CHAPTER 4   QUICKSTART

CONTENTS

4   QUICKSTART

4.1   Purpose of this Section and other Operating Manuals

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

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

–   B 40-042 e   Axioplan 2 imaging MOT, Operating Manual

–   B 40-080 e   Axiovert 200 M, Operating Manual

–   B 40-076 e   Axioskop 2 FS MOT, Operating Manual

4.1.1   Software

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

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☞   The installation of the software for the LSM 510 and the basic settings of the equipment components are carried out by Carl Zeiss service staff. This job includes the creation of a customized software configuration in line with the specific hardware components of the customer's microscope system.
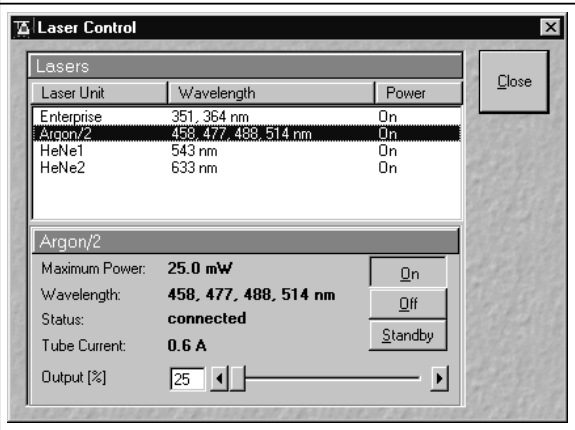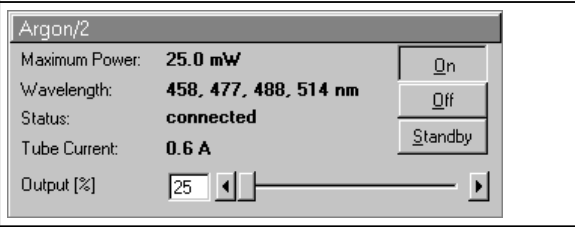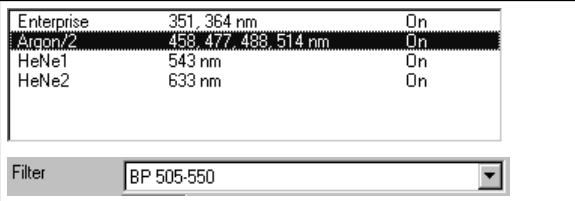
The LSM 5 software is menu-controlled and normally uses its own windows for the activation of the various functions; within these windows, further submenus (panels) can be displayed and removed.

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

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

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

4.1.2 Windows and Window Elements

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

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

4.1.3   Convention for the Text in this Manual

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

- names of windows,

- panels,

- input boxes,

- list / selection boxes,

- check boxes,

- menu items,

- names of buttons and

- keyboard keys,

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

4.1.4   Backup

System backup

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

User files backup

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

– Image database files: *.mdb (but not system_configuration_*.mdb

– LSM Image files: *.lsm

– Exported images: *.* (*.Tiff, *.LSM-Tiff, *.BMP, ...)

– Palette files: AIM \ Palette \ *.lut

– Filter files: AIM \ Filter \ *.krn

– Pinhole setting files: AIM \ PH*.pos

– Log files: AIM \ *.log

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

– Parameter file for pinhole setting: AIM \ *.set

– Parameter file after pinhole adjustment: AIM \ *.adj

– Scanner files: AIM \ bin \ *.bin

– Microscope stand database: AIM \ database \ system_configuration_*.mdb

4.1.5   Software Operation

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

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

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

4.2   Switching on the System

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

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

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

- Turn the **REMOTE CONTROL** switch to "**ON**" position (see Fig. 4-1).

    – This switches the entire system on.

    – Microscope and laser will be ready for operation after a short time.

    – Computer boots up.

    – Computer hardware system test runs.

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

The monitor shows a dialog box for selection of the operating system version.
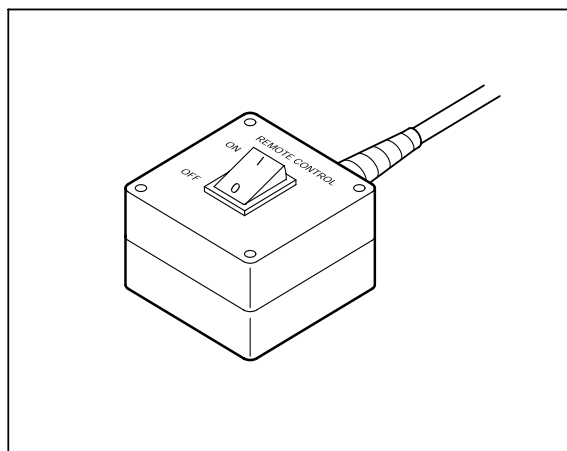
Fig. 4-1   **REMOTE CONTROL switch**
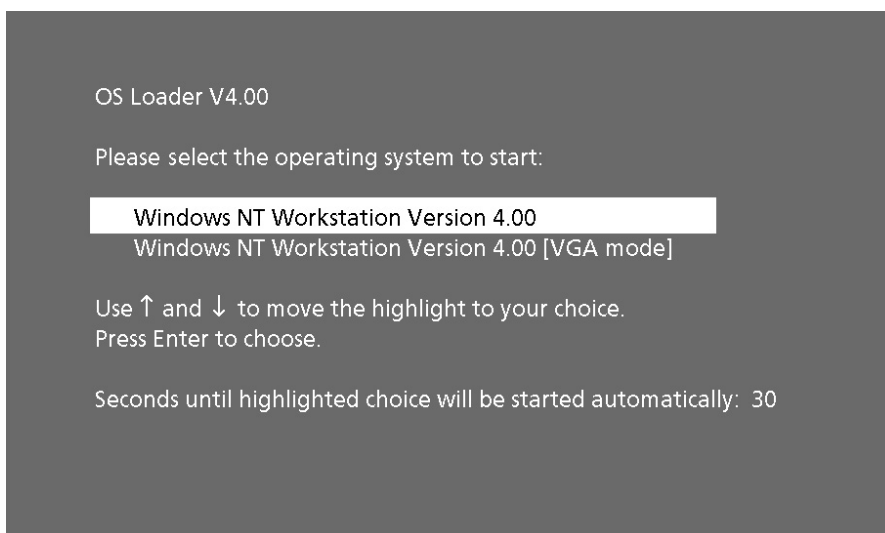
Fig. 4-2   **Selecting the operating system version**

Fig. 4-3   Begin Logon window

Fig. 4-4   Logon Information window

- Confirm the default setting of the "Windows NT Workstation Version 4.00" by pressing the **Enter** key.

  – WINDOWS NT operating system is being loaded.

  – The **Begin Logon** window appears on the screen.
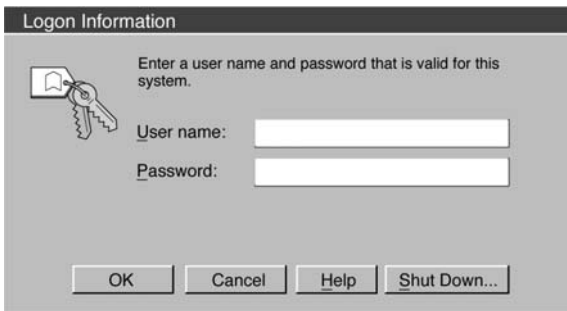
4.2.1   Log on to WINDOWS NT

- Press the three keys **Ctrl**, **Alt** and **Del** at the same time.

  – The **Logon Information** window appears on the screen, permitting you to log on to the WINDOWS NT 4.0 operating system.

- Enter the valid user name into the **User name** text box.

- Enter your password into the **Password** text box.

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

Change Filters icon

Stand Select icon

LSM 510 icon

LSM 510 Image Examiner icon

LSM 510 Image Browser icon
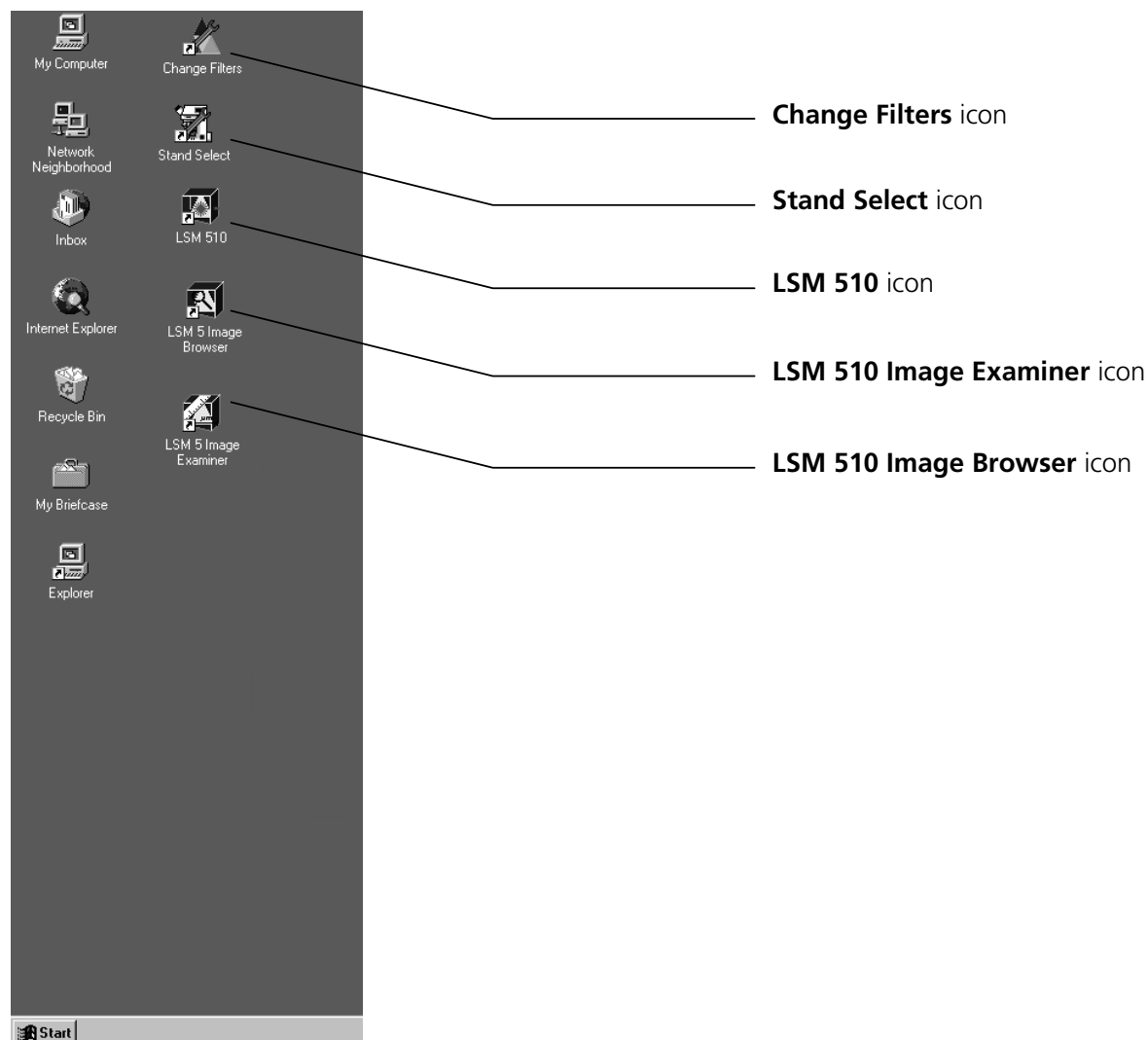
Fig. 4-5   WINDOWS NT operating system desktop
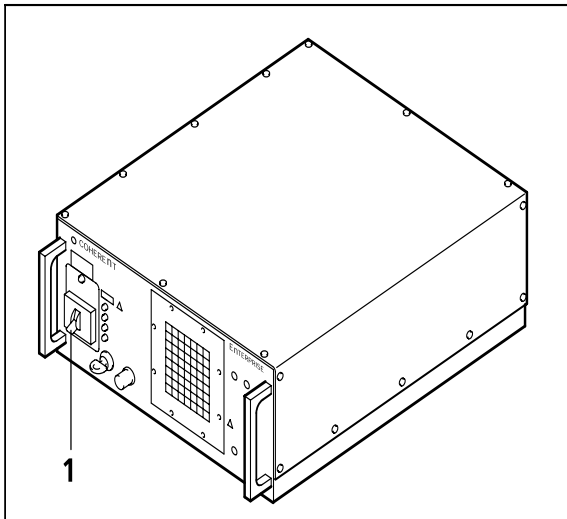
Fig. 4-6   Power supply of UV-Ar laser

Fig. 4-7   Starting the LSM 5 software

4.2.2   Switching on the Enterprise UV Laser

- If the UV laser is required, switch it on via the toggle switch (4-6/**1**) of the power supply.

    – It will be ready for operation after a few seconds.

4.2.3   Starting the LSM 5 Program

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

- Double-click on the **LSM 510** icon on the desktop of WINDOWS to start the LSM 5 software program (see Fig. 4-5).

    – The **LSM 510 Switchboard** menu appears on the screen (see Fig. 4-8).
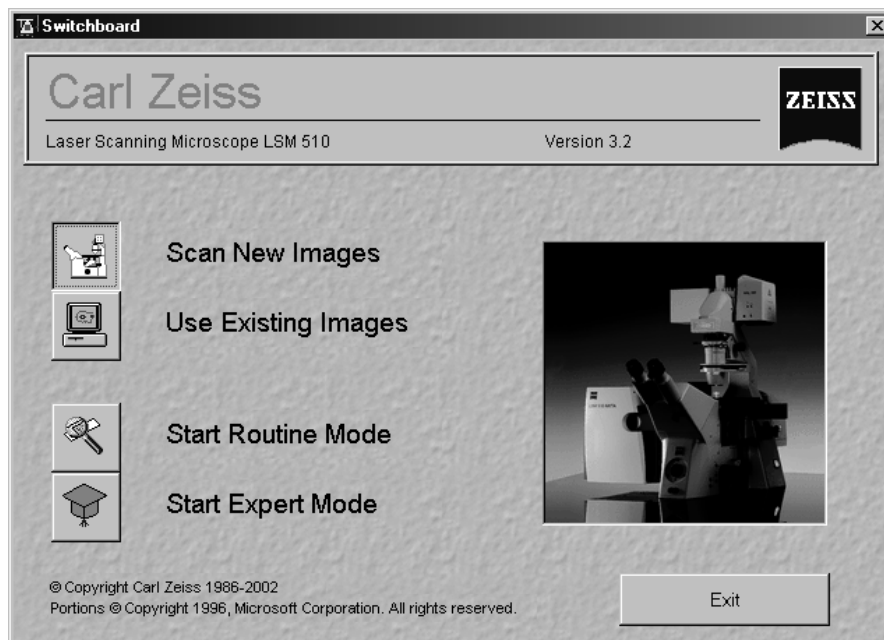
Fig. 4-8   LSM 510 Switchboard menu

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

– **Scan New Images**

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

– **Use Existing Images**

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

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

– **Start Routine Mode**

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

– **Start Expert Mode**

Use of this mode requires to be thoroughly familiar with the exact microscope procedures and interrelations.
You need to set all parameters and functions upon your own decision; this mode therefore provides you with the greatest flexibility of operation.
It is also possible, however, to call up stored configurations and to modify the parameters / settings if necessary.
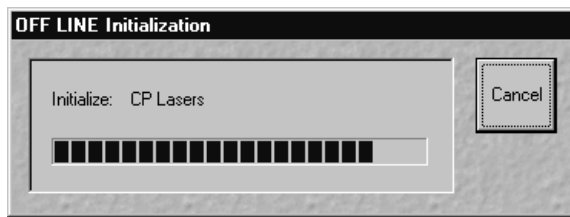
Fig. 4-9   OFF LINE Initialization window

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

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

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

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

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

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

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

4.3   Quick Start in the Expert Mode

Proceed as follows to generate images in the Expert Mode:

–   start the Expert Mode

–   test / change the microscope setting: objective, fluorescence / attenuation filters, illumination mode, diaphragms

–   normal setting of the microscope on the specimen with observation in brightfield or fluorescence contrast (KÖHLER-type illumination)

–   switch to **LSM** mode

–   setting of lasers: laser type, laser intensity (power)

–   configuration of beam path and channel assignment: tracks, multi-tracks, switching on / off of laser lines and intensity (excitation)

–   image creation: determine the scan method (line, frame) and scan parameters (image size, scan speed, pixel depth, scan direction, scan average, zoom, rotation, offset)

–   image optimization and storage

4.3.1   Start the Expert Mode

*   Double-click on the **LSM 510** icon on the WINDOWS NT operating system desktop.

    –   The **LSM 510 Switchboard** menu appears on the screen.

*   Click on the **Scan New Images** button and **Start Expert Mode** button in the **LSM 510 Switchboard** menu.

    –   The LSM will go through the initialization and open the **Main** menu labeled **LSM 510 Expert Mode**. The **Main** menu appears on the screen.

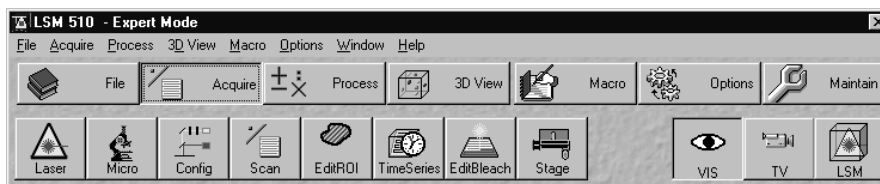**Fig. 4-10   LSM 510 Switchboard menu**

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

4.3.2 Set the Microscope

This step is used to set:

- microscope objective
- specimen position
- specimen focus

4.3.2.1 Axioplan 2 imaging MOT

- Click on the **Acquire** button in the toolbar of the **Main** menu.

- Move the tube slider on the microscope to the **VIS** position.
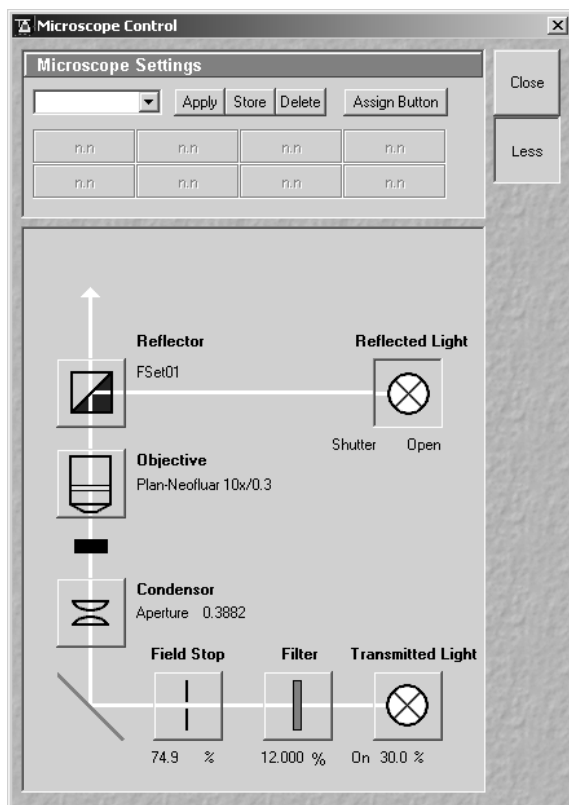
Fig. 4-12 Axioplan Control window

- Click on the **Micro** button in the **Acquire** subordinate toolbar.

  - The **Axioplan Control** window appears on the screen.

- Put the specimen on the stage - make sure the specimen is mounted securely and flat. For tests use the supplied Convallaria specimen.

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

- To view the specimen in transmitted light, set the **Reflector Turret** position to **None** and activate the **Transmitted Light** panel by clicking on the **Transmitted Light** button. Select the **On** button and control the intensity by the slider.

- Select an objective with low magnification by clicking in the **Objective** panel of the **Axioplan Control** window.

- Set the microscope to KÖHLER illumination manually (see Axioplan 2 imaging MOT operating manual).

- Select the specimen area to be examined by moving the XY-stage and focus exactly on the selected area.

- Close the **Axioplan Control** window. Move the tube slider on the microscope to the **LSM** position.

4.3.2.2   Axiovert 200 M

- Click on the **Acquire** button in the toolbar of the **Main** menu.

- Click on the **VIS** button in the **Acquire** subordinate toolbar.

- Click on the **Micro** button.

  - The **Axiovert Control** window appears on the screen.

- Put the specimen on the stage - make sure the specimen is mounted securely and flat. Use the supplied Convallaria specimen at first.

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

- To view the specimen in transmitted light, set the **Reflector Turret** position to **None** and activate the **Transmitted Light** panel by clicking on the **Transmitted Light** button. Select the **On** button and control the intensity by the slider.

- Select an objective with low magnification by clicking in the **Objective** panel of the **Axiovert Control** window.

- Set the microscope to KÖHLER illumination manually (see Axiovert 200 M operating manual).

- Select the specimen area to be examined by moving the XY-stage and focus exactly on the selected area.

- Close the **Axiovert Control** window. Click on the **LSM** button.

Fig. 4-13   Axiovert Control window

4.3.2.3   Axioskop 2 FS MOT

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

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

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

4.3.3 Turn on the Lasers
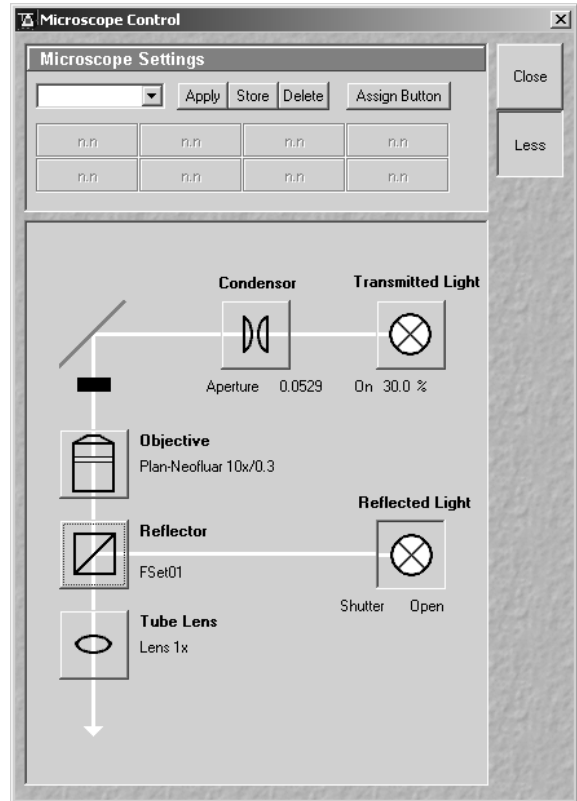
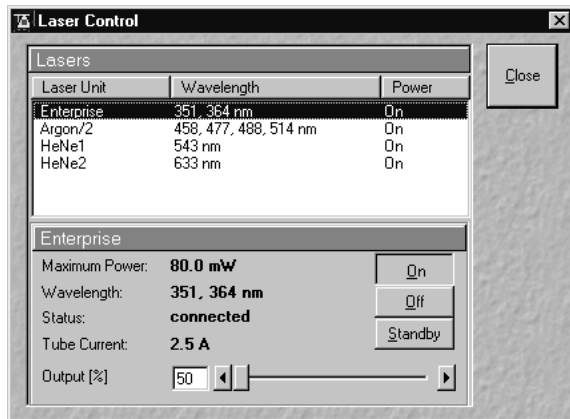This step is used to switch the lasers and set the intensity.

Fig. 4-14 **Laser Control window**

- Click on the **Laser** button in the **Acquire** subordinate toolbar of the **Main** menu.
  - A **Laser Control** window with a list of available lasers will appear. Depending on the lasers available in your system, your screen may differ from the one displayed.
- Using the mouse, click on the laser(s) featuring the appropriate wavelength to excite the dyes used to label your specimen.
- In the case of the argon and UV laser (Enterprise), click on the **Standby** button first.
  - **Warming Up** appears in the line **Status**.
  - After 2 minutes, when the warming-up phase is finished, the **Ready** message appears.

- Then click on the **On** button.
  - The laser is switched on.
- Use **Output [%]** scrollbar to set the intensity to 50 %.
- In the case of the HeNe laser, click on the **On** button directly.
- Close the window with the **Close** button.
- For precise measurements the system should warm up for 2 hours.
- Depending on the lasers available in your system, your screen may differ from the one displayed.

4.3.4   Set the Beam Path

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

- Click on the **Config** button in the **Acquire** subordinate toolbar of the **Main** menu.
  - The **Configuration Control** window appears on the screen. Your screen may differ from the one displayed.

- Click on the **Single Track** button, unless it has already been activated.

- Click on the **Config** button in the **Configuration Control** window.
  - The **Track Configurations** window appears on the screen.

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

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

- Click on the **Apply** button.

Fig. 4-15   **Configuration Control window**

Fig. 4-16   **Track Configurations window**

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

☞   If you click on the **Close** button, the **Track Configurations** window will be closed without any change being made to the Track Configuration.
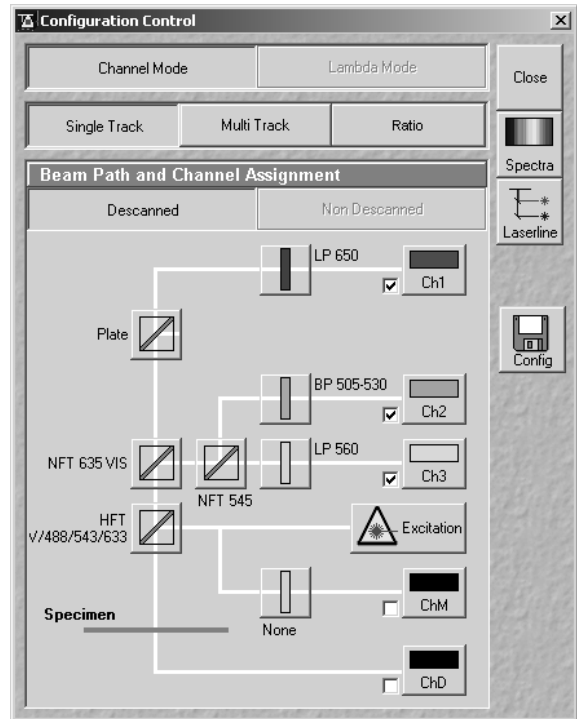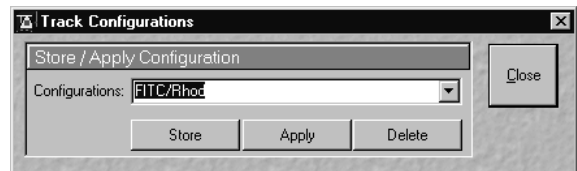
Fig. 4-17   **Scan Control window**

4.3.5   **Scan an Image**

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

- Click on the **Scan** button in the **Acquire** subordinate toolbar of the **Main** menu.

  – The **Scan Control** window appears on the screen.

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

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

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

- To scan an XY-image, click on the **Frame** button.

- Click on the **Find** button on the right-hand side of the **Scan Control** window.

  – An XY-image with automatically generated settings for brightness and contrast is produced.

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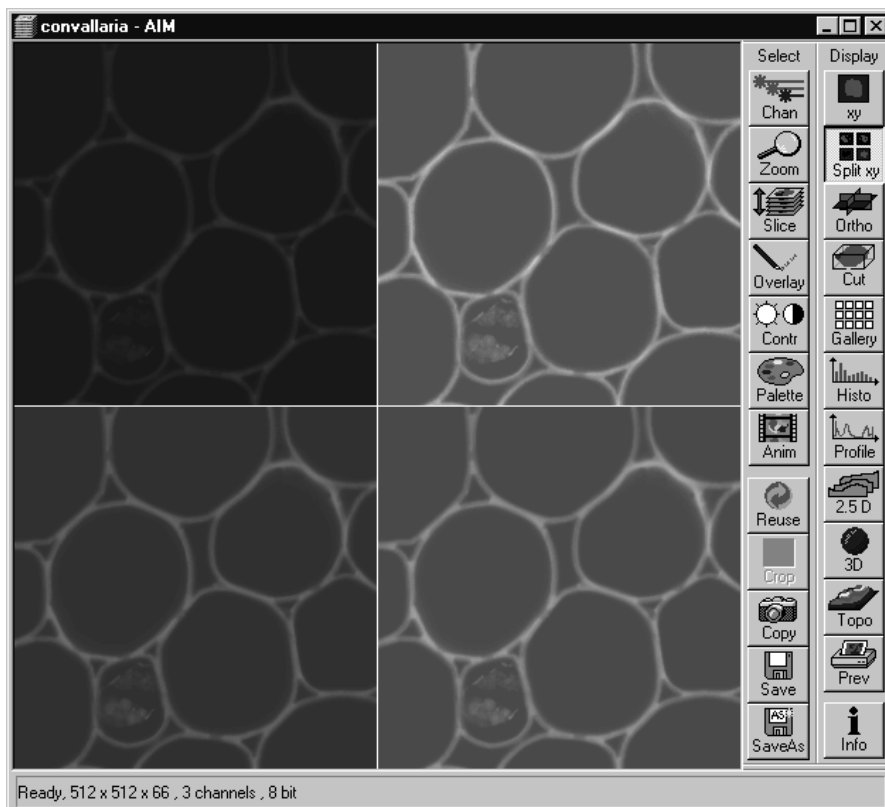

Fig. 4-18   Image Display window with Split xy mode

The scanned image can now be optimized for contrast, brightness and confocality.
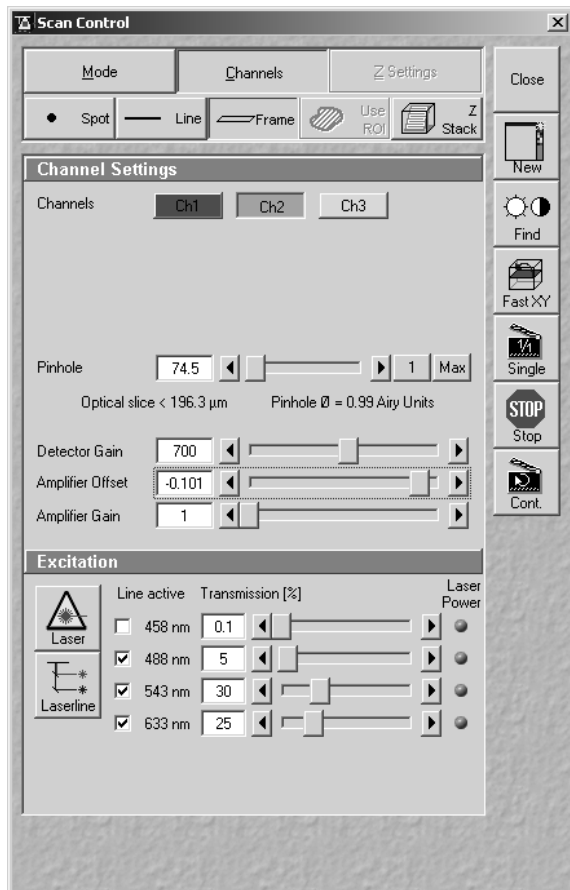
Fig. 4-19   Scan Control window

Proceed as follows for image optimization:

- Click on the **Channels** button in the **Scan Control** window.

  – The **Channel Settings** panel and the **Excitation of Track (...)** panel are displayed in the **Scan Control** window.

- Press the **Cont.** (continuous scan) button on the right-hand side of the window. This starts the continuous image acquisition, which can be interrupted by pressing the **Stop** button.

- Under the **Channel Settings** panel all buttons for each channel you have set up are displayed. Click **Ch1** (channel 1 of track 1), for example, if you want to adjust the first image displayed in the split mode window.

- Use the **Pinhole** slider to set the pinhole diameter.

  – The selected pinhole diameter should be small enough to still allow the Detector Gain setting and to provide sufficient image information. 1 Airy unit is a decent value to obtain a confocal XY-image (use the **1** button).

- If required, adjust the pinhole again (see **Main** menu, **Maintain** subordinate toolbar, **Pinhole** button).

- Use the **Detector Gain** slider to set image contrast and brightness. This adjustment is very sensitive. Try using the left and right arrows to make the adjustment instead of dragging the slider bar. Use the **Shift** and **Crtl** keys for changing to coarse and fine steps.

- To adjust the black level (background) use **Ampl. Offset**.

- Also, try adjusting the microscope by manual focusing. Sometimes you will find that there are other focal planes within the specimen which are brighter, and therefore the detector gain will need to be turned down.

- Once you have optimized a particular channel, you can switch to the next channel required and repeat the optimization.

- As soon as all channels are optimized, click on the **Stop** button.

- To further improve image quality you can slow down the scan speed, allowing more photons to integrate on the detector, or apply image averaging to remove random noise, or a combination of both. These adjustments are made by clicking on the **Mode** button on the **Scan Control** window.

- Set the **Scan Speed** in the **Speed** panel.

- Select the **Line** or **Frame** average **Mode**, the **Mean** or **Sum** average **Method** and the **Number** of averages in the **Depth, Scan Direction & Scan Average** panel accordingly by observing your image. The setting average of **16** (**Number 16**) should improve signal / noise dramatically; however, the image acquisition rate will be slower.

- When the image optimization has been finished, click on the **Single** button to generate a single image of the specimen.

- If your specimen is sensitive to photobleaching, you can attenuate the laser illumination by clicking on the **Channels** button in the **Scan Control** window. At the bottom of the window you can set the percentage of laser power (**Transmission [%]**) for each excitation wavelength. You will probably have to increase the **Detector Gain** if you decrease the laser power. This setting controls the transmission degree of the Acousto-Optical Tunable Filters (AOTF).

Fig. 4-20   Scan Control window

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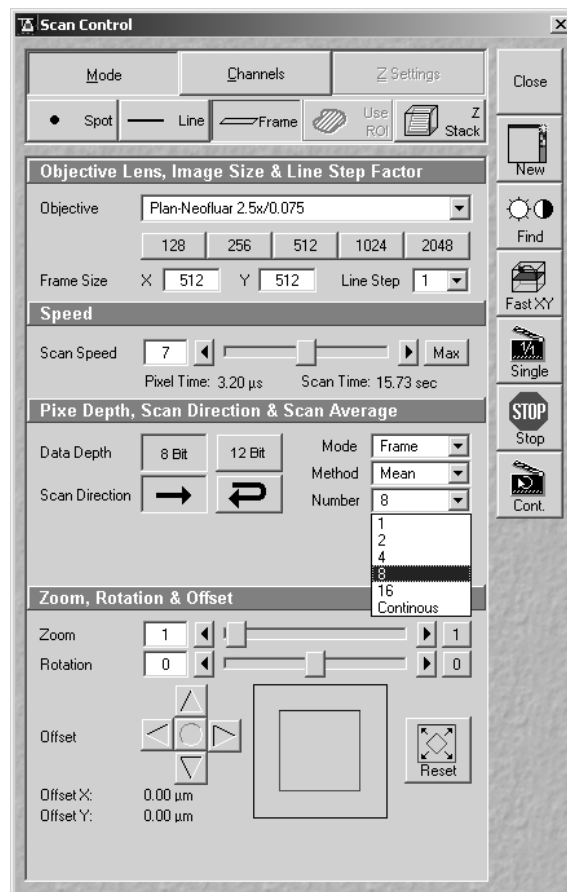
4.3.6 Store the Image

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

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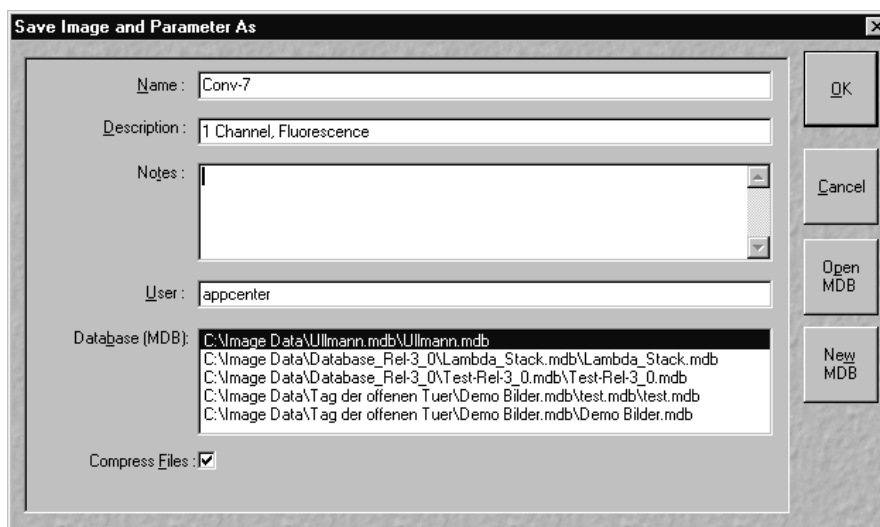 - The **Save Image and Parameter As** window appears on the screen.

Fig. 4-21   Save Image and Parameter As window

Proceed as follows to store images in an existing database:

- To select the required database, click on its name (blue cursor bar) in the **Database (MDB)** list box of the **Save Image and Parameter As** window.

- Enter a suitable name for the image in the **Name** input box. If required, enter further details on the image in the **Description** and **Notes** input boxes.

- Click on the **OK** button to add the image to the selected database.

Proceed as follows to store images in a new database:

- Click on the **New MDB** button in the **Save Image and Parameter As** window.

    – The **Create New Database** window will be opened.

- Enter a database name in the **File name** input box. The name can consist of as many characters as you like.

- Before clicking on the **Create** button in the **Create New Database** window, set the location in which the database will be created by selecting the drive in the **Create in** list box, and double-click on the required folder icon in the list displayed.

- Click on **Create**.

    – The new database will be created and displayed on the screen.

- As described above, enter a name and, if required, further image details in the **Save Image and Parameter As** window.

- Click on the **OK** button**.**

    – The image will be stored and included in the new database.

Fig. 4-22   Create New Database window

4.4   Shut-Down Procedure

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

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

4.4.1   Exiting the LSM Program

• Close all open windows of the LSM program by clicking on the closing icon ☒ in the top right corner of each window.

– This closes the respective window and removes the respective icons from the taskbar.

– After all dialog windows have been closed, the **LSM 510 Switchboard** window appears.

Fig. 4-23   LSM 510 Switchboard menu

• Click on the **Exit** button.

– This terminates the LSM program.

– The monitor screen shows the desktop of the WINDOWS NT operating system.

4.4.2   Shut Down the WINDOWS Operating System

• Move the cursor to the bottom margin of the screen.

– This opens the taskbar containing the **Start** button.

• Click on the **Start** button of the taskbar.

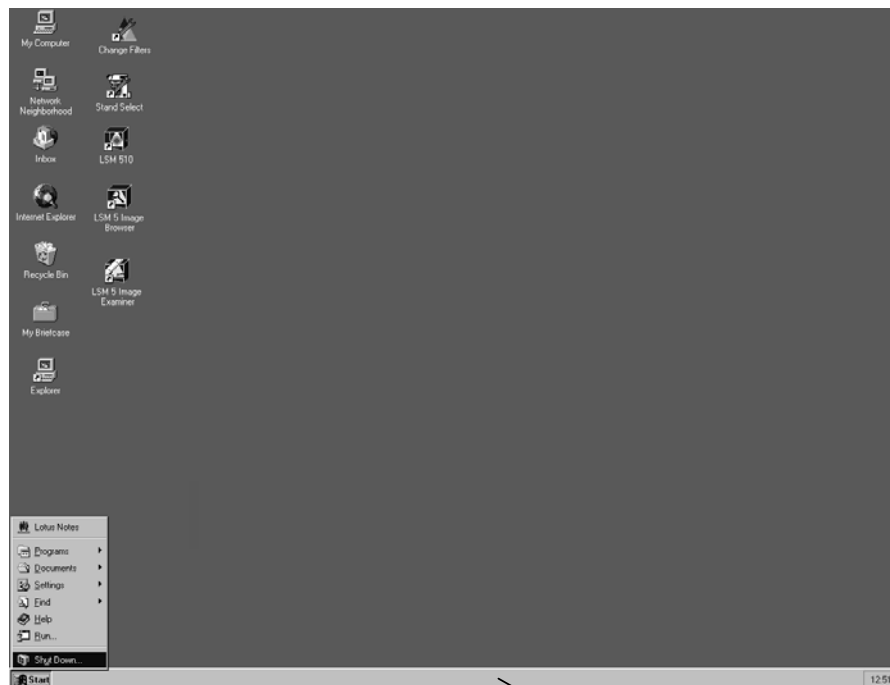– This opens a pop-up menu.

• Click on the **Shut Down** item.

Fig. 4-24   Start menu

Taskbar

Fig. 4-25   Shut Down window

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

• Unless already set by default, click on **Shut down the computer?**

• Click on the **Yes** button.

The screen now displays the message

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

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

4.4.3   Turning Power Off

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

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

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