

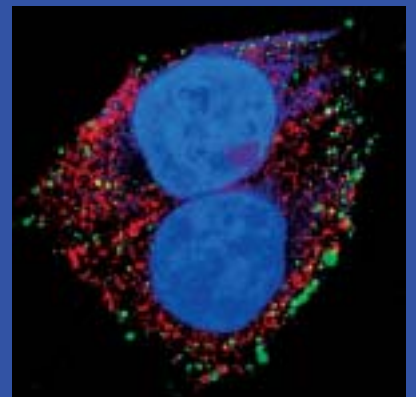
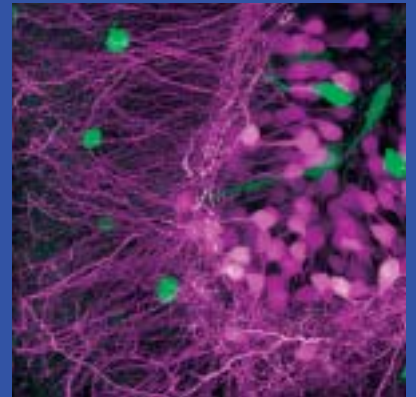
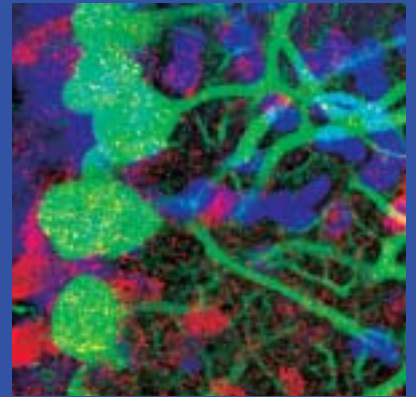


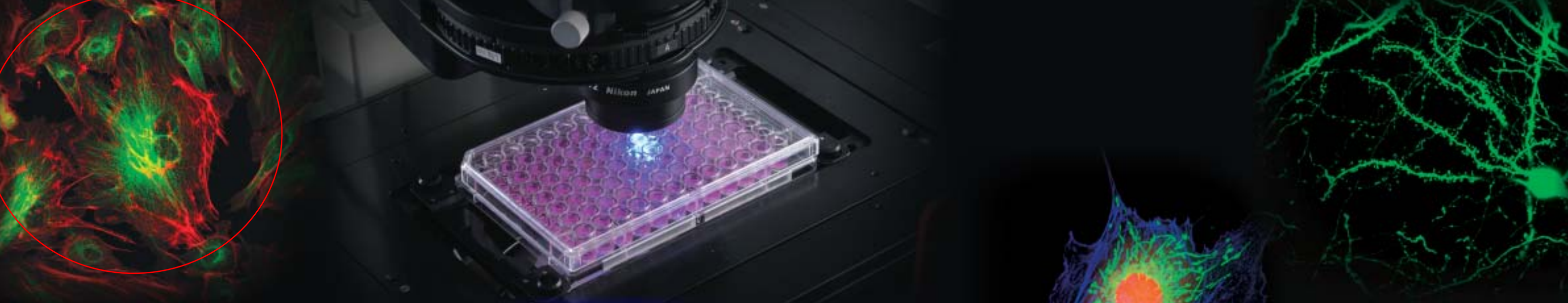
Modular Confocal Laser Microscope System

C1 series

DIGITALECLIPSE

C1si / C1plus





State-of-the-Art Resolution, Contrast, and Image Brightness

The Eyes of Science

Nikon has fused its first-class optics and electronics technology to develop the Eclipse C1 series modular confocal microscope systems that provide the highest-quality digital imaging with an ultra-compact and lightweight design. With basic C1plus and spectral imaging C1si, Nikon now has a confocal solution to suit a broad range of advanced research needs.

High-resolution image acquisition

Incorporating Nikon's renowned optical technologies—including VC objectives—in a confocal mechanism to eliminate flare from fluorescence images, the sophisticated C1 series allows high-resolution image capture. The system also supports multi-dimensional acquisition including XYZ imaging, time-lapse imaging, spectral imaging, and multi-point imaging.

Spectral image acquisition (C1si)

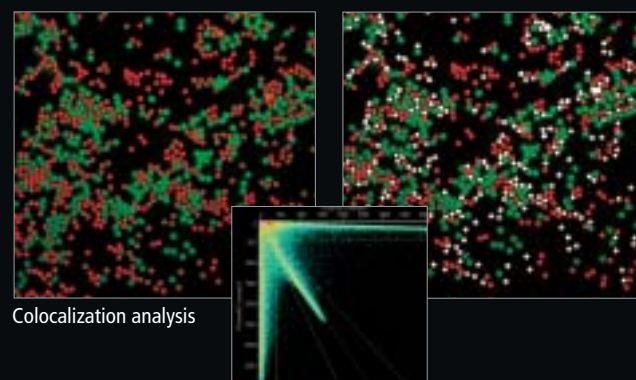
The C1si true spectral imaging confocal laser scanning microscope system is the powerful solution to autofluorescence, which is a problem when observing live specimens as it is difficult to distinguish autofluorescence from the target fluorescence. The spectral detector can acquire a broad wavelength range up to 320nm with a single scan to provide high-resolution images. The dedicated software allows unmixing of target fluorescence signals and autofluorescence. Also, the short scan time reduces damage to cells and improves time resolution.

Molecule dynamics with optical stimulation

The C1 series can handle a variety of the latest optical stimulation applications, including observation of photoconversion fluorescence proteins that change fluorescence characteristics when exposed to laser light, and observation of FRAP (fluorescence recovery after photobleaching) in the ROI (region of interest).

Powerful image analysis function

Nikon appreciates that the analysis of captured images is an important task. The latest analysis functions that the C1 series offers include analysis over time of intensity and spectra changes in ROI (region of interest), and colocalization analysis for studying intensity data between channels.



Modular confocal laser microscope system

DIGITAL ECLIPSE C1plus

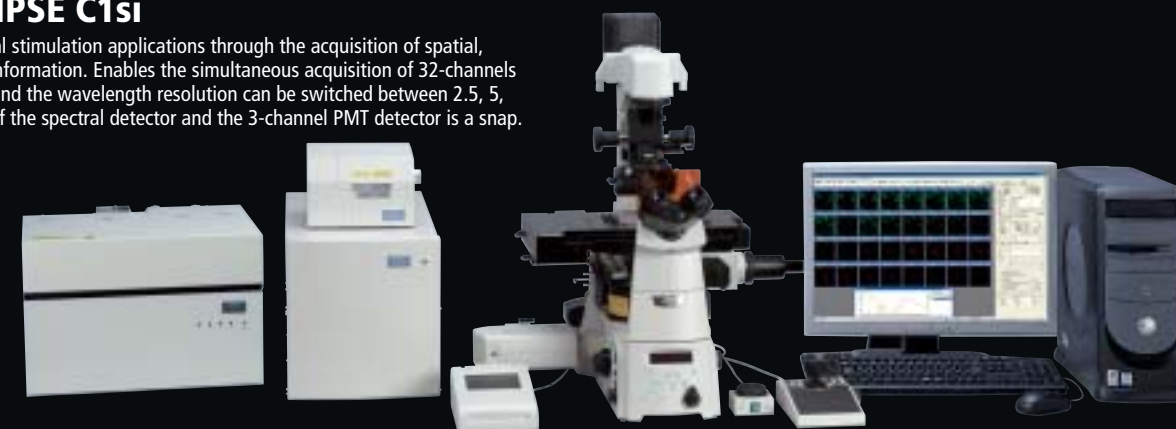
Employs high resolving power to capture images for spatial information—3D data, for example—and high-speed scanning for time-lapse information. Simultaneous 3-channel fluorescence, 3-channel plus DIC, and other advanced imaging is possible.



True spectral imaging confocal laser scanning microscope system

DIGITAL ECLIPSE C1si

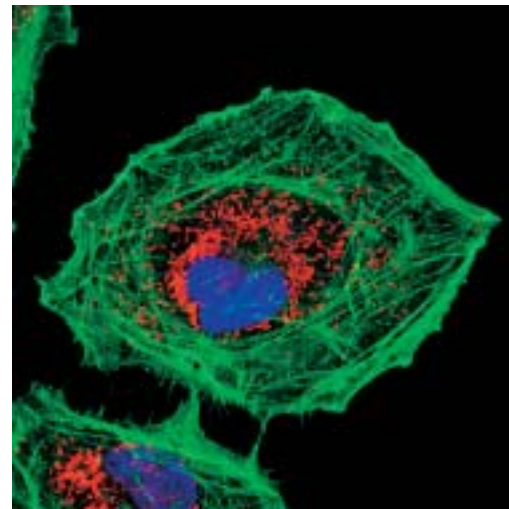
Supports the latest optical stimulation applications through the acquisition of spatial, time-lapse and spectral information. Enables the simultaneous acquisition of 32-channels of fluorescence spectra, and the wavelength resolution can be switched between 2.5, 5, and 10nm. Changeover of the spectral detector and the 3-channel PMT detector is a snap.



The highest quality optical performance

Unprecedented brightness and contrast

The Eclipse C1 series—the culmination of Nikon's many years of dedication as an optical equipment manufacturer—delivers optical performance of the highest level in this class of confocal systems. With the Eclipse C1 series, fluorescence images are rendered with unprecedented brightness, while DIC images are pin-sharp and of the highest possible contrast.



Actin/Mitochondria/DAPI



DIC image



Overlay of DIC and fluorescence images

Precision objectives for aberration-free confocal microscopy

CFI Plan Apochromat VC series

These objectives correct axial chromatic aberration on the whole visible light spectrum including 405nm (h line), making this series perfect for multi-stained confocal observations



CFI Plan Apo VC 100x oil/1.40 (left)
CFI Plan Apo VC 60x oil/1.40 (middle)
CFI Plan Apo VC 60x W/1.20 (right)

CFI Apochromat TIRF series

Among Nikon objectives, this series has the highest resolution. It boasts an unprecedented NA of 1.49 even when a standard coverslip and immersion oil are used.

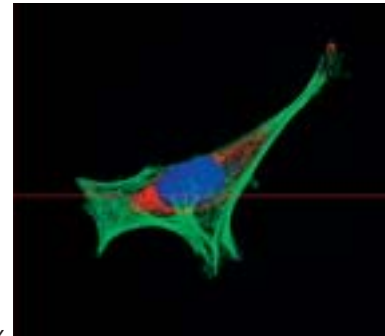


CFI Apo TIRF 60x oil/1.49 (left)
CFI Apo TIRF 100x oil/1.49 (right)

•Corrected shift in Z-axis direction with VC objective lens

With the conventional objective, DAPI fluorescence (blue) may shift in the Z-axis direction due to axial chromatic aberration. However, because the VC objective lens' axial chromatic aberration has been corrected up to the violet range, the DAPI fluorescence (blue) shift in the Z-axis direction is also corrected. The VC objective lens sample image on the right clearly shows that the DAPI-stained nucleus is positioned within the cell.

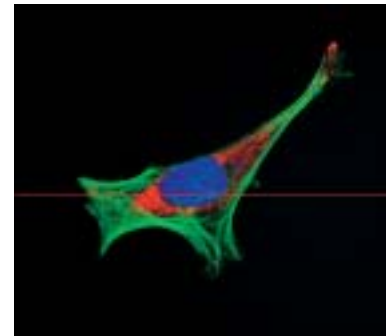
Conventional objective lens



XY

XZ

VC objective lens



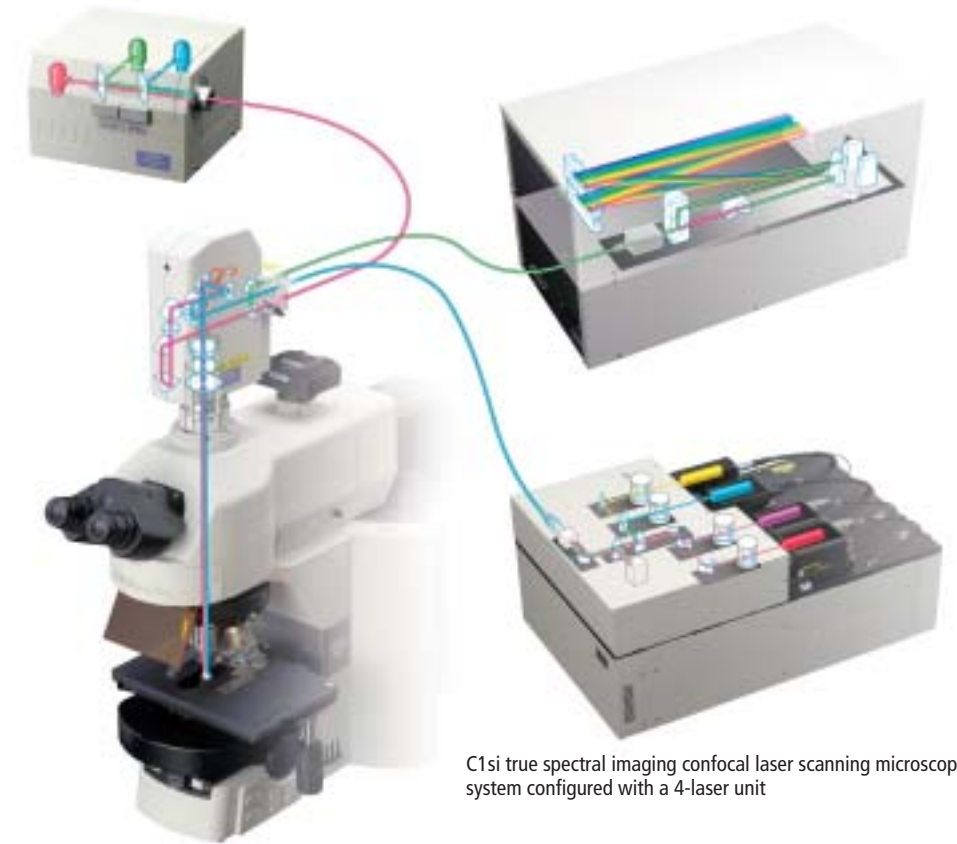
XY

XZ

Fluorescence image of actin (green: Alexa 488, excitation: 488nm), mitochondria (red: Mito Tracker Organe, excitation: 543nm) and nucleus (blue: DAPI, excitation: 408nm) of HeLa cell. Consecutive cross-sectional XY and XZ images acquired with a confocal laser microscope and CFI Plan Apo VC 100x oil objective lens.

Compact modular design

- Modular Components: Expansion and maintenance are easy.
- Pre-calibrated Modules: No need for calibration during set-up.
- Compact Design: Does not fill up bench space. The C1plus features the world's smallest and lightest scanning head.

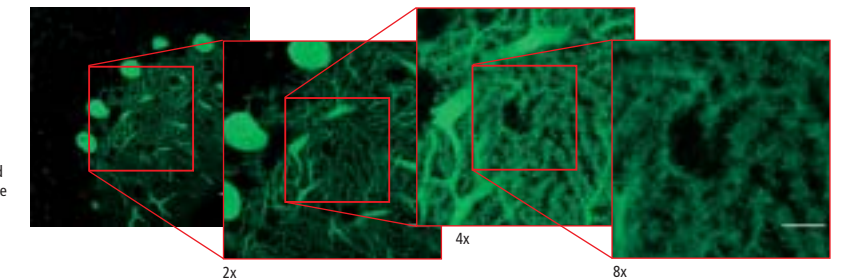


C1si true spectral imaging confocal laser scanning microscope system configured with a 4-laser unit

Advanced scanning features

• Up to 1000x optical zoom

The desired area can be optically zoomed. The GUI can be used to specify the area to be zoomed and rotate it.

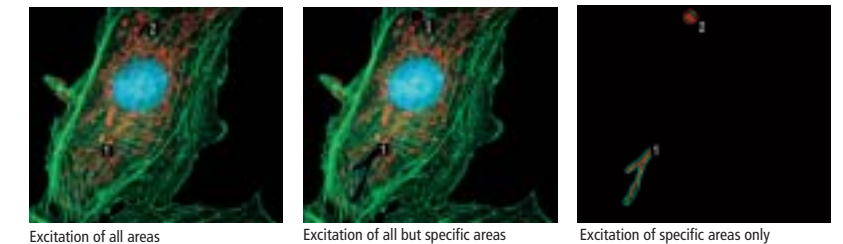


Specimen: Cerebellum Purkinje cell with mouse monoclonal anti-calbindin antibody stained with FITC
Specimen courtesy of Assistant Prof. Kazunori Toida, Department of Anatomy and Cell Biology, Institute of Health Biosciences, the University of Tokushima Graduate School

• ROI scanning with AOM/AOTF

Free shape scanning is possible with AOTF*(Acousto Optical Tunable Filter) and AOM** (Acousto Optical Modulator). It is effective for bleaching specific areas in FRAP/FLIP experiments or optical stimulation with a 405nm laser.

*Comes standard with the 4-laser unit
**Optional with the 3-laser unit



Excitation of all areas

Excitation of all but specific areas

Excitation of specific areas only

Broad selection of laser options

The C1 series accommodates a greater variety of lasers with wavelengths ranging from 405 to 640nm. It also supports solid-state lasers.

Leveraging the latest PC functions

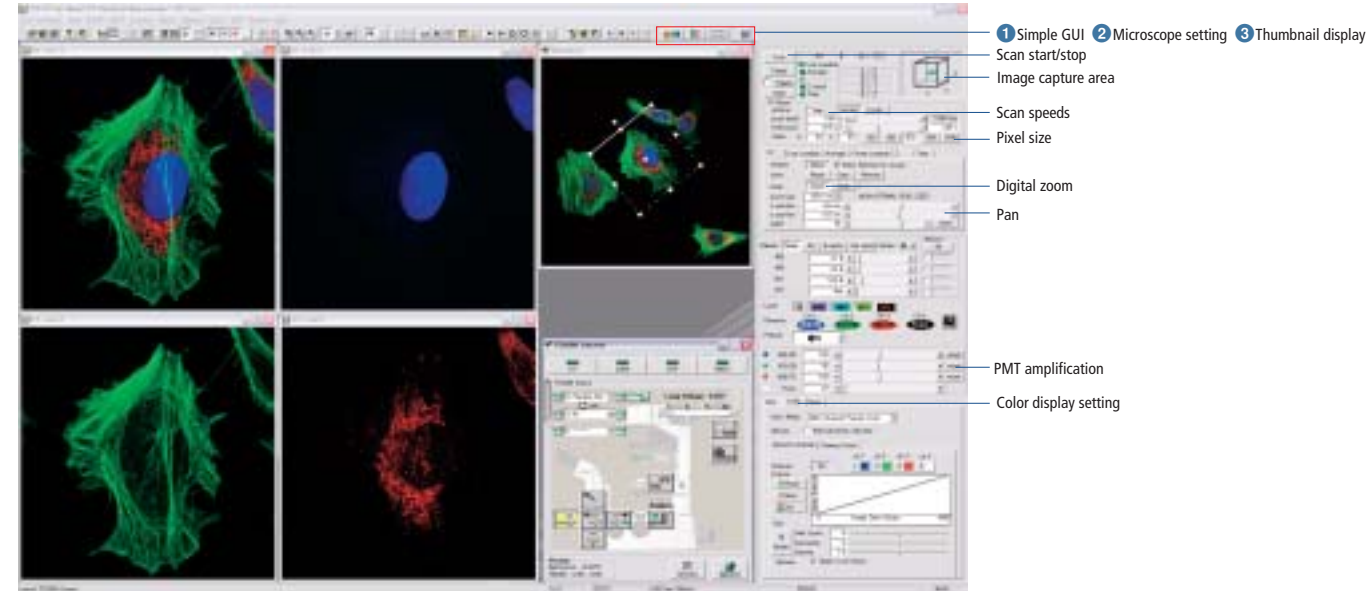
Because image processing is conducted with the controller instead of the PC, the user has greater freedom in selecting a PC. This makes it easy to upgrade the PC depending on requirements. Also, handling large amounts of data of up to four terabytes in size is possible, allowing large-size data acquisition, such as 3D time-lapse imaging.

Easy to use software for seamless image acquisition

All settings and procedures required for live image capture—fundamentals in confocal microscopy—can be viewed in a single window, eliminating the need for the operator to switch between many windows. The operation panel gives you an at-a-glance picture of all important settings including scan speed, pixel

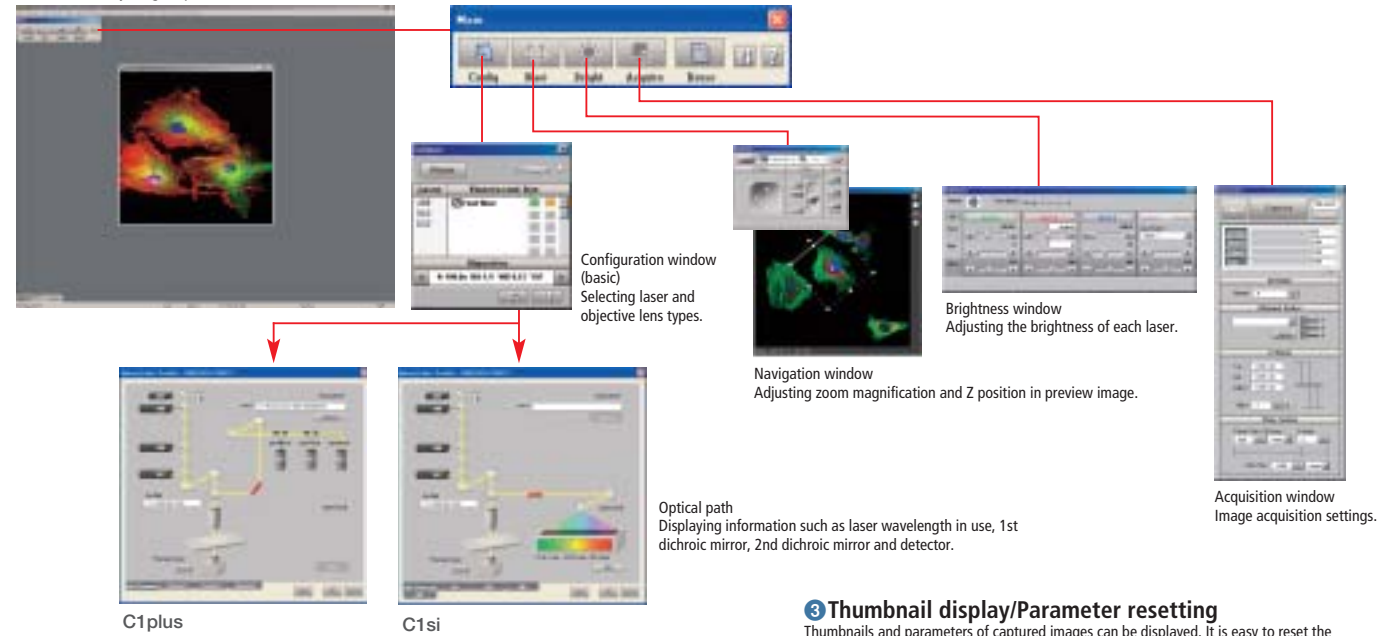
size, zoom/pan, PMT settings, pinhole, shutter, and color image look-up table. Scanning modes range from 2D (XY, XT), to 3D (XYZ, XYT), and even further to 4-dimensional (XYZT) scans.

At-a-glance setting panel



1 Simple GUI

Available for easy image capture.



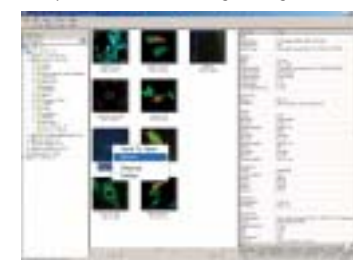
2 Microscope setting

Nikon motorized microscopes can be operated with the intuitive GUI.



3 Thumbnail display/Parameter resetting

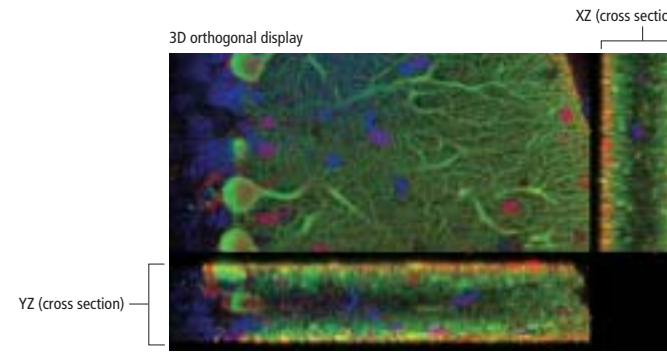
Thumbnails and parameters of captured images can be displayed. It is easy to reset the same parameters while confirming the images.



Versatile multi-dimensional imaging functions

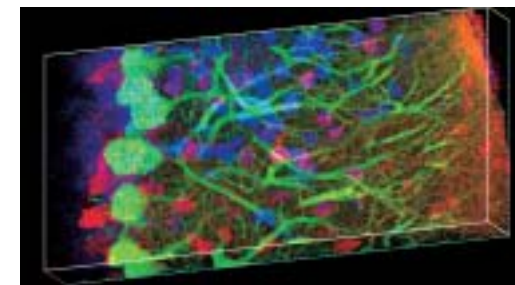
XYZ image

With precise Z-axis information of a specimen—a feat not possible with ordinary fluorescence microscopy—clear cross section images are possible.



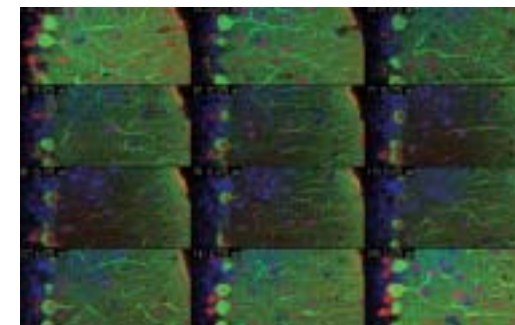
Volume rendering

Angles of 3D images can be freely changed.



3D tiled image

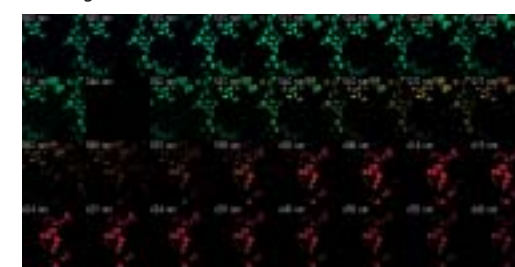
Images with different depths can be viewed simultaneously on a single screen.



Specimen: mouse's whole brain stained with mCB (mouse monoclonal anti-calbindin: green), rPV (rabbit polyclonal anti-parvalbumin: red) and nucleus (Hoechst: blue)
Specimen courtesy of Assistant Prof. Kazunori Toida, Department of Anatomy and Cell Biology, Institute of Health Biosciences, the University of Tokushima Graduate School

Spectral (XYλ) image (C1si)

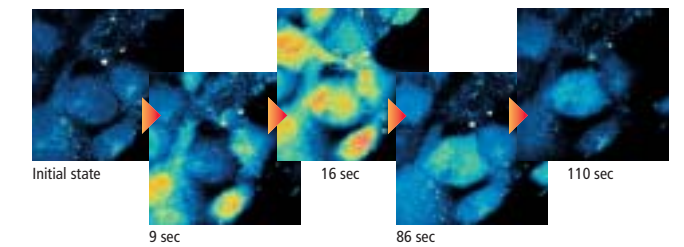
By using a spectral detector, spectral images with high spatial resolution can be captured with a single scan.



Specimen: respective expression of GFP, YFP and RFP in the nucleus of HeLa cell
Cells courtesy of Dr. Yoshihiro Yoneda and Dr. Takuya Saiwaki, Faculty of Medicine, Osaka University

Time-lapse (XYT) image

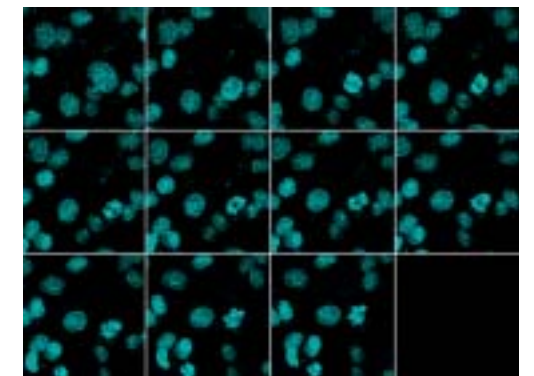
Changes over time can be analyzed using images with high spatial resolution. Intensity change analysis, ratio analysis and real time graph display during image acquisition is possible.



Specimen: visualized changes of calcium in cultured cells loaded with Fluo-4 AM

Time-lapse (XYZT) image

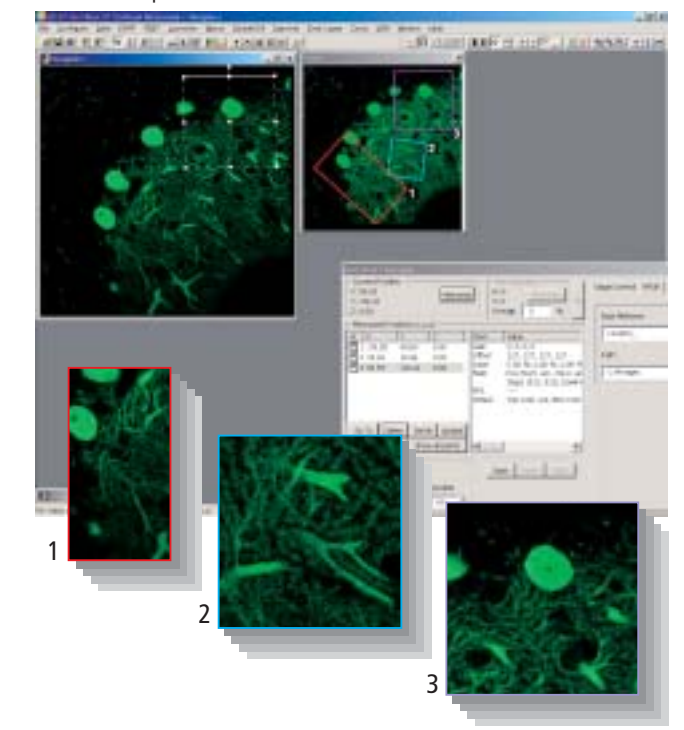
Two-dimensional (XY) or three-dimensional (XYZ) time-lapse images can be captured. The C1 series allows flexible interval time settings.



XYZT images of HeLa cells stained with Hoechst33342 are captured at 15-minute intervals.

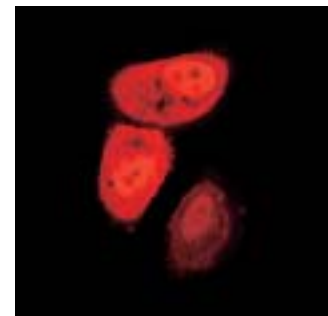
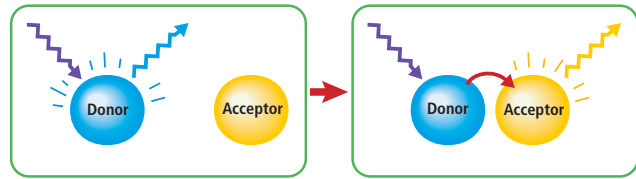
Multi-point imaging within the field of view

Time-lapse, Z-stack images of multiple points within the objective's field of view can be captured.

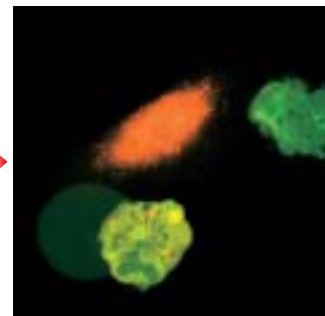


FRET (Fluorescence Resonant Energy Transfer)

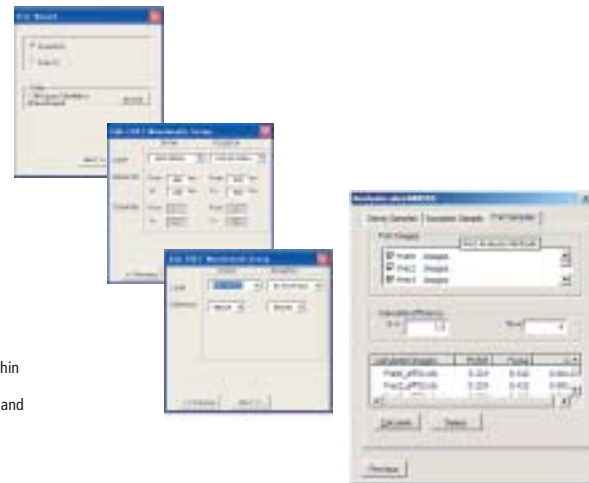
FRET is a physical phenomenon that occurs when there are at least two fluorescent molecules within 10nm distance and it can be observed when energy transfers from one fluorescent molecule to another. The C1 series enables imaging of the changes in three-dimensional protein structures as well as the interaction of two different proteins with high spatial resolution.



Before induction of apoptosis



After induction of apoptosis

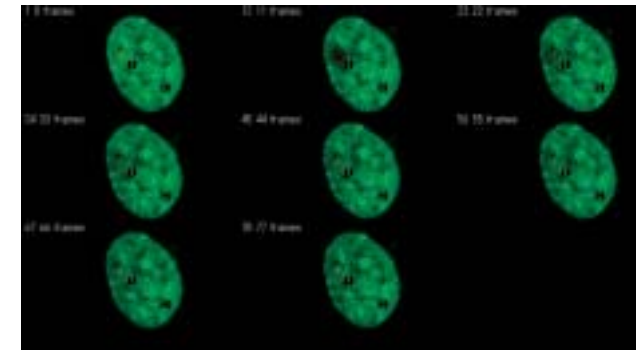
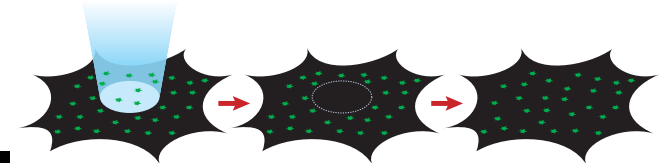


FRET analysis setting panel

Specimen: HeLa-S3 cell in which SCAT (sensor for activated caspase based on FRET) is expressed. CFP fluorescence (480nm: green) is detected within the cell undergoing apoptosis, and YFP fluorescence (545nm: red) is detected within the cell prior to apoptosis by FRET.
Specimen courtesy of Dr. Tokuko Haraguchi, Kobe Advanced ICT Research Center, National Institute of Information and Communications Technology

FRAP (Fluorescence Recovery after Photobleaching)

After bleaching ROI with strong laser exposure, the recovery process of fluorescence is observed to analyze movement of molecules. With the C1 series, only a specific area can be bleached by controlling the laser intensity with AOTF (comes standard with the 4-laser unit) and AOM (optional with the 3-laser unit).



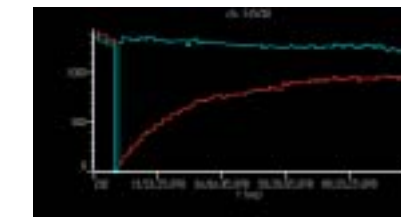
FRAP image

Specimen: after bleaching a part of a HeLa cell in which H1-GFP is expressed, the recovery of fluorescence intensity is observed in time-lapse recording. One image is displayed for every 11 images captured.

Specimen courtesy of Dr. Tokuko Haraguchi, Kobe Advanced ICT Research Center, National Institute of Information and Communications Technology

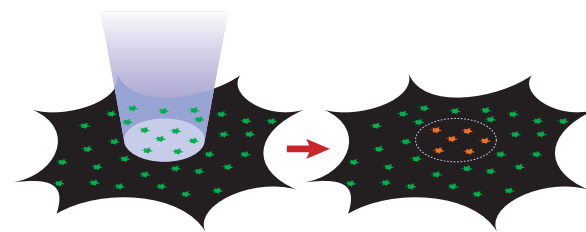


FRAP setting panel

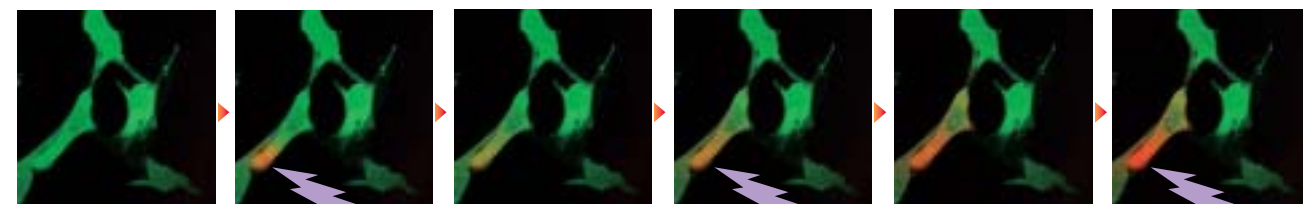
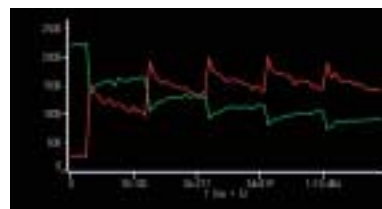


Kaede Photoconversion fluorescence protein

This photoconversion fluorescence protein originally emits green fluorescence that changes to red when exposed to ultraviolet light (408nm). By controlling the laser exposure position, area, intensity, timing and number of times, imaging the changing Kaede protein color in the cell is possible.



Kaede image (Overlay of DIC and Kaede fluorescence images)



405nm optical stimulation

405nm optical stimulation

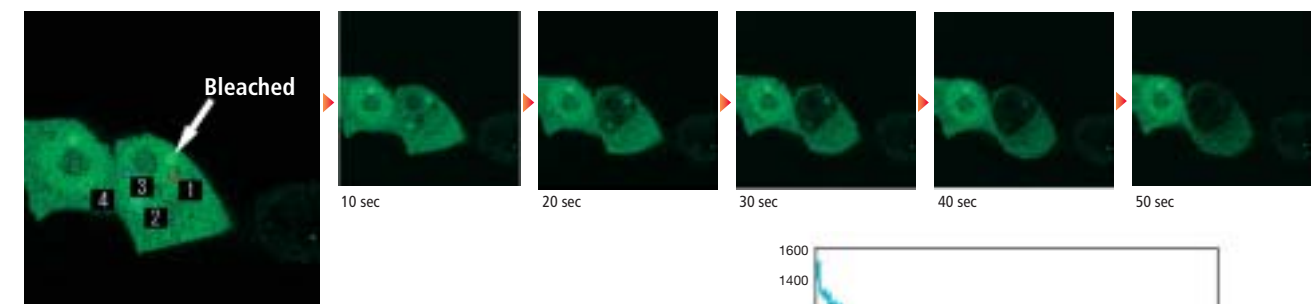
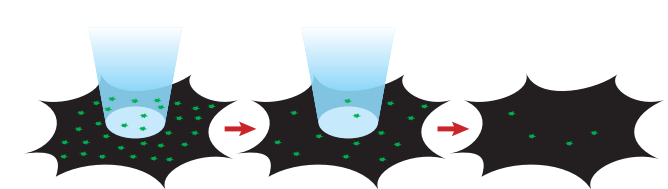
405nm optical stimulation

Specimen: fluorescent protein vector "CoralHue" Kaede" of Amalgaam Co., Ltd. expressed in HeLa cell. Fluorescence changes after optical stimulation by 405nm laser are captured over time. Diascopic DIC and fluorescence images of Kaede (green/red) are simultaneously acquired.
Cells courtesy of Amalgaam Co., Ltd.

i-FRAP (inverted FRAP) / FLIP (Fluorescence Loss in Photobleaching)

With i-FRAP, the area outside ROI is bleached to analyze movement of fluorescence molecules that leave ROI.

With FLIP, the movement of fluorescence molecules that enter the ROI from outside is analyzed by continuously bleaching ROI.



Initial state

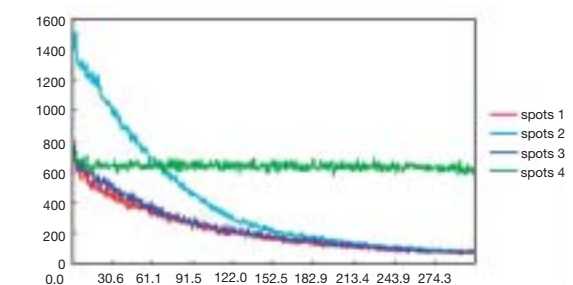
10 sec

20 sec

30 sec

40 sec

50 sec



Specimen: BDV (Borna disease virus) P protein fused with GFP expressed in human-derived glia cells infected by BDV. Images courtesy of Dr. Keizo Asanaga, Dept. of Virology, Research Institute for Microbial Diseases, Osaka University

True spectral imaging

In addition to the conventional fluorescence detector, the C1si 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 C1si captures minute changes of wavelength in true color and unmixes even overlapping spectra. Moreover, it has the capability to acquire spectra over a 320nm-wide wavelength range in a single scan, minimizing damage to living cells.

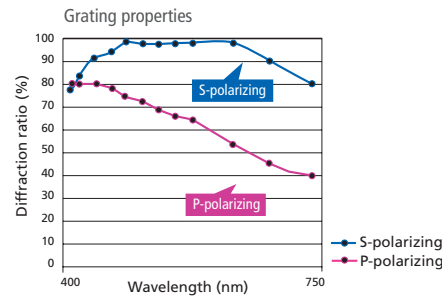
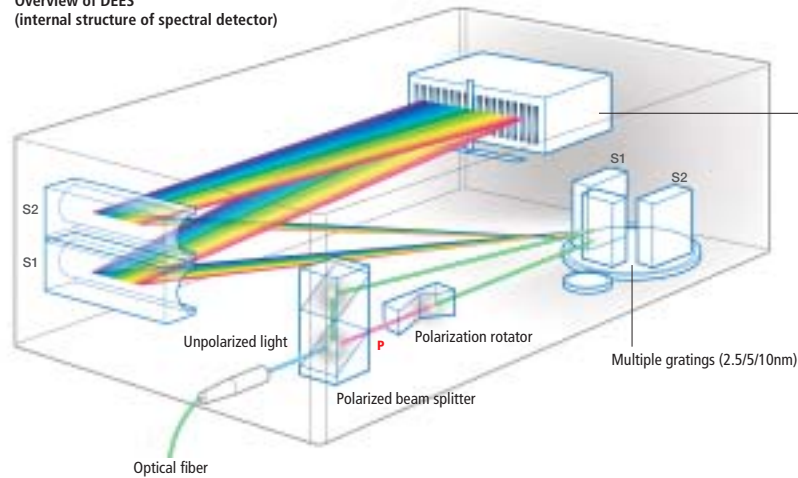
Brightness

Spectral detector with polarization control technology

Nikon original

Nikon's proprietary DEES (Diffraction Efficiency Enhancement System) for polarization correction is employed in the C1si's spectral detector to maximize brightness. By co-aligning the direction of polarization of fluorescent light, efficiency of the diffraction grating is optimized, resulting in exceptionally bright images. In particular, increasing the diffraction efficiency in the long wavelength range leads to improved brightness over the whole visible range from blue to red.

Overview of DEES (internal structure of spectral detector)



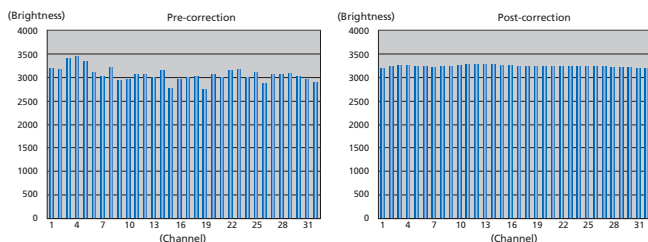
Multi-anode PMT
The spectral imaging detector utilizes a newly developed laser shielding mechanism. Coupled with a wavelength resolution independent of pinhole diameter, this mechanism successfully shuts out the reflected laser beam. The blocking mechanism can be moved freely with software, allowing users to block any laser wavelength, making the C1si compatible with virtually any laser selection.

Superb error and deviation correction

Nikon original

Accuracy of spectra is maintained with highly precise correction technologies, including wavelength correction using emission lines and luminosity correction utilizing a NIST (Nation Institutes of Standards and Technology) traceable light source. Also, multi-anode PMT sensitivity correction technology allows correction of sensitivity error and wavelength transmittance properties on a per-channel basis, allowing researchers to minimize measurement errors and deviations among different equipment.

Multi-anode PMT sensitivity correction



High-efficiency fluorescence transmission technology

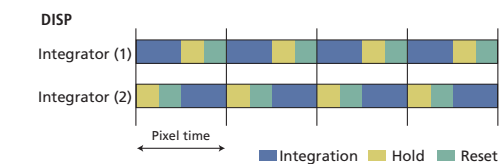
Nikon original

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.

Dual integration signal processing

Nikon original

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



Two integrators work in parallel when the optical signal is read to ensure there are no discrepancies.

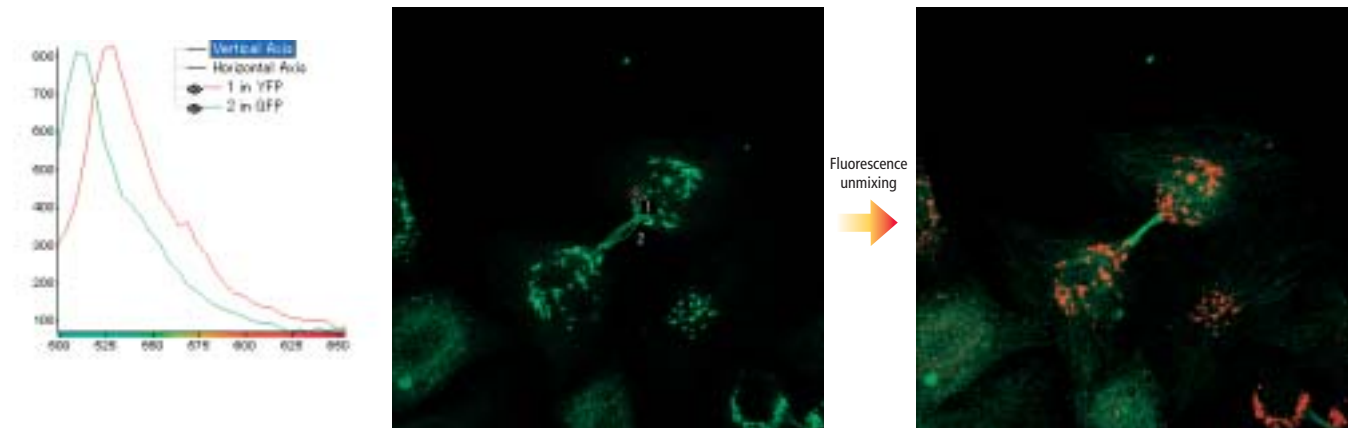
Fluorescence unmixing with no crosstalk

Fluorescence labels with closely overlapping spectra can be unmixed cleanly with no crosstalk.

Effortless fluorescence unmixing

Even without a given reference spectrum, simply specifying a ROI within the image and clicking the Simple Unmixing button allows separation of fluorescence spectra. The C1si 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 488nm laser (left). After fluorescence unmixing, GFP is indicated in green and YFP is indicated in red (right). The graph at 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?

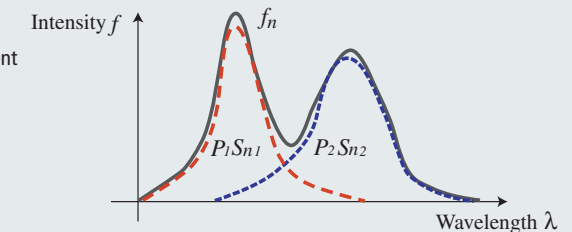
The spectrum obtained by actual measurement is a mix of spectral elements with a certain proportion. An imaging algorithm is used to compare the spectra of each pixel with reference curves for each spectral element. Each fluorescent probe in the specimen is displayed in a unique color in the final unmixed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

$$f_n = S_n * P$$

f_n = Wave pattern of spectrum obtained by actual measurement

S_n = Wave pattern of individual reference spectrum

P = Ratio of elements for each wave pattern



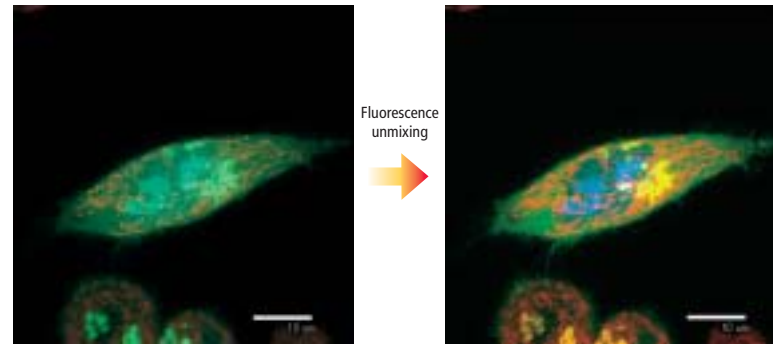
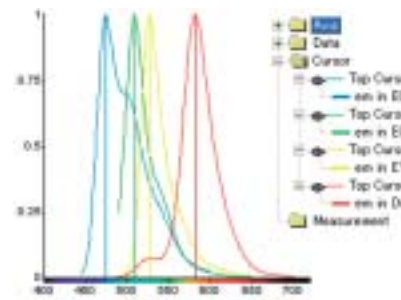
Reference wave pattern (S) is selected from the following three depending on the experiment.

- 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

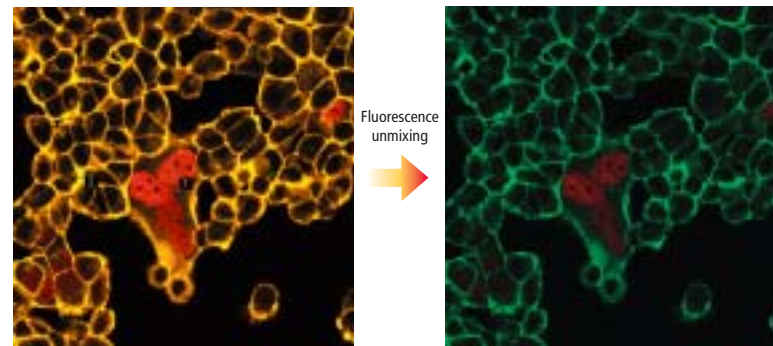
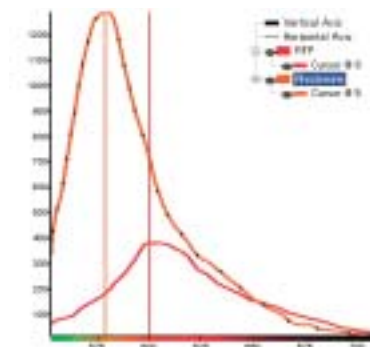
Because wavelength resolution and range are freely selectable, scanning of a fluorescence protein with a wide wavelength range from blue to red such as CFP/GFP/YFP/Ds Red is possible at one time. Reference data allows unmixing and display of each color.



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 408nm and 488nm 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.

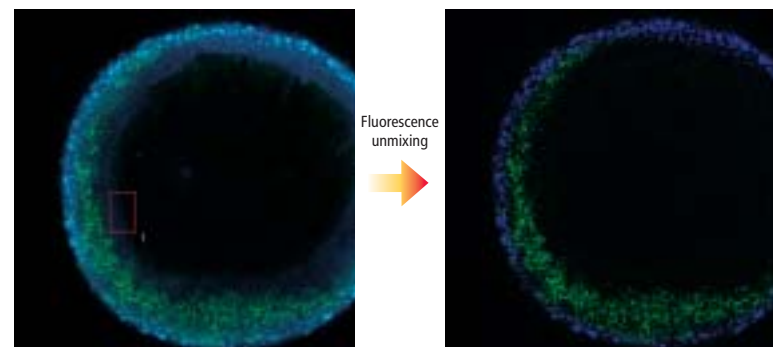
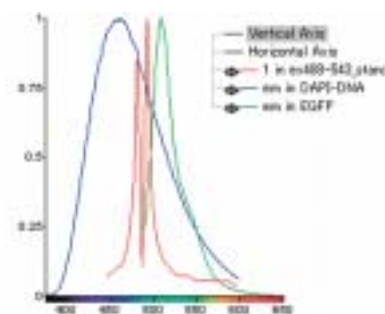


Spectra for ROI 1 and 2 corresponding to the image on the right. Rhodamine's fluorescence spectral peak is at approximately 579nm, while that for RFP is approximately 600nm. RFP's fluorescence is weaker than Rhodamine's, but their spectra are cleanly unmixed.

Specimen: actin of HeLa cell that has RFP expressed in the nucleus is stained with Rhodamine. Spectral image in the 550-630nm wavelength range captured at 2.5nm wavelength resolution with 543nm 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 autofluorescence 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 autofluorescence of cells, which until now was difficult.

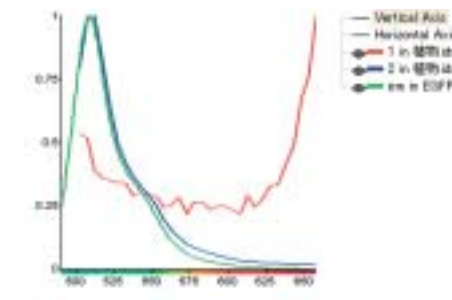


Specimen: Zebrafish egg stained with cadherin-GFP and DAPI. Spectral image captured with 408nm and 488nm laser exposure (left). After unmixing using reference spectra for autofluorescence (ROI1), GFP and DAPI, the autofluorescence in the image is eliminated (right). Specimen courtesy of Dr. Tohru Murakami,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

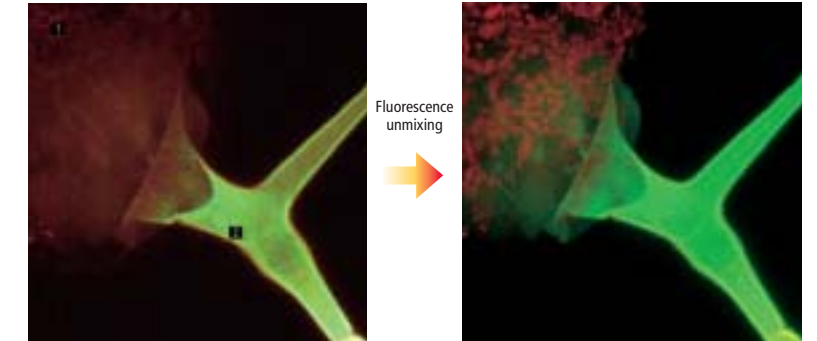
Reprint from Cell Imaging Press Vol. 3

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 C1si's spectral detector enables the slight color differences to be confirmed as wavelengths through sensitivity correction.



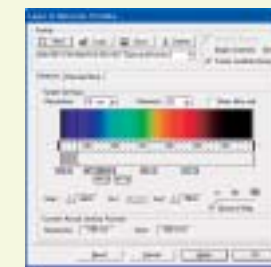
The correspondence of the spectral curve (blue) of ROI2 in the image and the reference curve (green) of eGFP proves that GFP is expressed in ROI2



Specimen: Arabidopsis proteoglycan and fused protein of GFP. Spectral image captured with 488nm laser exposure (left). Once the image is unmixed using reference spectra for autofluorescence (ROI1) and GFP, GFP is indicated in green and autofluorescence 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

Frame lambda

Using this function allows the user to acquire either wide continuous or bands of spectra by sequentially acquiring individual narrower spectra regions. The laser and the spectral range acquired can be individually set for each sequence in the series, the final single spectra is a result of combining all spectra in the sequence.



Frame lambda setting panel

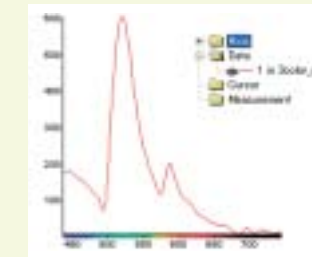
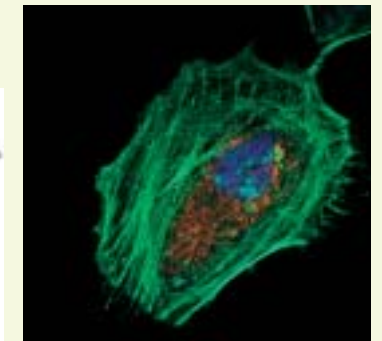
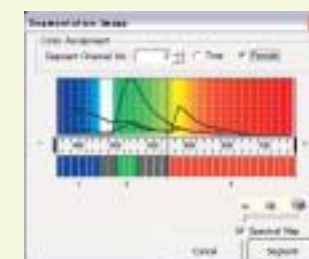


Image and spectral curve obtained with frame lambda function. 410-740nm spectral range captured with 408nm, 488nm and 561nm lasers is used.



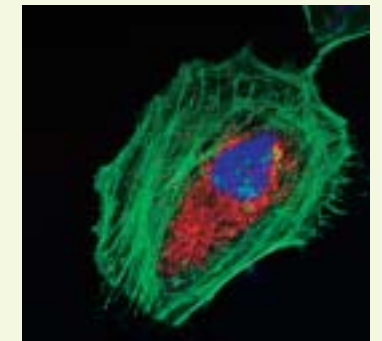
Segmentation

By spectral imaging with C1si, wavelength information of the entire range can be obtained in a single scan. Therefore, it is no longer necessary to acquire only the limited wavelength range or reshoot other ranges during the imaging session. Consequently, there is minimized photo-toxicity to the specimen. After spectral imaging, images that are filtered (segmented) with any desired wavelength range easily displayed.



Segmentation setting panel

Segmented image of the spectral image obtained with the frame lambda function



The spectral image of stained actin (Alexa 488), mitochondria (Mito Tracker Organe) and nucleus (DAPI) of HeLa cell, captured with the frame lambda function, is post-acquired in the three wavelength ranges of 420-480nm, 500-530nm, and 570nm and longer using the segmentation function.

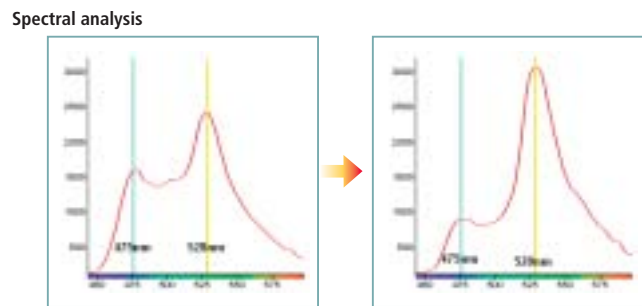
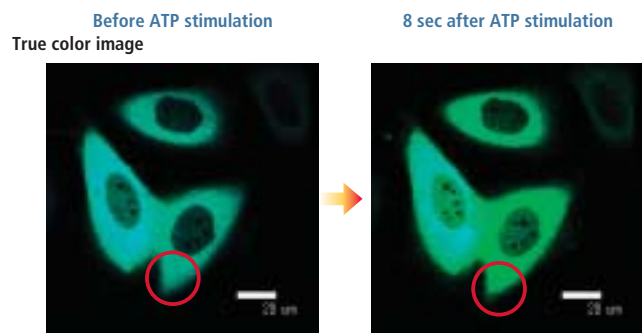
Specimen courtesy of Dr. Yoshihiro Yoneda and Dr. Takuya Saiwaki, Faculty of Medicine, Osaka University

True spectral FRET analysis

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

Acquisition of spectral image (XYTλ)

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



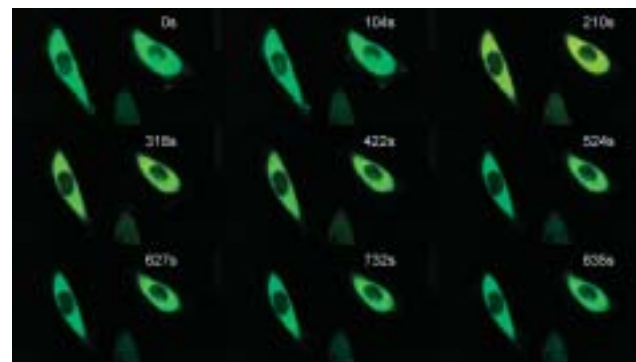
True color image and spectral analysis of CFP and YFP. Spectral curve in ROI. Left peak indicates CFP and right peak indicates YFP respectively. After ATP stimulation, peak of CFP drops and peak of YFP rises due to FRET.

Reprint from Cell Imaging Press Vol. 1

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.

Fluorescence unmixing

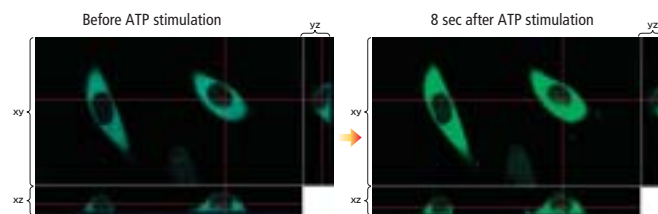
Spectral FRET analysis is possible by unmixing using reference data of CFP and YFP. Two-dimensional change (FRET) of intracellular Ca²⁺ concentration is easily determined from spectral data without acceptor bleaching.



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

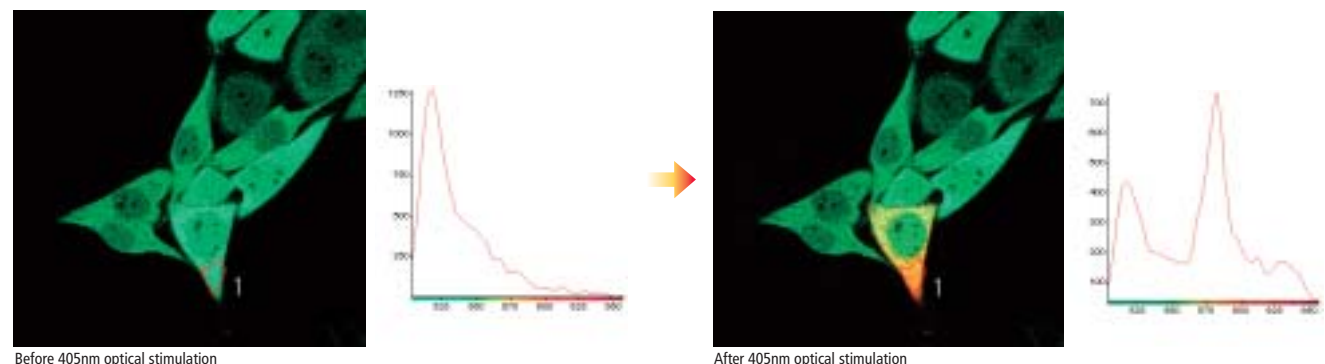
Five-dimensional analysis (XYZTλ)

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



True spectral Kaede analysis

Changes of Kaede fluorescence protein over time can be recorded as spectral changes. Not only color change from green to red but also slight spectral changes can be captured.



Specimen: fluorescent protein vector "CoralHue" Kaede of Amalgaam Co., Ltd. expressed in HeLa cell. Fluorescence in the 500-660nm range is obtained at 5nm wavelength resolution using the real-time spectral method. During this process, ROI is optically stimulated by 405nm laser. Spectral graphs show photoconversion of fluorescence from 520nm before optical stimulation to 580nm after stimulation. Specimen courtesy of Amalgaam Co., Ltd.

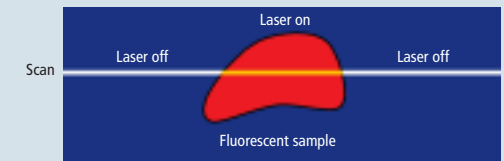
The system expandability of the C1 series meets the needs of today's research

To meet the requirements of constantly evolving live cell imaging, Nikon provides a wide array of microscopes, accessories and software. Outstanding expandability of the C1 series delivers optimal solutions for live cell observation.

● CLEM (Controlled Light Exposure Microscopy) system

What is CLEM?

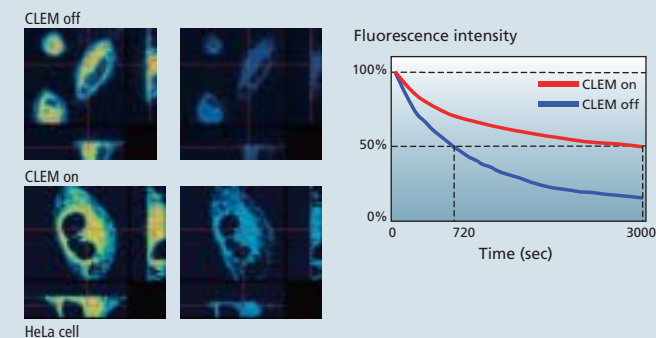
The CLEM system senses the existence of fluorescence in a specimen and exposes the laser in areas with fluorescence by controlling the laser pixel by pixel. Because the laser is not used where excitation light is not needed, laser exposure is minimized and live cell phototoxicity is drastically reduced.



Features

- Because the laser is switched off in areas that do not emit fluorescence, exposure light is reduced and the fading speed of fluorescence labels is decreased twofold to fourfold. Phototoxicity of cells is also reduced. (See diagram below)
- CLEM switches off the laser in areas with sufficient fluorescence and calculates pixel brightness. This extends the apparent dynamic range of fluorescence intensity, allowing an image with both extremely weak and bright fluorescence to be displayed without saturation.

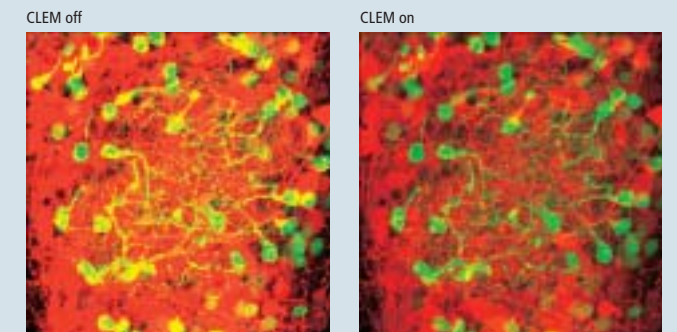
Reducing phototoxicity



Specimen: HeLa cells loaded with Rhodamine 123 are observed with CLEM on and off. The graph shows the fluorescence intensity change and scan time, and the reduction of fading by CLEM. Images courtesy of Dr. Takashi Sakurai, Photon Medical Research Center, Hamamatsu University School of Medicine

Reference
R.A. Hoebé, C.H. Van Oven, T.W.J. Gadella Jr, P.B. Dhonukshe1, C.J.F. Van Noorden & E.M.M. Manders, "Controlled light-exposure microscopy reduces photobleaching and phototoxicity in fluorescence live-cell imaging." Nature Biotechnology P1-5 (2007)

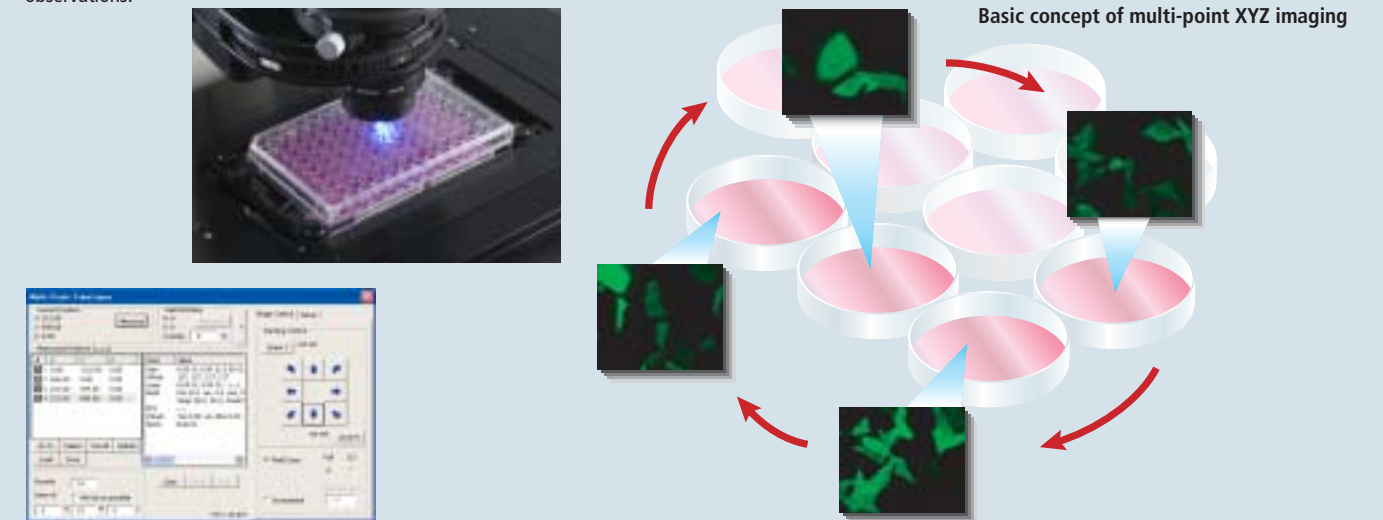
Extending dynamic range



Specimen: three-dimensional reconstruction image of juxtarglomerular cell of mouse olfactory bulb shows diversity of three-dimensional structure captured with confocal laser microscope. 50mm-thick coronal section is multiply immunostained with anti-calbindin antibody (mouse monoclonal antibody, FITC label, green) and anti-tyrosine hydroxylase antibody (mouse monoclonal antibody, Cy3 label, red). The image captured with CLEM on allows volume rendering without saturation even if the image has fluorescence intensity difference. In the image captured with CLEM off, saturation occurs in the volume rendering process. Specimen courtesy of Assistant Prof. Kazunori Toida, Department of Anatomy and Cell Biology, Institute of Health Biosciences, the University of Tokushima Graduate School

● Multi-point observation system

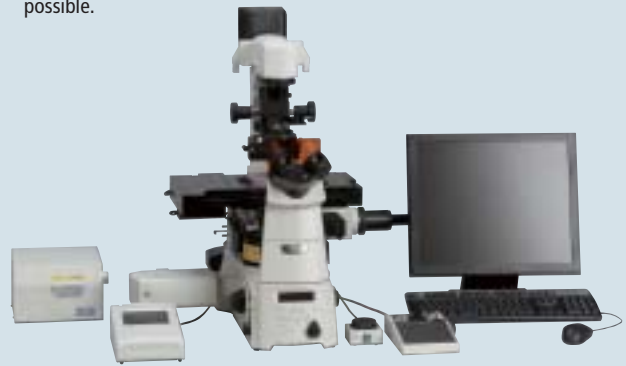
Use of optional motorized stage enables easy multi-point observation including multi-point time-lapse, multi-point XYZ and multi-point four-dimensional (XYZT) observations.



Multi-point setting panel

● Confocal microscope with Perfect Focus System

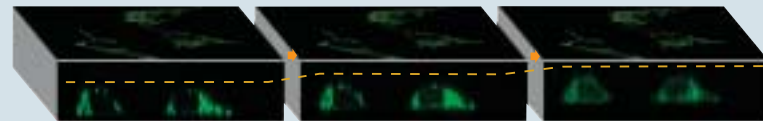
- Motorized inverted microscopes Ti-E and TE2000-E feature real-time focus maintaining system—Perfect Focus System (PFS)—continuously corrects focus drift during lengthy time-lapse observation and when reagents are being added.
- The spectra of a broad 320nm range can be obtained at one time with a spectral detector allowing stable time-lapse observation of the spectra.
- By using an optional stage incubator, lengthy time-lapse observations are possible.



Perfect Focus System with motorized sextuple DIC nosepiece (TI-E)

XZT recording of mitochondria by confocal microscopy

PFS off



PFS on



Specimen: HeLa cells stained with Rhodamine 123
Objective: CFI Plan Apo VC 60x water dipping, NA 1.20



Focus drift during long-term time-lapse imaging

Focus drift resulting from temperature change during long-term imaging is eliminated.



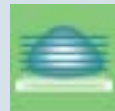
Focus drift when adding reagents

Focus drift resulting from sudden temperature change when adding reagents is eliminated, therefore improving the reliability of fluorescence intensity change measurement data.



Focus drift during multi-point time-lapse observation (using motorized XY stage)

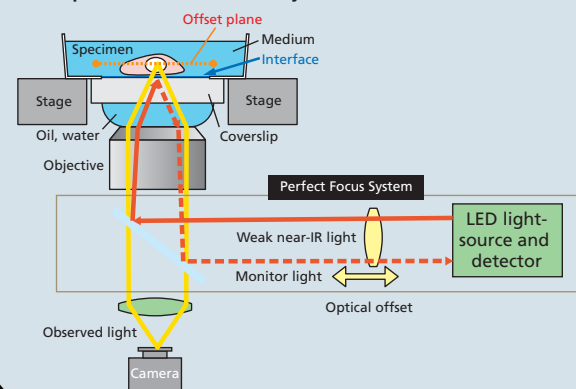
Focus drift caused by stage movement is eliminated.



Focus drift when capturing three-dimensional images

Focus position is maintained in real time, eliminating the need to take extra images in anticipation of focus drift. This reduces photobleaching and damage to live cells.

Concept of the Perfect Focus System



Sequential XZ sectional images taken by changing the Z-axis position. With PFS off, strong photobleaching occurs due to frequent (65) scanning in anticipation of focus drift. With PFS on, scanning is less frequent because of the elimination of focus drift, thereby reducing photobleaching

Reprint of Cell Imaging Press Vol. 2

● Stage incubation system INU series

Temperature of the stage, water bath, cover, and objective lens is controlled, allowing living cells to be maintained for a long period. A transparent glass heater prevents condensation, and loss of focus due to heat expansion on the stage surface is prevented, making this system ideal for lengthy time-lapse imaging applications.

Manufactured by Tokai Hit Co., Ltd.

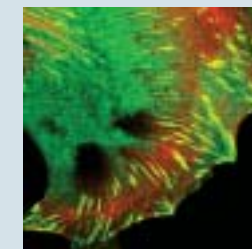


● Multi-mode imaging system—TIRF-C1

The laser TIRF system and the confocal microscope system can be mounted simultaneously on the inverted microscope Ti-E or TE2000-E. The laser TIRF system incorporates an epi-fluorescence module. Switching between the two light sources and adjustment of alignment are easy.

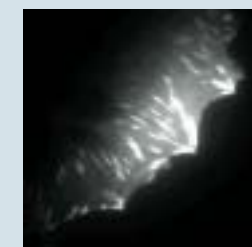


Digital camera is an option.



Confocal image

This shows the basal portion of the cell. A clear band of substantial F-actin (red) is shown at the leading edge of the cell, which is migrating toward the right side. Paxillin molecules are green. Stress fibers are facing the rear of the cell.



TIRF image

Strong and clear fluorescence derived from paxillin is observed in the evanescent field. The focal adhesions existing at the portion of cells in contact with the coverglass were clearly confirmed.

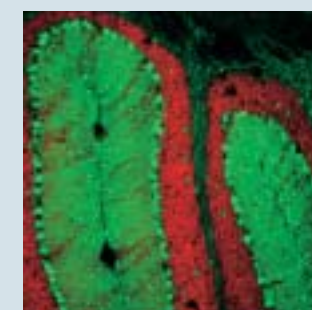
Specimen: mouse bone marrow stroma cell (ST2 cell). After fixing in 4% formaldehyde, cells were treated with 0.25% Triton X-100 before double staining with paxillin antibodies and TRITC-phalloidin. Images courtesy of Assistant Prof. Shuichi Obata, Kitasato University

● Confocal patch-clamp imaging system FN1-C1

Confocal observation with FN1 (fixed stage microscope) is highly beneficial in vivo imaging. The FN1 allows high-resolution imaging of deeper areas of cells with the excellent patch-clamp operability of FN1 and the performance of Plan 100x (NA 1.1) objectives. Also, elimination of autofluorescence in vivo, which until now has been difficult, can be easily achieved.



FN1-C1



Fluorescence image



DIC image

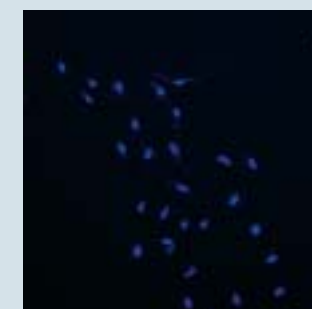
Specimen: GABA ERGIC neuron (green: GFP) and potassium channel protein (red: YFP) in a cerebellar slice. Images courtesy of Dr. Thomas Knöpfel, Team Leader, Laboratory for Neuronal Circuit Dynamics, Brain Science Institute, RIKEN

● Optional imaging software NIS-Elements series

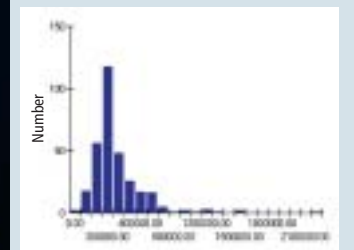
Nikon's original NIS-Elements imaging software enables advanced analysis using images captured by the C1 series confocal microscope. It allows object counting, measurement of area and brightness, and creation of histogram.



Operation panel

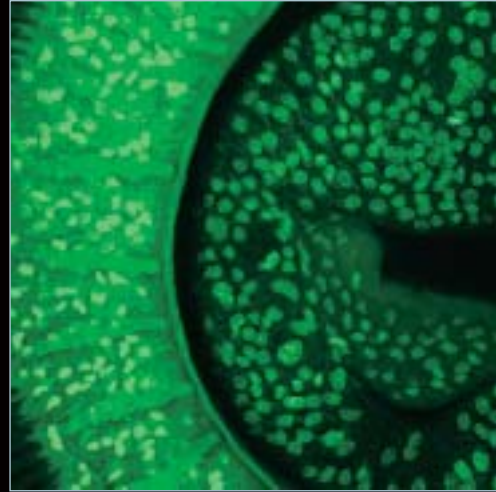


Intensity distribution measurement

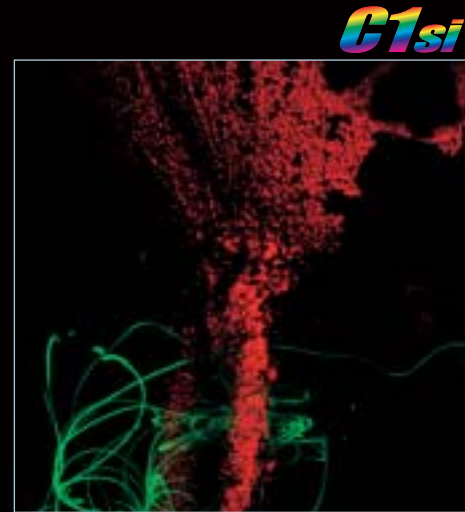


Nuclei of HeLa cells are stained with DAPI. The graph shows their intensity distribution and number.

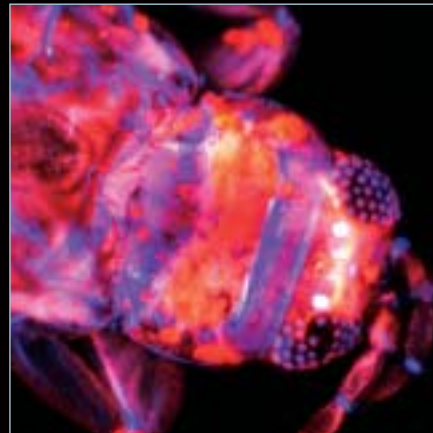
Confocal image gallery



Argulus acetabulum
Specimen courtesy of School of Environmental Sciences and Development,
North-West University, South Africa



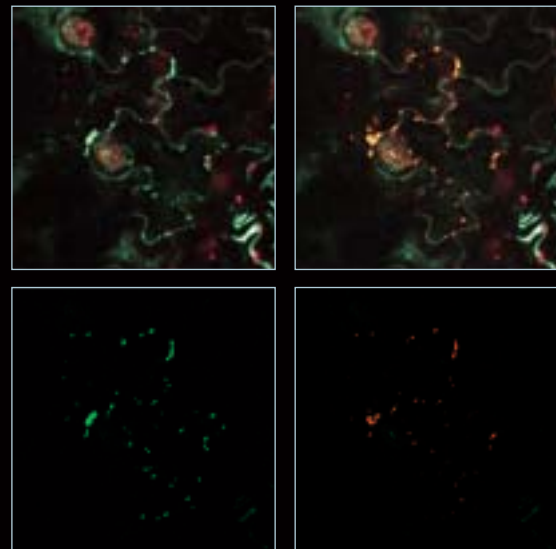
GFP expressed in the whole tail of drosophila sperm. Anterior pole of the egg is indicated in red (pseudo color) and GFP indicated in green after unmixing the autofluorescence spectrum of the egg and spectrum of sperm (GFP).
Images courtesy of Director and Professor Masatoshi Yamamoto, Drosophila Genetic Resource Center, Kyoto Institute of Technology



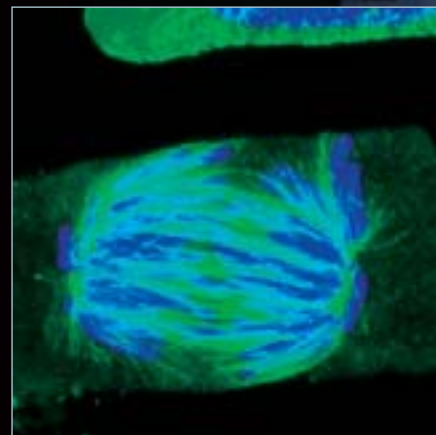
Autofluorescence of thrips, 408nm/488nm/543nm excitation
Image courtesy of Dr. Steve Cody, Ludwig Institute for Cancer Research



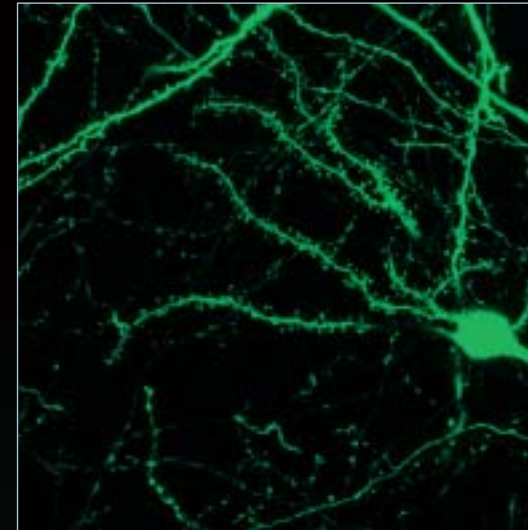
Fungus spore
Specimen courtesy of Prof. Rudi Verhoeven, Department of Plant Sciences, University of the Free State Bloemfontein, South Africa



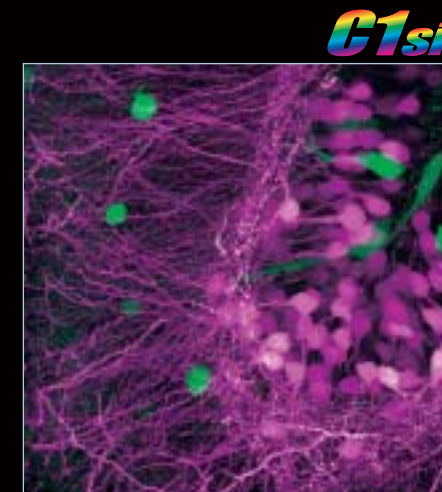
Kaede expression localized to the mitochondria within Arabidopsis leaf
Top: true color image before (left) and after (right) optical stimulation
Bottom: Kaede-green and Kaede-red after unmixing the above image using reference data of autofluorescence, Kaede-green and Kaede-red. Image before (left) and after (right) optical stimulation
Specimen courtesy of Assistant Professor Shinichi Arimura, Graduate School of Agricultural and Life Sciences, The University of Tokyo



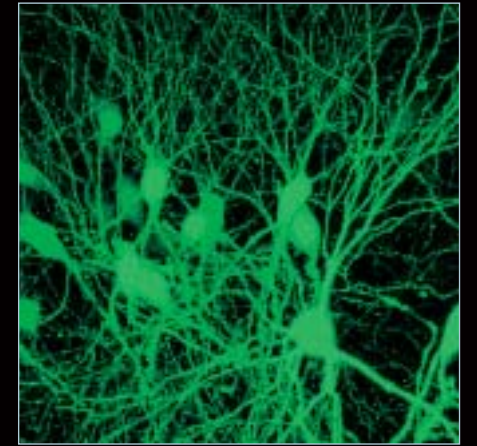
Cells of an onion root, Hoechst33258, OregonGreen488
Image courtesy of Dr. Yoshinobu Mineyuki, Department of Life Science, Graduate School of Life Science, University of Hyogo



Sliced hippocampus of a transgenic rat (image of a nerve in the spine)
Image courtesy of Dr. Hu Qian, Chinese Academy of Science



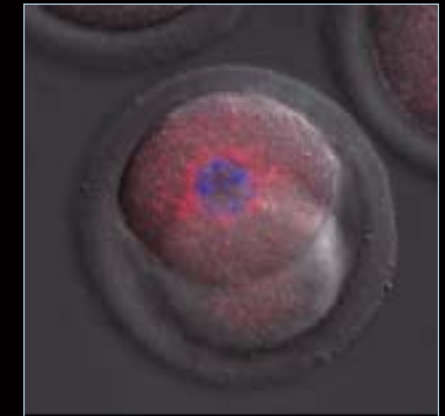
Three-dimensional reconstruction image of mouse hippocampus (GFP: inhibitory neurons, green, YFP: excitatory neurons, magenta) through volume rendering after spectra unmixing
Image courtesy of Dr. Masayuki Sekiguchi, Department of Degenerative Neurological Diseases, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan



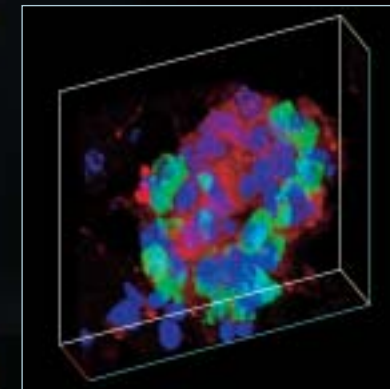
Three-dimensional reconstruction image of nerve through volume rendering after unmixing spectra of nerve (GFP) and peripheral tissue (autofluorescence) of the 188µm-thick sliced hippocampus image (spectral imaging with 5nm wavelength resolution, 500-660nm)
Specimen courtesy of Professor Shigeo Okabe and Tatsuya Umeda, Department of Cell Biology, School of Medicine, Tokyo Medical and Dental University



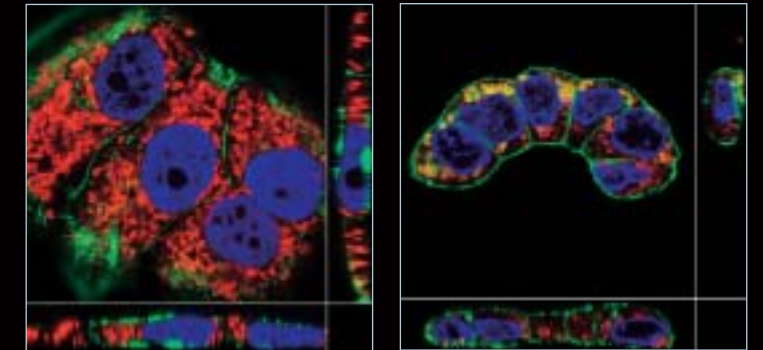
64-celled (32 cells) embryo of Branchiostoma belcheri stained with anti α tubulin antibody and DAPI
Image courtesy of Prof. Kinya Yasui, Assistant Prof. Kunifumi Tagawa, Marine Biological Laboratory, Hiroshima University Graduate School of Science



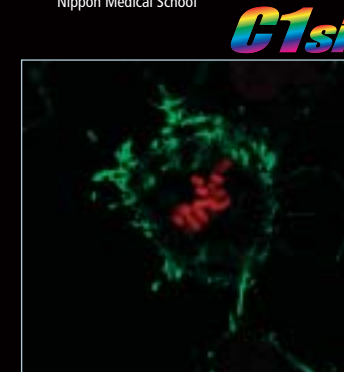
Living mouse egg, stained with Hoechst3342 (nucleus) and MitoTrackerOrange (mitochondria) and overlaid with DIC image
Image courtesy of Dr. Atsuo Ogura and Dr. Hiromi Miki, RIKEN Tsukuba Institute, RIKEN BioResource Center, BioResource Engineering Division



DAPI, Alexa 488, Alexa 546
Specimen courtesy of Dr. Ulf Ahlgren, Umea University, Sweden



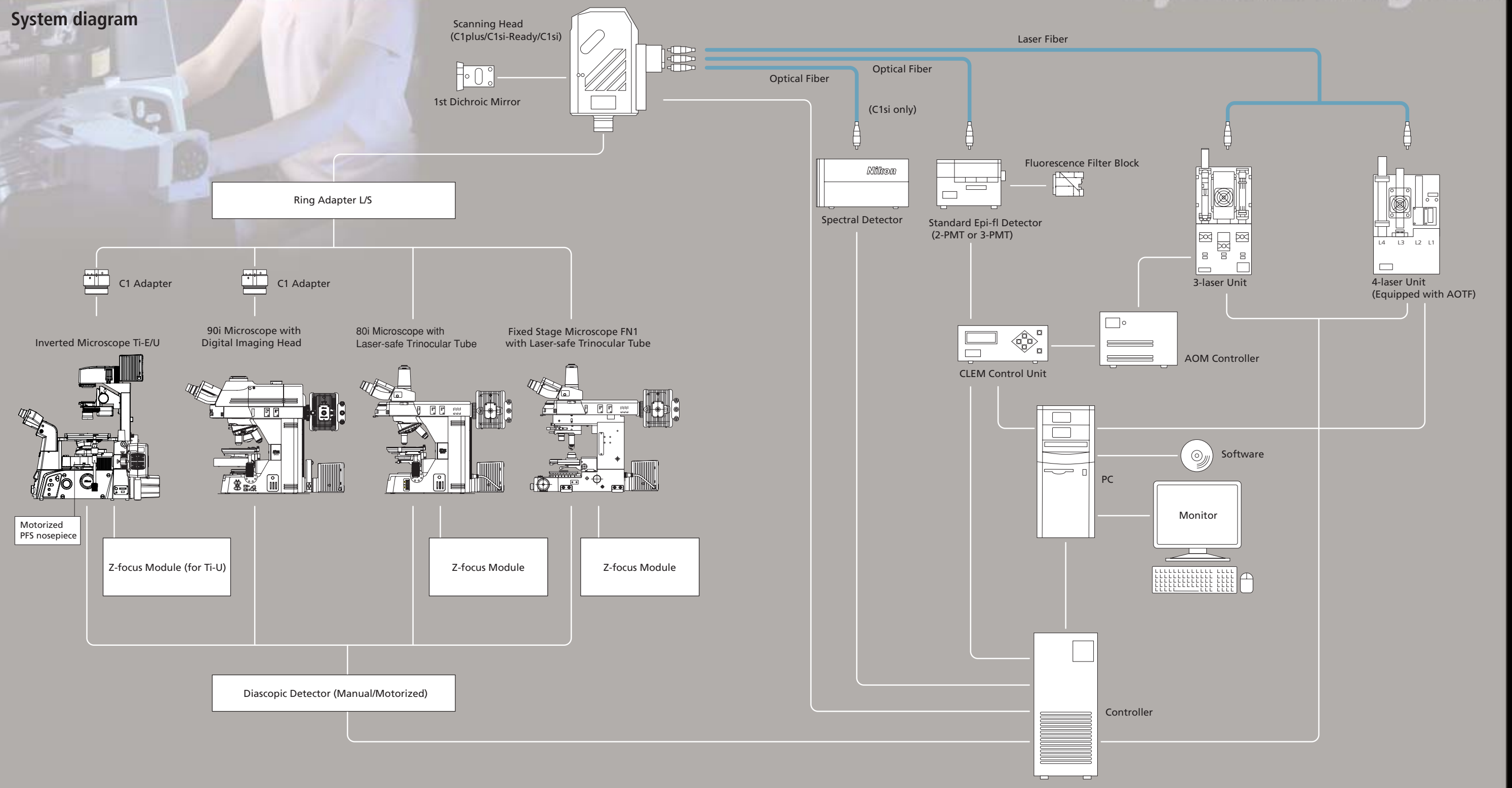
Comparison before (left) and after (right) exposure of anticancer agent to T47D breast cancer cell. Blue: nucleus, green: actin, red: mitochondria
Specimen courtesy of Dr. Mitsuhiro Kudo, Department of Pathology, Integrative Oncological Pathology, Nippon Medical School



Actin of HeLa cell that has YFP expressed in nucleus is stained with Alexa 488. Alexa 488 is indicated in green and YFP is indicated in red after unmixing using fluorescence reference data.
Specimen courtesy of Dr. Yoshihiro Yoneda and Dr. Takuya Saiwaki, Faculty of Medicine, Osaka University

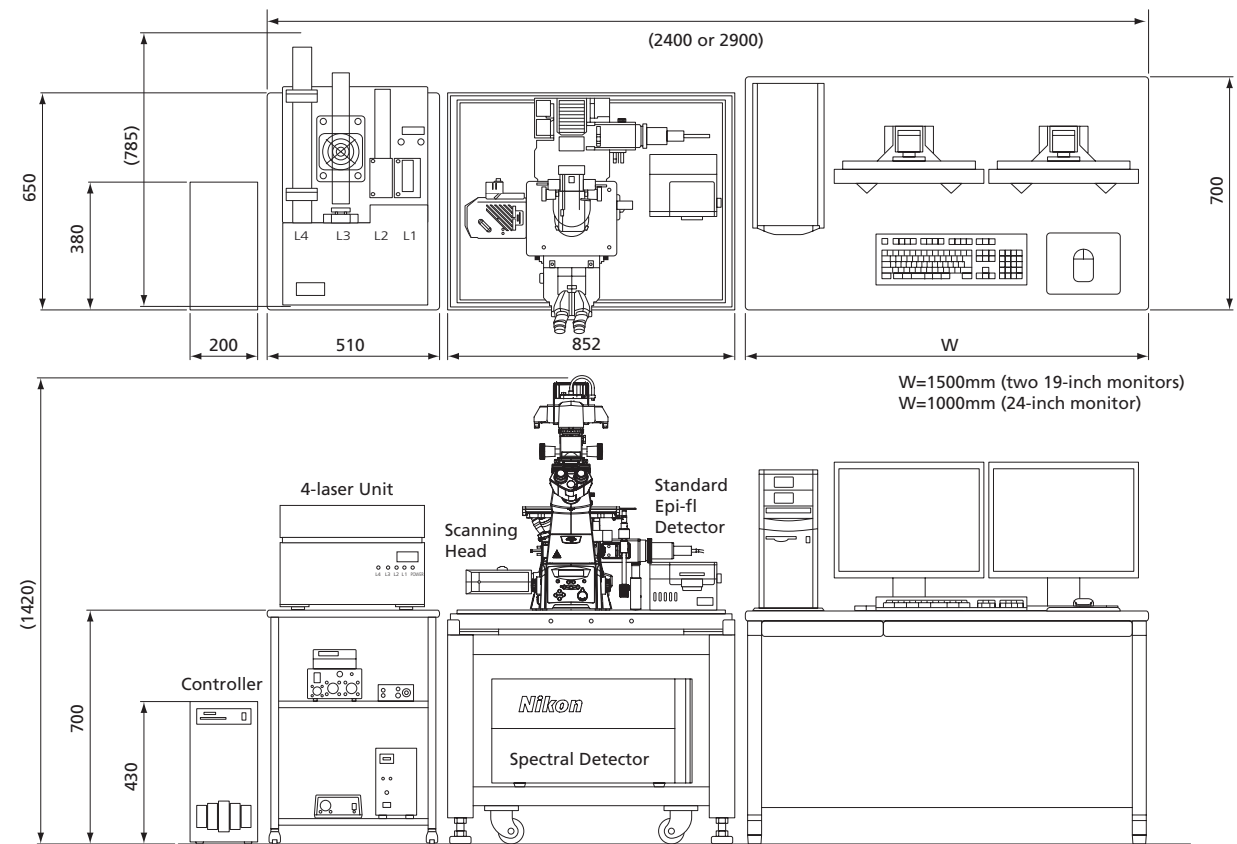
System diagram

System diagram



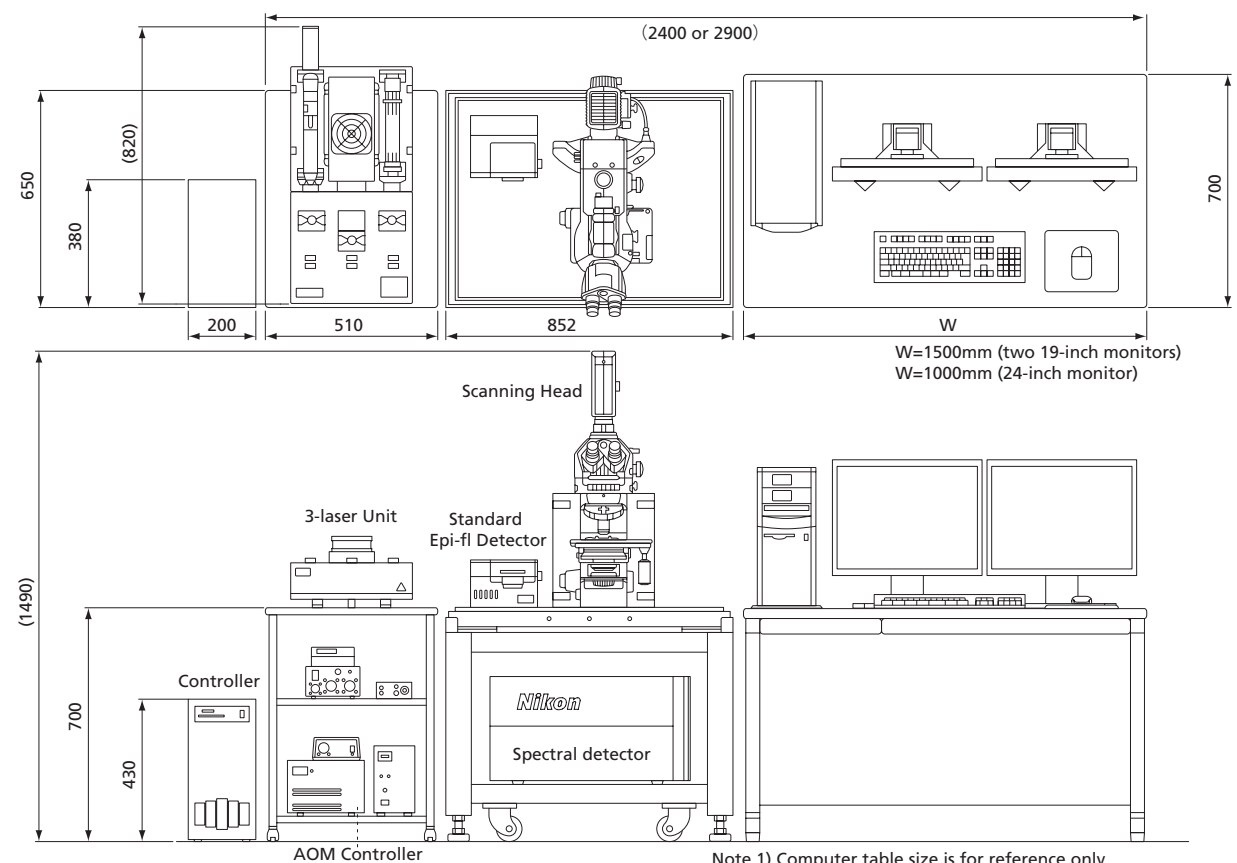
Recommended layout

Combination with the Inverted Microscope Ti-E/U with 4-laser Unit



Note 1) Computer table size is for reference only.
Note 2) Spectral detector is unnecessary for C1plus and C1si-Ready.

Combination with the Upright Microscope ECLIPSE 80i/90i with 3-laser Unit



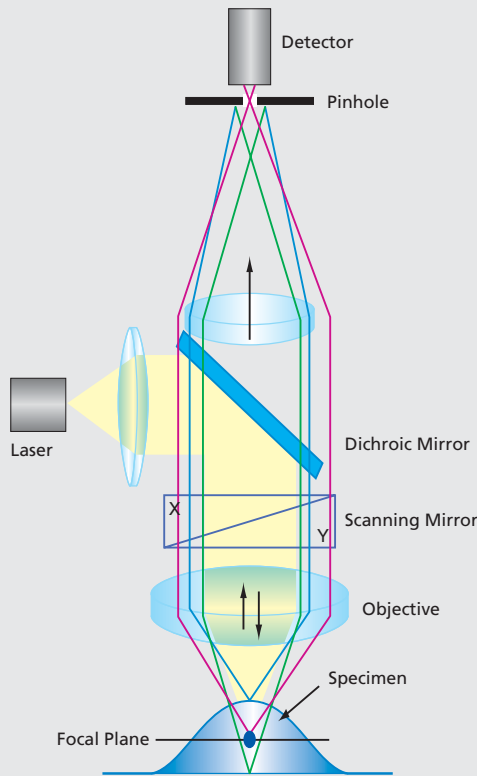
Note 1) Computer table size is for reference only.
Note 2) Spectral detector is unnecessary for C1plus and C1si-Ready.

Specifications

		C1 plus	C1si with 3-laser unit	C1si with 4-laser unit	
Laser light source	Laser wavelength	BD laser (405nm, 36mW, variable) Laser diode (440nm, 20mW, variable) Ar laser (488nm, 10mW) Ar laser (488nm, 25mW) Ar laser (488nm/514nm, 40mW) Solid-state laser (488nm, 20mW) G-HeNe laser (543nm, 2mW random polarization) Solid-state laser (561nm, 10mW) Y-HeNe laser (594nm, 2mW) R-HeNe laser (633nm, 5mW)		BD laser (405nm, 36mW, variable) Laser diode (440nm, 20mW, variable) Ar laser (488nm, 10mW) Ar laser (488nm, 25mW) Ar laser (457nm/477nm/488nm/514nm, 40mW) Solid-state laser (488nm, 20mW) G-HeNe laser (543nm, 1mW) Solid-state laser (561nm, 10mW) Semiconductor laser (640nm, 10mW)	
	Maximum loading number	4	3	4	
	Laser control	AOM/AOTF/Halving laser controller	AOM	AOTF	
	Laser shutter	Motorized mechanical shutter (each laser)			
Confocal pinhole	Variable	Motorized switching			
Standard fluorescence detector	Number of channels	2 channels/3 channels 457 (440), 408/514, 405/488, 457 (440)/514, 488/543, 488/594, 408/488/543, 405/488/561, 488/543/633	3 channels 457 (440), 408/514, 405/488, 457 (440)/514, 488/543, 488/594, 408/488/543, 405/488/561, 488/543/633	3 channels 457 (440), 405/488, 408/514, 457 (440)/514, 488/543, 408/488/543, 405/488/561, 405/488/543/640, 405/488/561/640, BS20/80	
		457 (440), 488, 405/488, 408/514, 457 (440)/514, 488/543, 488/594, 408/488/543, 408/488/561, 488/543/633	457 (440), 488, 405/488, 408/514, 457 (440)/514, 488/543, 488/594, 408/488/543, 408/488/561, 488/543/633	457 (440), 488, 405/488, 408/514, 457 (440)/514, 488/543, 405/488/543, 405/488/561, 488/543/640, 488/561/640, 405/488/543/640, 405/488/561/640	
Scanning specifications for a standard fluorescence detector	Display mode	160x16 to 2048x2048 pixels			
	Scanning speed	Standard: 1fps (512x512 pixels) Bi-directional scanning: 1.4fps (512x512 pixels)			
	Scanning mode	2D: X-Y, X-T 3D: X-Y-Z, X-Y-T 4D: X-Y-Z-T ROI scan (AOM necessary) Multi-point time-lapse within single screen (X-Y-Z-T-Point) Multi-point time-lapse (X-Y-Z-T-Point, motorized YX stage necessary) Point scan Scan rotation (-90 to 90°, 1° step)			
	Data acquisition for applications	FRET, FLAP, FLIP			
Spectral detector	CLEM compatibility	Compatible (when laser is controlled by optional AOM)	Compatible	Not compatible	
	Number of channels	—	32 channels		
	1st dichroic mirror	—	20/80 Beam Splitter		
	Corresponding wavelength	—	400-750nm		
	Wavelength resolution	—	2.5/5/10nm (switchable)		
Minimum wavelength step	—	0.2nm			
Scanning specifications for a spectral detector	Display mode	160x160 to 1024x1024 pixels			
	Scanning speed	Standard: 0.5fps (512x512 pixels, 32-channel simultaneous recording)			
	Scanning mode	3D: X-Y-λ 4D: X-Y-Z-λ, X-Y-t-λ 5D: X-Y-Z-t-λ Multi-point time-lapse within single screen (X-Y-Z-T-λ-Point) Multi-point time-lapse scan (X-Y-Z-T-λ-Point, motorized YX stage necessary) ROI scan (AOTF or AOM necessary) Point scan Scan rotation (-90 to 90°, 1° step)			
	Data acquisition for applications	FRET, FLAP, FLIP, etc.			
Diascopic detector	1 channel (motorized or manual)				
Optical zoom	1x-1000x (continuous variable)				
FOV	Square inscribed in a ∅18mm circle				
Image bit depth	12 bits				
Compatible microscopes	Upright type	ECLIPSE 80i/90i			
	Inverted type	ECLIPSE Ti-E/U, ECLIPSE TE2000-E/U/PFS			
	Fixed stage type	ECLIPSE FN1			
Z-axis control	Built-in microscope motor	ECLIPSE 90i, ECLIPSE Ti-E, ECLIPSE TE2000-E/PFS			
	External motor	Stepping motor, 50nm step			
Control computer	OS	Windows® XP Professional			
	Interface	Ethernet			
Analysis functions	2D, 3D, 4D, time-lapse, etc.		Spectra, Fluorescence unmixing, 2D, 3D, 4D, time-lapse, etc.		
Power	BD laser (408nm, 38mW/440nm, 15mW)	15W (single phase AC 100V, 0.15A, with earth)			
	Ar laser	1500W (single phase AC 100V, 15A, with earth)			
	Solid-state laser (488nm, 20mW)	140W (single phase AC 100V, 1.4A, with earth)			
	G-HeNe laser (543nm, 1/2mW)	40W (single phase AC 100V, 0.4A, with earth)			
	Y-HeNe laser (594nm, 2mW)	40W (single phase AC 100V, 0.4A, with earth)			
	Solid-state laser (561nm, 10mW)	40W (single phase AC 100V, 0.4A, with earth)			
	R-HeNe laser (633nm, 10mW)	40W (single phase AC 100V, 0.4A, with earth)			
	LD laser (640nm, 10mW)	15W (single phase AC 100V, 0.15A, with earth)			
	Confocal system	830W (single phase AC 100V, 8.3A, with earth) (PC, monitor, C1 controller, AOM controller)		910W (single phase AC 100V, 9.1A, with earth) (PC, monitor, C1 controller, 4-laser unit)	
	Fluorescence microscope	630W (Ti-E)			
Installation condition	Temperature 5-35°C, humidity 65% (RH) or less (non-condensing)				

Please ask Nikon or your local distributor about combining laser types.

Basic Principle of Confocal Microscopy



- Extremely high resolving power in the Z-axis direction (depth) makes confocal observation ideal for observing thick specimens such as embryos and eggs.
- Fluorescent-dyed specimens can be rendered in 3D.
- Extremely high S/N ratio images are obtainable.



Non-confocal microscope image



Confocal microscope image

With confocal pinhole observation, high S/N ratio images can be captured that have superior Z-axis resolution compared to that of ordinary fluorescence (non-confocal) observation. Minute structures deep within a thick specimen are clearly visible.

Images and specimens courtesy of:

Cover image (top): Mouse's whole brain stained with mCB (mouse monoclonal anti-calbindin; green), rPV (rabbit polyclonal anti-parvalbumin; red) and nucleus (Hoechst; blue)—Assistant Prof. Kazunori Toida, Department of Anatomy and Cell Biology, Institute of Health Biosciences, the University of Tokushima Graduate School

Cover image (middle): Three-dimensional reconstruction image of mouse hippocampus (GFP: inhibitory neurons, green, YFP: excitatory neurons, magenta) through volume rendering after spectra unmixing—Dr. Masayuki Sekiguchi, Department of Degenerative Neurological Diseases, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan

Cover image (bottom): HepG2 cells infected with dengue virus. Fluorescence reagent: Dengue virus (FITC), Nuclei (DAPI), Clathrin (Texas Red)—Ang Firzan and Dr. Justin Chu, Department of Microbiology, National University of Singapore

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.



Specifications and equipment are subject to change without any notice or obligation on the part of the manufacturer. March 2009 ©2008-09 NIKON CORPORATION



WARNING

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

* Monitor images are simulated.

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