OLYMPUS®

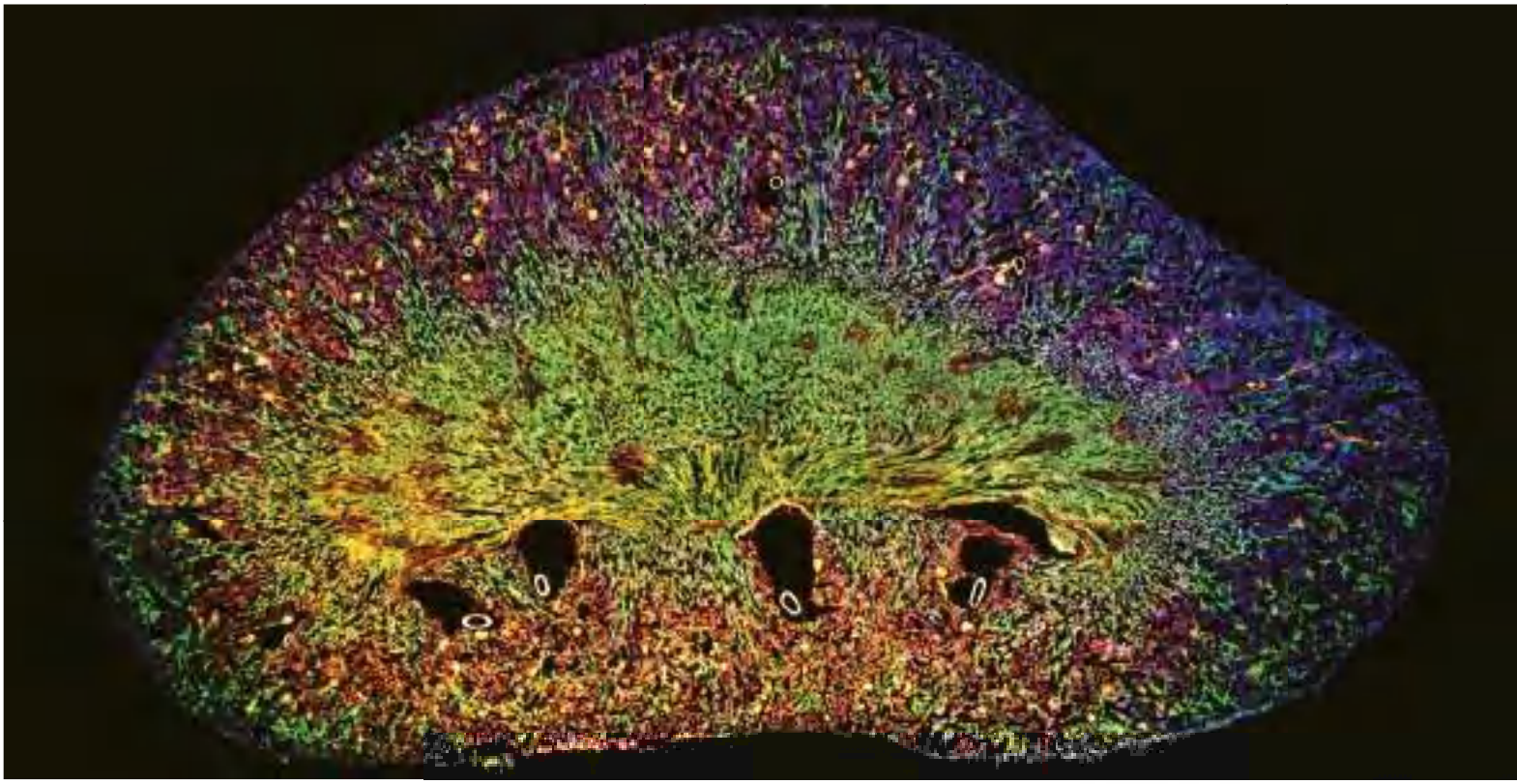
Your Vision, Our Future

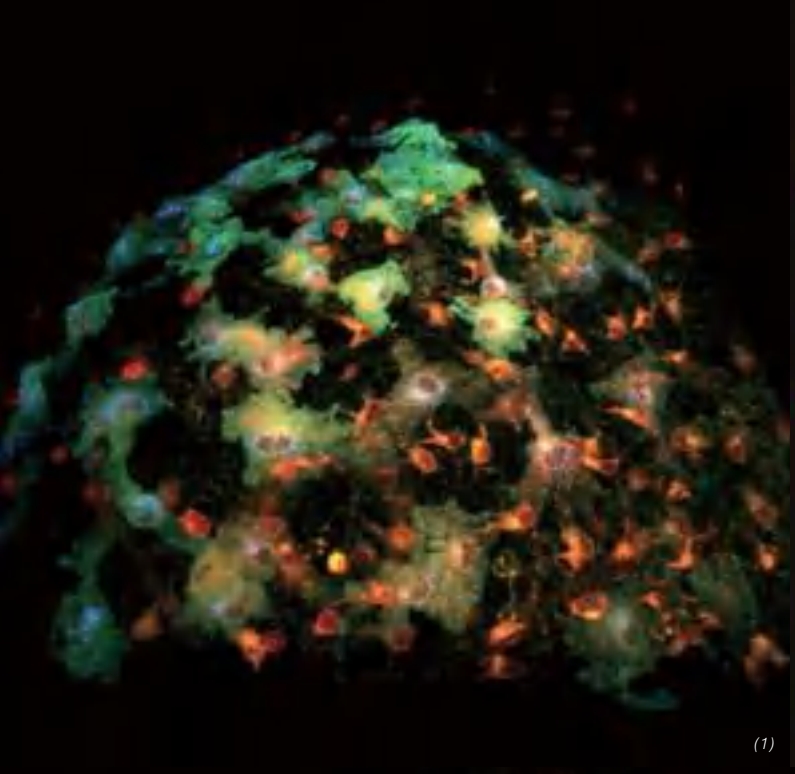
Confocal Laser Scanning Microscope

FV3000

FLUOVIEW

Next Generation FLUOVIEW for the Next Revolutions in Science





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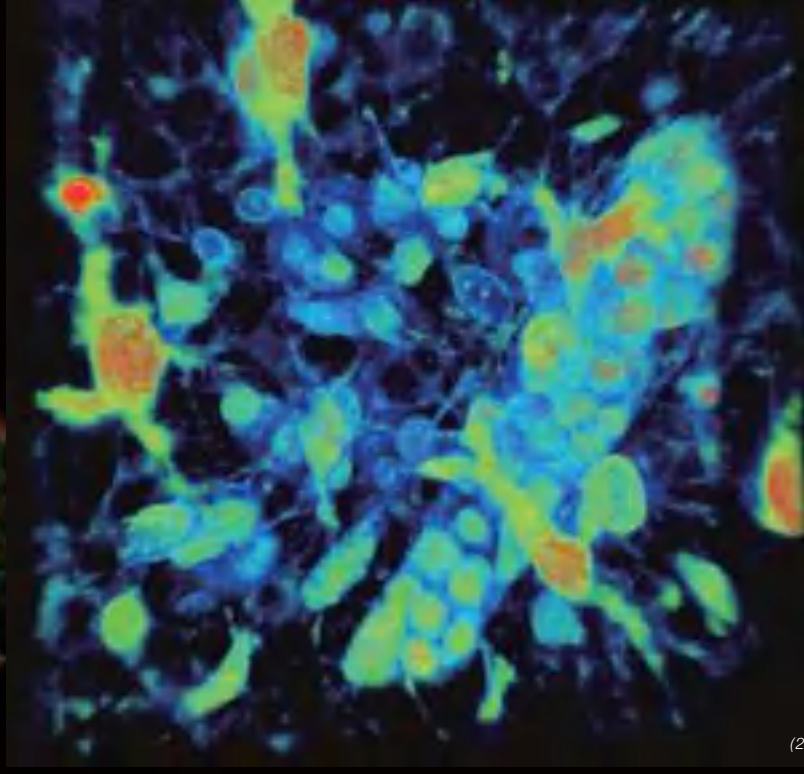

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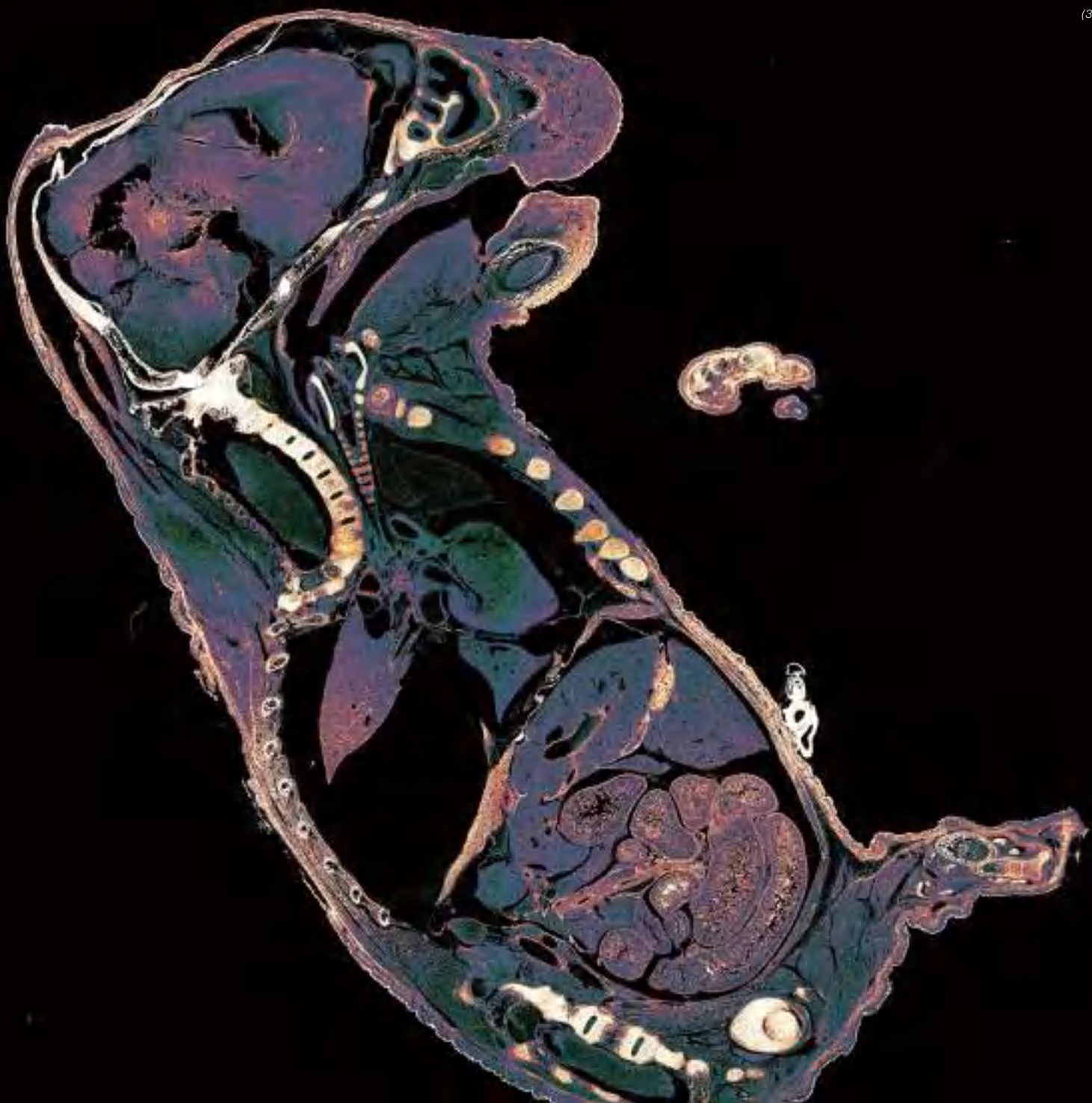


The FLUOVIEW FV3000 Series – FV3000 and FV3000RS

The Next Evolution of Confocal Laser Scanning Microscope Technology for Cell Biology, Cancer Research, Stem Cell Research, and Advanced Applications

The FLUOVIEW FV3000 Series is designed to meet some of the most difficult challenges in modern science. With the high sensitivity and speed required for live cell and tissue imaging and the ease of use and flexibility required for microplate imaging and complex screening protocols, the FV3000 Series supports complete workflows from live cell 2D–6D (x,y,λ,z,t,p) imaging through image processing, like deconvolution, and analysis. Particular attention has been paid to the needs of cell biology (pages 5–6), cancer research (pages 7–8), and stem cell research (page 9). The FV3000 is optimized for macro to micro imaging of cells, tissues, and small organisms.

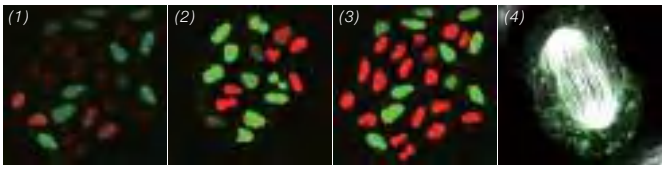
With Olympus' renowned optics at the heart of the system, the FV3000 features a new spectral detection concept for true multichannel spectral imaging with high sensitivity detection in multiple dynamic ranges so even dim signals can be separated. The optical path enables macro to micro imaging from 1.25X to 150X magnification combined with robust, intuitive automation to simplify complex experiments, including one-click cellSens macro analysis for cell counting and segmentation analysis. The precision of galvanometer scanning is combined with the speed of resonant scanning in the FV3000RS hybrid scanner so users can combine precision and high-speed imaging in one experiment.

Built for long service life and low operating costs, the FV3000 uses long-lasting all diode lasers and LED illumination. The system features a modular, upgradable design that includes 2-tier detection options, easily upgradeable laser configurations, and the stable and flexible IX83 microscope with a field-upgradable z drift compensator (IX3-ZDC2) for fast and robust live cell autofocus. With user-savable and selectable software workflows, the system adjusts to individual needs. The facility manager tracking software makes it easy to track system usage by user, making the FV3000 the ideal confocal system for years of productive science in single and multi-user environments.

The FV3000 Series: Meeting the Challenges of Cell Biology, Cancer Research, Stem Cell Research, and Advanced Applications

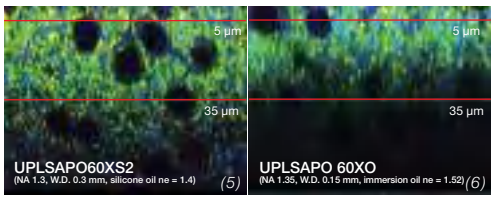
Cell Division, Proliferation, Counting, Cell Cycle, and Segmentation Analysis

Cell proliferation is a key aspect of cancer research. The FV3000 has tools for imaging and measuring these critical events.



Silicone Objectives Optimized for Live Tissue Observation

3D imaging has become an increasingly important part of cancer research. Olympus' exclusive silicone objectives provide clear and bright images at depth in live cells and tissues for accurate imaging and quantification.

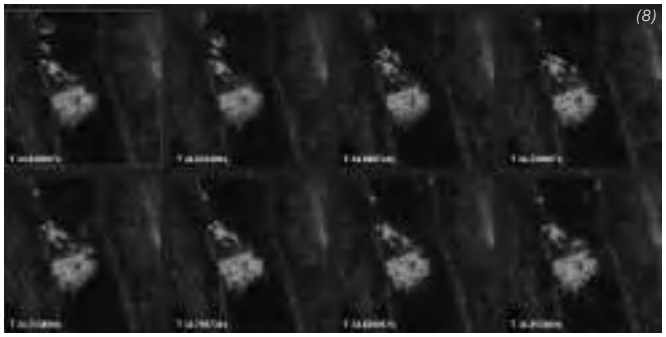


Macro to Micro and Whole Slide Imaging

Cell biology research demands the flexibility to image small organisms at the macro scale down to the micro at high resolution. The FV3000 Series features optics that enable macro to micro imaging for enhanced flexibility.

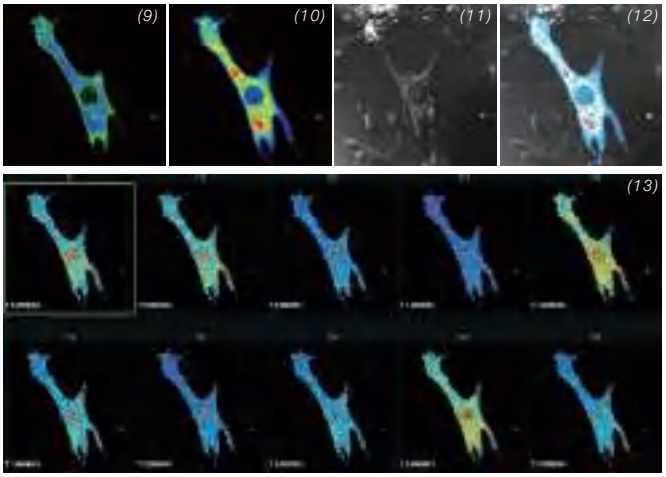
Microfluidics and High-Speed Blood Flow

Circulating tumor cells in peripheral blood and microfluidic device imaging can require high-speed imaging for accurate measurements. The FV3000RS provides high-speed imaging for critical velocity measurements to capture key events.



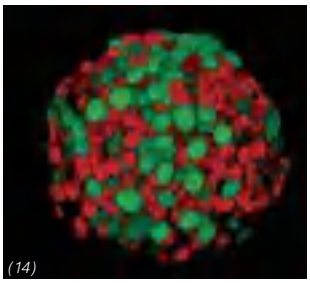
Fast Calcium Dynamics

Image calcium sparks and waves at speeds up to 438 frames per second. Slow heartbeats to visible rates and capture vast neuronal cell networks at full field of view at 30 frames per second.



Spheroid, Gel Matrix, Long-Term Time-Lapse, and Microplate Imaging

Long-term time-lapse imaging of live cells in 3D captures physiologically relevant information. As stem cells grow into spheroids and organoids, the FV3000 Series enables precise, stable time-lapse imaging with high sensitivity and low phototoxicity.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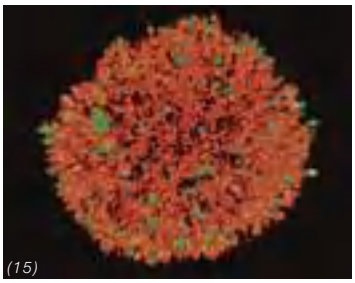
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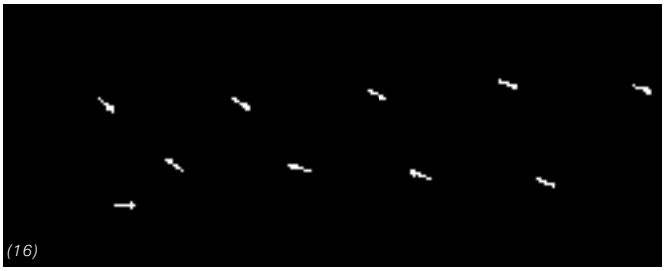
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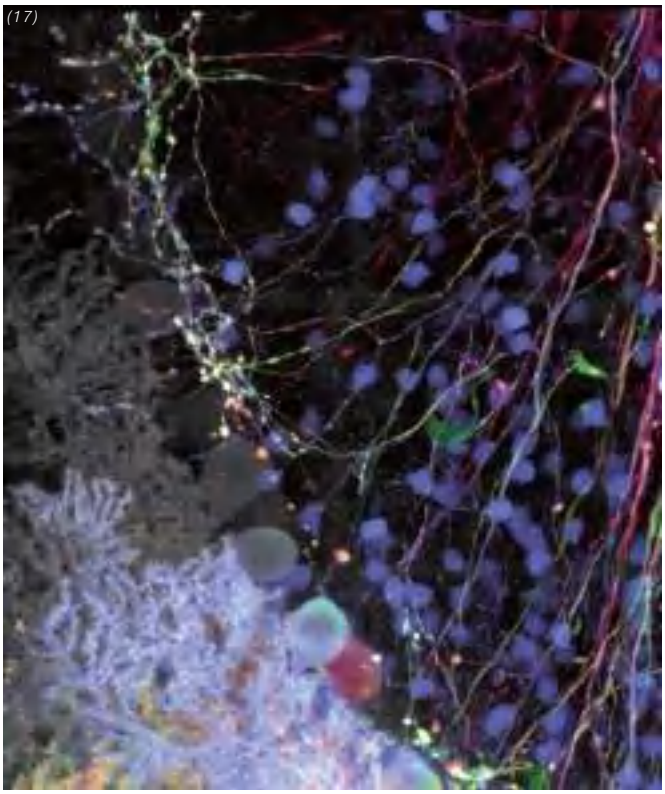
Spectral Unmixing

Complex overlapping fluorescent protein spectra can complicate a range of biological studies. The FV3000 Series efficiently separates signals for accurate measurements and localization.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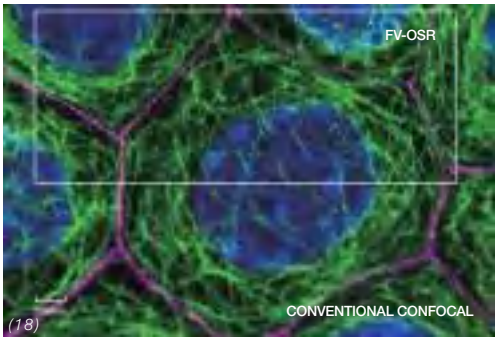
Super Resolution

Olympus' patented* confocal super resolution imaging provides an easy-to-use method for boosting resolution beyond the diffraction limit in fixed tissues.

*US8933418B/JP5784393B



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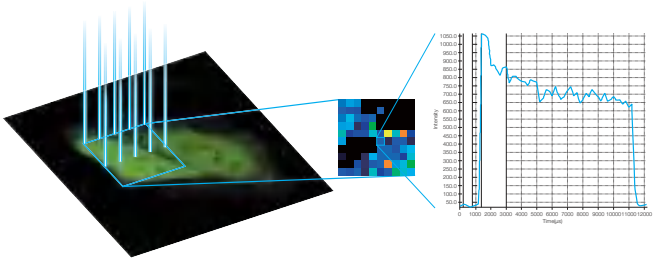
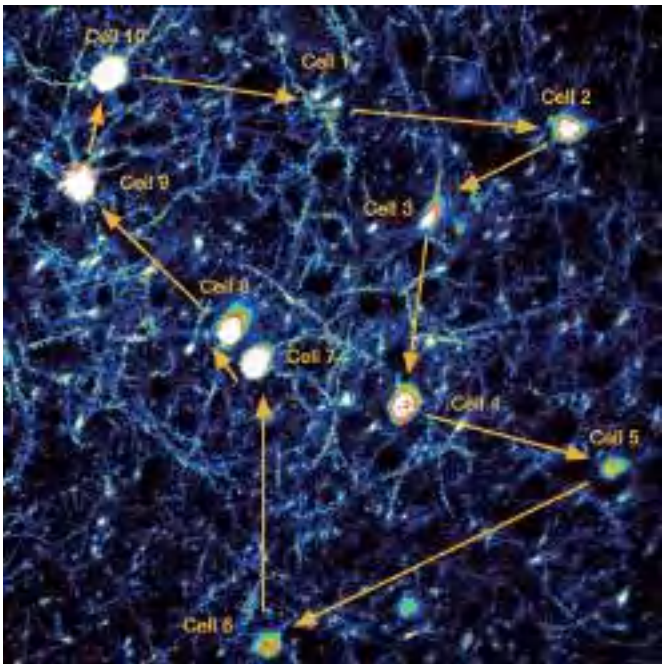
Photoconversion and Stimulation

Precise control of laser light stimulation timing and complex multipoint imaging and stimulation enable highly reproducible experiments for various studies.

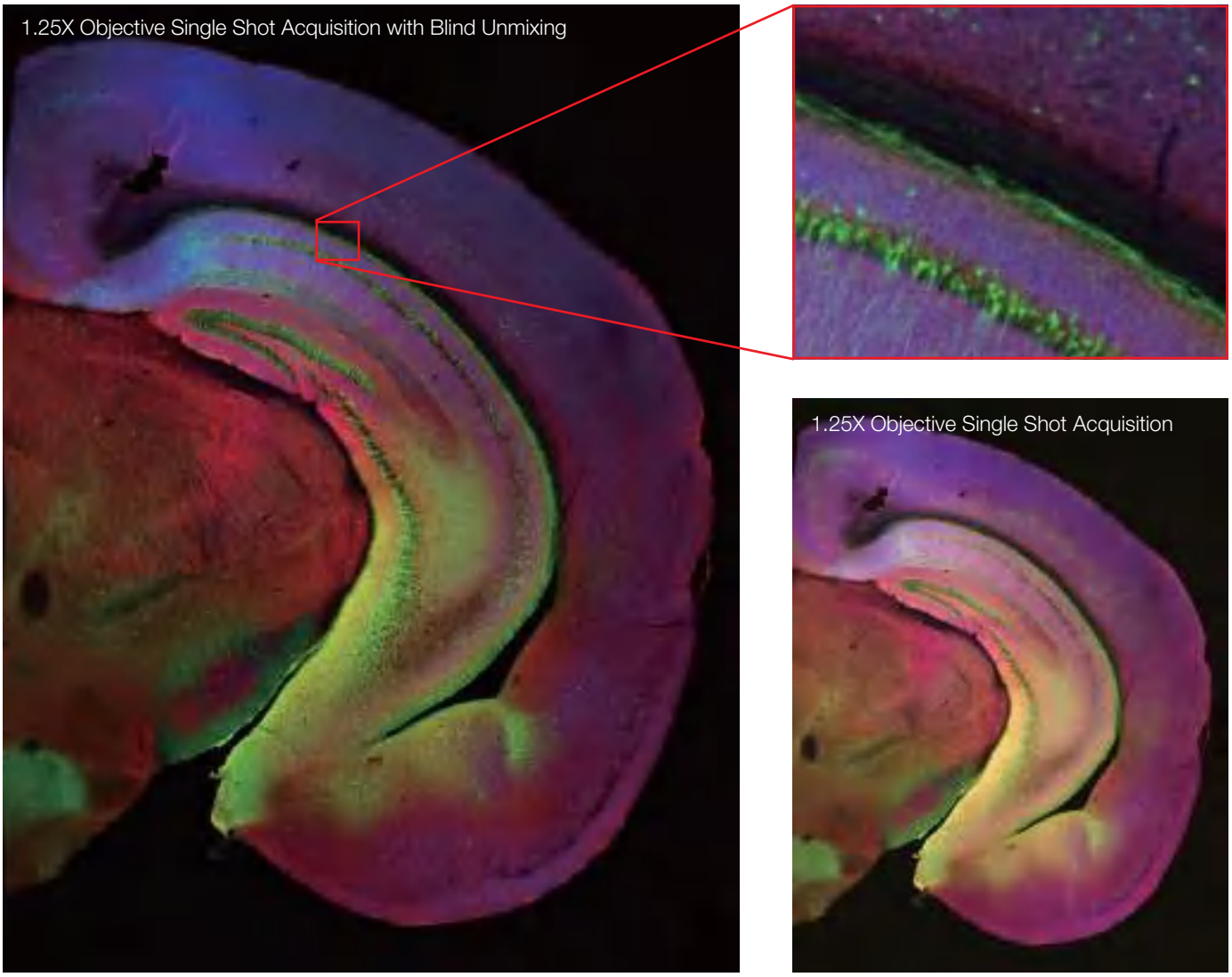




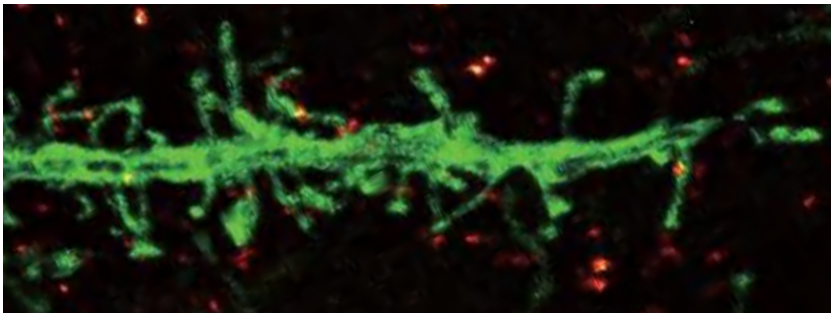
Solutions for Cell Biology: Image Dynamic *in vivo* Processes in Large and Small Organisms with Very Low to High Magnification

Macro to Micro and Whole Slide Imaging

Cell biology requires high sensitivity, and deals with live organisms such as zebrafish and C. elegans. Large pieces of tissue and small organisms may require both high speeds as well as large fields of view to see the entire organism in context. Accurately imaging a large field of view requires precise automation and excellent optics. The FV3000 System is designed to image large tissues and small organisms with accurate stage control, image stitching, and an optical design that facilitates very low to high magnification (1.25X up to 150X). Since autofluorescence can be an issue for cell biologists, the FV3000 was designed to be a fully spectral system capable of highly sensitive and accurate spectral background, autofluorescence, and overlapping spectra (e.g. GFP/YFP) separation.



Mouse brain hemisection embedded for Expansion Microscopy (pre-expansion). Secondary antibody labels against GFP (Alexa Fluor 488, neurons), SV2 (Alexa Fluor 565, Red) Homer (Alexa Fluor 647, Blue). Sample courtesy of Dr. Ed Boyden and Dr. Fei Chen, MIT.



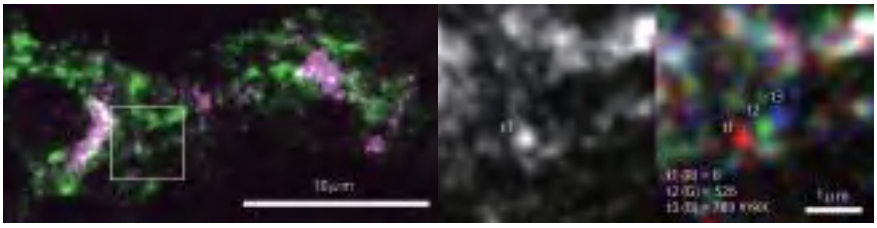
Dendrite (anti-GFP Alexa Fluor 488, green) and synaptic marker (SV2, Alexa Fluor 565, red) Olympus Super Resolution image processed with cellSens advanced contrained iterative deconvolution. Average Full Width Half Maximum measurements ~135 nm. Image acquired with 100X 1.35 NA silicone objective.
Sample courtesy of Dr. Ed Boyden and Dr. Fei Chen, MIT.

A new optical design means that even when using low magnification 30X silicone objectives with 1.05 NA, resolution can be boosted using Olympus super resolution technology—FV-OSR. Silicone objectives also help provide low spherical aberration on tissues and small organisms, so object measurements and distances are accurate. The resonant scanner also helps reduce phototoxicity and photobleaching compared to regular galvo scanners by reducing triplet states of excited fluorophores and reactive oxygen species.

Highly Dynamic Imaging

Small organisms are often favored as models for studying dynamic *in vivo* processes, so the FV3000RS is equipped with a very accurate resonant scanner, facilitating applications such as studying a beating heart, blood flow, calcium signaling, and other dynamic events at up to 438 frames per second. With the FV3000RS, switching between the high-precision galvanometer and high-speed resonance scanner is as simple as a mouse click. The resonance scanner maintains the same field of view so users won't get lost when switching between high-speed and high-precision scanning. Resonance images undergo post-processing with rolling average filtering for time gate image averaging while improving signal-to-noise. Ratio imaging can employ an Intensity Modulated Display (IMD) so real signal stands out above background noise. Selecting the spectral range is simple, and spectral unmixing is fast and automated.

Intensity Modulated Display of CFP/YFP ratio result during spontaneous contractions of *in vitro* cardiomyocyte.
Image data courtesy of Yusuke Nino and Atsushi Miyawaki, Cell Function Dynamics, Brain Science Institute of RIKEN.



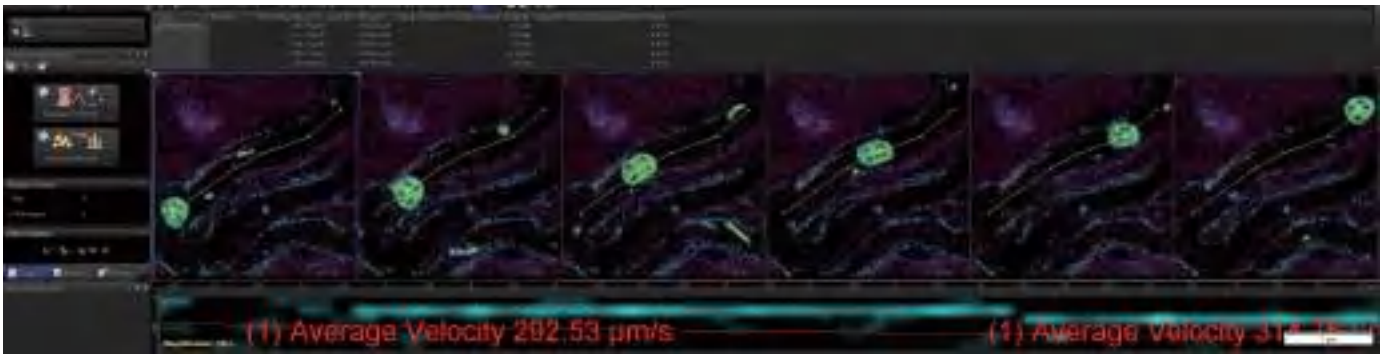
(Left) Rapid movement of endosomes was observed in the Drosophila embryo. Tracheal tip cells expressing GFP-tagged clathrin light chain (Green) and RFP-tagged cell adhesion protein p120 catenin (Magenda) were scanned with interval of 263 msec.
(Middle) Magnified view of the boxed region (GFP channel).
(Right) Overlay of three time points of the same region.
Color coded images show rapid endosomal movement.
Objective lens: UPLSAPO 60X, Scanning zoom: 7x,
Interval of frames: 263msec (Resonant scan averaged 4 times with sequential mode).
Image data courtesy of Shigeo Hayashi (Ph.D.),
Laboratory for Morphogenetic Signaling, RIKEN Center for Developmental Biology

Solutions for Cancer Research: Accurate 3D Cell and Tissue Imaging, High-Speed Blood Flow, Microfluidic Imaging, and Robust Analysis

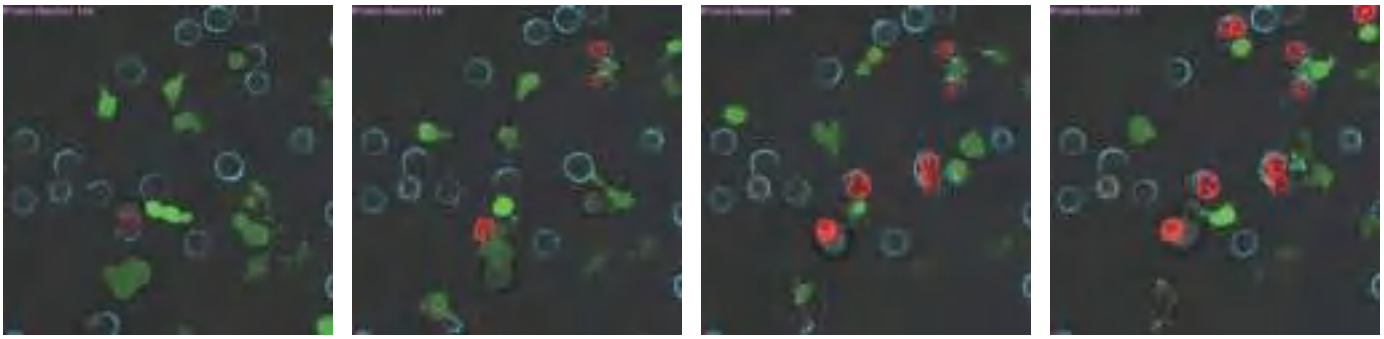
Cell Division, Proliferation, Counting, Cell Cycle, and Segmentation Analysis

The FV3000 Series incorporates the range of technologies necessary for cancer research imaging studies. In live cell cancer studies, sensitive fluorescence detection, optimized optics, and analytical tools such as cell counting and segmentation analysis are essential. With the emergence of microfluidics and a focus on circulating tumor cells, high-speed acquisitions can make the difference between success and failure in an experiment.

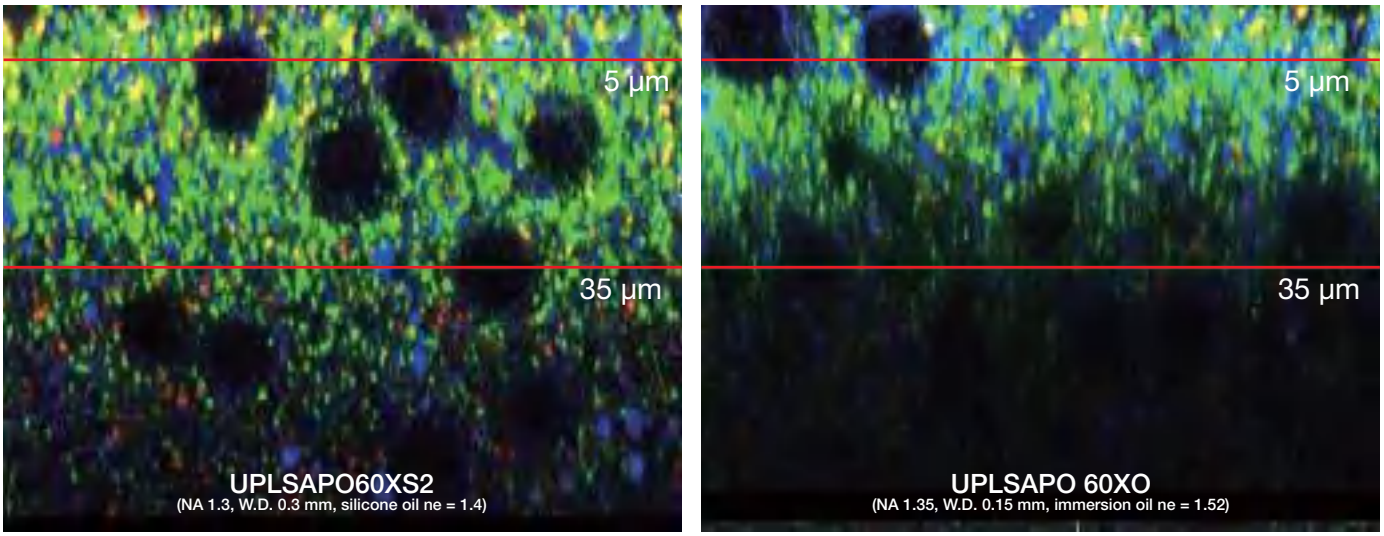
Accuracy and repeatability are equally important; cell cycle checkpoint times must be reliably tracked, 3D images of cells must correctly represent their shape and size, and images need to be bright and clear for segmentation analysis. Olympus' silicone objectives are optimized for tissue imaging. The FV3000 Series high-sensitivity cooled GaAsP detection unit with high signal-to-noise galvo and resonant scanning and robust software make imaging accurate and reproducible for reliable results.



Platelets bound to thrombosis in blood vessel of mouse. Images taken 30 fps in full frame by resonant scanner with 2 CH GaAsP PMTs.
Image data courtesy of Dr. Takuya Hiratsuka, Dr. Michiyuki Matsuda, Graduate School of Biostudies, Kyoto University.



NK-cell mediated cell killing after therapeutic anitbody application (blue). GFP labeled NK-cells (green). DAPI uptake marking dead cells (Red).
Image data courtesy of Dr. Yuji Mishima, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research.



Sca*l*eA2-treated neocortex
Image data courtesy of Motokazu Uchigashima, M.D., Ph.D., Masahiko Watanabe, M.D., Ph.D., Departments of Anatomy, Hokkaido University Graduate School of Medicine.

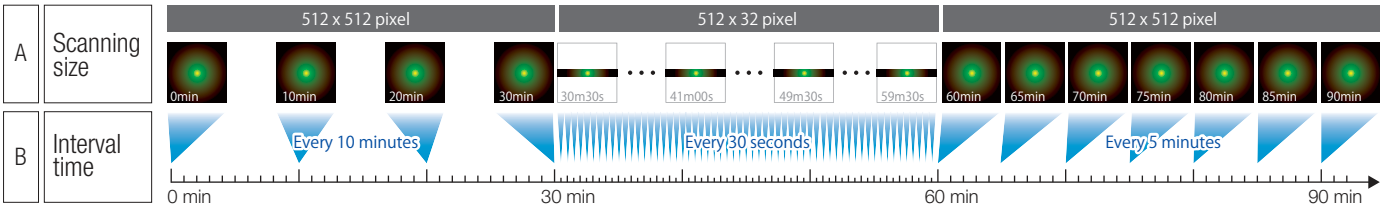
The system's sensitivity coupled with the laser power monitor and two freely selectable ranges for laser power help provide that apoptosis is part of the experiment and not caused by phototoxicity. The spectral sensitivity and accuracy enable researchers to conduct multi-color fluorescence labeling experiments with multiple biomarkers.

Complex Tasks Made Simple

Cancer research is complex but measuring proliferation with the FV3000 isn't. With cellSens macro capabilities, time-lapse images can be processed and counted and reports generated with a single mouse click. The layout of the acquisition software can be customized according to specific applications and immediately selected on startup, making workflows logical and tailored to a customer's needs. Specific experiment conditions can easily be reloaded, taking the guess work out of reproducing results.

Fucci cell cycle counting and expansion by cellSens.
Image data courtesy of Atsushi Miyawaki, Cell Function Dynamics, Brain Science Institute of RIKEN.

3D Time-lapse of mouse embryonic fibroblast labeled with silicone rhodamine docetaxol (Tubulin), imaged with 100X silicone objective and 30 fps resonant scanning followed by cellSens deconvolution. Image data courtesy of Dr. Markus Delling, Harvard University.

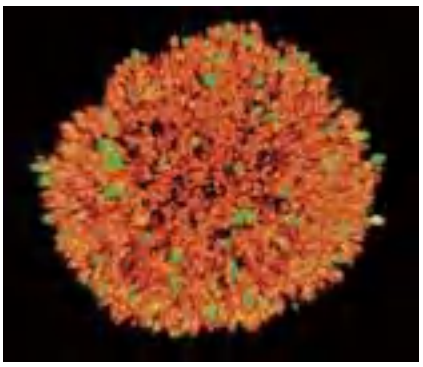


Sequence Manager allows for variable time-lapse

Solutions for Stem Cell Imaging: Z Drift Compensator, and Intuitive Software for Accurate Long-Term and Multipoint Time-Lapse Imaging in Microplates

Stem cell imaging requires increased levels of automation and long-term time-lapse capabilities. The FV3000 is designed to image cells over multiple days with accurate timing, low phototoxicity, and accurate focus. Multipoint time-lapse in microplates is routine in stem cell imaging, so the FV3000 can be enhanced with the IX3-ZDC2, Z drift compensator. The IX3-ZDC2 is designed to work with the well navigator, so each well stays in focus during an experiment. For long experiments, add the laser power monitor to maintain consistent laser exposure for excellent laser stability.

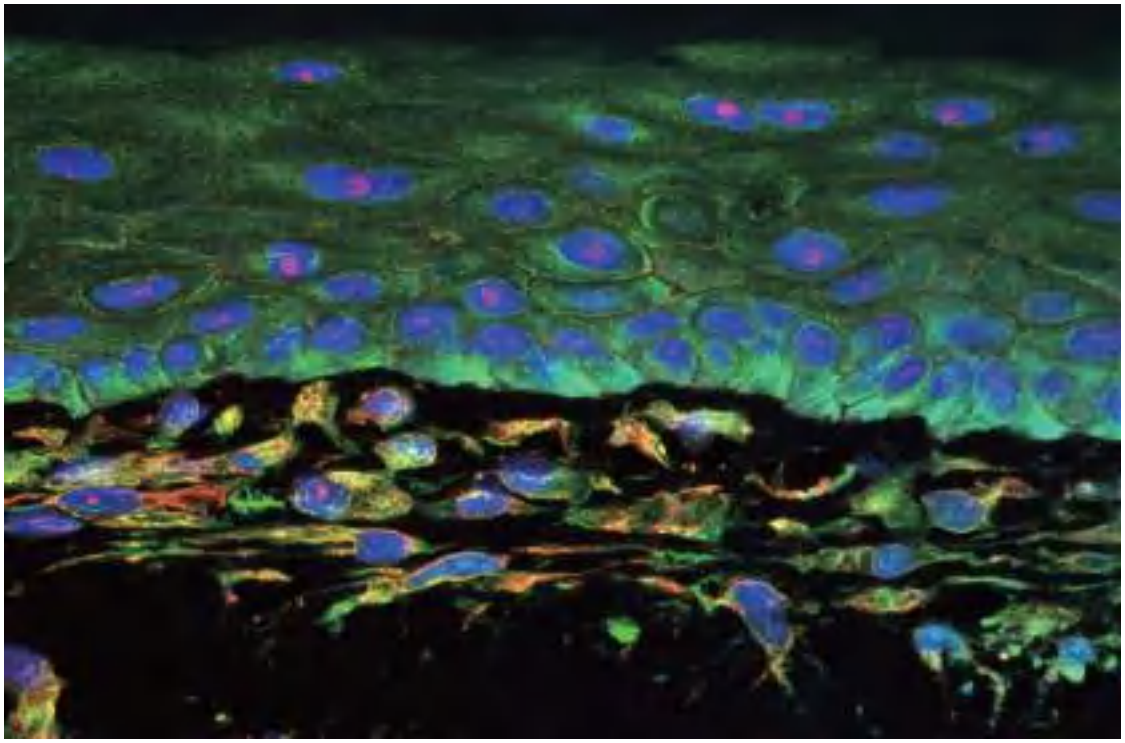
Users performing stem cell imaging benefit from high-sensitivity detection, silicone objectives, low phototoxicity from the resonant scanner, and the higher throughput from high-speed scanning. Precise stimulation control means photoconversion is simple and efficient, so cells can be reliably stimulated and imaged over multiple days for cell lineage tracking. Whether stem cell cultures are in microplates, single dishes, or microfluidic devices, the FV3000 software and automation makes workflows simple. The stage navigator includes well plate navigation and makes it easy to save, modify, and re-load frequently used plate settings and acquisition conditions. Users can quickly image individual lanes of microfluidic channels. The sequence manager makes it easy to set up long-term time-lapse imaging. Users can adjust the speed and timing of acquisitions while maintaining accurate timing. Quickly visualize and download publication and presentation-ready 3D and 4D image data with the intuitive rendering software included with the FV3000 software suite. Once imaging is completed, the macro functionality in cellSens analysis facilitates 2D cell counting and segmentation with a single mouse click.



A spheroid image of a NMuMG cell line expressing Fucci2.
Image data courtesy of Atsushi Miyawaki, Cell Function Dynamics, Brain Science Institute of RIKEN.

Multipoint time-lapse window

IX3-ZDC2



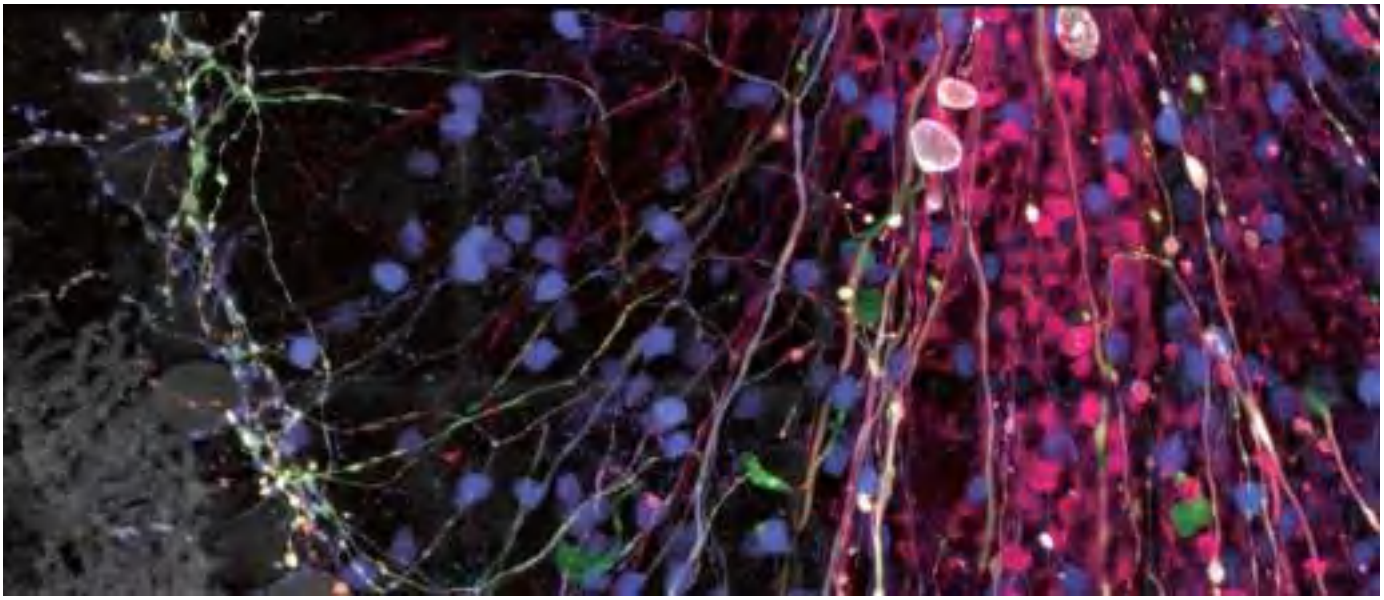
MatTek EpiDermFT Tissue Model: Immunofluorescence labeled with 6 targets of interest. 1. Abcam DRAQ5 ab108410, 2. Abcam Anti-GAPDH (Alexa Fluor 405) ab206372, 3. Abcam Anti-Tubulin (Alexa Fluor 488) ab1955883, 4. Abcam Anti-Fibrillarin (Alexa Fluor 568) ab202540, 5. Abcam Anti-Vimentin (Alexa Fluor 594) ab154207, 6. Abcam Anti-Ki67 (Alexa Fluor 647) ab 194724. sample courtesy of MatTek.

Solutions for Advanced Applications: Spectral Unmixing, Super Resolution, and Photostimulation

Both the FV3000 and FV3000RS have a range of standard and optional advanced application features including Olympus Super Resolution (FV-OSR), photostimulation, spectral unmixing, and an external beam combiner. With precise laser control and Olympus' patented super resolution method, the FV3000 Series can acquire images with a resolution down to 120 nm, similar to structured illumination methods. Spectral unmixing is robust for a range of applications while photoconversion and photostimulation are efficient and precise, enabling high-speed targeted path scanning and stimulation mapping studies.

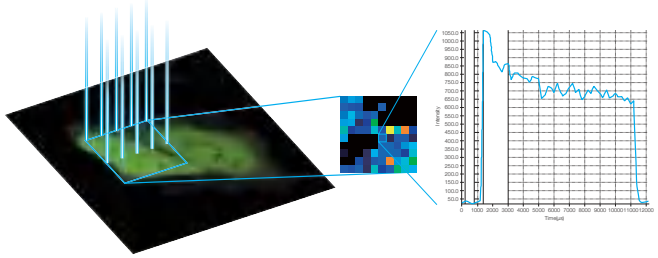
The Sequence Manager makes it easy to reliably achieve complex cell cycle imaging protocols. Advanced applications, such as random access or targeted path scanning, enable high signal-to-noise multipoint fluorescence measurements for *in vitro* neuronal cell signaling studies while real-time processing and triggering help provide accurate and coordinated timing control for TTL-driven perfusion devices, stimulators, or other 3rd party peripherals. Macro to micro functionality is easy with the FV3000 Series thanks to the stage navigator, automation built into the IX83 microscope, and the ability to save and reload software layouts, workflows, and experiment conditions.

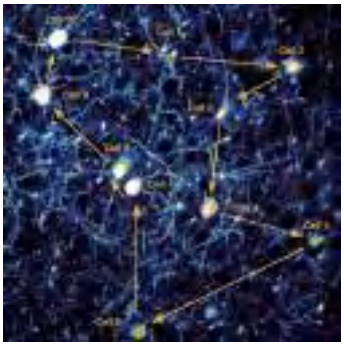
Spectral Umixing



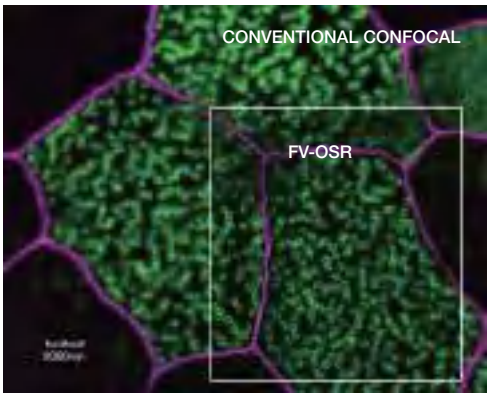
Brainbow AAV transfection of Purkinje cells, amplified with antibodies as described in Cai et al 2013. Visible are Purkinje cell somata, dendrites and axons, as well as some aspecific stainings of granule cells.

Photoconversion and Stimulation





Super Resolution



Trachea multi-ciliated epithelial cells (Culture)
Immunofluorescence microscopy: Odf2 staining (Alexa Fluor 488, green) of cilia at the upper part of the basal body (green). Staining for ZO-1 revealed the tight junctions (magenta).
Objective: UPLSAPO60XS
Image data courtesy of Hatsuho Kanoh, Elisa Herawati, Sachiko Tsukita,Ph. D. Graduate School of Frontier Biosciences and Graduate School of Medicine, Osaka University.

FV3000 with Galvanometer Scanner to FV3000RS with Resonant Hybrid Scanner: Flexible Configurations to Advance Science

High-Speed Resonant Scanning up to 438 Frames per Second
Page 13

Flexible Detection Lightpath with Wide Dynamic Range Photomultiplier Tubes (PMTs) or High Signal-to-Noise, Cooled GaAsP Spectral Detection Concept (2–4 Channels)
Page 14

Multichannel Spectral Detector with 16-Channel Unmixing
Page 14

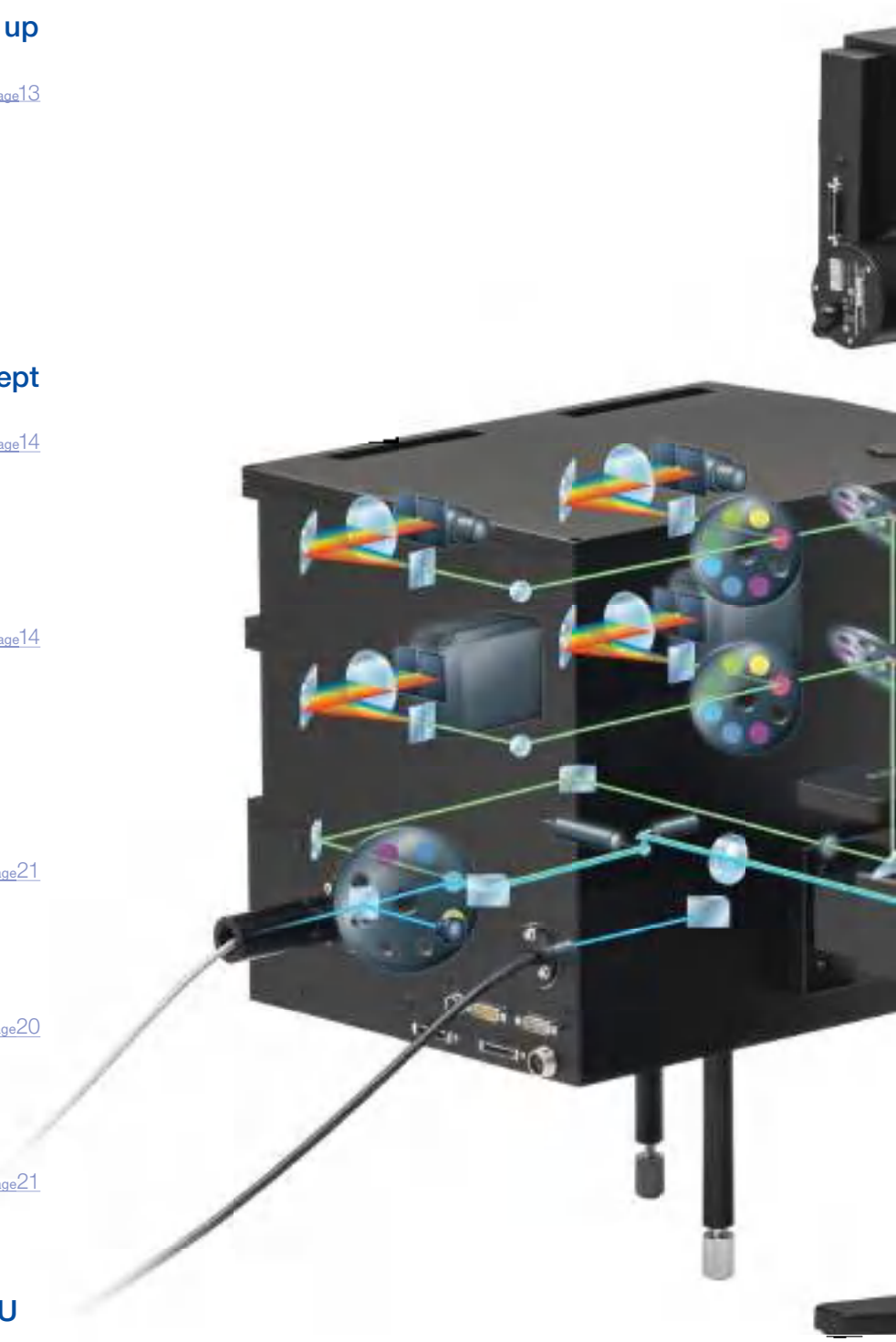
Combiner System Featuring Diode Lasers with a Range of Wavelengths
Page 21

Advanced Olympus Optics
Page 20

Z Drift Compensator—IX3-ZDC2
Page 21

Precise Ultrasonic Stage IX3-SSU for Multi-Area Imaging
Page 21

No Darkroom Required
Page 20



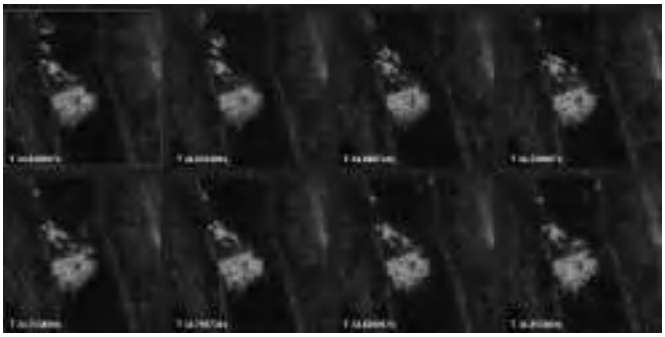
FV3000RS

The Right Mixture of Speed and Accuracy

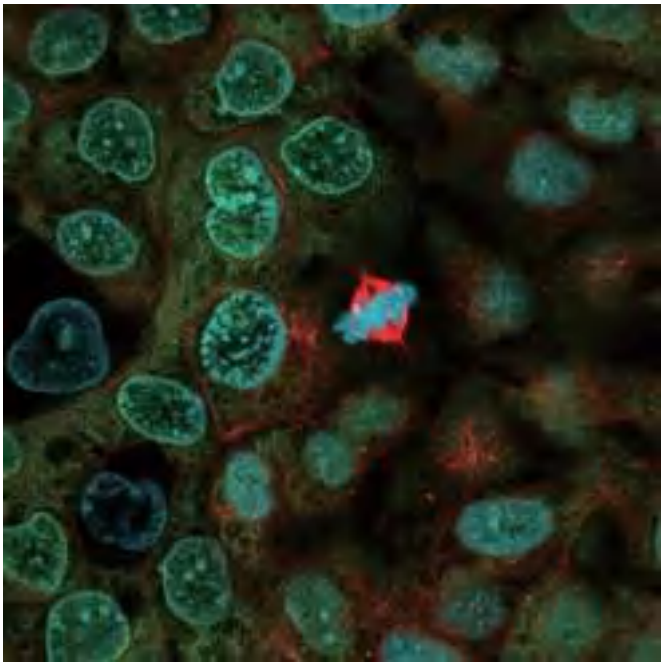
The FV3000 Series Scan Units

Galvanometer and Galvo/Resonant Hybrid Scanner

Users have their choice of two different types of scan units: galvanometer only with the FV3000 or galvanometer/resonant hybrid with the FV3000RS. The hybrid scan unit has galvanometer scanners for high-precision scanning, as well as a galvo/resonant scanner ideal for high-speed imaging. Galvanometer scanner enables Olympus super resolution technology (FV-OSR) yields resolutions down to 120 nm as well as high signal-to-noise, with precise tornado and multipoint stimulation and 100 ms switching time. Galvanometer scanning can achieve 16 frames per second at 2X zoom. The resonant scanner is capable of speeds ranging from 30 frames per second at 512 × 512 to 438 frames per second at 512 × 32.



Platelets bound to thrombosis in blood vessel of mouse. Images taken 30 fps in full frame by resonant scanner with 2 CH GaAsP PMTs.
Image data courtesy of Dr. Takuya Hiratsuka, Dr. Michiyuki Matsuda, Graduate School of Biostudies, Kyoto University.



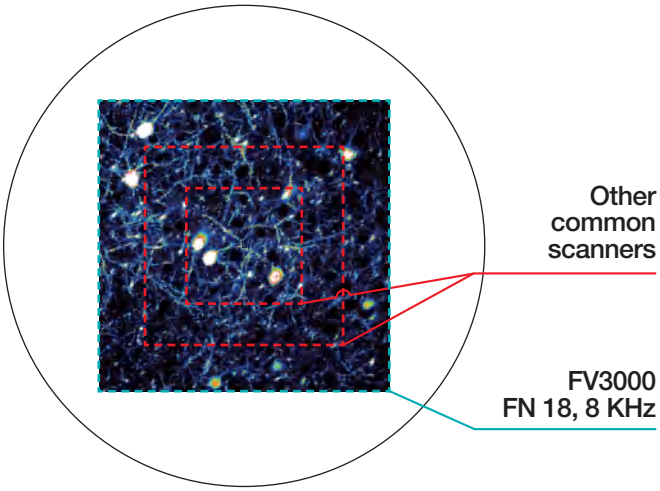
A431 cells fixed with methanol labeled with Abcam Anti-ERK1 + ERK2 antibody (Alexa Fluor 488) ab208564, and Anti-alpha Tubulin antibody (Alexa Fluor 594) ab195889 and DAPI.
Sample courtesy of Abcam.

Optimized for Live Cell Imaging

Resonant scanning greatly reduces photobleaching and phototoxicity compared to standard galvanometer scans by preventing the excitation of fluorophores into triplet states that create reactive oxygen species. These features make live cell experiments more robust and reliable. The FV3000 Series has complete high and low range laser intensity control enabling the system to use the minimum required amount of laser power on samples. The optional Laser Power Monitor provides consistent laser power during long-term time-lapse imaging across multiple days.

No Compromise between Speed and Field of View

Many high-speed scanning methods restrict the field of view, limiting their usefulness for examining large areas with multiple cells. The FV3000 Series' resonant scanner maintains a full 1X field of view, even at a video rate of 30 frames per second. Additional speed is generated by clipping the Y axis, even at 438 frames per second.



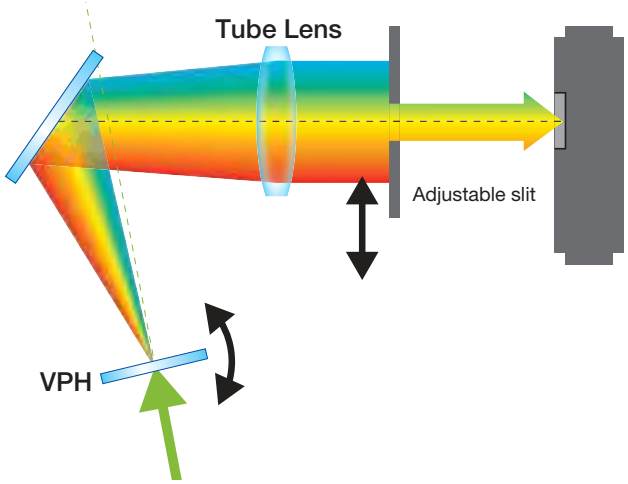
Most resonant scanners force a trade-off between speed and field of view. FLUOVIEW systems are optimized to maintain the field of view with even signal intensity so dynamic samples (e.g. calcium imaging) can be seen in the broad context of their cells and tissues.
The image above shows examples of the zoom factors required in other systems.

Introducing TruSpectral Detection

A Fully Spectral System with Sensitivity and Accuracy

The FV3000 Series employs Olympus' TruSpectral detection concept. Based on patented* Volume Phase Hologram (VPH) transmission and an adjustable slit to control light, the spectral detection in FV3000 and FV3000RS is highly efficient, enabling users to select the detection wavelength of each individual channel to 2 nm.

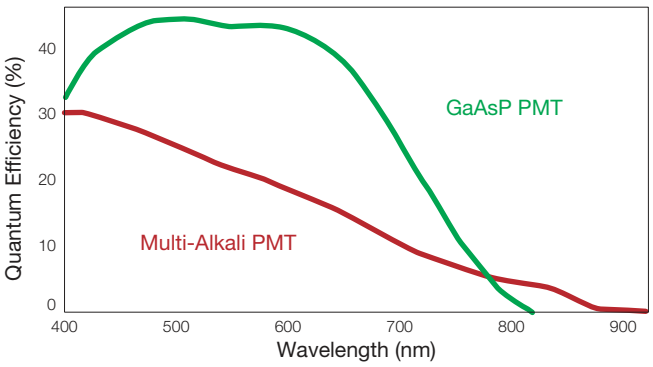
* US8530824B/JP5541972B/EP2395380A



High-Sensitivity Spectral Detector (HSD) with GaAsP Photomultiplier Tubes Enhances Quantum Efficiency

HSD makes it possible to view samples that were too dim to view with conventional equipment. The GaAsP PMT incorporates 2 channels with a maximum quantum efficiency of 45 %, and Peltier cooling reduces background noise by 20 % for high S/N ratio images under exceptionally low excitation light.

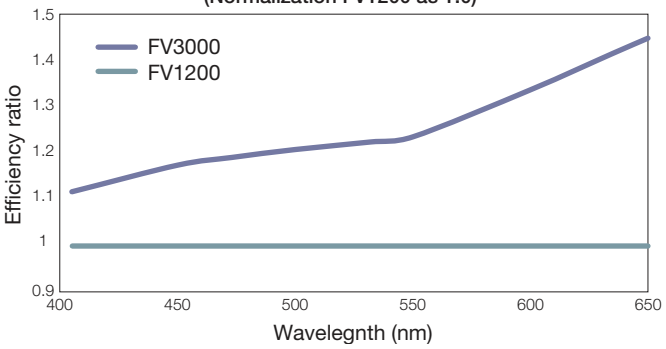
Standard Quantum Efficiencies of Detector Technologies



Efficient TruSpectral Detection System

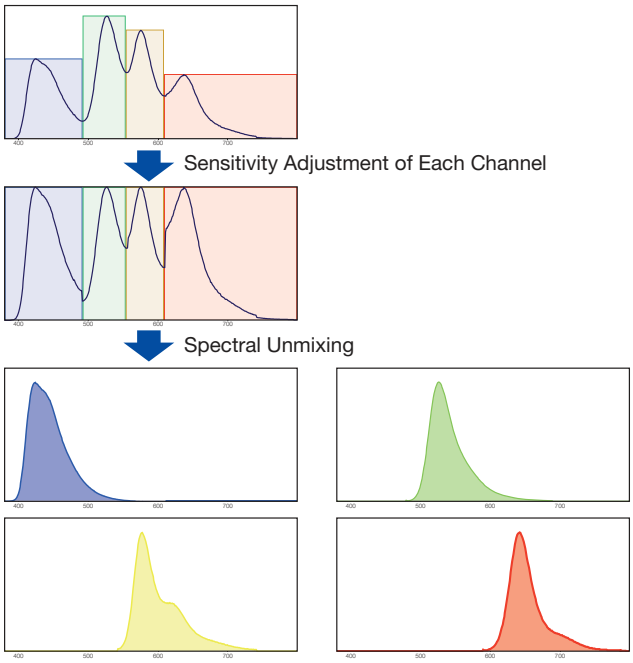
The FV3000 Series brings new levels of total system transmission efficiency, enabling every system to be completely spectral, improving overall sensitivity, and improving the signal-to-noise ratio for improved multi-color confocal imaging.

Transmission Efficiency Ratio of FV3000 to FV1200
(Normalization FV1200 as 1.0)



Multichannel TruSpectral Detection with 16-Channel Unmixing

TruSpectral's efficient design and software enable spectral detectors to run in multichannel mode for both live and post-processing spectral unmixing with a multichannel lambda mode. Multichannel mode facilitates constant spectral unmixing during live cell experiments, separating complex fluorescence during acquisition. With up to 4 different dynamic ranges from the 4 different channels of array, even bright and dim spectral signals can be separated by adjusting the sensitivity of each detector independently.



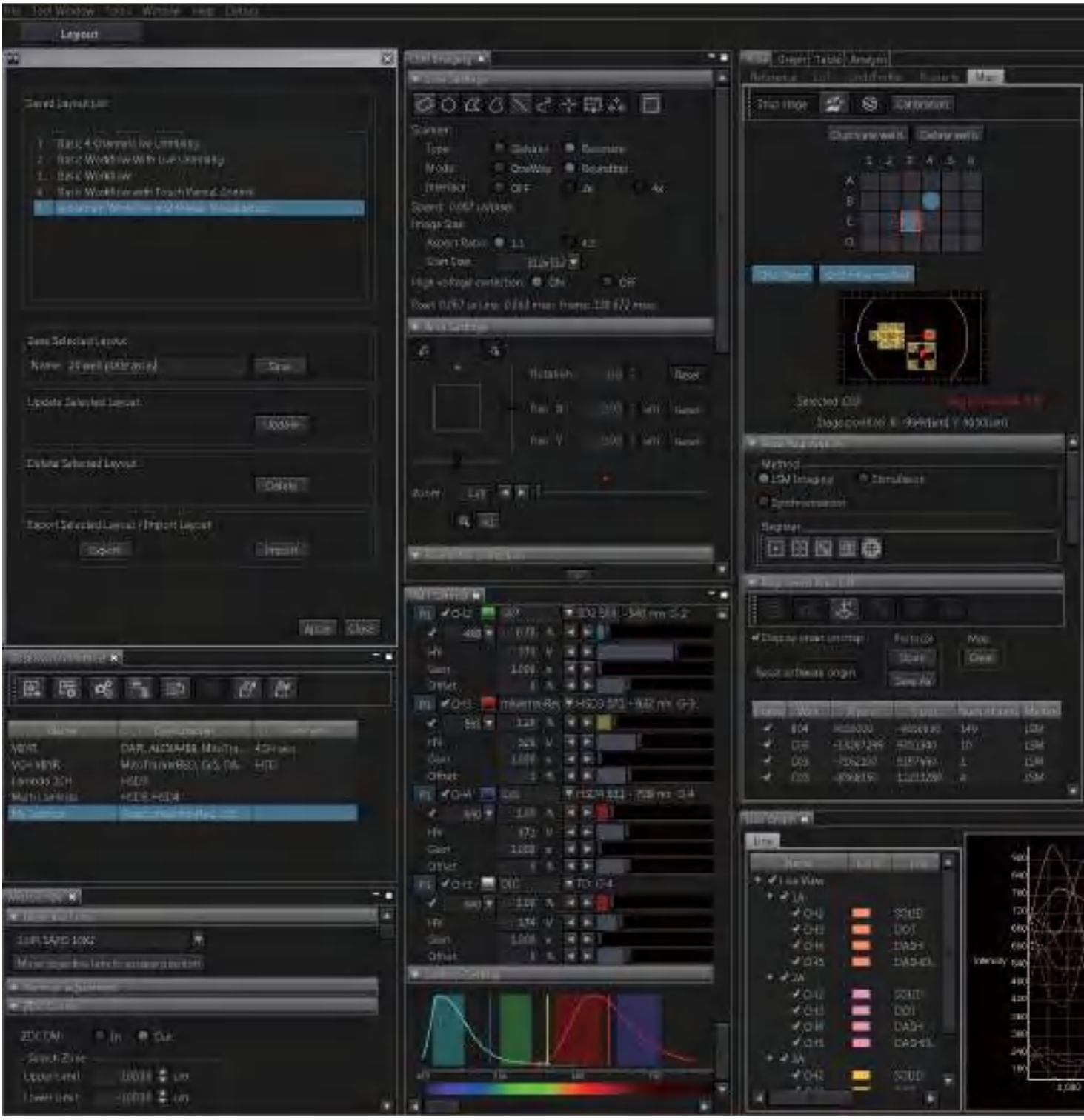
From Basic to Advanced Acquisition and Analysis, an Interface that Adapts to Your Workflow

Intuitive Workflow
Customizable and saveable layouts make it easy to tailor the interface to your workflow and experiment needs, from basic to complex.

1. Layout
Start by selecting your preferred display with specific tools for basic to complex acquisition.

2. Acquisition Condition
Reload settings that were ideal for your last experiment to provide consistency.

3. Acquisition
Activate basic to complex acquisitions with live ratio, intensity modulated display, quantitative region of interest (ROI) graphing or spectral unmixing display, and data backup for added security.

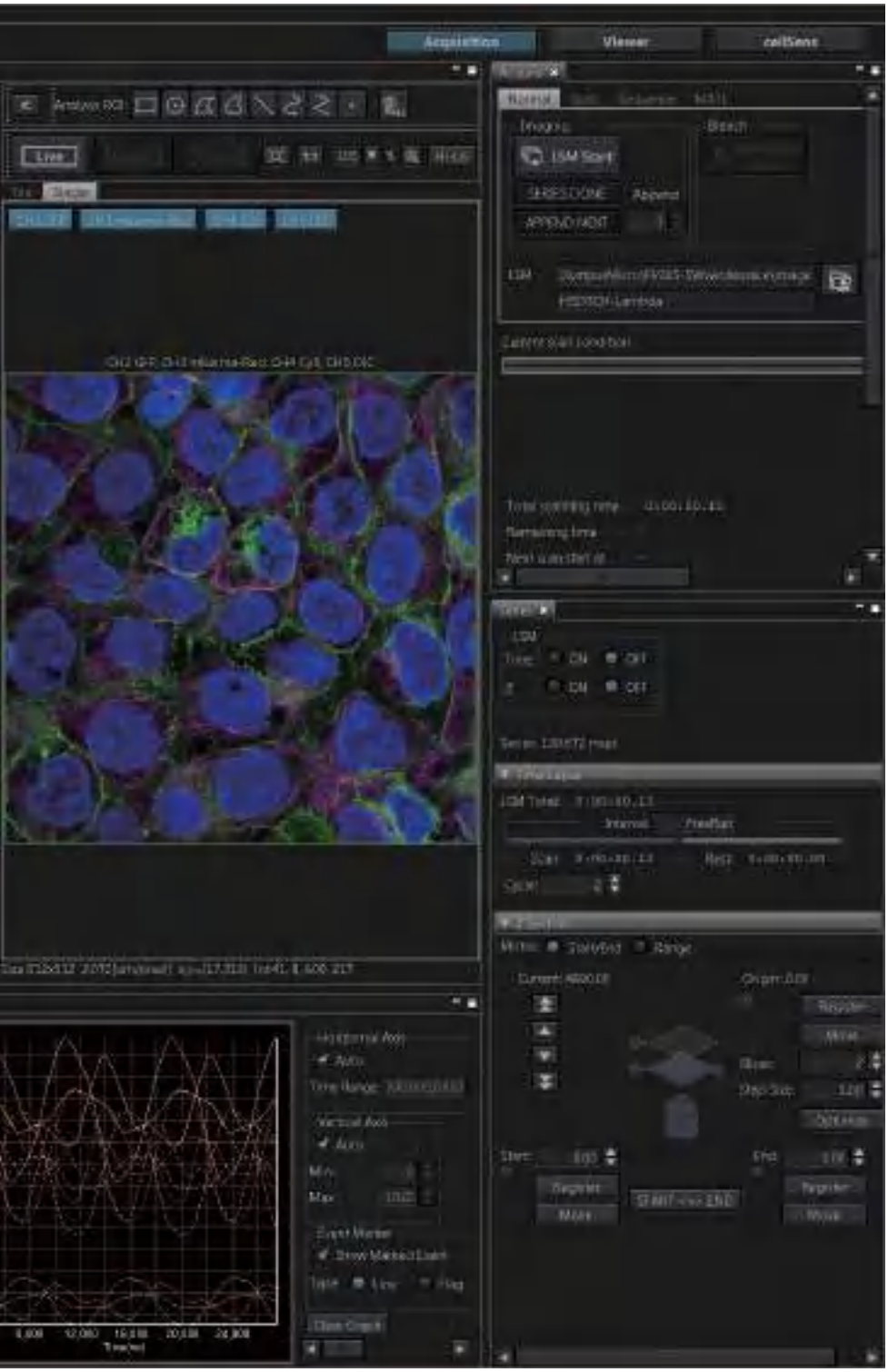


4.Viewer

Review data as it is generated. Generate 3D and 4D views and animations to explore and share data in depth.

5. Analysis

Extract data from images using online or offline processing. Analytical tools include Olympus super resolution technology (FV-OSR) and powerful cellSens software with features such as deconvolution, filtering, count and measure, and one-click macros.



Live Spectral Unmixing with TruSpectral Detection

Page17

Sequence Manager

Page17

Stage Control for Multipoint Time-Lapse and Microplate

Page17

Hard Drive Data Backup

Page17

One-Click Macro Analysis

Page17

Ratio Imaging and Intensity Modulated Display (IMD)

Page18

Rolling Average Processing

Page18

Deconvolution

Page18

FV-OSR (Olympus Super Resolution) Technology

Page19

Macro to Micro Observation

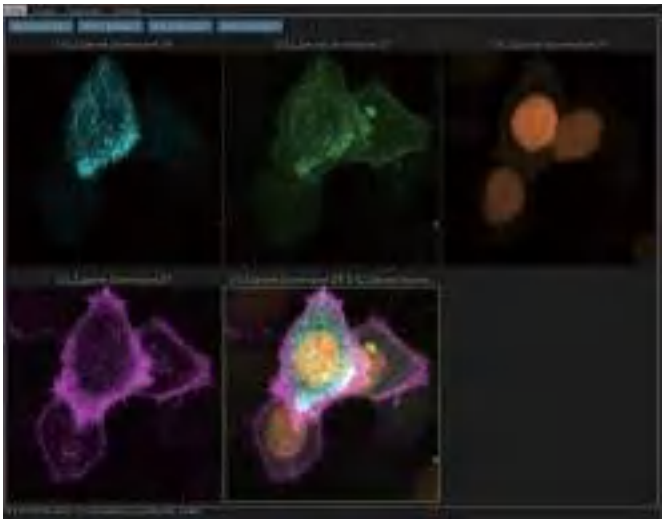
Page19

A431 cells fixed with 100 % methanol. Abcam Anti-Integrin alpha 2 antibody (Alexa Fluor 488) ab208770, and Anti-alpha Tubulin antibody (Alexa Fluor 594) ab195889 and DAPI.
Sample courtesy of Abcam.

Intuitive Stage Control, Live Spectral Unmixing, Real-Time Acquisition

Live Spectral Unmixing with TruSpectral Detection and Real-Time Processing

The power of TruSpectral detection plus multichannel mode means live spectral unmixing can be performed during image acquisition, providing real-time processing of complex overlapping spectra.

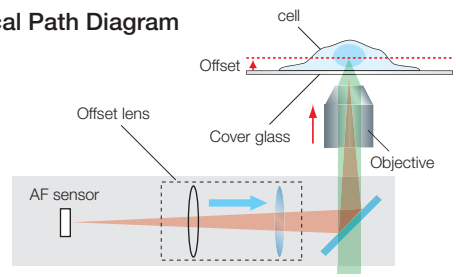


Live blind unmixing of CFP (endosomes, blue), mAmetrine (plasma membrane, green), mKO (nucleus, orange) and mKeima (F-actin, purple) during time-lapse imaging.
Image data courtesy of Dr. Kazuhiro Aoki, Dr. Michiyuki Matsuda, Graduate School of Medicine, Kyoto University.

Maintain Focus with Z Drift Compensation (ZDC) System

The IX3-ZDC2 uses minimally-phototoxic IR light (laser class 1) to identify the location of the sample plane. One-shot autofocus (AF) mode allows several focus positions to be set as desired for deeper samples, enabling efficient Z-stack acquisitions in multi position experiments. Continuous AF mode keeps the desired plane of observation precisely in focus, avoiding focus drift due to temperature changes or the addition of reagents, making it ideal for measurements that requires more stringent focusing. Furthermore, increased optical offset enables continuous AF over plastic vessels or with dry objectives. The IX3-ZDC2 is also compatible with silicone objectives (in AF mode).

IX3-ZDC2 Optical Path Diagram

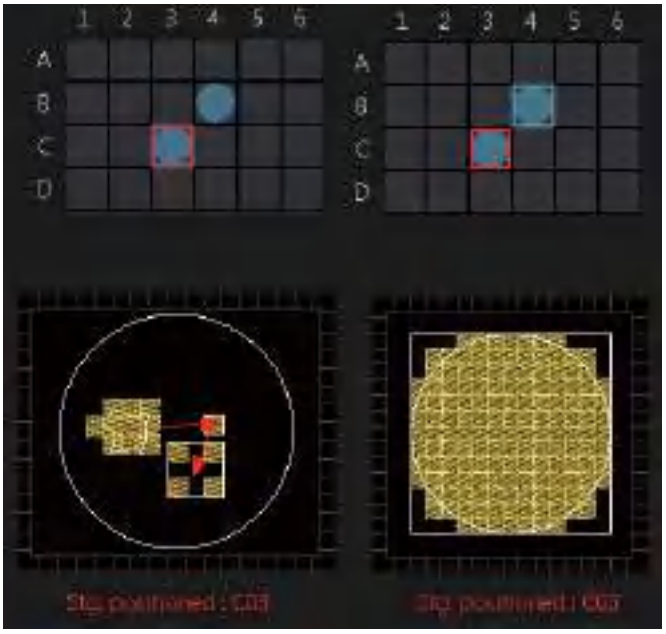


Precise Sequence Manager and Real-Time Acquisition

Complex protocols are handled with ease, and real-time control helps provide microsecond accuracy of scans with millisecond accuracy over days of time-lapse.

Stage Control for Multi-Area Time-Lapse, Microplate, and Stitching

Microplate imaging is important for many applications, and the Well Navigator provides sophisticated, intuitive controls for a wide range of cell culture vessels and custom plates. Multi-area time-lapse and stitching provide robust and accurate time-lapse data.

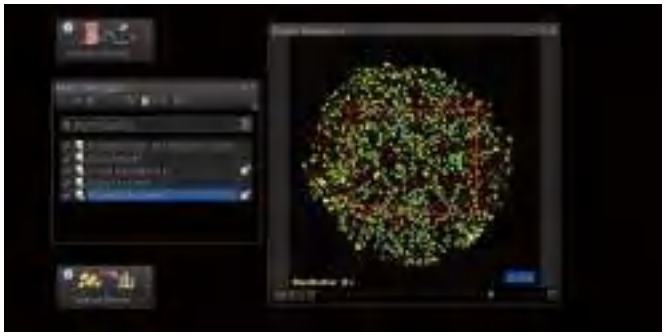


Hard Disk Recording

The microscope comes equipped with a hard-disk drive (HDD) recording function. The images captured are stored automatically in the HDD. Large volumes of data, such as those obtained from long-term time-lapse imaging can be stored.

Powerful One-Click Macro Analysis with cellSens

Images alone are not enough; with integrated cellSens Count and Measure analysis, the FV3000 Series can optimize images with deconvolution and analyze them with one-click macro functionality for a broad range of morphological measurements.

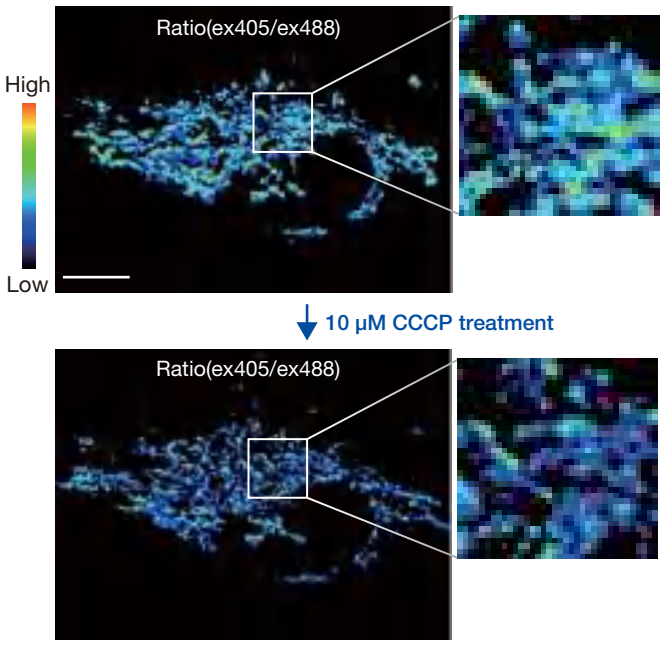


A spheroid image of a NMuMG cell line expressing Fucci2.
Image data courtesy of Atsushi Miyawaki, Cell Function Dynamics, Brain Science Institute of RIKEN.

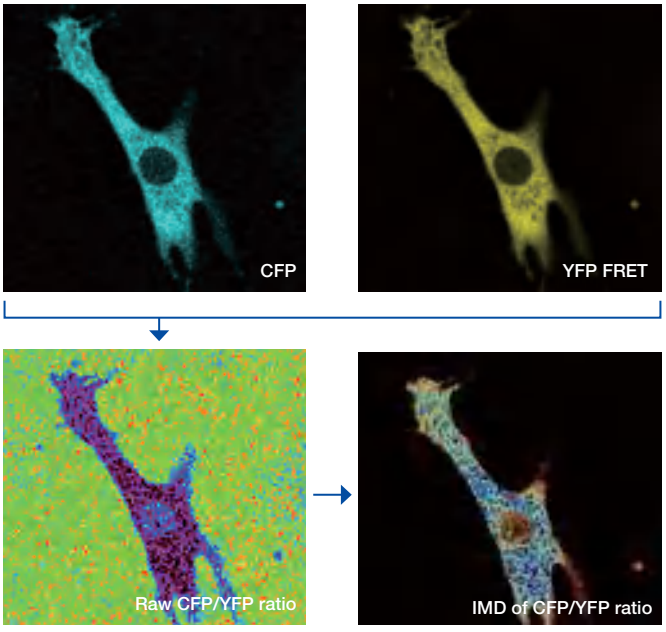
Additional Intuitive Features

Ratio Imaging and Intensity Modulated Display (IMD)

The FV3000RS includes an Intensity Modulated Display (IMD) function in the software that displays quantitative fluorescence ratio changes during both standard and high-speed acquisitions. This function is particularly useful for calcium and FRET imaging where a pure ratio display provides poor contrast in background areas.



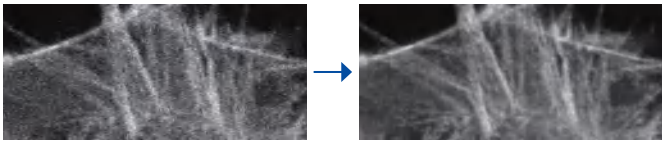
tsGFP1-mito reveals heterogeneity in mitochondrial thermogenesis in HeLa cells. The images of ratio (ex 405 nm/ex 488 nm) in tsGFP1-mito-expressing cells before and after CCCP treatment at 37 °C. Scale bars indicate 10 µm (whole image) and 3 µm (inset).
Image data courtesy of Shigeki Kiyonaka Ph,D, Yasuo Mori Ph,D Molecular Biology Field, Department of Synthetic Chemistry and Biological Chemistry, Kyoto University.



Cardiomyoctye
Image data courtesy of Yusuke Niino and Atsushi Miyawaki, Cell Function Dynamics, Brain Science Institute of RIKEN.

Rolling Average Processing

High-speed scanning at low laser power to avoid phototoxicity often decreases the signal-to-noise ratio. With rolling average post-processing, users have the flexibility to adjust high-speed time-lapse images while maintaining time scale and keeping the original data.

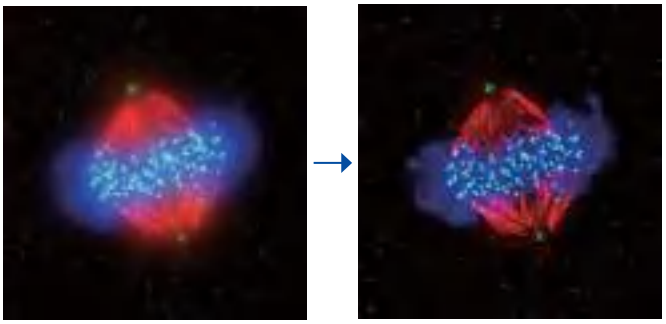


Raw 30 fps data acquired at low laser power (0.05 %, 488 nm).

Rolling average processing (10 frame) on 30 fps data acquired at low laser power.

Deconvolution

The optional Constrained Iterative (CI) Deconvolution Solution employs advanced CI algorithms to produce improved resolution, contrast, and dynamic range, with industry-leading speed. This proprietary post-processing tool is efficient for both CCD and confocal imaging and enhances the ability to differentiate between imaged objects.



Original Image

Deconvolved Image

Cell line: Human cervical cancer cell line HeLa cell
Immunostaining: Hec1 staining (green, Alexa Fluor 488), α-tubulin staining (red, Alexa Fluor 568),DAPI staining (blue)
Mitotic HeLa cell derived from human cervical cancer.
Mitotic spindle and kinetochores are stained with anti-α-tubulin (red) and anti-Hec1 (green) antibodies, respectively. Chromosomes interact with microtubules constituting mitotic spindle via kinetochores, protein structure assembled on centromere region of chromosomes.
Image data courtesy of Masanori Ikeda and Kozo Tanaka, Department of Molecular Oncology, Institute of Development, Aging and Cancer, Tohoku University.

Olympus Super Resolution Technology

Olympus Super Resolution (FV-OSR) Technology with Up to 4 Simultaneous Channels

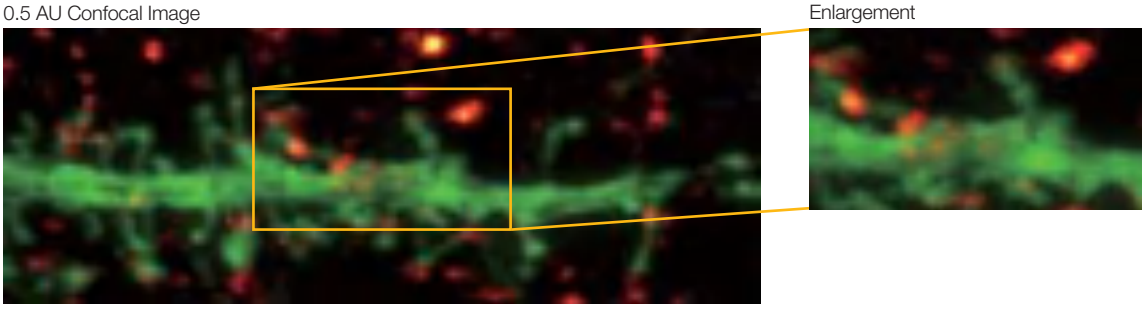
Olympus' widely applicable super resolution method requires no special fluorophores and works for a wide range of samples. Ideal for colocalization analysis, the FV-OSR can acquire 4 fluorescent signals either sequentially or simultaneously with a resolution of approximately 120 nm*, nearly doubling the resolution of typical confocal microscopy. The system is easy to use with minimal user training and can be added to any confocal system, making the FV-OSR a truly accessible method for achieving super resolution.

* Subject to objective magnification, numerical aperture, excitation and emission wavelength, and experiment conditions.

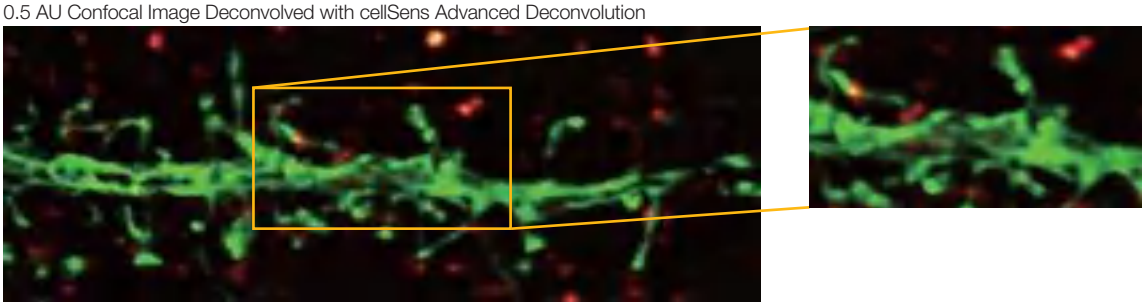
Beyond Deconvolving Confocal: Comparison of Confocal, Deconvolved Confocal and Deconvolved FV-OSR Images

0.5 AU Confocal Image

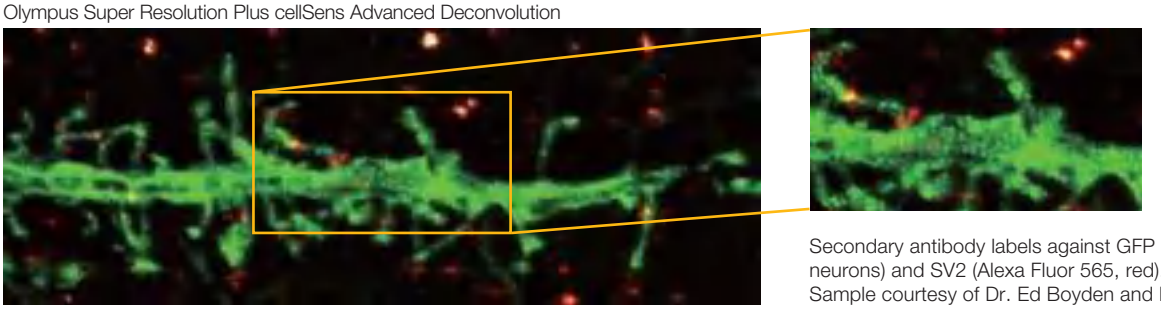
Enlargement



0.5 AU Confocal Image Deconvolved with cellSens Advanced Deconvolution



Olympus Super Resolution Plus cellSens Advanced Deconvolution



Secondary antibody labels against GFP (Alexa Fluor 488, neurons) and SV2 (Alexa Fluor 565, red).
Sample courtesy of Dr. Ed Boyden and Dr. Fei Chen, MIT.

Macro to Micro Observation

Finding areas of interest in samples can be challenging. The confocal optical design of the FV3000 Series supports macro to micro imaging so users can quickly switch from low magnification overview observation with 1.25X objectives to high-magnification, detailed observation with up to 150X objectives. Users can employ image stitching at both macro and micro levels to generate overview images that show samples in context.

A stitched image of a coronal section (30 µm thickness) from an adult YFP-H mouse cerebrum acquired with 20X objective (UPLSAPO20X).
Image data courtesy of Takako Kogure and Atsushi Miyawaki, Cell Function Dynamics, Brain Science Institute of RIKEN.

Superior Optics and a Rigid Frame Ideal for Live Cell Imaging

Silicone Immersion Objectives for Live Cell Imaging Deliver High-Resolution Observation at Depth

Olympus offers four high NA silicone immersion objectives that deliver excellent performance for live cell imaging. The refractive index of silicone oil (ne≈1.40) is close to that of living tissue (ne≈1.38), enabling high-resolution observations deep inside living tissue with minimal spherical aberration caused by refractive index mismatch. Silicone oil does not dry out or harden, so there is never a need to refill oil, making it ideal for extended time-lapse observations.

UPLSAPO30XS: For a broader view and greater depth

Magnification: 30X, NA: 1.05 (silicone oil immersion), W.D.: 0.8 mm, cover glass thickness: 0.13–0.19 mm, operating temperature: 23 –37 °C

UPLSAPO40XS : Complete the magnification range

Magnification: 40X, NA: 1.25 (silicone oil immersion), W.D.: 0.3 mm, cover glass thickness: 0.13–0.19 mm, operating temperature: 23 –37 °C

UPLSAPO60XS2: For 3D observations with superior resolution

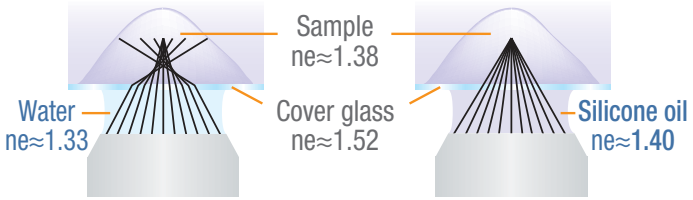
Magnification: 60X, NA: 1.30 (silicone oil immersion), W.D.: 0.3 mm, cover glass thickness: 0.15–0.19 mm, operating temperature: 23 –37 °C

UPLSAPO100XS: For greater depth in closely defined regions

Magnification: 100X, NA: 1.35 (silicone oil immersion), W.D.: 0.2 mm, cover glass thickness: 0.13–0.19 mm, operating temperature: 23 –37 °C

Refractive Index is Important with Deep Tissue Observation

In deep tissue observation, image quality depends on keeping the refractive index of the sample and immersion medium as close to each other as possible.



Water immersion objective

When working with a water immersion objective, the difference between the refractive index of the samples and water results in spherical aberration in deep tissue, causing resolution to deteriorate and fluorescence to become dim.

Silicone immersion objective

When working with a silicone immersion objective, the difference between the refractive index of the samples and silicone oil is minimal. So it achieves brighter fluorescence images with higher resolution for deep tissue.

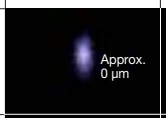
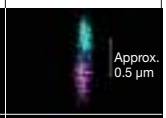
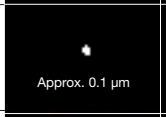
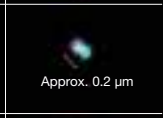
PLAPON60XOSC2: Enhance the Reliability of Colocalization Analysis with a Low Chromatic Aberration Objective

This oil immersion objective minimizes lateral and axial chromatic aberration in the 405–650 nm spectrum. Colocalization images are acquired reliably and images are measured with superior positional accuracy. The objective also compensates for chromatic aberration through near infrared up to 850 nm, making it the ideal choice for quantitative imaging.

Low Chromatic Aberration Objective

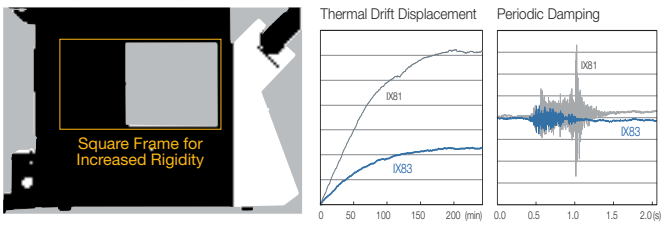
Magnification: 60X
NA: 1.4 (oil immersion)
W.D.: 0.12 mm
Chromatic aberration compensation range: 405–650 nm
Optical data provided for each objective.

Performance Comparison of PLAPON60XOSC2 and UPLSAPO60XO

	PLAPON 60XOSC2	UPLSAPO 60XO
Axial chromatic aberration (Z direction) Compared for PSF fluorescent beads (405 nm, 633 nm)		
Lateral chromatic aberration (X-Y direction) Compared for PSF fluorescent beads (405 nm, 488 nm, 633 nm)		
3D image Tubulin in Ptk2 cells labeled with two colors (405 nm, 635 nm) and compared		

Meeting the Requirements of Stability with the IX83

A Z-drive guide installed near the revolving nosepiece combines high thermal rigidity with the stability of a wraparound structure to significantly reduce the impact of heat and vibration and improve the quality of time-lapse imaging.



High Contrast under Bright Conditions

The umbra unit is designed specifically for fluorescence observation. It efficiently blocks out room light, enhances the contrast of fluorescence, and enables clear fluorescence observation under bright conditions.

Modular Units Designed for Your Applications

Scanners

Hybrid Scan Unit (Resonant/Galvanometer)
The hybrid scanner combines the capabilities of a galvanometer scanner with a resonant scanner for high-speed imaging in the full field of view at 30 fps and up to 438 fps at 512 × 32. The Sequence Manager makes it simple to automatically switch between resonant and galvanometer imaging in the same experiment.

Galvo Scan Unit
The galvanometer-only scanner provides precision scanning from 1 fps at 512 × 512 to 16 fps. High-speed multipoint stimulation or detection experiments can travel between multiple cells at over 100 Hz with data output as high as 500 kHz.

Spectral Detectors

High Sensitivity Spectral Detector (GaAsP PMT) with TruSpectral Technology
The 2-channel High Sensitivity Spectral Detector (HSD) employs the same Volume Phase Holographic (VPH) technology as the spectral detector (SD), with Peltier cooled GaAsP PMTs and a high quantum efficiency of 45 % and detection up to 750 nm. This unit can be combined with the 2-channel SD for a flexible dynamic range or a second 2-channel HSD unit for powerful 4-channel sensitivity.

Spectral Detector (Multialkali PMT) with TruSpectral Technology
The 2-channel SD employs efficient VPH transmission and an adjustable slit with 1–100 nm bandwidth from 400–800 nm detection. The multialkalai PMTs provide a broad dynamic range for detection up to 800 nm.

Laser Combiners

Main Laser Combiner
The main laser combiner is the heart of the laser system. Four standard lasers with an option to add a fifth laser or leave an open port to add an additional three diode lasers via the Sub Combiner.

Sub Laser Combiner
Add this optional combiner at any time with up to 3 diode lasers for a maximum of 7 laser lines in combination with the main laser combiner.

Illumination Units

The 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.

Light Source/U-HGLGPS
The pre-centered fluorescence illumination source requires no adjustment and has an average lifespan of 2,000 hours.

Transmitted Detector/FV31-LETD
This unit combines an external transmitted light photomultiplier detector and LED conventional illumination for both laser scanning and conventional transmitted light Nomarski DIC observation. Users can undertake simultaneous multichannel confocal fluorescence imaging and transmitted DIC acquisition.

Other Equipment

Choose from the following options with field-upgradable laser-based autofocus, fast and precise motorized stage control, analog input/output and TTL synchronization, and a convenient anti-vibration platform.

Z Drift Compensator/IX3-ZDC2
The IX3-ZDC2 uses minimally-phototoxic IR light to identify the location of the sample plane. The IX3-ZDC2 is also compatible with silicone objectives and plastic bottom vessels.

Ultrasonic Stage for IX3/IX3-SSU
With low thermal drift for improved accuracy, the ultrasonic stage can be controlled by both software and Touch Panel Control for fast, reliable multi-area imaging.

IO Interface Box/FV30-ANALOG
This unit supports electro-physiological experiments through analog inputs and TTL I/O support. The interface box converts voltage to images that can be treated in the same manner as fluorescence images.

Simple Anti-Vibration Plate/FV31-AVP
Designed to match the footprint of the FV3000, this simple anti-vibration plate provides a compact solution for those who do not need a full anti-vibration table.

Specifications

FLUOVIEW FV3000 Specifications

		FV3000	FV3000RS
Laser Light	Violet/Visible Light Laser	405 nm: 50 mW, 488 nm: 20 mW, 561 nm: 20 mW, 640 nm: 40 mW One optional laser port for sub laser combiner or optional laser unit	
Optional Laser	Sub Laser Combiner	Laser as follows (max. 3 laser units) 445 nm: 75 mW, 514 nm: 40 mW, 594 nm: 20 mW, connected to main laser combiner	
	Single Laser Unit	445 nm: 75 mW, 514 nm: 40 mW, or 594 nm: 20 mW, directly connected to main laser combiner	
Laser Light Control		Main laser combiner with implemented AOTF system, ultra-fast intensity modulation with individual laser lines, additional shutter control continuously variable (0.1 %–100 %, 0.1 % increments) 10 % or 100 % maximum laser power changer by ND filter	
Scanner	Scanning Method	2 silver-coated galvanometer scanning mirrors	2 silver-coated galvanometer scanning mirrors 1 silver-coated resonant and 1 silver-coated galvanometer scanning mirrors.
	Galvanometer Scanner (Normal Imaging)	Scanning Resolution: 64 × 64 to 4096 × 4096 pixels Scanning Speed (One Way): 512 × 512 with 1.1 s — 264 s. pixel time : 2 µs — 1000 µs. Scanning Speed (Round Trip): 512 × 512 with 63 ms - 250 ms 256 × 256 with 16 ms - 125 ms Optical Zoom: 1X – 50X in 0.01X increments Scan Rotation: Free rotation (360 degree) in steps of 0.1 degree Scanning Mode: PT, XT, XZ, XY, XZT, XYT, XYZ, XYλ, XYZT, XYλT, XYλZ, XYλZT ROI Scanning, rectangle clip, ellipse, polygon, free area, line, free line and point, tornado mode only for stimulation	
	Resonant Scanner (High-Speed Imaging)	–	Scanning Resolution: 512 × 32 to 512 × 512 pixels Scanning Speed: 30 fps at 512 × 512, 438 fps at 512 × 32 Optical Zoom: 1X – 8X in 0.01X increments Scanning Mode: XT, XZ, XY, XZT, XYT, XYZ, XYλ, XYZT, XYλT, XYZ, XYλZT ROI Scanning, Rectangle Clip, Line
	Pinhole	Single motorized pinhole, pinhole diameter ø50 – 800 µm (1 µm Steps)	
	Field Number (FN)	18	
	Dichromatic Mirror Turret	8 positions (high performance DMs and 10/90 mirror)	
	Optional Unit for Scanner	Laser Power monitor, optional laser port	
High Sensitivity-Spectral Detector	Detector Module	Cooled GaAsP photomultiplier, 2 channels	
	Spectral Method	Motorized Volume Phase Holographic transmission diffraction grating, motorized adjustable slit, selectable wavelength bandwidth: 1–100 nm, wavelength resolution: 2 nm	
	Dichromatic Mirror Turret	8 positions (high performance DMs and mirror)	
Spectral Detector	Detector Module	Multi-Alkali photomultiplier, 2 channels	
	Spectral Method	Motorized Volume Phase Holographic transmission diffraction grating, motorized adjustable slit selectable wavelength bandwidth: 1–100 nm, wavelength resolution: 2 nm	
	Dichromatic Mirror Turret	8 positions (high performance DMs and mirror)	
Microscope	Motorized Microscope	Inverted IX83 (IX83P2ZF) Integrated motorized focus module, minimum increment 0.01 µm	
System Control	Control Unit	OS: Windows 7 Professional 64-bit (English version), built-in dedicated I/F board and hardware sequencer for precise imaging timing	
	Display	30 or 32-inch monitor (WQUXGA 2560 × 1600)	
Fluorescence Illumination Unit		External fluorescence light source, fiber adapter to optical port of scan unit, motorized switching between LSM light path and fluorescence illumination	
Transmitted Light Detector Unit		Module with integrated external transmitted light photomultiplier detector and LED lamp, motorized switching	

Software

Basic Features	GUI designed for darkroom environment. User-arrangeable layout. Acquisition parameter reload features. Hard disk recording capability, adjust laser power and HV with Z-stack acquisition. Z-stack with alpha blending, maximum-intensity projection, iso-surface rendering.
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. 3D animation (maximum intensity projection method, α blending) 3D and 2D sequential operation function
Image Format	OIR image format 8/16-bit gray scale/index color, 24/ 32/ 48-bit color, JPEG/ BMP/ TIFF image functions, Olympus multi-tif format
Spectral Unmixing	Fluorescence spectral unmixing modes (up to 16 channels)
Image Analysis	Fluorescence intensity and time-lapse measurement
Statistical Processing	2D data histogram display
Optional Software	Motorized-stage control Mapping and multiplepoint stimulation Sequence manager Virtual channel acquisition Microplate navigation Remote development kit Super resolution imaging (FV-OSR)

World Wide Support

Installation generally takes one day to get systems up and running fast. We support our products via our global knowledge base. Olympus application specialists can assist you with choosing the features that will optimize your system for your applications. Confocal systems are an investment, and keeping the system running in the best performance is important. Our certified service teams can deploy rapid alignment procedures and system diagnostics to keep your system in top shape and diagnose any issues.

Image data are courtesy of the following institutions:

Mouse kidney (cover and page 2) and rat embryo sample (3, page 1) prepared by Dr. Mike Davidson. Images presented with lasting gratitude for his lifetime commitment to science and microscopy.

Whole mouse kidney captured in single shot with 1.25X objective. 10 µm section, TOMM20 ATTO 647N, Phalloidin Alexa Fluor 568, WGA Alexa Fluor 488, DAPI. (cover page and page 2)

3D rendered image of Xenopus endoderm labeled with malachite green and methylene blue. 3 channel image captures label and autofluorescence. (1, page 1)

3D rendered image of Xenopus endoderm labeled with malachite green and methylene blue. (2, page 1)

2 × 2 tiled image of whole rat embryo, 20 mm total field of view. H&E fluorescence with 640 nm laser diode. (3, page 1)

Growing HeLa cells expresses Fucci, a cell cycle indicator. Fluorescense image (1, page 3), Cell counting (2,3, page 3)
Asako Sakaue-Sawano, Atsushi Miyawaki, Cell Function Dynamics, Brain Science Institute of RIKEN.

3D Time-lapse of mouse embryonic fibroblast labeled with silicone rhodamine docetaxol (Tubulin, white), RFP centrin (green) imaged with 100X silicone objective and 30 fps resonant scanning followed by cellSens deconvolution. Dr. Markus Delling, Harvard University. (4, page3)

Sca*l*eA2-treated neocortex
Motokazu Uchigashima, M.D., Ph.D., Masahiko Watanabe, M.D., Ph.D., Departments of Anatomy, Hokkaido University Graduate School of Medicine. (5,6, page 3)

A stitched image of a coronal section (30 µm thickness) from an adult YFP-H mouse cerebrum acquired with 20X objective (UPLSAPO20X).
Takako Kogure and Atsushi Miyawaki, Cell Function Dynamics, Brain Science Institute of RIKEN. (7, page3)

Platelets bound to thrombosis in blood vessel of mouse. Images taken 30 fps in full frame by resonant scanner with 2 CH GaAsP PMTs.
Dr.Takuya Hiratsuka, Dr. Michiyuki Matsuda, Graduate School of Biostudies, Kyoto University. (8, page 3)

FRET spectral look up table display of cardiac myocyte (9, page 3), CFP spectral look up table display of cardiac myocyte (10, page 3) , Differential Interference Contrast (DIC) image of cardiac myocyte (11, page 3), Overlay (12, page 3), IMD ratio images of spontaneous Ca^{2+} oscillation in a beating rat cardiomyocyte expressing yellow cameleon. (13, page 3)
Yusuke Niino and Atsushi Miyawaki, Cell Function Dynamics, Brain Science Institute of RIKEN.

Fucci induced Spheroid of HT29 cell line
Yuji Mishima, Ph.D., Kiyohiko Hatake M.D., Ph.D. Clinical Chemotherapy, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research. (14, page 4)

A spheroid image of a NMuMG cell line expressing Fucci2.
Atsushi Miyawaki, Cell Function Dynamics, Brain Science Institute of RIKEN. (15, page 4)

FRET imaging by expressed Raichu-Cdc42 in cultured HT1080. Activated Cdc42 is observed to the cell moving direction.
Ms. Satsuki Fujiwara and Dr. Michiyuki Matsuda, Graduate school of Biostudies, Kyoto University. (16, page 4)

Brainbow AAV transfection of Purkinje cells, amplified with antibodies as described in Cai et al 2013. Visible are Purkinje cell somata, dendrites and axons, as well as some aspecific staining of granule cells. (17, page 4)

Cultured epithelial HeLa (EpH) cells.
Immunofluorescence microscopy: α-tubulin staining (Alexa Fluor 488, green), ZO-1 staining (Alexa Fluor 568, magenta)
Staining for ZO-1 revealed the tight junctions (TJs) (magenta).
Staining for α-tubulin showed an apical network of microtubules. This network associates with the TJ to form the “TJ-apical complex” (green).
Objective: UPLSAPO100XS
Image data Courtesy of
Hatsuho Kanoh, Tomoki Yano, Sachiko Tsukita,Ph.D. Graduate School of Frontier Biosciences and Graduate School of Medicine, Osaka University. (18, page 4)

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