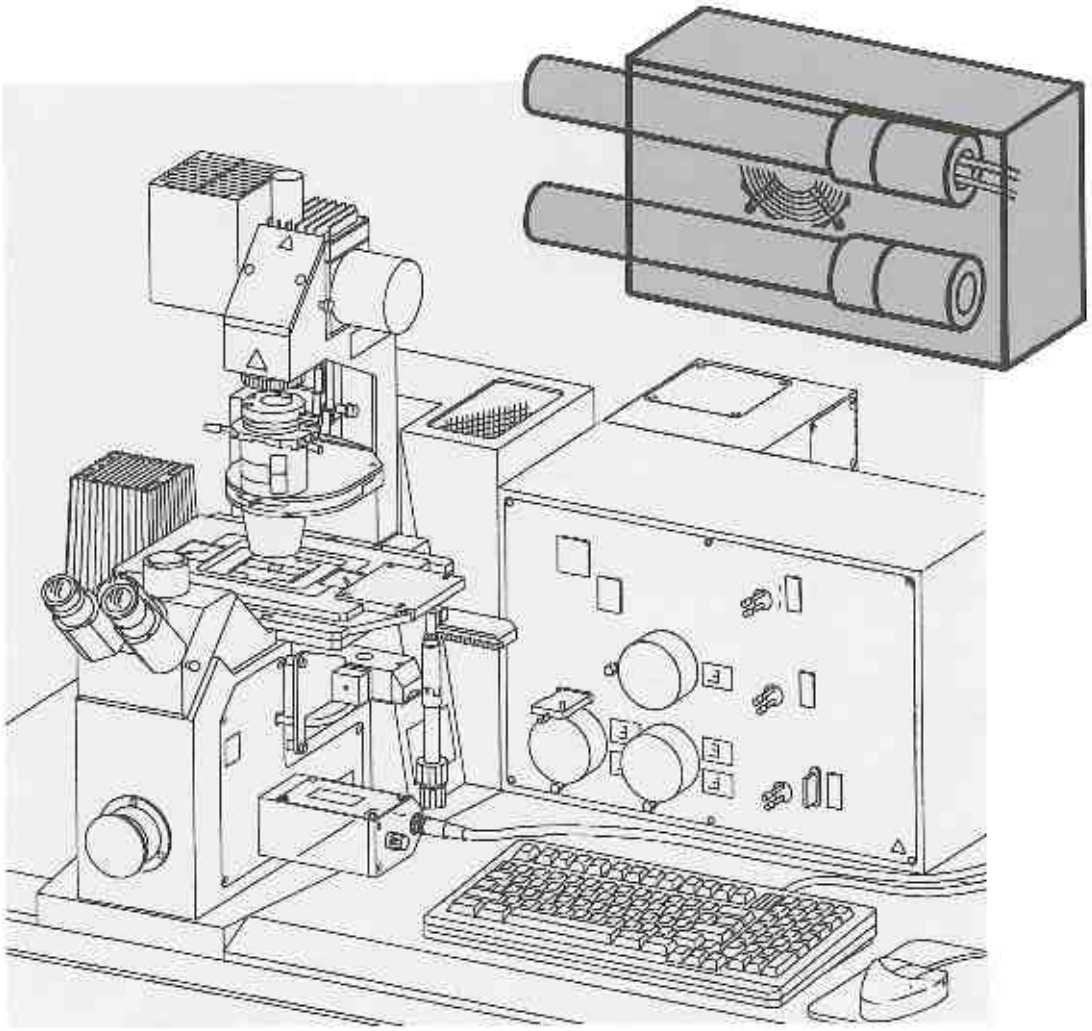
LSM 410 invert
Laser Scan Microscope



Operating manual

ZEISS

This manual is not covered by an update service.

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LSM 410 invert
LASER SCAN MICROSCOPE

How to make best use of the LSM 410 invert operating instructions

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

The WARNING symbol warns against hazards for the user that might arise when operating the laser or which might emanate 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 EXAMPLE symbol identifies those points in the text where a previously explained topic is presented with reference to a concrete example.

The APPLICATION symbol will help you to find a possible approach to a solution to your special application problem.

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

- If you want to quite generally know what topic areas are dealt with where, you can obtain a general overview by referring to the following outline of sections.
- To make it easy to get a general picture, you will find a detailed table of chapter contents at the start of every chapter. Here, you will see at a glance what topics are dealt with in detail.
- If you are completely new to the LSM 410 invert, then you are urgently advised to begin with Sections 1 and 2.
- If you already have an adequate knowledge of the system structure, you should nevertheless conscientiously work through Sections 4 and 5 to obtain information on operation and the application tasks. Don't try to find a solution to your problem by trial and error.
- If you have a fundamental knowledge of a subject matter, but you are not sure whether you are fully informed about all important possibilities, go over the problem chapter and, in doing so, pay attention to the corresponding subheadings.
- Use the page–oriented list of key words in Section 10 if you want to quickly find the sections containing concrete details of your problem.

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

1 This section contains general notes from the manufacturer on device safety and on operating safety and also contains information on possible hazards related to failure to observe the device documentation.

2 Here you will find a description of the individual system components. Any possible options are dealt with in the annex. Diverse block diagrams will swiftly convey an adequate knowledge of the system to you to enable you to optimally assess the performance capabilities of your LSM 410 invert.

3 This section contains a tabular list of meaningful laser combinations as well as the affiliated emission filter and colour splitter combinations.

4 In the section entitled "Operation", you will find fundamental information on commissioning by service personnel, on commissioning by operating personnel and notes on how to work with laser scan technology.

5 In this section you will find notes on user–oriented problem areas and examples of solution proposals or suggestions of how to optimally master your special microscopy task using the LSM 410 invert.

6 This section contains a description of the LSM software package (basic program and add–ons). At the same time, all functions and settings are presented in a systematic form and in the order in which they can be reached from the basic menu via the sub–menus and dialog boxes.

7 In this section you will find tips and practical hints on how to generate so–called macro functions yourself that are harmonised specially to your needs.

8 This section contains general notes on care operations that can be carried out by the operator on the LSM and also contains references to maintenance and service operations that should by carried out by the Technical Service.

9 In this section you will find detailed information on installation of the operating system and the graphical user interface, information on setting up Start/Ini files, information on how to adapt the LSM system to specific hardware and nationally specific settings and notes on how to perform an LSM program update.

10 Here you will find certificates and test reports concerning the LSM system and diverse lists which will substantially facilitate handling of the LSM documentation.

Country: _____

Order No.: _____

Serial No. (CZG): _____

Delivery date: _____

Special design: (Specification is available and is included with the report) ☐

Stand:

Axiovert (special design)	(45 13 03-9801)	☐
Axiovert 100 6-F H	(45 13 10)	☐
Axiovert 100 5 H/DIC	(45 13 11)	☐
Axiovert 135 (TV)	(45 13 14)	☐
Axiovert 6-F H (TV)	(45 13 14-9801)	☐
Axiovert 135 6-F H	(45 13 15)	☐
Axiovert 135 5 H/DIC	(45 13 16)	☐
Axiovert 135 M	(45 13 30)	☐
		☐
Optovar	(45 13 73 / 74)	☐

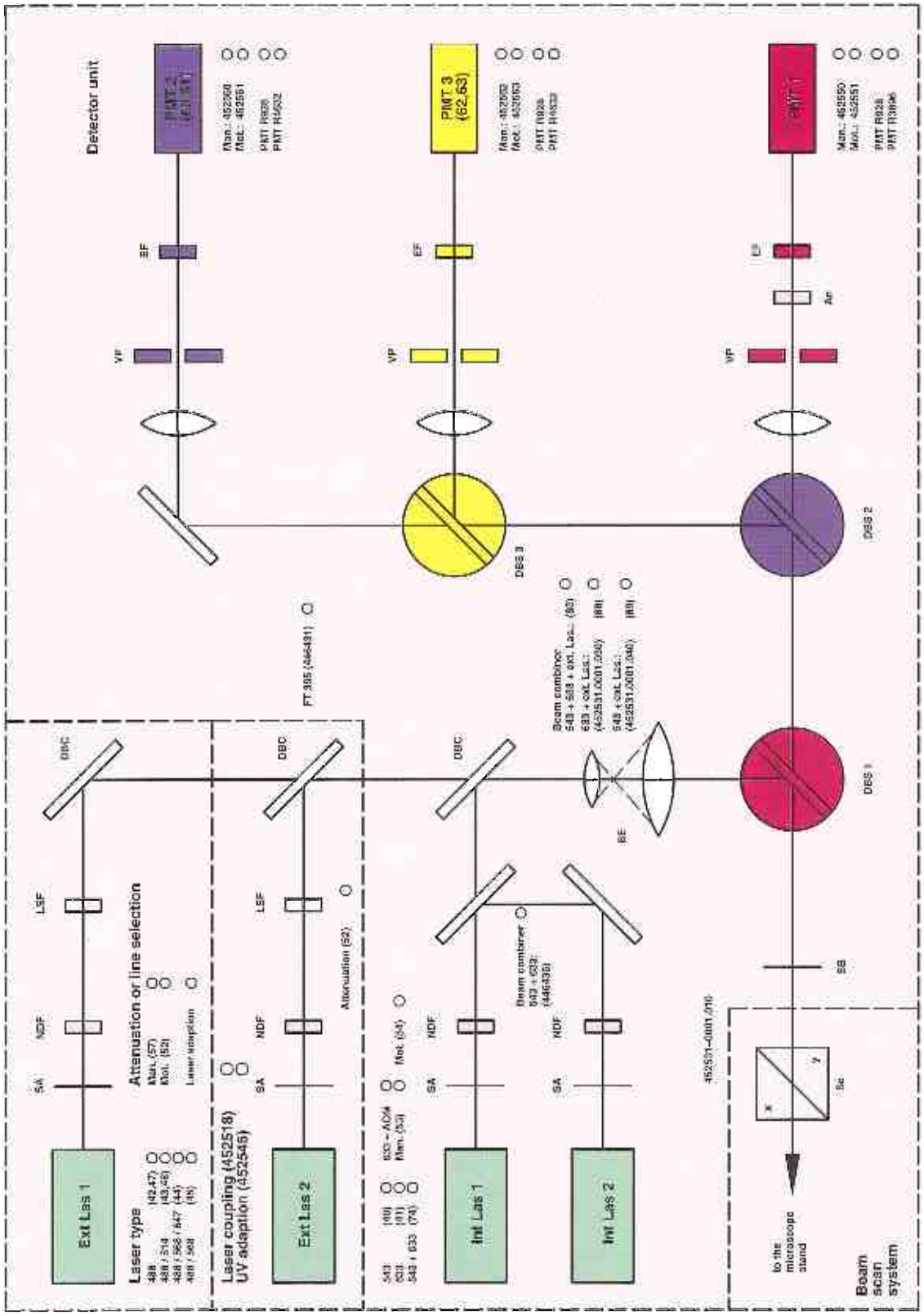
Operating voltage: 100 V ☐ 115 V ☐ 127 V ☐ 230 V ☐

Options:

Transmitted light detector:	Manual (45 25 65) ☐		Motor (45 25 67) ☐	
EISA computer		Yes ☐		No ☐
High resolution		Yes ☐		No ☐
TMC stage (41 90 23)		Yes ☐		No ☐
Laser tweezer		Yes ☐		No ☐

Software options:

3D	☐	Scanning stage (x,y)	☐
Time series	☐	Surface topography	☐
Remote	☐		



"Configuration variants"

CONTENTS

1 NOTES ON DEVICE SAFETY

1.1 General

The LSM 410 invert laser scan microscope including original accessories and compatible accessories from other manufacturers must only be used for the microscopy methods described in these operating instructions.

The manufacturer cannot assume any liability for any other application, possibly also involving individual modules or single parts. This also applies to all service or repair operations that are not carried out by authorised service personnel. Moreover, all guarantee/warranty claims will also be null and void for those parts that were not directly affected by repair.

1.2 Regulations

An extensive knowledge of the hardware/the system is indispensable for safe operation of the LSM 410 invert.

Read these operating instructions and all device publications belonging to the system conscientiously **before** operating the LSM 410 invert! You can obtain additional information on the delivered hardware configuration and on optional expansion possibilities from the manufacturer or via the service hotline.

☞ The LSM 410 invert was designed and tested in conformity with IEC publication 1010-1, "Safety regulations for electrical instrumentation and control and laboratory devices", taking applicable CSA and UL specifications into account, and was delivered in a safe condition (good manufacturing practice).

☞ As the system is largely operated via menus on a computer, you as the user should be acquainted with the principles of the MS DOS operating system and its graphical user interface WINDOWS. The corresponding manuals are supplied together with the programs.

☞ In accordance with WHO regulations, the LSM 410 invert is a device that belongs to laser hazard class 3b. The WHO recommendations concerning health and industrial protection when handling laser devices must be observed. The operator of the unit must also observe the legal accident prevention regulations.

1.3 Notes on setting up the microscope system

The LSM 410 invert is set up and assembled on the system base plate, and the unit is commissioned, including a basic introduction for the operating personnel, by authorised Carl Zeiss service personnel.

The LSM 410 invert laser scan microscope is delivered in three wooden crates:

- Crate 1: system table and, if applicable, external lasers
- Crate 2: stand unit mounted on a granite plate
- Crate 3: electronics rack, tower

Four persons are needed to set up the LSM 410 invert!

The LSM 410 invert 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 openings 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 included mains cables. The PE connection must not be detrimentally influenced 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 electronics rack.

The back panel of the electronics rack must only be removed by specialised Carl Zeiss personnel. This also applies to establishing or modifying cable connections on system components.

After installation or after conversion of the LSM system, authorised specialised personnel must carefully check that it is in a proper condition and, particularly, that covers protecting against laser radiation are present.

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, make sure that the formation of electrostatic charges by electronic components is avoided.
To avoid vibrations during operation, the LSM 410 invert 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 computer that operates with the DOS operating system under the WINDOWS graphical user interface.

Do make sure, though, that you receive your LSM system with the operating system installed, with initialisation and start files set up and with the LSM program incorporated in the autostart routine.

If you should ever replace the computer, the operating system, the start files and the user software must be reinstalled. If you have enough experience in handling DOS and WINDOWS, you can restore the prerequisites for execution of the LSM program on your new computer yourself (see also the information in Section 9).
If you are unsure, however, then enlist the aid of an authorised software specialist or Carl Zeiss service.

As standard, your computer has one hard disk drive and one diskette drive for 1.44 MB diskettes.

When working with the hard disk, it is important to know that it always becomes slower the more data it contains. Therefore, store data that you do not permanently need on diskette.

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 switch off your computer while data media are in operation and back up your files by creating back-up diskettes in good time.

1.5 Warning and information plates

The warning and information plates attached on the LSM 410 invert must be observed. Contact Carl Zeiss Germany or one of the service agencies if you should discover that the plates listed below are missing. You will receive a free replacement.

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

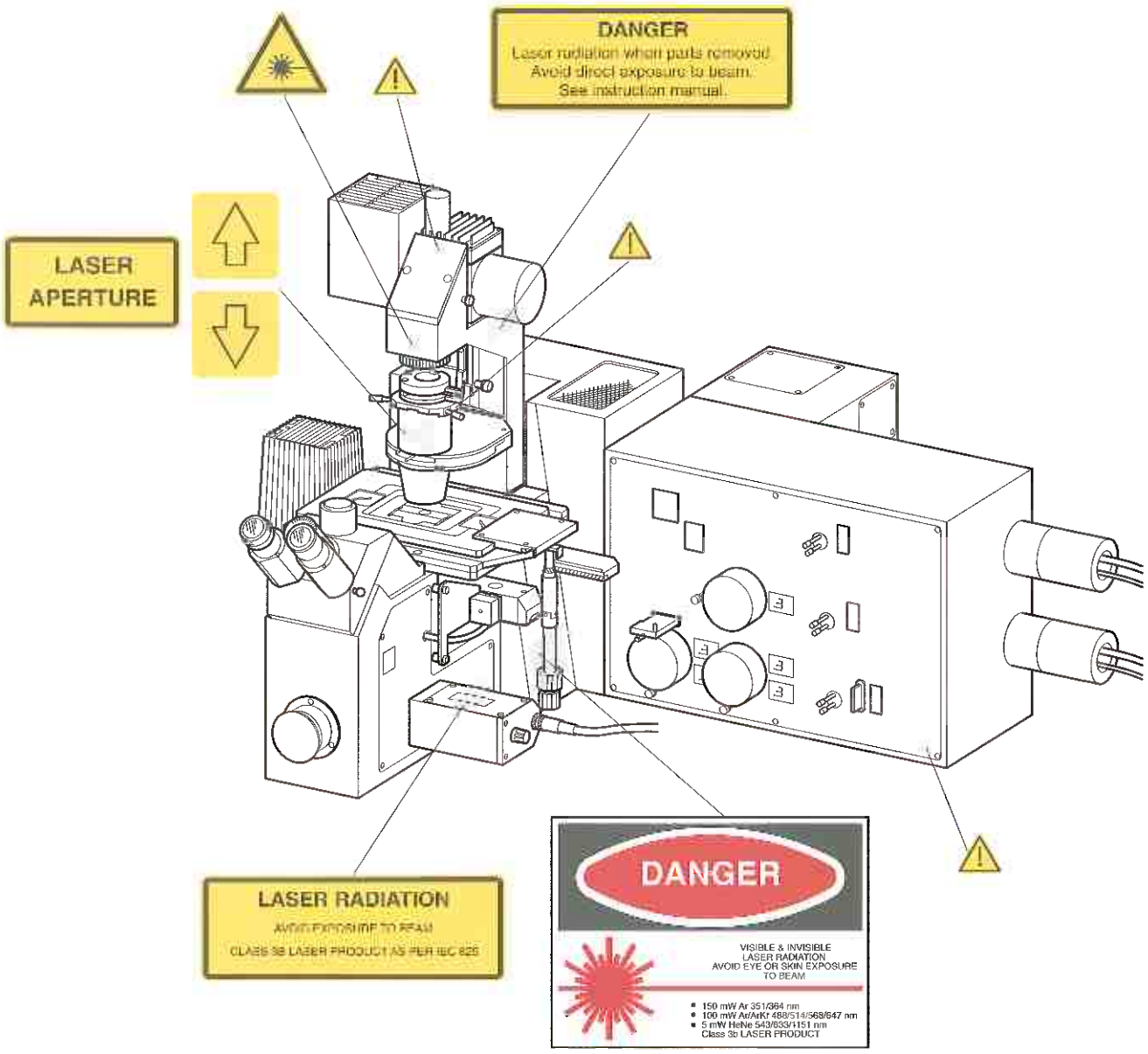


Figure 1-1 Warning and information plates on the front of the unit

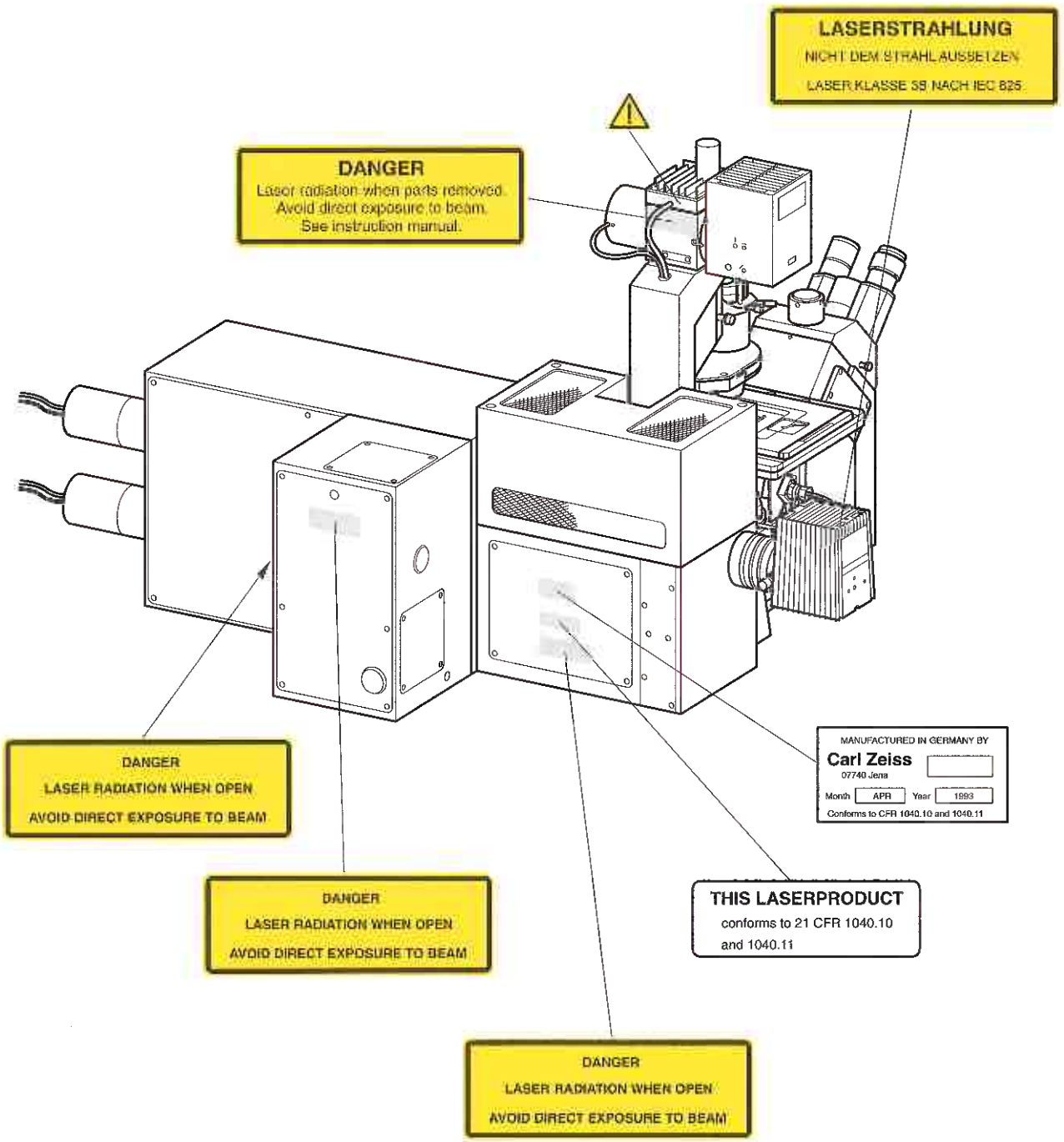


Figure 1-2 Warning and information plates on the rear of the unit

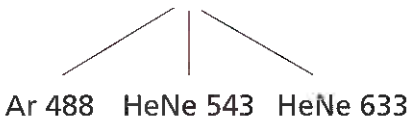
1.6 Note on handling the laser components

The LSM 410 invert is a unit that belongs to laser hazard class 3b and is marked as such (see Section1.5).
This moderate-risk class embraces medium-power lasers for which "Direct viewing into the laser beam" must be prevented.

The following laser types are currently recommended for use in the LSM 410 invert.

1 Ar 488
2 Ar 488/514
3 HeNe 543
4 HeNe 633
5 ArKr 488/568
6 ArKr 488/568/647
7 Ar 364 (UV)

Combinations of these are specified with a reference number as follows: e.g. 134 signifies



Please contact Carl Zeiss Germany (service hotline) if you intend to use a laser type with a wavelength other than the ones above.

If used properly, the LSM 410 invert will not pose any laser radiation risks for operating personnel. The laser 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 warning notes:

- If necessary – insofar as specified by law – inform the laser protection officer before commissioning the laser.
- Always store laser key switches and, if applicable, the keys belonging to further laser power supply units, in such a way that unauthorised persons are not able to operate the laser.
- Do not place any reflecting objects into the beam path.
- Never open any covers or panellings.
- Never look into the laser beam, not even to simply view the specimen or to look at it using optical instruments. **Risk of going blind!**
- Do not leave any screw positions for lenses open.

Suitable protective measures must be taken if gasses, dust and mist that are hazardous to health, secondary radiation or explosive objects may arise on the preparation as the result of laser radiation.

1.7 Notes on care, maintenance and service

The manufacturer of the unit cannot be held liable for damage resulting from operating errors, negligence or unauthorised 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.
This will also render all warranty claims null and void.

You are advised to conclude a service agreement with your next 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 authorised and trained for this purpose by the manufacturer.

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

Care operations to be carried out by operating personnel are limited tocleaning painted surfaces.

- cleaning painted surfaces
  To do this, use a clean cloth that has been moistened in a mixture of water and some detergent, using a lint-free cloth for drying; do **not** use any solvent, however.
- cleaning glass surfaces
  Rub glass surfaces that have become soiled or which are marked with fingerprints using a clean optical cleaning cloth.
  If soiling is persistent, dip the optical cleaning cloth into a mixture of distilled water and a little detergent beforehand.
  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 brush.

CONTENTS

2 DESCRIPTION OF THE LSM 410 INVERT MICROSCOPE SYSTEM

2.1 General information

2.1.1 Designation, purpose

Manufacturer's designation:	LSM 410 invert laser scan microscope
Abbreviated designation:	LSM 410 invert

The LSM 410 inverse laser scan microscope is based on the Axiovert 100, 135 or 135M microscope family.

These microscopes allow **conventional microscopy** using the usual contrast methods such as bright field, dark field, differential interference contrast, phase contrast, fluorescence and polarisation.

The microscope configuration can be combined from the basic units of the **Axiovert** microscope to suit individual requirements.

Please refer to the configuration sheets after the overview of sections for details of the currently delivered configuration.

In laser scan mode, **confocal laser scan microscopy** in reflected light (fluorescence or reflection) is possible.

It is possible to switch from conventional to LSM operation without conversion or adjustment. In doing so, the system is largely controlled by the **software**.

Image brightness, contrast, the zoom factor and all motor and electronic parameters are controlled from the WINDOWS graphical user interface.

The set system parameters or the parameters set for one particular experiment can be saved in a parameter file. This represents an inestimable advantage for multi-user operation because special settings can be retrieved swiftly by simply pressing a key. This saves time and guarantees reproducibility of measured results.

The settings in the parameter file are displayed in a so-called parameter window.

2.1.2 General view of the LSM system

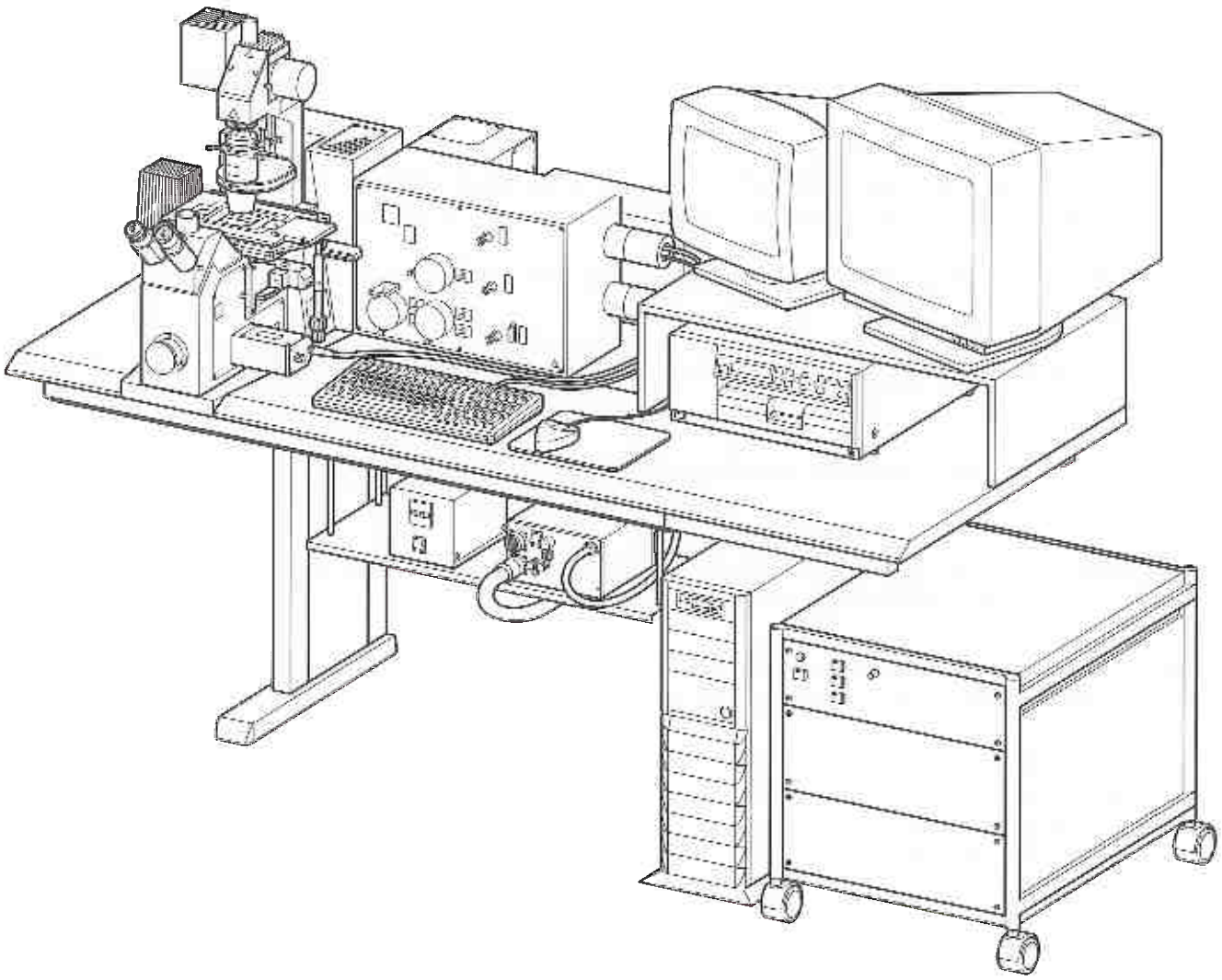


Figure 2-1 General view

2.2 Overview of the system

2.2.1 General

Thanks to its modular structure, the 410 invert laser scan microscope system offers optimum system configurations for a very wide range of applications. This involves the following function groups:

- **Basic LSM system** for the Axiovert 100, 135 and 135M microscopes with system base plate, beam scan system, detector unit, control computer including keyboard, mouse and colour monitor for display and control monitor; if applicable, set up on the vibration-damped system table.

- **Laser unit**, consisting of one or two internal lasers and of up to two additional external lasers. External lasers require a coupling unit.

- **Axiovert 100, 135 or 135M microscope equipment** for transmitted light, supplemented by the corresponding optical equipment and by various lighting units with the affiliated power supply units.

- **Hardware/software options** ranging from various laser combinations through emission filters/ colour splitter options, motor-controlled focusing, PC networking, UV laser scanning fluorescence microscope, image archiving on optical disk, black/white or colour documentation with a video printer to software packages for 3D reconstruction or for production of time series.

Recommended system configurations are, for example:

- The 410 invert LSM system with internal helium-neon laser, 543 nm (green), external argon ion laser, 488 nm, second photomultiplier, motor focusing in conjunction with the Axiovert 100 for transmitted light bright field, phase contrast and reflected light fluorescence.

- The 410 invert LSM system with internal helium-neon laser, 543 nm (green), external argon-krypton laser, 488/568/647 nm, third photomultiplier, motor focusing in conjunction with the Axiovert 135M, phase contrast and reflected light fluorescence as well as application packages for 3D reconstruction and time-resolved confocal microscopy.

- The 410 invert LSM system with two internal helium-neon laser, 543 nm (green) and 633 nm (red), external argon ion laser, 488 nm, third photomultiplier, motor focusing in conjunction with the Axiovert 135M H/DIC for transmitted light bright field, phase contrast and reflected light fluorescence and the application package for 3D reconstruction.

2.2.2 Schematic breakdown

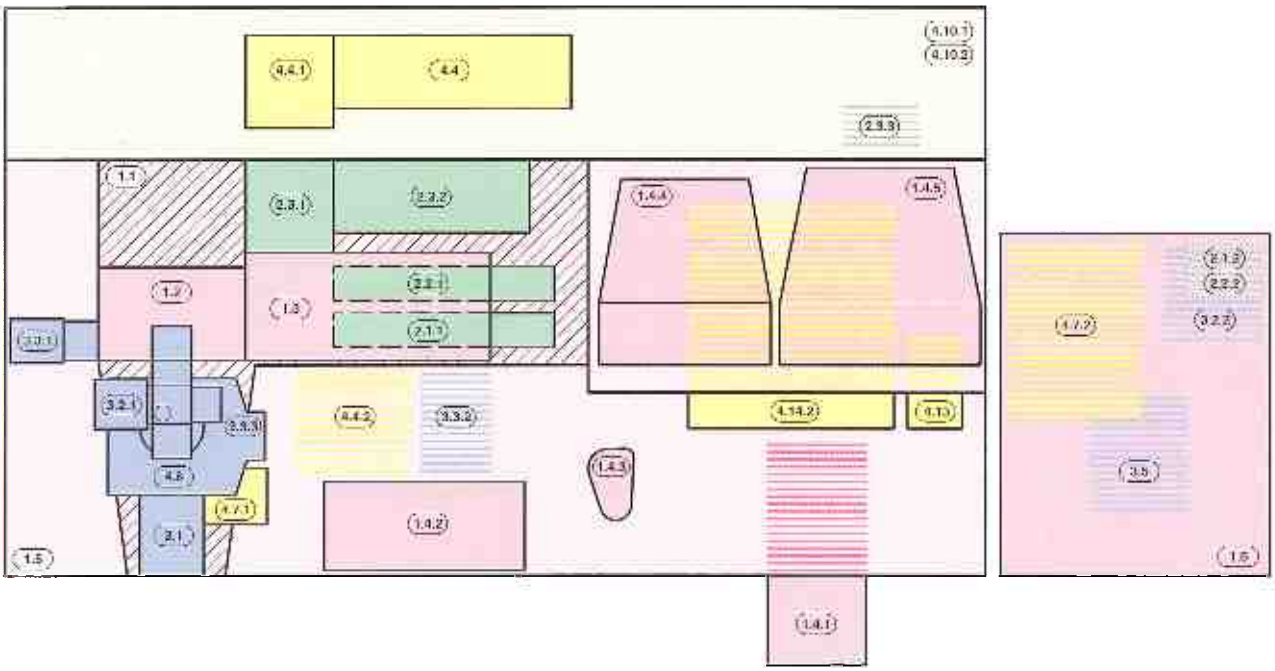


Figure 2-2 Schematic breakdown

1 Basic LSM system
1.1 System base plate
1.2 Beam scan system
1.3 Detector unit
1.4 Control computer
1.4.1 Tower with 240 MB hard disk
1.4.2 Keyboard
1.4.3 Mouse
1.4.4 Control monitor
1.4.5 17" image monitor
1.4.6 DOS/WINDOWS
1.5 System table
1.6 Elektronics rack

2 Laser unit
2.1 Internal laser 1
2.1.1 HeNe laser, 543 nm
2.1.2 Power supply unit, HeNe 1
2.2 Internal laser 2
2.2.1 HeNe laser, 633 nm
2.2.2 Power supply unit, HeNe 2
2.3 External laser
2.3.1 Laser coupling
2.3.2 Ar laser, 488 nm

3 Mikroscope equipment
3.1 Axiovert 100, 135, 135M
3.2 Lighting unit, 12 V 100 W
3.2.1 Lamp housing HAL
3.2.2 Power supply unit SNT 12 V 100 W
3.3 Lighting unit, HBO 50
3.3.1 Lamp housing HBO
3.3.2 Power supply unit for HBO 50
3.3.3 Reflector slide 3FL/4FL
3.3.4 Filter set (not illustrated)
3.4 Optical equipment (not illustrated)
3.5 Stand power supply unit

4 Hardware/software options
4.1 LSM system, high-resolution
4.1.1 20" image monitor, high-resol.
4.2 Transmitted light detector (not illustrated)
4.3 Internal laser
4.4 External laser
4.4.1 Laser coupling
4.4.2 External laser power supply unit
4.5 Multi-channel detection (additional photomultiplier)
4.6 Emission filter/colour splitter
4.7 Motor focusing
4.7.1 DC motor focusing
4.7.2 Motor control
4.8 Motor-driven specimen stage
4.8.1 Scanning stage
4.8.2 DC three-axis motor control
4.9 3D reconstruction/time series software
4.10 System tables for adaption of external lasers
4.10.1 System table for one external laser
4.10.2 System table for two external lasers
4.11 PC networking (not illustr.)
4.12 UV laser scanning fluorescence microscopy (not illus.)
4.13 Image archiving with optical disk
4.14 Video documentation
4.15 Differential interference contrast (DIC) (not illustrated)
4.16 Lens selection (not illustrated)
4.17 Antiflex unit (not illustrated)
4.18 TV connectors (not illustrated)
4.19 Laser coupling for external laser (not illustrated)
4.20 High-sensitivity photomultipliers (not illustrated)

2.2.3 Modules with order numbers, options

Ident No.	Designation	Order No.
	LSM 410 inverse laser scan microscope with two internal helium-neon lasers, 543 nm (green) and 633 nm (red), external argon ion laser, 488 nm, third PMT, motor focusing and "Axiovert" 100 H/DIC microscope for transmitted light bright field, phase contrast and reflected light fluorescence (220 V)	**492523 9805**
1	**Basic LSM system**	492500 9883
	LSM BioMed system (220 V) for Axiovert 100, 135 and 135M microscopes with	
1.1	System base plate	
1.2	Beam scan system	
1.3	Detector unit with confocal space filter	
1.4	IBM-compatible 80486 control computer, 66 MHz	
1.4.1	240 MB hard disk	
1.4.2	Keyboard	
1.4.3	Mouse	
1.4.4	15" control monitor	
1.4.5	17" image monitor	
1.4.6	DOS/WINDOWS	
1.5	LSM 4 system table with air/vibration damping	452590
2	**Laser unit**	
2.1	**Internal laser**	
2.1.1	Helium-neon laser, 543 nm (green) and 633 nm (red), internal, with fluorescence emission filters LP 570, BP 575-640 and RG 665 including 2x attenuation for internal lasers with filter wheel, complete, polarised	452574
–	Mount for emission filter before PMT 1 and colour splitter, motor-driven	452551
–	Colour splitter FT 510	446434
–	Colour splitter FT 560	446437
–	Colour splitter FT 655	446449
–	Slide-in colour splitter module	452555 9901
–	Colour splitter DBSP 488, 543	452585 9901
–	Neutral splitter 80/20/543	446446 9901
2.2	**External laser**	
2.2.1	Laser coupling	452518
2.2.2	Argon laser, 488 nm, 15 mW, for LSM 4 (220 V) including fluorescence emission filter LP 515	452544
–	Attenuation and line selection for external laser with filter wheel	452552
3	**Microscope equipment for transmitted light**	
3.1	**"Axiovert" 100 microscope stand with 5-fold lens turret H/DIC and integrated stabilised power supply DC 100...127-220...240V/0...12 V, 50...60 Hz, 200 VA**	451311
–	Binocular photo tube with 45ø/20 slide prism and 70/30 beam splitting	451322
–	Compound stage, 85x130, with holding frame, 76x26	451339
–	Dust protection cover UMSP/LSM	479321
3.2	**Lighting unit, 12 V 100 W**	
3.2.1	Lamp housing HAL with collector, reflector, lamp holder and thermal protection filter	447218
3.2.2	Power supply unit, stabilised, 12 V, 100 W Variable 1.5 V...12 V, 50...60 Hz	458417
3.2.3	Extension lead for lamp	479199 9049
–	Halogen lamp 12 V 100 W 2x	380079 9540

Ident No.	Designation		Order No.
3.3	**Lighting unit HBO 50**		
3.3.1	Lamp housing HBO		447220
–	Three-lens collector		447270
–	Holder for HBO 50		448006 9901
–	Mercury vapour short arc lamp HBO 50		381619
3.3.2	Power supply unit for HBO 50, 220–240 V, 50–60 Hz, 350 VA		392642
3.3.3	Reflector slide 4FL for the LSM 4		452531 9901
–	Filter set 09 Blue excitation 450-490		487909
–	Filter set 15 Green excitation H 546		487915
3.4	**Optical equipment**		
–	Lens: "Plan-Neofluar" 10x/0.30		440330
–	Lens: "Plan-Neofluar" 20x/0.50		440340
–	Lens: "Plan-Neofluar" 40x/1.30 Oil		440450
–	Lens: "Plan-Neofluar" 63x/1.25 Oil		440460
–	LD condenser 0.55 H, Ph 1, 2, DIC with iris diaphragm		451759
–	DIC prism 0.5-1.3/0.55		451396
–	Polariser A, rotating		453610
–	Eyepiece E-PL 10x/20 Spec.		444031 9901
–	Eyepiece E-PL 10x/20 Spec. foc.		444032 9902
–	Eyepiece cup	2x	444801
4	**Hardware/software options**		
4.2	**Transmitted light detector, motor-driven**		452567
4.5	**Multi-channel detection**		
–	Second PMT with filter wheel for emission filter		452561
–	Colour splitter FT 560		446437
–	Colour splitter FT 630		446448
–	Bandpass filter BP 590-610, D=18x3		467939
4.5.1	Third PMT with filter wheel for emission filter		452563
–	Full reflector		452584
–	Colour splitter FT 560		446437
–	Bandpass filter BP 510-525, D=18x4		467937
–	Interference green filter BP 515-565, d=18x2		467994 9904
4.7	**Motor focusing**		
4.7.1	DC motor focusing with transmission for "Axioskop", "Axioplan", "Axiotron" and "Axiovert" 100/135 microscopes		458306 9902
4.7.2	DC 3-axis motor control		457427
–	Connecting lead: "DC motor focusing/motor control"		457412 9002
–	Connecting lead "LSM stand-motor control"		457416 9001
4.9	**Software**		
–	3D reconstruction		480088 8040

Ident No.	Designation	Order No.
	Options	
4.1	**Option 1: LSM system, high-resolution** BioMed LSM system (220 V) for Axiovert 100, 135 and 135 M microscopes, but with high-resolution	492501 9882
4.1.1	20" image monitor (1024x1024)	
4.2	**Option 2: Transmitted light detector**	
	Transmitter light detector, manual	452565
	or	
	Transmitted light detector, motor-driven	452567
4.3	**Option 3: Internal laser**	
	One internal laser	
–	Helium-neon laser, 543 nm (green), polarised with emission filter LP 570	452540 9901
	or	
–	Helium-neon laser, 633 nm (red), with emission filter RG 665	452541
	Additionally required	
–	Attenuation for internal laser, manual	452553
	or	
–	Attenuation for internal laser with filter wheel	452554
	and	
–	Mounts for emission filter before PMT 1 and colour splitter, manualor	452550
	or	
–	Mounts for emission filter before PMT 1 and colour splitter, motor-driven	452551
	Two internal lasers	
–	Helium-neon laser, 543 nm (green) and 633 nm (red), internal with fluorescence emission filters LP 570, BP 575-640 and RG 665 including 2x attenuation for internal laser with filter wheel, complete, polarised	452574 9901
	Additionally required	
–	Mounts for emission filter before PMT 1 and colour splitter, manual	452550
	or	
–	Mounts for emission filter before PMT 1 and colour splitter, motor-driven	452551
4.4	**Option 4: External laser**	
–	Argon laser, 488 nm (blue), 15 mW (220 V) including fluorescence emission filter LP 515	452547 9011
	or	452547 9006
–	Argon laser, 488 nm (blue) 15 mW (110 V)	
–	Argon laser 488/514 nm, 25 mW (220 V) including attenuation filter and fluorescence emission filter LP 515 and OG 550	452548 9011
	or	
–	Argon laser 488/514 nm, 25 mW (110 V)	452548 9006
–	Argon-krypton laser, 488/568/647 nm including attenuation filter and fluorescence emission filter LP 515, LP 590, BP 515-540, BP 530-585, BP 590-610 and BP 670-810	452544
–	Argon-krypton laser, 488/568 nm including attenuation filter and fluorescence emission filter LP 515, LP 590 and BP 515-540	452545

Ident No.	Designation	Order No.
	Additionally required for all external lasers:	
4.4.1	Laser coupling	452518
–	Attenuation and line selection for external laser, manual	452557
	or	
–	Attenuation and line selection for external laser with filter wheel	452552
	In the case of multiline lasers, attenuation and line selection for external lasers with filter wheel (452552) is needed twice.	
4.5	**Option 5: Multi-channel detection** (additional photomultiplier)	
	Second PMT with mounts for emission filter, manual	452560
	or	
	Second PMT with filter wheel for emission filter	452561
	Third PMT with mounts for emission filter, manual	452562
	or	
	Third PMT with filter wheel for emission filter	452563
4.6	**Option 6: Emission filters/colour splitters**	
	Colour splitter FT 395	446431
	Colour splitter FT 460	446433
	Colour splitter FT 510	446434
	Colour splitter FT 560	446437
	Colour splitter FT 580	446435
	Colour splitter FT 630	446448
	Colour splitter FT 655	446449 9901
	Colour splitter DBSP 488, 543	452585 9901
	Colour splitter FT 488, 568	452586 9901
	Full reflector	452584
	Neutral splitter 80/20	446445
	Neutral splitter 80/20/543	446446 9901
	Bandpass filter BP 400-435, d=18x4	467936
	Bandpass filter BP 510-525, D=18x4	467937
	Interference green filter BP 515-565, d=18x2	467994 9904
	Bandpass filter BP 590-610, D=18x3	467939
	IF orange filter BP 575-640, 18x3	467920
	Please enquire about further filters	
4.7	**Option 7: Motor focusing**	
4.7.1	DC motor focusing with transmission for "Axioskop", "Axioplan", "Axiotron" and "Axiovert" 100/135 microscopes	458306 9902
4.7.2	DC 3-axis motor control	457427
–	Connecting cable: "DC motor focusing/motor control"	457412 9002
–	Connecting cable: "LSM stand-motor control"	457416 9001
	plus, if required	
	Control console for scanning table and motor focusing	452451

Ident No.	Designation	Order No.
4.8	**Option 8: Motor-driven specimen stage** (optoelectronically coded DC motors, travel range 100x90 mm, 0.25 µm increment, speed 20 mm/s)	
4.8.1	Scanning table DC 100x90	451740
	Additionally required	
–	Software for the LSM 3/LSM 4 scanning table (x, y)	480088 8042
	If motor focusing (option 7) is not ordered, the following is additionally required	
4.8.2	DC 3-axis motor control	457427
–	Adapter for 32 mm filter for LSM 4	452440 8430
	Plus, if required Control console for scanning table and motor focusing	
4.9	**Option 9: Software**	
–	Software for 3D reconstruction	480088 8040
–	Software for time series	480088 8041
4.10	**Option 10: System tables for adapting up to one external argon or argon-krypton laser**	
4.10.1	LSM 4 system table with air vibration damping	452590
	for adapting up to two external lasers, e.g. UV laser and argon laser	
4.10.2	Large vibration-isolated TMC system table with micro-g vibration isolation for mounting the external Spectra-Physics type 2017 UV laser for the LSM 4	419023
4.11	**Option 11: PC networking** Please enquire	
4.12	**Option 12: UV laser scanning fluorescence microscopy with one existing external UV laser (e.g. 364 nm)**	
–	UV laser 351/364 nm adaption and adapters for Spectra-Physics laser type 2017, for example	452546 9901
4.4.1	Laser coupling	452518
–	Colour splitter FT 395	446431
–	Line selection and attenuation for external laser with filter wheel	452552
4.10.2	Large vibration-isolated isolated TMC system table with micro-g vibration isolation for mounting the external Spectra-Physics type 2017 UV laser for the LSM 4 (instead of the system table 452590 from the basic configuration)	419023
–	**Lenses**	
	Lens: "Plan-Neofluar" 25x/0.80 Imm corr.	440544
	Lens: "Plan-Neofluar" 40x/1.30 Oil	440450
	Lens: "Plan-Neofluar" 63x/1.25 Oil	440460
	Lens: "Plan-Neofluar" 100x/0.30 Oil	440480
–	**Conventional reflected light fluorescence observation UV** Filter set 01 UV excitation H 365 This filter set can be inserted in the reflector slide 3FL (452530) or 4FL (452531) included in the basic configuration.	487901

Ident No.	Designation	Order No.
4.13	**Option 13: Image archiving with optical disk**	
–	Optical disk, 220 V (60 ms average access time)	412405
–	SCSI-2 cable, DB 50 minimal for Centronics 50 pl male	412406
–	SCSI cable for Adaptec controller	412407
–	Disk cartridge (5 1/4", ISO standard, 595 MB)	412408
	or	
–	SHARP magneto-optical disk drive JY 800 Internal version	412410 9001
–	Disk cartridge JY 801 MP, 1.2 GB 512 B/sector	412410 9002
4.14	**Option 14: Video documentation** **Colour documentation with colour video printer** **(In conjunction with the basic LSM system 492500 9883 and with the LSM system with high-resolution display 492501 9883)**	
4.14.1	**MITSUBISHI sublimation printer S 3410-30** for paper formats: DIN A4, special A4 with two interfaces: SCSI and Centronics including accessory/starter kit, connecting cable, Ink sheet, three colours, for A4/SA4: 100 prints/roll, Paper in DIN A4 oversize, 100 sheets/VPE	417725 9052
	or	
4.14.2	**Kodak sublimation printer for Windows 3.1** Miniature format, 140x100 mm printed area, 700 kB memory, 16.7 million colours, Centronics interface including Windows driver, 2 m Centronics cable and 120 printouts	417735 9001
4.14.3	**Documentation on miniature film** **Focus Graphics hard copy unit** for exposure of miniature films (35 mm, black/white, colour negative or slides) Suitable for connection to normal and high-resolution display boards, 15–85 kHz video line frequencies are synchronised automatically, RGB resolution up to 2000x1400	419195 9055
4.14.4	**Colour documentation with colour video printer** (not in conjunction with the high-resolution basic system 492501 9893) Colour video printer type UP-1800 EMP (108–132 V, 60 Hz, 200–240 V/50 Hz) Picture size: 119x87 mm (full size) Size can be reduced from 100 % to 70% or 50% Optionally 4 to 16 split possible Pixels: 772x584 (full size) Video memory: one full frame, 8 bits Colour levels: 3x256 for YMC (16.7 million colours) Printing time: 60 seconds – 75 seconds Composite video, RGB and Y/C inputs	417725 9053
	Connecting cable 1.5 m (BNC-BNC) 3x T-connector 3x	417708 479199 9029
	or	

Ident No.	Designation		Order No.
4.14.5	Colour video printer type UP-1850 EPM (108-132, 200–240 50/60 Hz) Picture size: 119x87 mm (full size) Size can be reduced from 100 % to 70% or 50% Optionally 4 to 16 split possible Pixels: 772x584 (full size) Video memory three full frames, 8 bits Colour levels: 3x256 for YMC (16.7 million colours) Printing time: 60 seconds – 75 seconds Composite video, RGB and Y/C inputs		417725 9054
	Connecting cable 1.5 m (BNC-BNC) T-connector	3x 3x	417708 479199 9029
4.14.6	Colour video printer type UP-5200 MDP (220 V/50 Hz) Picture size: 156x117.5 mm/4 and 9 split Pixels: 720x564 (normal scan mode), 756x582 pixels (wide scan mode) Video memory: one full frame, 8 bits Levels: 3x256 colour levels Printing time: approximately 60 seconds Inputs: video, composite video, RGB, components, RS 232 C Printing possible in black/white, colour and on film, monitoring possibility via a monitor		417725 9049
	Connecting cable 1.5 m (BNC-BNC) T-connector	3x 3x	417708 479199 9029
4.14.7	Colour video printer type UP-5250 MDP (220 V/50 Hz) Picture size: 156x117.5 mm/4 and 9 split Pixels: 720x564 (normal scan mode), 756x582 pixels (wide scan mode) Video memory: two full frames, 8 bits Levels: 3x256 colour levels Printing time: approximately 60 seconds Inputs: video, composite video, RGB, components, RS 232 C Printing possible in black/white, colour and on film, monitoring possibility via a monitor		417725 9050
	Connecting cable 1.5 m (BNC-BNC) T-connector	3x 3x	417708 479199 9029
	Please enquire about colour documentation with a colour video printer in conjunction with the LSM system featuring a high-resolution display 492501 9882.		
4.15	**Option 15: Differential interference contrast (DIC) for transmitted light (conventional) in addition to the basic configuration** (Prerequisite: Axiovert with H-DIC turret)		
–	Analyser, fixed		451393
	plus – depending on the required lens		
–	Lens: "Plan-Neofluar" 20x/0.50		440340
–	DIC slide 20x/0.50		444440
–	Lens: "Plan-Neofluar" 40x/0.75		440350
–	DIC slide 40x/0.75		444450
–	Lens: "Plan-Neofluar" 100x/1.30 Oil		440480
–	DIC slide 100x/1.30		444480
–	LD condenser 0.55 H, Ph 2, 3, DIC with iris diaphragm		451753
–	DIC prism 0.5-1.3/0.55		451396
	or		
–	LD condenser 0.55 H, Ph 1, 2, DIC with iris diaphragm		451759
–	DIC prism 0.5-1.3/0.55		451396

Ident No.	Designation	Order No.
	For maximum detailed resolution in transmitted light bright field and differential interference contrast (DIC)	
–	Lens: "Plan-Apochromat" 63x/1.40 Oil	440760
–	DIC slide 63x/1.40	444467
–	Condenser mount with iris	451355
–	Achromatic-aplanatic set condenser 0.32 Pol	445245 9902
–	Front lens 1.4 Pol	465268
–	DIC prism 0.5-1.4/1.4	445294
	Additionally required for transmitted light DIC with internal laser, 543 nm	
–	Polariser A, rotating	453610
4.16	**Option 16: Selection of lenses specially suitable for the LSM**	
–	Lens "Achroplan" 100x/1.25 Oil	440080
–	Lens: "Plan-Neofluar" 2.5x/0.075	440310
–	Lens: "Plan-Neofluar" 5x/0.15	440320
–	Lens: "Plan-Neofluar" 10x/0.30	440330
–	Lens: "Plan-Neofluar" 16x/0.50 Imm.	440530
–	Lens: "Plan-Neofluar" 20x/0.50	440340
–	Lens: "Plan-Neofluar" 25x/0.80 Imm. corr.	440544
–	Lens: "Plan-Neofluar" 40x/1.30 oil	440450
–	Lens: "Plan-Neofluar" 100x/1.30 oil	440480
–	Lens: "Plan-Apochromat" 20x/0.60	440640
–	Lens: "Plan-Apochromat" 40x/1.00 Oil iris	440756
–	Lens: "Plan-Apochromat" 63x/1.40 Oil	440760
–	Lens: "Plan-Neofluar" 40x/1.85 Pol See price list 22.02 for further lenses and DIC slides	440353
4.17	**Option 17: Antiflex unit**	
–	Lens: antiflex "Plan-Neofluar" 63x/1.25 oil Ph 3	440469
	Additionally required	
–	Polariser A, rotating	453610
	For antiflex with internal He-Ne laser, 543 nm (green)	
–	Polariser A, rotating	453610
	Remarks: the polariser is included with the transmitted light detector 452565 or 452567.	
	This lens contains a Ph 3 phase ring to seek and focus the specimen in transmitted light phase contrast. Subsequent fast switchover to reflected light laser scan operation leads directly to image generation in reflection contrast. The Ph 3 condenser phase ring must also be ordered if it is not included in the basic configuration.	
	Required for visual reflection contrast	
–	Filter set A Pol	487960
	plus	
–	Reflector 3FL for the LSM 4	452530
	or	
–	Reflector slide 4FL for the LSM 4 (if 452530 or 452531 of the basic configuration can no longer be used in the reflector slide 3FL or 4FL)	452531 9901

Ident No.	Designation	Order No.
4.18	**Option 18: TV connections**	
	1. In conjunction with the binocular photo tube 451321,451322 and 451325	
	a) Standard C connection thread	
–	Standard C 2/3" TV camera connector (factor 1x)	456105
	or	
–	Standard C 1/2" TV camera connector (factor 0.5x)	456106
	or	
–	Standard C 2/3" TV camera connector (factor 0.63x)	456107
	b) ENG bayonet connection	
–	2/3" ENG mount TV camera connector (factor 1x)	456115
	or	
–	2/3" ENG mount TV camera connector (factor 0.8x)	456117
	2. Lateral adapter for light and compact cameras	
–	TV connector for "Axiovert" 100/135 microscopes, lateral	451375
	Additionally required	
	a) Standard C connection thread	
–	Standard C 2/3" TV camera connector (factor 1x)	456105
	or	
–	Standard C 1/2" TV camera connector (factor 0.5x)	456106
	or	
–	Standard C 2/3" TV camera connector (factor 0.63x)	456107
	b) ENG bayonet connection	
–	2/3" ENG mount TV camera connector (factor 1x)	456115
	or	
–	2/3" ENG mount TV camera connector (factor 0.8x)	456117
4.19	**Option 19** **Fibre coupling for external lasers**	
	Polarisation-sustaining single mode fibre, 2 m long. **Input and output coupling optics with corresponding adjustment and adaption components.**	
	For the argon laser, 488 nm or 488/514 nm	
	Fibre coupling for argon laser, 488 nm or 488/514 nm	452525
	Adapter for fibre coupling for LSM 4	452527
	For the argon-krypton laser, 488/568 nm or 488/568/647 nm	
	Fibre coupling for argon-krypton laser, 2 and 3 wavelengths	452526
	Adapter for fibre coupling for LSM 4	452527

Ident No.	Designation	Order No.
4.20	**Option 20** **High-sensitivity photomultipliers** **(Upgrade for installed units)**	
	Version for motor-driven filter changer **R/FL 1 (first channel, 452551)** Retrofit kit LSM 4, PMT 1 (mot)	452551 9800
	R/FL 2 (second channel, 455261) or **R/FL 3 (third channel, 452563)** Retrofit kit LSM 4, PMT 2, PMT 3 (mot)	452561 9800
	Versions for manual filter changers **R/FL 1 (first channel, 452520)** Retrofit kit LSM 4, PMT 1 (man)	452550 9800
	R/FL 2 (second channel, 452560) or **R/FL 3 (third channel, 452562)** Retrofit kit LSM 4, PMT 2, PMT 3 (man)	452560 9800

2.3 Principle of operation

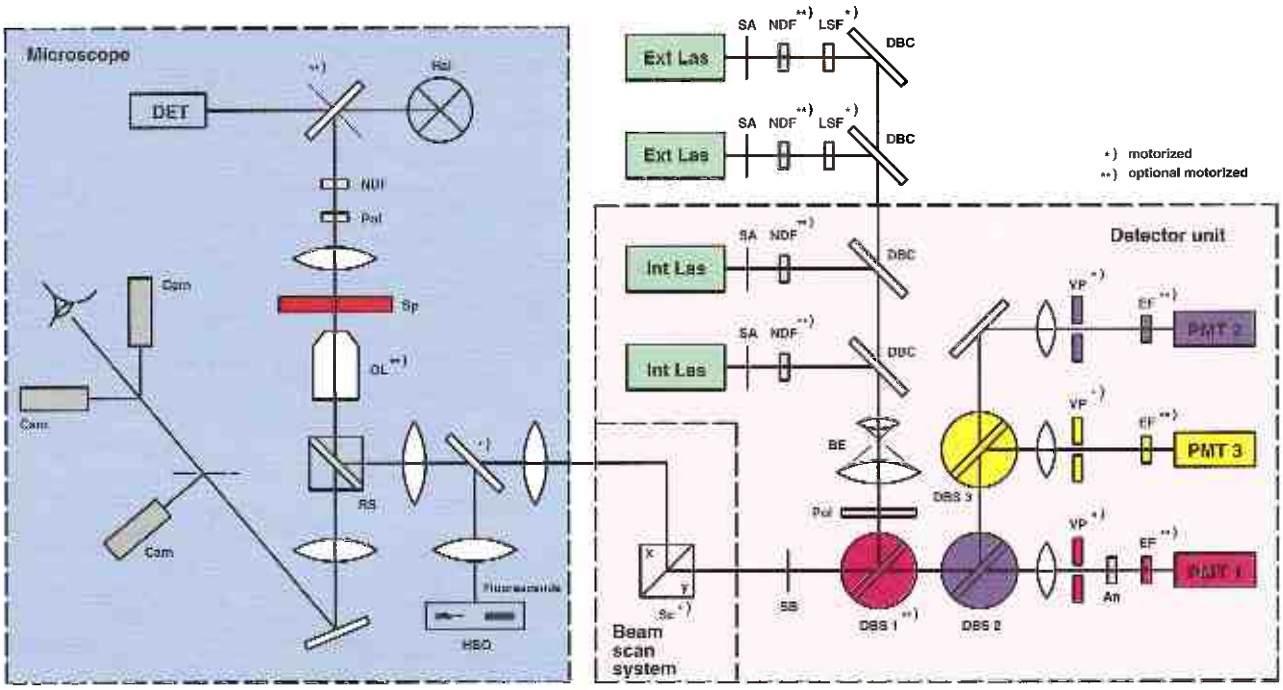


Figure 2-3 Beam path, schematic

HBO	High Pressure Mercury Lamp
An	Analyser
BE	Beam Expander
Cam	Camera
DBC	Dichroitic Beam Combiner
DBS	Dichroitic Beam Splitter
DET	Detector
EF	Emission Filter
Ext Las	External Laser
Hal	Halogen Lamp
Int Las	Internal Laser
LSF	Line Selection Filter
NDF	Neutral Dense Filter
OL	Objective Lens
PMT	Photomultiplier
Pol	Polarizer
RS	Reflector Slider
Sc	Scanner
SA	Safety lock A for laser light
SB	Safety lock B for laser light
Sp	Specimen
VP	Variable Pinhole

The laser scan microscope is shown schematically in the figure above. Refer to Section 5 for information on conventional microscopy using the usual contrast methods. Laser light, generated by the internal and/or external laser(s), is focused onto the specimen via the scanner and through the tube lens of the lens system and with limited diffraction. Emission light from the focusing plane and from planes above and below it passes through the scanner to a dichroitic beam splitter system (DBS), where the fluorescence emissions are split and meet up with the photomultipliers PMT 1 to maximally PMT 3. Computer-controlled variable pinholes (diaphragms) operate as so-called "space filters" and only allow light to pass that originates directly from the focusing plane, while light from other planes is effectively suppressed.

The basic LSM system includes a detector for fluorescence/reflection. A maximum of four detectors can be installed. Up to three detectors for fluorescence/reflection can be read out and displayed simultaneously. When an additional transmitted light detector (DET) is installed, up to two fluorescence channels with the transmitted light portion can be read out simultaneously and displayed on the image monitor.

2.4 Description of components

2.4.1 System table

The system table represents the central carrier unit for the LSM 410 microscope system.
Two variants are supplied:

- System table LSM 4 with air vibration damping (452590)
- TMC system table with micro-g vibration isolation (419023) for mounting an external UV laser (not illustrated).

Figure 2-4 shows the most frequent variant featuring three air vibration dampers as carrier elements for the granite plate. The microscope components are held on a practically vibration-free mount when the damping elements are pumped up correctly (the granite plate should then be level with the plate of the system table).

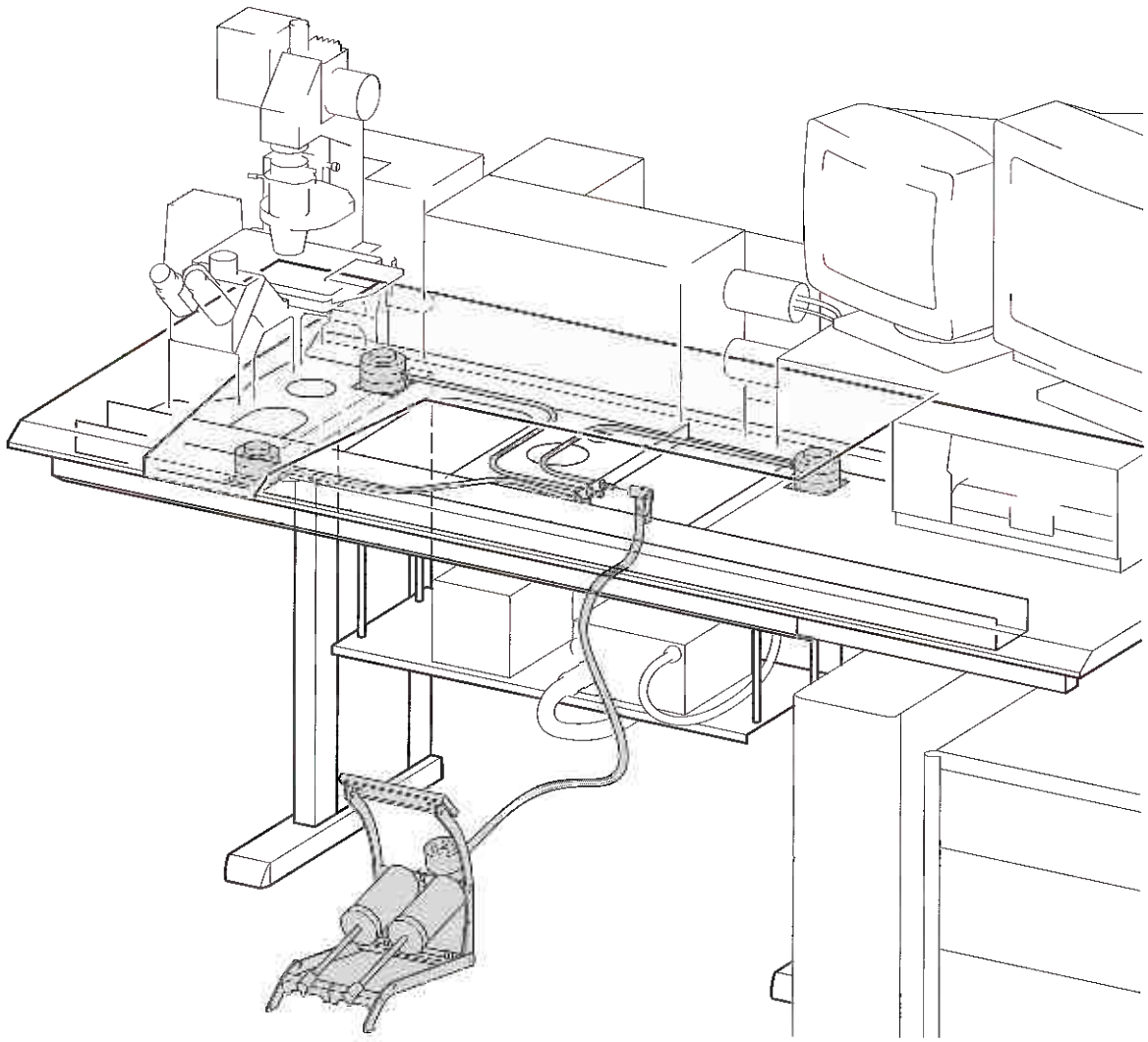


Figure 2-4 System table (452590)

2.4.2 Axiovert microscope

The LSM system was conceived to match the Axiovert 100 (135, 135M) microscope series. Thus, videography and photography are possible with all conventional microscopy methods.

With the exception of a few basic settings such as positioning the manual microscope stage (optional: motor-driven stage) and setting the reflector or blocking filter slide, which must be carried out manually, the microscope is completely processor-controlled. Interaction with the user is by menu prompting.

If you wish to inform yourself about details of the microscope itself, refer to the separately included operating instructions G 42-513-e, "Axiovert 100, Axiovert 135 and 135M, transmitted light and reflected light fluorescence".

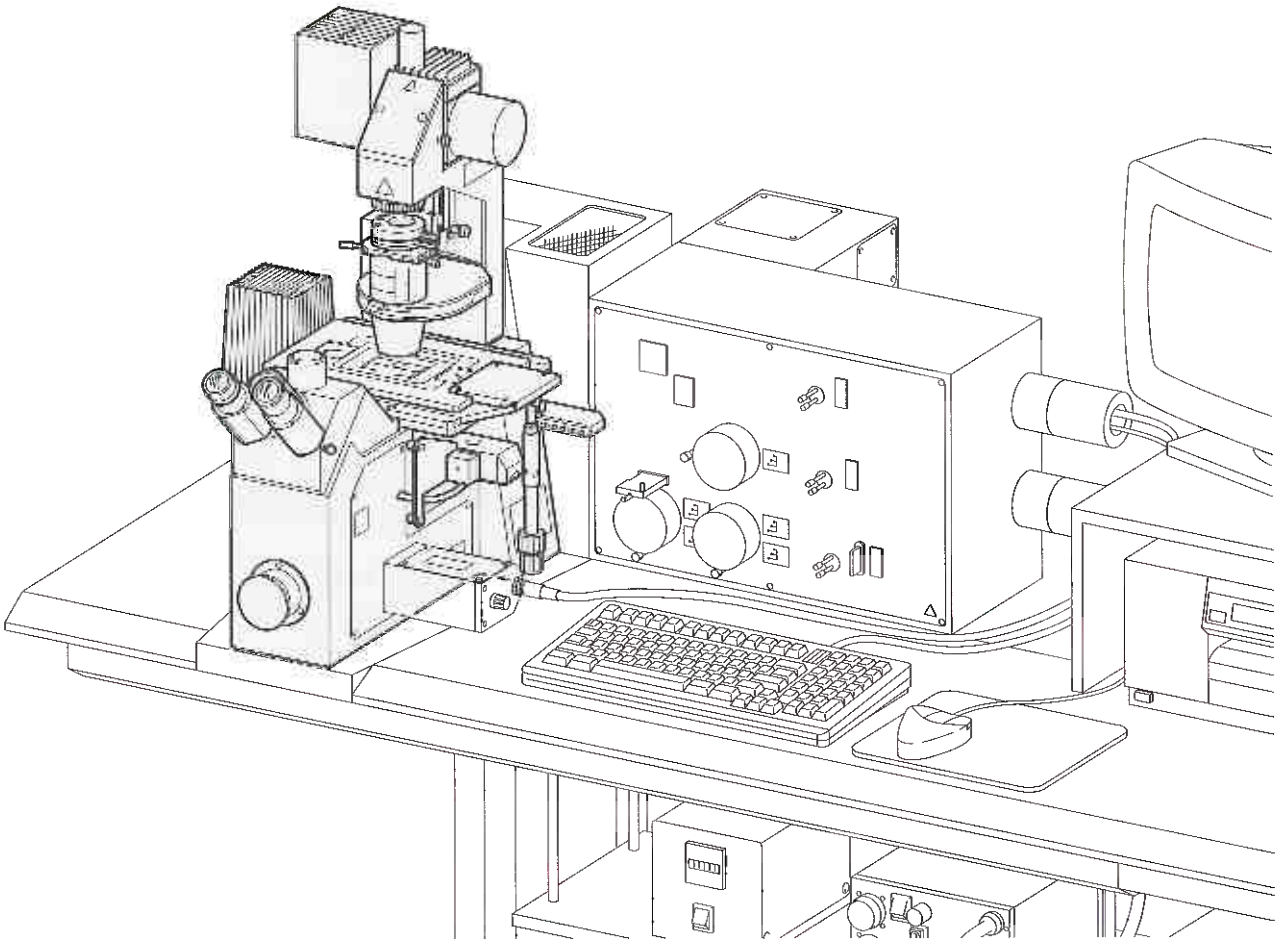


Figure 2-5 Axiovert microscope

2.4.3 Beam scan system

The LSM 410 invert operates with two electroplated scan reflectors that guide the laser light focused with limited diffraction over the specimen.
This enables special features such as zooming and rotating the image.

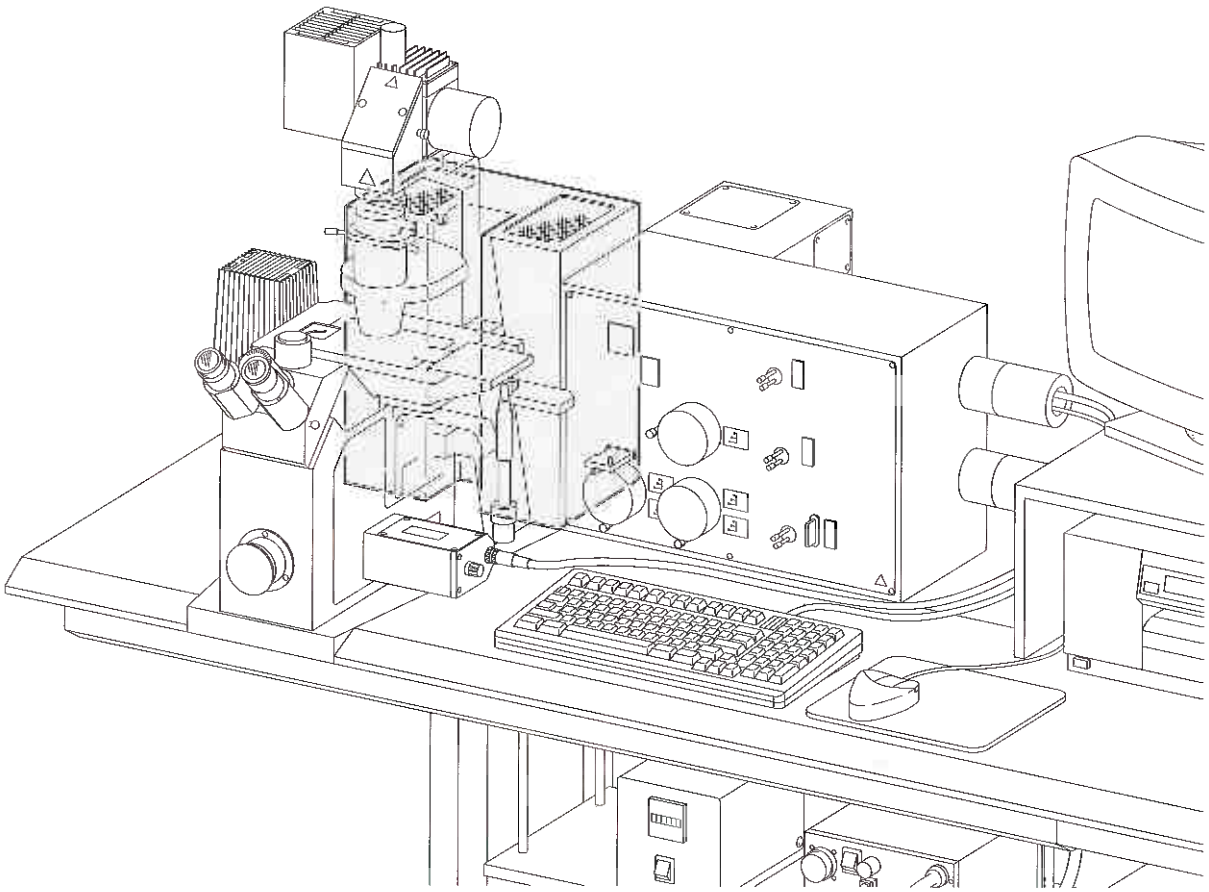


Figure 2-6 Beam scan system

2.4.4 Detector unit

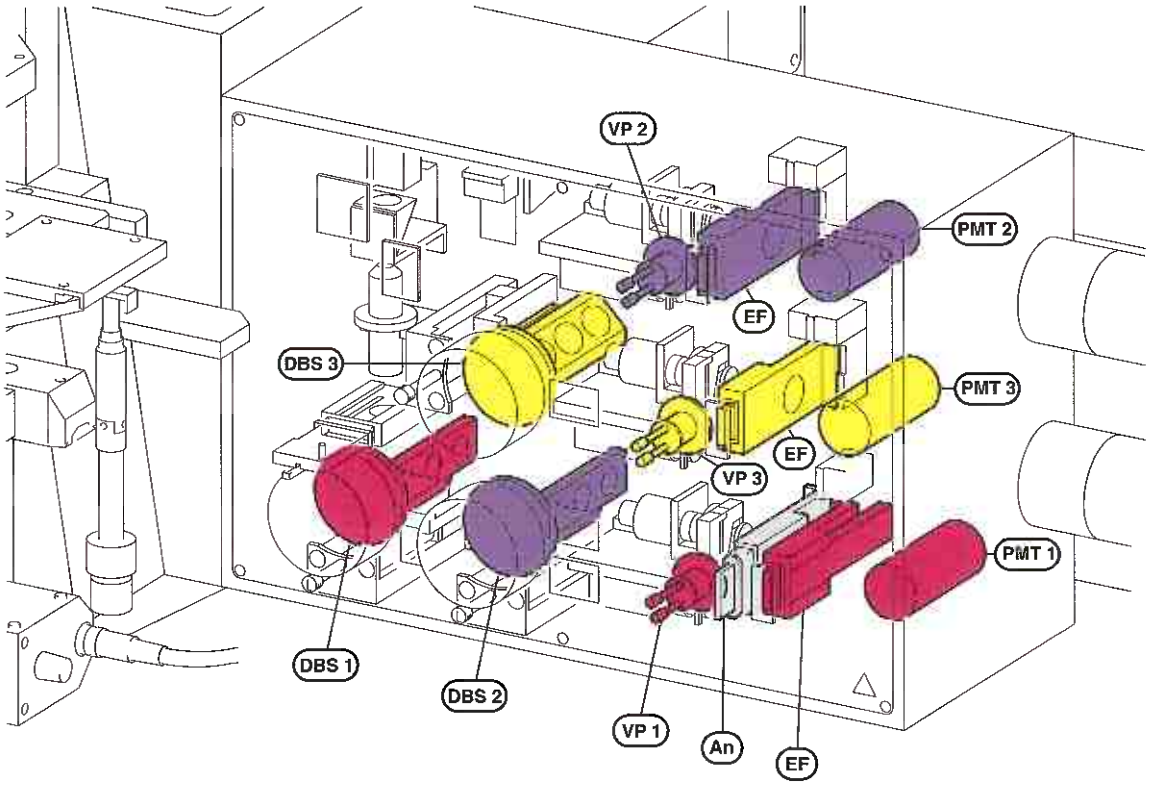


Figure 2-7 Detector unit

The LSM 410 can be equipped with a maximum of four detectors. The following are possible:

- one transmitted light detector,
- up to three fluorescence detectors.

One fluorescence detector

The fluorescence beam path contains the following elements:

- **Reflector slide** 3FL or 4FL (452530/31) with three or four positions (neutral splitter for reflected light, colour splitter for fluorescence, full reflector for laser scan mode).
  For safety reasons, it is coded to guarantee that laser light is not capable of reaching the observer.

- **Colour splitter** (DBS1) with three positions for different dichroitic splitters
  - Manual version (452550)
  - Motor-driven version (452551)

  replaceable by a further slide-in colour splitter module (452555) with different filter combinations.

- **Pinhole** (VP1 before PMT 1), computer-controlled diaphragm size, capable of centring with two adjusting screws.
- **Emission filter** (EF before PMT1) in the manual slide with four positions or in a motor-driven filter wheel with eight positions (filter wheel only in conjunction with the motor-driven colour splitter).
- **Analyser** (An) or special emission filter which can be placed over a one-position slide.

Two fluorescence detectors

In this case, the following elements are added to the ones already mentioned:

- **Second colour splitter** (DBS2) with three positions for different filter combinations
  - Manual-only version;
    replaceable by a further slide-in colour splitter module (452555) with different filter combinations.
- **Pinhole** (VP2 before PMT2), computer-controlled diaphragm size, capable of centring with two adjusting screws; its diameter can be adjusted independently of the pinhole for the first detector.
- **Emission filter** (EF before PMT2) in the manual slide with four positions or in a motor-driven filter wheel with eight positions. The manual slide is replaceable by a different slide with further emission filters.

The second detector is offered as one unit together with a colour splitter, pinhole and emission filter (see also option 5):
2nd PMT, manual filters (452560)
2nd PMT, with motor-drive filter wheel (452561)

Three fluorescence detectors

In this case, the following elements are added to the ones already mentioned (for the 1st and 2nd detectors):

- **Third colour splitter** (DBS3) with three positions for different filter combinations
  - Manual version;
    replaceable by an additional slide (452564) with different filter combinations.
- **Pinhole** (VP3 before PMT3), computer-controlled diaphragm size, capable of centring with two adjusting screws; adjustable diameter independently of the pinhole for the first two detector.
- **Emission filter** (EF before PMT3) in the manual slide with four positions or in a motor-driven filter wheel with eight positions. The manual slide is replaceable by a different slide with further emission filters.

The third detector is offered as a unit together with a colour splitter, pinhole and emission filter (see also option 5):
3rd PMT, manual filters (452562)
3rd PMT, with motor-drive filter wheel (452563)

2.4.5 Laser and laser coupling

A maximum of four lasers can be coupled into the LSM 410 invert.
From the large number of available possibilities, Carl Zeiss offers the following laser types either singly or in combinations:

1	Ar 488 nm	Ext.	Laser
2	Ar 488/514 nm		
3	HeNe 543 nm	Int.	
4	HeNe 633 nm		
5	ArKr 488/568 nm	Ext.	
6	ArKr 488/568/647 nm		
7	Ar 364 (UV) nm		

The chosen laser combination (Section 3.2, Overview table) is recognisable with reference to the number code:
134 means:
Ar 488 nm as external laser
HeNe 543 nm and HeNe 633 nm as internal laser.

One internal laser

When using only one laser, e.g.
HeNe 543 nm or HeNe 633 nm, the laser energy can be optionally attenuated either manually (452553) or by motor drive (452554).
When attenuating the laser energy manually, you can use two slides with three filter positions each on the detector housing.

Two internal lasers

When using **two** internal lasers, attenuation and line selection must be realised by motor drive (452574).

One external laser

One external laser can be operated (attenuation and line selection) either by motor drive (452552) via the operator control program or manually (452527).
When operating the laser manually, there are two slides with three filter positions each and one slide for the line selection filters on the detector housing.
In the motor-driven version, each slide is substituted by a filter wheel with eight positions.In the case of the Ar 364 UV laser, UV laser adaption is necessary. Attenuation and line selection are motor-driven.

Two external lasers

When using **two** external lasers, attenuation and line selection must be motor-driven (452552).

Attenuation filters (NDF)

Regardless of the chosen laser combination, the following six attenuation filters belong to each laser:

T = 0,32	T = 0,032	T = 0,0032
T = 0,1	T = 0,01	T = 0,001

In the manual version, the attenuation filters can be inserted in two slides with three positions each. In the motor-driven version, the filter wheels are installed at the works and can only be replayed by Carl Zeiss service.

Line selection filters (LSF)

The line selection filters are only needed for lasers with more than one wavelength and are included with the lasers:

Ar laser 488/514 nm

ILF 488	(452445 8001)
ILF 514	(452445 8002)

ArKr laser 488/568/647 nm

BP 485/20	(467974 0010)	488 nm
BP 530-585	(467988 8010)	568 nm
KP 600	(467928)	488+568 nm
LP 610	(454331 0013)	647 nm
BS 568	(467929)	488+647 nm
LP 515	(467864 8010)	568+647 nm

In the manual version, the line selection filters can be inserted in two slides with three positions each. In the motor-driven version, the filter wheels are installed at the works and can only be replaced by Carl Zeiss service.

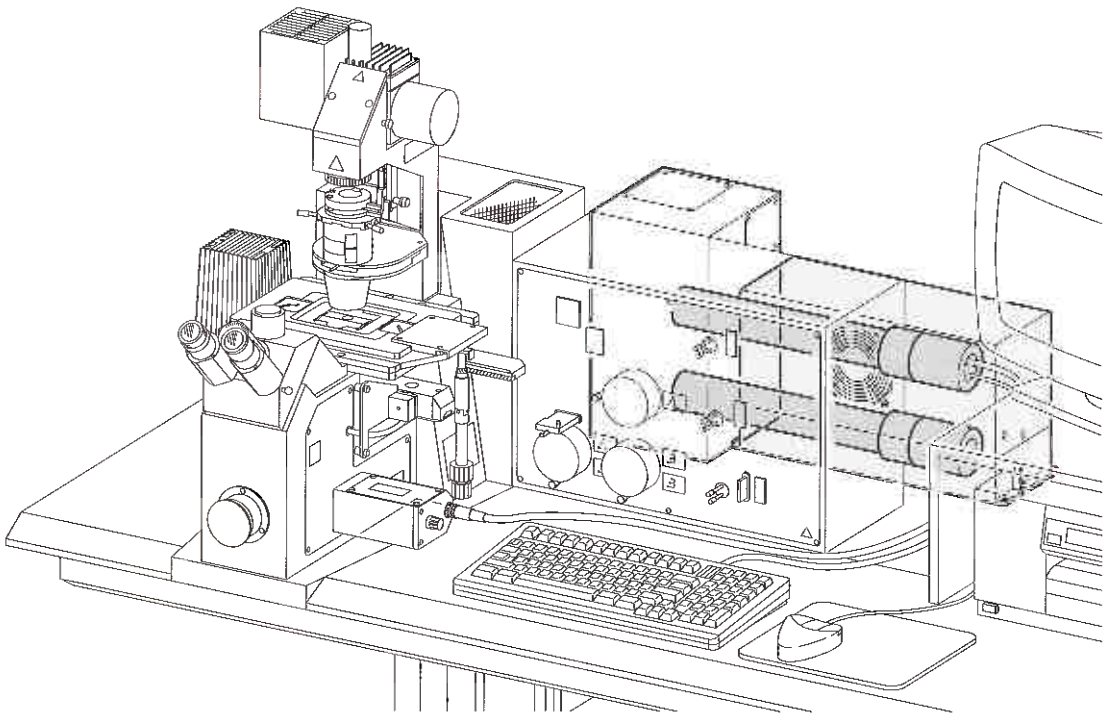


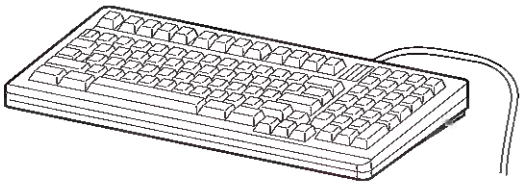
Figure 2-8 **Laser configuration and laser coupling**

2.4.6 Computer unit with keyboard and mouse

The central control computer consists of a tower.

Mouse
Type LOGITECH Ergonomic Mouse
6-pin connector, round
Including adapter for 9 or 25-pin serial connectors

Keyboard
US keyboard layout



Computer unit

Central control computer
IBM-compatible,
$3^1/_2$" diskette drive, 1.44 MB

Integrated video processor
Matrox processor unit and 4 MB video RAM
(high resolution system: 8 MB RAM)
RGB video signal

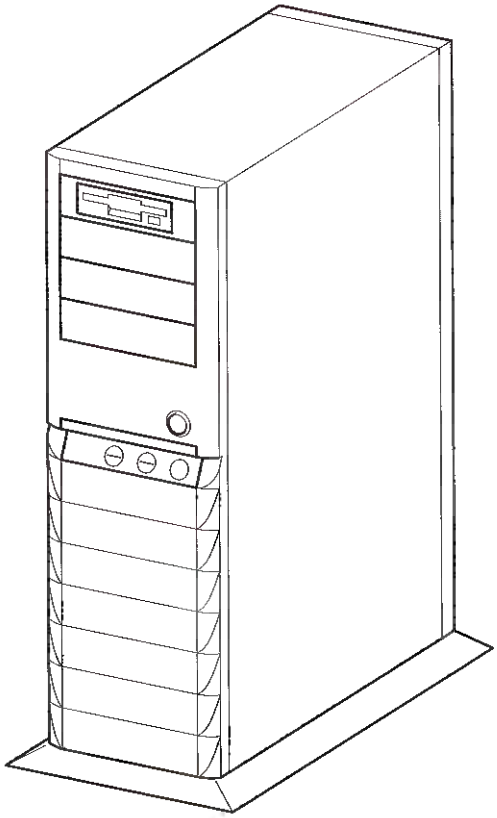
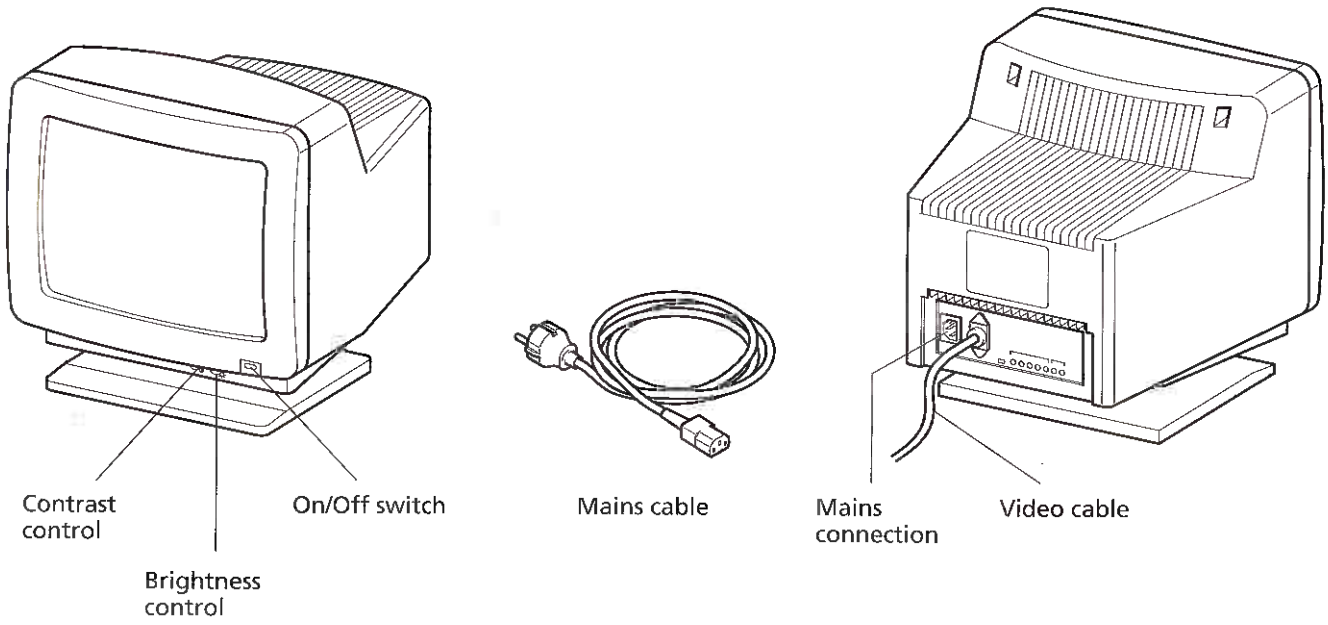


Figure 2-9 Computer unit

2.4.7 Operator control monitor

Type MITSUBISHI colour display monitor
Model No. FT3420 ETKL
100-120 V AC/220–240 V AC
50/60 Hz 1.6/0.8
A15" screen diagonal

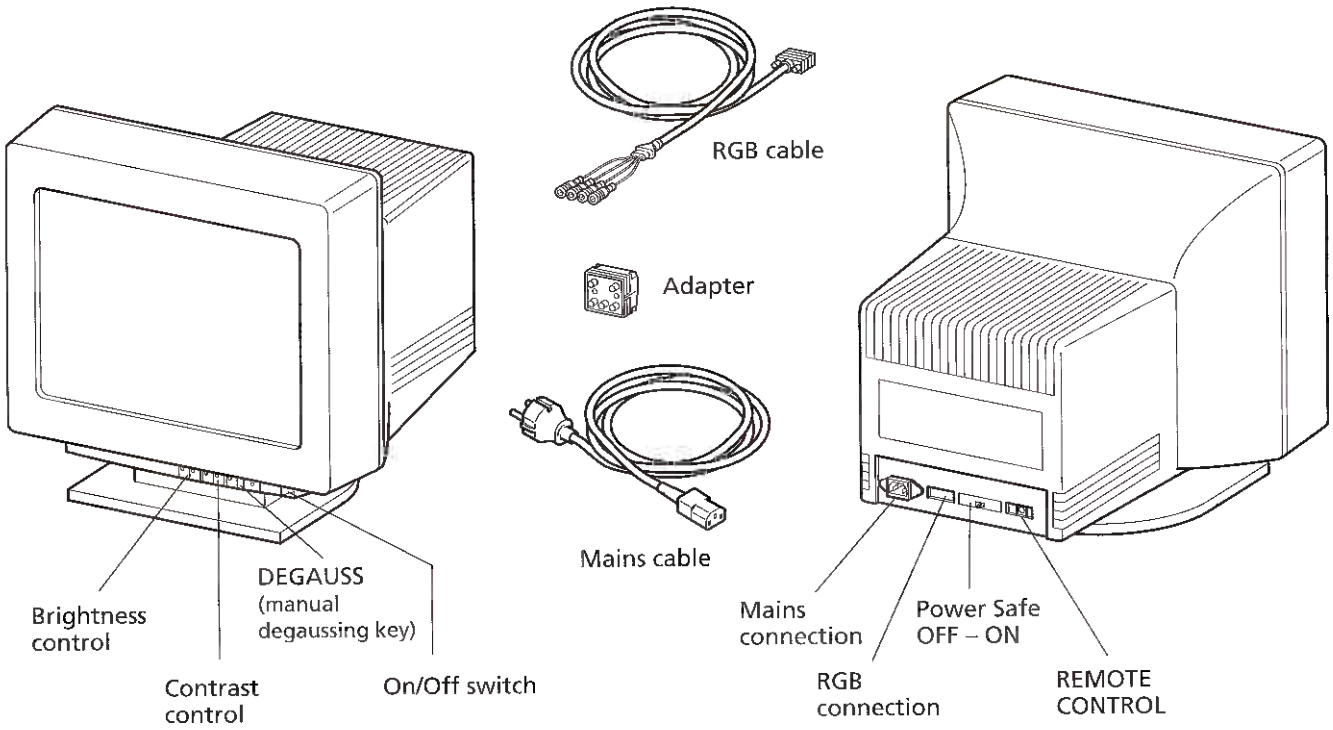


Please refer to the operating instructions of the MITSUBISHI 15" operator control monitor for further information.

Figure 2-10 Operator control monitor

2.4.8 17 " Image monitor

Type MITSUBISHI Diamond Scan 17FS
Model No. FFY7705SKTKL
100–120 V AC/220–240 V AC
50/60 Hz 2 A/1.2 A
17" screen diagonal (16" visible)
Resolution (HxV) 1280x1024



Please refer to the operating instructions of the MITSUBISHI 17" operator control monitor for further information.

Figure 2-11 Image monitor

CONTENTS

3 EMISSION FILTER AND COLOUR SPLITTER COMPONENTS, LASER COMBINATIONS

3.1 General

3.1.1 General configuration considerations

Owing to the large number of laser lines and fluorescence applications that can be used, configuration variants of emission filters and colour splitters must always be adapted to the specific application. The configuration variants listed in Section 3.2 cover the most usual filter combination possibilities. Please contact Carl Zeiss for details of even more special application tasks. Beforehand, you should decide the following:

- What fluorochromes are used?
- Are these used on the same specimen?
- Are they used simultaneously or in succession?

The following lasers are currently offered singly or in combinations:

1 Ar 488 nm
2 AR 488/514 nm
3 HeNe 543 nm
4 HeNe 633 nm
5 ArKr 488/568 nm
6 ArKr 488/568/647 nm
7 Ar 364 (UV) nm

In addition, however, lasers with different emission lines can also be used.

The colour splitter DBS1 depends on the decision as to which laser lines are to excite the specimen. The choice of this colour splitter is, however, also influenced by the choice of the fluorescence ranges you wish to verify with the photomultipliers.

The colour splitters DBS2 and DBS3 and the emission filters result from the required breakdown of the fluorescence light over the various PMTs.

- Short λ to PMT3
- Medium λ to PMT2
- Long λ to PMT1

when using three PMTs

- Short λ to PMT2
- Long λ to PMT1

when using two PMTs

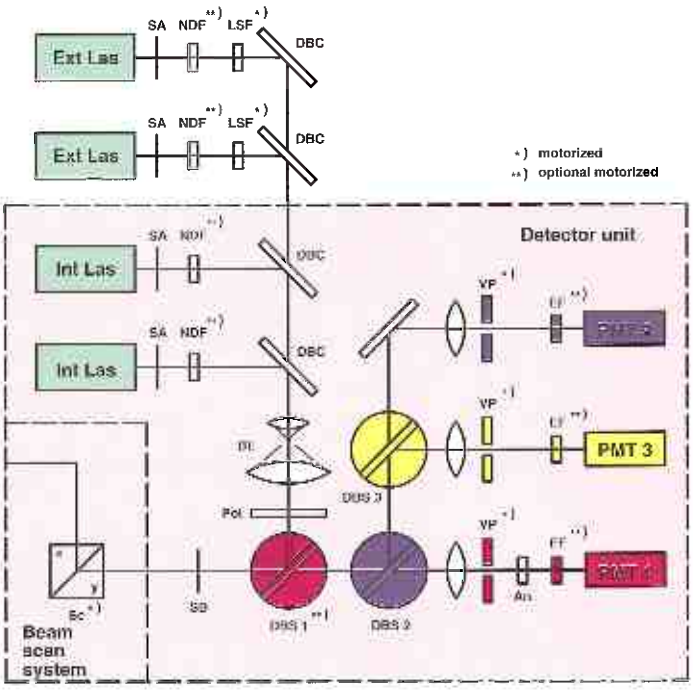


Figure 3-1 Detector unit configuration schematic

3.1.2 Dichroitic beam splitters and their use

FT 395	:	for single excitation by UV Ar line 395
FT 510	:	for single excitation by Ar line 488
FT 530	:	for single excitation by Ar line 514
FT 560	:	for single excitation by HeNe line 543 (see also DBSP 488/543)
FT 580	:	for single excitation by HeNe line 568 (see also DBSP 488/568)
FT 645	:	for single excitation by HeNe line 633
FT 655	:	for single excitation by ArKr line 647, also suitable for simultaneous excitation by blue (488) and red (633 or 647)
DBSP 488/543	:	double band beam splitter for simultaneous excitation by 488 and 543
DBSP 488/568	:	double band beam splitter for simultaneous excitation by 488and 568
DBSP 488/543/633	:	triple band beam splitter for simultaneous excitation by 488, 543 and 633
NT 80/20	:	for triple excitations and for double excitations for which no special double beam splitter is available; for reflections
NT 80/20-543	:	for all triple excitations and for double excitations for which no special beam splitter is available and where the green HeNe laser is to be used

When excitation is roughly broken down into four areas, the result is as follows:

- UV excitation ⇒ Blue emission
- Blue excitation ⇒ Green emission
- Green/yellow excitation ⇒ Orange/red emission
- Red excitation ⇒ Dark red emission

In simplified form, this results in the following for the beam splitters DBS2/3:

FT 510	splits blue and green emissions
FT 560	splits green and orange-coloured emissions
FT 630	splits orange/red and dark red emissions

3.1.3 Emission filters and their use

LP 397	:	long pass filter for single emission of UV excitation
BP 400-435	:	bandpass filter for UV emission and simultaneous or sequential recording of a secondary emission
LP 515	:	long pass filter for emission of blue (488) excited dyes
BP 510-525	:	bandpass filter specially for blue (488) fluorescence with simultaneous excitation by 488 and 543
BP 510-540	:	bandpass filter specially for blue (488) fluorescence with simultaneous excitation by 488 and 568. Also suitable for sequential recording of green and red fluorescence in specimens in which the red emission can also enter the green channel
BP 515-565	:	bandpass filter specially for blue (488) fluorescence; particularly also suitable for sequential recording of green and red fluorescence. Also useful for simultaneous recording of blue and red excitation
OG 550	:	long pass filter for the Ar line 514
LP 570	:	long pass filter for emission after excitation with HeNe 543. If the green emission comes through under double fluorescence, it is better to use the filter LP 590
LP 590	:	long pass filter for yellow (568) excited fluorescence
BP 575-640	:	bandpass filter for sequential recording of orange fluorescence excited with blue (488)
BP 590-610	:	bandpass filter for simultaneous recording of fluorescence excited with green (543) or with yellow (568) when the 488 and/or 633 line is used to excite a second or third fluorescence
RG 665	:	long pass filter for red (633) excited fluorescence
BP 670-810	:	long pass filter for red (647) excited fluorescence

3.2 Overview table of possible configuration variants

✓ : Filter that is already supplied with the laser
• : Beam splitter or filter that is recommended for the specified laser combination
* : Only required for the fully motor-driven version

One PMT

	Lasers							Dichroitic beam splitters DBS1								Emission filters								
# Ref. No.	Ar 488	Ar 488,514	HeNe 543	HeNe 633	ArKr 488,568	ArKr 488,568,647	UV 365	FT 510 (446434)	FT 530 (a.A.)	FT 560 (446437)	FT 580 (446435)	FT 645 (446441)	FT 655 (446449)	FT 395 (446431)	Zus. 452555 Slider	LP 397 (467860-9903)	LP 515 (467864)	BP 515-540 (467938)	OG 550 (467925)	LP 570 (467922)	LP 590 (467921)	BP 590-610 (467939)	RG 665 (467927)	BP 670-810 (467934)
1	x							•									✓							
2		x						•	•								✓		✓					
3			x							•										✓				
4				x								•											✓	
5					x			•			•						✓	✓			✓			
6						x		•			•		•				✓	✓			✓	✓		✓
13	x		x					•		•							✓			✓				
14	x			x				•				•					✓						✓	
23		x	x					•	•	•							✓		✓	✓				✓
24		x		x				•	•			•					✓		✓				✓	
34			x	x						•		•								✓			✓	
35			x		x			•		•	•						✓	✓		✓	✓			
36			x			x		•		•	•		•		•		✓	✓		✓	✓	✓		✓
45				x	x			•			•	•					✓	✓			✓		✓	
134	x		x	x				•		•		•					✓			✓			✓	
234		x	x	x				•	•	•		•			•		✓		✓	✓			✓	
345			x	x	x			•		•	•	•			•		✓	✓		✓	✓		✓	
7							x							•		✓								
17	x						x	•						•		✓	✓							
27		x					x	•	•					•		✓	✓		✓					
37			x				x			•				•		✓				✓				
47				x			x					•		•		✓							✓	
57					x		x	•			•			•		✓	✓	✓			✓			
67						x	x	•			•		•	•	•	✓	✓	✓			✓	✓		✓
137	x		x				x	•		•				•		✓	✓			✓				
147	x			x			x	•				•		•		✓	✓						✓	
237		x	x				x	•	•	•				•	•	✓	✓		✓	✓				
247		x		x			x	•	•			•		•	•	✓	✓		✓				✓	
347			x	x			x			•		•		•		✓				✓			✓	
357			x		x		x	•		•	•			•	•	✓	✓	✓		✓	✓			
367			x			x	x	•		•	•		•	•	•	✓	✓	✓		✓	✓	✓		✓
457				x	x		x	•			•	•		•	•	✓	✓	✓			✓		✓	
1347	x		x	x			x	•		•		•		•	•	✓	✓			✓			✓	
2347		x	x	x			x	•	•	•		•		•	•	✓	✓		✓	✓			✓	
3457			x	x	x		x	•		•	•	•		•	•	✓	✓	✓		✓	✓		✓	

Two PMTs

# Ref. No.	Lasers: Ar 488	Ar 488,514	HeNe 543	HeNe 633	ArKr 488,568	ArKr 488,568,647	UV 365	Dichroitic beam splitters DBS1: FT 510 (446434)	FT 530 (a.A.)	FT 560 (446437)	FT 645 (446441)	FT 655 (446449)	DBSP 488/543 (452585)	DBSP 488/568 (452586)	NT 80/20 (446445)	NT 80/20-543 (446446)	FT 395 (446431)	Zus. 452555 Slider	DBS2: FT 510 (446434)	FT 560 (446437)	FT 630 (446448)	Zus. 452555 Slider	Emission filters PMT1: LP 397 (467860-9904)	LP 515 (467864)	OG 550 (467925)	LP 570 (467922)	LP 590 (467921)	RG 665 (467927)	BP 670-810 (467934)	PMT2: BP 404-435 (467936)	BP 510-525 (467937)	BP 515-540 (467938)	BP 515-565 (467994-9904)	BP 590-610 (467939)
1	x							*											*					✓										
2		x						*	*											*				✓	✓									
3			x							*											•					✓			•					
4				x							*											•						✓						
5					x			*					*							*				✓			✓					✓		
6						x		*			*		*	*		*				*	*			✓			✓	✓				✓		✓
13	x		x					*			•									*				✓			✓							
14	x			x				*				•												✓				✓						
23		x	x					*	*			•								*				✓	✓		✓							
24		x		x				*	*				•											✓	✓			✓						
34			x	x						•	•					•				•						✓		✓						•
35			x		x			*				•	•							•						✓	✓					✓		
36			x			x		*			•	•		•		•				•	•			✓		✓	✓		✓			✓		✓
45				x	x			*			•		•	•		•				•	•			✓			✓	✓				✓		•
134	x		x	x				*			•	•			•	•				•	•			✓		✓		✓				•		•
234		x	x	x				*	*		•	•			•	•				•	•			✓	✓	✓		✓				•		•
345				x	x	x		*			•	•	•		•	•				•	•			✓		✓	✓	✓				✓		•
7							x							•										✓	•				•					
17	x						x	*						•		•								✓	✓									
27		x					x	*	*					•	•	•								✓	✓	✓								
37			x				x			•				•		•				•				✓			✓							
47				x			x				•			•		•				•				✓				✓						
57					x		x	*				•		•		•				•				✓	✓			✓						
67						x	x	*					•	•		•				*	*	*	*	✓	✓			✓	✓						
137	x		x				x	*				•		•		•				•	*			✓	✓		✓							
147	x			x			x	*				•		•		•				•				✓	✓			✓						
237		x	x				x	*	*			•		•		•				•	•			✓	✓	✓	✓							
247		x		x			x	*	*				•	•		•				•				✓	✓	✓								
347			x	x			x			•	•			•		•				•				✓			✓		✓					•
357			x		x		x	*				•	•	•		•				•	•			✓	✓		✓	✓				•		•
367			x			x	x	*				•	•	•		•	•			•	•	•		✓	✓		✓	✓		✓		•		✓
457				x	x		x	*				•	•	•		•	•			•	•	•		✓	✓			✓	✓			•		•
1347	x		x	x			x	*				•	•			•	•			•	•	•		✓	✓		✓		✓			•		•
2347		x	x	x			x	*	*			•	•			•	•			•	•	•		✓	✓	✓	✓		✓			•		•
3457			x	x	x		x	*				•	•	•		•	•			•	•	•		✓	✓		✓	✓	✓			•		•

Three PMTs

# Ref. No.	Emission filters PMT1: LP 397 (467860-9904)	LP 515 (467864)	OG 550 (467925)	LP 570 (467922)	LP 590 (467921)	RG 665 (467927)	BP 670-810 (467934)	PMT2: BP 510-525 (467937)	BP 515-540 (467938)	BP 515-565 (467994-9904)	BP 575-640 (467920)	BP 590-610 (467939)	PMT3: BP 404-435 (467936)	BP 510-525 (467937)	BP 515-540 (467938)	BP 515-565 (467994-9904)	Dichroitic beam splitters DBS1: FT 510 (446434)	FT 530 (a.A.)	FT 560 (446437)	FT 645 (446441)	FT 655 (446449)	DBSP 488/543 (452585)	DBSP 488/568 (452586)	NT 80/20 (446445)	NT 80/20-543 (446446)	FT 395 (446431)	Zus. 452555 Slider	DBS2: FT 560 (446437)	FT 630 (446448)	DBS3: FT 510 (446434)	FT 560 (446437)	Mirror (452584)
1		✓			•	•						•				•	•											•				
2		✓	✓		•	•						•				•	•	•										•				
3				✓		•						•				•			•									•	•			
4						✓														•												
5		✓			✓	•						•			✓		•				•							•	•			•
6		✓			✓		✓					✓			✓		•				•	•	•				•					
13		✓		✓		•						•		•			•				•							•	•			•
14		✓			•	✓						•				•	•				•							•	•			•
23		✓	✓	✓		•						•		•			•	•			•							•	•			•
24		✓	✓		•	✓						•				•	•	•			•							•	•			•
34				✓		✓						•							•									•	•			•
35		✓		✓	✓							•		•	✓		•					•	•					•	•			•
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345		✓		✓	✓	✓						•		•	✓		•				•	•	•				•	•	•			•
7		✓			•						•																	•				
17		✓	✓		•						•																	•				
27		✓	✓	✓	•						•																	•				
37		✓			✓			•				•																•				
47		✓			✓						•																	•				
57		✓	✓			✓						•																•				
67		✓	✓			✓	✓				•	✓		•														•				
137		✓	✓		✓			•				•																•				
147		✓	✓			✓					•																	•				
237		✓	✓	✓	✓			•				•																•				
247		✓	✓	✓		✓					•																	•				
347		✓			✓							•																•				
357		✓	✓		✓	✓		•	✓																			•				
367		✓	✓		✓	✓		✓	*	*		✓			✓													•				
457		✓	✓			✓	✓			•		•			✓													•				
1347		✓	✓		✓		✓		*			•																•				
2347		✓	✓	✓	✓		✓		*			•																•				
3457		✓	✓		✓	✓	✓		*	✓		•			✓													•				

CONTENTS

4 OPERATING THE MICROSCOPE SYSTEM

4.1 System setup by service

4.1.1 Delivery and setting up

Your LSM 410 invert is delivered in three wooden crates:

- Crate 1: system table and, if applicable, external laser (Ar)
- Crate 2: Axiovert stand unit, mounted on granite plate, including scan unit, operator control monitor, image monitor, keyboard and mouse
- Crate 3: electronics rack, tower

Further options are delivered in separate packaging.

Setting up, connection and commissioning and adjustment of an external laser are carried out by Carl Zeiss service personnel.

The LSM 410 invert must be set up so as to ensure that the clearance between the wall and the rear of the system is no less than 50 cm. This clearance is needed for adjustment and maintenance operations.

Setting up covers the following activities:

- Setting up the system table
- Setting up the stand unit
- Inserting the condenser and reflector slide
- Screwing in the lenses
- Setting up an external laser
- Setting up other optional modules
- Wiring the system

Before connecting the mains cables, check whether the mains voltage corresponds to the voltage specified on the rating plate of the electronics rack.

The back panel of the electronics rack must only be removed by specialised Carl Zeiss personnel. This also applies to the establishment or modification of cable connections on system components.

4.1.2 Wiring

As the LSM 410 invert is a microscope system that is combined specifically for you and your specific needs, a very large number of different component selections, and thus naturally also wiring, is possible.

Figure 4-1 shows the most frequently occurring variant including its affiliated wiring.

4.1.3 Commissioning

Prerequisites

The available configuration has been wired and checked. The main connection to the power supply mains has been established.

First-time commissioning of the LSM system is carried out by service personnel.
In this connection, functioning of the unit on the customer's premises is also verified.

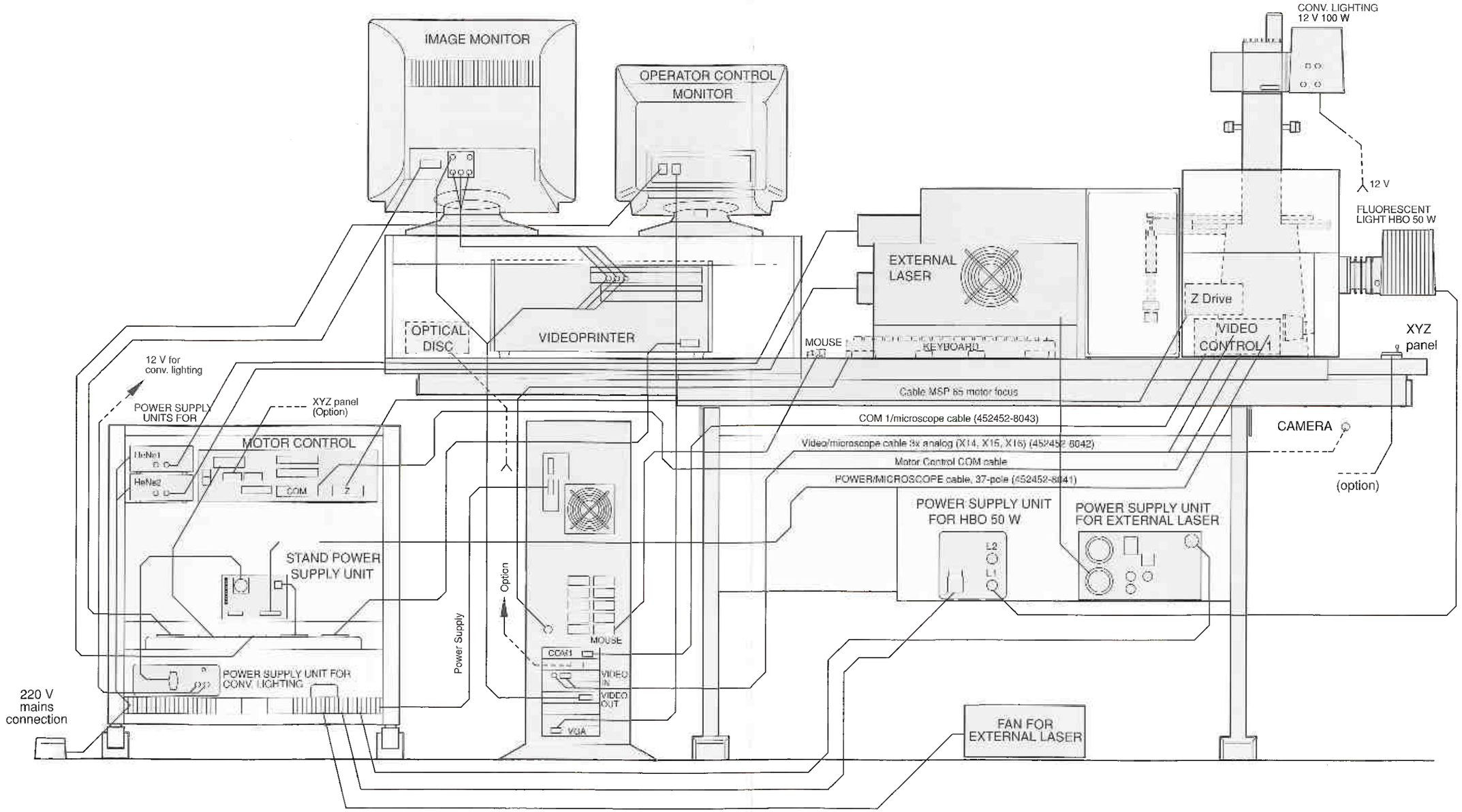


IMAGE MONITOR
OPERATOR CONTROL MONITOR
CONV. LIGHTING 12 V 100 W
12 V
FLUORESCENT LIGHT HBO 50 W
EXTERNAL LASER
Z Drive
VIDEO CONTROL 1
XYZ panel
OPTICAL DISC
VIDEOPRINTER
MOUSE
KEYBOARD
12 V for conv. lighting
POWER SUPPLY UNITS FOR
XYZ panel (Option)
Cable MSP 65 motor focus
COM 1/microscope cable (452452-8043)
Video/microscope cable 3x analog (X14, X15, X16) (452452-8042)
Motor Control COM cable
POWER/MICROSCOPE cable, 37-pole (452452-8041)
CAMERA
(option)
MOTOR CONTROL
HeNe1
HeNe2
COM
Z
STAND POWER SUPPLY UNIT
POWER SUPPLY UNIT FOR HBO 50 W
POWER SUPPLY UNIT FOR EXTERNAL LASER
Option
Power Supply
MOUSE
COM1
VIDEO IN
VIDEO OUT
VGA
POWER SUPPLY UNIT FOR CONV. LIGHTING
220 V mains connection
FAN FOR EXTERNAL LASER

Figure 4-1

4.2 Commissioning by operating personnel

4.2.1 Switching on the LSM system

Before switching on the LSM 410 invert microscope system, make sure that the microscope tower is vertical and the reflector slide is in the LSM position (right stop).

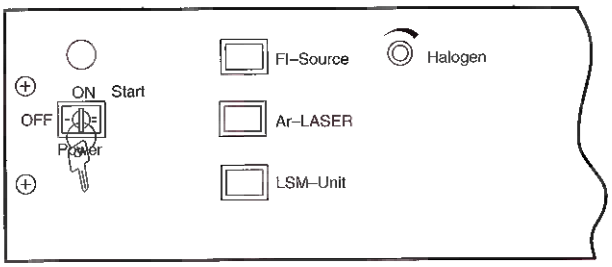


Figure 4-2

- Turn the POWER key switch on the electronics rack to "Start".
  - The switch returns to the "ON" position.
  - The green pilot lamp lights up.
  - The system produces three high signal tones.
- If you wish to operate under fluorescent light, then press the "Fl source" key.
  - The green pilot lamp lights up and the HBO lamp is triggered.

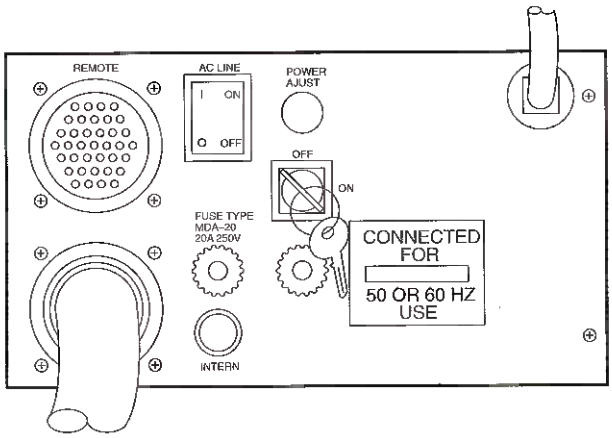


Figure 4-3

- If you wish to work with the argon laser, then press the "Ar laser" key.
- Switch on the Ar laser on the affiliated power supply unit as follows:
  - Set the red "AC line" switch to "ON".
    - The lamp in the switch lights up.
  - Set the key switch to "ON".
    - The yellow "INTERLOCK" lamp lights up approximately 60 seconds later.
    - The "POWER ADJUST" potentiometer is inactive. The power is controlled in a menu.

Do not switch on the LSM unit until approximately 120 seconds **after** switching on the HBO lamp or the external laser. Triggering of the lamp may cause the computer to crash.

- Now press the "LSM unit" key.
  - The lamp in the key lights up yellow.
  - The computer boots up.
  - The WINDOWS graphical user interface is activated (displayed briefly on the operator control monitor).
  - The Control Panel appears automatically by automatic starting of the LSM program.
  - A display of preset parameters may appear on the image monitor

    The configuration of the unit either corresponds to a presetting or to the configuration settings that applied the last time it was switched off.

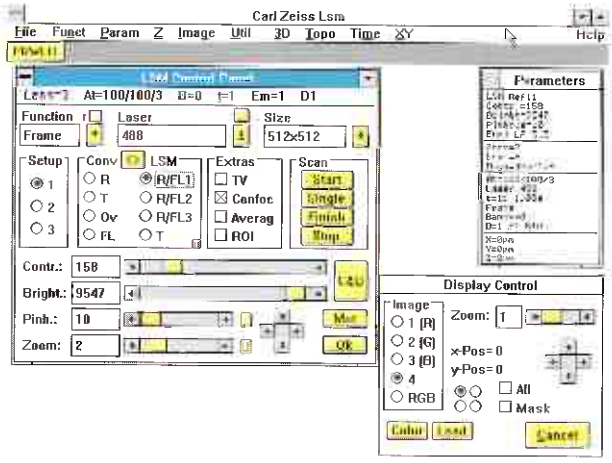


Figure 4-4

Acquaint yourself with the CONTROL PANEL before beginning work with the LSM (see Section 6).

4.2.2 Switching off the LSM system

- Close the Carl Zeiss LSM program by double clicking the system menu box.
  - Now only the "Program Manager" group icon appears on the monitor.

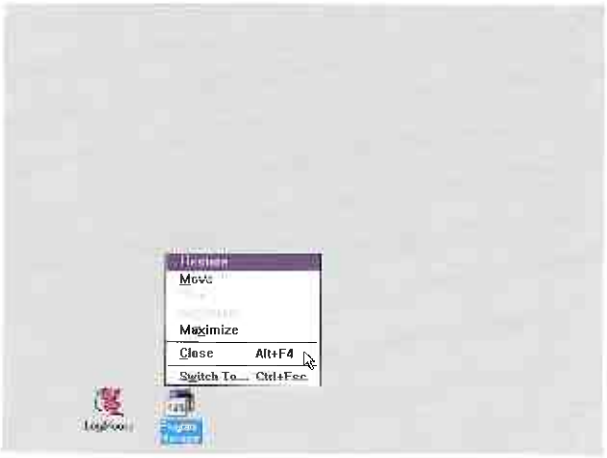


Figure 4-5

- Click once in the "Program Manager" group icon.
  - The adjacent pop-up menu is opened.

- Now click on "Close" once or press the "Alt+F4" keys.
  - A dialog box containing the following text appears on the screen:

```
This will end your Windows session
```

- Click on the Ok button.
  - WINDOWS is closed and the DOS operating system shows the C:\>_ prompt.

If you want to restart WINDOWS from the DOS operating system, then enter "Win" and press ENTER to confirm this.

- Now switch off the LSM unit by pressing the yellow key on the electronics rack.

If you switch off the computer while the LSM program is activated, the currently set operating parameters will be destroyed.

- If applicable, switch off the fluorescent lamp by pressing the "Fl source" key.
- If applicable, switch off the external laser.
  - Switch off the Ar laser by pressing the "Ar-LASER" key on the electronics rack.
  - Switch off the ArKr laser by setting the "AC LINE" switch to "OFF" and by setting the key switch to "OFF".
- Now deactivate the entire system power by setting the "POWER" key switch to "OFF".

CONTENTS

5 APPLICATION TASKS

5.1 Conventional microscopy

Please refer to the included operating instructions for details of how to handle the Axiovert microscope. The following notes on operation should only be looked upon as a rough guideline on how to proceed.
You should also acquaint yourself with the WINDOWS graphical user interface (see Chapter 6.2). Pay particular attention to the special features of the scrollbar in the ZEISS LSM program (see Section 6.2.4).

Prerequisites

The LSM system has been switched on as described in Section 4.3.1 and the Control Panel is activated on the operator control monitor.

General settings on the microscope

- Place your specimen (at the start, you are advised to use the included demo specimen) on the microscope stage.
  - In doing so, the cover glass must point down.
  - When using oil lenses, also place a drop of oil on the specimen.
- Make sure that the light path from the reflector slide to the eyepiece is unobstructed, i.e. check the following microscope components with reference to the microscope operating instructions:
  - Analyser
  - Polariser
  - Filter slide for reflected lighting
  - Reflector slide
  - Reticule
  - Lateral TV output
  - Control rod for switching between TV output and eyepiece
  - Eyepiece seal
- Only a few settings in the Control Panel are necessary for conventional microscopy.
  - Select a suitable lens (e.g. 20x) in the pop-up "Lens" menu.
  - Click the mouse under "Conv" in
    ○ **T** for transmitted light or in
    ○ **FL** for fluorescence.

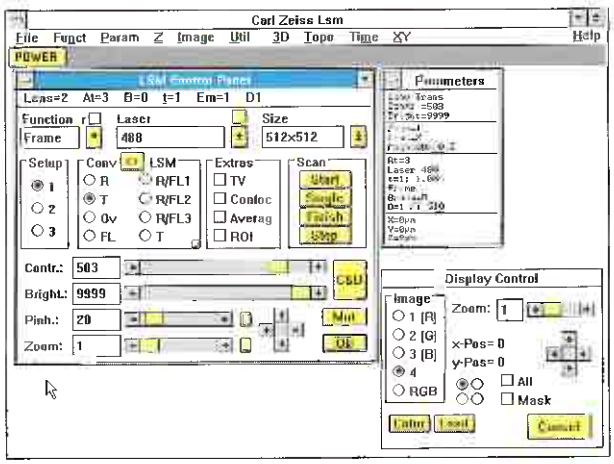


Figure 5-1

5.1.1 Transmitted light

Prerequisites

The settings described in Section 5.1 have been carried out.

- Slide the reflector slide out of the LSM position (right stop) into any position for transmitted light.
  - If no empty position is available, remove the filter slide completely or slide it into any fluorescence position but, in this case, the image will be discoloured.

Figure 5-2

- Turn the "Halogen" potentiometer on the electronics rack to the right.
  - Light shines on the specimen.
- Focus on a suitable position in the specimen.
- Now set lighting according to KÖHLER.
  - A sharply focused transmitted light image appears in the eyepiece.

5.1.2 Fluorescence

Prerequisites

The settings described in Section 5.1 have been carried out.
The fluorescence lamp ("Fl source" key on the electronics rack) has been switched on **before** the LSM unit.

Figure 5-3

- Set the reflector slide to a position for fluorescence excitation (ideal: filter set 09).
- Set the blocking slide before the reflector slide to an unblocked position.
- Focus on a suitable position in the specimen.
- Optimise the lighting as described in the instructions for FL lighting
  - A sharply focused reflected light fluorescence image appears in the eyepiece.

5.2 Laser Scan Microscopy

(1) General

Confocal microscopes can particularly be used profitably when thick specimens have to be analysed in fluorescence or when reflecting specimens with surface structures require analysis. Contrary to conventional microscopes, the confocal microscope only records information that originates from the focus plane. All other information (unfocussed images from other planes) is effectively isolated by a space filter. The filter consists of a small diaphragm (a pinhole) in a plane conjugated to the field (Figure 5-4).

The optical sections obtained in this way have thickness values down to 0.4 µm. The advantage is that the specimen is essentially not modified and therefore repeated measurements are possible with different parameters and increments. Multiply fluorescent-dyed specimens are particularly interesting in biology and justice is done to this with three simultaneously operating recording channels.

Besides great contrast enhancement in depth, such a unit is also of interest when it comes to spatially-visually realising three-dimensional structures. To this end, optical sections are recorded in series and, in doing so, the focus positions are moved to with a motor by computer control. The images initially consist of electronic data that can be stored on various storage media. Using a graphics card, the images can then be visualised on a monitor.

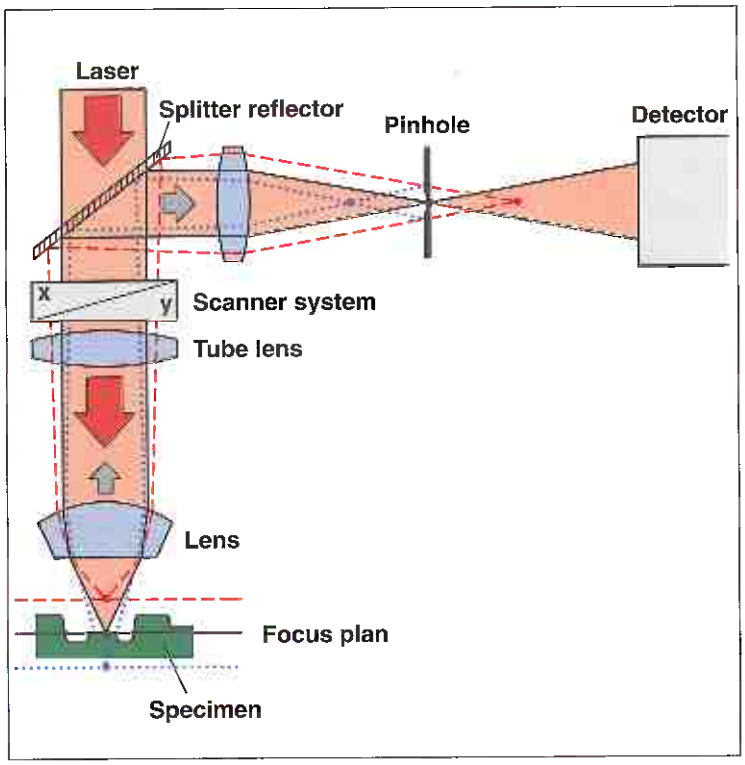


Figure 5-4

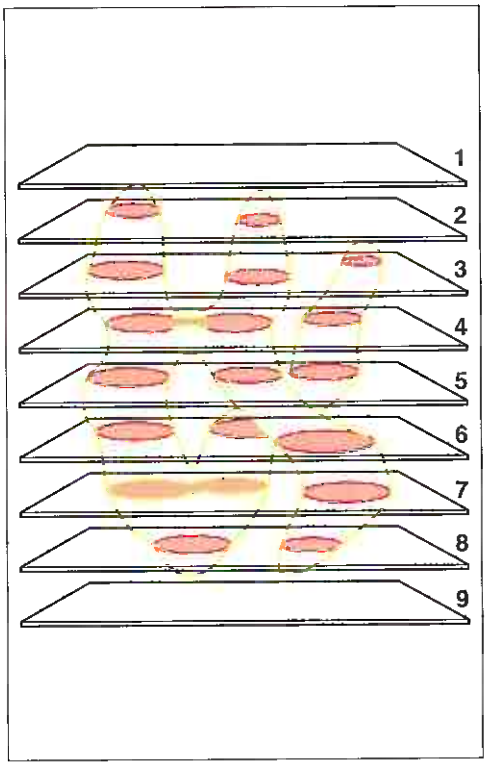


Figure 5-5

(2) Three-dimensional depiction of microscopic image series

Gallery

The simplest (and cheapest) method of depicting three-dimensional information is a gallery of optical sections in series. In this case, all sections in the series are placed in a row like on the page of a stamp collection album. The z position of the individual sections is displayed.

Extended depth of focus

The entire record can also be depicted in a projection. In doing so, the computer computes an image that is composed of all the focused optical sections that were produced when recording a series. The simplest method consists of projecting the brightest grey values in a point of the image along the z axis. The transparency method is more complex. In this case, the grey values in the sections of the series are weighted with different values denoting their "transparency", resulting in a depiction that corresponds rather to the surface. In any case, such a composite method supplies an image with a virtually infinite depth of focus. As has been said, only information originating from sharply focused planes contributes to the result. Therefore, if only half a micrometer is sharply imaged in depth in the eyepiece (e.g. using an oil lens), the entire depth of a section, e.g. 100 micrometers, can be sharply imaged when using this method.

Rotation animations

A projected image cannot only be computed as if the specimen were viewed vertically from above along the z axis, but can also be rotated to all conceivable angles. An effective means of depicting the third dimension is to compute a sequence of projections and, in doing so, to virtually rotate the specimen about a specific angle (e.g. 10ø), for example one full rotation about the y axis. If this sequence is now displayed fast enough on the monitor, the observer has the impression that a three-dimensional structure is rotating in the monitor. The reason for this is that our brains are simply too slow to recognise that the image is only a fast sequence of computed views.

Stereo images

In our normal environment, we observe things that are not too far away from two different directions. From these two different images, our brains reconstruct the three-dimensional world. As we are able to compute any chosen views with the computer, it is also possible to compute a pair of images that correspond to the images we see with our left and right eyes. Such a stereo pair can then be displayed adjacently on the monitor and, when using suitable aids, we do in actual fact see a three-dimensional image. Practised observers can even see the 3D image without using any aids. (The problem is that we bring images together at a specific distance by squinting but, at the same time, we have to focus the images. Our brains think they know the distance where two objects are when we adjust our eyes to a specific angle and then automatically focus on this distance.)
Another possibility consists of depicting one image in the red channel and one image in the green channel of the monitor one above the other. In this case, we only need to put on a specific pair of glasses containing one red and one green filter and the information denoting "image that must be viewed with the right eye" comes through the green filter and only arrives in the right eye. This is the anaglyph depiction that is also known from other fields.

Height colour coded extended depth of focus

The colour-coded height depiction is a direct continuation of the process of computing projections. In this case, a colour is assigned to the various planes in which the affiliated sections are then depicted. The result is a map that contains information about the z positions of the mapped structures. As on a conventional map, the elevations are red (mountain top) and the depressions are blue. To enable direct evaluation, a colour scale is also displayed to specify the actual height above the first section.

Orthogonal sections

Once a complete stack has been recorded, it is easy for the computer to compute a section (profile) at any point. The "orthogonal sections" function supplies a triplet of sectional images that are perpendicular to each other. In doing so, one point (Voxel) is selected from the three-dimensional data packet by clicking it with the mouse and the affiliated sections are automatically visible on the screen. Thus, you can obtain a very swift overview of the three-dimensional structure by "wandering" in the stack of sections.

Slanted sections

If you select a rotation and tilt angle in addition to the x, y and z coordinates, you have uniquely defined a plane surface. The section in such a plane can also be computed and displayed. For example, you can do this if you wish to show two specific structures whose joining line is not parallel to one of the three main axes. Since, when specifying five coordinates, we have problems imagining how this section will take place in the stack, the section plane (red) in the volume (green) is also displayed in a 3D cursor.

Image processing

As the data in the confocal laser scanning microscope is digital, all conceivable image processing and enhancement routines can be used to make the display clearer or to carry out measurements (see "Image" software description).

Image analysis

Density measurements are possible in addition to the usual measuring methods for determining lengths, areas and circumferences. In doing so, the grey values in the individual pixels are displayed or are used to compute histogram data. The simplest measurement of this kind is to read the grey value out of one single pixel. In the next step, the affiliated intensity profile is displayed for any chosen line in the image. (By the way, this can also be done online, i.e. the laser only moves along one line and the display is shown like in an oscillogram.) The next highest level is then two-dimensional brightness evaluation, i.e. average grey values and the maximum and minimum etc. in geometrically or interactively bordered areas. Using macro functions, such measurements can be automatically carried out swiftly and reproducibly on large quantities of data.

Further software packages such as topography and time series enhance the instrument for material research (surfaces) and physiology (kinetics).

(3) Image optimisation

As image optimisation is repeatedly necessary in all applications belonging to LSM technology, this is described in detail. In the description of the application tasks, reference is then only made to optimisation.

Figure 5-6

Prerequisites

- The following have been set in the main menu:
  Image → Color Tables → Greyscale.
- Scanning has been started.

Normally, the available quantity of light and multipliers are not optimally adapted to each other, i.e. the image on the image monitor is lacking in contrast.

- Select the following in the main menu:
  Image → Color Tables → Range Indicator,
  to display the brightness levels in colour.

Figure 5-7

If the brightness is too high, the display on the image monitor will appear red.

Figure 5-8

If the brightness is too low, the display on the image monitor will appear blue.

- Select the following in the main menu:
  Util → Auto B&C or press the F9 key
  - In this way, you will automatically see a good image which, however, can still be optimised.

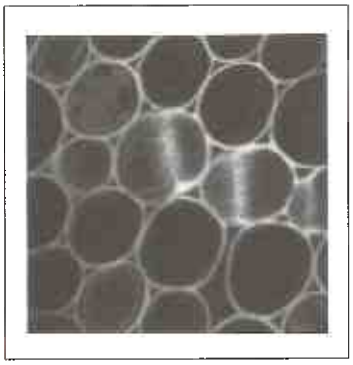


Figure 5-9

- Reduce the brightness by means of the scrollbar in the Control Panel until approximately half of the background appears blue where you do not expect a signal.
  - With Brightness, you control the electronic offset, which influences the overall brightness of the image.

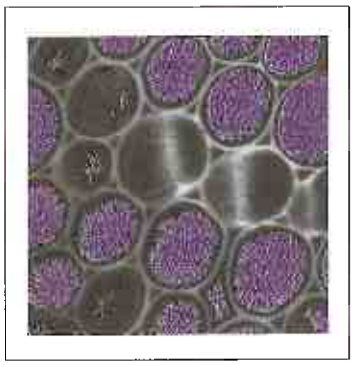


Figure 5-10

- By means of the scrollbar in the Control Panel, now increase the contrast until a few red dots still just appear in the image.
  - In this way, you control the high voltage on the photomultiplier (PMT) and the electronic preamplification factor.

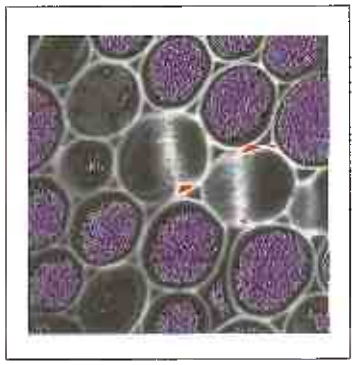


Figure 5-11

- Now select the following in the main menu:
  Image → Color Tables → Greyscale.

Using the Color key in the "Display Control" dialog box, you can toggle swiftly between "Greyscale" and "Range Indicator"
(see Section 6.3.3).

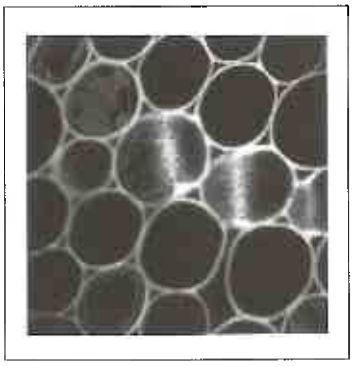


Figure 5-12

(4) Pinhole adjustment

Pinhole adjustment serves to further enhance the image in **confocal** microscopy applications (only one focus plane) once the image has been optimised via **Contrast** and **Brightness** by **non–confocal** microscopy.

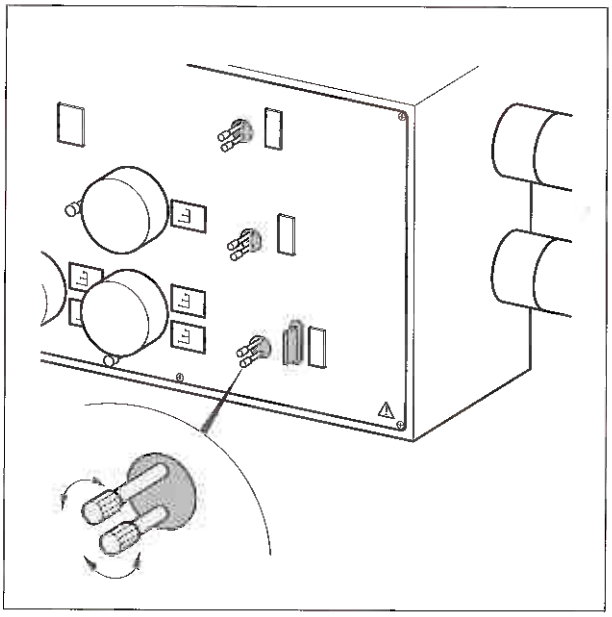


Figure 5-13

- Carry out pinhole adjustment as follows:
  - In the main menu
    Image → Color Tables → Range Indicator selected.
  - Set the pinhole in the Control Panel to 20.
  - Increase the contrast until a few red dots appear.
  - Optimise the top adjusting screw to a maximum red component.
  - Optimise the bottom adjusting screw to a maximum red component.

Reduce Contrast if the red component is generally too high.

- Repeat pinhole adjustment approximately 1–2 times: then, it will probably not be possible to enhance the image any further.
- If necessary, correct the **Contrast** once again in the **Control Panel**.
- Select the following in the main menu:
  Image → Color Tables → Greyscale.

If the adjustment of the position should be completely wrong, begin adjustment with a pinhole setting of 100, then continue with 50 and finally adjust with 20 as described above.

5.2.1 Single image display

Prerequisites

- The reflector slide is set to LSM position (right stop).
- The slide before the reflector slide is set to free passage.
- The specimen has been conventionally adjusted under fluorescent lighting.

Assumption

- The AR 488 laser is installed.

5.2.1.1 Single-channel display

(1) Fluorescence (FL)

a) Not confocal

- Carry out the following settings in the **Control Panel**:

Function	:	Frame
Setup	:	◉ 1
LSM	:	◉ R/FL1
r☐	:	Not activated
Laser	:	488
Extras	:	Not activated
Size	:	512x512
Lens	:	e. g. 3 (40x/1.3 is expedient)
At	:	3
B	:	0
t	:	1
Em	:	1 (LP 515)
D1	:	FT 510
Zoom	:	2
Contr.	:	┐
Bright.	:	├ At the moment, any setting possible
Pinh.	:	┘

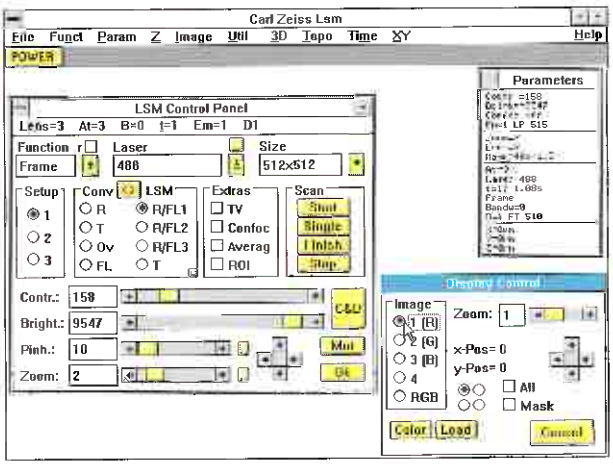


Figure 5-14

- Set the dichroitic beamsplitter DBS2 (if installed) to the "Free" position.
- In the **Display Control** window, select image: ◉ 1 (R) and Zoom: 1 (high resolution: 2).
- Start scanning by pressing Start.
  - The laser illuminates the specimen.
- Optimise the monitor image as described in Section 5.2 (3).
- Stop scanning by pressing the Stop button.

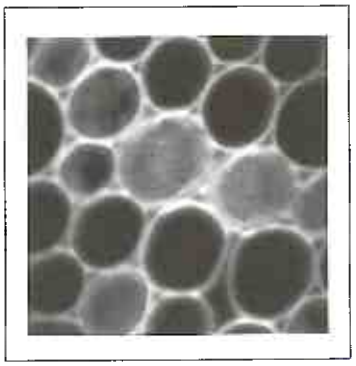


Figure 5-15

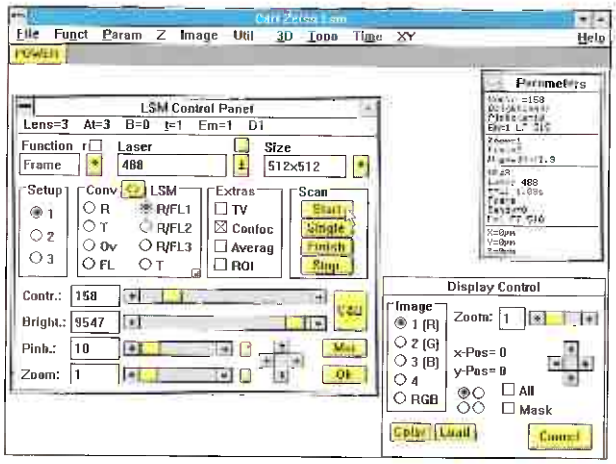


Figure 5-16

b) confocal

- Carry out the following settings on the **Control Panel:**

Function	:	Frame
Setup	:	◉ 1
LSM	:	◉ R/FL1
r ☐	:	not activated
Laser	:	488
⇨ Extras	:	☒ Confoc
Size	:	512x512
Lens	:	e. g. 3 (40x/1.3 is expedient)
At	:	3
B	:	0
t	:	1
Em	:	1 (LP 515)
D1	:	FT 510
Zoom	:	2
Contr.	:	] Any setting at the
Bright.	:	] moment
Pinh.	:	10

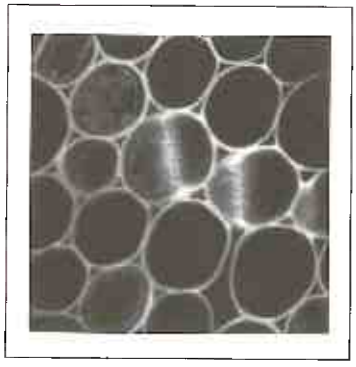


Figure 5-17

- Set the dichroitic beam splitter DBS2 (if available) to the "Free" position.
- In the **Display Control** Image window, select: ◉ 1 (R) and Zoom: 1 (high resolution: 2).
- Start scanning by selecting Start.
  - The laser illuminates the specimen.
- Optimise the monitor image as described in Section 5.2 (3).
- Carry out pinhole adjustment as described in Section 5.2 (4).
- Stop scanning by selecting Stop.

Pinhole adjustment can be carried out with greater ease by defining a narrow REGION OF INTEREST (ROI) in the centre of the image (dy ≈ 60). By virtue of the fact that the image is built faster within the ROI, changes in the pinhole adjustment are also visible much sooner.

The difference between **non–confocal** and **confocal** is elucidated by the following procedure.

- Generate a non–confocal single–channel image as described in Section (1).
- In the **Control Panel**, click on Options in ☒ROI.
  - The **Set ROI** dialog box appears on the operating monitor.
- Click Set ROI with the mouse.
  - The mouse pointer moves to the image monitor.
  - On the image monitor (green frame), the size and position of the ROI can now be defined with the mouse.
- Now press the right mouse button.
  - The mouse pointer moves back to the operator control monitor.

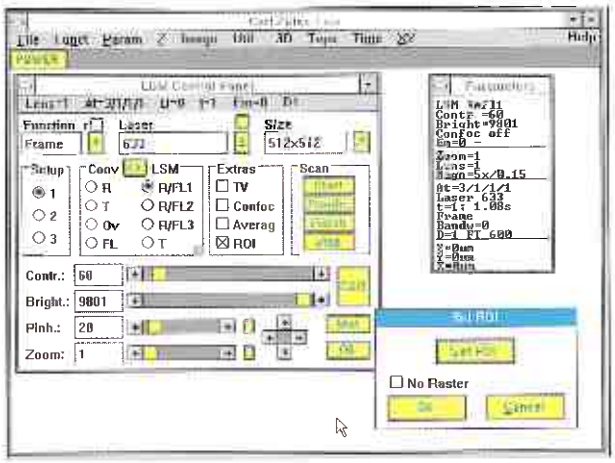


Figure 5-18

- In the **Set ROI** dialog box, now click in the OK box.
- Now select ☒Confoc and start scanning.
- Optimise the monitor image as described in Section 5.2 (3).
  - The monitor image is substantially better inside the ROI (confocal) than it is outside it.

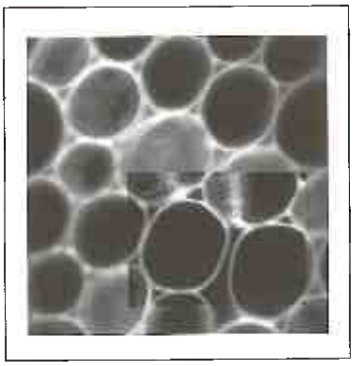


Figure 5-19

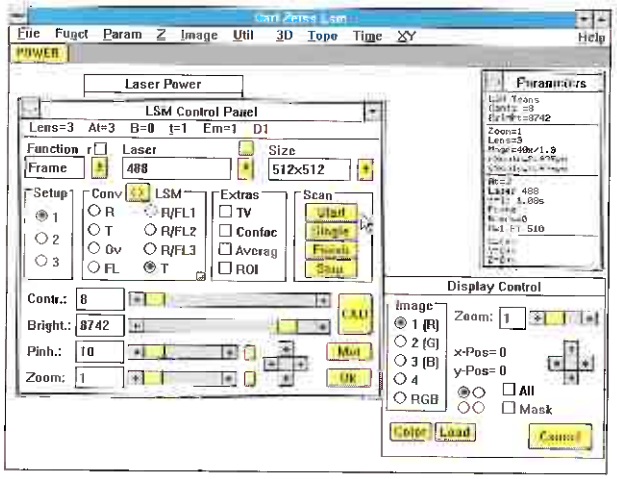


Figure 5-20

(2) Transmitted light (T)

- Carry out the following settings in the **Control Panel:**

Function	:	Frame
Setup	:	◉ 1
⇨ LSM	:	◉ T
r☐	:	not activated
Laser	:	488
Extras	:	keine aktiviert
Size	:	512x512
Lens	:	e. g. 3 (40x/1.3 is expedient)
At	:	3 (the attenuation may have to be increased)
B	:	0
t	:	1
Em	:	any
D1	:	FT 510
Zoom	:	2
Contr.	:	⎫
Bright.	:	⎬ At the moment, any setting possible
Pinh.	:	⎭

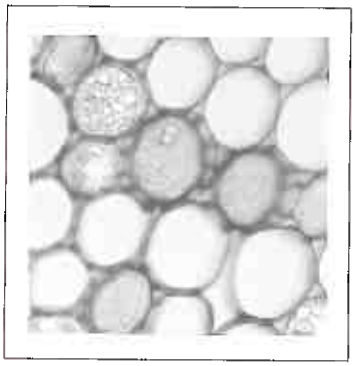


Figure 5-21

- In the **Display Control** window select image: ◉ 1 (R) and Zoom: 1 (high resolution: 2).
- Optimise the monitor image as described in Section 5.2 (3).
- Stop scanning by selecting Stop.

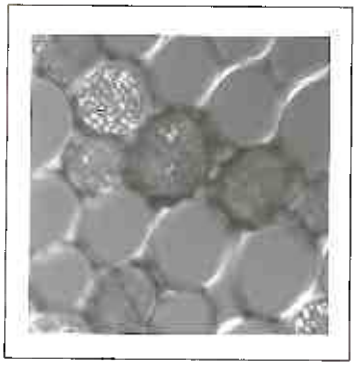


Figure 5-22

It goes without saying that all other transmitted light contrasting methods are possible, e.g.

- Phase contrast
- Differential interference contrast (DIC)
- Polarisation contrast (POL)
- Dark field

Figure 5-22 shows the specimen in DIC.

5.2.1.2 Multi-channel display

(1) Fluorescence and transmitted light (FL + T)

- Carry out the following settings in the **Control Panel**:

⇨ Function : Split 2
(in the **Display Control** window, the marking in the **Image** box changes to "4")

r☐ : Not activated
Laser : 488
Extras : ☒Confoc
Size : 512x512
Lens : 3 (40x/1.3 is expedient)
At : 3
B : 0
t : 1
D1 : FT 510
Zoom : 2

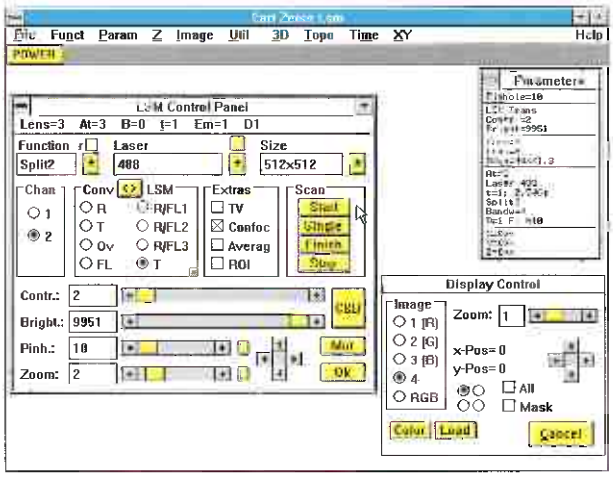


Figure 5-23

- By clicking Chan ◉1, now activate channel 1 with the following affiliations:
  - Channel 1 becomes the fluorescence channel by selecting ◉R/FL1,
  - Em: LP 515,
  - Pinh: 10,
  - At the moment any Contr./Bright.

- By clicking Chan ◉2, now activate channel 2 with the following affiliations:
  - Channel 2 becomes the transmitted light channel by selected ◉T.

- Start scanning.
  - The image for channel 2 (bottom half of the image) is generated.

- Optimise the monitor image as described in Section 5.2 (3).

- Now activate channel 1 again.
  - The image for channel 1 (top half of the image) is generated.

- Optimise the monitor image as described in Section 5.2 (3).

- Now carry out pinhole adjustment as described in Section 5.2 (4).

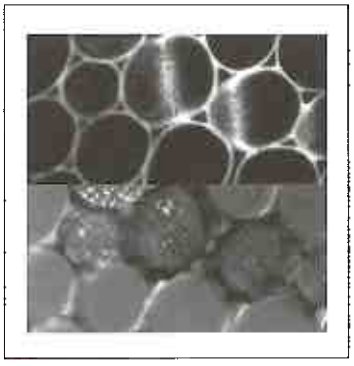


Figure 5-24

The images generated separately from channels 1 (R) and 2 (G) can now be superimposed to arrive at an overall image.

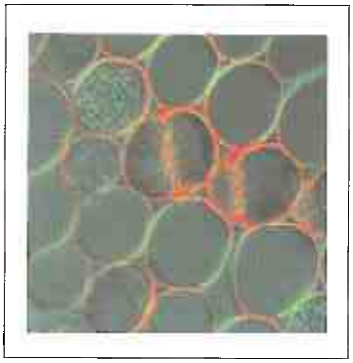


Figure 5-25

- Under Function, select: Overl2
  (in the **Display Control** window, the marking in the **Image** box changes to "RGB").
- Start scanning by selecting Start or by selecting Single.
  - The overall image superimposed from channels 1 (R) and 2 (G) appears on the image monitor.

Settings that refer to **one** specific channel only (e.g. CONTRAST, BRIGHTNESS, PINHOLE) can be changed when the respective channel is activated (Chan ◉ 1 or Chan ◉ 2).

(2)Double fluorescence (2FL)

- Carry out the following settings in the **Control Panel**:

  Function : Split 2
  (in the **Display Control** window, the marking in the **Image** box changes to "4")
  r☐ : not activated
  Laser : 488
  Extras : ☒Confoc
  Size : 512x512
  Lens : e. g. 3 (is expedient 40x/1.3)
  At : 3
  B : 0
  t : 1
  D1 : FT 510
  Zoom : 2

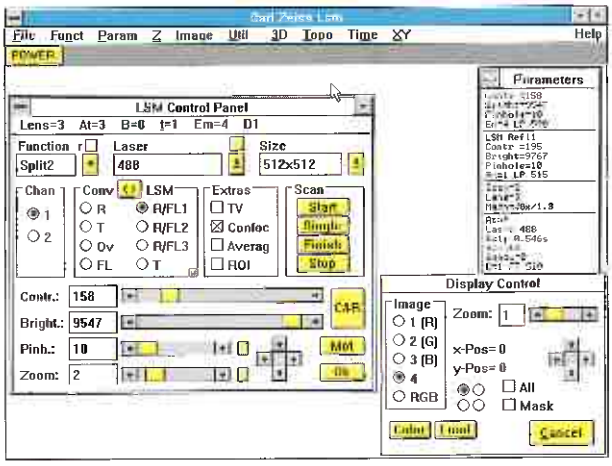


Figure 5-26

- By clicking Chan ◉1, now activate channel 1 with the following affiliations:
  - By selecting ◉R/FL1, channel 1 becomes the first fluorescence channel,
  - Em: LP 590 or LP 570,
  - Pinh: 10,
  - At the moment any value for Contr./Bright.

- By clicking Chan ◉2, now activate channel 2 with the following affiliations:
  - By selecting ◉R/FL2, channel 2 becomes the second fluorescence channel (if you have a three–channel unit, then select R/FL3),
  - Em: BP 515-565 or BP 515-540 or BP 510-525,
  - Pinh: 20 for second channel,
  - DBS2: FT 560,
  - If you have a three–channel unit, then set the DBS3 slide to the "Reflector" or "FT 560" setting.

- Now start scanning.
  - The image for channel 2 (bottom half of the image) is generated.

- Now optimise the image and carry out pinhole adjustment for channel 1 as described in Section 5.2 (3) and (4).

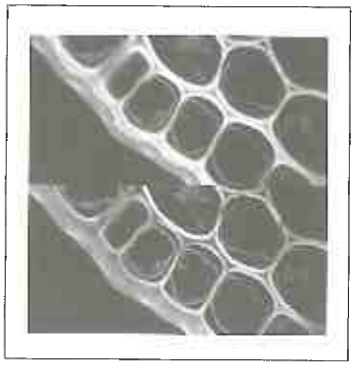


Figure 5-27

- Now select channel 1 (Chan 1) again.
  - The image for channel 1 (top half of the image) is generated.
- Now optimise the image and carry out pinhole adjustment for channel 1 (Chan 1) also.

You can see the images optimised for channels in Figure 5-27.

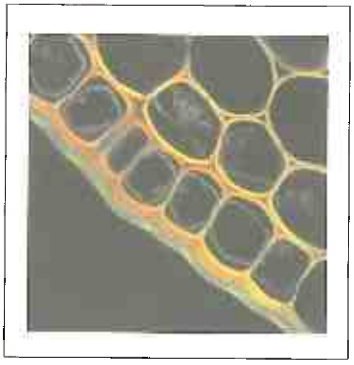


Figure 5-28

The images generated separately from channels 1 (R) and 2 (G) can now be superimposed in an overall image.

- In Function, select: Overl2
  (in the **Display Control** window, the marking in the **Image** box changes to "RGB").
- Start scanning by selecting Start or by selecting Single.
  - The overall image superimposed from channels 1 (R) and 2 (G) appears on the image monitor.

Note

In the event that you are using only **one** photomultiplier, you can generate a multi–channel fluorescence image as follows:

Function	:	Frame
r☐	:	not activated
Laser	:	488
Extras	:	☒Confoc
Size	:	512x512
Lens	:	e. g. 3 (40x/1.3 is expedient)
At	:	3 (the attenuation may have to be increased)
B	:	0
t	:	1
D1 (DBS1)	:	FT 510
Zoom	:	2
DBS2	:	in empty position

To make sure that an old image still in the video memory is not displayed on the image monitor, select the following in the main menu:
Image → Clear Buffer.

- Now activate Setup ⦿1 with the following settings:
  LSM : R/FL1,
  Em: LP 570 or LP 590,
  Pinh: 20,
  In the **Display Control** window, red channel selected: ⦿1(R).
- Now start scanning.
- Optimise the image and carry out pinhole adjustment as described in Section 5.2 (3) and (4).
- Stop scanning.

- Now activate Setup ⦿2 with the following settings:
  LSM : R/FL1,
  Em: BP 515-565,
  Pinh: 20,
  In the **Display Control** window, green channel selected: ⦿2(G).
- Now start scanning.
- Also optimise the image and carry out pinhole adjustment as described above.
- Stop scanning.

The monochrome images produced separately in the red/green channels can be superimposed in one colour image by clicking ⦿RGB in the **Display Control** window.

You can proceed in accordance with the same method for 3FL or 2FL+T.

Ratio function online

Prerequisites

- You have created a two–channel image under double fluorescence, displayed it in split mode and optimised this image.

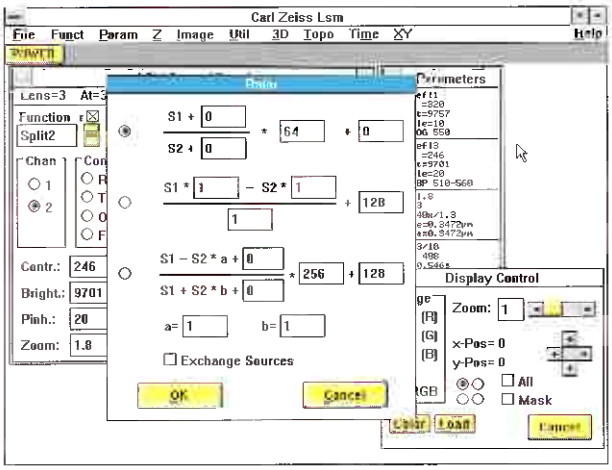


Figure 5-29

- Now activate r☒ in the **Control Panel**.
  - The **Ratio** window appears on the operator control monitor.

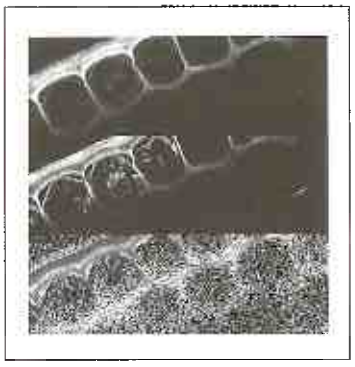


Figure 5-30

- Click the uppermost ratio function:

$$\frac{S1}{S2} \triangleq \frac{CHAN1}{CHAN2}$$

  - After pressing Ok to confirm, the adjacent image appears and the following affiliations apply:

Top third:	CHAN1
Middle third:	CHAN2
Bottom third:	Ratio

The ratio function can be used online, i.e. while scanning is active.

- Click the Stop button in the **Control Panel** to stop scanning.

- Select **Overl2** under Function.
  - In the **Display Control** window, the marker changes from ◉4 to ◉**RGB** RGB and the adjacent image appears on the image monitor with the following affiliations:
    CHAN1 in red
    CHAN2 in green
    Ratio in blue

Figure 5-31

The ratio function can be used for all z series and time functions.

Ratio and **Averaging** cannot be used simultaneously.

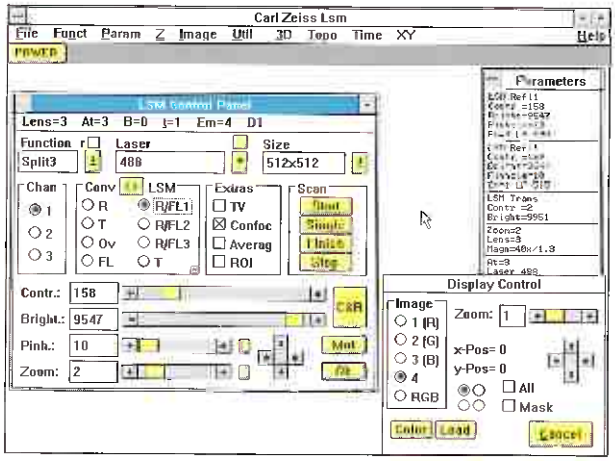


Figure 5-32

(3) Double fluorescence and transmitted light (2FL+T)

- Carry out the following settings in the **Control Panel:**

⇨ Function : Split 3
(in the **Display Control** window, the marking in the **Image** box changes to "4")
r☐ : not activated
Laser : 488
Extras : ☒Confoc activated
Size : 512x512
Lens : 3 (40x/1.3 is expedient)
At : 3
B : 0
t : 1
DBS1 : FT 510
Zoom : 2

- By clicking Chan ◉1, now activate channel 1 with the following affiliations:
  - Channel 1 becomes the first fluorescence channel by selecting ◉R/FL1,
  - Em: LP 590 or LP 570,
  - Pinh: 10,
  - At the moment, any Contr./Bright setting.
- By clicking Chan ◉2, now activate channel 2 with the following affiliations:
  - Channel 2 becomes the second fluorescence channel by selecting ◉R/FL2,
  - Em: BP 515-565 or BP 515-540 or BP 510-525,
  - DBS2: FT 560,
  - If you have a three channel unit, then set the DSB slide to "Reflector" or "FT 560".
- By clicking Chan ◉3 now activate channel 3 with the following affiliations:
  - Channel 3 becomes the transmitted light channel by selecting ◉T.
- Now start scanning.
  - The image for channel 3 (bottom third of the image) is generated.
- Now optimise the image for channel 3 as described in Section 5.2 (3).
- Now select channel 2 (Chan 2).
  - The image for channel 2 (middle third of the image) is generated.
- Now optimise the image and carry out pinhole adjustment as described in Section 5.2 (3) and (4).
- Now select channel 1 (Chan 1).
  - The image for channel 1 (top third of image) is generated.

- Now optimise the image and carry out pinhole adjustment as described in Section 5.2 (3) and (4).

You can see the images optimised separately for all three channels in 5-33.

Figure 5-33

The images generated separately from channels 1 (R) and 2 (G) and 3 (B) can now be superimposed to arrive at an overall image.

- Under Function, select: Overl3
  (in the **Display Control** window, the marking in the **Image** box changes to "RGB").
- Start scanning by selecting Start or by selecting Single.
  - The overall image superimposed from channels 1 (R), 2 (G) and 3 (B) appears on the image monitor.

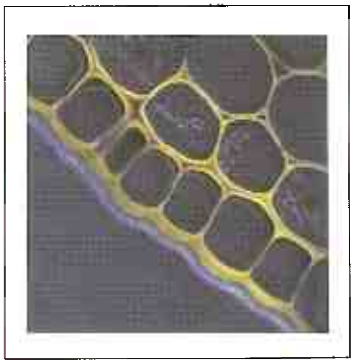


Figure 5-34

A modified colour display may be advantageous for certain applications.

- To do this, simply change the affiliations:
  Chan 1 → T : display in red
  Chan 2 → R/FL1 : display in green
  Chan 3 → R/FL3 : display in blue

Figure 5-35

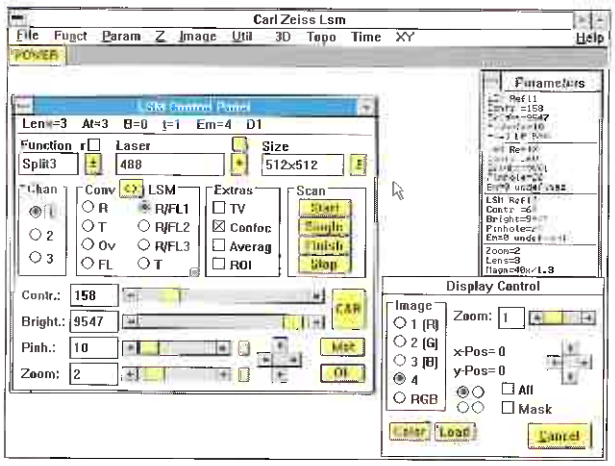


Figure 5-36

(4) Triple fluorescence (3FL)

- Carry out the following settings in the **Control Panel**:

Function	:	Split 3 (in the **Display Control** window, the marking in the **Image** box changes to "4")
r ☐	:	not activatd
Laser	:	488
Extras	:	☒Confoc
Size	:	512x512
Lens	:	e. g. 3 (40x/1.3 is expedient)
At	:	3
B	:	0
t	:	1
DBS1	:	FT 510
Zoom	:	2

- By clicking Chan ◉1, now activate channel 1 with the following affiliations:
  - Channel 1 becomes the first fluorescence channel by selecting ◉R/FL1.
  - Em: BP 670-810 or RG 665,
  - Pinh: 20,
  - At the moment, any Contr./Bright setting.

- By clicking Chan ◉2, now activate channel 2 with the following affiliations:
  - Channel 2 becomes the second fluorescence channel by selecting ◉R/FL2,
  - Em: BP 590-610,
  - DBS2: FT 630,
  - Pinh: 20.

- By clicking Chan ◉3, now activate channel 3 with the following affiliations:
  - Channel 3 becomes the third fluorescence channel by selecting ◉R/FL3,
  - Em: BP 515-540 or 510-525,
  - DBS3: FT 560,
  - Pinh: 20.

- Now start scanning.
  - The image for channel 3 (bottom third of the image) is generated.

- Now optimise the image and carry out pinhole adjustment for channel 3 as described in Section 5.2 (3) and (4) durch.

- Now select channel 2 (Chan 2).
  - The image for channel 2 (middle third of the image) is generated.

- Now optimise the image and carry out pinhole adjustment as described in Section 5.2 (3) and (4).
- Now select channel 1 (Chan 1).
  - The image for channel 1 (top third of image) is generated.
- Now optimise the image and carry out pinhole adjustment as described in Section 5.2 (3) and (4).

You can see the images optimised separately for all three channels in Figure 5-37.

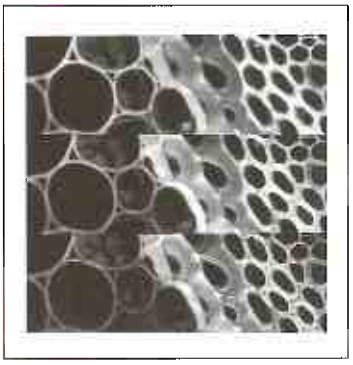


Figure 5-37

The images generated separately from channels 1 (R), 2 (G) and 3 (B) can now be superimposed in an overall image.

- Under Function, select: Overl3
  (in the **Display Control** window, the marking in the **Image** box changes to "RGB").
- Start scanning by selecting Start or by selecting Single.
  - The overall image superimposed from channels 1 (R), 2 (G) and 3 (B) appears on the image monitor.

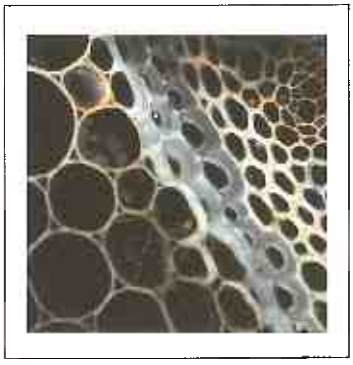


Figure 5-38

5.2.2 Image sequences

Owing to the quantities of data produced when generating image sequences, you should make sure that at least 25 MB free space is left on the hard disk.

(1) Recording a section stack

Note that a section stack can be recorded for all kinds of single image recordings.

Single image, single channel, fluorescence, confocal

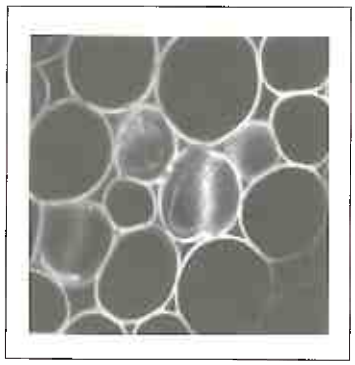


Figure 5-39

- Using the example specimen, produce an optimised LSM image as described in Section 5.2.1.1 (1) b).
- Search for a point in the specimen where the capillaries show "flats".
  - By means of Image → Color Tables → Greyscale, the image should approximately look like the adjacent one, i.e. one single optical section of the specimen is depicted.
- Now move the focusing drive lightly to and fro while scanning is still active.
  - Various sections become visible on the image monitor.
- Now turn the left focusing drive clockwise until you can see on the image monitor that you are just about to "leave" the specimen, i.e. the section plane through the specimen has been moved down.

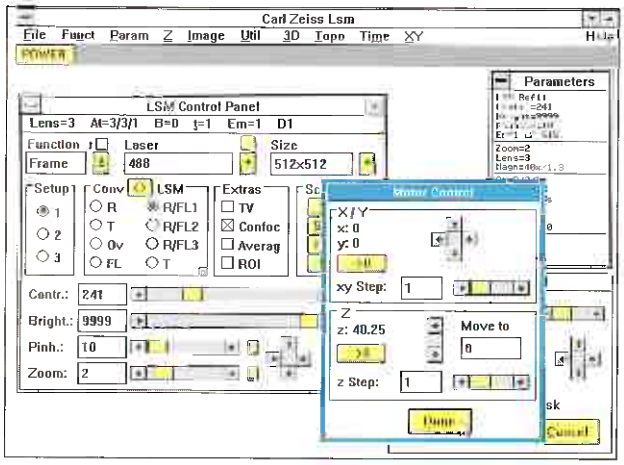


Figure 5-40

- Click the Mot key in the Control Panel.
  - The **Motor Control** dialog window is opened but, at the moment, only the bottom half relating to the z coordinates is of interest.
- Click on the → 0 button.
  - The current bottom edge of the specimen is defined as "0".

- Now turn the focusing drive counterclockwise until you just "leave" the top edge of the specimen.
  - The value then displayed under "2:" (e.g. 40.25 µm) then corresponds to the specimen thickness.
- Close the "Motor Control" window by pressing the Done button.
- Now position the focusing drive (clockwise) on the bottom end of the specimen.
- In the main menu, now select:
  z → Sectioning.
  - The adjacent dialog box appears.
- Now enter the following values:
  z Interval (µm) : 1
  Number of Sections : 40
  Current Section Pos : 1
  Refractive Correction : 1
- Click on ◉File in the Destination box.
  - The destination file: C:\SERIES\ is now additionally displayed.
- Now move the cursor to the parameter input box and enter your file name (up to 5 letters), e.g. "test" and press OK to confirm it.
  - During the scan that now takes place (40 images are scanned), the current message appears on the operator control monitor; the individual images can each be seen on the image monitor.

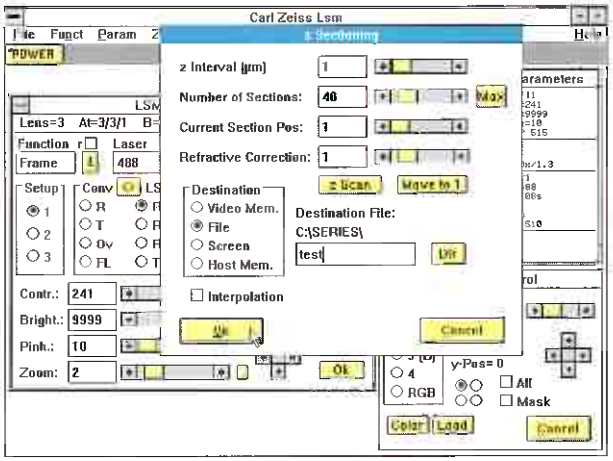


Figure 5-41

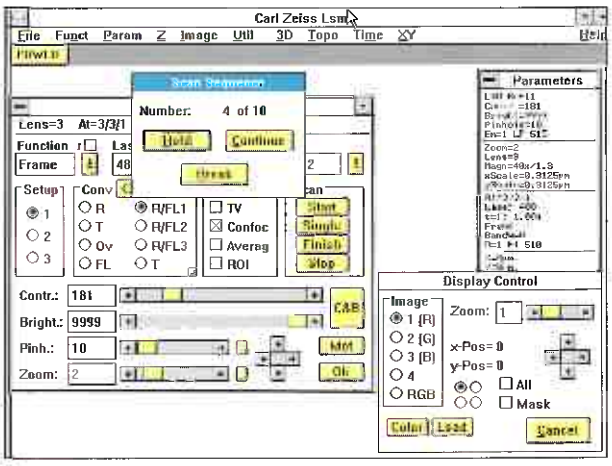


Figure 5-42

After the complete stack has been recorded, the focus returns to the idle position (i.e. to the position set **before** recording the stack).

(2) Retrieving single images from a sequence

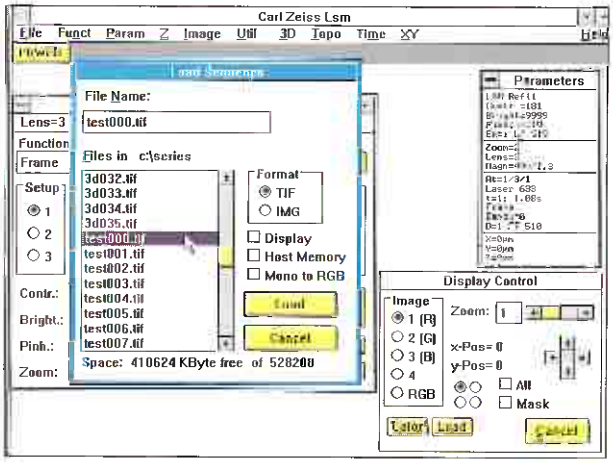


Figure 5-43

- In the main menu, select,
  File → Load Sequence...
  - The adjacent window appears.
- Now activate ☒Display.

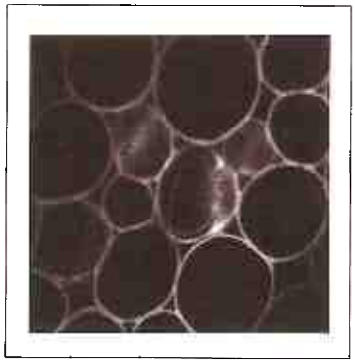


Figure 5-44

- By means of the scrollbar, now select the required single image and click on the image.
  - The selected image is now displayed on the image monitor.

(3) Loading sequences

- In the main menu, select,
  File → Load Sequence...
  - The adjacent window appears.
- Now activate ☒Display.
- Now select the required sequence with the scrollbar.

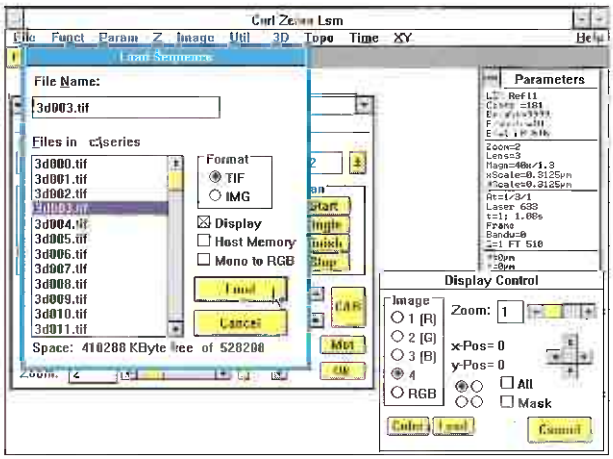


Figure 5-45

- Click on any image in the series, e.g. 3d003.tif.
  - The image is displayed on the image monitor.

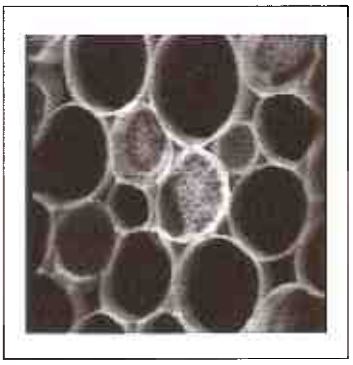


Figure 5-46

- Now confirm it by pressing the Load button or by double clicking with the mouse.
  - The complete image sequence is now loaded into the video memory and is acknowledged by a beep.

In the event that the video memory is too small for an image sequence, the data will be loaded automatically into the host memory; a message will appear if the host RAM is too small.

Loading can be observed because one image after the other is also displayed on the image monitor.

Once loading has been completed, the sequence stops and the image is shown on the image monitor.

Click on ☒ Host Memory if you want to load the selected sequence directly into main memory.

5.2.3 Image sequence applications

(1) Building up a gallery

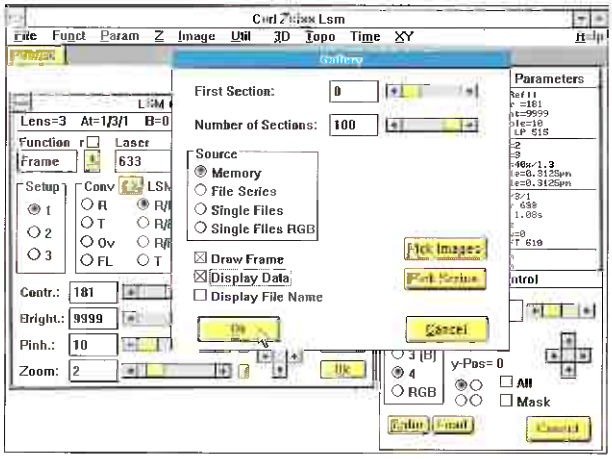


Figure 5-47

- Load a sequence into main memory as described in Section 5.2.2 (3).
- In the main menu, now select z → Gallery...
  - The adjacent window appears.
- Now enter the following data:
  Number of Sections: any number > actual number of images
  Source: ◉ Memory
  ☒ Draw Frame
  ☒ Display Data

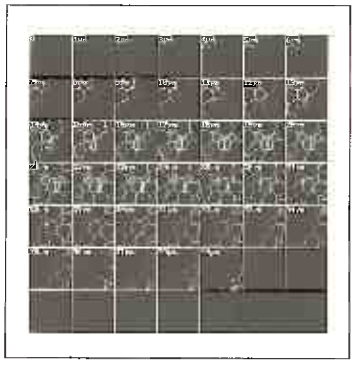


Figure 5-48

- Press Ok to confirm.
  - The series consisting of 36 images is displayed on the image monitor.

(2) Animation

- Load a sequence into main memory as described in Section 5.2.2 (3).

- In the main menu, now select:
  z → Animate...
  – The adjacent window appears.

- Now enter the following data:
  Duration: 1
  Current Slice: 0
  ○ Forward
  ◉ Backward
  ○ Reverse
  ☒ From Host
  ☒ Opt. Zoom
  Redu: 1
  Interv: 1

  Direction of the moving image sequence (any selection possible)

  Changes the image speed

- Now click on Start.
  – The image sequence runs.

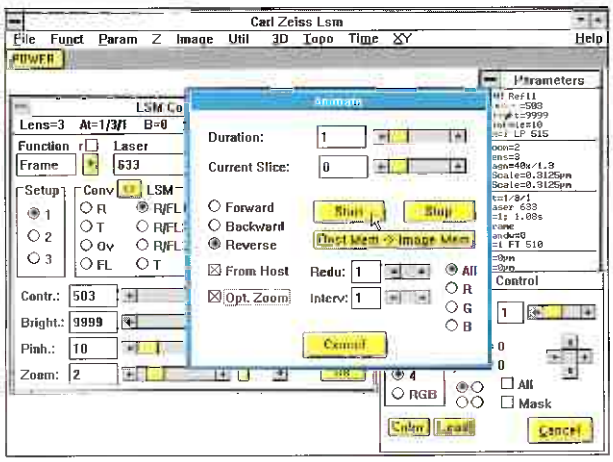


Figure 5-49

If you make a numeric entry in the Current Slice or click on the arrows with the mouse, you can also retrieve the images from memory one by one.

(3) 3D projection

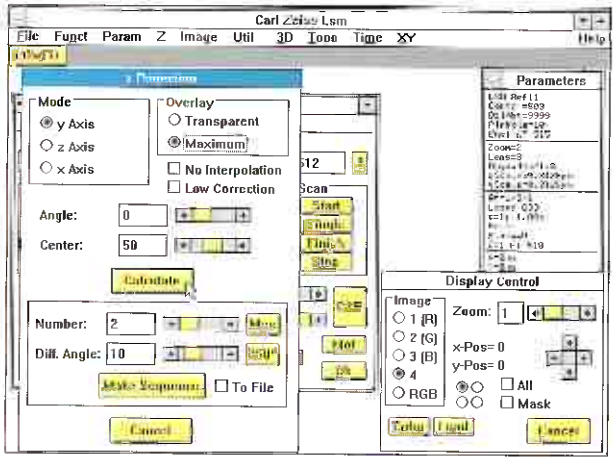


Figure 5-50

- Load a sequence into main memory as described in Section 5.2.2 (3).
- In the main menu, now select:
  3D → Projection...
  – The adjacent window appears.
- Now enter the following data:
  Mode: ◉ y Axis
  Overlay: ◉ Maximum
  ☐ No Interpolation
  ☐ Low Correction
  Angle: 0
  Center: 50

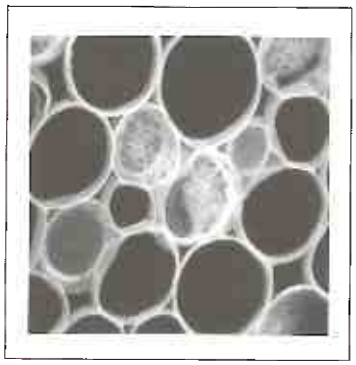


Figure 5-51

- Now confirm by pressing Calculate.
  – An image with a virtually infinite depth of focus is now generated on the image monitor.

(4) Colour height map

- Load a sequence into main memory as described in Section 5.2.2 (3).

- In the main menu, now select:
  3D → Depth Coding...
  - The adjacent window appears.

- Now enter the following data:
  Mode: ◉ Colored Scale
  (Colour scale display)
  Overlay: ◉ Maximum
  ☐ Rear View not activated
  Threshold: 5
  Intensity: 1,2
  Offset: 0

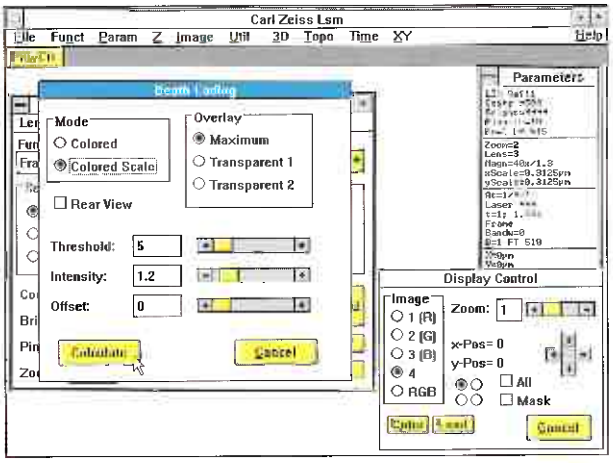


Figure 5-52

- Now confirm by pressing Calculate.
  - An image is now displayed on the image monitor in which the depth information is visible in a colour coded form. The colour scale is displayed in the top left corner of the image;
    Red ≙ front
    Blue ≙ rear

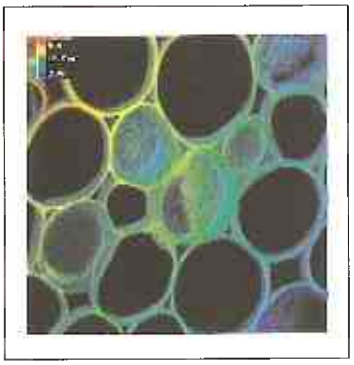


Figure 5-53

(5) 3D rotation

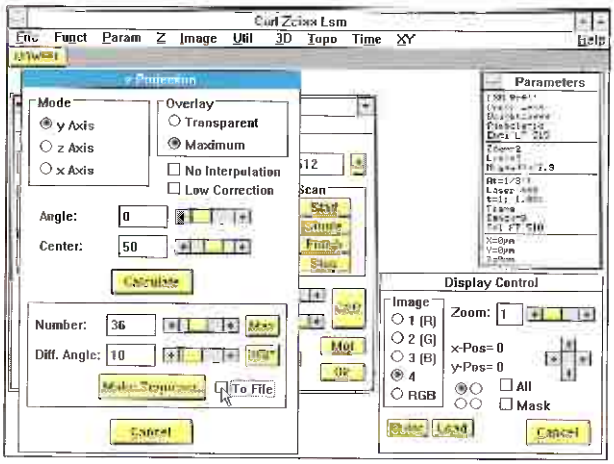


Figure 5-54

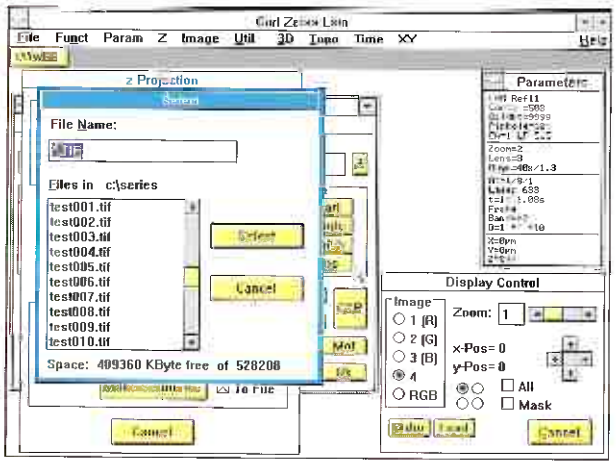


Figure 5-55

- Load a sequence into main memory as described in Section 5.2.2 (3).
- In the main menu, now select:
  3D → Projection...
  – The adjacent window appears.
- Now enter the following data:
  Mode: ◉ y Axis
  Overlay: ◉ Maximum
  ☐ No Interpolation
  ☐ Low Correction
  Angle: 0
  Center: 50
  Number: 36
  Diff. Angle: 10
- Now activate ☒ To File.
  – The adjacent window appears.
- In the File Name box, now enter the name for your rotation sequence: e.g. 3D.tif and press the Select button to confirm it.
  – The **z Projection** window appears again.
- Now confirm by pressing the Make Sequence button.
  – The sequence is now computed and saved to the hard disk.

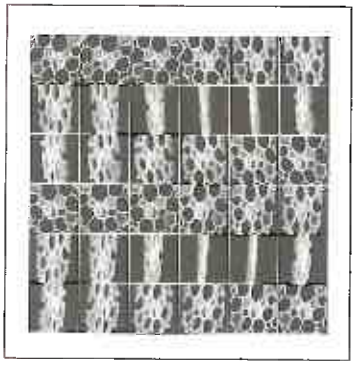


Figure 5-56

During recording of the sequence, a further small window is displayed to visualise the progress of computation.
Computation can be stopped at any time by pressing the Break button.

The 3D sequence can be called up by selecting the File → Load Sequence function and can be animated with z-Animate... or can be displayed as a sequence of images on the image monitor by selecting z-Gallery.

(6) Stereoscopy

- Load a sequence into main memory as described in Section 5.2.2 (3).

- In the main menu, now select:
  3D → Stereo Images.
  - The adjacent window appears.

- Now enter the following data:
  Mode: ◉ Red/Green Stereo Images
  Overlay: ◉ Maximum
  Diff. Angle: 2
  Basic Angle: 0

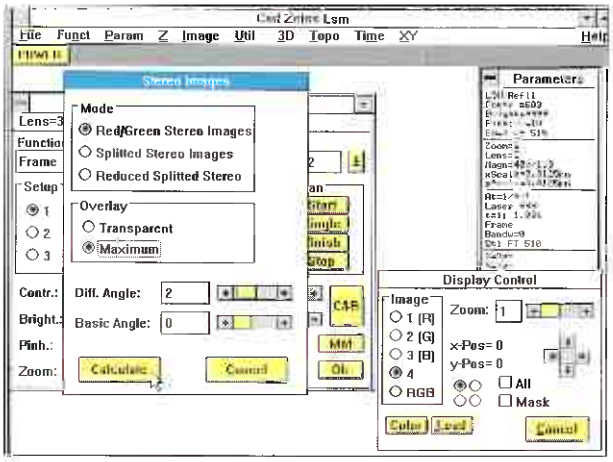


Figure 5-57

- Now confirm by pressing the Calculate button.
  - The image is built twice for the colours red and green and a stereoscopic image appears.

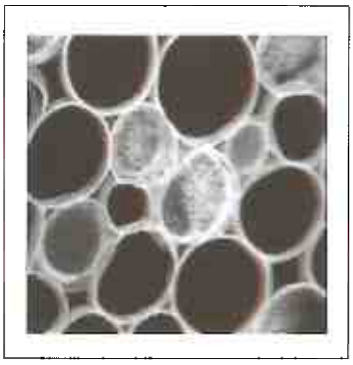


Figure 5-58

You can only see the stereoscopic image effect with red/green spectacles, however.

You can arrive at a modified display by selecting ◉ Reduced Splitted Stereo in the Mode field. No red/green spectacles are required in this case.

Figure 5-59

(7) Orthogonal sections

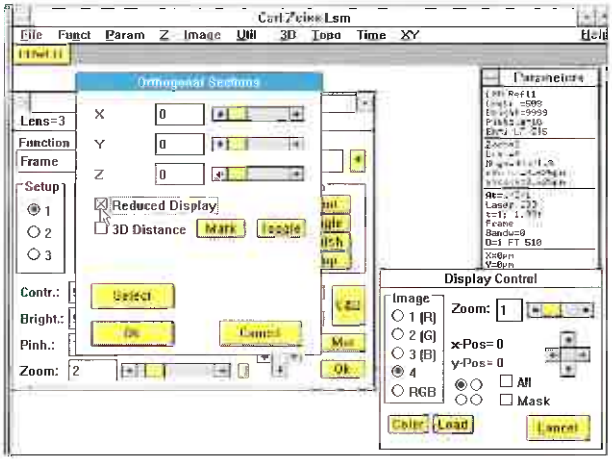


Figure 5-60

- Load a sequence into main memory as described in Section 5.2.2 (3).
- In the main menu, now select:
  3D → Orthogonal Sections.
  – The adjacent window appears.
- If necessary, activate ☒Reduced Display if the image is too large for the monitor.

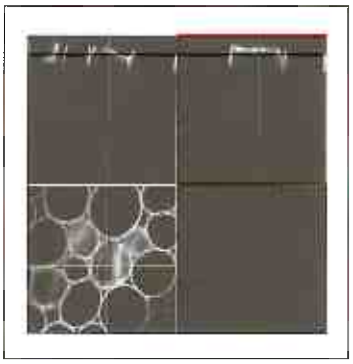


Figure 5-61

Three dimensional movement in the specimen is now possible by modifying the X, Y and Z parameters in the **Orthogonal Sections** dialog window.
Movement within the specimen is depicted by coloured lines within the section planes
xy plane (blue)
xz plane (green)
yz plane (red).

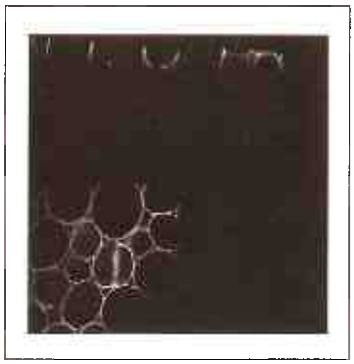


Figure 5-62

Please note that the values for x and y can be adjusted from 0 to 511.
Only values from 0 to 39 can be entered for z (in this example, the section stack embraces the single images from test000.tif to test039.tif).

If you click the Select button in the above example, the mouse pointer switches to the image monitor and you can directly modify the section plane by clicking the plane lines.

The following sketch showing three section planes in the specimen will elucidate the whole subject matter for you.

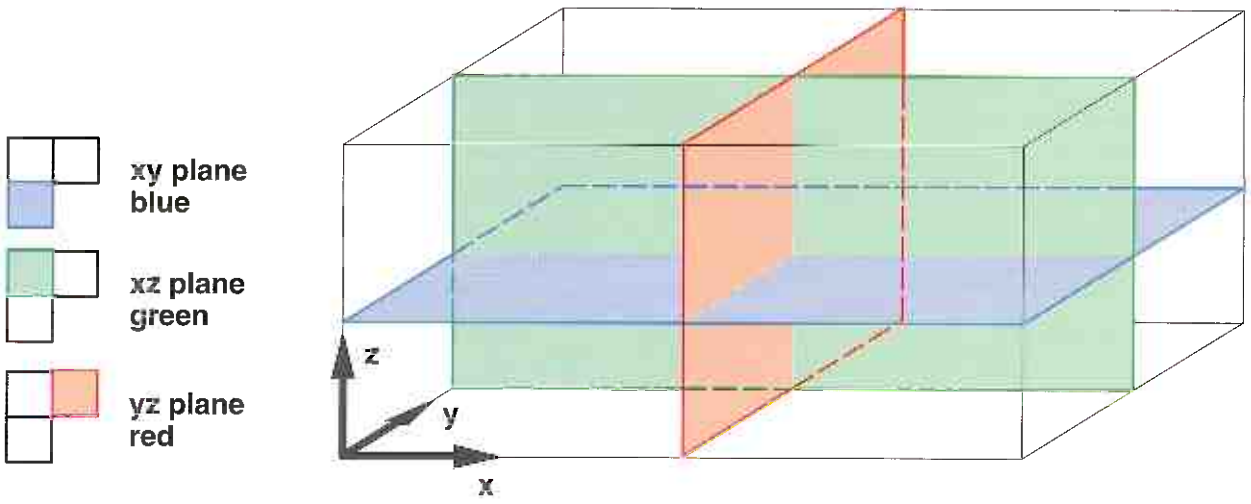


Figure 5-63

Length measurements of 3D diagonals are possible after activating ☒3D Distance.

In doing so, the length of the yellow measuring line is displayed directly in µm on the image monitor.

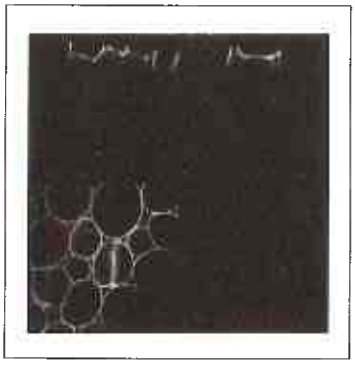


Figure 5-64

(8) Slanted sections

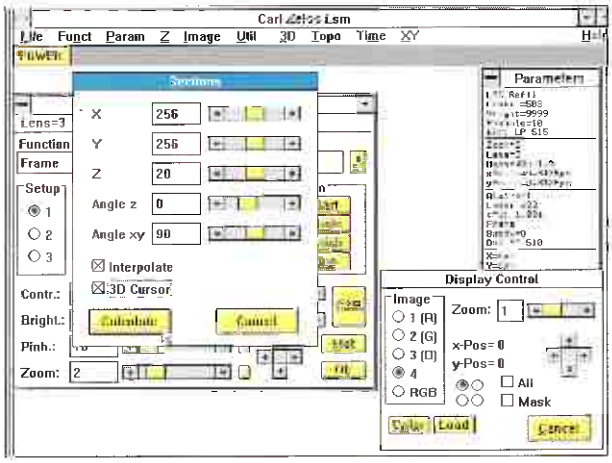


Figure 5-65

- Load a sequence into main memory as described in Section 5.2.2 (3).
- In the main menu, now select:
  3D → Sections...
  – The adjacent window appears.
- Activate
  ☒ Interpolate
  ☒ 3D Cursor
  and press the Calculate button to confirm.
  – The display with the 3D cursor shown now appears on the image monitor (Figure 5-66).

Figure 5-66

Figure 5-67

By modifying the values for Angle z (rotation angle of the section area about the z axis) and Angle xy (tilt angle of the section area), you can therefore position a section area with any inclination from any chosen 3D point.

5.2.4 Time functions

The time functions enable kinetic measurements, primarily to record concentration changes that are visualised via a fluorescence sample, e.g. calcium concentration (Fluo-3) or pH with SNARF etc.

These time-dependent processes can be recorded on

- complete images with Time Series,
- single lines with Time Scan,
- single points with Time Spot or with
- Online ROI measurements.

(1) Time series

Prerequisites

- In the Control Panel, carry out the same basic settings as described in Section 5.2.1.1 (1)b) and generate a single-channel confocal fluorescence image.
- Optimise the image and carry out pinhole adjustment as described in Section 5.2 (3) and (4).
  - A confocal LSM image appears on the image monitor.
- Now reduce the image by changing the entry in the Size box of the Control Panel from 512x512 to 512x100.
  - The adjacent image appears on the image monitor.

Figure 5-68

- The actual scan time for recording an image is the result of:
  - the number of lines,
  - the standard scan time (in the example, t = 1s) and
  - the number of averaging runs (average factor).

This time is displayed in the right column of the sub-menu.

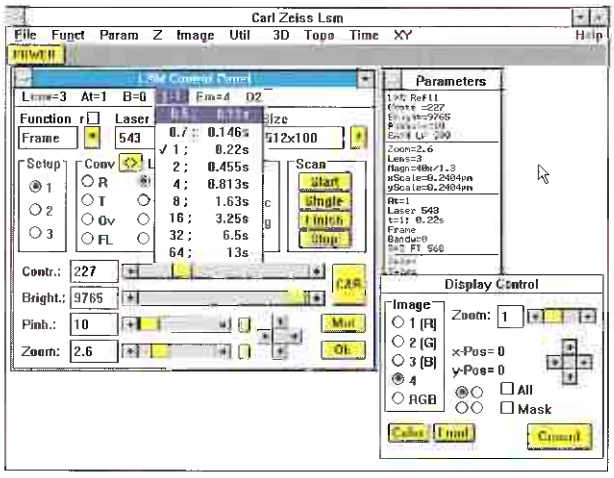


Figure 5-69

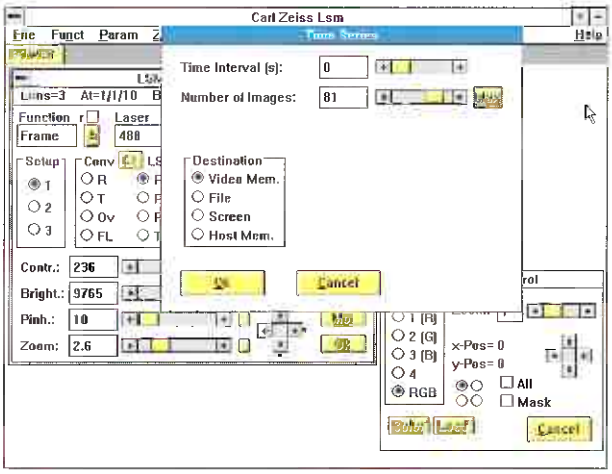


Figure 5-70

- In the main menu, now select:
  Time → Time Series....
  – The adjacent window appears.
- Enter the following data:
  Timer Intervall: 0 (fastest possible time)
  Number of Images: 10
  Destination: ◉ Video Mem
  If you click the Max box, the maximum number of images is computed automatically.
- Now confirm your entries by pressing Ok.
  – The time series is recorded and acknowledged with a beep.

If you want to try out this application with your demo specimen, then change the focus manually during recording. In this way, you can simulate a specimen that changes in time.

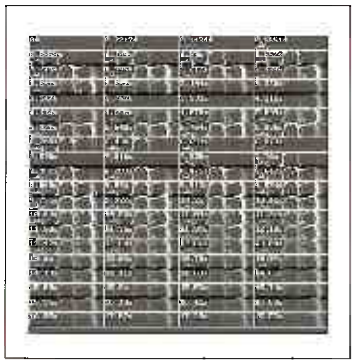


Figure 5-71

- Back up your time series stored in video memory by selecting
  File → Store Sequence.
- In the main menu, now select:
  Time → Gallery...
  – The adjacent image, in which the time-series images are listed, appears.

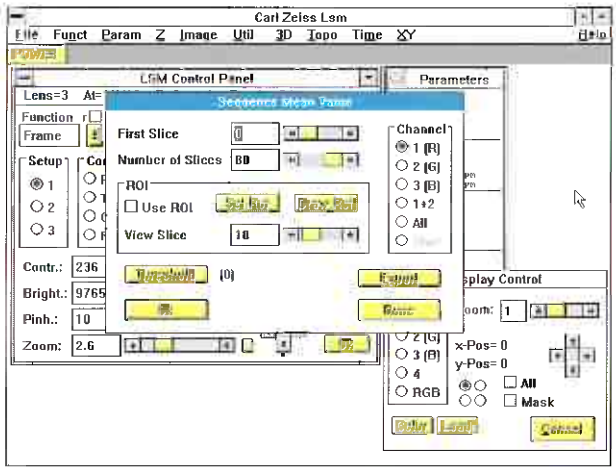


Figure 5-72

- Instead of Time → Gallery..., select the following in the main menu:
  Time → I(t) Diagram: Mean of ROI...
  – The **Sequence Mean Value** window appears.

– A diagram that shows the intensity as a function of time (i.e. the intensity is averaged over the entire image in each case) appears simultaneously on the image monitor.

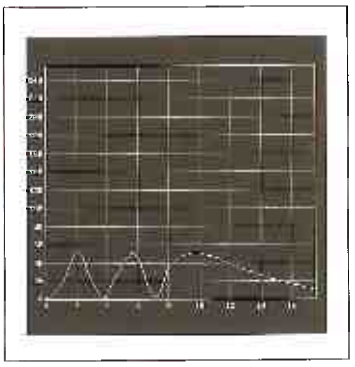


Figure 5-73

- If you are only interested in one specific area, then activate Draw ROI in the **Sequence Mean Value** window.
  – The **Area Measure** window appears.

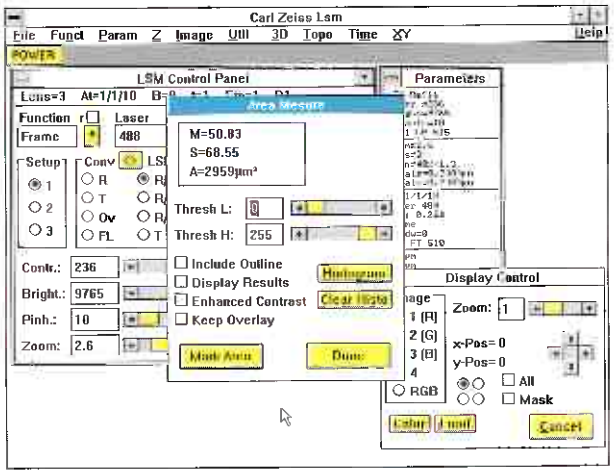


Figure 5-74

- Click on the Mark Area box.
  – The following instruction appears on the operator control monitor:

Instruction
Use the left mouse button to draw an outline. Then click with the left mouse button into the area. Pressing the right mouse button will abort 'Mark Area' and the whole area will be selected.

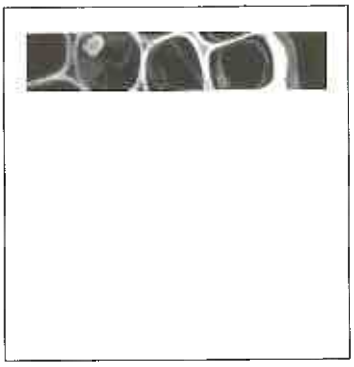


Figure 5-75

  – The cursor changes to the image monitor.

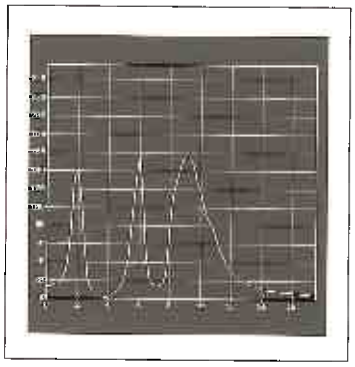


Figure 5-76

- As instructed above, surround the field you are interested in with the cursor and then click in the field with the left mouse button.
  - The cursor now moves back to the operator control monitor.
- Now click on Done.
  - The I (t) diagram of the small field you have selected appears.

If you wish to further process the I (t) information from the current diagram, you can save this data as an ASCII data file in the form of a table by clicking the Export button.

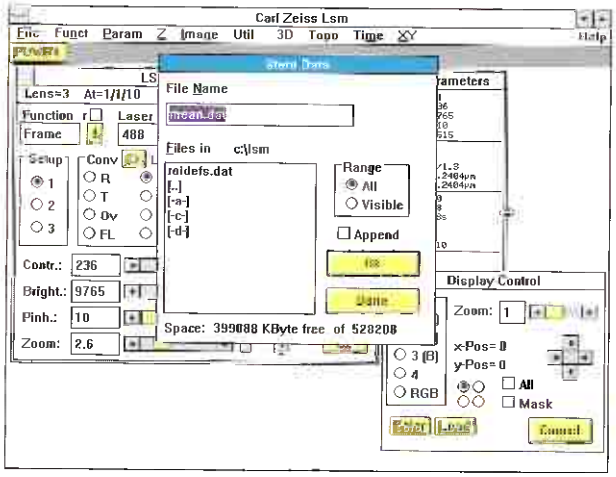


Figure 5-77

- Click on the Export box.
  - The **Store Data** window appears.
- In the File Name box, now enter the name you wish to use for the file and add the **.dat** extension.
- Select Ok to confirm your entry.
  - Your saved file can now be processed further in a different program.

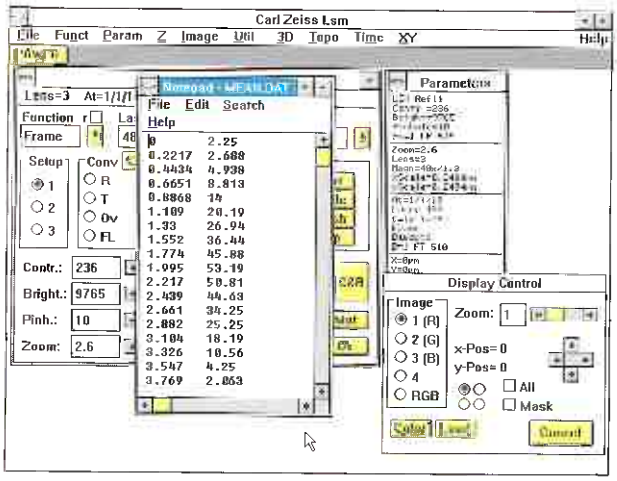


Figure 5-78

The data file you have saved can be displayed in the WINDOWS editor (Notepad).

(2) Time scan (intensity along a line as a function of time)

Prerequisites

- Make the same basic settings in the Control Panel as described in Section 5.2.1.1 (1)b) and then generate a one-channel confocal fluorescence image.
- Optimise the image and carry out pinhole adjustment as described in Section 5.2 (3) and (4).
  - A confocal LSM image appears on the image monitor.
- Now select the following in the main menu:
  Time → Time Scan...
  - The **Time Scan** window appears on the operator control monitor.
- Select Number of Columns: 3,
  i.e. 3x512 lines are scanned.
- Click on Set Cut.
  - The cursor changes to the image monitor.
  - The line displayed on the image monitor can now be clicked with the cursor there and moved to **any position**.
- Now click on Start.
  - The intensities along the cursor line are recorded.
  - The end of recording is acknowledged with a beep.

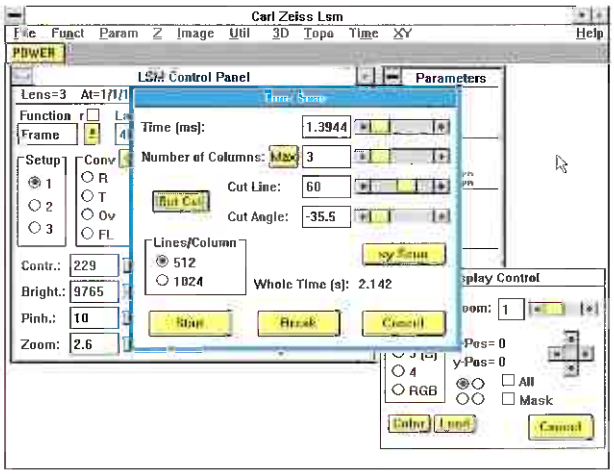


Figure 5-79

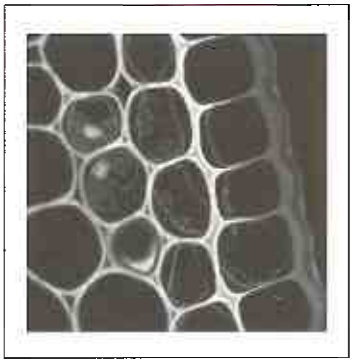


Figure 5-80

You can optionally display the recorded data with the Gallery function or the I (t) diagram.

- Select the following in the main menu:
  Time → Gallery...
  - The individual time line scans (3 of them have been selected above) are now displayed on the image monitor as a gallery.

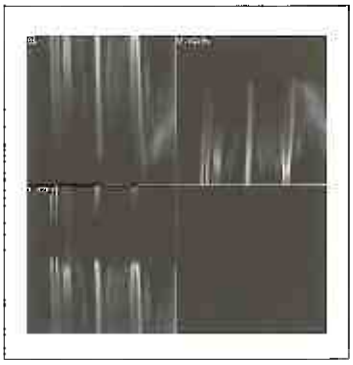


Figure 5-81

You can obtain a recording of the I (t) diagram in the same way as described in Section (1).

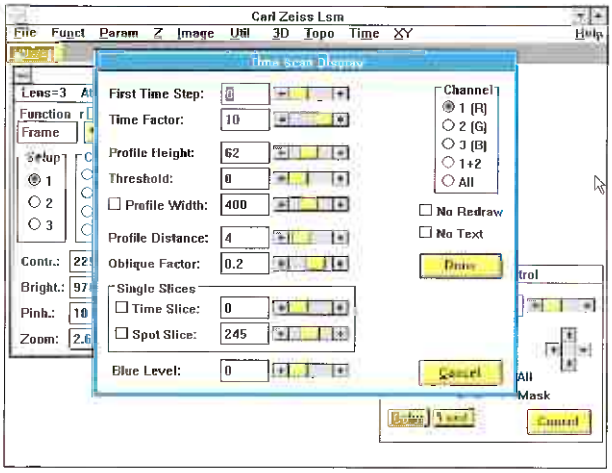


Figure 5-82

- Select the following in the main menu:
  Time → Display Time Scan...
  - The **Time Scan Display** window appears on the operator control monitor. In this window you can modify the intensity profile of your image with the various parameters.

Figure 5-83

- Here, the three-dimensional elevation represents the intensity of the time line scan.

The **Average Time Scan** function can be used to reduce noise or for data reduction. This involves a loss of time resolution, however.

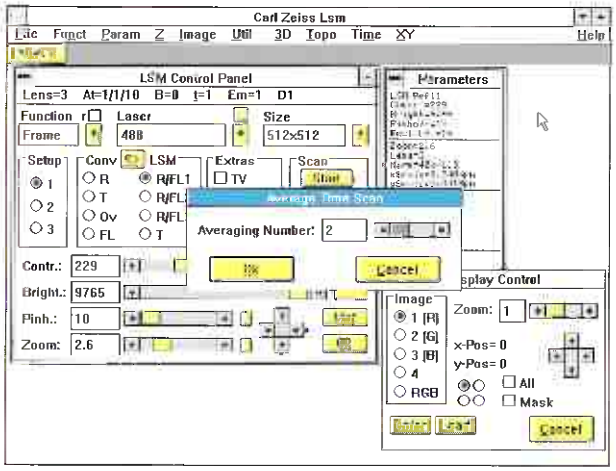


Figure 5-84

- Select the following in the main menu:
  Time → Average Time Scan...
  - The **Average Time Scan** window appears.
- Enter the number "2", for example, in the Averaging Number box.
  - In this way, two lines are each combined in **one** line.
- Select Ok to confirm.

The difference can be made clear with the I (t) function.

Recording the I (t) function without data reduction.

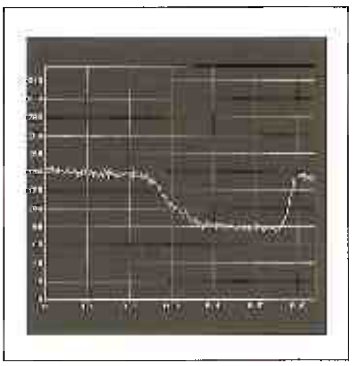


Figure 5-85

Recording the I (t) function with data reduction.

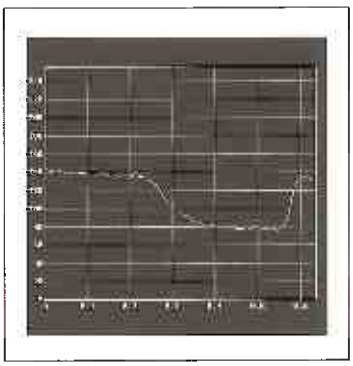


Figure 5-86

(3) Time Spot (intensity of one single image spot as a function of time)

Prerequisites

- Make the same basic settings in the Control Panel as described in Section 5.2.1.1 (1)b) and then generate a single-channel confocal fluorescence image.
- Optimise the image and carry out pinhole adjustment as described in Section 5.2 (3) and (4).

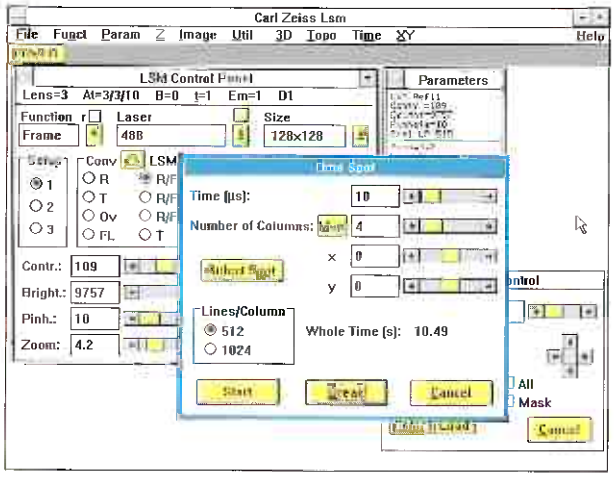


Figure 5-87

– A confocal LSM image appears on the image monitor.

- Now select the following in the main menu: Time → Time Spot...

– The **Time Spot** window appears on the operator control monitor.

- Enter the following values:
  Time: 10
  (6,4 µs is the fastest possible time)
  Lines/Column: ◉ 512
  (i.e. 512 lines are selected)

Regardless of the setting in the Size box in the Control Panel, each line contains 512 pixels.

Number of Colums: 4 (default)

The multiplication operation 512x512x4 = 1048576 produces the number of data points and, on the basis of this and with the chosen time factor of 10 µs, results in a whole time of 10.49 s for the complete data acquisition process.

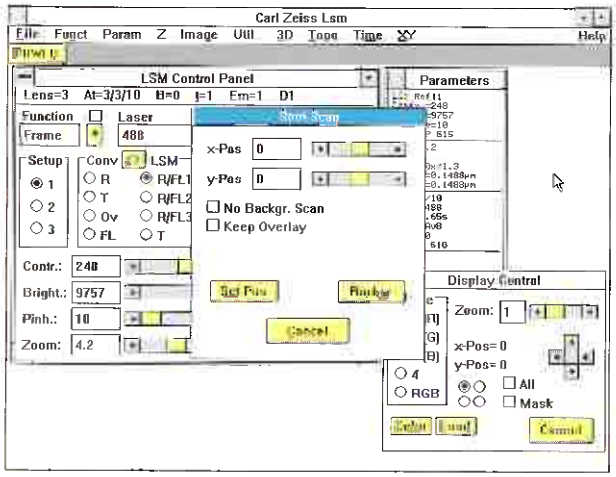


Figure 5-88

- Click on Select Spot.

– The **Spot Scan** window appears.

– A green cross, whose coordinates are defined as zero in the x/y direction, appears in the middle of the image monitor.

- Click on Set Pos.

– The cursor switches to the image monitor.

- Now click on the green cross and move it to required position in the image.
  e. g. x : 220
  y : 40
  - All coordinates of the point you have moved to are automatically transferred to the Spot Scan window.

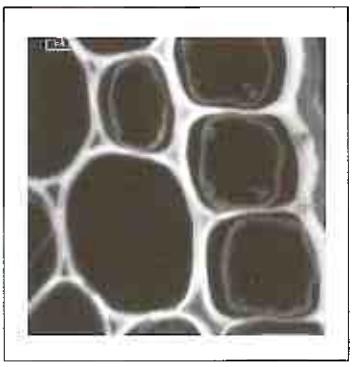


Figure 5-89

- Click the right mouse button.
  - The cursor returns to the Spot Scan window.

- Now select the following again in the main menu:
  Time → Time Spot...
  and click on Start.
  - The time change of the brightness at the selected point is recorded like a book text; the end of data recording is acknowledged by a beep.

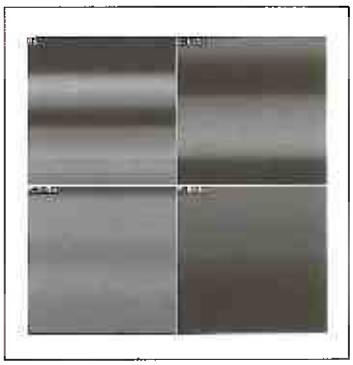


Figure 5-90

- Now select the following in the main menu:
  Time → Gallery
  and select Ok to confirm.
  - The four columns are now shown on the image monitor.

- Select the following in the main menu:
  Time → I(t) Diagram: Spot...
  - The **Spot Sequence Mean Value** window appears on the operator control monitor and the I (t) Diagram: Spot appears on the image monitor.

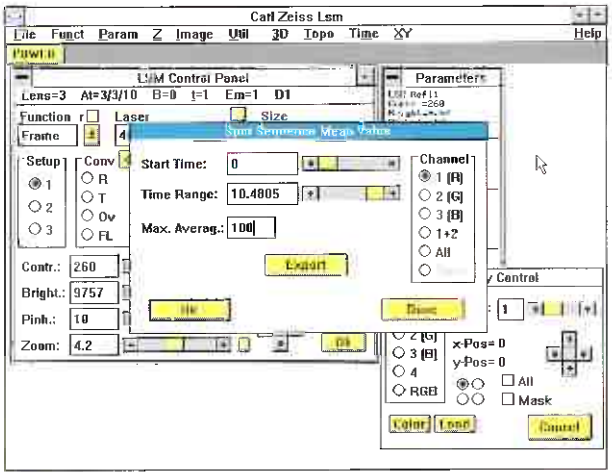


Figure 5-91

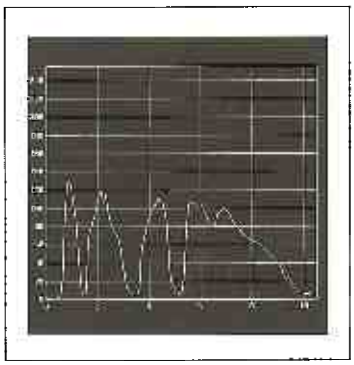


Figure 5-92

(4) Online ROI measurements

Prerequisites

- Make the same basic settings in the Control Panel as described in Section 5.2.1.1 (1)b) and then generate a single-channel confocal fluorescence image.
- Optimise the image and carry out pinhole adjustment as described in Section 5.2 (3) and (4).

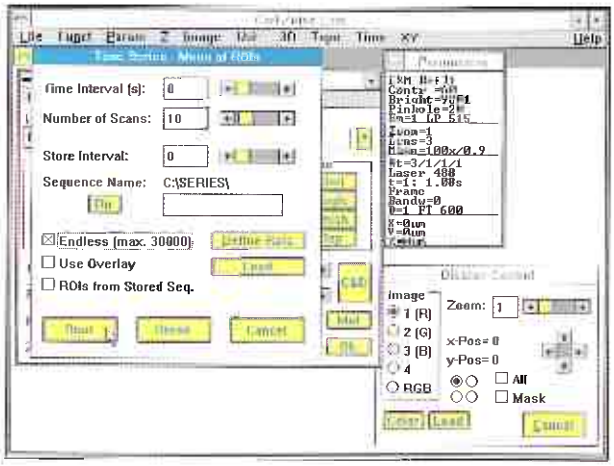


Figure 5-93

- A confocal LSM image appears on the image monitor.

- Now select the following in the main menu: Time → Scan Mean of ROIs...
  - The **Time Series – Mean of ROIs** window appears on the operator control monitor.

- Enter the following values:
  Time Interval: 0
  Number of Scans: 10
  Store Interval: 0
  ☒ Endless: activated

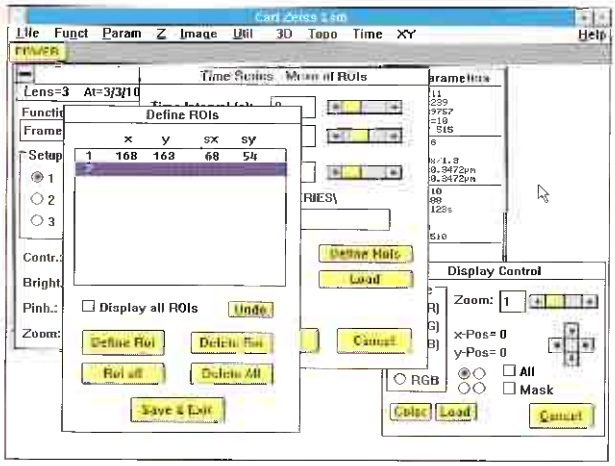


Figure 5-94

- Now click on Define Rois.
  - The **Define ROIs** window appears on the operator control monitor.

- Click on the Define Roi box.
  - The first ROI appears on the image monitor. You can modify it and position it with the cursor to suit your requirements.
  - If ☒ Display all ROIs is activated, all ROIs are displayed as defined by the user.

Figure 5-95

- Quit the Define ROIs window by clicking Safe & Exit.
  - The displayed ROIs disappear from the image monitor.

- In the **Time Series - Mean of ROIs** window, now click on Start.
  - Data recording begins with the set parameters.

If you attempt to implement the example given, you should focus to and fro with the specimen during data recording to ascertain a change in the example.

As you have activated ☒Endless beforehand, you should now stop data recording by selecting Break .

- Click on the Break box.
  - Data recording stops and the **Display Mean Values** window is displayed on the operator control monitor.

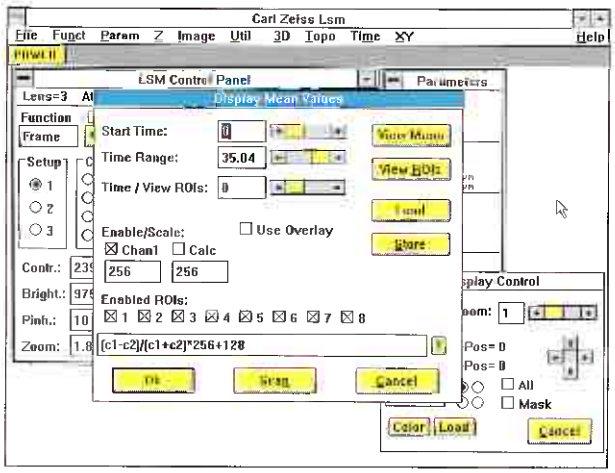


Figure 5-96

  - The adjacent image appears on the image monitor at the same time.

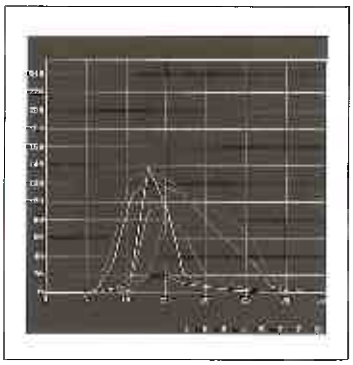


Figure 5-97

All time functions can be used on one, two or three channels without having any negative impact on the time resolution.
The same applies to use of the ratio function when simultaneously recording two channels.

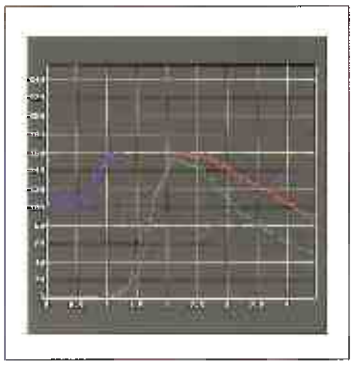


Figure 5-98

Result of a Time Scan measurement

Parameters used:

- 2-channel recording
- With online ratio
- 1.4 ms time resolution

Display:

- blue ≙ Ratio
- red ≙ Channel 1
- green ≙ Channel 2

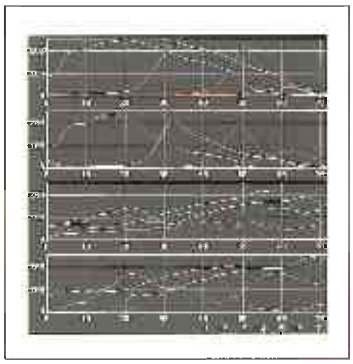


Figure 5-99

Result of a Scan Mean of ROIs measurement

Parameters used:

- 2-channel recording
- With online ratio
- 7 ROIs additionally defined (but only 4 displayed)

Display (from top to bottom):

- chan 1
- chan 2
- Ratio
- Arith
  Arithmetic operation performed as in the Display Mean Values window:

$$\frac{C1-C2}{C1+C2} \times 256 + 90$$

CONTENTS

6 SOFTWARE DESCRIPTION

6.1 General

The software of the 410 invert laser scan microscope consists of

- basic program (version 3.80) and
- options.

It is controlled by the DOS operating system under the WINDOWS graphical user interface.

The basic program contains the autostart, system control and operating system functions and the WINDOWS graphical user interface.
The programs are stored on the computer's hard disk, but are additionally supplied on 3 1/2" diskettes. Therefore, you are also able to install the LSM program on a different computer (see Section 9.1).

Optionally, the following add-ons are also available:

- 3D reconstruction (480088 8040) for creating 3D images from series of sections
- Time series (480088 8041) for recording time series
- Scanning table xy (480088 8042) for control of a motor-driven scanning table
- Surface topography (480088 8010) for displaying surface topographies
- PC networking

If these options are purchased later, they must be copied into the c:\LSM directory on the hard disk.

6.2 Introduction to the WINDOWS graphical user interface

The LSM software is based on the MICROSOFT WINDOWS™ graphical user interface. Using WINDOWS, several application programs can be run simultaneously and data can be transferred from one application program to another. With WINDOWS, created files can be sorted and managed intelligibly and it is possible to switch from one program to another without having to terminate the program just used.
All program commands are organised in menus, i.e. each application has its own menu.

For in-depth information on WINDOWS, please refer to the original MICROSOFT WINDOWS™ software manual.

A few functions essential to working with the LSM software are explained below.

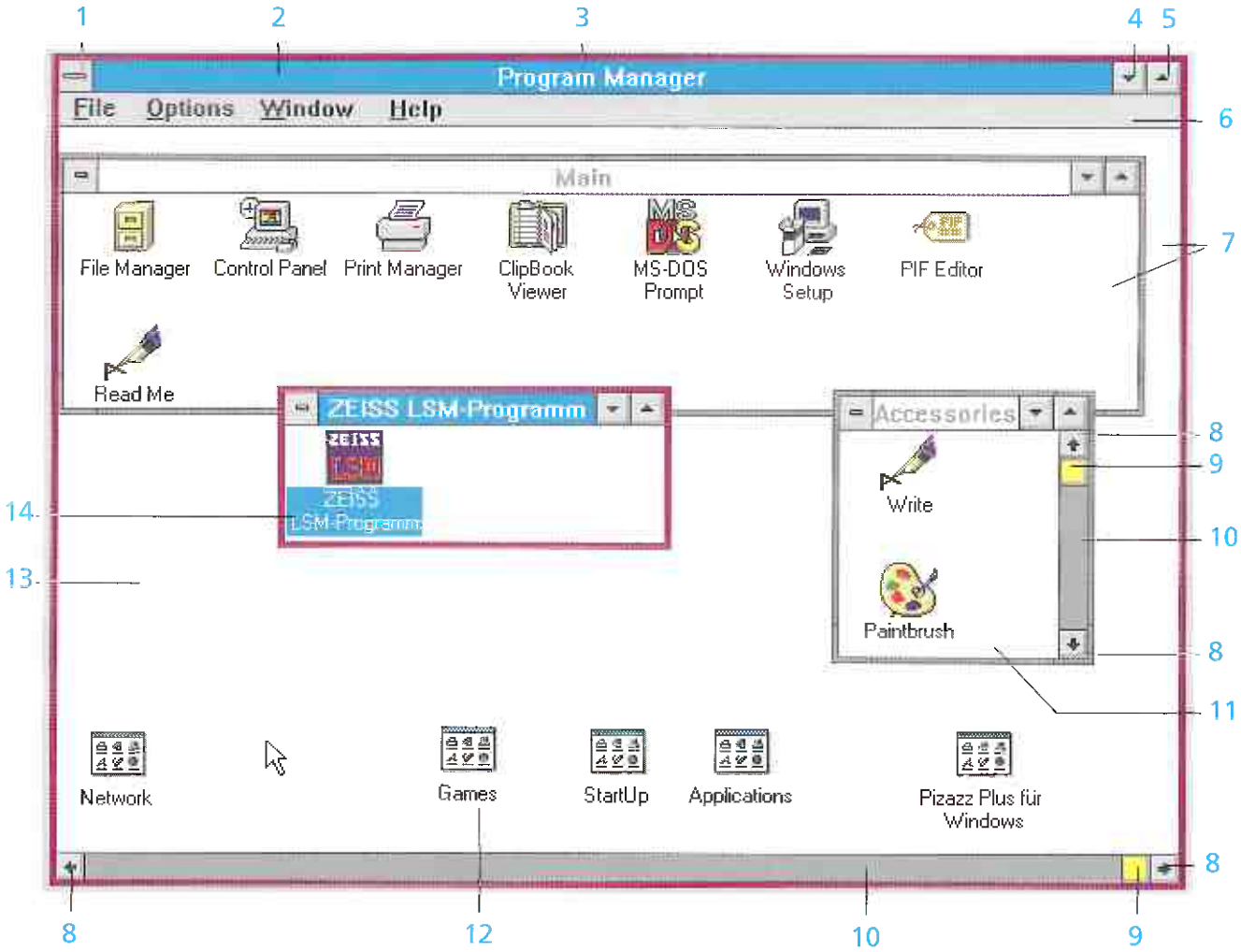


Figure 6-1

Each application program can be opened as a separate window. All windows have a few common basic elements, but not all elements can be found in every window. Using the mouse, you can move windows by simply clicking and dragging them, you can reduce them to icon size and you can enlarge them to full size, or can close windows and change their size. These functions can also be executed with the keyboard in the system menu; refer to the MICROSOFT WINDOWS™ manual.

1 In the **System Menu Box**, you can call up the < Close > and < Switch To... > functions.

2 When several windows are open, the **title bar of the active window** has a colour different to the colour of the inactive windows.

3 For each application window, the **program title** contains the name of the application program and the document. In a document window, it contains the name of a program group, a directory and the data file.

4 You can reduce an application window to icon size by pressing the **Icon button**.

5 You can enlarge an application window to screen size by pressing the **Full Screen Box** button.

6 The **menu bar** displays the available menus in an application.

7 The **window frames** of the active and inactive windows have different colours.

8 The **vertical and horizontal scroll arrows** move the contents of a window to enable you to see invisible portions of the window.

9 You can scroll the contents of a window even faster by means of the **scroll box**.

10 The scroll bar shows the direction in which the contents of a window can be moved; in doing so, a window may only contain one vertical, only one horizontal or both scroll bars. You can scroll the contents of a window at high speed by clicking on the scroll bar.

11 Programs are started by double-clicking the **program icon**.

12 You open the group window (14) by double-clicking on the **group icon**.

13 The texts and displays of an application are edited in the **workspace**.

14 **Group window**, opened with the activated program.

Mouse functions

WINDOWS can be operated with the mouse or on the keyboard. Combined use of the two is always advantageous.
The mouse should be moved on a flat surface (e.g. a mouse pad).

Click	Activate/deactivate/select an application or a box by pressing the mouse button briefly.
Double click	The mouse button must be pressed twice in fast succession for various applications.
Drag	Move a graphical element on the screen by clicking and simultaneously moving the mouse while keeping the mouse button pressed.

In addition to the above-mentioned mouse functions, a few special functions are realised in the ZEISS LSM program in conjunction with the scroll bar (see Section 6.2.4).

6.2.1 Working with menus

WINDOWS commands are listed in menus. From an open menu, you select a command.

Selecting a menu

In the menu bar, point with the mouse pointer to the name of the menu you require and click it to open the menu.

Leaving a menu

Click on the menu title or click anywhere outside the menu.

Selecting menu commands

Click on the name of the element you require. The element is highlighted.

Menu conventions

- Omission points (...) signify that a dialog box appears after selection of the command and that you must enter further information in the box.
- A tick mark (√) before the name means that the command is active.

6.2.2 Working with dialog boxes

WINDOWS prompts additional information for the application program in dialog boxes. Dialog boxes are also used to display warnings or to explain why a requested task could not be executed.

Entries

A few dialog boxes contain text boxes for entry of information such as a file name or a word you would like to find in a document.

❍ Entering a text or date
Move to the text box with the mouse pointer and enter the text or date, e.g. 11.05.94.

❍ Selecting data in the slider box (see also Section 6.2.4)
While pressing down the left button, drag the mouse pointer inside the slider box and release the button when the data you require appears.
If a parameter cannot be set to the required value with the slider, you must enter it on the keyboard.

- Marking a text box
  With the left mouse button, click on the position before the first character you wish to mark and, while keeping the mouse button pressed, drag the mouse over the selected text. End marking by releasing the left mouse button at the corresponding position in the text box. To quickly mark whole portions of text, click the left margin area of the first line and drag the mouse down. Mark individual words by double-clicking them.

- Selecting a specific element in a list box
  - Click on the scroll arrows at the side until the required element appears in the list box.
  - Click on the selected element.

  Some windows and dialog boxes have one scroll bar and two arrows with which you can display further texts.

- Selection by means of the scroll bar
  Point to the corresponding scroll arrow and keep the mouse button pressed until the required data is displayed. Alternatively, drag the scroll box in the scroll bar up or down until the required text appears.

Concluding entries

Once you have made all entries, select the "OK" command button. If you wish to cancel your work, click on the "Cancel" button or double-click on the system menu box.

6.2.3 Working with windows

Moving windows and icons

You can move windows and icons to various positions on the operating monitor:
Drag the icon or the title bar of the window to the new position. Release the mouse button once you have moved the window or the icon to the required position. If you wish to cancel the operation, you can press the ESC key at any time before releasing the mouse button.

Changing the shape and size of a window

Select the window whose size you wish to change. Point to one side of its frame or one corner that you would like to move. The pointer changes to a double arrow. Drag the frame side or the corner until the window has reached the required size. If you drag one frame side, the size of the window only changes on the side where you drag it. If you drag a corner, the size of the two adjacent sides changes. The chosen size and shape are outlined. Release the mouse button once you have changed the window to suit your requirements.

- Reducing a window to icon size
  Select the window you wish to reduce and click on the icon button with the downward-pointing triangle in the top right corner of the window.

- Enlarging a window to maximum size
  Select the window you wish to enlarge and click on the icon button with the upward-pointing triangle in the top right-hand corner of the window.

- Restoring a window from icon size
  Double click with the mouse button on the corresponding icon (e.g. ZEISS LSM) on the bottom edge of the window.

- Restoring an enlarged window to its previous size
  Click on the full screen/restore button for a file window with the two upward and downward-pointing triangles in the top right-hand corner.

6.2.4 Special features of the scroll bars in the ZEISS LSM program

With regard to setting a value with the slider, pay attention to the fact that sliders in the LSM program have an extended scope of functions that are normally not offered in other WINDOWS applications:

Rough setting

If you press on the left mouse button while the mouse arrow is in the slider box, the slider moves immediately to this position. It then follows all horizontal movements of the mouse until the mouse button is released again, even if the mouse pointer is no longer on the slider. In comparison with the usual behaviour under Windows, the advantage of this is that you do not have to constantly keep an eye on the mouse pointer.

Fine setting

If you press the right mouse button while the mouse arrow is in the slider box, you are working in the fine setting mode. In this case, the mouse pointer disappears and the slider reacts directly to movement of the mouse. The slider's position can be adjusted exactly in this way.
When you release the right mouse button, the mouse pointer appears in the slider box again. You complete the fine setting by double-clicking with the right mouse button, in which case the fine setting mode remains active until you press a mouse button again.
Fine setting with the right mouse button is also possible when two sliders have been combined in one cross-shaped control. In this case, two dimensions can be set simultaneously by means of horizontal and vertical movement.

Rough/fine setting

You can switch rapidly from rough to fine setting by pressing the right button briefly while keeping the left mouse button pressed.

6.3 Description of the ZEISS LSM program

6.3.1 Basic menu

After you switch on the computer, the DOS operating system, the WINDOWS graphical user interface and the LSM program are loaded and started automatically. The control monitor will highly likely show the following display which, however, depends on your start-up settings.

1 The **main menu** contains the various application programs and options. Their detailed functions are described below in the order in which they appear in the menu.

2 All LSM settings are entered in the **Control Panel**. This window can be cancelled or reduced to icon size.

3 The **Parameter window** contains the current settings; it can be cancelled.

4 Video memory and monitor display settings are entered in the **Display Control window**.

5 Macro command sequences can be defined/edited in the **Macro window**. If you do not work with this window, you are advised to cancel it.

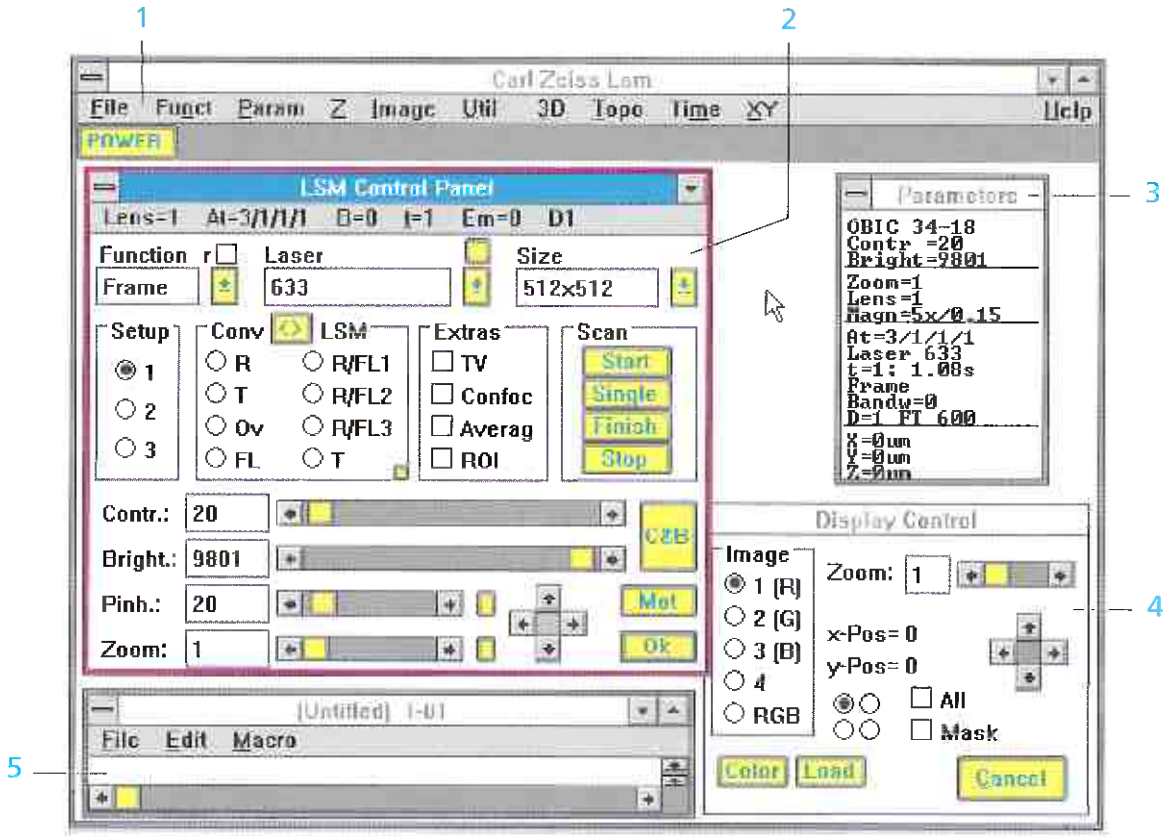


Figure 6-2

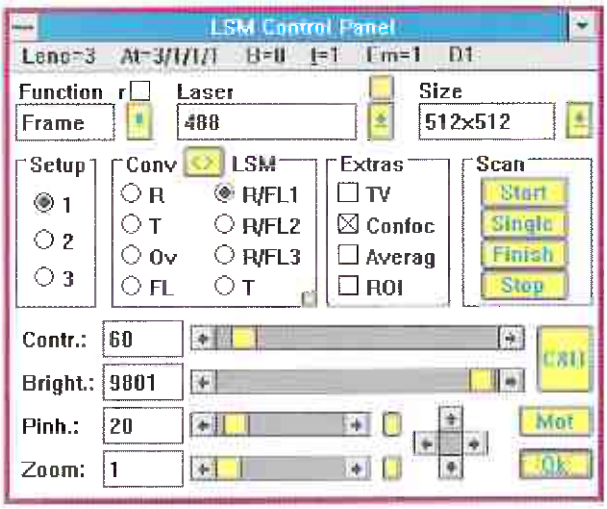


Figure 6-3

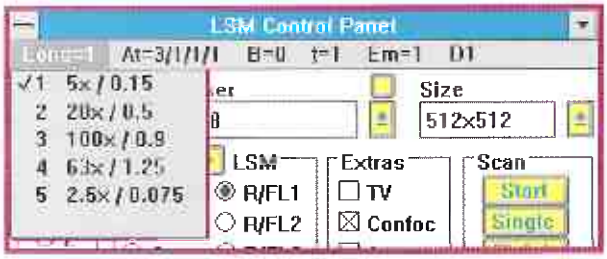


Figure 6-4

Figure 6-5

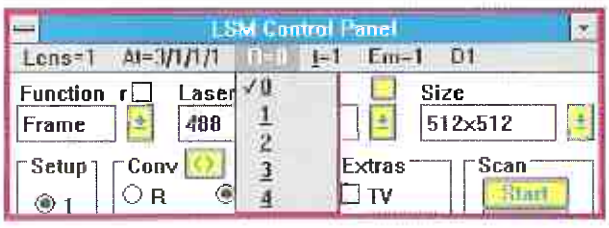


Figure 6-6

6.3.2 Control Panel

The LSM 410 invert laser scan microscope is controlled directly via the Control Panel. The Control Panel can be operated at any time provided a function that can currently not be cancelled is not running.
If the window is partly covered up by another window, click on any position in the panel to fetch it to the foreground.

Menus containing the allowed values are opened by clicking the entries in the menu line. You select the corresponding value by clicking one of these menu items. The selected value is then transferred to the menu line.

Lens
Position of the lens turret within the range from 1 – 5. When the menu is open, the affiliated magnification and aperture values are also displayed.
The turret configuration itself is entered in the main menu → Param/Objectives.

At
Optical attenuation filter for the laser output.
1 ≙ no attenuation
1000 ≙ attenuation to 1/1000 of the normal output.

B
Electronic bandwidth limiting by a low-pass filter.
0 ≙ minimum limiting
4 ≙ maximum limiting
(adapted bandwidth for the set t and Size parameters)

t
Scan time of one 512 x 512 image
Adjustment range:
In increments from 0.5 to 64 s (first column; in the second column you will find the time actually needed for each image, which also depends on the number of lines (size: pixels x lines) and on Average.

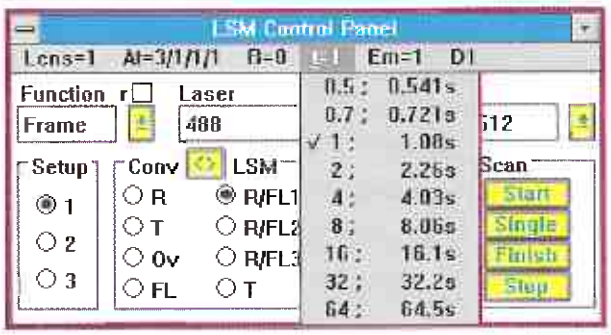


Figure 6-7

Em
Selection of the emission filters
(see Section 3.1.3)

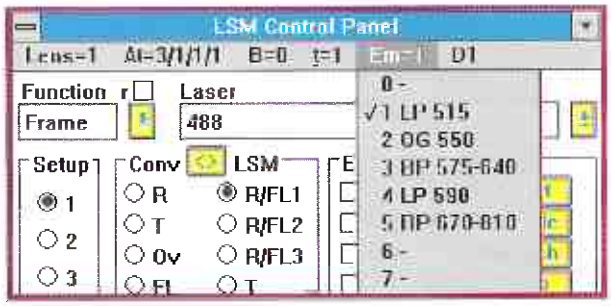


Figure 6-8

D
Selection of beam splitters
(see Section 3.1.2)

Figure 6-9

Function
On the image monitor, several images can be superimposed or displayed adjacently. The following possibilities can be selected in the fold-down menu:
Frame-Overl2-Overl3-Split2-Split3-Rotxx

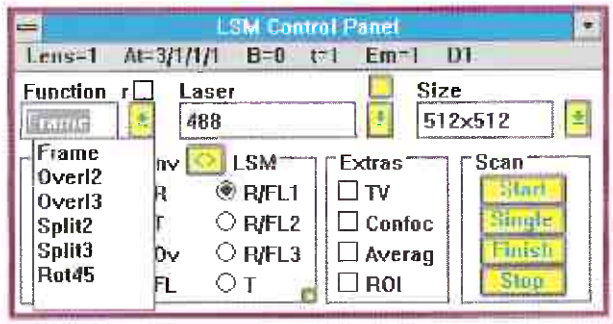


Figure 6-10

Meanings:

Frame
Display of one single image of the specimen on the image monitor.

Overl
Two or three images (R/FL1, R/FL2, R/FL3 or T) are superimposed. The value "RGB" is activated automatically in the **Image/Display Control** menu, in which case the images are then superimposed in red, green and blue.

Split
Two or three images are displayed one below the other on the image monitor.

Rotxx
Display of the scan rotated by xx degrees. The rotation angle xx is entered in the main menu and in Funct/Rotated Scan.

When you have a single-channel frame structure (FRAME), in the **Setup** box you can toggle swiftly between three defaults (1, 2 or 3). Each default embraces the following parameters:
Laser (Selection of laser lines), At (optical attenuation), B (bandwidth limiting), Contr. (contrast), Bright. (brightness), Pinh. (pinhole setting) and Sensor, Receiver (R/FL1, R/FL2, R/FL3, T). When you have a multiple-channel frame structure (Overlx, Splitx, Rotxx), **Chan** is displayed instead of **Setup**. In this way, you can assign the controls for Sensor Contr., Bright. and Pinh. to one of the two or three displayed channels. The following applies:

Mode	Overl2	Overl3	Split2	Split3
Channel 1	red	red	top	top
Channel 2	green	green	bottom	middle
Channel 3	-	blue	-	bottom

The values for **Laser, At** and **B** are not toggled here because this would be pointless in this mode of operation.

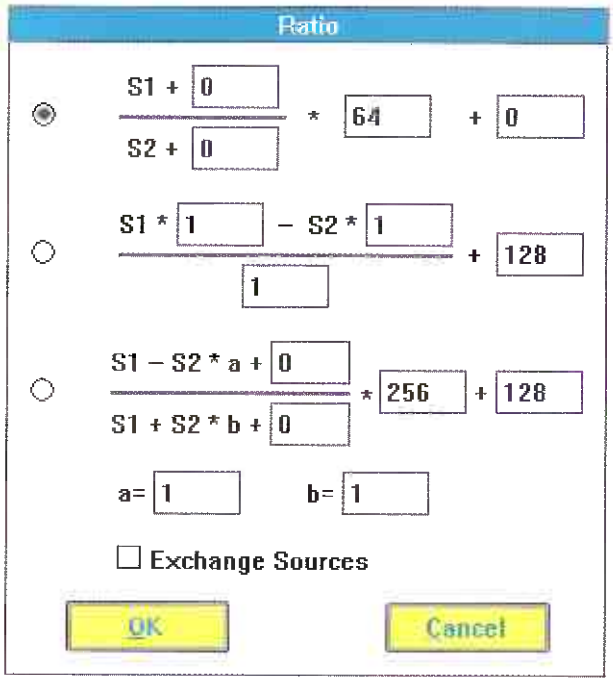


Figure 6-11

If you select the r☐ box on the right of **Function**, the adjacent "Ratio" window appears.
When you have a two-channel frame structure, you can compute a third channel on the basis of the two input channels S1 and S2 according to three different formulas.
This is possible without any delays during scanning.

Laser
The lasers or the spectral line are selected in the fold-down menu according to the hardware configuration:
488
488/514
543
633
488/568
488/568/647
365

Any combinations of the installed laser lines can be selected by activating and deactivating the corresponding check boxes, which become visible after clicking the laser box ☐ .

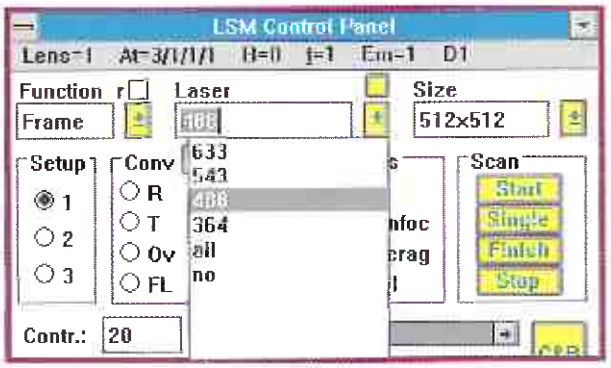


Figure 6-12

Conv/LSM

By means of the < > button, you can toggle swiftly between

- Conv (convent. microscopy without laser) and
- LSM (laser scan microscopy).

In doing so, the following operating states can be selected:

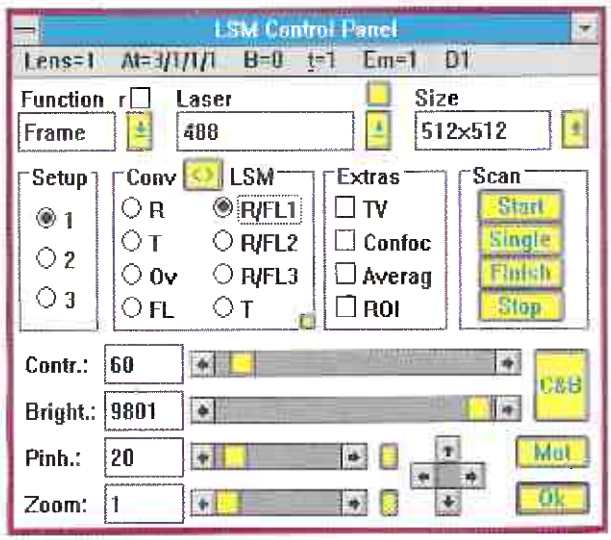


Figure 6-13

Conv

○	**R**	Conventional Reflected Mode
○	**T**	Conventional Transmission Mode
○	**Ov**	Conventional Overlay Mode (R + T)
○	**FL**	Conventional Fluorescence Mode

LSM

○	**R/FL1**	LSM Reflected Fluorescence Mode 1
○	**R/FL2**	LSM Reflected Fluorescence Mode 2
○	**R/FL3**	LSM Reflected Fluorescence Mode 3
○	**T**	LSM Transmission Mode 2

The **OBIC/External** window is opened with the small button in the bottom right-hand corner of the Conv/LSM box.
Here, the sensor inputs

- OBIC
- External
- R/FL3

which cannot be adjusted via the **Control Panel**, and the OBIC address are set.

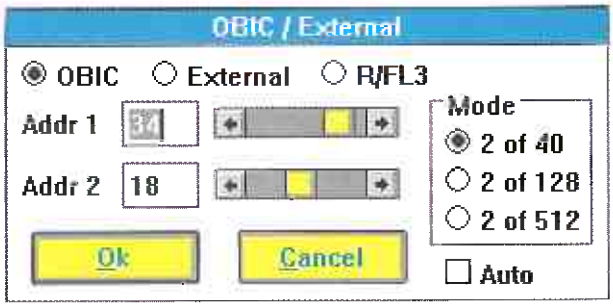


Figure 6-14

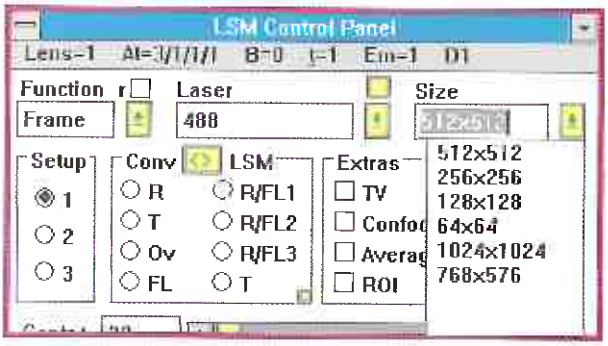


Figure 6-15

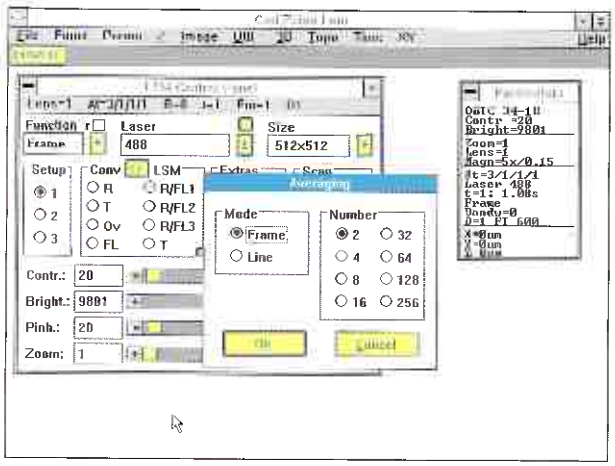


Figure 6-16

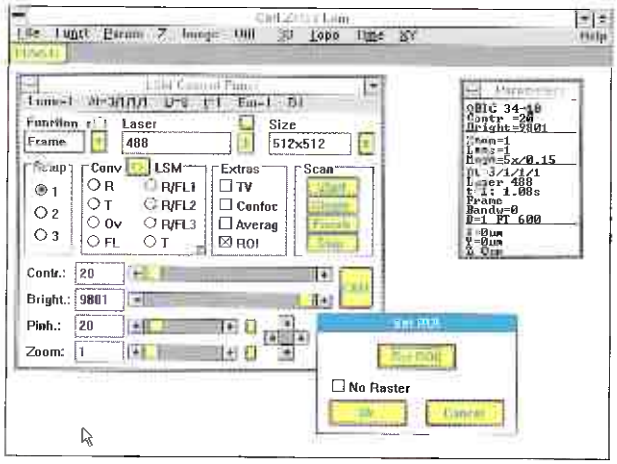


Figure 6-17

Size
A menu containing the default entries opens when you click the arrow on the right of the editing box.
By clicking a menu item, you select the value and copy it to the editing box. This allows you to select the number of pixels x and y.

Alternatively, you can click the editing box and enter values on the keyboard, in which case quantities other than the ones specified in the menu are possible.
In x (dots per line), you can enter any number that results in a multiple of 4.
You can enter any number in y (number of lines per frame). The maximum possible entry is 1024x1024.
(Refer also the time functions.)

Extras

☐ **TV**
toggles TV camera mode in conventional operation on or off.

☐ **Confoc**
activates or deactivates confocal imaging.

☐ **Averag**
When you click this option, the **Averaging** dialog box appears, in which you can set the type (Frame or Line) and amount (Number) of averaging.

☐ **ROI**
Use this to define a so-called "region of interest".
When you click ROI, the **Set ROI** dialog box appears. When you click the Set ROI key box, the user message
≪ Use left mouse button to drag ≫
appears instead (the cursor disappears from the control monitor, moving to the image monitor, where it is fixed by dragging the region of interest)
≪ Use right mouse button to exit ≫
(when you click the right mouse button, the cursor returns to the operating monitor)

Scan
Clicking on the corresponding button

Start

starts a continuous scan.

Single

starts one single scan.

Finish

ends the current scan and then aborts.

Stop

aborts a scan immediately.

Pinhole and Zoom
The values for Pinhole and Zoom can be entered directly in the respective editing box, in which case the slider then moves to the corresponding position; alternatively, move the box in the display bar with the cursor, in which case the value in the editing box changes in parallel with it:

Pinh. (pinhole size): 0 – 255
Zoom (zoom factor): 1.0 – 8.0

Contrast/Brightness
The values for Contrast and Brightness can be entered directly in the respective editing box, in which case the slider moves to the corresponding position; alternatively, use the cursor to move the box in the display bar, in which case the value in the editing field changes parallel to this.

Contr. (constrast): 0 – 999
Bright. (brightness): 0 – 9999

Fine adjustment of the values is possible by clicking the box in the display bar with the **right** mouse button.

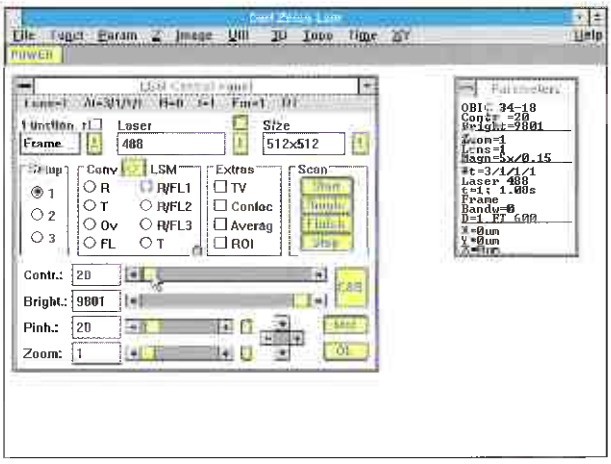


Figure 6-18

Contr./Bright. can also be set directly by moving the mouse.

- To do this, click the C&B box with the left mouse button.
  - The cursor disappears from the monitor and movement of the mouse in the x direction changes the contrast, while movement of the mouse in the y direction changes the brightness.
- Click with the right mouse button to end the mode.
  - The cursor then appears above the C&B box again.

Refer to Section 5 for information on suitable Contrast/Brightness settings.

Scan-Offset

The cross-shaped control can be used to vary the scan offset for fine adjustment of the scan box. To do this, you can click the left mouse button on the arrows or you can press the cursor keys after clicking. Clicking the left mouse button on the middle of the cross centres the scan offset again. If the scan offset is not 0, markings become visible on the cross to indicate the direction in which the scan offset has been shifted.
If you click the cross with the right mouse button and move the mouse while keeping the button pressed, the scan offset changes according to the movement of the mouse. In doing so, the mouse pointer becomes invisible. This function can also be set permanently by double-clicking with the right mouse button. The offset shift with the mouse remains active until a mouse button is pressed again.

Ok

The Ok button in the LSM Control Panel is currently only used when recording a macro, thus indicating that a slider setting is valid.

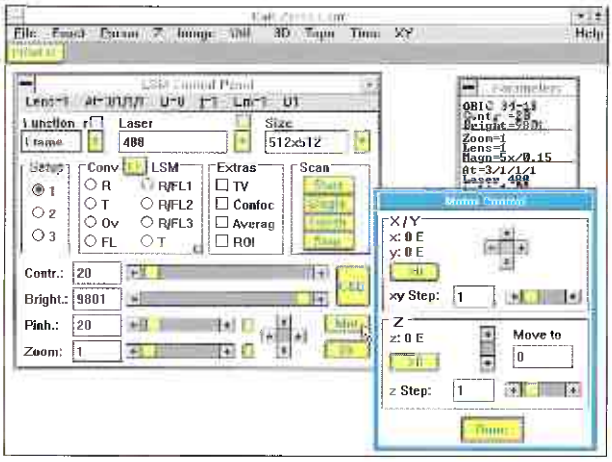


Figure 6-19

Motor Control

The **Motor Control** dialog window is opened by clicking the Mot button.

x/y

The scanning table can be moved here with the **cross-shaped control**. In doing so, the x and y positions are displayed.
The table can be moved to the 0/0 position by clicking the middle box. When you click the middle box with the right mouse button, the scanning table can be controlled according to the movement of the mouse.
The ->0 button sets the current **x/y** position to Zero. The sensitivity values of the scanning table can be set by means of **x/y Step** (by input or with the slider).

z

The z motor can be moved here with the **vertical slider**. By means of the right mouse button, the z motor can be positioned directly by moving the mouse up and down. When the arrows are clicked, the motor is moved by the distance set in z Step. In doing so, the z position is displayed.
A destination position for the z motor can be entered under **Move to**.
The ->0 button sets the z position to zero.

The dialog box is terminated again by pressing the Done button.

6.3.3 Main menu

Figure 6-20

The functions of the LSM program can be activated in the main menu. They are combined in the following sub-groups:

Basic functions (included in the scope of delivery)

File	File management functions. Frames and frame sequences can be called up and stored.
Funct	Special measuring functions such as line/spot scan, rotating the scanned area and measuring distances, angles or areas in the scan area. The Control Panel is also called up via Funct.
Param	selects, loads or stores the various working parameters.
Z	creates section stacks in the z direction, z profiles, intensity profiles, galleries and animations.
Image	Image processing functions such as digital filters, arithmetic and logical image operations, wrong colour displays and histogram evaluation.
Util	Test images and version information
Help	Help menus for user support

Options (separate software packages, which must be purchased separately)

3D	Display of image series in 3D form and of inclined sections etc.
Topo	Surface topography
Time	Recording and evaluation of image series in time sequence
XY	Control of a scanning table

Further function areas

Further, user-defined function areas (see **Funct/Install Macro**) may follow in the above menu line. Individual functions can also be added or removed to eliminate unused functions for clarity. This may alter the resulting user interface to such an extent that there may be differences in comparison with the figures shown in the manual.

(1) File

The File group embraces file management functions.
Here, individual images or image sequences can be loaded, stored or deleted.

Figure 6-21

Meanings:

Load Image...	:	loads one single image into the video RAM
Store Image...	:	stores one single image
Load Sequence...	:	loads an image sequence
Store Sequence...	:	stores an image sequence
View and Delete...	:	displays, loads or deletes individual images, sequences or other files
Print Image...	:	prints an image
Quit...	:	ends the LSM program
About LSM...	:	display LSM program information

(1.1) File → Load Image

The **Load Image** function loads one single image into the video RAM.

In doing so, you select the image file by entering its file name in the **File Name** editing box or by clicking the file name in the **Files in** box.

In the **Format** box, you can choose between TIFF and IMG files; IMG stands for a raw data file.

☒ **Display**
When Display is activated, images are displayed on the image monitor immediately after clicking them. In doing so, the dialog box remains open. By means of the cursor keys, you can then view the images in very swift succession.

☐ **Keep Overlay**
makes sure that the graphic in the overlay is not deleted when loading an image without an overlay.

☐ **ROI old Pos.**
makes sure that, when loading an ROI image, it is not loaded into the top left corner, but is placed in its original position in the image.

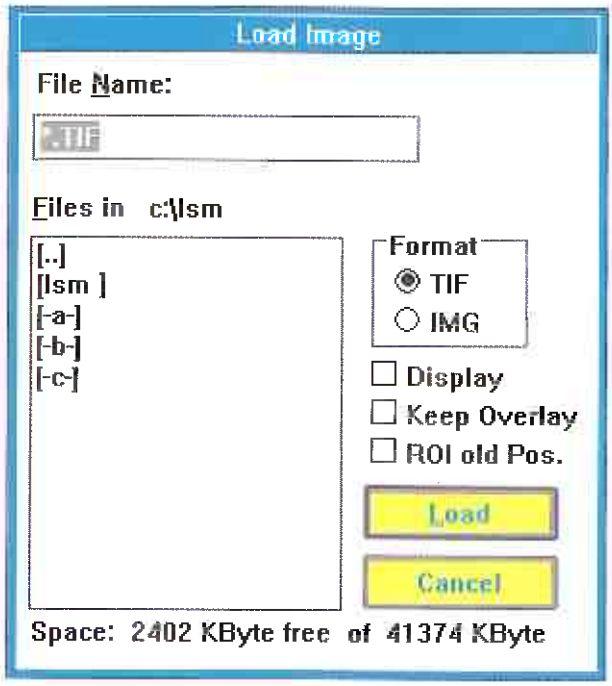


Figure 6-22

Clicking on the corresponding button

Load

executes the command.

Cancel

cancels the operation.

(1.2) File → Store Image

The **Store Image** function stores one single image.

The .tif extension is added automatically after entry of the file name in the **File Name** editing box or by clicking the file name.

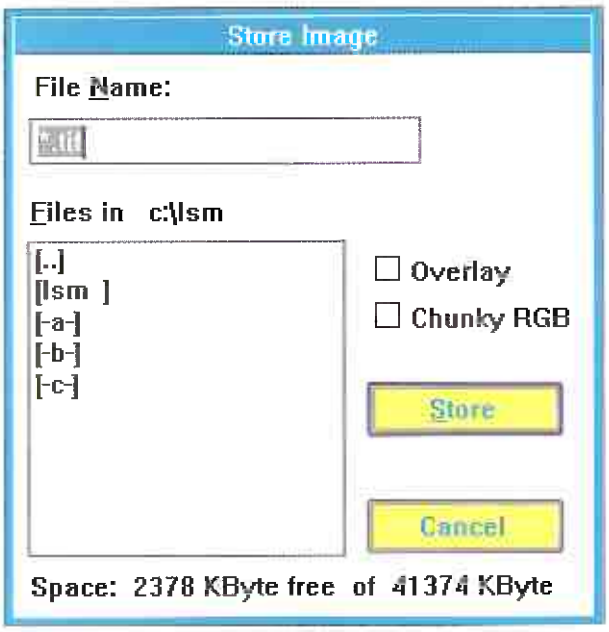


Figure 6-23

If one of the following extensions is explicitly specified when storing an image, the image is not stored in TIFF format, but in one of the following formats (monochrome only):

- RAW Raw data
- IMG LSM2 format
- KON Kontron format
- RAS Sun Raster format

☐ **Overlay**
stores the overlay together with the image.

☐ **Chunky RGB**
For TIFF files, there are two different methods of storing RGB images. Normally, the complete R, G and B images are stored one after the other. If Chunky RGB is selected, the R, G and B values are stored for each pixel. Various third-party programs use one or the other format. Therefore, you can adapt easily to your other programs.

Clicking on the corresponding button

Store
stores the image and closes the dialog window.

Cancel
cancels the operation.

(1.3) File → Load Sequence

The **Load Sequence** function loads the sequence of images.

In doing so, you select the image file by entering its name in the **File Name** editing field by clicking the file name in the **Files in** box.

In the **Format** box, you can choose between TIFF and IMG files.

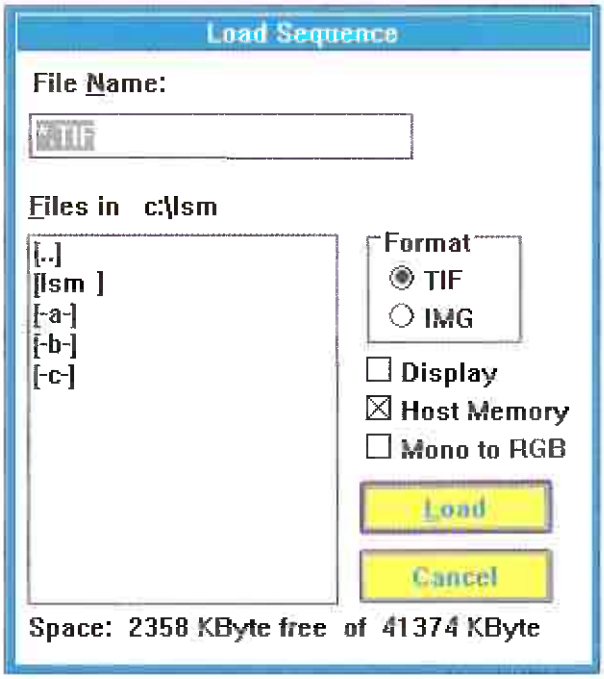


Figure 6-24

☒ **Display**
When Display is activated, the images are displayed on the image monitor immediately after clicking. In doing so, the dialog window remains open. With the cursor keys, the images can then be viewed in very swift succession. When the image monitor status display is activated, the parameters stored along with the display are also shown. When loading the sequence, these are only shown when Display is also activated.

☐ **Host Memory**
When ☐ **Host Memory** is not activated, the sequence is loaded into the video memory. If the video memory is inadequate, the complete sequence is then loaded into the host memory. A warning is issued if this memory volume does not suffice either.
If ☒ **Host Memory** is activated, the sequence is loaded into main memory immediately. A warning is issued if not enough memory volume is available.

☐ **Mono to RGB**
Several monochrome sequences can be combined in one RGB sequence.

Clicking on the corresponding button

Load

loads the sequence and closes the dialog window.

Cancel

cancels the operation.
Loading can be cancelled prematurely by pressing the ESC key. In this case, the sequence then only contains the images loaded up to this time.

(1.4) File

Store Sequence

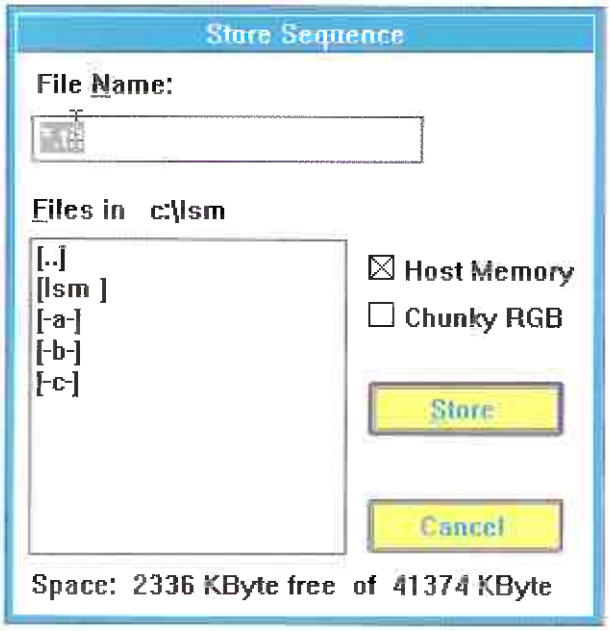


Figure 6-25

The **Store Sequence** function stores a sequence of images.

The .tif extension is added automatically after entry of the file name in the **File Name** editing box or by clicking the first image file name. If one of the following extensions is specified explicitly when storing a series of images, the image series is not stored in TIFF format, but in one of the following formats (monochrome only):

- RAW raw data
- IMG LSM2 format
- KON Kontron format
- RAS Sun Raster format

☐ **Host Memory**
The sequence that is currently in the main memory is stored.
If Host Memory is not activated, the sequence in the video memory is stored. The main memory and video memory may contain two different sequences. However, a sequence in the video memory is copied automatically to the main memory when a gallery or a 3D depiction is displayed. Pay attention to the fact that a sequence in the video memory is not protected and can be destroyed by a scan or a graphics function that does not operate exclusively in the overlay memory.

☐ **Chunky RGB**
For TIFF files, there are two different methods of storing RGB images. Normally, the entire R, G and B images are stored one after the other. If Chunky RGB is selected, the R, G and B values are stored for each pixel. Various third-party programs use one or the other format. You can therefore adapt easily to your other programs.

Clicking on the corresponding button

Store

stores the sequence and closes the dialog window.

Cancel

cancels the operation.

(1.5) File → View and Delete...

The **View and Delete** function allows you to display, load or delete individual images, sequences or other files.

Select the file by entering its name in the **File Name** editing box or by clicking the file name in the **Files in** box.

- ◉ **View on click/double click**
  Default: display of the file by single click/ double click
- ○ **Delete on double click**
  Default: deletion of the file by double click
- ○ **Delete on double click without conformation**
  Default: deletion of the file by double click (additional confirmation is off)

Clicking on the corresponding button

OK
displays the selected file in a Notepad window.

View
displays the selected image file on the image monitor.

Load
loads the selected file into a macro window.

Delete
deletes the selected file.

Close
closes the dialog box.

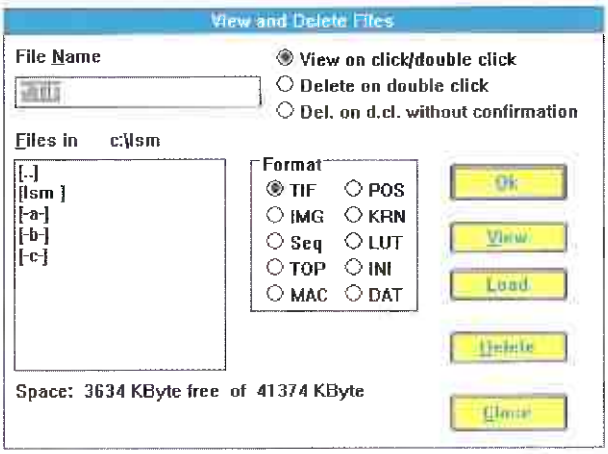


Figure 6-26

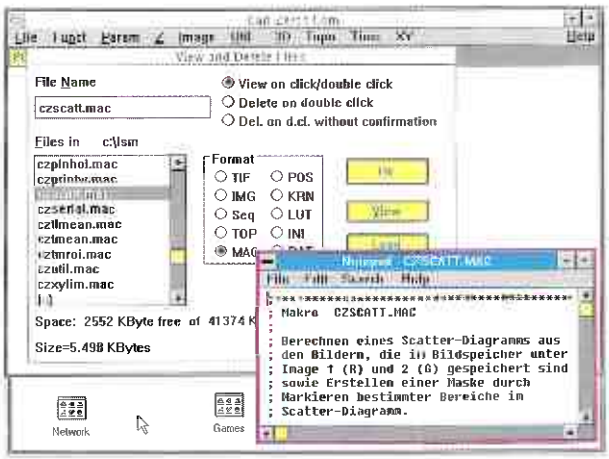


Figure 6-27

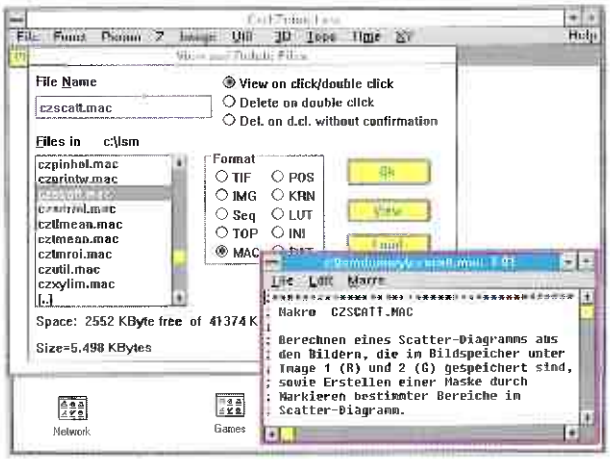


Figure 6-28

(1.6) File → Print Image

Using the **Print Image** function, the image currently loaded in the memory can be passed on to a connected printer if a WINDOWS driver has been loaded for this printer.

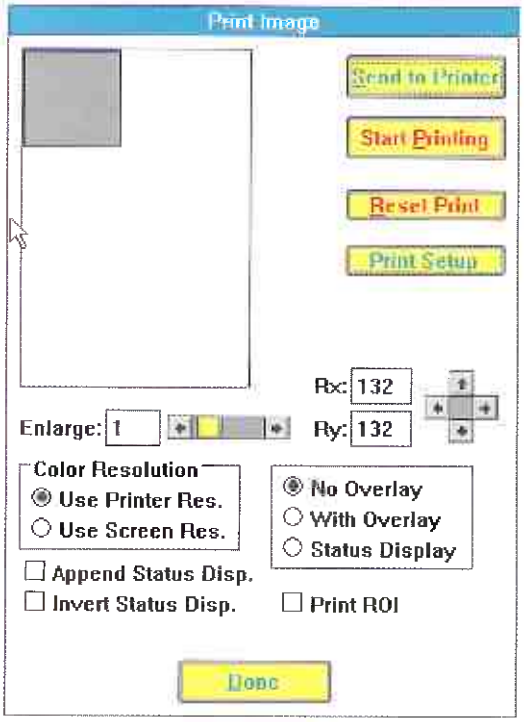


Figure 6-29

Image box
The rectangle represents the page to be printed. The image is marked in red at the point where it will then be printed. If you click on the image box with the left mouse button, you can move the image on the paper. You can also move the image with the **Rx** and **Ry** coordinate cross.

Enlarge
You can use this function to enlarge the image on paper.

Color Resolution box
◉ **Use Printer Res.** The resolution is matched
○ **Use Screen Res.** either to the printer or to the monitor.

☐ **Append Status Disp.**
The parameter list is printed out with the image.

☐ **Invert Status Disp.**
The parameter list is shown in black on a white background.

☐ **Print ROI**
Only the current region of interest is printed.

◉ **No Overlay**
○ **With Overlay**
○ **Status Display**

These options can be used to decide whether you wish to also print information from the Matrox overlay memory.

Send to Printer
forwards individual images to the printer buffer.

Start Printing
starts printing.

Reset Print
clears the printer buffer.

Print Setup
opens the printer driver to enable definition of settings.

Done
closes the dialog box.

(1.7) File → Quit

The **Quit** function ends the LSM program.

- The program returns to the **Program Manager**.

(1.8) File → About LSM

The **About LSM** function shows the version number of the LSM program and copyright information.

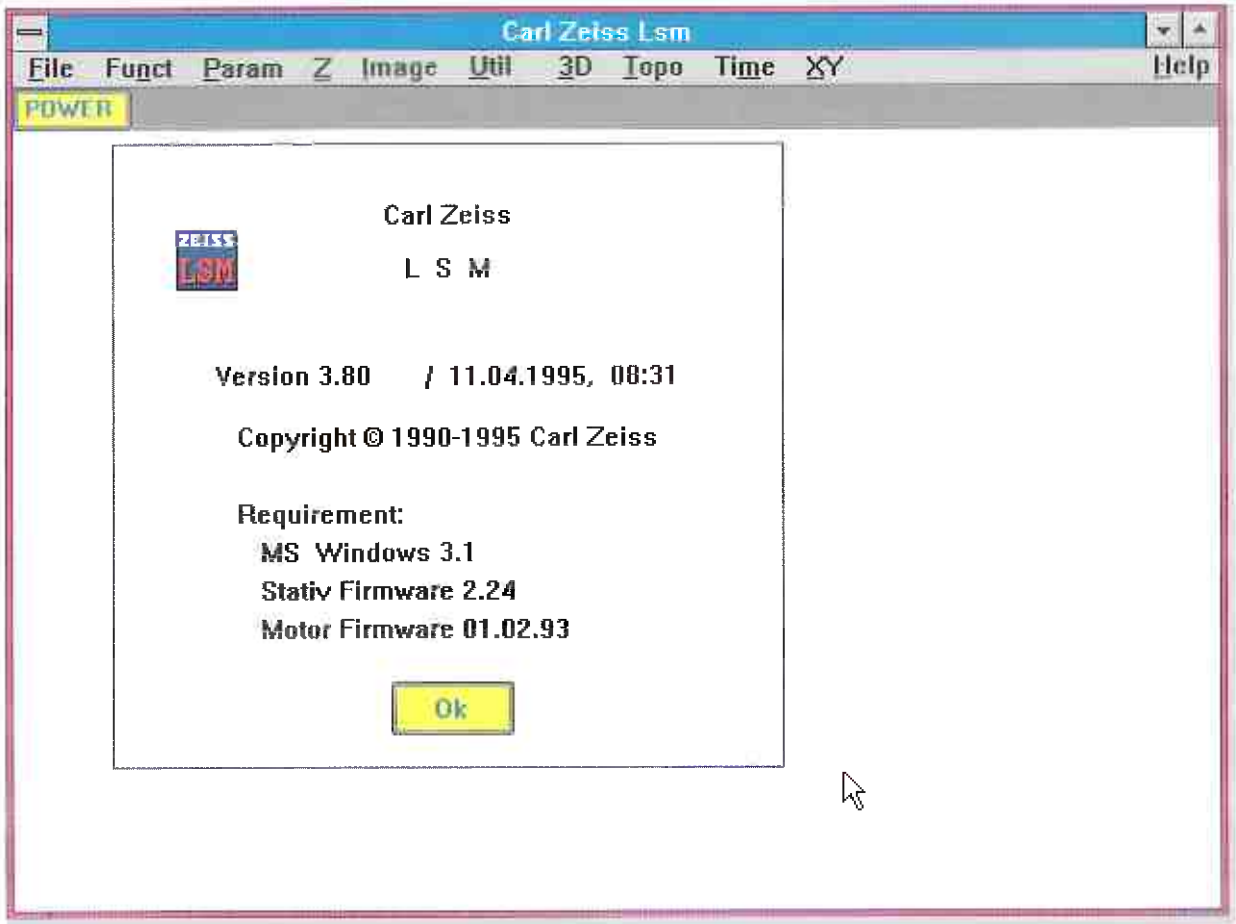


Figure 6-30

(2) Funct

The Funct function embraces basic functions such as line and dot scans, scan rotation and measuring functions.

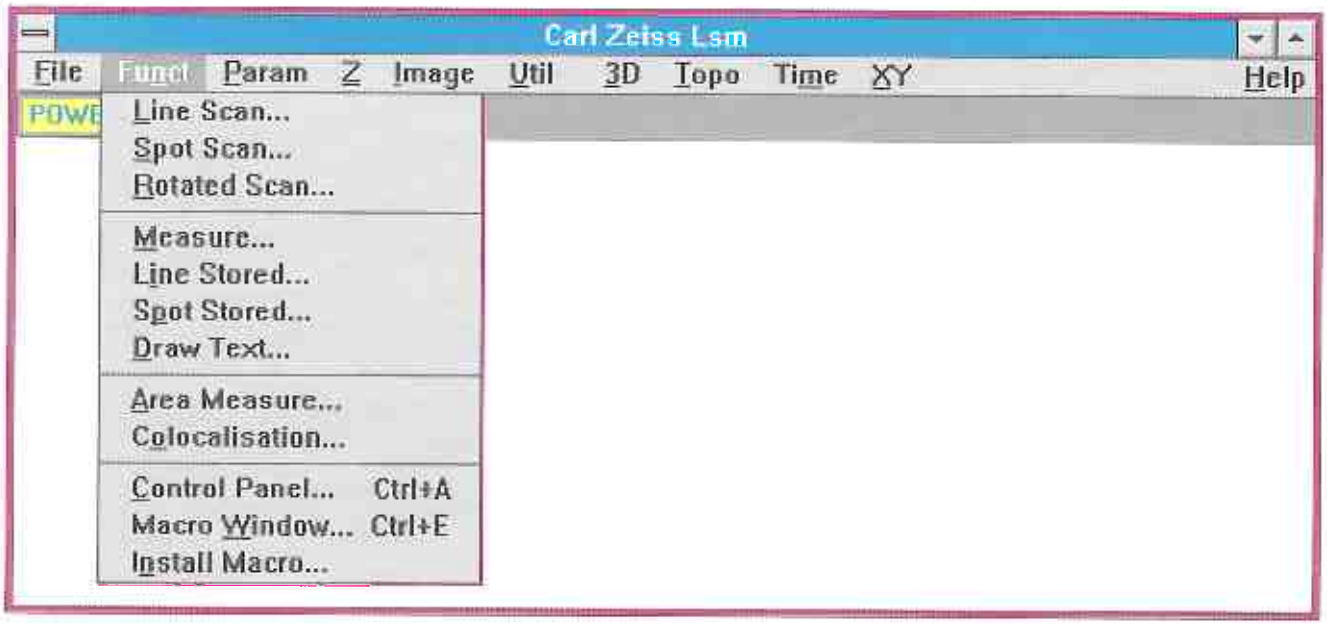


Figure 6-31

Meanings:

Line Scan...	:	displays a brightness profile along a straight line
Spot Scan...	:	displays the brightness value at a chosen spot in the image (x, y)
Rotated Scan...	:	rotates a scanned image in the xy plane
Measure...	:	measures distances, angles and simple surfaces
Line Stored...	:	displays a brightness profile along a line in the stored image
Spot Stored...	:	displays the brightness of a spot in the stored image
Draw Text...	:	inserts comment texts in the image
Area Measure...	:	measures the volume and the average intensity of any area
Colocalisation...	:	compares two images by computing a scatter diagram
Control Panel...	Ctrl+A:	opens the Control Panel window
Macro Window...	Ctrl+E:	opens the Macro window
Install Macro...	:	installs macros in the main menu or in the button bar

(2.1) Funct

Line Scan

The **Line Scan** function enables an on-line display of a brightness profile along a line.

The image is scanned in live mode along any line in the xy plane. The position of the scanned line is shown as a green line in the display. Five blue reference lines mark the 0 %, 25 %, 50 %, 75 % and 100 % intensities.

Min and **Max**
The displayed values define the intensity range shown.

Line
The selected line can be moved up/down with the scroll box.

Angle
The line can be tilted at any angle with respect to the horizontal (-89 bis +90°) with the scroll box.

☐ **No Backgr. Scan**
The image background is not read again.

☐ **Keep Overlay**
The overlay is retained when the function is ended by selecting Cancel.

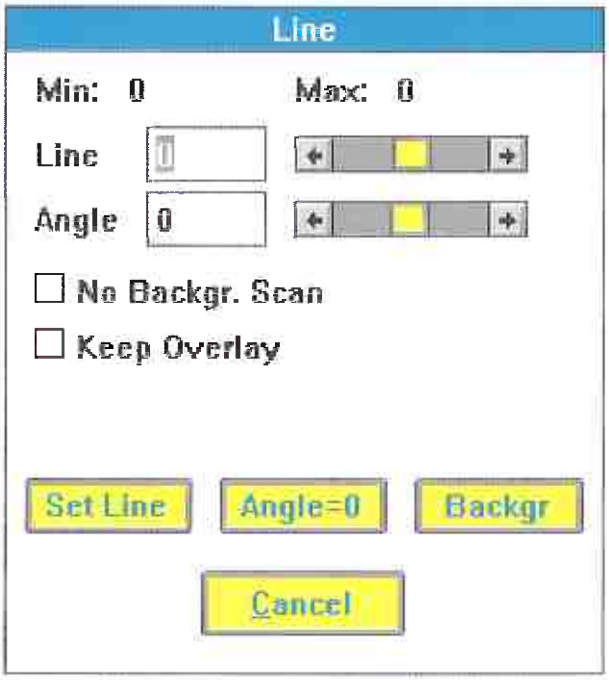


Figure 6-32

Clicking on the corresponding button

Set Line

allows you to also move the line with the mouse after clicking it.
To do this, move the arrow to the middle of the line, press the left mouse button and hold it down; the green line is moved up or down in parallel with it;
the line is rotated if it is not selected in the middle.

Angle=0

sets the angle to 0°.

Backgr

allows you to draw in a new image background if the object or the settings have changed.

Cancel

ends the function and closes the dialog window.

(2.2) **Funct** → **Spot Scan**

The **Spot Scan** function allows you to measure the brightness value at any chosen point (x, y).

Figure 6-33

You can set the coordinates of the chosen point between -256 and +256 with the two scroll boxes for **x-Pos** and **y-Pos**.

☐ **No Backgr. Scan**
The image background is not read again.

☐ **Keep Overlay**
The overlay is retained when the function is ended by selecting Cancel.

Clicking on the corresponding button

Set Pos
causes the cursor arrow to disappear. A cross, whose position can be moved with the mouse, appears on the image monitor;
at the same time, the following text is displayed in the dialog window:

```
Press left mouse button to select
Press right mouse button to exit
```

Backgr
allows you to draw in a new image background if the object or the settings have changed.

Cancel
ends the function and closes the dialog window.

(2.3) Funct → Rotated Scan

By means of the **Rotated Scan** function, an image with a resolution of 0.1 can be **scanned** after rotation about the optical axis. In doing so, the "Function" box in the **Control Panel** is set to Rotxx, in which case xx is the set angle. A line inserted in the image facilitates setting of the rotation angle. You can work in the manner to which you are accustomed with the rotated image. The scan is set automatically to 2 s if you have selected a time below 2 s.

Line
The selected line can be moved up/down with the scroll box (-256 to +256).

Angle
The line can be rotated with respect to the horizontal by means of the scroll box (-89 to +90°).

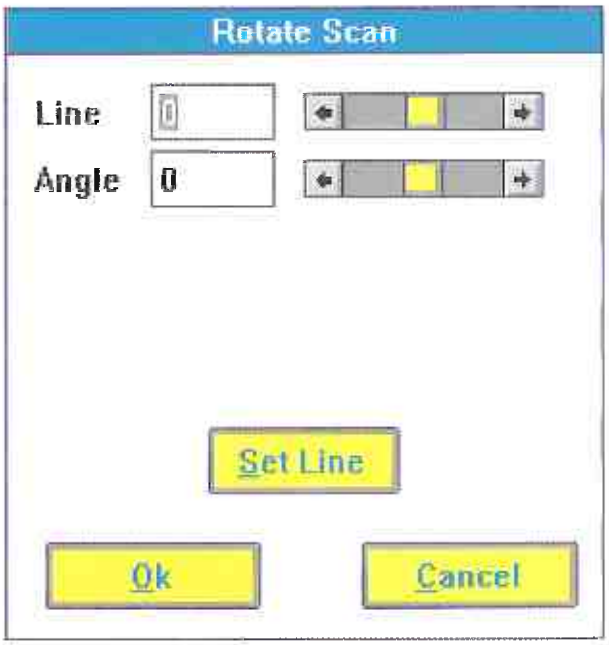


Figure 6-34

Clicking on the corresponding button

Set Line

allows you to also move the line with the mouse after clicking it.
To do this, move the arrow to the middle of the line, press and then hold down the left mouse button;
the green line is moved up or down in parallel; the line is tilted if it is not selected in the middle. When adjusting with the mouse, the resolution is 0.1°;
at the same time, the following text is displayed in the dialog window:

```
Use left mouse button to drag
Use right mouse button to exit
```

Ok

executes the function and closes the dialog window.

Cancel

ends the function and closes the dialog window.

(2.4) Funct
Measure

The **Measure** function allows you to measure distances, angles and simple surfaces.

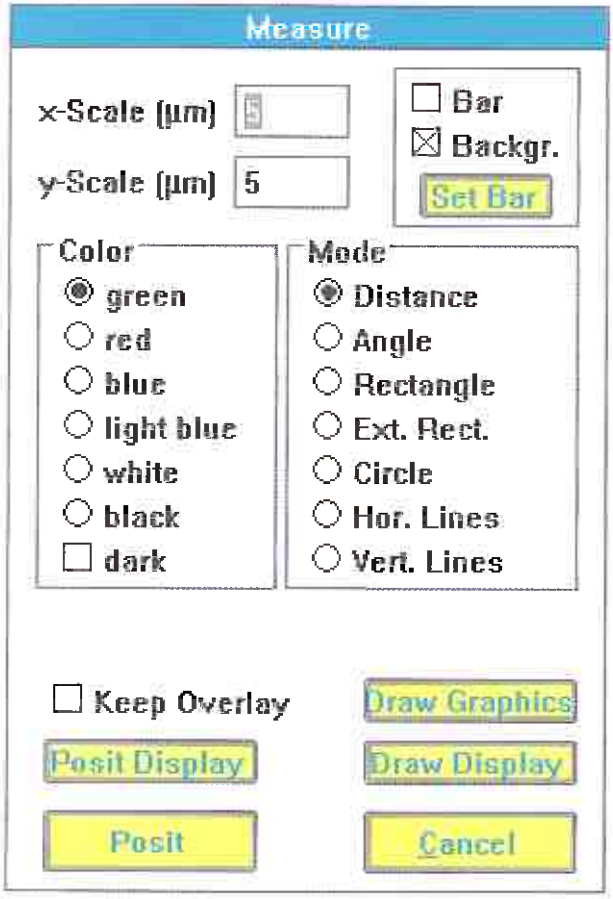


Figure 6-35

The values for
x-Scale (µm) ≙ pixel size in the x direction
y-Scale (µm) ≙ pixel size in the y direction
are set automatically.
However, the lens configuration must be specified correctly.

These quantities depend on the lens scale, the zoom factor and the number of pixels.

The colour of the measured elements can be adapted to suit the image to be measured.

Color box
- ◉ **green**
- ○ **red**
- ○ **blue**
- ○ **light blue**
- ○ **white**
- ○ **black**

☐ **dark**
The selected colour is displayed at half brightness.

☐ **Bar**
A measuring bar is inserted in the display.

☐ **Backgr.**
The text describing the measuring bar can be highlighted with a dark area.

Set Bar
After clicking, the position of the measuring bar can be moved interactively with the mouse.

Mode box
The required measurement mode can be selected

○ **Distance**	Distance measurement with two crosses
○ **Angle**	Angle measurement with a line
○ **Rectangle**	Rectangle (x, y, area)
○ **Ext. Rect.**	Rectangle with extended lines (x, y, area)
○ **Circle**	Circle (Radius, area)
○ **Hor. Lines**	Distance of a horizontal pair of lines
○ **Vert. Lines**	Distance of a vertical pair of lines

☐ **Keep Overlay**
The overlay is retained, even when the function is closed.

Clicking on the corresponding button

Posit Display
allows you to interactively display the measured values anywhere in the image.

Draw Graphics
writes the measured graphics permanently into the display.

Draw Display
transfers the measurement display permanently into the image.

Posit
causes the mouse cursor to appear on the image monitor after clicking on the button. At the same time, the following text is displayed in the dialog window:

```
Use left mouse button to drag
Use right mouse button to exit
```

The displayed elements can be moved or modified by clicking and holding down the mouse button.

Cancel
ends the function and closes the dialog window.

(2.5) Funct → Line Stored

The **Line Stored** function allows you to display a brightness profile along a line in the displayed image. In the image, the position of the scanned line is displayed as a green line. Five blue reference lines mark the 0 %, 25 %, 50 %, 75 % and 100 % intensities.

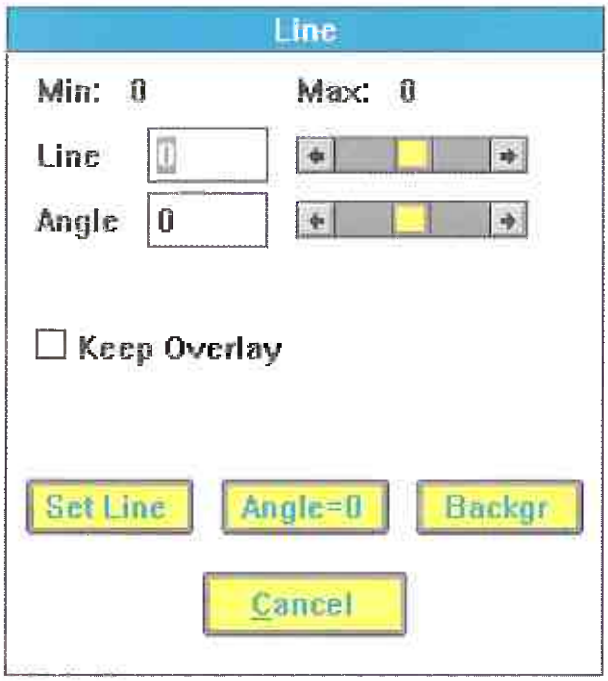


Figure 6-36

Min and **Max**
The displayed values define the intensity range shown.

Line
The selected line can be moved up/down with the scroll box.

Angle
The line can be tilted with respect to the horizontal (-89 to +90°) with the scroll box.

☐ **Keep Overlay**
The overlay is retained, even when the function is ended with Cancel.

Clicking on the corresponding button

Set Line
allows you to move the line with the mouse after clicking on it.
To do this, move the arrow to the middle of the line and then press the left mouse button and hold it down;
the green line is moved up or down in parallel with it;
the line is tilted if it is not selected in the middle.

Angle=0
sets the angle to 0°.

Backgr
allows you to insert a background scan.

Cancel
ends the function and closes the dialog window.

(2.6) Funct → Spot Stored

The **Spot Stored** function allows you to display the brightness value of a spot in the stored image.

The coordinates of the selected point can be set between 0 and 511 with the two scroll boxes for **x-Pos** and **y-Pos** (default: 256).

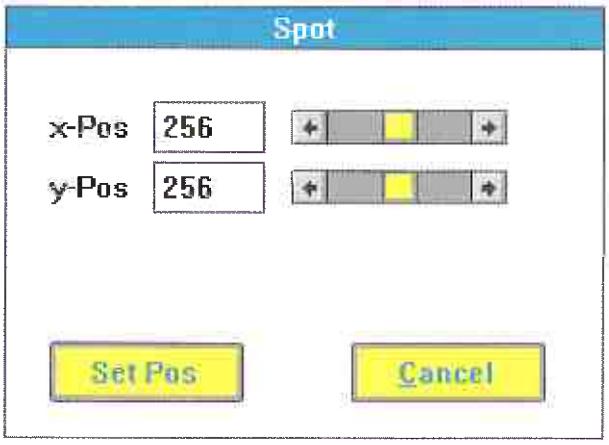


Figure 6-37

Clicking on the corresponding button

Set Pos

causes a cross to appear whose position can be moved with the mouse;
at the same time, the following text is displayed in the dialog window:

```
Use left mouse button to select
Use right mouse button to exit
```

Cancel

ends the function and closes the dialog window.

(2.7) Funct → Draw Text

The **Draw Text** function allows you to insert a comment text in the image.

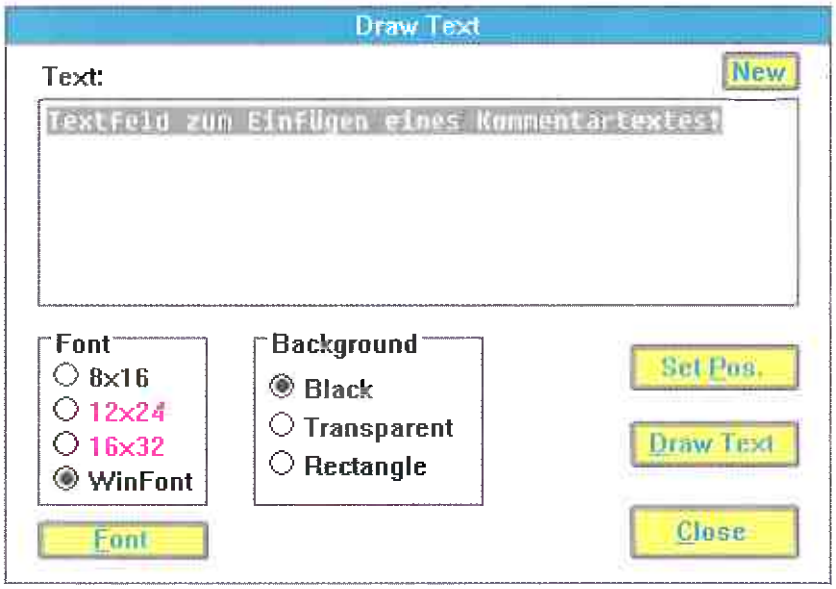


Figure 6-38

Size box
The size of the letters can be selected in pixels
- ◉ **8x16**
- ○ **12x24**
- ○ **16x32**

Background box
The colour and shape of the background can be selected
- ◉ **Black**
(Text with black background)
- ○ **Transparent**
(Text without background)
- ○ **Rectangle**
(Text appears in a black rectangle)

Clicking on the corresponding button

New
deletes the text in the window on the operating monitor.

Set Pos.
positions the comment text field in the image by clicking with the cursor (the position is indicated in the image by a rectangle). Hold down the mouse button and move the square with the button held down.

Draw Text
writes the text indelibly into the square of the image monitor.
If the area is not large enough, the text is then beyond the real edge.

Font
allows you to call up WINDOWS fonts; a further dialog box is displayed.

Close
ends the function and closes the dialog window.

(2.8) Funct → Area Measure

The **Area Measure** function allows you to measure the volume and the average intensity of an area of any shape.

The area to be measured is defined with a low (L) and a high (H) limit with the two scroll boxes **Thresh L** and **Thresh H**.

☐ **Include Outline**
The outline is included in area measurement.

☐ **Display Results**
The results of measurement are also shown on the image monitor.

☐ **Enhanced Contrast**
In the threshold setting, the remaining area of the image is always spread to full contrast.

☐ **Keep Overlay**
The overlay is retained when the function is ended.

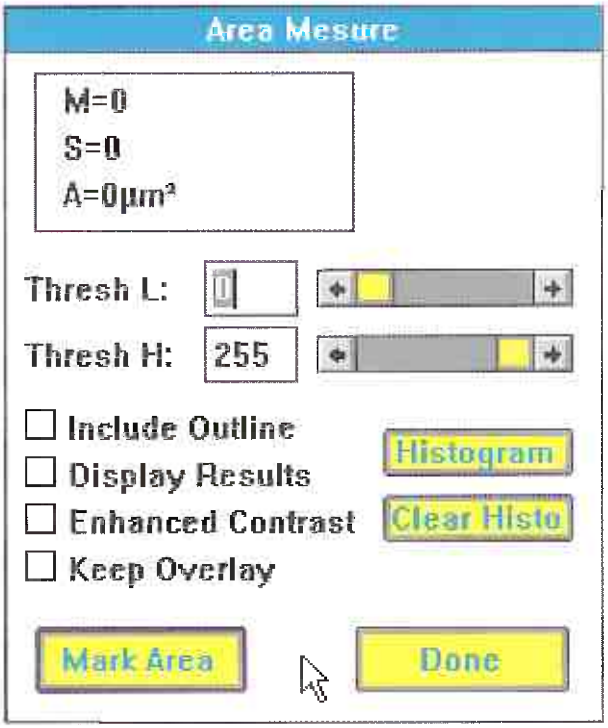


Figure 6-39

Clicking on the corresponding button

Histogram
shows the grey-value histogram of the marked area.

Clear Histo
clears the histogram.

Mark Area
defines the area to be measured by surrounding it with the mouse.
To do this, draw the border with the left mouse button pressed, then move the mouse pointer into the area to be measured and click it **with the left mouse button**. The area is then colour-highlighted briefly.

Done
ends the function and closes the dialog window.

(2.9) Funct
Colocalisation

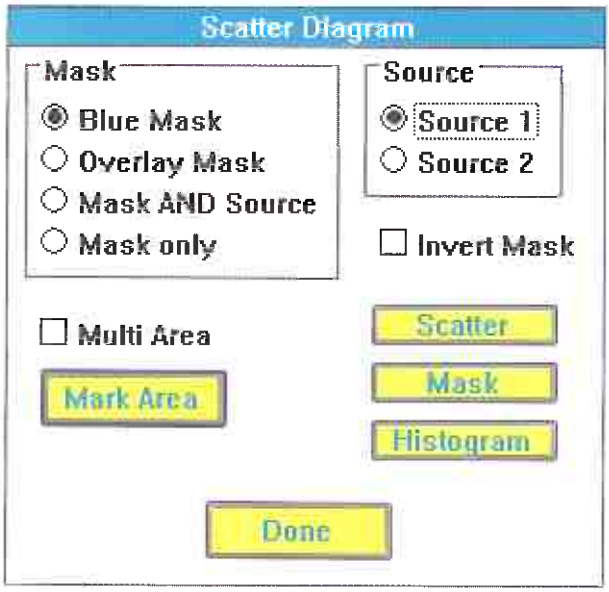


Figure 6-40

The **Colocalisation** function allows you to compare two images by calculating a scatter diagram (colocalisation).
The scatter diagram is computed from the images that are stored under Image 1 (R) and 2 (G) in the video memory. A 256x256 pixel scatter diagram is computed on the basis of these images and is displayed on the monitor with colour codings and double the size.
A mask can also be created by marking specific zones in the scatter diagram.

A scatter diagram is created as follows:
All pixels that are in the same position in both images are considered to be pairs. From each pixel pair (P1, P2) out of the source images, the brightness value of pixel P1 is interpreted as the x coordinate and the brightness value of P2 is interpreted as the y coordinate in the scatter diagram. The value of the pixel addressed in this ways is incremented by 1 in each case (up to max. 255). Thus, each pixel of the scatter diagram represents a numerator that expresses how often a specific pixel pair has occurred.
Identical images produce a sharp line running diagonally from the bottom left to top right because only the pixel pairs (0, 0), (1,1), (2,2,) to (255,255) can occur here. Discrepancies between the images lead to spots in the scatter diagram. By tracing these spots with the mouse, a mask can be computed that elucidates the position of the affiliated discrepancy in the image.

Mask box

A mask can be created by marking specific zones in the scatter diagram. This mask can be displayed in the blue channel (Blue Mask). It can be written into the overlay (Overlay Mask). The mask can be displayed on its own (Mask only) or with the selected source (Mask AND Source).

Source box

This box defines which of the two original images in the video memory levels 1 (R) and 2 (G) are to be displayed together with the mask when "Overlay Mask" and "Mask AND Source" are selected.

☐ **Invert Mask**
computes a complementary mask.

☐ **Multi Area**
is selected when it is intended to mark several separate area zones in the scatter diagram. Marking must then be concluded by selecting **Mask**.

Mark Area
By selecting **Mark Area**, by tracing the required area and by clicking the interior of this area, the area is defined. The mask is displayed automatically if Multi Area is not selected.

Scatter
returns you to the scatter diagram.

Mask
displays the mask after several areas have been marked with **Multi Area**.

Histogram
creates a grey-value histogram of the mask area.

Done
ends the function and closes the dialog window.

(2.10) Funct

Control Panel

The **Control Panel** function opens the **Control Panel** or moves the window to the foreground.

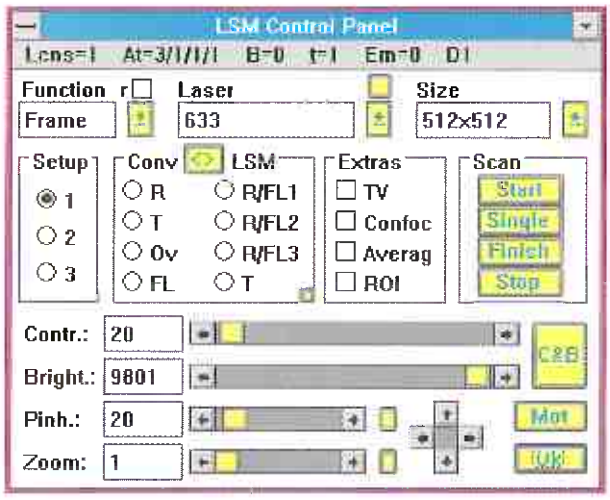


Figure 6-41

This function can also be activated directly by pressing Ctrl+A.

See Section 6.3.2 for a detailed description of the Control Panel.

(2.11) Funct

Macro Window...

The **Macro Window...** function opens the window **(Untitled) 1-01** or fetches the window to the foreground. The function can also be activated directly by pressing the Ctrl+E keys.

The macro window is a simple text editor in which macros can be edited.
The macro window has a menu of its own, which contains load and storage commands, editing commands and special macro commands.
You will find a brief description in Chapter 7. Refer to the On-Line Help for macros for in-depth information.

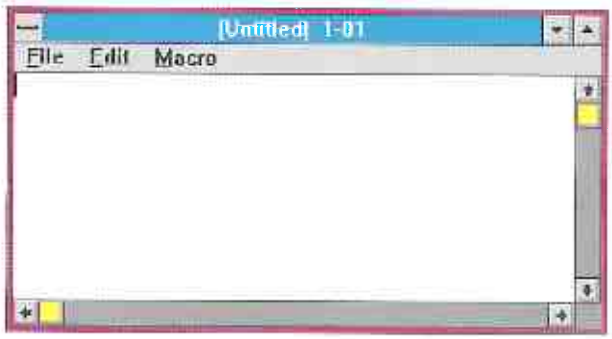


Figure 6-42

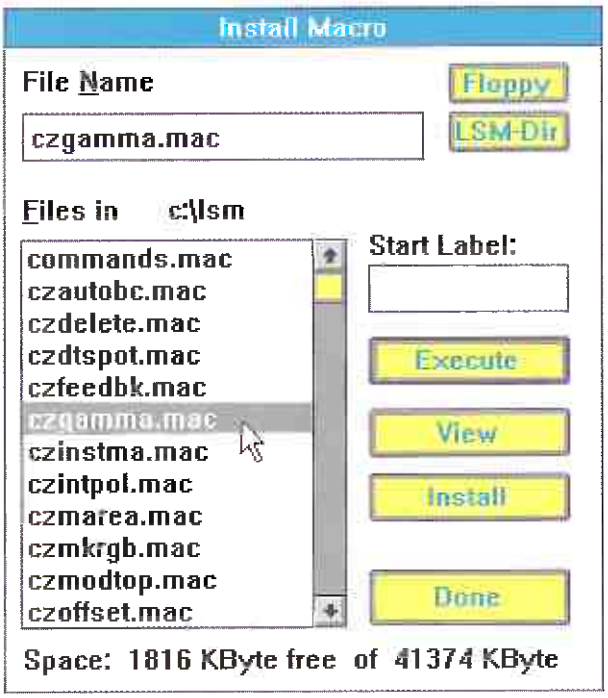


Figure 6-43

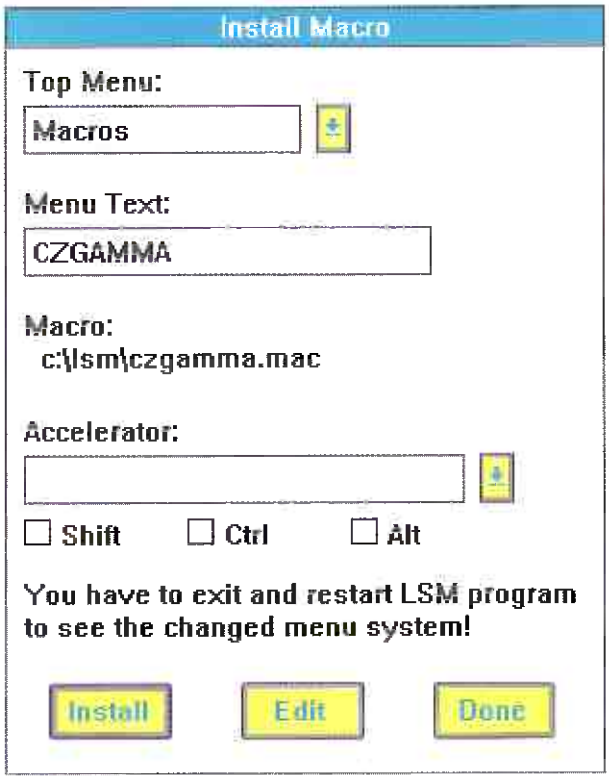


Figure 6-44

(2.12) **Funct** → **Install Macro...**

The **Install Macro...** function installs macros in the main LSM menu or in the button bar and allows you to display and directly execute a stored macro.

Floppy

The "A:\MACROS" path is set and the file list is displayed. During installation, macros on floppy disk (A:\MACROS directory) are copied automatically to the LSM directory.

LSM-Dir

The LSM path (normally: "C:\LSM") is set and the file list is displayed.

Execute

The macro selected in the file list is directly executed.

View

The macro selected in the file list is displayed in an editor.

Install

The macro selected in the file list can be installed in a newly opened dialog window (see Figure 6-44).

Top Menu:
The menu to which the new menu item is to be added can be selected here. If you enter a new menu name manually here, a new menu is created under this name. If the name "Button" is selected as the menu, a new button will be added to the button list.

(3.8) Param

Set Scan Phase

With an image scan time of 0.5 s, the **Set Scan Phase** function allows slight shifting of the phases to eliminate any unsharpness in the image.

Phase
The phase shifting value can be entered on the keyboard or by means of the scroll box (adjustment range: -50 to +50).

☐ **Auto**
The dialog box is always displayed automatically if 0.5 s has been selected for the scan time.

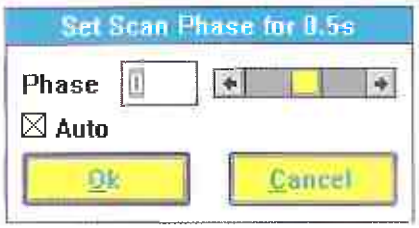


Figure 6-53

Clicking on the corresponding button

Ok
confirms the entered values.

Cancel
closes the dialog window.

(3.9) **Param** ⇨ **Load.....Parameters**

The **Load.....Parameters** functions allow you to load various parameter sets.

Load Default Parameters
are the basic settings defined at the works. They are loaded via the file **defpar.lsm.**

Load Exit Parameters
are the parameters that were used last and stored when the program was exited correctly. They are loaded from the file **lastexit.par.**

Load Start up Parameters
are the basic settings defined by the customer. They are loaded via the file **startup.par.**

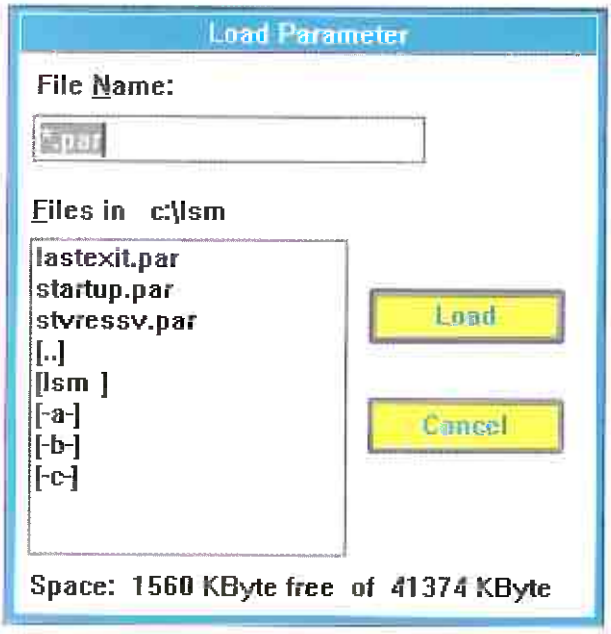


Figure 6-54

Load User Parameters...
are the individual settings that the user has stored in a file that bears the **.par** extension. They are selected in a menu by entering the file name in the **File Name** box or by clicking the file name in the **Files in** box.

Clicking on the corresponding button

Load
loads the selected file.

Cancel
closes the dialog window.

(3.10) Param

Store.....Parameters

The **Store.....Parameters** functions allow you to store the current parameters.

Store Start up Parameters
stores the current parameters as start-up and exit parameters.

Store User Parameters
stores the current parameters under a freely chosen name (up to 8 characters). The .par extension is added.

Clicking on the corresponding button

Store
stores the parameters in the corresponding file.

Cancel
closes the dialog window.

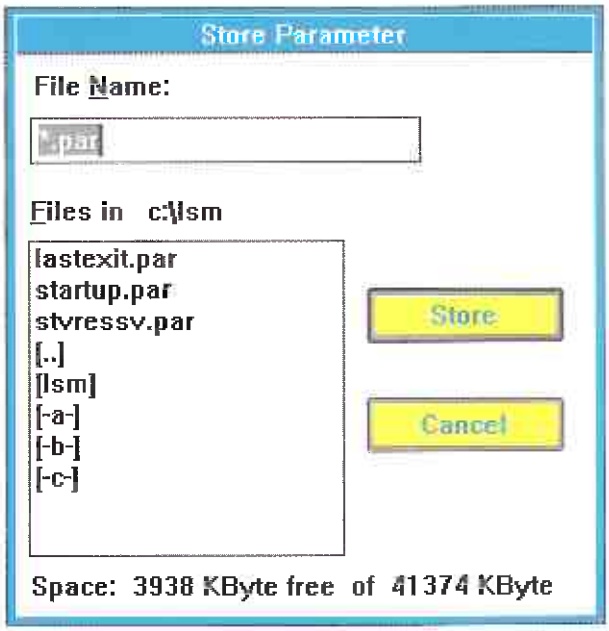


Figure 6-55

(3.11) **Param**

Start with Exit Parameters

When the **Start with Exit Parameters** function is selected, after the function has been clicked the unit automatically restarts with the parameters that were stored automatically the last time the program was ended.
To do this, however, the program must be ended by selecting **File/Quit** in the main menu.

When this option is not activated, the start-up parameters (startup.par file) are loaded when the unit is restarted.

(4) Z

The **Z functions** allow you to record and display confocal images with differing degrees of z depth of the specimen. Thus, 3D depictions of the specimen are possible in different ways.

All functions must be executed in the confocal mode.

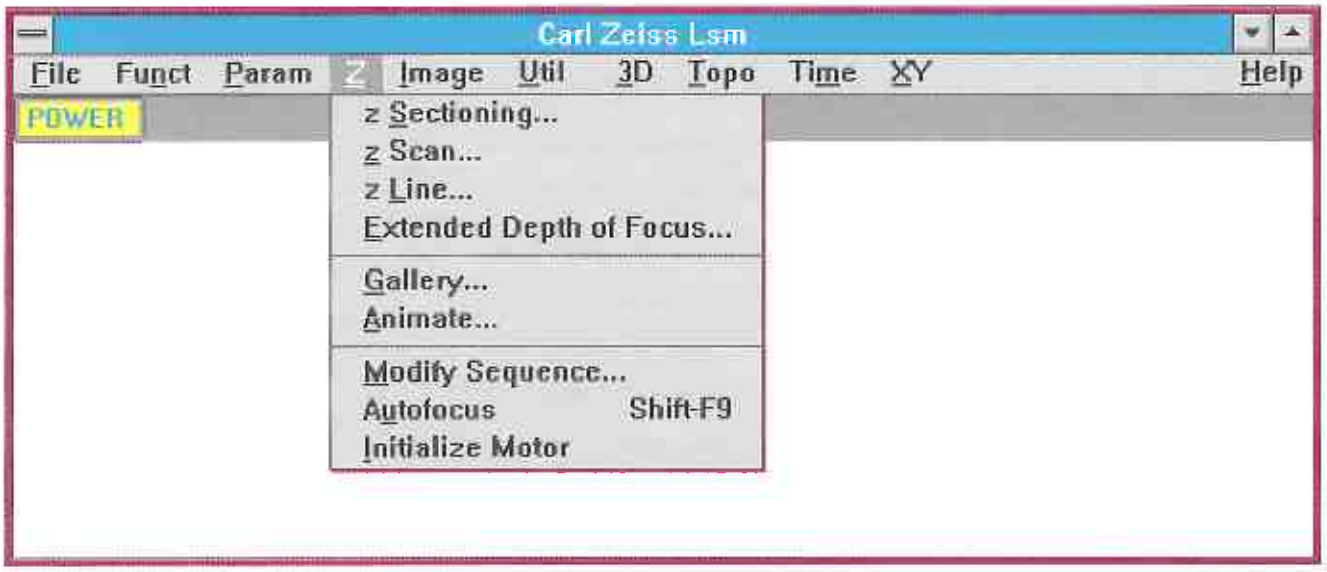


Figure 6-56

Meanings:

z Sectioning...	:	generates series of sections in the z direction
z Scan...	:	generates a scan in the z direction on a chosen line
z Line...	:	generates a scan in the z direction on a chosen point
Extended Depth of Focus...	:	generates an image with greater depth of focus by superposition
Gallery...	:	displays several images as a gallery
Animate...	:	generates a sequence of images similar to a film
Modify Sequence...	:	selects a sequence of images from a larger sequence
Autofocus	Shift F9:	activates the z autofocus
Initialize Motor	:	resets the motor control for x, y and z

(4.1) Z → z Sectioning

The **z Sectioning** function generates series of sections in the z direction by moving the table automatically from one step to another.

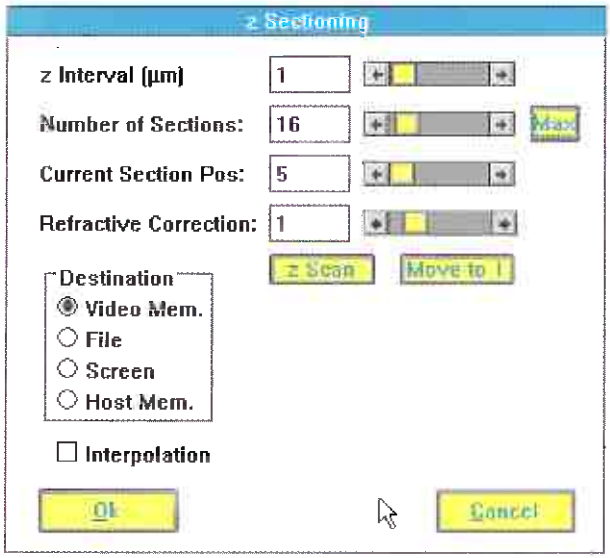


Figure 6-57

z Interval (µm)
Distance between the section planes (can be selected between 0 and 20 µm with the slider in increments of 0,05 µm).
Larger increments can be entered on the keyboard.

Number of Sections
(Adjustable between 1 and 200)

Max

sets the number of increments that still just fits into the video memory or the hard disk. If memory is already occupied, you are prompted as to whether or not you wish to release it beforehand because only in this way is precise determination possible.

Current Section Pos
Number of the current section position in the series (adjustable from 1 to 100), starting with Pos. 1.

Refractive Correction
Enter a refractive correction for the analysed specimen: n2/n1, e.g. air to crown glass 1.51/1.00 = 1.51 (adjustable from 0.5 to 3.0).

z Scan

After clicking, the section line can be moved with the mouse. Pressing the left mouse button produces the section at the chosen position. After sectioning, you can move an arrow on the image monitor with the mouse. The values for **z Interval (µm)** and **Current Section Pos** are altered by moving the continuous lines. The dashed line specifies the current z position.

Move to 1

After clicking, the z motor moves to the starting position of the stack (Current Section Pos: 1). If you press z Scan beforehand, you can set the destination position in the 2-scan image by moving the top continuous line.

Destination box

Select the storage medium in this box.

○ **Memory**
Scan the series into the video memory (max. 4 MB, i.e. 16 full frames of 256 kB each).

○ **File**
Store images on hard disk; in addition to the **Destination** box, the file dialog box

is displayed so you can enter a name for the image sequence.

○ **Screen**
Scan only; this option can be used for a test scan.

○ **Host Mem.**
scans the series into the host memory.

☐ **Interpolation**
This function allows continuous contrast and brightness boosting while recording a series with an absorbing sample by interpolation between the starting and end position.
When this check box is activated, the z motor moves immediately to the end position. The contrast and brightness settings can be defined manually or automatically by pressing AutoAdjust .When producing multiple scans, you must define the setting for all channels. You can set the contrast and brightness again by pressing Ok . The z motor moves to the starting position. After you have redefined the settings and pressed Ok the motor returns to its original position. By pressing Skip , you can switch to the other respective dialog box without saving the settings.

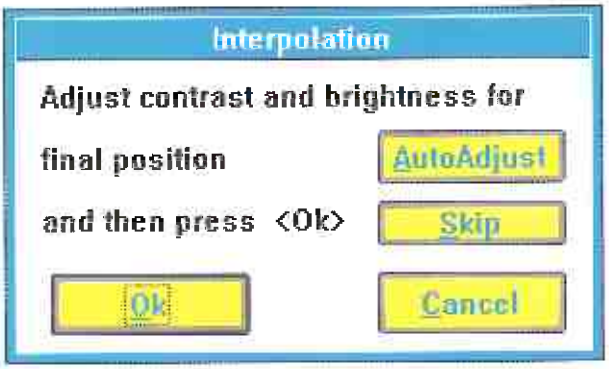


Figure 6-58

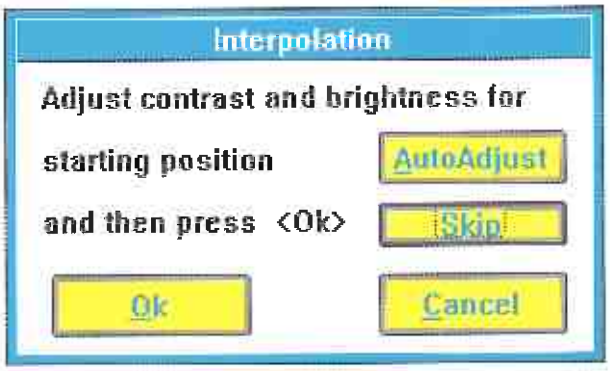


Figure 6-59

Clicking on the corresponding button

Ok
starts the function.

Cancel
closes the dialog window.

(4.2) Z → z Scan...

The z **Scan** function creates a section image that is defined by a freely chosen line in the xy plane and the z direction is scanned and displayed in the image monitor.

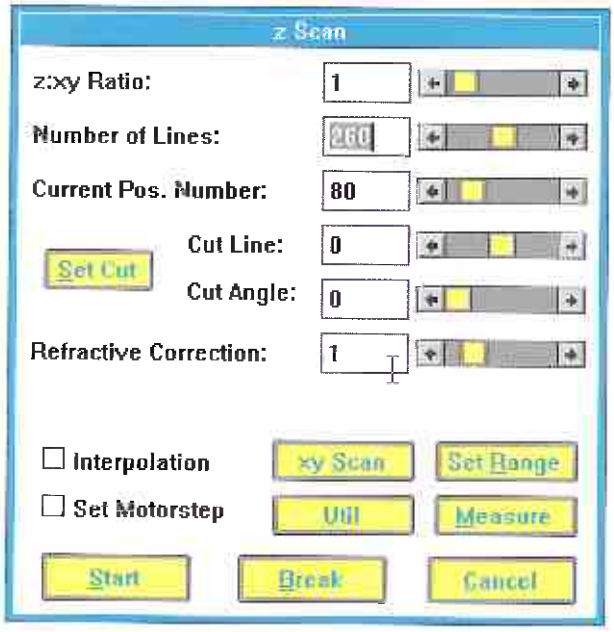


Figure 6-60

z:xy Ratio
Distance of the section lines in the z direction. Normally, this is the ratio to the increment in the xy plane.

When ☐ **Set Motorstep** is clicked, you can enter the z motor's increment directly in µm.

Number of Lines
Number of lines in the z direction to be scanned (variable between 1 and 512).

Current Pos. Number
Number of the current section position in the series (variable from 1 to 512).
You start at position 1.

Cut Line
With this option you can select a section:
-256: top edge of the image
0: image centre
256: bottom edge of the image
with 512 lines and 0° angle.

Cut Angle
Rotation angle of the line (variable from 0° to 359°).

Set Cut
Interactive selection of the cut on the monitor. You can return the mouse pointer to the operating monitor by pressing the right mouse button.

Refractive Correction
Enter a refractive correction for the specimen analysed: n2/n1
e.g. air to crown glass: 1.51/1.00 = 1.51 (variable from 0.5 to 3.0).

☐ **Interpolation**
This function allows continuous contrast and brightness boosting while recording a series with an absorbing sample by interpolation between the starting and end position.
When this check box is activated, the z motor moves immediately to the end position. The contrast and brightness settings can be defined manually or automatically by pressing AutoAdjust.

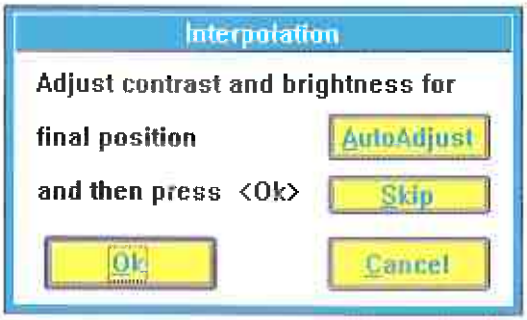


Figure 6-61

When producing multiple scans, you must define the setting for all channels. You can set the contrast and brightness again by pressing Ok. The z motor moves to the starting position. After you have redefined the settings and pressed Ok, the motor returns to its original position.

By pressing Skip, you can switch to the other respective dialog box without saving the settings.

Figure 6-62

☐ **Set Motorstep**
When this function is activated, in the **z:xy Ratio** box the increment of the z motor can be entered directly in µm on the keyboard.

xy Scan
If a scan has been rotated, it is displayed again in such a way that the section line appears horizontally.

Set Range
Two green lines specify the scan area. They can be shifted.

Measure
calls up the measuring function (see Item (2.4) Measure).

Start
starts scanning.

Break
stops scanning.

Cancel
closes the dialog window.

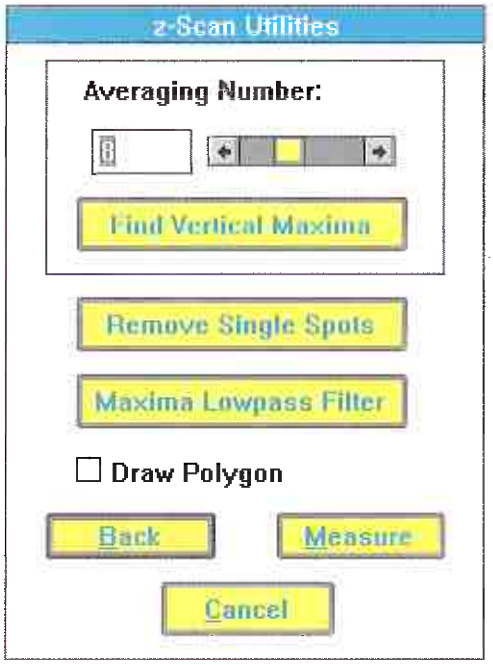


Figure 6-63

Util

calls up the **z-Scan Utilities** sub-dialog window to determine the maxima of each scan.

Find Vertical Maxima

The z position where the z scan exhibits a maximum is identified by a point. At the same time, several points can be averaged to determine the maximum (averaging number).

Remove Single Spots

Isolated points appearing as complete freak values in the general progression can be removed.

Maxima Lowpass Filter

The maxima points can be smoothed by filtering.

☐ **Draw Polygon**

The maxima points can be linked up in a line.

Back

returns you to the starting dialog window **z-Scan**.

Measure

opens the **Measure** dialog window (measuring menu for measuring tasks; Item (2.4)).

Cancel

closes the dialog window.

(4.3) Z → z Line

The z Line function performs a z scan on a freely chosen point (x, y). In doing so, the scanners are not moved and the specimen is scanned in the z direction with the focus motor.

z Interval (µm)
specifies the increment in µm (variable from 0 to 20 µm in increments of 0.05 µm).

Number of Sections
Max. 500.

Current Pos. Number
Number of the measured point that is to be assigned the current z position (from 1 to 500).

Refractive Correction
Enter a refractive correction for the specimen analysed
(from 0.5 to 3, in increments of 0.01)
n2/n1, e.g. air to crown glass 1.51/1.00 = 1.51.

Low Pass
Low pass filtering of the recorded curve (the filter can be used 0 to 9 times).

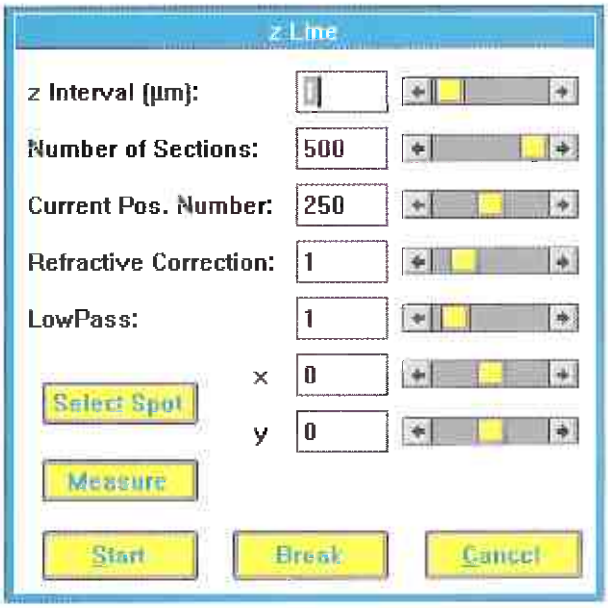


Figure 6-64

Clicking on the corresponding button

Select Spot

allows interactive definition of the measured point, in which case (x, y) represent the coordinates of the measured point (from -256 to +255).

As the phase offset between the moving and idle scanner cannot be compensated exactly, the position of the laser may deviate slightly from the displayed position. This is why you should keep an eye on the respective brightness value when positioning.

When the function is called up, the adjacent sub-menu appears to allow you to enter the (x, y) coordinates.

Measure

calls up the measurement functions (see Section (2.4)).

Start

activates scanning.

Break

stops scanning.

Cancel

closes the dialog window.

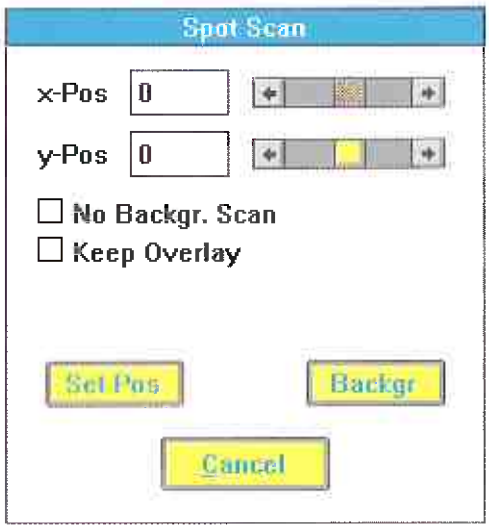


Figure 6-65

(4.4) Z → Extended Depth of Focus...

The **Extended Depth of Focus...** function allows you to overlay the images of a cut point to increase the depth of focus.

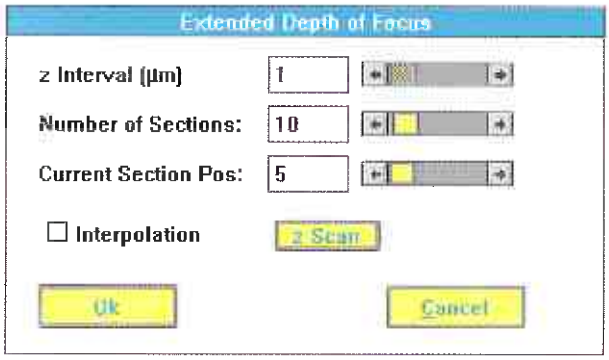


Figure 6-66

z Interval (µm)
specifies the increment in µm (variable from 0 to 20 µm in 0.05 µm increments).

Number of Sections
Max. 100.

Current Section Pos
Number of the current section position in the series (variable from 1 to 100). You start at position 1.

z Scan

When you click this box, the mouse pointer of the operating monitor is displayed and you can move the section line on the image monitor with the mouse. Pressing the left mouse button produces the section at the chosen position. After the section has been produced, you can move an arrow on the image monitor with the mouse.
By moving the continuous lines (move the arrow to the line and then press and hold down the left mouse button), the values for **z Interval** and **Current Section Pos** are modified on-line. The dashed lines specifies the current z position.

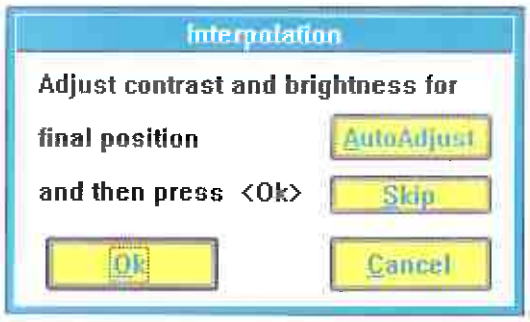


Figure 6-67

☐ **Interpolation**
This function allows continuous contrast and brightness boosting while recording a series with an absorbing sample by interpolation between the starting and end positions.

When this window is activated, the z motor moves immediately to the end position. The contrast and brightness can be set either manually or by selecting AutoAdjust . When carrying out a multiple-channel scan, the setting must be defined for all channels.

After you have set the contrast and brightness again and confirmed the settings by pressing Ok , the z motor moves to the starting position. The motor returns to its original position after setting the values again and pressing Ok .

Select Skip to switch to the other respective dialog box without saving the settings.

Ok
activates scanning.

Cancel
closes the dialog window.

Figure 6-68

(4.5) Z → Gallery...

The **Gallery...** function enables a simultaneous display of several images.

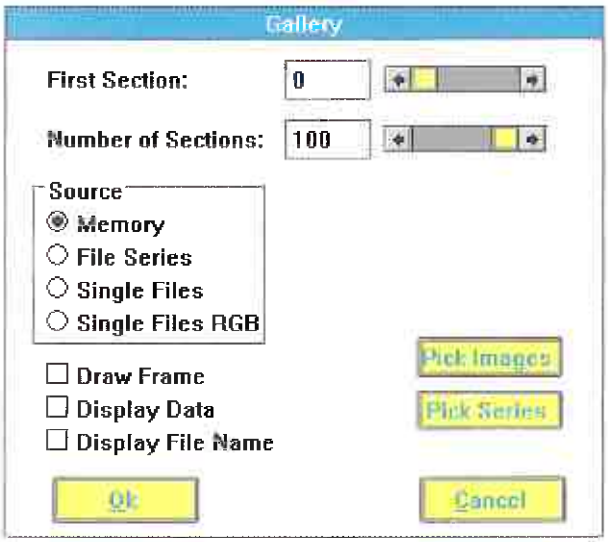


Figure 6-69

First Section
Number of the first section of a sequence to be displayed (ignored when Single Files is selected).

Number of Sections
Number of images to be displayed. This parameter also defines the reduction factor. This value is reduced automatically when a sequence that is lower than this value is displayed.

Source box

The section sequences are fetched as follows

◉ **Memory**
from the computer memory or in the case of
○ **File Series**
from the hard disk.
○ **Single Files**
allows a combination of individual images from the image directory by selecting Pick Images or Pick Series. In doing so, the first loaded image defines the image raster and colour depth (single-colour or RGB) of the entire gallery. In the event of a single-colour gallery, only one LUT can be displayed, namely the last one loaded. To display images with different LUTs in the right colours, select **Single Files RGB**. The selection is made with **Pick Images** or **Pick Series**.
○ **Single Files RGB**
All loaded images are arranged in an RGB image; in doing so, single-channel images are converted to an RGB image on the basis of the stored LUT (look-up table, colour table).

☐ **Draw Frame**
The images are separated by frames.

☐ **Display Data**
The images are assigned the corresponding data (times in time series or z sections in a z scan).

☐ **Display File Name**
The file name appears on the images.

Clicking on the corresponding button

Pick Images
selects individual images from the list of image files (same dialog window as in Figure 6-22).

Pick Series
selects individual images from the series directory (same dialog window as above).

Ok
produces the display when **Memory** or **File Series** has been selected

Cancel
closes the dialog window.

(4.6) Z → Animate...

The **Animate...** function runs a series of images like a film. The number of film images is limited by the size of the video memory (4 MB normal display, 8 MB high-resoluation diasplay).
An image with a size of 512x512 pixels with 256 grey levels requires 256 kB, i.e. up to 16 such images can be stored. A correspondingly larger number of smaller-size images can be stored (smaller amount of data).

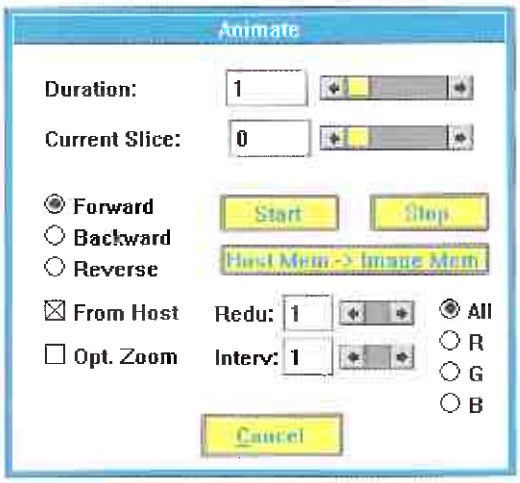


Figure 6-70

Duration
Display duration of each individual image (variable from 1 to 50); this influences the speed at which the series of images is able to run.

Current Slice
Manual selection of single images from the series (still image).

Run direction of the images in the film

◉ **Forward**
from low to high image numbers.
○ **Backward**
from high to low image numbers.
○ **Reverse**
forward and backward alternately.

☐ **From Host**
The animation runs with a sequence that is located in the main memory (host) instead of the video memory. Thus, large sequence can also be animated.
As the rate of data transfer from the host to the video memory is limited, the following data reduction possibilities can be used.

Redu:
The image is reduced by the set factor.
When the image is stopped, the image is always displayed at full resolution.

Interv:
The sequence is thinned; when Interv=3, for example, only every third image in the sequence is displayed.

☐ **Opt. Zoom**
When reducing, the zoom factor is increased automatically so that the animation always fills the screen.

◉ **All**
○ **R**
○ **G**
○ **B**

In RG or RGB sequences, either all channels can be displayed simultaneously or only one of them. When only one channel is displayed, this results in a speed increase of factor 2 (RG) or 3 (RGB).

Clicking on the corresponding button

Start
starts the automatic run.

Stop
stops the automatic run; the image selected with **Current Slice** is displayed on the monitor.

Host Mem -> Image Mem
transfers an image series from the main memory to the video memory for animation.
The quantity of data can be reduced by selecting "Redu", "Interv" and "All/R/G/B" so that a larger sequence will also fit into the 4 or 8 MB video memory. The sequence can then be played with up to 60 frames/second.

Pay attention to the fact that, when animating out of the host memory, in most cases a sequence in the video memory is partly destroyed. Therefore, you should always switch from animation out of the host to animation out of the video memory by selecting the "Host Mem –> Image Mem" button.

A further possibility of reducing data is provided by the **Modify Sequence...** command (4.7).

Cancel
stops the run, the display is reset to the setting in **Display Control** and the dialog window is closed.

The sequence should never be so large that WINDOWS has to swarp parts of it to the hard disk. This leads to the so-called "trashing" effect, i.e. always those images are swapped that are needed next and so a reasonable animation is no longer possible. As 4 kB blocks are always swapped to the hard disk, reduction does not result in any speed increase.

(4.7) Z → **Modify Sequence...**

The **Modify Sequence...** function allows a selection of a smaller sub-sequence out of a larger sequence. The display can also be modified (e.g. with Orthogonal Sections).

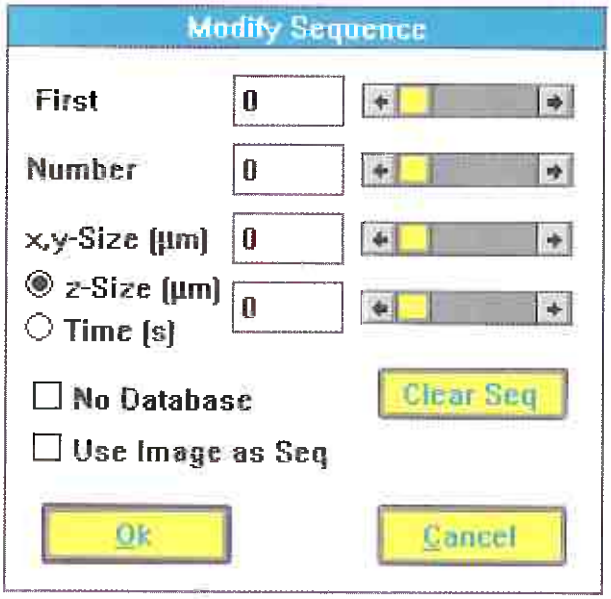


Figure 6-71

First
First image in the sequence (this can be selected out of series from 0 to 999).

Number
Number of images in the sequence (variable from 1 to 1000).

x,y-Size (µm)
The values are set automatically, but different values can also be selected if a different display is required.

◉ **z-Size (µm)** or ○ **Time (s)**
The values for the z sections in a z series or for the time sections in a time series are set automatically, but different values can also be selected if a different display is required.

☐ **No Database**
is activated if the sequence consists of processed images (no longer raw data).

☐ **Use Image as Seq**
can be selected to store an image in the sequence memory, in which case the functions for sequences (e.g. Time Scan) can then be executed on the image.

Clicking on the corresponding button

Clear Seq
clears a sequence from the host memory and releases the occupied memory.

Ok
runs the display.

Cancel
closes the dialog window.

(4.8) Z → **Autofocus**

The **Autofocus** function runs a z scan on a horizontal line in the middle of the image, then searches for the line in it with the highest contrast or, in confocal mode, with the highest brightness, and moves to this point. When higher magnification factors are selected, two runs are executed, a coarse and a fine run. The travel range is limited by the operating distance of the set lens. In the event of extreme deviations from the focus, the focus can no longer be found. The contrast and brightness should also be set so that neither total overshoot nor undershoot occurs.

This function can also be started by pressing the Shift+F9 keys.

(4.9) Z

Initialize Motor

The motor control for x, y and z is reset by activating the **Initialize Motor** function.

This function must be called up if the motor control has ever been switched off and switched on again during a session. The "Motor not ready" error message appears if a connection cannot be established to the motor control.
When the program is started, this function is called up automatically, but an error message is not issued if the motor control is not available.

(5) Image

The image processing functions embrace digital filters, arithmetic and logical image operations, wrong-colour displays and histogram evaluation. The video memory and overlay can also be cleared.

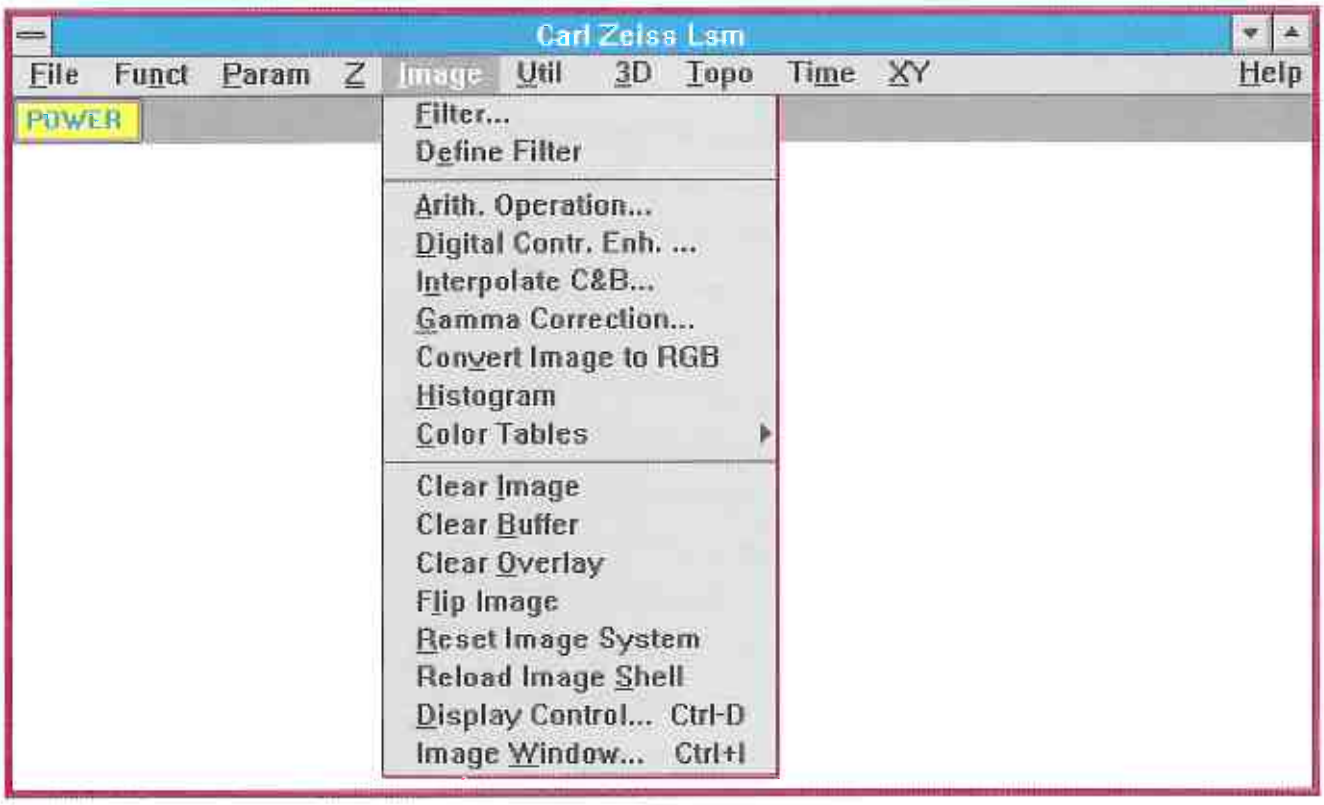


Figure 6-72

Meanings:

Filter...	:	later editing of scanned images with filter functions
Define Filter	:	definition of filters to suit the user's own needs
Arith. Operation...	:	arithmetic joining of images
Digital Contr. Enh. ...	:	later modification of contrast and brightness on the stored image
Interpolate C&B...	:	interpolation of contrast and brightness in a sequence stored on the hard disk
Gamma Correction...	:	generation of an LUT with a gamma correction
Convert Image to RGB	:	8-bit grey images with LUT and images with overlay are converted to an RGB image
Histogram	:	display of the stored image's brightness distribution on the image monitor
Color Tables	▶:	changeover to wrong-colour display
Clear Image	:	deletion of the currently visible image on the image monitor and in the memory
Clear Buffer	:	deletion of the scanned video memory contents
Clear Overlay	:	deletion of the overlay
Flip Image	:	mirroring of the image along the vertical middle line
Reset Image System	:	reset of the image processing board to a defined initial state
Reload Image Shell	:	reload image shell to the image processing board
Display Control... Ctrl-D:		activates the Display Control window

(5.1) Image → Filter...

The Filter... function enables later editing of scanned images with filter functions.

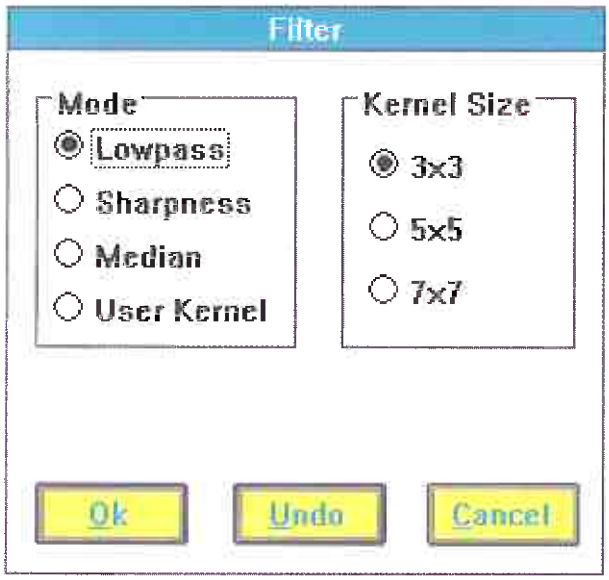


Figure 6-73

Mode box

◉ **Lowpass**
An activated low-pass filter reduces image noise, but image details may be lost.

○ **Sharpness**
improves the sharpness impression of the image. When selected, an additional parameter: **Divisor**: appears in the **Filter** dialog window (the value of the divisor is variable from 1 to 78)

○ **Median**
smoothes out freak values in a stored image without having a detrimental influence on fine structures as when using a low-pass filter.

○ **User Kernel**
User-defined filter functions can be loaded from a storage medium (hard disk, optical disk or floppy). After selection, the Load Kernel button appears in the **Filter** dialog window.
When Load Kernel is clicked, an additional menu for loading the filter matrix appears (redefine filter functions via **Image/Define Filter**).

Kernel Size box

◉ **3x3**
○ **5x5**
○ **7x7**

Allows you to select the size of the filter matrix but, in doing so, the effect of a filter also increases as the matrix size increases. The time needed for filtering therefore also increases.

Clicking on the corresponding button

Ok
starts the filter function.

Undo
reverses the last filter operation.

Cancel
closes the dialog window.

(5.2) Image → Define Filter

The **Define Filter** function allows you to define filters according to your own requirements.

You can make entries for the required filter matrix in the so-called editing window.

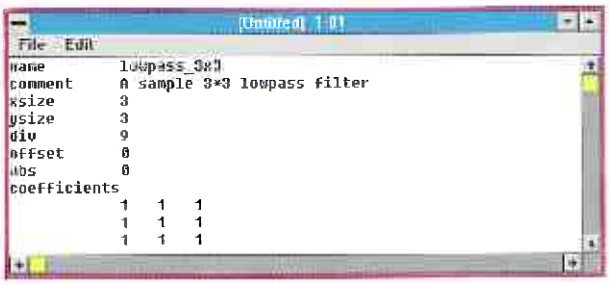


Figure 6-74

The entries have the following meanings:

name	: Filter name, any text
comment	: Additional comment, any text
xsize	: Number of columns
ysize	: Number of lines
div	: Divisor, which must only be used when **norm** is missing
norm	: Scaling factor, corresponds to **1/div**
offset	: is added to the result
abs	: 1: the amount is subtracted from the result
coefficients	: coefficients of the filter matrix; here, **ysize** lines, each with **xsize** integral numbers, must follow

(5.3) Image → Arith. Operation...

The **Arith. Operation...** function allows you to arithmetically join images.

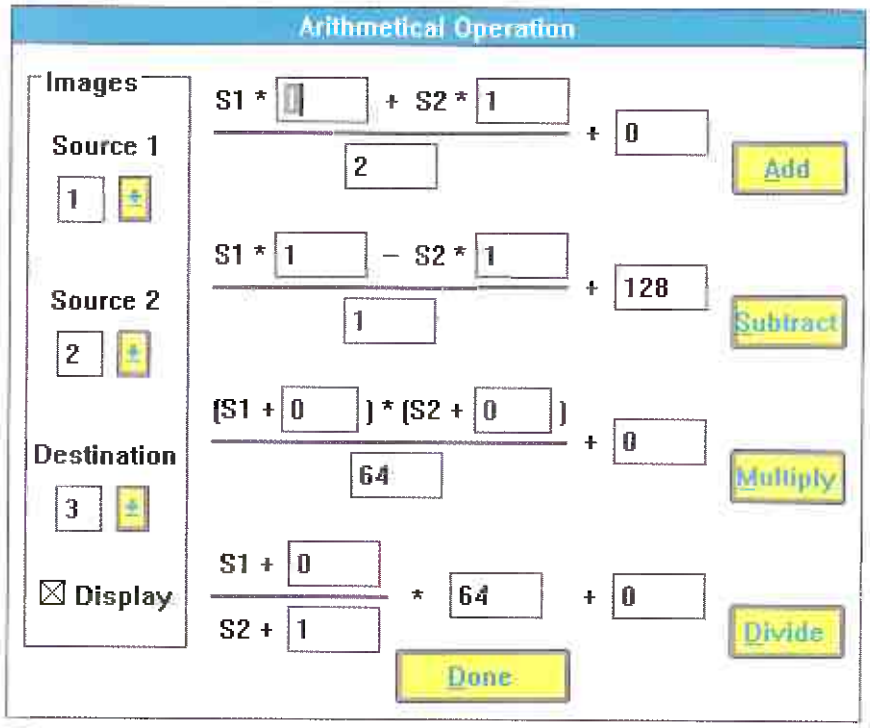


Figure 6-75

Images box

Source 1 and **Source 2**
Source of images in the memory (can be selected from 1 to 4 in the fold-down menu).

Destination
Video memory into which the edited image is written.

☐ **Display**
The operation is displayed immediately when the display is activated.

Clicking on the corresponding button

Add
adds images with freely selectable constants (divisor, factors and offset).

Subtract
subtracts images with freely selectable constants (divisor, factors and offset).

Multiply
multiplies images with freely selectable constants (divisor, factors and offset).

Divide
results in division of images with freely selectable constants (divisor, factors and offset).

Done
closes the dialog window.

(5.4) Image → Digital Contr. Enh. ...

The **Digital Contr. Enh. ...** function allows you to modify the contrast and brightness of a stored image later on.

Gain
The values for the gain can be entered either directly or by means of sliders (variable from 0.02 to 20, in increments of 0.02).

Offset
The values for the offset can be entered either directly or by means of sliders (variable from -255 through 0 to +255).

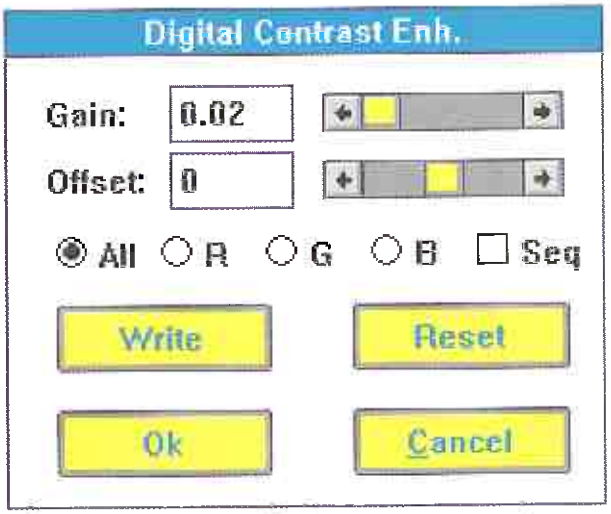


Figure 6-76

In the case of RGB images, all colour components can be optionally set together **(All)** or independently of one another **(R, G, B)**.
Before saving them, however, corrected images should be written into the video memory by pressing Write because TIF format does not provide for LUT in the case of RGB images.
If you activate the ☐ **Seq** checkbox, when pressing Write the complete video memory is always modified, and not only the current image or the ROI.

Clicking on the corresponding button

Write
writes the modified image into the video memory and, in doing so, the original image is overwritten.

Reset
sets **Gain** to 1 and **Offset** to 0.

Ok
closes the dialog window; the settings defined remain unchanged.

Cancel
closes the dialog window and the contrast and brightness are returned to their original values.

(5.5) Image → Interpolate C&B...

The **Interpolate C&B...** function allows you to interpolate the contrast (logarithmically) and brightness (linear) of a sequence stored on the hard disk.

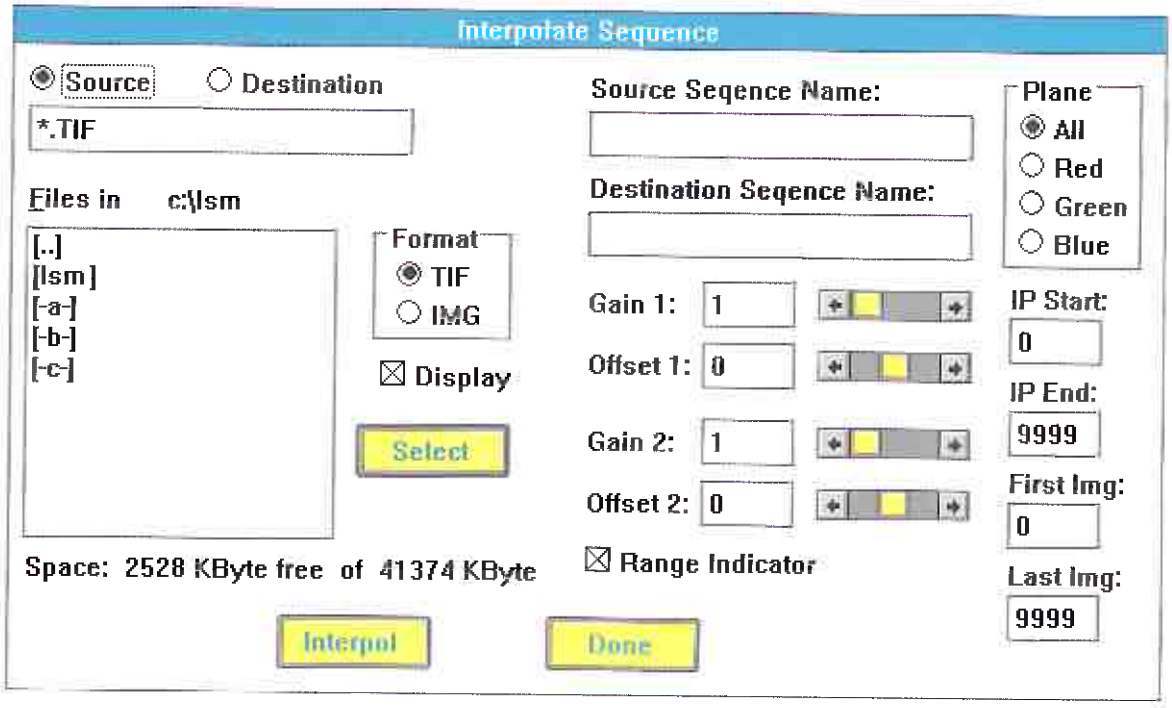


Figure 6-77

Plane box

- ◉ **All**
- ○ **Red**
- ○ **Green**
- ○ **Blue**

In the case of RGB sequences, the colour components can be interpolated together (plane: **all**) or independently of one another (plane: **red, green** or **blue**). To do this, the **Gain 1, Offset 1** and **Gain 2** and **Offset 2** values must be set again for each colour component.

Gain 1 and **Gain 2**
The values for the gain can be entered either directly or by means of sliders (variable from 0.1 to 20, in increments of 0.01).

Offset 1 and **Offset 2**
The values for the offset can either be entered directly or by means of sliders (variable from -255 through 0 to +255).

IP Start and **IP End**
The start and end of the sequence can be excluded from interpolation, i.e. all images ≤ **IP Start** are corrected with **Gain 1/Offset 1** and all images ≥ **IP End** are corrected with **Gain 2/Offset 2.**

First Img and **Last Img**
Correction can be limited to a part of the sequence, thus allowing interpolation in sections.

☐ **Range Indicator**
The range indicator displays portions of the image with overshoot (red) and undershoot (blue). This helps when optimising the contrast and brightness.

Clicking on the corresponding button

Interpol
runs the interpolation.

Done
closes the dialog window.

(5.6) Image → Gamma Correction...

The **Gamma Correction...** function allows you to create an LUT with a gamma correction.

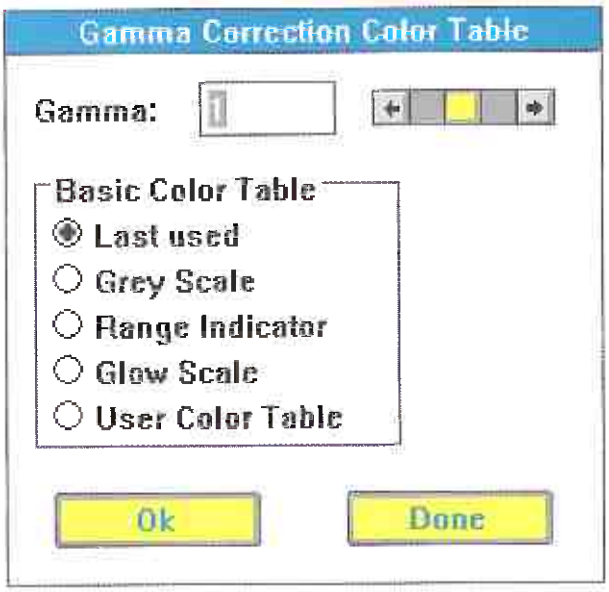


Figure 6-78

Gamma
Gamma correction values (variable from 0.1 to 2 in increments of 0.1).

Basic Color Table box

defines the required LUT:

- ◉ **Last used**
- ○ **Grey Scale**
- ○ **Range Indicator**
- ○ **Glow Scale**
- ○ **User Color Table**

Clicking on the corresponding button

Ok

runs the required correction.

Done

ends the function and closes the dialog window.

(5.7) **Image** 
Convert Image to RGB

The **Convert Image to RGB** function generates a 24-bit image from the monitor image.

(5.8) Image → Histogramm

The **Histogramm** function allows a display of the brightness distribution of the stored image on the image monitor.

Figure 6-79

NOTE The histogram overlay can be cleared with the **Image/Clear Overlay** function.

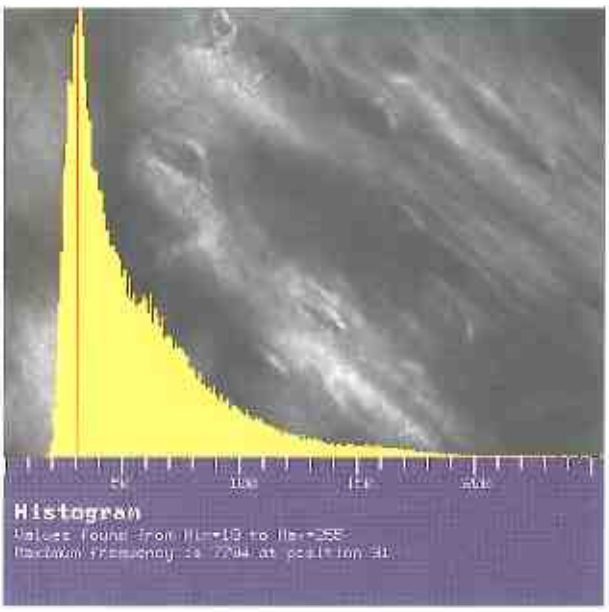


Figure 6-80

(5.9) Image → Color Tables

The **Color Tables** command opens a further window in which various wrong-colour modes can be selected.

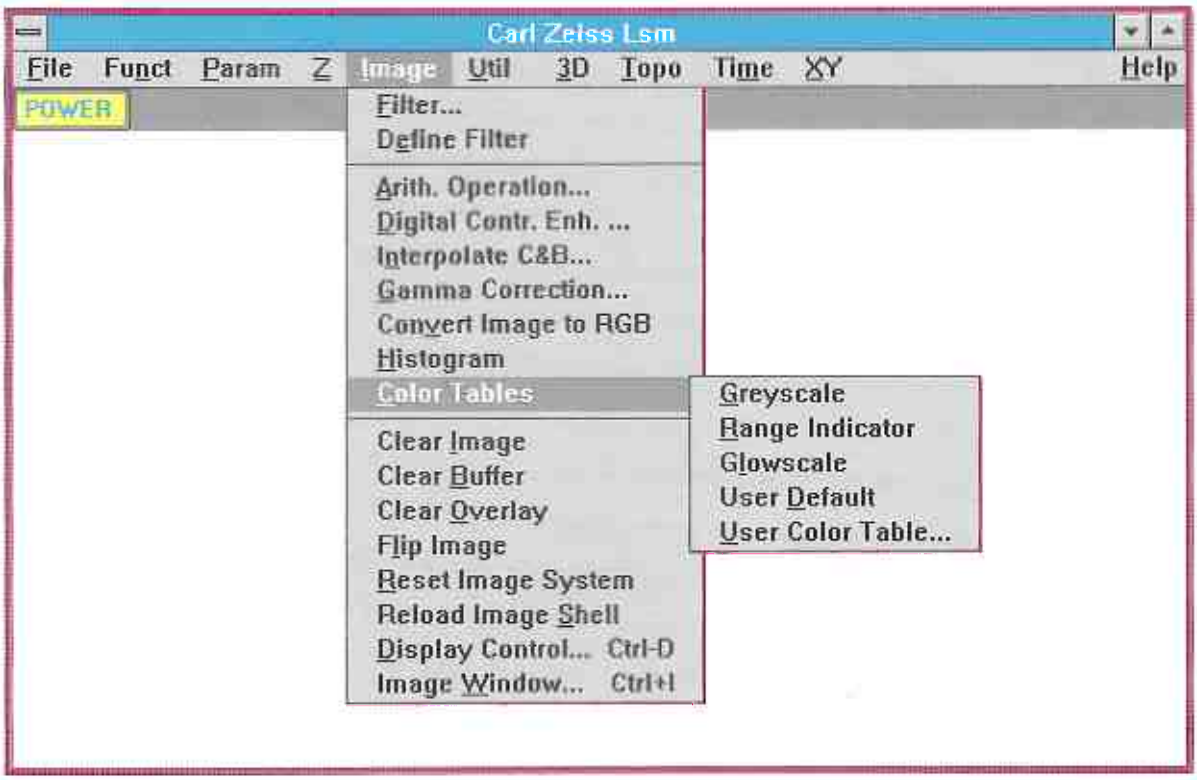


Figure 6-81

Meanings:

Greyscale
Normal monochrome display with 256 grey levels.

Range Indicator
The range indicator indicates portions of the image that have overshoot (red) and undershoot (blue). This helps to optimise the contrast and brightness.

Glowscale
Wrong-colour display preset in the program.

User Default
A colour table stored under DEFAULT.LUT is called up.

User Color Table...
In the wrong-colour display, each grey value (0...255) is assigned a specific RGB triplet.
This menu item opens the **Color Tables** dialog window.

Color Table
The color table allows you to generate any chosen wrong-colour display. The colour assignments may be restricted to any sub-ranges of the grey value scale. The range is defined by the starting point A and the end point B. The colour values are then interpolated between these points.

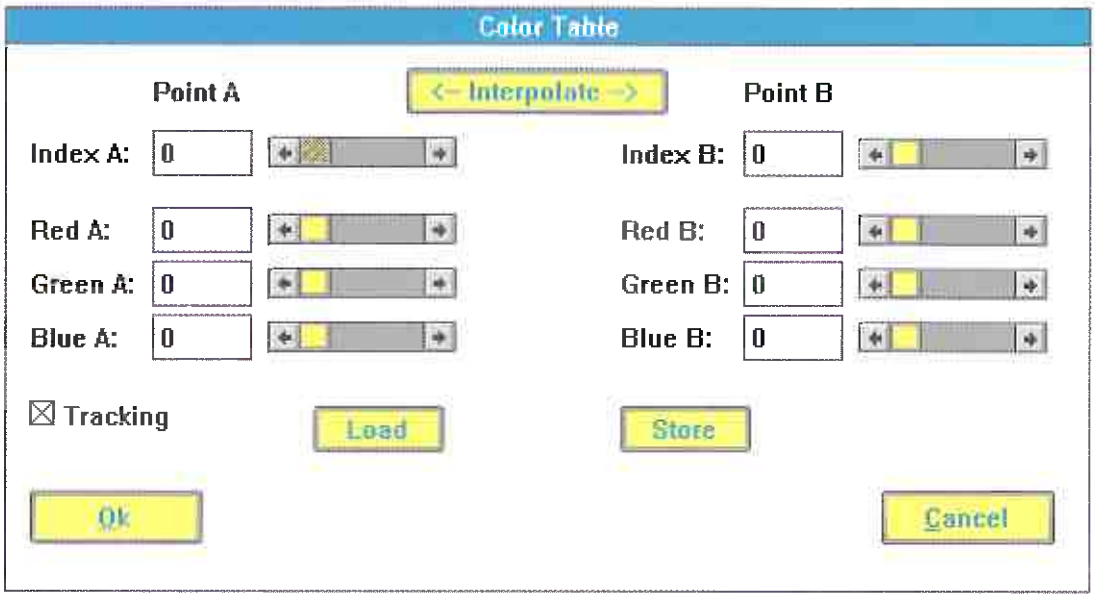


Figure 6-82

Index A and **Index B**
defines the range (variable from 0 to 255).

Red A, Green A, Blue A and **Red B, Green B, Blue B**
Colour components at points A or B (variable from 0 to 255).

<-Interpolate->
interpolates the transition colours from A to B.

☐ **Tracking**
When tracking is activated a change in the colour components is displayed on-line on the image monitor.

To arrive at a smooth progression of the LUT, it is advisable to always only change one of the two points A and B alternately, for example as follows:

Index A to 0,
set colour components of A.

Index B to 85,
set colour components of B and click Interpolate.

Index A tp 170,
set colour components of A and click Interpolate.

Index B to 255,
set colour components of B and click Interpolate.

In Tracking mode, you do not need to click Interpolate every time.

You can check the result in a bar displayed at the top of the image.

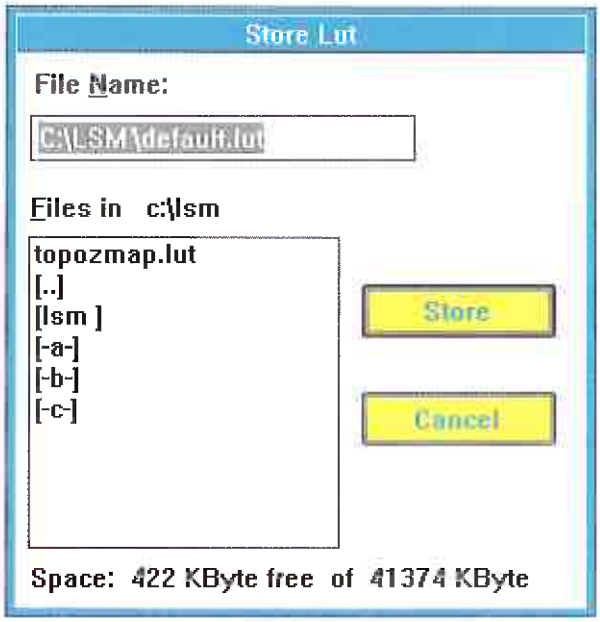


Figure 6-83

Store

stores a wrong-colour table. The adjacent submenu is opened. The name must not be more than 8 characters long The .LUT extension is added automatically.

Load

loads a wrong-colour table.

Ok

produces the wrong-colour display.

Cancel

closes the dialog window.

(5.10) Image

Clear Image

The **Clear Image** command clears the currently visible image on the image monitor and in the memory.

(5.11) Image

Clear Buffer

The **Clear Buffer** command clears the complete contents of the video memory.

(5.12) Image

Clear Overlay

The **Clear Overlay** clears the displayed overlay (e.g. a histogram).

(5.13) Image

Flip Image

The **Flip Image** command mirrors the image about the vertical middle line.

(5.14) Image

Reset Image System

The **Reset Image System** resets the image processing boards to a defined initial state. In doing so, the contents of the video memory are not cleared.

(5.15) Image

Reload Image Shell

The **Reload Image Shell** command reloads the image shell to the image processing board.

(5.16) Image

Display Control...

The **Display Control...** command activates the adjacent dialog window.

Image box

- ◉ **1 (R)**
- ○ **2 (G)**
- ○ **3 (B)**
- ○ **4**
- ○ **RGB**

The video memory is selected in this box. Up to 4 video memories are available and the video memories 1 (R), 2 (G) and 3 (B) are superimposed by clicking RGB. The individual contents of the memories are colour-coded (red, green and blue) with 256 colour levels each. The scan is placed in the selected memory.

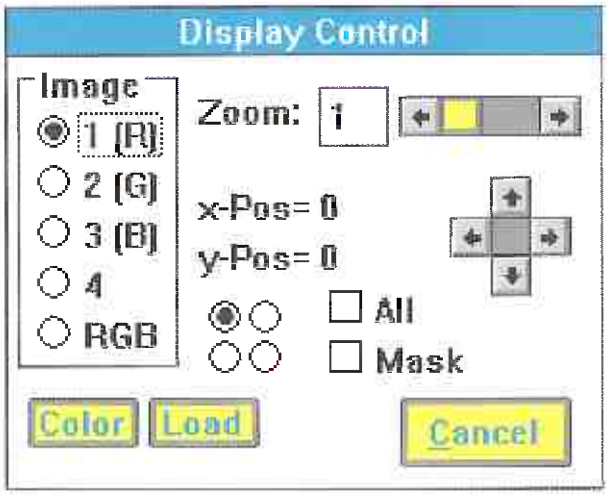


Figure 6-84

Zoom
This function enlarges or reduces the monitor image (image monitor). The values can be modified directly by means of the slider (variable from 1 to 16).

x-Pos=0 and **y-Pos=0**
The contents of the video memory can be moved on the image monitor by means of the **cross-shaped control**. The current position is displayed for x and y. To do this, click on the arrows with the left mouse button. Clicking with the left mouse button on the middle of the cross centres the image again. If you click on the middle with the right mouse button, the image is moved according to movement of the mouse. In doing so, the mouse pointer becomes invisible. This function can also be made permanent by double clicking with the right button. Movement is then active until one of the two mouse buttons is pressed.

◉ ○
○ ○

When you select one of these points, the image is moved to the top left/right or bottom left/right corner of an area measuring 1024x1024 pixels (when using 8 MB video memory, the area has a size of 2048x1024 pixels). The point of this setting possibility is as follows: If you produce a video printout of a monitor image, something is frequently cut off the left and top edges. This can be avoided by shifting the contents of the image slightly to the bottom right on the image monitor. In the case of RGB images, owing to hardware restrictions they can only be moved on a normal-resolution display if the image is not on the left edge of the area.
In the case of images up to a size of 512x512, you therefore also have a possibility of buffering 16 grey level images or 4-RGB images. However, you should pay attention to the fact that some operations may partly overwrite these images. This applies above all to filter operations and to loading or recording image series in the video memory.

☐ **All**
When this function is activated, the entire contents of the video memory are displayed.

☐ **Mask**
When 60 Hz is set, a mask is displayed that covers up the area that would no longer be printed out by an NTSC video printer.

Clicking on the corresponding button

Color
allows you to reset a wrong-colour table and, by multiple clicking, to select one of the standard LUTs or the user default LUT.

Load
allows you to load any LUTs; the "Load Lut" sub-menu is displayed (see also Image/Color/Tables).

Cancel
closes the dialog window.

(6) Util

The utilities embrace test images, version information and special settings.

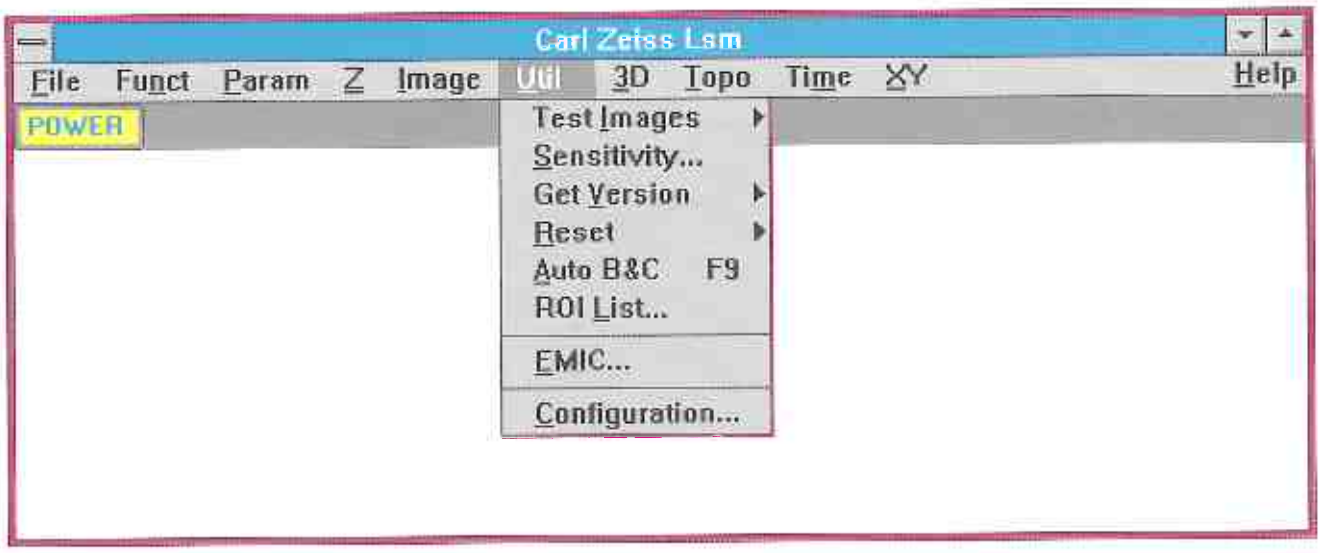


Figure 6-85

Meanings:

Test Images	▶ :	test images for adjusting the image monitor
Sensitivity...	:	display of gain values for quantitative brightness comparisons
Get Version	▶ :	display of status information relating to the hardware/software
Reset	▶ :	reset functions
Auto B&C	F9 :	automatic setting of contrast and brightness
ROI List...	:	definition of ROI lists
EMIC...	:	control functions for emission microscopy
Configuration...	:	accessible to service employees only!

(6.1) Util → Test Images

Test images for adjustment of the image monitor can be called up by means of the **Test Images** command and the affiliated fold-down menu.

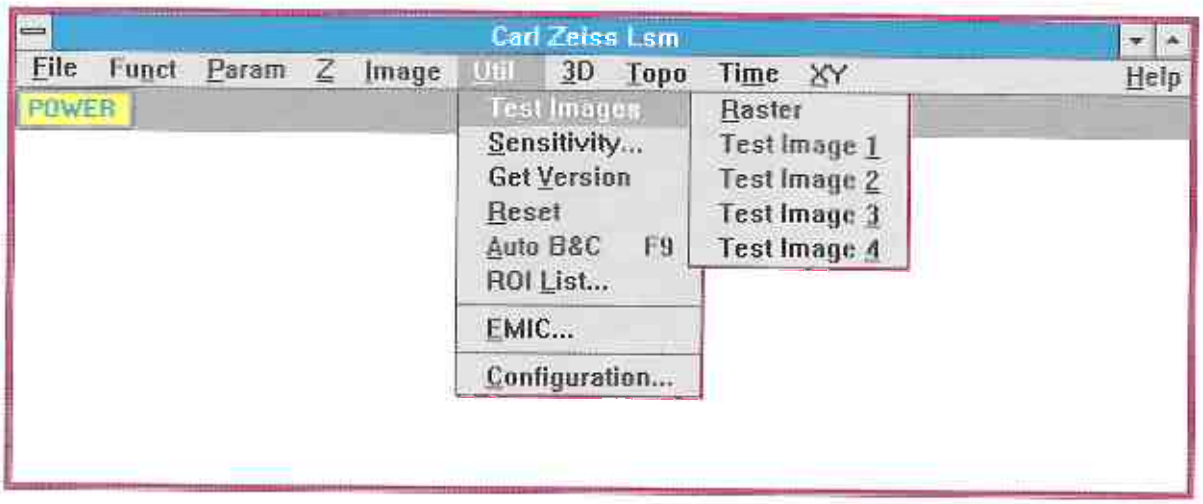


Figure 6-86

Raster
Grid and circle display in the overlay (red); this can be cleared again with Image/Clear Overlay.

Test Image 1
Test image 1 with 4x8 squares with a defined grey value distribution.

Test Image 2
Test image 2 with 16x16 rectangular areas containing the grey values 0 to 255.

Test Image 3
Test image 3 with a diagonally floating defined grey value distribution.

Test Image 4
Test image 4 with a floating defined grey value distribution from top to bottom.

(6.2) **Util** → **Sensitivity...**

The **Sensitivity...** function reads the gain values set by the contrast and brightness and displays them in a window.

It displays them as follows

in reflected illumination mode:

- **Voltage Gain** (electronic gain)
- **PMT-Voltage**
- **Sensitivity** (the sensitivity in relative units calculated on the basis of both values)

in transmitted light mode:

- **Voltage Gain** (electronic gain)
- **Sensitivity** (in relative units)

Therefore, this function allows a quantitative brightness comparison.

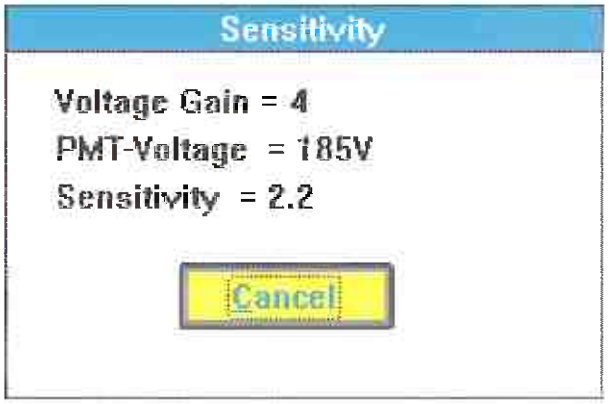


Figure 6-87

(6.3) Util → Get Version

Information about the hardware and software can be called up by selecting the **Get Version** command and by means of the affiliated fold-down menu.

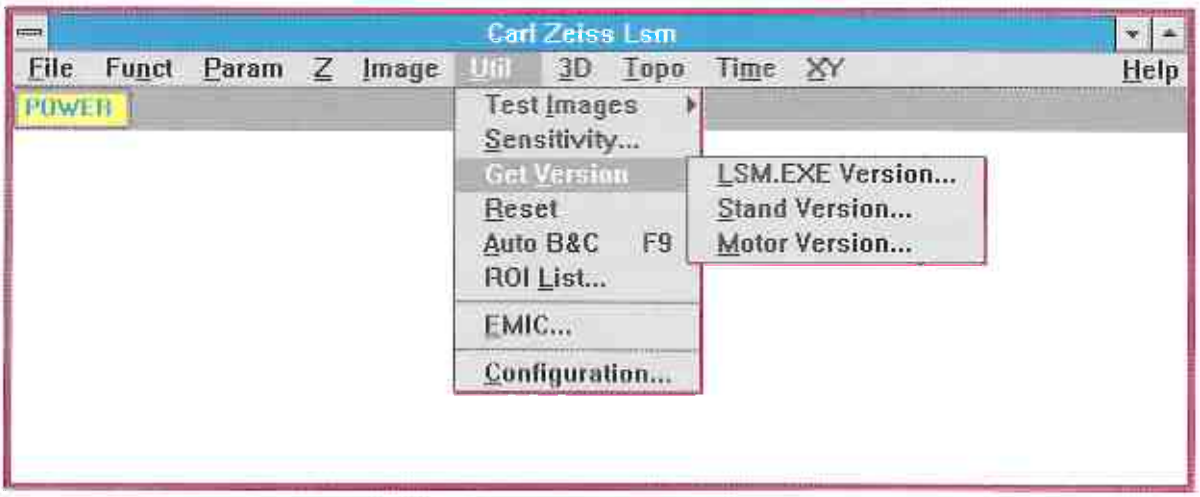


Figure 6-88

LSM.EXE Version...
Information about the Zeiss LSM program is displayed (same window as in Figure 6-30).

Stand Version...
reads the version number of the stand's firmware.

Motor Version...
displays information about the motor's software.

(6.4) Util → Reset

Reset functions can be run by means of the **Reset** command and the affiliated fold-down menu.

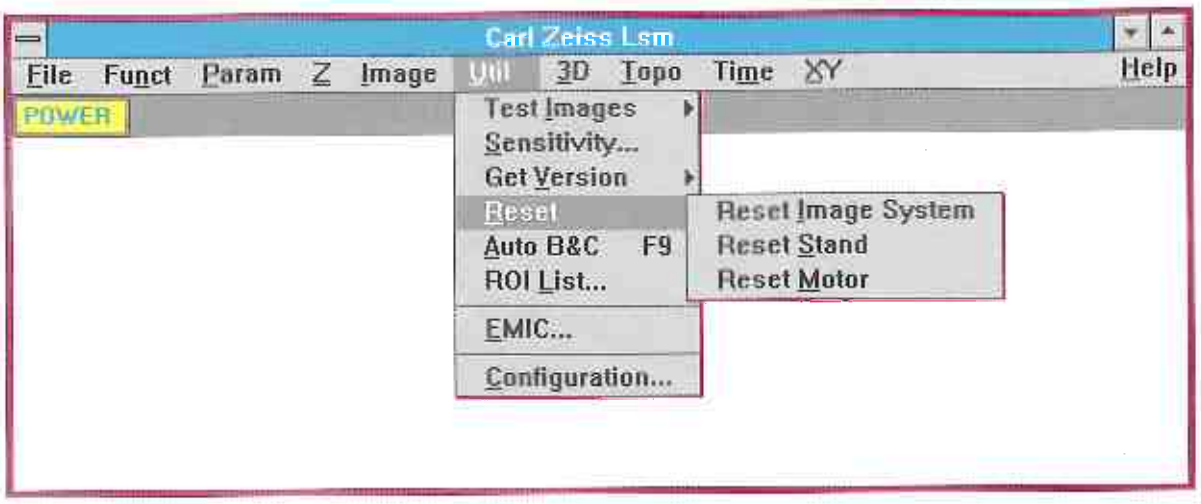


Figure 6-89

Reset Image System
resets the image system.

Reset Stand
hardware-resets the stand.

Reset Motor
Motor reset for x, y and z (same function as **Z/Initialize Motor**).

(6.5) Util

Auto B&C

By means of the **Auto B&C** command, the brightness B and contrast C are automatically displayed so that overshoot or undershoot just does not occur. When performing a multiple-channel scan, only the activated channel (Chan) is set.

You can arrive at an optimum setting as described in Section 5.2 (3).

(6.6) Util

ROI List...

By means of the **ROI List...** command, you can define, modify and store up to 10 ROIs and you can activate them again by clicking on the list.

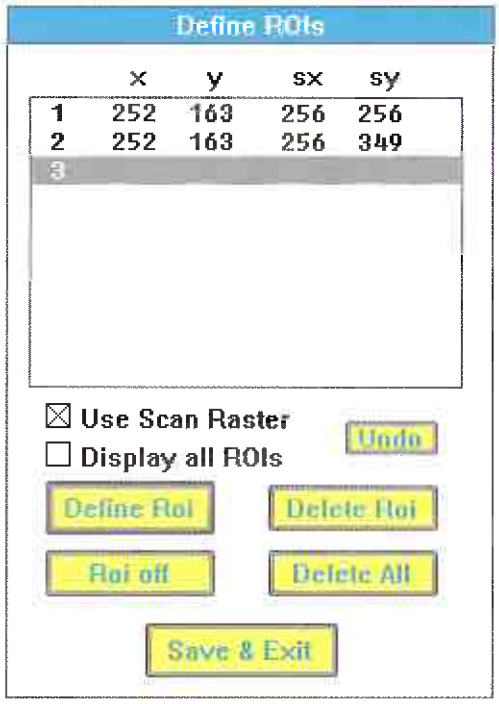


Figure 6-90

(6.7) Util → EMIC...

By means of the EMIC... command and the affiliated dialog window, you can control emission microscopy with a high-sensitivity cooled CCD camera.

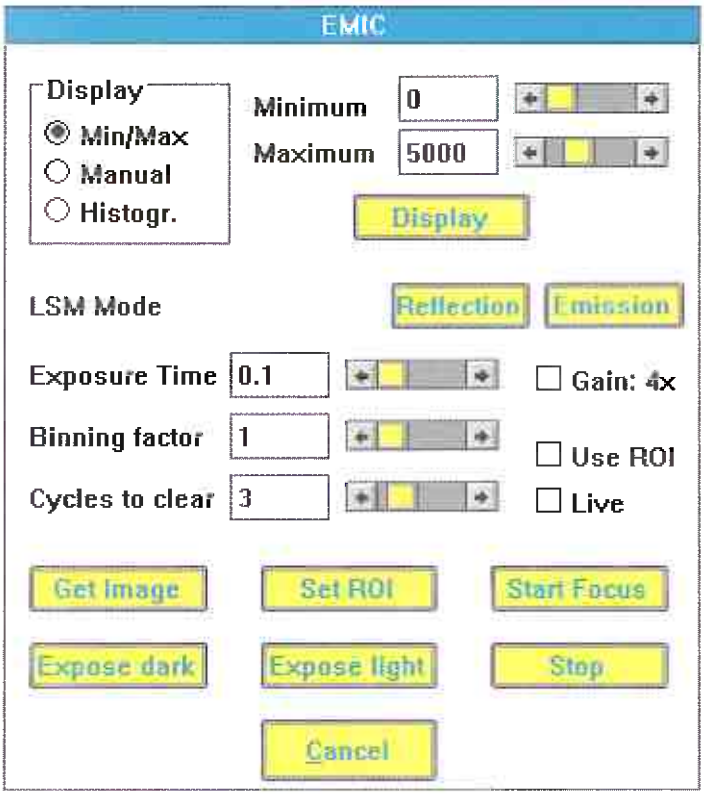


Figure 6-91

Cameras have a resolution of either 14, 15 or 16 bits.
However, only 8 bits are displayed. This is limited in the **display** box.

◉ **Min/Max**
linear between the automatically determined minimum and maximum.

○ **Manual**
linear between the manually entered minimum and maximum.

○ **Histogr.**
Display weighted with the histogram, not linear.

LSM Mode
Reflection | Emission

Exposure Times
0.01 to 10 s when reflection is selected.
1 to 600 s when emission is selected.

Binning factor Binning factor (variable from 1 to 100)
1: unchanged.
2: 2x2 pixels are averaged to one pixel.
3: 3x3 pixels are averaged to one pixel.

Cycles to clear
Clearing cycles of the CCD camera (variable from 0 to 20).

☐ **Gain: 4x**
The sensitivity is increased by a factor of 4, as is also the read-out time.

☐ **Use ROI**
The "region of interest" is displayed.

☐ **Live**
The Live display is activated.

Clicking on the corresponding button

Get Image
deactivates any activated **Use ROI** and/or **Live** boxes.

Set ROI
defines the "region of interest".

Start Focus
displays the "region of interest" live; the two **Use ROI** and **Live** boxes are activated automatically.

Expose dark
displays dark current.

Expose light
results in scanning of an image depending on how the two Use ROI and Live boxes are set.

Stop
deactivates the Live mode.

Cancel
closes the dialog window.

(6.8) Util → Configuration...

The LSM system is configured by means of the **Configuration...** command and the affiliated dialog window.

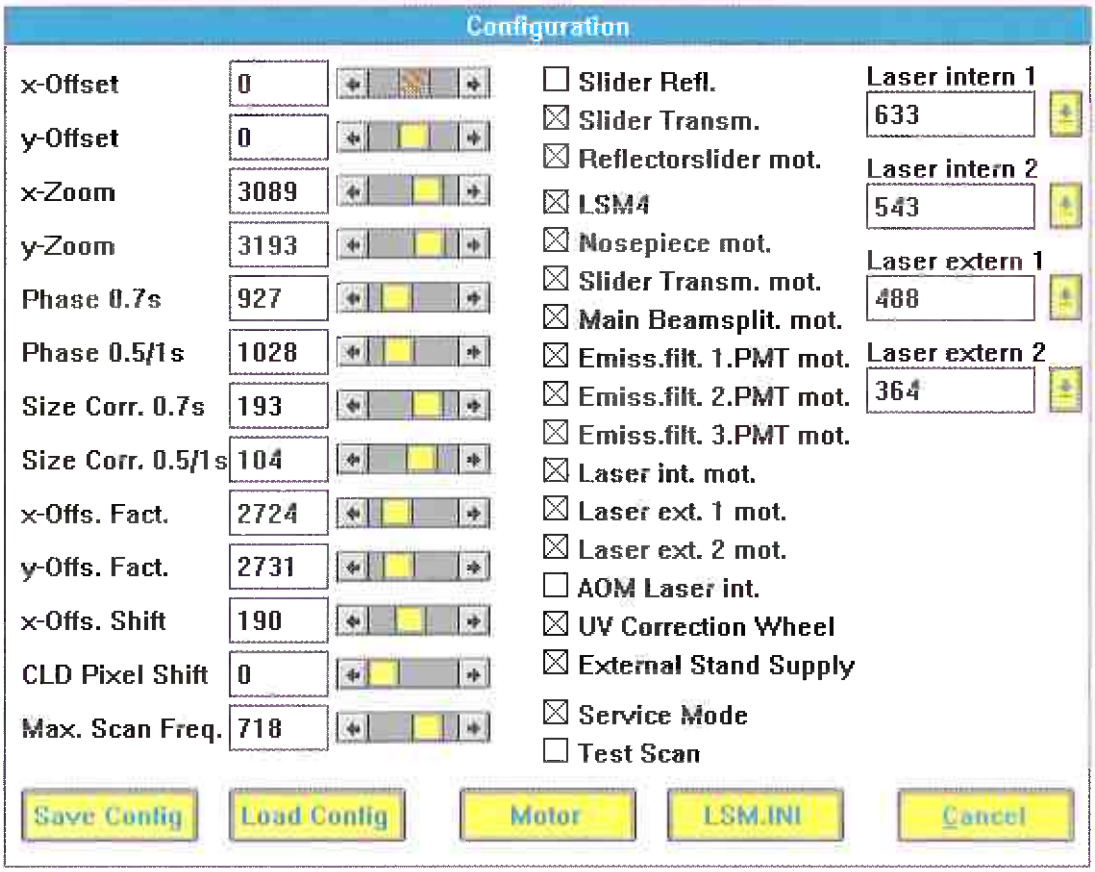


Figure 6-92

The **Configuration** dialog window is intended for service personnel only.

(7) 3D

The **3D** functions serve to record and play back series of images for 3D display of microscopic structures.
This option is obtainable by quoting the order number 480088-8040.

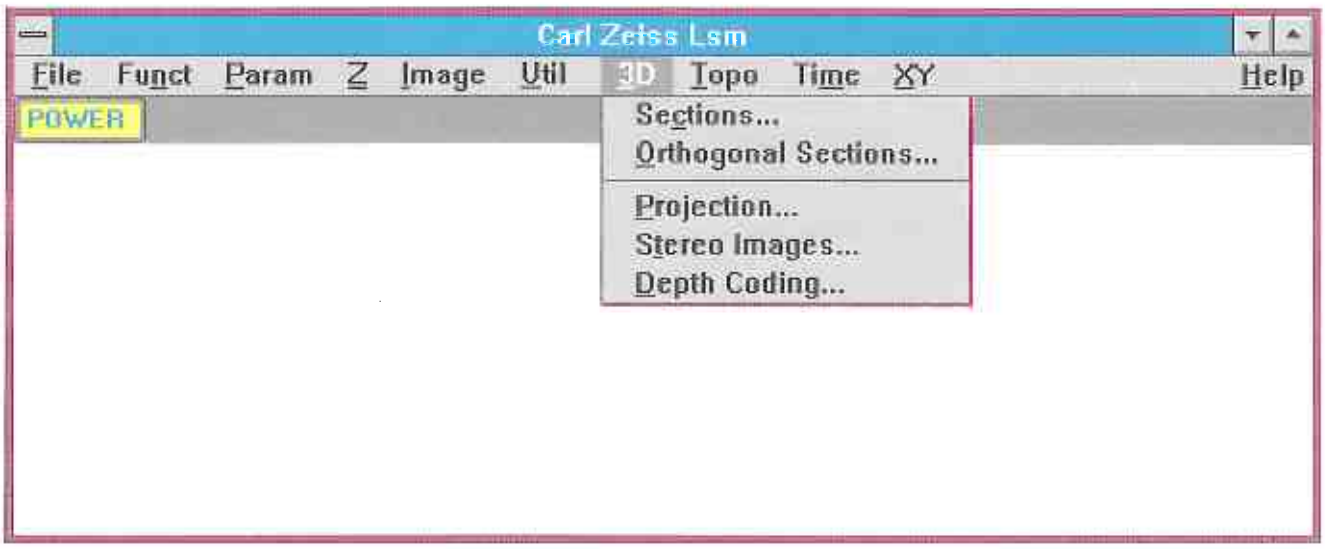


Figure 6-93

Meanings:

Sections...	:	section through a three-dimensional section stack
Orthogonal Sections...	:	display of orthogonal section areas parallel to the 3 principal planes
Projection...	:	computation of one single projection or of a projection series after rotation of the data package by the x, y or z axis
Stereo Images...	:	display of stereoscopic images, which can be viewed with red/green spectacles
Depth Coding...	:	elucidation of depth information by colour graduation

(7.1) 3D → Sections...

A section can be placed through a three-dimensional section stack by means of the **Sections...** command and the affiliated dialog window.

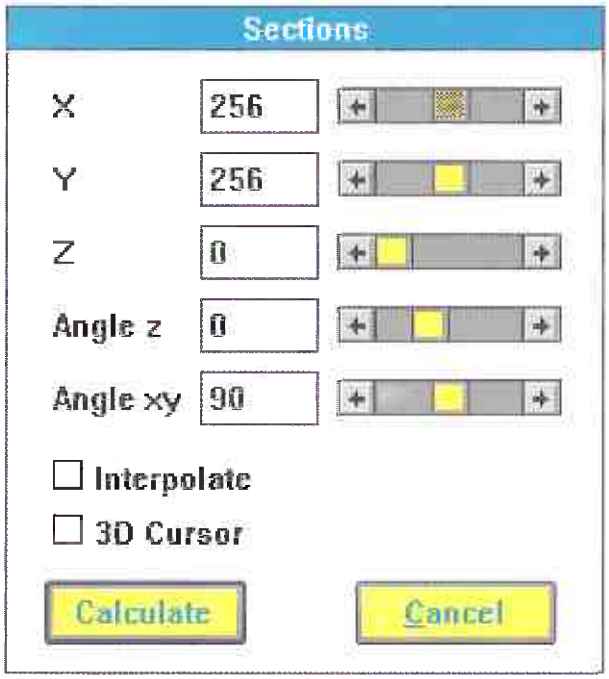


Figure 6-94

The section plane is defined by a point in the plane (x, y and z) and the tilt angle of the plane's normal with the z axis (angle z) and the rotation angle in the xy plane (angle xy).

☐ **Interpolate**
Activating this option results in interpolation between the section planes (increased time requirement).

☐ **3D Cursor**
Activating this option displays the three-dimensional cursor in the section plane.

Calculate
calculates the section plane.

Cancel
closes the dialog window.

(7.2) 3D → Orthogonal Sections...

By means of the **Orthogonal Sections...** command and the affiliated dialog window, orthogonal sections parallel to the principal planes xy, xz and yz can be selected and displayed. The section point (x, y and z) of these three planes can be chosen freely.

The coloured frames on the image monitor identify section areas parallel to the
xy plane blue
xz plane green
yz plane red.

The coloured lines on the image monitor indicate the section lines themselves.

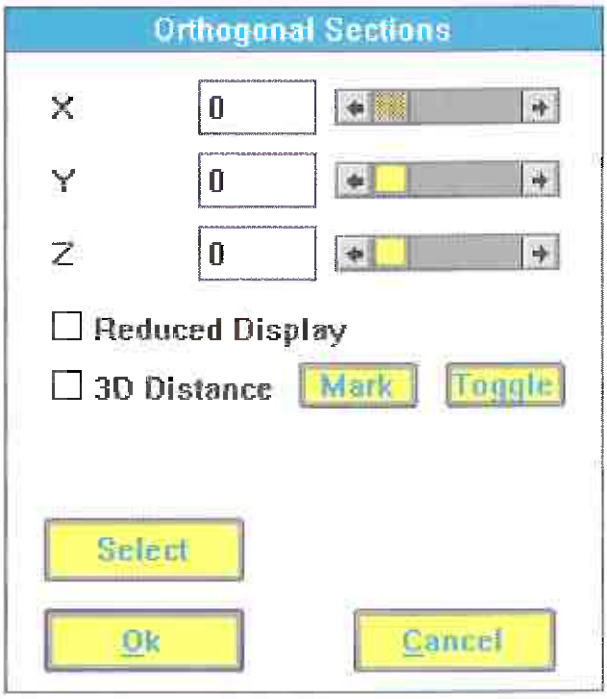


Figure 6-95

☐ **Reduced Display**
reduces the size of large images that no longer fit onto the image monitor.

☐ **3D Distance**
activates 3D distance measurement.
When distance measurement is activated, the 3D distance between the marked and displayed point in the fourth quadrant is output and a yellow line is displayed between the two points in all three quadrants.

Mark
marks the displayed point and activates distance measurement.

Toggle
swaps the marked point with the displayed point.

Select
causes the mouse cursor to appear on the image monitor after clicking. At the same time, the following text is displayed in the dialog window:

Press left mouse button to select
Press right mouse button to exit

Thus, a point can be set interactively in each of the three section images in relation to which the two other section images are displayed.

Ok
displays the section planes.

Cancel
ends the function and closes the dialog window.

(7.3) 3D → Projection...

By means of the **Projection...** command and the affiliated dialog window, one single projection or a series of projections can be calculated after rotation of the data package about the x, y or z axis.

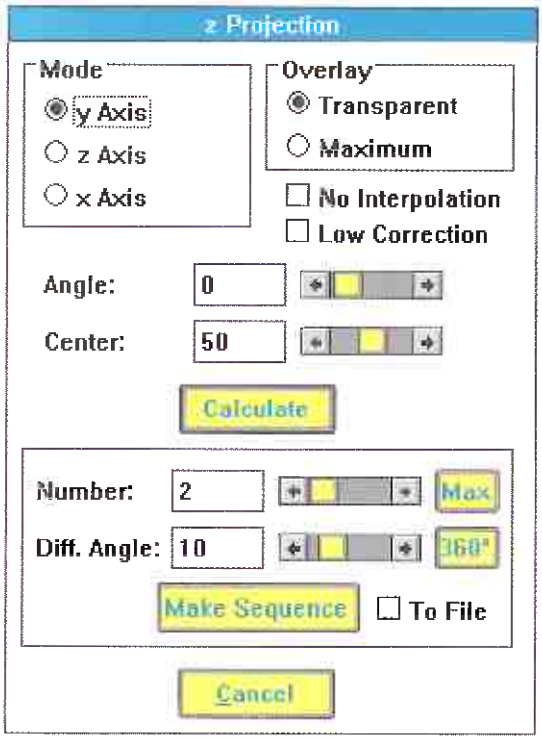


Figure 6-96

Mode box
- ◉ **y Axis**
- ○ **z Axis**
- ○ **x Axis**

Selects the axis about which the data package is to be rotated.

Overlay box
allows a selection between two different overlay methods.

○ **Transparent**
Transparent overlay with visibility curve (see also Figure 6-103).

◉ **Maximum**
The brightest point of the penetration line is displayed.

☐ **No Interpolation**
Interpolation is run when the resolution in the z direction is less than in the x/y direction. Interpolation can be deactivated by activating this function.

☐ **Low Correction**
When this function is activated, the artefact suppression correction is run.

Angle Rotation angle in degrees.

Center z position of the rotation axis in % (default: 50 %; middle of the data package).

Calculate calculates the single projection.

Cancel closes the dialog window.

The following is also necessary to calculate a series of projections (e.g. for an animation):

Number Number of projections for a sequence (variable from 0 to 100). The maximum possible number of a given video memory is taken when Max is pressed.

Diff. Angle Angle increment of a sequence. When Number is set, selecting 360° defines the difference angle so that the total angle for the sequence is 360° .

Make Sequence executes calculation of the sequence.

☐ **To File** A file selection list is displayed when this option is activated. When a file name is entered here, the projections are not stored in the video memory, but are saved onto disk as a file sequence, thus also enabling larger animations.

If you have selected the "Transparent" item in the "Overlay" box, the "Transparency" dialog box additionally appears, in which the overlay method can be specified in greater detail. Here, you can set the degree to which one single pixel is taken into account in the transparent overlay, depending on the respective pixel value. This is done via a curve in which the horizontal axis represents the grey value of the pixel and the vertical axis represents its degree of visibility.

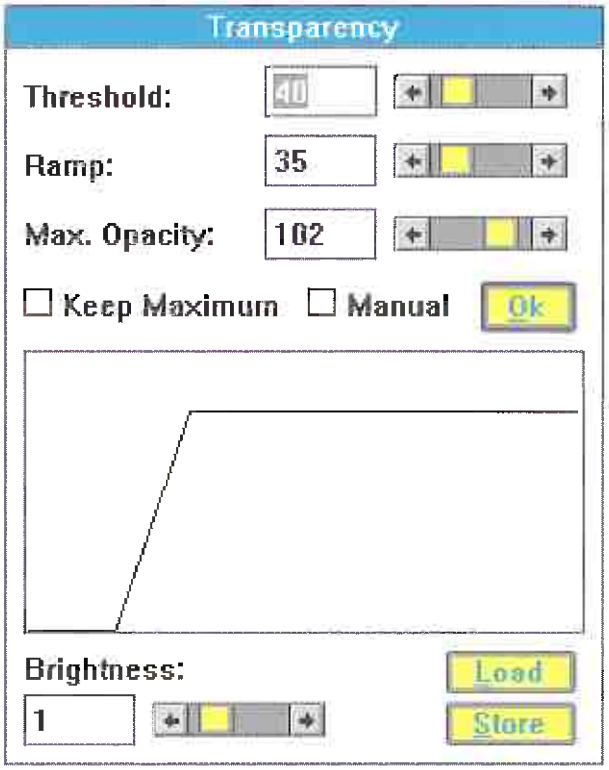


Figure 6-97

The curve denoting the degree of visibility can be entered manually (click on Manual and draw it with the left mouse button pressed) or with the aid of the following parameters:

Threshold
Pixel value at which the ramp rises (variable from 0 to 255).

Ramp
Slope of the ramp (variable from 0 to 255; 0 corresponds to a vertical rise).

Max. Opacity
Degree of visibility at the top corner of the ramp (variable from 0 to 128; 0 corresponds to the bottom edge in the diagram).

☐ **Keep Maximum**
Activating this option modifies the specification governing calculation of the projection.

☐ **Manual**
When making a manual input, the **Ramp**, **Threshold** and **Max. Opacity** parameters are irrelevant.

The transparency curve is displayed in the bottom part of the dialog window.

Brightness
The image can be brightened again by modifying the value (from 0.2 to 5).

Ok
accept the numeric inputs for the calculation.

Load
loads a file.

Store
stores the file.

(7.4) 3D → Stereo Images...

Stereoscopic images can be generated in a variety of ways by means of the **Stereo Images...** command and the affiliated dialog window.

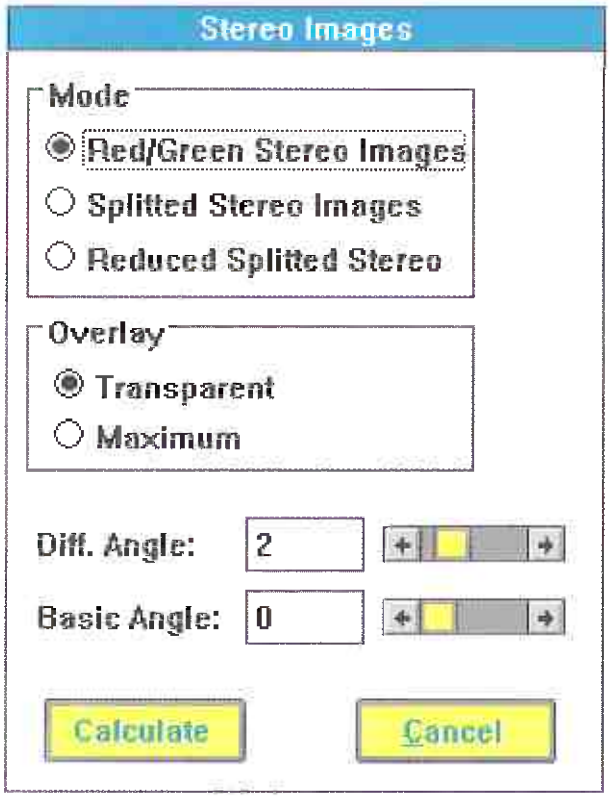


Figure 6-98

Mode box

◉ **Red/Green Stereo Images**
This displays a stereo image for red/green anaglyph observation using red/green spectacles.

○ **Splitted Stereo Images**
This displays a pair of stereo images for observation through a stereoscope. Coloured stereo images are also possible.

○ **Reduced Splitted Stereo**
Reduced display of a stereo image. 1:2 reduction, i.e. 4 pixels each are replaced by their average.

Overlay box

○ **Transparent**
Transparent overlay with visibility curve (see also Figure 6-99).

◉ **Maximum**
The brightest point of the penetrating line is displayed.

Diff. Angle
Stereo angle of the eyes (variable from 0 to 10°; sensible value: 3°).

Basic Angle
Direction angle at which the specimen is viewed; 0 from the front, 180° from the rear.

Calculate
calculates the stereo image.

Cancel
closes the dialog window.

If you have selected the "Transparent" item in the "Overlay" box, the "Transparency" dialog box additionally appears, in which the overlay method can be specified in greater detail. Here, you can set the degree to which one single pixel is taken into account in the transparent overlay, depending on the respective pixel value. This is done via a curve in which the horizontal axis represents the grey value of the pixel and the vertical axis represents its degree of visibility.

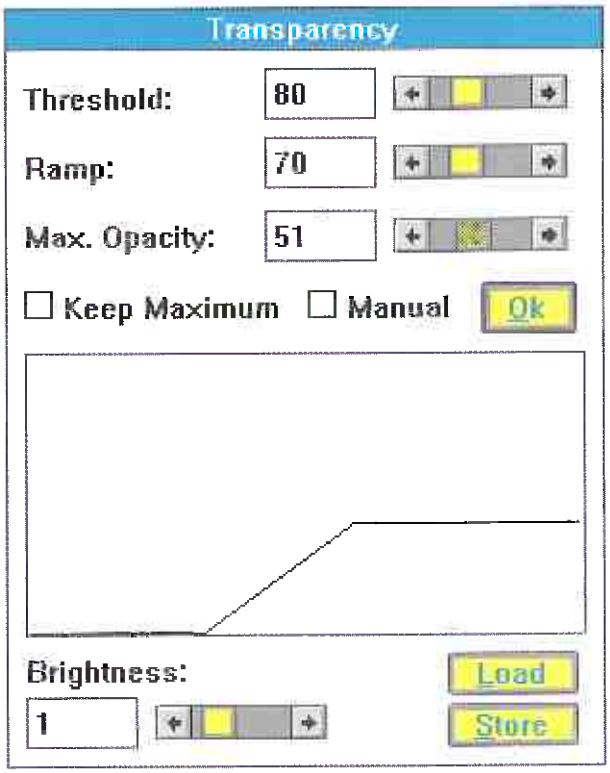


Figure 6-99

The curve denoting the degree of visibility can be entered manually (click on Manual and draw it with the left mouse button pressed) or with the aid of the following parameters:

Threshold
Pixel value at which the ramp rises
(variable from 0 to 255).

Ramp
Slope of the ramp
(variable from 0 to 255; 0 corresponds to a vertical rise).

Max. Opacity
Degree of visibility at the top corner of the ramp
(variable from 0 to 128; 0 corresponds to the bottom edge in the diagram).

☐ **Keep Maximum**
Activating this option modifies the specification governing calculation of the projection.

☐ **Manual**
When making a manual input, the **Ramp**, **Threshold** and **Max. Opacity** parameters are irrelevant.

The transparency curve is displayed in the bottom part of the dialog window.

Brightness
The image can be brightened again by modifying the value (from 0.2 to 5).

Ok
accept the numeric inputs for the calculation.

Load
loads a file.

Store
stores the file.

(7.5) 3D ⇨ Depth Coding...

By means of the **Depth Coding...** command and the affiliated dialog window, the depth information contained in a sequence can be coloured with the colours of the rainbow, in which case "red" stands for front and "blue" stands for rear.
The brightness is the brightness maximum of a transparent projection, multiplied by **Intensity** plus **Offset**.

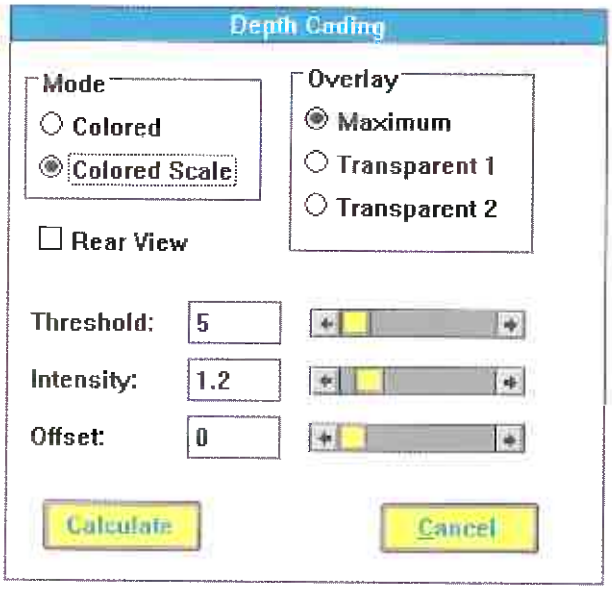


Figure 6-100

Mode box

○ **Colored**

◉ **Colored Scale**
displays a coloured scale in the image.

Overlay box

◉ **Maximum**
The colour is defined by the z position of the brightness maximum.

○ **Transparent 1**
The transparent projection is built up from the rear to the front. The colour is defined by the z position at which the original was last higher than or equal to **Threshold**.

○ **Transparent 2**
The transparent projection is built up from the rear to the front. In doing so, the resulting projection is compared against the original. The z position at which the original was last higher than or equal to the projection and higher than or equal to **Threshold** then defines the colour.

☐ **Rear View**
The image is viewed from the rear/below when this option is activated.

Threshold All brightness values below the Threshold (range: 0 to 255) are ignored or treated like 0 when determining the depth and the display.

Intensity defines the factor with which the brightness of the overlaid series affects the brightness of the depth-coded colour.

Offset adds a value between 0 and 255 to the brightness of the colour. The recognisability of the colour and thus of the depth in dark portions of an image can be improved with an offset.

Calculate calculates the image.

Cancel closes the dialog window.

(8) Topo

The Topo functions allow you to display the surface topography of a microscopic specimen.

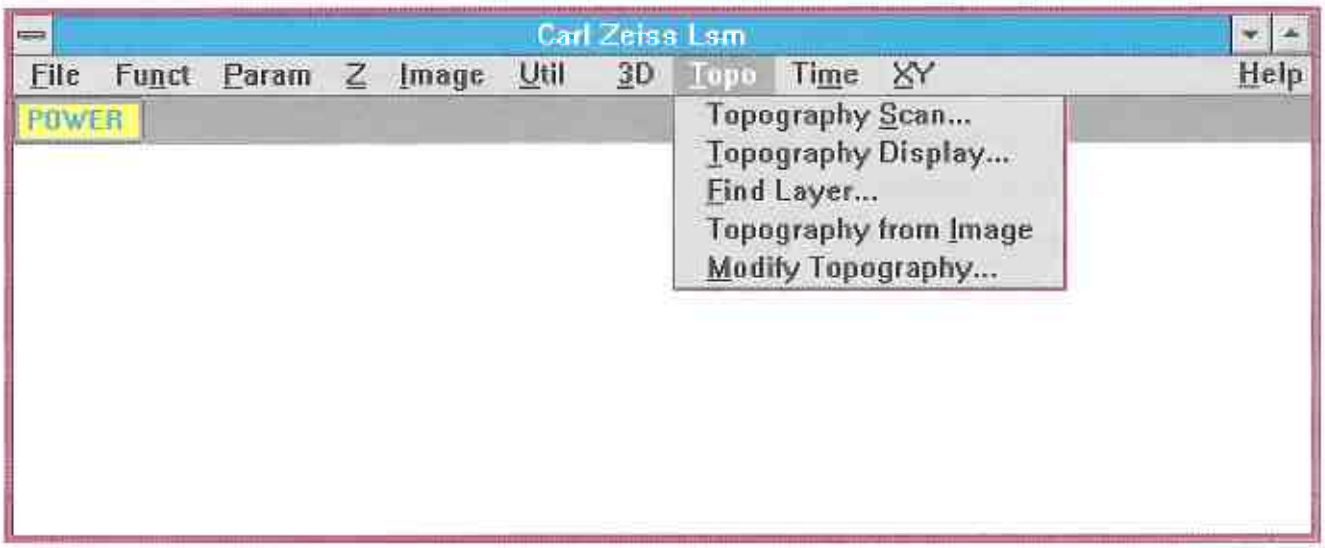


Figure 6-101

Meanings:

Topography Scan...	:	records a topography
Topography Display...	:	display and edits a topography
Find Layer	:	defines and measures layers
Topography from Image	:	converts any chosen image to a topographic image
Modify Topography	:	rotates the topography, allows to select ROIs and logs the data.

(8.1) Topo → Topography Scan...

By means of the **Topography Scan...** command and the affiliated dialog window, you can create a surface matrix on the basis of the brightness values.

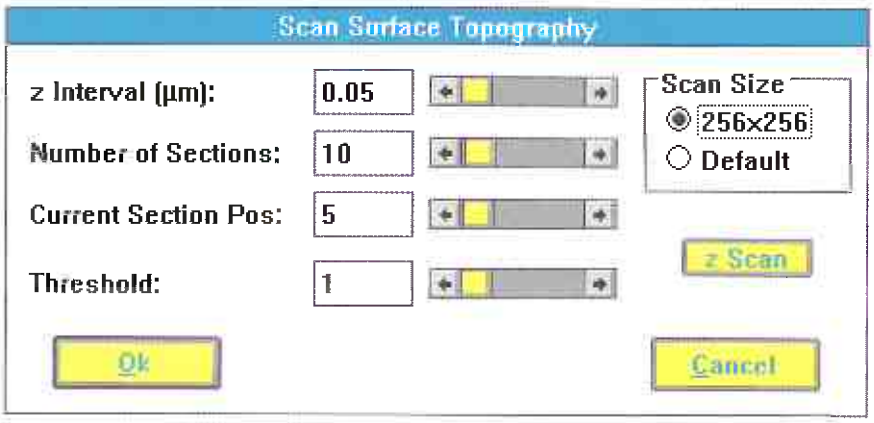


Figure 6-102

z Intervall (µm)
Distance between the section planes (variable from 0.05 to 20).

Number of Sections
Max. 200.

Current Section Pos
Number of the current position in the series (start = 1).

Threshold
Lowest grey value to be taken into account (0 to 255).

Scan Size box
◉ 256x256 The resolution is 256x256.
○ Default The image is scanned in the resolution that is set in the Control Panel. ROIs are also allowed.

z Scan
z scan for setting the above parameters.

Ok
starts the function.

Cancel
closes the dialog window.

(8.2) Topo → Topography Display...

By means of the **Topography Display...** command and the affiliated dialog window, you can create 3D displays with profile lines.

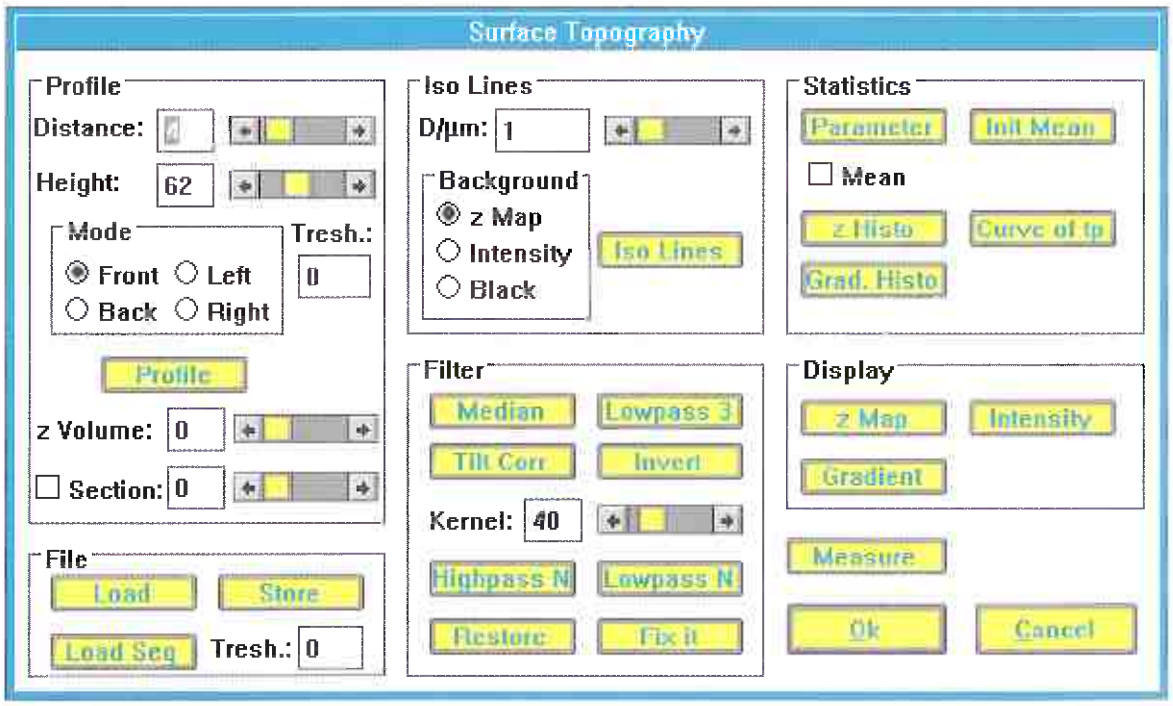


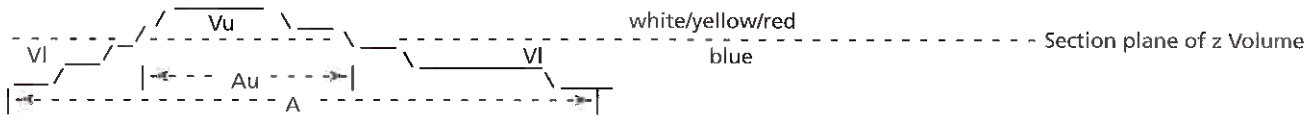
Figure 6-103

Profile box

Distance	Distance between two profile lines
Height	Max. height in pixel
Mode	Selection of the profile view
◉ Front	from the front
○ Left	from the left
○ Back	from the rear
○ Right	from the right
Thresh.	Height in µm at which the display begins
Profile	displays the profile
z Volume	defines the section plane for volume measurement
☐ Section	displays the profile additionally with one single profile line.

Up to 64 different height levels can be displayed with the profile lines. If you choose a maximum value for Height > 63, the highest ranges may be truncated. These ranges are marked in green. If you select the "z Volume" item, you can move a horizontal section plane in the z direction with the lower value.

In doing so, the area under the section plane is coloured in blue. At the same time, the z position is displayed in µm, the volume above and the hollow volume below the section plane (Vu, Vl), the area of the ranges located above the section plane (Au) and the ratio between this area and the total area in % (Au/A * 100 %: bearing ratio tp) are displayed.



Vu: Upper Volume
Vl: Lower Volume
Au: Upper Area or Profile Bearing Area
C:\lsm-menu\ Whole Area
tp = Au/A * 100%: Profile Bearing Area Ratio

File box

Load	loads the z and intensity matrix
Store	stores the z and intensity matrix
Load Seq.	loads an image sequence and creates a z and intensity matrix on the basis of it
Thresh.	is used when calling Load Seq. to suppress everything below the set threshold when converting to the topography.

Iso Lines box

D/µm	Height difference per line in µm
Background	
◉ z Map	Background z in colour coding
○ Intensity	Brightness maxima background
○ Black	Black background
Iso Lines	executes the function.

The z matrix is kept twice in the memory, once as the original and once as a working copy (in each case scaled to 250 values). The filter operations only affect the z matrix copy and can therefore be reversed easily with "Restore". However, only ever the original is stored. The original can, however, be replaced by the edited copy by selecting "Fix it". "Restore" is then no longer possible.
The median and low-pass filter are capable of improving the display with profile lines or height lines because the rough stages are smoothed and freak values at individual points are suppressed.
The variable low-pass filter is realised with a filter matrix of the size kxk and all matrix elements = 1.
When using a variable high-pass filter, a low-pass-filtered z matrix is subtracted from the original image:
Zh = Z - Z*T + z (z = average of the z matrix).
At the four edges, a strip that is k/2 pixels wide in each case is set to 0 because the filter does not deliver any correct results here. However, these areas are not included in calculation of the parameters.

Filter box

Median	Median over the z matrix
Lowpass 3	3x3 low-pass over the z matrix
Tilt Corr	Correction with compensation plane
Invert	Invert z matrix (peak → valley)
Kernel	Size of the filter matrix; k = 2...200
Highpass N	High-pass filter kxk (from 2x2 to 200x200)
Lowpass N	Low-pass filter kxk (from 2x2 to 200x200)
Restore	Reverse filtering
Fix it	Fix all editing operations

Statistics box

Parameter	Roughness parameters
Init Mean	Set averaging register to Zero
☐ Mean	Compute average values and standard deviation
z Histo	Histogram over the z matrix (original)
Grad. Histo	Histogram over the gradient
Curve of tp	Bearing curve (bearing proportion as a function z)

For the gradient histogram, at least the low-pass function should have been used once as otherwise coarse z graduation leads to a comb-like histogram.
The z histogram and the roughness parameters can be modified by filtering.
The "Variable Highpass" filter can be used as a wave filter, but then you lose the information at the edges. You can, however, also compute a compensation plane with "Tilt Correction" instead.

The values are calculated in accordance with the following formulas:
Average (mean height) $zm = (z1+z2+...) / n$
Standard deviation or average roughness value Rq (root-mean-square deviation)
$Rq = \sqrt{\{ [(z1-zm)^2 + (z2-zm)^2+...] / n)\}}$
Average roughness value Ra (arithmetical mean deviation)
$Ra = (|z1-zm| + |z2-zm|+...) / n$
Maximum peak-to-valley height Rt: $Rt = zmax - zmin$
Maximum peak-to-valley height Rmax: $Rmax = MAX (zmax1-zmin1, zmax2-zmin2, ... , zmax5-zmin5)$
Averaged peak-to-valley height Rz: $Rz (zmax1-zmin1 + zmax2-zmin2 + ... + zmax5-zmin5) / 5$
The numbers 1 to 5 refer to 5 strips into which the image is split.
Once the "Init Mean Values" function has been triggered, the roughness parameters are buffered for averaging each time the "Parameters" menu item is selected.
As soon as there are $n \geq 2$ parameter sets, the "Mean of n S" header line appears as well as two further columns containing the average values and the standard deviations.
Average $m = \Sigma x / n$
Standard deviation $S = \sqrt{\{ [\Sigma x^2 - (\Sigma x)^2 / n] / (n-1) \}}$

Display box

z Map	z in colour coding
Intensity	Brightness maxima
Gradient	Gradient (amount)
Measure	calls up the measuring function; on termination, you are returned to **Display Topography**
Ok	accepts a keyboard input; also activated by pressing the Return key
Cancel	closes the dialog box

(8.3) Topo → Find Layer...

By means of the **Find Layer...** command and the affiliated dialog window, various layers can be determined and measured on the basis of the maxima of z scans. In doing so, several local maxima can also be detected, indicating possible inner interfaces and thus layers.

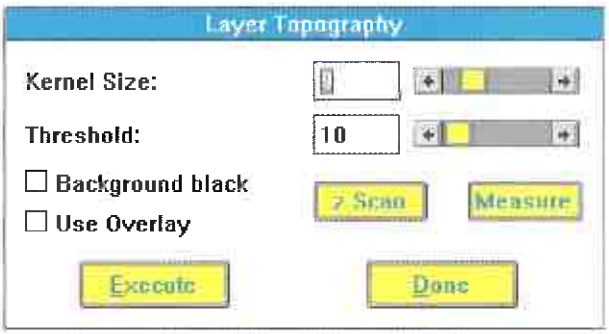


Figure 6-104

Kernel Size
depends on the thickness of the layers.

Threshold
cuts off background noise.

☐ **Background black**
When this option is activated, the original image is cleared; otherwise, the original image is written into the video memory with half brightness.

☐ **Use Overlay**
When this option is selected, only the line is drawn into the overlay and the background is darkened via an LUT.
It can be used to try out the effect of various settings without destroying the original image in each case.

z Scan
The **z Scan** dialog box appears (see also function (4.2)) and the z scan can be executed.

Measure
calls up the measuring functions to measure layer thicknesses (see also function (2.4)).

Execute
determines the layers from the executed z scan.

Done
cancels the function.

(8.4) **Topo** → **Topography from Image**

By using the **Topography from Image** command, any chosen image can be treated like a topography.

The dialog window is identical with the one that appears when you select the **Topography Display...** command.

(8.5) Topo → Modify Topography...

By means of the **Modify Topography...** command and the affiliated dialog window, a topography can be edited later.

This macro (CZMODTOP.MAC) is capable of rotating a topography file, of extracting a partial area (ROI), of calculating the roughness parameters on the basis of it and of storing them in an ASCII file.

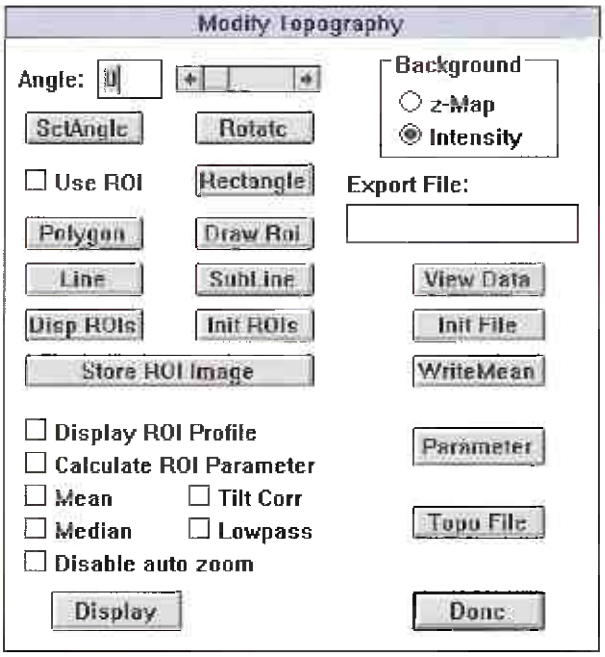


Figure 6-105

Angle:
allows you to enter the rotation angle of a rotating topography.

Set Angle
The rotation angle can also be defined by drawing a line.

Rotate
rotates the topography about the set angle. All ROIs already defined are also rotated.

☐ **Use ROI**
The zoomed section is defined interactively.

Rectangle
defines ROI as a rectangle.

Polygon
defines ROI as a polygon.

Draw Roi
defines ROI as a trace with the mouse.

Line
computes the topography along a line of any orientation.

SubLine
computes several sections of a line separately.

☐ **Tilt Corr**
When the Tilt Correction function is activated, the lines are correct with the compensation line.

Disp ROIs
draws all ROIs into the background image and numbers all of them consecutively.

Init ROIs
begins numbering at 1 again after Init ROIs.

Store ROI Image
stores the background image with ROIs.

☐ **Display ROI Profile**
A profile is displayed after definition of an ROI or calculation of the parameters. If the option is not activated, the background image defined under "Background" is displayed instead.

☐ **Calculate ROI Parameter**
After every definition of an ROI or a line, the parameters are calculated and are written into the export file.

☐ **Mean**
activates averaging when calculating the parameters.

☐ **Tilt Corr**
The topography is tilted so that its compensation plane or line becomes horizontal. This is done separately for each ROI and each (partial) line.

☐ **Median** ☐ **Lowpass**
Filtering is done with Median and/or Lowpass. To avoid edge errors this filtering is always done with the complete topography data; if the setting is changed, ROIs are deactivated automatically (☐ **Use ROI**).

☐ **Disable Auto Zoom**

Display
calls up the "Topography Display" dialog box.

Background box

○ z-Map	Height map	Selection of the background image
◉ Intensity	Intensity	

Export File:
Name of the export file; all new data is appended to an existing file as a line containing ASCII values. The individual values are separated by tabs. The first value is the number of the ROI.

Views Data
displays the data of the export file in the Notepad editor.

Init File
clears the export file.

Write Mean
The average values of the N last written parameters are calculated and are added to the export file. This line begins with MeanN.

Parameter
The parameters are calculated, displayed and, unless already done, are written into the export file. A profile or a background image is also displayed.

Topo File
loads or stores a topography file.

Done
ends the macro.

(9) Time

The **Time** functions enable recording of image series (or series of zooms) in time.

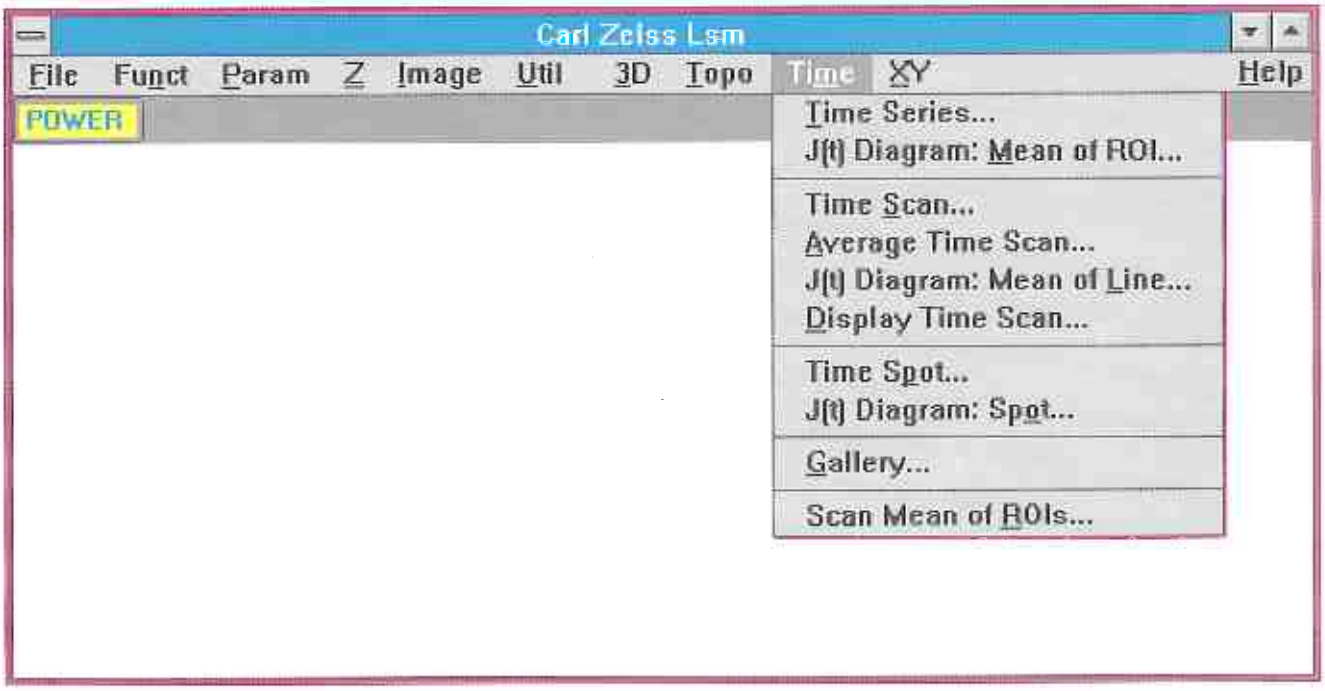


Figure 6-106

Meanings:

Time Series...	:	recording of image series (or series of zooms)
J(t) Diagram: Mean of ROI...	:	display of the intensity progression in an ROI
Time Scan...	:	recording of the time intensity along a line
Average Time Scan...	:	reduction of the time resolution (data reduction)
J(t) Diagram: Mean of Line...	:	display of the intensity along a line
Display Time Scan...	:	3D display of the time-variable profiles along an image line
Time Spot...	:	recording of the time intensity at one point of the image
J(t) Diagram: Spot...	:	display of the intensity at one spot
Gallery...	:	display of several images in a time series simultaneously on the image monitor
Scan Mean of Rois...	:	recording and display of the mean values of ROIs in their time dependence

(9.1) Time → Time Series...

Recordings of image series in time can be made by means of the **Time Series...** command.

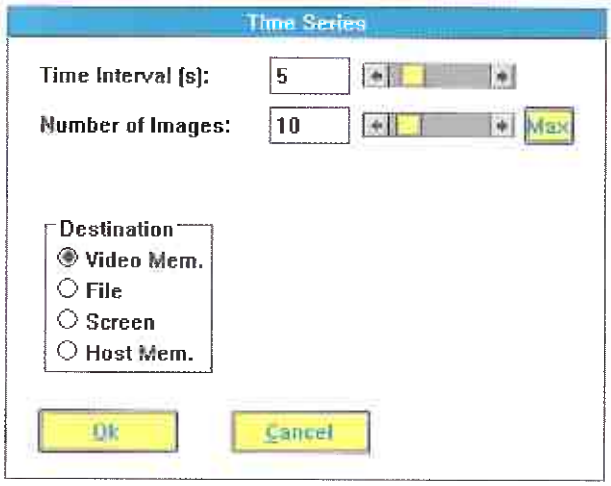


Figure 6-107

Time Interval (s)
Time interval between two scans. If this value is less than the scan time, the maximum scan speed is assumed. Three beeps are also issued as a warning. The value can be entered directly or can be modified by means of the slider.
When 0 is entered, the maximum scan speed is assumed.

Number of Images
Number of images to be scanned. The value can be entered directly or can be modified by means of the slider.

Max
Maximum number of images for the chosen image format and the available memory capacity.

Destination box

◉ Video Mem. scans the image series into the video memory (max. 4 MB, i.e. 16 full frames of 256 kB each; 8 MB when using a high-resolution image monitor, i.e. 32 full frames)

○ File The following is additionally displayed in the dialog window when this function is activated:

The name of the image sequence can be entered in the text entry box (up to 15 letters). When you click Dir, a further dialog window Select is opened, in which the destination drive a, b or c can be selected.

○ Screen scans and displays the image series, but without storing it; suitable for test scans.

○ Host Mem. The image series is stored in the host memory. When you select Max, the free memory is determined and the maximum number of images is displayed. If memory is already occupied, you are prompted to specify whether you wish to release it beforehand because only in this way is a precise determination possible.

Ok
executes the time series display.

Cancel
ends the function and closes the dialog window.

(9.1.1) Time ⇨ J(t) Diagram: Mean of ROI

By means of the **J(t) Diagram: Mean of ROI** function and the affiliated dialog box, the average values of a region of interest (ROI) are displayed in relation to their time dependence.

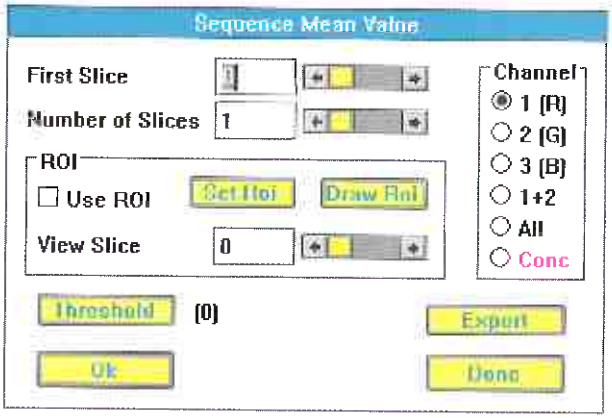


Figure 6-108

First Slice
Starting number of the image series.

Number of Slices
Number of images.

ROI box

☐ **Use ROI** The zoomed section is determined interactively.

Set ROI The zoomed section is set.

Draw ROI An ROI of any shape can be defined with the mouse. To conclude, click on the inside of the area.

View Slice A selected image is displayed.

Channel box

The R, G and B channels can be displayed singly or together (1+2 or All).

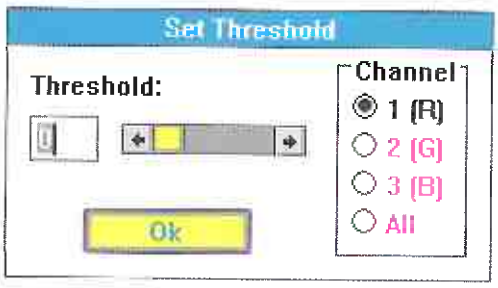


Figure 6-109

Threshold

When this button is clicked, a further dialog window is opened in which the threshold value can be entered.

Export

The mean values are stored as an ASCII file. A Store Data box appears for this purpose. Here, you can also choose between all data **(All)** and the data displayed on the monitor **(Visible)**. **Append** adds the data to a file without overwriting anything. The time information is also stored.

Ok

terminates setting of the values and executes averaging.

Done

ends the function and closes the dialog box.

(9.2) Time → Time Scan...

The time intensity progression on a line can be recorded by means of the **Time Scan...** function and the affiliated dialog window.

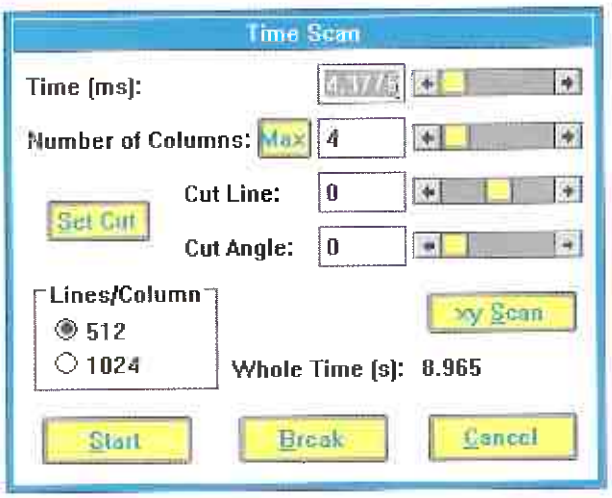


Figure 6-110

Time [ms]
Time for one line run.

Number of Columns
The successive line runs are combined in blocks (columns) of 512 or 1024 **(lines/column)**. 1024 lines/column is only meaningful when using a special memory expansion board. The total length of one section series is always an integral multiple of one block.
Depending on the line length and video memory capacity, the maximum possible number of blocks is obtained automatically by clicking Max.

Selecting the section in the image by defining its position and angle:

Cut Line
specifies the position of a horizontal line (0 through image centre, -256 top and +255 bottom edge of the image with 512 lines and 0°).

Cut Angel
specifies the angle of the line with the horizontal.

Set Cut
When this button is clicked, the section line can be moved and rotated with the mouse. Pressing the left mouse button produces the section at the selected position.

xy Scan
Image scan in the rotated coordinate system. The section line becomes the horizontal line.

Whole Time [s]
The entire time needed is specified.

Start
starts the scan.

Break
stops the scan.

Cancel
ends the function and closes the dialog window.

With a time interval ≤ 200 ms, the scan speed is chosen getting closely to the desired time interval. Times below 4.4 ms only allow two discrete time intervals (1.4 ms and 2.16 ms).
With a time interval of > 200 ms, the scan speed depends on the value set in the Control Panel and a pause is inserted after every line. A text such as max=8 can then also be displayed. This identifies the slowest scan speed that does not yet lead to a timeout. If a timeout occurs, an audible signal is sounded up to three times.
The 200 ms limit is imposed by the electronic scan circuitry. To arrive at larger intervals, scanning must be constantly aborted and restarted, which leads to a considerable time overhead.
If signals are highly noisy, it is better to limit the time interval to 200 ms and to reduce the noise and the quantity of data later on with "Average Time Scan".

(9.2.1) Time → Average Time Scan...

The amount of data can be reduced by means of the **Average Time Scan...** function and the affiliated dialog window.

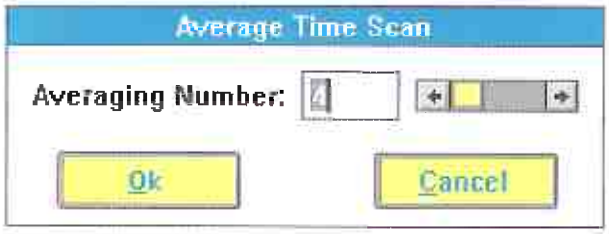


Figure 6-111

For a series of lines recorded with Time Scan, the time resolution is reduced by averaging the number of lines specified for the **Averaging Number** and by replacing it with a line.
If the number of columns is not a multiple of the **Averaging Number**, the last column is not filled completely with data. The rest of this column contains the value Zero.

(9.2.2) Time → J(t) Diagram: Mean of Line...

With the **J(t) Diagram: Mean of Line...** function and the affiliated dialog box, the average values of a line section are displayed in relation to their time dependence.

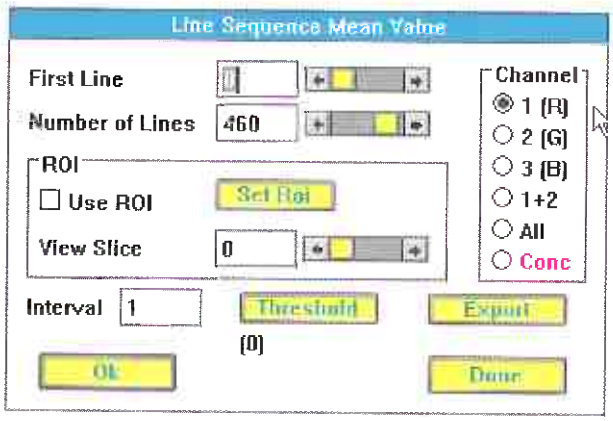


Figure 6-112

First Line
Starting number of the image series.

Number of Lines
Number of line sections.

ROI box

☐ **Use ROI** The image section is determined interactively.

Set ROI The image section is set.

View Slice A selected image is displayed.

Channel box

The R, G and B channels can be displayed singly or together (1+2 or All).

Interval
Not all average values need to be displayed; instead, the number entered here can be skipped.

Threshold

When this button is clicked, a further dialog window is opened in which the threshold value can be entered.

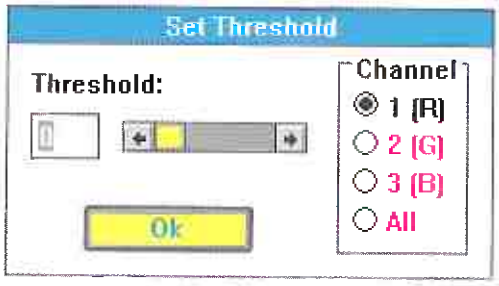


Figure 6-113

Export

The mean values are stored as an ASCII file. A Store Data box appears for this purpose. Here, you can also choose between all data **(All)** and the data displayed on the monitor **(Visible)**. **Append** adds the data to a file without overwriting anything. The time information is also stored.

Ok

terminates setting of the values and executes averaging.

Done

ends the function and closes the dialog box.

(9.2.3) Time
Display Time Scan...

By means of the **Display Time Scan...** command and the affiliated dialog box, you can display the profiles variable in time that have been obtained with **Time Scan** "three-dimensionally" along an image line.

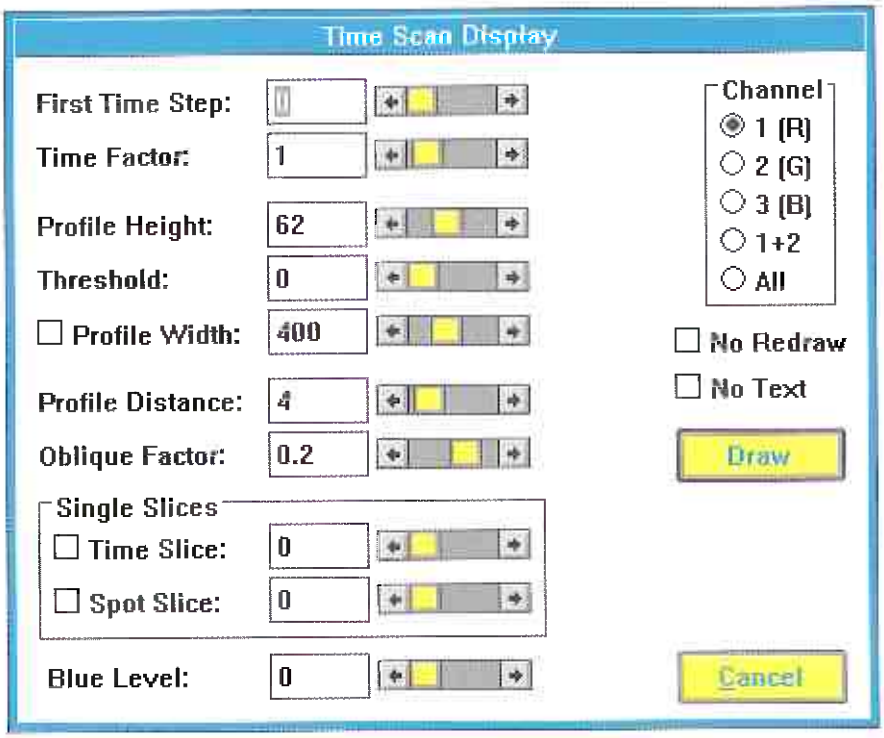


Figure 6-114

First Time Step
Time of the first displayed scan.

Time Factor
Time axis scaling.

Profile Height
Maximum profile height for the display.

Threshold
Threshold value for the display.

☐ **Profile Width**
Width of the displayed profile.

Profile Distance
Distance between two neighbouring profiles in the display (e.g. input of the value 4 means: only every fourth scan is displayed).

Oblique Factor
Inclination of the time axis with respect to the vertical.

Single Slices box

A selected cross-section from the three-dimensional display can be shown. The cross-section may represent the time progression at one point (Spot Slice) or an image series at one point in time (Time Slice).

Blue Level
As an aid to orientation, all displayed values under an intensity level can be displayed in blue.

Channel box

The R, G and B channels can be displayed individually or together (1+2 or All).

☐ **No Redraw**
The monitor display is not updated automatically in the event of changes.

☐ **No Text**
No text in the display.

Draw
updates the monitor image.

Cancel
ends the function and closes the dialog window.

(9.3) Time → Time Spot...

The time sequence of the intensity at one spot of the image can be registered by means of the **Time Spot...** function and the affiliated dialog window.

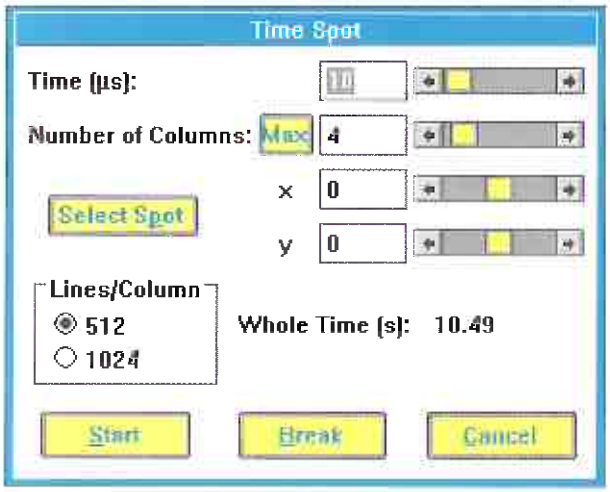


Figure 6-115

Time (µs)
specifies the time frame in µs.

Number of Columns
Number of columns for storing the images.

Max
When this button is clicked, the maximum possible number of columns is displayed.

Select Spot
Interactive definition of the measured point
x Enter the point's coordinates
y (variable from -256 to +255)

Lines/Column box

◉ 512 For organisation of image storage the number of lines/column can be selected.
○ 1024

Whole Time (s)
The entire time required is specified

Start
activates the scan.

Break
stops the scan.

Cancel
closes the dialog window.

(9.3.1) Time → J(t) Diagram: Spot...

With the **J(t) Diagram: Spot...** function and the affiliated dialog box, the time intensity progression at one spot of an image can be displayed.

Start Time
Time of starting.

Time Range
If the range set with Time Range contains more measured values than can be displayed (max. 460), the measured values are averaged.

Max. Averag.
For speed reasons, the maximum average value that can be set limits the number of averaging operations.

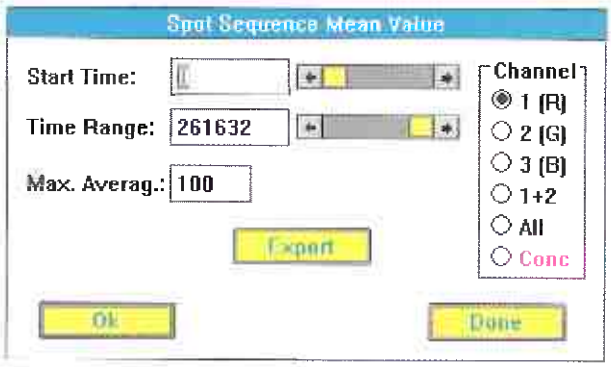


Figure 6-116

Channel box

The R, G and B channels can be displayed individually or together (1+2 or All).

Export
when exporting the data, only stores the displayed values together with the time.

Ok
produces the display.

Done
ends the function and closes the dialog box.

(9.4) Time → Gallery...

By means of the **Gallery...** command and the affiliated dialog box, several images in a time series can be displayed simultaneously on the image monitor.

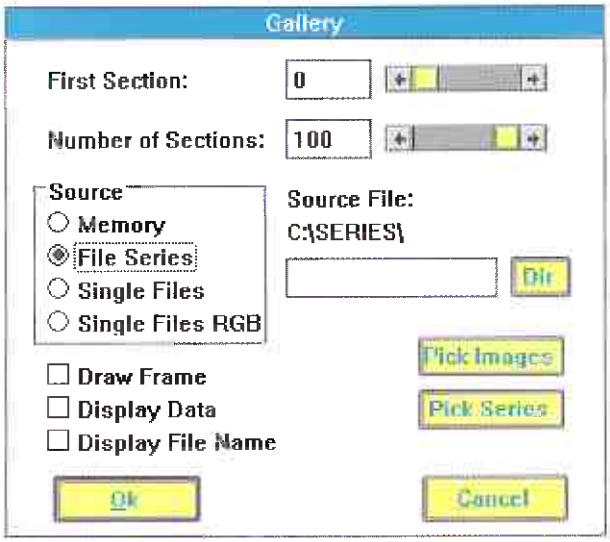


Figure 6-117

First Section
Number of the first image in a sequence to be displayed (ignored when Single Files is selected).

Number of Sections
Number of images to be displayed, which also defines the reduction factor. This value is reduced automatically when a sequence that is less than this value is displayed.

Source box

When

◉ **Memory**
is selected, image sequences are fetched from the ALU or when

○ **File Series**
is selected, they are fetched from the hard disk.

○ **Single Files**
allows combination of individual images from the image directory by selecting Pick Images or Pick Series. In doing so, the first loaded image defines the image raster and colour depth (monochrome or RGB) of the entire gallery. If a gallery is monochrome, only one LUT can be displayed, namely the one that was loaded last. Select **Single Files RGB** to display images with different LUTs in the right colours. Make the choice with **Pick Images** or **Pick Series**.

○ **Single Files RGB**
All loaded images are placed in an RGB image; in doing so, single-channel images are converted to an RGB image on the basis of the stored LUT (look-up table, colour table).

☐ **Draw Frame**
The images are separated by frames.

☐ **Display Data**
The images are assigned the corresponding data (times in time series, z sections in z scan).

☐ **Display File Name**
The file name appears on the images.

Clicking on the corresponding button

Pick Images
selects individual images from the list of image files (same dialog window as in Figure 6-22).

Pick Series
selects individual images from the series directory (same dialog window as above).

Ok
produces the display when **Memory** or **File Series** has been selected.

Cancel
closes the dialog window.

(9.5) Time → Scan Mean of ROIs...

By means of the **Scan Mean of ROIs...** command and the affiliated dialog box, a time series is scanned or loaded and, in doing so, the averages from up to 10 rectangular areas and up to 3 channels can be displayed as coloured curves on the image monitor.

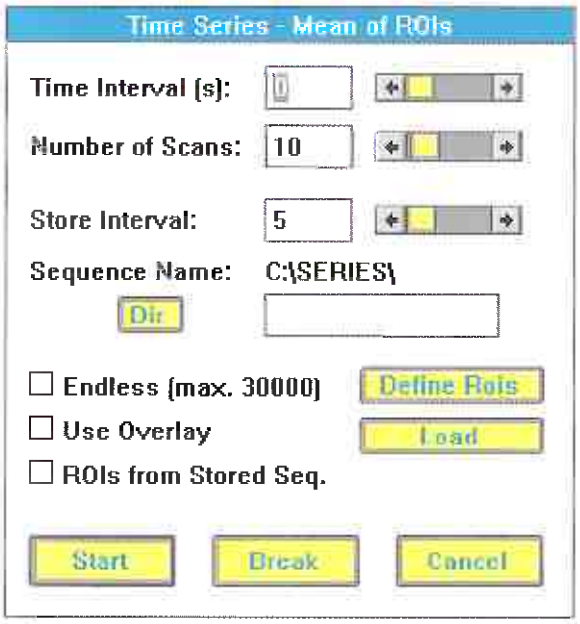


Figure 6-118

Time Interval (s)
When the value "0" is selected, the scan is run at the maximum frequency.

Number of Scans
Number of scans.

Store Interval
The first image and all images following at this interval are stored as a sequence.

Sequence Name
A sequence name can be entered directly or Dir can be pressed to call up the Select sub-menu.

☐ **Endless** (max. 30000)
When Endless is activated, the **Number of Scans** only serves to define the number of measured points displayed simultaneously on the image monitor (this number can still be changed during the scan!).

☐ **Use Overlay**
This option can be used to define whether the diagrams are to be placed in the normal video memory or as an overlay over a background image with reduced brightness.

☐ **ROIs from Stored Seq.**
The sequence is not scanned and, instead, a sequence already stored is used to determine the average values.

Define ROIs
displays the ROIs as a coloured frame. If there are background images, the image lying closest to the time set with "Time/View ROIs" is loaded as the background image.

Load
loads the files called up.

Start
starts data recording with the set parameters.

Break
stops the scan and displays the result.

Cancel
ends the function and closes the dialog box.

(10) XY

In conjunction with a scanning table, the **XY** function enables storage of positions in the XY and Z directions, after which the table can be moved to them again.

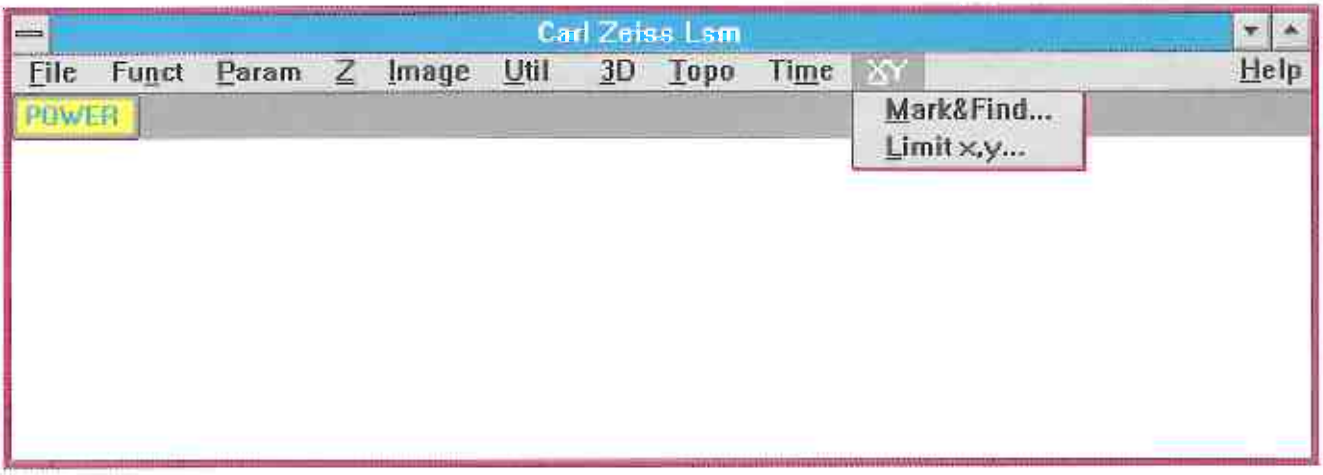


Figure 6-119

Meanings

Mark&Find...	:	mark and move to positions again
Limit x,y...	:	limit the travel range

(10.1) XY → Mark & Find...

By means of the **Mark & Find...** command and the affiliated dialog box, positions in the XY and Z directions can be stored and then moved to again with great precision.

The **Motor Control** dialog box has priority over the **Mark & Find** dialog box.

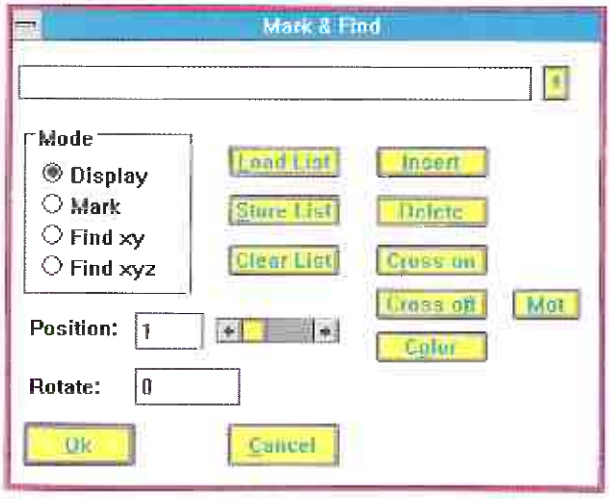


Figure 6-120

Mode box

◉ **Display**
displays the list of marked points.

○ **Mark**
Move the scanning table and focus to the required position via **Motor Control** and confirm the position with OK. The values are displayed and the position counter is incremented automatically.

○ **Find xy**
The stored positions are moved to automatically. To do this, enter the required number in Position.

○ **Find xyz**
The z coordinate is also moved to.

Load List — Stored positions can be loaded from a storage medium. The .POS extension is added to the file name automatically.

Store List — Positions can be stored on a storage medium. The .POS extension is added automatically to the file name.

Clear List — The positions stored in the RAM are cleared.

Insert — inserts an xyz position at the current point.

Delete — clears the current xyz position.

Cross on — displays a cross in the middle of the image.

Cross off — clears the cross.

Color — calls up a dialog box to set the colour of the cross.

Position — Enter the number of the marked position.

Rotate — rotates the x/y coordinate system with the chosen angle about the point (0,0). This can be used to compensate for rotation of the specimen.

Ok — The selected function is run.

Cancel — ends the function and closes the dialog window.

(10.2) XY → Limit x,y...

Limiting of the scanning table's travel range can be entered by means of the **Limit x,y...** command and the affiliated dialog box.

The limit can be set by entering the limits and by clicking Ok or by positioning on opposite corner points and clicking Corner 1 or Corner 2.
Travel commands that exceed the set limits are limited and a brief beep is issued.
The limit does not take effect, however, if the scanning table is controlled directly with the joystick or the mouse.
As the limits are always relative to the zero position, you should always move the known reference position before resetting x/y to zero or initialising the motor control.
The limit is not active after starting the program.

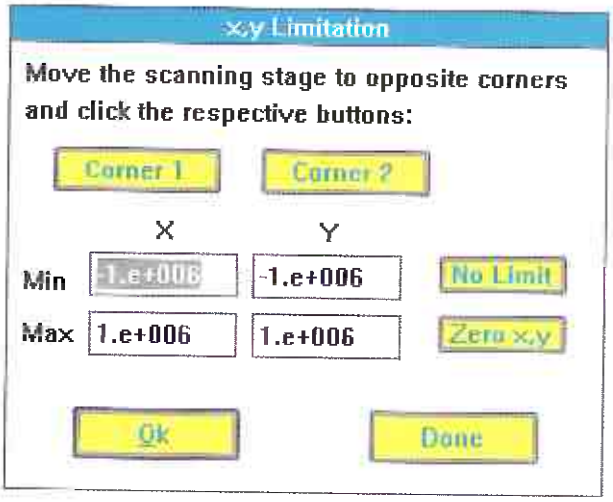


Figure 6-121

If an AUTOEXEC.MAC macro exists in the LSM directory and if it contains a CallMacro "czxylim" line, the CZXYLIM.MAC macro is called up automatically each time the program is started.

CONTENTS

7 MACROS

7.1 General

Macros are strings of commands that automate repetitive routine functions.

LSM commands from the Control Panel and many other commands can be combined in macros to produce complex program sequences. The macro program is contained in a text editor, but can also be executed directly from it. Therefore, it is an interpreter, the great advantage being that, when creating a macro, you can test its functions immediately and do not have to have the program compiled first by the computer.

A whole series of macros is already embedded in the LSM program. By means of the **Funct → Install Macro** function, you can select and install additionally included macros to suit your wishes (see also Section 6.3.3 (2.12)).

You should **not modify** macros and other files in the LSM directory that begin with CZ... because they might be overwritten by every update. In certain circumstances, modifications may also result in incomplete updating owing to the change in the date of creation. Therefore, make changes in a copy with a file name that **does not** begin with CZ.

Changes to macros or new macros should be realised by selecting **Funct → Macro Window**. Special macro elements, (macro commands, status variables, operators and functions) allow convenient and comprehensive macro programming.
Therefore, acquaint yourself with the macro elements and syntax. You can find information in the on-line help by selecting **Help → Macro Interpreter.**

7.2 Macro Window

The macro window is a simple text editor in which macros can be edited.

The window itself has a menu of its own, which contains

- Load and storage commands (File)
- Editor commands (Edit) and
- special macro commands (Macro).

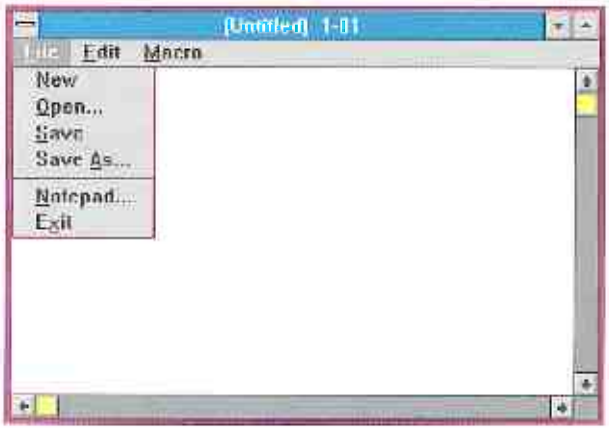


Figure 7-1

File

New	Clears the macro in the memory
Open...	Loads a macro; the "Open Edit" selection box appears
Save	Saves a macro
Save As...	Saves a macro under a new name; the "Save Edit" dialog window appears
Notepad...	Calls up the WINDOWS "Notepad" editor
Exit	Closes the macro window

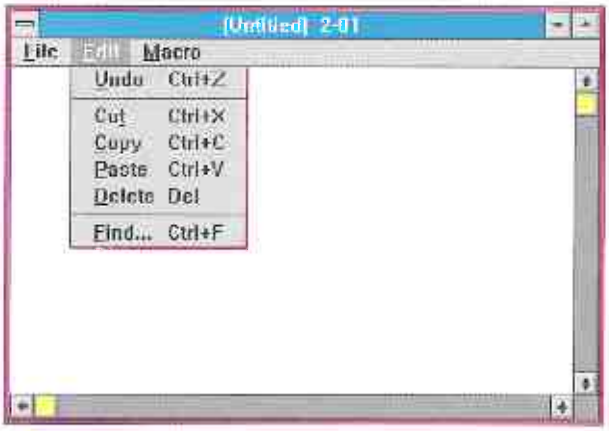


Figure 7-2

Edit

Undo	Reverses the last change
Cut	Copies the marked area to the clipboard and deletes it
Copy	Replaces the marked area by the contents of the clipboard
Delete	Deletes the marked area
Find...	Searches for a string; the "Find" dialog box appears

Macro

Figure 7-3

Execute/ Continue Macro	Executes or continues a macro
Start at Label	Macro begins at a specified label
Stop Macro	Aborts a macro
Trace	During execution of the macro, the cursor always shows the line currently being executed in the macro window
Record Macro	Activates the macro recorder. All operator control steps that can be recorded in a macro are recorded in the macro window in the form of macro commands and are appended to the end of the macro
Single Step	Runs a macro in steps (e.g. for testing).
Step Over	a subroutine is run through, and is then stopped.
Step Out	the macro runs from the current subroutine up to the point where you return to the main program.
Step to Cursor	the macro runs up to the line where the cursor is currently located.
Auxiliary Panel...	auxiliary panel for troubleshooting in macros.

7.3 Application example

(1) "Record Macro" is the most important specific macro function.

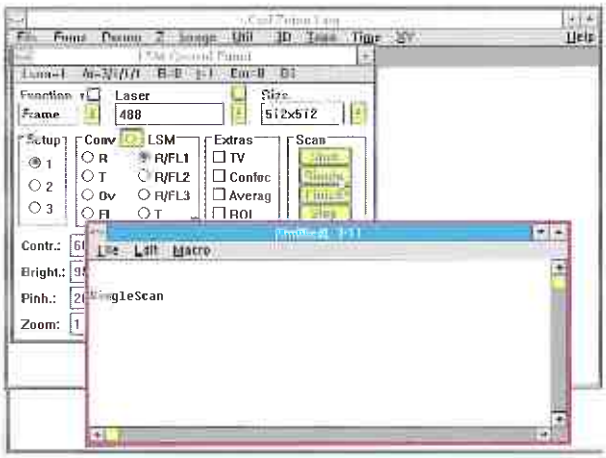


Figure 7-4

- Select Funct → Macro Window in the main menu.
  - The window **(Untitled) 1-01** appears.
- Now select Macro → Record Macro.
  - In doing so, you activate the recorder.
- Now click on the Single field in the **Control Panel**.
  - The "Single Scan" command line is now displayed in the macro window and you have now generated a very simple macro.

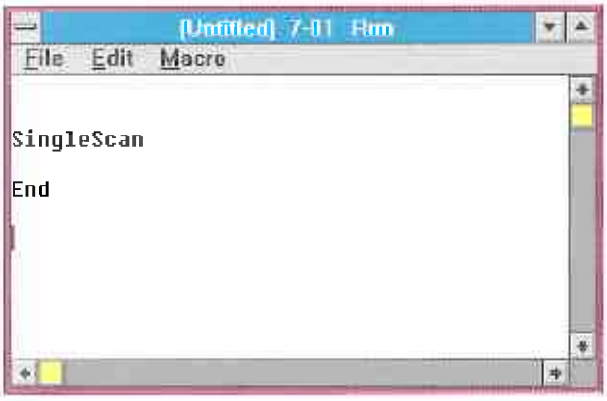


Figure 7-5

- To avoid adding further commands, click on the "Record Macro" line again to deactivate the recorder.
- Press the F5 key if you now wish to run the macro.
  - A single scan is executed.

A properly written program is terminated with an End command. In the macro language, this is the "End" command, which can be inserted in the window in the same way as in a document.

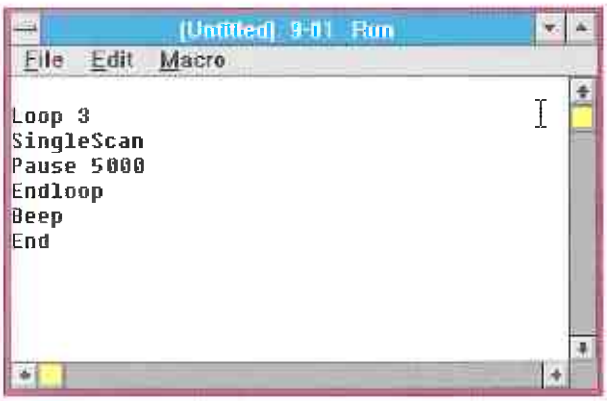


Figure 7-6

A further step would now consist of executing the single scan several times and of then indicating completion by and audible signal. For example, if shots are to be recorded with a pause of 5 seconds, the macro text must be edited as shown in the adjacent figure.

(2) One frequent problem is depicting a transmitted light image in grey and the fluorescence in the colours red (R), green (G) and blue (B).

- To do this, load a transmitted light image into Channel 4 and the fluorescent images into Channel 1 to 3 by selecting Image → Display Control.

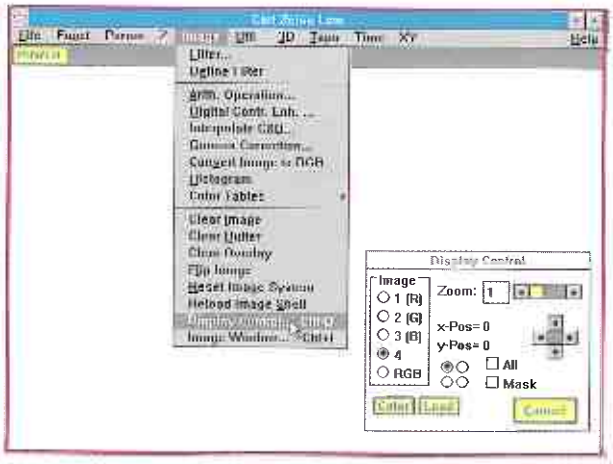


Figure 7-7

- Begin editing a new macro by selecting Funct → Macro Window.
  - The macro window **(Untitled) 1-01** is opened.

- Now activate the "Record Macro" function in the macro window.

- In the main menu, now select **Image → Arith. Operation...**
  - The Arithmetical Operation dialog window appears.

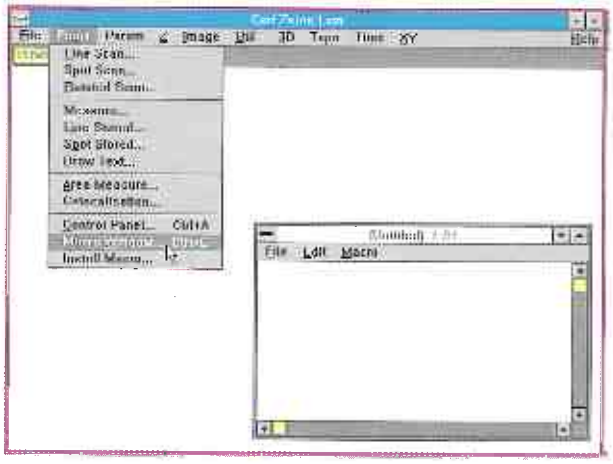


Figure 7-8

- Now edit the addition formula as shown adjacently.

- Enter the parameters
  Source 1 = 1
  Source 2 = 4
  Destination = 1 and activate Add.
  - The operation is executed and the line appears simultaneously in the macro editor.

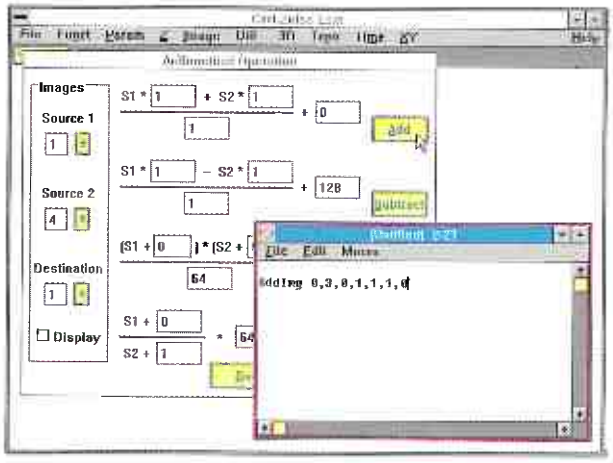


Figure 7-9

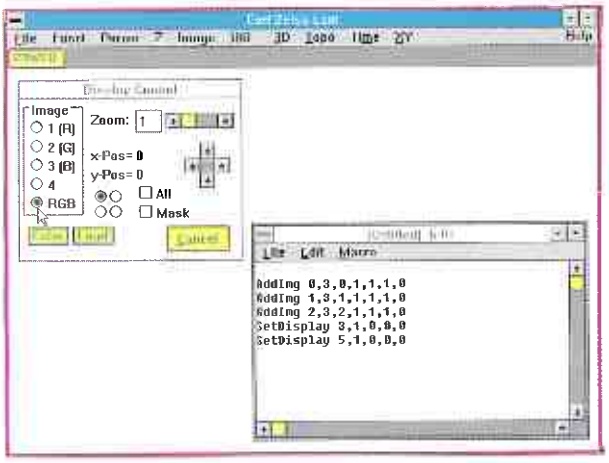


Figure 7-10

- Now enter the other commands
  Source 1 = 2
  Source 2 = 4
  Destination = 2 → Add

  Source 1 = 3
  Source 2 = 4
  Destination = 3 → Add

- To display the image as an RGB image, click on "RGB" in the Display Control Window.
  – The macro then appears as shown in the adjacent figure.

The macro can now be saved and a button can be generated in the LSM button bar (under the main menu) to swiftly call up the mixing operation at any time.

- Double click on the system menu box of the macro window.
  – The Save current changes: prompt appears.
- Select Yes to confirm.
  – The **Save Edit** dialog window appears.
- In the **File Name** box, enter a name for the macro you have generated (e.g. add.mac) and click on Save to confirm it.
  – The macro is now saved under this name.

- To install a button, select Funct → Install Macro.
  - The **Install Macro** dialog window is opened.
- Select add.mac and click on Install to confirm it.
  - The **Install Macro** window, into which you must now enter the installation parameters, then appears.
    - Under **Top Menu**, select where the macro is to be installed, e.g. "Button".
    - Under **Menu Text**, you can still edit the macro name.

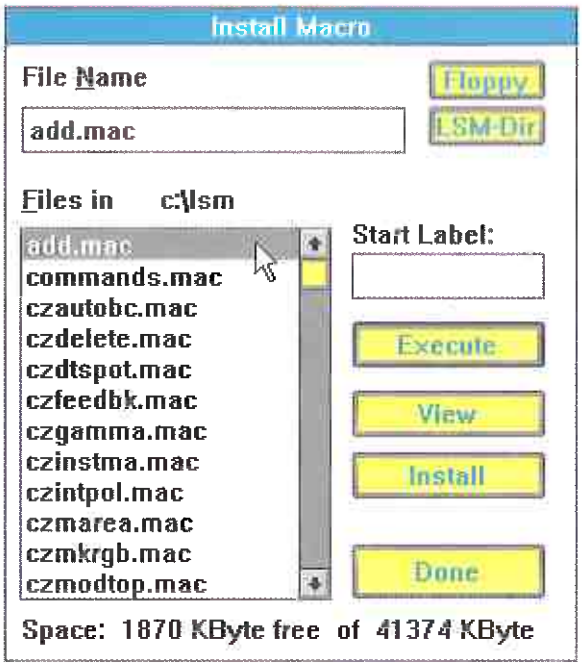


Figure 7-11

    - Under **Accelerator**, select the function key with which you want to start the macro, e.g. "F1".
- Now confirm your selection by clicking on Install.
- Now restart the LSM program
  - The macro is then installed as a button.

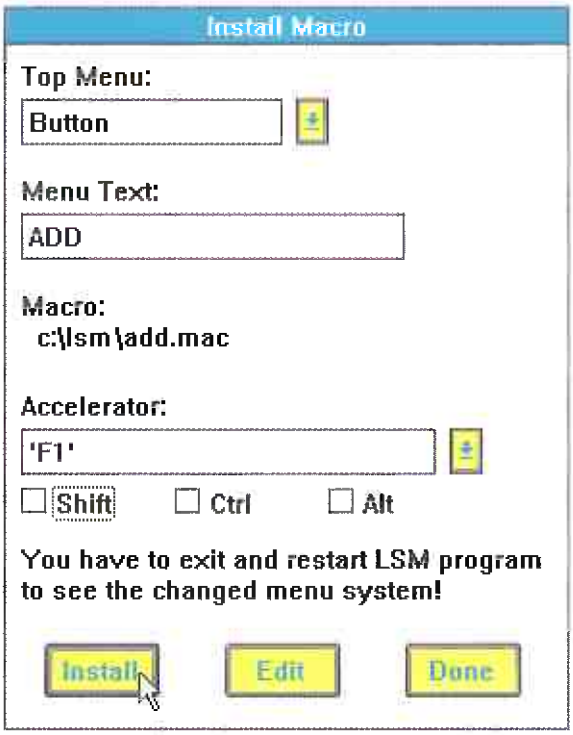


Figure 7-12

CONTENTS

8 CARE, MAINTENANCE, SERVICE

8.1 Care

Care of the laser scan microscope is limited to the following operations:

- Cover the Axiovert 135 microscope with the dust cover after every use.
- Whenever required, clean exposed optical parts.
- Conscientiously wipe off moisture deposits or precipitation of aggressive vapours using a dry cloth.
- Protect the unit against temperatures in excess of 50 °C, frost, moisture and chemically aggressive vapours/substances.
- Remove dust from optical surfaces with a rubber blower or with a natural hair brush. Degrease the brush in alcohol and then dry it. Remove stubborn soiling and fingerprints with a dust-free cloth or leather, if necessary after breathing onto the soiled surface.
  Clean the front surfaces of lenses with light petroleum, but do not use any alcohol.
- Use commercially available optical and spectacle cleaning cloths to remove extreme soiling (e.g. fingerprints) from optical surfaces; if necessary, moisten cloths lightly with petroleum ether.

When using the laser scan microscope in moist and warm climate zones, pay attention to the following notes:

- Store the LSM in dry and well ventilated rooms with a humidity < 85 %; store particularly sensitive modules and accessories such as lenses and eyepieces in dry cabinets.
- When storing the microscope or its parts in closed receptacles for longer periods of time, fungi can largely be avoided by placing absorbent substances soaked in fungicide in the receptacles.

Fine mechanical and optical devices are always at a risk of fungus infection under the following conditions:

- Relative humidity > 75 % for more than 3 days at temperatures from + 15 °C to +35 °C.
- Placing them in dark rooms where there is no movement of air and
- In the event of dust deposits and fingerprints on optical surfaces.

8.2 Maintenance

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

This service agreement comprises a so called "preventive maintenance" once a year in accordance to practice defined by Zeiss.

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 authorised and trained for this purpose by the manufacturer.

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

Troubleshooting on LSM system to be carried out by operating personnel is limited to only a few activities:

- Checking and if necessary changing various fuses
- Changing the 12 V 100 W halogen lamp
- Changing and adjusting the HBO 50 fluorescence lamp

(1) Checking and if necessary changing various fuses

Some units of the LSM system are equipped with power supply units, such as

- 12 V 100 W power supply unit for conventional lighting
- Power supply unit for HBO 50 fluorescence lampe
- Stand power supply unit
- Power supply unit for external laser
- Power supply unit for internal laser

After changing fuses it is not allowed to use fuses which are not identically with the original fuses relating to electrical data.
In case of necessity to change fuses again and again, there is every indication that internal faults have occured.
Immediately inform the service agency!

(2) Changing the 12 V 100 W halogen lamp

Carry out the following activities when replacing the lamp:

- Switch off the power supply unit.
- Remove the mains plug.
- Loosen hexagonal socket screw (8-1/3) and lift off lamp housing (8-1/4).
- Pull defective lamp bulb (8-1/2) out of lamp socket by simultaneously pressing down both springs (8-1/5).

While inserting the new lamp bulb, take care that protective sleeve (8-1/1) will be taken off **not before** lamp bulb is inserted.

- Attach lamp housing again and fix it.
- If necessary center the lamp unit and check homogeneous illumination.

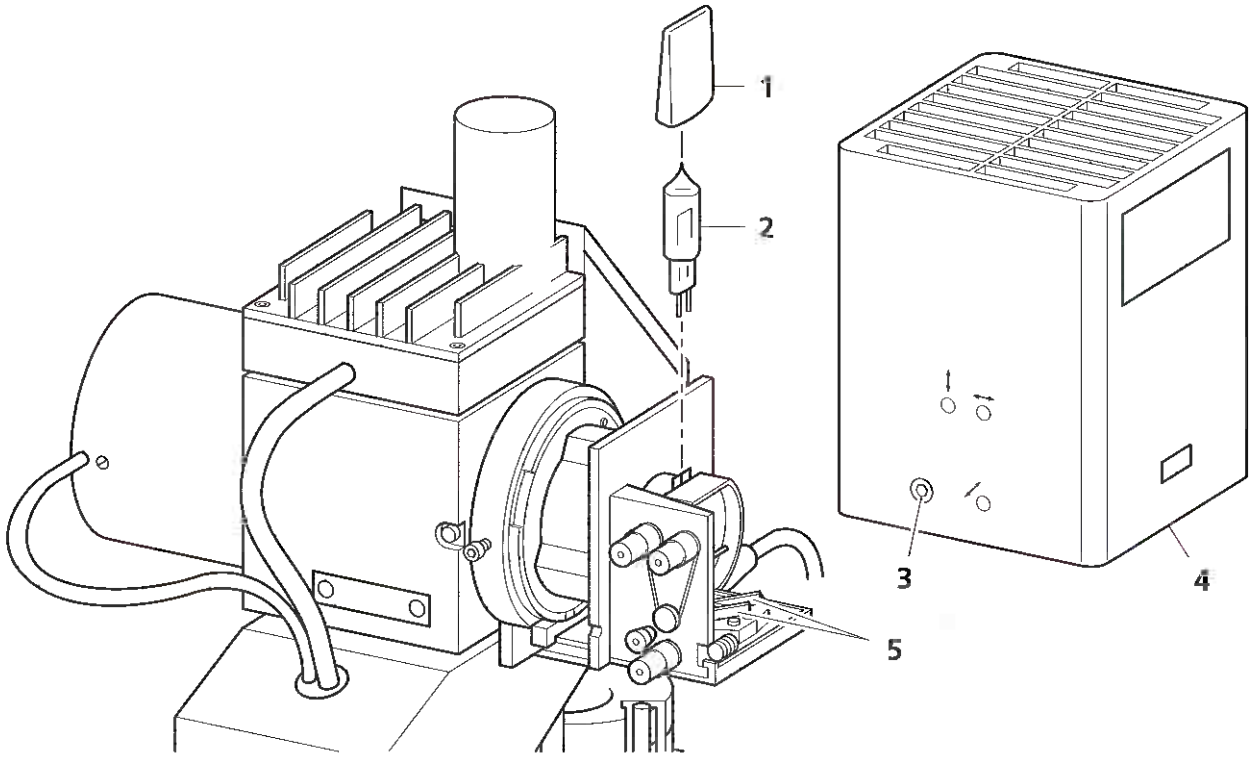


Figure 8-1 Changing the 12 V 100 W halogen lamp

(3) Changing and adjusting the HBO 50 fluorescence lamp

Safety notes for use

The HBO 50 is subject to a high pressure during operation. It must only be used in a closed microscope lamp.

- Cooling of the lamp housing must not be obstructed by covers.
- Before lamp replacement, the HBO 50 must cool down for approximately 15 minutes.
- The lamp emits UV radiation during operation. Avoid direct exposure of the eyes or skin. You are advised to wear protective goggles when handling the microscope lamp.

The HBO 50 must be replaced after its average useful life of 100 h has expired. There is an increasing risk of explosion once the average useful life has been exceeded.
You can read the operating time of the HBO 50 off the elapsed hours counter.

This warning plate on the rear of the lamp signifies:
Caution: hot surface!
Allow the lamp to cool down before touching it.

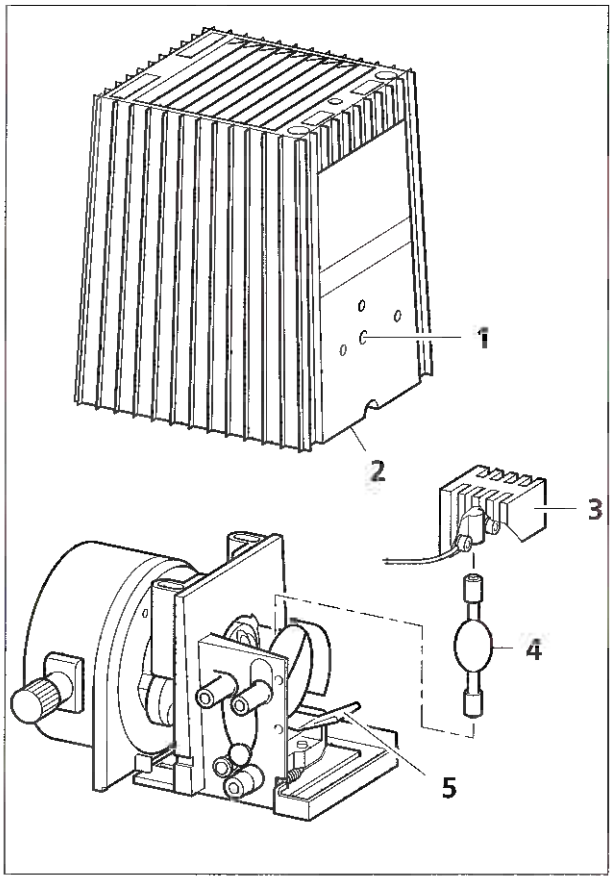


Figure 8-2 Replacing the HBO 50 burner

Burner replacement

Switch off the power supply unit and remove the connector of the lamp holder from the connection socket of the power supply unit. Allow the lamp and the lamp housing to cool down (at least 15 minutes).

- Detach the HBO 50 lamp from the microscope stand; to do this, undo the clamping screw on the microscope stand.
- Place the lamp on a flat working surface.
- Using the 3 mm ball-head screwdriver provided, undo the screw (8-2/1) and detach the lamp housing (8-2/2).
- Depress the spring lever (8-2/5), hold the burner (8-2/4) by the heatsink (8-2/3) and pull it out of the lamp holder.
- Place the heatsink with the burner on the working surface so that the clamping screw on the heatsink is accessible.

- Using the 3 mm ball-head screwdriver, undo the clamping screw on the heatsink and remove the used burner.

The cable on the heatsink must not be removed.

- Touch the new burner by the marked metal holder and insert it in the heatsink so that the reflecting part of the burner is at the bottom when inserted in the holder. The reflecting melt-off part of the burner must point to the side (so that it will not interfere with optical imaging).
- Carefully remove the clamping screw from the heatsink.
- Avoid fingerprints on the glass parts of the burner and, if necessary, remove fingerprints immediately.
- Depress the spring lever and insert the burner in the holder; in doing so, hold the burner by the heatsink only.
- Slowly relieve the spring lever, releasing the heatsink at the same time.

The heatsink must be aligned parallel with the lamp housing. The heatsink's position can be corrected by pressing the spring lever and by turning the heatsink.

- Fit the lamp housing onto the lamp base and tighten the screw (8-2/1).
- Fit the microscope lamp onto the microscope stand.
- Note down the elapsed hours counter reading on your power supply unit. The HBO 50 burner must be replaced once the rated useful life of 100 h has been reached.

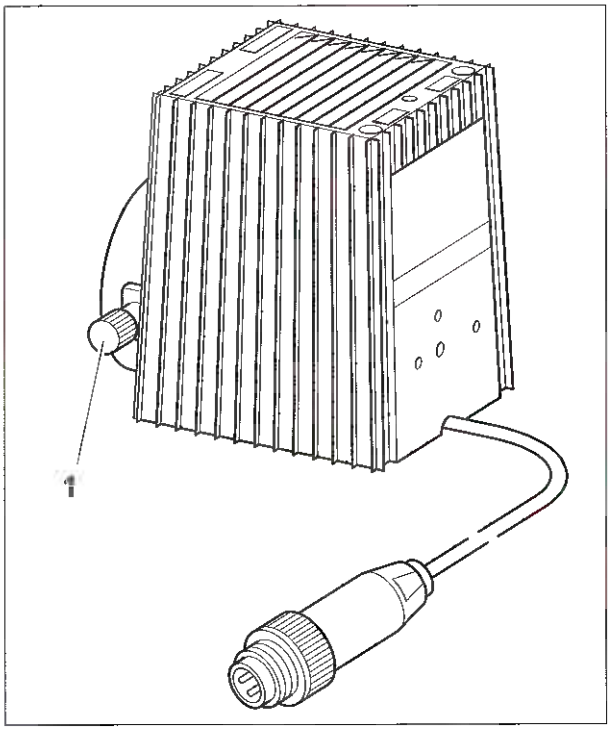


Figure 8-3 HBO 50 adjustment

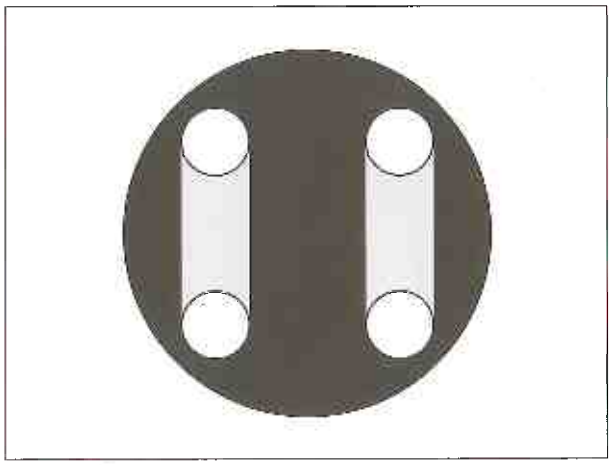


Figure 8-4 Burner imaging

Adjusting the lamp

Never look directly into the triggered lamp as otherwise you may suffer irreparable eye damage.
Wear eye protection, e.g. sunglasses, to protect your eyes when observing the bright light spot.

- Unscrew one lens and, via the free passage, check the image of the light source on a piece of paper in the specimen plane (on the specimen stage).
- By means of the knurled knob (8-3/1), focus the collector so that both light arcs are sharply focused.
- By means of the adjusting screw (↔), adjust the lamp axially to the reflector so that both arcs appear to be equally large analogously to Figure 8-4.
- Using the 3 mm ball-head screwdriver and the adjusting screws for height (↕) or side adjustment (↗), centrally position the arc image next to the arc analogously to Figure 8-4.
- Screw the lens back into the lens turret.

8.3 Service

The manufacturer of the unit cannot be held liable for damage resulting from operating errors, negligence or unauthorised 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.
This will also render all warranty claims null and void.

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 authorised and trained for this purpose by the manufacturer.

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

For servicing, contact your nearest regional representative or

Carl Zeiss Jena GmbH
Zeiss Gruppe
Unternehmensbereich Mikroskopie
Tatzendpromenade
D-07745 Jena

Telefon: (***49) Ø3641 / 64-2936
Telefax: (***49) Ø3641 / 64-3144

CONTENTS

9 SOFTWARE INSTALLATION AND ADAPTIONS, PROGRAM UPDATES

9.1 Software installation

9.1.1 Installing the DOS 6.2 operating system (OEM version)

The operating system must be installed before you use a computer for the first time. A so-called OEM version of MS-DOS 6.2 is supplied. This version is only available when purchasing a new PC.

This installation routine is simplified in comparison with the installation of update versions.

MS-DOS Version 6.2 has a separate installation program (setup) that carries out installation from the diskettes onto your hard disk automatically and features menu prompting.

If your dealer has already installed an operating system on a new computer (e.g. MS-DOS 4.01), when installing MS-DOS 6.2 (OEM version) the \DOS directory will be deleted or substituted by MS-DOS 6.2.

The fastest way to install MS-DOS 6.2 on your hard disk is as follows:

1. Insert the MS-DOS diskette "DISK 1" in the installation drive.
2. Select the drive in use as the current drive. For the drive a, for example, enter: **A:** and press the Enter key.
3. Enter the **setup** command on the keyboard and start it by pressing the Enter key.
4. Confirm the information about the computer (nationally specific information/key assignments), if correct.
5. Carry out all further installation steps in dialog mode.
6. After completing installation, remove all diskettes from the drives and press the Enter key. The computer now restarts and starts up with the new MS-DOS 6.2 operating system.

You can specially adapt MS-DOS at any time to your existing hardware and to your requirements by editing the **autoexec.bat** and **config.sys** system files. You are best advised, however, to leave such changes to a specialist because errors and mistakes may result in considerable problems. Thus, it may happen that the computer might no longer find programs or that the keyboard cannot be operated in the usual manner. In extreme cases, the computer can no longer be started in certain circumstances (see also Sections 9.1.3, 9.1.4 and 9.1.5).

Please refer to the manual entitled **WINDOWS FOR WORKGROUPS & DOS 6.2**, which is supplied together with the DOS program diskettes, for detailed information on how to install the update and OEM versions of MS-DOS 6.2.

9.1.2 Installing the WINDOWS 3.11 graphical user interface

The WINDOWS 3.11 graphical user interface represents a menu-controlled user interface that is a user-friendly addition to the DOS operating system.

Provided the hardware and software prerequisites are fulfilled (refer to the WINDOWS 3.11 manual), two methods of installing WINDOWS 3.11 are at your disposal:

- **Express Installation;** in this case, the program is installed largely automatically and you only need to answer a few questions relating to your printer and printer port, as well as specifying your name.
- **User-defined installation,** which should only be done by experienced users because this method of installation requires decisions on your part in relation to special configuration possibilities.

The fastest way to install WINDOWS 3.11 on your hard disk is as follows:

1. Insert WINDOWS disk 1 in the installation drive.
2. Define the drive in use as the current drive. For the drive a, for example, enter: **A:** and press the Enter key.
3. Enter the **setup** command on the keyboard and start it by pressing the Enter key.
4. Select Express Setup and follow the instructions issued by the SETUP installation program.

The installation program configures WINDOWS according to the information it has determined about the computer, monitor, mouse, keyboard, country, printer and, if applicable, network.

You can modify the WINDOWS environment and the hardware configuration at any time by means of the SETUP installation program, System Control or by way of the WIN.INI and SYSTEM.INI system files. The system files should only be modified by experienced users, however, because errors may lead to substantial problems when running the program.

Please refer to the manual entitled **WINDOWS FOR WORKGROUPS & DOS 6.2**, which is supplied together with the DOS program diskettes, for detailed information on how to install and use WINDOWS.

9.1.3 Setting up the initialisation and start files in the root path

This involves setting up the **autoexec.bat** and **config.sys** files in the WINDOWS user interface.

Prerequisites

WINDOWS has been started and the **Program Manager** and **Main** are open.

- Open the "Accessories" group icon by double clicking it.
- Using the scroll bar, move the contents of the "Accessories" group until the "Notepad" program icon is visible.
- Open the "Notepad" program icon by double clicking it.
  - The "Notepad-(Untitled)" window appears.
- Select "File Open..." in the editor's menu bar.
  - The "Open" window appears.
- In the "Directories" panel, select the root directory **c:** by double clicking it.
- Select "All Files (*.*)" in the fold-out menu of the "List Files of Type" box.
  - All files in the root directory are now displayed.
- Scroll with the scroll bar until you see the **autoexec.bat** file.
- Activate the file with the cursor.
  - In doing so, the **autoexec.bat** file is transferred to the "File Name" box.
- Press the OK button to confirm it.
  - The **autoexec.bat** start file generated automatically by DOS Version 6.2 now appears in the Notepad window.

```
@ECHO OFF
PROMPT $p$g
PATH C:\DOS
SET TEMP=C:\DOS
KEYB GR,,C:\DOS\KEYBOARD.SYS
```

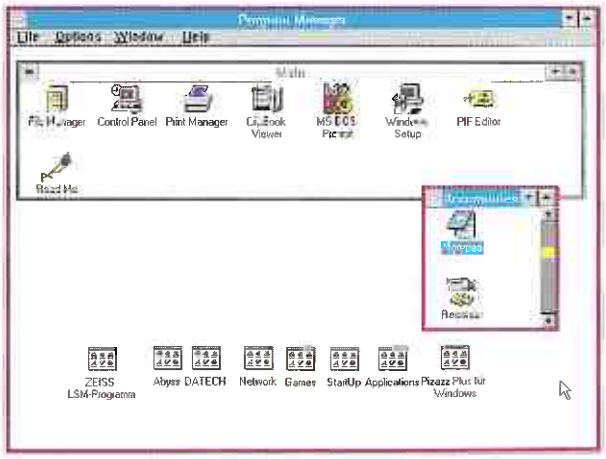


Figure 9-1

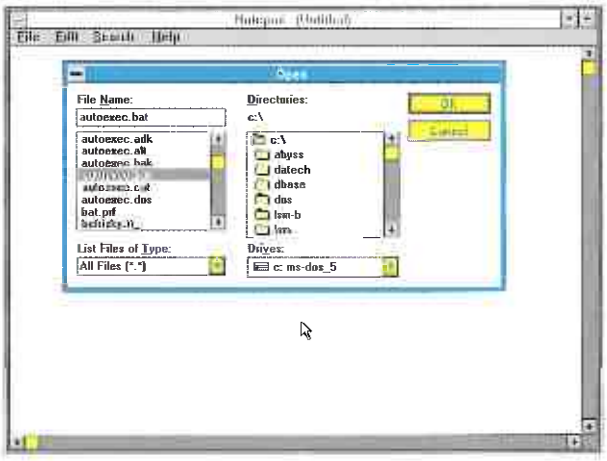


Figure 9-2

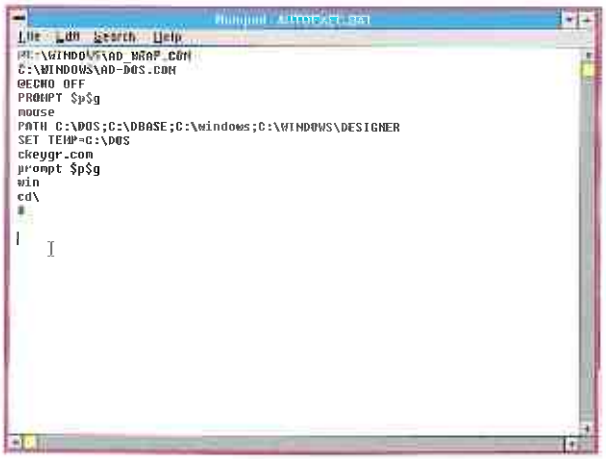


Figure 9-3

The automatically generated start file **autoexec.bat** now has to be adapted to your actual hardware conditions, i.e. you must add various commands.

You must now repeat the above steps for the **config.sys** file.
Begin with the Program Manager again. The **config.sys** initialisation file generated by DOS 6.2 is as follows:

```
DEVICE=C:\DOS\SETVER.EXE
DEVICE=C:\DOS\HIMEM.SYS
DOS=HIGH
COUNTRY:049,850,C:\DOS\COUNTRY.SYS
FILES=10
```

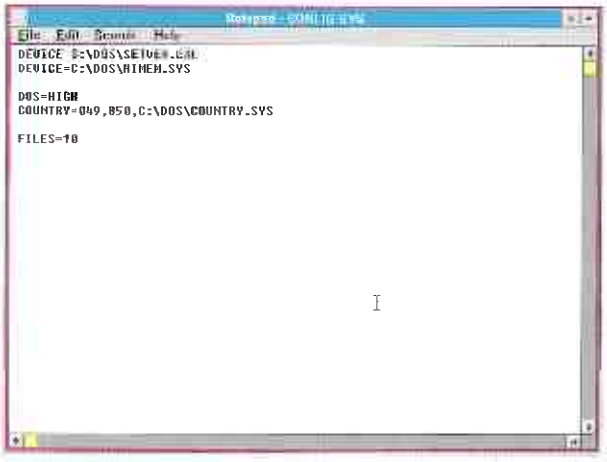


Figure 9-4

Now you may have to adapt this file also.

9.1.4 Making backups of the program, initialisation and start files

To protect your data and to ensure reproducibility of programs, you are advised to make so-called backup diskettes of all program diskettes supplied or of start/initialisation files you have created yourself. Only ever use the backup diskette for initialisation and never the original.

All original diskettes should be write-protected, i.e. both windows in the diskette should be open. Safeguard the backups you have made in the same way.

Get an adequate number of new diskettes before beginning work. You are likely to need the following:

- DOS operating system : 3
- WINDOWS user interface : 8
- Basic ZEISS LSM program including any LSM program options : 1
- Boot diskette including one backup : 2
- Start/initialisation files (autoexec.bat, config.sys, win.ini) : 1

→ 15 $3^1/_2$" diskettes

You create the program backups as follows:

- Format the presumably required number of diskettes as described in Section 9.3. When assigning data medium identifiers, pay attention to the fact that no more than 11 characters are allowed, no blanks are allowed and no special characters are permitted either.
  - After formatting diskettes, WINDOWS displays the File Manager again.
- Select "Disk/Copy Disk..." in the menu line.
- If necessary, select the drives for the source and destination disks.
- Confirm your selection by clicking the OK key.
  - The following message appears:

```
This operation will erase ALL
data from the destination disk.
Are you sure you want to
continue?
```

- Select Yes to confirm.
  - The following prompt appears:

```
Insert source disk.
```

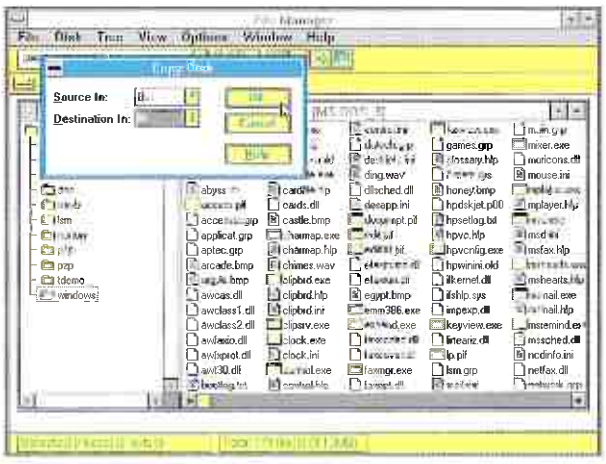


Figure 9-5

- If you have formatted a diskette beforehand, remove it from the drive and insert the source diskette.

- Select [OK] to confirm.
  - The source diskette is read to the computer's RAM.
  - During this time, the following message appears:

```
Now copying disk in
Drive B:
XX % completed
```

  - The next prompt appears when 50 % has been reached

```
Insert destination disk
```

- Remove the source diskette from the drive and insert the destination diskette in its place.

- Select [OK] to confirm.
  - The contents of the source diskette stored in the RAM are now copied to the destination diskette.
  - During this time, the following message appears:

```
Now copying disk in
Drive B:
XX % completed
```

  - The File Manager appears again once copying has been completed (51 % to 100 % completed).

- Create further program backups as described.

You create the Start/Ini backups as follows:

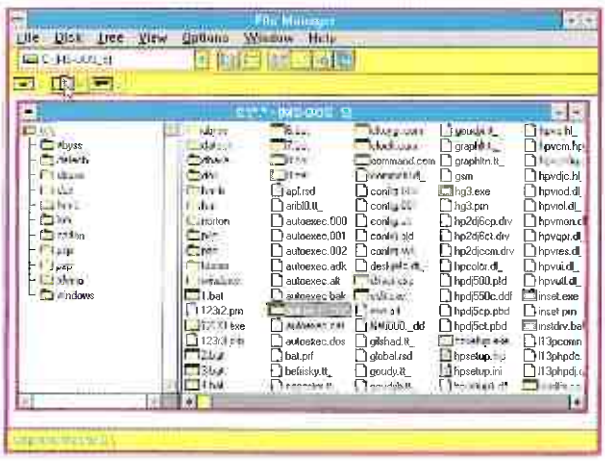


Figure 9-6

- Double click the root path c:\ in the open File Manager.

- Insert a formatted backup diskette in drive b.

- Click on the autoexec.bat key, hold down the mouse button and drag the corresponding symbol to drive b.
  - The following prompt appears:

```
Are you sure you want to copy
the selected files or directo-
ries to B:\?
```

- Select [Yes] to confirm.
  - The selected file is copied to the backup diskette inserted in drive b.
- Copy the **config.sys** ini file in the same way.
- To check that the file has been copied, open the drive icon b by double clicking it.
  - The copied files are displayed.
- Remove the start/ini backup diskette and mark it appropriately.

9.1.5 Producing a boot diskette for starting the system from a diskette drive

A so-called "bootable system diskette" contains the operating system files needed to start the computer as well as the drivers for the keyboard and the mouse.

A system diskette can only be used in drive a!

You create such a boot diskette by following the steps given below:

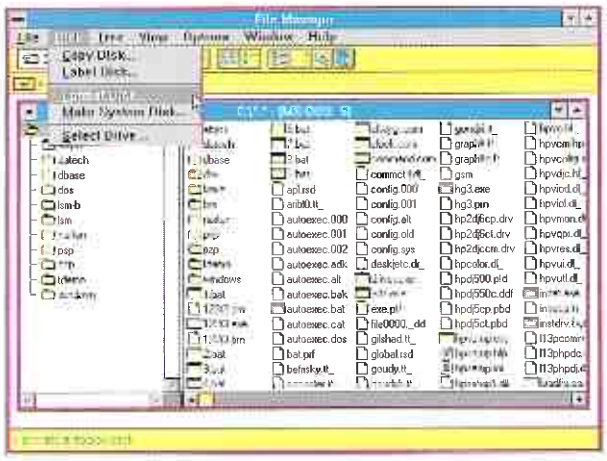


Figure 9-7

Prerequisites

Your computer is operable and WINDOWS has been activated.

- If necessary, open the "Main" window by double clicking it.
- Open the File Manager by double clicking it.
- Select drive c.
- In the menu bar, select the "Format Disk..." item in the "Disk" menu.
  - The "Format Disk" window appears.

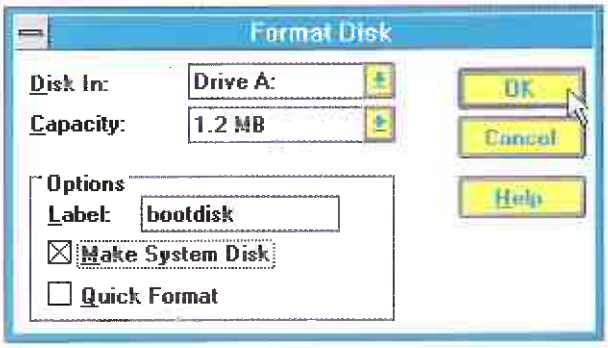


Figure 9-8

- Insert a new diskette in drive a.
- If necessary, select the drive containing the inserted diskette in the fold-down menu (in the example: "a").
- Under Options, enter the name of the diskette in the "Label" box, e.g. "bootdisk" (no more than 11 characters).
- Select "Make System Disk".
- Select OK to confirm.
  - A window containing the following text prompt appears:

```
Formatting will erase ALL data
from your disk. Are you sure you
want to format the disk in
drive A?
```

- Select [Yes] to confirm.
  - A window containing the following message follows:

```
Now formatting disk
            xx % completed
```

  - The following is then displayed briefly:

```
Copying system files
```

  - The adjacent window then appears.

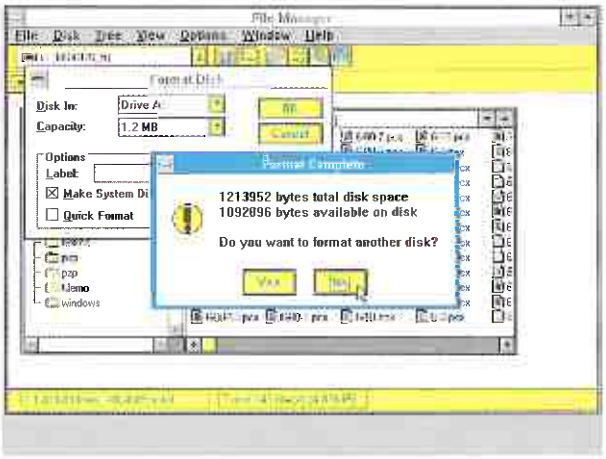


Figure 9-9

- Select [No] to acknowledge:
  - The system diskette containing the system files has been created and the program returns to the File Manager.
- In the "Windows" menu, open the pull-down menu and select "Cascade".

After you have created the system diskette, you must add the following files to it:

- autoexec.bat (Start file)
- config.sys (Configuration file)
- ckeygr.com (Keyboard driver)
- mouse.com (Mouse driver)

- Select drive a, followed by drive c, by double clicking.
  - The window for drive c is placed in front of the window for drive a.
- In the root directory c:\, click on the **ckeygr.com** file, hold down the mouse button and move the icon to the window of drive a.
  - A window containing the following prompt is opened:

```
Are you sure you want to copy the
selected files or directories to
A:\?
```

- Select [Yes] to acknowledge the prompt.
  - A message appears briefly, indicating that the file is being copied.
- Select the **autoexec.bat, config.sys and mouse.com** files from the root directory c:\ and copy them to a in the same way.

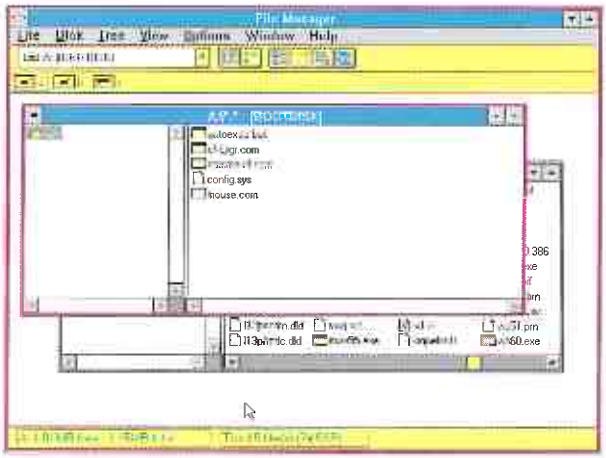


Figure 9-10

- After copying, click on the drive icon a to display the copied contents.

Of the files copied, the two files

- **autoexec.bat** and
- **config.sys**

now have to be edited, i.e. the files that were originally written for the drive c now have to be prepared for execution in the drive a.

- Close the File Manager and open the Program Manager.
- Open the "Accessories" group window by double clicking it.
- In the open "Accessories" group window, search for the "Notepad" icon.
- Open the "Notepad" program icon by double clicking it.
  - An empty "Notepad-(Untitled)" window is opened.
- Select "File/Open" in the menu line.
  - The "Open" window is displayed.
- Enter **autoexec.bat** in the "File Name" box.
- Select **"All Files (*.*)"** in the "List Files of Type" box.
- In the "Drives" box, select drive a in the fold-down menu.
  - This means that only the **autoexec.bat** file transferred to the diskette is edited and **not** the one saved in the root directory.

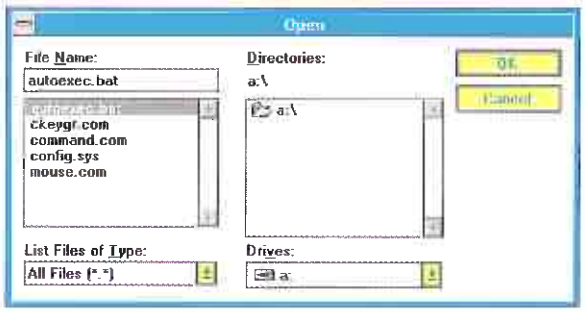


Figure 9-11

- Click on the OK button.
  - The ASCII file **autoexec.bat** is now displayed in the editor.

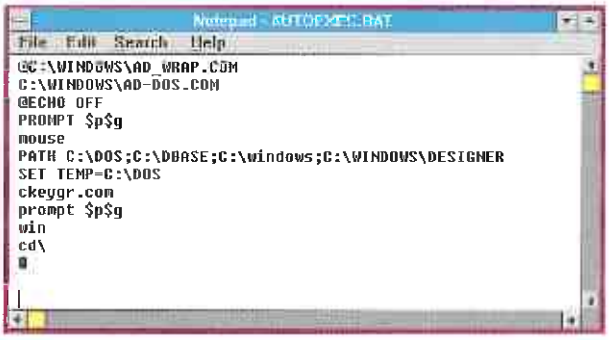


Figure 9-12

- Edit the **autoexec.bat** file as shown in the adjacent figure!

The **autoexec.bat** start file on the boot diskette now only contains the path (a:\) and the keyboard driver (ckeygr.com) and the mouse driver (mouse.com).

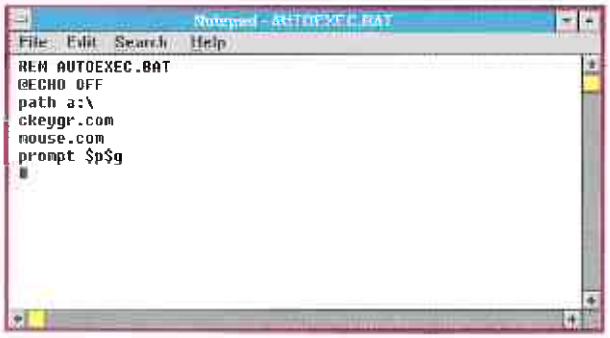


Figure 9-13

- Then edit the **config.sys** configuration file on the boot diskette in the same way.

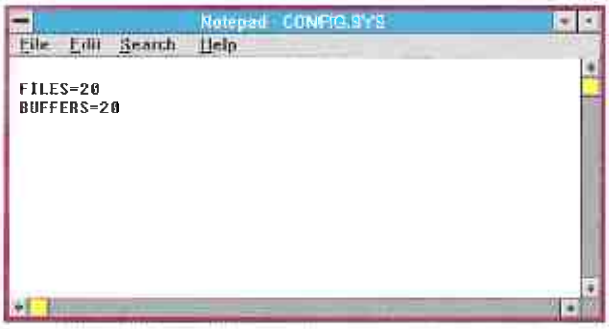


Figure 9-14

- Close the editor by double clicking.
  - The program returns to the Program Manager.

- Close the Program Manager by double clicking it.
- End WINDOWS.
  - The DOS 6.2 operating system shows the a:\>_ prompt.

You can now display the contents of the boot diskette in drive a by entering the DOS **dir** command:

```
Datenträger in Laufwerk A ist BOOTDISK
Datenträgernummer: 12FD-2A4F
Verzeichnis von A:\

COMMAND  COM     50031  06-11-91  12:00p
MOUSE    INI        28  11-09-94   9:54a
MOUSE    COM     56408  03-10-92  12:00p
AUTOEXEC BAT        75  11-09-94   9:21a
CONFIG   SYS        22  11-09-94   7:41a
CKEYGR   COM      3412  10-29-87   8:58a
         6 Datei(en)     109976 Byte
                        1030144 Byte frei
```

The boot diskette in drive a thus contains all start and initialisation files needed to start your system from diskette if the computer is no longer capable of accessing the data on its hard disk.

You can print out the information about your boot diskette as a hard copy by pressing the Print key, using it to identify your boot diskette.

- Remove the bootable system diskette from drive a, mark it and store it together with other backup diskettes.

Starting the system with the boot diskette is explained below. Carry out this operation once to make sure that your computer can be rebooted in the event of a crash:

- Insert the boot diskette in drive a.
- Restart the computer by one of the following methods:
  - by pressing the "Ctrl + Alt + Del" keys (warm start) or
  - by pressing the Reset key on the tower housing (cold start) or
  - by switching off the computer's power supply and by switching it on again.
- The system boots up and the following message appears:

```
A>REM AUTOEXEC.BAT

Keyboard Driver for G80-100HAD Version 1.2
Copyright (C) Cherry Mikroschalter GmbH 1987
Microsoft (R) Mouse Driver Version 8.20
Copyright (C) Microsoft Corp. 1983-1992. All rights reserved.
Mouse driver installed
A:\>
```

- Remove the boot diskette from drive a.
  - You can now reconfigure the computer with the aid of your backup diskettes.

- Insert the first backup diskette in drive a.
  - By entering the DOS **copy *.*c:** command, you can now copy all files from the backup diskette in drive a to drive c.
- Press the ENTER key (↵).
  - All backup files are displayed during copying.
  - The number of copied files appears once copying has been completed.
- The computer then displays the A:\> prompt again.
- Remove the backup diskette 1 and insert the next backup diskette.
  - You can redisplay the **copy *.* c:** command by pressing the function key F3. The command is activated again by pressing ENTER.
- Repeat copying the number of times needed to transfer all backup files back to drive c.
  - The computer displays A:\>_.
- Restart the computer by pressing the "Ctrl + Alt + Del" keys.
  - The computer boots up and starts WINDOWS or the ZEISS LSM program.

If an error has occurred at the WINDOWS level or in the user program, the computer will abort starting at this level.
In such cases, get a software specialist to remedy the error.

9.1.6 Installing the ZEISS LSM program under WINDOWS 3.11

Installation of the LSM program under the WINDOWS 3.11 graphical user interface embraces the following installation routines:

- Program installation from diskette and manual starting (test start) of the LSM program.
- Creating a program group (prerequisite for automatic starting) and creating the program icon for stating.
- Setting up automatic starting of the LSM program.

(1) Installation of the LSM program

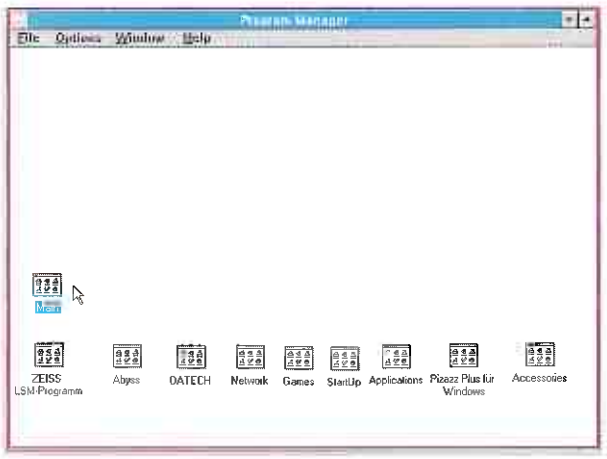


Figure 9-15

- Switch on the computer.
  - Booting in the DOS operating system begins.
  - The WINDOWS user interface is then activated (the WINDOWS icon is displayed briefly in full screen size).
  - WINDOWS then displays the **Program Manager**.

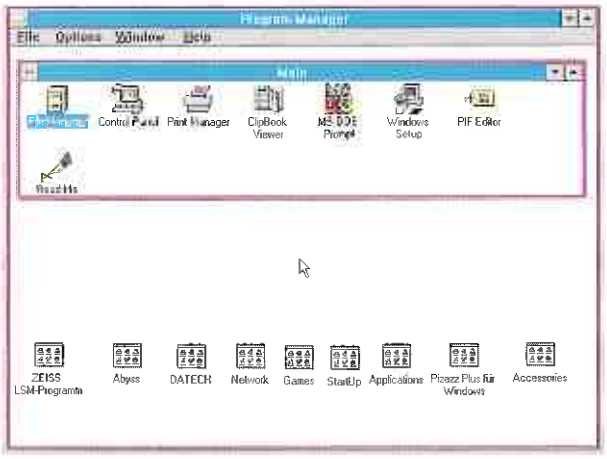


Figure 9-16

- Double click on **Main** to open it.
  - The **Main** group icon becomes an open group window.

- Double click on **File Manager** to open it.
  - The File Manager group icon becomes an open group window.
- Insert the ZEISS LSM program diskette in the computer's drive.
- According to the current hardware configuration, select the installation drive a or b by double clicking it

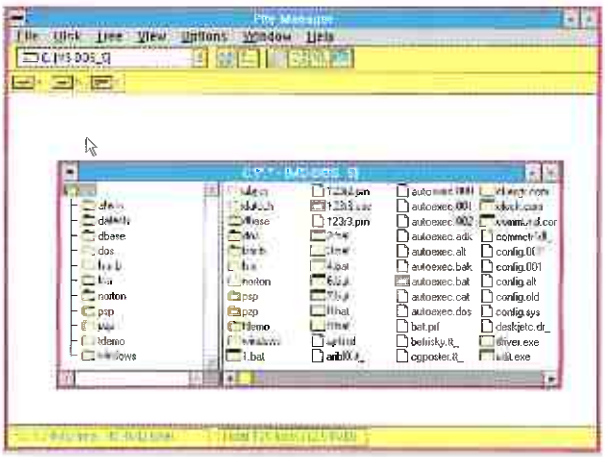


Figure 9-17

  - A further window showing the contents of the program diskette inserted in the installation drive appears on the screen, with the installation routine **install.bat** below it.

The LSM program requires approximately 1 MB of storage space.

- Double click on **install.bat.**
  - The LSM program is installed automatically on the hard disk.
    The files that are currently being installed are displayed on the screen at the same time; the computer then switches back to the **File Manager** (install.bat remains activated).

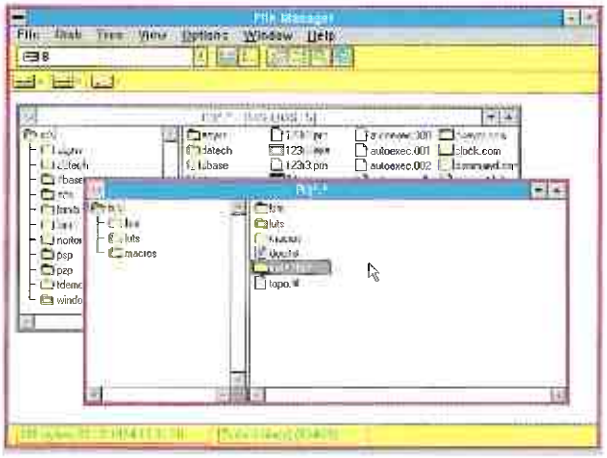


Figure 9-18

- Remove the program diskette from the drive.
- Select drive c.
  - The installation program is assigned to the "lsm" directory icon.
- Click on lsm.
  - All files in this directory are then displayed.
- Call up lsm.exe by double clicking it.
  - This manually starts the ZEISS LSM program.

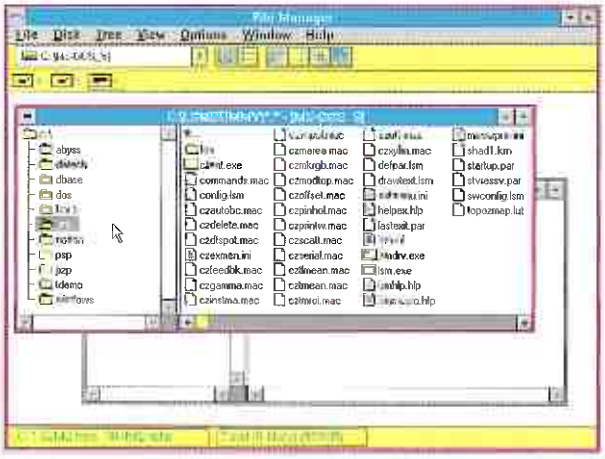


Figure 9-19

Figure 9-20

– The main LSM menu appears.

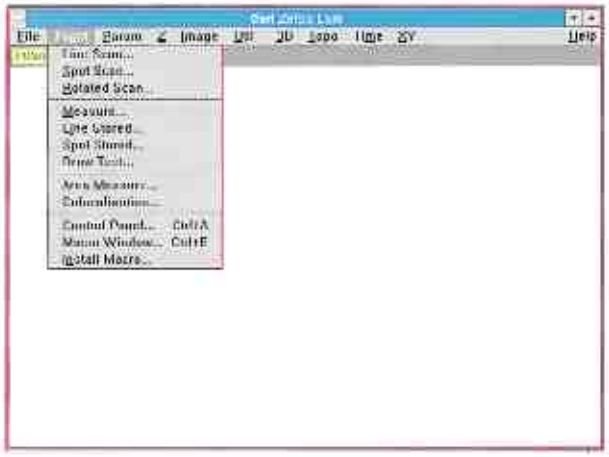


Figure 9-21

- Click on the Funct item in the main menu
  – The pull-down menu appears.

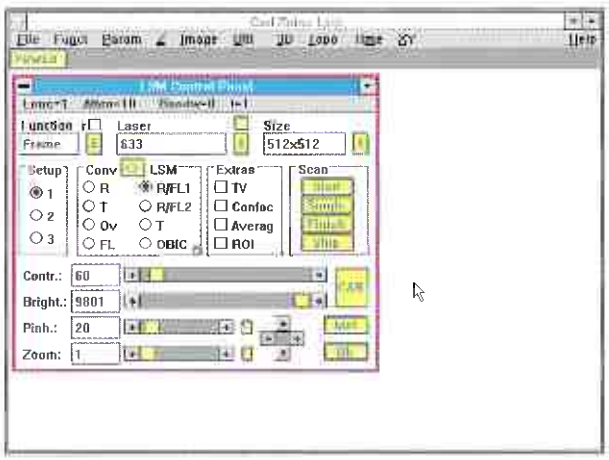


Figure 9-22

- Move the cursor to "Control Panel" and click on it.
  – The Control Panel of the LSM program appears.

You must set up a so-called "Autostart" group to make sure that the LSM program's Control Panel will appear automatically in future after switching on the computer. That is to say, you must create a "ZEISS LSM program" group and a program item for starting.

(2) Creating a program group and the program item for starting

- Close the "Control Panel" application window; this can be done in one of three ways:
  - By double clicking on the system menu box.
  - By clicking on the icon box button ▼.
  - By clicking on the "File" item and by selecting "Quit".
- Close the LSM program.
  - The program returns to the File Manager.
- Close the File Manager.
  - The program returns to the Program Manager.
- In the main menu, click on the "File" item.
  - The pull-down menu appears.
- Move the cursor to "New..." and click on it.
  - The "New Program Object" window appears.
- Select Program Group and confirm it by pressing OK.

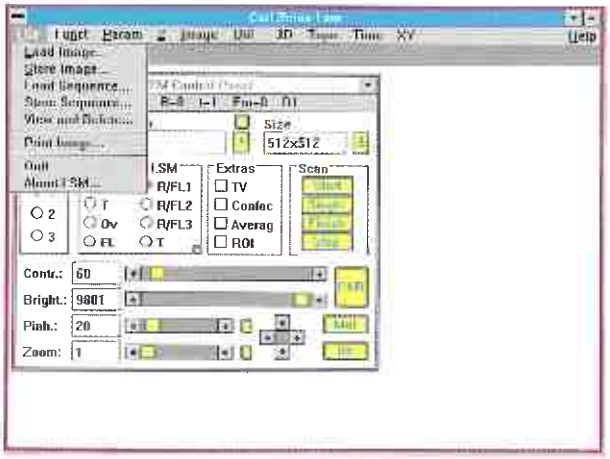


Figure 9-23

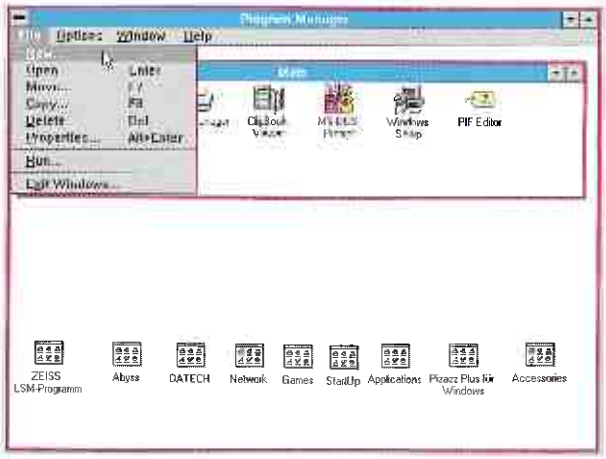


Figure 9-24

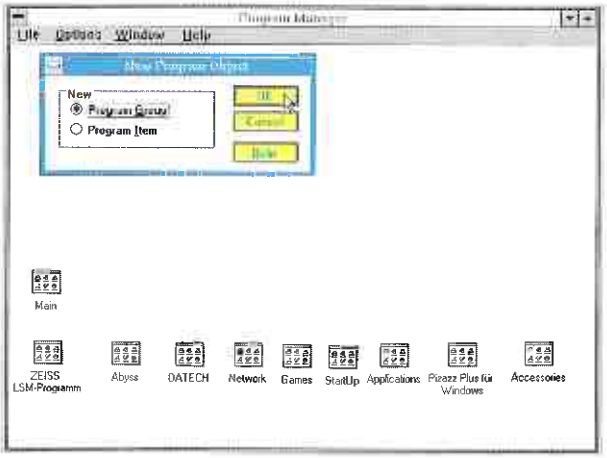


Figure 9-25

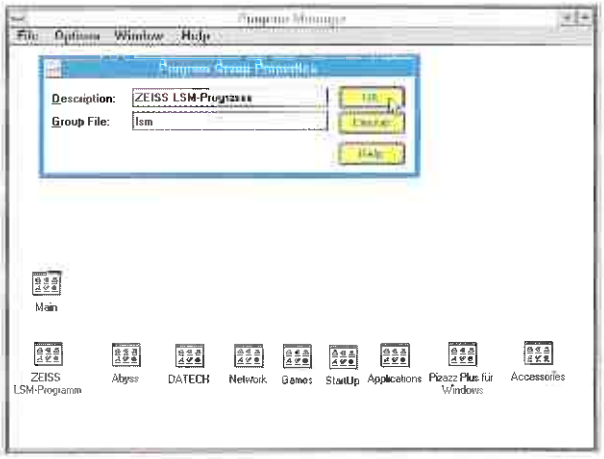


Figure 9-26

- Enter the program's name on the keyboard, e.g. "ZEISS LSM program".
- Position the cursor on the "Group File" box and enter the real name of the program stored under DOS: "lsm".

The group file name must not exceed a maximum length of eight characters. As this is a DOS file name, it must not contain any blanks either. None of the special characters are allowed either.

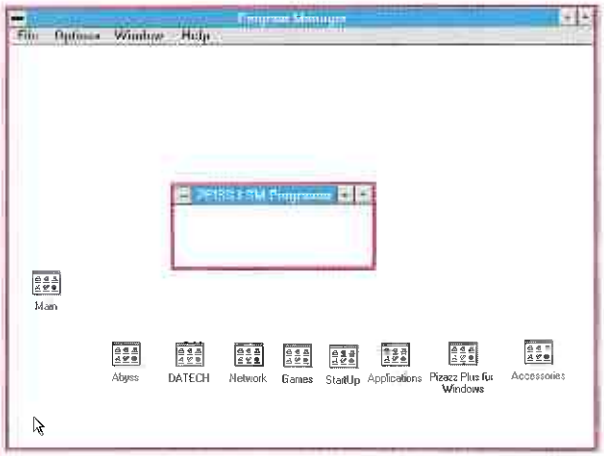


Figure 9-27

After you have entered "lsm", the program generates the complete group file name consisting of:

- drive parameter,
- path,
- starting file name.

```
C:\WINDOWS\LSM.GRP
```

This information can be viewed by selecting the "File" → "Properties..." menu item.

- Press OK to confirm the program properties.
  - The newly generated group window appears again.

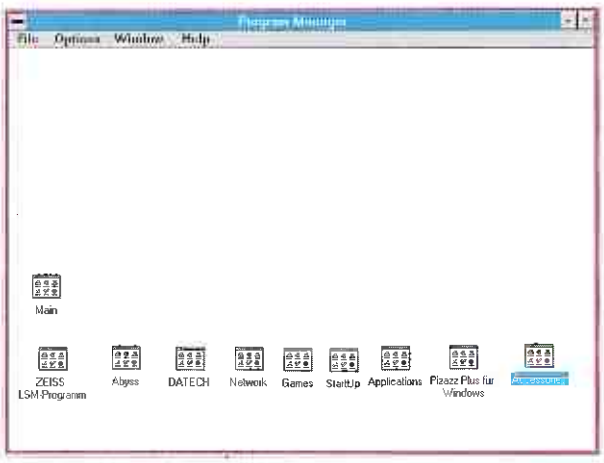


Figure 9-28

- Close the open "ZEISS LSM program" group window by double clicking on the system menu box or by clicking on the icon box button.
  - The group window is reduced to the program icon.

- Activate the "ZEISS LSM program" icon by one single click.
- Click on the "File" item in the Program Manager.
  - The pull-down menu appears.
- Move the cursor to "New..." and click on it.
  - The "New Program Object" window appears again.

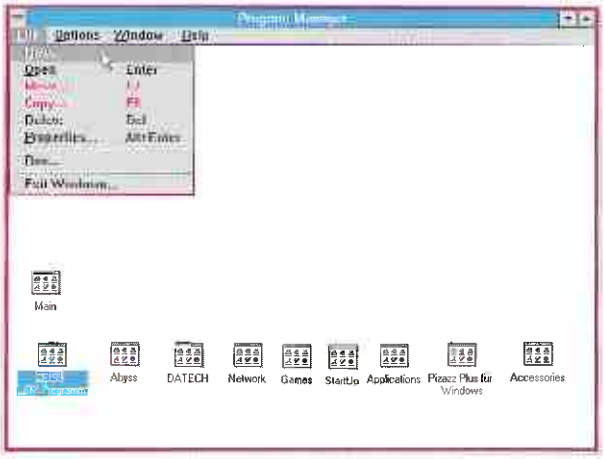


Figure 9-29

- In the "New Program Object" window, click on the "Program Item" option and press OK to confirm it.
  - The "Program Item Properties" window appears.
- On the keyboard, enter the name of the program in the Description box, e.g. "ZEISS LSM program" and then enter "c:\lsm\lsm.exe" in the Command Line box.
- If you enter the letter "S" in the Shortcut Key box, for example, the combination STRG + ALT + S , appears, i.e. you can start the LSM program later on by entering this keystroke combination.
- Click on the "Run Minimized" box and press Change Icon to confirm it.
  - The "Change Icon" window appears.

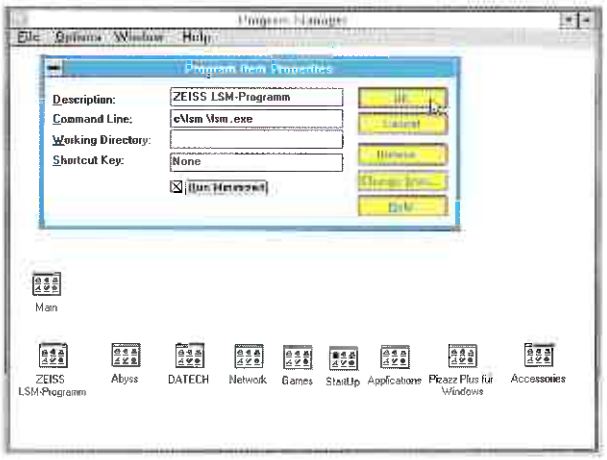


Figure 9-30

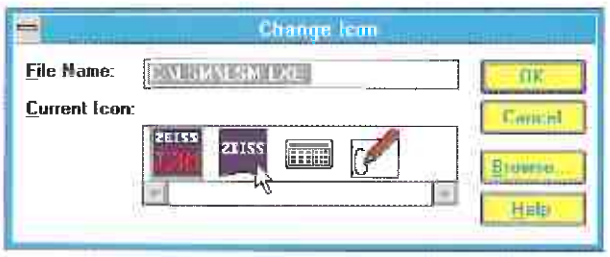


Figure 9-31

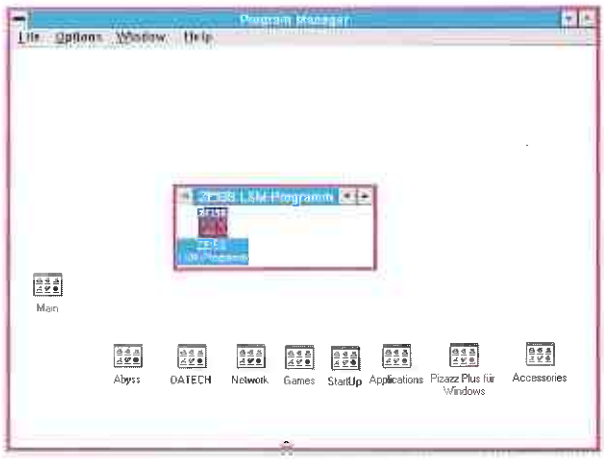


Figure 9-32

- Click on the required program icon and select [OK] to confirm it.
- Select [OK] to confirm again.
  - The program returns to the Program Manager and the ZEISS LSM program group is closed.
  - Open the ZEISS LSM program icon by double clicking it.
  - The program icon has now been placed in the LSM program window, i.e. the LSM program has now been integrated in the program group.

(3) Setting up automatic starting of the LSM program

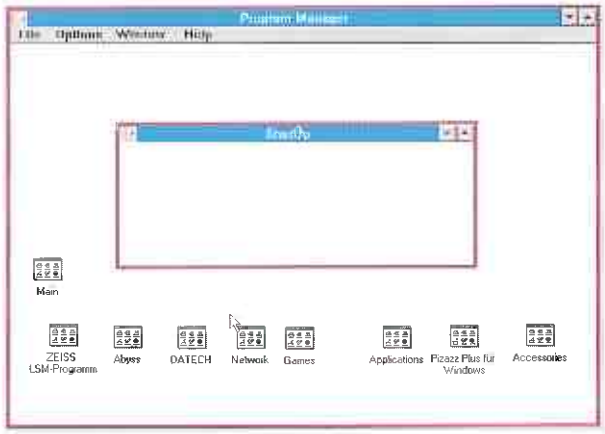


Figure 9-33

- Open the "StartUp" group icon by double clicking it.
- Click on the "ZEISS LSM program" group icon and drag it to the open "StartUp" window.
- Close all windows successively
  - StartUp window
  - ZEISS LSM program window
- Click on the system menu box in the Program Manager and select "Close".
  - The "Exit Windows" window follows.
- Select [OK] to confirm.
  - The system returns to the DOS operating system and displays the [C:\] prompt.
- Switch off the computer briefly.
- Switch on the computer again.
  - The computer boots up and switches automatically to the ZEISS LSM program, displaying the Control Panel.

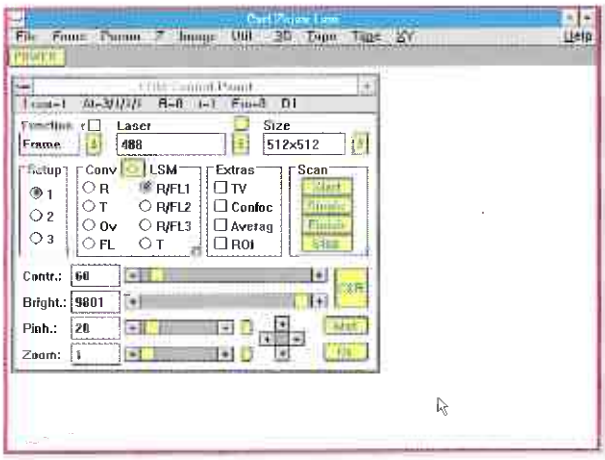


Figure 9-34

9.2 Hardware and nationally specific adaptions

Prerequisites

The computer is operable and the ZEISS LSM program has been activated.

- Close the ZEISS LSM program.
- Close the LSM program window.
- If the "Main" window is not already open, you made need to open the group icon by double clicking it.

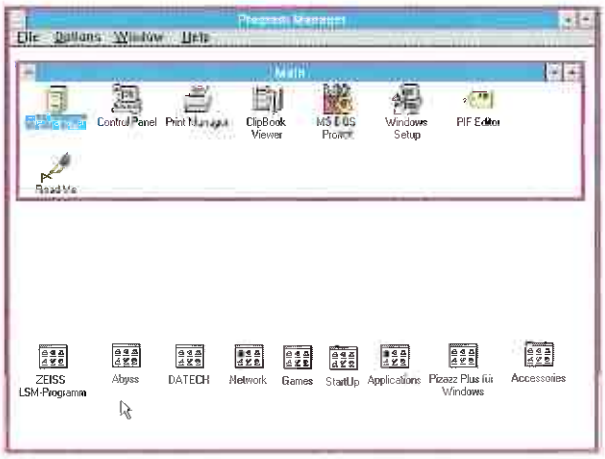


Figure 9-35

- Open the "Control Panel" program icon by double clicking it.
  - The "Control Panel" window is opened.
- Hardware settings:
  - Ports + printers
- Nationally specific settings:
  - International (country settings) + date/time

Figure 9-36

Ports

- Open the "Ports" program icon by double clicking it.

Depending on the version of computer you have, there may be from 1 to 4 ports.

- Select the required interface by clicking on it, e.g.:
  - COM1: port for serial printer
  - COM2: port for mouse
  - COM3: port for video printer
  - COM4: port for plotter

  All ports can be used as data output interfaces, e.g. for ISDN or similar.

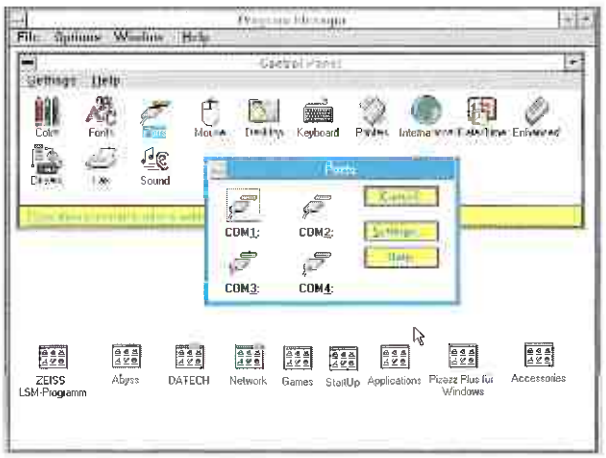


Figure 9-37

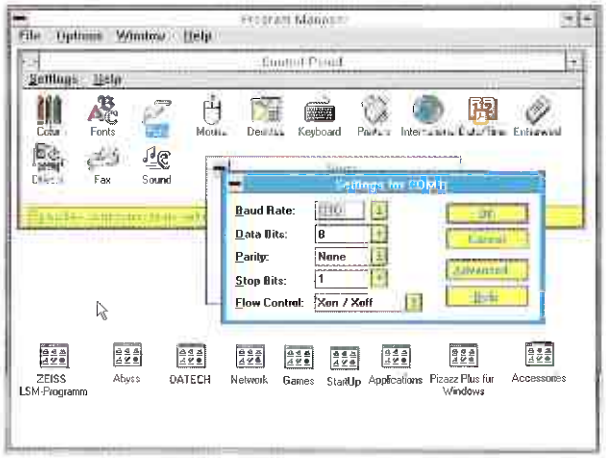


Figure 9-38

The COM1 interface (RS232) is used for **data transfer to the microscope**.

– Under no circumstances should the defaults be modified.

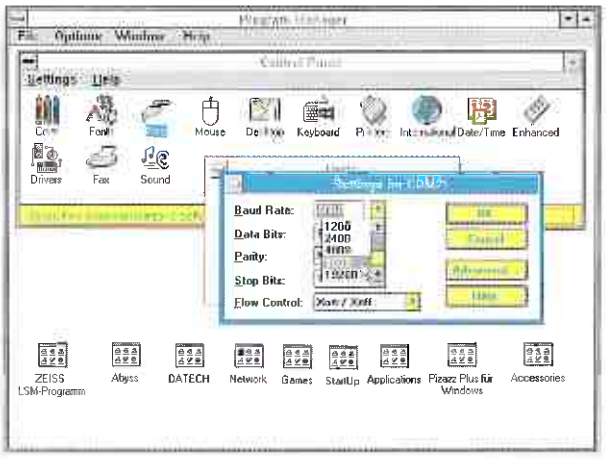


Figure 9-39

The COM2 interface (RS232) is used for **controlling the mouse**.

- In the "Ports" window, click on COM2 and then click on "Settings".
  – The window for setting COM2 appears; every value can be entered by means of pull-down menus.

Refer to the data sheet of the mouse used for details of the parameters.

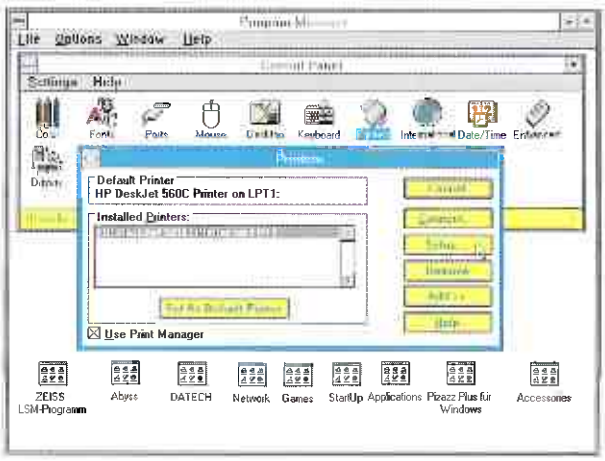


Figure 9-40

Printers

- Open the "Printers" program icon by double clicking on it.
  – The "Printers" window indicates which printer has been set in the LSM system.

To install any other printer, proceed as described in your WINDOWS manual.

International

- Select nationally specific parameters in the list boxes (fold-down menus).
- Set the date, time, currency or number formats in the sub-menus.
- Click on OK to confirm the Country settings.

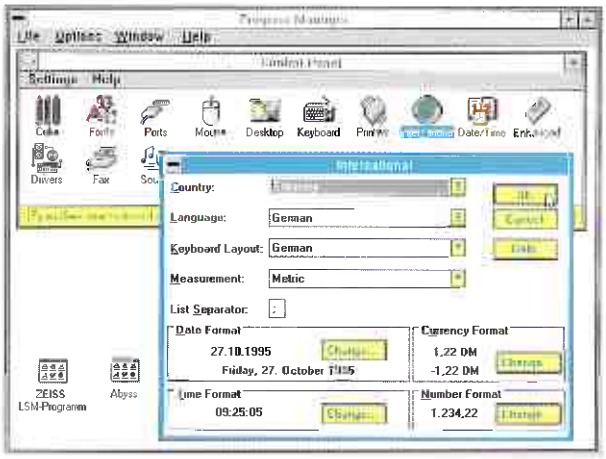


Figure 9-41

Date/Time

The date and time are displayed in accordance with the "International" (Country settings) options.

- Using the cursor, select the parameter you wish to modify and enter the required value on the keyboard.
- Click on OK to confirm entries.

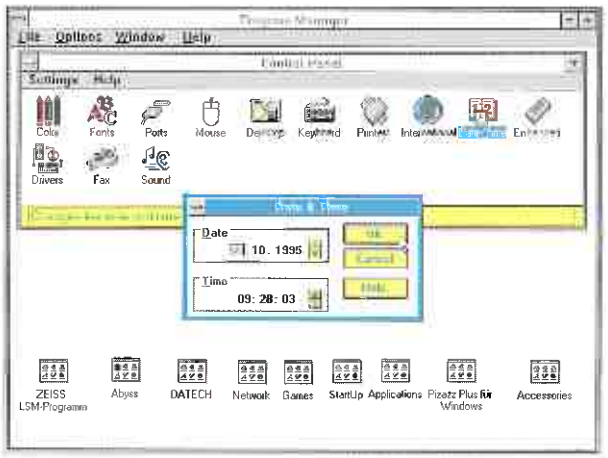


Figure 9-42

9.3 Formatting diskettes

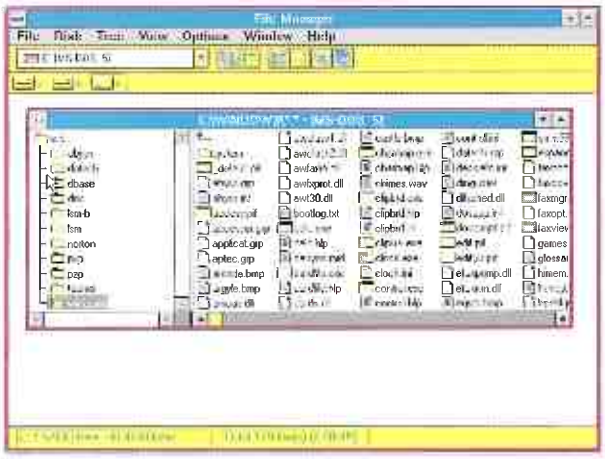


Figure 9-43

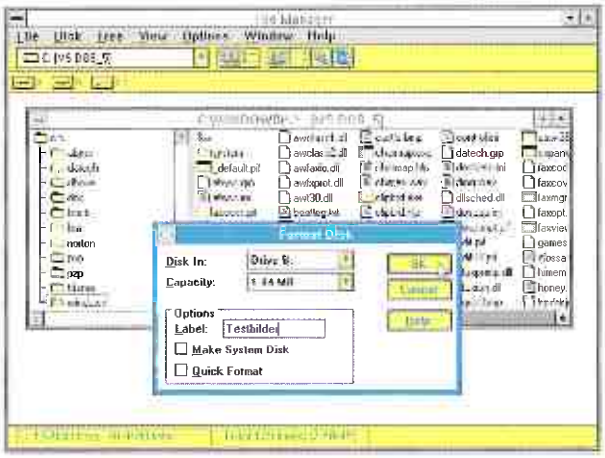


Figure 9-44

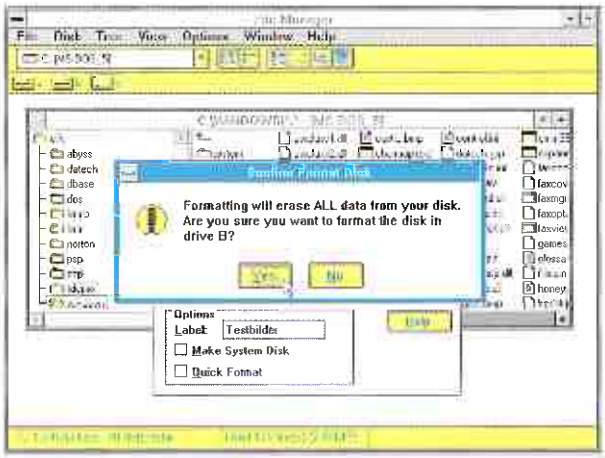


Figure 9-45

Prerequisites

The computer is operable and the ZEISS LSM program has been activated.

- Close the ZEISS LSM program.
- Close the LSM program window.
- If necessary, double click on the "Main" window to open it.
- Double click on the File Manager to open it.
  - The adjacent window appears.
- Insert a diskette in drive b.
- In the menu bar, select the "Disk" menu item and then select "Format Disk" in the pull-down menu that opens.
  - The "Format Disk" window appears.
- Select the drive.
  - The storage capacity of the inserted diskette is displayed.
- Move the cursor to the "Label" box and enter the name required for the diskette on the keyboard, e.g. "Testbilder". Mark "Make System Disk" only if you wish to create a special system/boot diskette, for example.
- Mark "Quick Format" only if you wish to reformat a diskette that has already been formatted.
- Click on the OK button.
  - The adjacent window appears.
- Click on the Yes button.

– The current formatting progress is displayed as a percentage. When formatting is complete, the following message is displayed briefly:

```
Creating root directory...
```

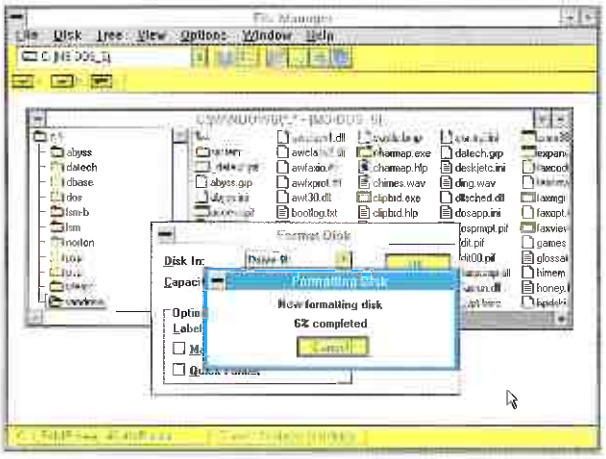


Figure 9-46

– The adjacent status message then appears.

- Confirm by pressing Yes or No depending on what you want.
  – If you select "Yes", the "Format Disk" window appears again.
  – If you select "No", the program returns to the File Manager.

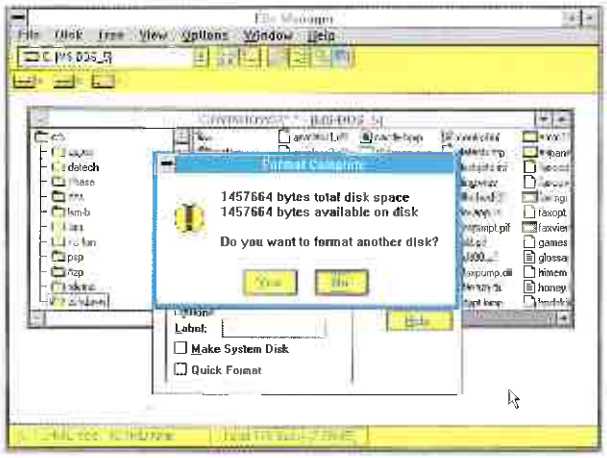


Figure 9-47

- Close the File Manager.
  – The program returns to the Program Manager.
  – Add further applications as required.

- Remove the formatted diskette from the drive and identify it with an adhesive label.

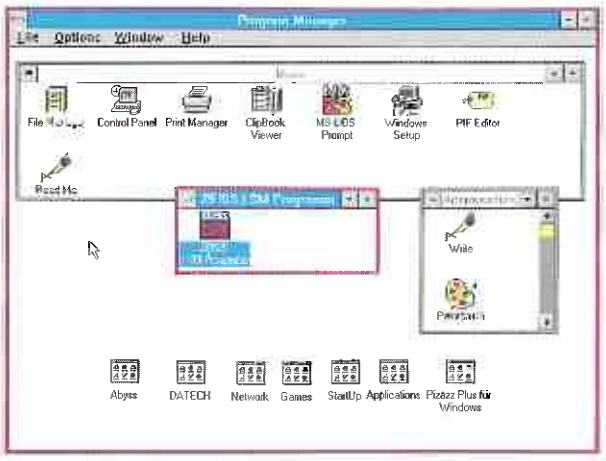


Figure 9-48

9.4 LSM program update

LSM

Rev. X.XX

Options: .....

Copyright Carl Zeiss 1990 – 1995

Figure 9-49

Prerequisites

Your computer is operable and WINDOWS is activated.

You will receive an update of the LSM program on a 3 1/2" diskette that is labelled as shown in the adjacent figure.

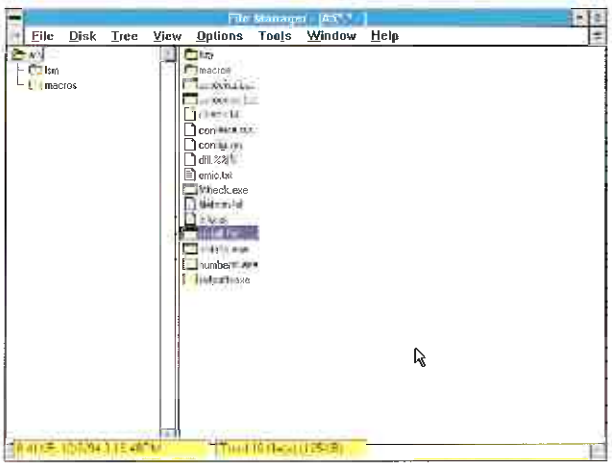


Figure 9-50

- Insert the diskette containing the program update in drive a.
- Double click on the File Manager.
- By means of Disk → Select Drive..., select drive a.
  - The adjacent directory of the update diskette appears.
- Double click on the **install.bat** file.
  - The following information then appears on the monitor:

```
                    Installation of LSM Program
Source:  A:
Destination:      C:\LSM

Select:
[1]     Update from a older to a newer LSM version.
[2]     Installation without replacing AUTOEXEC.BAT, CONFIG.SYS and the parameter files.
[3]     Full installation of LSM, all files will be replaced.
        Caution: AUTOEXEC.BAT, CONFIG.SYS and parameter files will be replaced.
        The older files will be saved as:
        "AUTOEXEC.BAT -> AUTOEXEC.OLD -> AUTOEXEC.001 -> AUTOEXEC.002"
        "CONFIG.SYS -> CONFIG.OLD -> CONFIG.001 -> CONFIG.002"
        "STARTUP.PAR -> OLDSTART.PAR, LASTEXIT.PAR -> OLDEXIT.PAR"
[4]     Full installation of LSM like before, but the EISA-Version of
        AUTOEXEC.BAT and CONFIG.SYS will be installed.
[5]     Change destination path.
[6]     Break

Press a number key, please!

>_
```

- Press the key "1".
  - The File Manager is then displayed.
- Close the File Manager by double clicking on the system menu box.
- Restart the ZEISS LSM program by double clicking on the icon.
  - The Control Panel is displayed.
  - The "FEEDBACK.TXT" Notepad window is also opened.
- Before working with your updated LSM software, kindly take the time to fill out the following status report.
- Send this information on floppy or as a hard copy to the address stated.

In doing so, you will make it possible for us to inform you about all current hardware and software changes.

Carl Zeiss Jena GmbH thanks you for your efforts.

FEEDBACK.TXT

Carl Zeiss LSM Support

Before working with your new LSM-Software please give us your information about yourself and the LSM-system in order to get any new and important information you need in future. Fill in and send the print or the file FEEDBACK.TXT (on a floppy) to:

Carl Zeiss Jena GmbH
Division of Microscopy
Product Management LSM
D-07740 Jena
Germany

LSM: 310..... 410..... other.....

Serial number:

Date of installation:

Image board resolution: standard..... hi.....

Lasers: 1. 2. 3. 4. 5.

Number of PMTs:

Computer & Software configuration:

3rd party equipment (printers, MOD etc):

Institution:

Main users name & address:

Research field:

Keywords:

Literature:

Annex

- Certification in accordance with DIN ISO 9001 / EN 29001 / EN 46001
- EC conformity declaration
- List of abbreviations
- References
- List of documentation/software belonging to the system
- List of key words

TÜV CERT

CERTIFICATE

DAR

The TÜV-Zertifizierungsgemeinschaft e.V.
hereby certifies that

Carl Zeiss Jena GmbH

D-07740 Jena

has established and applies
a quality system for

The whole company

Manufacturing of precision-mechanical, optical, electronic and medical products

An audit was performed, Report No. **4111**

Proof has been furnished that the requirements according to

DIN ISO 9001 / EN 29001 / EN 46001

are fulfilled.

The certificate is valid until

July 1997

Certificate Registration No.

09 100 4111

Bonn, 22.08.1994

TÜV CERT Executive Board

TÜV Rheinland

Cologne, 22.08.1994

TÜV CERT Certification Body of
TÜV Rheinland
Sicherheit und Umweltschutz GmbH

EG-Konformitätserklärung
EC Declaration of Conformity

Carl Zeiss Jena GmbH · Tatzendpromenade 1 a · 07740 Jena · Germany

Wir erklären hiermit die Übereinstimmung des genannten Gerätes mit den einschlägigen Bestimmungen der Richtlinie 89/336/EWG vom 3. Mai 1989 und des Gesetzes über die elektromagnetische Verträglichkeit vom 09. November 1992. Bei Änderungen am Produkt, die nicht von uns autorisiert wurden, verliert diese Erklärung ihre Gültigkeit.

We declare the compliance of the device concerned with the requirements of the Council Directive 89/336/EEC of May 3, 1989 and under the Law of EMC of November 9, 1992.
Any modification to the product, not authorized by us, will invalidate this declaration.

Gerätebezeichnung, Device name:

Laser-Scan-Mikroskop LSM 4

EG-Richtlinien/Normen, EC directives/standards:

EN 55011	(07/92)	**/CISPR 11**	**Klasse A**
EN 50082-2	(03/95)	**/IEC 801-2**	**8 kV (Luft)**
			4 kV (direkt)
		/IEC 801-3	**3 V/m**
		/IEC 801-4	**2 kV (Netzleistung)**
			1 kV (Steuerleistung)

Grundlage - Prüfbericht EMV Nr./Basis - Testreport EMC No.: 100/95

Das Gerät ist gekennzeichnet mit/The device is marked with CE.

Prüfung/Test: **EMV-Labor der Zeiss Jena GmbH**

Registriert/Registered: CZJ **MICE 028-95**

Jena, 22.11.1995

Carl Zeiss Jena GmbH

Carl Zeiss Jena GmbH Qualitätsmanagement

Dr. v. Falkenhausen

Unternehmensbereich Mikroskopie
Department of Microscopy

Dr. Frentzel

Qualitätsmanagement
Quality Management

Diese Erklärung bescheinigt die Übereinstimmung mit der Richtlinie und dem Gesetz. Gewährleistung und Haftung sind in unseren Allgemeinen Lieferbedingungen geregelt.

The declaration certifies the compliance with the Directive and the Law. Conditions of guarantee and liability are dealt within our General Conditions of Sale.

List of abbreviations

An	Analyser
Ar	Argon
At	Attenuation (Abschwächung)
B	Bandwidth
BE	Beam Expander
BP	Bandpass
C & B	Control & Brightness
Chan	Channel
confoc	confocal
conv	convential
corr	corrected
DBC	Dichroitic Beam Combiner
DBS	Dichroitic Beam Splitter
DET	Detector
DIC	Differential Interference Contrast
EF	Emission Filter
EN	Euronorm
ENG	Electronic News Gathering
ext Las	external Laser
FBAS	Composite Video Signal
FL, Fl	Fluorescence
FT	Colour Splitter
H	Horizontal
HAL	Halogen Lamp (transmitted light)
HBO	High Pressure Mercury Lamp
He	Helium

IEC	International Electronical Comission
IF	Interference
ILF	Interference Line Filter
int Las	internal Laser
ISO	International Organization for Standardization
KP	Short pass
Kr	Krypton
LD	Long Distance
LSM	Laser Scan Microscope
LP	Long pass
LSF	Line Selection Filter
Man.	manual
Mot.	motor-driven
NDF	Neutral Dense Filter
Ne	Neon
NT	Neutral Splitter
OBIC	Optical Beam Induced Current
OG	Orange Glass
Ov	Overlay
PC	Personal Computer
PL	Plane
PMT	Photomultiplier
Pol	Polarisation Contrast
R	Reflection
RGB	Red/Green/Blue Video Connector
ROI	Region of Interest
RS	Reflector Slider

Sc	Scanner
Sp	Specimen
T	Transmission
TV	Television
UV	Ultraviolet
V	Vertical
VP	Variable Pinhole
WHO	World Health Organization

References

Handbook of Biological Confocal Microscopy
James B. Pawley

1995 Plenum Press, New York

Image Analysis
Joyce-Loeble

1985
ISBN 0-9510 708 0 0

Confocal Microscopy
T. Wilson

1990
ISBN 0-12-757270-8

Visualization in Biomedical Microscopies
(3-D Imaging and Computer Applications)
A. Kriete

1992 VCC Verlagsgesellschaft mbH
ISBN 1-56081-222-2

List of the documentation/software belonging to the system

- Operating instructions B 40-050 e, Edition 10/95
  LSM 410 invert LASER SCAN MICROSCOPE
- Instructions for use G 42-513-e
  Axiovert 100, Axiovert 135 and 135 M, transmitted and reflected light fluorescence
- User's Guide
  WINDOWS FOR WORKGROUPS AND MS DOS 6.2
- OEM Version MS DOS 6.2 or later
  3 installation diskettes (1st. disk bootable)
- OEM Version MS WINDOWS 3.11 or later
  8 installation diskettes
- User's Guide EISA Configuration Utility
  1 diskette (configuration program)
- Operating instructions of the graphics card and driver software
- User's Guide
  LSM computer
  (Installation program and driver software)
- Operating instructions of the SCSI adapter

List of key words

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