High-Performance Laser Scanning Microscope for
Live Cell Imaging Combining Accuracy, Sensitivity and Laser Stimulation
The FLUOVIEW FV1200: High-quality Live Cell Imaging with High-level Reliability

The FLUOVIEW FV1200 biological laser scanning microscope builds on renowned Olympus optics, enhancing sensitivity through new galvanometer coating and GaAsP detector technology. With the new IX83 microscope, the FV1200 is optimized for some of the most challenging live cell imaging experiments, implementing real-time Z-drift compensation and touch panel control.

From high-resolution, confocal observation of fixed samples, with up to 5 simultaneous fluorescent detection channels, to high speed fluorescent measurements and simultaneous stimulation of living cells, the FV1200 offers advances in confocal system performance while providing the speed and sensitivity required for live cell imaging, with minimal risk of damage to living specimens.

What’s more, the FLUOVIEW FV1200 supports an array of optional functions—such as capability for measuring cellular molecular diffusion coefficients—extending the exceptional performance from visualization to stimulation, to precision measurement.
EXCELLENT PRECISION, SENSITIVITY AND STABILITY
FUĽOVÝV V1200 ENABLES PRECISE, BRIGHT IMAGING WITH MINIMUM PHOTOTOXICITY

Features of the NEW IX83

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

UIS2 Objectives
Olympus offers a line of high numerical aperture objectives with improved fluorescence S/N ratio, including objectives with silicone immersion, exceptional correction for chromatic aberration, total internal reflection fluorescence (TIRF), and oil- and water immersion objectives.

Switch Observation Methods with a Tap of the Touch Panel
A tap of the fingertip is all it takes to manage changes in magnification, switch between optical elements, and make adjustments to illumination. Not only does the controller make it a cinch to carry out complex microscope operations, but it can also save settings for observation modes.

The U-MCZ Controller Executes Procedures from a Preferred Position
The controller allows monitor observation to be executed in your preferred position and mode, while simple key arrangement allows confident control—even under darkroom conditions.

The U-HGLGPS Fluorescence Illumination Source Minimizes the Impact of Lamp Heat to Both Microscope and Specimen
Featuring a high-pressure mercury lamp with an average life of 2,000 hours, the user-friendly fluorescence illumination source incorporates a low chromatic aberration adapter that cleverly compensates when switching excitation wavelengths.

Scanners and Detection System

Choice of Main Scanner
Select the scanner to match the purpose at hand, with a choice of the spectral scan unit that achieves 2mm resolution for high-precision spectroscopy, and the filter scan unit incorporating high-quality filters.

High-performance Detection System
High performance and high S/N ratio optical performance are achieved through the smooth integration of a pupil projection lens, a high performance photomultiplier photomultiplier tube, silver-coated galvanometer scanning mirrors with high reflectance across a broad range of wavelengths, and an analog processing circuit that reduces noise to an absolute minimum. Furthermore, because the system enables image acquisition of this quality with only minimal laser power, phototoxicity is also significantly reduced.

High-sensitivity Detector
A high-sensitivity detector employing gallium phosphide (GaAsP) is also available as an option.
A STEP UP IN SENSITIVITY
THE FV1200 CAPTURES SUBTLE CHANGES IN LIVE CELLS, WITH HIGHLY SENSITIVE DETECTION IMMEDIATELY FOLLOWING PHOTOSTIMULATION

High Performance Across a Wide Range of Wavelengths
Galvanometer scanning mirrors on the main scanner feature an anti-oxidative silver coating that increases reflection efficiency for excitation and emission filters from 5% to 15% in the visible spectrum and by a maximum of 22% in the near-infrared spectrum. The standard, onboard multi-alkali photomultiplier tubes with a high dynamic range can also be combined with the optional, ultra high-sensitivity GaAsP photomultiplier tubes to further increase the freedom for experimental setups across a broad range of wavelengths.

Two Versions of Light Detection System that Set New Standards in Quality

**Spectral Based Detection**

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

- **Precise Spectral Imaging**
  The spectral detection unit uses a grating method that offers linear dispersion compared with prism nonlinear dispersion. The unit provides uniform 2nm wavelength resolution across the entire detection spectrum and high performance photomultiplier tube detectors. Fluorescence separation can be achieved through unmixing, even when cross-talk is generated by multiple fluorescent dyes with similar peaks. A standard third filter channel is provided without a grating allowing researchers greater flexibility and sensitivity.

**Filter Based Detection**

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

- **High-Performance Filters Deliver Outstanding Separation**
  Special coatings deliver exceptionally sharp transitions to a degree never achieved before, for acquisition of brighter fluorescence images.

The High Sensitivity GaAsP Detector Module

**Ultra-High Sensitivity Detector with GaAsP Photomultiplier Tubes Further Enhances Quantum Efficiency**

The ultra-high sensitivity detector makes it possible to view samples that were simply too dim to view with conventional equipment. The GaAsP PMT incorporates 2 channels and combines the images with a further 3 built-in channels as well as the channel transmitted from the detector. Maximum quantum efficiency is 45%. Peltier cooling holds noise down by 20%, and high S/N ratio images can be obtained under exceptionally low excitation light.

SIM Scanner Allows Simultaneous Photostimulation during Time-lapse Imaging

**Dedicated Scanner for Photostimulation**

Combination of the main scanner with a photostimulation scanner provide essential flexibility for tracking the diffusion or transport of fluorescence-labeled molecules or for marking specific live cells. The dual-fiber laser combiner makes it possible to use imaging lasers for photostimulation.

**Simultaneous Photostimulation and Imaging**

Lasers are used for both imaging and photostimulation.
ENHANCED RELIABILITY FOR LIVE CELL IMAGING MEETS DEMANDS FOR DEEPER 3D STRUCTURING, TIME-LAPSE IMAGING, AND PRECISION MEASUREMENT

Silicone Immersion Objectives for Live Cell Imaging Deliver High-resolution Observation At Depth

Silicone Immersion Objective

High-resolution Silicone Immersion Objective

Silicone immersion objectives can be designed with a larger numerical aperture (NA) than water immersion objectives, increasing image resolution and brightness.

Complete the range with the UPLSAPO40XS

This new objective with intermediate magnification and high NA performance supports continuous focus with the IX3-ZDC. Continuous high-resolution observation during extended time-lapse imaging.

UPLSAPO30XS: For Broader View and Greater Depth

Magnification: 60x
NA: 1.40 (silicone oil immersion)
W.D.: 0.12mm
Cover glass thickness: 0.15–0.19mm
Operation temperature: 23ºC–37ºC

UPLSAPO60XS: For 3D with Superior Resolution

Magnification: 60x
NA: 1.40 (silicone oil immersion)
W.D.: 0.3mm
Cover glass thickness: 0.15–0.19mm
Operation temperature: 23ºC–37ºC

SIL300C-300C: For Extended Time-lapse Imaging

Refractive index: ne=1.406, 23ºC
Net 30ºM
Low autofluorescence

Refractive Index is Important with Deep Tissue Observation

Water immersion objective

When working with a water immersion objective, the difference between the refractive index of the immersion objective and deep tissue causes resolution to deteriorate and spherical aberration in deep tissue.

Silicone immersion objective

When working with a silicone immersion objective, the difference between the refractive index of the immersion objective and deep tissue results in minimal spherical aberration and fluorescence to become dim.

So it achieves brighter fluorescence images with higher resolution for deep tissue.

Compatibility with a variety of sample and immersion mediums are close to that close to each other.

Enhance the Reliability of Colocalization Analysis, With the Low Chromatic Aberration Objective

Low Chromatic Aberration Objective

Acquire and Analyze Colocalization Imaging with the PLAPON60X0SC

This oil-immersion objective minimizes lateral and axial chromatic aberration in the 400–650nm spectrum, while supporting the reliable acquisition and measurement of colocalization images with superior positional accuracy. The objective also compensates for chromatic aberration through near infrared up to 850nm, making it an optimal choice for near infrared fluorescence observation.

PLAPON 60x0SC and UPLSAPO 60x0

<table>
<thead>
<tr>
<th>Objective</th>
<th>Axial chromatic aberration (Z direction)</th>
<th>Lateral chromatic aberration (X-Y direction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLAPON 60x0SC</td>
<td>Approximately ±0.5 µm</td>
<td>Approximately ±0.01 µm</td>
</tr>
<tr>
<td>UPLSAPO 60x0</td>
<td>Approximately ±0.1 µm</td>
<td>Approximately ±0.005 µm</td>
</tr>
</tbody>
</table>

3D image

When using oil immersion with two colors, minimal crosstalk and high performance.

Performance Comparison of PLAPON 60x0SC and UPLSAPO 60x0

X-Y: 46µm x 46µm (0.77 x 0.77 pixel)
Z: 21µm (52 pixels)

IX83: Two-deck System + IX3-ZDC

IKX-ZDC Optical Path Diagram

AF sensor
Offset lens
Cover glass
Objective

IKX-ZDC optics

Scanning unit

Z-drift Compensation System

The IX3-ZDC Z Drift Compensator Offers a Range of Functionality for Autofocus

The IX3-ZDC uses lowphototoxicity IR light to detect the correct focus position as set by the user. One-shot AF mode allows several focus positions to be set as desired for deeper samples, enabling efficient 2-stack acquisition in multi-position experiments. Continuous AF mode keeps the desired plane of observation precisely in focus, avoiding focus drift caused by temperature changes due to perfusion or reagent addition and making it ideal for measurements such as TRF that requires more stringent focusing.

ZDC One-shot Function Detects Focus Fast, Even in High Magnification Observation

IX3-ZDC focus detection and tracking can be performed via the innovative touch panel independent of software. There’s also a focus search function supported by a cell-safe, near-infrared laser enabling instantaneous focusing on samples and start scanning.

Rigidity

Tackle the Conflicting Requirements of Expandability and Rigidity with the IX3

A Z-drive guide installed near the revolving nosepiece combines high thermal rigidity with the further stability of a wraparound structure to significantly reduce the impact of heat and vibration and improve the quality of time-lapse imaging. Integration with the IX3-ZDC 2 drift compensator permits the imaging without focus drift or misalignment, even through temperature changes due to the addition of reagents or a perfusion device. Furthermore, combination with a motorized stage that enables multipoint registration, allows the achievement of high-precision multipoint time-lapse imaging.
Applications

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

Re-Use Function
Open previously configured scanning conditions and apply them to new or subsequent experiments.

Dark Application Skin
Use of the dark application skin minimizes the influence of the noise from the screen for the sample.

Image Acquisition by Application
User-friendly icons offer quick access to functions, for image acquisition according to the application (XYZ, XYI, XYZI, XYAL, XYAT).

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

Wide Choice of Scanning Modes
Several available scanning modes including ROI, point and high-speed bidirectional scanning.

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

Multi-dimensional Time-lapse Imaging with Outstanding Positional Accuracy
The FLUOVIEW FV1200 can be used for ideal multi-dimensional time-lapse imaging during confocal observation, using multi-area time-lapse software to control the motorized XY stage and iX3-ZDC Z-drift compensator.

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

Multi-dimensional Time-lapse Imaging
CO2 incubator control keeps the environment inside the tissue culture dish completely stable. The environment is precisely maintained at 37°C with 90% humidity and 5% CO2 concentration.

Human lymphoblast cells TK6
Courtesy of Masamitsu Honma, Dir. Biological Safety Research Center Div. of Genetics and Mutagenesis I, National Institute of Health Sciences
**Applications**

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

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

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

**Uncaging**
A 405nm laser is optional for uncaging with the SIM scanner system. Caged compounds can be uncaged point-by-point or within a region of interest, while the main scanner of the FV1200 captures images of the response with no time delay.

**Multi-Stimulation Software**

- **High Speed Multipoint Scans**
  User can designate the number of points on an image for light stimulation. Stimulation timing, duration and interval can be defined in the magnitude of µs and the user can program the experiment with continuous or pulse stimulation. The same software also provides features that allows extended multiple points surrounding one single point to cover a small area.

- **Mapping Scans**
  Light stimulation can be applied to a rectangular region of interest. Software control of stimulation of each point assures neighboring points will not be excited. This allows the user to observe reaction of sample more accurately. Changes in intensity from those points can be processed as a mapped image or graph.
Comparison of Diffusion Coefficients for EGFP Fusion Proteins Near to Cell Membranes and In Cytoplasm

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

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

FRAP Analysis

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

Diffusion Measurement Package Extends Analytical Capabilities

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

RICS—Raster Image Correlation Spectroscopy

Raster image correlation spectroscopy (RICS) is a new method for analyzing the diffusion and binding dynamics of molecules in an entire, single image. RICS uses a spatial correlation algorithm to calculate diffusion coefficients and the number of molecules in specified regions. Cross correlation RICS (c-RICS) characterizes molecular interactions using fluorescent-labeled molecules in two colors.

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**Accessory Units That Support an Array of Applications**

### Laser Systems

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

### Illumination Units

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

### Optional Upgrade Equipments for FV1200

- **Ultra-High Sensitivity Detector/GaAsP photomultiplier tube**: Achieves ultra-high sensitivity with low noise thanks to the gallium arsenide phoshide detector and the onboard Peltier cooling system.
- **4th Channel Detector Unit**: Attaches to the optional port of either the fiber or scanning type scanning unit and is used as a 4th confocal fluorescence detection channel. This is a fiber-based fluorescence detection unit.
- **Fiber Port for Fluorescence Output**: Continuous fluorescence emission can be introduced via fiber delivery system into external device. Fiber port equipped with FC connector (fiber delivery system not included).
- **SIM Scanner**: Second scanner dedicated for photostimulation, synchronized to the FV1200 main scanner for simultaneous photostimulation and confocal image acquisition. Independent fiber optic laser introduction port. Dichromatic mirror within motorized optical port of the scan unit required for introduction of laser into main scanner.
- **Motorized XY Stage**: This motorized stage supports well plates, 35mm diameter dishes, and slide chambers, and also comes complete with a universal sample holder.

Expandability

- **MitoTracker**: Expandability
- **ECFP**: Expandability
- **EYFP**: Expandability
- **HcRed1**: Expandability
- **458 Multi Argon**: Expandability
- **515 Multi Argon**: Expandability
- **Alexa Fluor 488**: Expandability
- **OregonGreen488**: Expandability
- **Rhodamine Green**: Expandability
- **Acridine Orange**: Expandability
- **Magnesium Green**: Expandability
- **GFP-uv**: Expandability
- **Calcium Green-1**: Expandability
- **Azami Green**: Expandability
- **Rhodamine Phalloidin**: Expandability
- **YOYO-1**: Expandability
- **Magnesium Orange**: Expandability
- **Fluo-4**: Expandability
- **Calcium Orange**: Expandability
- **EGFP**: Expandability
- **Kaede**: Expandability
- **furaRed**: Expandability
- **EYFP**: Expandability
- **FITC**: Expandability
- **Rhodamine Red-X**: Expandability
- **SNARF-1**: Expandability
- **DiO**: Expandability
- **MitoTracker Green**: Expandability
- **TOTO-3**: Expandability
- **HcRed1**: Expandability
- **Alexa Fluor 633**: Expandability
- **Alexa Fluor 647**: Expandability
- **TOTO-3**: Expandability

*Selected fluorescent dyes, while still shows the absorption maximum, graphs show the dye emission spectra.*
FLUOVIEW FV1200 system diagram

**FLUOVIEW FV1200 major specifications**

**Laser Light**
- Argon Dbl. Laser: Beam diameter 1.50–2.50 mm, 2–3W in 488nm and 514nm. Top and bottom illumination with wide beam type illumination. Single wavelength, double wavelength operation is available.
- Argon/HeNe Laser: Beam diameter 1.00–1.50 mm, 500mW in 488nm and 514nm. 2–3W in 633nm. Top and bottom illumination with wide beam type illumination. Single wavelength, double wavelength operation is available.
- Excimer Laser: Top and bottom illumination with wide beam type illumination. Single wavelength operation is available.

**Excitation Module**
- Optional excitation module: Spectral type or filter type detector system. 3D and 2D sequential operation function. 3D animation (maximum intensity projection method, SUM method).
- Power supply: 120–240V, 50/60Hz, 1.6A
- SIM Scanner: 350–700nm, 512 x 512, 1.1s, 1.6s, 2.7s, 3.3s, 3.9s, 5.9s, 11.3s, 27.4s, 54.0s.

**Fluorescence Detection**
- Fluorescence detection: 488nm, 492nm, 590nm, 633nm. 3D and 2D sequential operation function.
- Ultra-high Sensitivity Detector: Cooled GaAsP-PMT. 2 channels.
- SIM Scanner: 2 galvanometer scanning mirrors, pupil projection lens, built-in laser shutter, 1 laser port, Fiber introduction of near UV diode laser or visible light laser.

**Image Acquisition**
- USB2.0: Maximum speed 500Mbps
- 3D Visualization and Observation: Free orientation of cross section display. Comparison (maximum intensity projection method). With individual, with set to display stack cross section function.

**CFG (Configuration) parameters**
- DVD-ROM: 130 (W) x 224 (D) x 62 (H) 1.8 AC 100-120/240-240 V 50/60Hz 0.45A

**Recommended FV1200 system setup**
- IX83, BX61WI
- 100W mercury lamp
- U-HGLGPS
- HeNe(G) laser
- Optional Software: Review station software, Off-line FLUOVIEW software for data analysis.

**Image Processing**
- Diffusion-Measurement Package
- Multi Simulation Software
- Multi Area Time Lapse Software

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

**Objects for Fixed Stage Upright Microscope (using WP-UCE, WI-DIC/THRAJ)**

<table>
<thead>
<tr>
<th>Model</th>
<th>NA</th>
<th>FL</th>
<th>WD (mm)</th>
<th>Objective type</th>
<th>Correction ring</th>
<th>FV-DC15c position</th>
<th>FV-DC15d position</th>
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<tbody>
<tr>
<td>U-LUMPLN5X</td>
<td>0.10</td>
<td>20.00</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>WI-SSNP, URE-SSNP</td>
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<td>Oil</td>
<td>0.15–0.19</td>
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<td>U-LUMPLF20</td>
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<tr>
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**Objects for BX2 and IX3**

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<tr>
<th>Model</th>
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<th>WD (mm)</th>
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**Dimensions, weight and power consumption**

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<th>Power Consumption (W)</th>
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</tr>
<tr>
<td>100W</td>
<td>200–200</td>
<td>200–200</td>
<td>0.4</td>
<td>0.6</td>
<td>129 x 294 x 918</td>
</tr>
</tbody>
</table>

**Fluorescence Detection**
- Fluorescence detection: 488nm, 492nm, 590nm, 633nm. 3D and 2D sequential operation function.
- Ultra-high Sensitivity Detector: Cooled GaAsP-PMT. 2 channels.
- SIM Scanner: 2 galvanometer scanning mirrors, pupil projection lens, built-in laser shutter, 1 laser port, Fiber introduction of near UV diode laser or visible light laser.

**Image Acquisition**
- USB2.0: Maximum speed 500Mbps
- 3D Visualization and Observation: Free orientation of cross section display. Comparison (maximum intensity projection method). With individual, with set to display stack cross section function.

**Configuration**
- DVD-ROM: 130 (W) x 224 (D) x 62 (H) 1.8 AC 100-120/240-240 V 50/60Hz 0.45A