



Confocal Microscope C2+

# C2+

Confocal Microscope



Shedding New Light  
On **MICROSCOPY**

# Simple and Robust

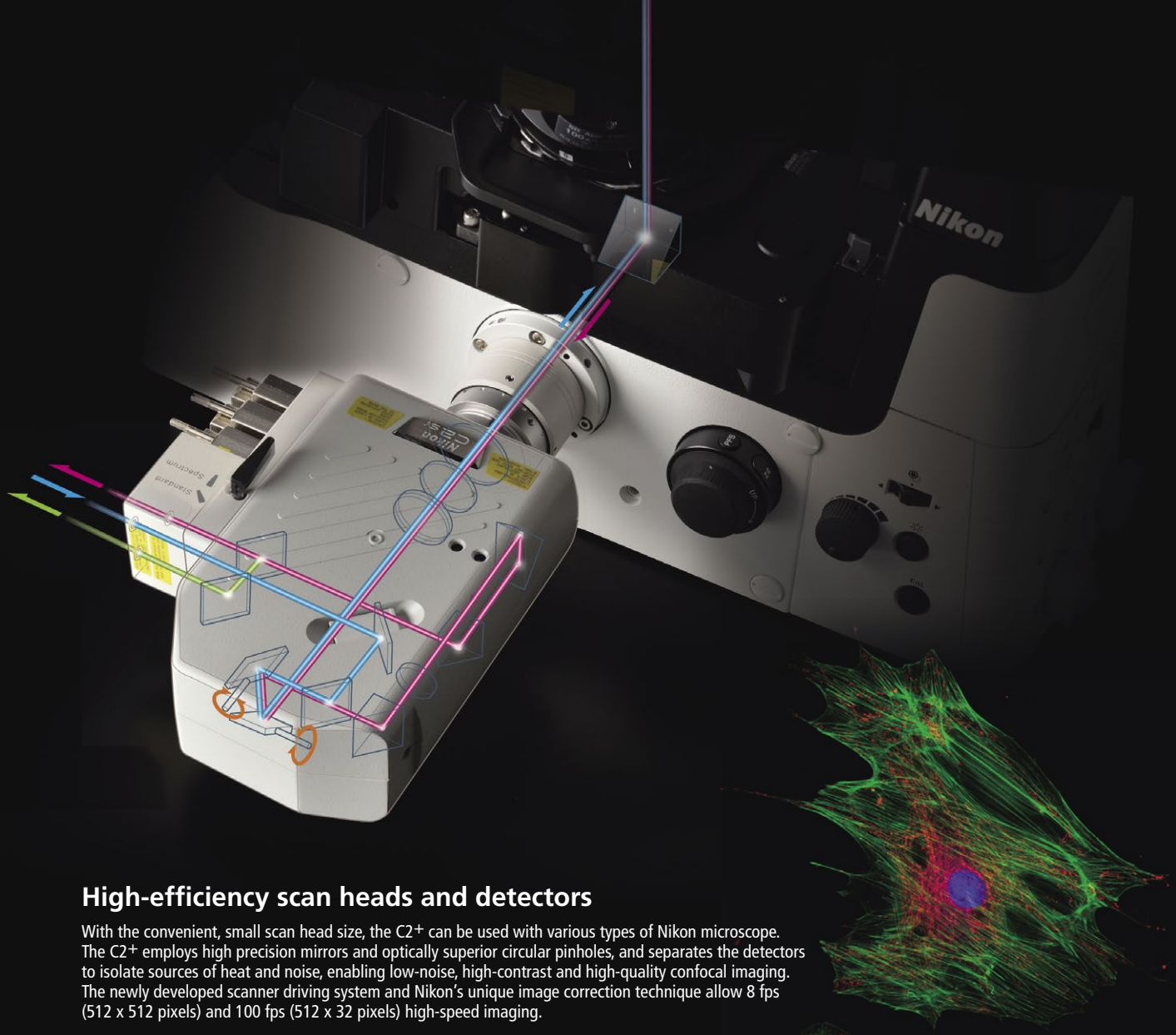
The C2+ confocal system is designed as an essential microscopy tool for the laboratory, providing powerful and robust imaging capabilities. The high-efficiency scan heads and detectors, coupled with Nikon's unrivaled optics, provide superior confocal images.

For research that requires spectral imaging capabilities, Nikon's C2si+ system provides dedicated spectral detector units in addition to the standard fluorescence detector unit. The C2-DUS spectral detector unit allows high-precision and high-speed 32-channel spectral imaging or high-sensitivity spectral imaging.

- The high-speed galvano scanners, operating at rates of up to 100 fps\*, enable even the fast-beating motion of cardiac muscles to be captured with precision.
- The system provides simultaneous acquisition of three fluorescent channels plus DIC in a single scan.

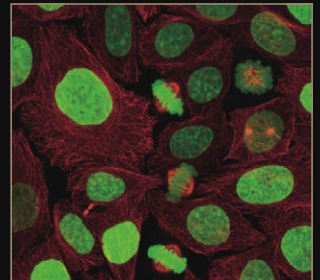
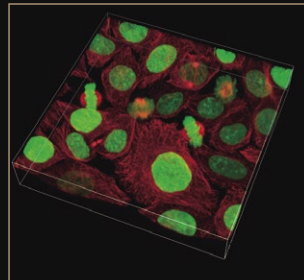
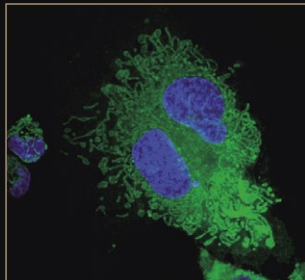
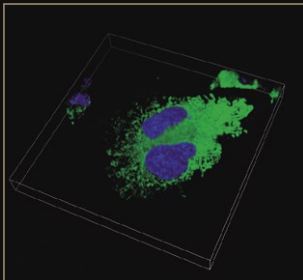
\* with 8x zoom or larger





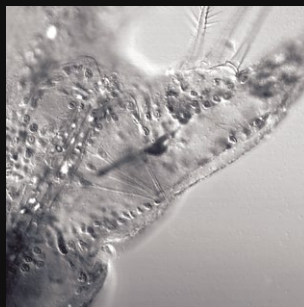
## High-efficiency scan heads and detectors

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-definition diascope DIC images

The C2+ can acquire simultaneous three-channel fluorescence or simultaneous three-channel and diascope 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-performance objectives for confocal imaging

## ■ Silicone immersion objectives

Silicone oil more closely matches the refractive index of live cells compared to water or oil, thereby minimizing spherical aberration issues common to live cell imaging. These lenses achieve exceptional resolving power even at great depths, making them well-suited for achieving bright, high-resolution images of thick specimens.



CFI Plan Apochromat  
Lambda S 25XC Sil



CFI Plan Apochromat  
Lambda S 40XC Sil



CFI SR HP Plan Apochromat  
Lambda S 100XC Sil

## ■ CFI Apochromat Lambda S Series

These high-numerical-aperture (NA) objectives provide chromatic aberration correction over a wavelength ranging from ultraviolet to infrared and are ideal for multicolor confocal imaging. In particular, the LWD Lambda S 40XC WI lens has an extremely wide chromatic aberration correction range from 405 nm to near-IR. The transmission property of these lenses is enhanced through the use of Nikon's exclusive Nano Crystal Coat technology.



CFI Apochromat LWD Lambda S 20XC WI  
CFI Apochromat LWD Lambda S 40XC WI

CFI Apochromat Lambda S 40XC WI  
CFI Apochromat Lambda S 60X Oil

## ■ 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. Correction collars enable optimization of point spread functions for varying imaging temperatures, ensuring highest quality confocal images whether you are imaging at room temperature or 37 degrees Celsius.



CFI Apochromat TIRF 60XC Oil/1.49  
CFI Apochromat TIRF 100XC Oil/1.49

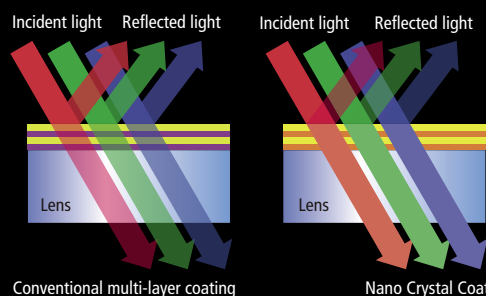
## ■ Recommended objective lenses

|  |                            |  |
|--|----------------------------|--|
| CFI Plan Apochromat Lambda 10X               | NA 0.45, W.D. 4.00 mm      |  |
| CFI Plan Apochromat VC 20X                   | NA 0.75, W.D. 1.00 mm      |  |
| CFI Apochromat LWD Lambda S 20XC WI          | NA 0.95, W.D. 0.95 mm      |  |
| CFI Plan Apochromat Lambda S 25XC Sil        | NA 1.05, W.D. 0.55 mm      |  |
| CFI Plan Apochromat Lambda 40XC              | NA 0.95, W.D. 0.21 mm      |  |
| CFI Apochromat Lambda S 40XC WI              | NA 1.25, W.D. 0.20-0.16    |  |
| CFI Apochromat LWD Lambda S 40XC WI          | NA 1.15, W.D. 0.60 mm      |  |
| CFI Plan Apochromat Lambda S 40XC Sil        | NA 1.25, W.D. 0.30 mm      |  |
| CFI Apochromat Lambda S 60X Oil              | NA 1.40, W.D. 0.14 mm      |  |
| CFI Plan Apochromat VC 60XC WI               | NA 1.20, W.D. 0.29 mm      |  |
| CFI Apochromat TIRF 60XC Oil                 | NA 1.49, W.D. 0.12 mm      |  |
| CFI Plan Apochromat IR 60XC WI               | NA 1.27, W.D. 0.18-0.16 mm |  |
| CFI Apochromat TIRF 100XC Oil                | NA 1.49, W.D. 0.12 mm      |  |
| CFI SR HP Plan Apochromat Lambda S 100XC Sil | NA 1.35, W.D. 0.30 mm      |  |

: Nano Crystal Coat-deposited

## Nano Crystal Coat technology

Nano Crystal Coat is an anti-reflective coating that assimilates ultra-fine crystallized particles of nanometer size. These crystallized particles eliminate reflections inside the lens throughout the spectrum of visible light waves in ways that far exceed the limits of conventional anti-reflective coating systems.



Conventional multi-layer coating

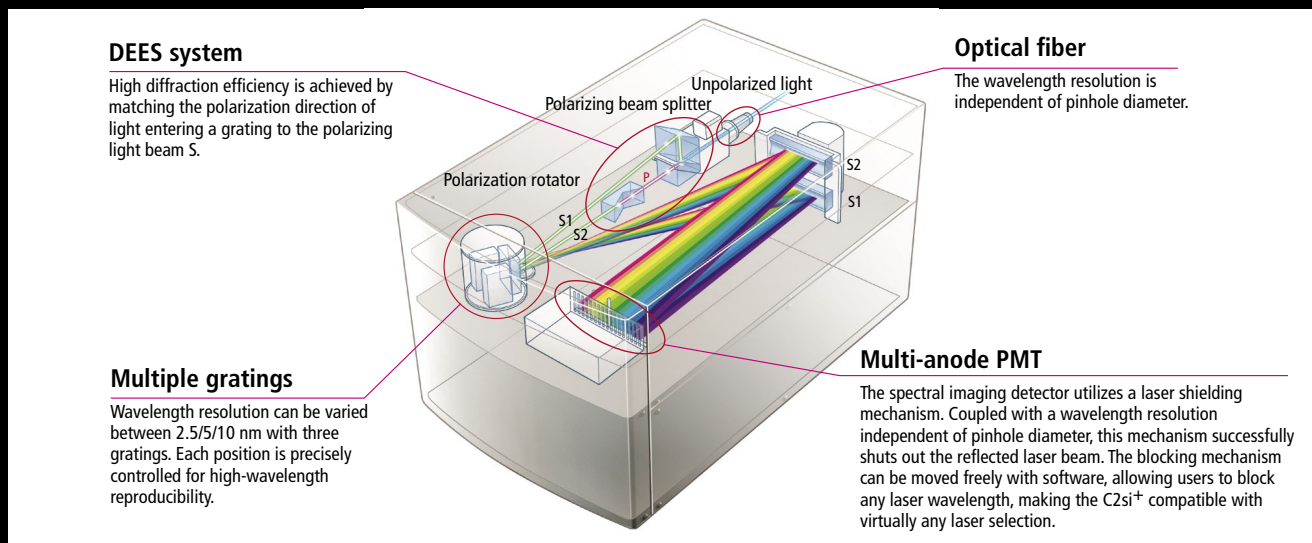
Nano Crystal Coat

# Enhanced Spectral Imaging

The C2si<sup>+</sup> provides 2 options for spectral imaging: a fast and accurate 32-channel spectral detector unit and the newly-developed high-sensitivity GaAsP detector unit. The C2si<sup>+</sup> allows not only clean separation of overlapping spectra of fluorescent labels in multi-stained specimens, but also unique user-definable "Virtual Filter" emission filter mode.

## IC2-DUS Spectral Detector Unit

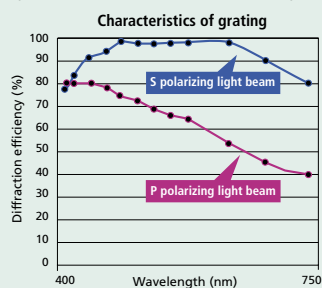
The C2-DUS allows for acquisition of 32 channels of fluorescence spectra over a 320 nm wavelength range with a single scan. By precisely unmixing the overlapping spectra at a high wavelength resolution of at least 2.5 nm, the C2-DUS facilitates the acquisition of detailed data.



## 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.

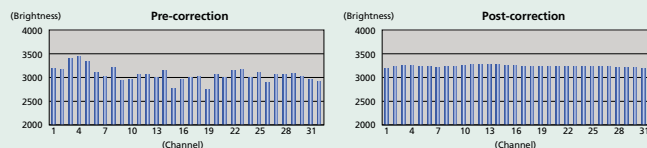


### High-efficiency fluorescence transmission technology

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

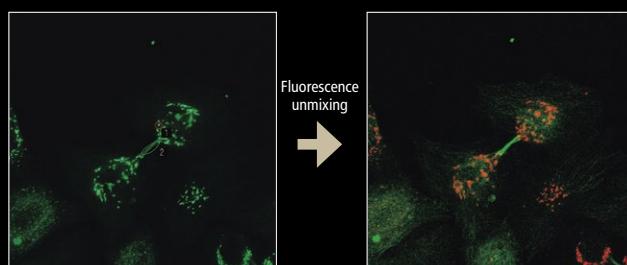
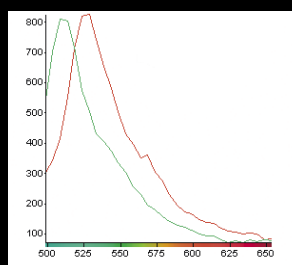
Three correction techniques allow for the acquisition of accurate spectra: interchannel sensitivity correction, which adjusts offset and sensitivity of each channel; spectral sensitivity correction, which adjusts diffraction grating spectral efficiency and detector spectral sensitivity; and correction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



## 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<sup>+</sup> contains a built-in

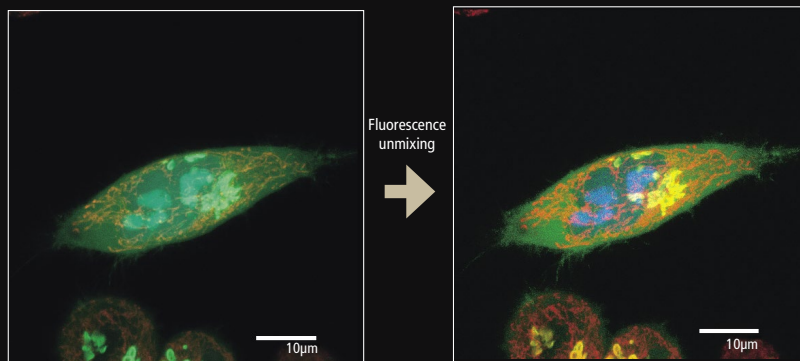
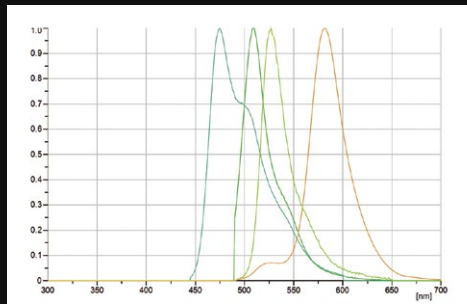
database of given spectral data provided by manufacturers of fluorescence dyes that can be specified as reference spectra for fluorescence unmixing. Users may also add spectral information for new labels to the database.



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: Drs. Sheng-Chung Lee and Han-Yi E. Chou, National Taiwan University College of Medicine, Institute of Molecular Medicine

## Unmixing of multiple fluorescence

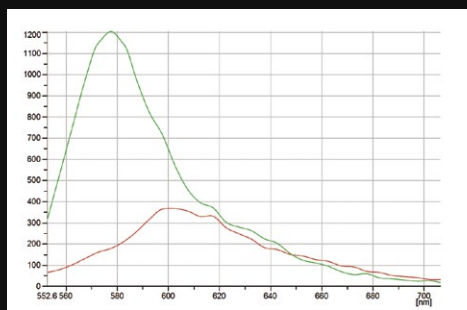
Because wavelength resolution and range are freely selectable, scanning of a fluorescence protein with a wide wavelength range from blue to red such as CFP/GFP/YFP/DsRed is possible at one time. Reference data allows unmixing and display of each color.



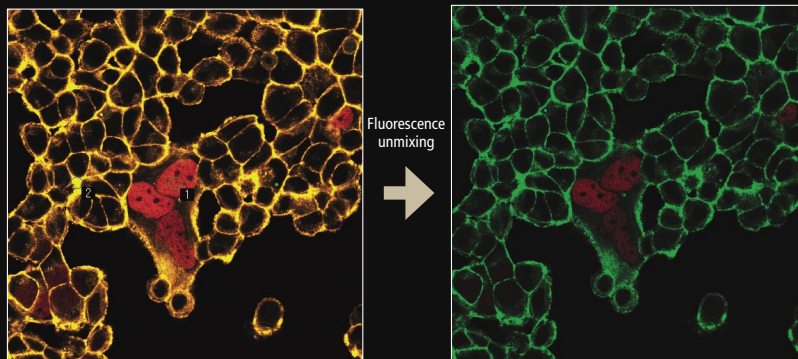
Specimen: HeLa cell in which nucleus is labeled with CFP, actin-related protein (Fascin) labeled with GFP, Golgi body labeled with YFP, and mitochondria labeled with DsRed. Spectral image captured with 408 nm and 488 nm laser exposure (left). The fluorescence spectra of the captured image are unmixed using reference spectra (right). Specimen courtesy of: Dr. Kaoru Katoh, 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.



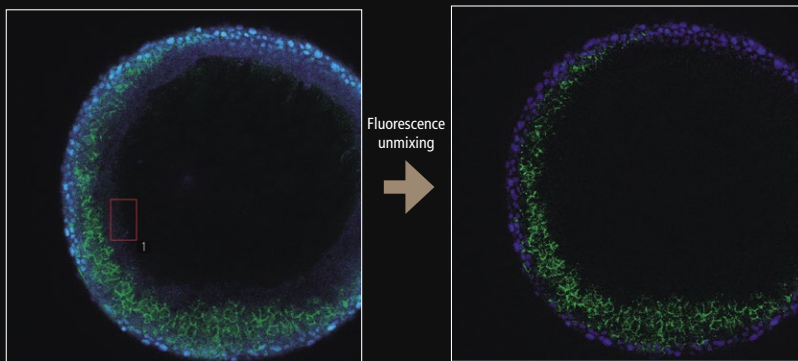
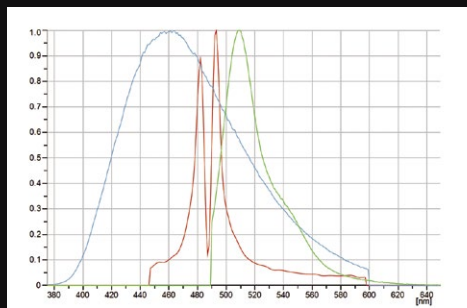
Spectra for ROI 1 and 2 corresponding to the image on the right. Rhodamine's fluorescence spectral peak is at approximately 579 nm, while that for RFP is approximately 600 nm. RFP's fluorescence is weaker than Rhodamine's, but their spectra are cleanly unmixed.



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.

## 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.

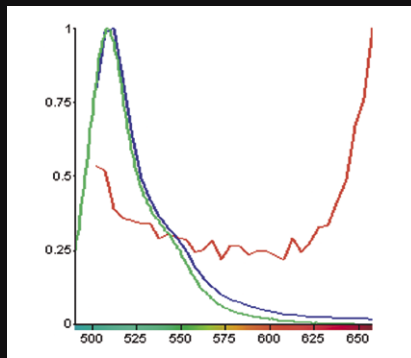


Specimen: Zebrafish egg stained with cadherin-GFP and DAPI. Spectral image captured with 408 nm and 488 nm laser exposures (left). After unmixing using reference spectra for auto-fluorescence (ROI1), GFP and DAPI, the auto-fluorescence in the image is eliminated (right).

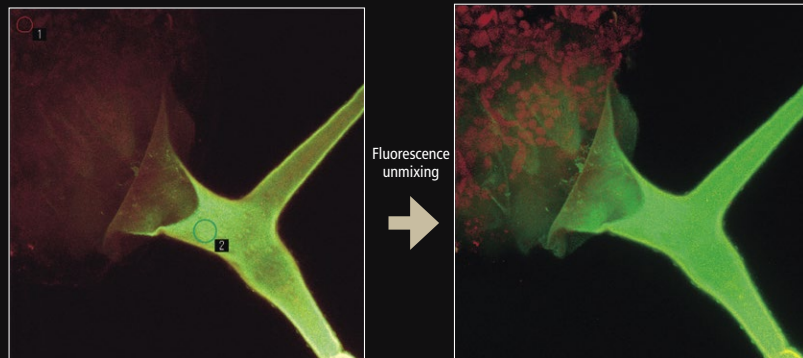
Specimen courtesy of: Dr. Tohru Murakami, Neuromuscular and Developmental Anatomy, Gunma University Graduate School of Medicine

## 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<sup>++</sup> 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).

## 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.

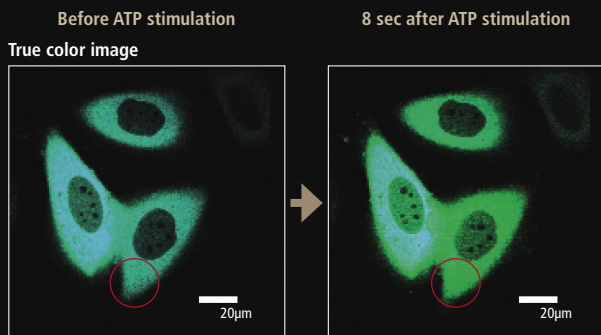
### Acquisition of spectral image (XYT $\lambda$ )

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

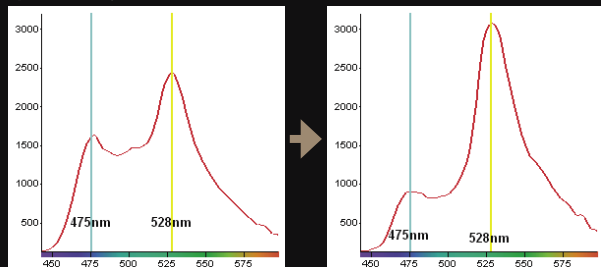
### Fluorescence unmixing

Spectral FRET analysis is possible by unmixing using reference data of CFP and YFP.

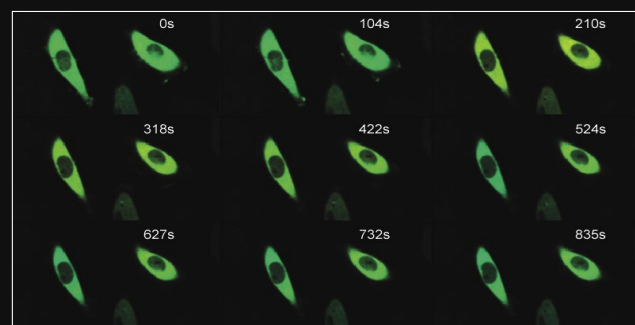
Two-dimensional change (FRET) of intracellular  $\text{Ca}^{2+}$  concentration is easily determined from spectral data without acceptor bleaching.



### Spectral analysis



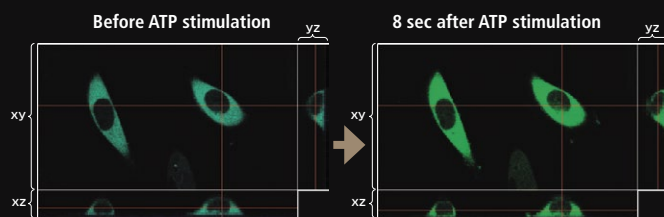
True color image and spectral analysis of CFP and YFP. Spectral curve in ROI. Left peak indicates CFP and right peak indicates YFP respectively. After ATP stimulation, peak of CFP drops and peak of YFP rises due to FRET.



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

### Five-dimensional analysis (XYTZ $\lambda$ )

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





# IC2-DUVB GaAsP Detector Unit

The C2-DUVB is the first high-sensitivity GaAsP detector unit developed for the C2<sup>+</sup> series. It is a compact fully tunable emission detector unit capable of spectral imaging with user-defined emission bandwidths in galvano imaging modalities.



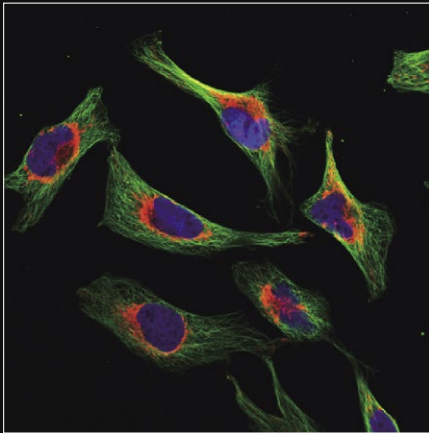
## High-sensitivity spectral image acquisition

With a GaAsP PMT, the C2-DUVB tunable emission detector delivers flexible detection of fluorescent signals with higher sensitivity.

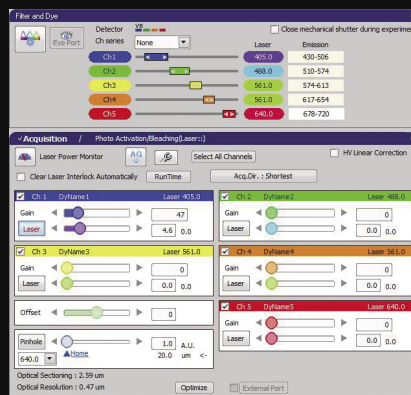
## Variable acquisition wavelength range

User-defined emission bands can collect images within a selected wavelength range, replacing the need for fixed bandwidth emission filters. Users can define the emission bandwidth range to as little as 10nm. Spectral images of multi-labeled specimens can be acquired by capturing a series of spectral images while changing detection wavelengths.

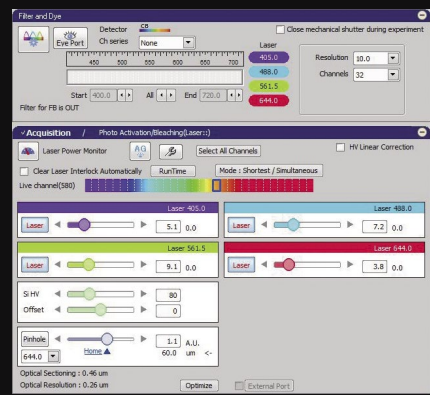
Based on the application, virtual bandpass mode and continuous bandpass modalities are selectable on the C2-DUVB.



VB (Variable Bandpass) mode  
Captured in 3 ch



VB (Variable Bandpass) mode allows maximum 5ch color image



CB (Continuous Bandpass) allows maximum 32 ch spectrum imaging

## Optional second channel detector

An optional second GaAsP PMT provides flexibility in detection. Users can divert selected wavelengths to the 2nd fixed bandwidth emission channel by inserting a dichroic mirror, while simultaneously utilizing the user-definable emission band on the first channel.

The second detector allows FRET, ratio imaging and other applications requiring simultaneous multi-channel imaging.

## Accurate spectral unmixing

Multi-channel images acquired with the C2-DUVB can be spectrally unmixed by using the spectra of reference samples, or the spectra within the acquired images.



# Dedicated software modules



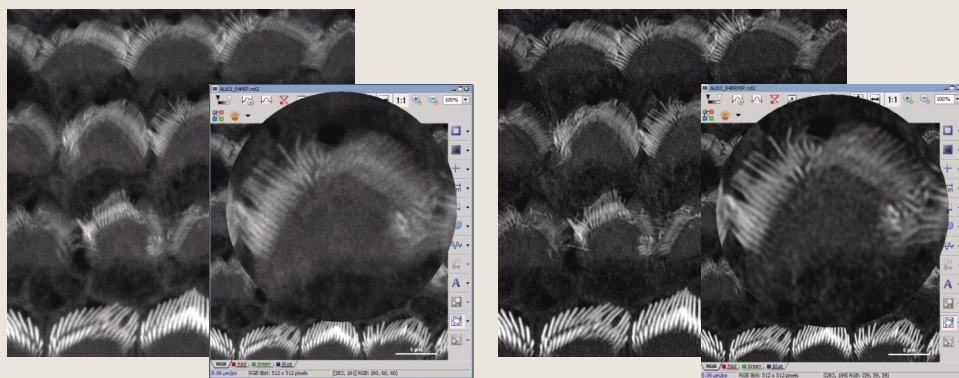
## NIS-Elements ER

### Automatic creation of high-resolution images

Higher resolution confocal images can be generated with a single click operation. The software assesses the captured image and automatically determines processing parameters to achieve enhanced resolution without sacrificing high imaging operability. In addition, the resolution of previously captured confocal images can be improved using the pre-processing technology.

### Improved resolution of confocal images

The NIS-Elements ER module increases conventional confocal resolution by up to two-fold, providing up to 120nm resolution in XY and 300nm in Z.



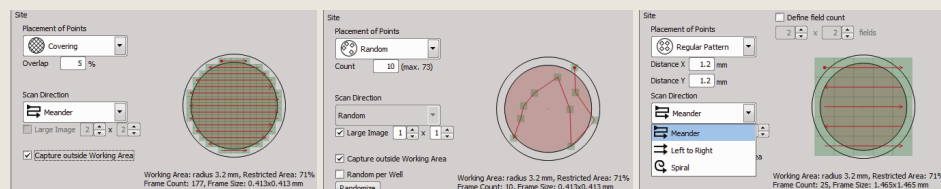
Apical surfaces of auditory epithelia of mouse cochlea were stained by Atto-565-phalloidin at postnatal day 2. Photographed with the cooperation of: Dr. Hideru Togashi, Division of Molecular and Cellular Biology, Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine



## High-Content Acquisition and Analysis

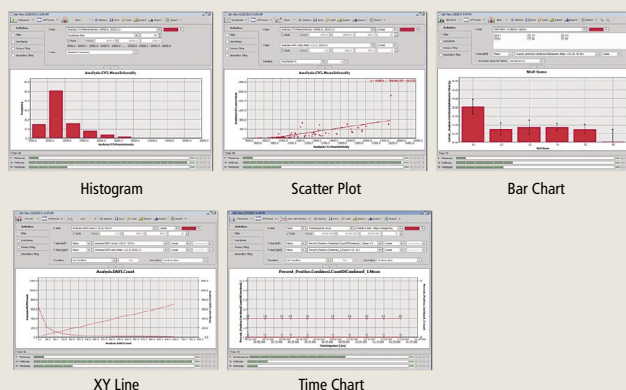
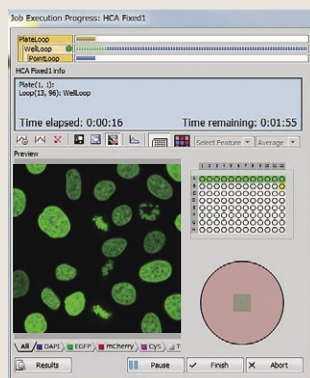
### Image acquisition capability of large number of samples

With fully-automated acquisition and analysis of a large number of high-content, multidimensional images following an easy stepwise workflow, HCA offers quick experimental setups and an immediate view of measurement data, well by well, during acquisition and via a heat map for trend observation and further analysis.

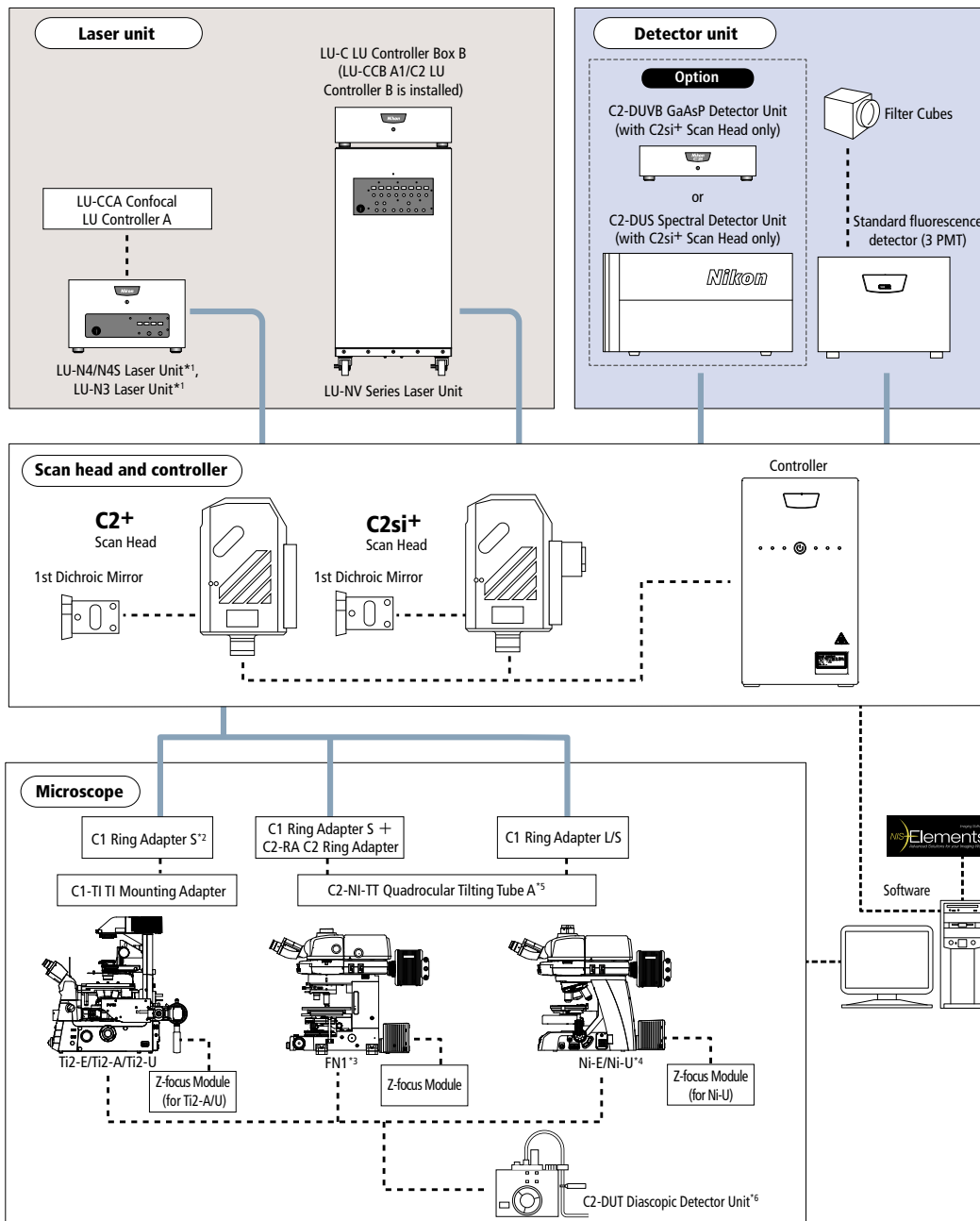


### Automated analysis during image acquisition

The current well-plate imaging point is displayed in real time along with a live image. Image analysis is conducted during image acquisition. Various formats are available for displaying results, including graphical display of analytical values such as histograms and scatter plots.



# System diagram



\*1 LU-N4S is designed for use with the C2si+ spectral imaging confocal microscope. LU-N4/N3 is not compatible with C2si+.

\*2 Not required when using a Stage-up Kit.

\*3 C-TT-C Trinocular Tube or NI-TT Quadrocular Tilting tube can be used.

\*4 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.

\*5 Required when using NI-TT Quadrocular Tilting tube or NI-TT-E Motorized Quadrocular Tilting tube.

\*6 Dedicated adapter is required depending on microscope model.

## Laser Units

### LU-N4/N4S 4-laser unit, LU-N3 3-laser unit

The LU-N4/LU-N4S/LU-N3 model provides one output fiber. With its compact design, this laser unit provides a space-saving solution for confocal microscope systems.

- The LU-N4S is compatible with spectral imaging.
- The LU-N4/LU-N4S is equipped with four lasers (405 nm, 488 nm, 561 nm, and 640 nm), while LU-N3 offers three lasers (405 nm, 488 nm, and 561 nm).



### LU-NV Series

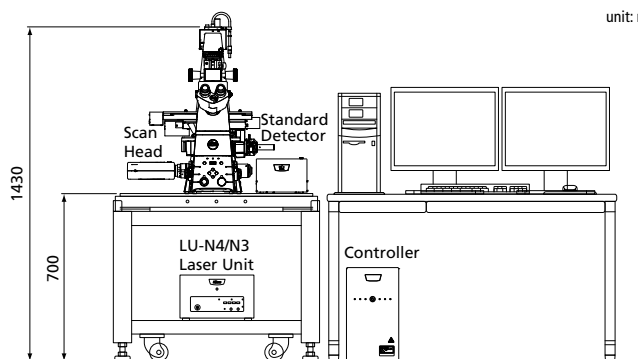
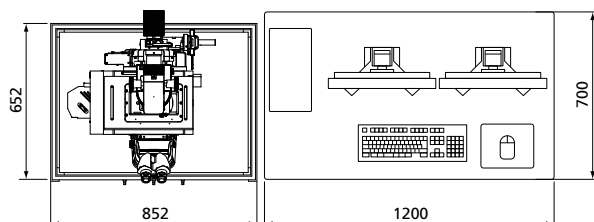
The LU-NV laser combiner can be configured with up to eight different lasers and seven output fibers, providing maximal flexibility. With its multiple output fibers and fiber switching system, the LU-NV can support multiple laser applications, such as C2+/C2si+ confocal, TIRF and photoactivation on a single microscope platform.

- Available lasers for the LU-NV: 405 nm, 445 nm, 458 nm, 488 nm, 514 nm, 532 nm, 561 nm, 594 nm, 640 nm and 647 nm.



# Recommended layout

unit: mm



Note) Computer table size is for reference only.

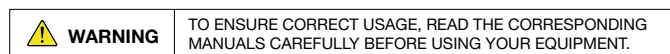
## Specifications

| C2+                          |   |
|------------------------------|---|
| Laser unit                   | LU-N3: installed laser 405 nm, 488 nm, and 561 nm, Cannot be used with C2-DUS spectral detector<br>LU-N4/N4S: installed laser 405 nm, 488 nm, 561 nm, and 640 nm, Use LU-N4S when using C2-DUS spectral detector<br>LU-NV series: mountable laser 405 nm, 445 nm, 458 nm, 488 nm, 514 nm, 532 nm, 561 nm, 594 nm, 640 nm and 647 nm   |
| Standard detector            | Wavelength: 400-750 nm, Detector: 3PMTs, Filter cube: 2 filter cubes  |
| Diascopic detector (option)  | Wavelength: 400-700 nm, Detector: 1 PMT   |
| Scan head                    | C2+/C2si+ scan head: when configuring with C2-DU3 standard fluorescence detector<br>Scanner: galvano scanner, Pixel size: 2048 x 2048<br>Scanning speed: Standard mode; 2 fps (512 x 512 pixels, bi-direction), 18 fps (512 x 32 pixels, bi-direction), Zoom: 1-1000x<br>Fast mode; 8 fps (512 x 512 pixels, bi-direction), 100 fps (512 x 32 pixels, bi-direction)*1, Zoom: 8-1000x  |
|                              | C2si+ scan head: when configuring with C2-DUS spectral detector<br>Scanner: galvano scanner, Pixel size: max. 1024 x 1024 pixels<br>Scanning speed: 0.5 fps (512 x 512 pixels, single direction), 7 fps (512 x 32 pixels, single direction)   |
| Scan mode                    | X-T, Y-T, X-Y, X-Y-T, X-Y-Z, X-Y-Z-T, Mirror Image (X, Y), Reciprocal image, Rotation image, ROI image, Line sequential acquisition   |
| Pinhole                      | Circular shape, 6 size  |
| Spectral detector (option)*2 | C2-DUS spectral detector unit<br>Number of channels: 32, Wavelength detection range: 400 - 750 nm, Spectral image acquisition speed: 2 fps (256 x 256 pixels)<br>Maximum pixel size: 1024 x 1024 (Spectral mode/Virtual filter mode), Wavelength resolution: 2.5/5.0/10.0 nm, Wavelength range variable in 0.25 nm steps  |
|                              | C2-DUVB GaAsP detector unit<br>Number of channels: 1 GaAsP PMT with variable emission plus 1 optional GaAsP PMT (C2-DUVB-OP) with a user-defined dichroic mirror and barrier filter, Wavelength detection range: 400 - 720 nm, narrowest: 10 nm, broadest: 320 nm, Maximum pixel size: 2048 x 2048 (CB mode/VB mode), Wavelength resolution: 10 nm, Wavelength range variable in 1 nm steps   |
| FOV                          | Square inscribed in a ø18 mm circle   |
| Image bit depth              | 12 bits   |
| Compatible microscopes       | Inverted microscope ECLIPSE Ti2-E/Ti2-A/Ti2-U, Upright microscope ECLIPSE Ni-E (focusing nosepiece type/focusing stage type)/Ni-U, Fixed stage microscope ECLIPSE FN1   |
| Z step                       | Ti2-E: 0.02 µm, FN1 stepping motor: 0.05 µm, Ni-E: 0.025 µm   |
| Software                     | Software: NIS-Elements C<br>Display/image processing/analysis<br>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: Windows 10 Pro 64 bit OS version 1809, CPU: Intel Xeon W-2123 (CPU3.6 Core4) or higher, RAM: 32GB or greater, HDD: Serial ATA 6 Gb/s (7,200 rpm) 146 GB or greater, Graphics: NVIDIA Quadro P600 or greater, Drive: Super Multi drive (Slimline), Expansion slots: PCI Express (x16) (for graphics) slot x2 etc., Interface: USB2.0 RS-232C, LAN port: 10/100/1000 network interface x1, Monitor: LCD2 monitor with 1600 x 1200 or higher resolution, dual monitor configuration is recommend |
| Dimensions and weight        | C2+/C2si+ scan head 145 (W) x 236.5 (H) x 70 (D) mm, Approx. 2 kg   |
|                              | Controller 225 (W) x 404 (H) x 382 (D) mm, Approx. 12 kg  |
|                              | Standard detector 225 (W) x 154 (H) x 224 (D) mm, Approx. 3.5 kg  |
|                              | Spectral detector C2-DUS: 289 (W) x 323 (H) x 593.5 (D) mm, Approx. 23 kg, C2-DUVB: 360 (W) x 91 (H) x 595.5 (D) mm, Approx. 9.5 kg   |

\*1 The described frame rate is NOT available with Rotation, CROP, ROI, Spectral imaging and Stimulation.

\*2 Only available with C2si+ scan head.

Specifications and equipment are subject to change without any notice or obligation on the part of the manufacturer. April 2021 ©2010-21 NIKON CORPORATION



Monitor images are simulated.

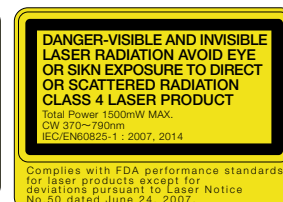
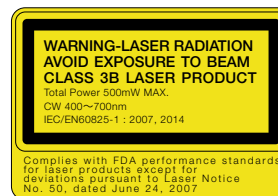
Some sample images in this brochure were captured using the C1 confocal microscope system.

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N.B. Export of the products\* in this brochure is controlled under the Japanese Foreign Exchange and Foreign Trade Law.

Appropriate export procedure shall be required in case of export from Japan.

\*Products: Hardware and its technical information (including software)



#### NIKON CORPORATION

Shinagawa Intercity Tower C, 2-15-3, Konan, Minato-ku, Tokyo 108-6290, Japan  
phone: +81-3-6433-3705 fax: +81-3-6433-3785

<https://www.healthcare.nikon.com/>

ISO 14001 Certified  
for NIKON CORPORATION

#### Nikon Instruments Inc.

1300 Walt Whitman Road, Melville, N.Y. 11747-3064, U.S.A.  
phone: +1-631-547-8500; +1-800-52-NIKON (within the U.S.A. only)  
fax: +1-631-547-0299

<https://www.microscope.healthcare.nikon.com/>

#### Nikon Europe B.V. (headoffice)

Tripolis 100, Burgerweeshuispad 101, 1076 ER Amsterdam, Netherlands  
phone: +31-20-7099-000 fax: +31-20-7099-298  
[https://www.microscope.healthcare.nikon.com/en\\_EU/](https://www.microscope.healthcare.nikon.com/en_EU/)

#### Nikon Instruments (Shanghai) Co., Ltd.

CHINA phone: +86-21-6841-2050 fax: +86-21-6841-2060  
(Beijing branch) phone: +86-10-5831-2028 fax: +86-10-5831-2026  
(Guangzhou branch) phone: +86-2-3882-0551 fax: +86-2-3882-0580  
[https://www.microscope.healthcare.nikon.com/zh\\_CN/](https://www.microscope.healthcare.nikon.com/zh_CN/)

#### Nikon Canada Inc.

CANADA phone: +1-905-625-9910 fax: +1-905-602-9953

#### Nikon France, Succursale de Nikon Europe B.V.

FRANCE phone: +33-1-4516-4516 fax: +33-1-4516-4505

#### Nikon Deutschland, Zweigniederlassung der Nikon Europe B.V.

GERMANY phone: +49-211-9414-888 fax: +49-211-9414-322

#### Nikon Italy, Branch of Nikon Europe B.V.

ITALY phone: +39-055-300-9601 fax: +39-055-300-993

#### Nikon Europe B.V., Amsterdam, Zweigniederlassung Schweiz (Egg/ZH)

SWITZERLAND phone: +41-43-277-2867 fax: +41-43-277-2861

#### Nikon UK, Branch of Nikon Europe B.V.

UNITED KINGDOM phone: +44-208-247-1717 fax: +44-208-541-4584

#### Nikon Österreich, Zweigniederlassung der Nikon Europe B.V.

AUSTRIA phone: +43-1-972-6111 fax: +43-1-972-6111-40

#### Nikon Singapore Pte Ltd

SINGAPORE phone: +65-6559-3651 fax: +65-6559-3668

#### Nikon Instruments Korea Co., Ltd.

KOREA phone: +82-2-6288-1900 fax: +82-2-555-4415