Olympus FV1000MPE Confocal and Multiphoton Laser Scanning Microscope

Confocal Laser Scanning Microscope singlephoton

A popular mode of optical microscopy in which a focused laser beam is scanned laterally along the x and y axes of a specimen in a raster pattern.
The emitted fluorescence (reflected light signal) is sensed by a photomultiplier tube and displayed in pixels on a computer monitor. The pixel display dimensions are determined by the sampling rate of the electronics and the dimensions of the raster.
Signal photons that are emitted away from the focal plane are blocked by a pinhole aperture located in a plane confocal with the specimen. This technique enables the specimen to be optically sectioned along the z axis.

Microscopy is basically a two-dimensional observation technique while biological samples are three-dimensional, of course. In order to map the entire volume, the specimen can be imaged in layers by moving the focal plane in precise steps through it with motorized microscope z-drives or piezo objective movers.

CLSM with unique SIM Scanner concept incorporates two independent, fully synchronized laser scanners in a single compact design for simultaneous laser light stimulation and high-resolution confocal observation. The new spectral optical detection system of the FV1000 provides superior linear spectral distribution throughout the wavelength range from 400 to 800 nm.

Unmatched 2nm wavelength resolution.

Excitation lasers: 405, 458, 488, 515, 559, 635.

Optical zoom: 1x - 50x in 0.5x increments ((1x - 10x in 0.1 increments).

3 internal PMT (photomultiplier) detectors for fluorescence detection + 1 external PMT detector for transmitted light, 3 laser ports.

External fluorescence light source with motorized shutter, fiber adaptation to optical port of scan unit. Motorized switching between LSM light path and fluorescence illumination.

For further details:
http://www.olympus-europa.com/microscopy/en/microscopy/systems/microscopy_systems.jsp

Simultaneous Laser Light Stimulation and Imaging (SIM Scanner)

The FV1000 incorporates 2 laser scanners in a single compact design for simultaneous confocal fluorescence observation and independent laser light stimulation.
Synchronization of these two functions ensures that rapid cellular reactions that occur during or immediately following stimulation are not overlooked.
Any region of interest can be specified for stimulation and scanning independently, with unrestricted control of variations in timing, duration and intensity. The circular “Tornado” scan provides highly efficient photo-bleaching and photo-activation in contrast to standard raster-scan patterns.

The SIM scanner makes the FV1000 the most suitable confocal microscope for a variety of applications, including FRAP, FLIP, photo-activation, photo-conversion, uncaging, laser ablation and many others.

DIC (differential interference contrast)

Reflected light microscopy is one of the most common techniques applied in the examination of opaque specimens that are usually highly reflective and, therefore, do not absorb or transmit a significant amount of the incident light.

Slopes, valleys, and other discontinuities on the surface of the specimen create optical path differences, which are transformed by reflected light DIC microscopy into amplitude or intensity variations that reveal a topographical profile.
Unlike the situation with transmitted light and semi-transparent phase specimens, the image created in reflected light DIC can often be interpreted as a true three-dimensional representation of the surface geometry, provided a clear distinction can be realized between raised and lowered regions in the specimen.

Spectral detection system for accurate separation of overlapping emission spectrums (spectral unmixing/deconvolution)

The original spectral detection system features two independent spectral detection channels, each configured with a diffraction grating and variable slit for high-resolution wavelength separation and high-speed bandwidth selection.
Accurate spectral unmixing of overlapping fluorescence emissions becomes possible with a linear resolution of 2nm throughout the visible spectrum (400 to 800nm).
Two different spectral deconvolution algorithms, Normal and Blind deconvolution, allow an easy and fast separation of different but overlapping fluorochromes within the specimen.
High-speed spectroscopy can be performed with a maximum speed of 100nm/msec.

Fluorescent probes

Many fluorescent dyes or fluorochromes can specifically interact with certain target molecules, like most nucleic acid stains (DAPI, propidium iodide, ethidium bromide) but often they are covalently linked to other molecules in order to yield specific probes.
In general, fluorescent probes are classified according to their excitation and emission characteristics, as well as their chemical and biological properties. Fluorescent probes belonging to each of the important bio-molecular classes, including nucleic acids, polysaccharides, lipids and proteins have been generated as tools in cell biology, where they fulfill various tasks like detection of binding events, staining, localization and tracing of target structures or measurement of ion concentrations to name just a few. Well known examples are fluorescently labelled antibodies or the biotin-streptavidin system.

Olympus FV1000MPE– Multiphoton Fluorescence Microscopy

Multiphoton fluorescence microscopy is a powerful research tool that combines the advanced optical techniques of laser scanning microscopy with long wavelength multiphoton fluorescence excitation to capture high-resolution, three-dimensional images of specimens tagged with highly specific fluorophores.

The technique features attractive advantages over confocal microscopy for imaging living cells and tissues with three-dimensionally resolved fluorescence imaging. Multiphoton excitation, which occurs only at the focal point of the microscope, minimizes the photo-bleaching and photo-damage that are the ultimate limiting factors in imaging live cells.
This advantage allows investigations on thick living tissue specimens that would not otherwise be possible with conventional imaging techniques.

The system is equipped with an AOM module (shutter) for high-speed control of the laser. Motorized beam-expander optics assures optimal illumination of the objective lens, and special gold-coated mirrors ensure the highest transmission values for the whole system.

MaiTai DeepSee Titanium-sapphire laser (690-1040nm).
Imaging of structures up to 1.1mm in brain slices, up to 350µm in lymph nodes and in-vivo complete mouse-digit.