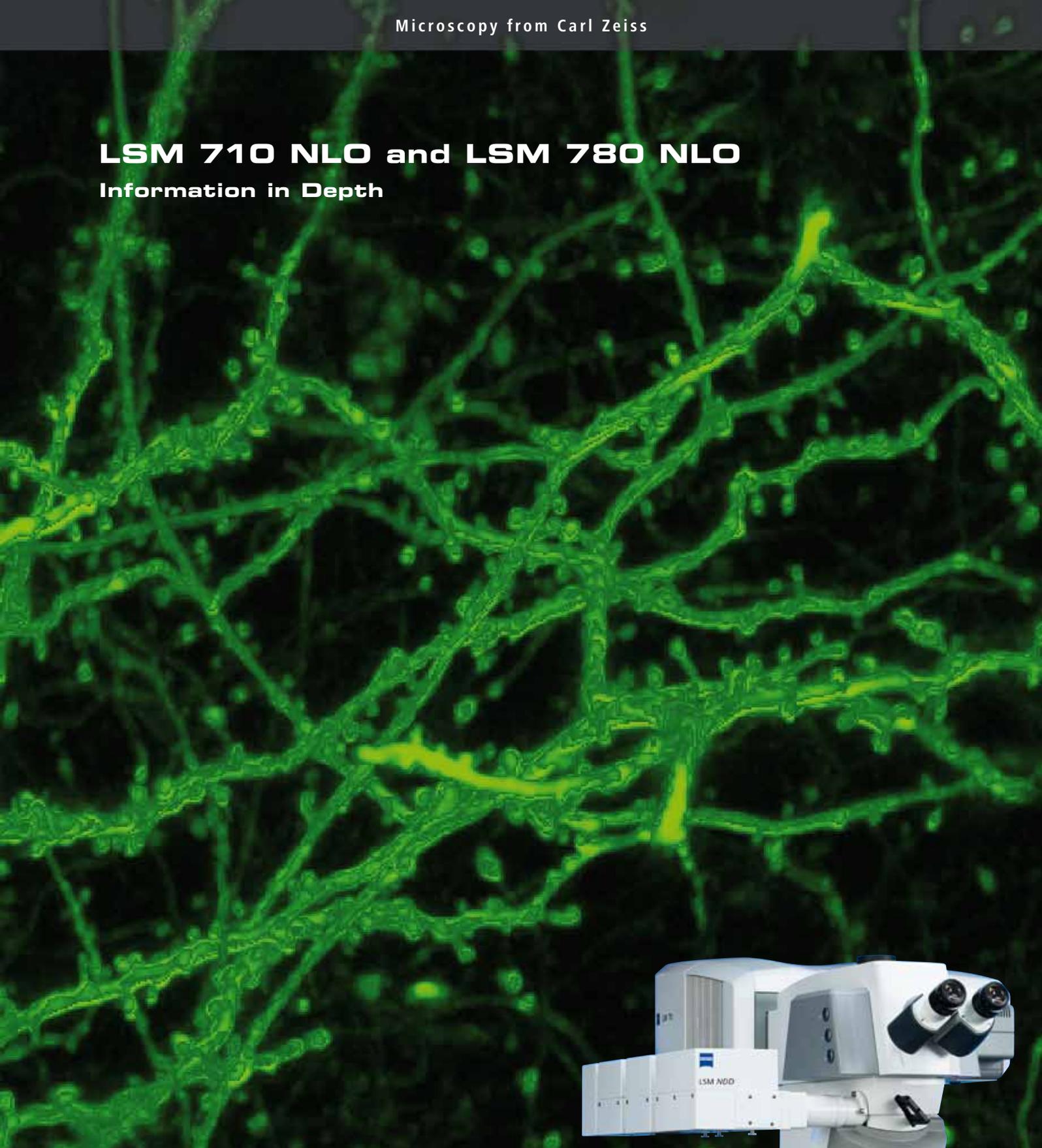


# LSM 710 NLO and LSM 780 NLO

Information in Depth



Innovative Systems for Multiphoton Microscopy



We make it visible.

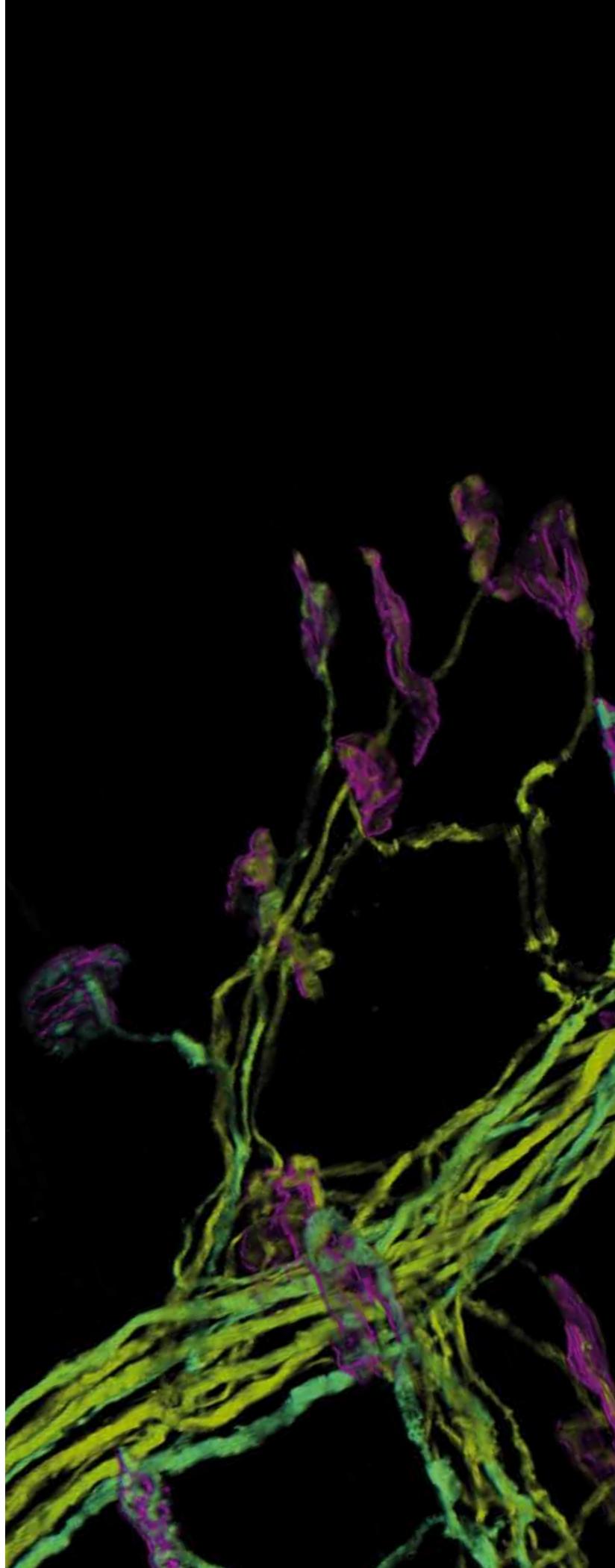
## Providing Support for Progress and Innovation

Biomedical sciences represent one of the most important, 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. Carl Zeiss creates ideal conditions for modern research by providing comprehensive professional consulting as well as systems and solutions tailored to users' exact needs.

*Title:  
Detailed view of dendrites and spines  
of YFP expressing neurons within  
mouse cortex.*

*Right:  
Motor neurons in the sternomastoid  
muscle of a postnatal day 9 mouse  
which constitutively express cyto-  
plasmic CFP and YFP in varying  
proportions under the thy1 promoter.  
Acetylcholine receptors are labeled  
with alpha-bungarotoxin - Alexa-647.  
Specimen provided by Stephen Turney,  
MCB, Harvard University, USA*





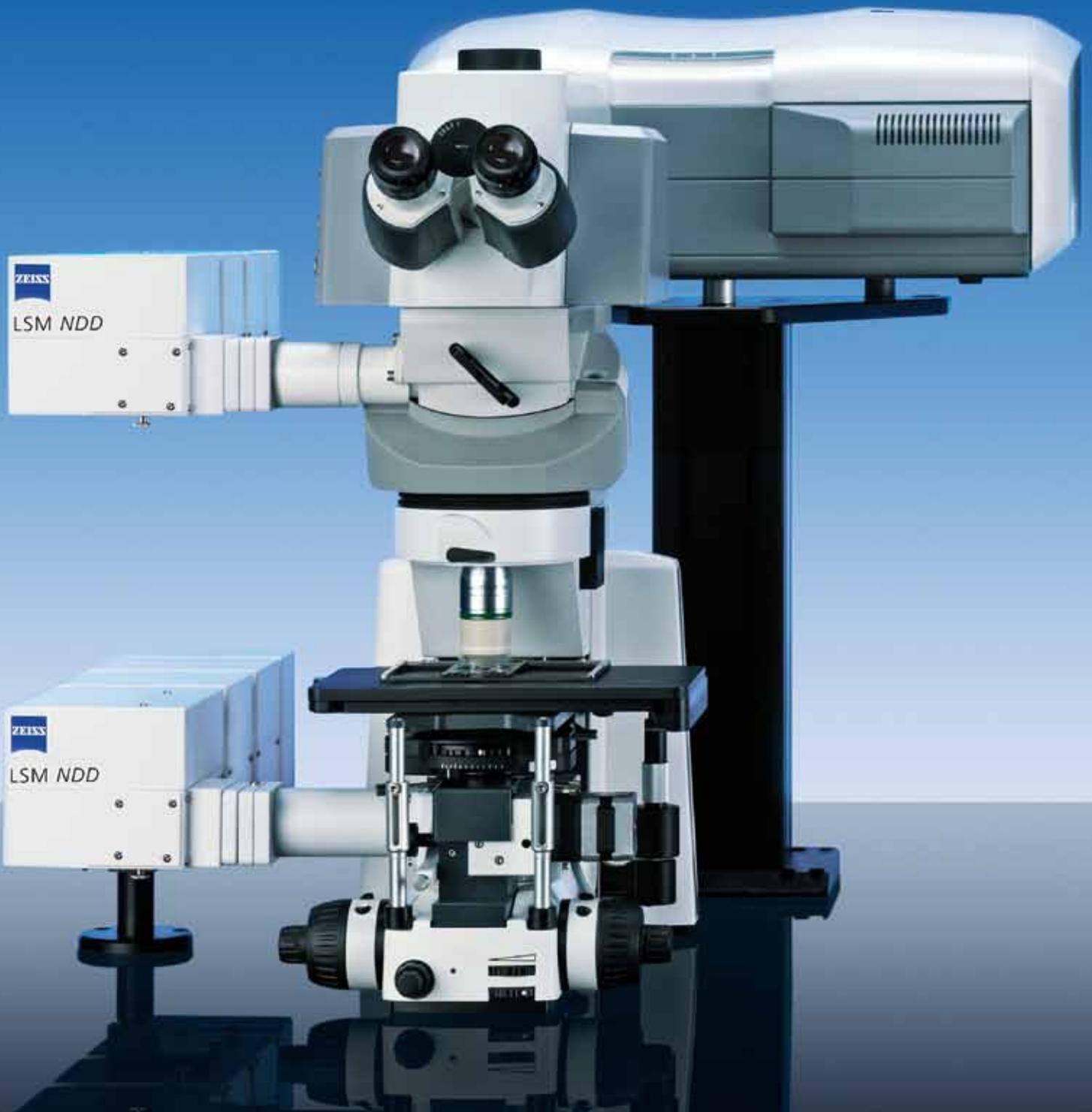
## Images in Depth

Ground-breaking research in fields such as neurophysiology, immunology, and developmental biology provides important insights into systematic connections in all life forms. Multiphoton microscopy is considered the best method in the field of minimal and non-invasive fluorescent microscopy today.

LSM 710 NLO and LSM 780 NLO allow scientists to generate images of very deep lying tissue with subcellular resolution in a gentle way.

## LSM 710 NLO on Upright Stands

Together with the Axio Examiner, the LSM 710 NLO and LSM 780 NLO represent the optimal multiphoton system for intravital imaging and electrophysiological research.



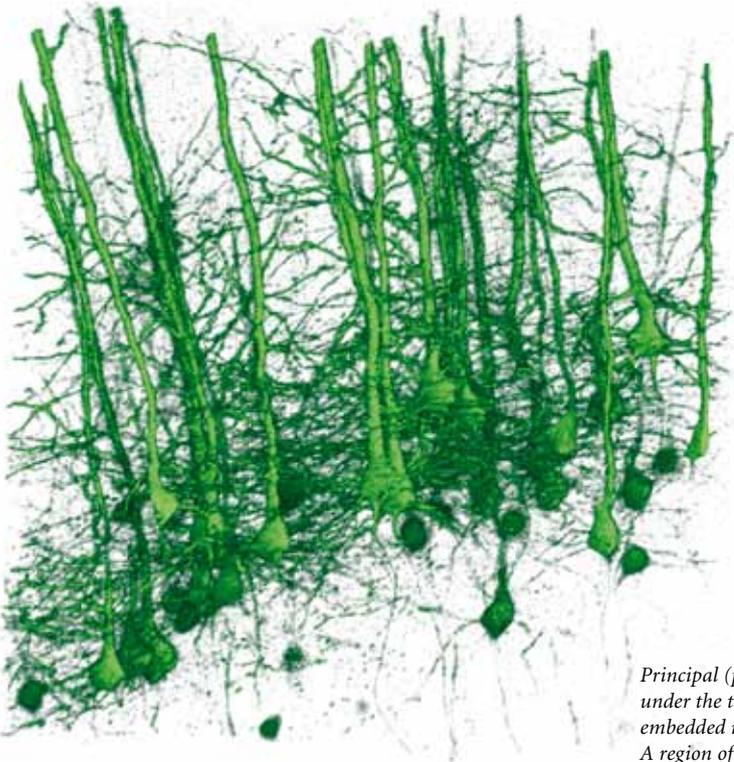
## LSM 710 NLO on Inverted Stands

In conjunction with the Axio Observer, the systems represent an incomparable multifunctional apparatus for the imaging of standard specimens and cells in culture.



# 3D Morphology

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

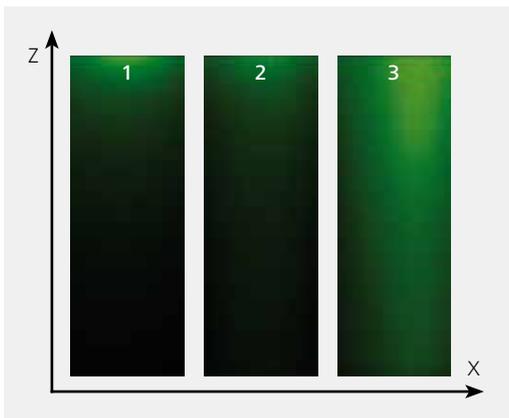


Knowing the morphology helps to understand functional inter-connections especially in brain tissue. Subcellular structures like spines and thin axonal processes demand imaging with high signal to noise ratio and of a large area or volume due to the wide spread cell processes.

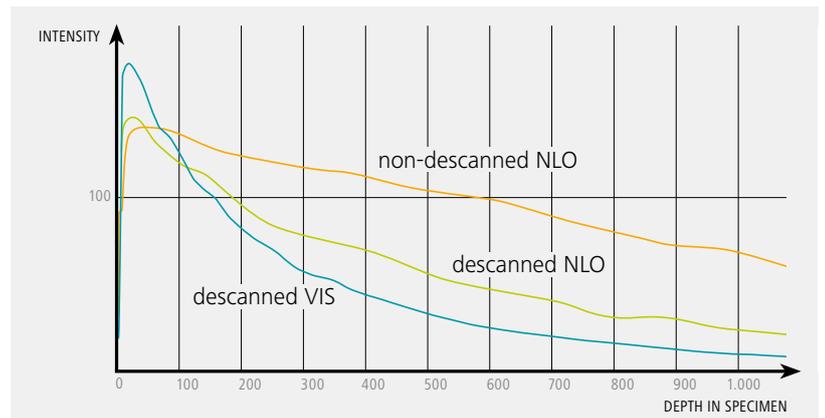
With the LSM 710 NLO or LSM 780 NLO these technical requirements are met by an extensive blocking of the excitation light and a highly efficient light guidance of the signals to the detectors. Completed with GaAsP type detectors with 2 times higher QE and lower dark noise compared to conventional PMT detectors, the systems transform fluorescent signals into 3-dimensional images that will reveal new and exciting knowledge.

*Principal (projection) neurons in cortex of a transgenic mouse that expresses YFP under the thyl1 promoter (adult, YFP-H line). The fixed forebrain was removed and 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).*

*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.*



# 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.

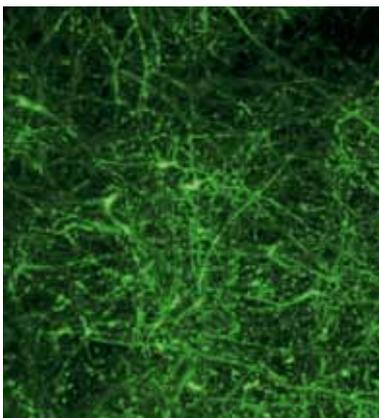


*Dendrites of cortical projection neurons of a transgenic mouse expressing YFP via the thyl1 promotor. This high-resolution image of the dendritic processes to a depth of  $430\ \mu\text{m}$  was made using multiphoton excitation of  $920\ \text{nm}$  in the living animal. Specimen provided by Stephen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

The point excitation with a pulsed IR laser is minimally invasive with a low level of phototoxicity, thereby creating the ideal conditions for the examination of living specimens. The infra-red excitation light penetrates deeper into tissue due to low scattering. This helps to visualize even subcellular structures in great depth. 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 systems also provide means to pursue very complex methods such as two-photon uncaging in connection with calcium imaging. This locally defined manipulation aids in the study of physiological 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



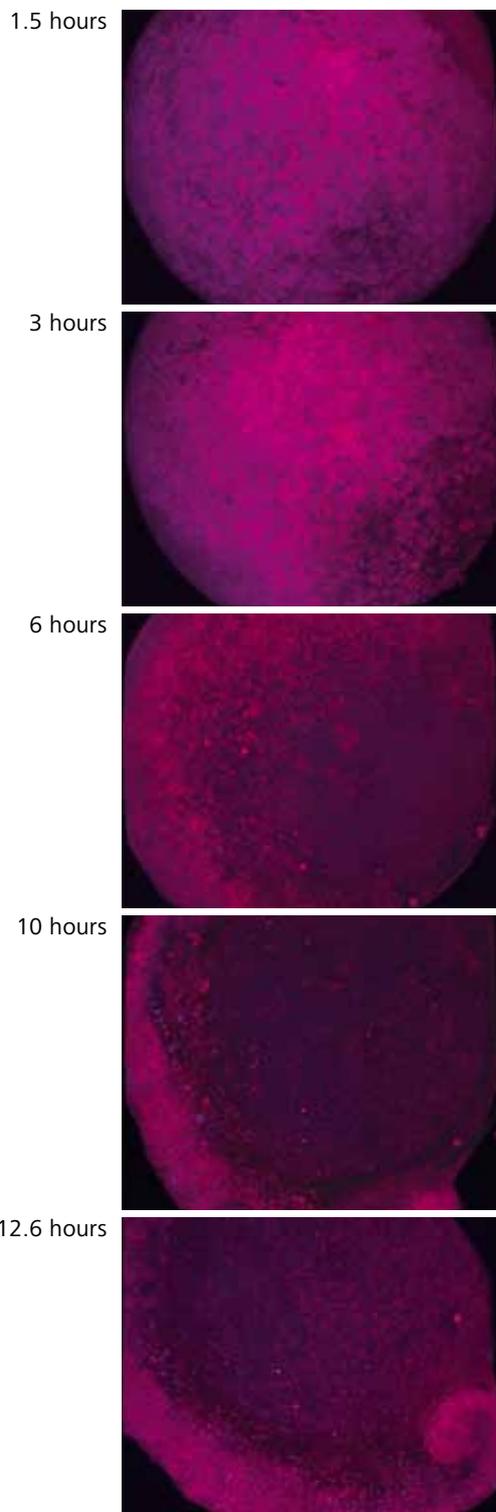
*Magnified section of the projection neurons' dendritic branches.*



*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  $20\times/1.0\ \text{NA}$  dipping objective and two photon excitation ( $880\ \text{nm}$ ). Stephen Turney, MCB, Harvard University, USA*

## 3D in Temporal Resolution

The point excitation of Multiphoton systems allows for a significant reduction in phototoxicity, as the light has an impairing effect only in the focus.



*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.*

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 and LSM 780 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.

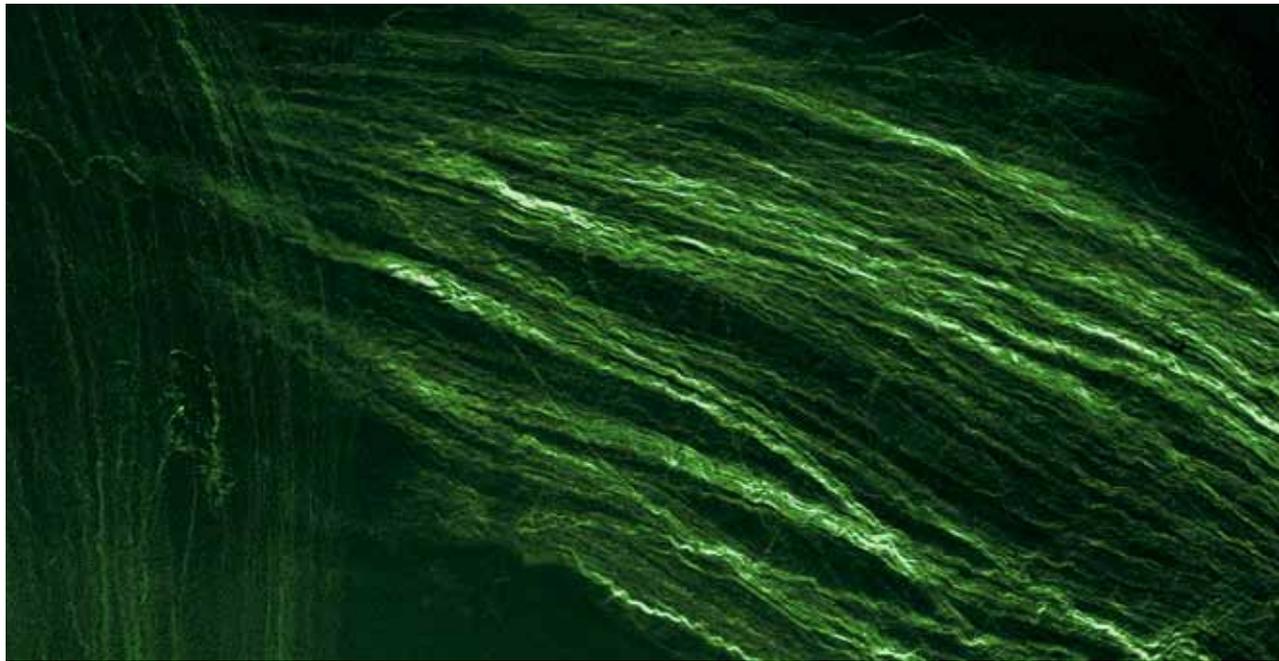
*“The LSM 710 NLO in conjunction with the new microscope Axio Examiner represents a very versatile system. When imaging embryonic stages, we are often troubled by abnormal developments caused by phototoxicity. Improved optics and detectors, especially the registration of emission signals in reflection 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*

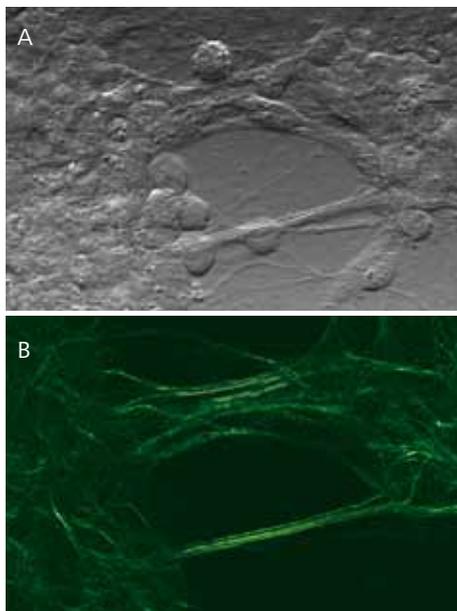
# SHG: Additional Contrast via Frequency Doubling

Second Harmonic Generation (SHG) is a non-linear photophysical effect that is used in non-linear microscopy to create additional contrast.

*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.*



*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*



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 or the LSM 780 NLO on Axio Examiner can be fully equipped for this application.

# 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 and LSM 780 NLO delivers true-to-detail and high-contrast images. The outstanding sensitivity of the systems is combined with innovative techniques to suppress laser light excitation. Improved non-descanned detectors (NDD) with extraordinary light collection efficiency and the GaAsP technology also guarantee excellent imaging results in thick tissue samples and living animals.

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

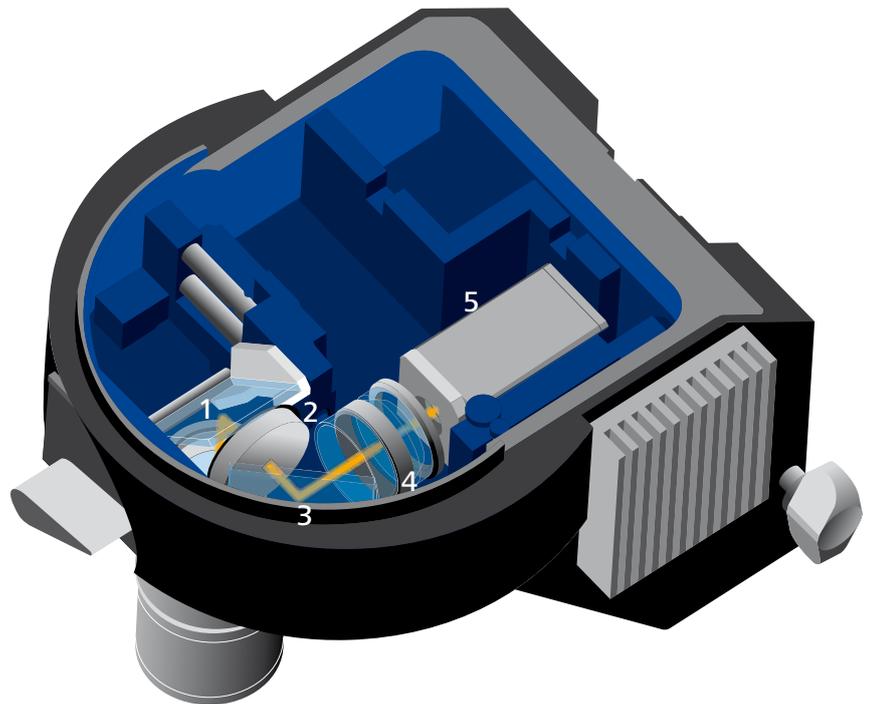
- low noise electronics with up to 30% longer sampling time per pixel via over-sampling for all detectors
- efficient light collection by means of innovative grating and spectral-recycling loop design
- NDD optimally positioned for the sample so as to capture scattered emission light
- GaAsP NDD detector and *BiG* (binary GaAsP): up to twice the detection sensitivity and 5 times lower dark noise compared to PMTs
- *BiG* also to be used in photon counting mode for very dim fluorescent samples



*BiG as non-descanned detector on Axio Observer*

## **GaAsP NDD Detector for Axio Examiner**

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





## **Axio Examiner — The Milestone for Intravital Microscopy**

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

To make the sample area easily accessible and to provide optimal viewing, the optical axis of the stand is right in front. With a sample area up to 10 cm, the Axio Examiner is especially suited for dealing with living animals. The components are controlled via the separate TFT Remote Control Panel or via controls positioned at the very front of the stand.

In conjunction with the LSM 7 family of multiphoton systems the motorization of the condenser carrier allows maintaining focus while imaging large Z-stacks with NDDs in transmission. The systems also support additional contrasting techniques such as DIC or Dodt contrast.

The W-Plan Apochromat 20x/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.

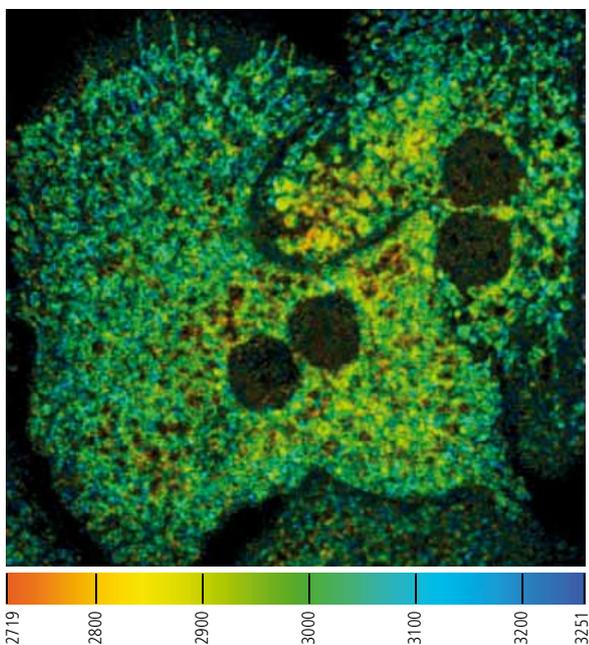
# Special Imaging Modes – More than “Just” Multiphoton Microscopy

Thanks to its excellent signal-to-noise ratio, the LSM 710 NLO and LSM 780 NLO offer additional possibilities for image acquisition and analysis.

The turnkey systems 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 multiphoton laser another method is available that allows molecules and even their spatial interaction to be traced. Fluorescence Lifetime Imaging Microscopy (FLIM) determines the lifetime of the emitted fluorescence. This makes it the ideal method for FRET experiments analyzing whether proteins are located less than 10 nm apart and thereby capable of interacting. FLIM can also be performed using *In Tune*, the pulsed tunable laser ranging from 488 to 640 nm.

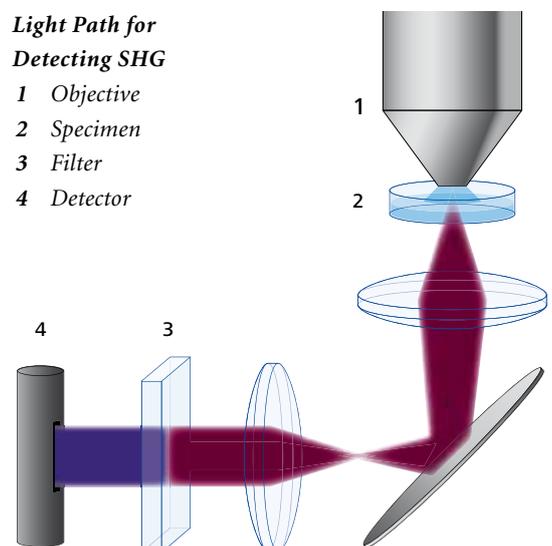
The special high NA condenser of the Axio Examiner, which also transmits in the UV range, and the highly sensitive NDDs in transmission, show very quickly and clearly a frequency doubling by means of anisotropic structures (SHG, Second Harmonic Generation). This additional signal can be recorded simultaneously with differential interference contrast. High dynamic range (HDR) imaging provides an excellent approach to balance the signal brightness for example between bright neuronal cell bodies and their thin processes.



Color coded FLIM image (ps) of hepatocytes stained for Cytochrome C (Alexa Fluor 488) and Mitochondria (Alexa Fluor 564). Lifetime image acquired using 568 nm for excitation. Specimen: R. Pick and R. Nitschke; University of Freiburg, Molecular Medicine and Life Imaging Center, Germany

### Light Path for Detecting SHG

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





## **LSM 7 MP - The Expert for Intravital Multiphoton Microscopy**

Efficient excitation combined with high detection sensitivity plus a variety of system set ups – install today, image tomorrow.

The combination of optical and electrophysiological methods helps studying changes in ion concentration within cells or visualizing changes in bioelectrical potentials of cell membranes which can be induced by a locally targeted release of neurotransmitters via photolysis. Effective optical manipulation is ideally performed sequentially or simultaneously using a second, independently set laser.

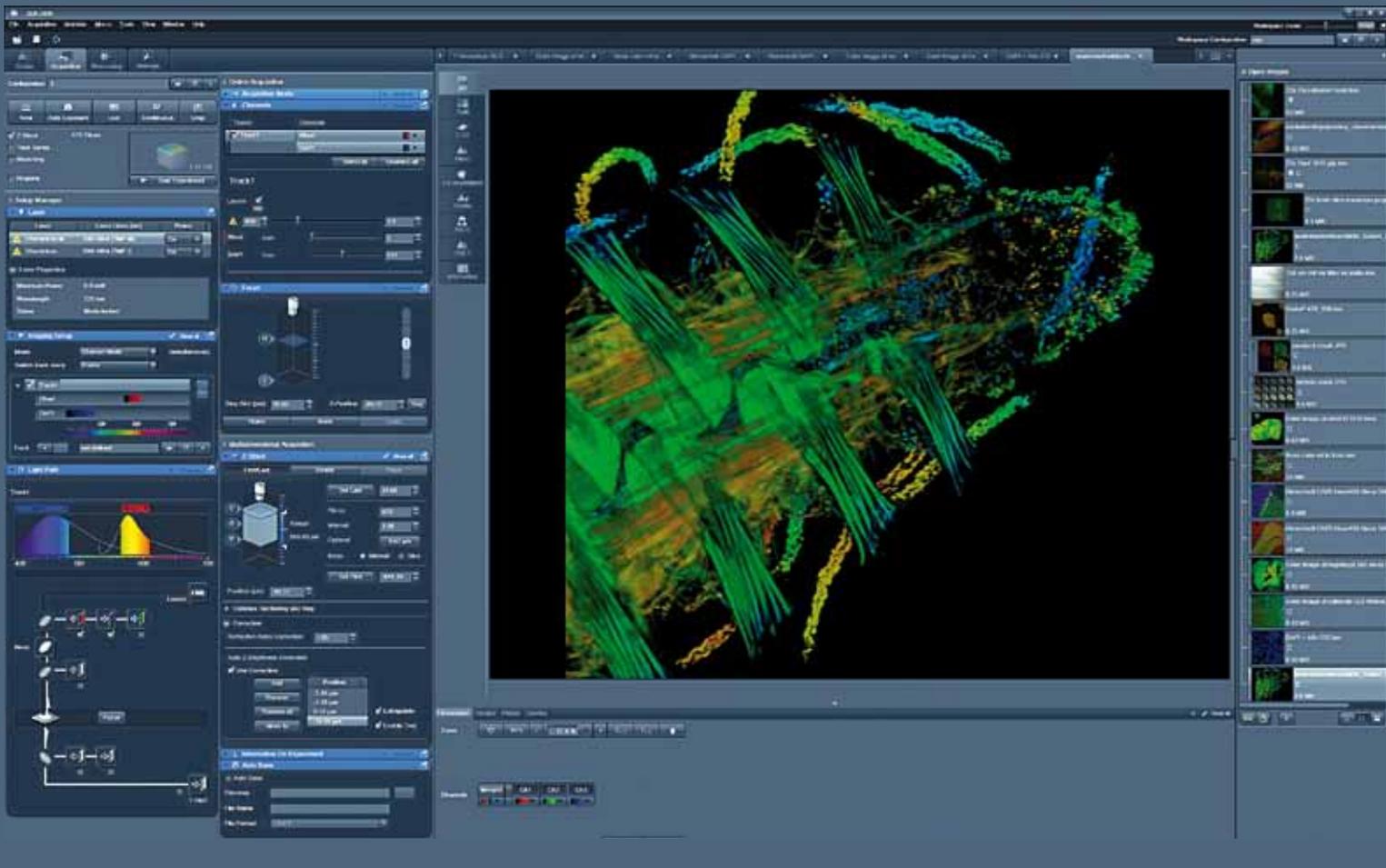
Concentrating on the essentials for multiphoton microscopy the LSM 7 MP is equipped with optimized optics reaching far into the near infrared for efficient excitation. It can be set up with one or two scanners for imaging combined with sequential or simultaneous manipulation.

A motorized stage for convenient animal positioning, incubation solutions and platforms for electrophysiology all team up to realize individual expert set ups for intravital imaging.

*“This is multiphoton microscopy made easy with all the obvious advantages. The system can supply exquisite 5D data from difficult samples.*

*It is an obvious answer to sites where multiphoton microscopy has become a priority.”*

*Dr. Dave Spiller, School of Biological Sciences,  
Center for Cell Imaging, University of Liverpool, UK*

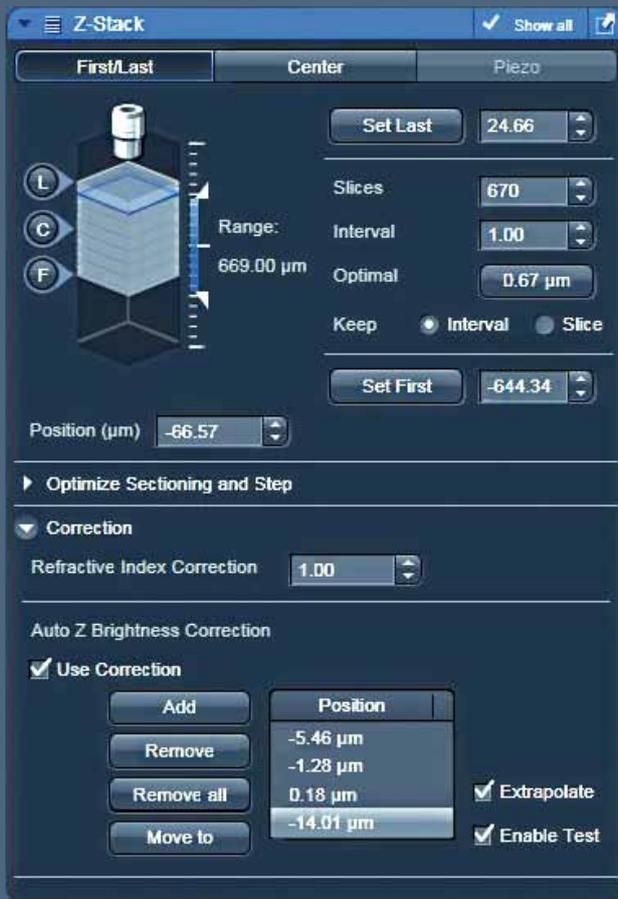


## ZEN – Efficient Navigation

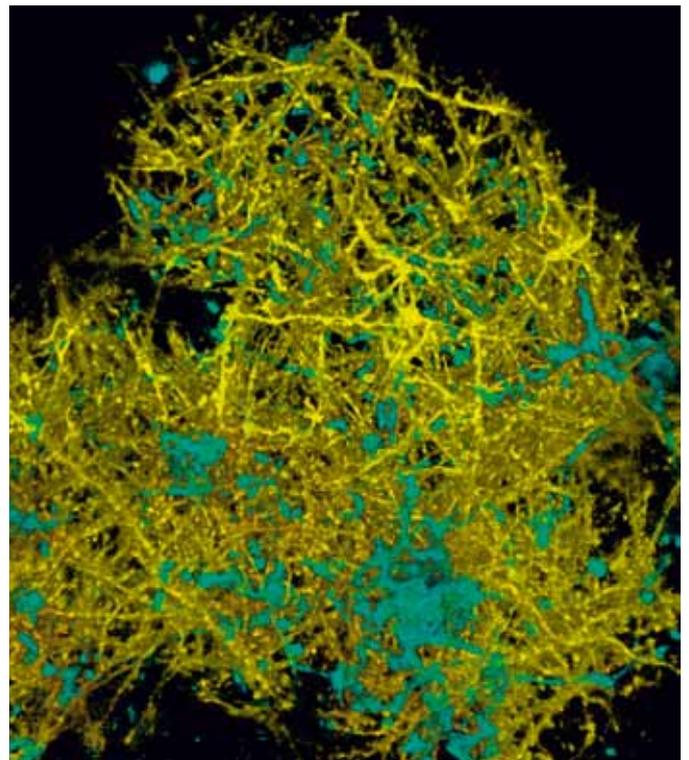
Using the reliable ZEN imaging software makes it easy to concentrate on what's most important.

The intuitive and simple interface, is optimized for a 30-inch widescreen monitor. It offers a number of very user-friendly functions. Its innovative concept for beginners and expert users keeps the software clear 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 are saved separately and retrieved at a moment's notice. Smart Set-up automatically sets the imaging parameters when knowing the dyes and markers employed providing an easy beginning for most imaging applications.

In addition there are various functions specifically important for multiphoton applications. As the multiphoton lasers including pre-chirping units are fully integrated into the software, wavelengths are set automatically when chosen for a specific task like sequential imaging with two different wavelengths or manipulating with one wavelength and imaging with another.



*Mouse cortex intravital: EYFP-expressing neurons (yellow) and microglial cells expressing EGFP (cyan). Transparency projection of 25 single images of 1.5 μm distance. Signals were additionally separated using the function Channel Unmixing. Specimen: F. Nadrigny, F. Kirchhoff, MPI of Experimental Medicine, Göttingen, Germany*



Within large Z-stacks equally bright images are generated using the automatic adjustment of imaging parameters like laser power and detector settings. For up to 10 different positions these parameters can be defined and reused with the general imaging settings.

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.

# LSM 710 NLO and LSM 780 NLO

## Technical Data

Microscopes	
<b>Stands</b>	Upright: Axio Imager.Z2, Axio Imager.M2, Axio Examiner, Axio Observer.Z1 with rear port
<b>Z drive</b>	Smallest increments: Axio Imager.Z2: <25 nm; Axio Imager.M2: <25 nm; Axio Observer.Z1: <25 nm; Axio Examiner: <30 nm; fast Piezo objective or stage focus accessory; Definite Focus unit for inverted stand
<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), 0.2 µm (Axio Imager) or 0,25 µm (Axio Examiner)
<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 for LSM 780 (45 % for GaAsP typical), 3 × lower dark noise compared to conventional PMTs; up to 10 individual, adjustable digital gains; prepared for lasers from UV to IR
<b>Scanners</b>	Two independent, galvanometric scan mirrors with ultra-short line and frame flyback; highly linear scanning process
<b>Scan resolution</b>	4 × 1 to 6144 × 6144 pixels; also for multiple channels; continuously variable
<b>Scanning speed</b>	15 × 2 speed stages; up to 8 frames/sec with 512 × 512 pixels (max. 250 frames/sec 512 × 16); up to 4000 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; 18 mm for Axio Examiner
<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 100 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; 32 × GaAsP for LSM 780 NLO; 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 or GaAsP NDD
<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>	(VIS, V, In Tune) 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 VIS-laser directly mountable in the scanning module; 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 manufacturers (including models with prechirp compensation); fast intensity control via AOM; NIR-optimized objectives and collimation; fiber coupling (single-mode polarization preserving) of external In Tune Laser, (488-640nm, <3nm width, pulsed) 1,5mW and prepared for UV laser (355nm, 60mW), 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; 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 Vista 32 or 64 bit; multi-user capable

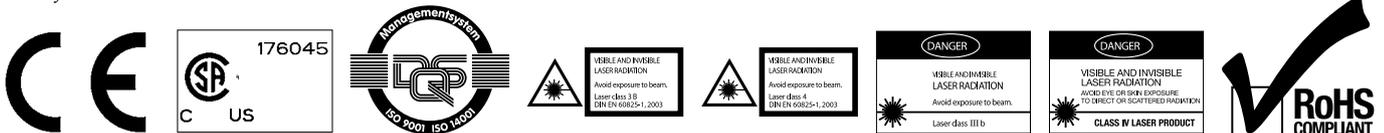
## ZEN Standard Software

<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/framewise, configurable); OSCiscan and 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 and for increased dynamics without global increase of laser exposure
<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 multicolor fluorescence 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

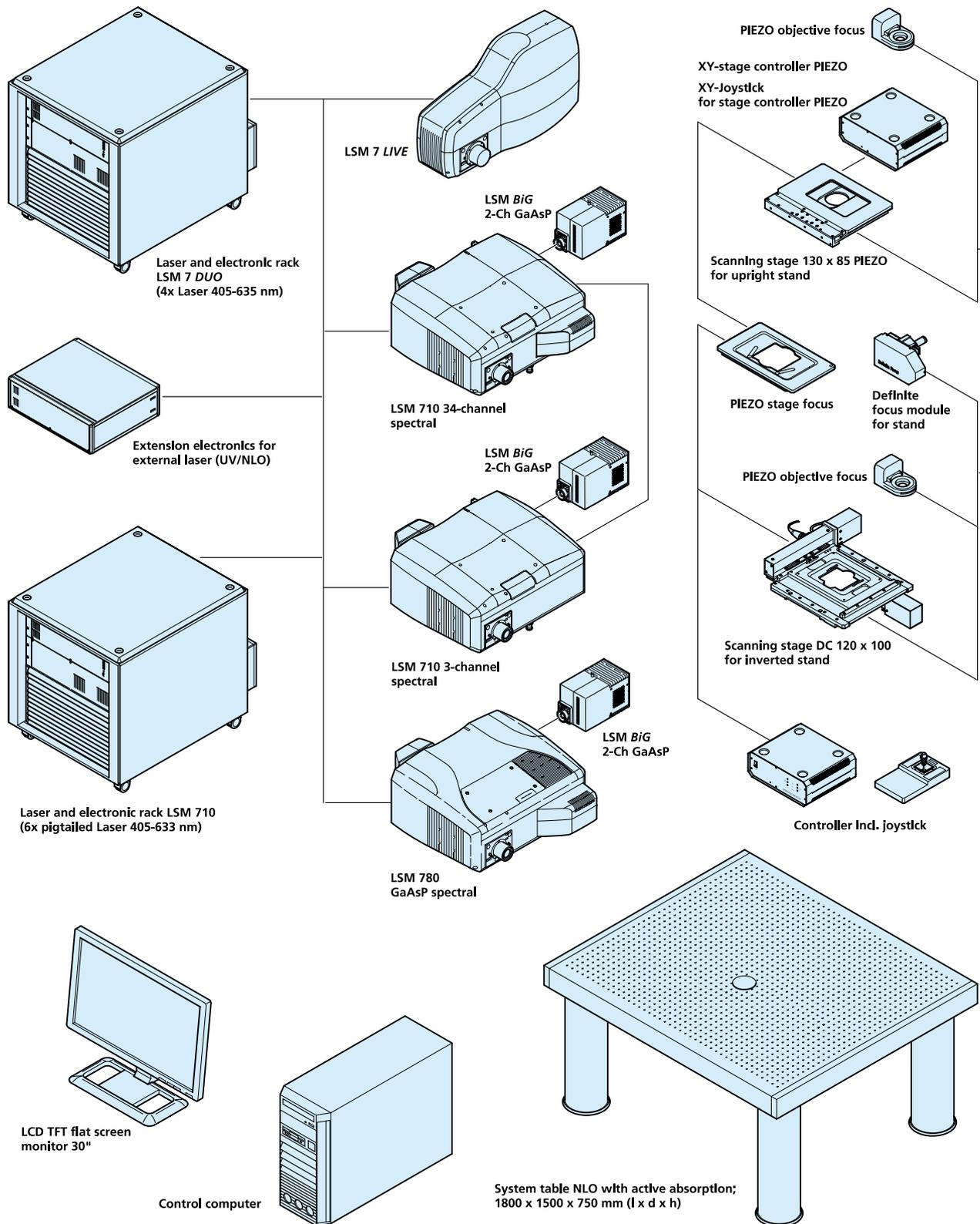
## Optional Software

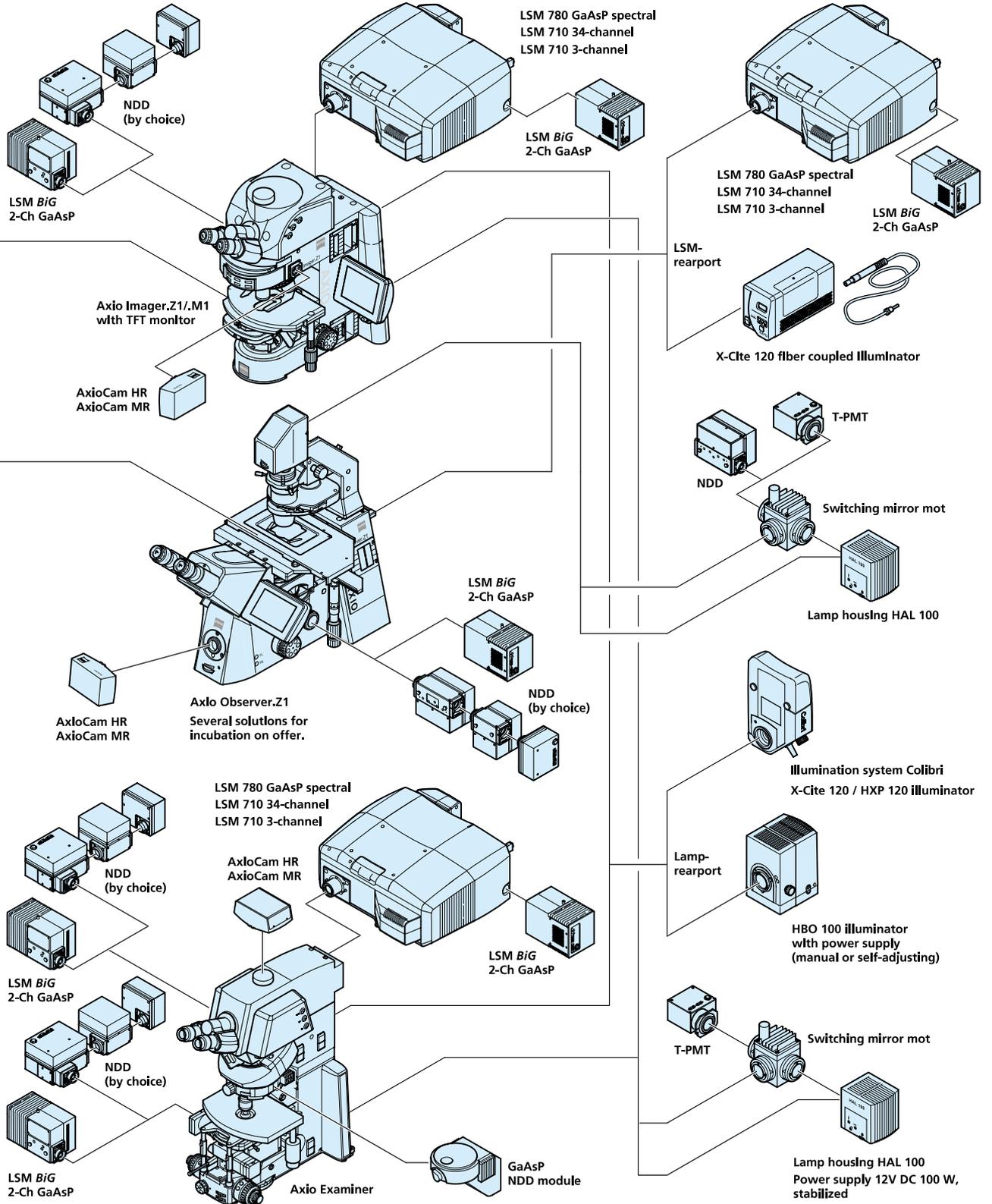
<b>LSM Image VisArt plus</b>	Fast 3D and 4D reconstruction; animation (different modes: shadow projection, transparency projection, surface rendering); package 3D for LSM with measurement functions upon request
<b>3D deconvolution</b>	Image restoration on the basis of calculated point-spread function (modes: nearest neighbor, maximum likelihood, constraint iterative)
<b>ROI-HDR</b>	High dynamic range imaging mode with intelligent local improvement of signal dynamics, free choice of gain or laser power modulation
<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>	Analysis of the intensity kinetics of FRAP (Fluorescence Recovery After Photobleaching) experiments.
<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>RICS image correlation</b>	Single molecule imaging and analysis for all LSM 710 and LSM 780 systems with PMT detectors (published by Gratton)

### Certifications:



# LSM 710 NLO and LSM 780 NLO System Overview







### **LSM 710 NLO and LSM 780 NLO**

- Cascading of up to 5 NDDs in reflected light and transmitted light position (depending on stand)
- High-performance GaAsP NDD detector and/or BiG
- Objective W-Plan Apochromat 20 ×/1,0NA
- Transmission PMT for simultaneous recording of differential interference contrast or Dodt contrast signals
- Automatic calibration of all opto-mechanical elements in the scanning head
- Fully SW-integrated femtosecond lasers
- Combination of NLO laser with VIS lasers (450–640 nm), UV laser and In Tune possible
- Individual collimating lenses for a precise overlay of all the excitation wavelengths in use
- High-speed scanning mirror with up to 8 fps

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