

# Confocal Laser Scanning Microscope



## ***Make/Model:***

- ZEISS LSM 510 Confor2 (2001 & upgraded in 2005).

## ***Features:***

- Microscope: Axiovert 200M with 25X and 63X water immersion objective lens
- Lasers: Diode (405nm), Argon (458, 477, 488 and 514nm), HeNe1(543nm) and HeNe2 (633nm).
- Software: LSM510/ConfoCor2, Version 3.2 SP2. High performance imaging system for 3D, movie capture and processing.

## ***Applications:***

- High resolution imaging equipment for fluorescence (or bright-field) microscopy of fixed or living cells. The multi channels and/or tracks could detect multiple dyes (fluorophores) simultaneously. Time series (movies), Z stacks (3D), FRET and FRAP, and emission fingerprint are some of the common confocal techniques that are broadly used by researchers to determine gene expression, subcellular localization, and protein-protein interactions.

# Zeiss LSM510 Meta Operation Manual

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Updated 2011-3-15

## Starting up the system

- [Note: Switch on Hg-lamp first ONLY when you need it—usually you DO NOT need it!]
- Switch ON the Remote Control.
- Switch ON the computer power.
- Log on the computer by entering your user name and password.

## Starting the LSM 5 software program



- Double click the **LSM 510** icon on the desktop of WINDOWS to start the LSM 5 software program.

The **LSM 510 Switchboard** window appears on the screen (Fig. 3).

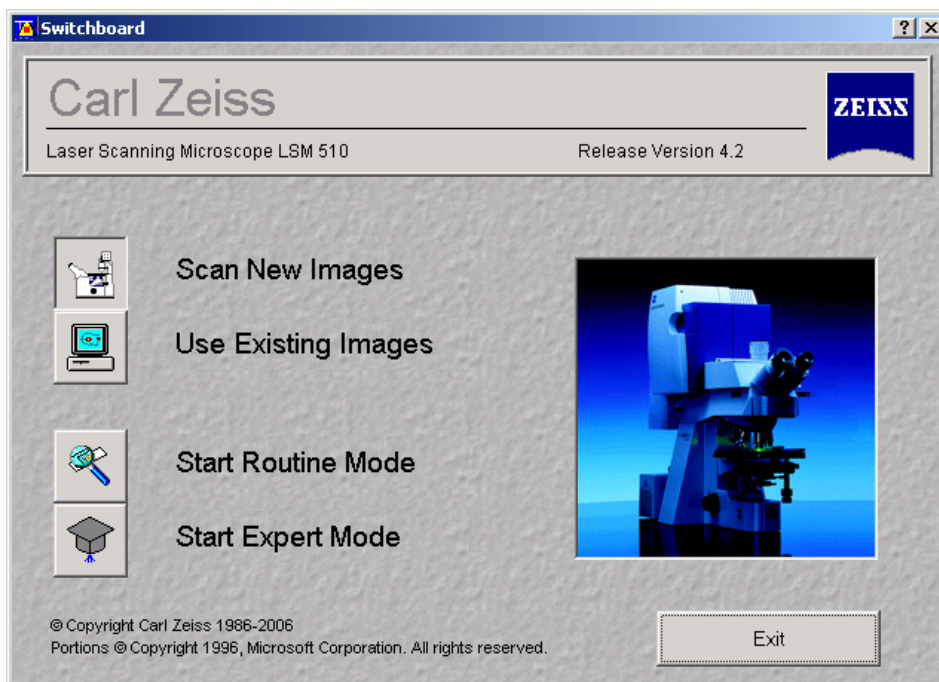


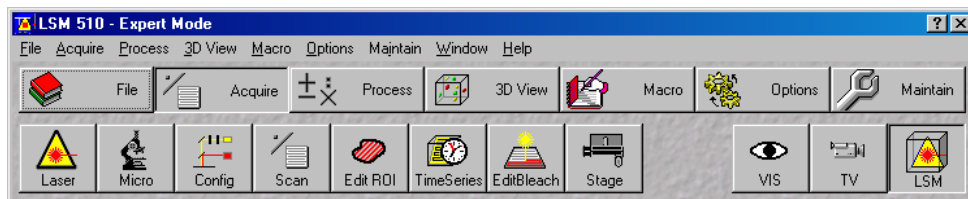
Fig. 3 LSM 510 Switchboard menu



- Click on the **Start Expert Mode** button in the **LSM 510 Switchboard** window.

The LSM 510 - Expert Mode **Main** menu appears on the screen.

Use of this mode requires to be thoroughly familiar with the exact microscope procedures and interrelations.



← **Main** menu (pull-down)

← **Main** menu toolbar

← Subordinate toolbar

Fig. 4 Main menu for Expert Mode

## Creating a database for acquired images



- Click on the **File** button in the **Main** menu toolbar.  
The **File** subordinate toolbar appears in the **Main** menu.

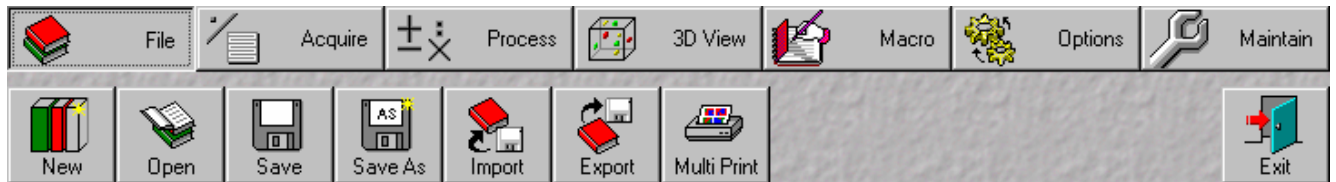
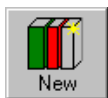


Fig. 5 Main menu with File subordinate toolbar



- Click on the **New** button in the **File** subordinate toolbar.  
The **Create New Database** window appears.

- Select drive **C:** or **D:** from pull down menu.

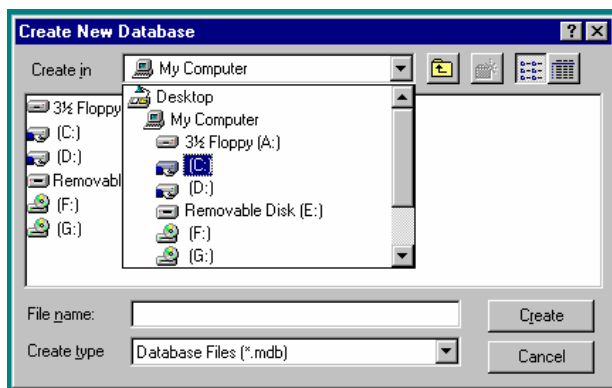


Fig. 6 Create New Database window

- Create a new directory if needed.

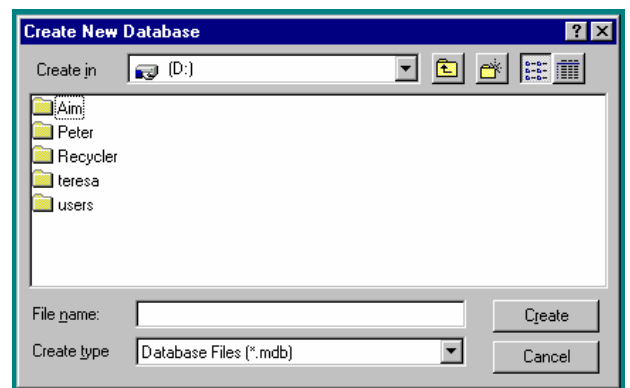
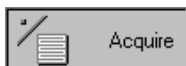


Fig. 7 Create New Database window

## Turning on the lasers



- Click on the **Acquire** button in the **Main** menu to open the **Acquire** subordinate toolbar.

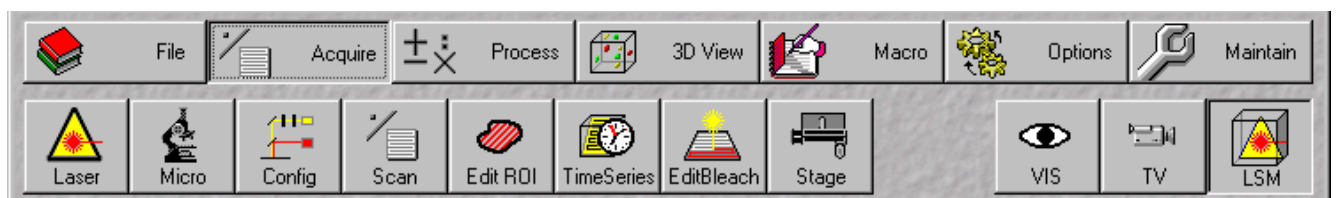
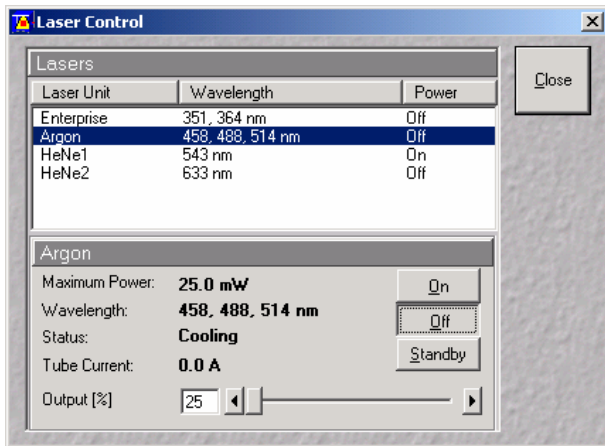


Fig. 8 Acquire subordinate toolbar



- Click on the **Laser** button to open the **Laser Control** window.



- Select the appropriate **Laser Unit** by clicking on the name of it.
- Click the **Standby** button to switch required laser(s) to Standby.
- When status is **Ready** click the **On** button.
- Set **Output [%]** so that the **Tube Current** is about **4 A** (50% of the output power).

Fig. 9 Laser Control window

## Setting the microscope

### Changing between direct observation or laser scanning

The **VIS**, **TV** and **LSM** buttons switch the beam path and indicate which beam path has been set in the binocular tube of the microscope:



- Click on the **VIS** button to set the microscope for direct observation via the eyepieces of the binocular tube, lasers are off.



- Click on the **LSM** button to set the microscope screen observation via laser excitation using the LSM 510 and software evaluation.

### Setting the microscope and storing the settings

- Click on the **VIS** button for direct observation.



- Click on the **Micro** button in the **Acquire** subordinate toolbar to open the **Microscope Control** window of the used microscope.

The **Microscope Control** window appears (Fig. 10).

- Open the graphical pop-up menu by clicking on the **Objective** button (Fig. 10).
- Click on the objective you want to select. The selected objective will automatically move into the beam path.

- Open the graphical pop-up menu by clicking on the **Transmitted Light** button (Fig. 11).
- Click on the **On** button. Set the intensity of the Halogen illuminator using the slider.
- Click on **Close** to close the pop-up menu.
- Place specimen on microscope stage. The cover slip must be facing the objective.
- Use the focusing drive of the microscope to focus the required object plane.
- Select specimen detail by moving the stage in X and Y using the XY stage fine motion control.

only when you use UV-light.

- Click on the **Reflected Light** button to open the shutter of the HBO 100 mercury lamp.
- Click on the **Reflector** button and select the desired filter set by clicking on it.

Microscope settings can be stored and up to 8 buttons assigned for fast retrieval and adjustment using the **Microscope Settings** panel.

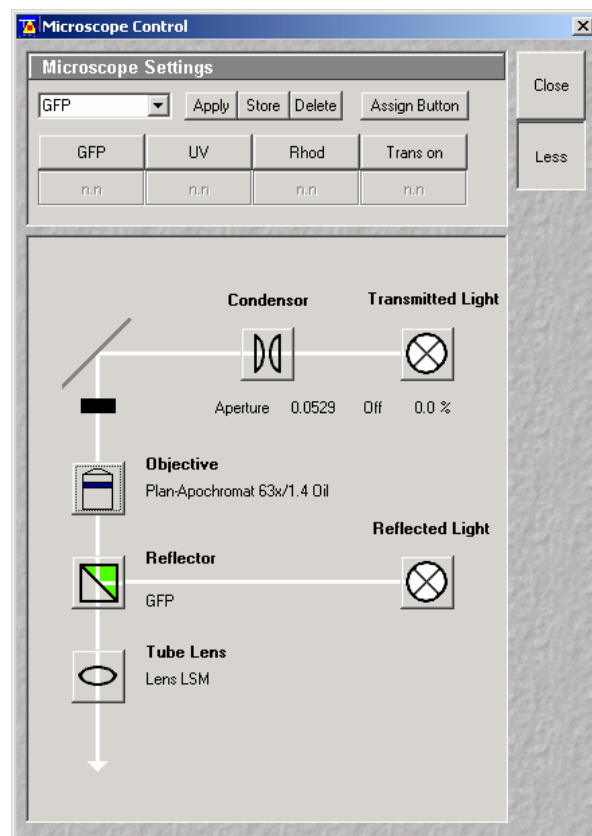
The **Store** button permits existing microscope configurations to be stored under any name.

The **Apply** button permits existing stored microscope configurations to be loaded.

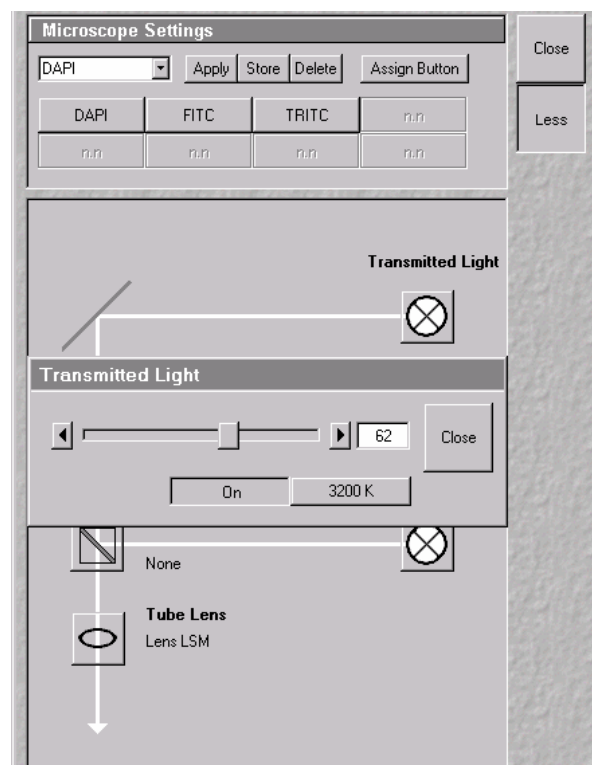
The **Delete** button permits existing microscope configurations to be deleted.

The **Assign** button permits the assignment of a microscope configuration to a button.

**Note:** Depending on the microscope configuration, settings must be done manually if necessary.



**Fig. 10** Microscope Control window, e.g.: Axio Observer



**Fig. 11** Microscope Control window with Transmitted Light pop-up menu

## Configuring the beam path and lasers

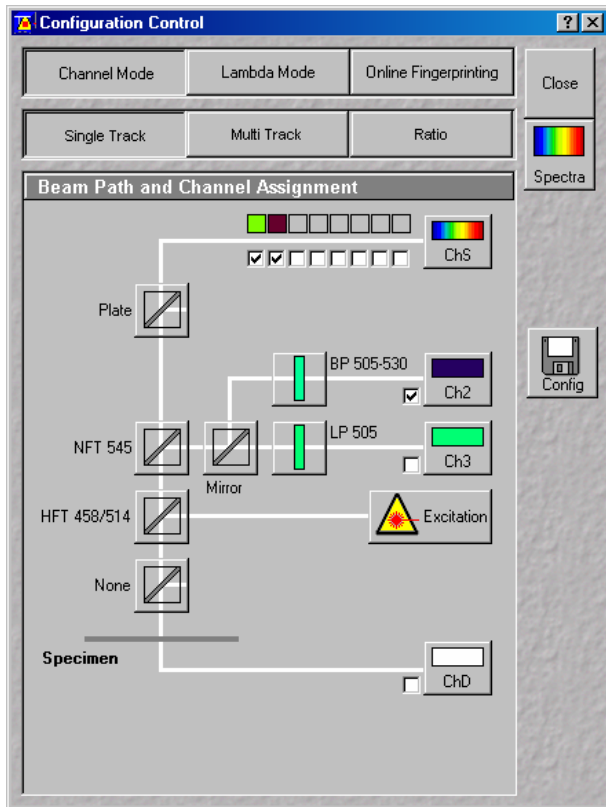


Fig. 12 Configuration Control window for Single Track

- Click on the **LSM** button in the **Acquire** subordinate toolbar for laser scanning.

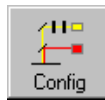
### Choosing the configuration

#### Single Track

- Use for **single**, double and triple labeling; simultaneous scanning only
- Advantage: faster image acquisition
- Disadvantage: potential cross talk between channels

#### Multi Track

- Use for double and triple labeling; sequential scanning, line by line or frame by frame
- Advantage: when one track is active, only one detector and one laser is switched on. This reduces cross talk.
- Disadvantage: slower image acquisition



- Click on the **Config** button in the **Acquire** subordinate toolbar to open the **Configuration Control** window (Fig. 12).

## Setting for single track configuration in Channel Mode

- Select **Channel Mode** (Fig. 12).
- Click on the **Single Track** button in the **Configuration Control** window (Fig. 12).
- Click on the **Descanned** button if available.

The **Beam Path and Channel Assignment** panel of the **Configuration Control** window displays the selected track configuration which is used for the scan procedure.

- You can change the settings of this panel using the following function elements:



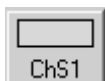
Activation / deactivation of the excitation wavelengths (check box) and setting of excitation intensities (slider). Open the **Laser Control** window via the **Laser** button.



Selection of the main dichroic beam splitter (HFT) or secondary dichroic beam splitter (NFT) position through selection from the relevant list box.



Selection of an emission filter through selection from the relevant list box.



Activation / deactivation (via check box) of the selected channel (Ch 1-4, monitor diode ChM, META detectors ChS1-8, transmission ChD) for the scanning procedure by assigning an existing color icon or defining a new one.



- Select the appropriate filters and activate the channels.
- Click the **Excitation** button to select the laser lines and set the attenuation values (transmission in %) in the displayed window.

For the configuration of the beam path, please refer to the application-specific configurations depending on the used dyes and markers and the existing instrument configuration.



- Clicking on the **Spectr** button opens the **Detection Spectra & Laser Lines ...** window (Fig. 13) to display the activated laser lines for excitation (colored vertical lines) and channels (colored horizontal bars).



- Clicking on the **Config** button opens the **Track Configurations** window (Fig. 14) to load, store or delete track configurations.
- For storing a new track configuration enter a desired name in the first line of the **Configurations** list box and click an **Store**.
- For loading an existing configuration select it in the list box and click on **Apply**.
- For deleting an existing configuration select it in the list box and click on **Delete**.

### Setting for multi track configuration in Channel Mode

The **Multi Track** function permits several tracks to be defined as one configuration for the scan procedure, to be stored under any name, reloaded or deleted.

The maximum of four tracks with up to 8 channels can be defined simultaneously and then scanned one after the other. Each track is a separate unit and can be configured independently of the other tracks with regard to detection channels, laser power, emission filters and dichroic beam splitters.

- Select **Channel Mode** (Fig. 15).

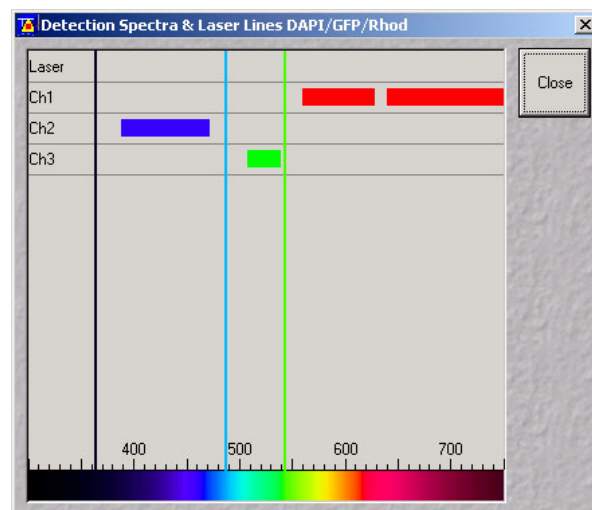


Fig. 13 Detection Spectra & Laser Lines ... window

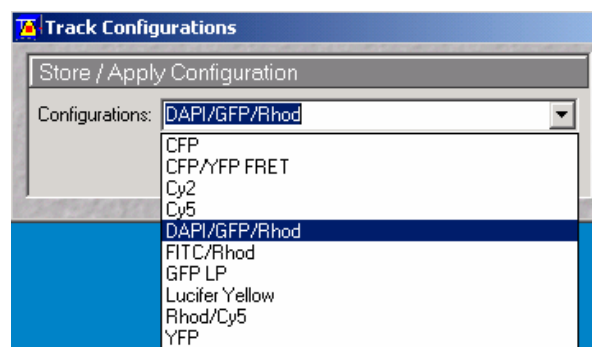


Fig. 14 Track Configurations window

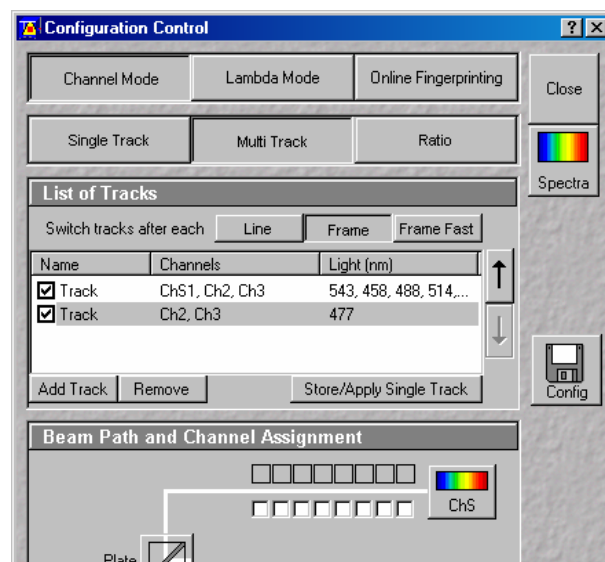


Fig. 15 Configuration Control window for Multi Track



- 
- Click on the **Multi Track** button in the **Configuration Control** window (Fig. 15).
  - Click on the **Descanned** button if available.

The following functions are available in the **List of Tracks** panel (Fig. 15).

#### Modes

Switch tracks after each

##### **Line** button

Tracks are switched during scanning line by line. The following settings can be changed between tracks: Laser line and intensity and channels incl. settings for gain and offset.

Switch tracks after each

##### **Frame** button

Tracks are switched during scanning frame by frame. The following settings can be changed between tracks: Laser line and intensity, all filters and beam splitters, the channels incl. settings for gain and offset and the pinhole position and diameter.

##### **Frame Fast** button

The scanning procedure can be made faster. Only the laser line and intensity and the **Amplifier Offset** are switched, but no other hardware components. The tracks are all matched to the first track with regard to emission filters, dichroic beam splitters, setting of Detector Gain, pinhole position and diameter. When **Line** button is selected, the same rules apply as for **Frame Fast**.

#### Settings

##### **Add Track** button

An additional track is added to the configuration list. A maximum of four tracks can be used. One track with a default configuration is added, i.e.: Ch 1 channel is activated, all laser lines are switched off, emission filters and dichroic beam splitters are set in accordance with the configuration last used.

##### **Remove** button

The single track previously marked in the **List of Tracks** panel in the Name column is deleted.

##### **Store/Apply Single Track** button

Opens the **Track Configurations** window. A selected track defined in a Channel Mode Configuration can also be stored as a single track for single tracking applications. Also, it's possible to load a single track into a multi tracking configuration.



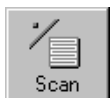
A click on this arrow button will move the selected track (highlighted in blue) one position upwards in the list box.



A click on this arrow button will move the selected track (highlighted in blue) one position downwards in the list box.

- Configure each desired track for **Multi Track** function as described for **Single Track**.
  - For storing/applying or deleting a **Channel Mode Configuration** use the **Config** button.
-

## Scanning an image



- Click on the **Scan** button in the **Acquire** subordinate toolbar to open the **Scan Control** window (Fig. 16).

### Setting the parameters for scanning

- Select **Mode** in the **Scan Control** window.
- Select the **Frame Size** as predefined number of pixels or enter your own values (e.g. 300 x 600) in the **Objective Lens, Image Size & Line Step Factor** panel. Click on the **Optimal** button for calculation of appropriate number of pixels depending on N.A. and  $\lambda$ .

The number of pixels influences the image resolution!

**Note:** When using an Axioskop 2 FS MOT, indicate the objective that is in use in the **Scan Control** window. This ensures correct calculation of pinhole size, frame size, speed, and Z stack parameters.

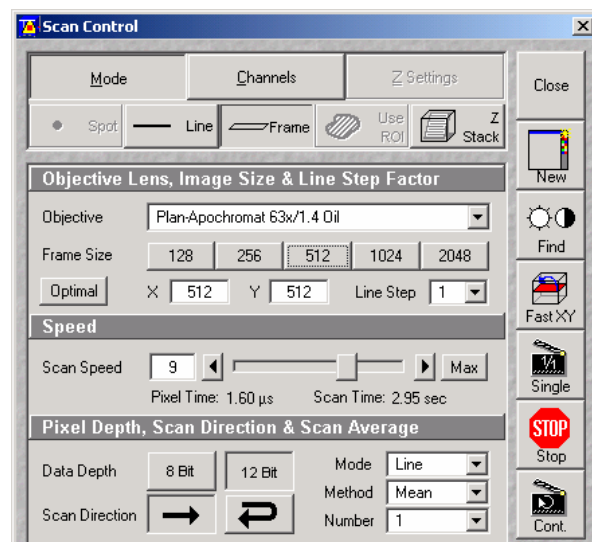


Fig. 16 Scan Control window, Mode settings

### Adjusting the scan speed

- Use the slider in the **Speed** panel (Fig. 16) to adjust the scan speed.

A higher speed with averaging results in the best signal to noise ratio. Scan speed 8 usually produces good results. Use speed 6 or 7 for superior images.

### Choosing the dynamic range

- Select the dynamic range 8 or 12 Bit (per pixel) in the **Pixel Depth, Scan Direction & Scan Average** panel (Fig. 16).

8 Bit corresponds to 256 gray levels; 12 Bit to 4096 levels. Publication quality images should be acquired using 12 Bit data depth. 12 Bit is also recommended when doing quantitative measurements or when imaging low fluorescence intensities.

### Setting the scan averaging

Averaging improves the image by increasing the signal : noise ratio. It can be achieved line by line or frame by frame. Frame averaging helps reduce photobleaching, but should not be applied for moving samples.

- Select the **Line** or **Frame** mode for averaging.
- Select the desired scan average method **Mean** or **Sum** in the **Method** selection box.

If you are using the **Mean** method, the image information is generated by adding up all scans pixel by pixel and then calculating the mean value.

In the **Sum** method, the pixel values of all scans are only added up, without a mean value being calculated.

- Select the Number for averaging.

**Continuous** averaging is possible in the **Frame** mode. In this case a **Finish** button for ending continuous averaging is displayed instead of the **Cont.** button.

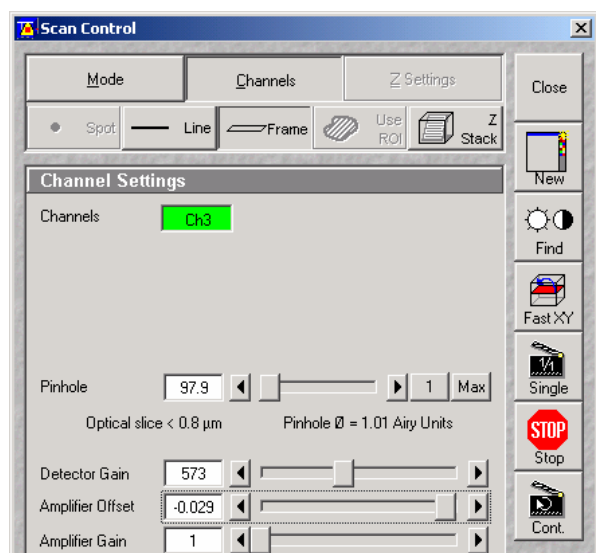


Fig. 17 Scan Control window, Channel settings

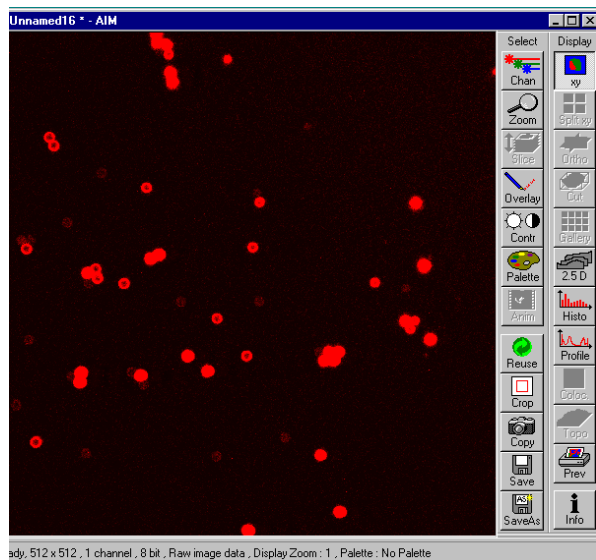


Fig. 18 Image window

## Adjusting the pinhole

- Select **Channels** in the **Scan Control** window.
- Set the **Pinhole** size to **1** (Airy unit) for best compromise between depth discrimination and efficiency.

Pinhole adjustment changes the **Optical Slice**. When collecting multi channel images, adjust the pinholes such that each channel has the same **Optical Size**. This is important for colocalization studies.

## Image acquisition

Once you have set up your parameter as defined in the above section, you can acquire a frame image of your specimen.

- Use one of the **Find**, **Fast XY**, **Single** or **Cont.** buttons to start the scanning procedure and acquire an image.

Scanned images are shown in separate windows.

- Click on the **Stop** button to stop the current scan procedure if wanted.



Select **Find** to automatically pre-adjust of detector sensitivity.



Select **Fast** for continuous and fast scanning – useful to find and change the focus.



Select **Single** to record a single image.



Select **Cont.** to continuously scan with the preset scan speed.



Select **Stop** to stop the current scan procedure.

## Image optimization

### Choosing a lookup table

- Select **Palette** in the **Image** window of the scanned image (Fig. 19).

The **Color Palette** window appears.

- In the **Color Palette List** panel, click on the **Range Indicator** item (Fig. 20).

The scanned image appears in a false-color presentation (Fig. 19).

If the image is too bright, it appears red on the screen. Red = saturation (maximum grey value).

If the image is not bright enough, it appears blue on the screen. Blue = zero (minimum grey value).

### Adjusting the laser intensity

- Set the **Pinhole** to **1** Airy Unit (Fig. 21).
- Set the **Detector Gain** high.
- When the image is saturated, decrease laser power in the **Excitation** panel (Fig. 21) using the slider.

### Adjusting gain and offset

- Increase the **Amplifier Offset** until all blue pixels disappear, and then make it slightly positive (Fig. 21).
- Reduce the **Detector Gain** until the red pixels only just disappear.

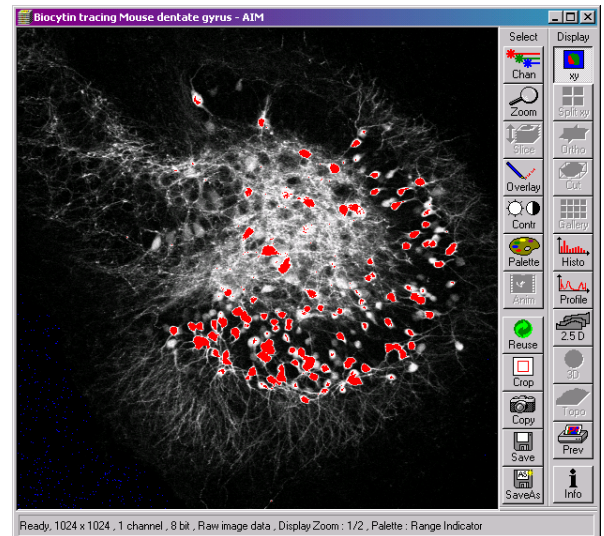


Fig. 19 Image window

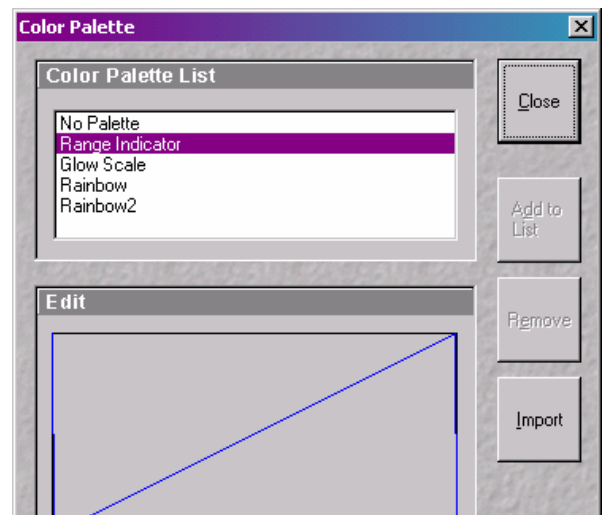


Fig. 20 Color Palette window

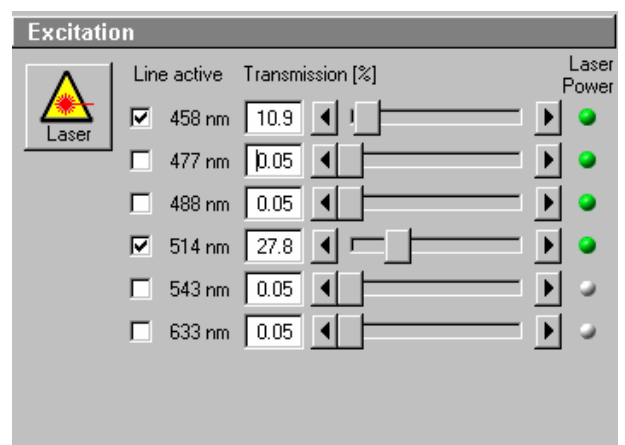


Fig. 21 Scan Control window, Channel settings

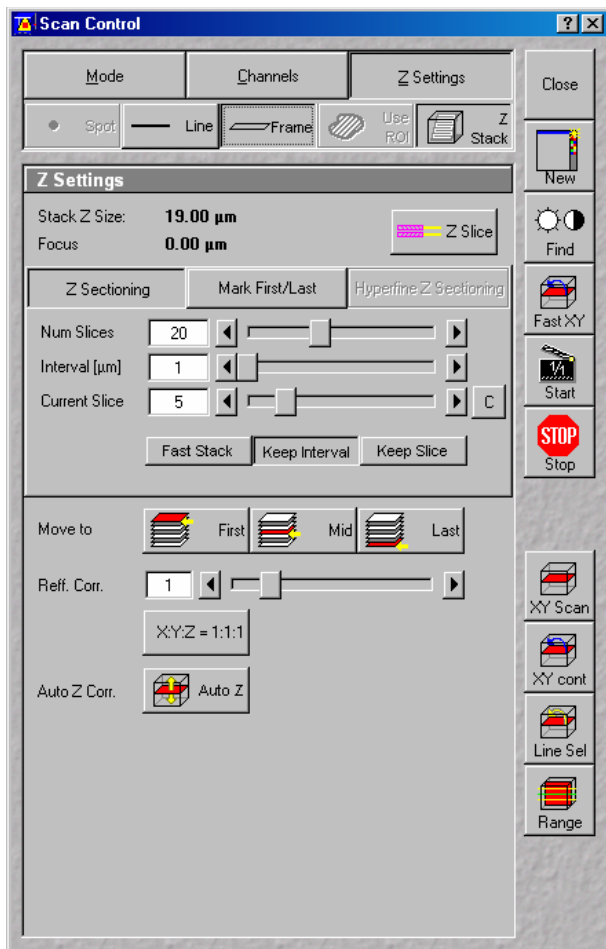


Fig. 22 Scan Control window, Z Stack settings

## Scanning a Z stack

- Select **Z Stack** in the **Scan Control** window.
- Select **Frame**
- Select **Mark First/Last** on the **Z Settings** panel.
- Click on the **XY cont** button.

A continuous XY-scan of the set focus position will be performed.

- Use the focusing drive of the microscope to focus on the upper position of the specimen area where the Z Stack is to start.
- Click on the **Mark First** button to set the upper position of the Z Stack.
- Then focus on the lower specimen area where the recording of the Z Stack is to end.
- Click on the **Mark Last** button to set this lower position.
- Click on **X:Y:Z=1:1:1** button to set the Z-interval in such a way that the voxel has identical dimensions in the X-, Y- and Z-directions (cube).
- Click on the **Start** button to start the recording of the Z Stack.

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## Storing an image

- Click on the **Save** or **Save As** button in the **Image** window or in the **File** subordinate toolbar of the **Main** menu.

The **Save Image and Parameter As** window appears.

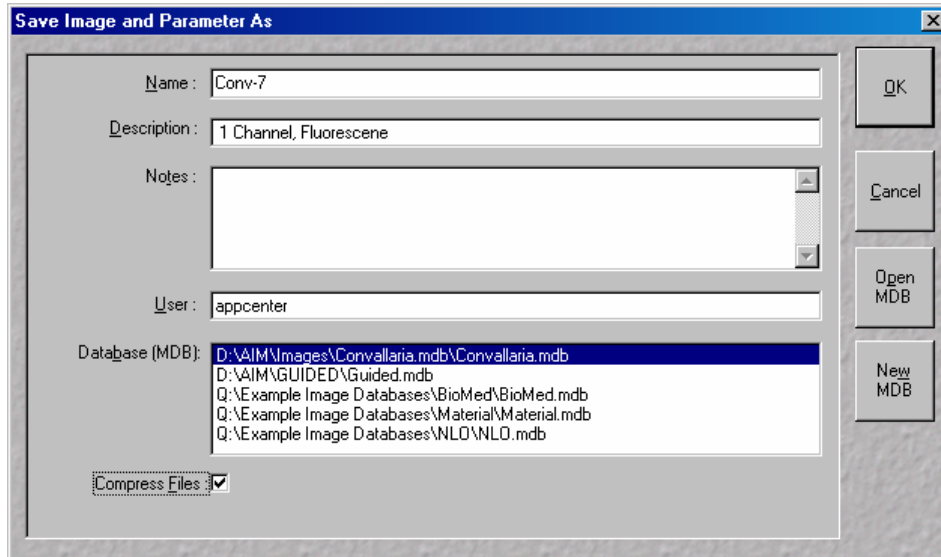


Fig. 23 Save Image and Parameter As window

- Enter file name, description and notes in the appropriate text boxes.
- Click on the **OK** button.

## Switching off the system

- Switch off the laser. If Arg is used, set at Stdb by if another user is arriving in the next 2 hrs, otherwise WAITE for ~5mins to let cooling fan turnoff (no fan noise).
  - Exit Zeiss software.
  - Shut down Windows/computer.
  - Switch OFF Remote Control.
  - [Switch off mercury Arc lamp if use.]
  - Clean lens and table.
  - Sign on the Logbook.
-