CHAPTER 9 ANNEX

CONTENTS

Page

9 Annex 9-3

9.1 Beampath Configuration Guide for Fluorescence Microscopy with the LSM 5 9-3
9.1.1 Optical elements in the Configuration Control window 9-3
9.1.1.1 Main Dichroic Beam Splitter (HFT) 9-3
9.1.1.2 Secondary Dichroic Beam Splitter (NFT) 9-3
9.1.1.3 Emission Filters (EF) 9-4
9.1.1.4 Miscellaneous 9-4
9.1.2 Setup of Single Tracks Using Single Detectors 9-5
9.1.3 Multitracking Configuration 9-7
9.1.4 Beam path Configuration for Multi Photon Excitation 9-8

9.2 Recommendations for excitation laser lines and emission filters of dyes 9-9

9.3 Configurations Overview 9-11
9.3.1 LSM 510 META 9-11
9.3.2 LSM 510 Basic Configurations 9-12
9.3.3 LSM 510 Upgraded Configurations - UV 9-13
9.3.4 LSM 510 Upgraded Configurations - NLO 9-14

9.4 Filter Change in the Detection Beam Path of Channels 1 and 2 9-15

9.5 Detaching / Attaching the Scanning Module from / to Microscope Stands 9-16

9.6 Hints on the Use of the HRZ 200 Fine Focusing Stage 9-18
9.6.1 General Description 9-18
9.6.2 Application Fields 9-18
9.6.3 Additional Information on the Operation 9-18

9.7 Piezo Objective Focussing Device - MIPOS 3 SG 9-21

9.8 Specifications of Trigger-Interface LSM 510 9-23

9.9 Monitor Diode 9-26

9.10 NLO Non Linear Optics Laser for LSM 510 NLO 9-33
9.10.1 Laser Control Window 9-33
9.10.2 Configuration Control Window 9-34
9.10.3 Pinhole and Collimator Settings 9-34

9.11 Non Descanned Detection (NDD) 9-35

9.12 AxioCam High Resolution Digital Cameras 9-36
9.12.1 High Resolution Microscopy Camera AxioCam HRm Rev.2 9-36
9.12.2 High Resolution Microscopy Camera AxioCam HRc 9-37
9.12.3 Microscope camera port adapters for the AxioCam 9-38

9.13 List of Key Words 9-39

9 ANNEX

9.1 Beampath Configuration Guide for Fluorescence Microscopy with the LSM 5

9.1.1 Optical elements in the Configuration Control window

All wavelength values given in Nanometer [nm].

9.1.1.1 Main Dichroic Beam Splitter (HFT)

- A **HFT XXX[/YYY/...]** deflects the indicated laser lines onto the specimen and allows the emitted fluorescent light to pass through.
  Example: HFT 458/514, HFT UV/488/543/633 (deflects also UV excitation light)
- A **HFT KP XXX** (KP = Short Pass) is a special type of a main dichroic used for IR multi photon excitation. The **HFT KP 650**, deflects laser light with a wavelength longer as 650 nm onto the specimen and allows fluorescent emission light in the visible range below 650 nm to pass through.
  Example: HFT KP 650
- A **HFT KP XXX_YYY** is a combination of a HFT YYY and HFT KP XXX used for simultaneous IR multi photon and single photon excitation.
  Example: HFT KP 700_488.

9.1.1.2 Secondary Dichroic Beam Splitter (NFT)

- The **NFT XXX** is used to split the emitted light which will be guided into separate channels. Light with shorter wavelength than XXX nm is deflected, light with longer wavelength passes the NFT. A cascade of NFTs allows to distribute the emission light to more than two channels/detectors.
- The **NFT KP YYY** solits emission light the other way round: it transmits light shorter than YYY nm and deflects above YYY nm.

9.1.1.3 Emission Filters (EF)

- A **LP XXX** (Long Pass) transmits emission light with wavelengths longer than the indicated threshold value XXX.
- A **KP XXX** (Short Pass) transmits emission light with wavelengths shorter than the indicated threshold value XXX.
- A **BP XXX-YYY** (Band Pass) transmits emission light within the indicated wavelength band.
- A **BP XXX/BB** has a transmission band for emission light with a center wavelength of XXX nm and a width of BB nm.
- The **BG 39** (Blue Green glass) blocks infrared excitation light by absorption.
- **BP ... IR** (Band Pass – Infra Red) is a band pass suitable for detection of IR excited dyes. It blocks the IR light.

9.1.1.4 Miscellaneous

- **Plates** do transmit light 100%. They are used for a correct beam guidance and will be set automatically.
- **Mirrors** do deflect 100% over the whole spectral range and can be used to guide the emission light to selected detectors.

9.1.2 Setup of Single Tracks Using Single Detectors

- Switch on the suitable lasers for excitation of the dyes in the specimen. For the UV laser and the Argon laser set the tube current of the laser to a value of app. 50% (**Excitation, Laser, Output [%]**). Example: for Alexa 488 and CY 3 switch on **Argon** (blue excitation) and **HeNe1** (green excitation).
- Activate the proper laser lines in the **Line Active** check box, set **Transmission [%]** for each active line.
  Example: Select 488 to 5% and 543 to 100%
- Select a main dichroic beamsplitter (HFT) which deflects the selected laser lines to the specimen.
  Example: HFT 488/543

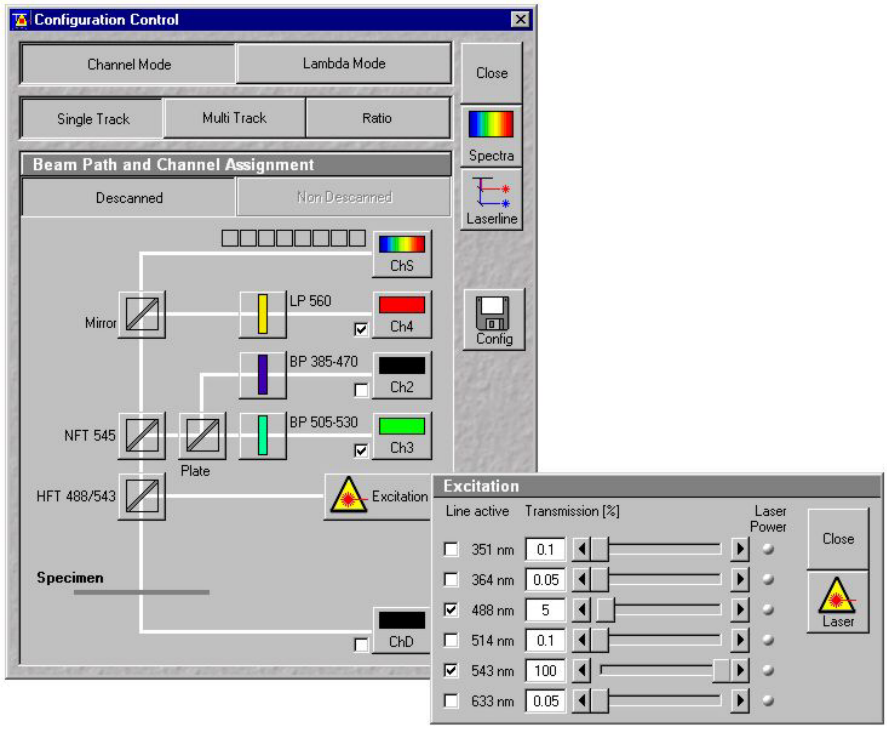


Fig. 9-1 Configuration Control window and Excitation panel

- Check the available emission filters (EM 1- 4) for transmission of fluorescent light from the specimen, in order to identify the channels for detection.
  Example: BP 505-530 in channel Ch 3 for acquisition of green emission and LP 560 in channel Ch 1 for acquisition of red emission

- Use the secondary beam splitters (NFT 1/2/3) to split and guide the emitted fluorescent light to the detectors (PMTs) of the selected channels (see above).
  Example: an NFT 545 in NFT 1 position will allow light longer then 545nm to pass to Ch 1 and deflects light shorter then 545 towards channel Ch 3 (if available) and channel Ch 2.
  Note: on switching from 'None' to a beam splitter in the NFT 1 position the system will automatically set 'Plate' in NFT 3 position.
- Select the proper emission filters in front of the channels and activate channels.
  Example: select LP 560 in front of Ch 1, and BP 505-530 in front of Ch 3].
- Make sure that the active detection bands do not include any of the active laser lines
  Example: do not use a BP 505-550 for detection of green emission when using the 543 nm line for green excitation

Additional hints:

- Do not forget to turn on detectors.
- Assign appropriate colors to these activated channels. Example: Ch 1 - red (for Cy3 emission, Ch 3 - green (for Alexa488 emission)
- The Spectra dialog is a big help for checking if the configuration of the beam path was successful. It shows activated laser lines and for each channel the emission range that can be "seen" by the detector indicated by the corresponding channel color. A gray bar indicates an emission range that is guided into a channel, but the detector is not turned on.
- When simultaneously detecting more than one fluorescent dye use channel Ch 1 for detection of the emission with long wavelength, then channels Ch 4 and Ch 3 (if available) for medium wavelengths and channel Ch 2 for short wavelengths.
- Use NFT 3 for separating emission into channels Ch 1 and Ch 4 and NFT 2 for separating emission into channels Ch 2 and Ch 3

9.1.3 Multitracking Configuration

Multitracking is the method of choice for multi fluorescence imaging. It has the advantage to avoid artifacts based on emission crosstalk that occurs when using simultaneous excitation and detection. Laser lines are switched very fast and channels recorded quasi-simultaneously.

The configuration of Multiple Tracks follows the same rules described above for single track configuration. The main difference is that each track is configured to excite and detected only one fluorescent dye to prevent cross talking (or two dyes with non overlapping emission spectra).

- Create a single track for both, Alexa 488 and CY 3 detection separately (see above).
- Open the Multi Track configuration window. The system displays the Single Track setup as track one.
- Add a new track.
- Click on track one, deactivate Ch 1 (red emission detection) and switch off the green laser line (543nm) in the **Line Active** check box in the **Excitation** control window.
- Click on track two, deactivate Ch 2 (green emission detection) and switch the blue laser line (488) off.
- To extend the detection band for the green light it is now reasonable to use the BP 505-550 instead of BP 505-530 in track one. This is now possible since the green laser line is turned off during detection of the green fluorescence emission.
- Use the Spectra window to check the proper settings for each individual track as described above.

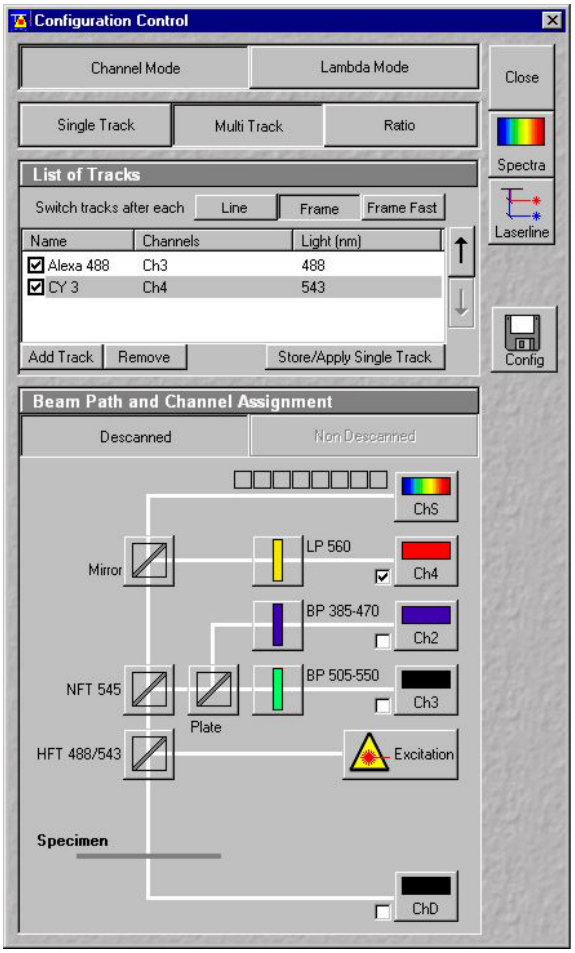


Fig. 9-2 Configuration Control window

Line and Frame Mode of Multitracking

- Settings can be used for **Line** or **Frame** wise Multitracking.
- In Line mode the lines are scanned in turns for all tracks with the corresponding laser lines turned on exclusively. Preferred for living samples with moving objects. Acquisition time can be reduced using bidirectional scan mode.
- In Frame mode whole frames are scanned in turns for all tracks with the corresponding laser lines turned on exclusively. This mode can be advantageous for dyes that tend to bleach and need time to recover.
- There are parameters that can be changed quickly in a line wise: Amplifier Gain, Amplifier Offset, Laser Line Attenuation,
- Any other changes of track settings of the selected tracks, e. g. different filters, dichroics or Detector Gain settings, need a bit more time to be changed, and require Frame mode.
- There is a fast Frame mode, that requires identical settings of these parameters.
- In our example it is now possible to use the BP 505-550 also in track two. There is no function of this BP in track two, but it guarantees equal settings in both tracks/channels, which now allows line wise Multitracking.

9.1.4 Beam path Configuration for Multi Photon Excitation

- The beam path configuration for multi photon excitation follows the same rules as described for a single and multi track configuration (see above).
- Use a KP 650 or KP 700 as main dichroic beam splitter to deflect the IR excitation light (700-900 nm) onto the specimen.
- On detection side, set always a BG 39 in the beam path or use a IR suitable band pass filters (BP XXX-YYY IR) to prevent a bleed through of IR excitation light to the detector.

9.2 Recommendations for excitation laser lines and emission filters of dyes

Dye	Laser line/HFT	Emission/EM
DAPI	364 or 405	> 385/420, max. at 461
EBFP	364 or 405	> 385/420, max. at 447
Hoechst	364 or 405	> 385/420, max. at 440
Fluoro-Gold	405 or 458	> 420/475, max. at 536
ECFP	405 or 458	> 420/475, max. at 501
Lucifer Yellow	458	> 475, max. at 536
EGFP	477 or 488	> 505, max. at 507/516
FM 1-43™	477 or 488	> 505, max. at 598
Alexa Fluor 488™	488	> 505, max. at 520
Calcium Green	488	> 505, max. at 531
Cy2™	488	> 505, max. at 508
DiO (DiOC18(3))	488	> 505, max. at 508
Fluo-3	488	> 505, max. at 520
Fluorescein (FITC)	488	> 505, max. at 520
Cy3™	514	> 530, max. at 566
EYFP	514	> 530, max. at 535
Oregon Green	514	> 530, max. at 535
SYTOX Green	514	> 530, max. at 536
FM 4-46	514 or 543	> 560, max. at 640
Alexa Fluor 546™	543	> 560, max. at 572
Calcium Orange	543	> 560, max. at 575
DiI (DiIC18(3))	543	> 560, max. at 565
DsRed	543	> 560, max. at 583
Tetramethylrhodamine (TRITC)	543	> 560, max. at 576
Rhodamine B	543	> 560/585, max. at 625
Texas Red™	543 or 568	> 560/585, max. at 620
Alexa Fluor 633™	633	> 650, max. at 654
Cy5™	633	> 650, max. at 666

Here you can note your specific combinations:

Dyes	Laser/HFT	EM1	NFT	EM2

Example:

Dyes	Laser/HFT	EM1	NFT	EM2
FITC/Cy3	488/543	BP 505-530	545	LP 560

9.3 Configurations Overview

9.3.1 LSM 510 META

Configuration	3 META	13 META	15 META	18 META
Main beam splitter / available laser lines	NT 80/20 UV/488/543/633 477/543 488/543 458/514 514/633 458 488	NT 80/20 UV/488/543/633 KP 700/488 KP 700/543 458/514 458 488 KP 650	NT 80/20 UV/488/543/633 UV/488 458/514 477/543 458 UV (375) 488	NT 80/20 UV/488/543/633 405/488/543 405/514 458/514 488/543 458 488
Secondary beam splitter 1	none mirror 545 570 635 VIS none KP 545 plate	none mirror 490 515 545 635 VIS KP 545 plate	none mirror 490 515 545 635 VIS KP 545 plate	none mirror 490 515 545 635 VIS KP 545 plate
Secondary beam splitter 2	mirror 515 545 plate	mirror 490 545 BG39	mirror 490 545 plate	mirror 490 515 545
Secondary beam splitter 3	none plate none mirror	none plate BG39 mirror	none plate none mirror	none plate none mirror

9.3.2 LSM 510 Basic Configurations

Configuration	1	2	3	4
Main beam splitter / available laser lines	NT 80/20 458/514 458/543 488/543 458 488 514 477/543	NT 80/20 UV/488/543/633 458/514 458/543 458 488/548 514/633 488	NT 80/20 UV/488/543/633 477/543 488/543 458/514 458 514/633 488	NT 80/20 UV/488/568/633 488/568 488 568 633 none none
Secondary beam splitter 1	none mirror 515 545 none none* none plate	none mirror 515 545 635 VIS none* none plate	none mirror 545 570 635 VIS none* none plate	none mirror 570 635 VIS none* none none plate
Secondary beam splitter 2	mirror	mirror	mirror 515 545 plate	mirror 570 plate none
Secondary beam splitter 3	none plate none mirror	none plate none mirror	none plate none mirror	none plate none mirror

***) Position used for beam splitter NFT 610 of SNARF filter sets**

****) Position used for beam splitter NFT 450 of Indo-1 filter sets**

9.3.3 LSM 510 Upgraded Configurations - UV

Configuration	5	9	11	15	16	18
Main beam splitter / available laser lines	NT 80/20 UV/488 UV/543 458/514 488/543 UV (375) 477/543 458	NT 80/20 UV/488/543/633 UV/488 458/514 488/543 UV (375) 477/543 458	NT 80/20 UV/488/543/633 UV/488 UV/568 488/568 UV (375) 488 568	NT 80/20 UV/488/543/633 UV/488 458/514 477/543 458 UV (375) 488	NT 80/20 UV/488/543/633 UV/488 458/514 477/543 488/543 UV (375) 458	NT 80/20 UV/488/543/633 405/488/543 405/514 458/514 488/543 458 488
Secondary beam splitter 1	none mirror 490 515 545 none** none* plate	none mirror 490 515 545 570 none plate	none mirror 490 545 570 635 VIS plate none	none mirror 490 515 545 635 VIS plate none*	none mirror 490 515 545 635 VIS plate none*	none mirror 490 515 545 635 VIS none* plate
Secondary beam splitter 2	mirror	Mirror 490 none* plate**	mirror 490 none* plate**	mirror 490 545 plate**	mirror 490 545 plate**	mirror 490 515 545
Secondary beam splitter 3	none plate none mirror	None Plate None Mirror	none plate 635 VIS mirror	none plate none mirror	none plate 635 ViS mirror	none plate none mirror

***) Position used for beam splitter NFT 610 of SNARF filter sets**

****) Position used for beam splitter NFT 450 of Indo-1 filter sets**

9.3.4 LSM 510 Upgraded Configurations - NLO

Configuration	12	13
Main beam splitter / available laser lines	**NT 80/20** **KP 700/488** **KP 700/514** **KP 700/543** **458/514** **488/543** **488** **KP 650**	**NT 80/20** **UV/488/543/633** **KP 700/488** **KP 700/543** **458/514** **458** **488** **KP 650**
Secondary beam splitter 1	**none** **mirror** **490** **515** **545** **none** **none*/**** **plate**	**None** **mirror** **490** **515** **545** **635 VIS** **none*/**** **plate**
Secondary beam splitter 2	**mirror**	**mirror** **490** **545** **BG39**
Secondary beam splitter 3	**none** **plate** **BG39** **mirror**	**none** **plate** **BG39** **mirror**

***) Position used for beam splitter NFT 610 of SNARF filter sets**

****) Position used for beam splitter NFT 450 of Indo-1 filter sets**

9.4 Filter Change in the Detection Beam Path of Channels 1 and 2

For optimum investigation of specimens it is useful to employ filter wheels permitting the motor-controlled change between different filters for narrow-band or broad-band detection depending on the wavelength. The number of filters is limited by the capacity of the filter wheel. The change of the filter wheel as a whole involves complete readjustment.

The filter wheels of channels 1 (upper cover cap) and 2 (lower cover cap on the right side) of the Scanning Module have a change position in which a filter, including its mount, can be changed in a reproducible position without requiring readjustment. The filters can be rotated in their cells, and with the light path being eccentric relative to the filter center, the best transmission area of the filter for the respective wavelength or pass range can be found by rotating the filter. This is very important for the investigation of specimens of low emission.

Filter change

- By software control, move filter wheel (9-3/**5**) to the change position.
- Pull cover cap (9-3/**1**) off the Scanning Module.
- Use the filter tool (9-3/**2**) to pull the filter mount (9-3/**4**) with the filter (9-3/**3**) out of the guide well.
- Change filter to suit the application.

☞ The filter is rotatable in its mount, allowing adjustment for finding the best transmission area of the filter for the wavelength used.

- Enter the designation of this particular filter into the System Software database.

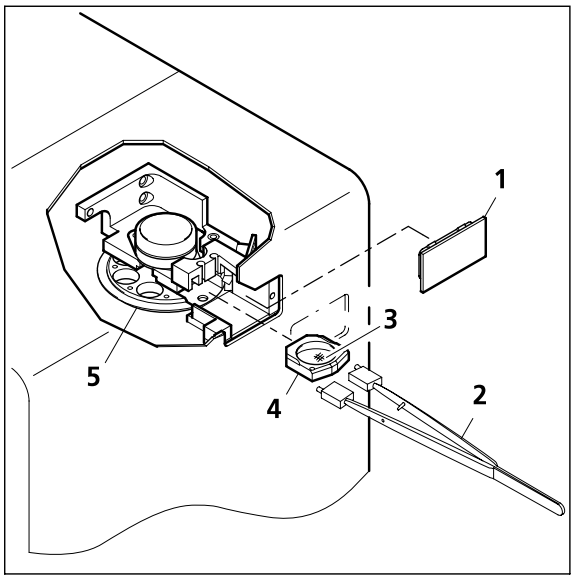


Fig. 9-3 Filter change

9.5 Detaching / Attaching the Scanning Module from / to Microscope Stands

Tool needed: 3 mm Allen key

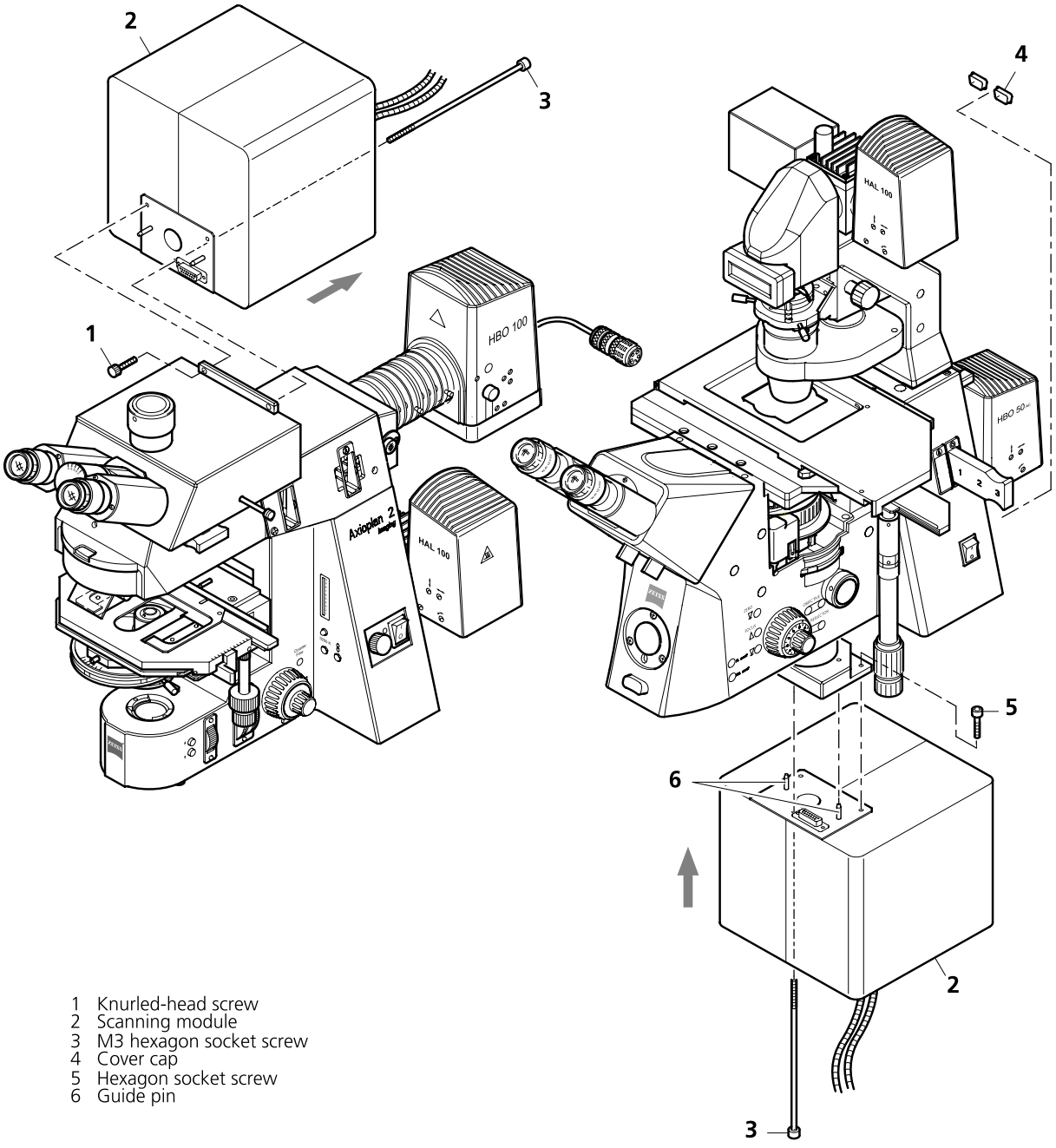
☞ The user can remove the Scanning Module from one microscope and attach it to another within a few minutes. **No adjustment** is required after the change-over. Described below is the change-over from an Axioplan 2 to an Axiovert 100 M in baseport configuration.

⚠ **Before** the change-over, **shut down** the system as described in chapter 4 in order to avoid damage to the system and loss of data.

- Turn out both knurled-head screws (9-4/**1**) at the Scanning Module (9-4/**2**) fitted to the Axioplan 2.
- Turn out M3 hexagon socket screw (9-4/**3**) with the Allen key.
- Cautiously pull Scanning Module off the Axioplan 2 stand.
- Attach Scanning Module to the baseport of the Axiovert 100 M, minding the guide pins (9-4/**6**), and secure it with the M3 hexagon socket screw (9-4/**3**).
- Fasten Scanning Module to the baseport with two hexagon socket screws (9-4/**5**), using an offset Allen key.

☞ As the Scanning Module is heavy, weighing about 14 kg, it is easier if the changeover is carried out by two persons.

- Pull off covering caps (9-4/**4**) from the CAN-BUS and RS232 interface ports at the rear of the Axiovert, remove the two cables 457411-9011 (CAN-BUS) and 457411-9012 (RS232) from the Axioplan, plug them into the Axiovert and secure them there.
- Switch the LSM 510 on with the REMOTE CONTROL switch.
- Click on the **Stand select** icon to update the system database with the new database of the Axiovert 100 M microscope.
- Restart the LSM program.



1 Knurled-head screw
2 Scanning module
3 M3 hexagon socket screw
4 Cover cap
5 Hexagon socket screw
6 Guide pin

Fig. 9-4 Change-over of the Scanning Module

9.6 Hints on the Use of the HRZ 200 Fine Focusing Stage

9.6.1 General Description

The HRZ 200 fine focusing stage is a compact attachment for the Axioplan 2 MOT and Axiovert 100 M microscope stages, which allows the particularly fast and high-precision fine focusing of the object. The HRZ 200 permits fine focusing over a range of 200 µm, with the smallest step width being less than 10 nm, reproducibility better than 40 nm, and the maximum speed amounting to 10 Hz. The stage allows the use of specimens with a weight of less than 100 g.

The HRZ 200 is not used if manual coarse focusing is performed. To position the objective in relation to the optical Z-axis, the standard XY-microscope stage is used.

The HRZ 200 features a mount for standard object carriers of 76 mm x 26 mm x 1 mm and a milled-out receptacle for ∅ 36 mm x 1 mm Petri dishes.

9.6.2 Application Fields

- High-precision fine focusing and translation of the object along the optical axis.
- Fast and high-precision mounting of one-dimensional Z-line sections.
- Fast and high-precision mounting of two-dimensional R-Z-longitudinal sections.
- Fast and high-precision mounting of XY-Z-Stacks for the three-dimensional reconstruction of the object.
- Exact measurement of Point-Spread-Functions for deconvolution.

9.6.3 Additional Information on the Operation

The HRZ 200 fine-focusing stage is a high-precision, sensitive accessory for the LSM 510 from Carl Zeiss and must therefore be treated carefully.

High mechanical stress, such as the use of specimens weighing more than 100 g or the application of pressure or knocks on the movable stage tongue, can result in damage and therefore in failure of the stage function.

To be able to fully utilize the outstanding precision attainable with the fine focusing stage, anything which could interfere with its operation, especially mechanical knocks and impact of the LSM components, should be avoided. We would recommend you to always use the actively vibration-damped Kinetics stage (available as accessory under the order number 1007 508 or 1007 512) as the base for the setup of LSM systems containing the HRZ 200 stage.

The specifications of the stage are obtained only after a heating phase of approx. 30 minutes. Furthermore, the installation conditions for the LSM system must be observed.

The maximum reproducibility (better than 40 nm) for moving to an absolute position in Z is achieved by always moving to the required position from below.

Fine focusing is performed mechanically via an inclined position of the stage tongue. Therefore, the lifting range Z at the location of the image field depends on the position of the HRZ in relation to the optical axis. This means: if the user shifts the object on the microscope stage to the right via the HRZ 200, the lift will be different from the one in the zero position of the stage (max. 200 µm) and also from the one after a shift of the stage to the left.

The HRZ has been developed to enable minute increments at a high precision. It is possible to have either a large travel range at a low precision or a low travel range at a high precision. The entire travel range of ± 100 µm can only be passed without intermittent "Levelling" if step width >1 µm is selected.

If the LSM system is equipped with a motorized scanning stage, this shift is read back to Δx and the lift is calibrated automatically if the zero position of the HRZ has been matched to the zero position of the scanning stage via an initialization run. For this, activate the **Stage** button of the **Acquire** toolbar. Then position the scanning stage in such a way that the optical axis of the microscope corresponds to the zero position of the HRZ, i.e. to the center of the specimen holder in the stage tongue. Then perform initialization by pressing the **HRZ Null** button. This step must be repeated after every new start of the system. Also see the notes on the operation of the motorized scanning stages.

If the system is equipped with a manual microscope stage, the user has the option of performing the calibration by entering the Δx shift in mm via the **Calibration** slider.

The shift is read off from the microscope stages. In the case of the manual Axioplan 2 stage, Δx can be read directly from the scale adhered to the front of the stage. In the case of the manual Axiovert 100 stage, a scale is located on the right of the knob, where the 45 mm Δx shift relative to the zero position of the microscope stage can be read off. The Δx value is positive for both stages if shift from the zero position is made to the right and negative if the shift is made to the left.

On account of the inclined position of the stage tongue, the object is also shifted laterally during the fine focusing motion. This lateral shift is negligibly small if, as recommended by us, specimen carriers with thickness 1.0 mm are used exclusively. Otherwise, the marked lateral shift of the object during fine focusing can result in image distortion. For the same reason, Petri dishes without fixation ring must be used exclusively.

The nosepiece of the Axiovert stand is moved to the load position prior to switching off the LSM system and the HRZ 200 is then moved to the lowest position to avoid damage of the objective or object by a possible collision. The user must refocus after start-up of the system. Before an objective change in the Axiovert 100 or the Axioplan 2, the nosepiece and the microscope stage must be moved to the load position by the user, and then back to the work position to prevent the objectives from hitting the HRZ components. This is performed automatically if the objectives are changed menu-controlled via the relevant buttons of the LSM program.

The HRZ 200 for the Axiovert 100 M (1013 186) or for the Axioplan 2 MOT (1013 187) can be attached to the following standard microscope stages:

- mechanical stage 85 x 130 for Axiovert (451339)
- scanning stage DC 100 x 90 for Axiovert (451740)
- mechanical stages 75 x 50 for Axioplan (453505, 453502-9904, 453507)
- scanning stage DC 4" x 4" for Axioplan (453585-9901)

In the case of the last configuration, the object plane is shifted upwards so that KÖHLER illumination and classical transmitted-light microscopy will no longer be possible because the condenser cannot be moved sufficiently close to the object.

The user will not have to deal with any other restrictions.

9.7 Piezo Objective Focussing Device - MIPOS 3 SG

For upright stands Axioplan 2 imaging MOT, Axioskop 2 mot plus, Axioskop 2 FS MOT

Range: 80 µm

Minimum step size: 5 nm

Speed:

		Piezo objective focussing device	HRZ 200	Piezo / HRZ
Slices	**Step size [µm]**	**xz-lines / s**	**xz-lines / s**	
20	1	10	2.8	x 3.6
20	0.5	10	2.8	x 3.6
10	1	20	5.7	x 3.5

Objectives:

– W0.8/M27; Diameter max. 29 mm => NO C-Apochromats 40x/63x

– Modified Achroplan 40x / 0.8 W with reduced length to compensate for piezo height

Technical data:

part no. thread M25xo.75 ..... O-303-01
RMS (W0.8x1/36") O-304-01

motion ..... 100/80 µm
(typical value measured with -10 V to 150 V) (open loop/closed loop)

operating voltage.....-10 to 150 V

capacitance .....7.2 µF
(typical value for small electrical field strength) .....

resonant frequency..... 700 Hz
(without load / objective mass 140 g)

resolution open loop..... 0.13 nm
(measured with-103-18 amplifier)

stiffness.....1.4 N/µm

connector.....LEMOSA

cable length..... 1 m

weight..... 115 g

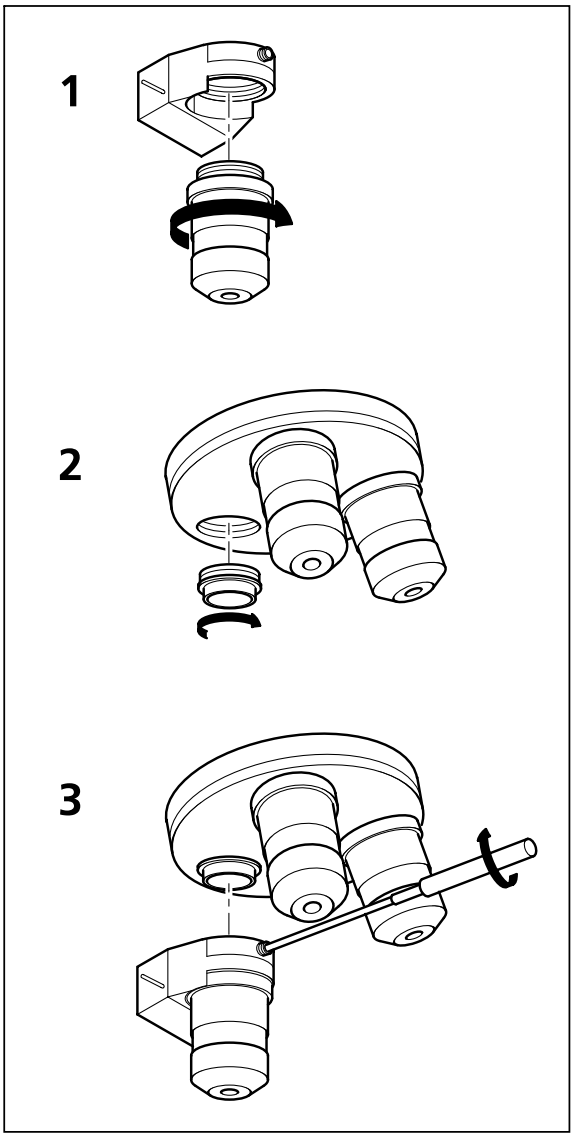


Fig. 9-5 Installation of the Piezo Objective Focusing Device

Installation:

- Screw in your microscope objective into Piezo Objective Focusing Device (see Fig. 9-5/**1**).
- Screw the thread-ring into your microscope (see Fig. 9-5/**2**).
- Easy clamp the Piezo Objective Focusing Device on the thread-ring (see Fig. 9-5/**3**).

9.8 Specifications of Trigger-Interface LSM 510

Application:

With the LSM 510 you can control various actions externally using Trigger-In or force external devices to work at a defined time depending on an action using Trigger-Out during time series.
These actions are: Scan-Start / Stop, Bleach, Change of Scan-Interval, end of a countdown or even a mouse-click on a button.

Interface:

- Front plate Scanner-Interface (Scan-IF) inside
- Electronic-Box (Scan-Control-Module) of LSM 510:
- Connector 'User I/O', 26-pin shrunk SUB-D

Number:

- 4x Trigger-In, 4x Trigger-Out

Type/Voltage Range:

- TTL (HCMOS), 0.0 - 5.0 V

Load:

- In: 22 kOhm input impedance
- Out: ± 4 mA

Trigger pulse description:

- Level detection:
  - Low level: 0.0 - 1.0 V
  - High level: 3.0 - 5.0 V
- Slew rate:
  - rising edge: 1 µs
  - falling edge: 1 µs
- Pulse width (always positive pulses / high level):
  - Trigger-In: ≥ 20 ms (Speed 10 - 5)
    ≥ 31 ms (Speed 4)
    ≥ 62 ms (Speed 3)
    ≥ 123 ms (Speed 2)
    ≥ 246 ms (Speed 1)
  - Trigger-Out: ca. 100 ms
- Pulse frequency:
  - Trigger-In: 2x pulse width
  - Trigger-Out: > pulse width
- Valid edge:
  - Trigger-In: Rising edge
  - Trigger-Out: Falling edge

Caution:

- Never apply more than 5 V or negative voltages to avoid any damage.
- In and outputs are not galvanically decoupled.
- Therefore proper measures for galvanic decoupling of external devices have to be taken (opto-coupler etc.).
- Do not connect pins labeled ‘reserved’ (see table below). Otherwise, at least the interface can be damaged.

Pin assignment:

No.	Name	Direction	Description
1	Trig1O	Out	Trigger Output #1
2	Trig2O	Out	Trigger Output #2
3	Trig3O	Out	Trigger Output #3
4	Trig4O	Out	Trigger Output #4
5 ... 8	-	-	reserved
9	GND	-	Ground (0 V)
10	Trig1I	In	Trigger Input #1
11	Trig2I	In	Trigger Input #2
12	Trig3I	In	Trigger Input #3
13	Trig4I	In	Trigger Input #4
14 ... 17	-	-	reserved
18	GND	-	Ground (0 V)
19 ... 25	-	-	reserved
26	GND	-	Ground (0 V)

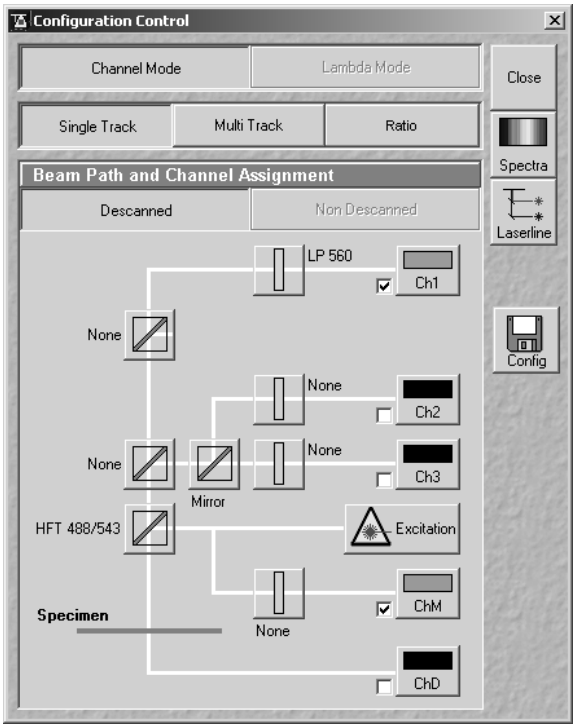


Fig. 9-6 Configuration Control window

9.9 Monitor Diode

The monitor diode is placed in the excitation ray path of the LSM 510 behind the beam splitter combining the visible and the UV ray path and in front of the main beam splitter. Therefore it allows to check the laser input in terms of power and noise. With the attenuation filter wheel in front of the diode it is possible to attenuate the laser power reaching the diode. It is not possible to select one line out of a few excitation wavelengths to be detected by the diode.

Proceed as follows to activate the diode as a detector:

- Click on the corresponding button in the **Configuration Control** window of the LSM 510 software (Expert Mode).
- Choose either **Frame** or **Line** scan.

- Change to the **Scan Control** window and press **Cont.**; the system will scan with the diode as a channel.
- Choose the right amplification of the signal obtained by using the special neutral density filters in front of the diode or / and by using the setting of the **Amplifier Gain** and **Amplifier Offset** value.
  (Scan Control - Channels ChM-1).

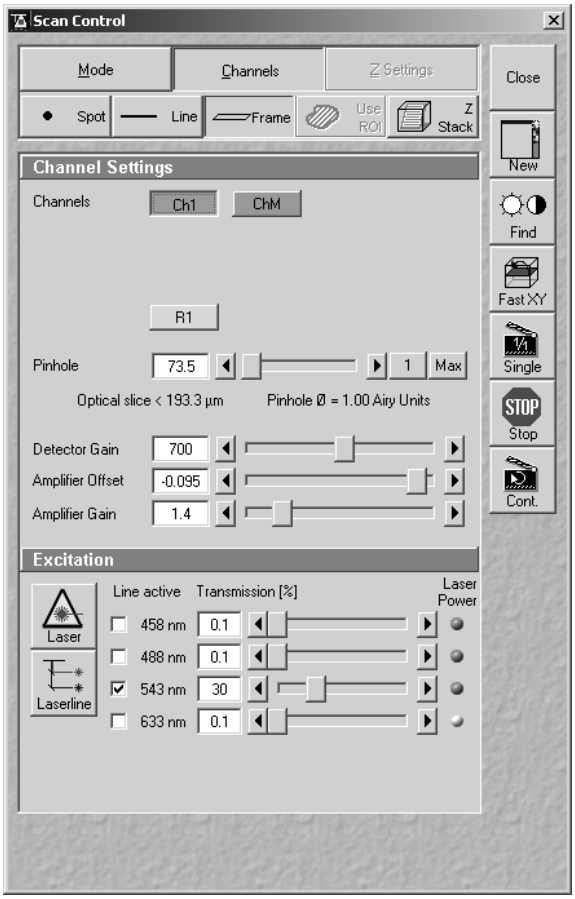


Fig. 9-7 Scan Control window

Application examples:

a) Checking the laser power

This function is not automated so far. To qualitatively measure the laser power, the diode can be used in such a way that the gray level obtained in the **Line Scan** mode at a certain setting of the whole system is stored as a text overlay together with the image (manually done by user). As the diode setting (Ampl. Gain, Ampl. Offset, ND filter) is stored together with the image, the setting is automatically reloaded when using the **REUSE** button. If deviations can be observed it is easy to set the laser power to the old value by means of the AOTF transmission.

b) Noise Reduction by Ratio

Contrary to the PMT signal, the signal of the monitor diode is not modulated by any specimen information. Thus it can be used to ratio the PMT signal to get rid of the laser noise (due to any laser as a physical fact) and thereby improve the signal to noise ratio of the fluorescence or reflectance image. The major condition which has to be fulfilled to use the monitor diode for this purpose is that the dominating source of noise is laser noise. The signal of the monitor diode will always be dominated by laser noise (independent of the power set at the laser, or the transmission set at the AOTF), whereas the dominating source of noise in the PMT signal can also be the shot noise of light (shot noise especially occurs in low light fluorescence application; as rule of thumb it can be noted that the shot noise is limiting the signal to noise ratio, if the PMT voltage has to be set to a value > 400 V).

☞ Any kind of noise which can not be observed in both channels at a time will be amplified and not reduced by the ratio process. Low or high frequency laser noise is the only source of noise which is correlated in the PMT signal and the signal of the monitor diode.

Low or high frequency laser noise is mainly introduced if the Ar, ArKr lasers are used at a tube current lower than 8 A (Ar-Vis, ArKr) or 20 A (Ar-UV) respectively.

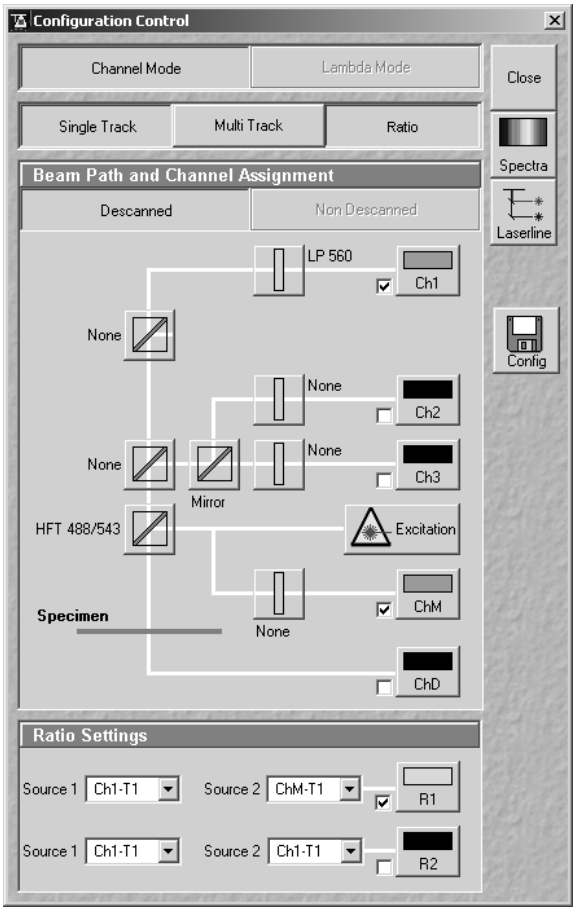


Fig. 9-8 Configuration Control window

To use the monitor diode for ratio application, proceed as follows:

- Click on the **Ratio** button.
- Activate the ratio channel R1 or R2 in the **Ratio Settings** panel in the **Configuration Control** window in addition to the monitor diode channel (ChM-1) and one PMT channel.
- Choose the appropriate PMT channel as source 1 in the **Ratio Settings** panel and ChM-1 as source 2. If this numbering is changed (inverted), the ratio image will show an inversion of gray levels if compared to the PMT image.

☞ It is not possible to do the ratio between an on-line ratio image generated with two PMT channels (as in ion-concentration sensitive ratio imaging) and the signal of the monitor diode.

The following image is an example of the reduction of correlated noise. The low frequency noise has been generated artificially.

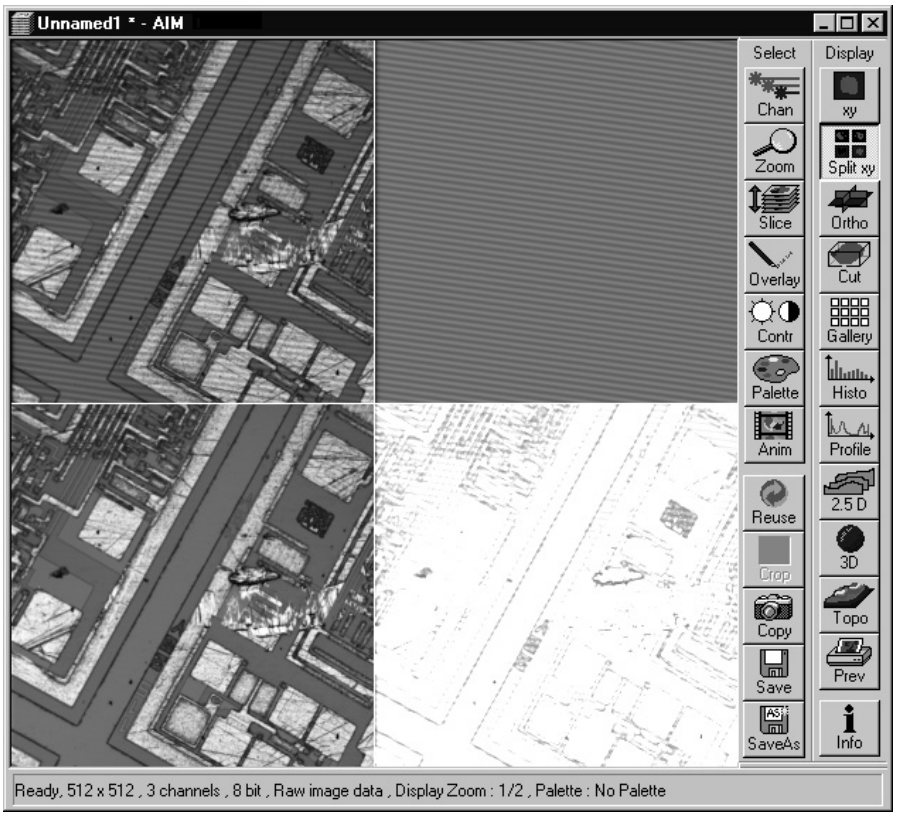


Fig. 9-9 Image Display window

The image in the upper left corner shows the PMT image plus noise, the image beneath this (upper right corner) shows the signal of the diode expanded to 512 x 512 pixels (noise without object information). The two images below show the ratio of the PMT and diode signal (left) and the sum of all signals (right). The sum-image does not contain any information and can therefore be neglected.

To get a ratio image like the one shown here, Detector Gain, Amplifier Gain, Amplifier Offset of the PMT channel, Gain and Offset of the diode channel, Gain and Offset of the ratio channel must be set in the correct way.

Each of the parameters summarized effects either the amplification of the ratio image, or the contrast of the ratio image, or the quality of the noise reduction.

The single steps to find the right setting of all the parameters to be set are listed in the following:

- Activate the Range Indicator.
- Adjustment of **Amplifier Offset**: the Offset of the PMT channel and diode channel have to fit to each other to guarantee the best noise reduction.

The best way to do the adjustment is the following:

- Choose different colors in the **Configuration Control** window for PMT and diode channel.
- Activate **Line Scan**.
- Switch off all laser lines in the **Excitation** window.
- Activate **Cont.**
- Set values for **Ampl. Gain** to 1 in each channel.
- Set the lines visible to the same level as close to the ground level as possible; the values you find for the Offset in each channel should be negative.

A final adjustment of the offset adjustment is done by visually evaluating the noise reduction in the ratio image. As the Offset value of the PMT channel influences the range setting of the ratio image much less than the Offset value of the diode channel, the fine tuning should be done via the PMT offset, if required.

☞ As mentioned before, the calculation of the ratio image is very sensitive to different signal offsets in the two channels used. As the offset is influenced by the scan speed as well as by the Amplifier Gain used, the offset calibration is not valid any more if the scan speed is changed, or the Ampl. Gain is set to a new value respectively. In most cases a new fine tuning is necessary. If this doesn't work, the complete calibration process has to be repeated.

Another possibility to calibrate the offset values is to set the values to -0.1 as default for both channels, then perform steps 3 and 4 and finally adjust the noise reduction by varying the PMT offset value.

If the ratio application is used and the offset has been set to the best reduction of noise in the ratio image, it is not allowed to change the offset of the PMT channel to change the reduction of background fluorescence, for example. This can be done only if the diode offset is corrected afterwards.

Adjustment of Detector Gain

The Gain of the PMT should be set with the help of the range indicator function. No 'red ' and no 'blue' pixels should occur in the image of the PMT.

Amplifier Gain

The diode signal is set to the right range (gray level between 50 and 200 - 8 bit image / 750 and 3500 - 12 bit image) with the help of gray filters and amplifier gain. The use of a lower filter density should be prioritized against the use of a high gain value.

The value of the amplifier gain of both channels (PMT and diode) should be set to one, if possible. Because of an increasing amplifier noise, parallel to the gain factor, a gain value of more than 2 should be avoided. The most important thing is to avoid pixels below the zero level and beyond the maximum range respectively.

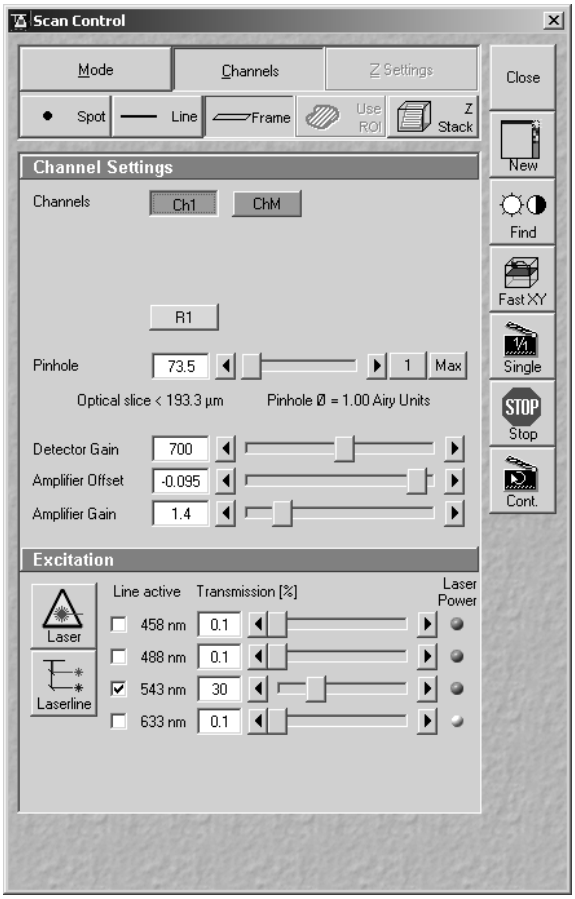


Fig. 9-10 Scan Control window

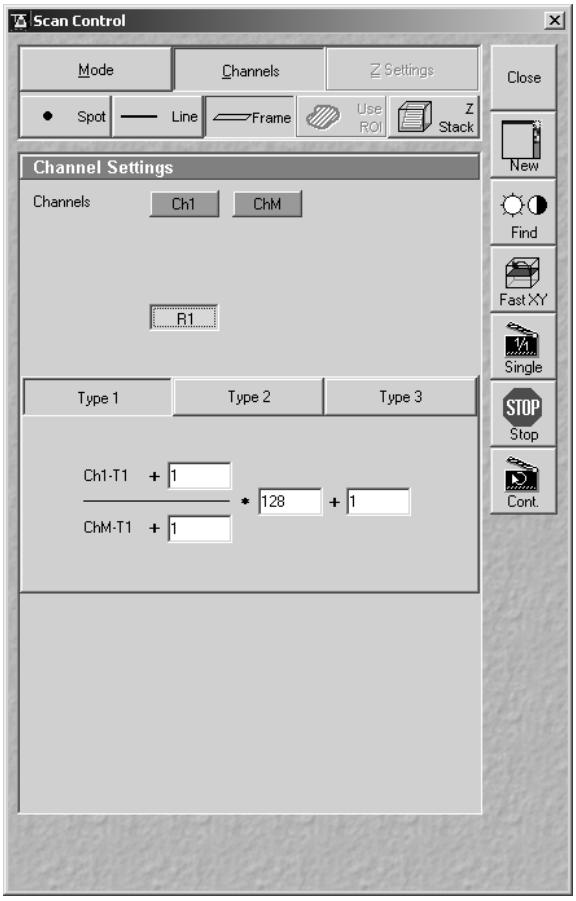


Fig. 9-11 Scan Control window

Gain and offset in Ratio channel

If the setting of the PMT channel is finished, the range of the ratio channel is adjusted by the parameters in the corresponding formula. Three types of formulas are offered when the **R1-1** button is pushed. The only formula needed for a ratio image with the monitor diode is type 1:

$$\frac{S1+n}{S2+m} * x+y$$

The values for n and m have to be zero, as well as the value for y. Any deviation from zero will decrease the contrast of the ratio image.

Only the value of x shall be influenced by the user. Dependent on the choice of data depth (8 or 12 bit), x is between 0 and 256 (8 bit) or between 0 and 4096 (12 bit).

Default settings are 150 and 3000 respectively. With the help of the range indicator the default value is changed until pixel overflow ('red pixels') is no more available

☞ Any new value can be set by hand-typing and pressing the **ENTER** key while the scan is running.

Any change in the setting parameters of PMT and diode signal will make a new Gain x in the ratio formula necessary.

If the adjustment of all parameters is finished, only the ratio image can be scanned or displayed by switching off the PMT channel and the diode channel in the **Configuration Control** window and leaving only the Ratio Channel turned on. As a result, only the ratio image is displayed; which can still be influenced by the settings in PMT and diode channel.

9.10 NLO Non Linear Optics Laser for LSM 510 NLO

When the optional NLO laser (Titanium-Sapphire-Laser in the near infrared range - NIR) is used, some specialties in the operation of the **Laser Control** window and the configuration of the system in the **Configuration Control** window must be taken into consideration.

9.10.1 Laser Control Window

The **Laser Control** window features no remote control functions for the NLO laser.

On activation of the **On** button, the laser is not switched on directly, but the software is only informed that the laser is switched on and the laser safety shutter can be opened.

The laser wavelength to be used must be matched with the hardware and entered in the software.

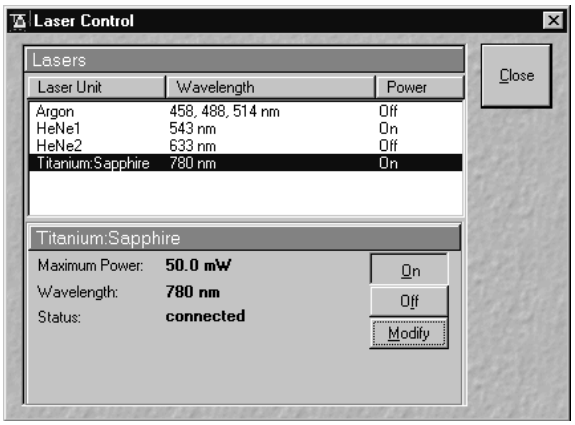


Fig. 9-12 Configuration Control window

- Allow the use of the **Titanium:Sapphire** laser with a click on the **Lasers** panel of the **Scan Control** window. The **Titanium:Sapphire** panel is displayed.
- Click on **On** to activate the laser for the software.
- Click on the **Modify** button. The **Laser Modify Control** window is displayed.
- Enter the wavelength set on the laser in the **Edit Laser Wavelength** input box (no laser tuning).
- The **Fine Tuning AO-Frequency** slider enables you to fine-tune the AO-frequency (Acousto Optical) during the continuous scanning procedure. This should only slightly influence the intensity of the signal because the automatic presetting is of high precision.
- Click on **Store** to confirm the setting. The **Laser Modify Control** window is closed and the **Laser Control** window updated.

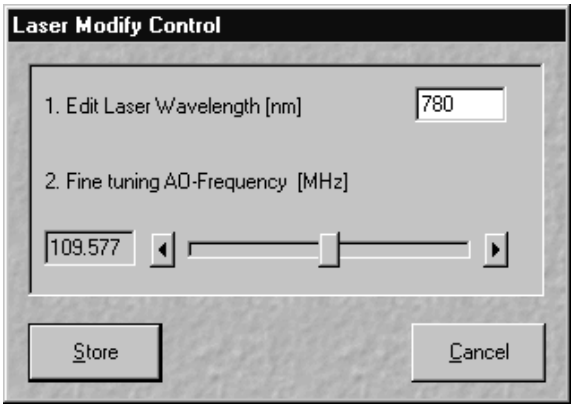


Fig. 9-13 Laser Modify Control window

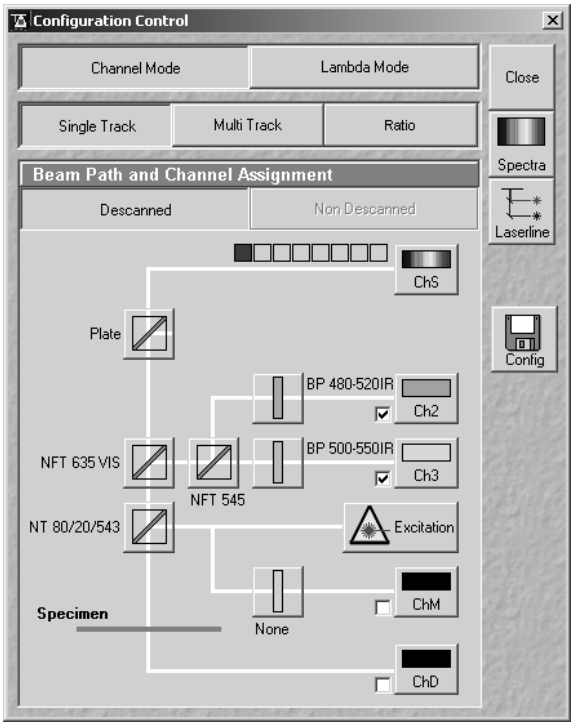


Fig. 9-14 Configuration Control window

9.10.2 Configuration Control Window

Application of the NLO laser requires special main dichroic beam splitters and the relevant emission filters to be activated in the **Configuration Control** window.

NLO-excited signals can be detected in every channel by taking care on the blocking of the NIR laser excitation light.

The following filters are especially designed for detection of NLO-excited fluorescence signals:

- **HFT KP 680**
  main dichroic beam splitter reflecting NIR excitation longer than 680 nm,
  transmission for shorter wavelengths
- **HFT KP 680 / 488 (514, 543)**
  main dichroic beam splitter reflecting NIR excitation longer than 680 nm,
  transmission for shorter wavelengths and reflecting 488 nm excitation for simultaneous detection of NIR and VIS excitation
- **KP 685** (short pass filter)
  transmitting wavelengths shorter than 685 nm
- **BP 500-550 IR**
  special bandpass for NLO blocking also in the NIR range (extension IR),
  can be used without additional BG 39
- **BG 39**
  highly efficient block filter for NIR, for combination with normal emission filters without IR extension

9.10.3 Pinhole and Collimator Settings

- The pinhole can be fully opened for maximum detection efficiency due to the focal excitation capabilities of the NLO effect (see **Scan Control** window, **Channel Settings**).
- In the **Pinhole & Collimator Control** window, the **NIR** collimator can be used to align the overlap of the excitation planes within the object for VIS as well as NIR excitation light wavelength in the **Collimator** panel (see **Maintain** menu, **Pinhole** button).

9.11 Non Descanned Detection (NDD)

The application of Non-Descanned Detection with the LSM 510 is only useful in combination with the optional NLO laser.

The Non-Descanned Detection modules can be used on the reflected or transmitted-light beam path or simultaneously on both beam paths. This means that the maximum of four NDD channels can be configured. If two NDD channels have been assigned to the transmitted-light beam path, no transmission PMT can be implemented.

In Non-Descanned Detection, the radiation emitted by the specimen is conducted directly on the relevant detector without passing the scanner mirror again.

Non-Descanned Detection is set and configured in the **Configuration Contro**l window by activating / deactivating the buttons **Descanned Detection** and **Non-Descanned Detection** while the NDD module is being connected.

- Click on the **Non-Descanned Detection** button to change to Non-Descanned Detection.
- Configure the NDD channels analog to the Descanned Detection mode.

☞ The configuration of multitracks is also possible for NDD applications, though not in combination with the standard channels.

- Pull out or push in the pushrod (9-16/**1**) to close or open the shutter for the HBO 100 illuminator.

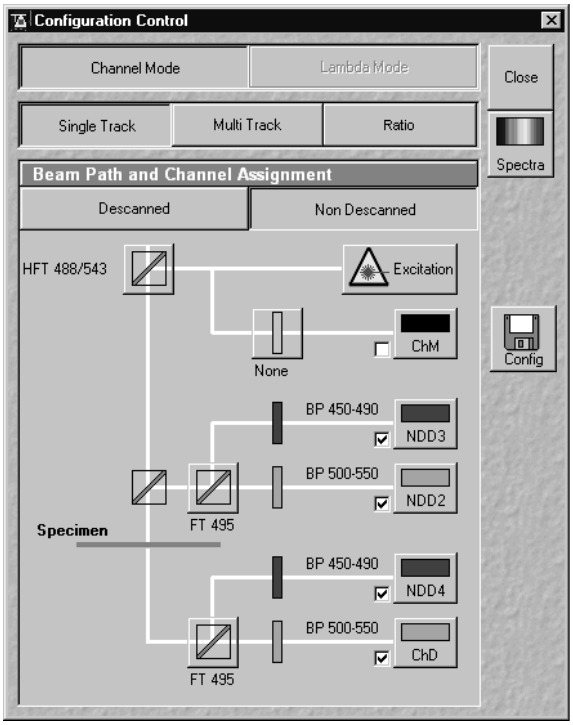


Fig. 9-15 Configuration Control window

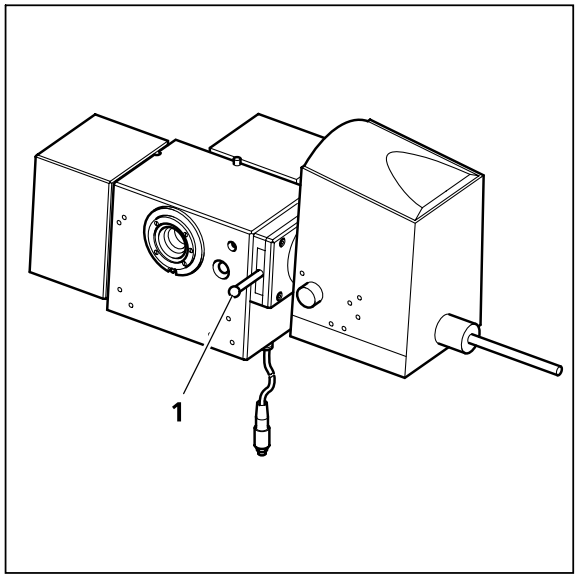


Fig. 9-16 Non descanned detection module with HBO 100 lamp

9.12 AxioCam High Resolution Digital Cameras

9.12.1 High Resolution Microscopy Camera AxioCam HRm Rev.2

Cat. No	000000-0445-553, incl. digital interface and cable
High Range Monochrome	
Number of Pixels:	1388 (H) x 1040 (V) = 1.4 Mega pixel
Chip size:	8.9 mm x 6.7 mm, equivalent to 2/3"
Spectral range:	With BK-7 protection glass up to 1000 nm, with IR barrier filter BG40 limited to about 350 nm to 700 nm
Selectable Resolution by Binning or Microscanning	

H	x	V	Acquisition Time (s) @ 20 ms exposure
694	x	520	0.07 (13 images / s
1388	x	1040	0.2 (5 images / s)
2776	x	2080	
4164	x	3120	

Dynamic Range:	Better than 2000 : 1 @ 8 e readout noise
Integration Time:	1 ms to several minutes
Cooling:	Single stage Peltier cooling
Optical Interface:	C-Mount
Size:	about 11 cm x 8 cm x 6.5 cm (2.3" x 3.2" x 2.6")
Registration:	GS, CE, cUL
Power Supply:	12 V DC, 1 A, 230 V/110 V, autodetecting

9.12.2 High Resolution Microscopy Camera AxioCam HRc

Cat. No	000000-0412-312, incl. digital interface and cable
High Range Color	
Number of Pixels:	1300 (H) x 1030 (V) = 1.3 Mega pixel
Chip size:	8.7 mm x 6.9 mm equivalent to 2/3"
Spectral range:	Limited by IR barrier filter BG40, about 400 nm to 700 nm

Selectable Resolution by Binning or Microscanning

H	x	V	Acquisition Time (s) @ 20 ms exposure
432	x	342	0.07 (Color interpolation)
1300	x	1030	0.2 (Color interpolation)
1300	x	1030	0.7 (full resolution for color channels)
2600	x	2060	
3900	x	3090	

Dynamic Range:	Typical 2000 : 1 @ 9 e readout noise
Integration Time:	1 ms to several minutes
Cooling:	One stage Peltier cooling
Optical Interface:	C-Mount
Size:	about 11 cm x 8 cm x 6.5 cm (2.3" x 3.2" x 2.6")
Registration:	GS, CE, cUL
Power Supply:	12 V DC, 1 A, 230 V/110 V, autodetecting

9.12.3 Microscope camera port adapters for the AxioCam

Adapter Video V200 C 2/3" 0.63x at frontport Axiovert 200M Cat. No 000000-1071-171

This adapter is **needed for attachment** of the high-resolution AxioCam microscope cameras **on the Axiovert 200M**

Adapter Video 60 C 2/3" 0.63x Cat. No 000000-1069-414

This adapter is **needed for attachment** of the high-resolution AxioCam microscope cameras **on the Axioplan 2, 2i** and **Axioskop 2, 2FS & 2plus**.

For an additional documentation port to be connected to the camera port of the tubes with interface 60:

Double video adapter Cat. No 000000-1058-640

For connection to interface 60, 2 switching positions for switching to 100% mirror or to interface for P&C modules.

Adapter Video 44 C 2/3" 0.63x Cat. No 452997-0000-000

This adapter is **needed for attachment** of the high-resolution AxioCam microscope cameras **on the Axiovert 100M BP / SP**.

☞ **No other cameras are supported by the LSM Software!**

9.13 List of Key Words

Numbers

2.5 D 5-290
3D 5-292
3D for LSM
terms and definitions 8-3
3D View 5-20, 5-178

A

About LSM 510 5-238
ACE Automatic Component Extraction 5-349
Acquire 5-20, 5-44
Add image sequences 8-22
Alpha rendering method 8-60
And calculation 8-49
Automatic object measurement 8-66
Ampl. Gain 5-356, 5-358
Ampl. Offset 5-356, 5-358
Analysis of images 5-239
Animation 5-259
Area 5-276
Average 5-89
Axioplan Control 5-49
Axioskop 2 FS Control 5-60
Axiovert Control 5-56

B

Beam path 5-68, 5-70
Beam Path 5-66, 5-71, 5-352, 5-360
Bleach 5-135
Bleach Parameter 5-138
Bleach Regions 5-138

C

Calculation
And 8-49
Or 8-50
Xor 8-51

C

Camera detection 5-83
Carl Zeiss Vision image sequences 8-15
Channel 5-91, 5-242
Channel
add, remove 8-8, 8-20
delete 8-21
Channel Assignment 5-66, 5-71, 5-352, 5-360
Closing 8-43
Coded 5-342
Collimator adjustment 5-230
Color Palette 5-256
Configuration Control 5-61
Configurations overview 9-11, 9-12
Contrast 5-159, 5-252
Contrast
change contrast 8-26
enhance 8-31
Copy 5-263
Copy
content of Display window 8-19
Crop 5-262
Cut 5-272

D

Database 5-21
Delete function 5-33
Export of images 5-39
Filter function 5-30
Form mode 5-25
Gallery mode 5-27
Import of images 5-38
Multi Print 5-40
New 5-21
Open 5-23
Saving an image 5-34
Subset function 5-28
Table mode 5-28
Deconvolution 5-174

D

Delete
- images ... 8-21
- object in specified size range ... 8-54

Depth Coding ... 5-178
Desktop ... 4-9, 5-15
Detaching the Scanning Module ... 9-16
Detector Gain ... 5-356, 5-358
Dialog boxes ... 8-6
Dichroic beam splitters ... 5-67
Dilation ... 8-37, 8-39, 8-41, 8-43
Display of images ... 5-239
Display window ... 8-6, 8-10
Dyes ... 9-9

E

Edit Menu ... 8-8
Emission filter ... 5-67; 9-9
Erosion ... 8-36, 8-37, 8-38, 8-41, 8-43
Excitation ... 5-70, 5-80, 5-93
Excitation laser lines ... 9-9
Excitation of Bleach Track ... 5-139
Exit the Expert Mode ... 5-43
Exiting the LSM program ... 4-24, 5-363
Expert Mode ... 4-12, 4-13, 5-18
- exit ... 5-43
- quick start ... 4-13

F

File ... 5-20, 5-21
File menu ... 8-7
Fills holes ... 8-55
Filter ... 5-68, 5-156, 7-26
Filter change ... 9-15
Find ... 5-233, 5-354, 5-360
Fingerprinting ... 5-347
Fingerprinting ... 5-81
Frame ... 5-84, 5-87
- creation of a ... 5-93

Full Screen ... 5-236

F

Functions
- Add Channel ... 8-20
- Arithmetics-Add ... 8-22
- Arithmetics-Subtract ... 8-24
- Automatic Object Measurementl ... 8-75
- Boolean-And ... 8-49
- Boolean-Mask ... 8-53
- Boolean-Not ... 8-52
- Boolean-Or ... 8-50
- Boolean-Xor ... 8-51
- Condition ... 8-73
- Contrast-Automatic ... 8-29
- Contrast-Interactive ... 8-26
- Contrast-Linearize ... 8-31
- Copy ... 8-19
- Delete All Images ... 8-21
- Delete Channel ... 8-21
- Edit Channels ... 8-20
- Exit ... 8-18
- Fill Holes ... 8-55
- Morphology ... 8-34
- Morphology-Close ... 8-43
- Morphology-Dilate ... 8-39
- Morphology-Erode ... 8-38
- Morphology-Open ... 8-41
- Object Features ... 8-66
- Open Image ... 8-14
- Print ... 8-18
- Render-Alpha ... 8-60
- Render-Surface ... 8-56
- Save Display As ... 8-17
- Save Image As ... 8-16
- Scrap ... 8-54
- Segment-Automatic ... 8-47
- Segment-Interactive ... 8-45
- Set Channel Colour ... 8-13
- Smooth (Gauss) ... 8-33
- Volume Features ... 8-70

G

Gallery ... 5-273, 8-5, 8-10
Gauss filter ... 8-33
Gradient shading method ... 8-56
Grey value segmentation ... 8-45, 8-47

H

Help 5-238
Help menu.....8-9
HFT 5-67
Histogram.....5-275
Holes to fill 8-55
HRZ 200 ..... 5-103, 7-16; 9-18
Hyperfine Z Sectioning 5-103

I

Image
Delete function 5-33
Display and analysis.....5-239
Export of an 5-39
Import of an.....5-38
Information.....5-336
Load an.....5-23
Saving an 5-34
Image Acquisition 5-44
Image Browser.....7-30
Image Examiner 7-31
Image optimization 5-351, 5-361
Image sequence.....8-3
channels 8-4
Image Size 5-87
Information of the image 5-336
Interpolation.....5-161
Ion Concentration.....5-168

L

Lambda Mode 5-78
Laser attenuation.....5-70
Laser Control 5-45
Line 5-84, 7-17
Line Selection 5-99
Line Step Factor 5-87
Line, creation of a.....5-107
Log on to WINDOWS NT..... 4-8, 5-14
Lowpass filter 5-156
LSM 510 Switchboard. 4-11, 4-24, 5-17, 5-363

M

Macro
editing and debugging 5-194
Macro 5-20, 5-189
Main components of the system 8-5
Main menu.....5-19
Main window 8-5
Maintain.....5-20, 5-217
Admin 5-234
collimator adjustment.....5-230
digital signal processor 5-232
objektive.....5-224
Parameter 5-234
pinhole adjustment 5-227
Reboot.....5-234
set find 5-233
spline.....5-234
Test Grid.....5-235
TSP Trace 5-234
Mark First/Last 5-102
Marker 5-127, 5-130
Mathematical morphology 8-34
Measurement concept 8-64
Measurement menu 8-9
Measurement process.....8-65
Max 5-342
Mean 5-337, 5-343
Mean ROI 5-133
Median filter.....5-157
Microscope Control 5-48
Mode 5-87
Monitor diode 9-26
Monochrome image display 5-244
Multi Track 5-61
Multiple-channel.....5-360
multitracking 5-71, 5-72
Non Descanned 5-82

N

Negation of image.....8-52
NFT 5-67

N

Non Descanned Detection 9-35
Non Linear Optics Laser 9-33

O

Objective 5-224
object features (densitometric) 8-69
Objective Lens 5-87
Object features (geometric) 8-68
Offset 5-90
Opening 8-41
Operation 4-3, 5-9
Options 5-20, 5-203
  dye database 5-205, 5-206
  export to 5-203
  load routine mode 5-204
  settings 5-207
Orthogonal sections 5-267
Or calculation 8-50
Overlay 5-248

P

Palette 5-256, 5-357, 5-358
Parfocality 5-226, 7-28
Pinhole 5-356
Pinhole adjustment 5-227
Pixel Depth 5-89**,** 5-359
Player 8-11
  control elements 8-12
PMT photomultiplier 5-358
Preview print 5-334
Print 5-40, 5-334
Print
  content of Display window 8-18
Process 5-20, 5-148
  add 5-148
  channel shift 5-162
  contrast 5-159
  copy 5-154
  duplication 5-154
  filter 5-155

P

Process
  interpolate 5-160
  multiply 5-152
  ratio 5-153
  subtract 5-151
Process Menu 8-8
Profile 5-287
Projection 5-181
Properties 8-4

R

Range 5-101
Range Indicator 5-357, 5-358
Ratio 5-153
Ratio channel 9-32
Ratio Settings 5-76, 5-77
Recording configuration 5-61, 5-71, 5-74, 5-75
Refresh 5-29
Region Of Interest 5-106
  edit 5-116
Reuse 5-261
ROI 5-106
  edit 5-116, 5-138
Rotation 5-90
Routine Mode 4-11, 5-17, 7-3
  acquisition of a frame 7-7
  acquisition of a time series 7-20
  acquisition of a Z Stack 7-12
  Standard Examination Methods 7-4
  User Defined Examination Methods 7-4
Running down the operating system 4-25, 5-364

S

Save 5-264
Save
  content of Display window 8-17
  image 8-16
  image sequence 8-16
Save As 5-264
Saving an image 5-22, 5-34

S

Scan Average....5-89, 5-359
Scan Control....5-84
Scan Direction....5-89
Scan speed....5-359
Scanning Modes....5-73
Scatter diagram....5-280
Segmentation
automatic....8-47
interactive....8-45
Select
object in specified size range....8-54
Settings....5-207
Settings for excitation....5-73
Sharpness filter....5-157
Shut-Down Procedure....4-24, 5-363
Single channel....5-351
Single Track....5-61
single tracking....5-72
Single tracking....5-66
Slice....5-247, 8-3
Software....4-3, 5-9
Speed....5-88
spline....5-110
Spline....5-234
Spline scan....5-111, 5-234
Split xy....5-94, 5-266, 5-362
Spot....5-84
Spot, creation of a....5-113
Stage....5-140
Starting the LSM 510 program....4-10, 5-16
Stereo....5-185
Structures
dilate....8-39
erode....8-38
Subset....5-274
Subtract image sequences....8-24
Switching on the Enterprise UV laser 4-10, 5-16
Switching on the system....4-7, 5-13

T

Tile Scan....5-144
Time Delay....5-125
Time Interval....5-125
Time Series....5-120
of a Frame....5-129
of a Z Stack....5-131
of a Z Stack over Lambda....5-132
with Mean ROI....5-133
Toolbar....5-19, 5-237
Tool bar....8-5, 8-9
Tools....7-26
change filters....7-26
LSM Image Browser....7-30
LSM Image Examiner....7-31
stand select....7-29
Topography....5-301
3D measurement functions....5-329
data processing....5-302, 5-304
display modes....5-307, 5-310
measurement functions....5-317
Track....5-61
Track configuration....5-64, 5-65
Track Configuration....5-61
Tracks panel, List of....5-72
Transmission....5-352
Trigger-Interface LSM 510....9-23
Turning power off....4-26, 5-365

U

User-defined filter....5-158

V

Value
enter new value....8-28
mask grey value....8-53
scale a range of grey values....8-31
scale grey value....8-29
Vertical scale of histogram....8-28
View Menu....8-9
Volume features (densitometric)....8-72

V

Volume features (geometric) 8-72

W

Window 5-236
full screen 5-236
toolbar 5-237
Windows Menu 8-9

X

Xor calculation 8-51
xy display 5-265

Z

Zeiss TIFF image sequence 8-14
Z Sectioning 5-98
Z Settings 5-96
Z Stack 5-96
creation of a 5-105
Zoom 5-90, 5-245