LSM 510\textsuperscript{Mk4} and LSM 510 META\textsuperscript{Mk4}
Laser Scanning Microscopes

Microscopy from Carl Zeiss

Your First Choice
in Cell Biology Research

We make it visible.
Mark 1 as the basic version of the LSM 510 was introduced in 1997 as the first confocal system to be designed as a very compact but versatile cube with full motorization, up to four fluorescence channels, individual pinholes, exchangeable emission filters and six-channel AOTF.

Mark 2 of 1998–2000 introduced improvements like programmable DSP for ROI scanning and bleaching, fast 77 frames per second, intelligent scan modes such as spline scan and the LSM 510 NLO (multiphoton) and ConfoCor 2 combi (Fluorescence Correlation Spectroscopy) extensions.

Mark 3 version from 2001–2004 added the first-ever spectral confocal, the LSM 510 META. Included was intelligent software such as automatic component extraction and improved sensitivity with 1250 V high-gain PMTs. In addition, Mark 3 offered a broader choice of lasers, including modern diode versions e.g. for 405 or 561 nm.

Mark 4 as the current version of the LSM 510 is characterised by extended capabilities of a realtime electronics to control multiple scan systems and imaging modes. This provides state-of-the-art solutions like LSM 5 DUO, LSM 510 DuoScan or the brand-new ConfoCor 3. The Mark 4 also offers increased excitation flexibility for the ever-growing range of fluorescent proteins.

As with its forerunners, the LSM 510 Mk4 version is an extremely powerful, up-to-date confocal system that will meet the scientific community’s demands for many years to come.
Whichever member of the LSM 510 family you choose, you can be sure of the best possible solution for your LSM application.
LSM 510
Perfect 3D Multifluorescence
A solution for users who require a less opulent yet universal Laser Scanning Microscope.

LSM 510 META
Instant Spectral Imaging
Undoubtedly the leading light in the LSM 510 range.

LSM 510 NLO
In-depth Insights
Especially designed for non-linear optics, i.e. multiphoton imaging.

LSM 510 ConfoCor 3
Single Molecule Detection
The perfect solution for single molecule fluorescence correlation spectroscopy.
LSM 510 DuoScan
Intracellular Analysis

Ideal for photomanipulation techniques such as FRET or FRAP.
The advantage of confocal light microscopy is that it captures the light reflected or emitted by a single plane of a specimen.

A pinhole conjugated to the focal plane obstructs the light coming from objects outside that plane so that only light from in-focus objects can reach the detector. A laser beam scans the specimen pixel by pixel and line by line. The pixel data are then assembled to form an image that represents an optical section through the specimen and is distinguished by high contrast and high resolution in X, Y and Z. Several images generated with the focal plane shifted in small steps can be combined into a 3D image stack, which is then available for digital processing.
3D Visualization

You can enjoy entirely new insights into the spatial structures of a specimen through the extensive 3D visualization modes offered by the LSM 5 Image VisArt software package. Fast 3D and 4D reconstruction functionality and a variety of projection and animation options give you an entirely new understanding of interrelationships – for research and training purposes. For even higher resolution levels you can use the deconvolution functions implemented on the basis of calculated point spread functions (nearest neighbor, maximum likelihood and constraint iterative).

Multifluorescence

If you want to optimize your multifluorescence analysis, the LSM 510 META gives you the unique possibility of combining the META detector with other single detectors. This enables you to configure the spectral range of the META detector as required, and at the same time maximize the signal yield via the single detector. The fact that the pinholes of each detector can also be individually adjusted and positioned offers you an easy way of perfecting each and every experiment.
System Components
Quite Simply a Perfect Match

The basic design of the LSM 510 META system is unsurpassed in the way it implements the principle of confocal microscopy. As a result, multicolor images can be captured without compromising resolution and efficiency.

Microscopes
Every LSM 510 META is based on a ZEISS high-performance research microscope. Which instrument you choose – the upright Axio Imager.Z1, the inverted Axio Observer.Z1 or the fixed stage Axioskop 2 FS MOT – depends on your specific applications. All of them are equipped with IC’S optics, unsurpassed for image quality, and fully supported with great precision by the LSM software.

Objectives
IC’S objectives from Carl Zeiss are highly regarded for the excellence of their performance and come in a wide range of types and specifications. Simply select the objectives that provide the best possible combination of resolving power, aperture, working distance and correction for your specific applications.

Laser Module
The LSM 510 is equipped with different lasers emitting a number of lines in the UV and visible spectral ranges for excitation of fluorescent dyes and proteins. It is also possible to use direct-coupled tunable short-pulse lasers for multiphoton excitation. The excitation light is precisely controlled down to a single pixel by means of an acousto-optical tunable filter (AOTF) or an acousto-optical modulator (AOM). This ensures the best possible specimen preservation and makes targeted photobleaching possible, e.g. for FRAP and FLIP experiments.

Scanning Module
A unique scanning module is the core component of the LSM 510. It contains motor-driven collimators, scanning mirrors, individually adjustable and positionable pinholes, and highly sensitive detectors (including the META detector). A highly efficient optical grating provides an innovative way of separating the fluorescent emissions onto the 32 channels of the META detector. This enables the spectral signature to be acquired for each pixel of the scanned image.

Control Computer and Software
The easy-to-use LSM software enables you to control all system components. The Windows-based operating system provides multitasking capability and easy linking to existing computer networks. All components have been carefully selected and tested. The high-performance graphics card with OpenGL capability ensures fast presentation of 2D and 3D graphics and animations.

Electronics Module
The LSM 510 is controlled by realtime electronics. This results in fast and flexible synchronization of the scanners, the AOTF and the detectors. It also enables sophisticated functions such as fast multitracking, spot scan, spline scan, or ROI scan and bleaching for FRAP, uncaging and photoactivation.
The LSM 510 is the perfect match of a highly integrated scanning module and a fully motorized high-end research microscope – either with the upright Axio Imager or Axioskop 2 FS mot, or the inverted Axio Observer. Each of these microscopes is easy to operate with the LSM software.

The compact, highly integrated scanning module can easily be attached and removed quickly to allow for a trouble-free change from one microscope to another. This ensures you enjoy optimum working conditions in any application. The Axio Observer allows the scanning module to be fitted to a side or base port (i.e. below the microscope) – the choice is yours. The base port configuration offers a maximum amount of freedom on the specimen stage. It is ideal for working with micro-manipulators and incubation chambers. This configuration is also preferable for detecting extremely faint signals because of its ultrashort light path.
Four Incident-Light Channels
Looking Good in Any Light

Perfect Results for Multifluorescence and Reflection

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 offering individual diameter and XY adjustments. The pinhole of channel 1 can be adjusted along Z so that the inevitable chromatic difference of focus between ultraviolet, visible and infrared light can be perfectly compensated for at any time. In short, each pinhole can be adjusted to ensure the optimum setting for any emission wavelength. The pinholes are fully motorized and can be easily controlled using the LSM software. The automatic adjustment program can be activated at the click of a mouse.

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

Leaf tissue with vascular bundle, double fluorescence and differential interference contrast (DIC)
Crosstalk is the most frequent problem occurring in multifluorescence imaging. Whereas it is easy to avoid emission crosstalk of dyes by means of selective excitation with the ZEISS multitracking function, it used to be virtually impossible to use dyes with overlapping excitation bands.

In 2001, however, the LSM 510 META solved this problem by means of the award-winning META detector technology. In 2002 Carl Zeiss introduced Automatic Component Extraction (ACE), a software function that automatically unmixes fluorescence signals to remove excitation crosstalk.

Today, this ZEISS ACE technology is also available for the single detector-based LSM 510 system and solves the problem of excitation crosstalk in dual-labeling experiments. MultiChannel unmixing is the perfect tool for tricky dual-labeling experiments.
Quantitative Colocalization
Finding the Needle in the Haystack

The LSM 510 enables you to easily perform quantitative colocalization analysis with a previously unattainable degree of reliability and precision. The image display, scattergram and data table are interactively linked to the ROI and thresholding tools.

If, for example, you select an area in the scattergram, existing colocalizations will be shown immediately. The data table, histogram and image are interlinked in the same way. You can hardly have a more intuitive or precise analysis of your data.

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 the colocalization analysis results
- Quantitative colocalization analysis for up to 99 ROIs, including:
  - Area and average gray level intensity
  - Colocalization degree
  - Colocalization coefficient
  - Pearson’s correlation coefficient
  - Manders’ overlap coefficient
- Analysis results exportable

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

Correct use of first-class tools:
Image display, scattergram and data table interactively linked to the ROI and thresholding tools
You know just how big a difference there is between seeing a lot and clear detection. The limits of conventional multifluorescence microscopy are always reached when the emission signals of the dyes overlap. The LSM 510 META solves this problem – as the name itself suggests. meta in Greek means “going beyond”, and that is precisely what this system does. It goes beyond what is currently available and takes you much further than the traditional limits.

The LSM 510 META represents a new generation of laser scanning microscopes. Leaving the old standards far behind, this system gives you brilliant images with previously unattainable information content. As a result, you can not just see a lot more, but detect things much more clearly.
Online Fingerprinting  
Keeping Tabs on Dynamic Processes

No need to wait till the end of the scanning procedure to assess dynamic processes in a living cell. Carl Zeiss now offers you immediate results via the Online Fingerprinting function. This achievement was made possible though close cooperation with research scientists to further advance the Emission Fingerprinting technique.

In the Online Fingerprinting dialogue you select your reference spectra prior to scanning. Each spectrum is unmixed during scanning and the result displayed immediately. All this means that the time required to induce a reaction by applying a stimulus is easy to determine. You no longer need to focus on the technique of your application but can fully concentrate on analyzing your work.

CFP, CGFP, GFP and YFP in cultivated cells after Emission Fingerprinting  
Specimen: Dr. A. Miyawaki, Riken Institute, Tokyo, Japan

Online Fingerprinting:  
All the required settings from excitation to emission are made in a single menu.
Carl Zeiss brought linear unmixing technology to microscopy. It permits the precise separation of fluorochromes with highly overlapping emission spectra and the isolation of the powerful autofluorescent signals present in many living specimens.

One highlight of the LSM 510 META is its time-saving, specimen-preserving parallel data acquisition capability in multiple META channels. Since up to 32 channels can be acquired in only 1.2 seconds at full resolution, you can carry out spectral imaging with dynamic samples and precise unmixing – even online or where unknown signal spectra are involved.

The tools the LSM 510 META offers to remove signal crosstalk include Emission Fingerprinting, Automatic Component Extraction and reliable reference spectra for fast Online Fingerprinting.
The LSM 510 NLO, available with or without META detector, is the ideal solution for sensitive in-depth analysis of live specimens — and even whole organisms. One outstanding feature is its unparalleled depth selectivity. Even low concentrations of fluorochromes can be detected through precisely tunable multiphoton excitation and non-descanned detection. With the help of the depth-selective excitation unique to the LSM 510 NLO your bleaching experiments can be carried out successfully with pinpoint precision. Whether you are interested in thick tissue sections or live specimens, in structural or functional analysis of ultra-fine nerve cells in neurobiology or of whole embryos in developmental biology, the LSM 510 NLO is in a class of its own for in-depth insight.

Multiphoton microscopy takes live cell experiments a decisive step further by enabling deep penetration into a living tissue without using damaging laser power levels. The LSM 510 DuoScan systems give you even greater freedom in this kind of experiment.

CA1 pyramidal cell in a mouse hippocampus. Such a highly detailed resolution is only possible through the depth penetration ability of the multiphoton method. Specimen: M. Fuhrmann, Center for Neuropathology and Prion Research, LMU Munich, Germany
LSM DuoScan
Unrestricted Versatility in Photomnipulation

The LSM DuoScan puts an end to experimental restrictions. This is the system to combine ultra-precise point scanning, spectral imaging, 3D-Imaging, with flexible sample manipulation. Controlled through an integrated software interface and real-time electronics, featuring a shared microscope platform and a common laser module, the LSM DuoScan photomnipulation attachment creates a unique, highly efficient configuration.

No matter which basic ZEISS LSM 510 configuration you own, the LSM DuoScan enables you to run all experiments requiring sample manipulations with freely defined ROIs. The LSM DuoScan allows applications such as FRAP, FLIP or FLAP in the visible wavelength range, photoconversion and photoactivation with violet light at 405 nm, or uncaging in the UV range.
PA-GFP, Dronpa and Kaede
When Manipulation is a Good Thing

You can push back the frontiers of biomedical research through flexible sample manipulation experiments, e.g. photoactivation and photoconversion, conducted with great precision and at high-resolution time scales.

The recently developed fluorescent proteins PA-GFP, Dronpa and Kaede enable you to study dynamic processes directly. The two independent scanner groups in the LSM 510 DuoScan give you a great deal of flexibility for such photoactivation and photoconversion experiments.

**PA-GFP + Dronpa:**
Dronpa is a fluorescent protein that can be optically stimulated to switch between a fluorescent and a non-fluorescent state.

**Kaede is a fluorescent protein whose fluorescence changes from green to red when irradiated with ultraviolet light.**

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**Dronpa-transfected cultured cell, repeatedly activated by pulses of 405 nm light and imaged fast with 488 nm excitation.**

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**PA-GFP + Dronpa:**
Dronpa is a fluorescent protein that can be optically stimulated to switch between a fluorescent and a non-fluorescent state.
The new ConfoCor 3 is more than an imaging module. It allows you to trace single molecules in a non-invasive manner with high speed and precision.
Unique Combination
Blinking Movers Detected and Analyzed

Combine a ZEISS ConfoCor 3 with the LSM 510 META and you have a fully integrated, and spectroscopic imaging platform for single-molecule detection. This combined system analyzes minute signal fluctuations and quantifies them in terms of molecule concentration and diffusion times. For samples in solution or in a cell, the ConfoCor 3 delivers statistically significant and reliable data thanks to its high sensitivity and automated functionality. To trigger repeated measurements and obtain real-time results, all you have to do is select your measurements accurately.

Convincing Benefits

This combined system gives you an integrated platform for confocal imaging and spectroscopic analysis. High-performance detectors deliver extremely high levels of sensitivity and time resolution. The real-time analysis function is based on intelligent algorithms. Last but not least, high information density is achieved through assessing the localization, concentration, interaction and speed of molecules in a single measurement.
Software
Bringing in New Options

The following software package options are available:

■ **3D Visualisation and Image Improvement Tools**
  • 3D for LSM
  • Deconvolution
  • Image VisArt plus

■ **Time Series and ROI Analyzing Tools**
  • Physiology/Ion concentration
  • FRET plus
  • FRAP Wizard/Kinetic Analysis

■ **Acquisition Programming Tools**
  • Visual Macro Editor
  • VBA Macro Recorder/Editor
  • Multiple Time Series
3D Visualisation and Image Improvement Tools
Specializing in Making It Visual

3D for LSM, Deconvolution (DCV), and Image VisArt plus: This set of software options makes expert 3D image processing possible.
- Visualization of 3D images
- Analysis and measurement of 3D images
- Image restoration of 3D image data
- Computation of point spread function
- Visualization of computed image data
- Fast 3D/4D reconstruction and animation
- Shadow, transparency and surface rendering techniques
- Presentation and animation of images

Image VisArt plus software:
Lachrymal gland of the mouse, 3D visualization.
Specimen Dr. B. Zimmermann, University of Potsdam, Germany
Physiology/Ion Concentration
Opting for a Complete Recording and Analysis Tool

This optional package allows you to display and analyze ion concentration:

- Online and offline ratioing for ratiometric dyes
- Online and offline F/F₀ for single wavelength dyes
- Calibration for single wavelength or ratiometric dyes
  - In situ and in vitro
  - including background compensation
  - Titration or Grynkiewicz-based
- Interactive scaling of data series and graphic display

Physiology software: Salivary gland of a fly.
Time series of Ca²⁺ concentration (Fluo-4, green) and membrane potential (TMRE, red).
Specimen: Dr. B. Zimmermann, University of Potsdam, Germany
Interactions between proteins provide useful hints about functional relationships in cell physiology. Fluorescence Resonance Energy Transfer (FRET) is a sensitive analytical method that enables you to detect and quantify protein interaction.

Two proteins of interest are marked with different fluorescent dyes. The emission wavelength of one dye (the donor) overlaps 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 activated to emit detectable light.

FRET plus, the new LSM software module, provides a variety of acquisition and analysis techniques that support all the recognized methods used in FRET experiments. This means you can obtain fast, reproducible results with stored configurations. You can repeat experiments conveniently and reliably for statistical analysis.

An elegant and well-established way of detecting FRET is known as acceptor bleaching. Here, you select a specific region within the specimen and eliminate acceptor fluorescence with high laser intensity (FRAP).

Calcium imaging using the FRET indicator Yellow Cameleon 2.
FRAP
Detecting the Dynamics of Protein Diffusion

There is no standstill in a living cell. All the cell components make up a dynamic equilibrium. Fluorescence Recovery After Photobleaching (FRAP) enables the movements of each component in this equilibrium to be analyzed.

The new FRAP tool in the LSM Software contains an interactive FRAP Guide that will take you step by step through an entire FRAP experiment – from configuring the irradiation process to selecting the bleaching regions and laser intensities. Even inexperienced users can conduct successful FRAP experiments and safely record quantitative data with the help of this FRAP Guide, which also contains additional explanations and advice on key steps and parameters.

The new tool also permits initial data preparation for modeling using mathematical functions. Comparisons with kinetic models allow first conclusions to be drawn on the movements of the proteins observed.

In a FRAP experiment, a defined region in a cell expressing e.g. a GFP fusion protein is bleached by brief but intense laser irradiation. The recovery of fluorescence is documented by time-lapse shots and measured.
Confocal microscopy technology is becoming increasingly sophisticated. But each advance also adds to the complexity of the procedures. The Visual Macro Editor, Macro Editor VBA, and Multiple Time Series software options provide flexibility and customization at any level.

The new Visual Macro Editor provided by the LSM software is a function that allows you to create, edit and save every step of any experiment right up to data extraction. Editing individual steps by inserting, shifting and copying icons is an incredibly simple and clear process. You can even start your next experiment while the LSM completes the one that is running. The use of macros as routine tools makes it easy to precisely reproduce even comprehensive and complex scan procedures. The bottom line is that you can take an observed change and verify it as a fact.

Automatic time lapse acquisition of a complex series with integrated focusing steps.
### Various Applications

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Reversible site-selective labeling of membrane proteins in live cells.

Kogure T., Karasawa S., Araki T., Saito K., Kinjo M., Miyawaki A.
A fluorescent variant of a protein from the stony coral Montipora facilitates dual-color single-laser fluorescence cross-correlation spectroscopy.


Quantum dot ligands provide new insights into erbB/HER receptor-mediated signal transduction.

“The power of the ZEISS system is not only in its sensitivity, its software and its user-friendliness, but also in the technical enhancements for spectral selection.”

William C. Hyun,
Comprehensive Cancer Center,
University of California, San Francisco, USA

“The new scan modes of the system offer a completely new quality of analysis. The interpretation of the data is far more reliable than with any conventional system based on filter sets and band pass acquisition.”

Dr. Frank-D. Böhmer,
Molecular Cell Biology Research Unit,
Friedrich Schiller University, Jena, Germany
# Specifications

## LSM 510\textsuperscript{Mk4} and LSM 510 META\textsuperscript{Mk4}

### Microscopes

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<tr>
<th>Models</th>
<th>Upright: Axio Imager.Z1/M1, Axioskop 2 FS MOT. Inverted: Axio Observer.Z1</th>
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</thead>
<tbody>
<tr>
<td>Z drive</td>
<td>DC motor with optoelectronic coding, smallest increment ( \leq 25 ) or ( \leq 50 ) nm; accessory: Fast piezoelectric focusing drive acting on objective</td>
</tr>
<tr>
<td>Fine focusing</td>
<td>Accessory: Piezoelectric drive acting on stage or objective; total travel approx. 250 µm, smallest increment &lt; 10 nm</td>
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<tr>
<td>XY stage (option)</td>
<td>Motor-driven XY scanning stage with Mark&amp;Find and Tile Scan (Mosaic Scan) functions; smallest increment 1 µm</td>
</tr>
<tr>
<td>Accessories</td>
<td>AxioCam Digital Microscope Camera; integration of incubation chambers, micromanipulators, etc.</td>
</tr>
</tbody>
</table>

### Scanning Modules LSM 510 / LSM 510 META

| Models       | Various configurations with two, three or four confocal channels, or two channels and polychromatic multichannel detector, prepared for lasers from UV to near infrared |
| Scanner      | Two independent galvanometric scanning mirrors, real-time controlled, with ultrashort line and frame flyback |
| Scan resolution | 4x1 to 2048x2048 pixels, also for several channels, continuously variable |
| Scanning speed | 13 x 2 speed stages; up to 5 frames/s with 512x512 pixels (max. 77 frames/s with 512x32 pixels), 0.38 ms for a line of 512 pixels |
| Scan zoom    | 0.7x to 40x, digitally variable in steps of 0.1 |
| Scan rotation | Free 360° rotation in steps of 1°, free X/Y offset |
| Scan field   | 18 mm field diagonal (max.) in the intermediate image plane, homogeneous field illumination |
| Pinholes     | Pre-adjusted pinholes, individual variation of size and position for each reflected-light channel |
| Detection    | Simultaneous for up to four confocal reflected-light channels, each with a highly sensitive photomultiplier; META detector for fast acquisition of lambda stacks with up to 32 channels in 1.2 s; optional transmitted-light channel with photomultiplier |
| Data depth   | 8 or 12 bits, individual 12- bit A/D converted for each channel |

### Laser Modules LSM 510 / LSM 510 META

| VIS Laser Module | Polarization-preserving single-mode fiber, temperature-stabilized VIS-AOTF (Acousto-Optical Tunable Filter) for simultaneous intensity control of up to six visible-light laser lines, switching time < 5 µs; diode laser (405 nm) 50 mW, Ar laser (458, 477, 488, 514 nm) 30 or 45 mW, HeNe laser (543 nm) 1 mW, DPSS laser (561 nm) 10 mW; HeNe laser (594 nm) 2 mW and HeNe laser (633 nm) 5 mW (end-of-life specification) |
| UV Laser Module | Polarization-preserving single-mode fiber, temperature-stabilized UV-AOTF for simultaneous intensity control of two ultraviolet laser lines, switching time < 5 µs; Ar laser (351, 364 nm) 80 mW (end-of-life specification) |
| Multiphoton option | Direct coupling of pulsed NIR (near-infrared) lasers into the scanning module; support of various makes. Fast change of laser intensity by means of AOM (Acousto-Optical Modulator). NIR-optimized objectives. |

### Scanning Module LSM DuoScan

| Scanner      | Two independent galvanometric scanning mirrors, real-time controlled, with ultrashort line and frame flyback |
| Scanning speed | 13 x 2 speed stages; up to 5 regions/s with 512x512 pixels (max. 77 regions/s with 512x32 pixels), 0.38 ms for a line of 512 pixels |
| Scan zoom    | 0.7x to 40x, digitally variable in steps of 0.1 |
| Scan rotation | Free 360° rotation in steps of 1°, free X/Y offset |
| Scan field   | 18 mm field diagonal (max.) in the intermediate image plane, homogeneous field illumination |
**Laser Modules LSM DuoScan**

Variable beam splitting
Additional outlet from existing VIS or V Laser Module with polarization-preserving single-mode fiber; splitting proportion between the outlets freely variable through the software; for 405, 488 or 532nm laser lines

**VIS Laser Module**
Polarization-preserving single-mode fiber, temperature-stabilized VIS-AOTF for simultaneous intensity control; switching time < 5 µs; all lasers of maintenance-free diode or solid-state type without significant heat dissipation. 405nm laser diode, 50 mW; 488nm laser diode, 100 mW; 532nm DPSS laser, 75 mW

**UV Laser Module**
Polarization-preserving single-mode fiber, temperature-stabilized UV-AOTF for simultaneous intensity control of two ultraviolet laser lines, switching time < 5 µs; Ar laser (351, 364 nm), 80 mW

**Electronics Module**

**LSM 510 Control**
Controls the microscope, laser modules, scanning module and other accessories; Control through real-time computer and Gigabit Ethernet Communication

**Computer I**
Standard PC with main and hard disk memory space for practical requirements; ergonomic flat-panel displays of 19” (4:3); Windows XP OS

**Computer II**
High-end PC with abundant main memory space and ultrafast RAID 0 hard disk system; ergonomic flat-panel displays of 19” (4:3), many accessories; Windows XP OS

**Standard Software**

**System configuration**
Convenient control and configuration of all motor-driven microscope functions and of the laser and scanning modules; saving and restoration of application-specific configurations

**ReUse function**
Restoration of acquisition parameters with a mouse click

**Acquisition modes**
Line, Frame, Z-stack, time-lapse series and combinations: xy, xyz, xyt, xz, xt, xzt; on-line computation and visualization of ratio images. Averaging and summation.

**Auto-Z function**
On-line adaptation of acquisition parameters for Z-stacks for uniform brightness distribution

**Zoom Crop function**
Convenient selection of scanning areas (Zoom, Crop, Offset)

**ROI Bleach**
Localized photobleaching in up to 99 bleaching ROIs for such applications as FRAP (Fluorescence Recovery After Photobleaching) or Uncaging, up to 99 ROIs (Regions of Interest) of any shape, and laser blanking with single-pixel accuracy

**Multitracking**
Acquisition of multiple fluorescence signals by fast change of the excitation lines

**Visualization**
Orthogonal view (xy, xz, yz in one display), cut view (3D section at freely definable solid angles), 2.5D view for time-lapse series of line scans, projections (stereo, maximum, transparency projection) for single images and series (animations), depth coding (false-color view of height information). Brightness and contrast adjustment; off-line interpolation for Z-stacks, selection and modification of color look-up tables (LUTs), drawing functions for documentation

**Image analysis**
Modern tools for colocalization and histogram analysis with various parameters and options, profile measurement along straight lines and curves of any kind, measurement of lengths, angles, areas, intensities, etc.

**Image operations**
Addition, subtraction, multiplication, division, ratio, shift, filters (low-pass, median, high pass, etc; user-definable)

**Image archiving, export, import**
LSM image database with convenient functions for managing the images and the associated acquisition parameters; Multiprint function for compiling 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.

**Image Browser**
Free software package for visualization, processing, sorting, printing and export/import of LSM 5 images

**Software Options for all Systems**

**LSM Image VisArt plus**
Fast 3D and 4D reconstruction and animation (various modes: Shadow projection, transparency projection, surface rendering)

**Multiple Time Series**
Multiple time series with varied application configurations, autofocus and bleaching functions

**Physiology**
Comprehensive analysis software for time-lapse series, graphical Mean-of-ROI analyses, on-line and off-line calibration of ion concentrations

**FRET plus**
Analysis of experiments with the Sensitized Emission or Acceptor Photobleaching methods

**FRAP**
User guiding for, and analysis of FRAP and FLIP experiments, with calculation of the quantitative parameters

**VBA Macro Editor**
Recording and editing of routines for the automation of scanning and analysis functions

**Visual Macro Editor**
Graphical compilation of routines for scanning and analysis functions

**3D for LSM**
3D visualization and 3D measurement of volume data records

**3D Deconvolution**
Image restoration based on computed point spread functions
System Overview

- Laser module RGB (458, 477, 488, 514, 543/561, 594, 633 nm)
- Laser Enterprise II 653 (80 mW, 351 nm, 364 nm) UV Laser module
- LSM DuoScan
- ConfoCor 3
- NLO kit for direct coupling
- Upgrade kits LSM 510 to LSM 510 META
- VIS, VIS/UV, VIS/NLO or VIS/405 scan module LSM S10
- System electronic rack
- Laser module LSM 5 LV/FVE (with variable output coupling)
- Laboratory cart
- Plug-in unit for external laser
- 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
- System table with breadboard
  - Width 1000x750mm (1200x950 overall)
  - Narrow 750x1000mm (950x1200 overall)
- LCD TFT flat screen monitor 19”
- Control computer

Option:
- Fiber decoupling channel 4 Fluorescence Correlation Spectroscopy (FCS)
### Glossary and Functions

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ACE</td>
<td>Automatic Component Extraction</td>
</tr>
<tr>
<td>ADC</td>
<td>Analog-to-Digital Converter</td>
</tr>
<tr>
<td>AOM</td>
<td>Acousto Optical Modulator</td>
</tr>
<tr>
<td>AOTF</td>
<td>Acousto Optical Tunable Filter</td>
</tr>
<tr>
<td>CFP</td>
<td>Cyan Fluorescent Protein</td>
</tr>
<tr>
<td>DIC</td>
<td>Differential Interference Contrast (Nomarski)</td>
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<tr>
<td>FCS</td>
<td>Fluorescence Correlation Spectroscopy</td>
</tr>
<tr>
<td>FLIM</td>
<td>Fluorescence Lifetime Imaging Microscopy</td>
</tr>
<tr>
<td>FRAP</td>
<td>Fluorescence Recovery After Photobleaching</td>
</tr>
<tr>
<td>FRET</td>
<td>Fluorescence Resonance Energy Transfer</td>
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<tr>
<td>GFP</td>
<td>Green Fluorescent Protein</td>
</tr>
<tr>
<td>NLO</td>
<td>Non-Linear Optics (multiphoton imaging)</td>
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<tr>
<td>ROI</td>
<td>Region Of Interest</td>
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<tr>
<td>YFP</td>
<td>Yellow Fluorescent Protein</td>
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**Automatic Component Extraction**
Statistical procedure for the detection of single dye spectra in a Lambda Stack.

**Emission Fingerprinting (patent pending)**
Method available with the LSM 510 META for the recording, analysis and separation of emission signals in multifluorescence images; also suitable for widely overlapping spectra.

**Lambda Stack**
Image stack with information in x, y and λ; combinable with z and/or time series; for the determination of spectral signatures at any specimen location.

**Linear Unmixing**
Mathematical procedure for the spectral deconvolution of multiple emission signals.

**Metatracking**
Scanning mode available with the LSM 510 META, similar to Multitracking, but with additional fast switching between detection settings.

**Multitracking**
Scanning mode available with the LSM 5, generates multifluorescence images without crosstalk of emission signals, by means of fast switching between excitations, and quasi-simultaneous detection.

**RealROI (rROI) Scan**
Scanning mode in which freely definable specimen areas are excited and imaged; guarantees maximum specimen protection thanks to exact blanking of the laser lines outside the selected specimen areas.

**ROI Bleaching**
Defined photobleaching of several, freely defined specimen areas, e.g. for FRAP, Uncaging, or Photoactivation experiments.

**Spline Scan**
Scanning along a freehand-defined line for recording fast (physiological) processes, e.g. along neurons.

**Spot Scan**
Scanning mode in which the signal intensity at a confocal point can be tracked with extremely high temporal resolution.

**Step Scan**
Fast overview scan in which intermediate lines are added by interpolation.

**Tile Scan**
Records an overview image consisting of a number of tiled partial images for the recording of larger objects with improved resolution.
The LSM 510 META wins the renowned R&D 100 award for technical developments.

LSM 510
US Patents: 5127730, 6037583, 6167173, 6278555, 6462345, 6486458, 6563632, 6631226, 6848825, 6941247
German Patents: 19702752C2, 19702753C2, 19758744C2, 19758745C2, 19758746C2, 69131176T2

LSM 510 META
US Patents: 6403332, 6747737, 6750036, 6858852, 6891613, 6958811, 7009699
German Patents: 19915137C2, 1003318084, 1003852684

LSM 510 NLO
US Patents: 5034613, 6344653, 6403332, 6521999, 6867915, 7119898
German Patents: 19919091C2, 69032621T3, 69034117T2

ConfoCor 3
US Patent: 6591223

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