FLUOVIEW website

www.olympusfluoview.com

- **OLYMPUS CORPOARATION is ISO9001/ISO14001 certified.**
- **Illumination devices for microscope have suggested lifetimes. Periodic inspections are required. Please visit our web site for details.**

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- Images on the PC monitors are simulated.
- Specifications and appearances are subject to change without any notice or obligation on the part of the manufacturer.

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Confocal Laser Scanning
Biological Microscope

FV1000

FLUOVIEW

FLUOVIEW—Always Evolving

FLUOVIEW—From

Olympus is Open

FLUOVIEW—More Advanced than Ever

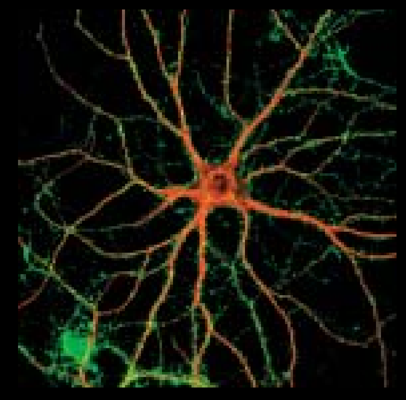
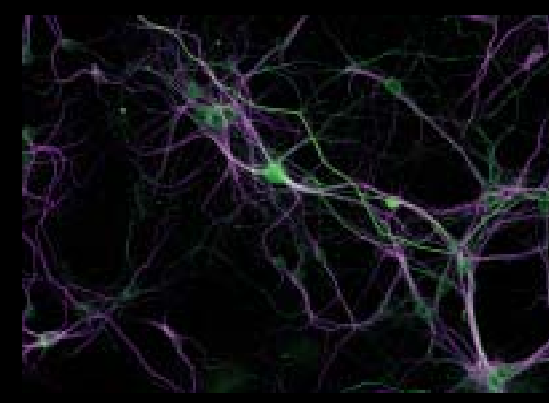
The Olympus FLUOVIEW FV1000 confocal laser scanning microscope delivers efficient and reliable performance together with the high resolution required for multi-dimensional observation of cell and tissue morphology, and precise molecular localization. The FV1000 incorporates the industry's first dedicated laser light stimulation scanner to achieve simultaneous targeted laser stimulation and imaging for real-time visualization of rapid cell responses. The FV1000 also measures diffusion coefficients of intracellular molecules, quantifying molecular kinetics. Quite simply, the FLUOVIEW FV1000 represents a new plateau, bringing "imaging to analysis."

Olympus continues to drive forward the development of FLUOVIEW microscopes, using input from researchers to meet their evolving demands and bringing "imaging to analysis."

Quality Performance with Innovative Design
FV10i

Imaging to Analysis

ing up New Worlds

Advanced FLUOVIEW Systems Enhance the Power of Your Research

Technology

Superb Optical Systems Set the Standard for Accuracy and Sensitivity.

Two types of detectors deliver enhanced accuracy and sensitivity, and are paired with a new objective with low chromatic aberration, to deliver even better precision for colocalization analysis.
These optical advances boost the overall system capabilities and raise performance to a new level.

Application

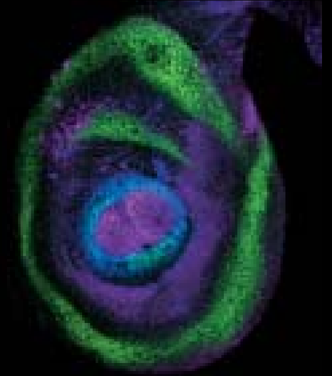
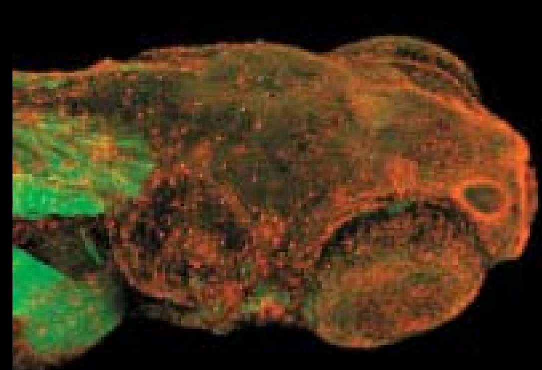
Imaging, Stimulation and Measurement— Advanced Analytical Methods for Quantification.

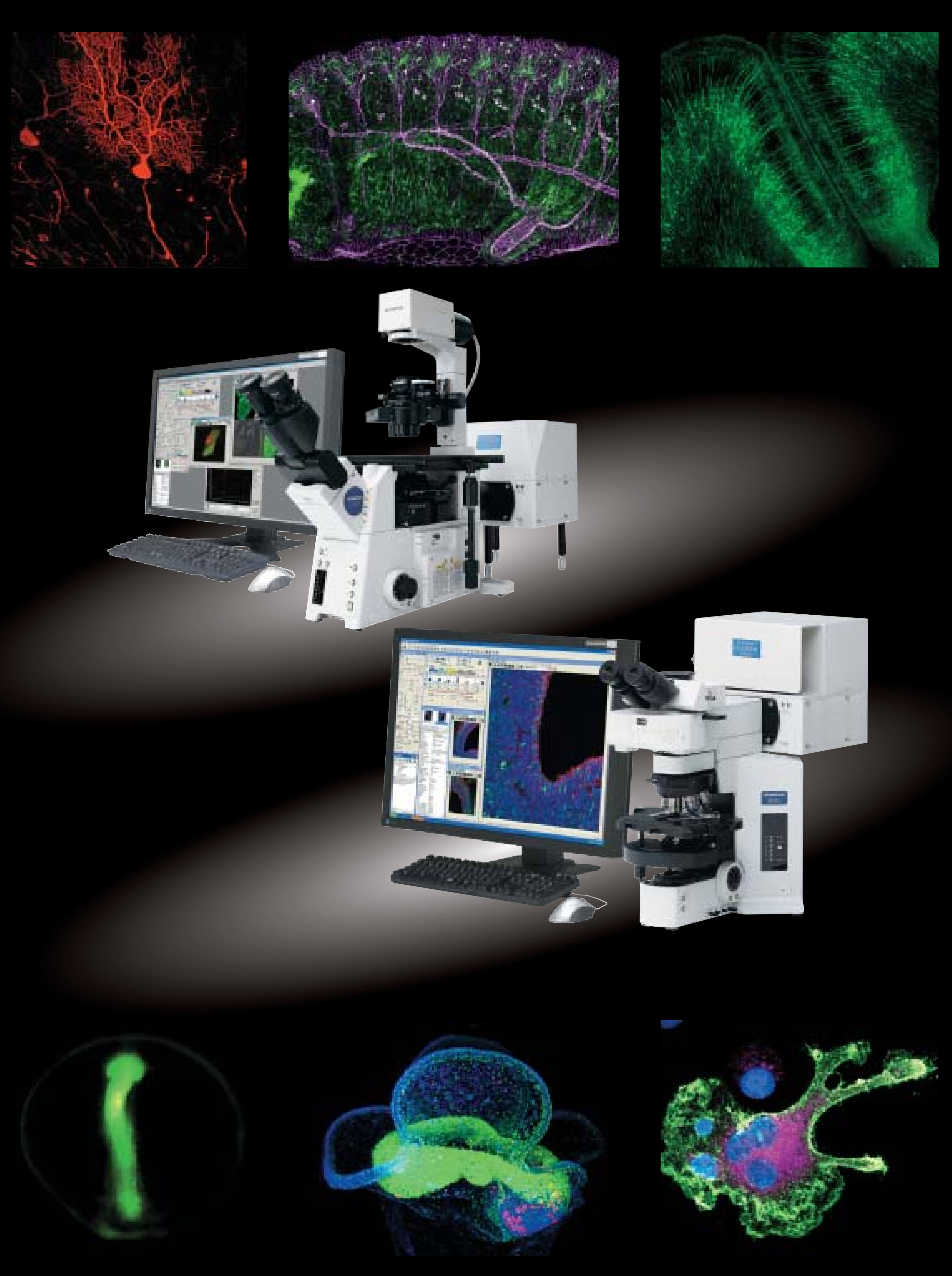
Now equipped to measure the diffusion coefficients of intracellular molecules, for quantification of the dynamic interactions of molecules inside live cell.
FLUOVIEW opens up new worlds of measurement.

Expandability

Evolving Systems Meet the Demands of Your Application.

Upgradeable system with optional hardware and software to meet the demands of your research.



Excellent Precision, Sensitivity and Stability.
FLUOVIEW Enables Precise, Bright Imaging with Minimum Phototox

Main scanner
Barrier filter
Grating
Grating
Confocal pinhole
Laser combiner
LD635
LD559
LD473
LD405
AOTF
AOTF
Broadband fiber *
Broadband fiber

Laser combiner/Fiber

Diode Laser

Greater stability, longer service life and lower operating cost are achieved using diode lasers.

Laser Feedback Control

Scanner unit is equipped with laser power monitor for feedback control enhancing stable laser output.

Laser Compatibility

Diode laser :
405 nm, 440 nm, 473 nm, 559 nm, 635 nm
Gas laser :
Multi-line Ar laser (458 nm, 488 nm, 515 nm)
HeNe(G) laser (543 nm)

Broadband Fiber

Broadband fiber connection for 405–635 nm lasers, to achieve an ideal point light source with minimal color shift and position shift between images.

Laser Combiner

Two versions available.

•Single fiber-type combiner is used for main scanner FV1000 with up to six lasers, ranging from 405 to 635 nm.
•Dual fiber-type combiner is used for laser light stimulation with main and SIM scanner FV1000.

Scanners/Detection

High Sensitivity Detection System

High-sensitivity and high S/N ratio optical performance is achieved through the integration of a pupil projection lens, use of a high sensitivity photomultiplier tube and an analog processing circuit with minimal noise. Enables high S/N ratio image acquisition with minimal laser power to reduce phototoxicity.

Up to Four PMT Channels

Three integrated confocal PMT detectors, and optional module with fourth confocal PMT expandable up to four PMT channels.

Spectral Scanning Unit
Filter Scanning Unit

Two Versions of Light Detection System

• Spectral detection for high-precision spectroscopy with 2 nm resolution.
• Filter detection equipped with high quality filter wheels.

icity.

SIM Scanner *

* Option

PMT

PMT

PMT

Microscope

Galvanometer scanning mirrors

Specimen

UIS2 objectives

Galvanometer scanning mirrors

Pupil projection lens

Optical System

Motorized Microscopes

Compatible with Olympus IX81 inverted microscope, BX61WI focusing nosepiece and fixed-stage upright microscope, and BX61 upright microscope.

IX81

BX61

UIS2 Objectives

Olympus UIS2 objectives offer world-leading, infinity-corrected optics that deliver unsurpassed optical performance over a wide range of wavelengths.

High S/N Ratio Objectives with Suppressed Autofluorescence

Olympus offers a line of high numerical aperture objectives with improved fluorescence S/N ratio, including objectives with exceptional correction for chromatic aberration, oil- and water-immersion objectives, and total internal reflection fluorescence (TIRF) objectives.

Samples and Specimens

Supports a Wide Range of Samples and Specimens

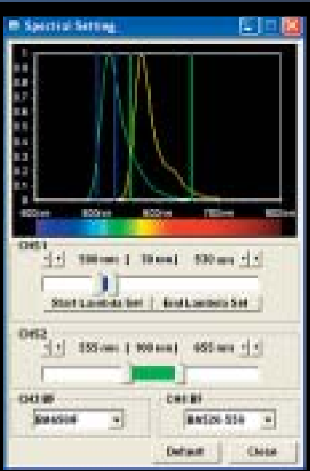
Tissue culture dishes, slide chambers, microplates and glass slides can be used with live cells and fixed specimens.

Two Versions of Light Detection System that Set New Standards for Optical Performance.

Spectral Based Detection

Flexibility and High Sensitivity

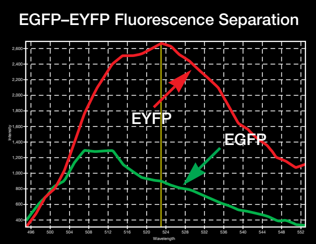
Spectral detection using gratings for 2 nm wavelength resolution and image acquisition matched to fluorescence wavelength peaks. User adjustable bandwidth of emission spectrum for acquiring bright images with minimal cross-talk.

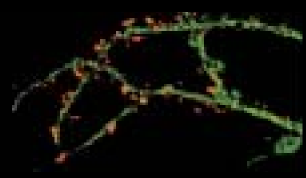


Precise Spectral Imaging

The spectral detection unit uses a grating method that offers linear dispersion compared with prism dispersion. The unit provides 2 nm wavelength resolution to high-sensitivity photomultiplier tube detectors. Fluorescence separation can be achieved through unmixing, even when cross-talk is generated by multiple fluorescent dyes with similar peaks.

EGFP–EYFP Fluorescence Separation





EGFP (dendrite) — EYFP (synapse)
XYλ
Wavelength detection range: 495 nm–561 nm in 2 nm steps
Excitation wavelength: 488 nm

Courtesy of: Dr. Shigeo Okabe
Department of Anatomy and Cell Biology,
Tokyo Medical and Dental University

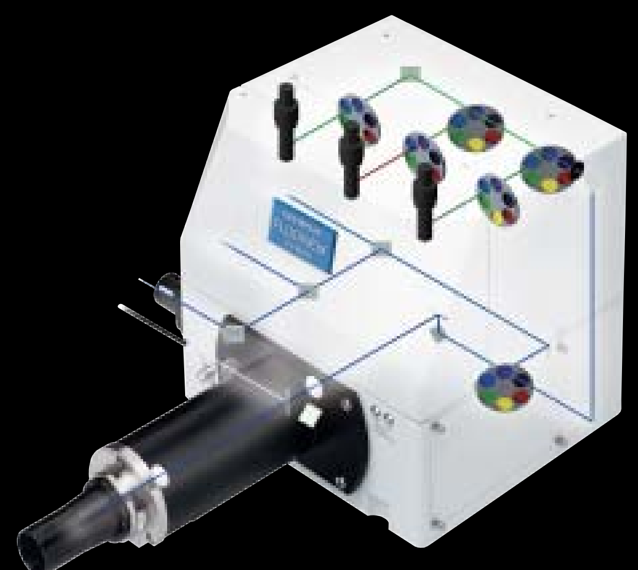
Filter Based Detection

Enhanced Sensitivity

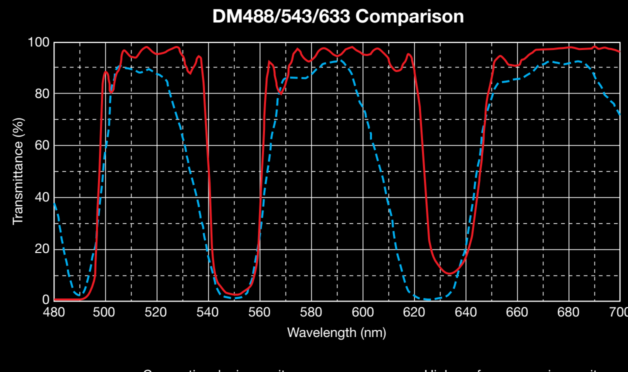
Three-channel scan unit with detection system featuring hard coated filter base. High-transmittance and high S/N ratio optical performance is achieved through integration of a pupil projection lens within the optics, the use of a high sensitivity photomultiplier and an analog processing circuit with minimal noise.

High-Performance Filters Deliver Outstanding Separation

Special coatings deliver exceptionally sharp transitions to a degree never achieved before, for acquisition of brighter fluorescence images.



DM488/543/633 Comparison



SIM Scanner Unit for Simultaneous Laser Light Stimulation and Imaging.

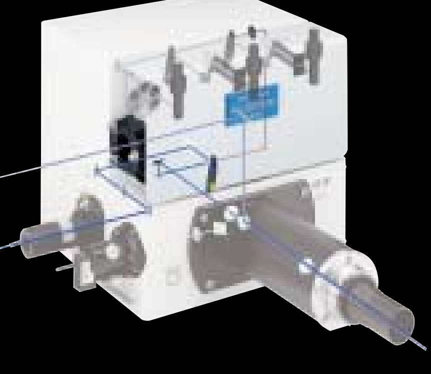
SIM (Simultaneous) Scanner Unit

Combines the main scanner with a dedicated laser light stimulation scanner for investigating the trafficking of fluorescent-labeled molecules and marking of specific live cells.

Simultaneous Laser Light Stimulation and Imaging

Performs simultaneous laser light stimulation and imaging to acquire images of immediate cell responses to stimulation in photobleaching experiments.

Lasers are used for both imaging and laser light stimulation.

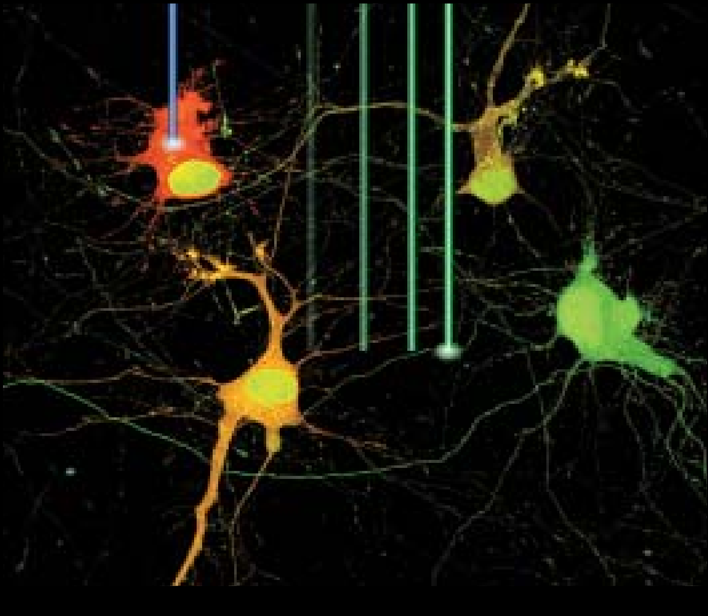


Branching of laser in laser combiner.

LD635
LD559
LD473
LD405
AOTF
AOTF

Modifiable Stimulation Area During Imaging

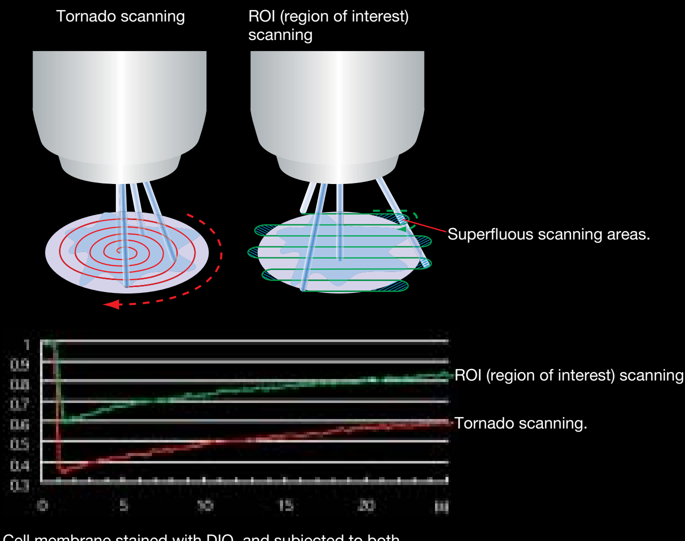
The stimulation area can be moved to a different position on the cell during imaging, providing a powerful tool for photoactivation and photoconversion experiments.



Unique "Tornado" Scanning for Efficient Bleaching

Conventional raster scanning does not always complete photobleaching quickly. Tornado scanning greatly improves bleaching efficiency by significantly reducing unnecessary scanning.

*Tornado scanning only available for SIM scanner.



Cell membrane stained with DIO, and subjected to both conventional ROI and tornado scanning.

Wide Choice of Bleaching Modes

Various scan modes can be used for both the observation area and stimulation area. Enables free-form bleaching of designated points, lines, free-lines, rectangles and circles.

Multi-Purpose Laser Combiner

All lasers can be used for both Imaging and laser light stimulation.

LD405 / LD635 / AOTF / AOTF / LD473 / LD559

Laser Sharing with Main Scanner

Dual fiber laser combiner provides laser sharing between the SIM scanner and main scanner, eliminating the need to add a separate laser for stimulation.

New Objective with Low Chromatic Aberration Delivers World-Leading Imaging Performance.

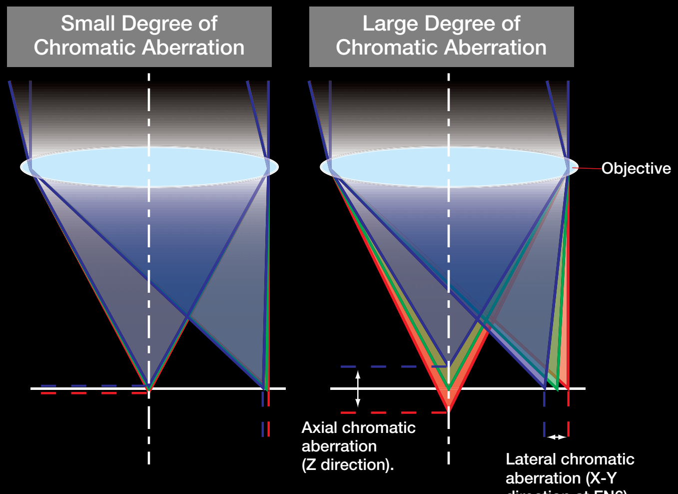
NEW Low Chromatic Aberration Objective

Best Reliability for Colocalization Analysis

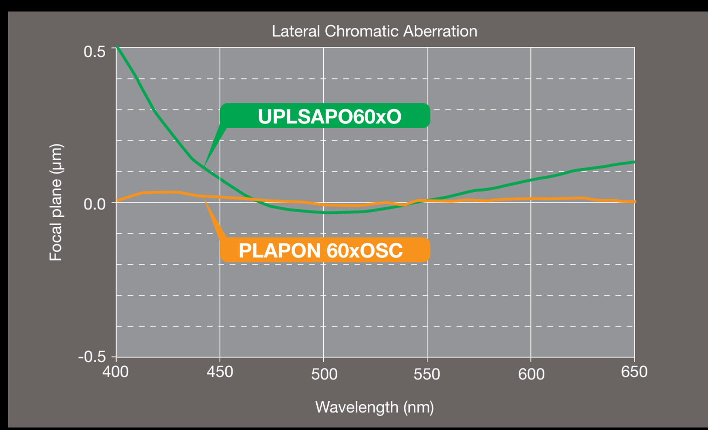
A new high NA oil-immersion objective minimizes chromatic aberration in the 405–650 nm region for enhanced imaging performance and image resolution at 405 nm. Delivers a high degree of correction for both lateral and axial chromatic aberration, for acquisition of 2D and 3D images with excellent and reliable accuracy, and improved colocalization analysis. The objective also compensates for chromatic aberration in the near infrared up to 850 nm.

Low Chromatic Aberration Objective: PLAPON60xOSC
Magnification: 60x
NA: 1.4 (oil immersion)
W.D.: 0.12 mm
Chromatic aberration compensation range: 405–650 nm
Optical data provided for each objective.

Lateral and Axial Chromatic Aberration



Chromatic Aberration Comparison for PLAPON 60xOSC and UPLSAPO 60xO



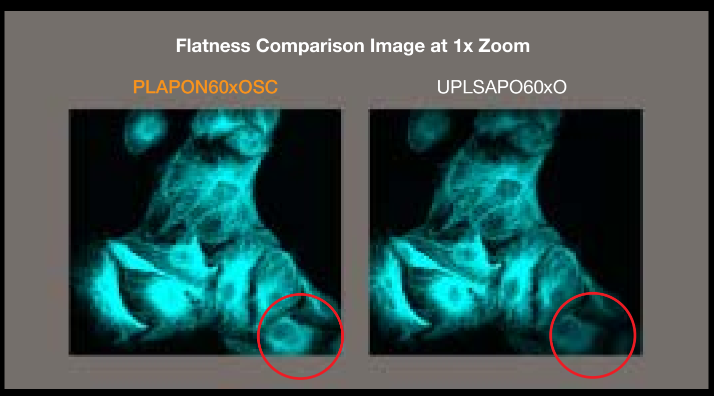
*Chromatic aberration values are design values and are not guaranteed values.

Performance Comparison of PLAPON 60xOSC and UPLSAPO 60xO

	PLAPON60xOSC	UPLSAPO60xO
Axial chromatic aberration (Z direction) Compared for PSF fluorescent beads (405 nm, 633 nm).	Approx. 0 μm	Approx. 0.5 μm
Lateral chromatic aberration (X-Y direction) Compared for PSF fluorescent beads (405 nm, 488 nm, 633 nm).	Approx. 0.1 μm	Approx. 0.2 μm
3D image Tubulin in Ptk2 cells labeled with two colors (405 nm, 635 nm) and compared.		

Improved Flatness and Resolution at 405 nm

Better flatness reduces the number of images for tiling.



Exceptional Resolution for Imaging of Cytoplasmic Membrane and Areas Deep Within Living Specimen.

TIRFM (Total Internal Reflection Fluorescence Microscope) System

Switchable between Confocal and TIRFM Imaging

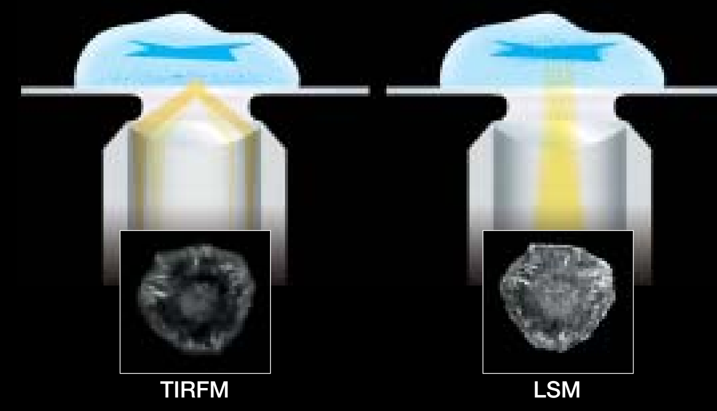
Switchable between confocal and TIRFM imaging for localization of proteins on the cytoplasmic membrane surface and acquisition of sectioning images within cells.

Software Control of TIRF Illumination

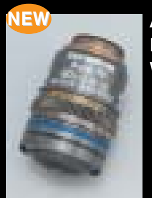
Built-in laser provides TIRF illumination. Software can be used to tune the angle of incidence of excitation light and calculates the penetration depth of the evanescent wave based on the TIRF objective used.

High-Numerical Aperture Objectives for TIRF Illumination

A line of high-numerical aperture (NA) objectives is available for TIRF illumination.



GFP—Pak—K298A in HeLa cells.
Courtesy of Dr.J M Dong of sGSK-NRP laboratory, Singapore



APON60xOTIRF
NA : 1.49 (oil immersion)
WD: 0.1 mm

UAPON100xOTIRF
NA : 1.49 (oil immersion)
WD: 0.1 mm

UAPON150xOTIRF
NA : 1.45 (oil immersion)
WD: 0.08 mm

Apo100xOHR
NA : 1.65 (oil immersion)
WD: 0.1 mm
(Customized cover glass and immersion oil)

FV1000MPE Multiphoton Excitation System

Brighter and Deeper Imaging with Finer Resolution

The FV1000 is upgradeable to multiphoton excitation capability by adding a dedicated laser and multiphoton optical system. Optical design is optimized for multiphoton principles for brighter imaging of features deep within living specimens, at higher resolutions than previously possible.

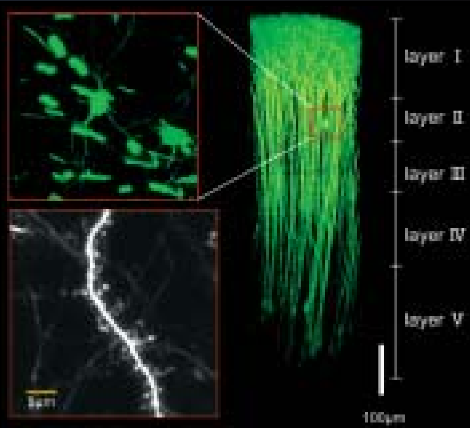
Special Multiphoton Objective with Outstanding Brightness and Resolution

Olympus offers a high NA water-immersion objective designed for a wide field of view, with improved transmittance at near-infrared wavelengths. A correction collar compensates for spherical aberration caused by differences between the refractive indices of water and specimens, forming the optimal focal spot even in deep areas, without loss of energy density. The objective is designed to collect scattered light over a wide field of view for maximum image brightness.

XLPLN25xWMP
Magnifications : 25x
NA : 1.05 (water immersion)
W.D. : 2.0 mm

Multiphoton Laser Light Stimulation

Adding a multiphoton laser to the SIM scanner enables multiphoton laser light stimulation or uncaging confined to the focal volume.



3-dimensionally constructed images of neurons expressing EYFP in the cerebral neocortex of a mouse under anesthesia.

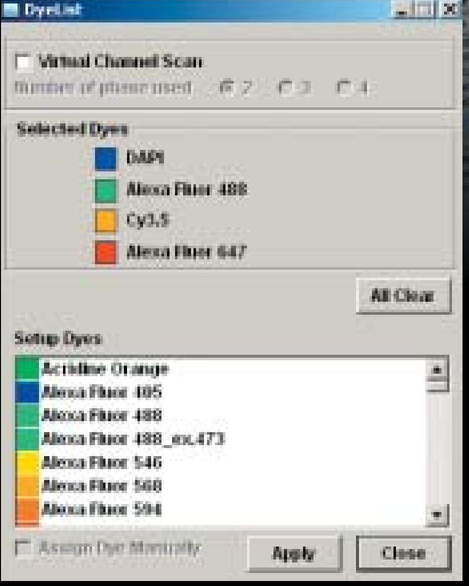
Courtesy of:
Hiroaki Waki, Tomomi Nemoto, and Junichi Nabekura
National Institute for Physiological Sciences, National Institutes of Natural Sciences, Japan

* The FLUOVIEW FV1000MPE is a class 4 laser product.

User-Friendly Software to Support Your Research.

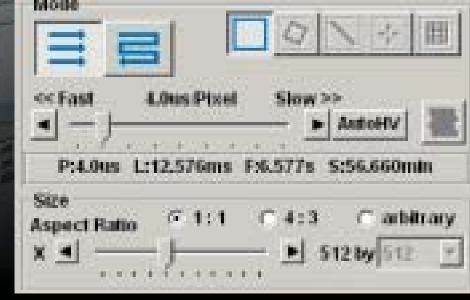
Configurable Emission Wavelength

Select the dye name to set the optimal filters and laser lines.



Wide Choice of Scanning Modes

Several available scanning modes including ROI, point and high-speed bidirectional scanning.



Configurable Excitation Laser Power

Easily adjust the optimum laser power for each specimen (live cells and fixed specimens).

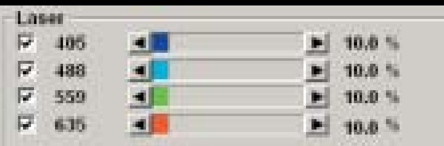
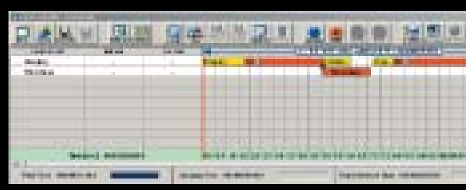


Image Acquisition by Application

User-friendly icons offer quick access to functions, for image acquisition according to the application (XYZ, XYT, XYZT, XYλ, XYλT).

Time Controller

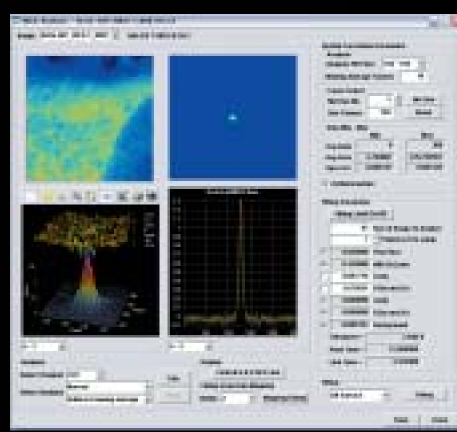
Precisely synchronizes different experimental protocols including FRAP, FLIP and FRET by acceptor photo-bleaching and time-lapse. Save and open settings for later use.



Optional Software with Broad Functionality.

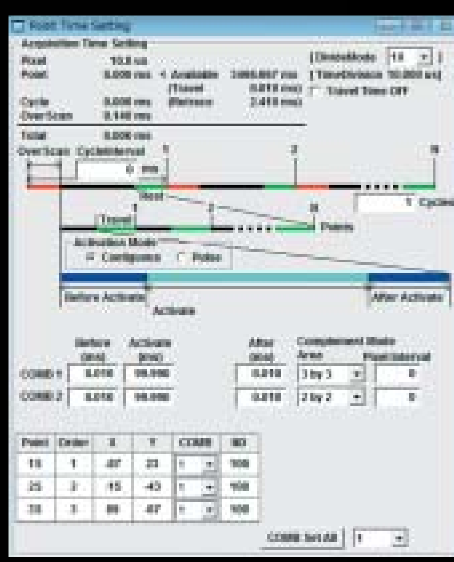
Diffusion Measurement Package

For analysis of intracellular molecular interactions, signal transduction and other processes, by determining standard diffusion coefficients. Supports a wide range of diffusion analysis using point FCS, RICS and FRAP.



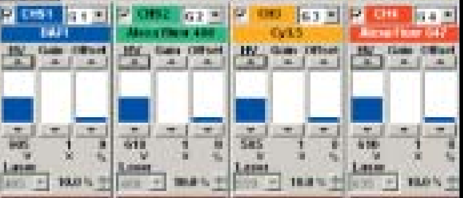
Multi Stimulation Software

Configure multiple stimulation points and conditions for laser light stimulation synchronized with imaging, for detailed analysis of the connectivity of cells within the stimulation area.



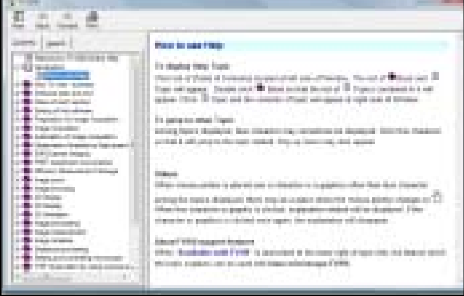
Re-Use Function

Open previously configured scanning conditions and apply them to new or subsequent experiments.



Help Guide

Comprehensive help guide describes the functions and usage for each command, and overall sequence of operations.



Multi-Area Time-Lapse Software

Multi-Area Time-Lapse

Software control of the motorized XY stage enables multiple measurement points in glass slides, 35 mm dishes or individual microplate wells. Repeated imaging of multiple cells improves the statistical power of time-lapse experiments.

Mosaic Imaging

A motorized XY stage is programmed with the use of a high-magnification objective to acquire continuous images from adjacent fields of view, to assemble a single, high resolution image covering a wide area. Three-dimensional images can also be assembled using XYZ acquisition.

Broad Application Support and Sophisticated Experiment Control.

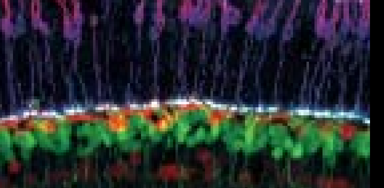
Measurement
Diffusion measurement and molecular interaction analysis.

Light Stimulation
FRAP/FLIP/Photoactivation/Photoconversion/Uncaging.

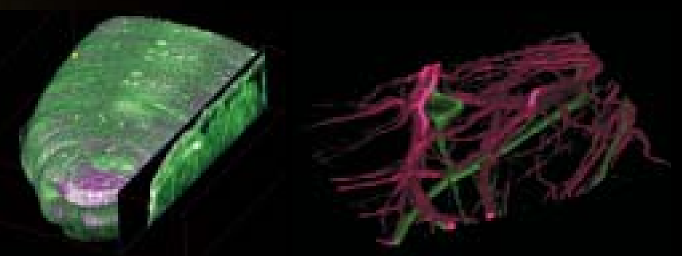
Multi-Dimensional Time-Lapse
Long-term and multiple point.

3D Mosaic Imaging
High resolution images stitched to cover a large area.

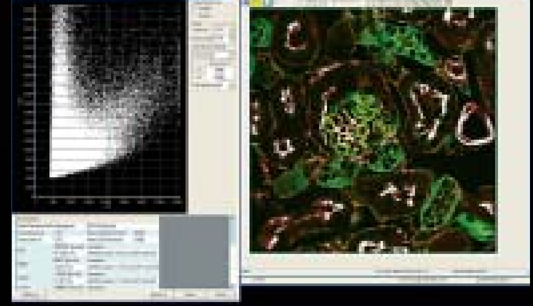
Multi-Color Imaging
Full range of laser wavelengths for imaging of diverse fluorescent dyes and proteins.



3D/4D Volume Rendering
One-click 3D/4D image construction from acquired XYZ/T images.
Change the angle of 3D image with a single click.



Colocalization
Configurable threshold values for fluorescence intensities on the scatterplot.
Accurate colocalization statistics and visualization of colocalized area on image.



FRET
Configuration wizard simplifies the setting of FRET experimental procedures.
Optimal laser excitation wavelengths for CFP/YFP FRET.

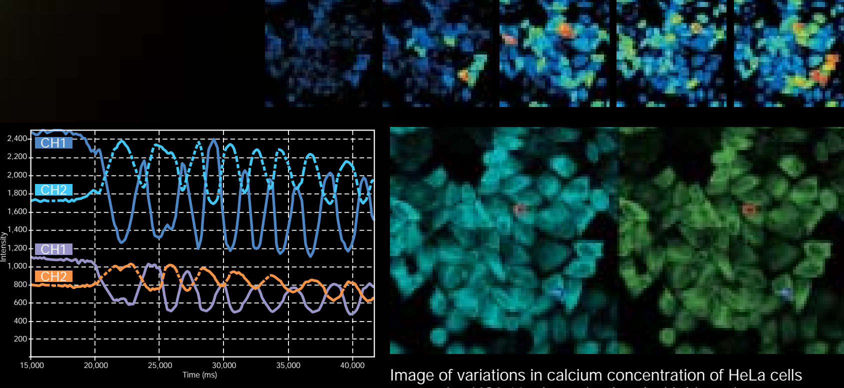
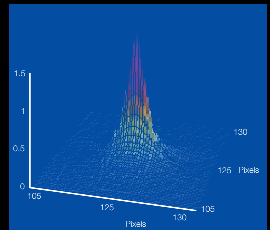


Image of variations in calcium concentration of HeLa cells expressing YC3.60 when stimulated with histamine.

Reference:
Takeharu Nagai, Shuichi Yamada, Takashi Tominaga, Michinori Ichikawa, and Atsushi Miyawaki 10554-10559, PNAS, July 20, 2004, vol. 101, no.29



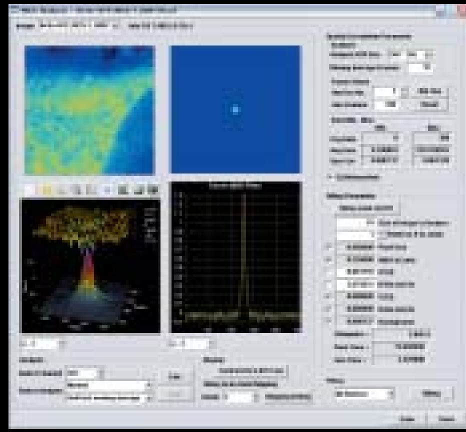
Diffusion Measurement Package

This optional software module enables data acquisition and analysis to investigate the molecular interaction and concentrations by calculating the diffusion coefficients of molecules within the cell.
Diverse analysis methods (RICS/ccRICS, point FCS/point FCCS and FRAP) cover a wide range of molecular sizes and speeds.

RICS—Raster Imaging Correlation Spectroscopy

Raster image correlation spectroscopy (RICS) is a new method for analyzing the diffusion and binding dynamics of molecules in an entire, single image. RICS uses a spatial correlation algorithm to calculate diffusion coefficients and the number of molecules in specified regions.

Cross correlation RICS (ccRICS) characterizes molecular interactions using fluorescent-labeled molecules in two colors.

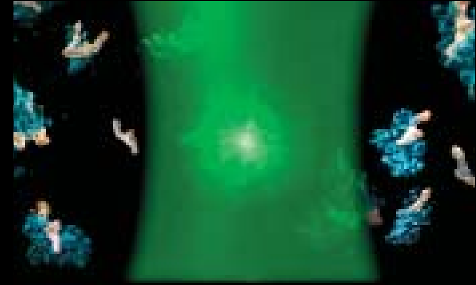


point FCS—Point scan Fluorescence Correlation Spectroscopy

point scan fluorescence correlation spectroscopy (point FCS) analyzes intensity fluctuations caused by diffusion or binding/unbinding interactions of a protein complex. point FCS uses an auto correlation function to carry out operations on fluorescence signals obtained by continuous scanning of a single pixel on the screen.

point scan fluorescence cross-correlation spectroscopy (point FCCS) analyzes the fluctuation of fluorescent-labeled molecules in two colors. The coincidence of fluctuations occurring in two detection channels shows that the two proteins are part of the same complex.

point FCS and point FCCS can now be performed with a standard detector, eliminating the need for a special high-sensitivity detector.



FRAP Analysis

The Axelrod analytical algorithm is installed as a FRAP analysis method. The algorithm is used to calculate diffusion coefficients and the proportions of diffusing molecules.

Analytical methods according to molecule diffusion speeds

	Small molecules in solution	Proteins in solution	Diffusion of proteins in cell	Lateral diffusion in cell membrane	Protein trafficking	Molecular complex formation, aggregation
Diffusion Coefficient ($\mu m^2/s$)	> 100	~ 100	1 ~ 100	< 0.1	< 0.01	<< 0.001

Capable range of measurement

point FCS

RICS

FRAP

RICS Application and Principles

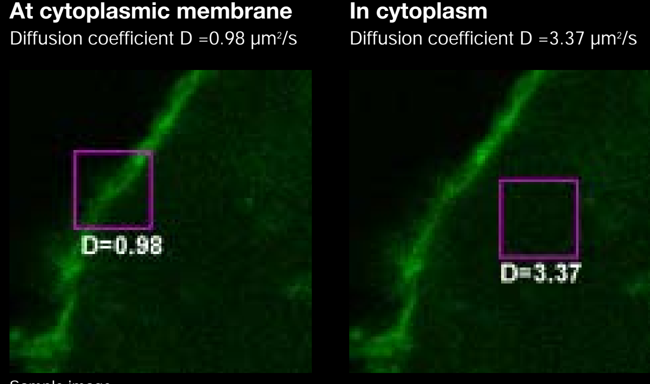
Comparison of Diffusion Coefficients for EGFP Fusion Proteins Near to Cell Membranes and In Cytoplasm

RICS can be used to designate and analyze regions of interest based on acquired images.
EGFP is fused at protein kinase C (PKC) for visualization, using live cells to analyze the translocation with RICS. The diffusion coefficient close to cell membranes was confirmed to be lower than in cytoplasm, after stimulation with phorbol myristate acetate (PMA). This is thought to be from the mutual interaction between PKC and cell membrane molecules in cell membranes.

In addition to localization of molecules, RICS analysis can simultaneously determine changes in diffusion coefficient, for detailed analysis of various intracellular signaling proteins.

At cytoplasmic membrane
Diffusion coefficient D =0.98 μm²/s

In cytoplasm
Diffusion coefficient D =3.37 μm²/s

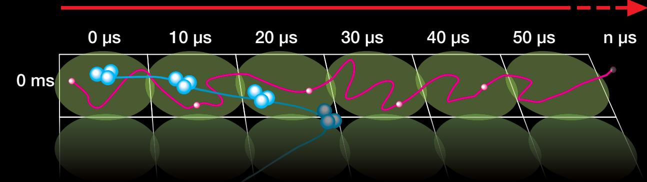


Sample image:
HeLa cells expressing EGFP fusion PKC (after PMA stimulation)

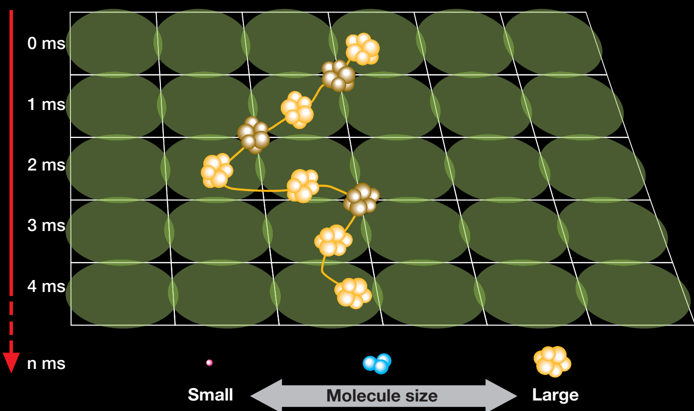
RICS Principle

Molecules of different sizes diffuse at different speeds within cells. Small molecules move faster, compared with large molecules that move relatively slowly. The FV1000 acquires information on the movement of these diffusing fluorescent-labeled molecules as image data, together with morphological information about the cell. The image data obtained for each pixel was sampled at different times, so the data for each pixel is affected by the passage of time, in addition to its spatial XY information. By analyzing this image data with a new statistical algorithm for spatial correlation, the diffusion coefficients and molecule counts can be calculated for molecules moving within the cell.

Scan in X-Axis Direction



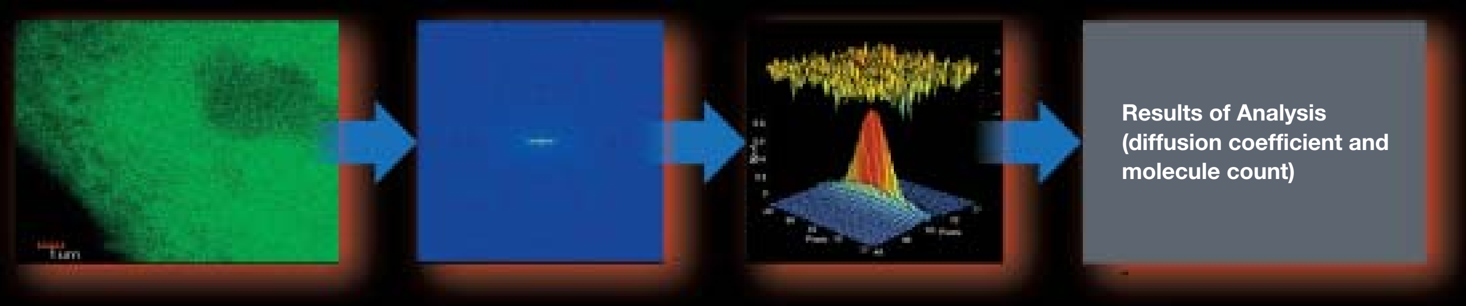
Scan in Y-Axis Direction



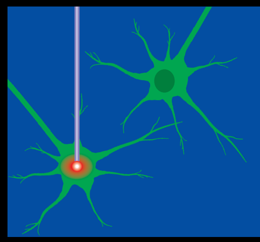
Spatial Correlation Algorithm

When the spatial correlation algorithm is applied between pixels, a higher correlation is obtained as the speed of movement of the molecule nears the scanning speed. When calculating the spatial correlation in the X-direction, because the scanning speed in the X-direction is fast, a higher correlation is obtained for fast-moving molecules than for slow-moving molecules. When the scanning speed in the Y-direction is slow, a higher correlation is obtained for slow-moving molecules. RICS using LSM images scans in both X- and Y-directions, so it can be used to analyze the movements of a wide range of molecules, both fast and slow.

RICS Analysis Method



LSM Image — Spatial Correlation — Theoretical Formula Used for Fitting Calculation

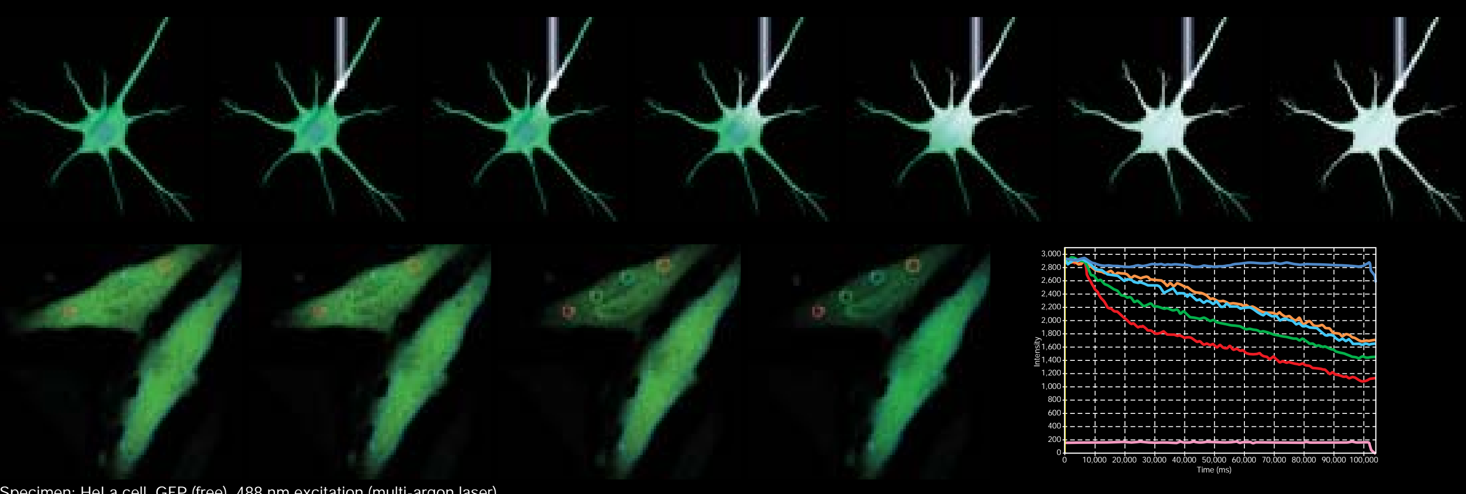


Laser Light Stimulation

The SIM scanner system combines the main scanner with a laser light stimulation scanner.
Control of the two independent beams enables simultaneous stimulation and imaging, to capture reactions during stimulation.
Multi-stimulation software is used to continuously stimulate multiple points with laser light for simultaneous imaging of the effects of stimulation on the cell.

FLIP—Fluorescence Loss in Photobleaching

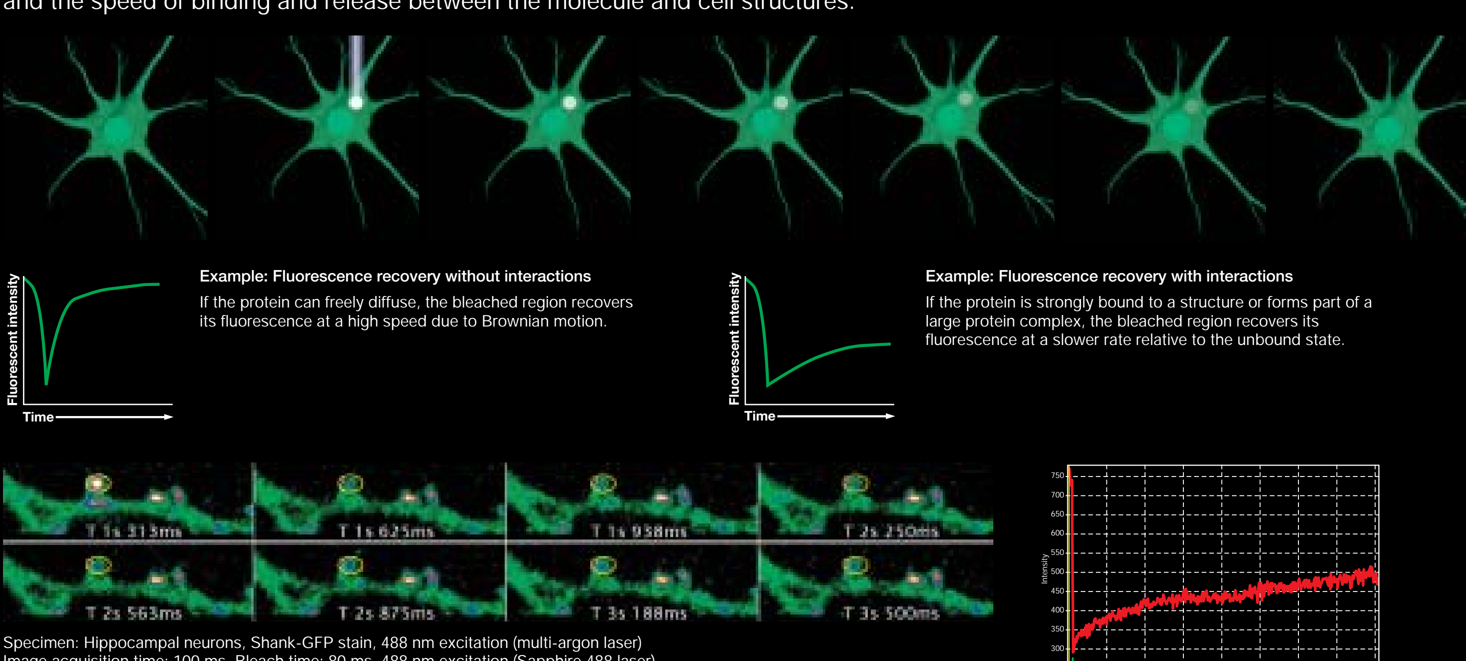
Fluorescence loss in photobleaching (FLIP) combines imaging with continuous bleaching of a specific region to observe the diffusion of a target protein within a cell. The changes in the image over time make it possible to observe the location of structural bodies that inhibit the diffusion of the molecule.



Specimen: HeLa cell, GFP (free), 488 nm excitation (multi-argon laser)
Image acquisition time: 100 ms/ bleach time: 100 s continuously, 405 nm bleaching

FRAP—Fluorescence Recovery after Photobleaching

Exposure of fluorescent-labeled target proteins to strong laser light causes their fluorescence to fade locally. Fluorescence recovery after photobleaching (FRAP) is used to observe the gradual recovery of fluorescence intensity caused by protein diffusion from the area surrounding the bleached region. By examining the resulting images, it is possible to characterize the diffusion speed of the molecule, and the speed of binding and release between the molecule and cell structures.



Example: Fluorescence recovery without interactions

If the protein can freely diffuse, the bleached region recovers its fluorescence at a high speed due to Brownian motion.

Example: Fluorescence recovery with interactions

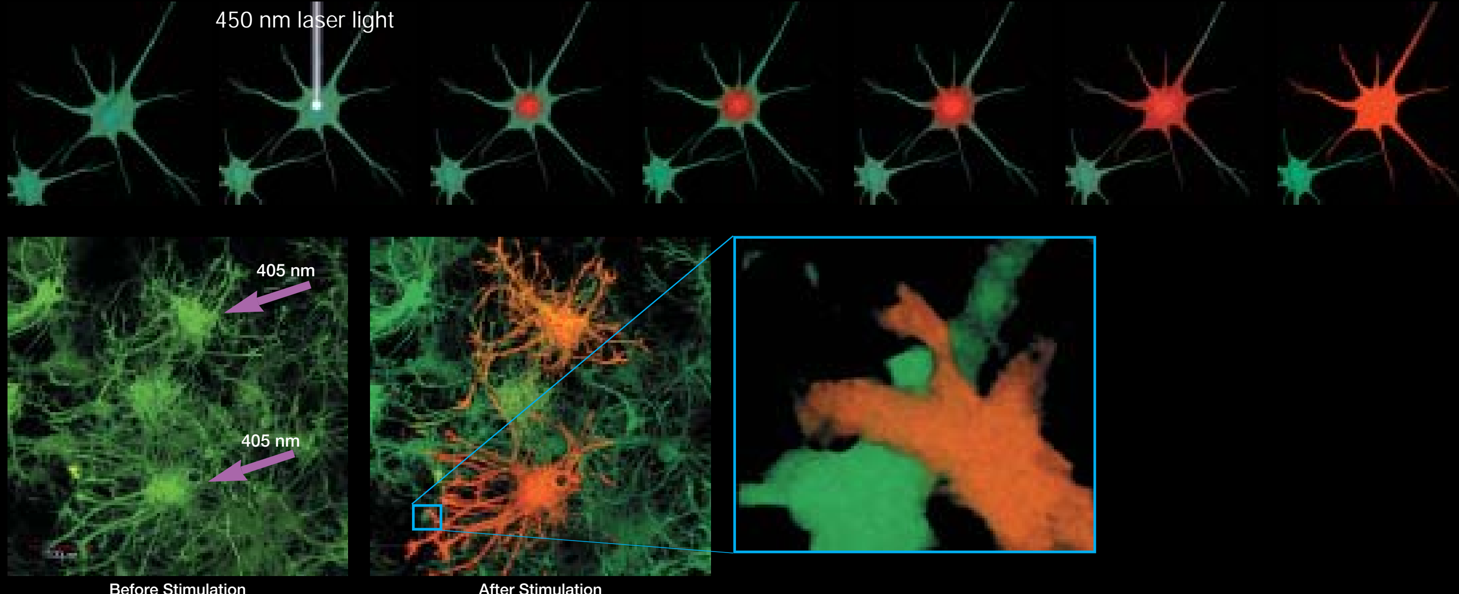
If the protein is strongly bound to a structure or forms part of a large protein complex, the bleached region recovers its fluorescence at a slower rate relative to the unbound state.

Specimen: Hippocampal neurons, Shank-GFP stain, 488 nm excitation (multi-argon laser)
Image acquisition time: 100 ms Bleach time: 80 ms, 488 nm excitation (Sapphire 488 laser)

Data courtesy of: Dr. Shigeo Okabe
Department of Anatomy and Cell Biology, Tokyo Medical and Dental University

Photoconversion

The Kaede protein is a typical photoconvertible protein, which is a specialized fluorescent protein that changes color when exposed to light of a specific wavelength. When the Kaede protein is exposed to laser light, its fluorescence changes from green to red. This phenomenon can be used to mark individual Kaede-expressing target cells among a group of cells, by exposing them to laser light.

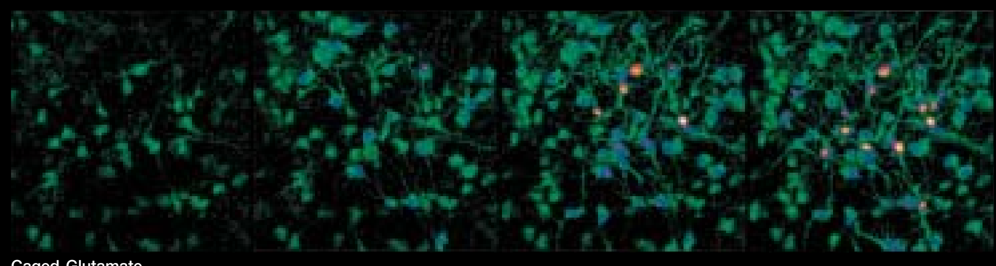


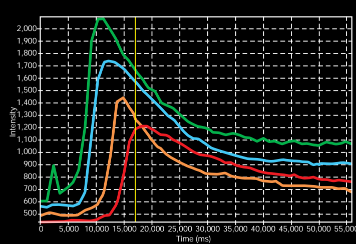
Kaede-expressing astroglia cells are stacked on the Kaede-expressing neurons. By illuminating two colonies with a 405 nm laser, the Kaede color can be photoconverted from green to red. The glial cells in contact with the neurons are observed while they are forming colonies and extending their processes, and the nuclei of these colonies can also be observed. The SIM scanner FV1000 makes it easy to change cell colors from green to red while conducting an observation, and to control neutral colors between red and green.

Data courtesy of: Dr. Hiroshi Hama, Ms. Ryoko Ando and Dr. Atsushi Miyawaki, RIKEN Brain Science Institute Laboratory for Cell Function Dynamics

Uncaging

A 405nm laser is optional for uncaging with the SIM scanner system. Caged compounds can be uncaged point-by-point or within a region of interest, while the main scanner of the FV1000 captures images of the response with no time delay.



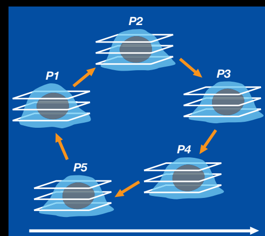


Caged-Glutamate
Fluorescent calcium indicator Fluo-3 in HeLa cells. Image acquisition at 1-second intervals
Using the caged compound Bhcmoc-Glutamate, an increase in calcium ion concentration inside the cell can be observed in response to glutamate stimulation, released via 405 nm laser illumination.

Data courtesy of:
Dr. Hiroshi Hama, Dr. Atsushi Miyawaki, RIKEN Brain Science Institute Laboratory for Cell Function Dynamics
Caged compound Bhcmoc-Glutamate presented by Dr. Toshiaki Furuta, Department of Science, Toho University

Multi-Point Laser Light Stimulation

Using multi-stimulation software, the user can configure continuous laser light stimulation of multiple points with simultaneous imaging, which is effective for applications such as uncaging experiments involving laser light stimulation of several spines in neurons.

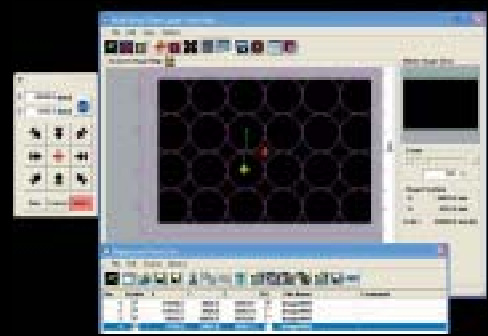


Multi-Dimensional Time-Lapse

The FV1000 can be used for ideal multi-dimensional time-lapse imaging during confocal observation, using multi-area time-lapse software to control the motorized XY stage and focus compensation.

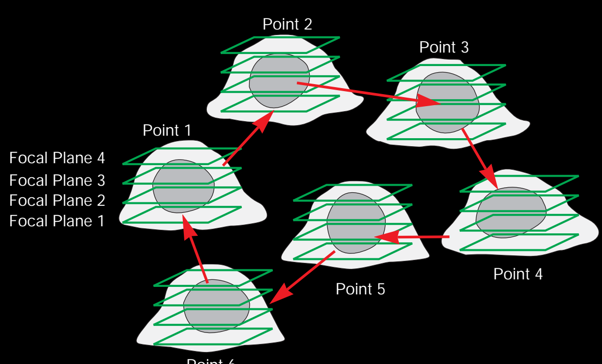
Significantly Improved Long Time-Lapse Throughput

Equipped with motorized XY stage for repeated image acquisition from multiple points scattered across a wide area. The system efficiently analyzes changes over time of cells in several different areas capturing, large amounts of data during a single experiment to increase the efficiency of experiments. Microplates can be used to run parallel experiments, which significantly improves throughput for experiments that require long-term observation.



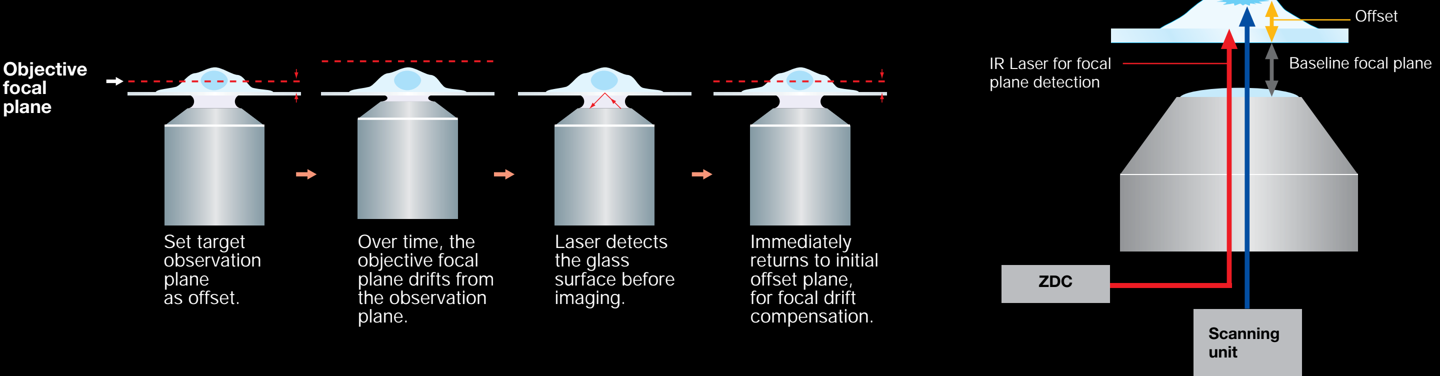
Supports repeated image acquisition from multiple areas in a single microplate well.

Multi-Point Time-Lapse Software



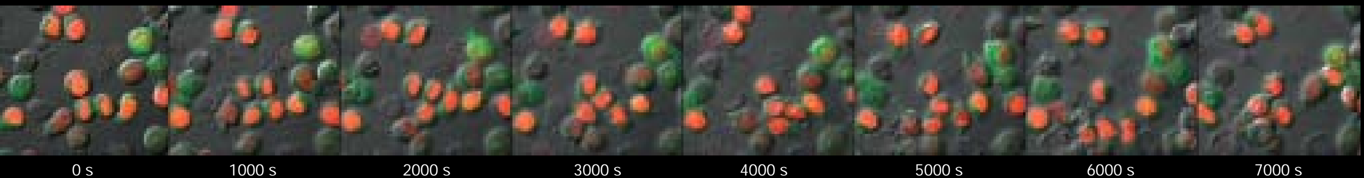
Focal Drift Compensation for Long Time-Lapse Imaging

The IX81-ZDC Zero Drift Compensation system corrects loss of focus caused by temperature changes around the microscope and other factors during long time-lapse observation. The thermal drift compensation eliminates the need to take images at several Z planes, minimizing live cell exposure to irradiation.



Maintain Cell Activity Over A Long Period

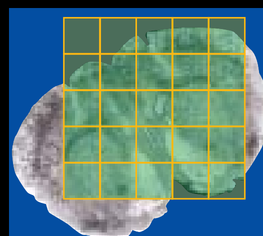
Proprietary CO_2 incubator control keeps the environment inside the tissue culture dish completely stable. The environment is precisely maintained at 37°C with 90% humidity and 5% CO_2 concentration.



0 s 1000 s 2000 s 3000 s 4000 s 5000 s 6000 s 7000 s

Human lymphoblast cells TK6

Courtesy of: Masamitsu Honma, Dir.
Biological Safety Research Center Div. of Genetics and Mutagenesis I, National Institute of Health Sciences

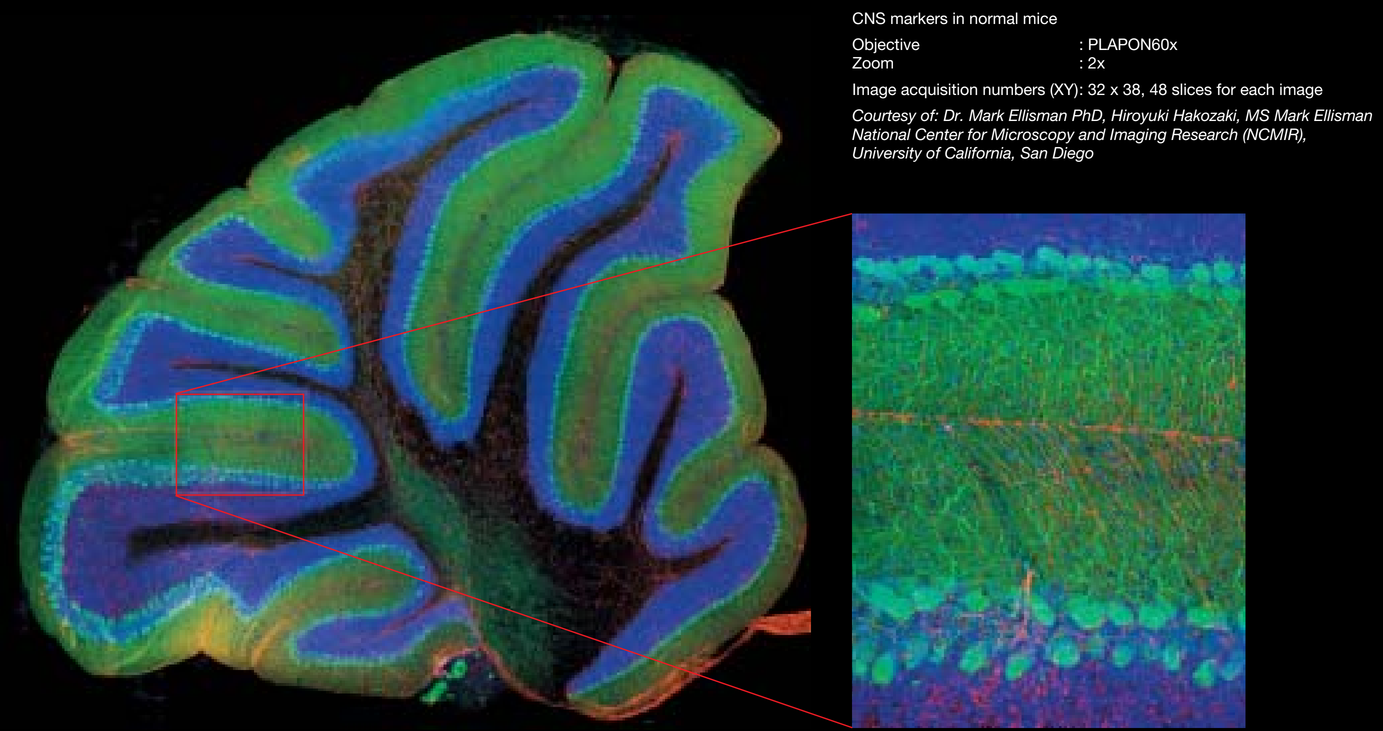


3D Mosaic Imaging

Mosaic imaging is performed using a high-magnification objective to acquire continuous 3D (XYZ) images of adjacent fields of view using the motorized stage, utilizing proprietary software to assemble the images. The entire process from image acquisition to tiling can be fully automated.

Mosaic Imaging for 3D XYZ Construction

Composite images are quickly and easily prepared using the stitching function, to form an image over a wide area. 3D construction can also be performed by acquiring images in the X, Y and Z directions. Tiled images can be enlarged in sections without losing resolution.



CNS markers in normal mice

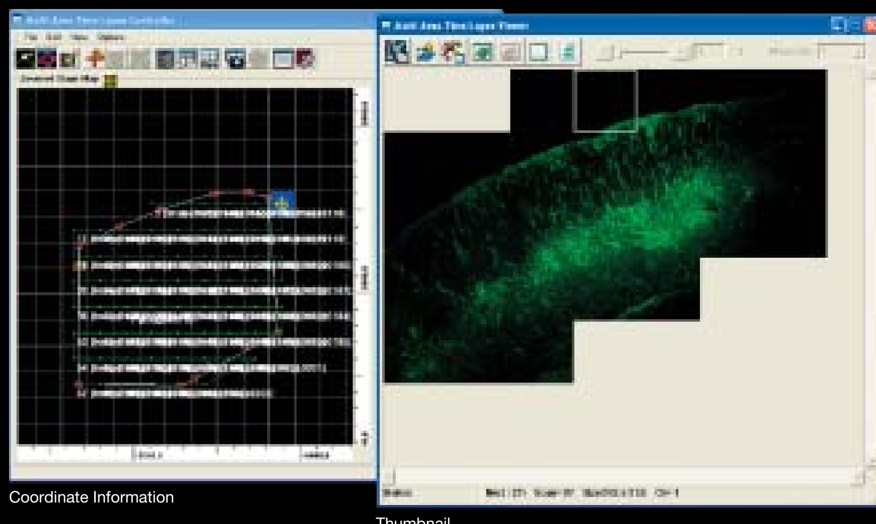
Objective : PLAPON60x
Zoom : 2x

Image acquisition numbers (XY): 32 x 38, 48 slices for each image

Courtesy of: Dr. Mark Ellisman PhD, Hiroyuki Hakozaki, MS Mark Ellisman National Center for Microscopy and Imaging Research (NCMIR), University of California, San Diego

Automated from 3D Image Acquisition to Mosaic Imaging

Multi-area time-lapse software automates the process from 3D image acquisition (using the motorized XY stage) to stitching. The software can be used to easily register wide areas, and the thumbnail display provides a view of the entire image acquired during the mosaic imaging process.



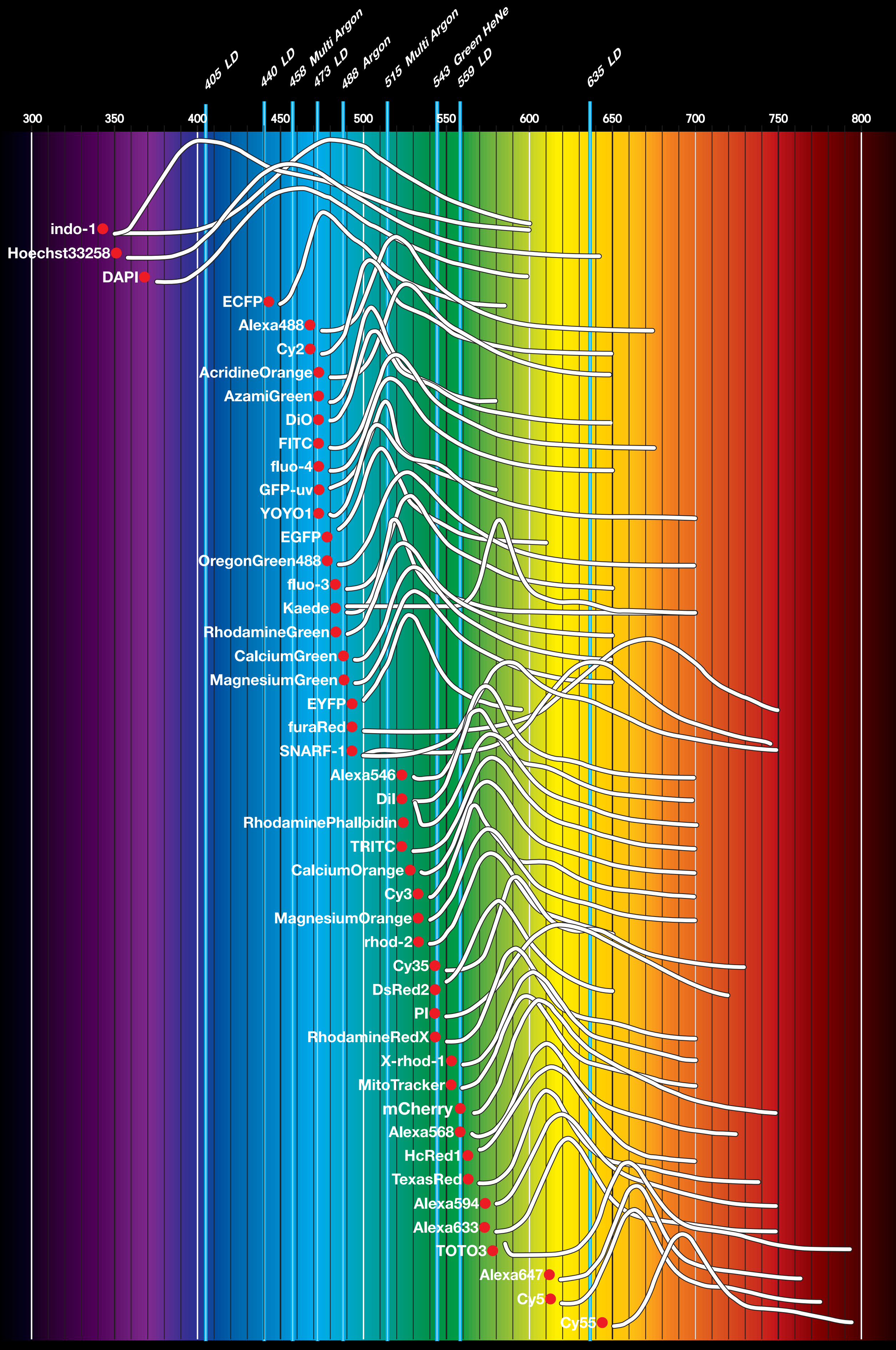
Coordinate Information

Thumbnail

Expandability to Support Diverse Application.

Application	Standard Functions	Optional Functions
Molecular interaction and molecular concentration analysis	—	**Intracellular diffusion measurement** Calculation of diffusion coefficients for intracellular molecules, and analysis of molecular binding and changes in molecular density. Supports a wide range of methods (RICS/ccRICS, point FCS/point FCCS and FRAP). *Software Required: Diffusion measurement package*
Laser Light stimulation	Acquires images while rapidly switching the built-in laser between imaging and laser light stimulation. Features tornado scanning for high-efficiency bleaching using laser light stimulation.	**SIM scanner system** Performs simultaneous imaging and laser light stimulation. Provides detailed settings for laser light stimulation including position and timing. Features tornado scanning for high-efficiency bleaching using laser light stimulation. *Equipment Required: SIM scanner, laser combiner (dual fiber version)*
		Multi-point laser light stimulation system Register multiple points for laser light stimulation, and program the respective stimulation order, stimulation time and type of stimulation (continuous laser light or pulse laser light). *Software Required: Multi-stimulation software*
Multi-dimensional time-lapse imaging	—	**Long time-lapse system** Microscopes equipped with zero drift compensation (ZDC) acquire each image at a set focus plane. The microscope CO_2 incubator maintains cell activity for a long period for continuous imaging. *Equipment Required: IX81-ZDC microscope, CO_2 incubator*
		Multi-point scanning system Register multiple points for repeated image acquisition. Efficiently observe multiple cells in parallel on 35-mm dishes, microplates or chamber slides. *Software and Equipment Required:* *Multi-area time-lapse software, motorized XY stage***
3D mosaic imaging	—	**3D mosaic imaging system** Continuous imaging of adjacent fields of view and mosaic imaging to form a composite image. Acquisition of adjacent Z-series images for 3D mosaic imaging. *Software and Equipment Required:* *Multipoint time-lapse software, motorized XY stage***
TIRFM	—	**TIRFM imaging** Uses the laser from the laser combiner to provide evanescent illumination, for imaging the movement of molecules near the glass surface, such as cell membranes and adhesion factors. *Software and Equipment Required: TIRFM unit*, TIRF objective, high-sensitivity CCD camera**, CCD camera control software***
FRET	Provides FRET analysis functions. Diode laser offers exceptional stability and long life. Supports FRET efficiency measurements using acceptor photobleach method.	**CFP-YFP FRET** Ratio imaging and sensitized emission. Available 440 nm diode laser is optimized for CFP-YFP FRET experiments methods. Diode laser offers exceptional stability and long life. *Equipment Required: LD 440 nm Laser*
Multi-color imaging	Three-channel detector for simultaneous acquisition of fluorescence images from three different dyes. Sequential mode for acquisition of fluorescence images without cross-talk. Fluorescence can also be separated using unmixing (only available on spectral scan unit).	**Imaging blue dyes** Available 405-nm laser for image acquisition of multi-stained samples labeled with V-excitation fluorescent dyes such as DAPI, Hoechst and Alexa 405. *Equipment Required: LD 405 nm laser*
		Simultaneous four-color imaging Fourth channel detector can be easily added to simultaneously acquire images of four colors. *Equipment Required: 4-channel detector*
Colocalization analysis	Easily determine if labeled substances are present locally in the same locations. Calculate of Pearson coefficients, overlap coefficients and colocalization indices.	**High-accuracy colocalization analysis** New 60x oil-immersion objective offers image acquisition with exceptional positional accuracy coefficient. *Equipment Required: PLAPON 60xOSC*

* SIM scanner and TIRFM scanner cannot be installed on the same system.
** For more information about peripheral equipment, contact your Olympus dealer.



405 LD
440 LD
458 Multi Argon
473 LD
488 Argon
515 Multi Argon
543 Green HeNe
559 LD
635 LD
300
350
400
450
500
550
600
650
700
750
800
indo-1
Hoechst33258
DAPI
ECFP
Alexa488
Cy2
AcridineOrange
AzamiGreen
DiO
FITC
fluo-4
GFP-uv
YOYO1
EGFP
OregonGreen488
fluo-3
Kaede
RhodamineGreen
CalciumGreen
MagnesiumGreen
EYFP
furaRed
SNARF-1
Alexa546
DiI
RhodaminePhalloidin
TRITC
CalciumOrange
Cy3
MagnesiumOrange
rhod-2
Cy35
DsRed2
PI
RhodamineRedX
X-rhod-1
MitoTracker
mCherry
Alexa568
HcRed1
TexasRed
Alexa594
Alexa633
TOTO3
Alexa647
Cy5
Cy55

Scanning Units

Two types of scanning units, filter-based and spectral detection, are provided. The design is all-in-one, integrating the scanning unit, tube lens and pupil projection lens. Use of the microscope fluorescence illuminator light path ensures that expandability of the microscope itself is not limited. Visible, UV and IR laser introduction ports are provided, as well as a feedback control system.

Scanning Unit for IX81 Inverted Microscope
Dedicated mirror unit cassette is required.

Scanning Unit for BX61/BX61WI Upright Microscopes
Fluorescence illuminator integrated with scanning unit.

Laser Systems

The multi-combiner enables combinations with all of the following diode lasers: 405 nm, 440 nm, 473 nm, 559 nm and 635 nm. The system can also be equipped with conventional Multi-line Ar laser and HeNe(G) laser.

Dual Type
The multi-combiner outputs laser light with two fibers. Light can be used both for observation and laser light stimulation.

Single Type
Single channel laser output. AOTF is standard equipment.

Illumination Units

Conventional illumination modules are designed for long-duration time-lapse experiments. Since light is introduced through fiber delivery systems, no heat is transferred to the microscope.

Fluorescence Illumination Unit
Stand with Mercury lamp house, motorized shutter, and fiber delivery system for conventional fluorescence observation. Light introduction via fiber optic port.

Transmitted Light Detection Unit
External transmitted light photomultiplier detector and 100 W Halogen conventional illumination, integrated for both laser scanning and conventional transmitted light Nomarski DIC observation. Motorized exchange between transmitted light illumination and laser detection. Simultaneous multi-channel confocal fluorescence image and transmitted DIC acquisition enabled.

Optional Upgrade Equipments for FV1000

4th Channel Detector Unit
Attaches to the optional port of either the filter or spectral type scanning unit and is used as a 4th confocal fluorescence detection channel. This is a filter-based fluorescence detection unit.

SIM Scanner
Second scanner dedicated for laser light stimulation, synchronized to the FV1000 main scanner for simultaneous laser light stimulation and confocal image acquisition. Independent fiber optic laser introduction port. Dichromatic mirror within motorized optical port of the scan unit required for introduction of laser into main scanner.

TIRFM Unit
Enables control of the necessary volume of excitation light using FV1000 software. This unit enables TIRF imaging using the laser light source used with Confocal.

Fiber Port for Fluorescence Output
Confocal fluorescence emission can be introduced via fiber delivery system into external device. Fiber port equipped with FC connector (fiber delivery system not included).

FV1000 System Diagram

Fluorescence illumination unit

A

LD635 laser
635 nm

LD559 laser
559 nm

HeNeG laser
543 nm
Select either laser

Multi Ar laser
458, 488, 515 nm

LD473 laser
473 nm
Select either laser

LD440 laser*
440 nm

LD405 laser*
405 nm

AOTF Laser combiner
(Single-fiber type)
B

AOTF Laser combiner
(Dual-fiber type)
B
C

IR laser*
D

Transmitted light detection unit
E

Fiber port for fluorescence output*

4th channel detector unit*
F

Scanning unit for IX81
(Spectral type or Filter type detector system)

E F G D B A

CO2 incubator *

Motorized XY stage *

IX81
IX81-ZDC
Inverted motorized microscope

Scanning unit for BX61WI, BX61
(Spectral type or Filter type detector system)

F G D B A E

BX61WI
BX61
Upright motorized microscope

Cover *

G

TIRFM unit *

SIM Scanner*

C

FV Power supply *

Microscope control unit

FV power supply unit

FV control unit

Software
- Basic software
- Review station software *
- Diffusion Measurement Package *
- Multi Stimulation Software *
- Multi Area Time Lapse Software *

Monitor

*Optional unit

IX81-ZDC
Focal drift compensation for long time-lapse imaging.
* Requires IX81 microscope. For information about ZDC-compatible objectives, contact your Olympus dealer.

CO_2 Incubator/ MIU-IBC-IF-2, MIU-IBC-I-2
Highly precise incubator control keeps the environment inside a laboratory dish completely stable, at just below 37°C temperature, 90% moisture and 5% CO_2 concentration; in this way, live cell activity can be maintained for approximately two days.
* Not available in some areas

High-Precision Motorized Stage/ PRIOR H117
Multi-point time-lapse photography using a 35 mm glass-bottom dish is easy to perform with this motorized stage, which can reproduce previously-set positions with extreme precision. It also allows efficient photographing of multiple cells and detection of individual cells showing expected reactions.

Main Specifications

		Spectral Version	Filter Version
Laser Light	Ultraviolet/Visible Light Laser	LD lasers: 405 nm: 50 mW, 440 nm: 25 mW, 473 nm: 15 mW, 559 nm: 15 mW, 635 nm, 20 mW Multi-line Ar laser (458 nm, 488 nm, 515 nm, Total 30 mW), HeNe(G) laser (543 nm, 1 mW)	
	AOTF Laser Combiner	Visible light laser platform with implemented AOTF system, Ultra-fast intensity modulation with individual laser lines, additional shutter control Continuously variable (0.1%–100%, 0.1% increment), REX: Capable of laser intensity adjustment and laser wavelength selection for each region	
	Fiber	Broadband type (400 nm–650 nm)	
Scanning and Detection	Scanner Module	Standard 3 laser ports, VIS – UV – IR Excitation dichromatic mirror turret, 6 position (High performance DMs and 20/80 half mirror), Dual galvanometer mirror scanner (X, Y) Motorized optical port for fluorescence illumination and optional module adaptation, Adaptation to microscope fluorescence condenser	
	Detector Module	Standard 3 confocal Channels (3 photomultiplier detectors) Additional optional output port light path available for optional units 6 position beamsplitter turrets with CH1 and CH2 CH1 and CH2 equipped with independent grating and slit for fast and flexible spectral detection Selectable wavelength bandwidth: 1–100 nm Wavelength resolution: 2 nm Wavelength switching speed: 100 nm/msec CH3 with 6 position barrier filter turret	Standard 3 confocal Channels (3 photomultiplier detectors) Additional optional output port light path available for optional units 6 position beamsplitter turrets with CH1 and CH2 CH1 to CH3 each with 6 position barrier filter turret (High performance filters)
	Filters	High performance sputtered filters, dichromatic mirrors and barrier filters	
	Scanning Method	2 galvanometer scanning mirrors	
	Scanning Modes	Scanning speed: 512 x 512 (1.1 sec., 1.6 sec., 2.7 sec., 3.3 sec., 3.9 sec., 5.9 sec., 11.3 sec., 27.4 sec., 54.0 sec.) 256 x 256 bidirectional scanning (0.064 sec., 0.129 sec.)	
		X,Y,T,Z,λ Line scanning: Straight line with free orientation, free line, Point scanning	X,Y,T,Z Line scanning: Straight line with free orientation, free line, Point scanning
	Photo Detection Method	2 detection modes: Analog integration and hybrid photon counting	
	Pinhole	Single motorized pinhole pinhole diameter ø50–300 µm (1 µm step)	Single motorized pinhole pinhole diameter ø50–800 µm (1 µm step)
	Field Number (NA)	18	
	Optical Zoom	1x–50x in 0.1x increment	
	Z-drive	Integrated motorized focus module of the microscope, minimum increment 0.01 µm or 10 nm	
	Transmitted Light Detector unit	Module with integrated external transmitted light photomultiplier detector and 100 W Halogen lamp, motorized switching, fiber adaptation to microscope frame	
Microscope	Motorized Microscope	Inverted IX81, Upright BX61, Upright focusing nosepiece & fixed stage BX61WI	
	Fluorescence Illumination Unit	External fluorescence light source with motorized shutter, fiber adaptation to optical port of scan unit Motorized switching between LSM light path and fluorescence illumination	
System Control	PC	PC-AT compatible, OS: Windows XP Professional (English version), Windows Vista (English version), Memory: 2.0 GB or larger, CPU:Core2Duo 3.0 GHz, Hard disk: 500 GB or larger, Media: DVD Super Multi Drive, FV1000 Special I/F board (built-in PC), Graphic board: conformity with Open GL	
	Power Supply Unit	Galvo control boards, scanning mirrors and gratings, Real time controller	Galvo control boards, scanning mirrors
	Display	SXGA 1280X1024, dual 19 inch (or larger) monitors or WQUXGA 2560 x 1600, 29.8 inch monitor	
Optional Unit	SIM Scanner	2 galvanometer scanning mirrors, pupil projection lens, built-in laser shutter, 1 laser port, Fiber introduction of near UV diode laser or visible light laser, Optional: 2nd AOTF laser combiner	
	TIRFM Unit	Available laser: 405–633 nm. Motorized penetration ratio adjustment. Automatic optical setting for TIRFM objectives	
	4th CH Detector	Module with photomultiplier detector, barrier filter turret, beamsplitter turret mounted with 3rd CH light path	
	Fiber Port for Fluorescence	Output port equipped with FC fiber connector (compatible fiber core 100–125 µm)	

Software	
Image Acquisition	Normal scan: 64 x 64, 128 x 128, 256 x 256, 320 x 320, 512 x 512, 640 x 640, 800 x 800, 1024 x 1024, 1600 x 1600, 2048 x 2048, 4096 x 4096 Clip rectangle scan ,Clip ellipse scan ,Polygon clip scan,line scan ,free line scan,Point scan, Real-time image
	2-dimension: XY, XZ, XT and Xλ 3-dimension: XYZ, XYT, XYλ, XZT, XTλ and XZλ 4-dimension: XYZT, XZTλ and XYTλ 5–dimension: XYZTλ
Programmable Scan Controller	Time Controller function
2D Image Display	Each image display: Single-channel side-by-side, merge, cropping, live tiling, live tile, series (Z/T/λ), LUT: individual color setting, pseudo-color, comment: graphic and text input
3D Visualization and Observation	Interactive volume rendering: volume rendering display, projection display, animation displayed (save as OIF, AVI or MOV format) Free orientation of cross section display 3D animation (maximum intensity projection method, SUM method) 3D and 2D sequential operation function
Image Format	OIB/ OIF image format 8/ 16 bit gray scale/index color, 24/ 32/ 48 bit color, JPEG/ BMP/ TIFF/ AVI/ MOV image functions Olympus multi-tif format
Spectral Unmixing	2 Fluorescence spectral unmixing modes (normal and blind mode)
Image Processing	Filter type: Sharpen, Average, DIC Sobel, Median, Shading, Laplacian Calculations: inter-image, mathematical and logical, DIC background leveling
Image Analysis	Fluorescence intensity, area and perimeter measurement, time-lapse measurement
Statistical Processing	2D data histogram display, colocalization
Optional Software	Review station software, Off-line FLUOVIEW software for date analysis. Motorized stage control software, Diffusion measurement package, Multi stimulation software, Multi area time-lapse software

Objectives for BX2 and IX2

(using U-UCD8A-2, IX2-LWUCDA2 and U-DICTS)

Description	NA	W.D. (mm)	Cover glass thickness (mm)	Immersion	Correction ring	Condenser for BX2 U-UCD8A-2 optical element	Condenser for IX2 IX2-LWUCDA2 optical element	U-DICTS position
UPLSAPO4X	0.16	13	—					
UPLSAPO10X2	0.40	3.1	0.17			U-DIC10	IX2-DIC10	normal
UPLSAPO20X	0.75	0.6	0.17			U-DIC20	IX2-DIC20	normal
UPLSAPO20XO	0.85	0.17	—	Oil		U-DIC20	IX2-DIC20	normal
UPLSAPO40X2	0.95	0.18	0.11-0.23		○	U-DIC40	IX2-DIC40	normal
UPLSAPO60XO	1.35	0.15	0.17	Oil		U-DIC60	IX2-DIC60	BFP1
UPLSAPO60XW	1.20	0.28	0.13-0.21	Water	○	U-DIC60	IX2-DIC60	normal
UPLSAPO100XO	1.40	0.12	0.17	Oil		U-DIC100	IX2-DIC100	normal
PLAPON60XO	1.42	0.15	0.17	Oil		U-DIC60	IX2-DIC60	BFP1
PLAPON60XOSC	1.40	0.12	0.17	Oil		U-DIC60	IX2-DIC60	BFP1
UPLFLN40XO	1.30	0.2	0.17	Oil		U-DIC40	IX2-DIC40	BFP1
APON60XOTIRF	1.49	0.1	0.13-0.19	Oil	○	U-DIC60	IX2-DIC60	BFP1
UAPON100XOTIRF	1.49	0.1	0.13-0.19	Oil	○	U-DIC100	IX2-DIC100	normal
UAPON150XOTIRF	1.45	0.08	0.13-0.19	Oil	○	U-DIC100	IX2-DIC100	normal
Apo100XOHR	1.65	0.1	0.15	Oil		U-DIC100	IX2-DIC100	normal

Objectives for fixed stage upright microscope

(using WI-UCD, WI-DICTHRA2)

Objectives	NA	W.D. (mm)	DIC prism	Revolving nosepiece
MPLN5X	0.10	20.00	–	WI-SSNP, WI-SRE3
UMPLFLN10XW	0.30	3.50	WI-DIC10HR	WI-SSNP, WI-SRE3
UMPLFLN20XW	0.50	3.50	WI-DIC20HR	WI-SSNP, WI-SRE3
LUMPLFLN40XW	0.80	3.30	WI-DIC40HR	WI-SSNP, WI-SRE3
LUMPLFLN60XW	1.00	2.00	WI-DIC60HR	WI-SSNP, WI-SRE3
LUMFLN60XW	1.10	1.5	WI-DIC60HR	WI-SSNP, WI-SRE3
XLUMPLFLN20XW	1.00 *	2.0	WI-DICXLU20HR	WI-SNPXLU2

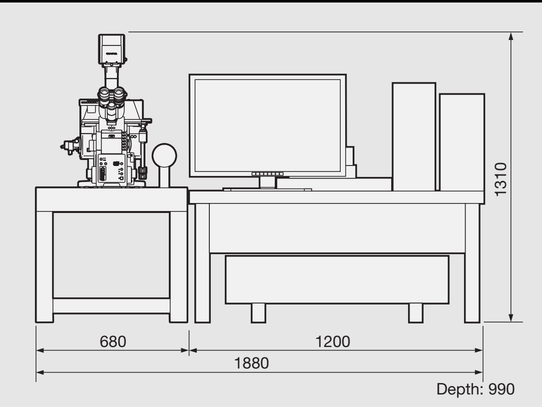
* Note: These conditions are not met in confocal microscopy

Dimensions, Weight and Power Consumption

		Dimensions (mm)	Weight (kg)	Power consumption
Microscope with scan unit	BX61/BX61WI IX81	320 (W) x 580 (D) x 565 (H) 350 (W) x 750 (D) x 640 (H)	41 51	—
Fluorescence illumination unit	Lamp Power supply	180 (W) x 320 (D) x 235 (H) 90 (W) x 270 (D) x 180 (H)	6.7 3.0	AC 100-240 V 50/60 Hz 1.6 A
Transmitted light detection unit		170 (W) x 330 (D) x 130 (H)	5.9	—
Microscope control unit		125 (W) x 332 (D) x 216 (H)	5.2	AC 100-120/220-240 V 50/60 Hz 3.5 A/1.5 A
FV Power supply unit		180 (W) x 328 (D) x 424 (H)	7.5	AC 100-120/220-240 V 50/60 Hz 4.0 A/2.0 A
FV control unit (PC)		180 (W) x 420 (D) x 360 (H)	10.5	AC 100/240 V 50/60 Hz 497.5 W
Display	19 inch, dual (value per monitor)	363 (W) x 216 (D) x 389.5–489.5 (H)	5.9	AC100-120/200-240 V 50/60 Hz 0.65 A/0.4 A
	29.8 inch	689 (W) x 254.7 (D) x 511.5–629.5(H)	15.7	AC100-120/200-240 V 50/60Hz 1.8 A/0.8 A
Power supply unit for laser combiner		210 (W) x 300(D) x 100 (H)	4.0	AC 100-120/200-240 V 50/60 Hz 2.0 A/1.0 A
Laser combiner (with Ar laser heads)		514 (W) x 504 (D) x 236 (H)	45	—
Laser combiner (without Ar laser heads)		514 (W) x 364 (D) x 236 (H)	40	—
LD559 laser power supply		200 (W) x 330 (D) x 52 (H)	1.2	AC 100-240 V 50/60 Hz 30 W
Multi Ar laser power supply		162 (W) x 287 (D) x 91 (H)	4.4	AC 100-240 V 50/60 Hz 20 A
HeNe(G) laser power supply		130 (W) x 224 (D) x 62 (H)	1.8	AC 100-120 V 50/60 Hz 0.45 A

Recommended FV1000 system setup (IX81, BX61, BX61WI)

(unit: mm)

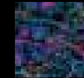


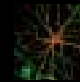
*1 This product corresponds to regulated goods as stipulated in the "Foreign Exchange and Foreign Trade Control Law". An export license from the Japanese government is required when exporting or leaving Japan with this product.

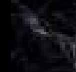
*2 The performance and safety of this device is not guaranteed if it is disassembled or modified.

*3 This device is designed for use in industrial environments for the EMC performance. (IEC61326-1 Class A device) Using it in a residential environment may affect other equipment in the environment.

Images are courtesy of the following institutions:

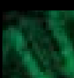

"Brainbow" mouse brain stem
Courtesy of the laboratories of Jeff W. Lichtman and Joshua R. Sanes Harvard University MCB Department and the Center for Brain Science


Hippocampal neurons
Courtesy of Dr. Shigeo Okabe
Department of Cellular Neurobiology, Graduate School of Medicine, The University of Tokyo


Cultured nerve cells derived from the mouse hippocampus
Courtesy of Dr. Koji Ikegami, Dr. Mitsutoshi Setou
Molecular Geriatric Medicine, Mitsubishi Kagaku Institute of Life Sciences

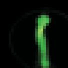
Cerebellum Purkinje cell
Courtesy of Dr. Tetsuro Kashiwabara, Assistant Professor; and Dr. Akira Mizoguchi, Professor;
Neuroregenerative medicine course, Mie University School of Medicine

Drosophila, Stage 14
Courtesy of Dr. Tetsuya Kojima
Laboratory of Innovational Biology, Department of Integrated Biosciences Graduate School of Frontier Sciences, University of Tokyo

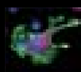

Mouse brain section
Courtesy of Mr. Masayuki Sekiguchi (Section Chief)
Department of Degenerative Neurological Diseases, National Institute of Neuroscience, National Center of Neurology and Psychiatry

Rudimentary limbs of larva in latter part of 3rd instar
Courtesy of Dr. Tetsuya Kojima
Laboratory of Innovational Biology, Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo

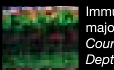
Zebrafish
Courtesy of Dr. Toru Murakami,
Department of Neuromuscular & Developmental Anatomy, Gunma University Graduate School of Medicine


Medaka embryogenesis (somite stage)
Courtesy of Minoru Tanaka, Hiromi Kurokawa
National Institute for Basic Biology Laboratory of Molecular Genetics for Reproduction

Pilidium larva of Micrura alaskensis
Courtesy of Dr. Svetlana Maslakova of the University of Washington and Dr. Mikhail V Matz of the Whitney Laboratory for Marine Bioscience, University of Florida.


Osteoclast induced from rat monocyte in rat kidney
Courtesy of Dr. Keiko Suzuki,
Department of Pharmacology, Showa University School of Dentistry

Fucci–Sliced mouse brain, expressing S/G2/M phases
Courtesy of Dr. Hiroshi Kurokawa, Ms. Asako Sakaue-Sawano and Dr. Atsushi Miyawaki
RIKEN Brain Science Institute Laboratory for Cell Function Dynamics


Immunolabeling of a transgenic mouse retina showing the major retinal cells types
Courtesy of Dr. Rachel Wong, Mr. Josh Morgan
Dept. Biological Structure, University of Washington, Seattle.

Wild-type embryo in stage 17 of drosophila
Courtesy of Dr. Tetsuya Kojima
Laboratory of Innovational Biology, Department of Integrated Biosciences
Graduate School of Frontier Sciences, University of Tokyo

Alpha Blend method (Cultured nerve cells derived from the mouse hippocampus)
Courtesy of Dr. Koji Ikegami, Dr. Mitsutoshi Setou
Molecular Geriatric Medicine, Mitsubishi Kagaku Institute of Life Sciences