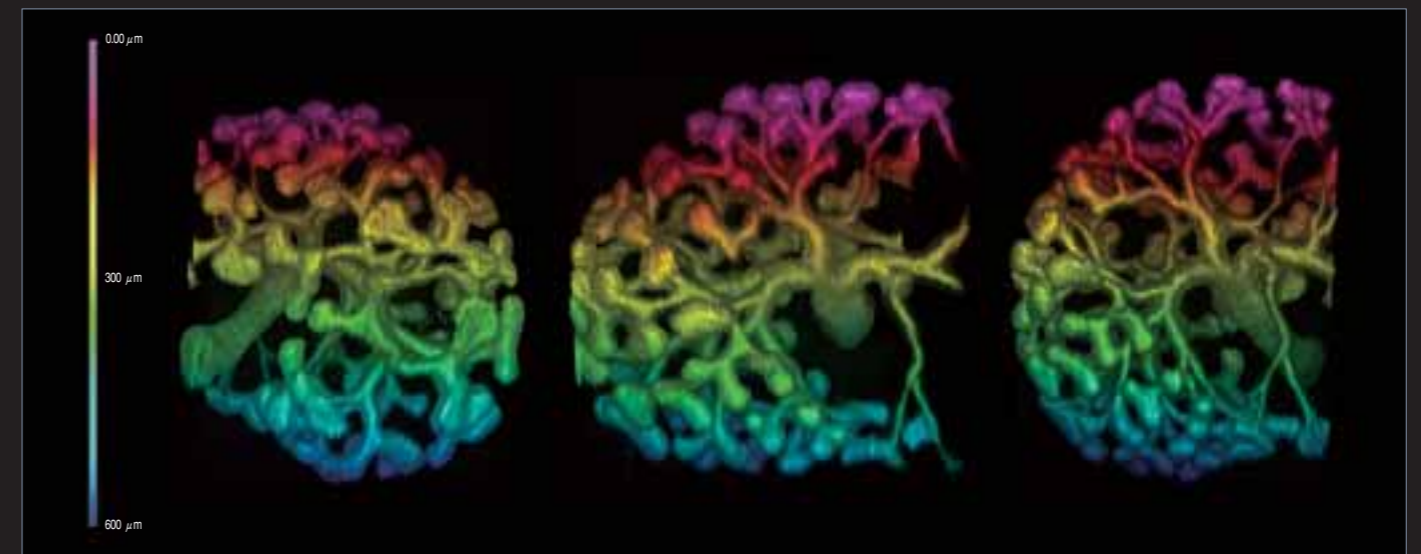
With multiphoton excitation, fluorophores have a considerably broader profile of the absorption spectra than with single photon excitation. Therefore simultaneous excitation of multiple fluorophores with single excitation wavelength is possible. Additionally, the wavelength of pulsed laser for multiphoton excitation can be changed and the user can select a suitable and well-balanced wavelength for the excitation of multiple fluorophores. A1R MP's new 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.



The entire embryo was cultured for approximately 44 hours after transfecting 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.

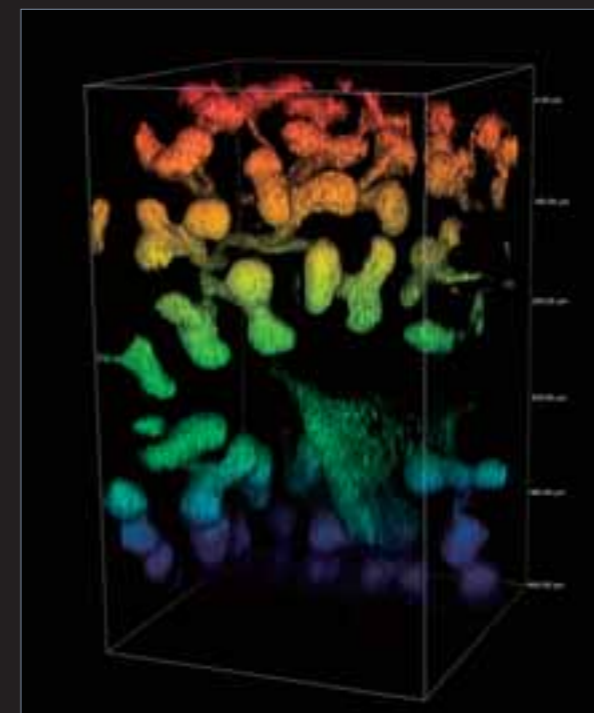
Photographed with the cooperation of: Dr. Noriko Osumi, Dr. Masanori Takahashi, Division of Developmental Neuroscience, United Center for Advanced Research and Translational Medicine (ART), Tohoku University Graduate School of Medicine

Multiphoton imaging gallery



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 μm step for 550 μm).
Objective: CFI Apo 25xW MP
Scan zoom: 1x
Z step size: 1 μm
IR excitation wavelength: 930 nm
Image resolution: 1024x1024 pixels
Image volume: 460 μm (length) x 460 μm (width) x 600 μm (height)

Photographed with the cooperation of Dr. Frank Costantini and Dr. Liza Pon, Columbia University Medical Center, New York



Mouse kidney glomeruli labeled with GFP, using depth-code pseudocolor volume rendering to reference Z depths (pseudocolored by depth - 1 μm step for 530 μm).
Objective: CFI Apo LWD 40xWI λS
Z step size: 1 μm
IR excitation wavelength: 910 nm

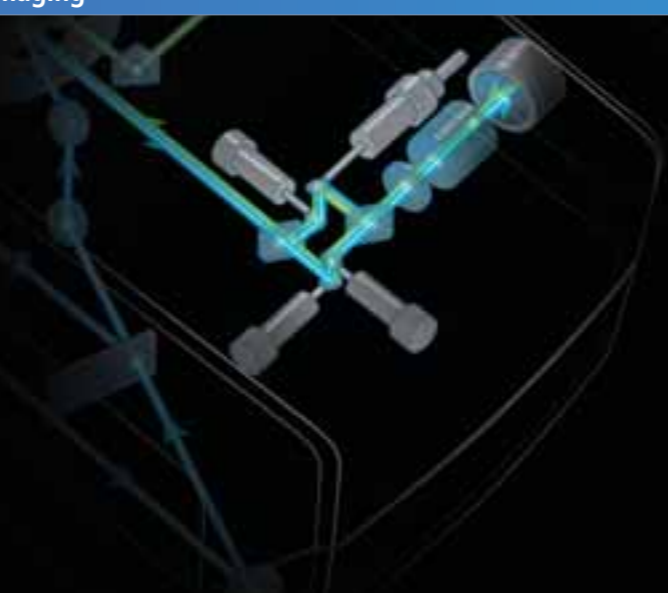
A1R MP achieves the most advanced multiphoton imaging

Up to 420 fps, the world's fastest multiphoton confocal imaging

The Nikon resonant scanner is the world's fastest point scanning technology. Unique to this design is a resonant scan mirror capable of imaging much larger fields of view than other resonant scanners, and at much higher speeds than traditional galvanometer raster scanners. The NDD*1 for multiphoton microscopy makes it possible to image fast and deep through the thickest specimens. 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.

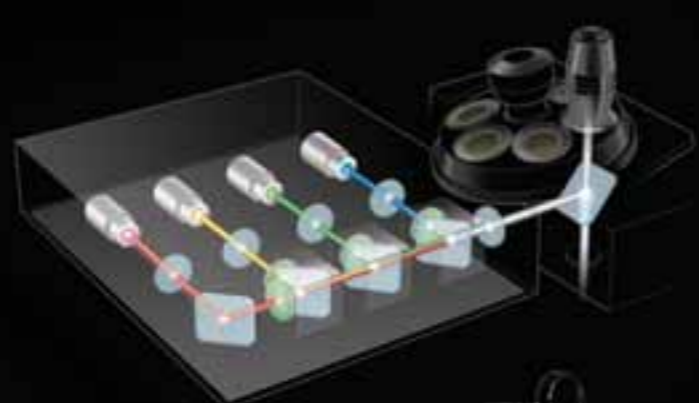
*NDD (Non-Descanned Detector)

Unlike traditional confocal imaging, where emitted light from the specimen passes through a pinhole and is descanned before being detected, multiphoton excitation eliminates the need for an emission pinhole. The A1RMP features a 4 channel NDD array. By locating the NDD close to the back aperture of the objective, more of the scattered fluorescence emissions can be collected, improving the sensitivity of the instrument.

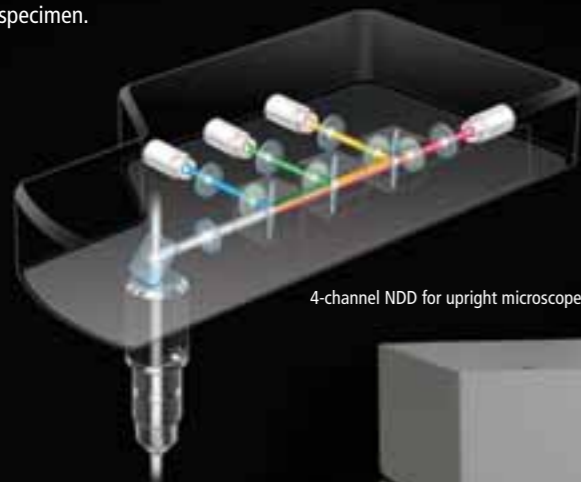


New NDD with unmixing function

Because the fluorescence emissions from deep within a specimen are highly scattered in multiphoton excitation, the conventional detector using a pinhole cannot provide bright fluorescent images. The A1R MP's NDD features a four channel detector array with a wide sensitive area that is located close to the back aperture of the objective to detect maximum of scattered emission signals from deep within living specimens. 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. The contribution of background autofluorescence is also eliminated, enabling high-contrast image capture from deep within the specimen.



4-channel NDD for inverted microscope



4-channel NDD for upright microscope



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

Newly introduced high NA objectives have been developed which highly correct chromatic aberrations over a wide wavelength range, from ultra violet 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 2.0mm working distance. It also has a ring 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 ultra-low refractive index nano-particle 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.

Objectives

CFI75 Apo 25xW MP	NA 1.10	WD 2.0	Nano Crystal Coat
CFI Apo LWD 40xWI λ S	NA 1.15	WD 0.6	Nano Crystal Coat
CFI Apo 40xWI λ S	NA 1.25	WD 0.18	Nano Crystal Coat
CFI Plan Apo IR 60xWI	NA 1.27	WD 0.17	Nano Crystal Coat
CFI Plan Fluor 20xA MI	NA 0.75	WD 0.35	



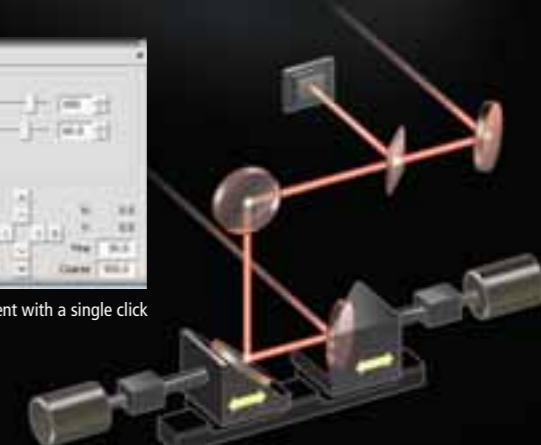
Auto laser alignment when changing multiphoton excitation wavelength

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 A1R MP's newly developed 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

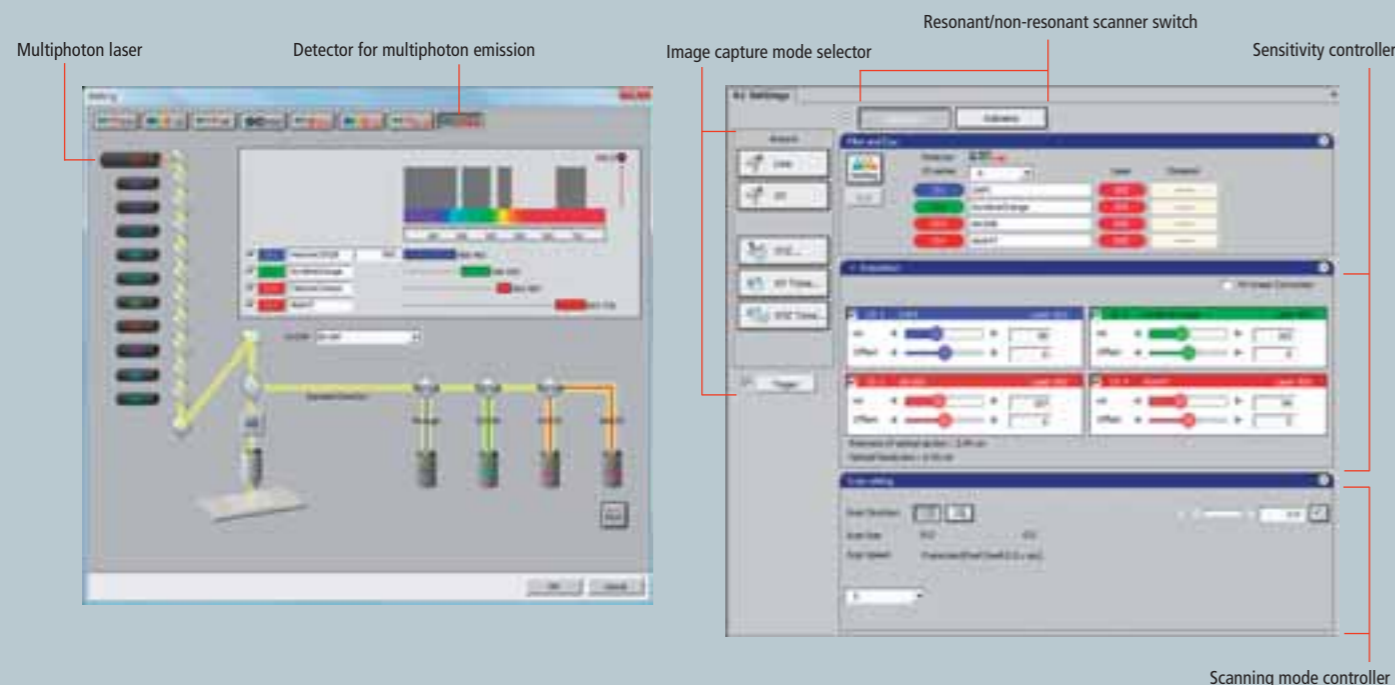


Intuitive, easy-to-use software for multiphoton imaging

NIS-Elements C Acquisition and Analysis software

Simple operations common with Nikon A1 series confocal microscope

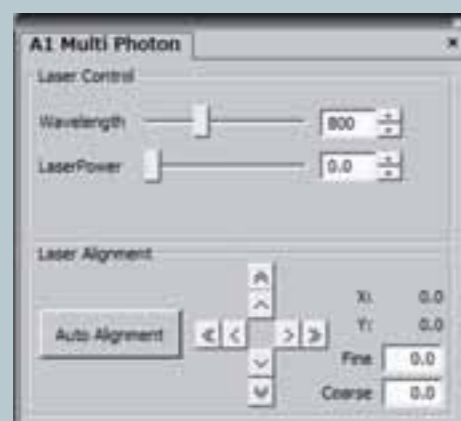
- All necessary operations for image capture are displayed in one window.
- Lasers and detectors for visible laser excitation can be switched simply by selecting fluorescent probe to be used.
- One-touch switching of high speed resonant scanner and high-resolution non-resonant scanner
- Simultaneous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



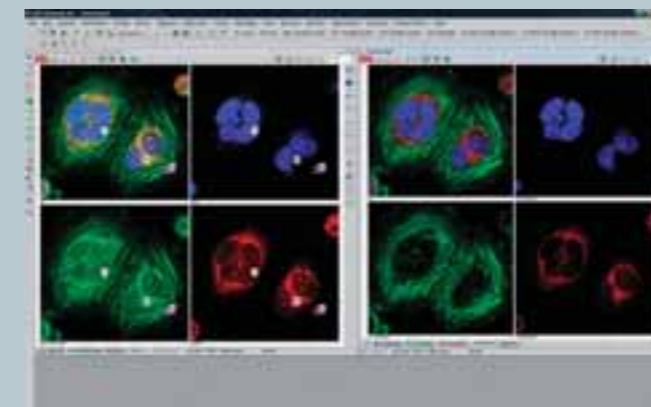
Z-intensity control function

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.

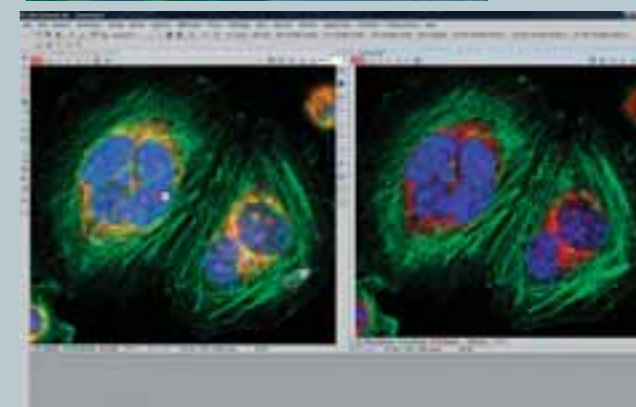
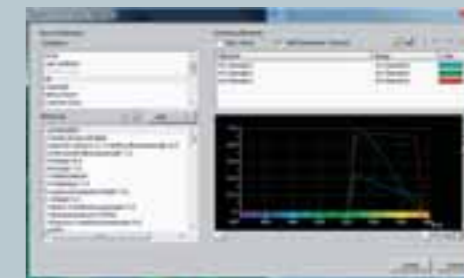


Channel unmixing function

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)

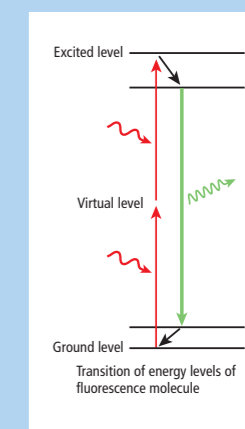
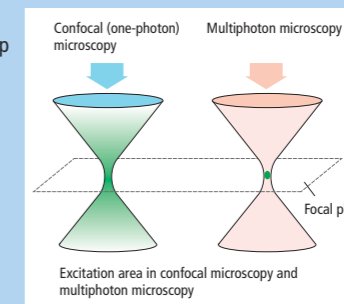
External trigger control

The A1R MP controller has an onboard 8 channel trigger port for connecting optional equipment for in-and-out (I/O) triggering applications. This is effective for synchronizing frame and scanning times with electrophysiology recordings, or to externally trigger the confocal to scan.

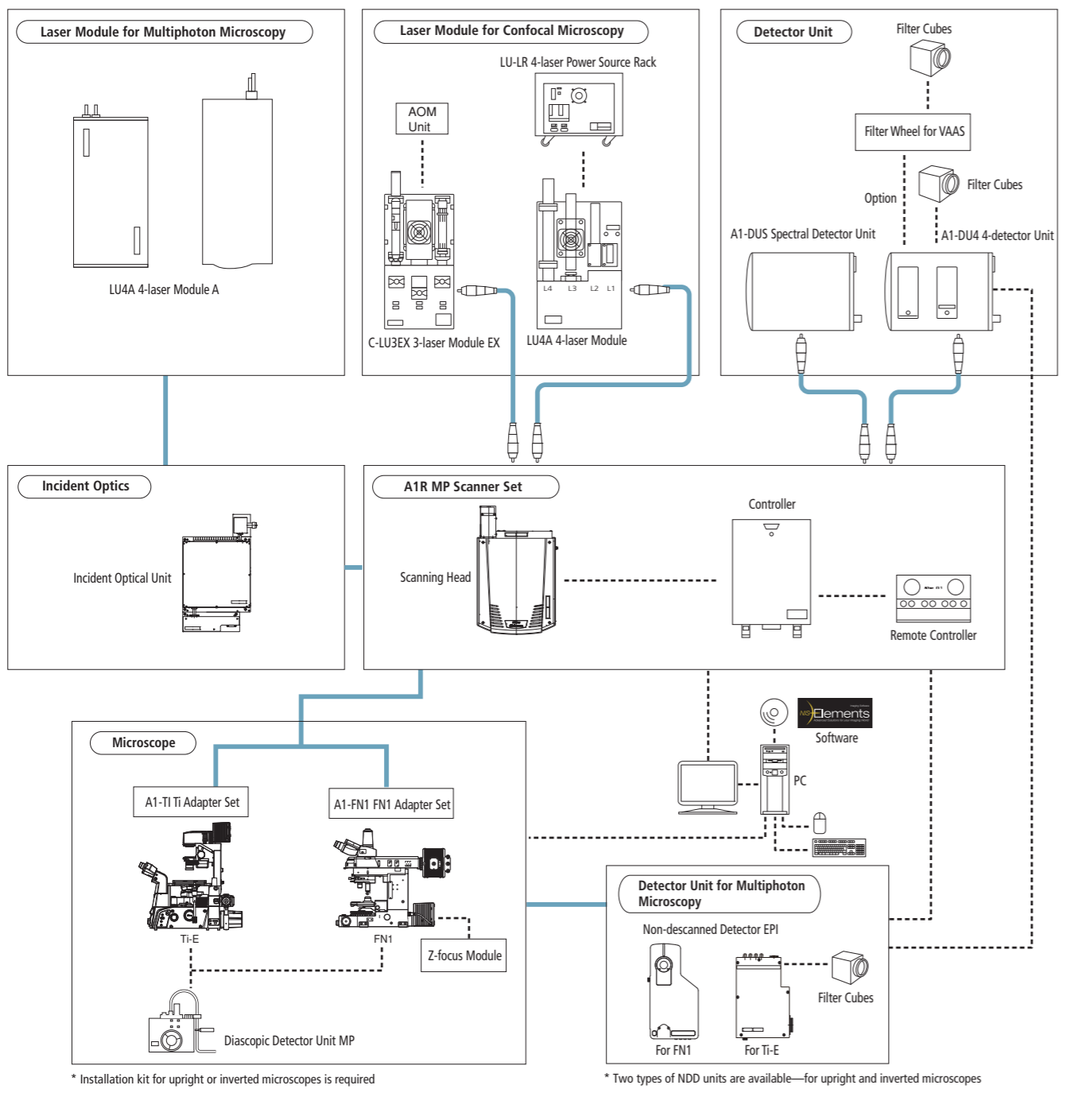


Principle of multiphoton excitation

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 precompensation "prechirping" system incorporated in the multiphoton laser and the use of the nondescanned multiphoton detector (NDD) allows fluorescence imaging deeper into a specimen than is possible with standard confocal technique.



System diagram



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.



Mai Tai HP DeepSee, Newport Corp., Spectra-Physics Lasers Division (Nikon specifications)



Chameleon Vision II, Coherent Inc. (Nikon specifications)

Specifications

A1R MP	
Input/output port	3 laser input ports 4 signal output ports (for 4-PMT detector, spectral detector, VAAS, optional detector ¹⁾)
Laser for confocal microscopy	Compatible laser Modulation Laser unit
Laser for multiphoton microscopy	Compatible laser Modulation Incident optics
Standard 4-channel detector	Wavelength Detector Filter cube
Diascopic detector	Wavelength Detector
NDD for multiphoton microscopy	Wavelength Detector Filter cube Unmixing
Image bit depth	4096 gray intensity levels (12 bit)
Scanning head	Scanning Dichroic mirror Pinhole
Spectral detector (option)	Wavelength detection range Number of channels Spectral image acquisition speed Wavelength resolution Unmixing
Compatible microscopes	ECLIPSE Ti-E inverted microscope, ECLIPSE FN1 fixed stage microscope
Z step	Ti-E: 0.025 μm, FN1 stepping motor: 0.05 μm
Option	Motorized XY stage (for Ti-E), High-speed Z stage (for Ti-E), High-speed piezo objective-positioning system (for FN1), VAAS
Software	Display/image generation Image format Application
Control computer	OS CPU Memory Hard disk Data transfer Monitor
Vibration isolated table	1800 (W) x 1500 (D) mm recommended, or 1500 (W) x 1500 (D) mm

¹ FCS/FCCS/FLIM is possible in combination with third-party systems. ² Special 1st DM for 478 is required. Please consult your local distributor. ³ Under development. ⁴ Photo activation by a laser for multiphoton microscopy is scheduled to be available in 2011.

Operation conditions

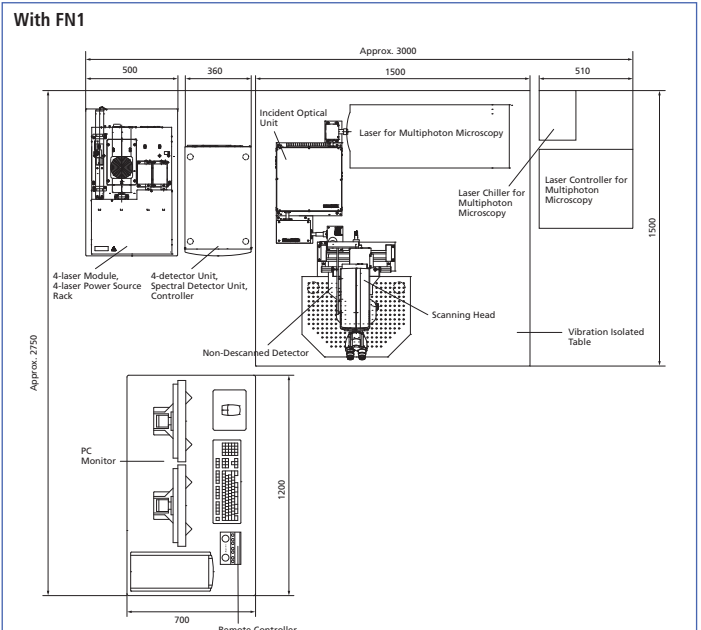
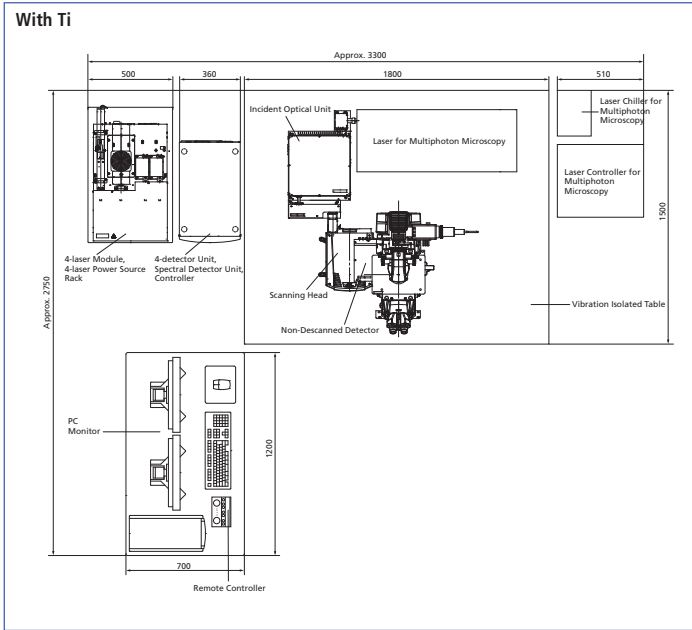
- Temperature: 20 °C to 25 °C (± 1 °C), with 24-hour air conditioning
- Humidity: 75 % (RH) or less, with no condensation
- Completely dark room or light shield for microscope

Power source

A1R MP	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	12.5 A
		220 VAC	6.8 A
	Except Ar laser (457 nm, 488 nm, 514 nm)	120 VAC	2.5 A
		220 VAC	1.4 A
Microscope	Laser for multiphoton microscopy (laser, water chiller, others)	120 VAC	19.2 A
		220 VAC	10.5 A
	Inverted microscope Ti-E with HUB-A and epi-fluorescence illuminator	120 VAC	4.4 A
		220 VAC	2.4 A

Layout

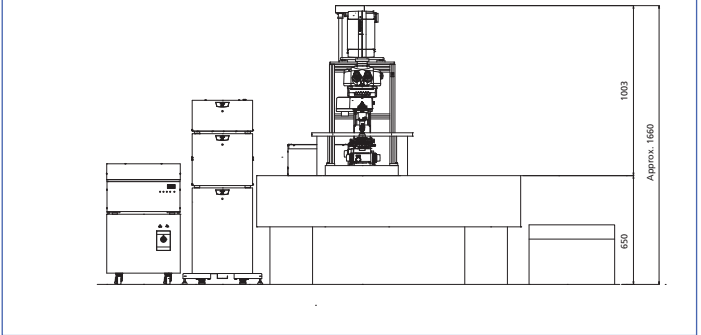
Unit: mm



Dimensions and weight

Scanning head	276 (W) x 183 (H) x 453 (D) mm	Approx. 13 kg
Incident optical unit	363 (W) x 186 (H) x 404 (D) mm	Approx. 11 kg
Controller	360 (W) x 580 (H) x 600 (D) mm	Approx. 45 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 325 (H) x 595 (D) mm	Approx. 26 kg
Non-descanned detector unit (for Ti)	349.9 (W) x 54.5 (H) x 280.5 (D) mm	Approx. 5 kg
Non-descanned detector unit (for FN1)	214 (W) x 85 (H) x 425 (D) mm	Approx. 6 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)

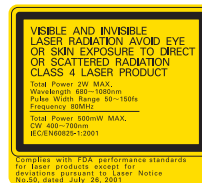
Dimensions exclude projections.



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

WARNING TO ENSURE CORRECT USAGE, READ THE CORRESPONDING MANUALS CAREFULLY BEFORE USING YOUR EQUIPMENT.

Monitor images are simulated.
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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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