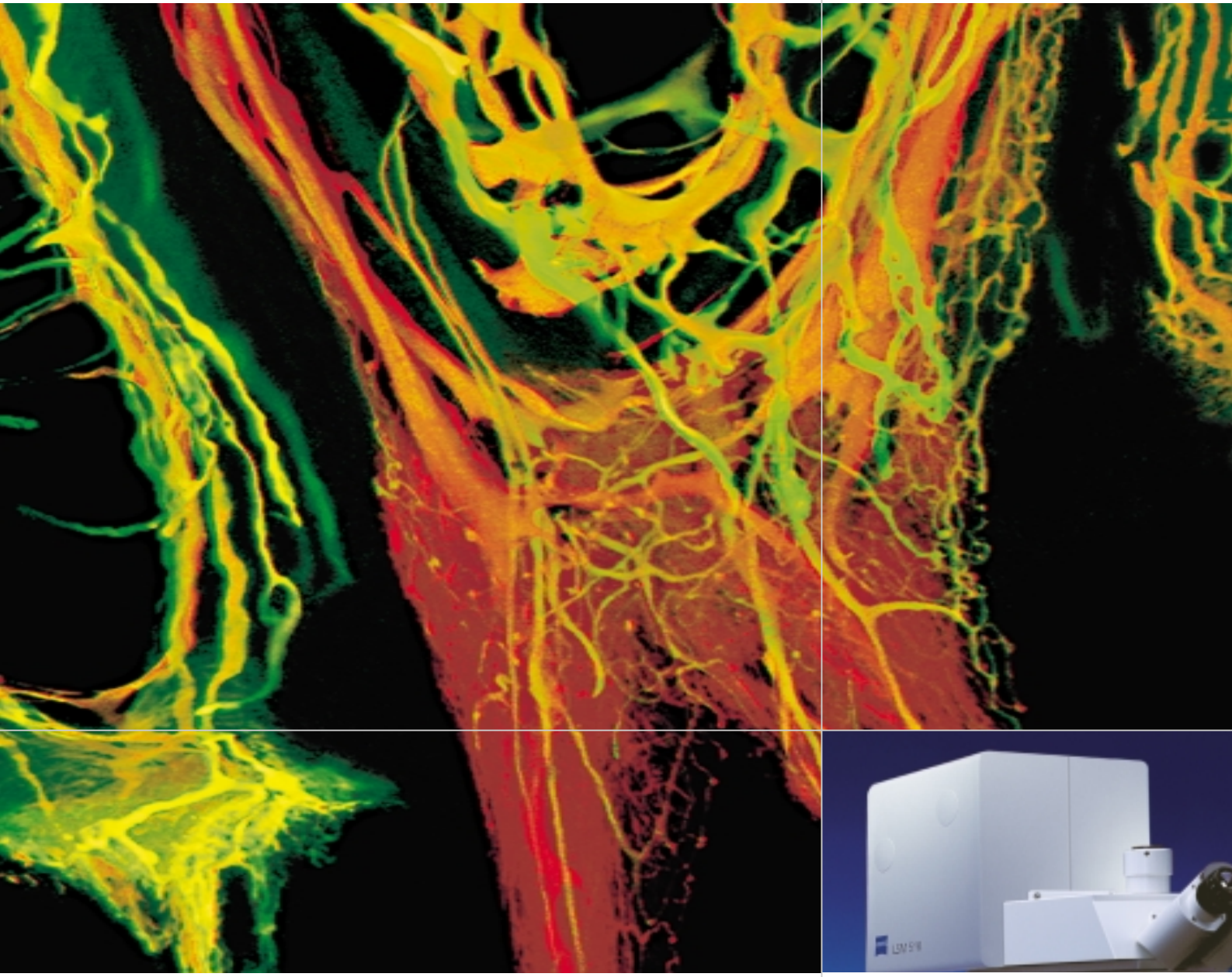
LSM 510

Laser Scanning Microscope



Cellular Dynamics Optimally Detected

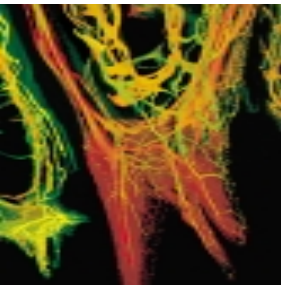
ZEISS

A Perfect Match:
Highly Integrated Scanning Module and Fully Motorized Microscopes

The LSM 510 system comes with the upright Axioplan 2 imaging or Axioskop 2 FS mot, or the inverted Axiovert 200 M, all being absolutely high-end research microscopes. All are fully motorized and can be operated conveniently via the LSM software.

The compact, highly integrated Scanning Module can be attached and removed quickly and easily, allowing problem-free change between the two microscopes and providing optimum conditions for any application. The Axiovert 200 M offers the choice of fitting the Scanning Module either to a side port or to a base port (i.e. below the microscope).

The base port configuration offers maximum freedom on the specimen stage. It is ideal for work with micromanipulators and incubation chambers. Due to the extra-short light path, this configuration is preferable for the detection of extremely faint signals.



Section through a jaw and a tooth of an adult ICR mouse, FITC/TRITC gelatin, projection of 44 optical slices making up a stack of 250 µm thickness. Specimen: Dr. Hashimoto, Dept. of Anatomy, and Dr. Kusakabe, Dept. of Animal Experimental Research, Jikei University, Tokyo, Japan

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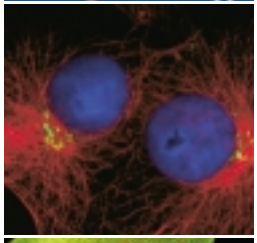

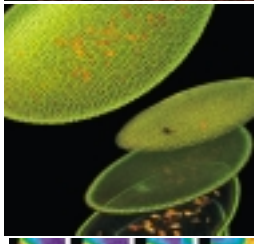

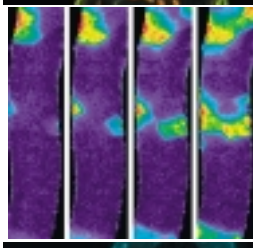

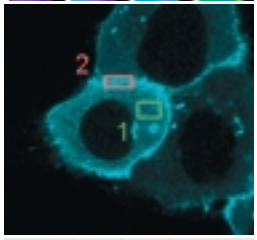

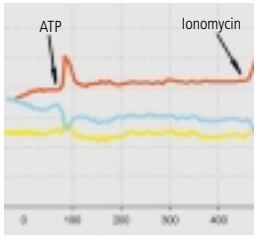

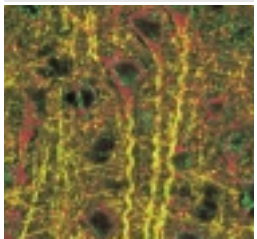

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HAL 100
HAL 100
LSM 510

LSM 510

The Confocal Principle

Maximum Resolution

The excitation light emitted by the laser modules is reflected by a main dichroic beamsplitter and focused into the specimen by the objective.

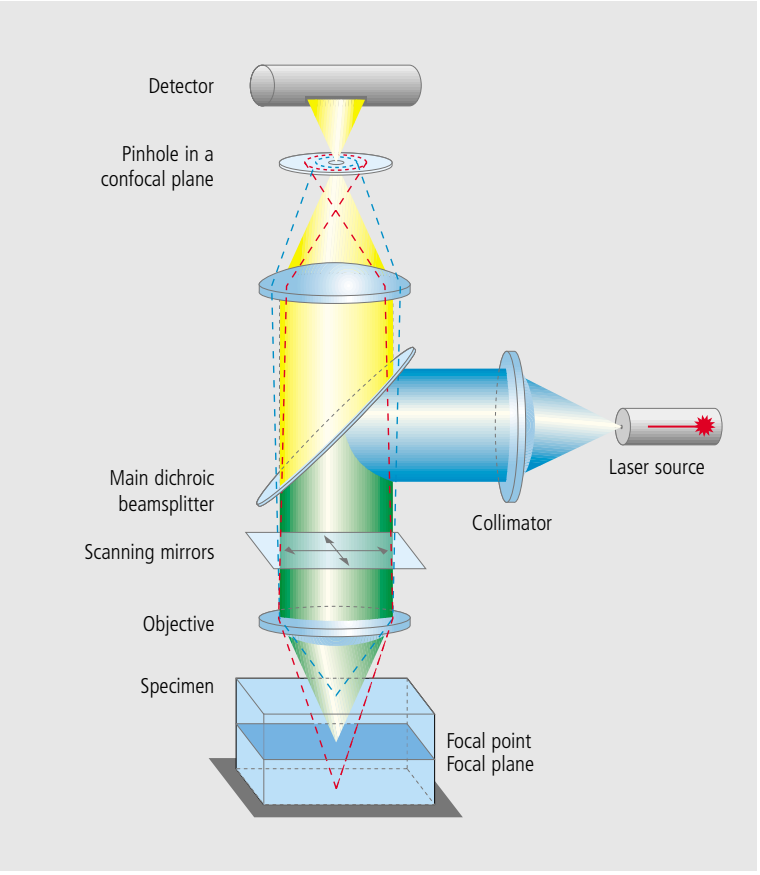
The excitation intensity is greatest at the focal point (see illustration below), yet fluorescent dyes are excited throughout the light cone, emitting fluorescence also above (blue lines) and below (red lines) of the focal plane.

The light returned by the specimen is collected by the objective and focused on to a tiny diaphragm, the pinhole.

As the focal point in the specimen and the pinhole are located in conjugate planes (i.e. they are confocal), light coming from the focal point only can pass the pinhole and be detected by the photomultiplier behind it. Light emitted above (blue lines) or below (red lines) the focal plane is efficiently suppressed by the confocal pinhole. As a result, there is a well-defined image of the focal point that is largely free from scattered light.

Using precisely controlled mirrors, the focused excitation light is scanned through the specimen point by point; the resulting image represents an optical section through the specimen.

Beam path of the confocal laser scanning microscope



Beam path in the LSM 510 Scanning Module (schematic)

1 *Optical fibers conducting laser light*
2 *Collimators*
3 *Beam combiner*
4 *Main dichroic beamsplitter*
5 *Scanner*
6 *Scanning lens*
7 *Secondary dichroic beamsplitter*
8 *Secondary dichroic beamsplitter*
9 *Secondary dichroic beamsplitter*
10 *Pinholes*
11 *Emission filters*
12 *Photomultipliers*
13 *Neutral density filters*
14 *Monitor diode*

The Scanning Module of the LSM 510

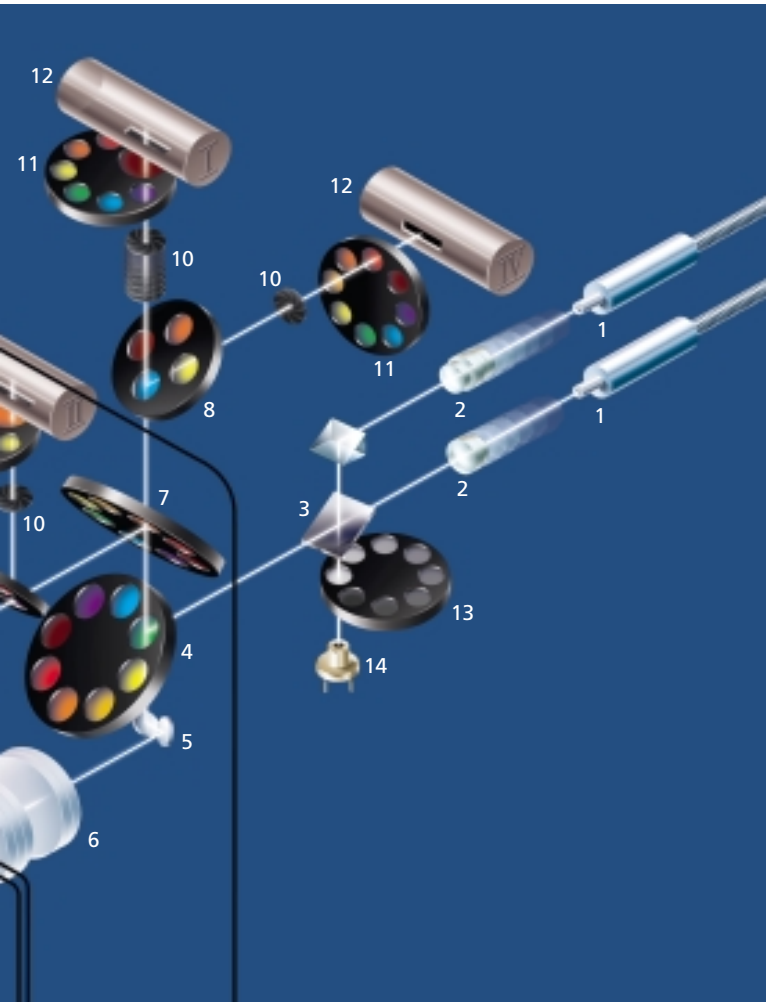
Ingeniously Compact and Efficient

The highly integrated, compact Scanning Module is the core of the LSM 510. Short light paths and optimized components ensure maximum light yield.

The Beam Path

The visible and ultraviolet laser beams emerging from the laser modules are conducted to the Scanning Module by individual optical fibers (1). After passing adjustable collimators (2), the laser beams are combined (3) and reflected by the main dichroic beam splitter (4) on to the scanner (5). The scanner mirrors deflect the beam across the specimen in X and Y; thanks to the highly corrected scanning lens (6), the beam forms a diffraction-limited focal spot. The light reflected, or the fluorescent radiation emitted by the specimen again passes the main dichroic beam splitter (4), after which it can be spectrally separated by three secondary dichroic beam splitters (7, 8, 9). Each of the four confocal channels (I to IV) has its own pinhole diaphragm (10) whose diameter and position can be adjusted individually. Various emission filters (11) precisely defined for all relevant spectral ranges ensure that the highly sensitive photomultiplier tubes (12) detect photons of the desired wavelengths only.

As an option, a small portion of the incident laser light, after beam combination (3), can be reflected on to a monitor diode (14) and attenuated with neutral filters (13) if necessary.



The Scanning Module
is available in configurations with two, three or four confocal channels, designed for the use of different laser lines from ultraviolet to infrared, and equipped with corresponding filter combinations, so that users can select precisely the system needed for their application.

The Collimators
for visible and ultraviolet laser radiation are movable to make the focal planes of the various wavelengths exactly coincide. This is a must for reliable spatial analyses; unless excitations come from one and the same plane, the signals detected also come from different planes.

The Scanner
consists of two symmetrical, independent scanning mirrors. Therefore, the scanning field can be placed anywhere within the field of view and rotated by any angle desired. The LSM 510 thus saves the trouble of rotating the specimen, which is hardly practicable at high magnifications.

The Pinholes
can be adjusted separately for each channel – the only way to achieve maximum image resolution, allowing the user to set the optimum interval between successive optical sections for different wavelengths.

The Monitor Diode
detects the intensity of the excitation light. High-frequency laser intensity fluctuations can thus be corrected in real time.

DSP and AOTF

Flexible Scanning Strategies

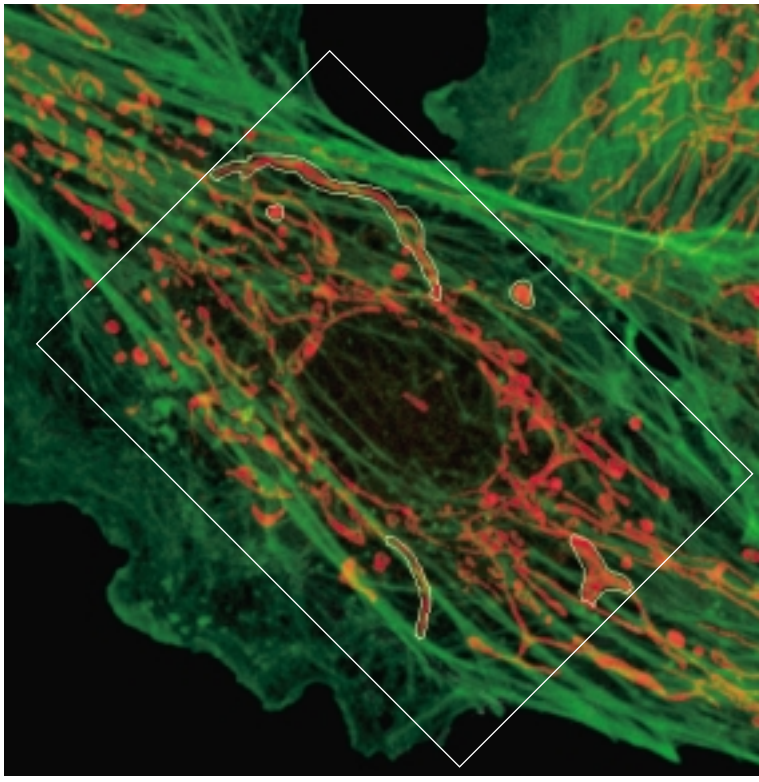
The Digital Signal Processor (DSP) provides almost unlimited possibilities. It controls data acquisition, scanner operation and the acousto-optical tunable filter (AOTF).

Everything is possible

Perform fast line scans. Scan tiniest spots, or large frames approximating the microscope's field of view.
Different scanning speeds, averaging, zoom and offset functions are available for all scanning modes and can be selected with a mouse click. With the two symmetrical scanning mirrors controlled by the DSP, scanning lines and fields can be freely rotated and scaled to fit the frame to the specimen. Moreover, you can even define any number of irregularly shaped "regions of interest" interactively on the screen. Simply use the mouse to mark the structures to be imaged, and let the DSP and the AOTF do the rest.

AOTFs for pinpoint accuracy

The acousto-optic tunable filters incorporated in the LSM 510 not only provide exact control of laser intensity but also turn the laser beam on or off at exactly the right locations, with pinpoint accuracy. The intensity of each laser line can be set in 0.1% increments, i. e. almost continuously. The visible-light AOTF simultaneously controls up to six different, individually adjustable laser lines. The UV AOTF controls two ultraviolet laser lines at a time.



*Bovine endothelium cells;
actin filaments (BODIPY FL),
mitochondria (MitoTracker Red);
some mitochondria are marked
by user-definable regions of interest.*

*Fluorescent gel;
rotated scanning fields and lines,
and freely defined regions of interest
show the performance capabilities
of DSP and AOTF.*

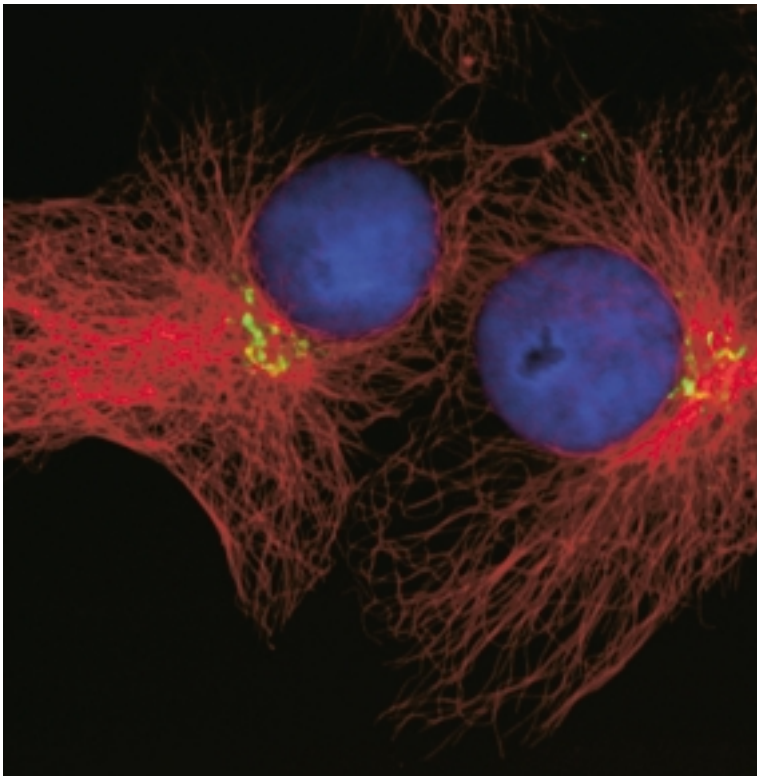
Four Confocal Incident-Light Channels plus Transmitted-Light Channel

Best Reception

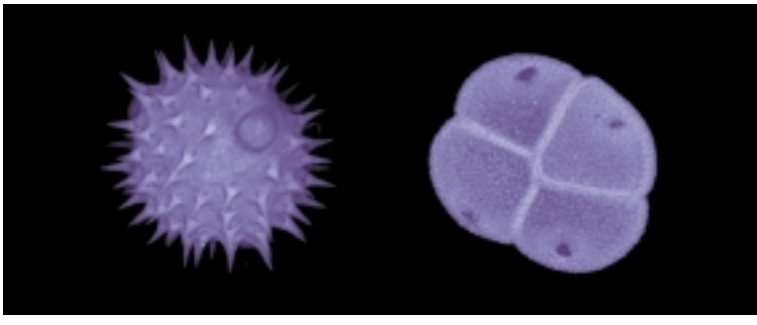
Whether fluorescence, reflection or transmission – the LSM 510 provides the right solution to every application.

Multifluorescence or reflection – simply perfect

Up to four simultaneous confocal detection channels are available for fluorescence or reflected light observations. Each channel is equipped with a photomultiplier that responds with high sensitivity to the entire spectrum, and a separate pinhole with individual diameter and XY adjustments. The pinhole of channel 1 can be adjusted along Z, to allow perfect compensation at any time of the inevitable chromatic difference of focus between ultraviolet, visible and infrared light. In short, each pinhole can be given the optimum setting for any emission wavelength. The pinholes are fully motorized and controlled conveniently through the software. With a mouse click you can activate an automatic adjustment program.

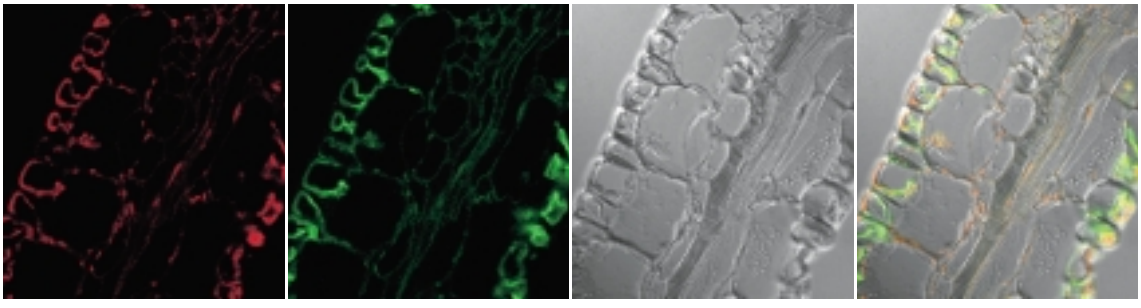


L929 cells, triple fluorescence. Specimen: Dr. Pepperkok, EMBL Heidelberg, Germany



Cereal pollen grains, reflected-light image, projection of a stack of 127 optical slices

Leaf tissue with vascular bundle, double fluorescence and differential interference contrast (DIC)



Multitracking for Clear Separation

Unique Capabilities

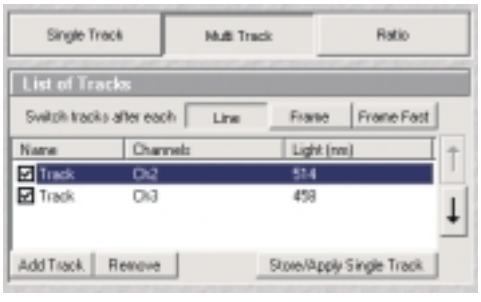
The risk of crosstalk between channels increases with the number of dyes used in an experiment. This is a critical problem especially in colocalization studies. Frame- or linewise, Multitracking is an elegant solution with a high degree of certainty.

Unique capabilities

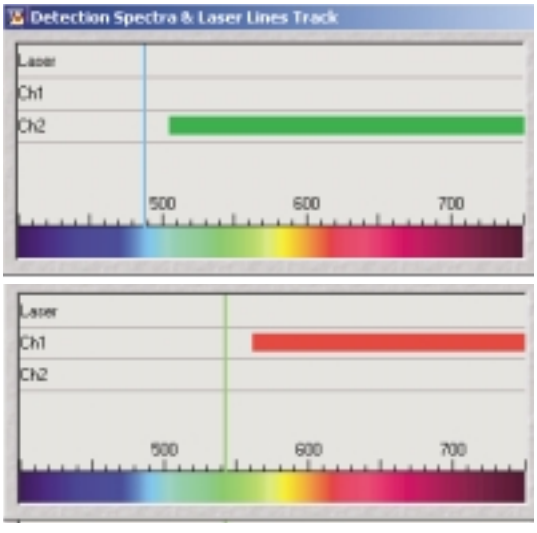
The LSM 510 allows the user to define illuminating parameters and detection settings for the respective dye in several tracks. A list of such tracks can be stored in Multitracking and used for the automatic acquisition of multifluorescence images. Images from up to eight channels can thus be captured in a single run. The DSP-controlled AOTF efficiently blanks and unblanks the laser lines between the tracks. Due to the selective excitation and detection of the dyes, signal crosstalk is prevented reliably.

Multitracking is also beneficial in the case of very faint signals. Here, the researcher can now use long-pass emission filters to utilize the entire emission spectrum of the dyes. The pinhole diameter can be set individually for each track to match the signal intensity. This allows optimum adjustment to different dye intensities. As an added advantage in critical colocalization studies, it is possible to adapt optical slice thickness.

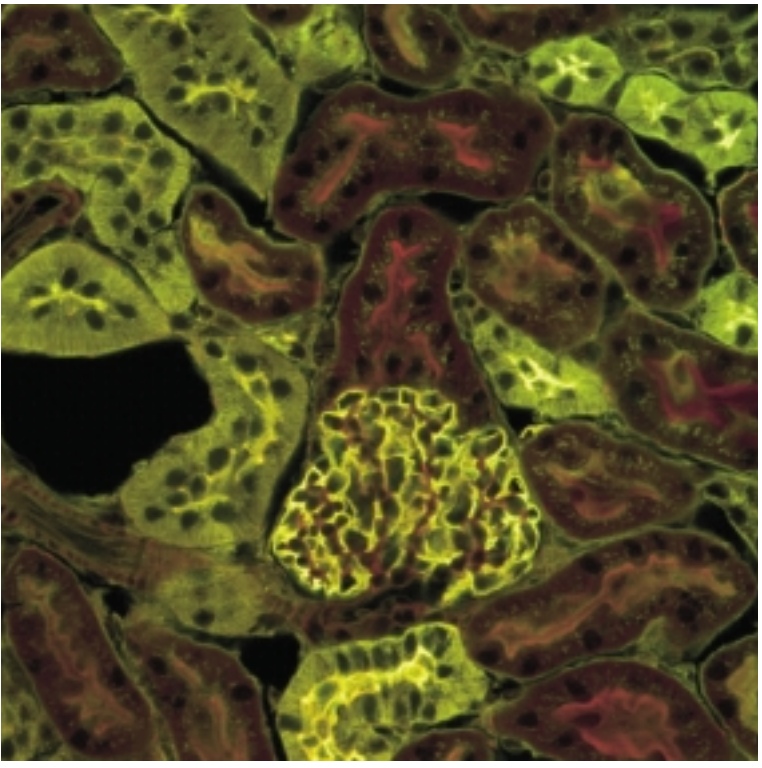
Setting up a Multitracking routine is easy as pie: Select the laser lines, main dichroic beamsplitters and emission filters for each channel in the Configuration Control dialog window. Once compiled, a list of tracks can be saved and activated later whenever needed.

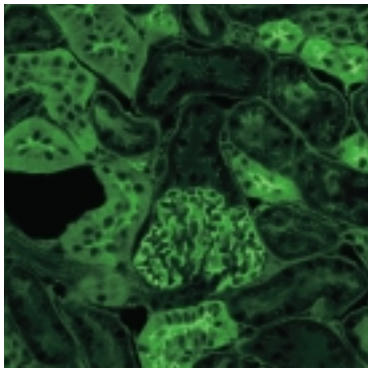


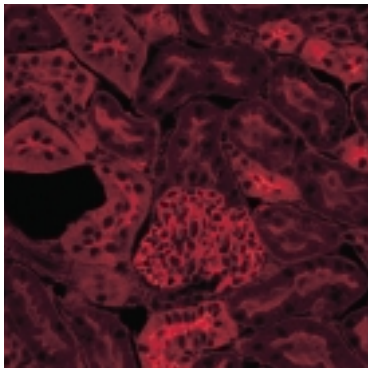
The Spectra function allows the selected laser lines and filters to be displayed in a clear overview. Example of a configuration for detecting Cy2 (green) and Alexa 568 (red) as a Multitrack with long-pass filters.



Kidney of a mouse, labelled with Alexa Fluor 488 WGA (green) and Alexa Fluor 568 phalloidin (red). Specimen: Molecular Probes, Netherlands

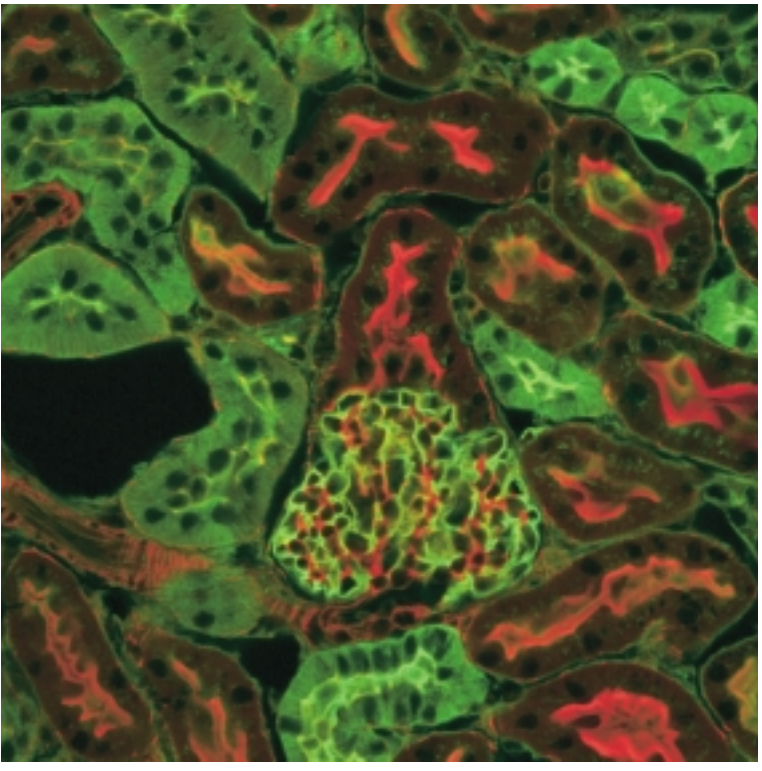


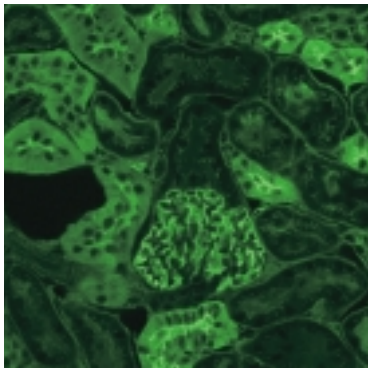


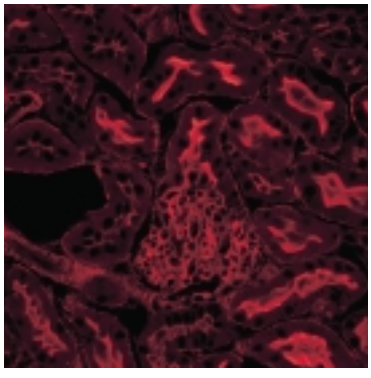


The simultaneous recording of Alexa Fluor 488 and 568 shows strong bleed-through of the Alexa 488 signal (middle) into the Alexa 568 channel (right).

Superposition of the images obtained from the two channels clearly illustrates the difference: Simultaneous recording results in massive signal crosstalk, which wrongly suggests a colocalization (yellow).







The same specimen recorded by Multitracking (with the same laser intensity). The channels are clearly separated without any bleed-through.

With Multitracking, the signals from the various channels are clearly separated, and optimum signal yield is guaranteed.

The Third Dimension

Controlled Coordinates

One of the main tasks of confocal microscopy is the three-dimensional analysis of a specimen. By means of pinholes acting as confocal diaphragms, optical sections are produced, which are free from stray light and can be combined into a projection image that is in focus at all levels.

Optical sections (slices) through a Drosophila embryo, and projection of a stack of 47 slice images.

One pinhole often is not enough

For correct three-dimensional measurements, individually adjustable pinholes offer big benefits: The thickness of an optical section (slice) is a function of the pinhole diameter and the light wavelength. With a single pinhole for all channels, the signals detected at the various wavelengths come from specimen slices of different thicknesses. Three-dimensional reconstructions would result in misleading spatial relationships. Only with channels having individual pinholes it is possible to precisely match the optical slice thickness to every wavelength. With the LSM 510 software, this matching is particularly easy: All relevant data are automatically converted for the resultant slice thickness, displayed in a graph, and optimized at the touch of a button.

Instantaneous preview

The Preview function of the LSM 510 is a valuable time saver. You can change parameters interactively and see the results immediately. And as soon as you see the very thing you are after, have the software compute the complete set of data.

Display of optical slice thicknesses for different wavelengths, with identical pinhole diameters.

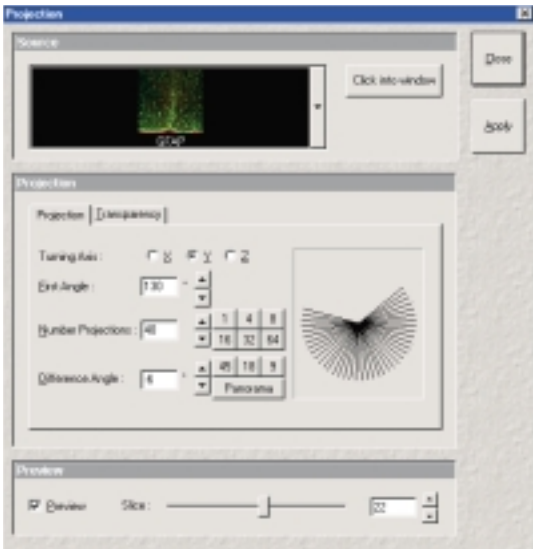
Image analysis with 3D for LSM, Image VisArt and Deconvolution

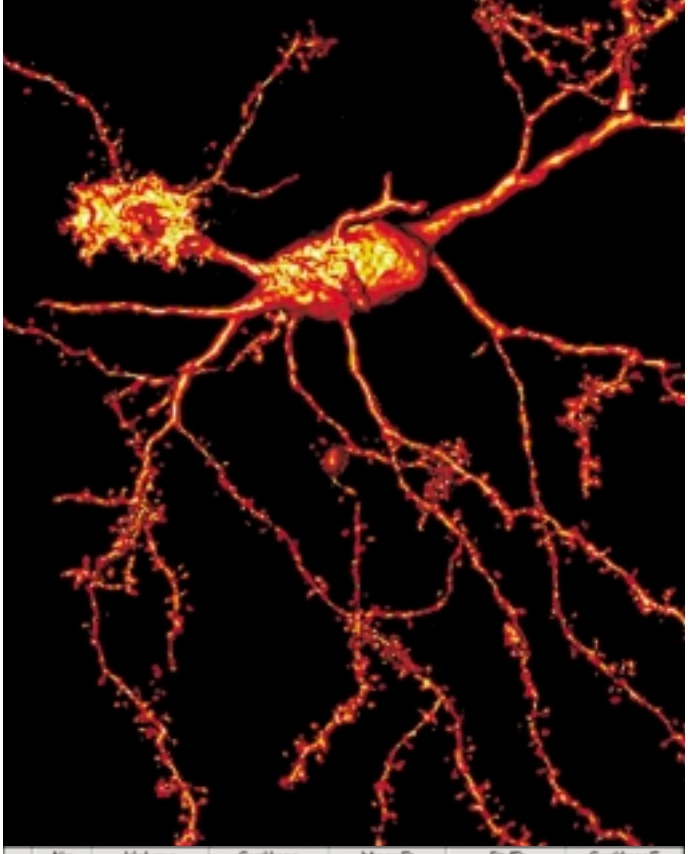
If you want still greater analyzing power from your LSM 510, use one of the optional LSM software packages. Specially tailored to the capabilities of the system, they provide the following options:

– Automatic measurement functions in the 3D space, for the quantitative analysis of image data such as volumes, areas, diameters, mean gray level etc.
– 3D reconstructions of image data from freely selectable viewing angles by means of surface and alpha rendering
– Processing of image sequences with up to eight data channels
– 3D Deconvolution

The 3D programs share the overall system's ease of operation. For orientation, and for comparison of results with input data, all image processing steps are presented in a gallery. With the tool bar and the menu commands provided, you will obtain reliable results quickly and conveniently.

"Projection" function window. Viewing angle, number of projections and transparency conditions are freely selectable, and a quick "Preview" of the result is possible.



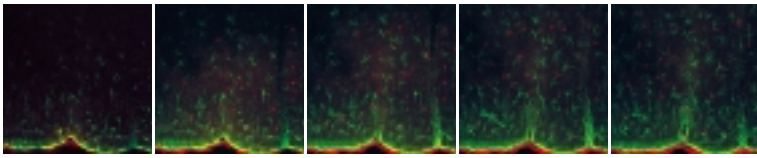


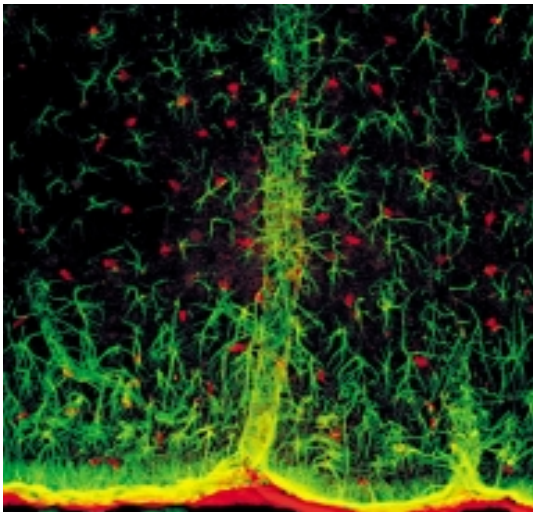
No	Volume micrometer^3	SurfArea micrometer^2	MeanD grey	StdD grey	SurfAreaF micrometer^2
1	23921.92	116807.29	48.39	50.96	115655.50

VolCount	VolVolume micrometer^3	VolVolumeP %	VolSurfArea micrometer^2
1	23921.92	4.34	116807.29

Table showing the quantitative results of a segmentation

The slices of a stack in gallery presentation





Section through a rat's brain; pial surface with blood vessel, glia-fibrillary acid protein (Cy2), glutathione peroxidase (Cy3). Specimen: Dr. Bidmon, Institute of Brain Research, University of Düsseldorf, Germany

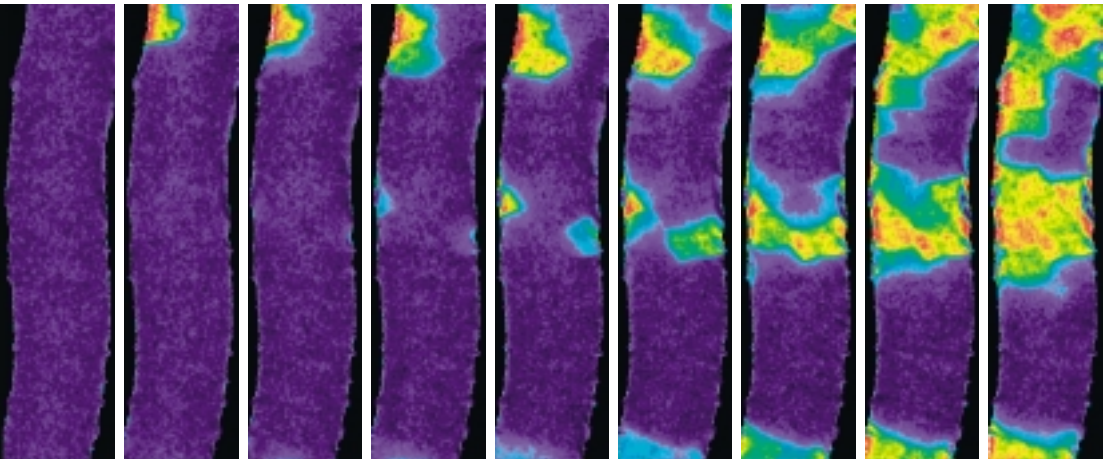
The Physiology Software

Fast Processes Analyzed with Certainty

In physiological examinations, the superb advantages of the LSM 510 are particularly obvious. This is mainly due to the system's extremely fast and efficient scanning modes. On-line ratio calculations permit direct data display while image acquisition is still running. To make this possible, the system uses preset analysis formulae. Various modes are available for the calibration of dyes in concentration analyses. With these performance features, the LSM 510 is suitable for every dye and its specific fluorescence properties.

Display and analysis of ion concentrations:

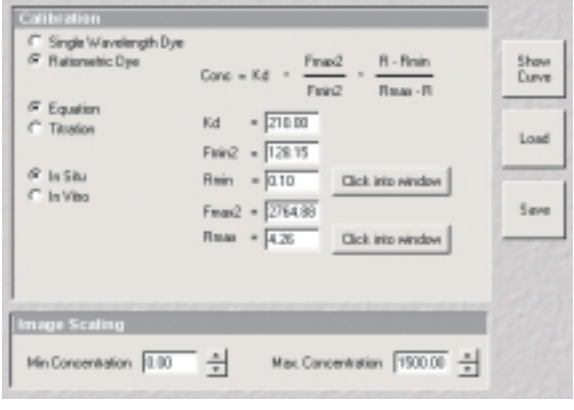
On-line and off-line ratio for ratiometric dyes
On-line and off-line F/F_0 for single-wavelength dyes
Calibration for single-wavelength and ratiometric dyes • in situ and in vitro • including background correction • after titration with various curve fittings • according to Grynkiewicz
Interactive scaling of image data series
Interactive graphic display of the measured data from ROIs

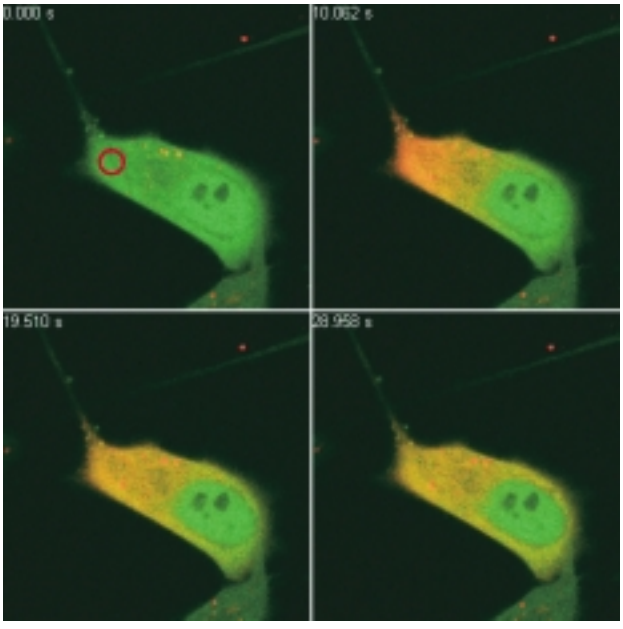


Hormone-induced calcium changes in the salivary gland of an insect, visualized with Fluo-4. Specimen: Dr. B. Zimmermann, Dr. B. Walz, University of Potsdam, Germany

Time series can be configured freely and include the option to define in/out control triggers in a convenient menu.

Software dialog for the interactive calibration of ion-sensitive dyes



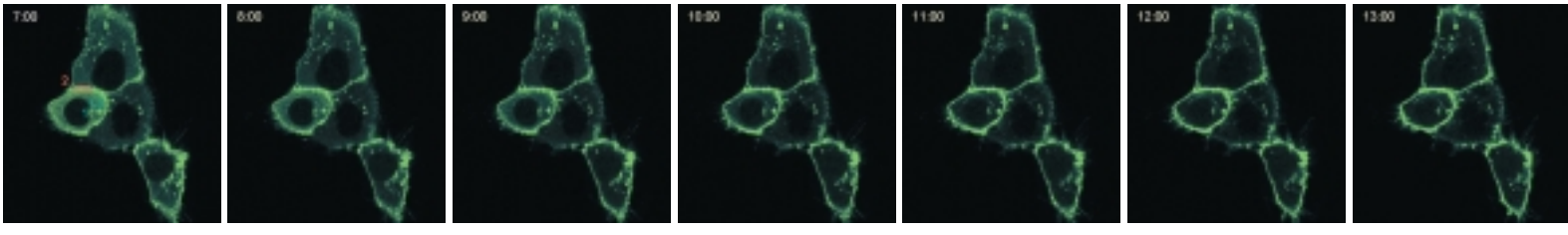


Local activation of the "Kaede" marker (Japanese: maple leaf -> color change green/red) in cultured cells. Initially, Kaede is activated in the selected ROI at the top of the cell only (laser pulse 405 nm), but then distributed by transport throughout the cytosol. Other parts of the cell and neighboring cells remain unaffected.

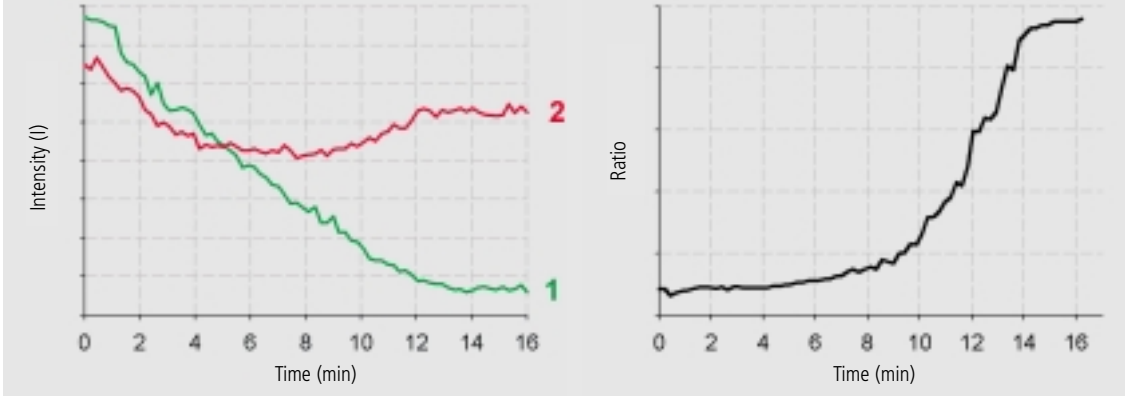
By freely defining Regions of Interest (ROIs), you can have the system examine the details of the specimen you are interested in. During image acquisition, either the series of images or the intensity curves in the ROIs, or both, can be displayed.

Investigating protein movements

Time lapse series of an experiment in HeLa cells transfected with PKC-GFP. Stimulation of the cells with PMA at the time t=1 causes a redistribution of PKC from the cytosol to the plasma membrane. Specimen: Dr. S. Yamamoto, Hamamatsu Medical University, Japan



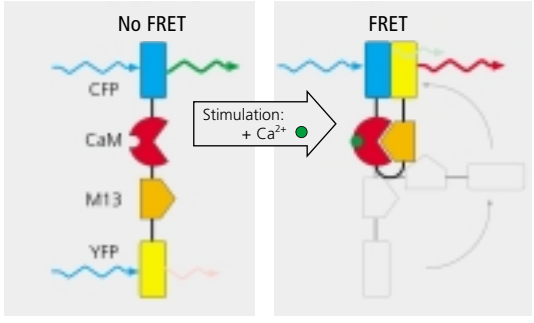
The individual intensities (left) and the ratio of intensities in the two ROIs marked above (right), plotted in corresponding colors.



FRET and FRAP

Molecular Interactions Made Visible

FRET (Fluorescence Resonance Energy Transfer) is an analytical method to investigate and quantify protein-protein interactions. Two proteins of interest are marked with different fluorescent dyes, with the emission wavelength of one dye (the donor) overlapping the excitation wavelength of the other (the acceptor). If the two molecules are spaced closely enough (<10 nm), the donor transfers its energy to the acceptor without any emission, whereas the acceptor is set off to emit light which can be detected.

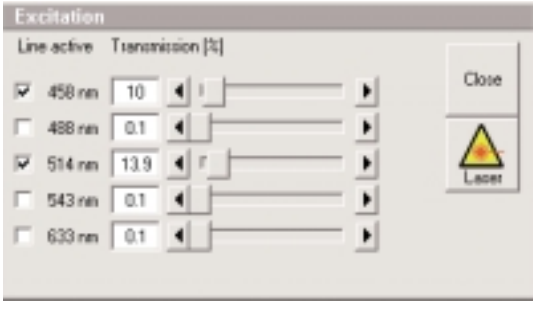


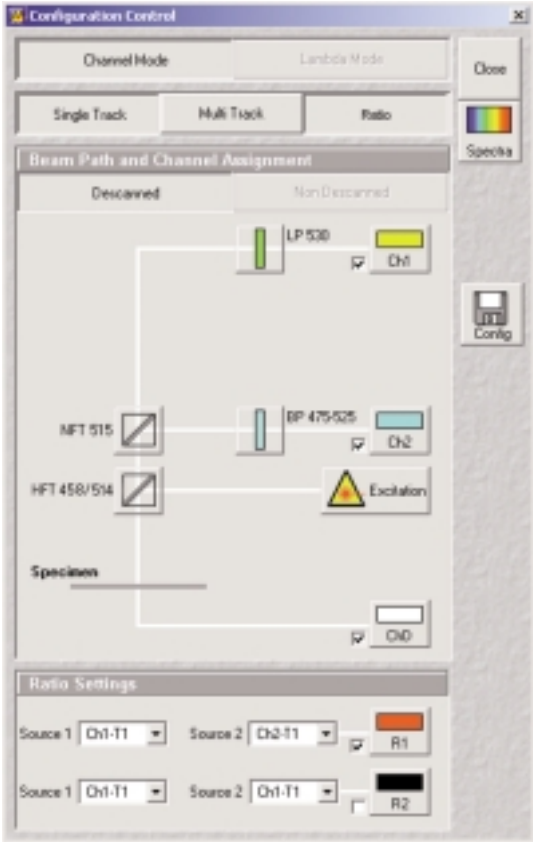
CaM: Calmodulin
M13: Calmodulin-binding domain

The experiment: Calcium imaging using the FRET indicator Yellow Cameleon 2

The LSM 510 with its many hardware and software functions is an ideal environment for FRET studies. This refers not only to the broad range of laser lines to choose from for optimum dye excitation but also to specially matched dichroic beamsplitters and emission filters for best possible detection.

Through separately defined channels, the user can keep track of ratio measurements on the screen in real time.

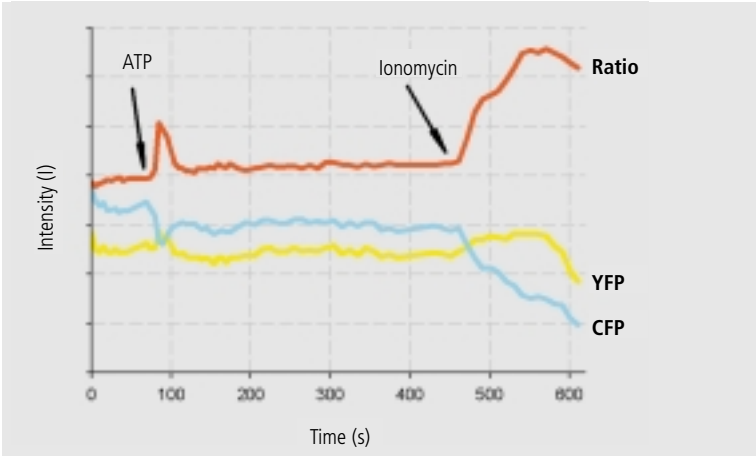




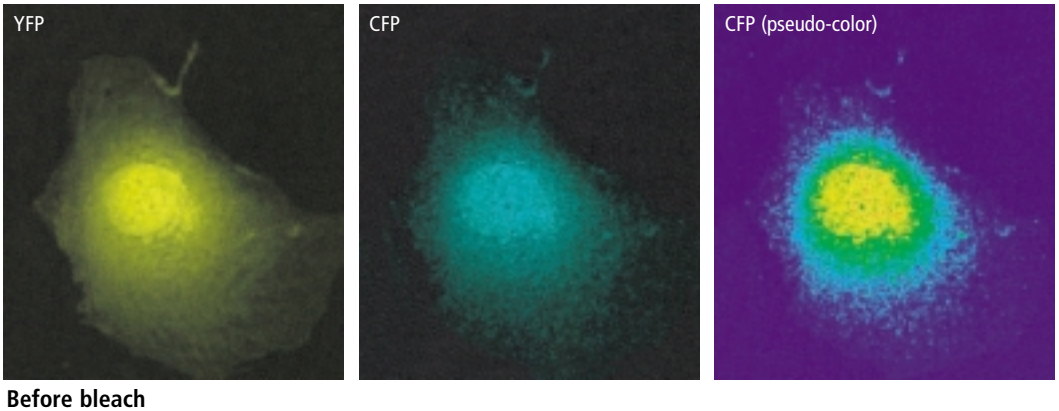
Possible beam path configuration for the simultaneous detection of CFP and YFP for FRET investigations. Continuous variation of laser intensities by means of an AOTF. One or two ratio channels can be defined in addition, and the result displayed during the measurement.

For some time now, FRET method has been established also as a sensor system for investigating enzyme activities, ion concentrations and interactions between messenger substances in cells. One of the FRET sensors used to detect changes in intracellular calcium concentration is Yellow Cameleon 2.

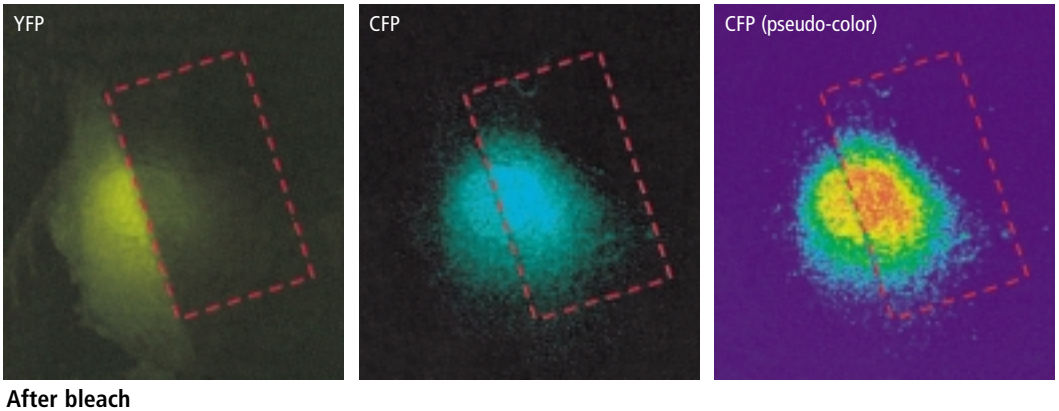
The time series function, optionally combined with Multitracking, allows complete FRET experiment runs to be configured conveniently and reproducibly. An elegant and well-established way of detecting FRET is known as acceptor bleaching, in which the operator selects a certain region within the specimen, and eliminates acceptor fluorescence with high laser intensity (FRAP).



Intensity changes of Yellow Cameleon 2 as a reaction to physiological stimuli in a FRET time series experiment.



Before bleach



After bleach

The acceptor-bleach method demonstrated with CFP/YFP. As a proof of FRET, the signal intensity of the donor (CFP) is increasing when the acceptor (YFP) is locally bleached (red box).
Specimen:
B. Giese, G. Müller-Newen, RWTH-Aachen, Germany

Quantitative Colocalization

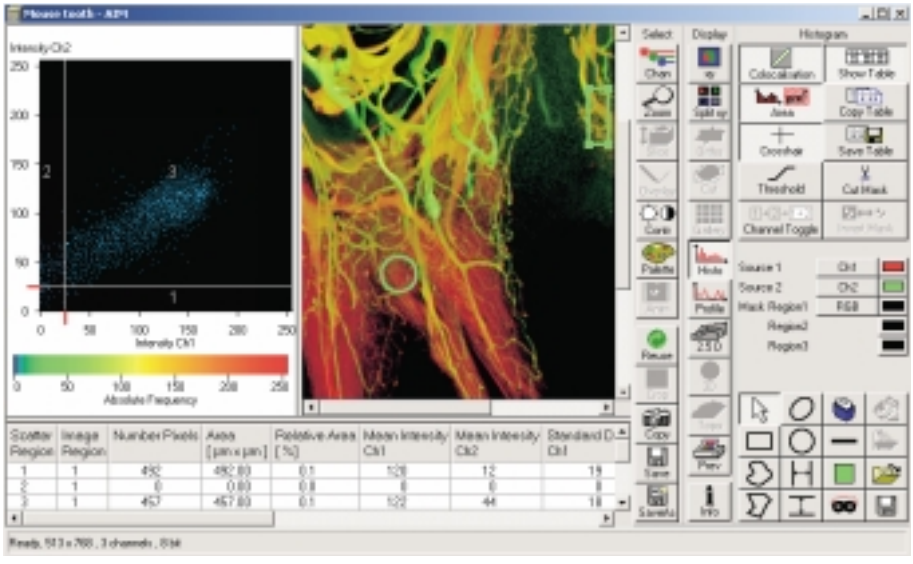
Finding the Needle in the Haystack

The LSM 510 enables you to easily perform quantitative colocalization analysis with a reliability and precision never achieved before. Image display, scattergram and data table are interactively linked to the ROI and thresholding tools.

For example, select an area in the scattergram, and the existence of colocalizations will be shown immediately. Data table, histogram and image are interlinked in the same way. Data analysis can hardly be any more intuitive and precise.

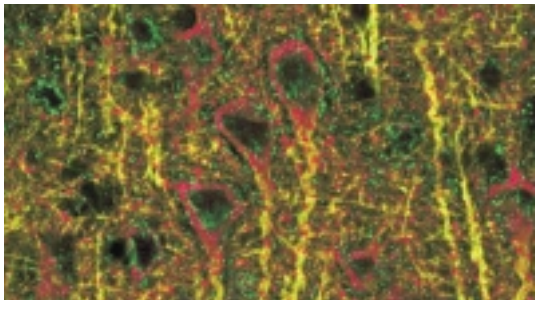
Display and analysis of colocalization experiments:

Interactively linked image displays, scattergrams and data tables
Interactive or automated determination of thresholds
Overlay of image channels with results of the colocalization analysis
Quantitative colocalization analysis for up to 99 ROIs, with: • area and average gray level intensity • colocalization degree • colocalization coefficient • Pearson's correlation coefficient • Manders' overlap coefficient
Export of analysis results



Use first-class tools correctly:
Image display, scattergram and data table are interactively linked to the ROI and thresholding tools.

Conventional qualitative (color-coded) colocalization analysis is often misleading in complex specimens. Only quantitative tools (see screenshot on the left) make things clear. Cerebral cortex of a rat: mitochondria and microtubuli. Specimen: Dr. J. Lindenau, Institute of Medical Neurobiology, Magdeburg University, Germany



Software with Expert and Routine Modes

Ingeniously Simple

The LSM software controls the microscope, the scanning module, the lasers, the AOTFs, the DSP and the computer. Thanks to its ingenious architecture, the complex interplay of all components remains hidden below the neatly organized user interface, which can be operated with straightforward intuition.

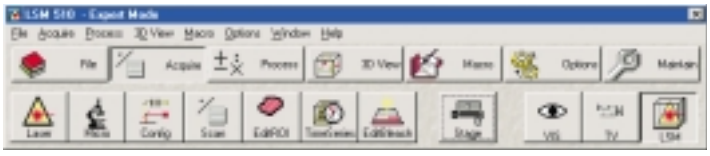
Simply ingenious

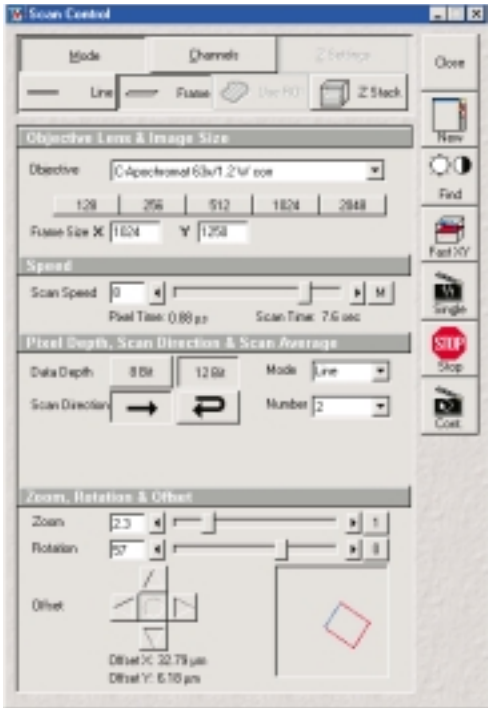
In the Expert Mode, users can avail themselves of the full scope of LSM 510 capabilities. This means almost unlimited freedom in the selection of functions and parameters, so that optimum results can be achieved, no matter how difficult or complex the application. Many auxiliary functions provide support and convenience: The Find function automatically identifies suitable contrast and brightness settings; Z intervals and pinhole diameters are optimized with a mouse click; the ReUse function reactivates all settings originally used for recording an image; pinhole adjustment can be automated, etc.

The LSM 510 is a genuine multi-user system. Every user may define and save their individual configurations optimally suited to specific applications in the Routine Mode.

The system allows any number of images to be acquired and displayed on the screen simultaneously without intermediate storage. You can directly compare the images before deciding which of them to save. You can choose between many export formats for the convenient transfer of images to other programs. To export measured data and tables to other programs, simply use the Copy and Paste commands.

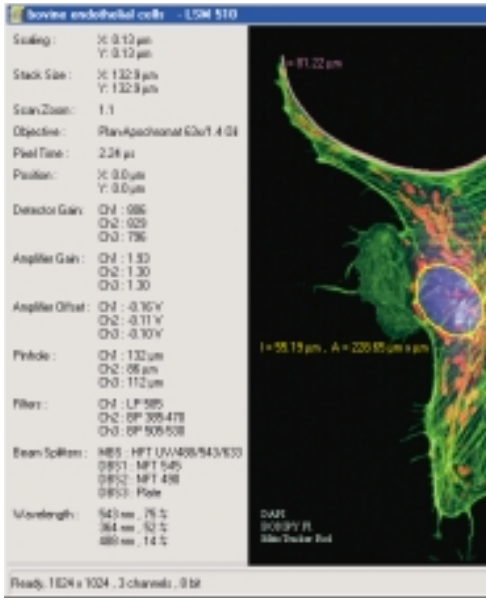
Main menu of the LSM 510 Expert Mode with the "Acquire" submenu activated, which contains all functions for recording new images.





"Scan Control" function window. Here you can define scanning parameters such as resolution, speed, zoom, offset and rotation of the scanning field, 8 or 12 bit digitization, photomultiplier sensitivity, pinhole diameters, laser line intensities and many others.

For maximum reproducibility, all relevant data (pinhole settings, the objective used, the zoom factor, the configuration of the beam path including the intensities of the various laser lines, the color rendition selected) are saved automatically in a database together with the images.



Bovine endothelium cells: nucleus (DAPI), F-actin (BODIPY-FL), mitochondria (MitoTracker Red); activation of several functions of the image menu.

Specification
LSM 510 System Components

Microscopes

Models	Upright: Axioplan 2 imaging mot, Axioskop 2 FS mot Inverted: Axiovert 200 M BP (base port) or SP (side port)
Z drive	DC motor with optoelectronic coding, smallest increment 25 or 50 nm; fast piezo focusing attachment
HRZ 200 (option)	High-precision galvanometric fine focusing stage, total lift 200 µm, smallest increment < 10 nm
XY stage (option)	Motorized XY scanning stage with Mark & Find and Tile Scan (Mosaic Scan) functions, smallest increment 1 nm
Accessories	AxioCam digital microscope camera, integration of incubation chambers, micromanipulators, etc.

Scanning Module

Models	Various models with two, three or four confocal channels, prepared for lasers from UV to NIR
Scanner	Two independent galvanometric scanning mirrors, DSP-controlled, providing ultrafast line and frame flyback
Scanning resolution	1 x 4 to 2048 x 2048 pixels, also for several channels, continuously adjustable
Scanning speed	13 x 2 speed stages, up to 5 frames/s with 512 x 512 pixels (max. 77 frames/s with 512 x 32 pixels), 0.38 ms per line of 512 pixels
Scanning zoom	0.7 x to 40 x, variable in digital increments of 0.1
Scanning rotation	Free 360° rotation, variable in increments of 1°, free XY offset
Scanning field	Homogeneously illuminated field of 18 mm diagonal (max.) in the primary image plane
Pinholes	Preadjusted pinholes for all epi-illumination channels, each with individual size and position adjustments
Detection	Simultaneous for up to four confocal epi-illumination channels, each with a highly sensitive photomultiplier tube; one optional transmitted-light channel with photomultiplier tube; one optional monitor diode for measuring the excitation intensity
Data depth	8 bit or 12 bit; one individual 12-bit A/D converter per channel

Laser Modules

VIS laser module	Polarization-preserving single-mode fiber, thermally stabilized visual-light AOTF (acousto-optic tunable filter) for simultaneous intensity control of up to six visible laser lines, switching time < 5 µs, AOTF reprogramming via LSM software. Diode laser (405 nm), 25 mW; Ar laser (458, 477, 488, 514 nm), 30 mW; ArKr laser (488, 568 nm), 30 mW; HeNe laser (543 nm), 1 mW; HeNe laser (633 nm), 5 mW (end-of-lifetime specification)
UV laser module	Polarization-preserving single-mode fiber, thermally stabilized UV AOTF (acousto-optic tunable filter) for simultaneous intensity control of two ultraviolet laser lines, switching time < 5 µs. Ar laser (351, 364 nm), 80 mW; optional Ar laser (413 nm), 40 mW (end-of-lifetime specification)
Multiphoton option	Direct or fiber coupling of pulsed NIR lasers into the scanning module; various makes are supported. Grating Dispersion Compensator (GDC) and Post Fiber Compressor (PFC) for optimum pulse shaping. Fast change of laser intensity by means of an Acousto-Optic Modulator (AOM). Up to 4 external detectors for Non-Descanned Detection (NDD). Objectives optimized for use in the NIR range.

Electronics Module

LSM 510 Control	Controls the microscope, the VIS and UV laser modules, the scanning module, and further accessories; monitors data acquisition via a digital signal processor (DSP); controls data exchange between DSP and computer via an ultrawide SCSI
Computer	High-end PC with ample RAM and hard disk storage capacity; ergonomic high-resolution monitor or TFT flat-panel display, many accessories; Windows 2000/NT 4.0 operating system with multi-user capability

Standard Software

System configuration	Convenient control and configuration of all motorized microscope functions, and of the laser and scanning modules; saving and restoration of application-specific configurations
ReUse function	Restoration of acquisition parameters per mouse click
Acquisition modes	Spot, Line/Spline, Frame, Z Stack, Time series and combinations: XY, XYZ, XYT, XYZT, XZ, XT, XZT, Spot-T On-line computation and presentation of ratio images Averaging and summation (linewise or framewise, configurable) Step Scan (for higher frame rates, configurable)
Auto-Z function	On-line adaptation of Z Stack acquisition parameters for uniform brightness distribution
Crop function	Convenient selection of scanning ranges (simultaneous zoom, offset and rotation)
RealROI scan	Scanning of up to 99 ROIs (regions of interest) of any shape, with pixel-accurate laser blanking
ROI bleach	Localized photobleaching of up to 99 bleaching ROIs for applications such as FRAP (Fluorescence Recovery After Photobleaching) or uncaging
Spline scan	Scanning along a freehand defined line
Multitracking	Acquisition of multiple fluorescences with fast change of excitation lines to minimize signal crosstalk
Presentation	Orthogonal view (XY, XZ, YZ in a single presentation); cut view (3D section made under a freely definable spatial angle); 2.5D view for time lapse series of line scans Projections (stereo, maximum, transparency) for single frames and series (animations) Depth coding (pseudo-color presentation of height information) Brightness and contrast adjustments; off-line interpolation for Z stacks Selection and modification of color lookup tables (LUTs) Drawing functions for documentation
Analysis	Advanced tools for colocalization and histogram analysis with individual parameters and options Profile measurement of straight lines and curves of any shape Measurement of lengths, angles, areas, intensities, etc.
Image operations	Addition, subtraction, multiplication, division, ratio, shift, filters (low-pass, median, high-pass, etc., user-definable)
Data archiving, export, import	LSM image database with convenient functions for managing images together with their acquisition parameters Multiprint function for creating assembled image and data views More than 20 file formats (TIF, BMP, JPG, PSD, PCX, GIF, AVI, Quicktime, ...) for compatibility with all common image processing programs

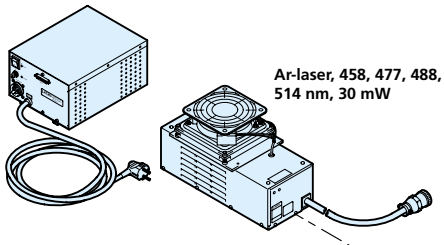
Software Options

LSM Image VisArt	Fast 3D and 4D reconstruction and animation (various modes: shadow projection, transparency projection, surface rendering)
3D Deconvolution	Image restoration based on computed point spread functions (modes: nearest neighbor, maximum likelihood, constrained iterative)
Multiple Time Series	Complex time lapse series with change of application-specific configurations, autofocus and bleaching functions
3D for LSM	3D presentation and measurement of volume data records
Physiology package	Extensive software for the analysis of time lapse series, graphical mean-of-ROI analysis, on-line and off-line calibration of ion concentrations
Topography package	Visualization of 3D surfaces (fast rendering modes), plus many measurement functions (roughness, surface area, volume)
VBA Macro Editor	Recording and editing of routines for the automation of scanning and analysis functions

Image Browser

Free software package for display, editing, sorting, printing, exporting and importing LSM 5 images

LSM 510 System Overview

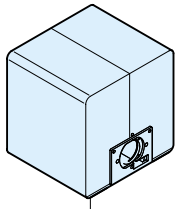


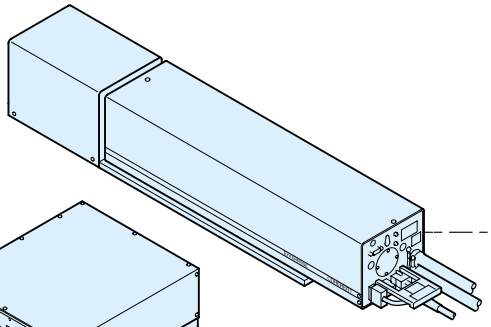
Ar-laser, 458, 477, 488, 514 nm, 30 mW

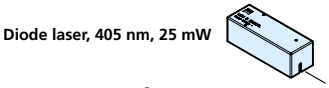
NLO kit for fiber coupling

NLO kit for direct coupling

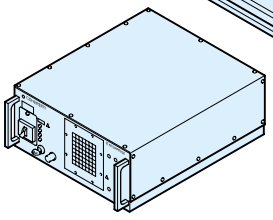
VIS Scan module LSM 510
VIS/UV Scan module LSM 510
VIS/NLO Scan module LSM 510
VIS/405 Scan module LSM 510





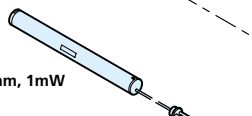


Diode laser, 405 nm, 25 mW

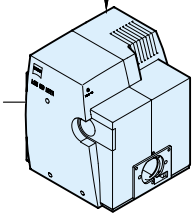


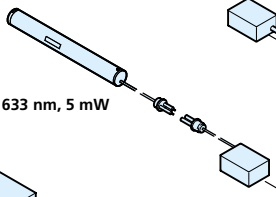
Laser Enterprise II 653 (80 mW, 351, 364 nm)
Laser module kit

Upgrade kits LSM 510 to LSM 510 META

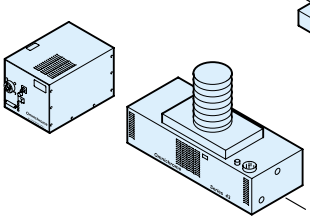


HeNe laser, 543 nm, 1mW

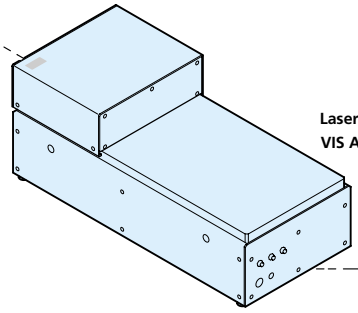




HeNe laser, 633 nm, 5 mW



Ar-Kr-laser, 488, 568 nm, 30 mW

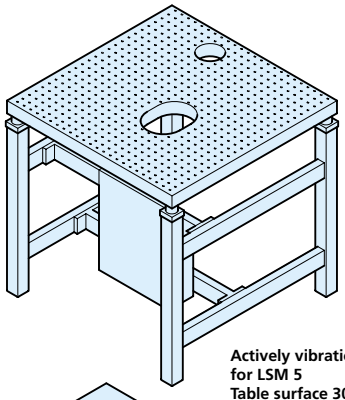


Laser module VIS
VIS AOTF (6 channels)

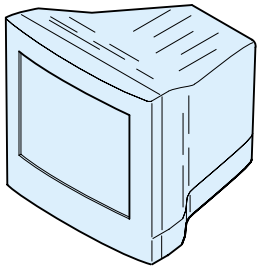
VIS Scan module LSM 510 META
VIS/UV Scan module LSM 510 META
VIS/NLO Scan module LSM 510 META
VIS/405 Scan module LSM 510 META

Fiber decoupling, channel 4

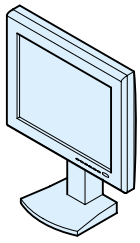
Options:
-Fluorescence Lifetime Imaging Microscopy (FLIM)
-Fluorescence Correlation Spectroscopy (FCS)



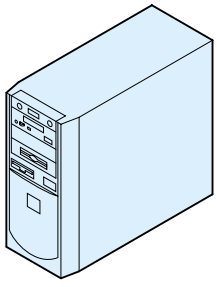
Actively vibration-absorbing system table
for LSM 5
Table surface 30" x 30"



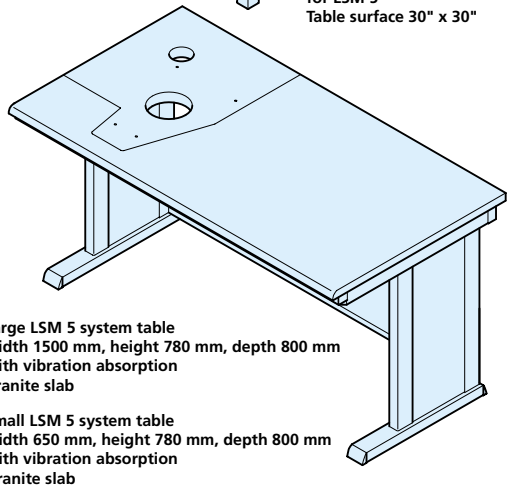
Monitor 21" (50 cm)



LCD TFT-Flatscreen 18"

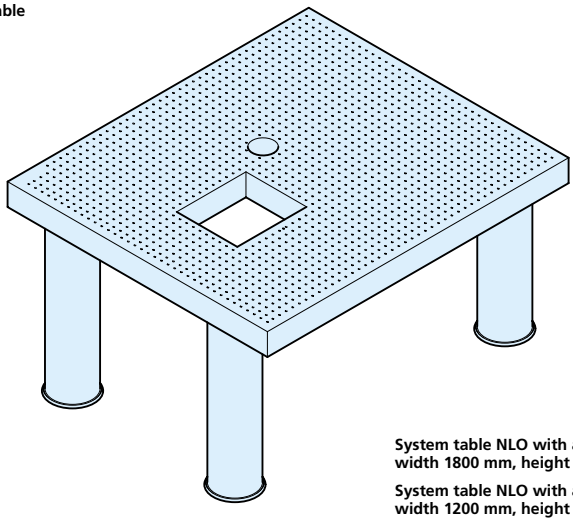


Host computer



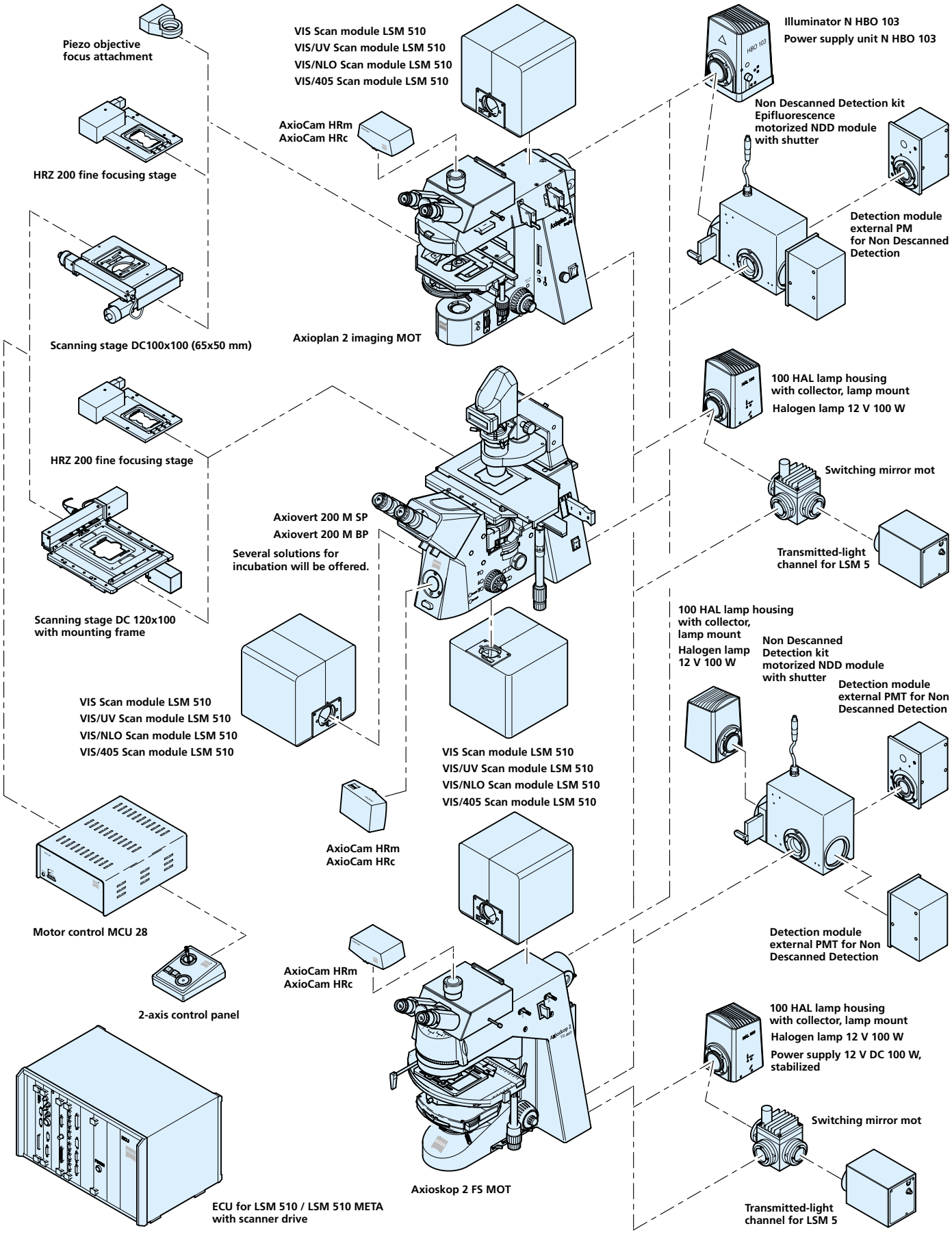
Large LSM 5 system table
width 1500 mm, height 780 mm, depth 800 mm
with vibration absorption
Granite slab

Small LSM 5 system table
width 650 mm, height 780 mm, depth 800 mm
with vibration absorption
Granite slab



System table NLO with active absorption
width 1800 mm, height 750 mm, depth 1400 mm

System table NLO with active absorption
width 1200 mm, height 750 mm, depth 1400 mm


Piezo objective
focus attachment
HRZ 200 fine focusing stage
Scanning stage DC100x100 (65x50 mm)
VIS Scan module LSM 510
VIS/UV Scan module LSM 510
VIS/NLO Scan module LSM 510
VIS/405 Scan module LSM 510
AxioCam HRm
AxioCam HRc
Axioplan 2 imaging MOT
Illuminator N HBO 103
Power supply unit N HBO 103
Non Descanned Detection kit
Epifluorescence
motorized NDD module
with shutter
Detection module
external PM
for Non Descanned
Detection
HRZ 200 fine focusing stage
Scanning stage DC 120x100
with mounting frame
Axiovert 200 M SP
Axiovert 200 M BP
Several solutions for
incubation will be offered.
100 HAL lamp housing
with collector, lamp mount
Halogen lamp 12 V 100 W
Switching mirror mot
Transmitted-light
channel for LSM 5
VIS Scan module LSM 510
VIS/UV Scan module LSM 510
VIS/NLO Scan module LSM 510
VIS/405 Scan module LSM 510
AxioCam HRm
AxioCam HRc
100 HAL lamp housing
with collector,
lamp mount
Halogen lamp
12 V 100 W
Non Descanned
Detection kit
motorized NDD module
with shutter
Detection module
external PMT for Non
Descanned Detection
Motor control MCU 28
2-axis control panel
AxioCam HRm
AxioCam HRc
Detection module
external PMT for Non
Descanned Detection
100 HAL lamp housing
with collector, lamp mount
Halogen lamp 12 V 100 W
Power supply 12 V DC 100 W,
stabilized
Switching mirror mot
Transmitted-light
channel for LSM 5
Axioskop 2 FS MOT
ECU for LSM 510 / LSM 510 META
with scanner drive

Our Services

Thanks to many years of experience in the development of laser scanning microscopes, we are able to offer you a system with perfectly matched components that can be combined and extended. You can profit from the application-oriented design of the fifth generation of laser scanning microscopes from Carl Zeiss.

LSM 510 systems already installed can easily be upgraded with the new spectral detection module into the LSM 510 META at the customer's site.

The existing optical, mechanical and electronic interfaces enable step-by-step upgrading for further techniques, for example the measurement of molecule interactions with FCS (Fluorescence Correlation Spectroscopy), multiphoton microscopy or FLIM (Fluorescence Lifetime Imaging).

New scanning and analysis techniques are made available quickly and easily via software upgrades.

Our experts are continuously developing new software and hardware modules to meet your challenging application requirements. Over the past two decades, your applications expertise, combined with our know-how in scientific instrument design, have helped us to transform the laser scanning microscope from a 3D imaging device into a very versatile and flexible imaging and analysis center.

This makes the LSM 510 a rewarding long-term investment.

Professional support

The laser scanning system you purchase should be tailored to the range of your applications. Especially in a multi-user environment, making the right decision is a complex task, with many different requirements to be matched.

Our LSM team specialists are familiar with all available confocal systems, including components from other manufacturers. They can help you **to select the right system.**

We are committed to supporting you in your efforts **with specific advice on applications and technology** for your examination methods.

Reliable service

To ensure smooth operation of your LSM 510, we offer you the following services:

Our regional consultants and technicians will provide reliable services and technical support to assist you in your research.

After every system installation you are offered **a comprehensive introduction to LSM applications.**

Furthermore, Carl Zeiss offers **training courses and workshops,** which provide in-depth know-how about practical topics and applications in laser scanning microscopy.

1. *Primary culture. Fura Red and Fluo-3.*
   Specimen: Bitplane, Zurich, Switzerland

2. *Acinous pancreas. Fluorescence and transmitted light.*
   Specimen: Dr. Nietschke, Freiburg, Germany

3. *Drosophila embryo, early stadium.*
   Double fluorescence: FITC (green) marks actin filaments, PI (red) RNA.
   Specimen: Dr. E. Schejter, Tel Aviv, Israel

4. *3T3 cell culture. TMRE, calcium green.*
   Specimen: Dr. Sparagna, UCT, Farmington, USA

5. *Cytokeratins and desmoplacin.*
   Specimen: Dr. Kartenberg, Heidelberg, Germany

6. *Cell walls and chloroplasts. Double fluorescence.*

7. *Cell nuclei, cytokeratins and desmoplacin.*
   Specimen: Dr. Kartenberg, Heidelberg, Germany

8. *Drosophila embryo, cytoskeleton.*
   Specimen: Dr. Zhang, Duke University, Durham, USA

Glossary and Functions

AOTF Acousto Optical Tunable Filter

CFP Cyan Fluorescent Protein

DIC Differential Interference Contrast (Nomarski)

DSP Digital Signal Processor

FCS Fluorescence Correlation Spectroscopy

FLIM Fluorescence Lifetime Imaging Microscopy

FRAP Fluorescence Recovery After Photobleaching

FRET Fluorescence Resonance Energy Transfer

GFP Green Fluorescent Protein

NLO Non-Linear Optics (multiphoton imaging)

ROI Region of Interest

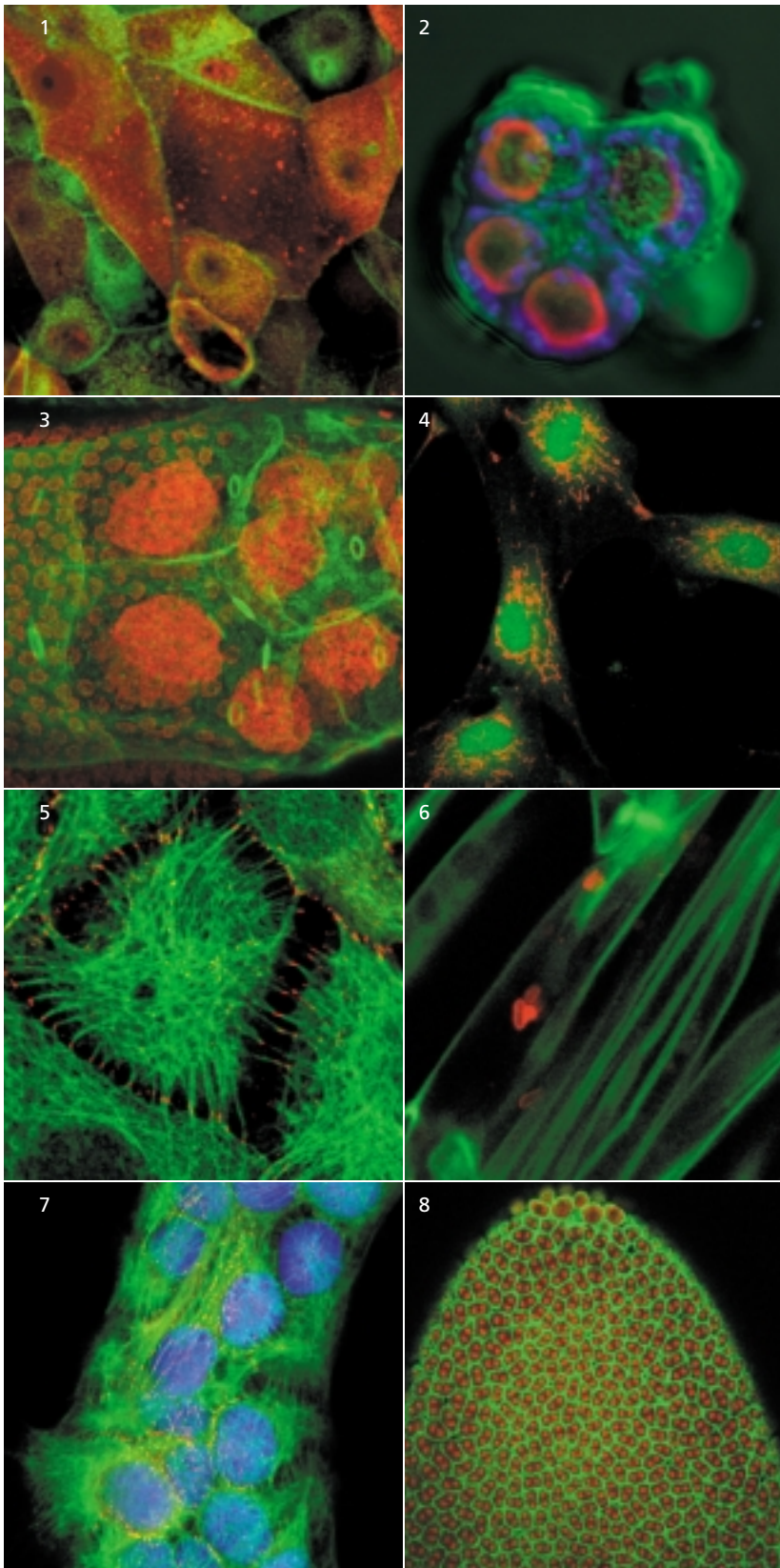
YFP Yellow Fluorescent Protein

Multitracking
Scanning mode that generates multi-fluorescence images without crosstalk of emission signals by means of fast switching between excitations.

ROI Scan & Bleach
Scanning and photobleaching of freely definable specimen areas, e.g., for FRAP, uncaging or photoactivation experiments.

Spline / Step / Tile Scan
Flexible scanning modes: freehand-defined line (Spline), interpolation of lines (Step), overview mosaic (Tile).

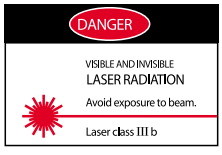
Brilliant Images: Laser-sharp



Designed and made by the pioneer of laser scanning microscopy, the **LSM 510** is

- the result of more than 150 years of innovation in optics and 20 years of experience in all relevant areas of laser scanning microscopy,
- the mature synthesis of confocal microscopy and the high-performance, extensively motorized Axioplan 2 imaging, Axioskop 2 FS mot and Axiovert 200 M research microscopes.

Everything from Carl Zeiss.



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