LSM 510 and LSM 510 META Laser Scanning Microscopes

Operating Manual
Release 3.2

ZEISS

Knowledge of this manual is required for the operation of the instrument. Would you therefore please make yourself familiar with the contents of this manual and pay special attention to hints concerning the safe operation of the instrument.

The specifications are subject to change; the manual is not covered by an update service.

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How to make best use of the LSM 510 operating instructions

A few symbols in these operating instructions will help you to recognize the nature and purpose of information immediately:

The WARNING symbol warns against hazards for the user that might arise when operating the laser.

This WARNING symbol warns against hazards from dangerously high voltages.

The CAUTION symbol warns against faults and hazards that might arise during operation and which might cause damage to the unit.

☞ The NOTE symbol will help you to optimally solve your work problem. It represents a practical tip which will help you to find out which settings and methods are capable of improving or accelerating a procedure.

The HOT SURFACE symbol warns against hazards for the user that might arise when touching the lamp housing during operation.

The MAINS PLUG symbol remembers service personal to pull the mains plug before opening the device housing.

Depending on the problem, these operating instructions will supply you with various possibilities:

- If you want to know where to find certain general areas of information, refer to the following outline of sections to get a general overview.
- You will find a detailed table of contents at the start of every chapter. There you will see at a glance what topics are dealt with in detail.

Always remember: The time you invest in getting acquainted with the product will pay for itself many times over in your application task.

Scope of Equipment Supplied

Country:	.....
Order number:	.....
Serial number:	.....
Delivery date:	.....
Custom configuration:	.....

Axioskop 2 FS mot	000000-1056-2308	☐
Axioplan 2 imaging MOT (ie)	000000-1166-265	☐
Axiovert 200 M SP	000000-1115-092	☐
Axiovert 200 M BP	000000-1115-094	☐
Axiovert 200 M BP/SP	000000-1115-289	☐

Objectives: .....
.....
.....

Confocal Laser Scanning Module LSM 510		
Configuration 1	000000-1219-975	☐
Configuration 2	000000-1219-977	☐
Configuration 3	000000-1219-978	☐
Configuration 4	000000-1219-979	☐
Configuration 5	000000-1219-980	☐
Configuration 9	000000-1219-981	☐
Configuration 11	000000-1219-982	☐
Configuration 12	000000-1219-983	☐
Configuration 13	000000-1219-984	☐
Configuration 15	000000-1219-985	☐
Configuration 16	000000-1219-986	☐
Configuration 18	000000-1219-987	☐
Configuration 3 META	000000-1219-995	☐
Configuration 13 META	000000-1219-997	☐
Configuration 15 META	000000-1220-000	☐
Configuration 18 META	000000-1220-004	☐

Control computer	000000-0438-360	☐
21“ monitors	000000-1060-089	☐
TFT monitors	000000-0435-035	☐

The license to the LSM control software is included in each configuration 1...18 (META).

Optional software:		
Image VisArt option	000000-1207-934	☐
3D for LSM option	000000-1207-919	☐
3D Deconvolution option	000000-1207-920	☐
Physiology option	000000-1207-930	☐
Topography option	000000-1207-933	☐
Multiple Time Series option	000000-1207-926	☐
Macro Reader and Editor option	000000-1207-925	☐
StitchArt option	000000-1207-932	☐
Canon S 830 D Photo printer	000000-0445-508	☐
Kodak XLS 8650 PS printer	000000-1113-131	☐
Transparency exposure device (D)	412410-9011-000	☐
Large system table	453031-0000-000	☐
Small system table	453032-0000-000	☐
System baseplate	000000-1171-342	☐
Active anti-vibration table 30" x 30"	000000-1177-841	☐
Active anti-vibration table 1200 mm x 1400 mm	000000-1198-394	☐
Active anti-vibration table 1800 mm x 1400 mm	000000-1150-353	☐
UV laser	412410-9015-000	☐
XY scanning stage for Axiovert 200 M BP	000000-1113-509	☐
XY scanning stage for Axioplan 2 imaging MOT	000000-1027-823	☐
Piezo objective drive W = 0.8"	000000-1210-045	☐
High resolution Z stage HRZ 200 for Axiovert 200 M	000000-1115-248	☐
High resolution Z stage HRZ 200 for Axioplan 2 imaging MOT (ie)	000000-1115-245	☐
Set of INDO filters	447960-0000-000	☐
Set of SNARF filters	447961-0000-000	☐
AxioCam HRm	000000-0445-553	☐
AxioCam HRc	000000-0412-312	☐

The LSM 510 in the configuration as checked above

was installed and handed to the customer in functional condition

on .....

by .....

Phone: .....

Fax: .....

The customer has been instructed on how to operate and maintain the equipment.

(Place)....., (date) .....

.....
Carl Zeiss Jena GmbH
Microscopy Division

.....
Customer

One copy to be kept by customer
One copy to be kept by Carl Zeiss

CHAPTER 1 NOTES ON DEVICE SAFETY

CONTENTS

1 NOTES ON DEVICE SAFETY

1.1 General

The LSM 510 laser scanning microscope, including its original accessories and compatible accessories from other manufacturers, may only be used for the purposes and microscopy techniques described in this manual (intended use).

The manufacturer will not assume liability for any malfunction or damage caused by any thing other than the intended use of the LSM 510 or individual modules or parts of it, nor by any repair or other service operation performed or attempted by persons other than duly authorized service staff. Any such action will invalidate any claim under warranty, including parts not directly affected by such action. This also includes the modification of the system computer with new cards, etc. by the user.

1.2 Regulations

Extensive knowledge of the hardware/the system is indispensable for safe operation of the LSM 510.

☞ Read these operating instructions and all device publications belonging to the system conscientiously **before** operating the LSM 510! You can obtain additional information on the hardware configuration delivered and on optional system extensions from the manufacturer or via the service hotline.

⇒ The LSM 510 has been designed, built and tested in conformity with the standards DIN EN 61010-1 (IEC 1010-1) "Safety requirements for electrical instrumentation and control and laboratory apparatus", and DIN EN 60825-1 (IEC publication 825-1) "Safety of laser equipment", and taking relevant CSA and UL specifications into account.

⇒ As the system is largely operated via menus on a computer, you should be acquainted with the principles of the operating system and its WINDOWS NT 4.0 and WINDOWS 2000 graphical user interface. The respective manuals are supplied together with the programs.

⇒ In accordance with WHO regulations, the LSM 510 is a device that belongs to laser hazard class 3 B. WHO recommendations concerning health and industrial protection when handling laser devices must be observed. The operator of the unit must also observe all and any relevant statutory accident prevention regulations.

1.3 Notes on Setting up the Microscope System

☞ Setting up, assembly on the system base plate and commissioning of the LSM 510 must be performed by authorized Carl Zeiss service staff, who are also advised to give the customer's operators a basic introduction to operation and maintenance.

The LSM 510 laser scanning microscope is delivered in several crates:

- Crate 1: microscope stand, laser scanning module, control unit
- Crate 2: computer
- Crate 3: monitor
- Crate 4: large system table
- Crate 5: second microscope stand
- Crate 6: small system table
- Crate 7: scan module META
- Crate 8: upgrade kit META

The LSM 510 must be set up so as to ensure that the minimum clearance between the wall and the rear of the system is no less than 0.5 m. This clearance is needed for adjustment and maintenance operations.

Do not set up the unit in the proximity of heat sources such as radiators or direct sunlight. To avoid heat build-ups, the ventilation louvers on the microscope system must not be covered up.

The unit must be connected to a properly installed socket outlet with earthing contact by means of the mains cables supplied. Continuity of PE connection must not be affected by the use of extension leads.

Before connecting the mains cables, please check whether your mains voltage corresponds to the voltage specified on the rating plate of the laser module.

For reasons of laser safety, the TV port on the microscope must either be equipped with a camera or covered by a cap.

Maintenance, repairs, modifications, removal or exchange of components, or other interference with the equipment beyond the operations described in this manual may only be carried out by the manufacturer Carl Zeiss or by persons expressly authorized by us to do so. This applies especially to the microscope system, the laser scanning module, lasers, the PC system, the power supply units, cable connections and other system components.
Please note that the LSM 510 is a high-precision opto-electronic instrument. Inexpert handling may easily impair it's function or even damage it.

After installation or after conversion of the LSM system, authorized specialized staff must carefully check that it is in a proper condition and, particularly, that covers protecting against laser radiation are provided.

Tube openings or other unused mounts should always be protected against dust and moisture with the corresponding device components or with termination covers/blind plugs.

By establishing a corresponding workplace environment, please ensure that the formation of electrostatic charges by electronic components is avoided.

To avoid vibrations during operation, the LSM 510 should only be operated in conjunction with the system table (vibration damping).

1.4 Notes on Handling the Computer and Data Media

The computer used as standard in your LSM system is an IBM-compatible high-end pentium computer with WINDOWS NT 4.0 or WINDOWS 2000 operating system.

As standard, your computer has one hard disk drive, one drive for 1.44 MB diskettes and one CD-ROM drive. An CD reader/writer is installed.

☞ Do make sure, though, that you receive your LSM system with the operating system installed, with initialization and start files set up and with the LSM program also installed.

☞ When working with the hard disk, it is important to know that the more data it contains, the slower its operation will become. Therefore, data that you do not need permanently should be stored on a diskette or CD-ROM.

⚠ When handling diskettes, avoid data losses by protecting them against extreme temperatures, moisture and magnetic fields. The data on a diskette is stored in the form of magnetic signals. To some extent, monitors, telephones or even lamps generate magnetic fields that might destroy this data. Also, never open the metal cover on diskette cases. A diskette´s surface can also be destroyed by touching it.

⚠ Never turn your computer off before you have exited the LSM program and run down the WINDOWS NT operating system. Otherwise, the program and/or data files may get lost.

⚠ When handling discs of the CD reader/writer, do not touch the data side of the disc (the side of the disc with no label or printing).
Do not apply paper labels or write on any part of the disc, data side or label side. If dust or fingerprints get on the disc, wipe it with a soft cloth from the center to the edge, but do not use benzine, paint thinner, record cleaner, or static repellent. This can damage the disc.
Do not place the disc in any place where it is exposed to direct sunlight or high temperatures.

1.5 Notes on Care, Maintenance and Service

The manufacturer of the unit cannot be held liable for damage resulting from operating errors, negligence or unauthorized tampering with the device system, particularly as the result of removal or replacement of parts of the unit or as the result of the use of unsuitable accessories from other manufacturers.
Any such action will also render all warranty claims null and void.

You are well advised to arrange a service agreement with your nearest Zeiss representative to guarantee perfect functioning of the microscope system in the long term.

Modifications and conversion work on the components of the system must only be carried out by the manufacturer, by the service agency or by persons authorized and trained for this purpose by the manufacturer.

Damaged units or parts may only be repaired or maintained by the responsible service agency.

Care operations that may be carried out by operating staff are limited to cleaning painted and glass surfaces.

- Cleaning painted surfaces
  To do this, use a clean cloth that has been moistened in a mixture of water and some detergent; do not use any solvent, however. Dry with a lint-free cloth.
- Cleaning glass surfaces
  Glass surfaces that have become soiled or which are marked with fingerprints may be rubbed with a clean optical cleaning cloth.
  If soiling is persistent, dip the optical cleaning cloth into a mixture of distilled water and a small quantity of detergent.
  To complete cleaning, lightly breathe on the glass surface and rub it dry with a clean cloth. Lint or dust is best removed with a clean hairbrush.

The air filter mat at the bottom of the LSM 510 Control Unit must be cleaned every six months. Filter mats can be ordered from our Service Department.

1.6 Notes on Handling the Laser Components

The LSM 510 is a laser hazard class 3 B instrument and is marked as such.
This moderate-risk class embraces medium-power lasers. You must take care not to expose yourself to the radiation of such lasers. In particular, never look into the laser beam!

The following laser types are currently recommended for use in the LSM 510.

1 Ar 351/364 (UV)
2 Ar/ML 458/477/488/514
3 HeNe 543
4 ArKr 488/568
5 HeNe 633

☞ Please contact Carl Zeiss if you intend to use a laser type with a wavelength other than the ones above.

If used properly, the LSM 510 will not pose any laser radiation risks for operating staff. The dangerous laser radiation area is limited to the beam path and to a distance of up to around 10 cm from the specimen. Nevertheless, you should observe the following warnings:

- If necessary - insofar as specified by law - inform the laser protection officer before commissioning the laser.
- Always store laser key switches and, if applicable, keys for further laser power supply units, where they are inaccessible to persons not authorized to operate the laser.
- Do not place any reflecting objects into the beam path.
- Never open any covers or panelings.
- Never look into the laser beam, not even to simply view the specimen, whether with the aid of optical instruments or without. **Otherwise you risk going blind!**
- Do not leave any screw positions of the nosepiece uncovered.

☞ Suitable protective measures must be taken if gases, dust or vapors hazardous to health, secondary radiation or explosive objects should arise on the specimen as a result of laser radiation.

1.7 Warning and Information Labels

The warning and information labels attached on the LSM 510 must be observed. Check whether all of the labels shown below are provided on your instrument, and contact Carl Zeiss Germany or one of the service agencies if you should discover that any of the labels should be missing. You will receive a free replacement.

The ⚠ label means: "Do not remove securing screw as otherwise laser beam will escape. For use by service only!"

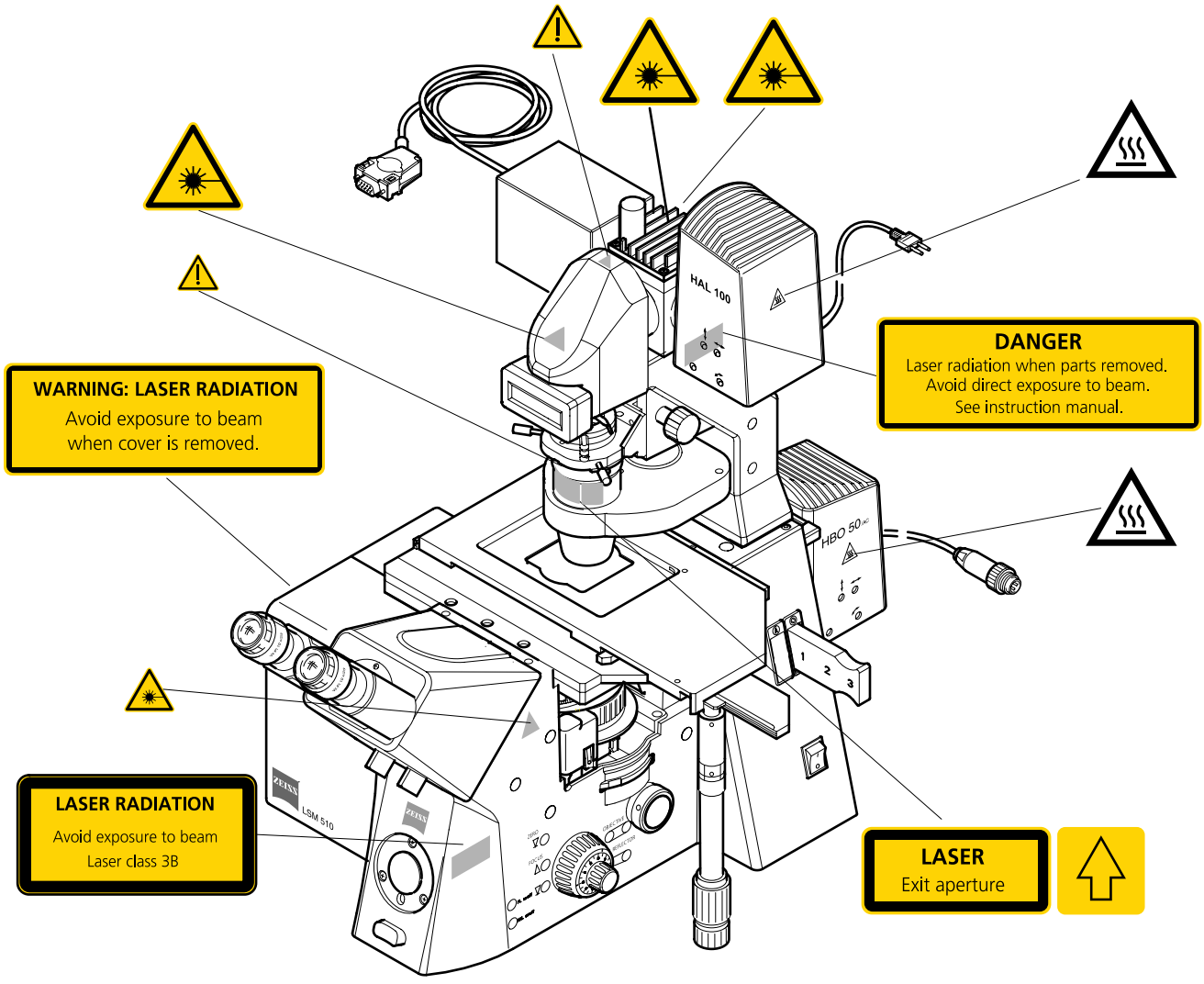


Fig. 1-1 Warning and information labels on the Axiovert 200 M microscope with the LSM 510 scanning module

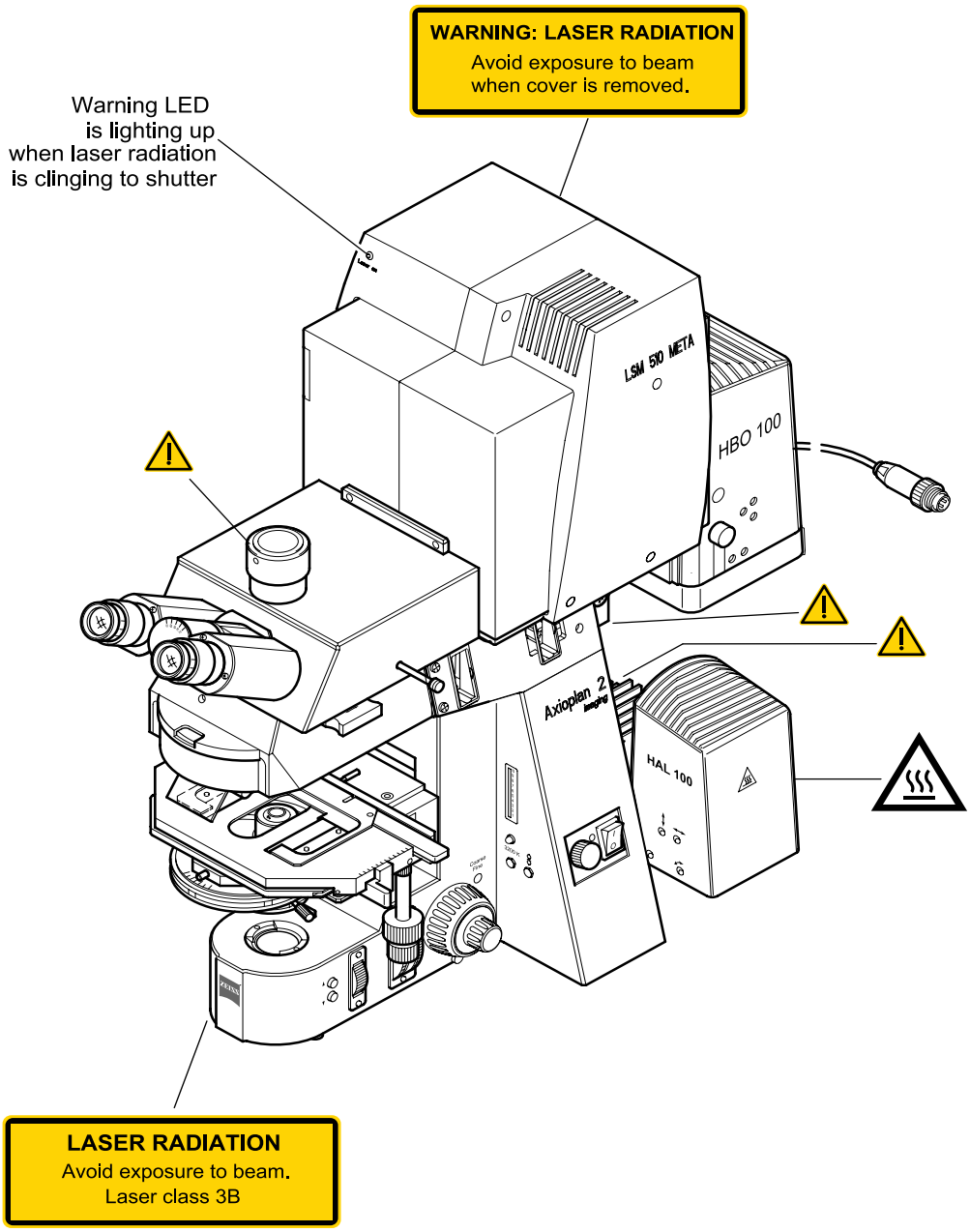


Fig. 1-2 **Warning and information labels on the Axioplan 2 imaging MOT microscope with LSM 510 META scanning module**

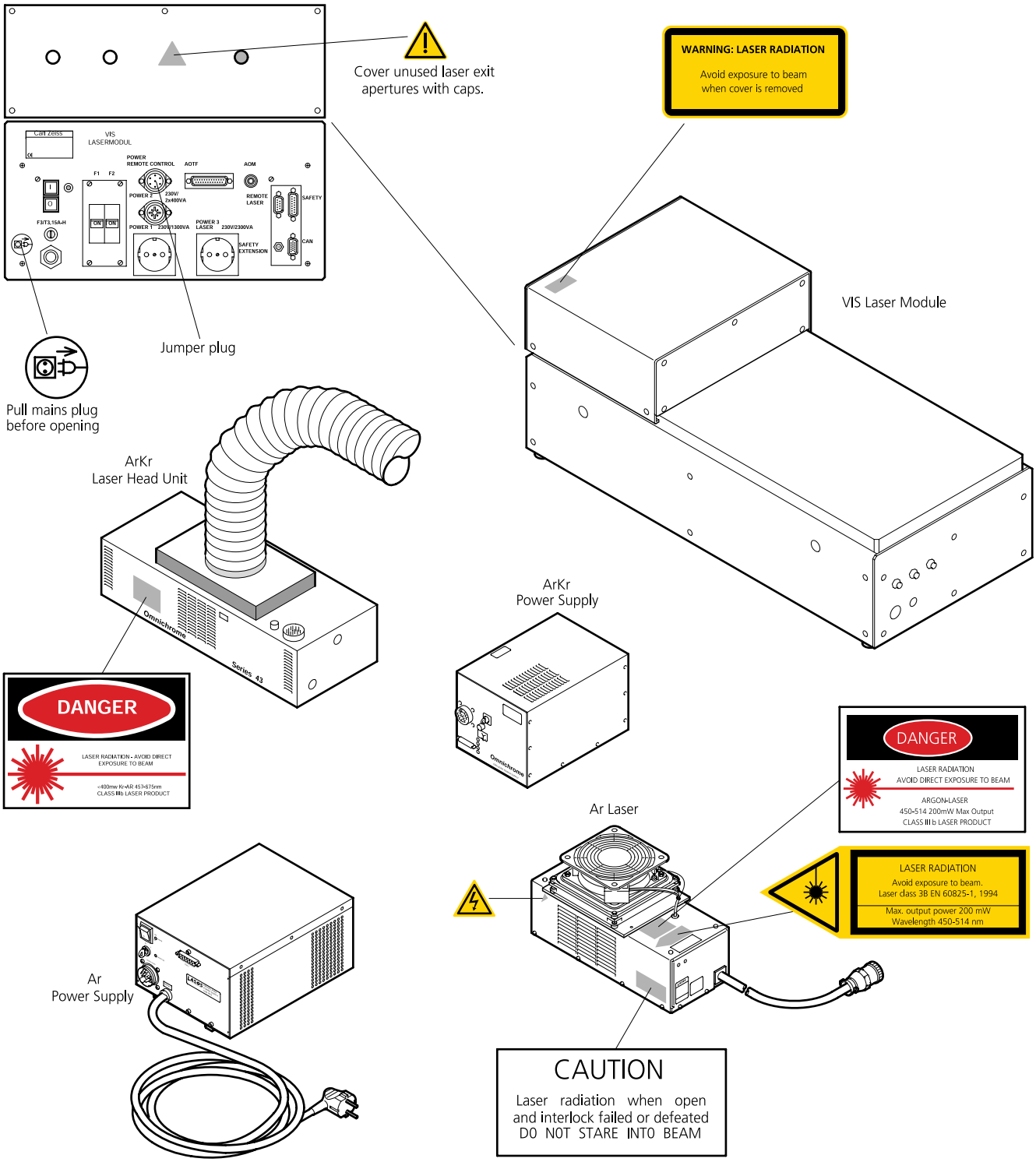


Fig. 1-3 Warning and information labels on laser components (page 1)

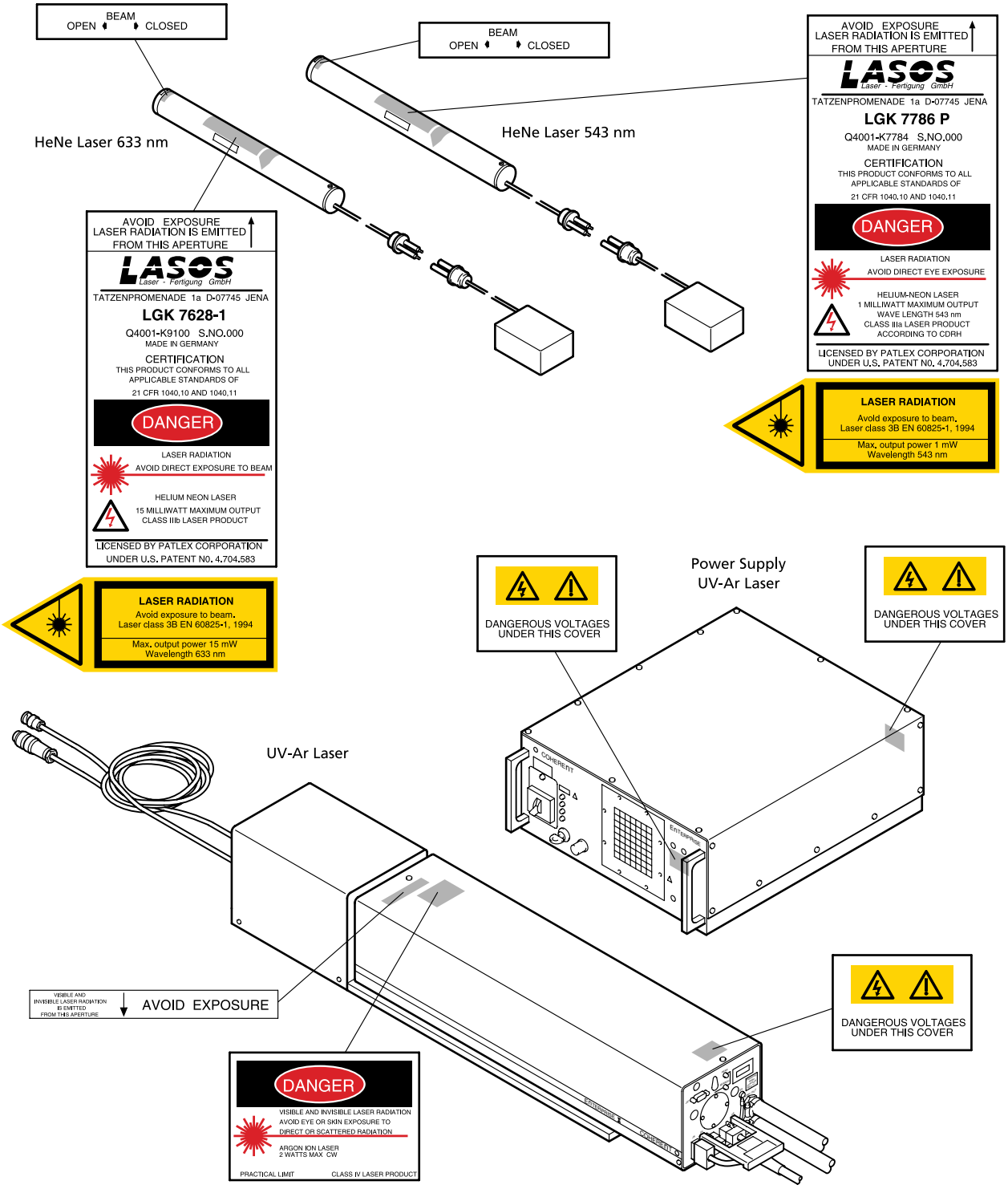


Fig. 1-3 Warning and information labels on laser components (page 2)

CHAPTER 2 LSM 510 - SETUP REQUIREMENTS

CONTENTS

2 LSM 510 - SETUP REQUIREMENTS

2.1 Space Requirements

2.1.1 LSM (one microscope, large system table): 300 × 250 cm

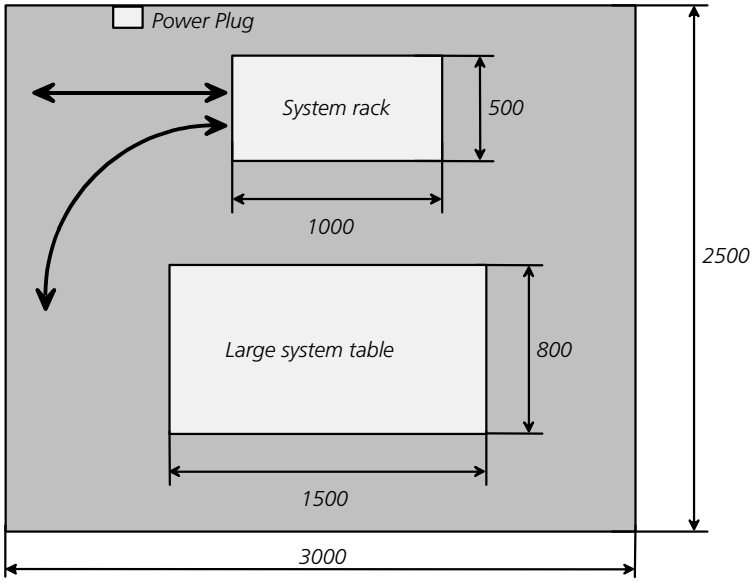


Fig. 2-1

The system rack contains the laser module (Helium-Neon laser 543 nm, 633 nm and Argon ion laser), the power supply for the Argon ion laser, for HBO lamp and halogen lamp, the electronic control unit (ECU) and the MCU28 unit (if a motorized XY stage is applied).

2.1.2 LSM with Ar UV Laser

☞ We recommend placing the cooling unit of the Ar laser (UV) in a separate room to prevent heat accumulation and vibration. Length of the water hose: 400 cm

One microscope:

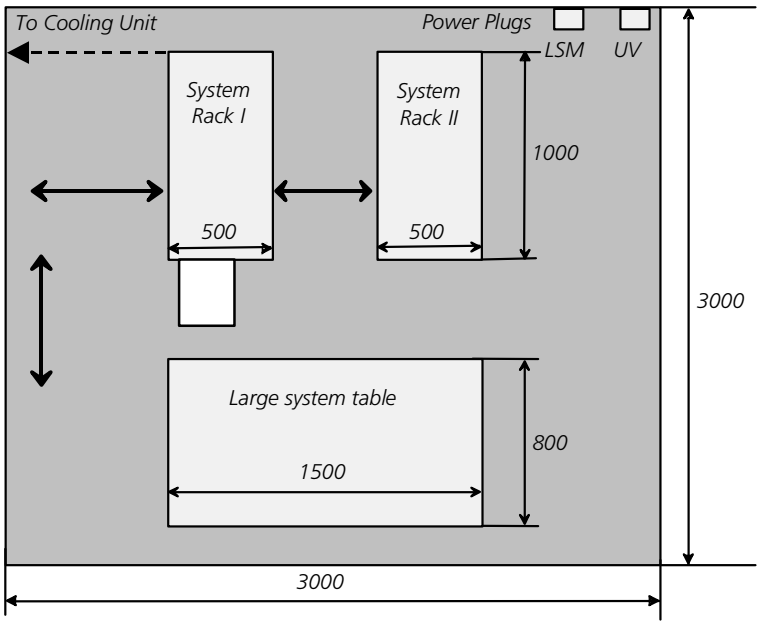


Fig. 2-2

Two microscopes:

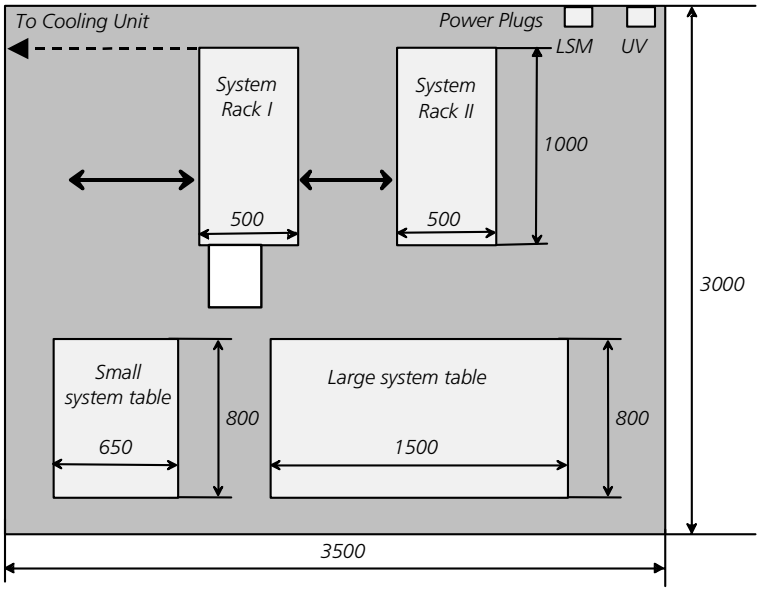


Fig. 2-3

The system rack I contains the VIS laser module (Helium-Neon laser 543 nm, 633 nm and Argon ion laser) and the Argon UV laser module. The system rack II contains the power supplies for lasers, for HBO and halogen lamps, the electronic control unit (ECU) and the MCU28 unit (if a motorized XY stage is applied).

2.1.3 LSM prepared for Two Photon Lasers (NLO)

2.1.3.1 Coherent "Mira 900" Direct-coupling with Inverted Stand (Upright Stand also possible)

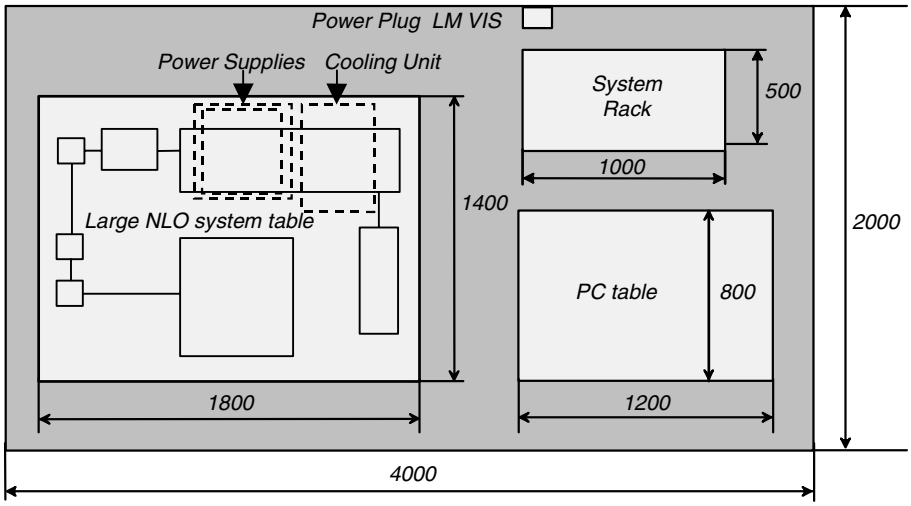


Fig. 2-4

2.1.3.2 Spectra Physics "MaiTai" Direct-coupling with Upright Stand (Upright Stand also possible)

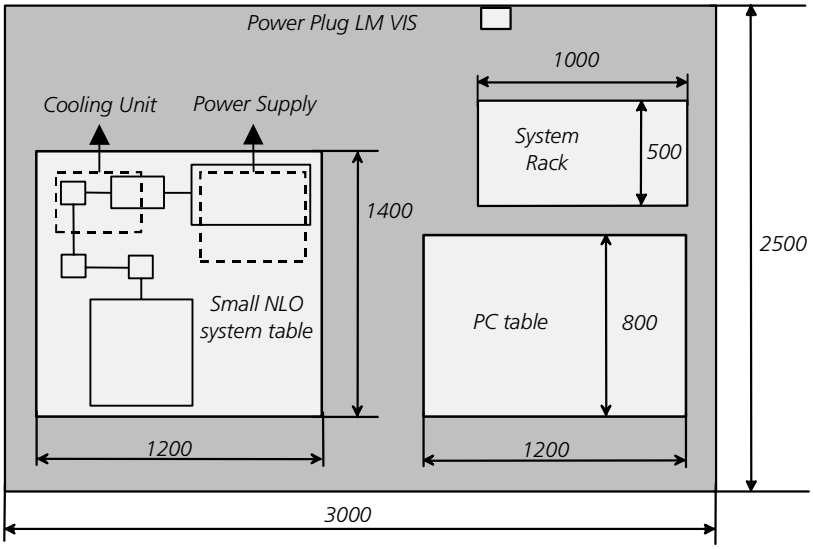


Fig. 2-5

2.1.3.3 Coherent "Mira" Fiber-coupling with Inverted Stand (Upright Stand also possible)

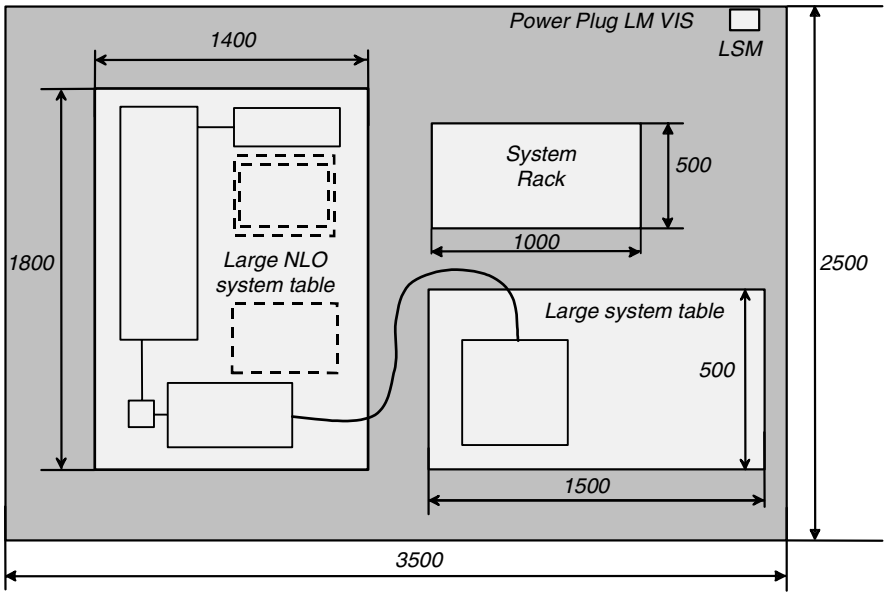


Fig. 2-6

2.1.3.4 Coherent "Chameleon" Direct-coupling with Upright Stand (Inverted Stand also possible)

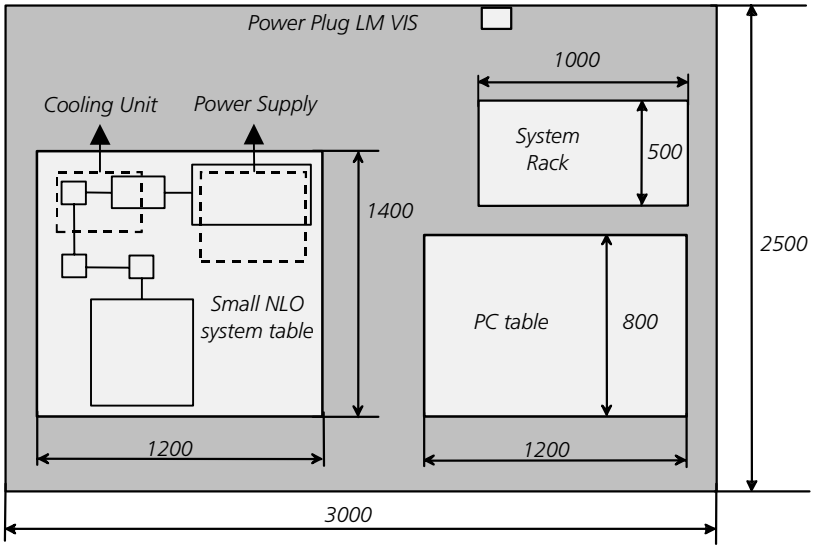


Fig. 2-7

2.2 Power Requirements

☞ The LSM 510 comes with a mains power supply cord and plug, either CEE red (230 V, 16 A, 3 phases), or CEE yellow (115 V, 32 A, 3 phases), and with the matching mains socket outlet.

Line voltage	230 V AC: 220...240 V AC (±10 %)	115 V AC: 100...125 V AC (±10 %)
Line frequency	50...60 Hz	50...60 Hz
LSM incl. VIS laser		
– Max. current	2 phases at 16 A Phase 1 = 1.8 kVA max. Phase 2 = 2 kVA max.	2 phases at 25 A Phase 1 = 1.8 kVA max. Phase 2 = 2 kVA max.
– Power consumption	2000 VA per phase	2000 VA per phase
– Power plug	CEE red (230 V, 16 A): 3 phases+N+PE, phases 1 and 2 connected	CEE yellow (115 V, 32 A): 3 phases+N+PE, phases 1 and 2 connected
Argon UV laser		
- Line Voltage	208...240 V AC (±10 %) 50 / 60 Hz	208...240 VAC (±10 %) 50 / 60 Hz
– Max. current	1 phase at 63 A Note: For Line Voltage 220 V the connector and power plug are rated for 63 Amps, However wiring and fuse should be rated for 32 Amps.	1 phase at: 208 V: 34 Amps 230 V: 31 Amps 240 V: 29 Amps
– Power consumption	7000 VA	7000 VA
Class of protection	I	I
Type of protection	IP 20	IP 20
Overvoltage category	II	II
Pollution degree	2	2

☞ If the line voltage in your country is 115 V AC, you need to order an additional 2.5 kW step-up-transformer, part no. 234.366, to be able to run the ArKr laser. Reason: The ArKr laser requires a 220 V input.

Power distribution inside the Laser Module VIS:

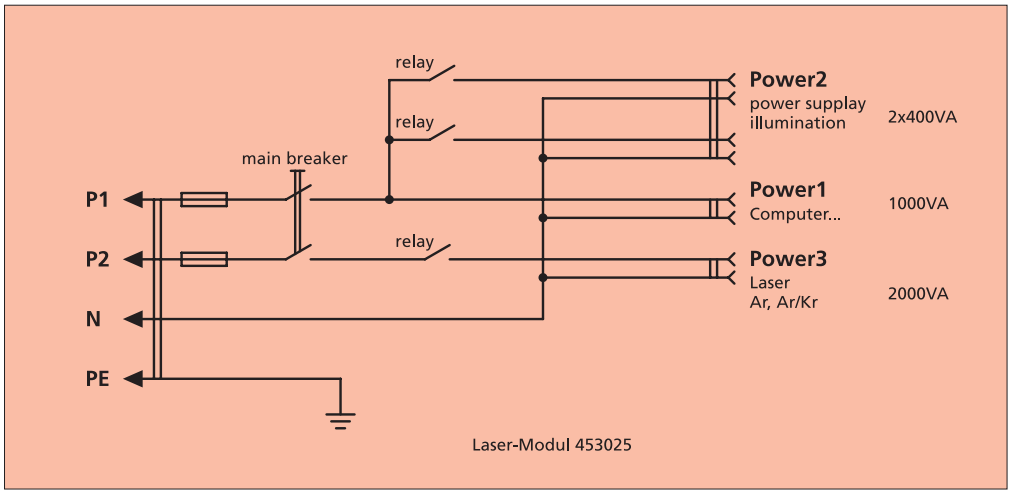


Fig. 2-8

2.2.1 Phase 1 (LSM)

feeds the following units:

Laser Module
HeNe 2×
via Power 1 (5-socket adapter)
- Computer + monitor
- Microscope
- MCU28
- Scanning Module

via Power 2:
- HAL lamp
- HBO lamp

2.2.2 Phase 2 (LSM, Power 3)

feeds the following units:

Ar laser	2000 VA
or ArKr laser	2000 VA

2.2.3 Separate Connection

Ar laser (UV)	7000 VA

2.3 Physical Dimensions

	Length (cm)	Width (cm)	Height (cm)	Weight (kg)
Large system table	150	80	78	100
Small system table	65	80	78	60
Active anti-vibration table	75	75	75	125
Active anti-vibration table (NLO)	120	140	75	200
For Mai Tai Laser or Chameleon				
Active anti-vibration table (NLO)	180	140	75	400
For Mira or Tsunami Laser				
Scanning Module LSM 510	25	20	25	15
Scanning Module LSM 510 META	28	27	30.5	13
Microscope	50	35	50	20
Laser Module, VIS(ible light)	90	40	50	60
Laser Module, UV	140	20	20	60
Electronics box	50	30	30	10
Power supply for Ar, ArKr	30	30	20	10
Power supply for Ar (UV)	50	50	30	30
Cooling unit for Ar (UV)	80	45	50	30
Water hose for Ar (UV)	700			
Fiber optic cable, VIS(ible)	200			
Fiber optic cable, UV	200			
Cables	250			
SCSI cable	200			

2.4 Dimension of Shipment Crates

Crate containing	Length (cm)	Width (cm)	Height (cm)	Weight (kg)
Large system table	160	85	95	120
Small system table	90	75	80	80
Active anti-vibration table	145	115	115	150
Active anti-vibration table (NLO) For Mai Tai Laser or Chameleon	145	160	110	330
Active anti-vibration table (NLO) For Mira or Tsunami Laser	200	160	110	460
LSM	190	85	120	350
Monitor, computer	120	80	90	80
UV laser unit	125	55	50	100
UV cooling unit	120	60	90	50
META scan head	52	47	47	13
META upgrade kit	64.5	60.5	42.5	20

2.5 Environmental Requirements

1. Operation, specified performance	T = 22 °C ± 3 °C without interruption (24 h a day independently whether system is operated or switched-off)
2. Operation, reduced performance	T = 10 °C to 35 °C, any conditions different from 1. and 5.
3. Storage, less than 16 h	T = -40 °C to 55 °C
4. Storage, less than 6 h	T = -55 °C to 70 °C
5. Temperature gradient	± 0.5 °C/h
6. Warm up time	1 h, for high-precision and/or long-term measurements ≥ 2 h
7. Relative humidity	< 65 % at 30 °C
8. Operation altitude	max. 2000 m

2.6 Vibrations

Vibrations under operation conditions (with system table)	**Shipping shock (LSM 510 box)**
5 µm pp at 5 Hz 10 µm pp at 10 Hz 10 µm pp at 20 Hz	3 g

2.7 Laser Specifications

2.7.1 Coherent Enterprise 653 II: 352, 364 nm, 80 mW, Laser Class 3 B

Line voltage	208...240 V
Line frequency	50...60 Hz
Max. current	1 phase at: 208 V: 34 Amps 230 V: 31 Amps 240 V: 29 Amps
Power consumption	7000 VA
With heat exchanger LP5:	
Water flow	8.0 l/min (max. 16 l/min)
Water pressure	1.4...4.2 kg/cm²
Water temperature	10...60 °C at 8.0 l/min

2.7.2 Point Source i-flex 2000: 405 nm, 25 mW, Laser Class 3 B

Line voltage	100...240 V
Line frequency	50...60 Hz
Power consumption	30 VA

2.7.3 LASOS LGK 7786 P / Power supply 7460 A: 543 nm, 1 mW, Laser Class 3 B

Line voltage	115/230 V with factory setting
Line frequency	50...60 Hz
Power consumption	20 VA

2.7.4 LASOS LGK 7628-1: 633 nm, 5 mW, Laser Class 3 B

Line voltage	100...240 V with factory setting
Line frequency	50...60 Hz
Power consumption	20 VA

LASOS LGK 7812 ML-4 / LGN 7812: 458, 477, 488, 514 nm, 30 mW, Laser Class 3 B

Line voltage	100...240 V with factory setting
Line frequency	50...60 Hz
Max. current	1 phases at 25 A
Power consumption	2000 VA
Cooling fan	on top of laser head

2.7.6 Melles Griot 643-YB-A02 / Power supply 171B: 488, 568 nm, 30 mW, Laser Class 3 B

Line voltage	100...240 V with factory setting
Line frequency	50...60 Hz
Max. current	1 phase at 16 A
Power consumption	2000 VA

2.7.7 AOTF

☞ In the unlikely case of complete utilization of the acousto-optical tunable filter (100 % intensity of all AOTF-supported lines) the tolerable limits of the EMV regulations could be slightly exceeded in the MHz range.

2.8 Microscopes

Upright Axioplan 2 imaging MOT

Upright Axiotron 2 mot

Inverted Axiovert 200 M BP or SP

Upright Axioskop 2 FS MOT

Upright Axioskop 2 MAT mot

All Zeiss ICS objectives and accessories can be accommodated.

Z motor	DC servomotor, opto-electronically coded
	Least Z interval: 50 nm (Axioplan 2 imaging MOT, Axiovert 200 M BP or SP)
	100 nm (Axioskop 2 FS MOT)
HRZ 200	Galvanometer-driven precision focusing stage
	Max. travel 200 µm; resolution 6 nm; accuracy 40 nm
	Allows continuous Z-scan at up to 10 Hz
Piezo Objective focus	Piezo-driven single objective drive
	Max. travel 100 µm; resolution 5 nm
	Allows continuous Z-scan at up to 20 Hz

2.9 Scanning Module

	2 individually driven galvanometric scanners
Scanning speed	Up to ~5 frames/sec (512 × 512 pixels)
Field resolution	Max. 2048 × 2048 pixels (individually adjustable for each axis)
Field of view	10 × 10 mm² with a 1.25× objective
Zoom	1× ... 40×, continuous control
Channels	a) Up to 4 channels simultaneously **or** b) 3 traditional confocal channels and 1 META channel
	4 confocal reflection/fluorescence channels (PMT) **or** 3 PMT and 1 META
	1 transmitted light channel (PMT) and 3 NDD **or** 4 NDD
	1 reference monitor diode
	Cooled PMTs (option, forthcoming)
	Fiber-optic adaptation of external detectors (option, forthcoming)
Dynamic range	12-bit DAC for each detection channel
Pinholes	4 individual variable pinholes (one per confocal channel)
	Computer controlled automatic alignment

2.10 Laser Module VIS (405, 458, 477, 488, 514, 543, 633 nm)

Single-mode polarization preserving fiber

Laser beam attenuation for all lasers by VIS-AOTF

HeNe laser (543 nm, 1 mW)

HeNe laser (633 nm, 5 mW)

Diode laser (405 nm, 25 mW)

Ar laser (458, 477, 488, 514 nm, 30 mW)

ArKr laser (488, 568 nm, 30 mW)

Fuses and automatic circuit breakers

for 230 V:	G-type fuse 5 × 20 mm; slow-blow 3.15 A / H / 250 V, acc. to IEC 127
	2 circuit breakers; C 10 A
for 110 V:	G-type fuse 5 × 20 mm; slow-blow 3.15 A / H / 250 V, acc. to IEC 127
	Circuit breaker; B 25 A
	Circuit breaker; C 25 A
	Circuit breaker; B 16 A
	Circuit breaker; B 10 A

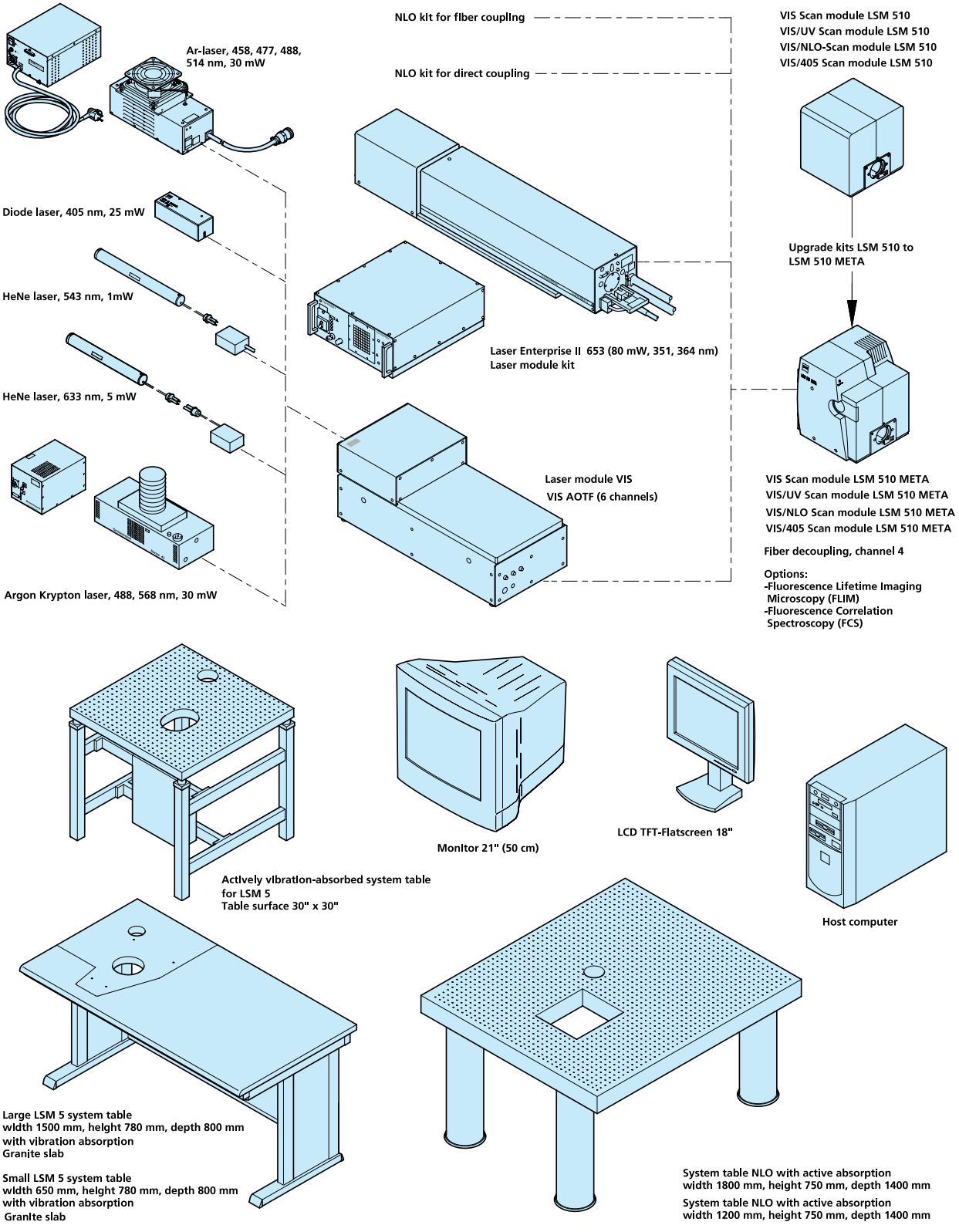
2.11 Laser Module UV (351, 364 nm)

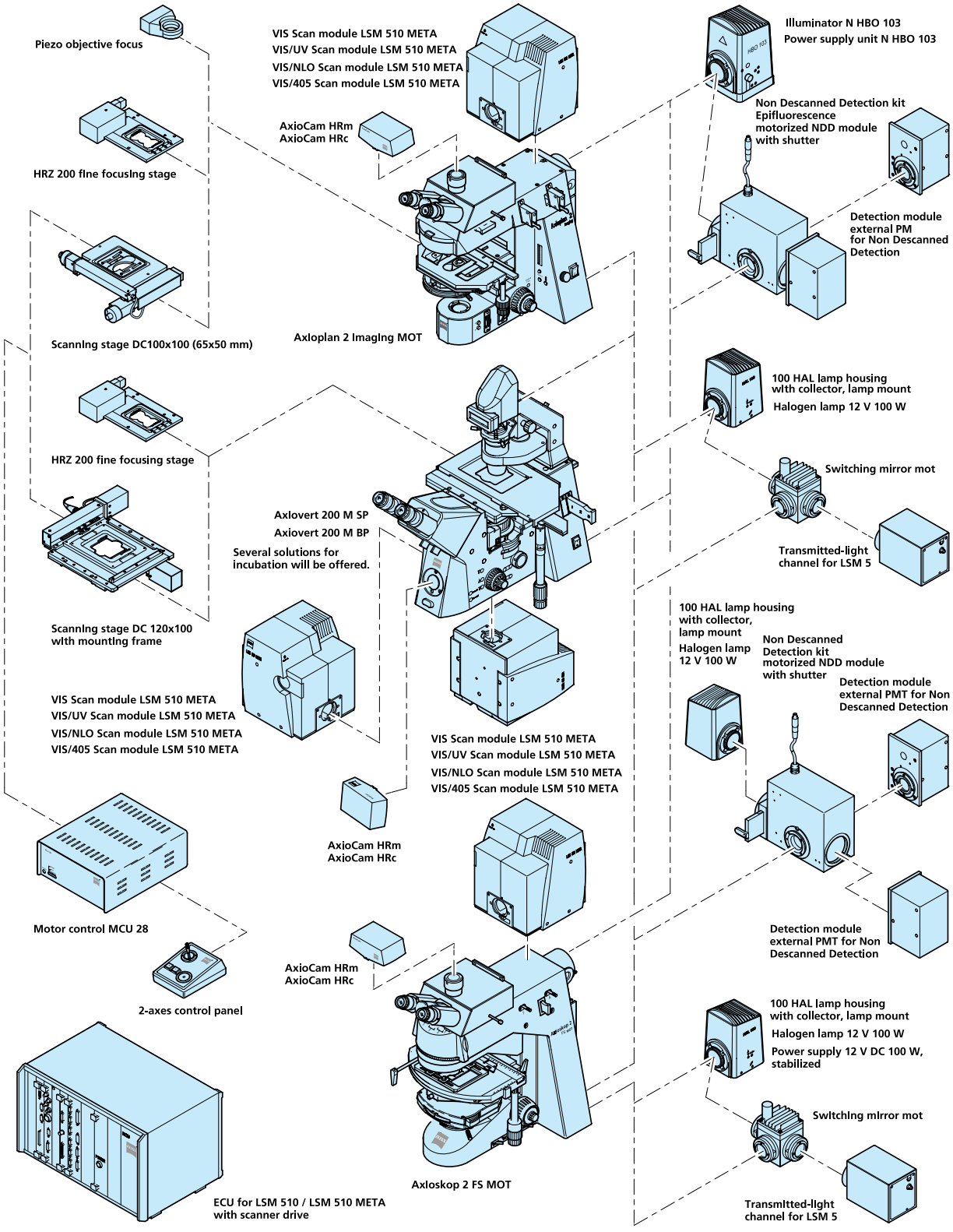
Single-mode polarization preserving fiber

Laser beam attenuation for all lasers by UV-AOTF

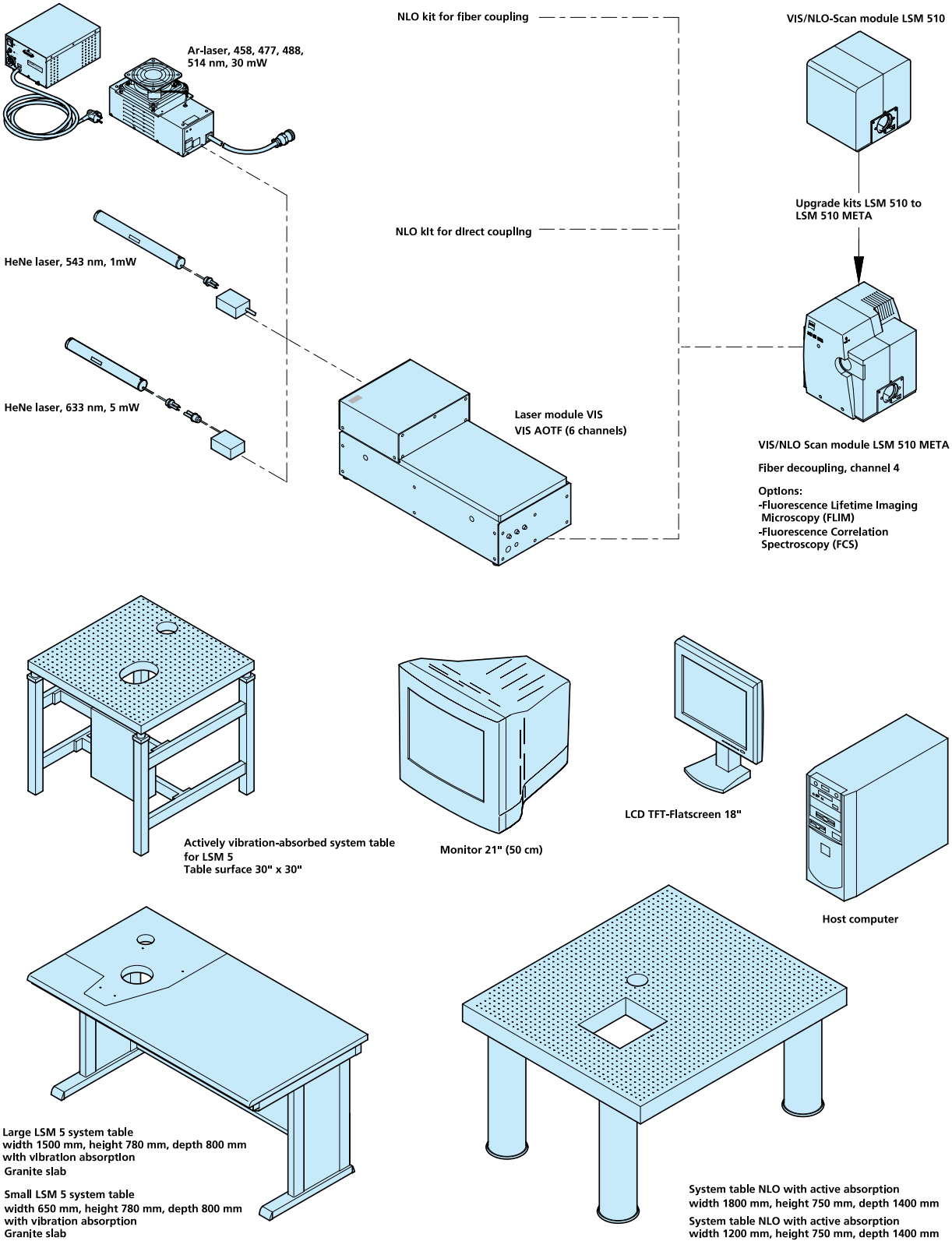
Ar laser (351, 364 nm, 80 mW)

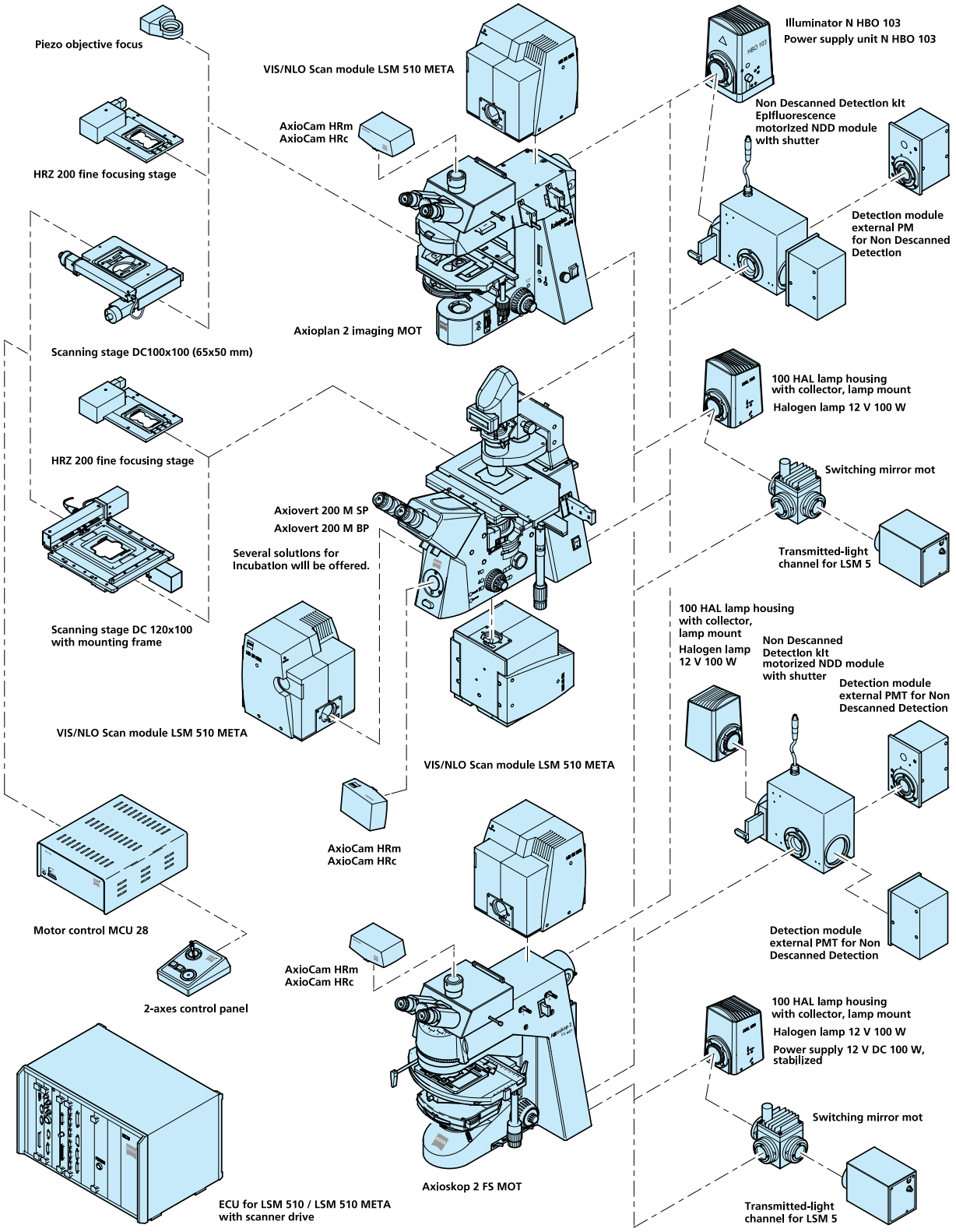
2.12 System Overview LSM 510 META




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

2.13 System Overview LSM 510 META - NLO




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

CHAPTER 3 INTRODUCTION TO LASER SCANNING MICROSCOPY

CONTENTS

3 INTRODUCTION TO LASER SCANNING MICROSCOPY

3.1 Principle of Laser Scanning Microscopy

To yield information on their inner structure by conventional transmitted-light microscopy, specimens have to be very thin and translucent; otherwise image definition will be poor. In many cases it is a problem to satisfy these requirements.

The essential considerations have led to trailblazing changes in conventional microscopy and supplied a successful solution to the above problem.

- Unlike the practice of even illumination in conventional microscopy, the LSM technique projects the light of a point light source (a laser) through a high-NA objective onto a certain object plane of interest as a nearly diffraction-limited focus. However, if not for another "trick", the stray light produced outside the object plane, or the fluorescence of fluorescent specimens, would disturb the in-focus image of object point of interest, resulting in a blurred image of poor contrast. The problem therefore is how to capture only the light coming immediately from the object point in focus, while obstructing the light coming from out-of-focus areas of the specimen.
- The light reflected, or the fluorescence light produced, at the focus of the high-NA objective is projected onto a variable pinhole diaphragm by the same objective and a tube lens. The focus inside the specimen and the pinhole are situated at optically conjugate points (**confocal imaging**)**.** The decisive advantage of this arrangement is the fact that essentially no other light than that coming from the object plane of interest can pass the narrow pinhole and be registered by a detector. Unwanted light coming from other specimen areas is focused outside the pinhole, which passes only a small fraction of it. The smaller the pinhole, the less stray light or fluorescence from out-of-focus areas will get on the detector. The image point thus generated is largely free from blur caused by unwanted light.

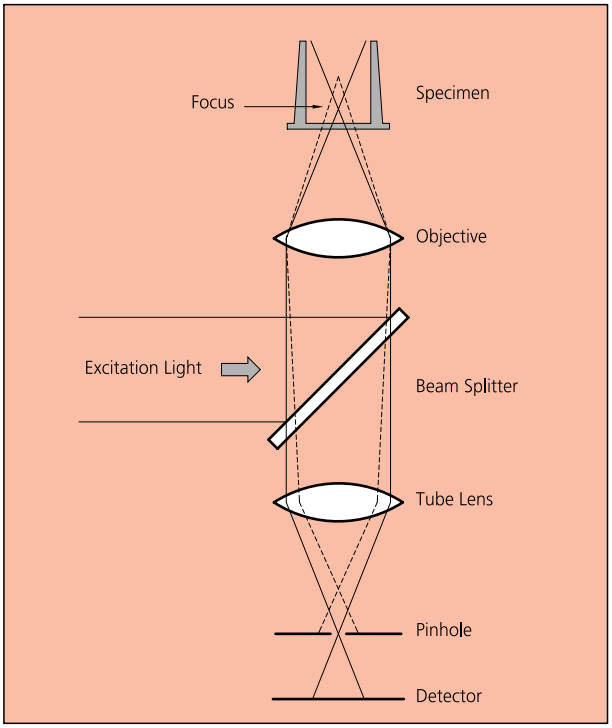


Fig 3-1 Principle of confocal imaging

- In order to obtain an image of the selected object plane as a whole, it is necessary to scan the object plane in a point-by-point, line-by-line raster by means of an XY light deflection system. The detectors - as a rule, photomultipliers - convert the optical information into electric signals. This allows the image of any object plane to be generated and stored within less than a second. By a defined focusing (Z axis) movement it is possible to look at any object plane of interest. By scanning a succession of object planes in a specimen, a stack of slice images can be produced.

This way, the LSM technique in conjunction with ICS optics (Infinity Color-Corrected System) has brought decisive improvements over conventional microscopy in terms of resolving power and confocal depth contrast:
Object features in the order of 0.2 µm can be resolved, and height differences of less than 0.1 µm made visible, without the use of interference methods.

3.2 Three-Dimensional Presentations of LSM Image Stacks

One of the advantages of the LSM technique is that it can present structures in three dimensions. This opens up many ways to process images. Outlined below are some of the possible methods to extract spatial information from stacks of slice images.

- **Gallery**
  The simplest presentation of 3D information is a gallery showing the individual slice images (sections) of a stack arranged side by side, with each slice apart from the next by a defined, selectable interval on the Z axis.
- **Virtually infinite depth of focus**
  The entire set of data can be imaged as a single projection. The computer establishes an image composed of all in-focus optical sections. The image produced by this so-called composite method has a virtually infinite depth of focus, since the result is made up of information from in-focus planes only.
- **Rotary animation**
  A sequence of projections is computed, with the specimen being apparently rotated by a certain angle from image to image, for example by a full turn about an axis. If such a sequence is displayed on the monitor screen in rapid succession, the visual effect is that of a rotating three-dimensional object.
- **Stereo image pairs**
  The computer establishes a pair of images corresponding to those we see with the right and the left eye, respectively. The two images forming the stereo pair can be shown on the monitor side by side. They can be seen as a 3D image with suitable optical aids. Another possibility is to present both images in registration, with one image in the red channel and the other in the green one (anaglyph). Viewed through red and green color filters in a spectacle frame, which only pass the image intended for the respective eye, the two images form a 3D image in the brain

- **Color-coded height slices**
  Each level, i.e. each slice is assigned a different color. For direct evaluation, a color scale is shown, indicating the actual height above the bottom slice.
- **Orthogonal sections**
  This computation produces a triplet of mutually perpendicular sectional images.
- **Oblique sections**
  A section through the stack is made along an oblique plane defined by the selection of five coordinates, i.e. X, Y, Z, angle of rotation, and angle of tilt.
- **Topography** (option)
  A computing program for surface topography presentations (as required in materials research) is available.
- **Physiology** (option)
  With a special software, kinetic processes can be tracked, which is especially of interest to physiology.
- **Image VisArt** (option)
  Three-dimensional display of floating transparent structures (cells) or opaque structures (material)
- **3D Deconvolution** (option)

3.3 Optical Diagram of the LSM 510 (Schematic)

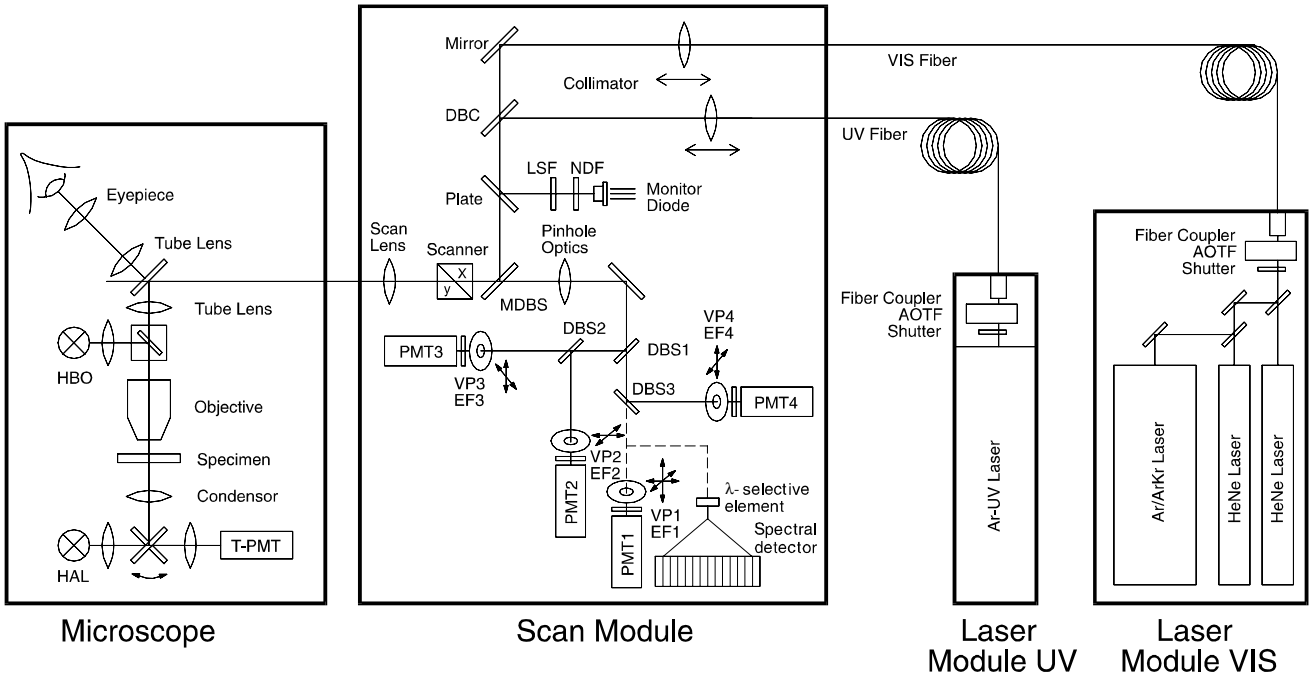


Fig. 3-2 Optical path, schematic (4-channel configuration)

AOTF	Acousto Optical Tunable Filter
DBC	Dichroic Beam Combiner
DBS	Dichroic Beam Splitter
EF	Emission Filter
HAL	Halogen Lamp
HBO	Mercury Vapor Short-Arc Lamp
LSF	Line Selection Filter
MDBS	Main Dichroic Beam Splitter
NDF	Neutral Density Filter
VP	Variable Pinhole
PMT	Photomultiplier
T-PMT	Transmission-Photomultiplier

The diagram above is a schematic representation of the LSM system.

Laser light is focused onto the specimen through an objective in a diffraction-limited mode. Light emitted at the focal plane and at planes below and above it is directed via an XY scanner onto a main dichroic beam splitter (MDBS), which separates the emissions from the excitation light. The fluorescences are separated from each other by a series of dichroic beam splitters (DBS1 ... maximally DBS4) and directed to individual photomultipliers (PMT1 ... maximally PMT4).

3.4 Performance Features of the LSM 510

3.4.1 Optical and Mechanical Aspects

The highly integrated system design makes for the shortest possible optical paths, top-grade optical precision and high stability. The compact scanning module can be fitted to an inverted (Axiovert 200 M BP or SP) or upright (Axioplan 2 imaging MOT) microscope in less than three minutes. On the Axiovert, the scanning module may be mounted either to the base port directly below the microscope or to the side port.

The spectral range available extends from the UV to the IR region.

For the VIS (visible-light) Laser Module, the user can select from up to five lasers with wavelengths of 633, 568, 543, 514, 488, 477, 458 and 405 nm. The UV Laser Module provides wavelengths of 351 and 364 nm. Coupling of the laser light is through polarization-preserving single-mode optical fibers. One variable beam collimator each for the UV and visible ranges provides optimum adaptation of the respective laser wavelength to the objective used and, thus, optimum correction for Z aberrations.

Acousto-optical tunable filters (AOTF) adjust the necessary brightness for up to 6 desired laser lines within microseconds.

A monitor diode permanently registers the laser output; it can be used for the on-line checking of the intensity of the exciting light. This check is also possible selectively for the different wavelengths if a line selection filter is inserted.

The four simultaneous image acquisition channels, usable for reflection or fluorescence, and an additional transmitted-light channel are ideal for the investigation of multiple fluorescence specimens. Separately in each of the four channels, the diameters of the pinholes and their XY positions can be optimized, and the desired emission filter placed into the beam path, by servo-motor control. In the case of pinhole VP1, this adjustment also includes positioning along Z. In the simultaneous registration of multiple fluorescences, identical optical sections can be obtained in each confocal channel. This is of importance, e.g., with the FISH method (fluorescence in-situ hybridization) used for genome analysis in cytogenetic studies.

The microscope's transmitted-light channel is equipped with a photomultiplier, too. It is therefore possible to superimpose a multiple fluorescence image on a brightfield, differential interference or phase image.

A fiber-optic cable connection to external special detectors, such as cooled PMTs or spectrometers, is under development.

In addition to the emission filters for all standard and special applications, available in motor-controlled filter wheels, the user can easily install his own emission filters in two of the channels.

The high-NA C-APOCHROMAT objectives specially developed for the LSM technique reach the physical limit in resolving power, and can be used throughout the 350...700 nm spectral range with the same high quality, producing brilliant images.

A two-mirror scanner system, controlled by a digital signal processor (DSP), offers several advantages. The large deflection angle of the scanning mirrors allows a wide area to be scanned. With a 1.25× objective, the object area scanned is 10×10 mm².

The scanning field size can be freely selected between 4×1 and 2048×2048 pixels.

It is possible to rotate the XY scanning field through 360° and carry out XY scans without having to rotate the specimen itself under laser radiation load.

Selection of the specimen detail of interest for zooming is fast and convenient, and the zoomed image is automatically centered. This saves the job of specimen centration with the microscope stage.
Using a bi-directional scanning facility will double the scanning rate to approx. 5 frames/sec (at 512 × 512 pixels); if two different laser wavelengths are used for the two scanning directions (wavelength 1 for left-to-right, and wavelength 2 for right-to-left scanning), two fluorochrome dyes can be viewed and documented in a quasi-simultaneous mode. This will absolutely prevent "bleeding".

3.4.2 Microscope Equipment of the LSM 510 System

The LSM 510 system is equipped either with the inverted Axiovert 200 M BP or SP microscope or with the upright Axioplan 2 imaging MOT, Axiotron 2 or Axioskop 2 FS mot microscopes.

Only the differences from the delivered operating manual "Axiovert 200 M" will be explained here.

(1) Stand

a) The motorized objective nosepiece 5× H DIC is firmly fixed to the stand, where no operating elements can be found for the nosepiece. Operation will be performed via LSM 5 software control. The "Restriction of the nosepiece height to protect the objectives during motorized objective change" is inactivated. The nosepiece will be moved down automatically before each motorized objective change.

b) The reflector mount is motorized and provided with the Axiovert 200 reflector turret. The reflector turret has 5 positions: One transmitting light position, which is identical to the LSM position, and four further positions for fluorescence filter sets (reflector modules). If you want to use more than five conventional fluorescence filter sets, it is advisable to use a further reflector turret. When changing the reflector turret position you must make sure that the turret will click into position, since otherwise the image area will be cut.

c) The stand has a motorized focusing drive (fine coarse). Sensitivity of the focusing drive is adjusted to the delivered objectives by the manufacturer. If you want to use other objectives, sensitivity and parfocality can be adjusted via the Axioset program.

d) The stand features an integrated power supply for the internal motors and stand electronics. The power supply can be switched on at the right side of the stand. External power supply units will be used for the mercury vapor short arc lamp.

e) The analyzer slider for conventional DIC methods will be operated from the right side and is located just below the nosepiece.
When the rod is pushed in, the analyzer is located in the beam path. In the LSM-mode, the analyzer must **not** be located in the beam path, and the analyzer rod must be pulled out.

f) The stands dispose of five additional ports: two side ports, front ports and base ports.
The side port or the front is equipped with the LSM 5 special interface, one of the others with the TV interface. The LSM 510 scanning module can be mounted to the special interface port. Different camera systems can be adapted to the TV interface using the TV adapters 452982/83/92/94/95/97/98-0000-000.

(2) Specimen stages and fine focus drives

a) Mechanical stage
The stage with coaxial drive must be mounted on the right side of the stand.

b) Scanning stage

c) HRZ 200 fine focusing stage

d) Piezo objective focus drive

(3) Transmitted-light illumination

a) The illuminator support contains a security circuit which activates a shutter preventing laser light from reaching the stand when the support is moved to the back. A complementary shutter built in the stand prevents laser light from reaching the eyepieces during the scanning mode.

b) The illuminator support is equipped with a rotary polarizer. The Axiovert description contains the adjustment for the DIC mode during conventional observation.
For scanning in the transmitted-light DIC mode, the polarizer in the transmitted light support works like an analyzer and must be adjusted in such a manner that direct laser light will be blocked.
The conventional analyzer slider in the stand must not be located in the beam path because the laser light is already polarized.

c) A fully motorized, LSM 5 software-controlled switching mirror is mounted as an option on the illuminator support. Alternatively, the light is directed to the LSM 5 T-light detector or enables conventional transmitted-light observation.

d) The focusing screen for conventional transmitted-light is located in a support in front of the halogen lamp housing.

e) Further information on the halogen lamp and the condensers is provided in the Axiovert operating manual.

(4) Reflected light fluorescence

With the exception of the reflector slider, all the Axiovert fluorescence accessories can be used.
Further information is provided in the Axiovert operation manual.

(5) Imaging optics

Optovar sliders cannot be used.
The analyzer for the conventional DIC mode will be operated from the right side and is located just below the nosepiece.
Use of sliders with auxiliary objects (473704/14-0000-000) is not possible.

(6) Photo equipment

The stand does not feature an integrated SLR-port, but microscope cameras as described in the Axiovert operation manual can be used.

(7) TV adaptation

The TV port at the side and the tubes can be used as described in the Axiovert operation manual.

The TV interface side port or base port can be used with TV adapters 44 or LSM adapters.

3.4.3 Computer Hardware and Software

The LSM 510 is controlled via a standard high-end Pentium PC. Linking to the electronic control system is made via an ultrafast SCSi interface. The PC comes with the 32-bit WINDOWS NT 4.0 or WINDOWS 2000 operating system.

The instrument is fully motorized, permitting fast change-over between methods as well as automatic operation. Parameters once set or complex examination sequences once established can be saved and reproduced; therefore, complete application programs can be loaded and performed by pushbutton control.

The software of the LSM 510 has two levels. On the simple operator interface level, a result will be achieved after a few prompts; graphical prompting of the user in conjunction with automatic setting of many parameters is an ideal tool for daily routine jobs. The expert level offers perfect facilities for individual settings of functions and parameters.

Conversion of the light signals into a digital image is effected by means of four 12-bit A/D converters, each of which can generate 4096 brightness levels.

The software provides an enormously wide range of image processing functions, including all standard 2D/3D (stereo, projection) functions identical to sophisticated 3D reconstruction capabilities (surface and alpha rendering), digital processing of voxels and 3D measurement functions (surface areas, volumes).

As all files and images are recorded in MS Access databases, elegant image database editing is just as easy as transferring the records to other programs.

CHAPTER 4 QUICKSTART

CONTENTS

4 QUICKSTART

4.1 Purpose of this Section and other Operating Manuals

This section describes the operation of the LSM 510 and LSM 510 META Laser Scanning Microscopes exemplified by typical applications in conjunction with the LSM 5 software and its graphic user environment.

When starting up and operating the microscope system, mind the operating instruction manuals for the Axioplan 2 imaging MOT, Axiovert 200 M and Axioskop 2 FS microscopes:

- B 40-042 e Axioplan 2 imaging MOT, Operating Manual
- B 40-080 e Axiovert 200 M, Operating Manual
- B 40-076 e Axioskop 2 FS MOT, Operating Manual

4.1.1 Software

The LSM 5 software, Version 3.2, controls the microscope, the scanning and laser modules, tools (filters, stand, Axioset) and the image acquisition process, and displays and analyzes the images. It is based on the network-capable graphic 32-bit Microsoft ® WINDOWS NT 4.0 operating system and WINDOWS 2000, respectively.

☞ The installation of the software for the LSM 510 and the basic settings of the equipment components are carried out by Carl Zeiss service staff. This job includes the creation of a customized software configuration in line with the specific hardware components of the customer's microscope system.

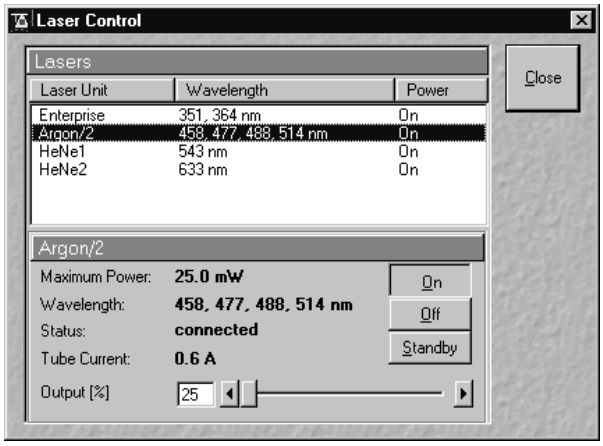
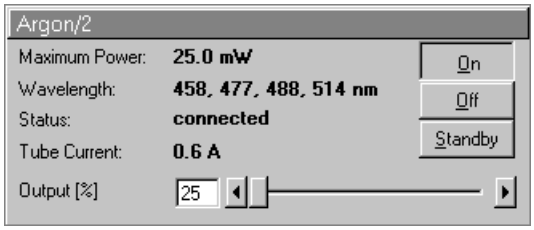
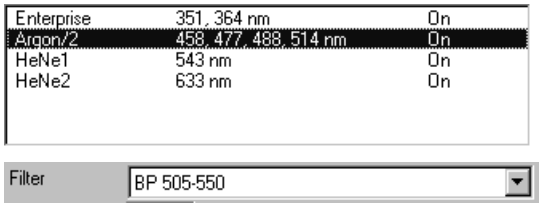
The LSM 5 software is menu-controlled and normally uses its own windows for the activation of the various functions; within these windows, further submenus (panels) can be displayed and removed.

Images of the specimens to be examined, created by scanning, are displayed in separate **Image Display** windows.

Theoretically, the number of simultaneously opened windows for software operation or image display is unlimited, but should not be too excessive so that an overview is still possible.

Identical functions, e.g. **Laser Control**, can be performed in several software windows. Changes made by the software are recorded immediately and are automatically transferred to all the other windows concerned.

4.1.2 Windows and Window Elements

Window element	Description / Explanation
	Window (e.g.: **Laser Control** window) – Window displayed after activation of a function button (e.g.: **Laser** button in the toolbar of the Expert Mode).
	Panel (e.g.: **Argon** panel) – Limited function range within a window
	List box or selection box – Selection of one of the displayed options at a click of the mouse. – Open the box by clicking on the arrow button.
	Input box – Input of text or numeric values via the keyboard.
	Scrollbar with slider – Setting of numbers in the relevant input box by moving the slider or clicking on the arrow buttons or clicking on the slider and moving via the arrow keys of the keyboard. Press the **Shift** or **Ctrl** key while clicking on the arrow button to change the numeric values in coarse or fine steps.

Window element	Description / Explanation
	Check box – Activates / deactivates setting options.
	Button – Selection / performance of a function via mouse click.

4.1.3 Convention for the Text in this Manual

All the originally used terms of the software interface, e.g.

- names of windows,
- panels,
- input boxes,
- list / selection boxes,
- check boxes,
- menu items,
- names of buttons and
- keyboard keys,

are displayed in **bold letters** to allow easier identification.

4.1.4 Backup

System backup

- A complete backup is contained on the enclosed backup CD-ROM.

User files backup

The following user-generated files need to be included in a backup procedure (keep directory structure):

- Image database files: *.mdb (but not system_configuration_*.mdb
- LSM Image files: *.lsm
- Exported images: *.* (*.Tiff, *.LSM-Tiff, *.BMP, ...)
- Palette files: AIM \ Palette \ *.lut
- Filter files: AIM \ Filter \ *.krn
- Pinhole setting files: AIM \ PH*.pos
- Log files: AIM \ *.log

The following files generated during the system integration should also be included in a backup procedure:

- Parameter file for pinhole setting: AIM \ *.set
- Parameter file after pinhole adjustment: AIM \ *.adj
- Scanner files: AIM \ bin \ *.bin
- Microscope stand database: AIM \ database \ system_configuration_*.mdb

4.1.5 Software Operation

The LSM 5 software can be operated using the mouse, the PC keyboard, or both.

The operation of the mouse and the keyboard is identical to that of the Microsoft ® WINDOWS operating system and is therefore not dealt with in detail in this manual.

If required, see the Microsoft manual or online help for relevant information.

4.2 Switching on the System

The LSM system is turned on with the **REMOTE CONTROL** switch. This switches all the system components on except for the "Enterprise" UV laser.

If the UV laser shall be used, it can be switched on after the start of the WINDOWS ® NT operating system - but must always be switched on before the LSM 5 software is started.

If **REMOTE CONTROL** switch is not used, turn the system on with the "**I**" button on the laser module; in addition, the jumper plug supplied must be connected to the **POWER REMOTE CONTROL** terminal.

- Turn the **REMOTE CONTROL** switch to "**ON**" position (see Fig. 4-1).
  - This switches the entire system on.
  - Microscope and laser will be ready for operation after a short time.
  - Computer boots up.
  - Computer hardware system test runs.

☞ Drive "A" of the computer must not contain a floppy disk.

The monitor shows a dialog box for selection of the operating system version.

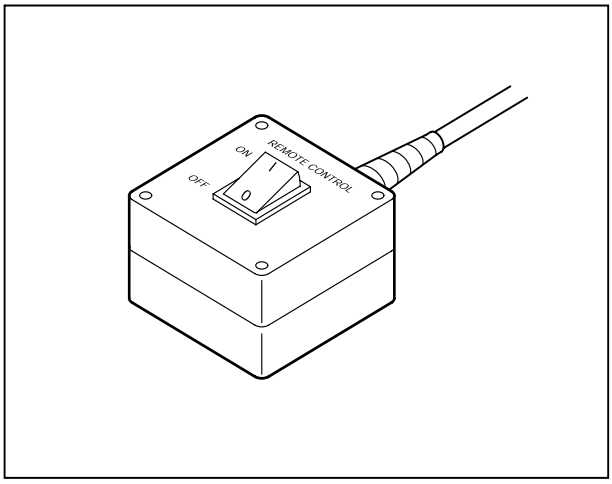


Fig. 4-1 REMOTE CONTROL switch

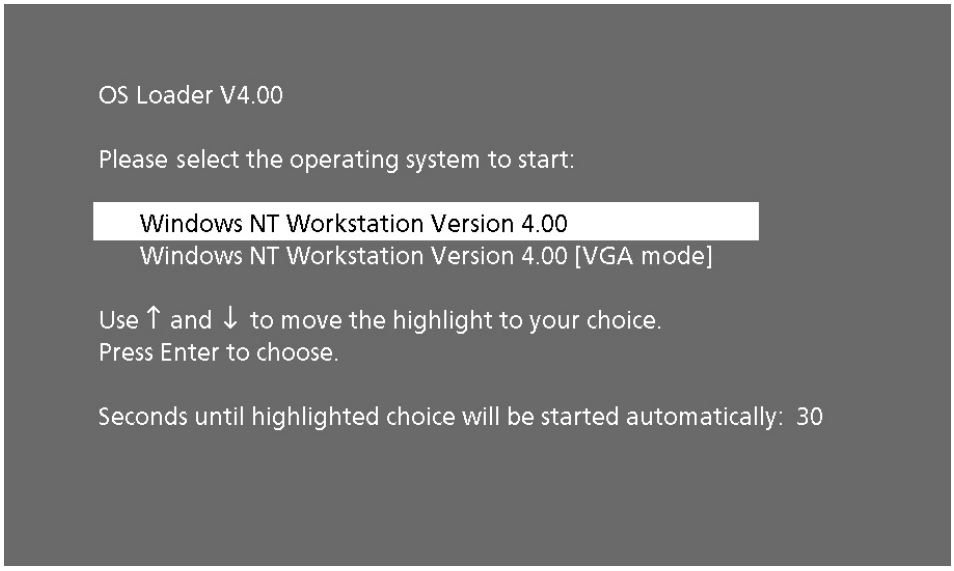


Fig. 4-2 Selecting the operating system version

Fig. 4-3 Begin Logon window

- Confirm the default setting of the "Windows NT Workstation Version 4.00" by pressing the **Enter** key.
  - WINDOWS NT operating system is being loaded.
  - The **Begin Logon** window appears on the screen.

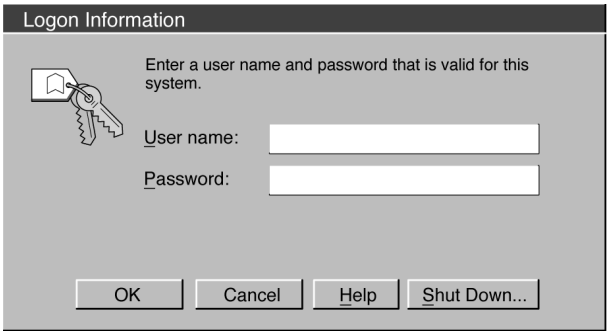


Fig. 4-4 Logon Information window

4.2.1 Log on to WINDOWS NT

- Press the three keys **Ctrl**, **Alt** and **Del** at the same time.
  - The **Logon Information** window appears on the screen, permitting you to log on to the WINDOWS NT 4.0 operating system.
- Enter the valid user name into the **User name** text box.
- Enter your password into the **Password** text box.

- After entries, confirm by clicking the **OK** button or **Enter**.
  - The WINDOWS NT operating system desktop appears on the screen, showing a number of icons.

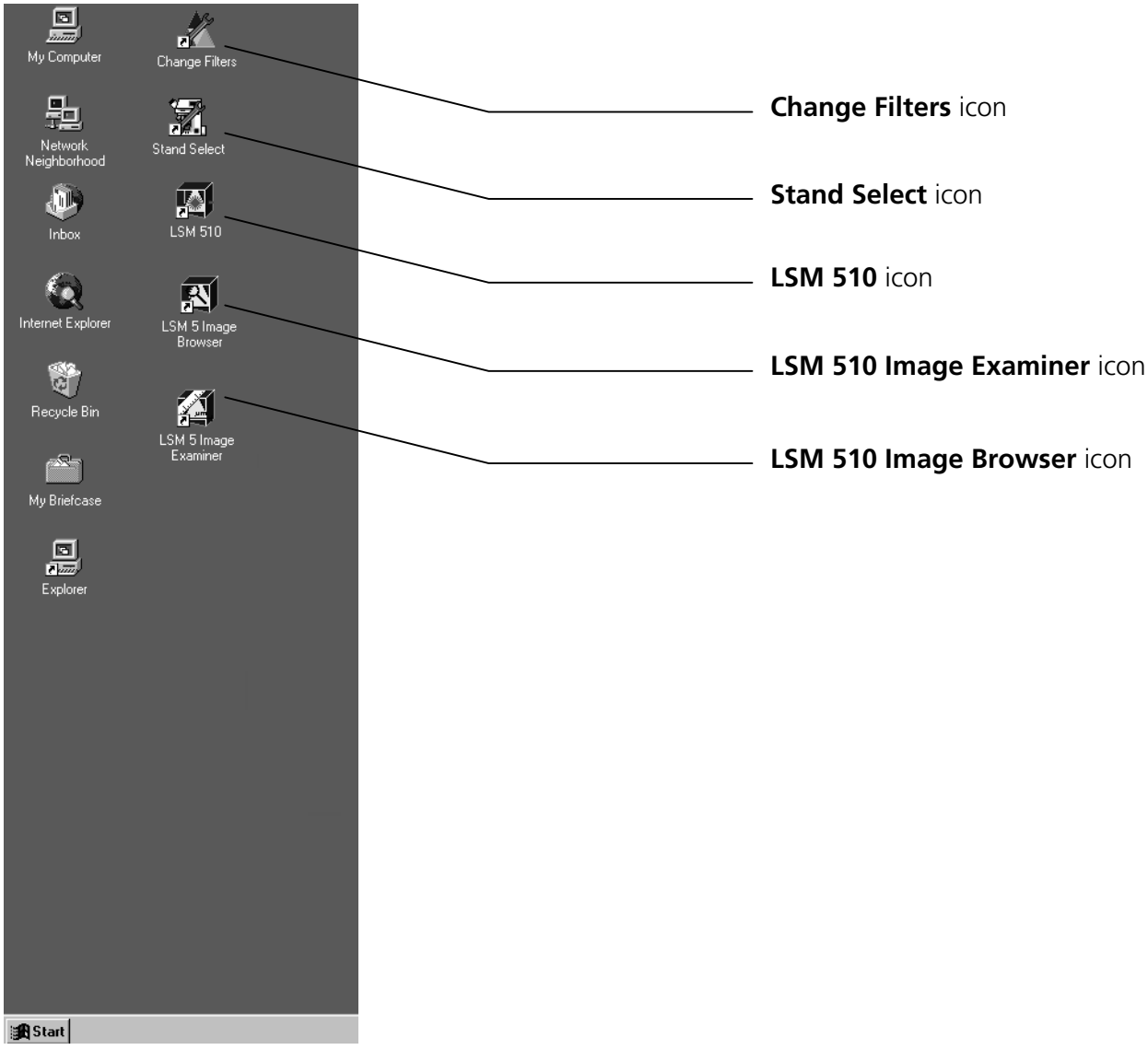


Fig. 4-5 WINDOWS NT operating system desktop

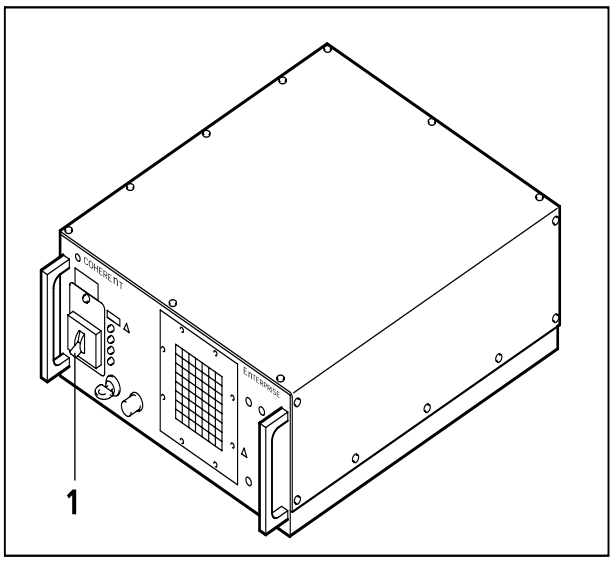


Fig. 4-6 Power supply of UV-Ar laser

4.2.2 Switching on the Enterprise UV Laser

- If the UV laser is required, switch it on via the toggle switch (4-6/**1**) of the power supply.
  - It will be ready for operation after a few seconds.

Fig. 4-7 Starting the LSM 5 software

4.2.3 Starting the LSM 5 Program

The LSM 5 software program can be operated in two different modes (with or without connected instrument system). In the on-line mode, the entire program package (image recording and analysis) is available, while only a part of the software functions (image analysis only of already stored images) and no hardware functions are available in the off-line mode. Of course, the off-line mode can also be started when the instrument system is connected. In that case, it is not necessary that the lasers and the microscope are switched on.

- Double-click on the **LSM 510** icon on the desktop of WINDOWS to start the LSM 5 software program (see Fig. 4-5).
  - The **LSM 510 Switchboard** menu appears on the screen (see Fig. 4-8).

Fig. 4-8 LSM 510 Switchboard menu

The **LSM 510 Switchboard** menu presents the following items for selection:

- **Scan New Images**

  Clicking on this button activates the complete LSM hardware (on-line mode).

- **Use Existing Images**

  This item allows you to process and analyze previously acquired images with the LSM 5 software. In this mode, control of the hardware (laser module ...) is not possible (off-line mode).

☞ Please note that the **Scan New Images** button must be activated before setting up the Routine Mode or the Expert Mode. Otherwise, the hardware can not be controlled by the LSM 5 software.

- **Start Routine Mode**

  Click on this button if you want to work with pre-configured system settings (typical applications).

– **Start Expert Mode**

Use of this mode requires to be thoroughly familiar with the exact microscope procedures and interrelations.
You need to set all parameters and functions upon your own decision; this mode therefore provides you with the greatest flexibility of operation.
It is also possible, however, to call up stored configurations and to modify the parameters / settings if necessary.

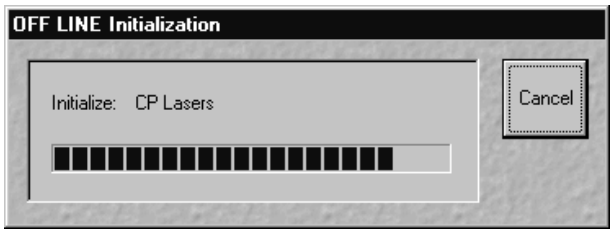


Fig. 4-9 OFF LINE Initialization window

After the start of the **Expert Mode** or the **Routine Mode**, instrument initialization is performed and can be monitored in the **Initialization** window and interrupted with a click on the **Cancel** button, if required.

Depending on the selected option (**Scan New Images** or **Use existing Images**), initialization is performed in the offline or online mode.

☞ Existing images can only be loaded and processed in the **Expert Mode**.

If you want to change from the **Expert Mode** to the **Routine Mode** and vice versa, close all the windows first.

Some printers (for example KODAK Thermo Printer) will produce an error message "hard key not found" in case the printer is not switched on.

Remedy: turn on the printer before starting the LSM 5 software.

Don't switch off the KODAK printer during the scanning process.

4.3 Quick Start in the Expert Mode

Proceed as follows to generate images in the Expert Mode:

- start the Expert Mode
- test / change the microscope setting: objective, fluorescence / attenuation filters, illumination mode, diaphragms
- normal setting of the microscope on the specimen with observation in brightfield or fluorescence contrast (KÖHLER-type illumination)
- switch to **LSM** mode
- setting of lasers: laser type, laser intensity (power)
- configuration of beam path and channel assignment: tracks, multi-tracks, switching on / off of laser lines and intensity (excitation)
- image creation: determine the scan method (line, frame) and scan parameters (image size, scan speed, pixel depth, scan direction, scan average, zoom, rotation, offset)
- image optimization and storage

4.3.1 Start the Expert Mode

- Double-click on the **LSM 510** icon on the WINDOWS NT operating system desktop.
  - The **LSM 510 Switchboard** menu appears on the screen.
- Click on the **Scan New Images** button and **Start Expert Mode** button in the **LSM 510 Switchboard** menu.
  - The LSM will go through the initialization and open the **Main** menu labeled **LSM 510 Expert Mode**. The **Main** menu appears on the screen.

Fig. 4-10 LSM 510 Switchboard menu

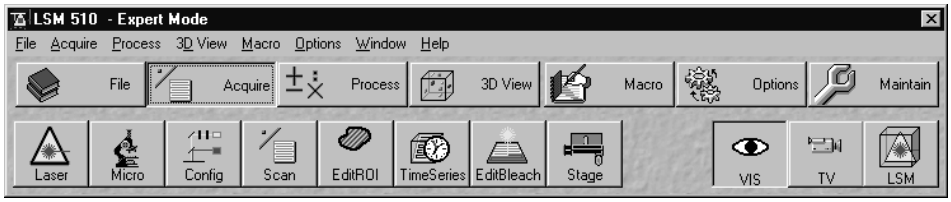


Fig. 4-11 Main menu of LSM 510 - Expert Mode

4.3.2 Set the Microscope

This step is used to set:

- microscope objective
- specimen position
- specimen focus

4.3.2.1 Axioplan 2 imaging MOT

- Click on the **Acquire** button in the toolbar of the **Main** menu.
- Move the tube slider on the microscope to the **VIS** position.

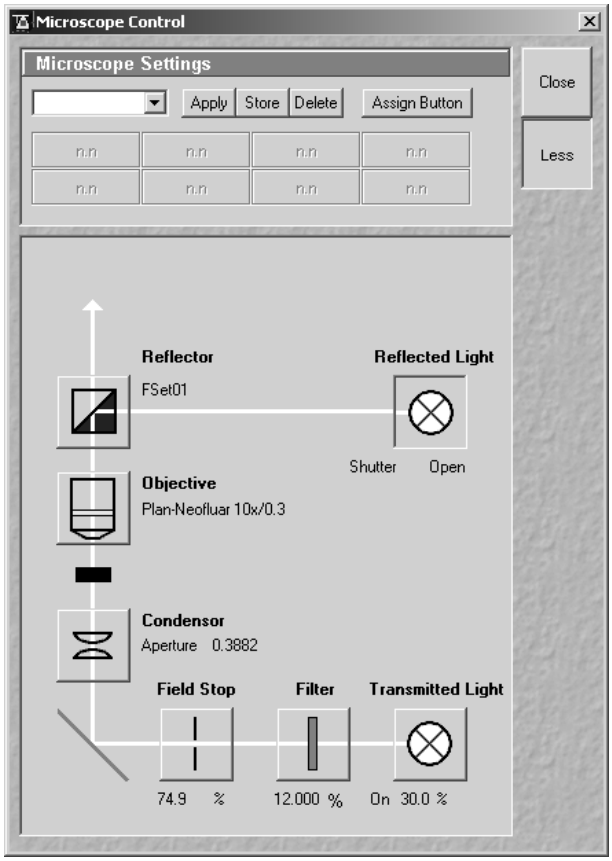


Fig. 4-12 Axioplan Control window

- Click on the **Micro** button in the **Acquire** subordinate toolbar.
  - The **Axioplan Control** window appears on the screen.
- Put the specimen on the stage - make sure the specimen is mounted securely and flat. For tests use the supplied Convallaria specimen.

You can view the specimen in either fluorescence (reflected light) or transmitted light.

- To view the specimen in transmitted light, set the **Reflector Turret** position to **None** and activate the **Transmitted Light** panel by clicking on the **Transmitted Light** button. Select the **On** button and control the intensity by the slider.
- Select an objective with low magnification by clicking in the **Objective** panel of the **Axioplan Control** window.
- Set the microscope to KÖHLER illumination manually (see Axioplan 2 imaging MOT operating manual).
- Select the specimen area to be examined by moving the XY-stage and focus exactly on the selected area.
- Close the **Axioplan Control** window. Move the tube slider on the microscope to the **LSM** position.

4.3.2.2 Axiovert 200 M

- Click on the **Acquire** button in the toolbar of the **Main** menu.
- Click on the **VIS** button in the **Acquire** subordinate toolbar.
- Click on the **Micro** button.
  - The **Axiovert Control** window appears on the screen.
- Put the specimen on the stage - make sure the specimen is mounted securely and flat. Use the supplied Convallaria specimen at first.

You can view the specimen in either fluorescence (reflected light) or transmitted light.

- To view the specimen in transmitted light, set the **Reflector Turret** position to **None** and activate the **Transmitted Light** panel by clicking on the **Transmitted Light** button. Select the **On** button and control the intensity by the slider.
- Select an objective with low magnification by clicking in the **Objective** panel of the **Axiovert Control** window.
- Set the microscope to KÖHLER illumination manually (see Axiovert 200 M operating manual).
- Select the specimen area to be examined by moving the XY-stage and focus exactly on the selected area.
- Close the **Axiovert Control** window. Click on the **LSM** button.

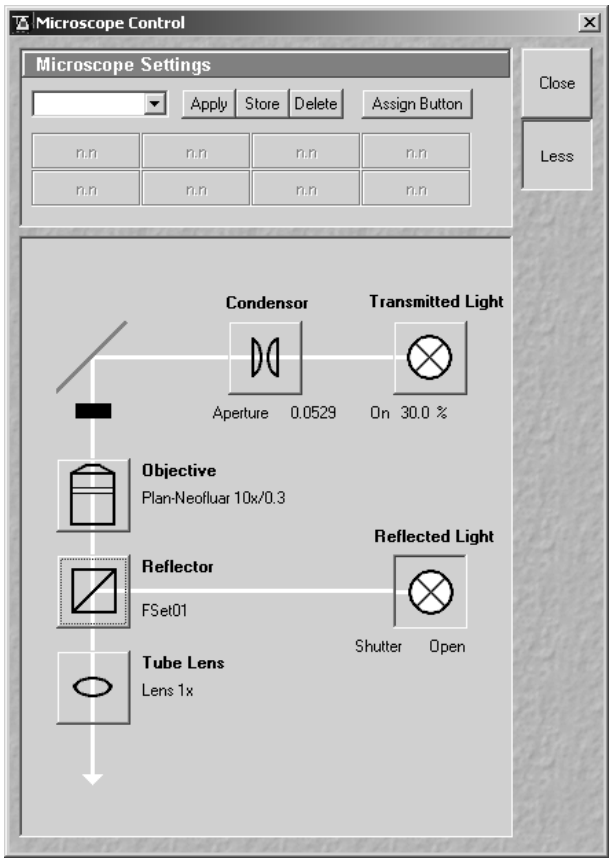


Fig. 4-13 Axiovert Control window

4.3.2.3 Axioskop 2 FS MOT

For setting the Axioskop 2 FS MOT, proceed in the same way as described for Axioplan 2 imaging MOT.

Since the Axioskop 2 FS MOT is not motorized (except for the z motor drive), all microscope settings have to be made manually.

The **Micro** button in the **Acquire** subordinate toolbar of the **Main** menu is therefore not available.

4.3.3 Turn on the Lasers

This step is used to switch the lasers and set the intensity.

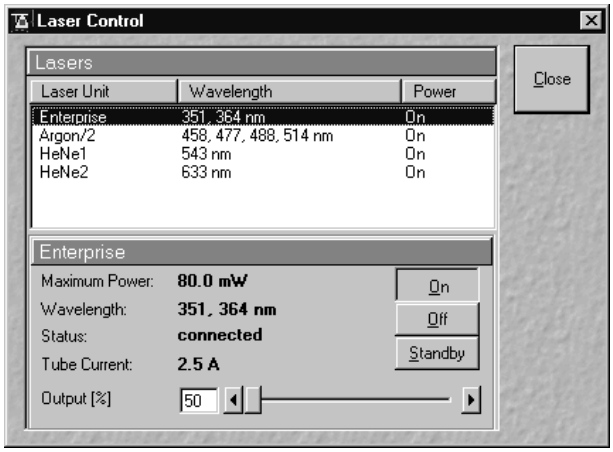


Fig. 4-14 Laser Control window

- Click on the **Laser** button in the **Acquire** subordinate toolbar of the **Main** menu.
  - A **Laser Control** window with a list of available lasers will appear. Depending on the lasers available in your system, your screen may differ from the one displayed.
- Using the mouse, click on the laser(s) featuring the appropriate wavelength to excite the dyes used to label your specimen.
- In the case of the argon and UV laser (Enterprise), click on the **Standby** button first.
  - **Warming Up** appears in the line **Status**.
  - After 2 minutes, when the warming-up phase is finished, the **Ready** message appears.
- Then click on the **On** button.
  - The laser is switched on.
- Use **Output [%]** scrollbar to set the intensity to 50 %.
- In the case of the HeNe laser, click on the **On** button directly.
- Close the window with the **Close** button.
- For precise measurements the system should warm up for 2 hours.
- Depending on the lasers available in your system, your screen may differ from the one displayed.

4.3.4 Set the Beam Path

This step is used to specify beam path parameters by using a predefined **Track Configuration**.

- Click on the **Config** button in the **Acquire** subordinate toolbar of the **Main** menu.
  - The **Configuration Control** window appears on the screen. Your screen may differ from the one displayed.
- Click on the **Single Track** button, unless it has already been activated.
- Click on the **Config** button in the **Configuration Control** window.
  - The **Track Configurations** window appears on the screen.

Stored standard configurations (Tracks) are available in the **Track Configurations** window, which can be used for fast and easy image acquisition.

- The list of configurations will appear by clicking on the button. Choose the **FITC/Rhod** configuration from the list.
- Click on the **Apply** button.

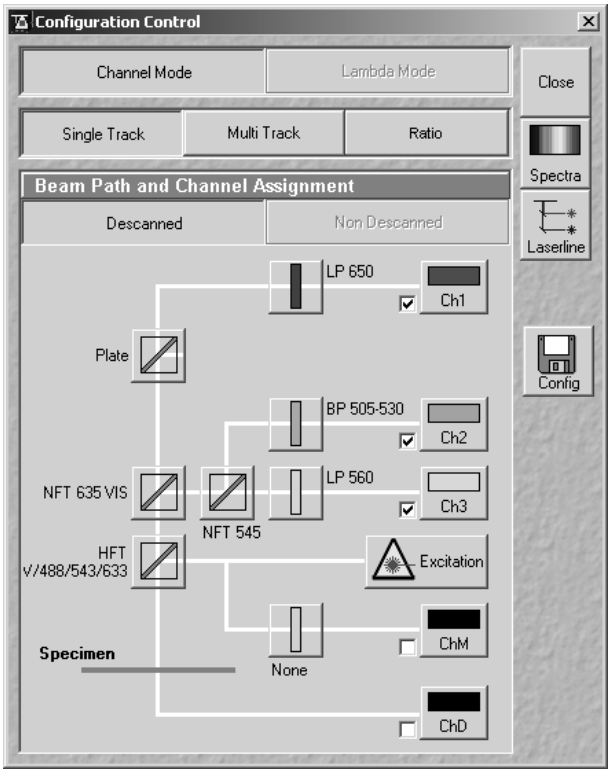


Fig. 4-15 Configuration Control window

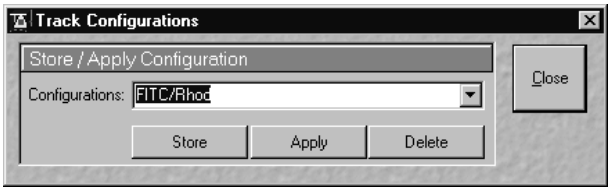


Fig. 4-16 Track Configurations window

All the settings of the selected standard configuration, such as beam path, excitation wavelength and intensity, AOTF attenuation (Acousto-Optical Tunable Filters), Gain, Offset and Data Depth, are loaded via the software and displayed in the relevant windows and panels. The **Track Configurations** window is closed automatically.

☞ If you click on the **Close** button, the **Track Configurations** window will be closed without any change being made to the Track Configuration.

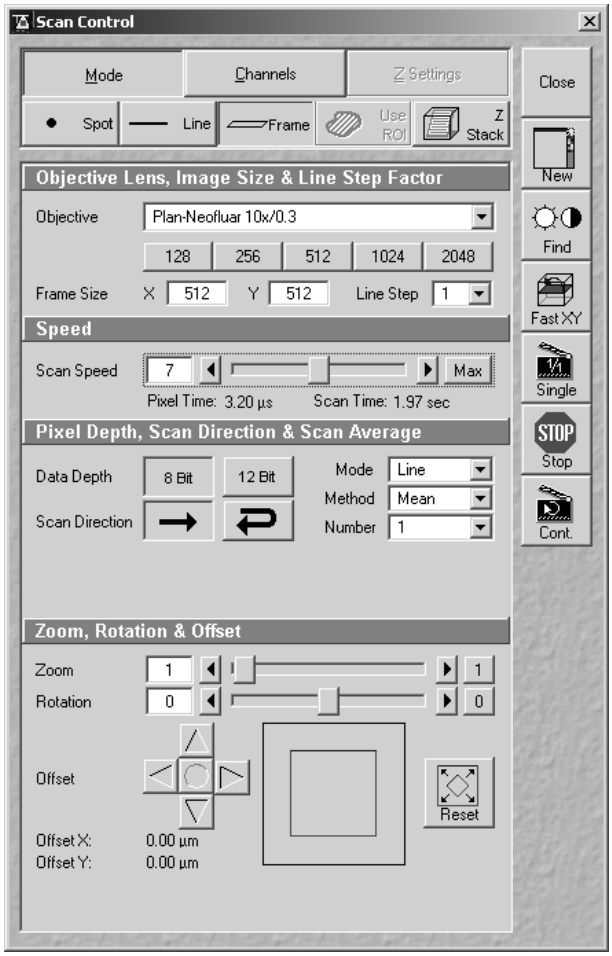


Fig. 4-17 Scan Control window

4.3.5 Scan an Image

This step is used to specify parameters and execute image acquisition.

- Click on the **Scan** button in the **Acquire** subordinate toolbar of the **Main** menu.
  - The **Scan Control** window appears on the screen.

The microscope must be in the LSM mode (press **LSM** button and move the relevant slider(s) on the microscope stand to the **LSM** position).

On the right-hand side of the **Scan Control** window various buttons appear.

We will use the: **Find**, **Single**, **Cont.**, (**Stop**) scan buttons.

- To scan an XY-image, click on the **Frame** button.
- Click on the **Find** button on the right-hand side of the **Scan Control** window.
  - An XY-image with automatically generated settings for brightness and contrast is produced.

- A specimen with 2 labels (FITC, Rhod.) with defined channels is easier to view in split screen where each channel is arranged side by side. You can toggle between **xy** and **Split xy** in the **Image Display** window.

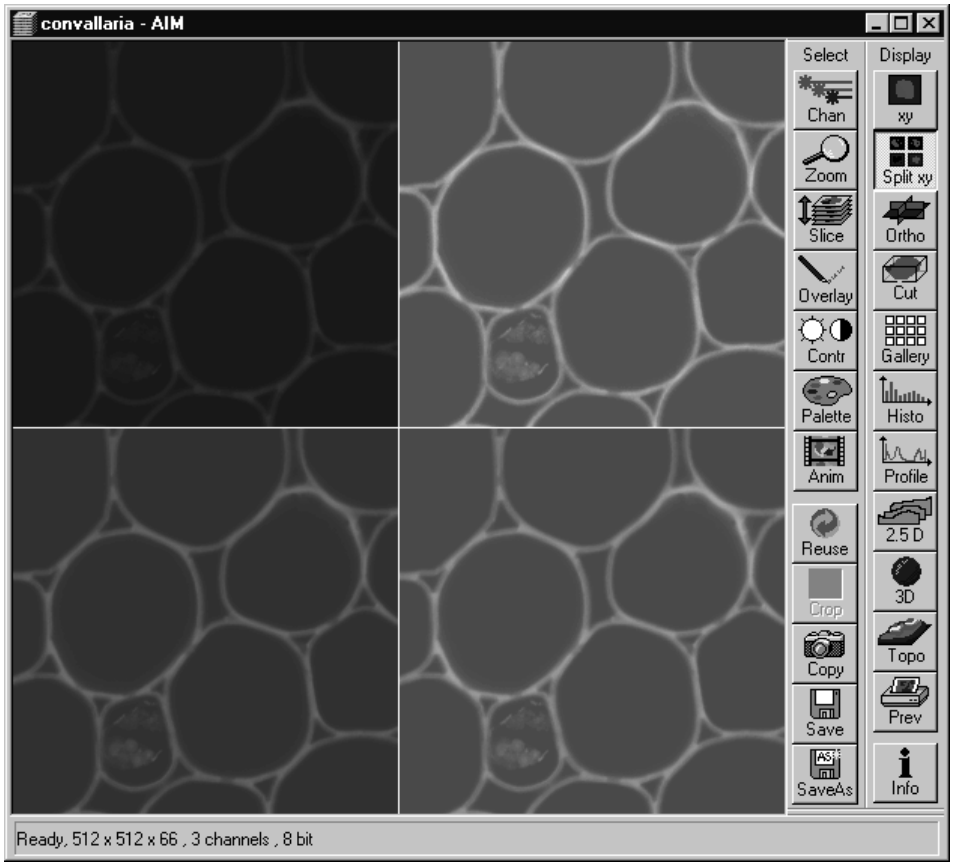


Fig. 4-18 Image Display window with Split xy mode

The scanned image can now be optimized for contrast, brightness and confocality.

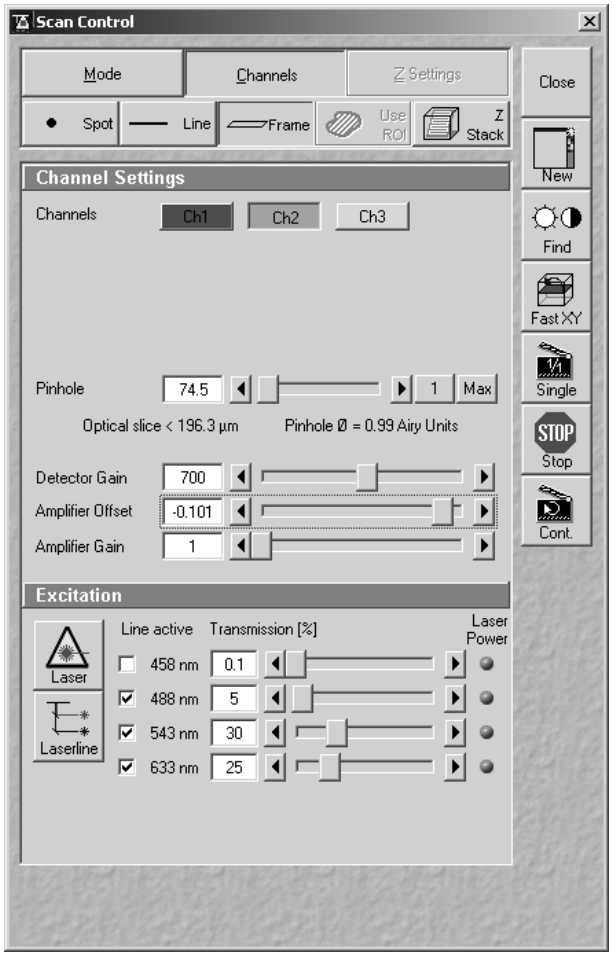


Fig. 4-19 Scan Control window

Proceed as follows for image optimization:

- Click on the **Channels** button in the **Scan Control** window.
  - The **Channel Settings** panel and the **Excitation of Track (...)** panel are displayed in the **Scan Control** window.
- Press the **Cont.** (continuous scan) button on the right-hand side of the window. This starts the continuous image acquisition, which can be interrupted by pressing the **Stop** button.
- Under the **Channel Settings** panel all buttons for each channel you have set up are displayed. Click **Ch1** (channel 1 of track 1), for example, if you want to adjust the first image displayed in the split mode window.
- Use the **Pinhole** slider to set the pinhole diameter.
  - The selected pinhole diameter should be small enough to still allow the Detector Gain setting and to provide sufficient image information. 1 Airy unit is a decent value to obtain a confocal XY-image (use the **1** button).
- If required, adjust the pinhole again (see **Main** menu, **Maintain** subordinate toolbar, **Pinhole** button).

- Use the **Detector Gain** slider to set image contrast and brightness. This adjustment is very sensitive. Try using the left and right arrows to make the adjustment instead of dragging the slider bar. Use the **Shift** and **Crtl** keys for changing to coarse and fine steps.
- To adjust the black level (background) use **Ampl. Offset**.
- Also, try adjusting the microscope by manual focusing. Sometimes you will find that there are other focal planes within the specimen which are brighter, and therefore the detector gain will need to be turned down.
- Once you have optimized a particular channel, you can switch to the next channel required and repeat the optimization.
- As soon as all channels are optimized, click on the **Stop** button.

- To further improve image quality you can slow down the scan speed, allowing more photons to integrate on the detector, or apply image averaging to remove random noise, or a combination of both. These adjustments are made by clicking on the **Mode** button on the **Scan Control** window.
- Set the **Scan Speed** in the **Speed** panel.
- Select the **Line** or **Frame** average **Mode**, the **Mean** or **Sum** average **Method** and the **Number** of averages in the **Depth, Scan Direction & Scan Average** panel accordingly by observing your image. The setting average of **16** (**Number 16**) should improve signal / noise dramatically; however, the image acquisition rate will be slower.
- When the image optimization has been finished, click on the **Single** button to generate a single image of the specimen.
- If your specimen is sensitive to photobleaching, you can attenuate the laser illumination by clicking on the **Channels** button in the **Scan Control** window. At the bottom of the window you can set the percentage of laser power (**Transmission [%]**) for each excitation wavelength. You will probably have to increase the **Detector Gain** if you decrease the laser power. This setting controls the transmission degree of the Acousto-Optical Tunable Filters (AOTF).

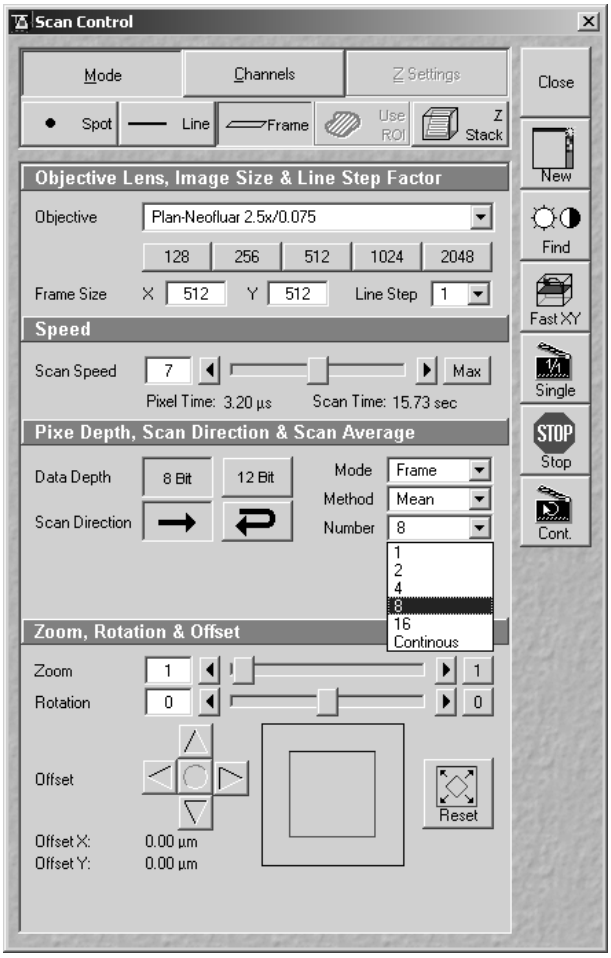


Fig. 4-20 Scan Control window

☞ Try to use as low laser light intensity as possible to prevent sample bleaching. For that purpose increase the detector gain to a value of approximately 800 V.

4.3.6 Store the Image

This step is used to activate an existing database or to create a new database in which the acquired image is stored with the used settings and comments.

- To save the image, click on the **Save As** button on the right-hand side of the **Image Display** window.
  - The **Save Image and Parameter As** window appears on the screen.

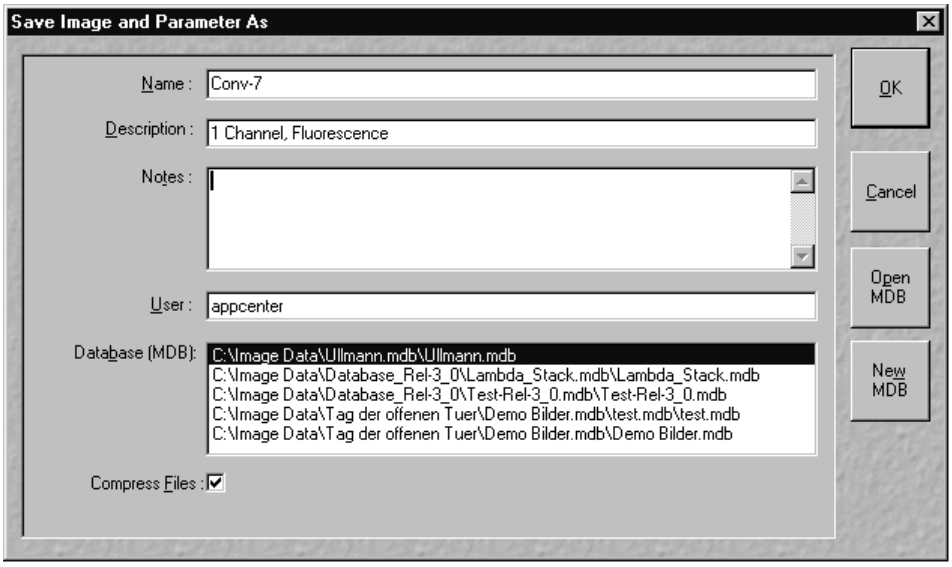


Fig. 4-21 Save Image and Parameter As window

Proceed as follows to store images in an existing database:

- To select the required database, click on its name (blue cursor bar) in the **Database (MDB)** list box of the **Save Image and Parameter As** window.
- Enter a suitable name for the image in the **Name** input box. If required, enter further details on the image in the **Description** and **Notes** input boxes.
- Click on the **OK** button to add the image to the selected database.

Proceed as follows to store images in a new database:

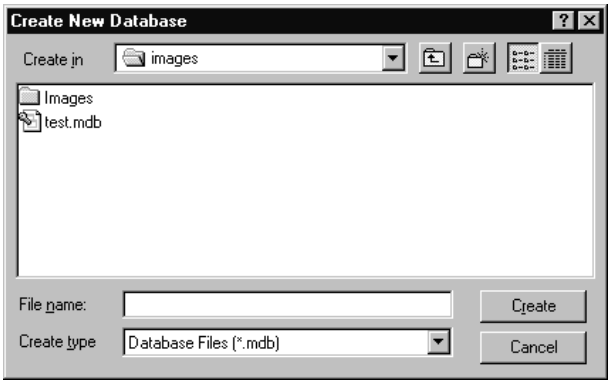


Fig. 4-22 Create New Database window

- Click on the **New MDB** button in the **Save Image and Parameter As** window.
  - The **Create New Database** window will be opened.
- Enter a database name in the **File name** input box. The name can consist of as many characters as you like.
- Before clicking on the **Create** button in the **Create New Database** window, set the location in which the database will be created by selecting the drive in the **Create in** list box, and double-click on the required folder icon in the list displayed.
- Click on **Create**.
  - The new database will be created and displayed on the screen.
- As described above, enter a name and, if required, further image details in the **Save Image and Parameter As** window.
- Click on the **OK** button**.**
  - The image will be stored and included in the new database.

4.4 Shut-Down Procedure

Never shut down the computer by its main switch while your LSM program is still active, or else you will lose the currently set operating parameters and the images just scanned.

In the **Settings for user** dialog window, which can be activated with the **Options / Settings** buttons, activate **Laser off** or **Exit** in the **Shutdown** tab. The lasers will then automatically be switched off when you exit the LSM program.

4.4.1 Exiting the LSM Program

- Close all open windows of the LSM program by clicking on the closing icon ☒ in the top right corner of each window.
  - This closes the respective window and removes the respective icons from the taskbar.
  - After all dialog windows have been closed, the **LSM 510 Switchboard** window appears.

Fig. 4-23 LSM 510 Switchboard menu

- Click on the **Exit** button.
  - This terminates the LSM program.
  - The monitor screen shows the desktop of the WINDOWS NT operating system.

4.4.2 Shut Down the WINDOWS Operating System

- Move the cursor to the bottom margin of the screen.
  - This opens the taskbar containing the **Start** button.
- Click on the **Start** button of the taskbar.
  - This opens a pop-up menu.
- Click on the **Shut Down** item.

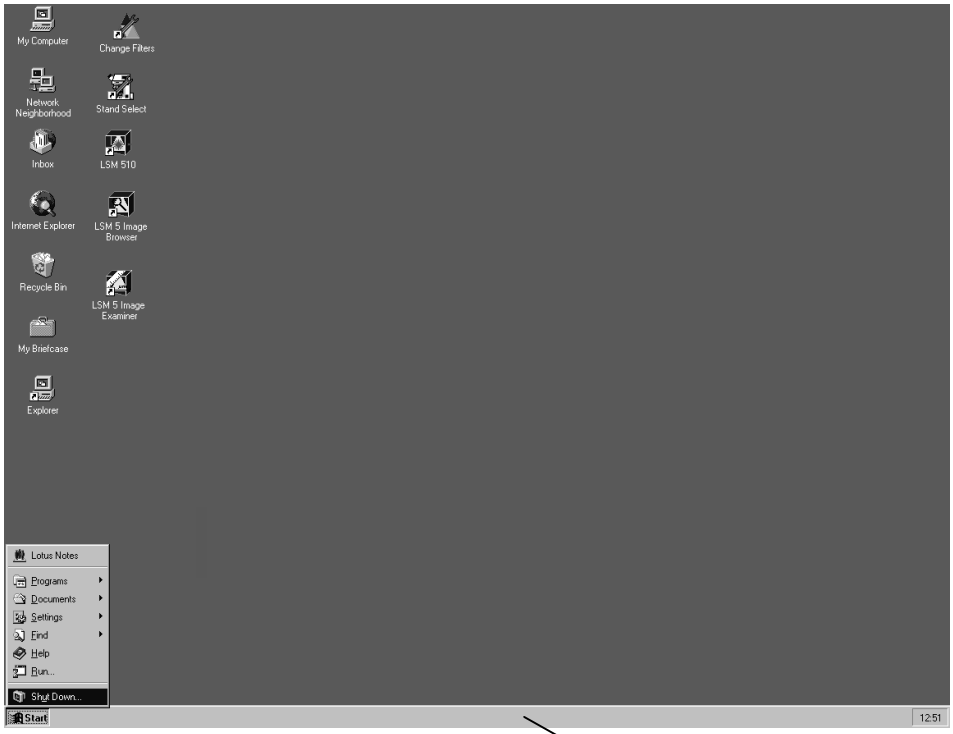


Fig. 4-24 Start menu

Fig. 4-25 Shut Down window

– This opens the **Shut Down Windows** window, in which you can select between **Shut down, Restart** and **Login**.

- Unless already set by default, click on **Shut down the computer?**
- Click on the **Yes** button.

The screen now displays the message

Shutdown in Progress - Please wait while the system writes unsaved data to the disk.

About 20 seconds after WINDOWS NT has been run down, the **Shutdown Computer** window appears which tells you that you can now turn off your computer.

4.4.3 Turning Power Off

☞ Please bear in mind that a cooling phase of at least 5 minutes is required between switching off of the laser via the software and switching off of the entire system via the REMOTE CONTROL main switch or the Power Supply switch of the Enterprise UV laser.

⚠ Throw the REMOTE CONTROL main switch and the power supply switch of the Ar Laser to position "**OFF**" after 5 minutes.

– This puts your LSM 510 microscope system, including the computer, off power.