Nikon

C2+

Confocal Microscope

Simple and Robust

Confocal Microscope C2+

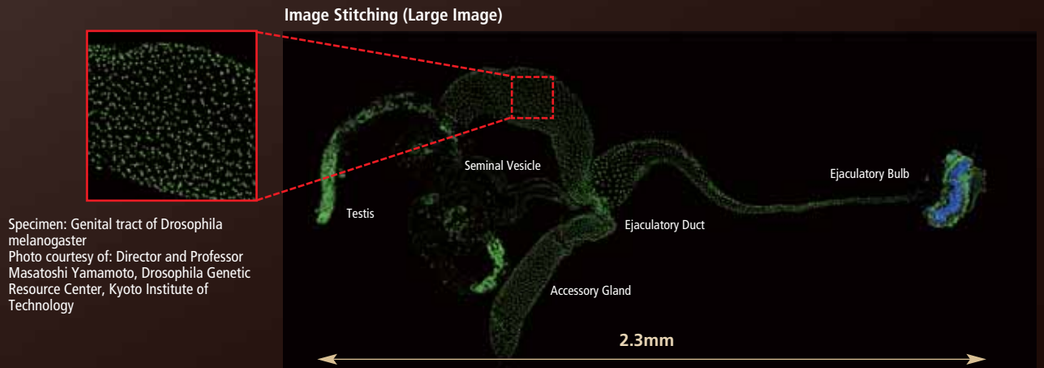
An essential microscopy laboratory instrument…

The C2+ confocal microscope system is part of a new generation of Nikon confocal instruments designed to be essential laboratory microscopy tools. Built on a reputation of incredible stability and operational simplicity coupled with superior optical technologies and high-speed image acquisition of up to 100 fps*, the C2+ is the perfect tool for a new microscope, or as a new accessory to an existing Nikon imaging system.

* With 8x zoom or larger

Large field-of-view imaging and three-dimensional reconstruction

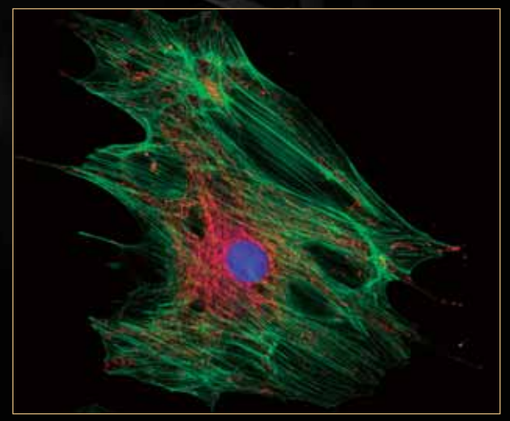
Confocal image acquisition, using high-numerical aperture and high-magnification objectives, together with XY stage control and advanced image stitching with Nikon NIS-Elements software, enables high-resolution images of large areas of a specimen to be produced. In addition, the microscope's high-precision Z-axis control allows assemblage of Z stack images for three-dimensional image reconstruction.



Specimen: Genital tract of Drosophila melanogaster
Photo courtesy of: Director and Professor Masatoshi Yamamoto, Drosophila Genetic Resource Center, Kyoto Institute of Technology

Image Quality

Nikon's unprecedented optics and highly efficient optical design provide the brightest and sharpest images, at the longest working distances.



High-efficiency scanning heads and detectors

With the convenient, small scan head size, the C2$^+$ can be used with various types of Nikon microscope. The C2$^+$ employs high precision mirrors and optically superior circular pinholes, and separates the detectors to isolate sources of heat and noise, enabling low-noise, high-contrast and high-quality confocal imaging.
The newly developed scanner driving system and Nikon's unique image correction technique allow 8 fps (512 x 512 pixels) and 100 fps (512 x 32 pixels) high-speed imaging.

High-performance optics

CFI Apochromat λS Series

These high-numerical aperture (NA) objectives are ideal for confocal imaging with correction of chromatic aberrations over a wide wavelength range from ultraviolet. In particular, the LWD 40xWI lens corrects up to infrared. Transmission is increased through the use of Nikon's exclusive Nano Crystal Coat technology.

CFI Apochromat TIRF Series

These objectives boast an unprecedented NA of 1.49 (using a standard coverslip and immersion oil), the highest resolution among Nikon objectives. The temperature correction ring adjusts for image quality affected by temperature change in the range of 23°C to 37°C.

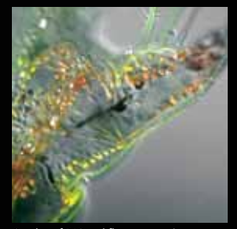
CFI Apochromat 40xWI λS, NA1.25 (left)
CFI Apochromat LWD 40xWI λS, NA1.15 (middle)
CFI Apochromat 60x Oil λS, NA1.4 (right)

CFI Apochromat TIRF 60x oil/1.49 (left)
CFI Apochromat TIRF 100x oil/1.49 (right)

High-definition diascopic DIC images

The C2$^+$ can acquire simultaneous three-channel fluorescence or simultaneous three-channel and diascopic DIC observation. High-quality DIC images and fluorescence images can be superimposed to aid in morphological analysis.

DIC image



Overlay of DIC and fluorescence images

High functionality

High-performance imaging software NIS-Elements offers a variety of image processing and analysis functions. It also enables data extraction from acquired images. In addition, NIS-Elements allows for intuitive operation of Nikon microscopes and other third-party peripheral devices, such as EMCCD cameras and filter wheels, to broaden the range of experiments possible.

Multimode capability

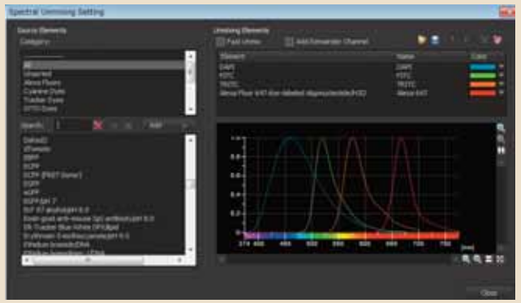
Various imaging methods, such as confocal, widefield, TIRF, photoactivation, as well as processing, analysis and presentation of acquired images, are available in one software package. Users can easily learn how to control different imaging systems with a common interface and workflow.

Setting: Easy-to-recognize display for setting lasers, detectors, etc.

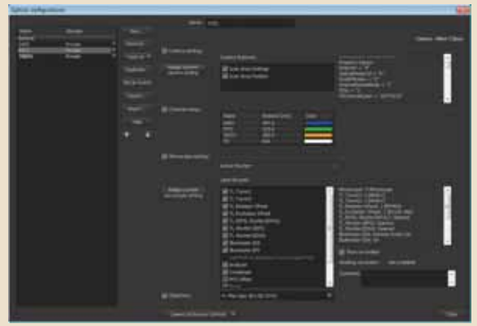
Simple GUI: Simple display of fundamental image acquisition settings

Scanning parameter settings

nDtime, nDXYZ: Intuitive settings for time schedule and Z series parameters, etc.



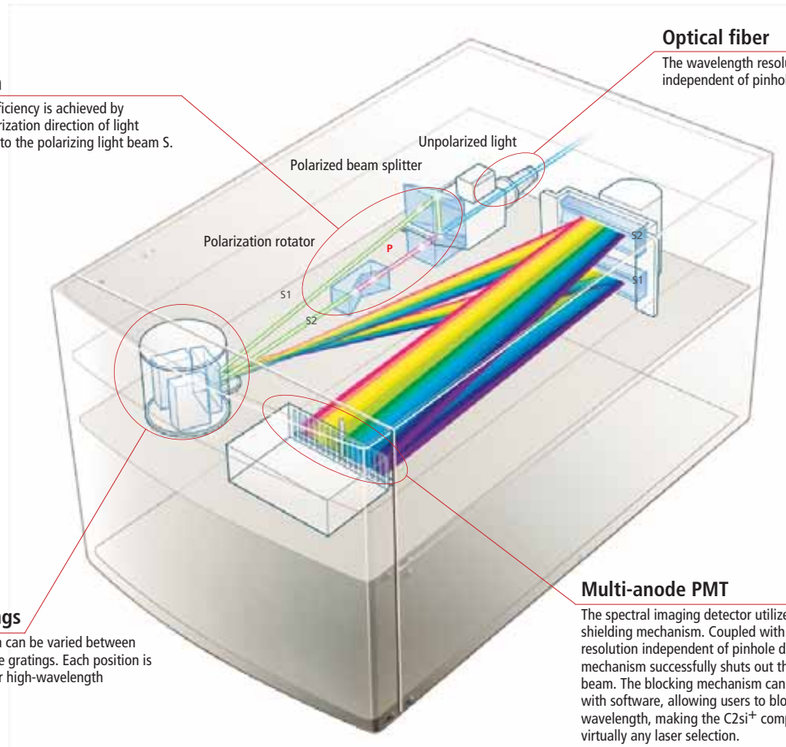
Spectral analysis GUI: Numerous functions for analysis and unmixing of acquired spectrums are provided, while spectral profiles of general dyes and fluorescent proteins are preprogrammed.



Optical Config: Multiple setting parameters (such as camera settings and channel setup) can be extracted from acquired images and registered for reuse.

True Spectral Imaging

In addition to the conventional three-channel fluorescence detector, the C2si+ true spectral imaging confocal laser scanning microscope is equipped with a dedicated spectral detector. By switching between these detectors, accurate spectral data of fluorescence signals can be obtained. The C2si+ captures minute changes of wavelength in true color and even unmixes overlapping spectra. Moreover, it has the capability to acquire spectra over a 320 nm-wide wavelength range in a single scan, minimizing damage to living cells.



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 2.5/5/10 nm with three gratings. Each position is precisely controlled for high-wavelength reproducibility.

Multi-anode PMT

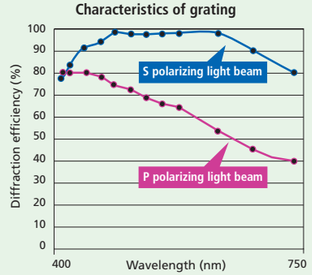
The spectral imaging detector utilizes a laser shielding mechanism. Coupled with a wavelength resolution independent of pinhole diameter, this mechanism successfully shuts out the reflected laser beam. The blocking mechanism can be moved freely with software, allowing users to block any laser wavelength, making the C2si+ compatible with virtually any laser selection.

High-quality spectral data acquisition

Diffraction Efficiency Enhancement System (DEES)

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

Characteristics of grating



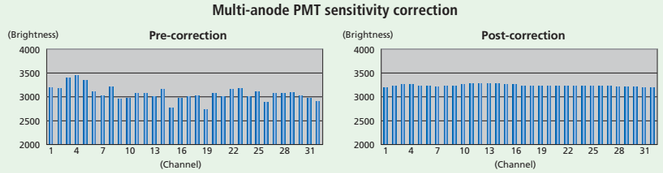
High-efficiency fluorescence transmission technology

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

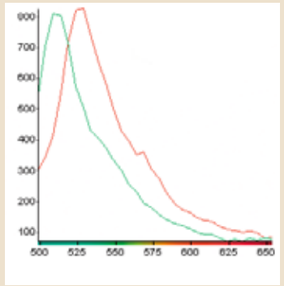
Three correction techniques allow for the acquisition of accurate spectra: interchannel sensitivity correction, which adjusts offset and sensitivity of each channel; spectral sensitivity correction, which adjusts diffraction grating spectral efficiency and detector spectral sensitivity; and correction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

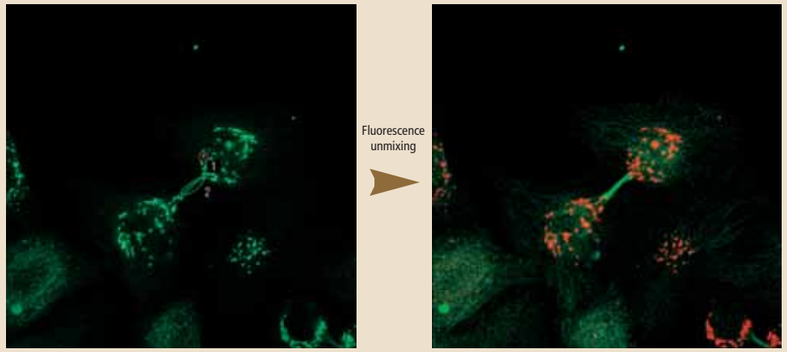
Multi-anode PMT sensitivity correction



Effortless fluorescence unmixing

Fluorescence labels with closely overlapping spectra can be unmixed cleanly with no crosstalk. Even without a given reference spectrum, simply specifying a Region of Interest (ROI) within the image and clicking the Simple Unmixing button allows separation of fluorescence spectra. The C2si+ contains a built-in database of given spectral data provided by manufacturers of fluorescence dyes that can be specified as reference spectra for fluorescence unmixing. Users may also add spectral information for new labels to the database.





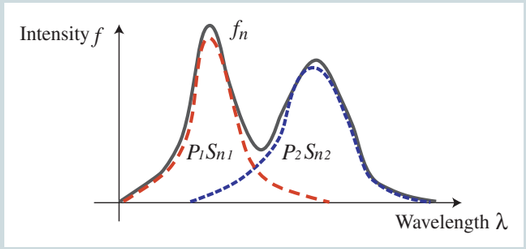
Specimen: HeLa cell in which GFP (Tubulin) and YFP (Golgi) are expressed. Spectral image captured with a 488 nm laser (left). After fluorescence unmixing, GFP is indicated in green and YFP is indicated in red (right). The graph (left) shows the spectral curve in the ROI.
Specimen courtesy of Dr. Sheng-Chung Lee, Dr. Han-Yi E. Chou, National Taiwan University College of Medicine, Institute of Molecular Medicine

What is spectral unmixing?

The spectrum obtained by actual measurement is a mix of spectral elements with a certain proportion. An imaging algorithm is used to compare the spectra of each pixel with reference curves for each spectral element. Each fluorescent probe in the specimen is displayed in a unique color in the final unmixed image.

$fn = Sn * P$

fn = Wave pattern of spectrum obtained by actual measurement
Sn = Wave pattern of individual reference spectrum
P = Ratio of elements for each wave pattern

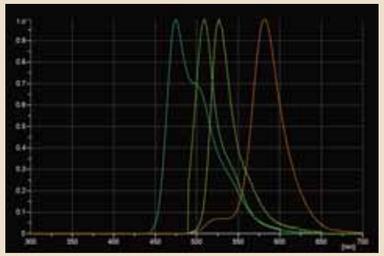


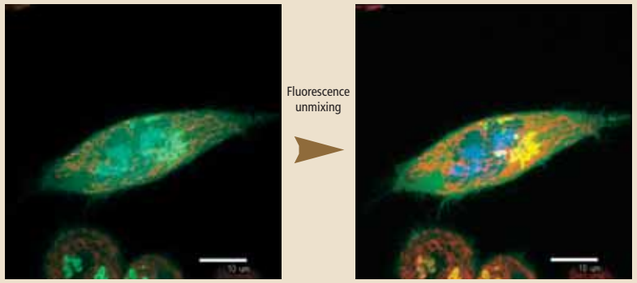
Reference wave pattern (S) is selected from the following three depending on the experiment.

1 Spectrum obtained by actual measurement of the zone with less crosstalk in the captured image
2 Data obtained by another actual measurement using only one probe
3 Spectral data provided by probe maker

Unmixing of multiple fluorescence

Because wavelength resolution and range are freely selectable, scanning of a fluorescence protein with a wide wavelength range from blue to red such as CFP/GFP/YFP/Ds Red is possible at one time. Reference data allows unmixing and display of each color.

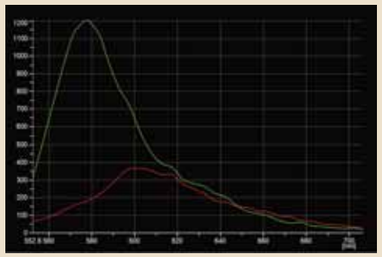




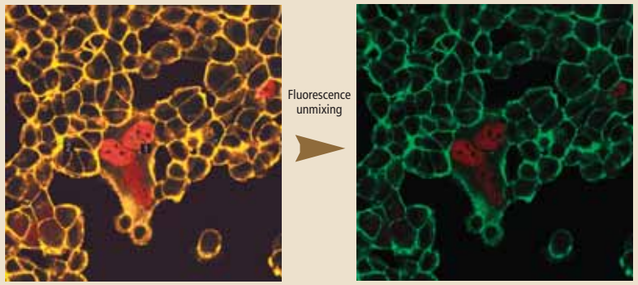
Specimen: HeLa cell in which nucleus is labeled with CFP, actin-related protein (Fascin) labeled with GFP, Golgi body labeled with YFP, and mitochondria labeled with DsRed. Spectral image captured with 408 nm and 488 nm laser exposure (left).
The fluorescence spectra of the captured image are unmixed using reference spectra (right).
Specimen courtesy of Dr. Kaoru Kato and Dr. Masamitsu Kanada, Neuroscience Research Institute, The National Institute of Advanced Industrial Science and Technology (AIST)

Unmixing red fluorochromes

Red fluorochromes, which had previously posed a challenge, are now simple to unmix.



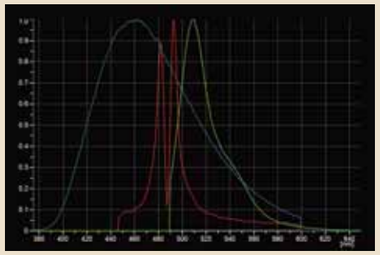
Spectra for ROI 1 and 2 corresponding to the image on the right
Rhodamine's fluorescence spectral peak is at approximately 579 nm, while that for RFP is approximately 600 nm. RFP's fluorescence is weaker than Rhodamine's, but their spectra are cleanly unmixed.

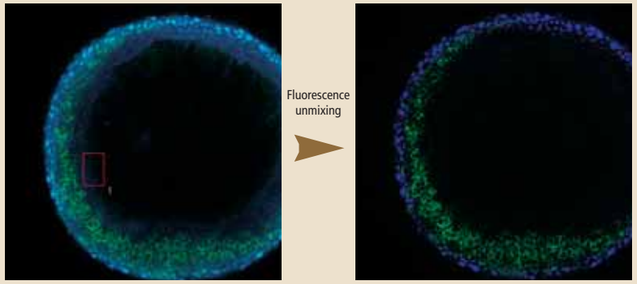


Specimen: actin of HeLa cell that has RFP expressed in the nucleus is stained with Rhodamine. Spectral image in the 550-630 nm wavelength range captured at 2.5 nm wavelength resolutions with 543 nm laser exposure (left). RFP indicated in red and Rhodamine indicated in green (right) in the image after fluorescence unmixing.
Specimen courtesy of Dr. Yoshihiro Yoneda and Dr. Takuya Saiwaki, Faculty of Medicine, Osaka University

Unmixing auto-fluorescence of multi-stained samples

Fluorescence unmixing makes it possible not only to separate closely overlapping fluorescence spectra such as CFP and YFP but also to eliminate auto-fluorescence of cells, which until now was difficult.

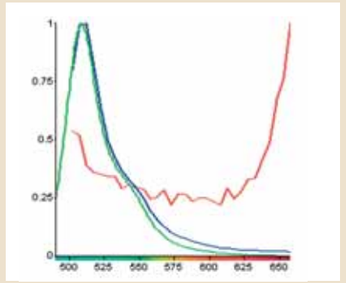




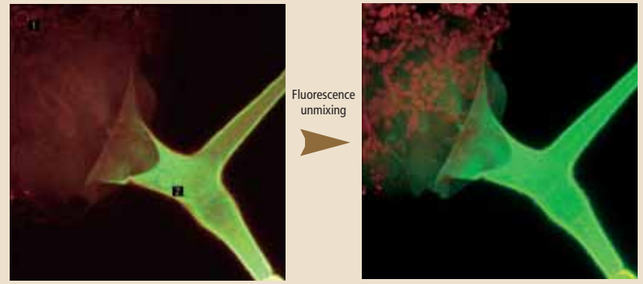
Specimen: Zebrafish egg stained with cadherin-GFP and DAPI. Spectral image captured with 408 nm and 488 nm laser exposures (left). After unmixing using reference spectra for auto-fluorescence (ROI1), GFP and DAPI, the auto-fluorescence in the image is eliminated (right).
Specimen courtesy of Dr. Tohru Murakami, Neuromuscular and Developmental Anatomy, Gunma University Graduate School of Medicine

Confirmation of GFP expression

In conventional confocal observation, fluorescence is visualized as fluorescence intensity in a certain wavelength range. The spectral detector allows the confirmation of detailed wavelength characteristics of the fluorescence. The C2si^{+}' spectral detector enables the slight color differences to be confirmed as wavelengths through sensitivity correction.



The correspondence of the spectral curve (blue) of ROI2 in the image and the reference curve (green) of eGFP proves that GFP is expressed in ROI2



Specimen: Arabidopsis proteoglycan and fused protein of GFP. Spectral image captured with 488 nm laser exposure (left). Once the image is unmixed using reference spectra for auto-fluorescence (ROI1) and GFP, GFP is indicated in green and auto-fluorescence is indicated in red (right).
Specimen courtesy of Assistant Prof. Toshihisa Kotake, Laboratory of Developmental Biology, Department of Life Science, Graduate School of Science and Engineering, Saitama University

True spectral FRET analysis

FRET (Fluorescence Resonance Energy Transfer) analysis using true spectral imaging allows three-dimensional analysis with high signal-to-noise (S/N) ratio and high-spatial resolution as well as easy determination of FRET by real-time detection of spectral changes derived by FRET.

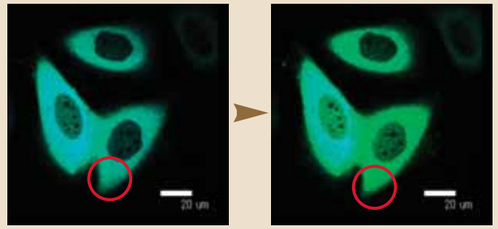
Also, even when spectra of donor and acceptor are overlapped like CFP and YFP, unmixing using reference data enables detection of detailed intensity changes and ratio analysis of fluorescence signals (YFP/CFP) without bleed through.

Acquisition of spectral image (XYTλ)

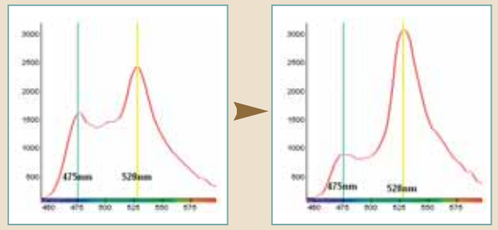
Spectral image in the 460-620 nm range captured at 5 nm wavelength resolution using a spectral detector enables observation of fluorescence wavelength changes.

Before ATP stimulation — 8 sec after ATP stimulation

True color image



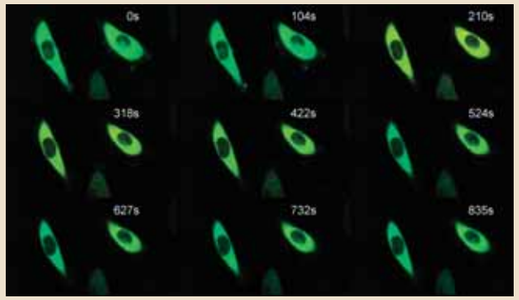
Spectral analysis



True color image and spectral analysis of CFP and YFP. Spectral curve in ROI. Left peak indicates CFP and right peak indicates YFP respectively. After ATP stimulation, peak of CFP drops and peak of YFP rises due to FRET.

Fluorescence unmixing

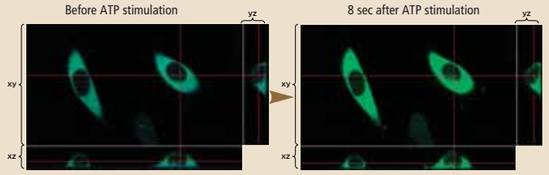
Spectral FRET analysis is possible by unmixing using reference data of CFP and YFP. Two-dimensional change (FRET) of intracellular Ca^{2+} concentration is easily determined from spectral data without acceptor bleaching.



FRET image after spectral unmixing. CFP is indicated in blue and YPF indicated in green.

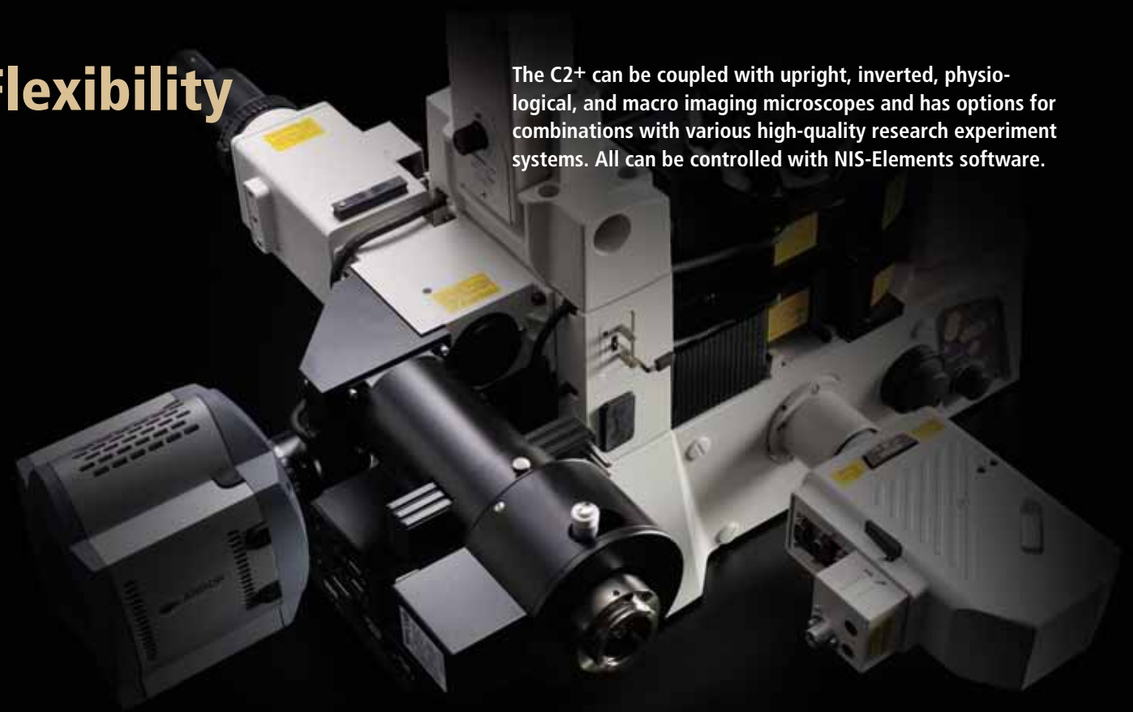
Five-dimensional analysis (XYZTλ)

Time-lapse changes (T) and spectra (λ) in three-dimensional space (XYZ) can be analyzed.



Flexibility

The C2+ can be coupled with upright, inverted, physiological, and macro imaging microscopes and has options for combinations with various high-quality research experiment systems. All can be controlled with NIS-Elements software.

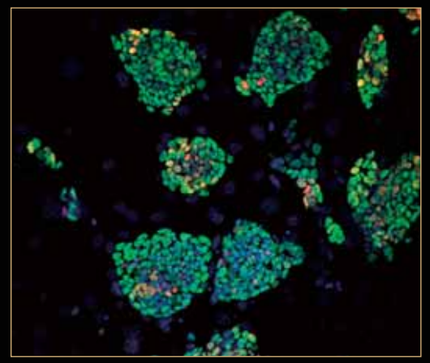


TIRF/Photoactivation-C2+ Multimode imaging system

Optional TIRF laser illumination module and a photoactivation module can be integrated to enable both imaging of single molecules with an extremely high S/N ratio, and imaging of the fluorescence characteristic changes of photoactivated and photo-convertible fluorescent protein.

AZ-C2+ Macro confocal microscope system

With a high-definition large field of view, specimens larger than 1cm can be acquired with an unprecedentedly high S/N ratio. The AZ-C2+ allows for imaging of whole-mount specimens, such as embryos, in a single acquisition, up to 4000x4000 pixel resolution, and it can also acquire 32-channel spectral data with the C2si+. It offers a combination of low and high magnification objective lenses, optical zoom and a confocal scanning zoom function, enabling continuous imaging from macro to micro.



TT2 ES cells
Anti-Nanog antibody (Cy3), anti-Oct3/4 antibody (Alexa488) and DAPI localized in cell nuclei
Photographed with the cooperation of: Hiroshi Kiyonari, Laboratory for Animal Resources and Genetic Engineering, RIKEN Center for Developmental Biology

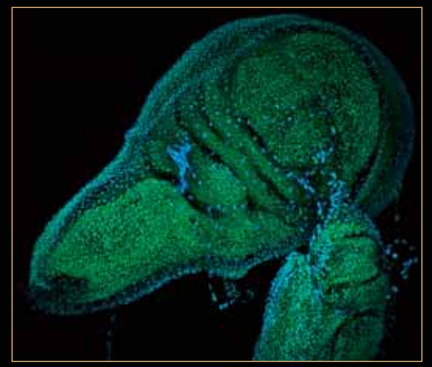
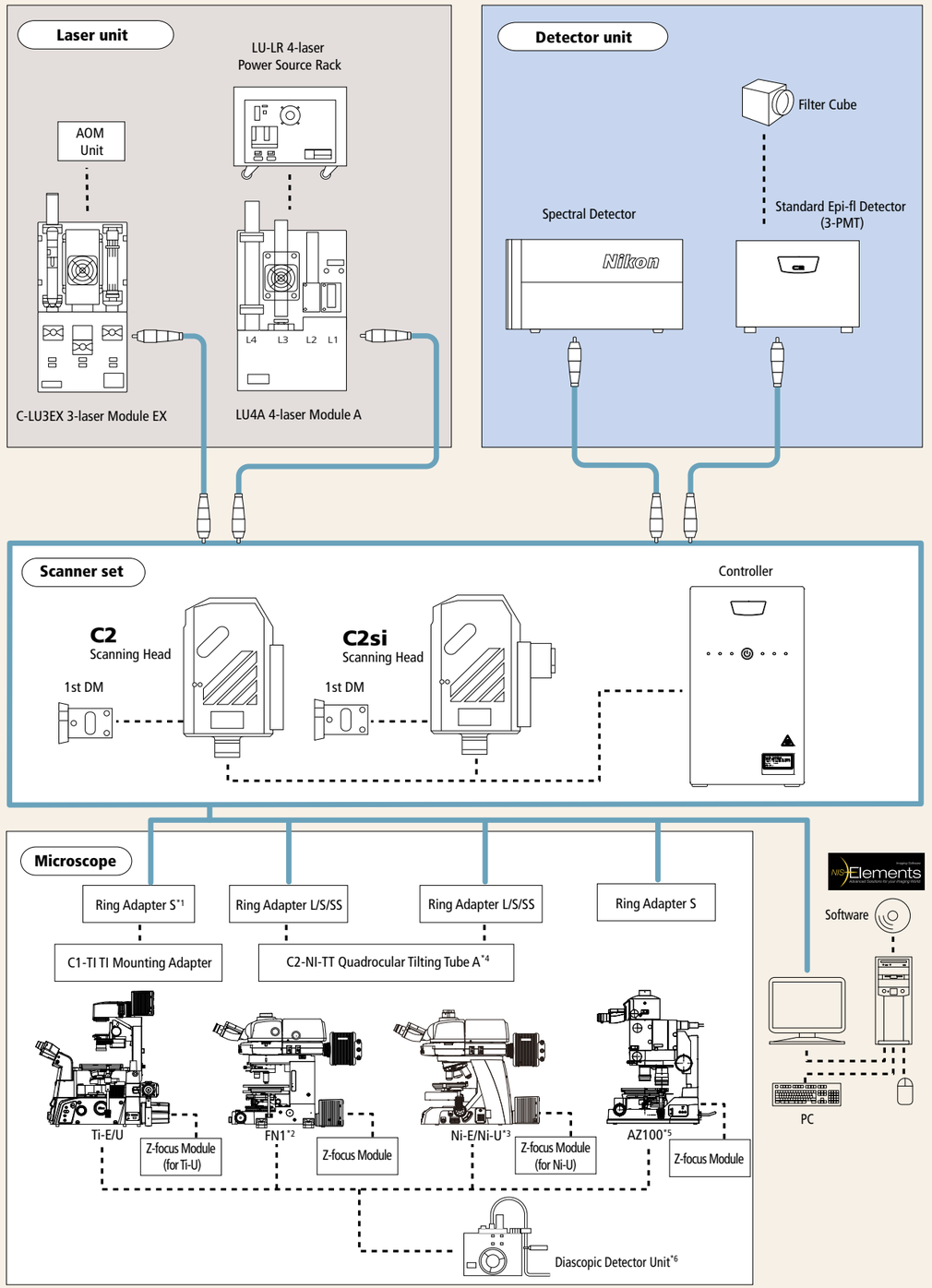


Photo courtesy of: Director and Professor Masatoshi Yamamoto, Drosophila Genetic Resource Center, Kyoto Institute of Technology

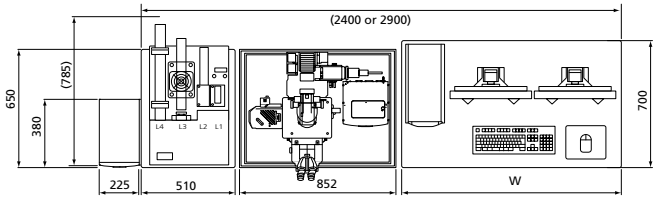
System diagram



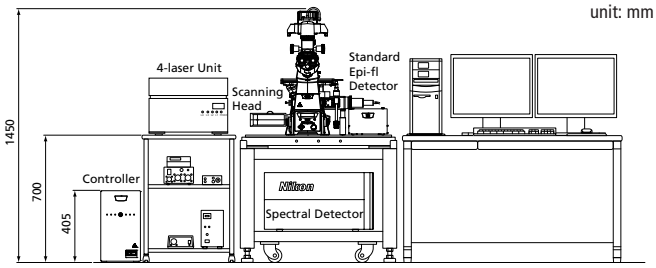
*1 Not required when using a Stage-up Kit.
*2 C-TT-C Trinocular Tube, C1 Y-TT Trinocular Tube or NI-TT Quadrocular Tilting tube can be used.
*3 With Ni-U, NI-TT Quadrocular Tilting tube or C-TT-C Trinocular Tube can be used.
With Ni-E, NI-TT-E Motorized Quadrocular Tilting tube, NI-TT Quadrocular Tilting tube or C-TT-C Trinocular Tube (for Ni-E focusing stage only) can be used.
*4 Required when using NI-TT Quadrocular Tilting tube or NI-TT-E Motorized Quadrocular Tilting tube.
*5 Use AZ-TE100LS Ergonomic Trinocular Tube 100 LS and AZ100 Stage Cover.
*6 Dedicated adapter is required depending on microscope model.

Recommended layout

unit: mm



W=1500mm (two 19-inch monitors)
W=1000mm (24-inch monitor)



Note) Computer table size is for reference only.

Specifications

	C2+
Compatible laser*1	405 nm/440 nm, 488 nm/Ar laser (457 nm, 477 nm, 488 nm, 514 nm), 543 nm/561 nm/594 nm, 633 nm/638 nm/640 nm
Laser unit	C-LU3EX 3-laser Module EX (AOM or manual modulation), LU4A 4-laser Module A (AOTF modulation)
Standard detector	Wavelength: 400-750 nm, Detector: 3PMT, Filter cube: 2 filter cubes
Diascopic detector (option)	Wavelength: 400-700 nm, Detector: 1 PMT
Scanning head (galvano)	With Standard detector: Pixel size: 2048 x 2048 Scanning speed: Standard mode: 2 fps (512 x 512 pixels, bi-direction), 17 fps (512 x 32 pixels, bi-direction), Zoom: 1-1000x Fast mode: 8 fps (512 x 512 pixels, bi-direction), 100 fps (512 x 32 pixels, bi-direction)*2, Zoom: 8-1000x
	With spectral detector: Pixel size: max. 1024 x 1024 pixels Scanning speed: 0.5 fps (512 x 512 pixels, single direction), max. 6 fps (64 x 64 pixels, single direction)
Scanning mode	X-Y, XY rotation, zoom, ROI, XYZ, time lapse, X-Z, stimulation, multipoint, image stitching (large image)
Pinhole	Circular shape, 6 size
Spectral detector (with galvano scanner) (option)	Number of channels: 32 channels, Wavelength: 400-750 nm, Wavelength resolution: 2.5 nm, 5 nm, 10 nm, wavelength range variable in 0.25 nm steps, Unmixing: High-speed unmixing, precision unmixing
FOV	Square inscribed in a ø18 mm circle
Image bit depth	12 bits
Compatible microscopes	ECLIPSE Ti-E/Ti-U inverted microscope, ECLIPSE Ni-E (focusing nosepiece type/stage focusing type)/Ni-U upright microscope, ECLIPSE FN1 fixed stage microscope, AZ100 multi-purpose zoom microscope
Z step	Ti-E: 0.025 µm, FN1 stepping motor: 0.05 µm, Ni-E: 0.025 µm
NIS-Elements C software	Display/image processing/analysis 2D/3D/4D analysis, time-lapse analysis, 3D volume rendering/orthogonal, spatial filters, image stitching, multipoint time-lapse, spectral unmixing, real-time unmixing, virtual filters, deconvolution, AVI image file output
	Application: FRAP, FLIP, FRET, photoactivation, colocalization, three-dimensional time-lapse imaging, multipoint time-lapse imaging
Control Computer	OS: Microsoft Windows 7 Professional 64 bit SP1 (Japanese/English), CPU: Intel Xeon X3565 (3.20 GHz/8 MB/1066 MHz/Quad Core) or higher, Memory: 4 GB, Hard disk: 146 GB SATA 3 Gb/s (7200 rpm), Data transfer: LAN, Network interface: Gigabit Ethernet, Monitor: 1600 x 1200 or higher resolution, dual monitor configuration is recommended

*1 Compatible lasers and usable wavelengths differ depending on the laser unit in use.

*2 The described frame rate is NOT available with Rotation, CROP, ROI, Spectral imaging and Stimulation.

Specifications and equipment are subject to change without any notice or obligation on the part of the manufacturer. December 2011

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

Monitor images are simulated.
Some sample images in this brochure were captured using the C1 confocal microscope system.

N.B. Export of the products* in this brochure is controlled under the Japanese Foreign Exchange and Foreign Trade Law.
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*Products: Hardware and its technical information (including software)

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Printed in Japan (1112-00)T
Code No. 2CE-SCHH-3
This brochure is printed on recycled paper made from 40% used material.

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