AX R MP with NSPARC Multiphoton Confocal Microscope

Niko

Nikon

AX-MP

LLL



## AX R MP with NSPARC

Multiphoton Confocal Microscope

Shedding New Light On MICROSCOPY

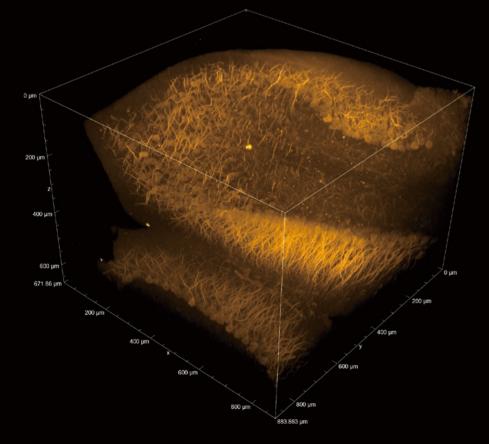
# LOOK DEEPER

## Explore the hidden depths of whole organisms in panoramic views

Nikon's multiphoton confocal microscopes, which clearly visualize fine structures deep within living organisms, have evolved even further. The AX R MP is equipped with a high-speed resonant scanner with 2K resolution and can capture in a single scan dynamics that span a wide area with superior spatial and temporal resolution. In addition, the innovative NSPARC super-resolution detector utilizes a newly developed SPPC array detector to collect a two-dimensional image at each scanned point, achieving a significant improvement in resolution. This enables macro-to-micro imaging with a single microscope system.

#### For broad areas of research

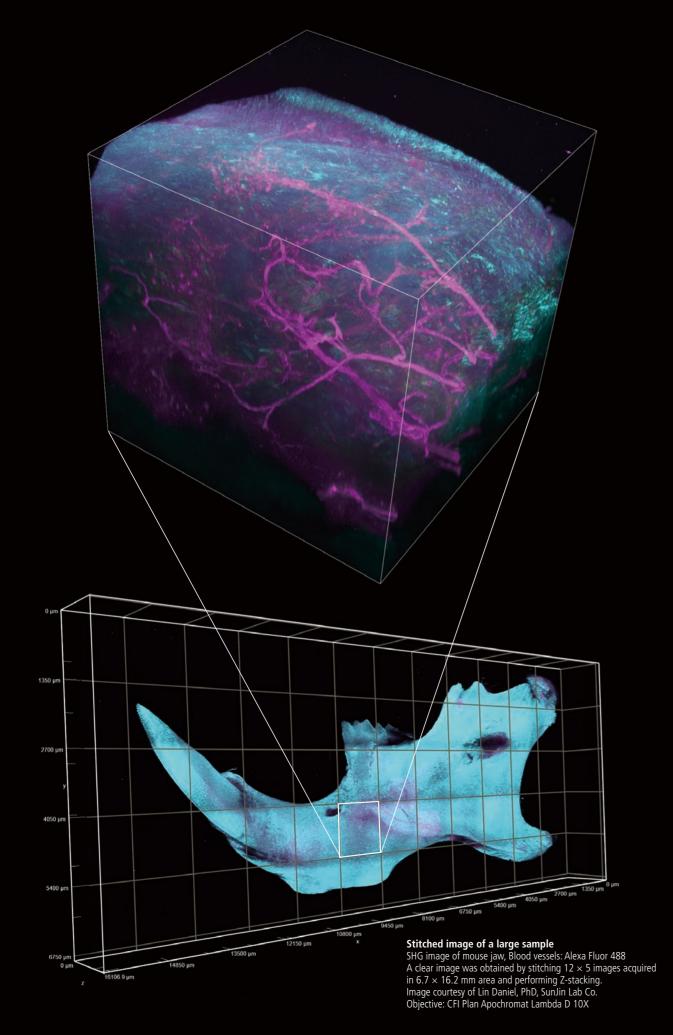






- High resolution
- High sensitivity
- Flexibility
- Super resolution

Resonant and Galvano scanners: FOV 22 mm Resonant scanner: 720 fps (at 2048 x 16 pixels) Resonant scanner: 2K x 2K, Galvano scanner: 8K x 8K Improved SNR with new detectors and electronics Two types of stands and tilting nosepiece Spatial information of 0.2 Airy units for each SPPC



# Capture wide views at high speeds

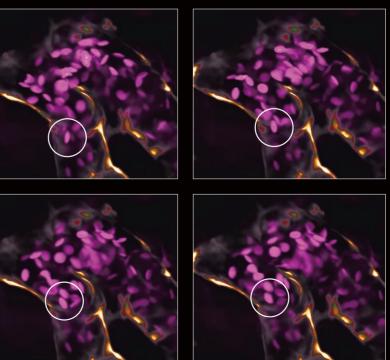
Featuring a field-of-view with a diagonal of 22 mm for both resonant and Galvano scanners, the AX R MP captures more data per single frame at any magnification. This is incredibly beneficial for faster acquisition of large specimens, or a wider perspective for time-lapse imaging.

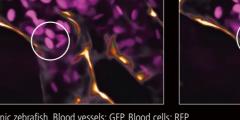
#### High magnification acquisition over a wide area

The larger FOV of the AX R MP can benefit imaging in several ways: - More of the sample is visible in each frame and more data can be collected, without changing objectives - More details are visible while keeping the same FOV when using higher resolution/magnification lenses Ultimately this means that you can save time, especially when producing higher quality stitched images.

#### High-speed scanning that reliably captures dynamic events

The resonant scanner of the AX R MP provides high-speed imaging while maintaining a large field of view of 22 mm. Total acquisition time is exponentially reduced compared to Galvano-based imaging. This also means a shorter illumination time and less phototoxicity in the sample. By setting an ROI, ultra-fast imaging of up to 720 fps (2048 x 16 pixels) can be achieved.



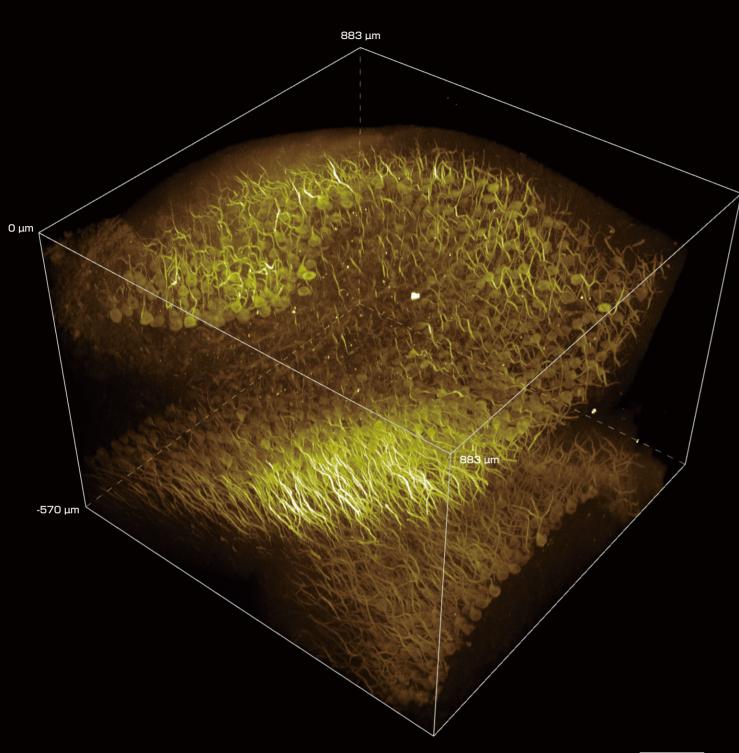


Embryonic zebrafish, Blood vessels: GFP, Blood cells: RFP Individual blood cells are identified in high resolution, and blood flow is imaged at a high speed of 28 fps (2048 x 546 pixels) Images courtesy of Erika Dreikorn and Dr. Beth Roman, Department of Human Genetics, University of Pittsburgh Graduate School of Public Health Objective: CFI75 Apochromat LWD 20XC W





inle video



MIP image of mouse brain, Prukinje: GFP

Z-stack imaging at an excitation wavelength of 920 nm using a 2K resonant scanner. Individual nerve cells in the depths are visualized with high S/N ratio Images courtesy of Dr. Laurence Dubreil, Dr. Julien Pichon and Pr Marie-Anne Colle, PAnTher UMR703 INRAE/Oniris, Nantes France Objective: CFI75 Apochromat LWD 20XC W



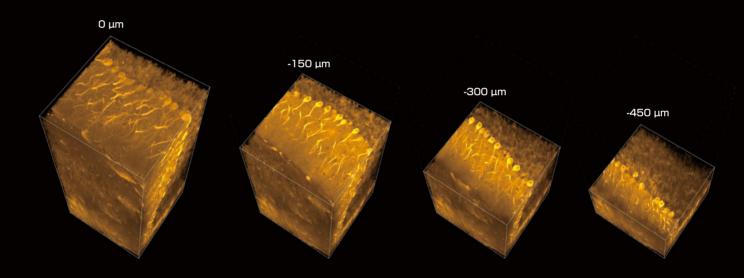
Sample video

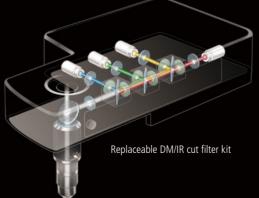
# Bright, high-definition imaging of deep structures

Multiphoton resonant imaging of up to 2K x 2K provides high resolution images even to deep areas within specimens. Fluorescence from deep areas can be reliably captured by suppressing signal loss with a highsensitivity detector.

### High resolution deep imaging for intravital microscopy

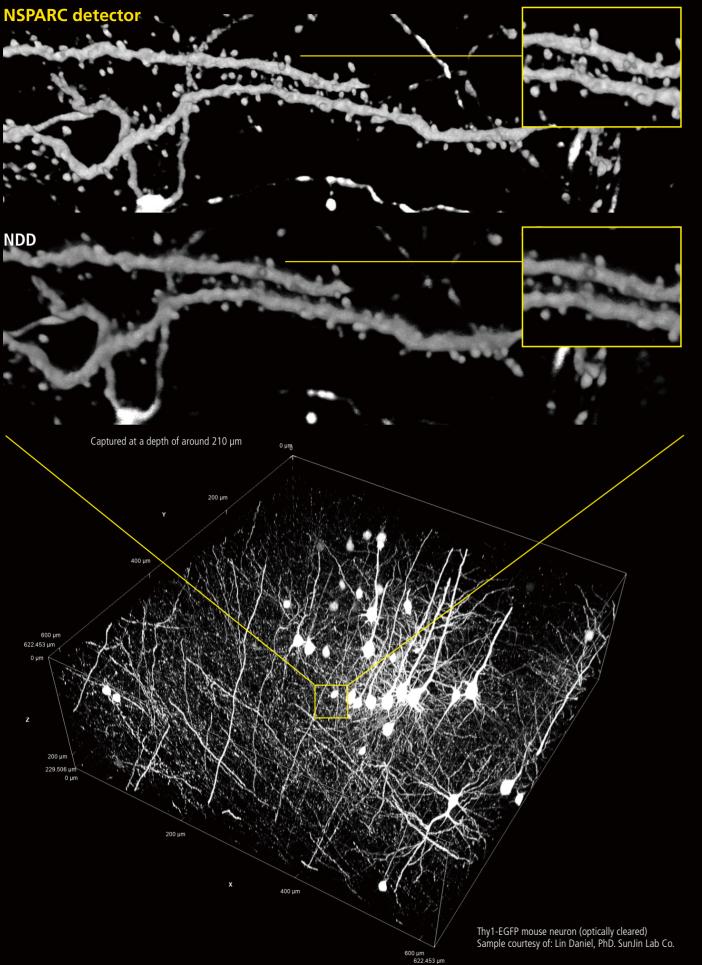
The AX R MP's two selectable scanners, resonant and Galvano, allow users flexibility in acquisition, and provide both high-speed and high-resolution solutions. The Galvano scanner is capable of obtaining 8192 x 8192 pixel high resolution images, with a pixel density that enables Nyquist sampling at any magnification. The high-speed resonant scanner supports high resolution imaging with pixel densities of up to 2048 x 2048. Both can visualize morphological changes in deeper regions in fine detail.





deep areas The AX R MP's non-descanned detectors (NDDs) are placed in flexible configurations near the back aperture of the objective for maximum emission collection. Flexible configurations from 2 to 4 channels in multi-alkali PMT or GaAsP PMT, depending on target emission wavelengths, are also standard options.

### High-sensitivity detection of signals from



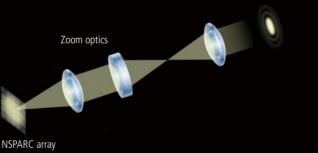
## Realizes deep imaging with super resolution

The newly developed NSPARC (Nikon SPatial ARray Confocal) super-resolution detector collects two-dimensional information for each pixel by utilizing an array detector. NSPARC technology enables high-resolution image acquisition with increased signal-to-noise ratio (S/N), providing more spatial information than NDDs. Super-resolution images and excellent S/N can be achieved even in deep areas, enabling fine detailed structures to be acquired there. NSPARC's sensitive detection will benefit a wide range of sampling, contributing to more accurate studies.

#### NSPARC spatial array detector technology

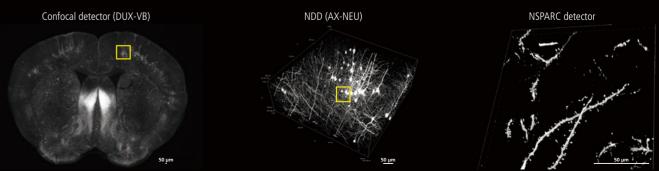
The NSPARC detector comprises an array detector with 25 Single Pixel Photon Counters (SPPCs) that enables collection of two-dimensional spatial information at each scanned pixel, rather than collecting only one intensity value per pixel. By controlling zoom optics, the size of the illumination spot projected onto the detector array can be adjusted to 1 airy unit, and 0.2 airy units worth of information can be acquired with each SPPC. This allows the NSPARC detector to reconstruct images with higher spatial resolution than traditional NDDs.

With NSPARC detection, the fluorescence emission light is directed through optical lenses to the detector array, where the projected light can fill the array.



### Accommodates macro to micro imaging of large samples

With its large FOV and expansive space under the objective, the AX R MP enables image acquisition of varied specimens and sample sizes. Combined with the NSPARC detector, the AX R MP allows imaging from macro to micro. Imaging of both large samples using traditional detection and super-resolution imaging of fine structures can be achieved within a single experiment. It can also be used while switching to and from confocal detectors, depending on the condition of the sample.



detector.



Each spot (pixel) from NSPARC contains spatial information. which can be used to reconstruct fine details in a resulting

The AX R MP with NSPARC supports the entire range of imaging applications, from macro imaging of a sample by image stitching to micro imaging using the super-resolution



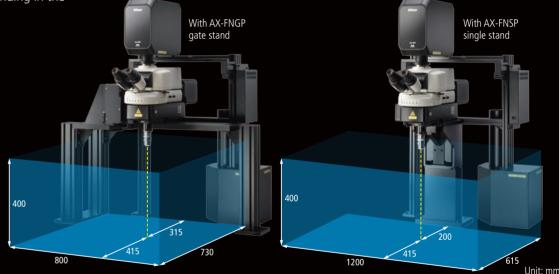
## Large space for a wide range of sample setups

The motorized upright microscope dedicated for AX R MP provides a clearance of 40 cm under the objective. Two types of motorized stand are available, both providing a large amount of free space around the sample without the need for customization, and improves sample positioning flexibility and accessibility to samples. Also, the angle of the objective is adjustable, providing even more flexibility and enabling the sample to be observed in its natural posture.

#### Ample space around the sample

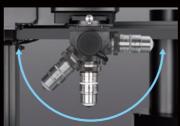
The stage is separated from the microscope to ensure a large space under the objective. Two types of stand are available for different observation purposes. The gate stand has a large space extending in the longitudinal direction, and the single stand has a large space extending in the

lateral direction.



#### Observe samples in their natural postures

Nikon has developed the new CFI75 single tilting nosepiece that can adjust the objective to different angles. It enables observation of a sample in the lateral and oblique directions without changing its orientation, reducing the load on the sample. The Piezo Z device (optional) allows for highly accurate, high-speed Z imaging





Horizontal rotation (±100°)

Vertical rotation (±90°)



Z axis coarse movement (±3 mm) Piezo Z device stroke: 450 µm

#### Highly accurate sample positioning

The dedicated motorized stage enables highly precise movement of samples such as tissue sections and culture dishes, within the range of  $\pm$ 34 mm (X) and  $\pm$ 27 mm (Y), using a joystick.

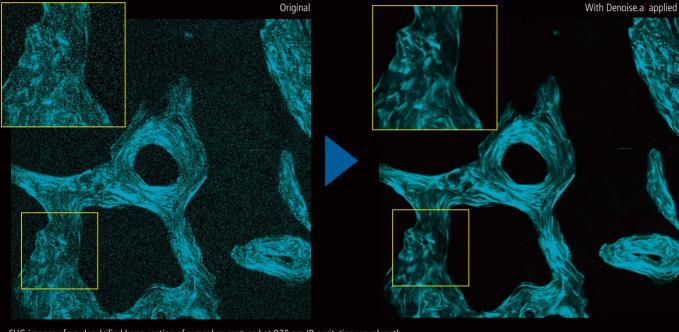


## Software for deep, wide imaging

NIS-Elements C control software enables centralization of workflow from image acquisition to analysis, making it easy to customize experiment templates that combine multiple settings.

#### Al software innovations designed to assist

The optional software module NIS.ai is equipped with image processing tools and customization functions. Utilizing deep learning and AI technology, it automates image acquisition and generation of optimal images for analysis.



SHG images of un-decalcified bone section of a monkey captured at 920 nm IR excitation wavelength Image courtesy of Dr. Tadahiro limura and Dr. Takanori Sato of the Department of Pharmacology, Faculty and Graduate School of Dental Medicine, Hokkaido University

Denoise.ai, a standard module in the NIS-Elements C and C-ER imaging software, automatically removes Poisson shot noise from resonant confocal images. Resonant scanning results in ultrashort (tens of nanoseconds) dwell times that are extremely favorable for reducing phototoxicity and increasing specimen viability for long term imaging. While resonant scanning at very short exposure times usually requires line averaging to reduce Poisson shot noise contributions, users instead can employ Denoise.al to eliminate the noise component. Denoise al can recognize and remove the shot noise components of images, increasing clarity and allowing for shorter exposure times and longer time-lapse experiments, while maintaining viability.

Application note

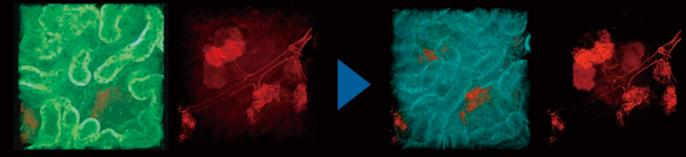
#### High-resolution image capture with a single click

NIS-Elements C-ER assesses captured images and automatically determines processing parameters to achieve enhanced resolution. Higher resolution confocal images (up to 120 nm resolution in XY and 300 nm in Z)\* can be easily generated with a single click. \* For confocal imaging.

### Unmix wavelength crosstalk

Multiphoton excitation makes simultaneous excitation of multiple fluorescent probes with a single IR wavelength possible. When there is significant crosstalk in images acquired via multiple channels, fluorescent separation (spectral unmixing) allows clear separation of dyes.

#### Acquired image

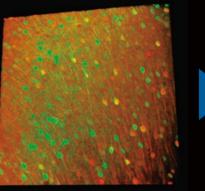


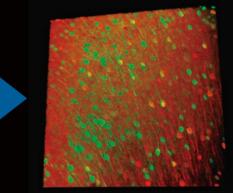
All channels merged

Red only

Multicolor fluorescent images of mouse kidney Red: blood vessels (Alexa Fluor 594), Cyan: SHG, Green: Autofluorescence

Acquired image

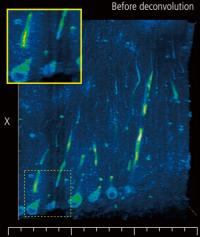


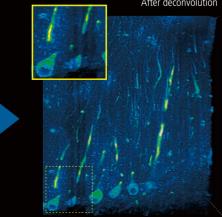


All channels merged

#### Deconvolution improves image guality in deep areas

Deconvolution processing is effective in capturing detailed structures in deep areas because it reduces image elongation in the optical axis direction.





300 um

#### 0 µm

#### Unmixed image

#### Unmixed image

Two AAV- (AAV-Camk2-Cre and AAV-hSyn-GFP) were injected into the cerebral cortex of an Ai14 mouse to express GFP and TdTomato in neurons. Red: AAV-Camk2-Cre neurons (TdTomato) Green: AAV-hSyn-GFP neurons (GFP) Image courtesy of Dr. Aya Ishida, RIKEN Center for Brain Science, Laboratory for Brain Development and Disorders

#### After deconvolution

Slices of the cerebellum of a LC3GFP mouse. The right side of each image is the surface layer and left side is at a depth of about 300 µm. Blue: cerebellum (autofluorescence) Green: Purkinje cells Image courtesy of Dr. Laurence Dubreil, Dr. Julien Pichon and Pr. Marie-Anne Colle, PAnTher UMR703 INRAE/ Oniris, Nantes France

# Bright images, high quality

A wide range of high NA objectives that correct chromatic aberrations up to the near infrared wavelength range and support multiphoton excitation imaging is provided.

### CFI75 Apochromat LWD 20XC W

Supports large field of view observation with an FOV of 22 mm. This objective makes it possible to observe deep inside the sample, with a long working distance of 2.8 mm. This low-magnification water-immersion objective is bright up to the periphery of its wide field of view.

#### CFI90 20XC Glyc

This objective is capable of correcting the refractive index of the immersion liquid from 1.44 to 1.50. It has a large field of view, high NA (1.00), and long working distance (8.20 mm). It also corrects chromatic aberration up to 1300 nm.

#### CFI75 Apochromat 20XC W 1300

This objective has a long working distance (2.0 mm) and high NA (1.10), and corrects chromatic aberration up to 1300 nm. Since it can also correct spherical aberration due to depth, it is suitable for deep multiphoton imaging.

#### **CFI Apochromat Lambda S 40XC WI**

This objective has the highest NA (1.25) among water immersion objectives. It is bright and has high resolution, and is suitable for confocal live cell imaging.



PO LWD 20X/1.00M

∞/0-0.17 WD 2.8

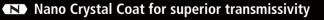
Nikon

#### CFI Plan Apochromat 10XC Glyc

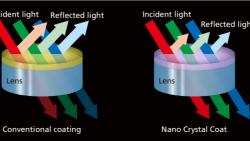
Since this objective is able to correct the refractive index of the immersion liquid from 1.33 to 1.51, it supports various tissue-clearing technologies. It makes 3D observation of deeper areas in living tissue possible.

#### **CFI Apochromat LWD Lambda S 20XC WI**

This is a high-performance, highly versatile objective that boasts a high NA (0.95), a large view field, and a long working distance (0.95 mm).

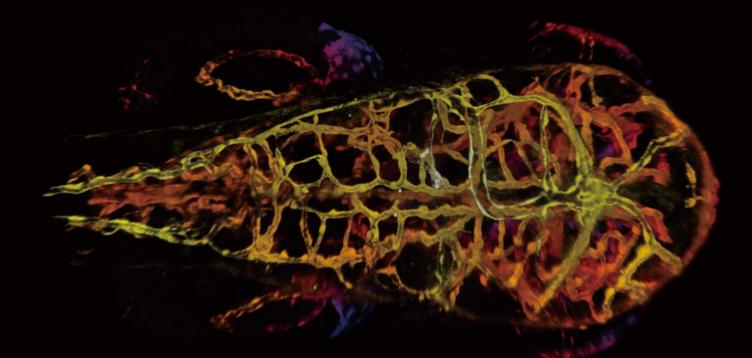


Nikon's exclusive Nano Crystal Coat is an anti-reflective coating consisting of ultrafine crystalline particles. This forms a coarse structure that enables lower refractive indices, facilitating the passage of light through the lens rather than reflecting it, thus providing superior light transmission.



## Support for visible light imaging

The AX R MP supports observation not only at infrared wavelengths, but also at visible wavelengths. It enables both multiphoton imaging and confocal imaging with a single microscope. It also enables simultaneous photostimulation and imaging using two different wavelengths.



#### Opti-Microscan photostimulator (optional)

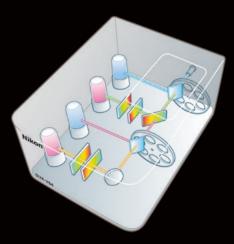
Photostimulation using wavelengths\* of 405 nm, 488 nm, and 561 nm enables simultaneous visible light stimulation and IR imaging. Stimulation modes include simultaneous, sequential, and manual stimulation. \*Limited by the specifications of the filter cube used.

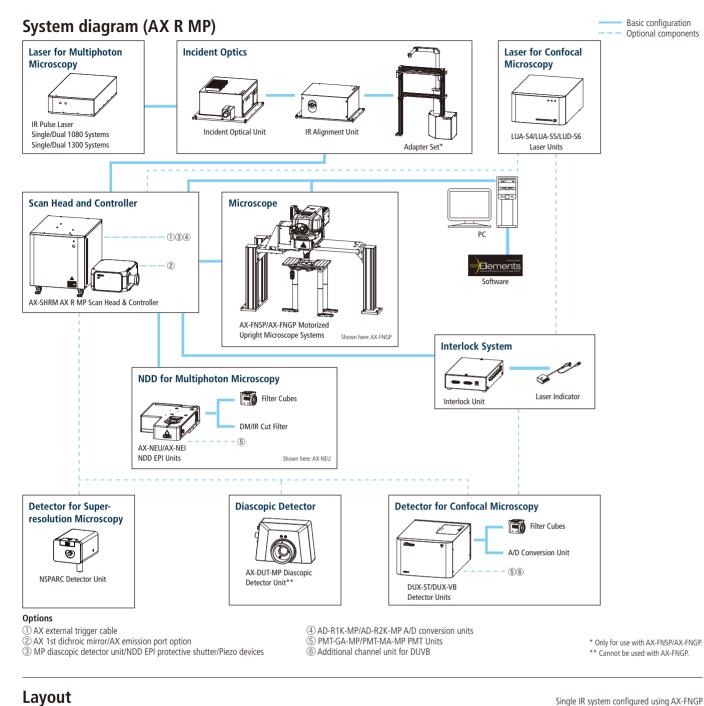
#### DUX-VB high-sensitivity visible light detector unit

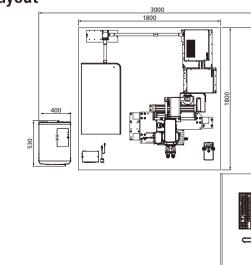
The transmission wavelength band of the LVF (Linear Variable Filter) employed in the DUX-VB gradually changes depending on its location, enabling continuous tuning of the wavelength detection setting within a range of 400 nm to 750 nm.

From 2 to 4 channels can be selected, and high sensitivity GaAsP PMT can be used for all channels.

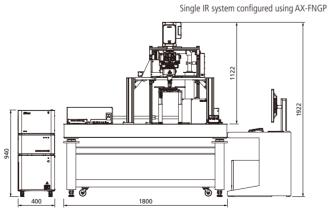








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Unit: mm

#### Specifications (dedicated AX-FN motorized upright microscope)

		AX-FN
Main body	Optical system	Infinity optical system
	Microscope stands	AX-FNSP Single Stand
	Focusing	AX-FN Focusing Nosepier Motorized coaxial coarse/fi Focusing stroke: Up 13 mm refocus mechanism Focal plane: 400 mm above
	Controls	AX-FNCTL Control Box     AX-FNHC Hub Controller Stage Joystick, Motorized DSC Zooming Port)
Tubes		Pupillary distance: 50-75 m 100/0/0, 0/100/0, 0/0/100 • NI-TT2 Quadrocular Tiltir • NI-TT2-E Motorized Quad
Eyepieces (F.O.V. (mm))		• CFI 10X (22) • CFI 12.5X
Photodetector		AX-NEU Non-descanned
Nosepieces		<ul> <li>FN-S2N CFI60 Sliding Not can be attached</li> <li>FN-S2N-2 CFI90 2 Place S prism slider can be attach</li> <li>FN-MN-H CFI75 Holder*5</li> <li>FN-MN-H2 CFI90 Holder</li> <li>AX-FNTN-H CFI75 Single</li> </ul>
Stages	Adapter	AX-FNSA Stage Adapter, adjustable to 2 positions vibration isolated table)
	Stage	FN-3PS2 XY stage, Cross     AX-FNS-E Motorized XY
Epi-fluorescent illuminator	Illumination unit	NI-FLEI-2 Epi-fluorescence
	Light source	D-LEDI Fluorescent LED II
	Filter cube turret	6 mountable filter cubes, s • NI-FLT6 Epi-fluorescence • NI-FLT6-I Intelligent Epi-f • NI-FLT6-E Motorized Epi-
	Photostimulation device	AX-FNBPU Stimulation B simultaneous stimulation
Diascopic illuminator	Illumination unit	AX-FNDIA Diascopic Unit 4 filter slider attachable, Co stroke: Up 2.5 mm/Down 1 Turret mountable
	Light source	Halogen Lamp (12V100W) • NI-LH Precentered Lamp • FN-LH Precentered Lamp High Luminescence White • LV-LL LED Light Source
	Shutter	NI-SH-E Motorized Shutter
	Condenser	• FN-C LWD condenser, O.
	Polarizer Turret	NI-PT Polarizer Turret, Vis polarizer attachable
Observation methods		Brightfield, Epi-fluorescenc
Power consumption		100W
Weight (approx.)		66 kg (fully motorized fluo with diascopic illuminator)

\*3 DIC prism slider cannot be attached

\*1 Based on the focus position \*2 Software controlled value

\*4 FOV 12, Usable objectives: CFI75 LWD 16X W, CFI75 Apochromat LWD 20XC W, CFI75 Apochromat 25XC W, CFI75 Apochromat 25XC W 1300 \*5 Cannot be used with diascopic illumination. The FN-MN-H cannot be used with diascopic illumination only when the 400 µm objective piezo positioner (PI) is attached.

ISP	AX-FNGP	
11.5	AX-FNGP Gate Stand	
ce Unit Tine focusing n/Down 2 mm <sup>*1, *2</sup> , Min	imum step: 0.02 μm, Motorized escape and	
e the surface of the vibr	ation isolated table	
	ng Nosepiece Unit, Diascopic Illumination System, Turret, Motorized Quadrocular Tilting Tube 2 and	
nm, Inclination angle: 15 via DSC Zooming Port ng Tube 2, With interloc drocular Tilting Tube 2, '		
(16) • CFI 15X (14.5) • C	FI UW 10X (25)	
EPI Upright Detector		
sepiece, Forward-backv	vard sliding type, two positions, DIC prism slider	
hed to the front objectiv	m slider can be attached	
, supporting both manua	al and motorized XY stages. Stage height: ize (400 mm/200 mm from the surface of the	
s travel 29.5 (X) x 29.5 ( stage, Cross travel ±34	() mm, with 2 auxiliary plates (X) x ±27 (Y) mm	
e attachment		
llumination System		
hutter function Cube Turret fluorescence Cube Turre -fluorescence Cube Turre		
ack Port, 6 mountable f n imaging can be switch	ilter cubes, Fluorescence imaging and ed	
t ondenser holder 1.8 mm, NI-PT Polarizer		
house bhouse LED Illuminator		
ter		
.D. 8.2 mm, NA: 0.78		
sible or infrared		
ce, DIC, IR-DIC	V	
rescence system,	66 kg (fully motorized fluorescence system)	

#### Specifications (AX R MP)

		AX R MP	
Scan head	Туре	AX-SHRM AX R MP Scan Head & Controller	
	FOV	ø22 mm	
	Standard image acquisition	Galvano scanner	
		Pixel size: max. 8192 x 8192 pixels	
		Scanning speed: max. 240 fps (512 x 16 pixels), 10 fps (512 x 512 pixels)	
	High-speed image acquisition	Resonant scanner	
		Pixel size: max. 2048 x 2048 pixels	
		Scanning speed: max. 720 fps (2048 x 16 pixels for 2K, 1024 x 16 pixels for 1K), 30 fps (2048 x 512 pixels for 2K, 1024 x 512 pixels for 1K)	
	Scan mode	Line scanning, bi-direction scanning and averaging	
	Simultaneous acquisition	Max. 5 channels (including a diascopic detector channel)	
	IR laser wavelength range	700-1080 nm (1080 system), 820-1300 nm (1300 system)	
	Dichroic mirror	Position: 6	
	Pinhole	6-153 μm variable	
	Zoom	1-1000X continuously variable	
	Input/output port	2 laser input ports 2 signal output ports	
Laser for multiphoton microscopy	Single 1080 system	Mai Tai HP/eHP DeepSee, Chameleon Vision II, Axon 920	
	Dual 1080 system	Chameleon Vision II + Axon 920, Axon 920 + Axon 1064	
	Single 1300 system	InSight X3, Chameleon Discovery NX	
	Dual 1300 system	InSight X3 Dual Option, Chameleon Discovery NX, Chameleon Discovery NX + Axon 920	
	Incident optics	700-1080 nm (1080 system), 820-1300 nm (1300 system), auto alignment	
	Modulation	Method: AOM (Acousto-Optic Modulator) device Control: power control, ROI exposure control	
Laser for confocal microscopy (option)	4-laser unit	405 nm, 488 nm, 561 nm and 640 nm lasers are installed	
	5-laser unit	405 nm, 488 nm, 561 nm, 594 nm and 640 nm lasers are installed	
	6-laser unit	405 nm, 445 nm, 488 nm, 515 nm, 561 nm and 640 nm lasers are installed	
NDD for multiphoton microscopy	NDD EPI unit AX-NEI (for Ti2-E) and AX-NEU (for AX-FNSP/FNGP)	Detectable wavelength range: 400-650 nm (1080 system), 400-750 nm (1300 system) Detectors: 2 GaAsP PMTs (4 GaAsP PMTs, or 3 GaAsP PMTs + 1 multi-alkali PMT are possible by adding options)	

Visible stimulation/ IR imaging (option)	Opti-Microscan Photostimulator	Stimulation wavelength: 40 Excitation wavelength for ir Stimulation speed: Max. 1 r Stimulation modes: simulta Stimulation area: square ins no number limit
Diascopic detector (option)	AX-DUT-MP*1 (for AX-FNSP/Ti2-E)	Detectable wavelength ran Detector: Multi-alkali PMT
Detector for confocal/ multiphoton microscopy (option)	DUX-VB detector unit	Detectable wavelength ran Detection width: 10 nm to Maximum pixel size: 8192 x Wavelength resolution: 5 n Compatible with Galvano a 2 or 4 channels (Multi-alkal
	DUX-ST detector unit* <sup>2</sup>	Detectable wavelength ran 2 or 4 channels (Multi-alkal
	NSPARC Detector Unit	Equipped with SPPC (Single Up to 7 barrier filters can be 525/50, 593/46, 700/75) With galvano scanner: Can 8192 pixels With resonant scanner: Car of 128 to 1024 pixels <sup>*3</sup>
Compatible microscopes		Dedicated AX-FNSP/AX-FN inverted microscope
Z step		AX-FNSP/FNGP: 0.02 µm, T
Option	Motorized XYZ	Motorized XY stage (for AX High-speed piezo objective
	Nosepiece for AX-FNSP/FNGP	AX-FNTN-H CFI75 single til
Software	Acquisition/analysis	Imaging software (equipped NIS-Elements C-ER
	Display/image generation	2D analysis, 3D volume ren
	Image format	JP2, JPG, TIFF, BMP, GIF, PN
		FRAP, FLIP, FRET(option), ph
	Application	colocalization
Control computer	Application OS	colocalization
Control computer Recommended insta	OS	

\*1 Cannot be mounted on AX-FNGP

\*2 Must be used with a confocal laser.

\*3 Resolution of 2048 pixels cannot be set.

\*4 FOV12, Compatible objectives: CFI75 LWD 16X W, CFI75 Apochromat LWD 20XC W, CFI75 Apochromat 25XC W and CFI75 Apochromat 25XC W 1300

05 nm, 488 nm, 561 nm; imaging: 800-1080 nm (1080 system), 820-1080 nm (1300 system) ms (point stimulation), Max. 20 µs/pixel (ROI stimulation) aneous, sequential, manual iscribed within a 22 mm-diameter circle, stimulation ROI: arbitrary pattern,

nge: 400-920 nm

nge: 400-650 nm (with IR laser), 400-750 nm (with visible laser); 320 nm x 8192 (with Galvano scanner) nm, wavelength range variable in 1 nm steps and resonant scanners ali PMT or GaAsP PMT options)

nge: 400-650 nm (with IR laser), 400-750 nm (with visible laser); Ili PMT or GaAsP PMT options)

le Pixel Photon Counter) array detector be mounted (Mountable filter: QuadBand446/523/600/677, 452/45,

be used with X resolution of 64 to 8192 pixels, Y resolution of 2 to

an be used with X resolution of 256, 512 and 1024 pixels, Y resolution

NGP motorized upright microscope system, ECLIPSE Ti2-E motorized

Ti2-E: 0.02 µm

X-FNSP/FNGP/Ti2-E), High-speed piezo Z stage (for Ti2-E), e-positioning system (for AX-FNSP/FNGP)

Iting nosepiece\*4

ed with Denoise.ai noise reduction function): NIS-Elements C or

ndering/orthogonal, 4D analysis, spectral unmixing

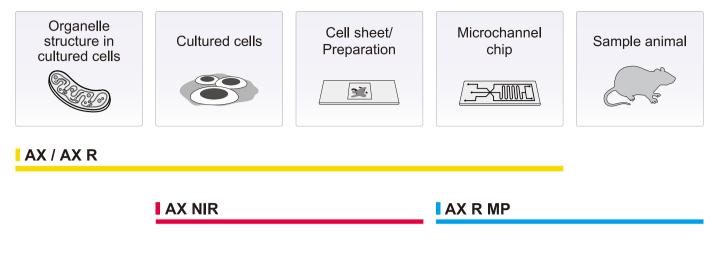
NG, ND2, JFF, JTF, AVI, ICS/IDS

hotoactivation, 3D time-lapse imaging, multipoint time-lapse imaging,

licrosoft Windows<sup>®</sup> 11 Pro

1°C, air conditioning at all hours no condensation)

### AX series



AX / AX R with NSPARC

AX R MP with NSPARC

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

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