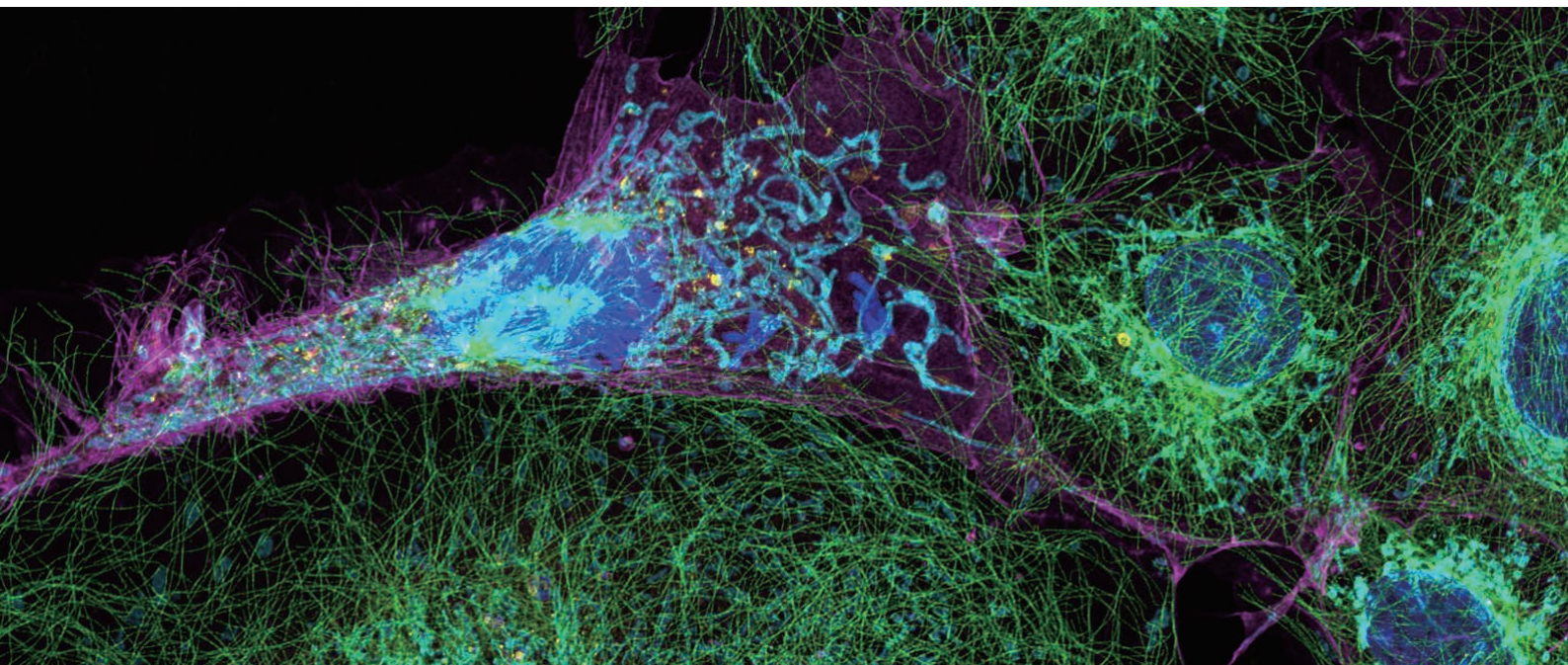
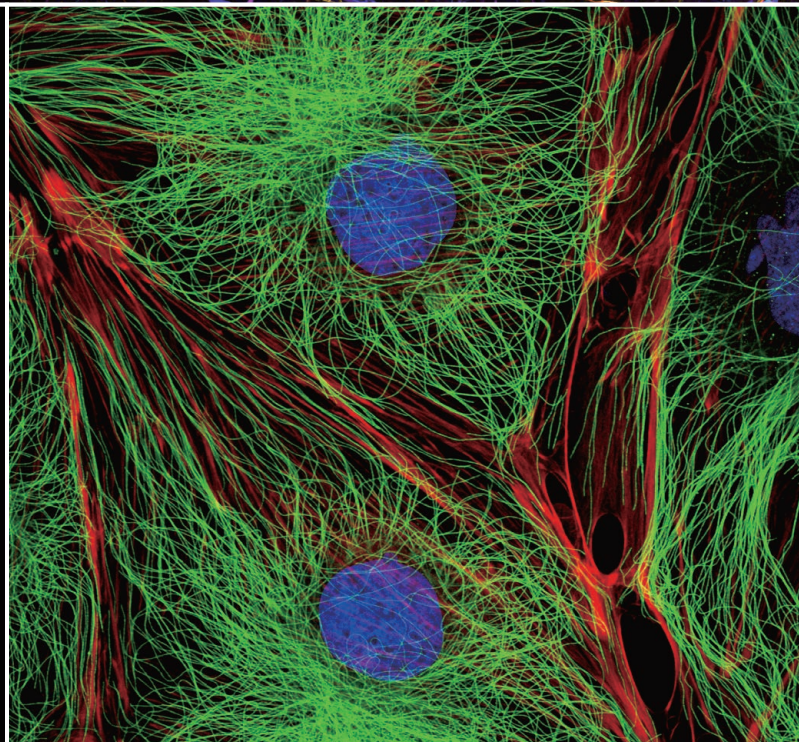
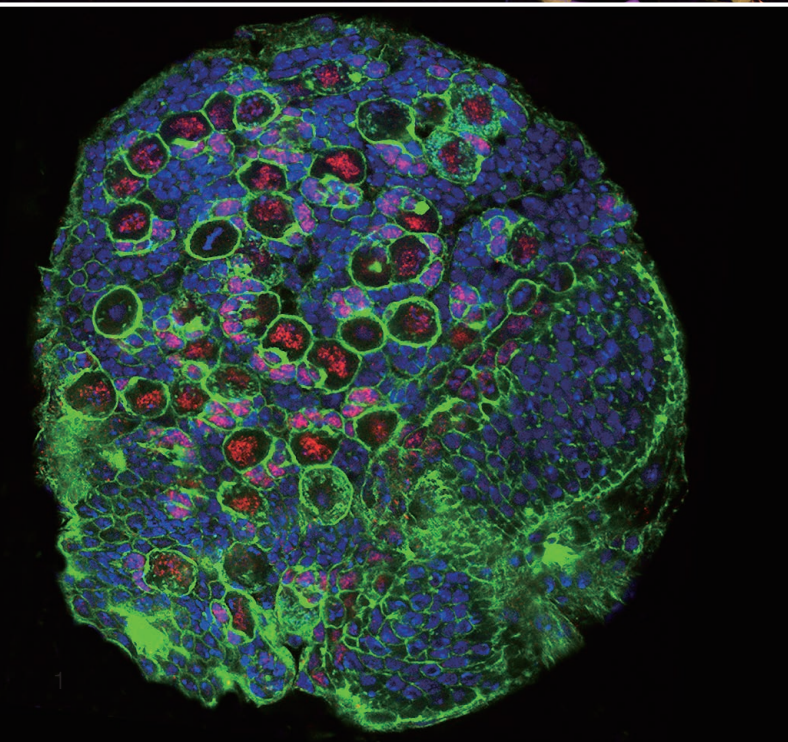
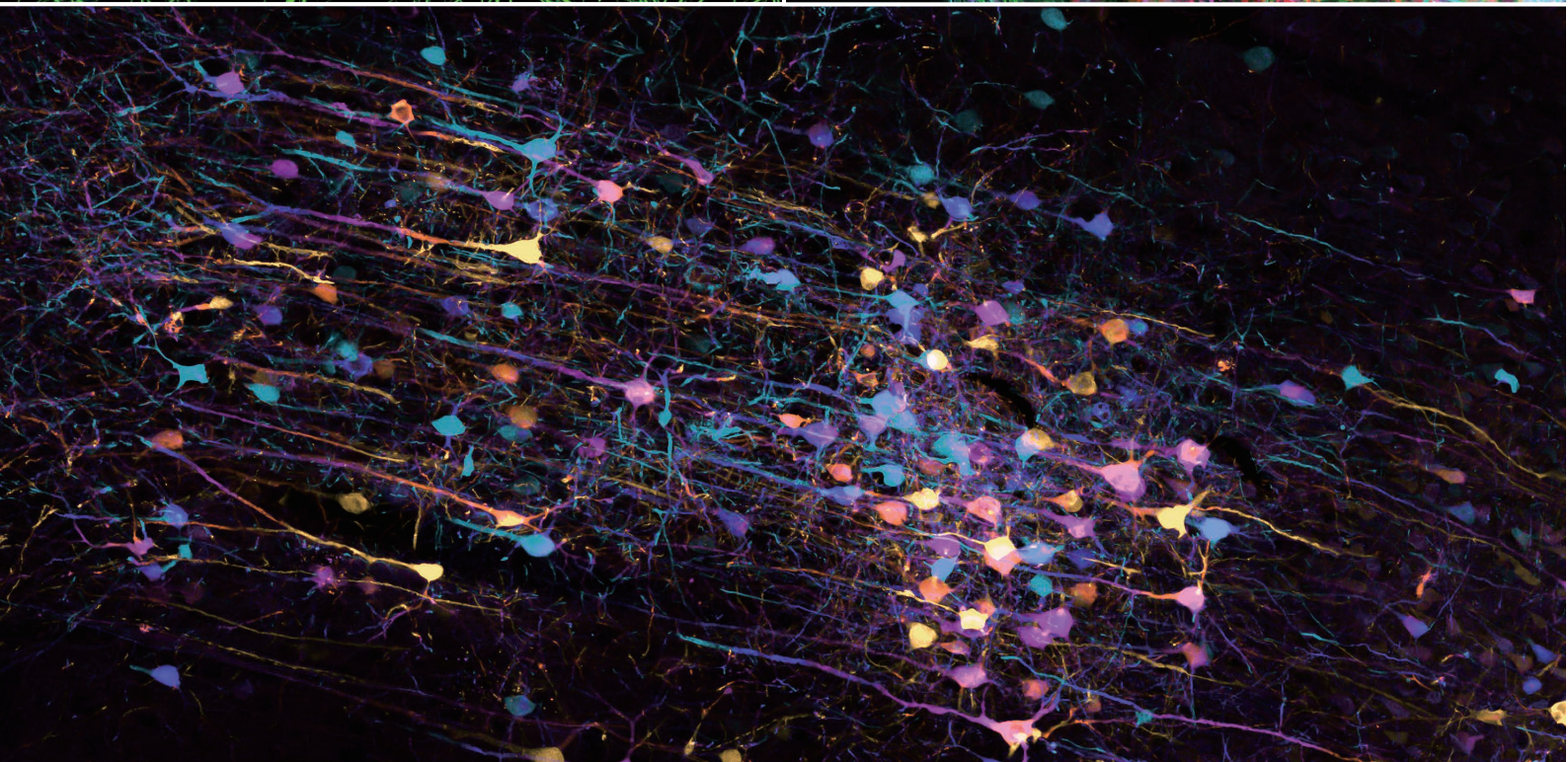
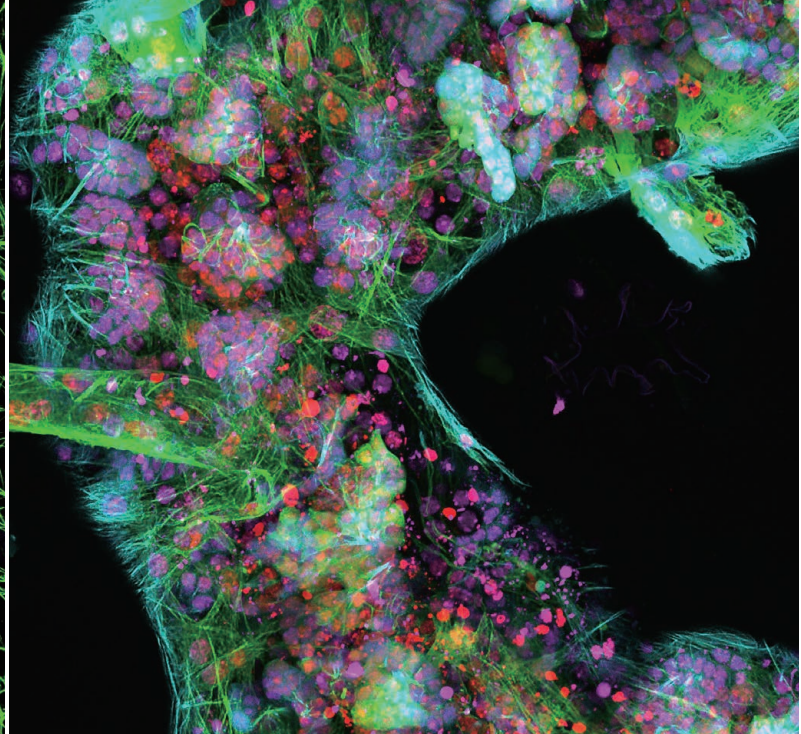
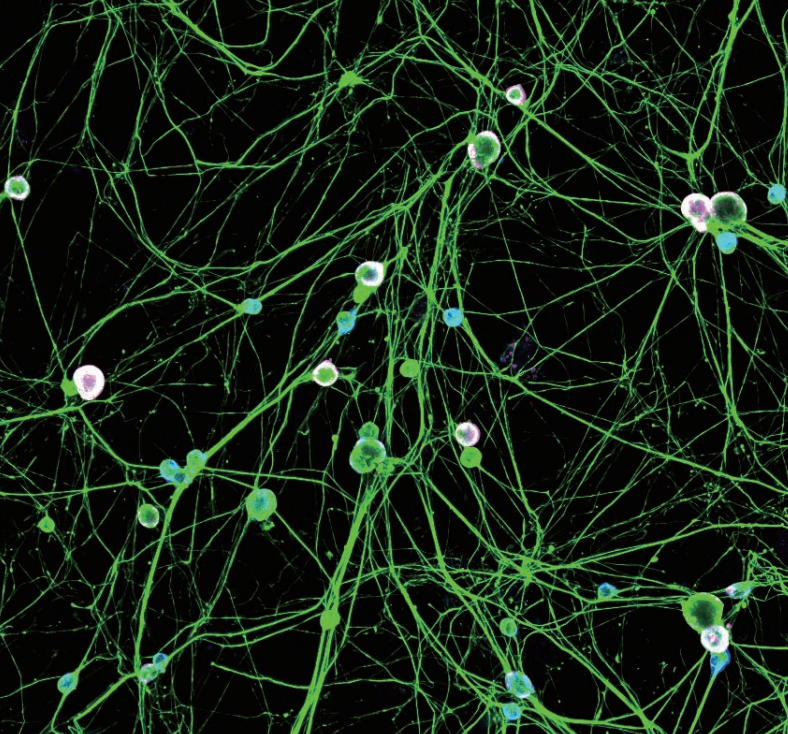


Next Generation FLUOVIEW for the Next Revolutions in Science





# FLUOVIEW FV3000 Laser Scanning Microscope

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 to image processing, such as deconvolution, and analysis.

With Olympus' renowned optics at the heart of the system, the FV3000 laser scanning microscope features TruSpectral detection technology for multichannel spectral imaging with high-sensitivity detection in multiple dynamic ranges, so even dim signals can be separated. The FV3000 Red system further expands the multichannel imaging capabilities into the near-infrared (NIR) by combining NIR-specialized high sensitivity detectors and lasers for multiplex imaging from violet to NIR. The precise galvanometer scanner combined with a silver-coated resonant scanner in the FV3000RS system enables users to combine precision and high-speed imaging in their experiments. The optical path enables macro-to-micro imaging from 1.25X to 150X magnification, enabling users to easily observe fine detail within the overall context of the tissue. Higher resolution imaging is possible using either TruSight deconvolution or Olympus Super Resolution (OSR). Simplify complex experiments with robust, intuitive automation, such as one-click cellSens macro analysis for cell counting and segmentation analysis. TruAI deep-learning technology provides efficient segmentation and accurate results.

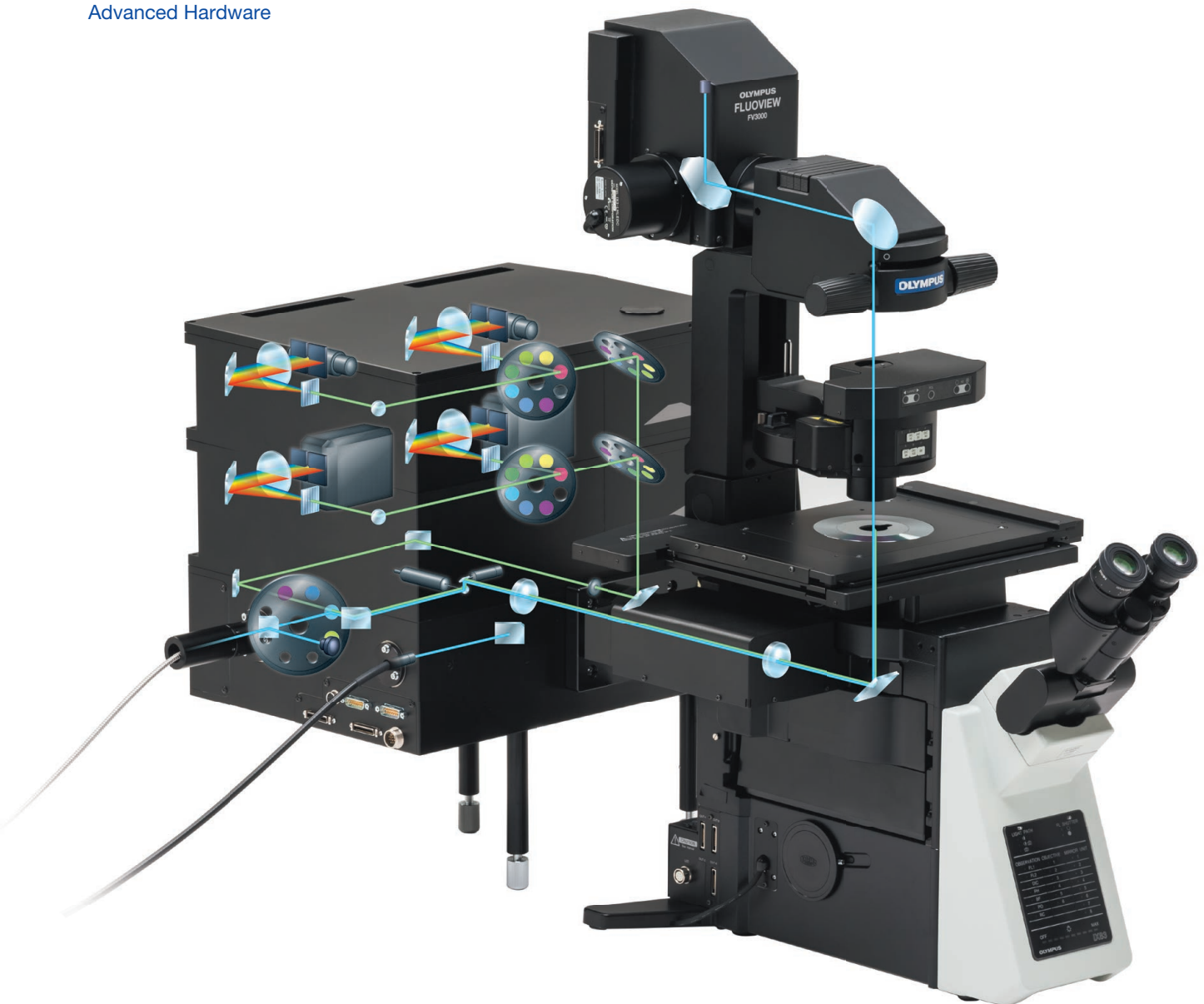
NoviSight 3D cell analysis software also includes advanced statistical tools to advance your discovery.

With user-savable and selectable software workflows, the FV3000 system adjusts to individual needs. The microscope's software makes it easy for facility managers to track the system usage of every user, improving efficiency in multiuser environments.



# Meeting Your Application Challenges

Advanced Hardware

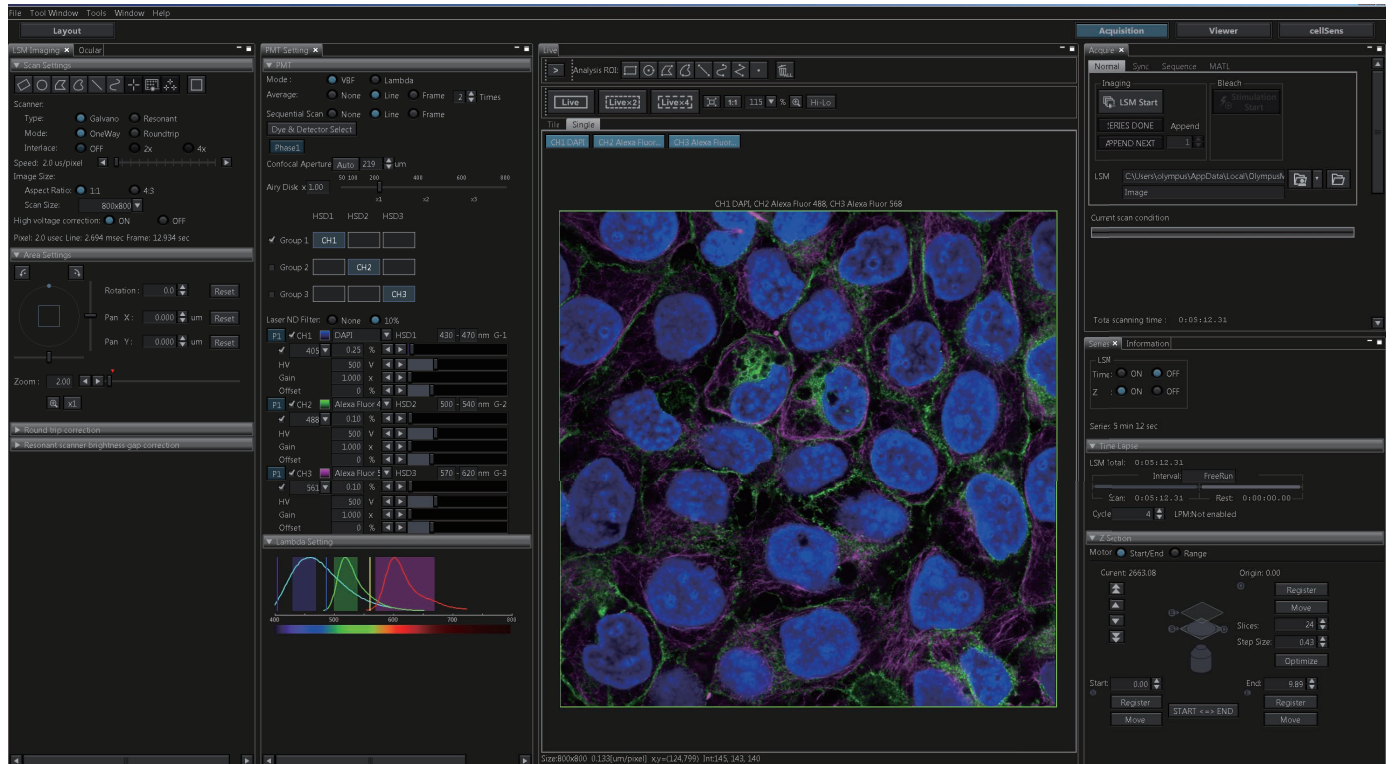


High-Sensitivity  
Multiplex Imaging  
from Violet to NIR

Macro-to-Micro  
Imaging and  
Super Resolution

Accurate Time-  
Lapse Imaging

## Adapt the User Interface for Your Workflows



### 1. Layout

- Select the layout appropriate for your experiment

### 2. Acquisition Condition

- Auto-setting by dyes
- Reload the settings from previous experiments

### 3. Acquisition

- Acquire multidimensional images
- Acquire multi-area images
- Acquire images using a complex protocol

### 4. Viewer

- Review images during or after acquisition using multiple display methods

### 5. Analysis

- Various image analysis menus are available
- One-click macro analysis

Increase Productivity with High-Speed Imaging

Superior Objectives

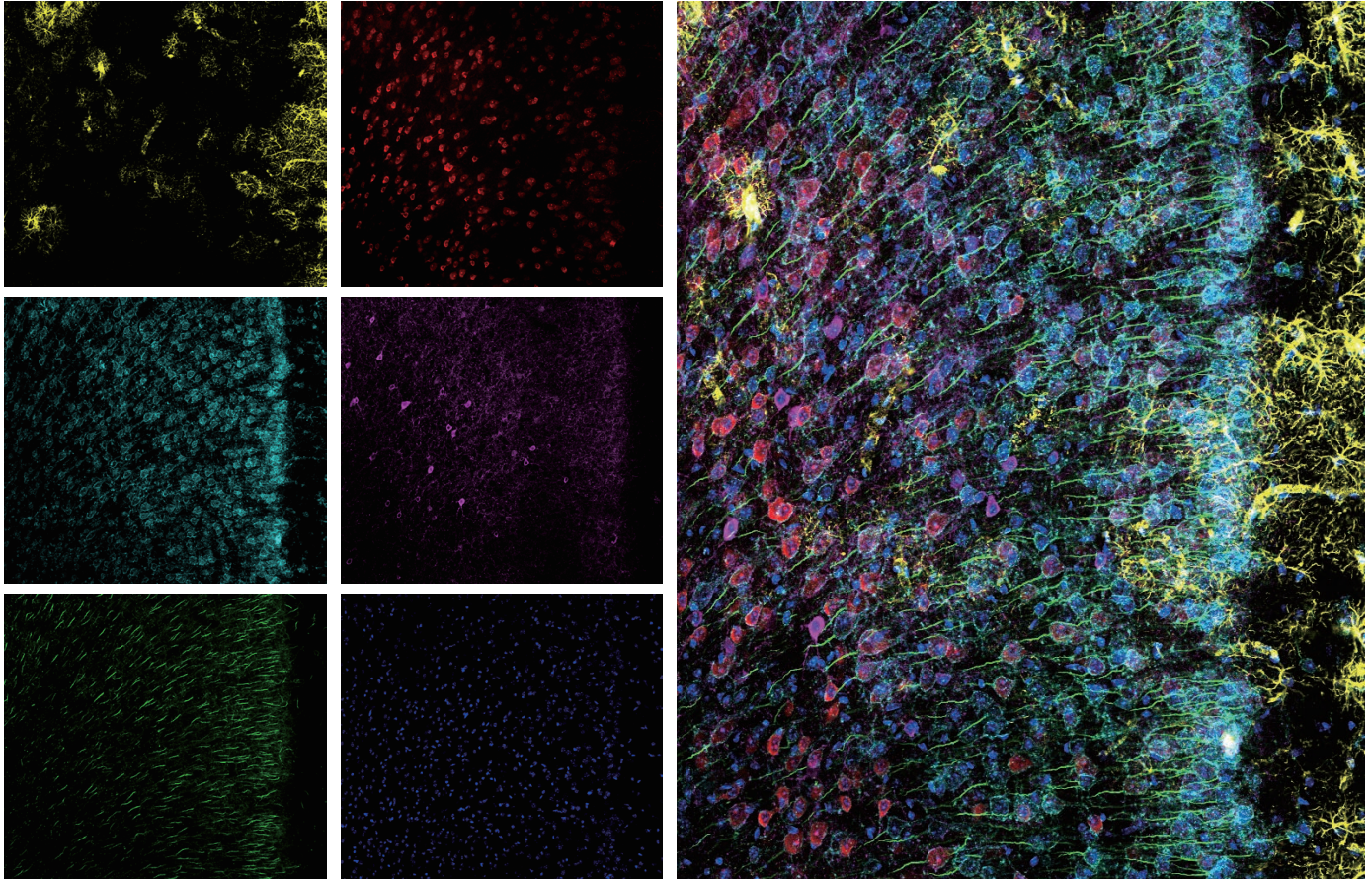
- X Line
- A Line

Advanced Solutions

- TruAI Deep Learning
- 3D Cell Analysis

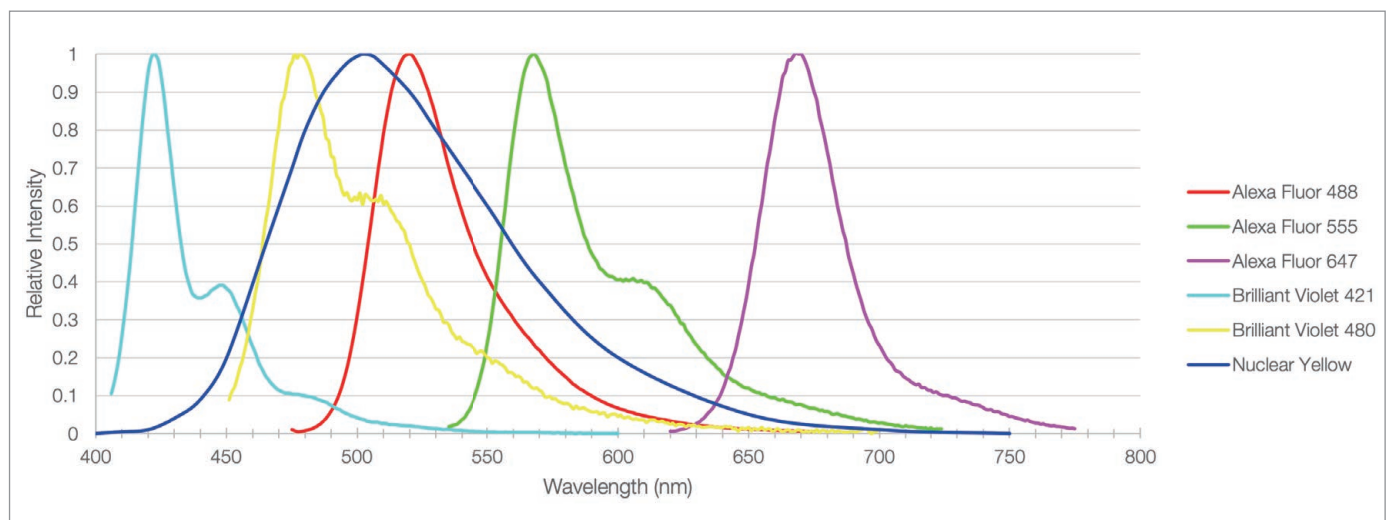
## » Application: High-Sensitivity Multiplex Fluorescence

Acquiring multicolor fluorescent images is important for analyzing the fine internal structure of cells and tissues and confirming protein expression. The FV3000 system's TruSpectral detectors enable users to select the detection wavelength on each channel to optimize signal detection for each individual fluorophore. The variable barrier filter mode provides simultaneous four-channel image acquisition, and up to 16 channels sequentially. The system's online or offline lambda scanning mode facilitates the separation of complex fluorescence signals by defining characteristic spectral emission profiles for each fluorophore, enabling accurate spectral unmixing of complex overlapping fluorescent signals.



Mouse mPFC labeled with glial fibrillary acidic protein (GFAP; astrocyte marker; yellow), calmodulin-dependent protein kinase II (CaMKII; pyramidal neuron marker; red), amphoterin-induced protein 1 precursor (AMIGO-1; neuronal membrane marker; cyan), parvalbumin (PV; inhibitory neuron marker; purple), ankyrin-G (AnkG; axon initial segment marker; green), and nuclear yellow (nuclei marker; blue).

Image data courtesy of Stephanie Shiers, Ph.D. Candidate, and Theodore J. Price, Ph.D., Price Lab, Eugene McDermott Professor, Director, Center for Advanced Pain Studies, Department of Neurobiology, School of Behavioral and Brain Sciences, University of Texas at Dallas.

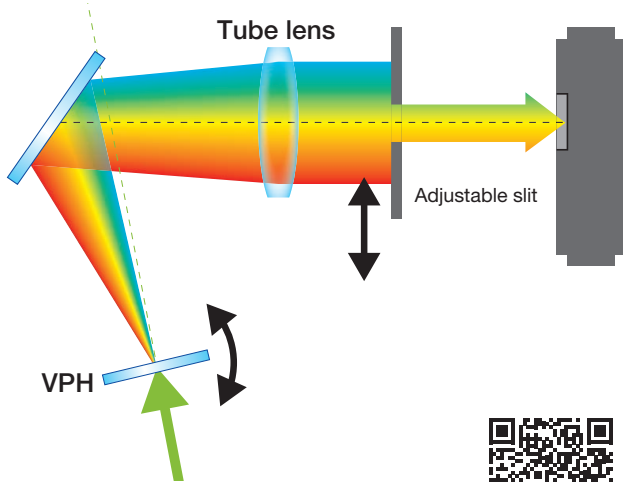


Emission spectra of the six fluorophores used to label mouse medial prefrontal cortex sections. Even though the spectrum of each fluorophore is overlapping, the cross talk can be minimized by optimizing detection wavelength by TruSpectral detectors.

# High-Sensitivity Multiplex Imaging

## A Fully Spectral System with Sensitivity and Accuracy

The FV3000 series employs Olympus' TruSpectral detection technology. Based on patented volume phase hologram (VPH) transmission and an adjustable slit to emitted light, the spectral detection is highly efficient, enabling users to select the detection wavelength of each individual channel at the spectral resolution of 1 nm.

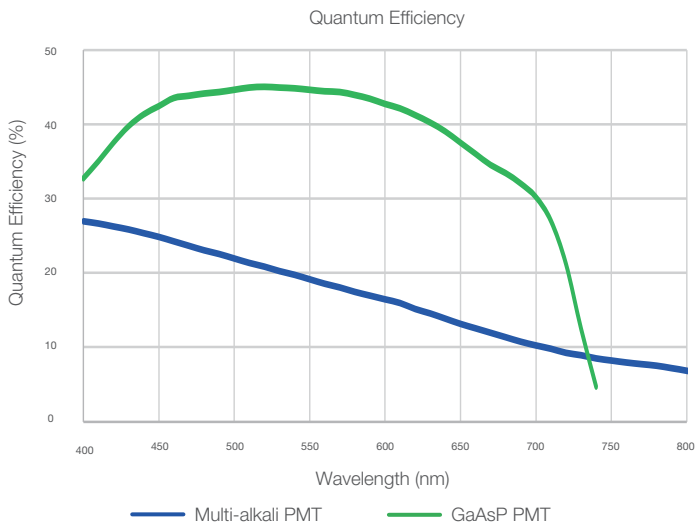


[www.olympus-lifescience.com/video/fv3000\\_truspectral](http://www.olympus-lifescience.com/video/fv3000_truspectral)

## High-Sensitivity Spectral Detector (HSD) with Cooled GaAsP Photomultiplier Tubes Enhance Quantum Efficiency

The GaAsP PMTs in the FV3000 system's high sensitivity detector enable you to view samples whose emission is too weak to view with conventional detection methods. The GaAsP PMT unit incorporates two channels with a maximum quantum efficiency of 45% and Peltier cooling that reduces background noise by 20% for high S/N ratio images under very low excitation light.

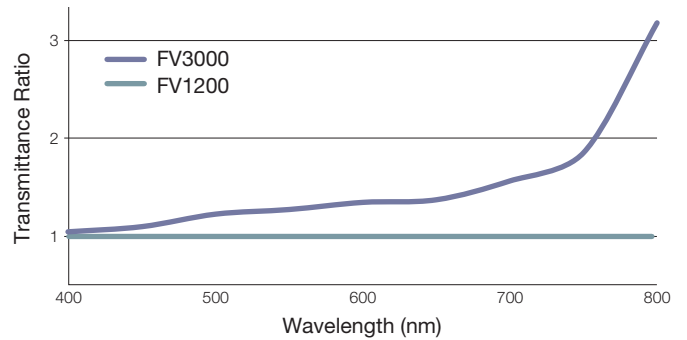
## The Standard Quantum Efficiencies of Detector Technologies



## Efficient TruSpectral Detection System

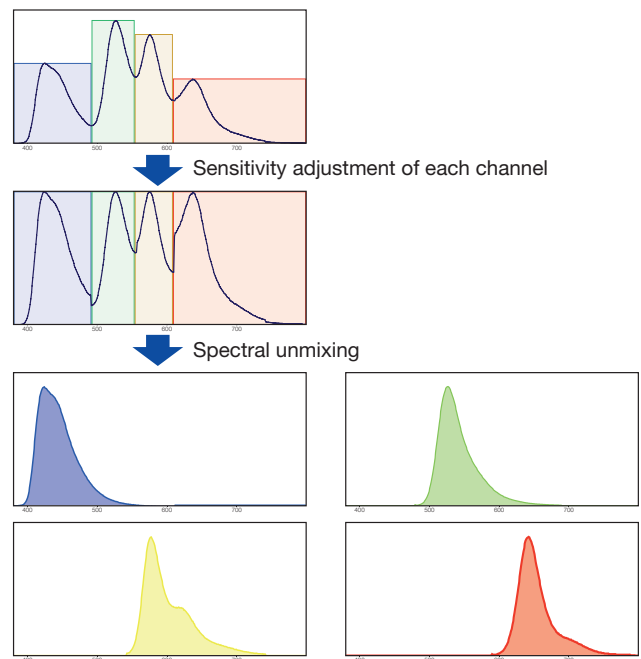
All the detectors in the FV3000 series have spectral imaging capability. TruSpectral detection delivers up to three-fold improved overall transmission and sensitivity with a high signal-to-noise ratio, resulting in excellent multicolor confocal imaging capabilities.

## Transmittance Ratio of FV3000 to FV1200 in Detection Path



## Multichannel TruSpectral Detection with Sixteen-Channel Unmixing

TruSpectral technology'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. The multichannel mode facilitates constant spectral unmixing during live cell experiments, separating complex fluorescence during acquisition. With up to four dynamic ranges from the four detection channels, bright and dim spectral signals can be separated by independently adjusting the sensitivity of each detector.

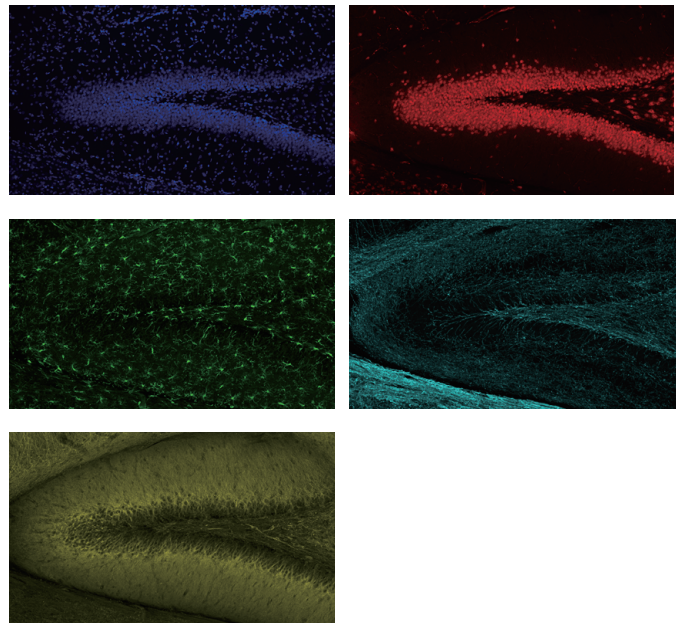
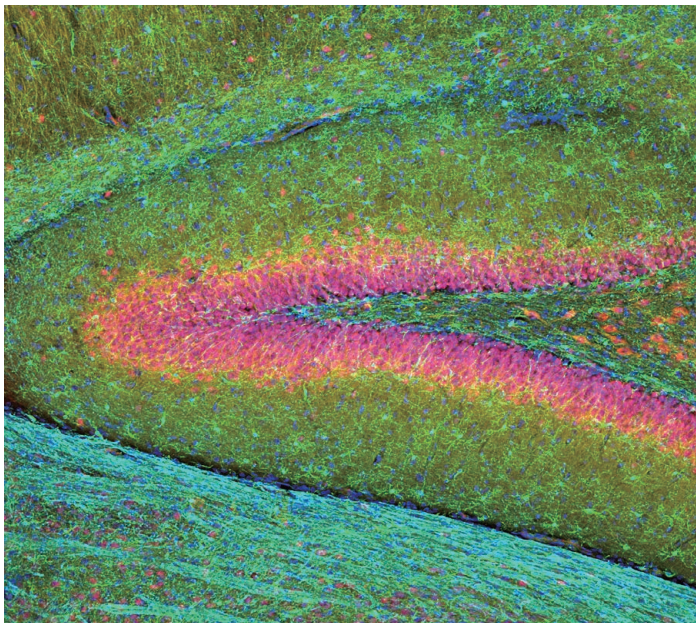


## » Application: FV3000 Red System for NIR Solutions

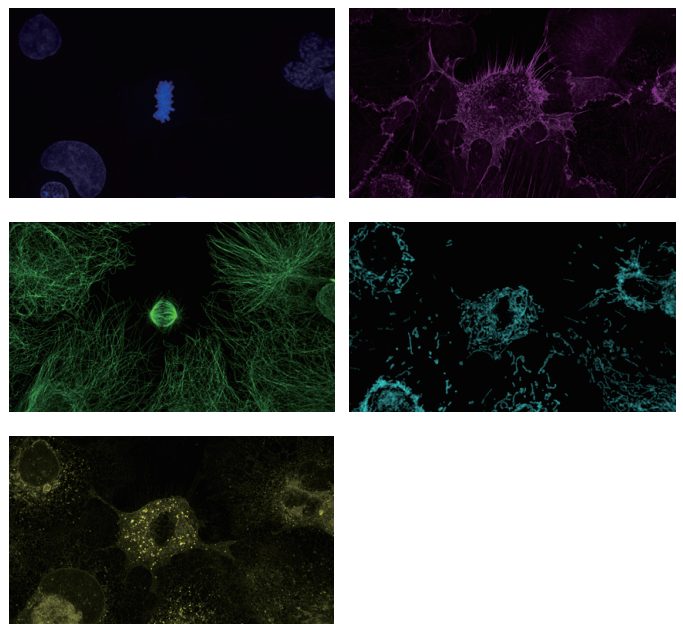
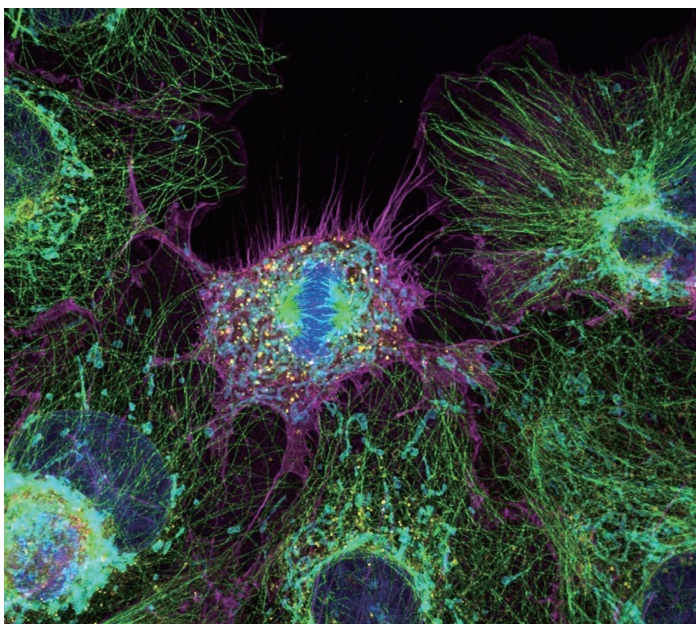
Near-infrared (NIR) fluorescence expands the possibilities for multiplexing, gentle live cell imaging, and deeper imaging, while at same time reducing autofluorescence.

The FV3000 Red system's detection optical path greatly improves near-infrared transmittance. Moreover, it is equipped with NIR lasers, such as 730 nm or 785 nm wavelengths, and 1–2 channels of NIR-dedicated GaAs detectors (~890 nm), which enable six-channel multiplexed imaging from 400 nm to 890 nm.

Simultaneous imaging of up-conversion fluorescence of nanoparticles and conventional fluorescence is now possible by introducing longer wavelengths lasers such as 808 nm and 980 nm.



Rat brain slice labeled with Hoechst (blue), anti-IBA1 (Alexa Fluor 488; green), anti-MAP2 (Alexa Fluor 594, yellow), anti-FOX3/NeuN (Alexa Fluor 647; red), and anti-MBP (Alexa Fluor 750; cyan). Images were acquired using a UPLXAPO10X objective with 405 nm, 488 nm, 561 nm, and 730 nm laser lines on GaAsP and GaAs detectors. Maximum intensity projection in Z with TruSight deconvolution processing. Sample courtesy of EnCor Biotechnology.

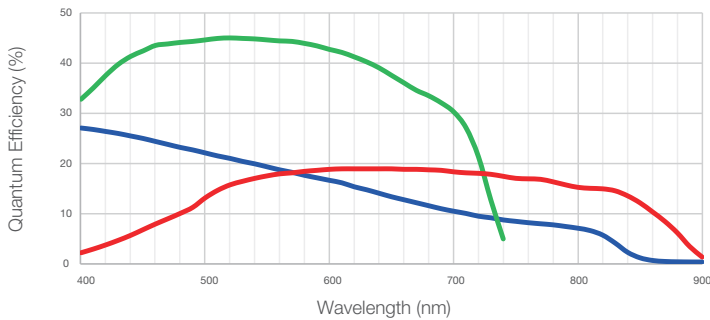


Cos-7 cells labeled with DAPI(blue), anti-Tubulin (Alexa Fluor 488; green), Concalavalin A (Alexa Fluor 594; yellow), SiR-Actin (magenta) and anti-TOMM20 (Alexa Fluor 750; cyan). Images were acquired using a UPLAPO60XOHR objective and 405 nm, 488 nm, 561 nm, 640 nm, and 730 nm laser lines on GaAsP and GaAs detectors. Maximum intensity projection in Z with TruSight deconvolution processing. Sample courtesy of Dr. Jana Döhner, Dr. Urs Ziegler, University of Zürich.



# FV3000 Red System for NIR Solutions

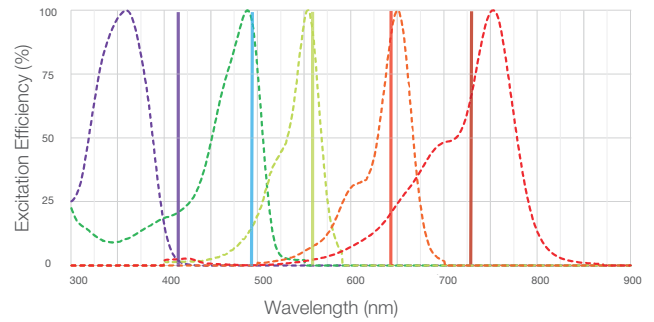
## Cooled GaAs PMT Detector for NIR Imaging



— Multi-alkali PMT    — GaAsP PMT    — GaAs PMT

GaAs photomultiplier tube (PMT) has a higher quantum efficiency at 700 nm–890 nm range compared with a conventional GaAsP PMT or multi-alkali PMT.

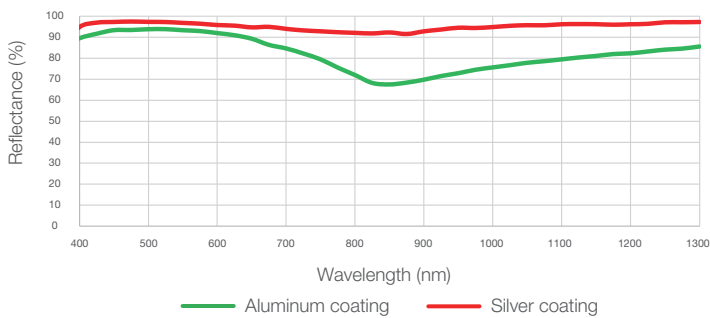
## NIR Laser Diodes



— DAPI    — GFP    — RFP    — Alexa Fluor 647    — Alexa Fluor 750

NIR laser diodes help reduce crosstalk caused by dye selection. These laser diodes (LD730/785) are designed to be driven in continuous wave (CW) mode, offering stability, low maintenance, and a longer lifetime.

## High-Transmittance Optics for NIR Imaging



— Aluminum coating    — Silver coating

Efficient NIR imaging requires high-quality optics. All the FV3000 system's optical elements have a high transmission from 400 nm to 1300 nm, including the galvanometer and resonant scanner, which are coated in silver rather than the conventional aluminum coating.

## TruFocus Red Z-Drift Compensator



TRU<sup>FOCUS</sup>

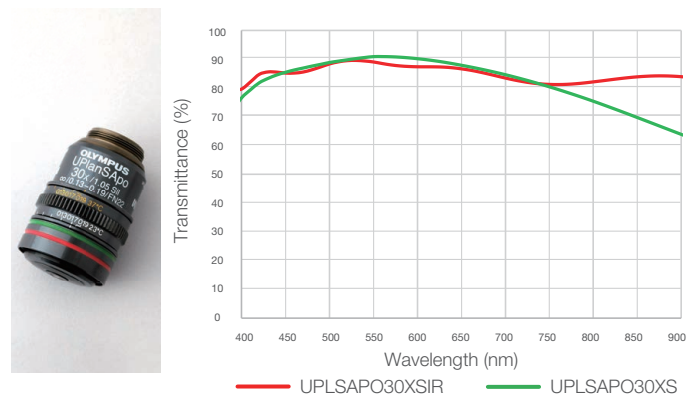
The TruFocus Red uses a very low intensity 830 nm diode laser to identify the coverslip surface and maintain the focus position of the sample. The unit works well for both live cell and multiwell plate imaging and covers all wavelengths from visible to the NIR, including fluorescent proteins like iRFP.

## Multiplexing with X Line High-Performance Objectives



Award-winning X Line objectives work well for NIR imaging as they are corrected for chromatic aberrations between 400–1000 nm. They also have a higher numerical aperture, excellent flatness, and very high transmittance from UV to NIR, increasing the multiplexing capabilities

## Deep-Tissue Observation with Silicone Oil Immersion Objectives in the NIR

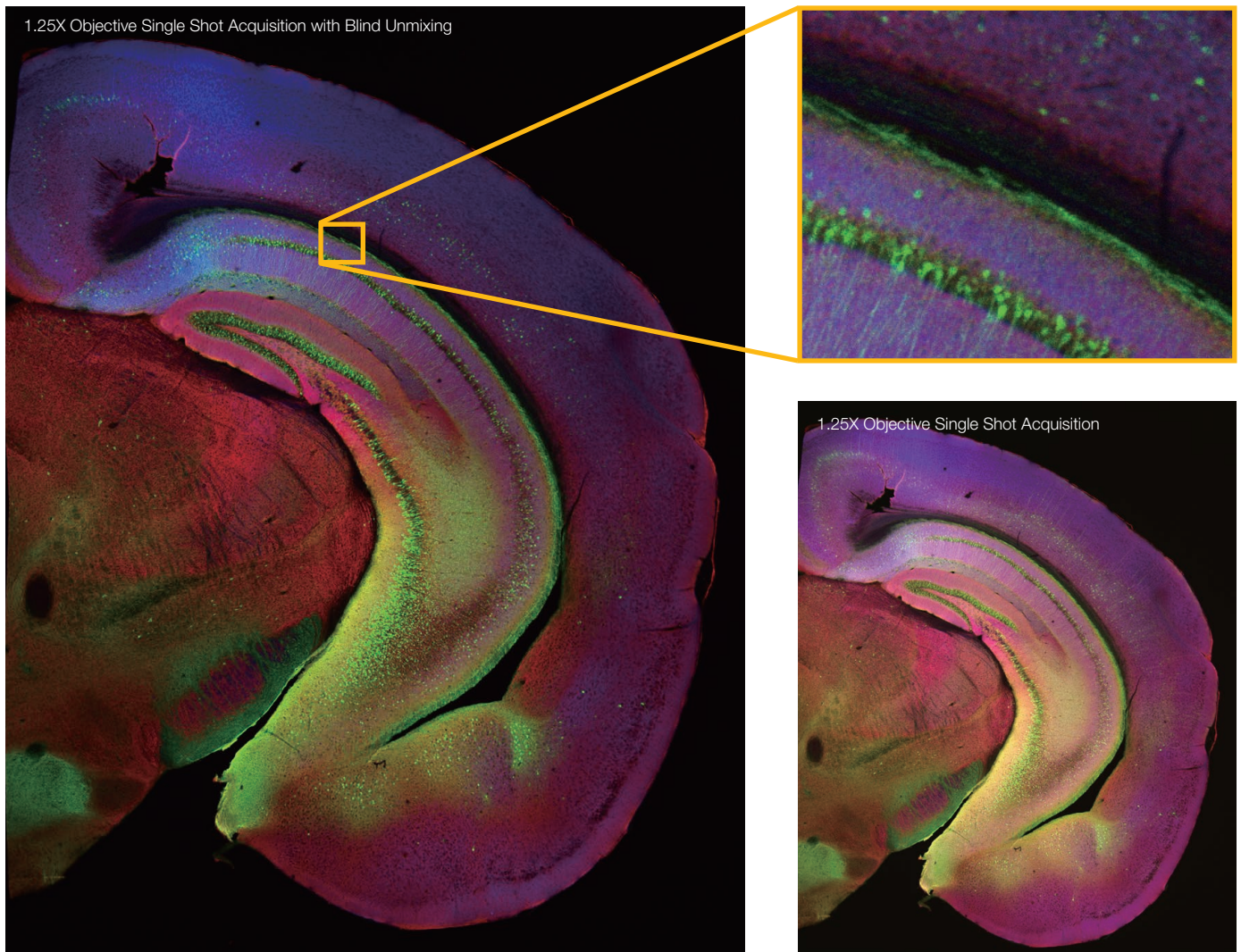


— UPLSAPO30XSIR    — UPLSAPO30XS

One advantage of imaging in the NIR range is its ability to penetrate deeper into the sample. This is because longer wavelengths of light are scattered less by biological samples. Combined with silicone oil immersion objectives (ne~1.40), spherical aberration is significantly minimized, enabling higher S/N images, even deep within living tissues.

## » Application: Macro-to-Micro Imaging and Super Resolution

Life science research applications require users to observe regions of interest within the context of the larger tissue structure. A large field of view is critical to being able to see a cell's components in relation to the entire tissue sample. The FV3000 microscope's optical light path facilitates macro-to-micro observation from 1.25X to 150X. Image stitching enhances these applications even more by enabling users to quickly locate target cells on the macro image and follow up with higher resolution imaging of the cell's fine structures. For even greater resolution, Olympus Super Resolution technology (FV-OSR) can be coupled with this approach to provide optimal macro-to-micro imaging performance.



Mouse brain hemisection embedded for expansion microscopy (pre-expansion), labeled with secondary antibodies against GFP (Alexa Fluor 488, green), SV2 (Alexa Fluor 565, red) Homer (Alexa Fluor 647, blue). Sample courtesy of Dr. Ed Boyden and Dr. Fei Chen, MIT.

### Objectives for Macro Observation

	NA	FOV
PLAPON1.25X	0.04	14.4 mm
PLAPON2X	0.08	9.0 mm
UPLXAPO4X	0.16	4.5 mm

### Objectives for Micro Observation

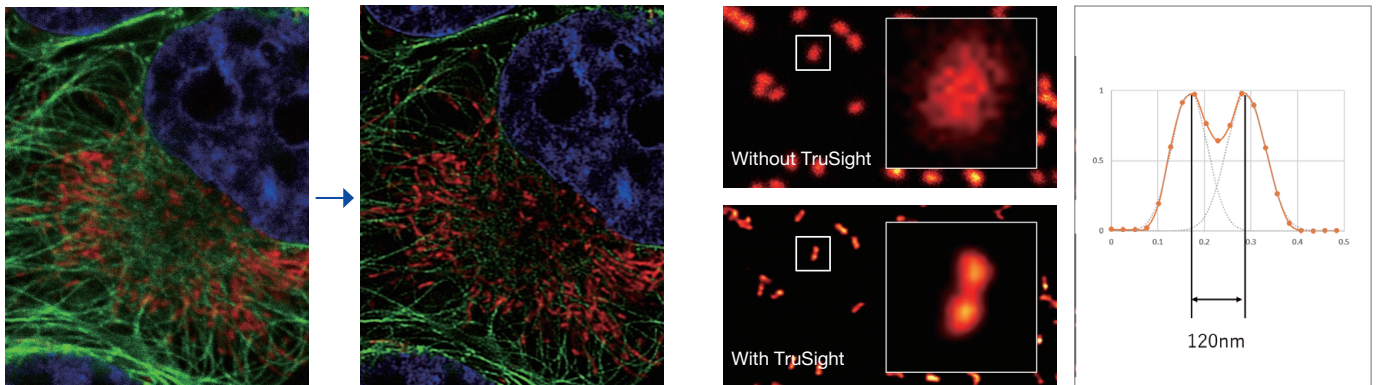
	NA	Theoretical Resolution (XY)
UPLXAPO40XO	1.4	169 nm
UPLXAPO60XO	1.42	166 nm
UPLXAPO100XO	1.45	163 nm
UPLAPO60XOHR	1.5	157 nm
UPLAPO100XOHR	1.5	157 nm
APON100XHOTIRF	1.7	139 nm

\*Theoretical resolution is calculated at 488 nm excitation and confocal aperture 1 AU.

# Macro-to-Micro Imaging and Super Resolution

## TruSight Deconvolution

TruSight deconvolution image processing improves the sharpness and clarity of images by reducing optical blur and noise. The optimized image processing mode for optical characteristics enables the restoration of confocal images acquired using the FV3000 microscope. TruSight deconvolution can also reduce noise for Olympus Super Resolution (FV-OSR) acquired images. TruSight deconvolution uses a GPU for high-speed image processing.



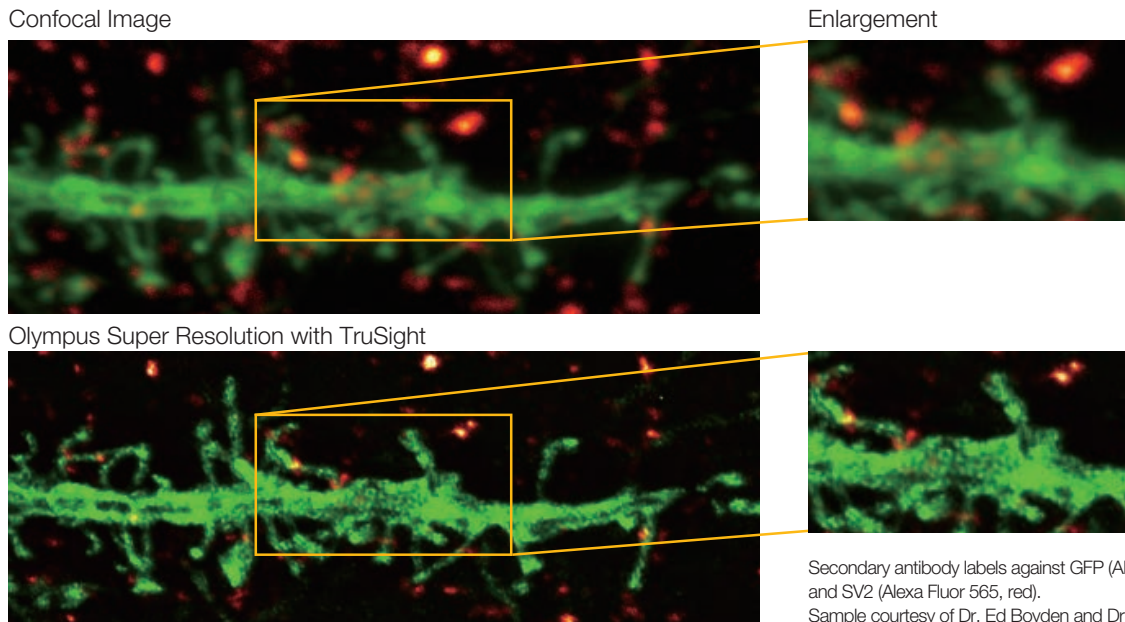
cLSM image without TruSight      With TruSight  
 HeLa cell. Blue: nuclear (DAPI), green: microtubule (Alexa Fluor 488), red: mitochondria (MitoTracker Red)

Image of GATTA-SIM nanoruler (SIM 120B, GATTA quant GmbH) acquired by using UPLAPO60XOHR objective (NA: 1.5) a 0.8 AU pinhole size. The fluorescent markers at both ends of the nanorulers can be resolved by TruSight deconvolution.

## Olympus Super Resolution (FV-OSR) Technology with Up to Four 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 Olympus Super Resolution imaging module can acquire four fluorescent signals either sequentially or simultaneously with a resolution of approximately  $120 \text{ nm}^*1$ , nearly doubling the resolution of typical confocal microscopy. The imaging module is easy to use with minimal user training and can be added to any FV3000 system, making it a truly accessible method for achieving super resolution.

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



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.

## Example of Actual Measurement of FWHM Using $\Phi 40 \text{ nm}$ Fluorescent Beads<sup>2</sup>

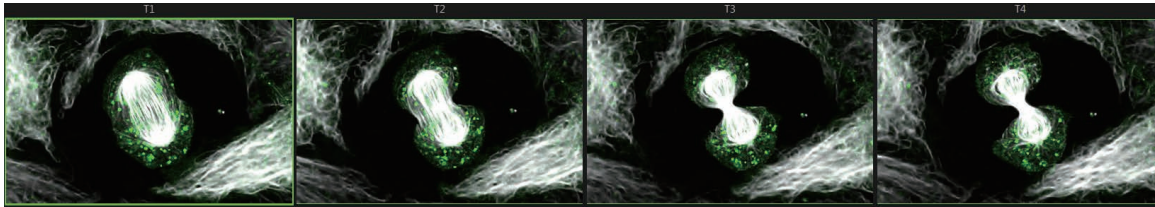
	Confocal (1 AU)	TruSight	FV-OSR
Lateral projection			
Axial projection			
Lateral FWHM [nm]	202	120	120
Axial FWHM [nm]	465	292	405

<sup>1</sup> Subject to objective magnification, numerical aperture, excitation and emission wavelength, and experiment conditions.

<sup>2</sup> Images are captured by UPLAPO60XOHR (NA1.5) / ex 488 nm /  $\Phi 40 \text{ nm}$  beads

## » Application: Time-Lapse Imaging

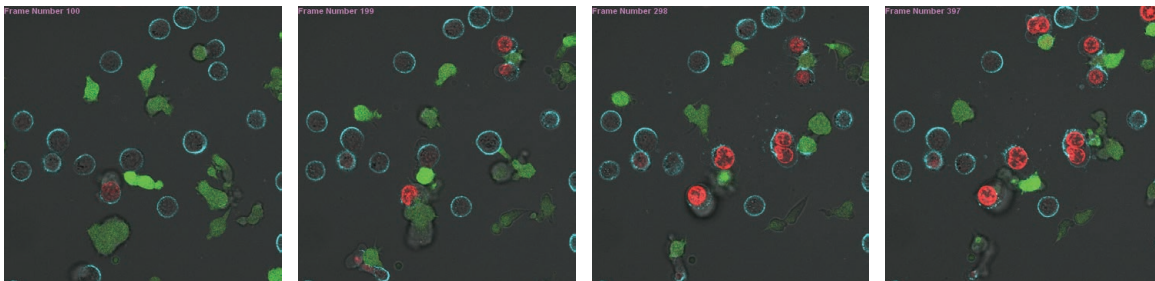
Time-lapse imaging experiments require focus to be maintained throughout with less phototoxicity to the sample. Olympus' TruFocus Z-drift compensator helps each position stay in focus during an experiment despite changes in temperature or the addition of reagents. Additionally, the FV3000 system's high-sensitivity detector requires reduced laser intensity, and the resonant scanner reduces the laser illumination time, reducing phototoxicity for more physiologically accurate imaging data.



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



[www.olympus-lifescience.com/fv3000-cell-division-resonant/fv3000-cell-division-resonant](http://www.olympus-lifescience.com/fv3000-cell-division-resonant/fv3000-cell-division-resonant)



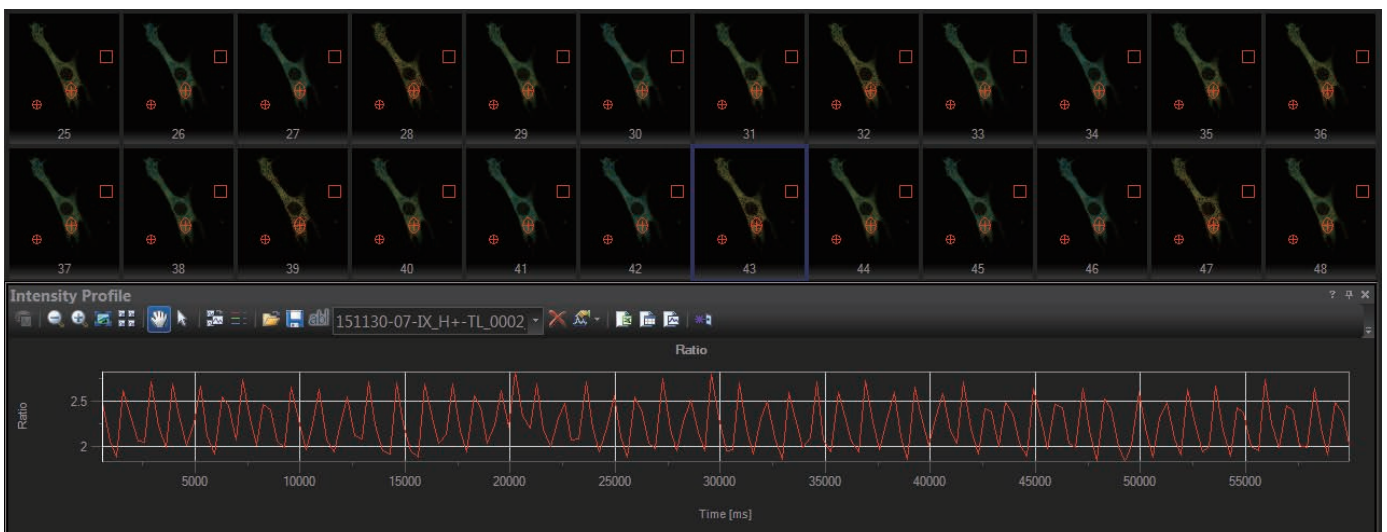
NK-cell mediated cell killing after therapeutic antibody application (blue). GFP labeled NK-cells (green). DAPI uptake marking dead cells (Red). Image data courtesy of Dr. Yuji Mishima, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research.



[www.olympus-lifescience.com/fv3000-dudi-158v-xyt-0001\\_00000](http://www.olympus-lifescience.com/fv3000-dudi-158v-xyt-0001_00000)

## » Application: High-Speed Imaging

High-speed imaging is required to observe fast dynamic phenomena, such as a beating heart, blood flow, or calcium ion dynamics inside cells. The FV3000RS hybrid scan unit uses a galvanometer scanner for precision scanning as well as a resonant scanner that is ideal for high-speed imaging of live physiological events. The resonant scanner is capable of speeds starting from 30 fps at FN18, up to 438 fps using clip scanning. Users can switch between the galvanometer scanner and resonance scanner with a single click and use the sequence manager to record each action for complex experiments.



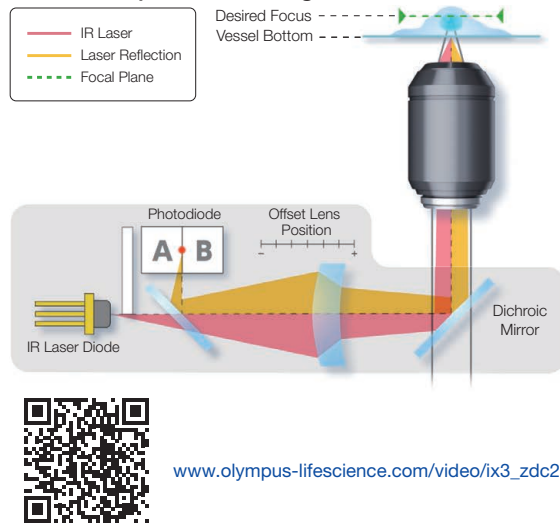
Intensity Modulated Display of the CFP/YFP ratio result during spontaneous contractions of an *in vitro* cardiomyocyte. Image data courtesy of Yusuke Nino and Atsushi Miyawaki, Cell Function Dynamics, Brain Science Institute of RIKEN.

# Accurate Time-Lapse Imaging

## Maintain Focus with TruFocus Z-Drift Compensation

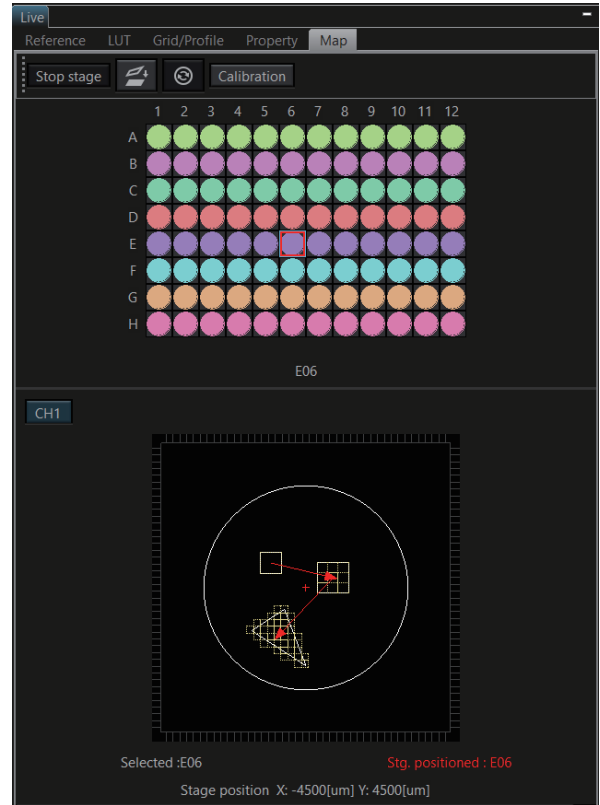
The TruFocus ZDC system uses minimally-phototoxic infrared light (laser class 1) to identify the location of the sample plane. One-shot autofocus (AF) mode enables several focus positions to be set as desired for thicker samples, enabling efficient Z-stack acquisitions in multiposition experiments. The continuous AF mode keeps the desired plane of observation precisely in focus, avoiding focus drift caused by temperature changes or the addition of reagents, making it ideal for measurements that require more stringent focusing. Furthermore, the increased optical offset enables continuous AF with plastic vessels or with dry objectives. The TruFocus ZDC system is also compatible with silicone oil objectives, enabling accurate time-lapse imaging.

### TruFocus Optical Path Diagram



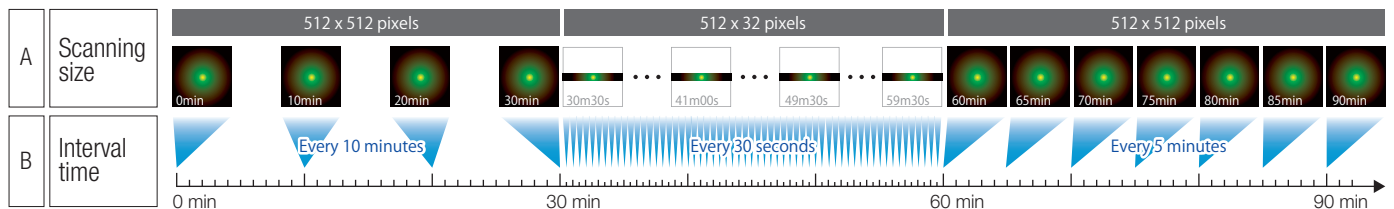
## Stage Control for Multi-Area Time-Lapse, Microplate, and Stitching

Multi-area time-lapse and stitching provide robust and accurate time-lapse data and enable users to generate detailed overview images to see their data in context. The well navigator function provides sophisticated, intuitive controls for a wide range of commercially available microplates as well as custom designed cell culture dishes.



## Reduce Complexity with the Sequence Manger

With the Sequence Manager software module, complex protocols are handled with ease and accurate timing. Multiday time-lapse experiments are controlled with microsecond scan accuracy and millisecond sequence execution accuracy. Various protocols, such as time-lapse with different time intervals, switching between high and low magnification, switching between galvo and resonant scanners, and photo-stimulation between imaging by FRAP or FRET (acceptor photobleaching), can be performed.

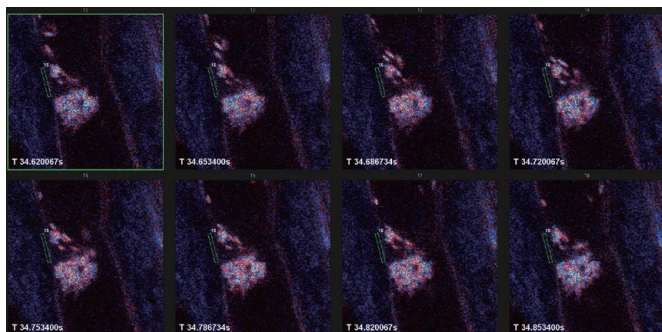


The Sequence Manager enables variable time-lapse experiments with microsecond scanning accuracy.

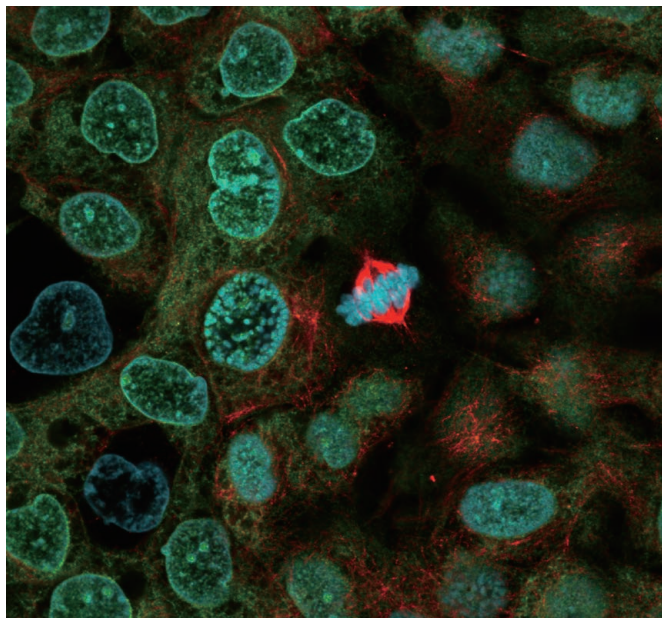
# Increase Productivity with High-Speed Imaging

## Galvanometer and Hybrid Galvo/Resonant Scanners

Users have their choice of two different types of scan units: galvanometer only with the FV3000 system or hybrid galvanometer/resonant with the FV3000RS system. The hybrid scan unit has a galvanometer scanner for high-precision scanning, as well as a resonant scanner that is ideal for high-speed imaging. With the galvanometer scanner and Olympus super resolution technology (FV-OSR), users can obtain resolutions down to 120 nm with a high signal-to-noise ratio. The galvanometer scanner also features flexible scanning options, including precise tornado scanning as well as multipoint stimulation with 100 ms switching time. The galvanometer scanner can image up to 16 frames per second. By switching to the resonant scanner, users can capture 30 frames per second with a full field of view at 512 × 512 pixels. At 512 × 32 pixels, the resonant scanner can capture up to 438 frames per second to capture critical live physiological events such as calcium ion flux.



Platelets bound to a thrombosis in the blood vessel of a mouse. Images taken at 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.



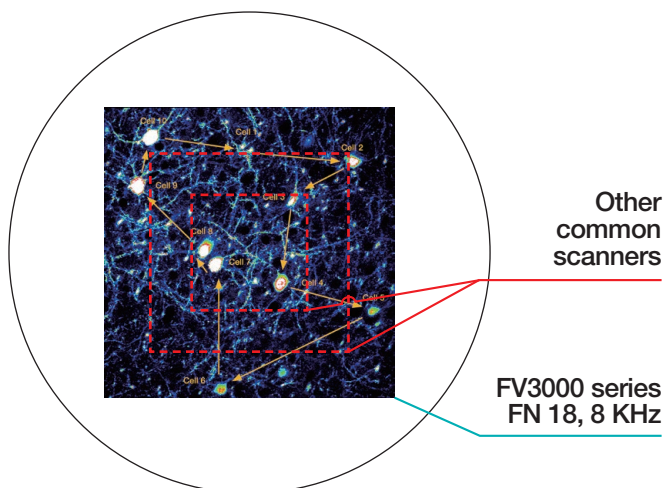
A431 cells fixed with methanol labeled with Abcam Anti-ERK1 + ERK2 antibody (Alexa Fluor 488) ab208564 and Anti-alpha Tubulin antibody (Alexa Fluor 594) ab195889 and DAPI. Sample courtesy of Abcam.

## 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 laser intensity control from low to high range, 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. By clipping the Y axis, additional speeds up to 438 frames per second can be achieved.

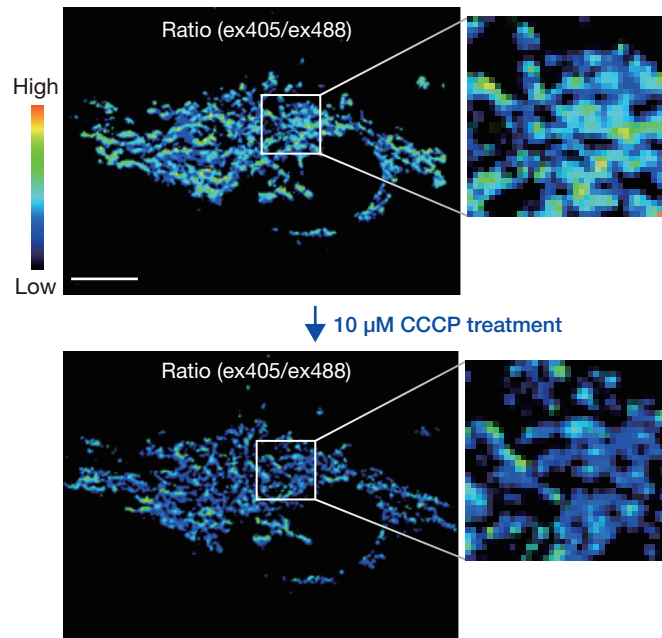


Most resonant scanners force a trade-off between speed and field of view. FLUOVIEW systems are optimized to maintain the field of view with even signal intensity so dynamic samples (e.g., calcium imaging) can be seen in the broad context of their cells and tissues.

The image above shows examples of the clipped fields of view required in other resonant scanning systems.

## Ratio Imaging and Intensity Modulated Display (IMD)

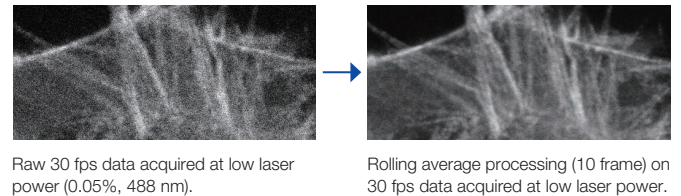
The FV3000 microscope's ratio imaging analysis function includes an intensity-modulated display (IMD) function in the software that displays quantitative fluorescence ratio changes during both standard and high-speed acquisitions. This function is particularly useful for calcium and FRET imaging where a pure ratio display provides poor contrast in background areas.



tsGFP1-mito reveals heterogeneity in mitochondrial thermogenesis in HeLa cells. Ratio images (ex 405 nm/ex 488 nm) in tsGFP1-mito-expressing cells shown 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.

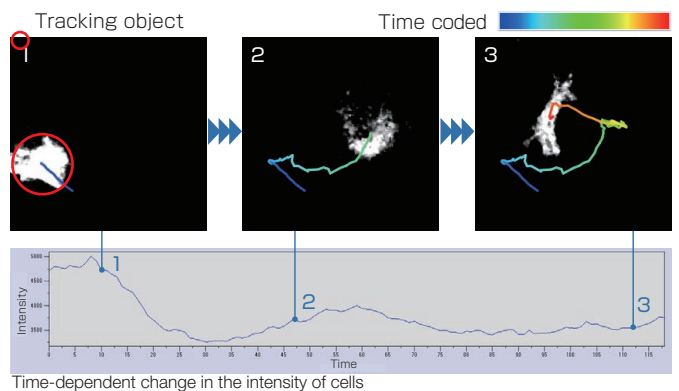
## Rolling Average Processing

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 the time scale and keeping the original data.



## Object Tracking

In time-lapse imaging, moving objects can be automatically detected, tracked, and analyzed. cellSens software's tracking function provides a powerful and intuitive tool to quantify dynamic processes such as cell movement and division.

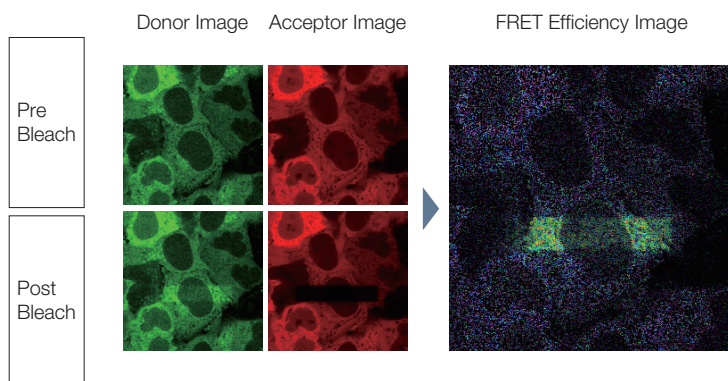


## Life Science Analysis

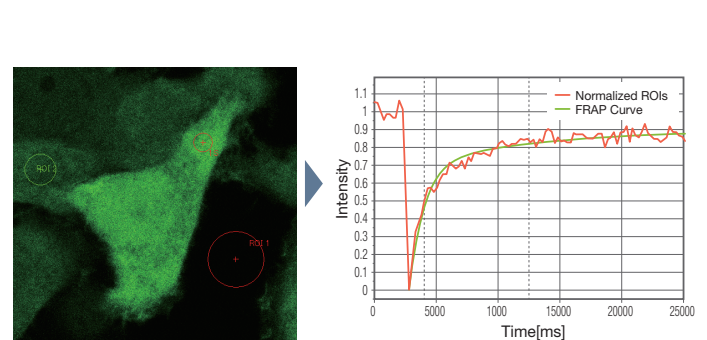
The cellSens Life Science Analysis module enables analysis of images from FRAP or FRET experiments. The FRAP module includes single as well as double exponential fitting. This module helps to calculate  $\tau/2$  and mobile/immobile fractions.

The FRET module enables ratio imaging processing and measurement of FRET efficiency from acceptor photobleaching and sensitizes emission. Several computational methods, including Gordon (1998), Xia (2011), and Elangovan (2003) are available.

### Example of FRET analysis (acceptor photobleaching)



### Example of FRAP analysis



## » Application: Deep Tissue Observation with Silicone Oil Objectives

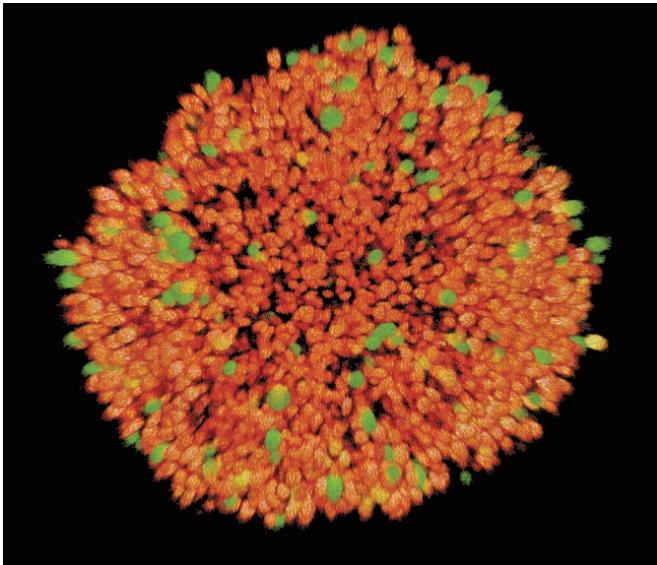
Olympus silicone oil immersion objectives deliver excellent performance for live cell imaging. The refractive index of silicone oil is close to that of living tissue, enabling high-resolution observation deep inside living tissue with minimal spherical aberration. This refractive index match delivers an ideal focal volume, resulting in perfect volume reconstruction and enabling high-resolution confocal imaging of large living organisms. The FV3000 system's 3D construction software enables simultaneous image acquisition and real-time 3D rendering to visualize the sample in its entirety.



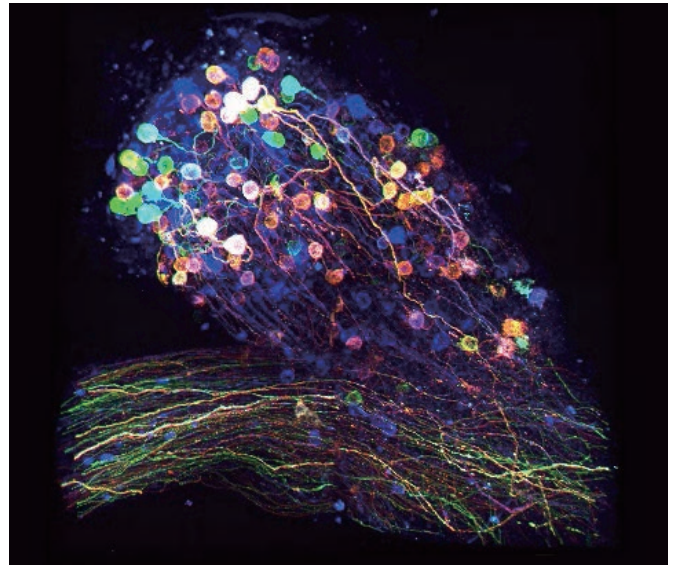
[www.olympus-lifescience.com/fv3000-spheroid-animation](http://www.olympus-lifescience.com/fv3000-spheroid-animation)



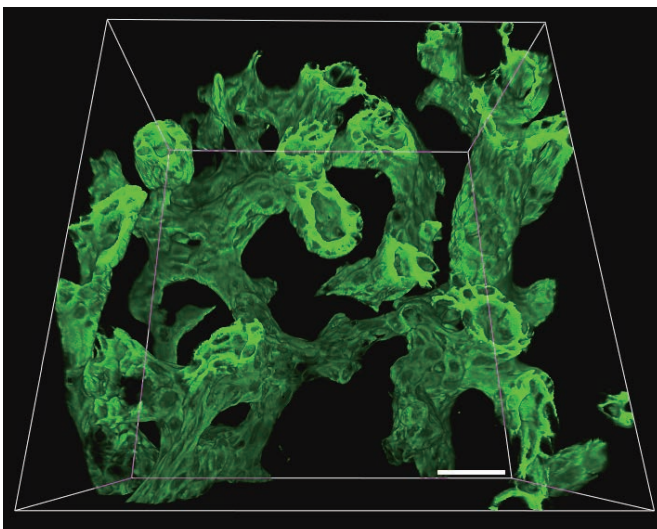
[https://static3.olympus-lifescience.com/data/Video/Library/3Dimageofchickciliaryganglionclearedbytissueclearingreagent\\_480.mp4?rev=64AE](https://static3.olympus-lifescience.com/data/Video/Library/3Dimageofchickciliaryganglionclearedbytissueclearingreagent_480.mp4?rev=64AE)



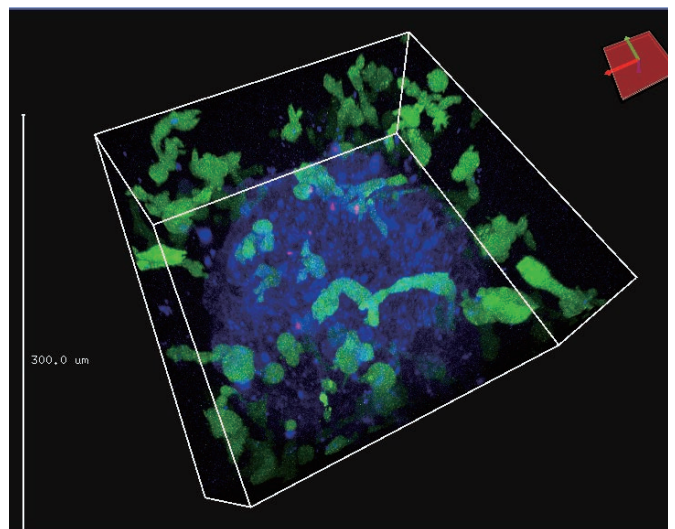
A spheroid image of an NMuMG cell line expressing Fucci2. Image data courtesy of Atsushi Miyawaki, Cell Function Dynamics, Brain Science Institute of RIKEN.



3D image of chick ciliary ganglion cleared by tissue clearing reagent. Image data courtesy of Dr. Ryo Egawa, Tohoku University Graduate School of Life Science.



3D observation of biliary tree structures in mouse liver. Image data courtesy of Dr. Hajime Okada, Division of Mammalian Development, Genetic Strains Research Center, National Institute of Genetics, and Dr. Tohru Itoh, Laboratory of Stem Cell Therapy, Institute for Quantitative Biosciences, The University of Tokyo.



NK cell line KHYG-1 (green) changing shape while attacking and killing HT-29 tumor cells labeled with cetuximab (blue). PI uptake (red) indicates cell death. Image data courtesy of Dr. Yuji Mishima, The Cancer Chemotherapy Center of JFCR.



<https://www.olympus-lifescience.com/applications/cleared-mouse-liver/>



[https://www.olympus-lifescience.com/applications/spheroid\\_3d\\_imaging/](https://www.olympus-lifescience.com/applications/spheroid_3d_imaging/)



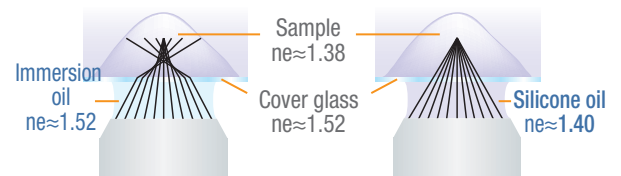
## A Line: Silicone Oil Immersion Objectives for Live Cell Imaging Deliver High-Resolution Observation at Depth

Olympus offers four high NA silicone oil immersion objectives that deliver excellent performance for live cell imaging. The refractive index of silicone oil ( $n_e \approx 1.40$ ) is close to that of living tissue ( $n_e \approx 1.38$ ), 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 the need to refill the oil is eliminated, making it ideal for extended time-lapse observations.

Objectives	Working Distance (WD) [mm]	Numerical Aperture (NA)
UPLSAPO30XS	0.8	1.05
UPLSAPO40XS	0.3	1.25
UPLSAPO60XS2	0.3	1.3
UPLSAPO100XS	0.2	1.35

### 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.

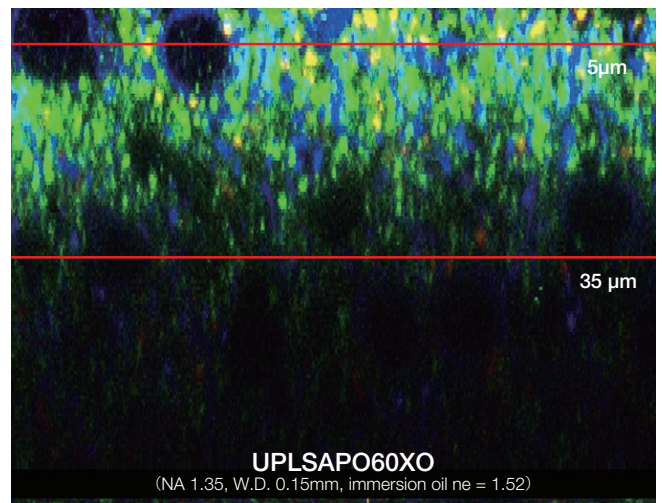
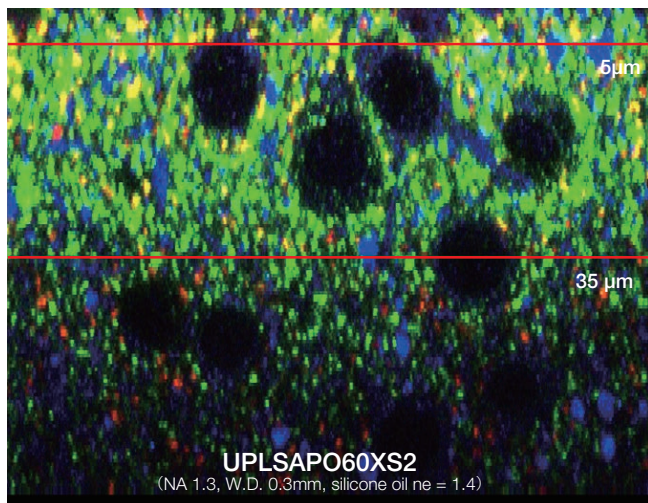


#### Oil immersion objective

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

#### Silicone oil immersion objective

When working with a silicone oil 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 observation.



ScaleA2-treated neocortex acquired with UPLSAPO60XS2 and UPLSAPO60XO objectives (predecessor of the UPLXAPO60XO). Image data courtesy of Motokazu Uchigashima, M.D., Ph.D., Masahiko Watanabe, M.D., Ph.D., Department of Anatomy, Hokkaido University Graduate School of Medicine.

## A Line PLAPON60XOSC2:

### Enhance the Reliability of Colocalization Analysis with a Super-Corrected Apochromat Objective

This oil immersion objective minimizes chromatic aberration to the utmost limit in the 405–650 nm spectrum. 0.1 μm or less axial chromatic aberration is guaranteed, and every objective is delivered with its measured data sheet. Furthermore, various aberrations are compensated even at 405 nm, and high-quality homogeneous 405 nm images can be achieved. This objective is used for rigorous colocalization analysis or super resolution.



#### Super-Corrected Apochromat Objective

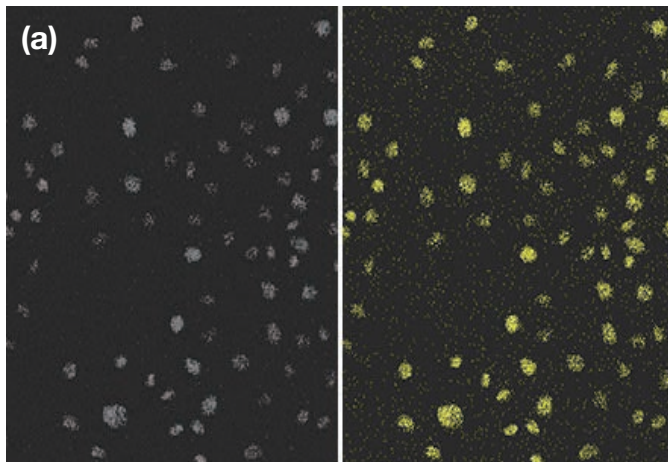
Magnification: 60X  
 NA: 1.4 (oil immersion)  
 W.D.: 0.12 mm  
 Chromatic aberration compensation range:  
 405–650 nm  
 Optical data provided for each objective.

### Performance Comparison of the PLAPON60XOSC2 and UPLXAPO60XO

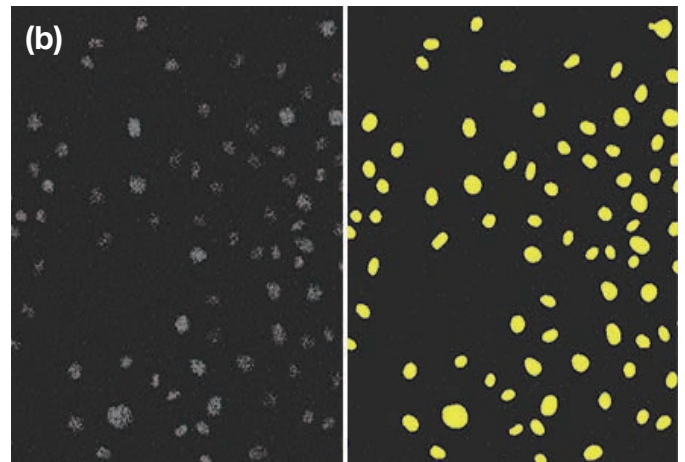
	PLAPON 60XOSC2	UPLXAPO 60XO
On axis vertical chromatic aberration (Z direction)	Approx. 0 μm	Approx. 0.2 μm
Off axis lateral chromatic aberration (X-Y direction)	Approx. 0.05 μm	Approx. 0.15 μm

Comparison of chromatic aberration measured by the FLUOVIEW FV3000 microscope using TetraSpeck Microsphere. Cyan: 405 nm excitation, Magenta: 640 nm excitation.

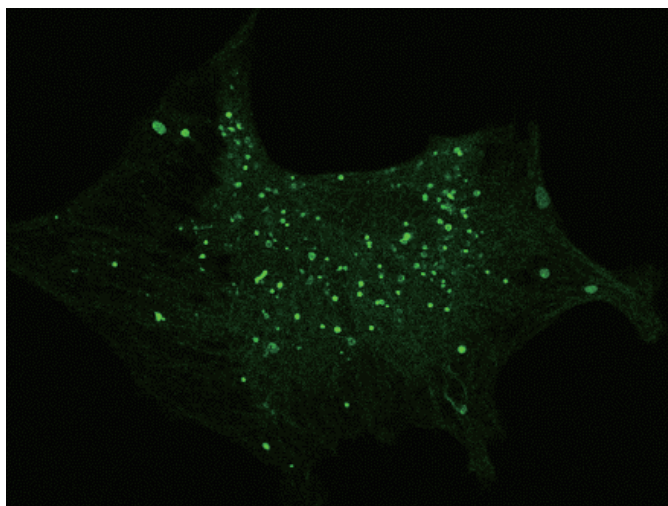
Experiments often require extracting and analyzing data from microscope images. For accurate image analysis, segmentation—especially thresholds based on intensity values or color—is used to extract the analysis targets from the images. But this can be time-consuming and affect the sample condition. TruAI technology's next-generation image analysis using deep learning helps address these challenges.



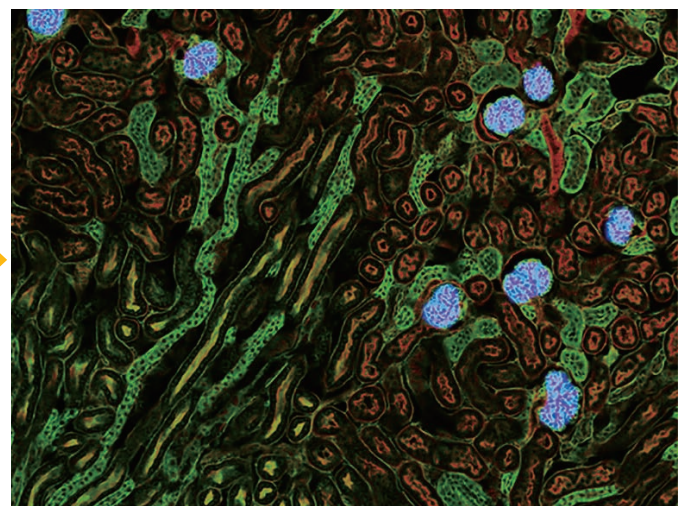
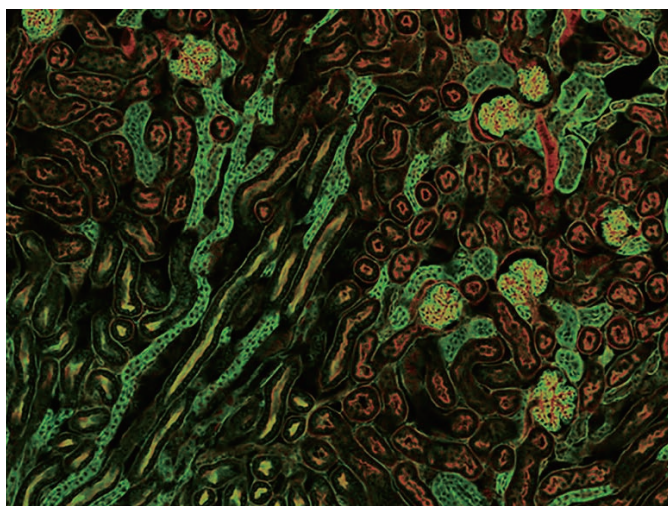
The result of detecting nuclei (right) with the conventional method, from fluorescence images (left) with extremely poor SNR due to weak excitation light. You can see that the detection accuracy is low.



The result of detecting nuclei (right) using TruAI from a fluorescence image (left) with extremely poor SNR due to weak excitation light. You can see that the accuracy is much higher accuracy than (a)



Autophagosome of mouse fibroblast (left), and autophagosomes recognized by TruAI (right).



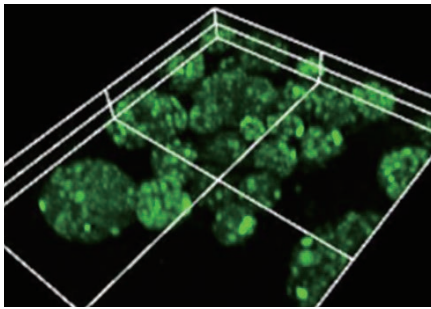
Prediction of glomeruli positions on a mouse kidney section using TruAI (blue). TruAI captures and detects the glomeruli features (right).

# NoviSight 3D Cell Analysis Software

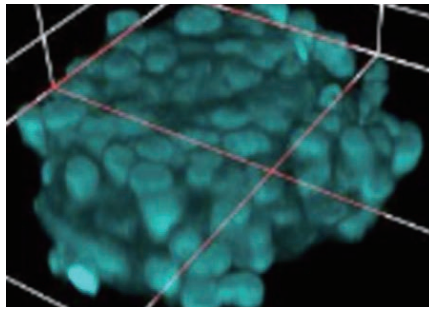
NoviSight 3D cell analysis software eases the analysis of 3D images of spheroids and organoids in microplates that are acquired using the FV3000 laser scanning microscope. The software's True 3D technology enables you to easily measure and count 3D objects in spheroids and organoids. By improving your observation efficiency as well as the accuracy and speed of your 3D analysis, NoviSight software increases the speed of discovery.

## Fast, Precise Object Detection

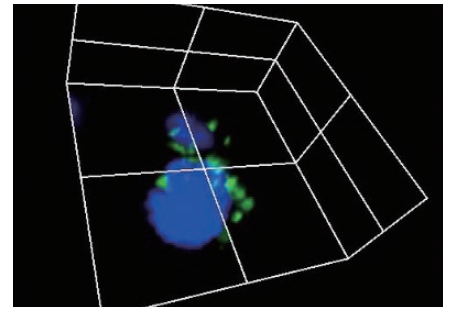
With numerous detection algorithms designed for 3D samples, NoviSight software can quantify cell activity and interactions in three dimensions. With precise detection, the software analyzes objects of interests to provide morphology and spatiotemporal parameters in 3D space.



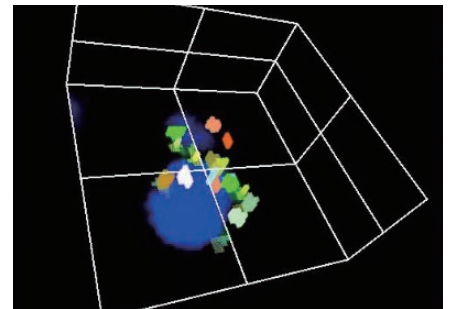
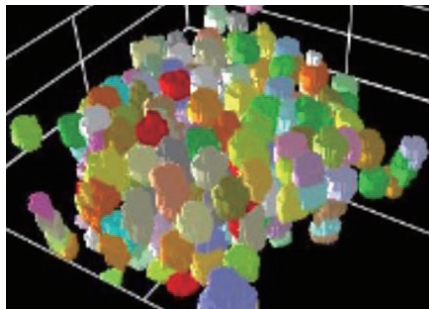
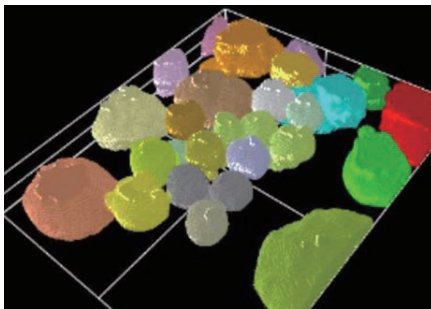
Spheroids



Nuclei

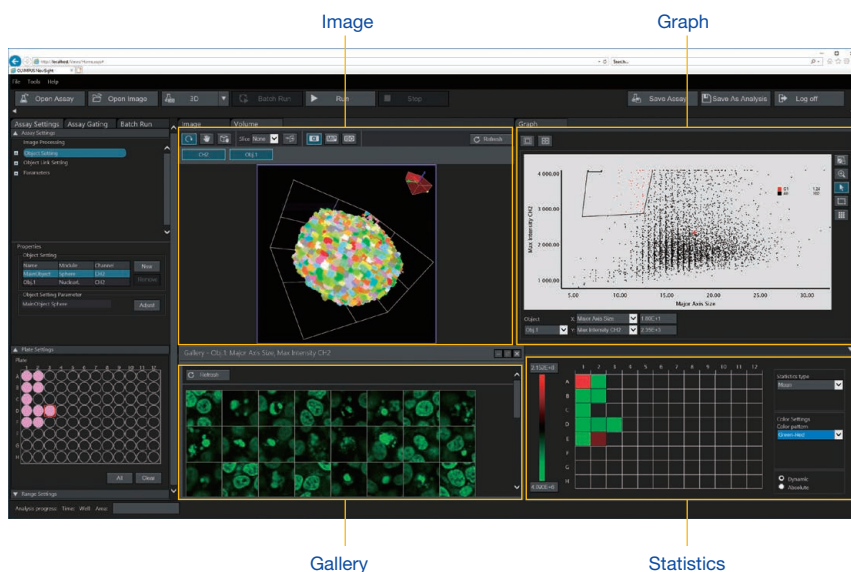


Organelle (Autophagosome)



## Intuitive User Interface Reduces Your Analysis Time

All the data you need—recognition, analysis, and statistical results—are in one convenient location. The original images are paired with the quantitative data for easy validation and interpretation. The data is easily exported as a CSV or FCS file for further analysis.



### Image

Get 2D or 3D views of your samples. Locate objects in 2D within an image plane or switch to 3D to explore the entire spheroid.

### Graph

The scatter plot makes it easier to classify objects. You can click and select an individual point, which will then bring up an image of that object.

### Gallery

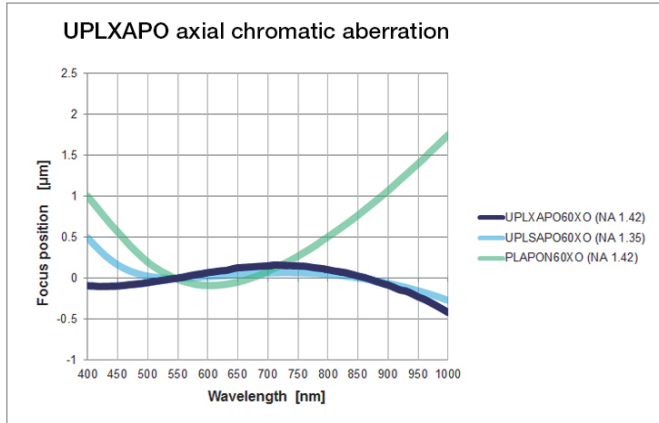
Observe the details in each region of an object at a glance. Visualize how classification is working by highlighting two areas in the scatter plot, such as morphology differences in the nuclei, and display the resulting galleries side by side.

### Statistics

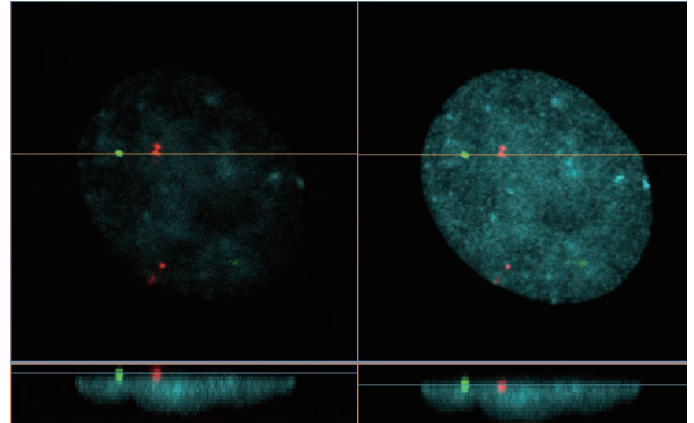
View quantitative results numerically or displayed on a heat map.

## X Line: Breaking Barriers to Improve Confocal Image Quality

Olympus X Line objectives provide improvements in three important areas: a higher numerical aperture (NA) to acquire brighter and higher resolution images, expanded image flatness for smoother image stitching and efficient image analysis, and a wide chromatic aberration correction range covering 400 nm–1000 nm wavelengths. X Line objectives can acquire multicolor images with high accuracy and precision.

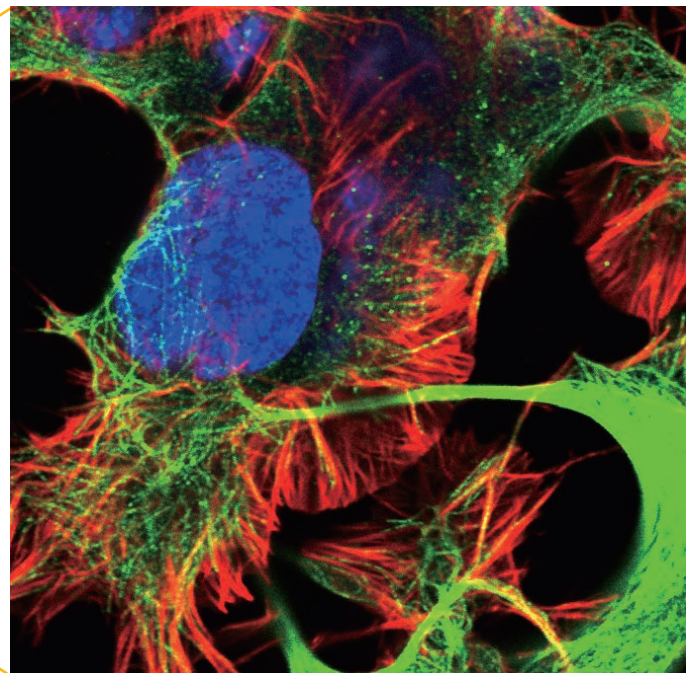
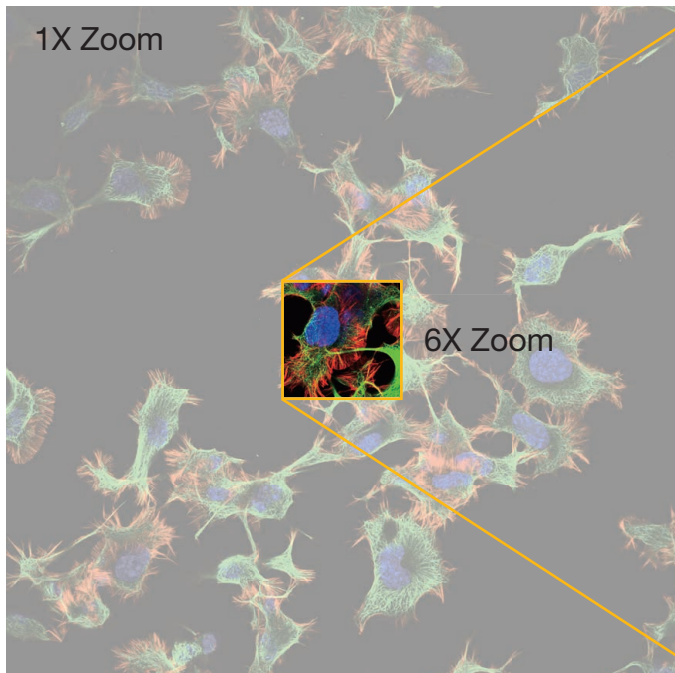


Comparing the focus position in the 400-1000 nm wavelength range



Conventional objectives

X Line



UPLXAP40X0 X Line objective (NA 1.40)

## Selection Guide for X Line Objectives

Objectives	Working Distance (WD) [mm]	Numerical Aperture (NA)
UPLXAPO4X	13	0.16
UPLXAPO10X	3.1	0.4
UPLXAPO20X	0.6	0.8
UPLXAPO40X	0.18	0.95
UPLXAPO40XO	0.13	1.4
UPLXAPO60XO	0.15	1.42
UPLXAPO100XO	0.13	1.45
UPLXAPO60XOPH	0.15	1.42
UPLXAPO100XOPH	0.13	1.45

# Choose the Frame That Suits Your Application

## Inverted microscope

- Used to observe cells cultured in a vessel.
- TruFocus Z-drift compensation helps ensure the sample remains in focus during time-lapse observations.
- Maintain the environmental conditions of cultured cells by adding a stage-top or full enclosure incubator.



## Upright microscope (configured for imaging)

- Optimized for fixed tissue and glass slide specimens.
- Motorized nosepiece precisely maintains the focus position.
- Motorized seven-position nosepiece and condenser enable automated transitions from low to high magnification.



## Upright microscope (configured for electrophysiology)

- Ample space around the objectives enables patch-clamp devices to be installed.
- Add additional space by lowering the stage position for experiments that require large sample handling.
- Swing and slider nosepieces are available so objectives can be easily changed without interfering with the patch-clamp set-up.
- Trigger I/O is possible using the FV30-ANALOG to synchronize with electrophysiology equipment.



# 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 × 512. 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 one-way scanning from 1 fps at 512 × 512, and up to 16 fps with bi-directional scanning. 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 two-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 two-channel SD for a flexible dynamic range or a second two-channel HSD unit for powerful four-channel sensitivity.

### Spectral Detector (Multialkali PMT) with TruSpectral Technology

The two-channel SD employs efficient VPH transmission and an adjustable slit with 1–100 nm bandwidth from 400–800 nm detection. The multialkali 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. The combiner accommodates 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.

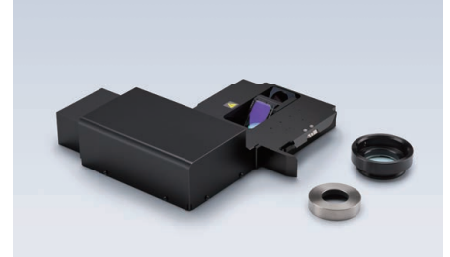


### Transmitted Detector

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

Available upgrade options: field-upgradable laser-based autofocus, fast and precise motorized stage control, analog input/output and TTL synchronization, and a convenient antivibration platform.



### TruFocus/IX3-ZDC2 Z-Drift Compensation System

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



### Umbra unit/FV31-SPCOV

The umbra unit is designed for fluorescence observation under bright room conditions. It efficiently blocks out room light and enables clear fluorescence observation without the need for a dark room.



### Remote correction collar unit/IX3-RCC

The remote correction collar unit enables the user to easily adjust the correction collar manually to improve image quality.

# Specifications

## FLUOVIEW FV3000 Laser Confocal Microscope Specifications

		FV3000	FV3000RS
Laser Light	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	
	Single NIR Laser Unit	730 nm: 30 mW, 785 nm: 100 mW, connected to scanner via optional port	
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 degrees) 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	
Spectral Detector	Detector Module	Cooled GaAsP photomultiplier (high-sensitivity type) or 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)	
NIR Detector	Detector Module	GaAs photomultiplier tube, 1 channel or 2 channels with filter cube	
Fluorescence Illumination Unit	External fluorescence light source, fiber adaptor 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		

## Microscope

	Inverted frame	Upright frame (for imaging)	Upright frame (for electrophysiology)
Microscope Frame	Motorized inverted microscope IX83P2ZF	Motorized fixed stage upright microscope BX63L	
Revolving Nosepiece	Motorized sextuple revolving nosepiece	Motorized septuple revolving nosepiece	Coded swing nosepiece Coded slider nosepiece
Condenser	Motorized long working distance condenser	Motorized universal condenser	Manual long working distance condenser
Focus Stroke	Built-in motorized nosepiece focus Stroke: minimum increment: 0.01 μm		

## 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, 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 display. 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	Region and line measurements, Intensity plot over time/Z, Colocalization analysis
Statistical Processing	2D data histogram display
Optional Software	Motorized-stage control / Mapping and multipoint stimulation / Sequence manager / Virtual channel acquisition / Microplate navigation / Remote development kit / Super resolution imaging (FV-OSR) / Digital camera control function / TruSight Deconvolution / FRET&FRAP analysis / Automatic object measurement and classification / Object tracking / TruAI deep-learning technology / NoviSight 3D cell analysis

**Image data are courtesy of the following institutions:**

Cos-7 cells labeled with DAPI (blue), anti-Tubulin (Alexa Fluor 488; green), Concalavalin A (Alexa Fluor 594; yellow), SiR-Actin (magenta) and anti-TOMM20 (Alexa Fluor 750; cyan). Images were acquired using a UPLXAPO60XO objective and 405 nm, 488 nm, 561 nm, 640 nm, and 730 nm laser lines on GaAsP and GaAs detectors. Maximum intensity projection in Z with TruSight deconvolution processing. (cover)

Sample courtesy of Dr. Jana Döhner, Dr. Urs Ziegler, University of Zürich.

Immunocytochemistry (peripherin; green; neuronal cell bodies and axons) in combination with in situ hybridization (CALCA mRNA; red and P2RX3 mRNA; blue) of cultured mouse dorsal root ganglion neurons. (top left, page 1)  
Image data courtesy of Stephanie Shiers, Price Lab, University of Texas at Dallas.

Live culture of Drosophila (fruit fly) brains. Microtubule marker which marks neurons in the brain, and histone marker which marks nuclei. (top right, page 1)  
Image data courtesy of Martin Hailstone, Oxford University.

Mouse brain tissue section was labelled with trans-synaptic viral tracer to investigate neural pathways that link the visual systems in the mouse. The varying colours reflect the changing depths of neuronal processes. (center, page 1)  
Image data courtesy of Arthur Chien, Macquarie University.

Fruit fly 3rd instar larvae brain lobe. Neuroblasts and ganglion mother cells are red and pink, respectively. Actin is green. DNA is blue. Wolbachia bacteria are green dots within cells. (bottom left, page 1)  
Image data courtesy of Anton Strunov, Medical University of Vienna.

5 µm sagittal cryosection of E14.5 mouse embryo, stained with TSA reagents. (page 2)

Image data courtesy of Dr. Guan Yang and Prof. Xiao Yang, Genetic Laboratory of Development and Diseases, Beijing Institute of Biotechnology, AMMS.



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- **EVIDENT CORPORATION is ISO9001 certified.**
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