

# LSM 710 NLO

Information in Depth



Innovative Systems for Multiphoton Microscopy



We make it visible.

## **Providing Support for Progress and Innovation**

Biomedical sciences represent one of the most important and future-oriented fields of research. Taking advantage of increasingly powerful technologies, they lead to a deeper understanding of the complex mechanisms that form the foundation of living systems at the molecular, cellular, and tissue levels.

For more than 160 years, Carl Zeiss has supplied the scientific community with the finest technological instruments and the expertise needed for their optimal use. Zeiss creates ideal conditions for modern research by providing comprehensive professional consulting as well as systems and solutions tailored to users' exact needs.



## Images in Depth

Ground-breaking research in fields such as neurophysiology, immunology, and developmental biology provide important insights into systematic connections in all life forms. With the help of innovative technological developments such as multiphoton microscopy, it is now possible to perform research on and especially in living organisms with an increasingly minimal level of functional invasiveness. Multiphoton microscopy is considered the best method in the field of minimal and non-invasive fluorescent microscopy today. The LSM 710 NLO allows scientists to generate images of very deep-lying tissue with subcellular resolution in a gentle way.



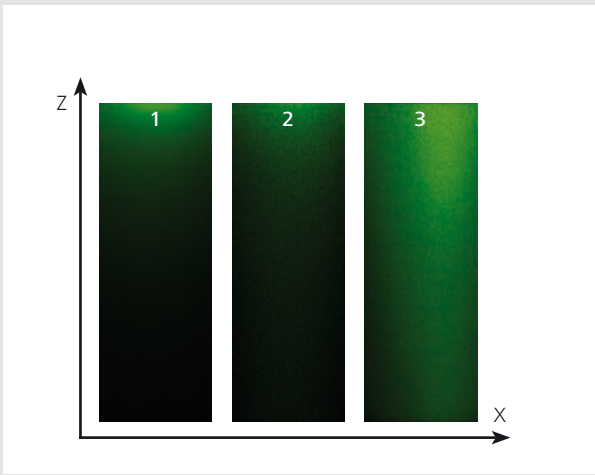
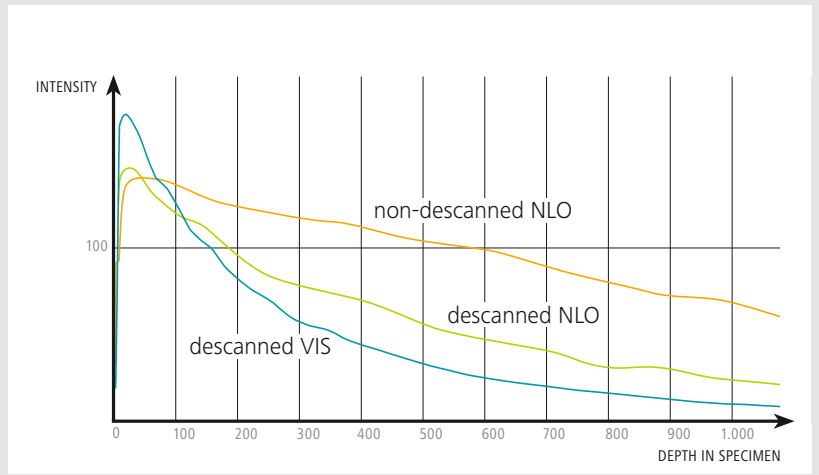


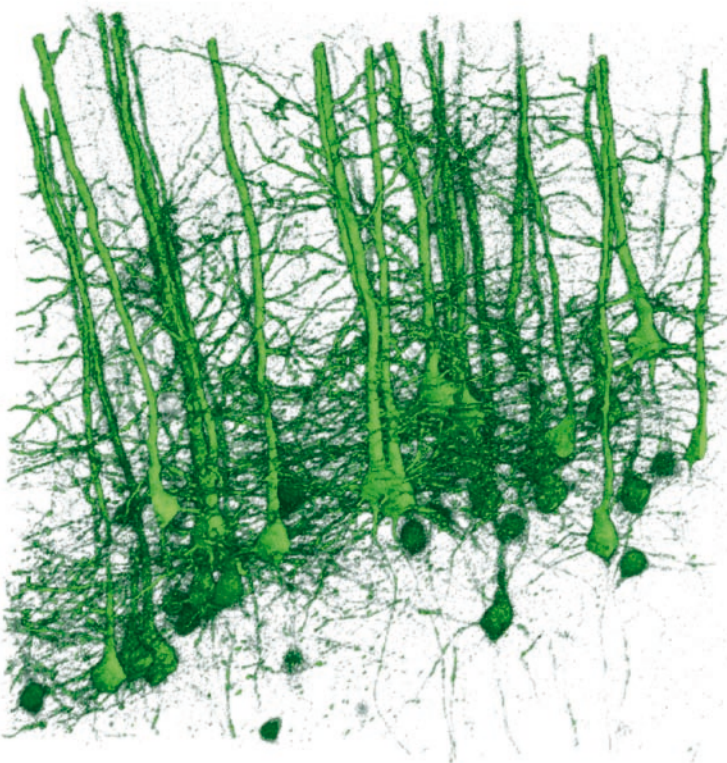
Illustration of the XZ level of a homogeneous colored sample after laser excitation in the visible range (1), using a multiphoton laser (2), and alternative detection using NDD (3).



A comparison of the intensity distribution along the Z axis shows the noticeably better excitation in deeper layers of the specimen using the multiphoton laser. It also shows the more efficient signal acquisition using non-descanned detectors.

## 3D Morphology

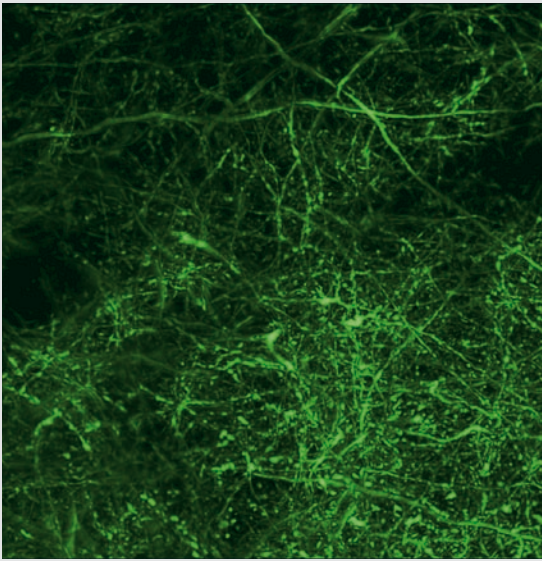
High-resolution 3D imaging of tissues and cell structures forms the very basis of our understanding of their morphological composition and functionality.



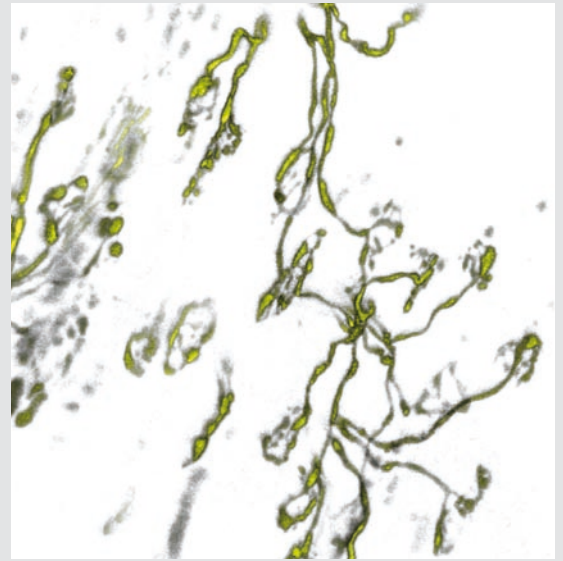
The detection technology of the LSM 710 NLO was optimized further so as to facilitate even better imaging results, for example via efficient optics for light collection, perfect detection geometry, and the special GaAsP non-descanned detector. This detector has a very low level of dark noise and a high quantum efficiency, which results in an outstanding signal-to-noise ratio. This, in turn, permits the imaging of very fine structures in subcellular areas even in the case of critical samples like neurons deep inside a brain.

Thanks to these technological improvements, the system makes it possible to create high-resolution 3D reconstructions out of even highly dispersive samples. This enables scientists to perform optimal research on the morphological components and 3D structure of different cell types.

*Principal (projection) neurons in cortex of a transgenic mouse that expresses YFP under the thy1 promoter (adult, YFP-H line). The brain was fixed with 3% PFA and the forebrain was removed by transverse section. The forebrain was then embedded in 8% agarose with the caudal portion (cut surface) facing up. A region of the cortex was imaged from the cut surface to a depth of 260 microns using multiphoton excitation (930 nm).*



*Neuromuscular junctions in sternomastoid muscle of an adult transgenic mouse that expresses YFP in all motor neurons. Image was acquired in a living animal using the Zeiss W-Plan Apochromat 20×/1.0 NA dipping objective and two photon excitation (880 nm).  
Stephen Turney, MCB, Harvard University, USA*



*Magnified section of the projection neurons' dendritic branches. Spines are clearly visible.*

## Intravital Imaging

In order to understand interactions and functional connections of cells within organisms, it is necessary to perform minimal-impact research on the living specimen.

The LSM 710 NLO offers all the prerequisites for intravital imaging with subcellular resolution. The point excitation of a pulsed IR laser is minimally invasive with a low level of phototoxicity, thereby creating the ideal conditions for the examination of living specimens in the gentlest manner possible. The parallel use of different channels and dyes allows for the observation of up to five signal types and thus the interactions between many different structures.

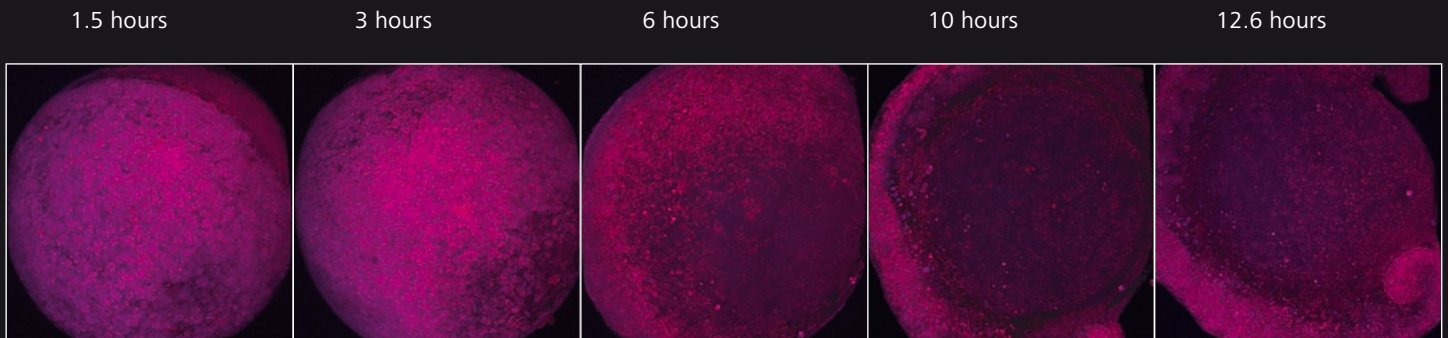
The innovative system also allows scientists to pursue very complex methods such as two-photon uncaging in connection with calcium imaging. This locally defined manipulation aids in the study of cellular processes and interactions.

*"Multiphoton imaging requires an efficient NDD light path. The LSM 710 NLO offers many improvements that result in brighter images and deeper tissue penetration. Also, the configuration of NDD modules is very flexible, allowing simultaneous acquisition of many channels for multicolor imaging."*

Dr. Stephen Turney, MCB, Harvard University, Boston, USA



*Dendrites of cortical projection neurons of a transgenic mouse expressing YFP via the thyl1 promotor. This high-resolution image of the dendritic processes to a depth of of 430 μm was made using multiphoton excitation of 920 nm in the living animal. Specimen provided by Stephen Turney, MCB, Harvard University, USA*



*3D reconstruction of a Zebrafish embryo expressing a genetically encoded Ca<sup>2+</sup> indicator, Cameleon. Early developmental stages of the embryo were observed for 13 hours at 25 °C. Excitation at 850 nm, timestamp post fertilization.*

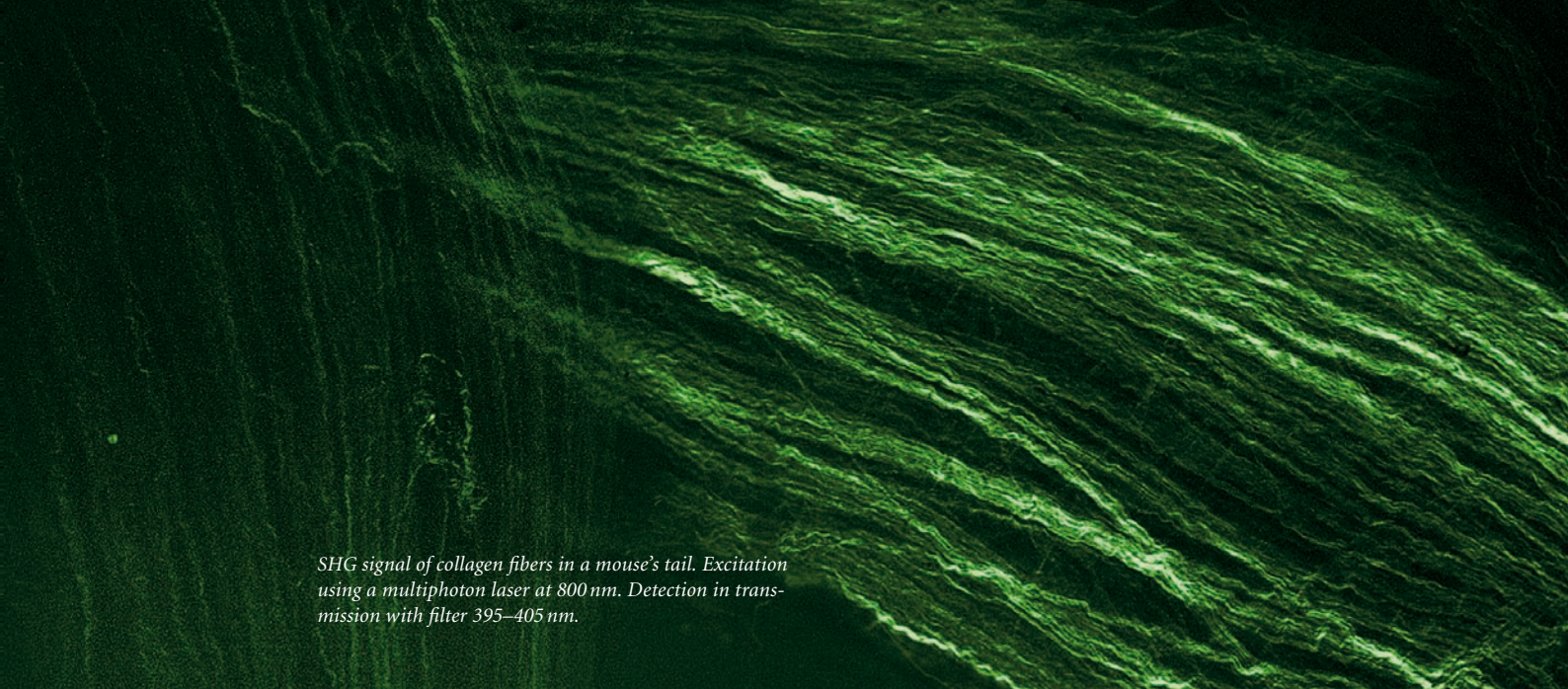
## 3D in Temporal Resolution

The LSM 710 NLO offers a decisive advantage in the long-term observation of biological processes: the device's point excitation allows for a significant reduction in phototoxicity, as the light has an impairing effect only in the focus.

When used in embryology, this process allows scientists to observe developmental processes such as cell organization and cell distribution in a detailed manner. With the help of practical markers or by means of the photoactivation of special fluorescent proteins, it is possible to track individually targeted cells and investigate their interactions in a physiological 3D space – for example in the investigation of immuno-active cells moving through the body. The LSM 710 NLO permits the optimal observation of the behavior of these cells whether it be in an artificial 3D collagen matrix or in vivo for as long a period as possible.

*"The LSM 710 NLO in conjunction with the new microscope Axio Examiner represents a very versatile system. When imaging embryonic stages, we are often troubled by abnormal developments caused by phototoxicity. Improved optics and detectors, especially the registration of emission signals in reflection and transmission, allow a reduction of the laser intensity for excitation, which is crucial for normal development."*

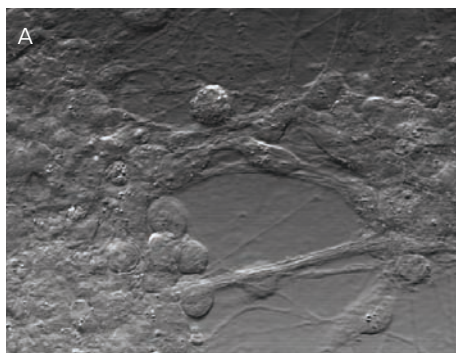
Dr. Hideaki Mizuno, Brain Science Institute, Riken, Wako, Japan



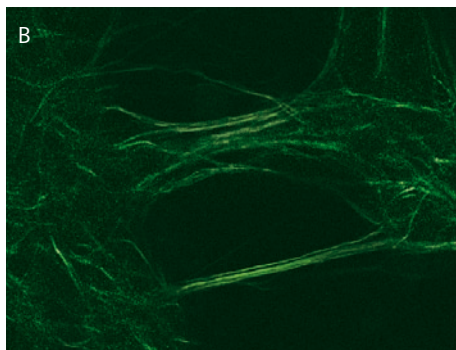
SHG signal of collagen fibers in a mouse's tail. Excitation using a multiphoton laser at 800 nm. Detection in transmission with filter 395–405 nm.

## SHG: Additional Contrast via Frequency Doubling

Second Harmonic Generation, or SHG for short, is a non-linear photophysical effect that is used in non-linear microscopy to create additional contrast.



In this process, two photons of a strong incident laser are driven through polarizable tissue and transformed into a single photon with doubled energy and frequency levels. The key advantage of SHG is that it requires no dyes, seeing as the image contrast is already structurally intrinsic to the sample. This makes Second Harmonic Imaging the ideal method with which to investigate living cells and tissues. The additional contrast provides crucial information on the structure and/or changes found in certain proteins. As a result of its special optics, the LSM 710 NLO on the Axio Examiner is ideally equipped for this procedure.



*Second Harmonic Imaging of embryonic stem (ES) cell-derived mouse motor neurons in vitro. The motor neurons were established in a long-term co-culture (5 days) with either ES cell-derived or primary glial cells. The image is a composite of SHG (B) and oblique illumination contrast (A) signals acquired simultaneously using low-intensity multiphoton excitation (800 nm). Specimen provided by Monica Carrasco, MCB, Harvard University, USA*



ZEISS

LSM 710



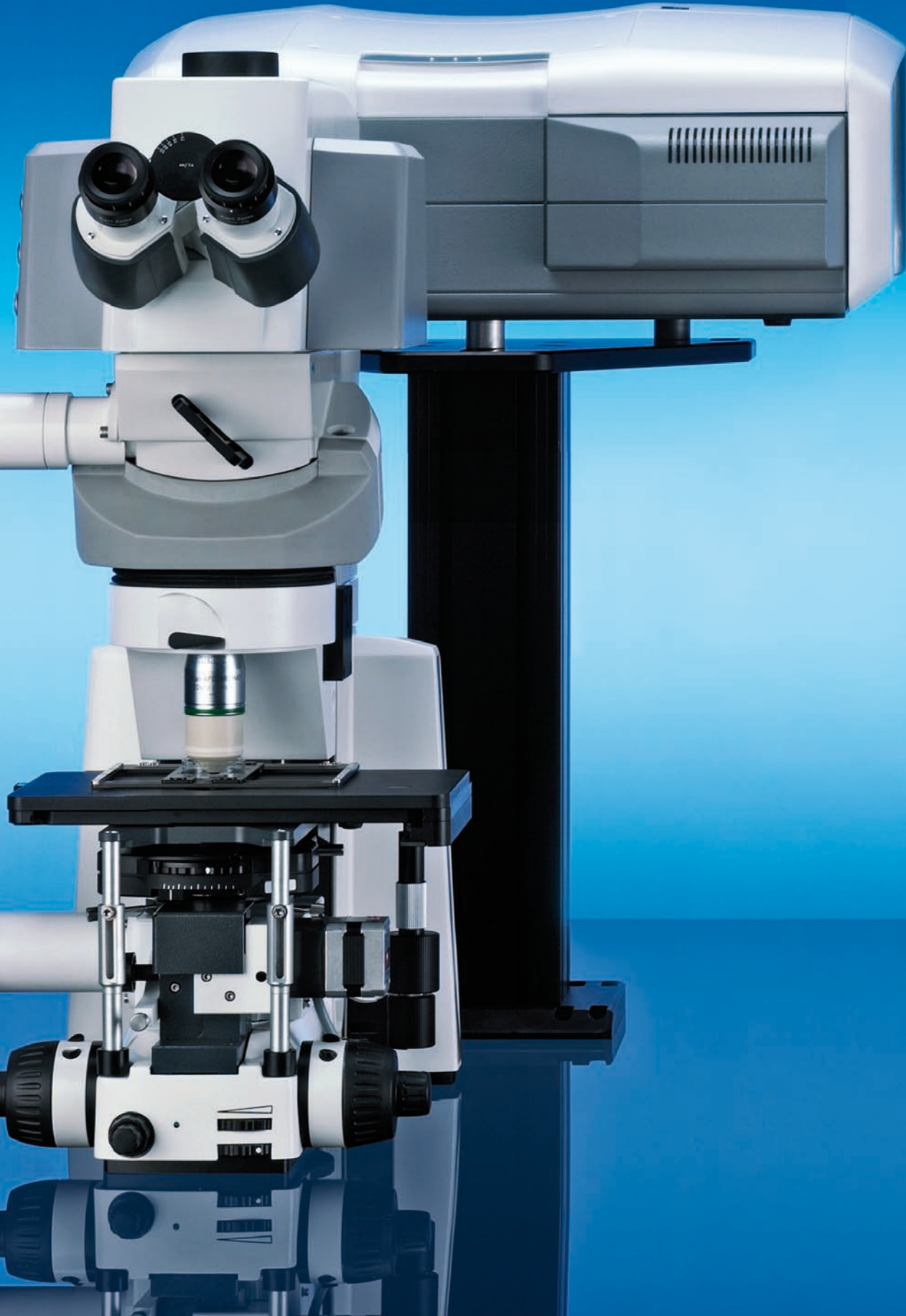
**Discover the New Sensitivity:  
LSM 710 NLO**



## LSM 710 NLO on Upright Stands

Together with the Axio Examiner, the LSM 710 NLO represents the most optimal system for intravital imaging and electrophysiological research in conjunction with multiphoton microscopy.





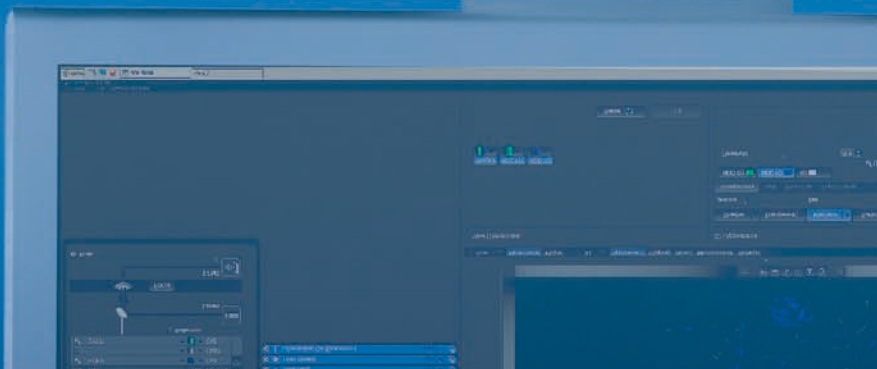
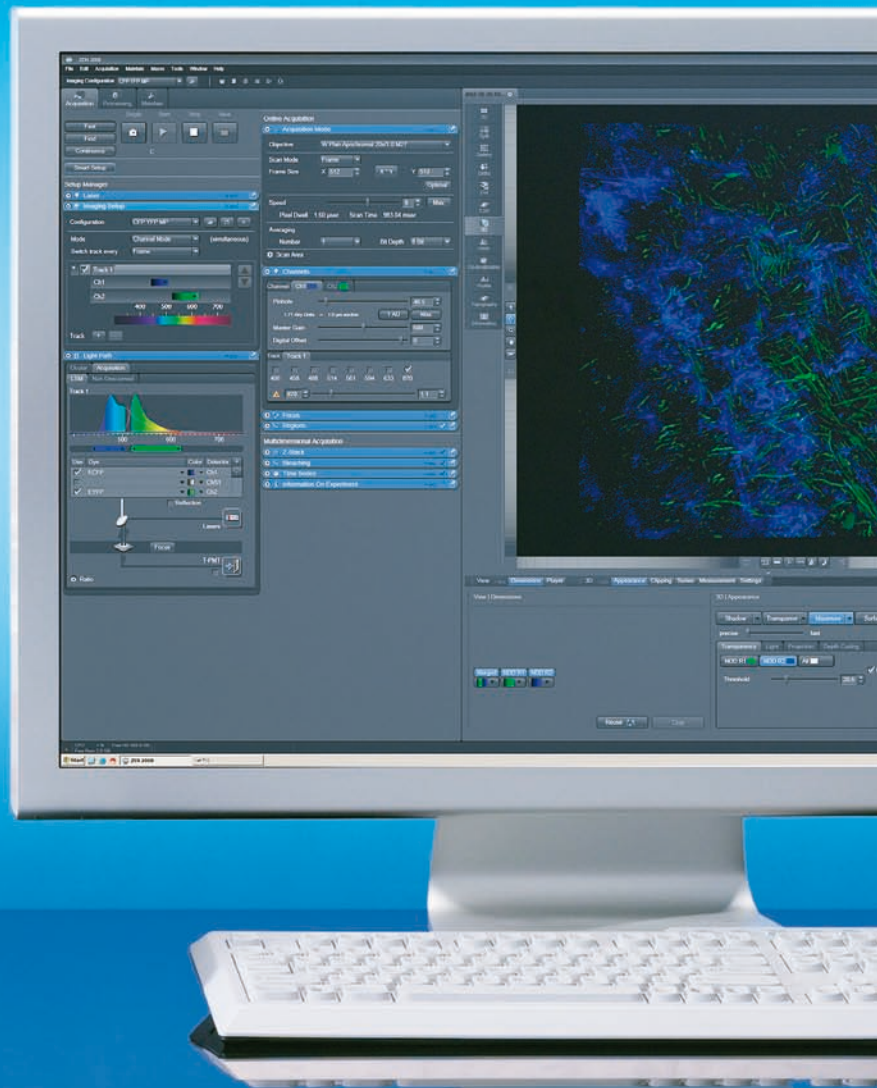
# LSM 710 NLO on Inv

In conjunction with the Axio Observer, the functional apparatus for the imaging of sta



# erted Stands

system represents an incomparable multi-  
ndard specimens and cells in culture.



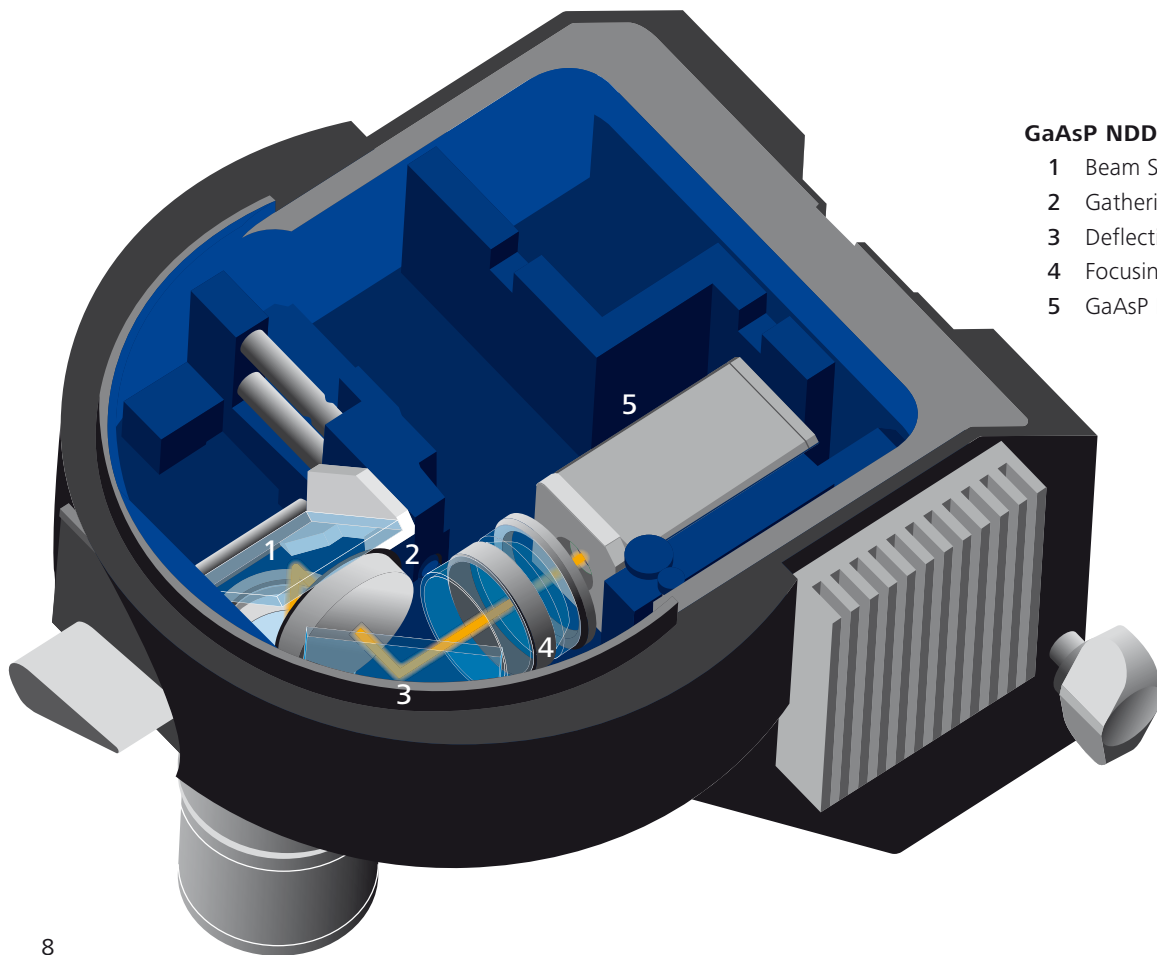
# Sensitivity Is The Key

The fundamental prerequisite for all demanding applications in laser-scanning microscopy is high sensitivity in detection with low detector dark noise.

Whether it's intravital imaging, long-term observation of developmental processes, or high-resolution 3D imaging, the LSM 710 NLO delivers true-to-detail and high-contrast images. The outstanding sensitivity of the system was combined with innovative techniques to suppress laser light excitation. Improved non-descanned detectors, extraordinary light collection efficiency from optimal optics design, and – last but not least – the GaAsP NDD detector also guarantee excellent imaging results in thick tissue samples and living animals.

In order to attain this capability, a whole range of innovations was implemented into the system, including the following:

- low noise electronics with up to 30% longer sampling time per pixel via over-sampling
- best light collection efficiency by means of innovative grating and spectral-recycling loop design
- NDD with new electronics and optics optimally positioned for the sample so as to capture scattered emission light
- GaAsP NDD detector: signal reflection directly at the lens with up to twice as high detection efficiency



## GaAsP NDD Detector for Axio Examiner

- 1 Beam Splitter
- 2 Gathering Lens
- 3 Deflection Mirror
- 4 Focusing Lens
- 5 GaAsP Detector



## **Axiolux Examiner — A Milestone for Intravital Microscopy**

Access to samples is one of the decisive factors when using intravital microscopy stands. The Axiolux Examiner provides optimal conditions for this area in particular.

In order to make the sample area as accessible as possible and to allow for optimal viewing, the optical axis of the new stand was shifted all the way forward. With a sample area up to 10 cm high, the Axiolux Examiner is especially suited for dealing with living animals. The motorized components are controlled via the separate TFT Remote Control Panel or via the manual controls positioned at the front of the stand. The system can be set to optimally meet individual needs by choosing holders for either 1, 2, or 4 lenses. The zero-current mode also allows for extremely precise electrophysiological measurements.

When used in conjunction with the LSM 710 NLO, the system offers additional advantages such as the motorization of the condenser carrier in order to maintain focus while imaging large Z-stacks with NDDs in transmission. The high value for light guidance at the optical ports and within the NDDs assures the largest possible light collection efficiency. Moreover, the system also supports additional contrasting techniques such as DIC or Dodt contrast.

# Special Imaging Modes — More Than “Just” Multiphoton Microscopy

Thanks to its excellent signal-to-noise ratio, the LSM 710 NLO offers additional possibilities for image acquisition and analysis that go beyond conventional imaging.

The system is the first turnkey system to offer Image-Correlation Spectroscopy (ICS), a technique developed by E. Gratton and P. Wiseman. ICS requires no special hardware and its analysis is done in the normal scanned image. ICS also produces a real image as a result. In this way, for example, information about the number, aggregation, and the diffusion coefficient of many quick-moving fluorescent molecules in a sample can be obtained.

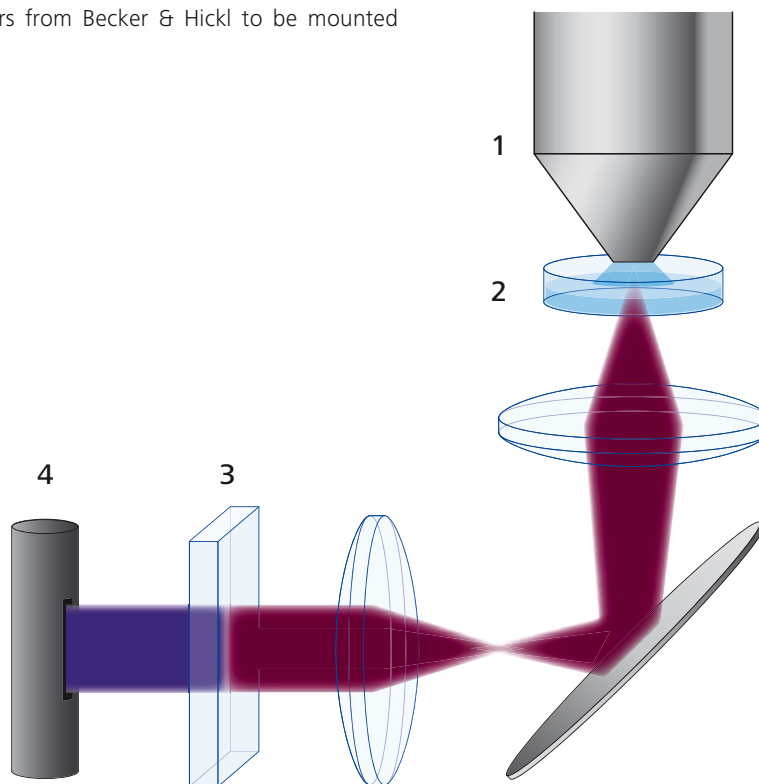
With the pulsed laser on the LSM 710 NLO, another method is available that allows molecules and even their spatial interaction to be traced. Fluorescence Lifetime Imaging Microscopy (FLIM) allows the lifetime of the emitted fluorescence to be determined, which makes it the ideal method for undertaking FRET experiments analyzing whether proteins are located less than 10 nm apart and thereby capable of interacting. The LSM 710 NLO allows for matching FLIM detectors from Becker & Hickl to be mounted instead of NDDs.

The special lens of the new condenser, which also transmits in the UV range, and the high sensitivity of the NDDs when it comes to capturing signals in transmission with the Axio Examiner, show very quickly and clearly a frequency doubling by means of anisotropic structures (SHG, Second Harmonic Generation). This additional signal can be recorded also simultaneously with differential interference contrast.

The coupling of the lasers with independently adjustable and motorized collimating lenses, allows scientists to obtain a precise overlay of the excitation levels even when combining UV manipulation (405 nm) and multiphoton imaging.

## Light Path for Detecting SHG

- 1 Objective
- 2 Specimen
- 3 Filter
- 4 Detector







## Top of the Line Objectives

Carl Zeiss has developed special lenses with extraordinary qualities for intravital and multiphoton microscopy.

The W-Plan Apochromat 20×/1.0 is the classic objective for electrophysiology with multiphoton imaging. A high numerical aperture and a 1.9 mm working distance with low magnification are the decisive qualities for both applications. With up to 30% more light collection efficiency than comparable objectives and an exceedingly high transmission up to a wavelength of 1100 nm, it is among the best in its class. Moreover, like all immersion objectives used in electrophysiology, it offers a large front angle in order to place the patch pipette on the cell under examination with minimal effort.

The W-Plan Apochromat 40×/1,0 and 63×/1,0 are additional special objectives used in electrophysiology. Their construction and level of quality make them also suitable for the high demands of confocal microscopy. In order to produce identical optical slices or to obtain high-resolution data on a single sample point using various excitation wavelengths in the infrared, these objectives offer an additional color correction in this range. When it comes to extremely complicated and challenging applications, scientists shouldn't have to compromise in their choice of objectives – Carl Zeiss has the right solutions.



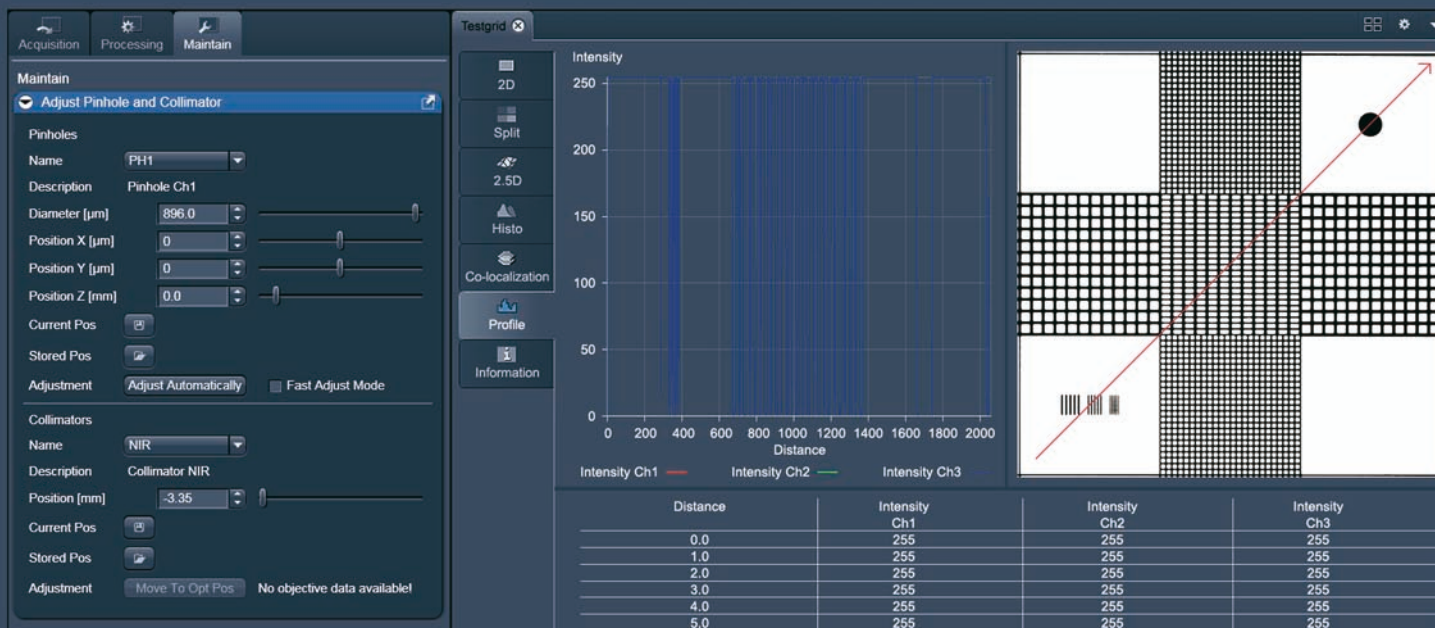
## ZEN – Efficient Navigation

Using the reliable ZEN imaging software while working with the LSM 710 NLO makes it easy to concentrate on what's most important.



The interface, which has been optimized for the 30-inch widescreen monitor, is simple, intuitive, and offers a number of very user-friendly functions. Its innovative Basic Pro concept makes it possible to keep the software simple and easy-to-use without limiting important functionality. Using the workspace zoom, the size of the display can be adjusted as needed. User- and experiment-specific settings can be saved separately and retrieved at a moment's notice. The Smart Setup, in particular, makes it much easier to operate the system, as it automatically sets the imaging parameters when knowing the dyes and markers employed.

*Smart Setup: Just choose the dyes that are in your sample, and ZEN automatically sets the rest. Depending on the application, you can select whether the image acquisition should be conducted as quickly or as exactly as possible.*



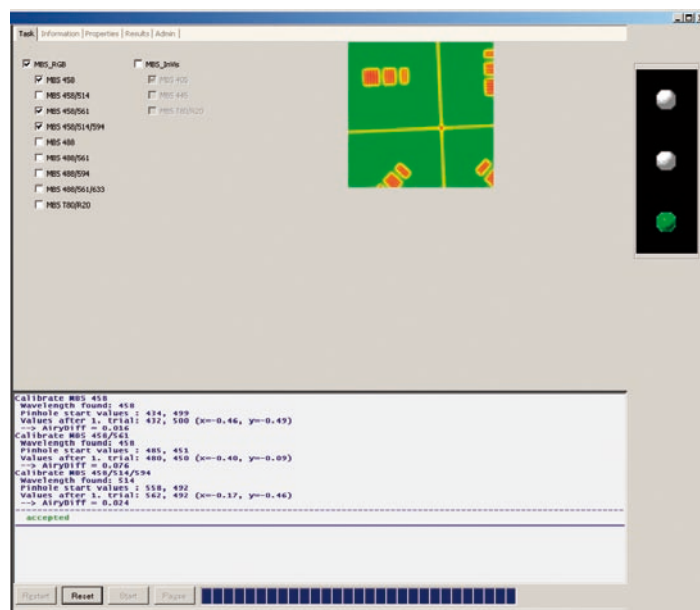
## Reliable Long Term Performance

The LSM 710 NLO offers a quick and easy general system maintenance tool.

Systems that are constantly in use and operated by many different users are at particular risk of losing their optimal calibration over longer periods of time. This can adversely affect results gathered in both comparative studies and standard applications. The maintenance tool allows the operator to automatically re-calibrate the system at any time. Moreover, the software indicates immediately whether the system is functioning optimally. As a result, the user can make sure that the same imaging settings are used when conducting comparative studies.

A special objective facilitates this uniquely easy adjustment function, which itself guarantees that the LSM 710 NLO maintains its high-standard and stable performance.

*The Maintenance Tool conducts fully automated calibration of the scanner and the opto-mechanical elements of the beam path. A series of lights on the right-hand edge of the monitor screen indicates whether the system is functioning optimally.*



# Technical Data LSM 710 NLO

## MICROSCOPES

<b>Stands</b>	Upright: Axio Imager.Z1, Axio Imager.M1, Axio Examiner*, with rear port   (*available summer 2008)
<b>Z drive</b>	Smallest increments: Axio Imager.Z1: < 25 nm; Axio Imager.M1: < 25 nm; Axio Observer.Z1: < 25 nm; Axio Examiner*: < 30 nm; fast Piezo objective or stage focus accessory; Definite Focus unit for inverted stand   (*available summer 2008)
<b>XY stage (option)</b>	Motorized XY-scanning stage, with Mark & Find function (xyz) and Tile Scan (mosaic scan); smallest increments 1 µm (Axio Observer) or 0.2 µm (Axio Imager)
<b>Accessories</b>	Digital microscope camera AxioCam; integration of incubation chambers

## SCANNING MODULE

<b>Models</b>	Scanning module with 2, 3 or 34 spectral detection channels; high QE, 3 × lower dark noise; up to 10 individual, adjustable digital gains; prepared for lasers from V (405) to IR
<b>Scanners</b>	Two independent, galvanometric scan mirrors with ultra-short line and frame flyback
<b>Scan resolution</b>	4 × 1 to 6144 × 6144 pixels; also for multiple channels; continuously variable
<b>Scanning speed</b>	14 × 2 speed stages; up to 12.5 frames/sec with 256 × 256 pixels; 5 frames/sec with 512 × 512 pixels (max. 77 frames/sec 512 × 32); min 0.38 ms for a line of 512 pixels; up to 2619 lines per second
<b>Scan zoom</b>	0.6 × to 40 ×; digital variable in steps of 0.1 (on Axio Examiner 0.67 × to 40 ×)
<b>Scan rotation</b>	Free rotation (360 degrees), in steps of 1 degree variable; free xy offset
<b>Scan field</b>	20 mm field diagonal (max.) in the intermediate plan, with full pupil illumination
<b>Pinholes</b>	Master-pinhole pre-adjusted in size and position, individually variable for multi-tracking and short wavelengths (e.g. 405 nm)
<b>Beam path</b>	Exchangeable TwinGate main beam splitter with up to 50 combinations of excitation wavelengths and outstanding laser light suppression; optional laser notch filters for fluorescence imaging on mirror-like substrates (on request); outcoupling for external detection modules (e.g., FCS, B&H FLIM); low-loss spectral separation with Recycling Loop for the internal detection
<b>Spectral detection</b>	Standard: 2, 3 or 34 simultaneous confocal fluorescence channels with highly sensitive low dark noise PMTs; spectral detection range freely selectable (resolution down to 3 nm); additionally two incident light channels with APDs for imaging and single photon measurements; transmitted light channel with PMT; cascaded non-descanned detectors (NDD) with PMT and GaAsP NDD unit for Axio Examiner
<b>Data depth</b>	8-bit, 12-bit or 16-bit selectable; up to 37 channels simultaneously detectable

## LASER INSERTS

<b>Laser inserts (VIS, V)</b>	Pigtail-coupled lasers with polarization preserving single-mode fibers; stabilized VIS-AOTF for simultaneous intensity control; switching time < 5 µs, or direct modulation; up to 6 V/VIS-laser directly mountable into the scanhead; diode laser (405 nm, CW/pulsed) 30 mW; diode laser (440 nm, CW+pulsed) 25 mW; Ar-laser (458, 488, 514 nm) 25 mW or 35 mW; HeNe-laser (543 nm) 1 mW; DPSS-laser (561 nm) 20 mW; HeNe-laser (594 nm) 2 mW; HeNe-laser (633 nm) 5 mW (pre-fiber manufacturer specification)
<b>External lasers (NLO, VIS, V)</b>	Prepared laser ports for system extensions; direct coupling of pulsed NIR lasers of various makes (incl. models with prechirp compensation); fast intensity control via AOM; NIR-optimized objectives and collimation; fiber coupling (single-mode polarization preserving) of external manipulation lasers of high power in the VIS range 488–561 nm (e.g., LSM 7 DUO-systems)

## ELECTRONICS MODULE

<b>Realtime electronics</b>	Control of the microscope, the lasers, the scan module and other accessory components; control of the data acquisition and synchronization by real-time electronics; over-sampling read out logic for best sensitivity and 2 × better SNR; data communication between real-time electronics and user PC via Gigabit-Ethernet interface with the possibility of online data analysis during image acquisition
<b>User PC</b>	Workstation PC with abundant main and hard disk memory space; ergonomic, high-resolving 16:10 TFT flat panel display; various accessories; operating system Windows XP or VISTA (depending on availability); multi-user capable

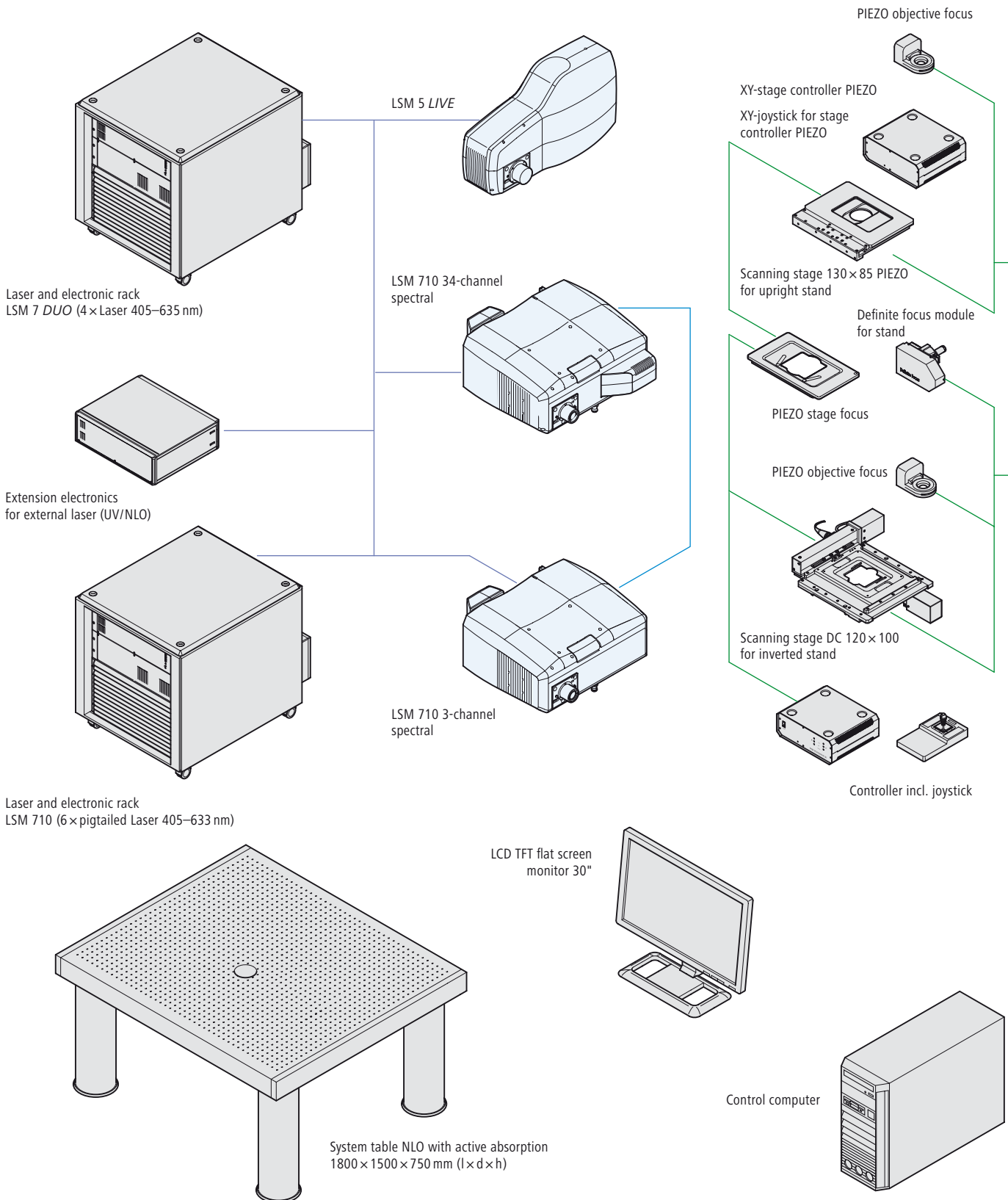
## STANDARD SOFTWARE (ZEN)

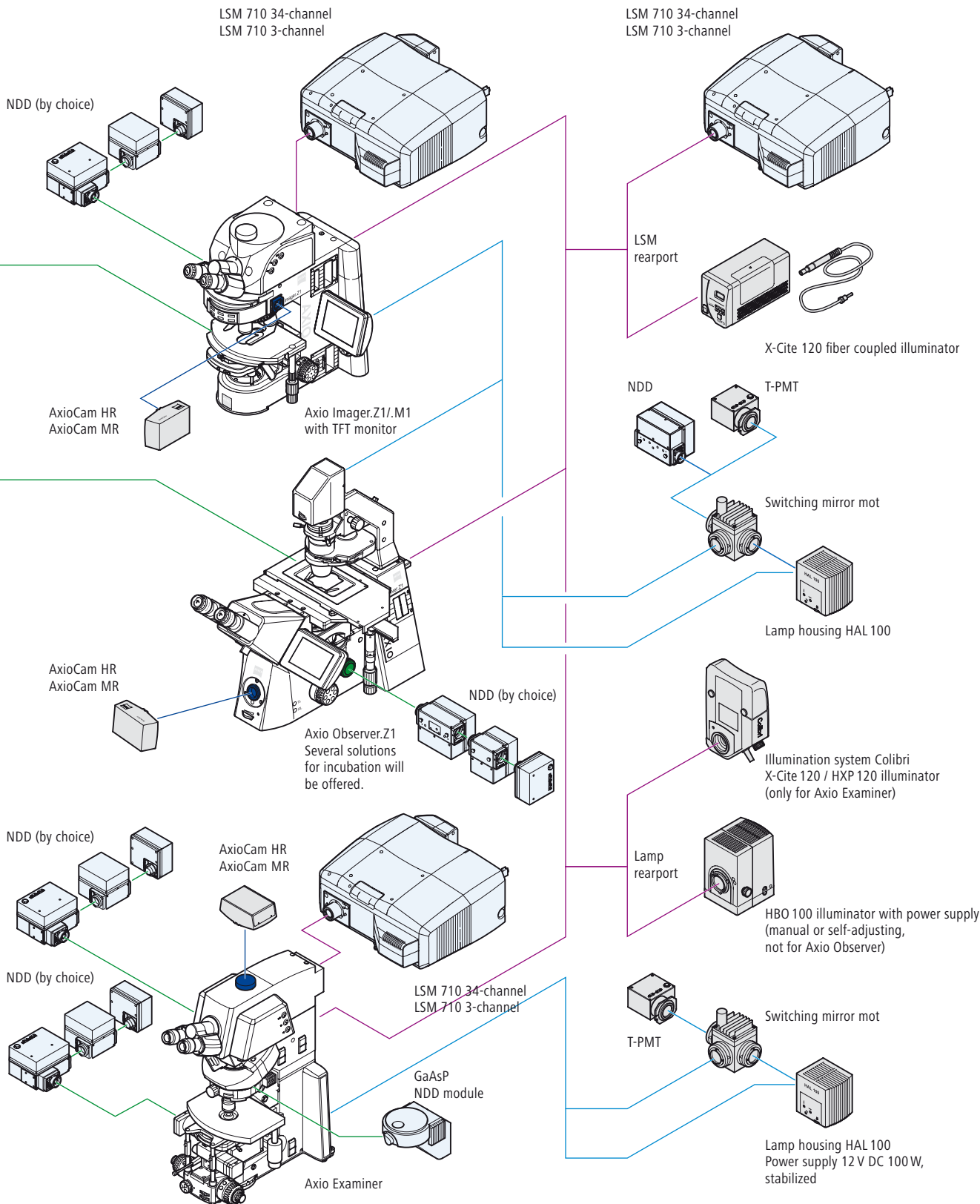
<b>System configuration</b>	Workspace for comfortable configuration of all motorized functions of the scanning module, the lasers and the microscope; saving and restoring of application-specific configurations (ReUse)
<b>System self-test</b>	Calibration and testing tool for the automatic verification and optimal adjustment of the system
<b>Acquisition modes, Smart Setup</b>	Spot, line/spline, frame, z-stack, lambda stack, time series and all combinations (xyz λ t); online calculation and display of ratio images; averaging and summation (line/frame-wise, configurable); step scan (for higher frame rates); smart acquisition setup by selection of dyes
<b>Crop function</b>	Convenient and simultaneous selection of scanning areas (zoom, offset, rotation)
<b>RealROI scan, spline scan</b>	Scanning of up to 99 arbitrarily shaped ROIs (Regions of Interest); pixel-precise switching of the laser; ROI definition in z (volume); scan along a freely defined line
<b>ROI bleach</b>	Localized bleaching of up to 99 bleach ROIs for applications such as FRAP (Fluorescence Recovery After Photobleaching) or uncaging; use of different speeds for bleaching and image acquisition; use of different laser lines for different ROIs
<b>Multitracking</b>	Fast change of excitation lines at sequential acquisition of multicolor fluorescence for reduction of signal crosstalk
<b>Lambda scan</b>	Parallel or sequential acquisition of image stacks with spectral information for each pixel
<b>Linear unmixing</b>	Generation of crosstalk-free multifluorescence images with simultaneous excitation; spectral unmixing – online or offline, automatically or interactively; advanced logic with reliability figure
<b>Visualization</b>	XY, orthogonal (xy, xz, yz); cut (3D section); 2.5D for time series of line scans; projections (maximum intensity); animations; depth coding (false colors); brightness; contrast and gamma settings; color selection tables and modification (LUT); drawing functions
<b>Image analysis and operations</b>	Colocalization and histogram analysis with individual parameters; profile measurements on any line; measurement of lengths, angles, surfaces, intensities etc; operations: addition, subtraction, multiplication, division, ratio, shift, filtering (low pass, median, high-pass, etc; also customizable)
<b>Image archiving, exporting &amp; importing</b>	Functions for managing of images and respective recording parameters; multi-print function; over 20 file formats (TIF, BMP, JPG, PSD, PCX, GIF, AVI, Quicktime, etc) for export

## OPTIONAL SOFTWARE

<b>LSM Image VisArt plus</b>	Fast 3D and 4D reconstruction; animation (different modes: shadow projection, transparency projection, surface rendering); package 3D for LSM with measurement functions upon request
<b>3D deconvolution</b>	Image restoration on the basis of calculated point-spread function (modes: nearest neighbor, maximum likelihood, constraint iterative)
<b>Physiology/ Ion concentration</b>	Extensive analysis software for time series images; graphical mean of ROI analysis; online and off-line calibration of ion concentrations
<b>FRET plus</b>	Recording of FRET (Fluorescence Resonance Energy Transfer) image data with subsequent evaluation; supports both the methods acceptor photobleaching and sensitized emission
<b>FRAP</b>	Wizard for recording of FRAP (Fluorescence Recovery After Photobleaching) experiments with subsequent analysis of the intensity kinetics
<b>Visual macro editor</b>	Creation and editing of macros based on representative symbols for programming of routine image acquisitions; package multiple time series with enhanced programming functions upon request
<b>VBA macro editor</b>	Recording and editing of routines for the automation of scanning and analysis functions
<b>Topography package</b>	Visualization of 3D surfaces (fast rendering modes) plus numerous measurement functions (roughness, surfaces, volumes)
<b>StitchArt plus</b>	Mosaic scan for large surfaces (multiple XZ profiles and XYZ stacks) in brightfield mode
<b>ICS image correlation spectroscopy (PMT)</b>	Single molecule imaging and analysis for all LSM 710 systems with PMT detectors (publ. by Gratton)

# System Overview LSM 710 NLO





## The LSM 710 NLO at a Glance

- Cascading possible for up to 5 reflected light and 5 transmitted NDDs (depending on stand)
- Additional high-performance GaAsP NDD detector for Axio Examiner
- Objective W-Plan Apochromat 20×/1,0 NA
- Transmission PMT for the simultaneous illustration of the differential interference contrast or Dodt contrast in addition to the fluorescence signals
- Automatic calibration of the scanner and all of the opto-mechanical elements of the scanning head
- Fully SW-integrated femtosecond laser from various manufacturers (Newport Spectra Physics and Coherent)
- Combination of NLO laser with VIS lasers (450–640 nm) and a UV laser (405 or 440 nm) possible
- Individual collimating lenses for a precise overlay of all the excitation wavelengths in use
- High-speed scanning mirror with 5 fps at 512×512 pixels

## Patents

### LSM 710

**US Patents:** 5127730, 6037583, 6167173, 6278555, 6377344, 6462345, 6631226, 6848825, 6941247

**German Patents:** 19702753, 19702752, 69131176

**EP Patent:** 0977069

### LSM 710 mit Array Detection

**US Patents:** 6403332, 6747737, 6750036, 6858852, 6891613, 6958811, 7009699

**German Patents:** 19915137, 10038526, 10033180

### LSM 710 NLO

**US Patents:** 5034613, 617804, 6403332, 6867915, 7119898

**German Patents:** 69032621, 69034117

### LSM 7 DUO

**US Patents:** 6037583, 6462345, 6848825, 6888148, 6947127

**EP Patent:** 1617264





## Perfection Is No Miracle

The precision and performance of our instruments derives from our ongoing pursuit of technological perfection. Our products have provided the stepping-stones for many important discoveries and scientific breakthroughs.

**Carl Zeiss MicroImaging GmbH**  
07740 Jena, Germany

BioSciences  
Phone : +49 3641 64 3400  
Telefax: +49 3641 64 3144  
E-Mail : [micro@zeiss.de](mailto:micro@zeiss.de)

[www.zeiss.de/lsm](http://www.zeiss.de/lsm)