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 a new galvanometer coating and GaAsP detector technology. With the new IX83 microscope, the FV1200 has been optimised for some of the most challenging live cell imaging experiments, implementing real-time Z-drift compensation and touch panel control.

From the high-resolution, confocal observation of fixed samples, with up to 5 simultaneous fluorescent detection channels, through to high-speed fluorescent measurements and the 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 the ability to measure cellular molecular diffusion coefficients – extending the exceptional performance from visualisation and stimulation through to precision measurement.
 Greater stability, longer service life and lower operating costs are achieved using diode lasers.

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

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

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

**Laser combiner**
- Dual fibre-type combiner for observation and simultaneous photostimulation
- Single fibre-type combiner for observation and sequential photostimulation

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

**High S/N ratio objectives with suppressed autofluorescence**
- Olympus offers a range 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.

**Features of the NEW IX83**
- Discover improved expandability and rigidity with the IX83
  - The Z-drive guide with high thermal rigidity is installed near the revolving nosepiece to further augment the stability of the IX83 in the face of heat and vibration, and improve the results of time-lapse imaging. Furthermore, when combined with the IX3-ZDC Z-drift compensator and the motorised stage, high-precision multipoint time-lapse imaging is made possible without the risk of focus drift or misalignment.

**Switch observation methods with a tap of the touch panel**
- A single tap 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, 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 minimises the impact of lamp heat to both microscope and specimen**
- Featuring a high-pressure mercury lamp with an average life of 2,000 hours, this user-friendly fluorescence illumination source incorporates a low chromatic aberration adapter that cleverly compensates when switching between excitation wavelengths.
A STEP UP IN SENSITIVITY
THE FV1200 CAPTURES SUBTLE CHANGES IN LIVE CELLS, WITH HIGHLY SENSITIVE DETECTION IMMEDIATELY FOLLOWING PHOTO STIMULATION

High performance across a wide range of wavelengths
Galvanometer scanning mirrors on the main scanner feature an anti-oxidative silver coating that increases reflectivity 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 set-ups across a broad range of wavelengths.

Two versions of light detection system that set new quality standards

**Spectral-based detection**
Spectral detection using gratings for 2 nm wavelength resolution and image acquisition matched to fluorescence wavelength peaks. User-adjustable bandwidth of emission spectrum for acquiring bright images with minimal crosstalk. Precise spectral imaging

The spectral detection unit uses a grating method that offers linear dispersion compared with prism non-linear dispersion. The unit provides a uniform 2 nm wavelength resolution across the entire detection spectrum and high-performance photomultiplier tube detectors. Fluorescence separation can be achieved through unmixing, even when crosstalk 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 the integration of a pupil projection lens within the optics, and the use of a high-performance photomultiplier and an analogue 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%. Patented 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**
The combination of the main and photostimulation scanner provides essential flexibility for tracking the diffusion or the transport of fluorescence-labeled molecules or for marking specific live cells. The dual-fibre laser combiner makes it possible to use imaging lasers for photostimulation.

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

Lasers are used for both imaging and photostimulation.
Silicone immersion objectives for live cell imaging deliver high-resolution observation at depth

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.

**Magnification**: 40x
**NA**: 1.25 (silicone oil immersion)
**W.D.**: 0.13 mm
**Cover glass thickness**: 0.15–0.19 mm
**Operation temperature**: 23 ºC–37 ºC

**UPLSAPO60XS**: for 3D with superior resolution

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

**SIL300CS-30CC**: for extended time-lapse imaging

**Magnification**: 30x
**NA**: 1.05 (silicone oil immersion)
**W.D.**: 0.8 mm
**Cover glass thickness**: 0.13–0.19 mm
**Operation temperature**: 23 ºC–37 ºC

**UPLSAPO30XS**: for a broader view and greater depth

**Magnification**: 30x
**NA**: 1.05 (silicone oil immersion)
**W.D.**: 0.3 mm
**Cover glass thickness**: 0.15–0.19 mm
**Operation temperature**: 23 ºC–37 ºC

**PLAPON60XOSC**
Acquire and analyse colocalisation imaging with the 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 instant focusing on samples.

**Enhance the reliability of colocalisation analysis with the low chromatic aberration objective**

**Low chromatic aberration objective**

Acquire and analyse colocalisation imaging with the PLAPON60XOSC.
This oil immersion minimises lateral and axial chromatic aberration in the 405–650 nm spectrum, while supporting the reliable acquisition and measurement of colocalisation images with superior positional accuracy. The objective also compensates for chromatic aberration through near infrared of up to 850 nm, making it an optimal choice for near infrared fluorescence observation.

**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 PLAPON 60xOSC and UPLSAPO 60xO**

<table>
<thead>
<tr>
<th>Objective</th>
<th>Axial chromatic aberration (Z-direction)</th>
<th>Lateral chromatic aberration (X-Y direction)</th>
<th>3D image</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLAPON 60xOSC</td>
<td>Approx. 6.1 µm</td>
<td>Approx. 6.1 µm</td>
<td>Tubulin in Ptk2 cells labelled with two colors</td>
</tr>
<tr>
<td>UPLSAPO 60xO</td>
<td>Approx. 0.5 µm</td>
<td>Approx. 0.5 µm</td>
<td>Tubulin in Ptk2 cells labelled with two colors</td>
</tr>
</tbody>
</table>

**Maintain high-precision focus through extended time-lapse imaging**

**IX3-ZDC compensation system**

The IX3-ZDC Z-drift compensator offers a range of functions for autofocusing.

The IX3-ZDC uses low phototoxicity 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 Z-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 instant focusing on samples.

**Rigidity**

Tackle the conflicting requirements of expandability and rigidity with the IX3-ZDC. A Z-drive guide installed near the revolving nosepiece combines high thermal rigidity with the stability of a wrap-around structure to significantly reduce the impact of heat and vibration and improve the quality of time-lapse imaging. Integration with the IX3-ZDC Z-drift compensator permits imaging without focus drift or misalignment, even through temperature changes due to the addition of reagents or a perfusion device. Furthermore, combination with a motorised stage that enables multipoint registration makes high-precision multipoint time-lapse imaging possible.
USER-FRIENDLY SOFTWARE TO SUPPORT YOUR RESEARCH

Image acquisition by application
User-friendly icons offer quick access to functions, for image acquisition according to the application (XYZ, XYT, XYZT, XYZA, XY).  

Time controller
Precisely synchronises different experimental protocols including FRAP, FLIP and FRET through acceptor photobleaching and time lapse. Save and reopen settings for later use.

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

Dark application skin
The use of the dark application skin mode minimises the influence of the screen brightness for the imaging process.

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

Wide choice of scanning modes
Several scanning modes available, including ROI, point and high-speed bi-directional scanning.

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

Applications

MULTI-DIMENSIONAL TIME LAPSE

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 Motorised XY stage and IX3-ZDC Z-drift compensator.

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

Multi-point time-lapse software
Supports repeated image acquisition from multiple areas in a single microplate well.

Multi-dimensional time lapse imaging
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 Motorised XY stage and IX3-ZDC Z-drift compensator.

 Maintain cell activity over a long period
CO₂ incubator control keeps the environment inside the tissue culture dish completely stable. The environment is maintained precisely at 37 °C, with 90% humidity and 5% CO₂ concentration.

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

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 in order to capture reactions during stimulation. Multi-stimulation software is used to continuously stimulate multiple points with laser light for the simultaneous imaging of the effects of stimulation on the cell.

**FLIP – fluorescence loss in photobleaching**

Fluorescence loss in photobleaching (FLIP) combines imaging with the 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 fluorescently labelled 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 characterise the diffusion speed of the molecule, and the speed of binding and release between the molecule and cell structures.

**Uncaging**

A 405 nm 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 PV1200 captures images of the response with no time delay.

**Multi-stimulation software**

High-speed multipoint scans

Users can designate the number of points on an image for light stimulation. Stimulation timing, duration and intervals can be defined in the magnitude of µs and the user can programme 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 the stimulation of each point assures that neighbouring points will not be excited. This allows the user to observe the reaction of a 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 with 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 arise from the mutual interaction between PKC and cell membrane molecules in cell membranes.

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

FRAP analysis

The Axelrod analytical algorithm is used 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 molecular interactions and concentrations by calculating the diffusion coefficients of molecules within the cell. Diverse analytical methods (RICS/ccRICS, point FCS/point FCCS and FRAP) cover a wide range of molecular sizes and speeds.

RICCS – raster image correlation spectroscopy

Raster image correlation spectroscopy (RICS) is a new method for analyzing the diffusion and binding dynamics of molecules in one complete image. RICS uses a spatial correlation algorithm to calculate diffusion coefficients and the number of molecules in specified regions. Cross correlation RICS (ccRICS) characterizes molecular interactions using fluorescence labeled molecules in two colors.

High-level magnification with high resolution for the broad-scope imaging of large-scale specimens

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

Mosaic imaging for 3D XYZ construction

Composite images are quickly and easily prepared using the stitching function, to form an image over a wide area. 3D construction can also be performed by acquiring images in the X, Y and Z directions. Tiled images can be enlarged in sections without losing resolution. Particularly useful for “connectome” or “brain mapping” or similar projects requiring large-area scanning at high resolution. Tiling functions include true stitching and smoothing options for improved seamless images.

Automated from 3D image acquisition to mosaic imaging

Multi-area time-lapse software automates the process from 3D image acquisition (using the Motorised XY stage) to stitching. The software can be used to easily register wide areas, and the thumbnail display provides a view of the entire image acquired during the mosaic imaging process.
Laser systems
The multi-combiner enables combinations with all of the following diode lasers: 405 nm, 488 nm, 515 nm, 561 nm, 647 nm, 670 nm, and 785 nm. The system can also be equipped with the conventional multiline Ar laser and HeNe(G) laser.

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

Optional upgrade equipment for FV1200
Ultra high-sensitivity detector/GaAsP photomultiplier tubes
Achieves ultra high sensitivity with low noise thanks to the gallium arsenide phosphide (GaAsP) detector and the on-board Peltier cooling system.

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

SIM Scanner
Second dedicated scanner for photostimulation, synchronised to the FV1200 main scanner for simultaneous photostimulation and confocal image acquisition. Independent fibre-optic laser introduction port. Dicromatic mirror within motorised optical port of the scan unit required for introduction of laser into main scanner.

CD® stage top incubator
Precise controls maintain a constant environment within the dish or well plate, controlling temperature, humidity and CO2 concentration. (Manufactured by Terasaki Co., Ltd.)

Motorised XY stage
This motorised stage supports wall plates, 35 mm diameter dishes, and 6-well chambers, and also comes complete with a universal sample holder.

Transmitted light detection unit
External transmitted light photomultiplier detector and 100 W halogen conventional illumination, integrated for both laser scanning and conventional transmitted light nonconfocal DC observation. Motorised exchange between transmitted light illumination and laser detection. Simultaneous multichannel confocal fluorescence image and transmitted DC acquisition enabled.

AN ARRAY OF APPLICATIONS

About ZDC-compatible objectives, contact your Olympus dealer.

* Requires IX83 microscope. For information about ZDC-compatible objectives, contact your Olympus dealer.

** Available in the near future.

Expandability

Access to all dyes and fluorophores.