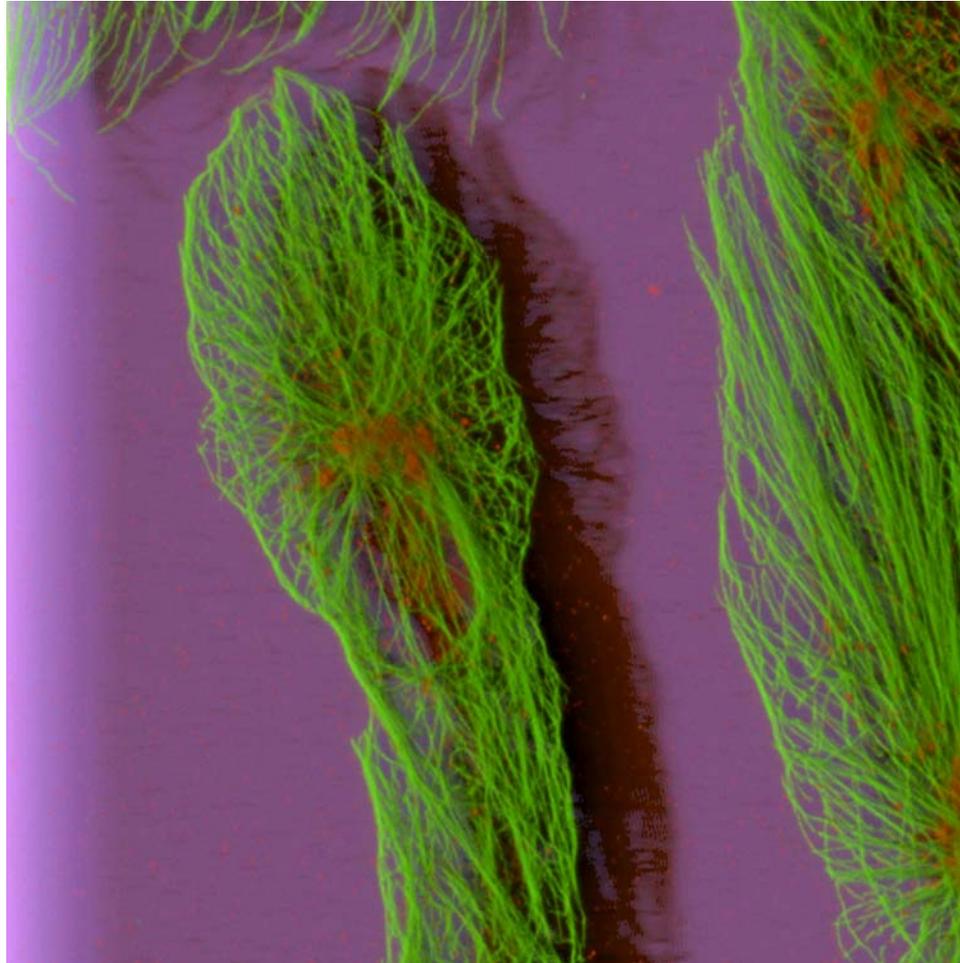


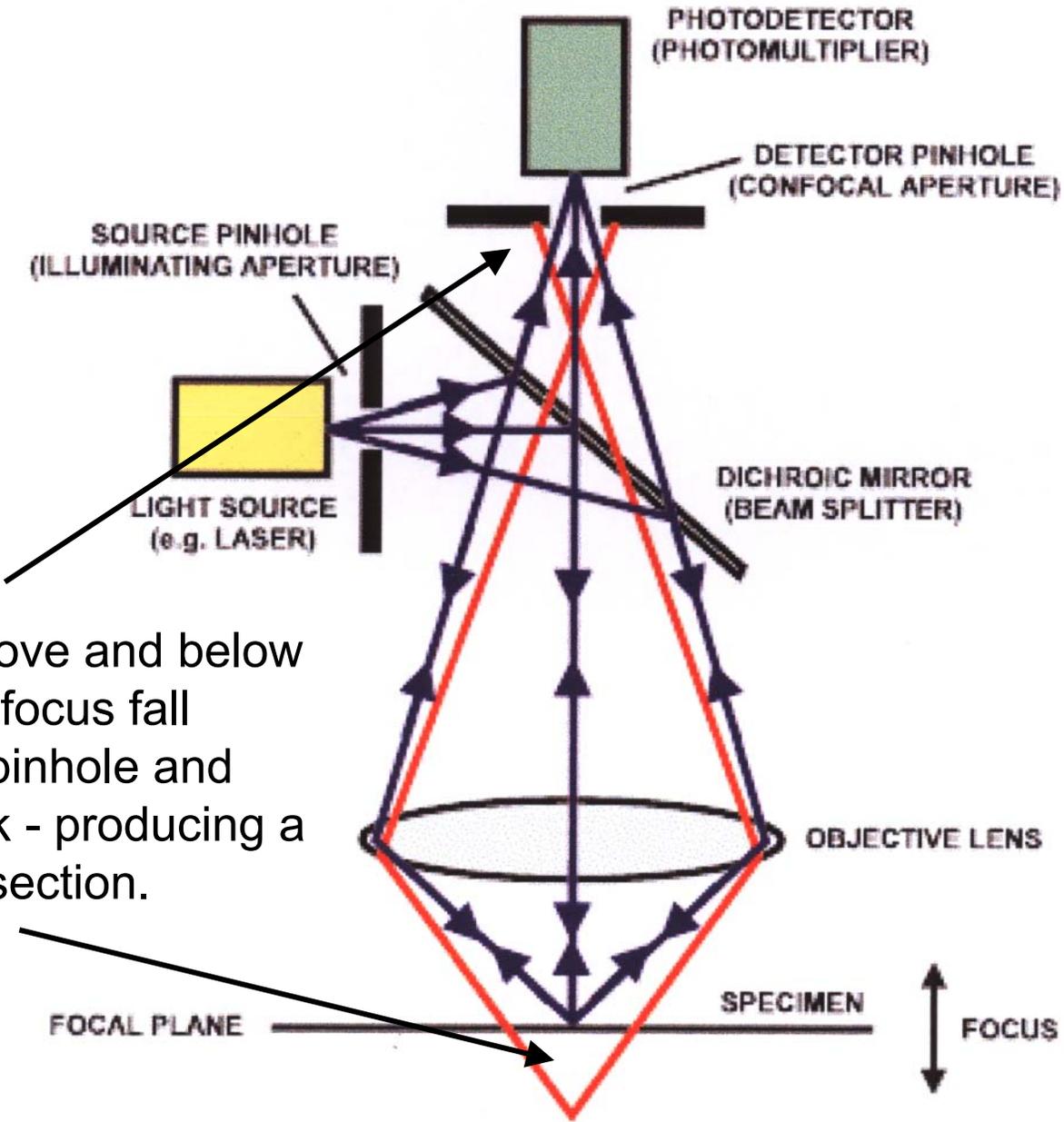
Confocal microscopy

Zeiss LSM 510 and Zeiss LSM 510 META

Visualisation of biological structures in 3D



Confocal Microscope

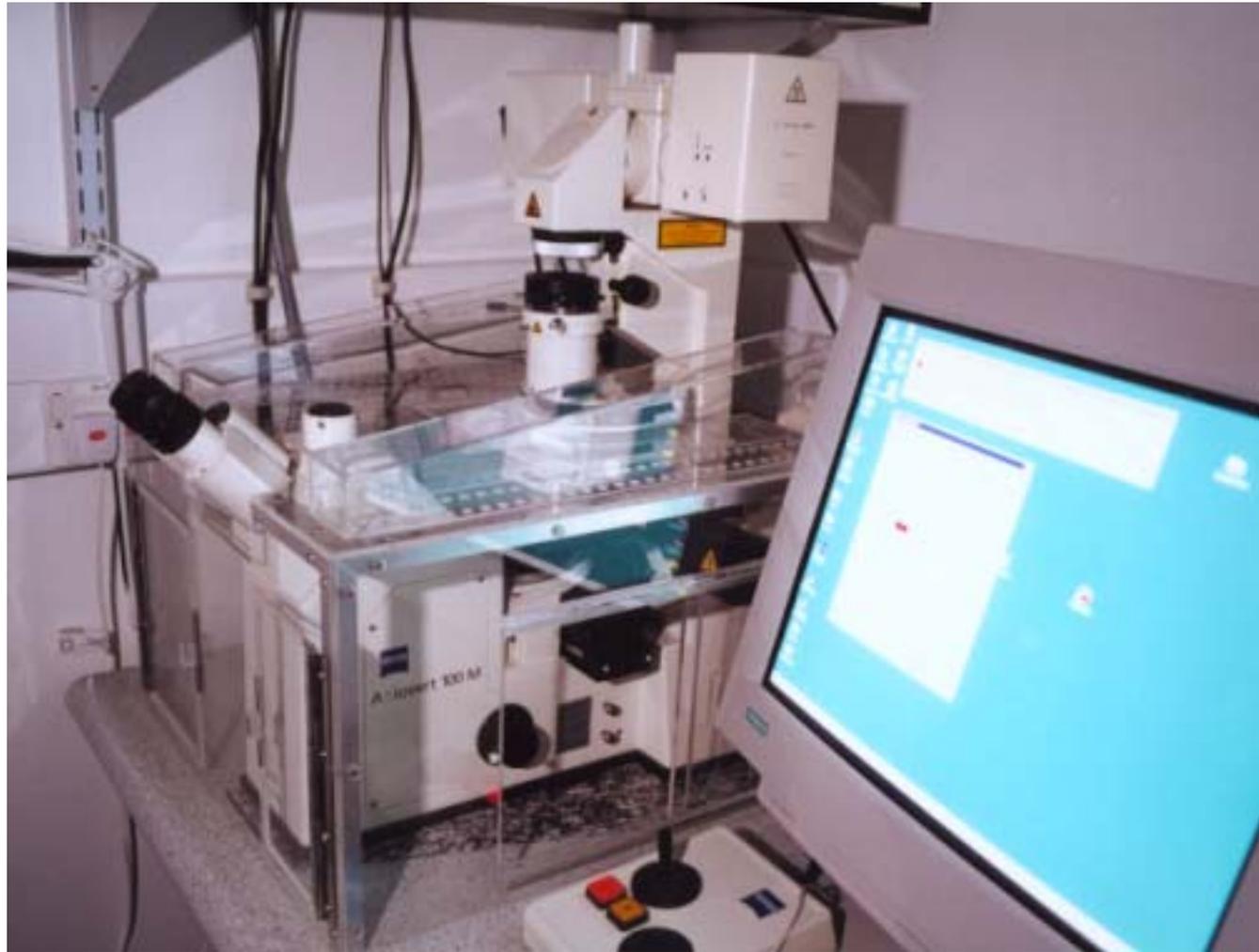


Features above and below the plane of focus fall outside the pinhole and appear black - producing a true optical section.

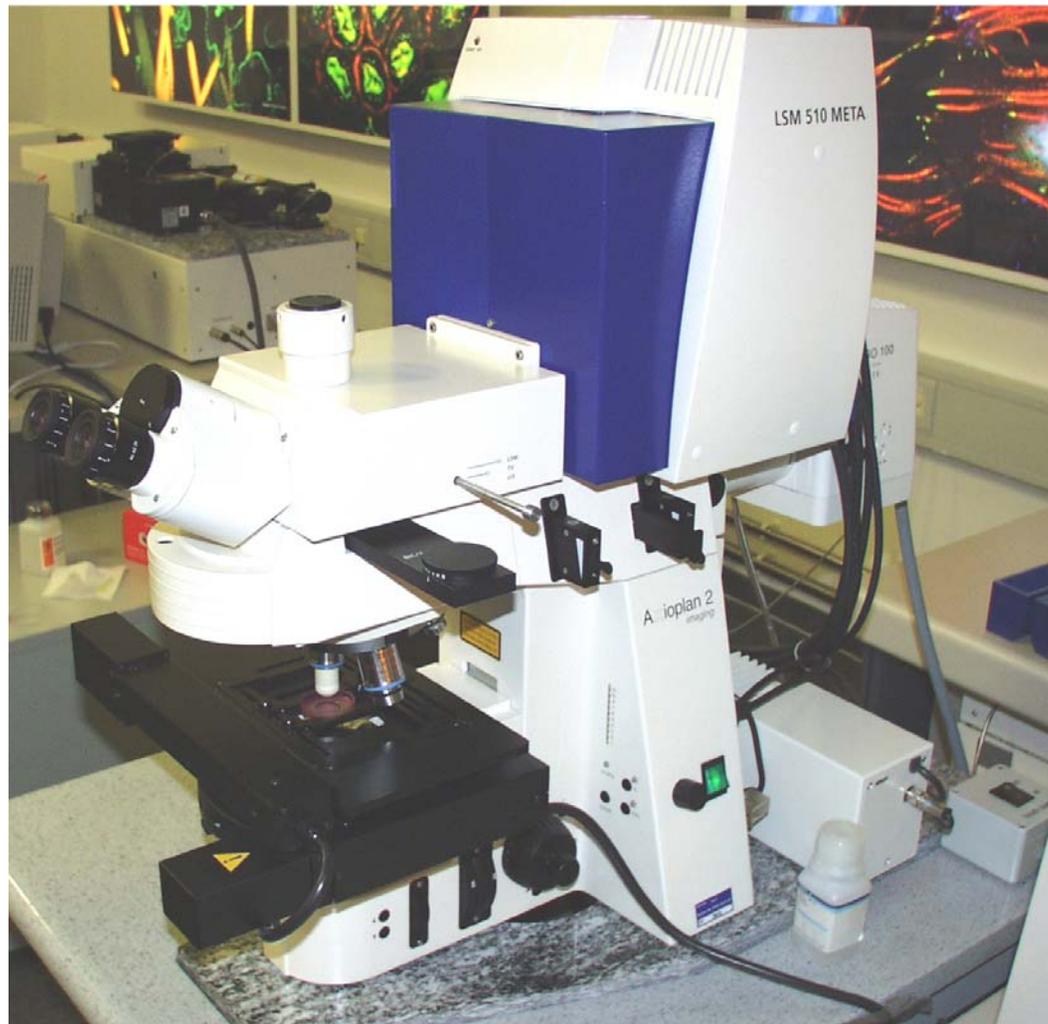
Upright Zeiss LSM 510 confocal microscope



Inverted Zeiss LSM 510 confocal microscope



Upright Zeiss LSM 510 META confocal microscope



Contents

- Starting the Zeiss LSM 510 microscope, software and laser Selecting an objective and focusing the microscope
- Configuring the laser scanning and detection for confocal image acquisition
- Acquiring a Z- and Time - Series
- Data storage

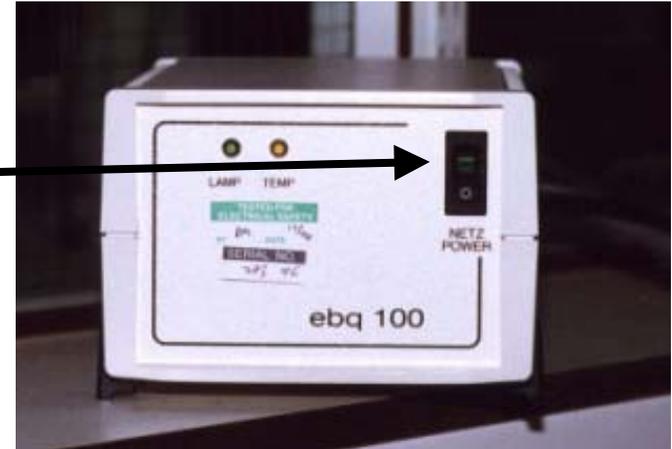
Descriptions also include the LSM 510 META

Contents

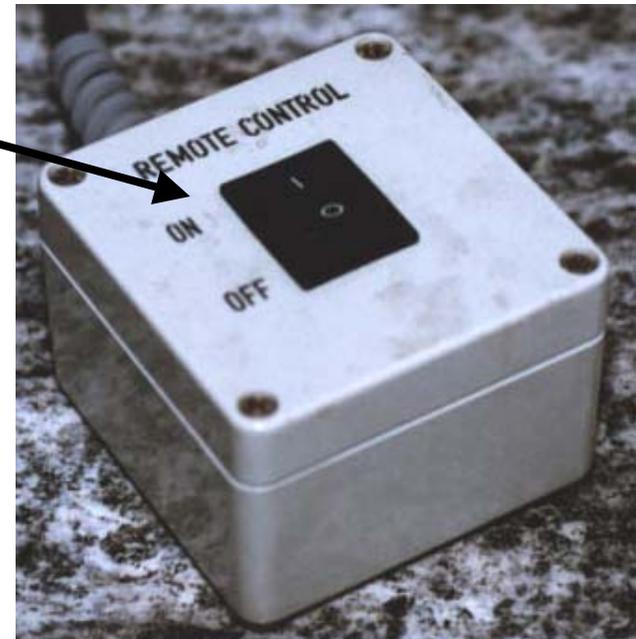
- Starting the Zeiss LSM 510 microscope, software and laser
- Selecting an objective and focusing the microscope
- Configuring the laser scanning and detection for confocal image acquisition
- Acquiring a Z- and Time - Series
- Data storage

Start the Zeiss LSM 510 Confocal Microscope

1) First switch on the mercury lamp

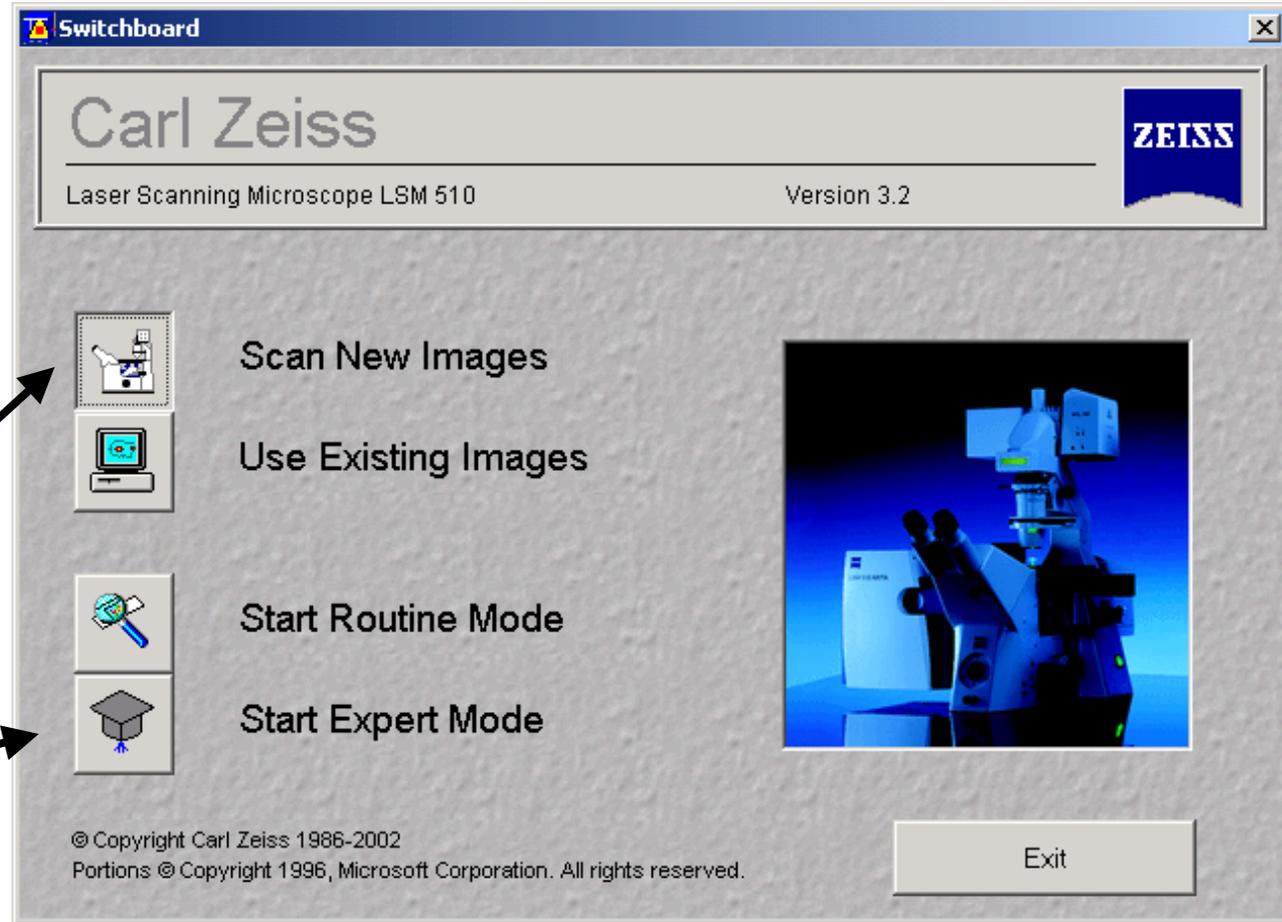


2) Turn on the remote control switch



3) Wait for the computer to boot up and Login by simultaneously pressing the Ctrl, Alt and Delete keys

Starting the LSM 510 software

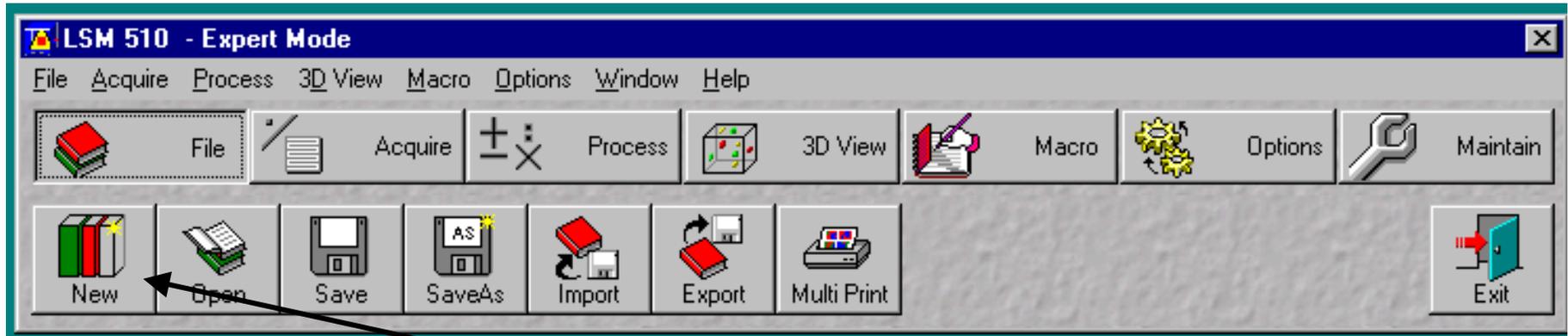


1) Double click
the LSM 510
icon

2) Select "Scan
New Images"

3) Select "Start
Expert Mode"

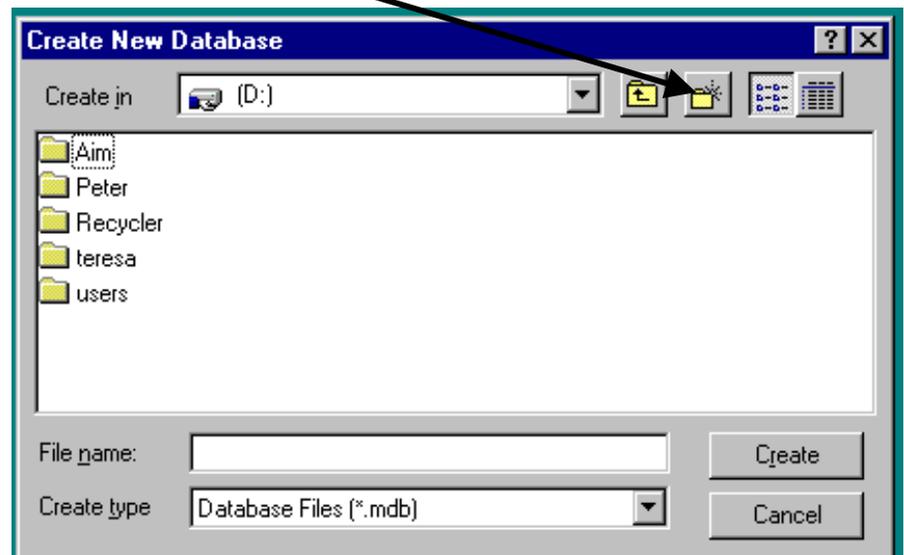
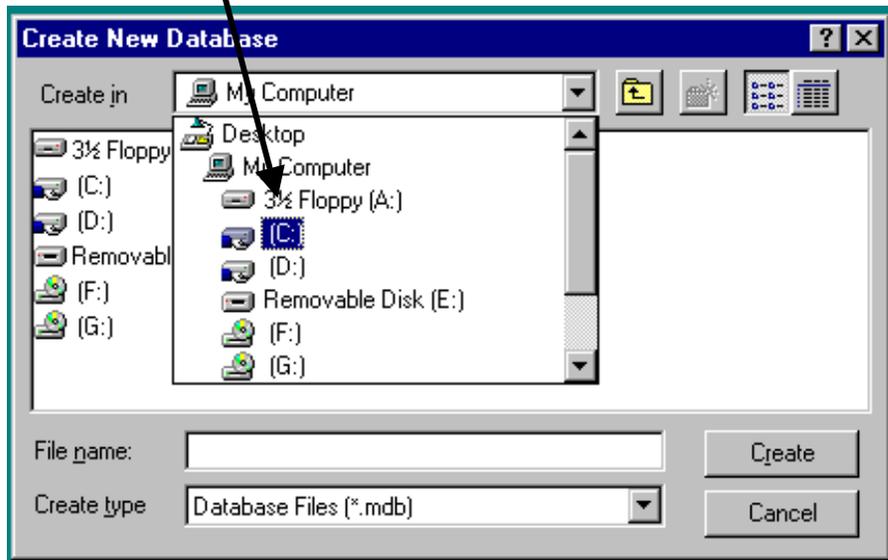
Creating a database for acquired images



1) In the main menu *File* select *New* database

2) Select drive C or D: from pull down menu

3) Create a new directory for each session



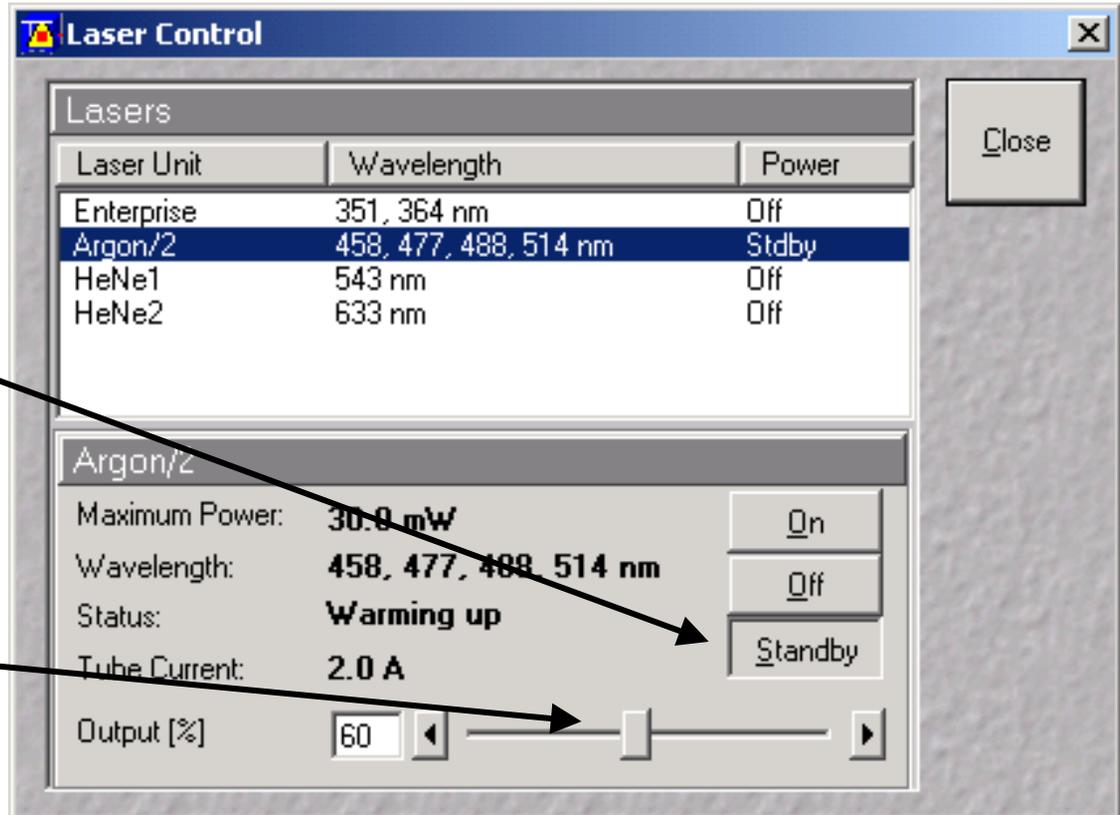
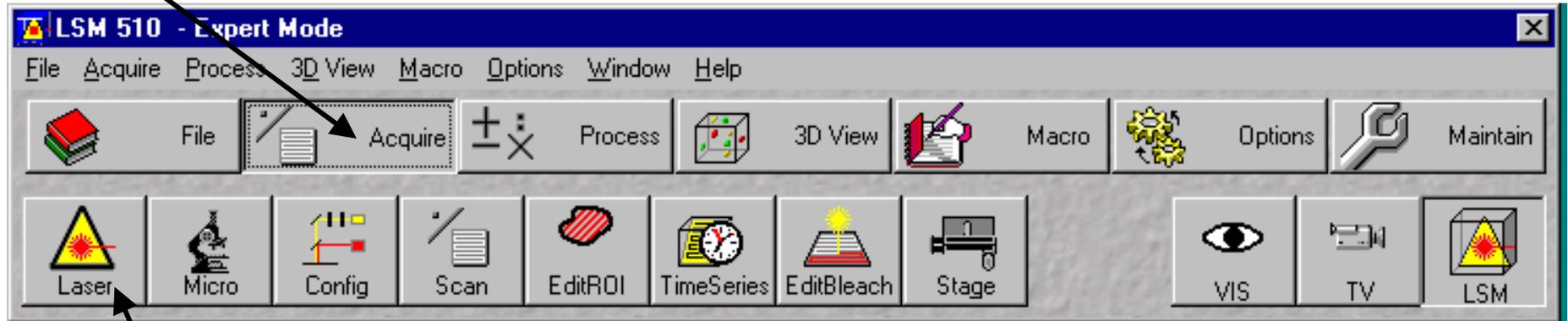
Turning on the lasers

1) Select Acquire

2) Select *Laser*

3) Switch required laser/s to *Standby* or *On*

Argon power should be set to about 60%



Change between direct observation and laser scanning

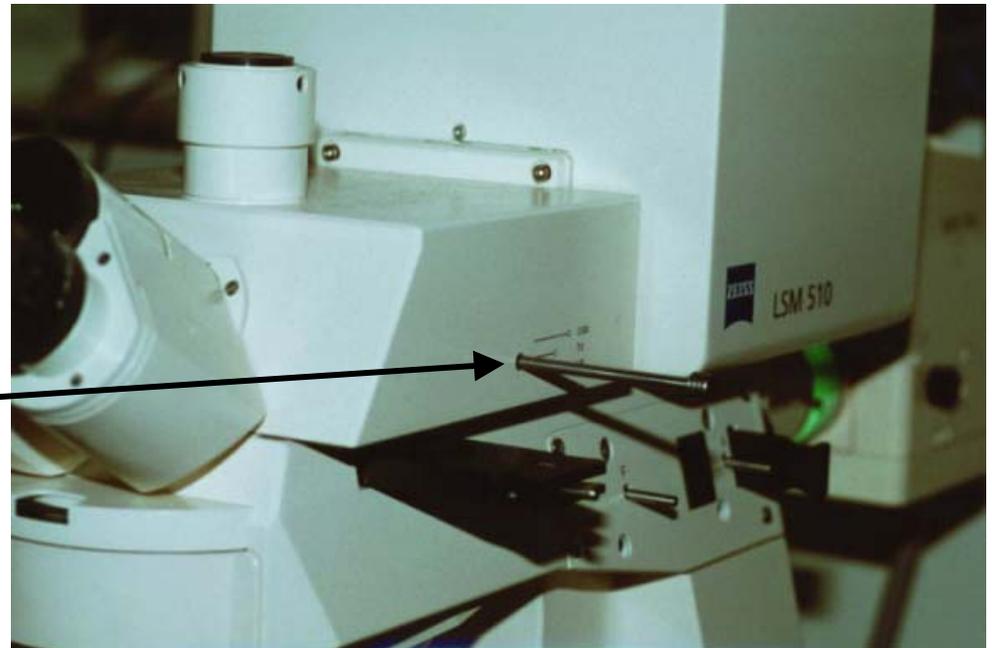
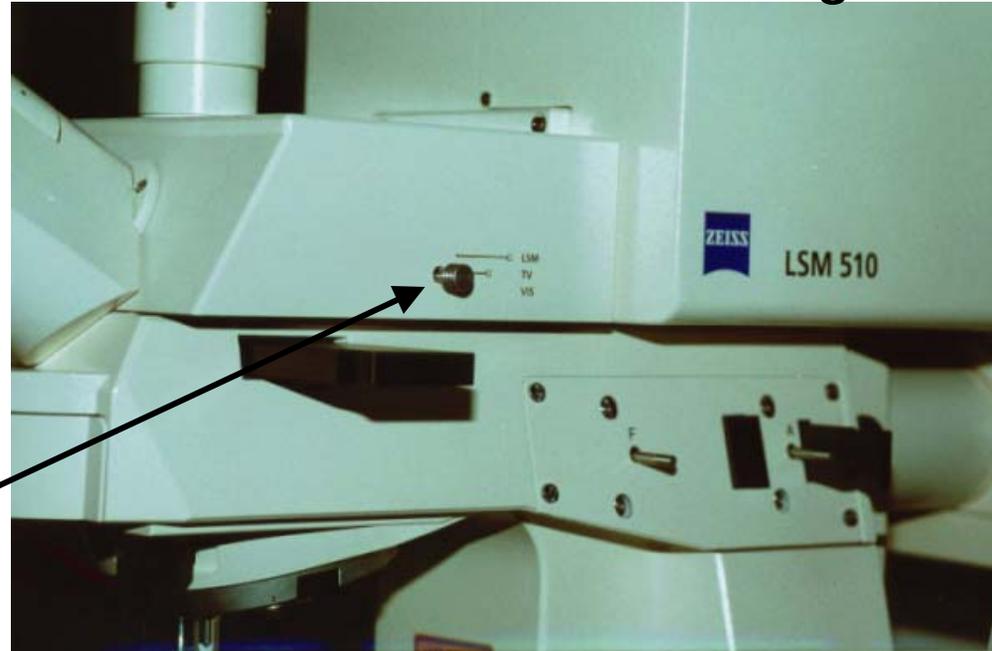
Upright Microscopes:
Axioplan 2 imaging and
Axioskop 2 FS

For direct observation
of transmitted light and
fluorescence:

Set slider to “VIS”
(push it in)

For laser scanning
image acquisition:

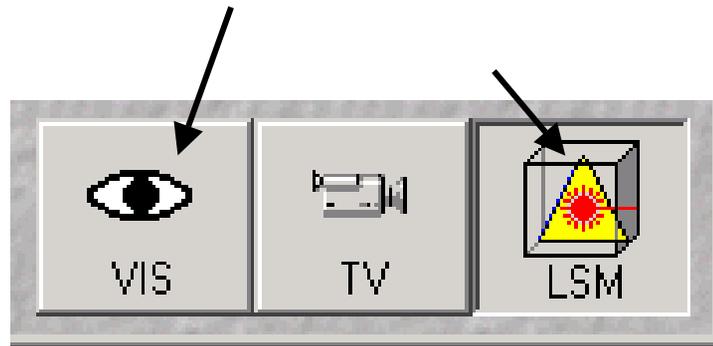
Set slider to “LSM”
(pull slider out)



Change between direct observation and laser scanning

Inverted Microscope: Axiovert 200 M

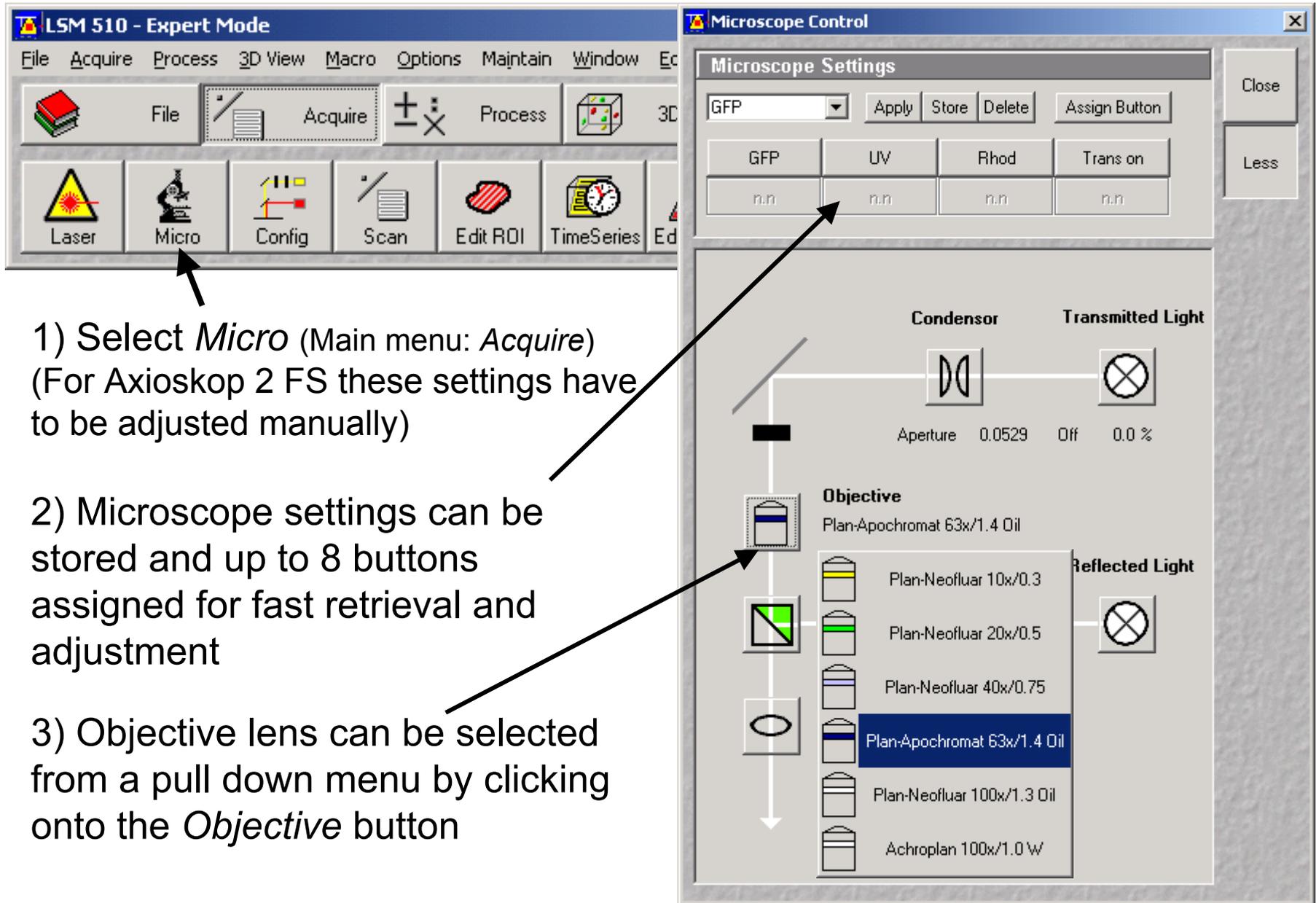
Toggle between Vis and LSM button in main menu, automatic switching between direct observation and laser scanning (no slider)



Contents

- Starting the Zeiss LSM 510 microscope, software and laser
- **Selecting an objective and focusing the microscope**
- Configuring the laser scanning and detection for confocal image acquisition
- Acquiring a Z and Time -Series
- Data storage

Selecting an objective and focusing the microscope



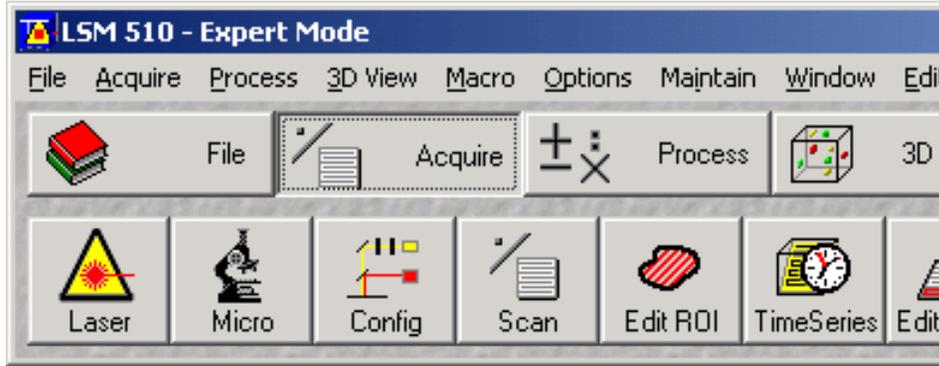
The image shows two windows from the LSM 510 software. The left window, titled 'LSM 510 - Expert Mode', has a menu bar with 'File', 'Acquire', 'Process', '3D View', 'Macro', 'Options', 'Maintain', and 'Window'. Below the menu is a toolbar with icons for 'File', 'Acquire', 'Process', and '3D View'. A second row of icons includes 'Laser', 'Micro', 'Config', 'Scan', 'Edit ROI', and 'TimeSeries'. An arrow points to the 'Micro' icon. The right window, titled 'Microscope Control', contains 'Microscope Settings' with a dropdown menu set to 'GFP' and buttons for 'Apply', 'Store', 'Delete', and 'Assign Button'. Below this is a table of settings for GFP, UV, Rhod, and Trans on. A diagram shows the optical path with 'Condensor', 'Transmitted Light', and 'Reflected Light' sections. An 'Objective' list is open, showing various lenses, with 'Plan-Apochromat 63x/1.4 Oil' selected. Arrows from the text point to the 'Micro' icon and the selected objective.

1) Select *Micro* (Main menu: *Acquire*)
(For Axioskop 2 FS these settings have to be adjusted manually)

2) Microscope settings can be stored and up to 8 buttons assigned for fast retrieval and adjustment

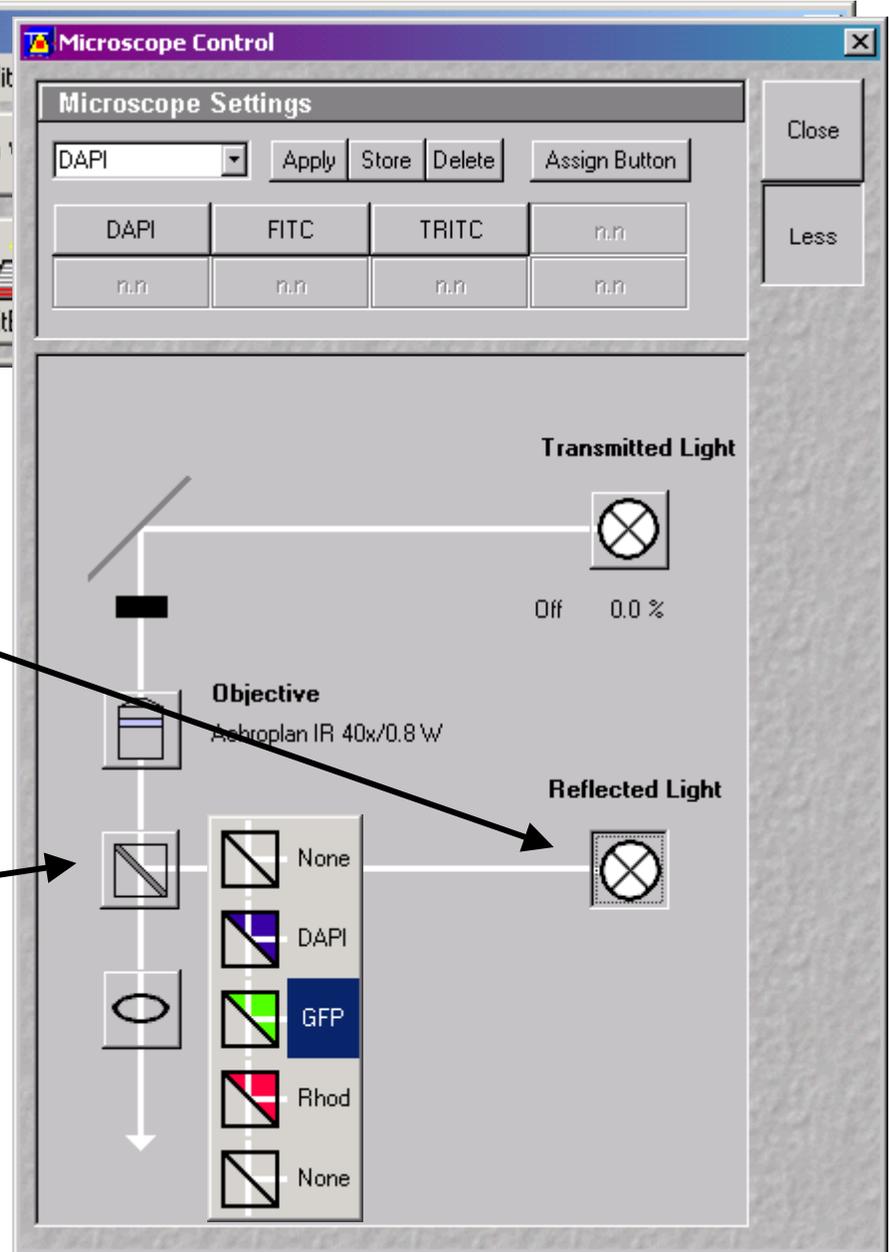
3) Objective lens can be selected from a pull down menu by clicking onto the *Objective* button

Focusing the microscope in fluorescence mode



Click onto *Reflected Light* to open the shutter of the HBO lamp

Fluorescence is observed by selecting the appropriate filter set in the pull down menu of *Reflector*



Focusing the microscope in transmitted mode

The image shows the LSM 510 Expert Mode software interface. The main window has a menu bar (File, Acquire, Process, 3D View, Macro, Options, Maintain, Window) and a toolbar with icons for File, Acquire, Process, Laser, Micro, Config, Scan, Edit ROI, and TimeSeries. A 'Microscope Control' dialog box is open, showing 'Microscope Settings' with a dropdown menu set to 'DAPI' and buttons for 'Apply', 'Store', 'Delete', and 'Assign Button'. Below this is a table of filter settings:

DAPI	FITC	TRITC	n.n
n.n	n.n	n.n	n.n

The 'Transmitted Light' section includes a diagram of a light path with a slider and a 'Close' button. Below the slider, there are buttons for 'On' and '3200 K'. The 'None' option is selected for the reflector cube, and the 'Lens LSM' is selected for the tube lens.

Click onto *Transmitted Light* and move the slider to set the intensity of the HAL illumination

Use no reflector cube in the reflector turret, chose *None*

Contents

- Starting the Zeiss LSM 510 microscope, software and laser
- Selecting an objective and focusing the microscope
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- Data storage

Choosing the configuration

SINGLE TRACK

Use for **single**, double and triple labelling

Simultaneous scanning only

ADVANTAGES

Faster image acquisition

DISADVANTAGES

Cross talk between channels

MULTI TRACK

Use for double or triple labelling

Sequential scanning, line by line or frame by frame

ADVANTAGES

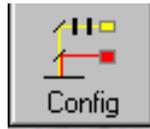
When one track is active, only one detector and one laser is switched on. This dramatically reduces crosstalk.

DISADVANTAGES

Slower image acquisition

Configuration of the filters and storage of the track configurations

SINGLE TRACK - lasers scan simultaneously

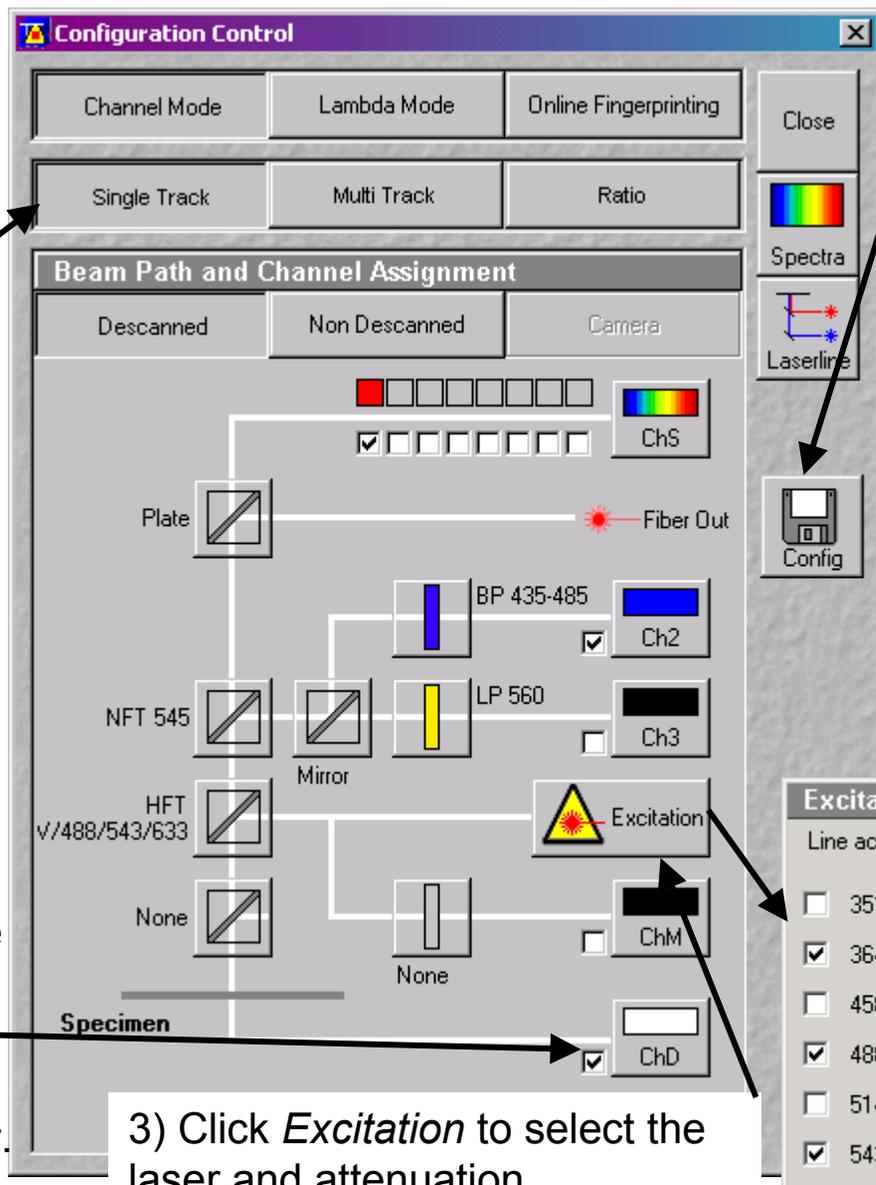


1) Select *Config* in the *Acquire* menu

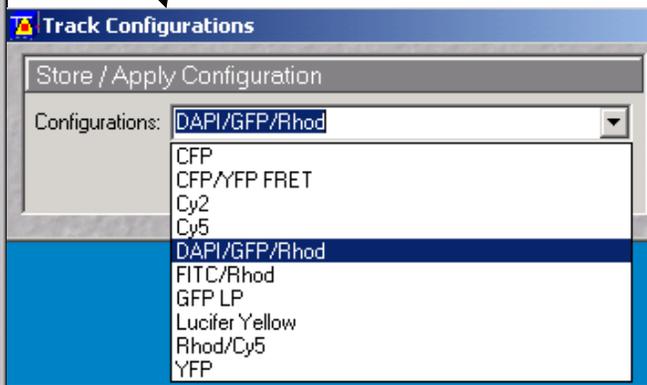
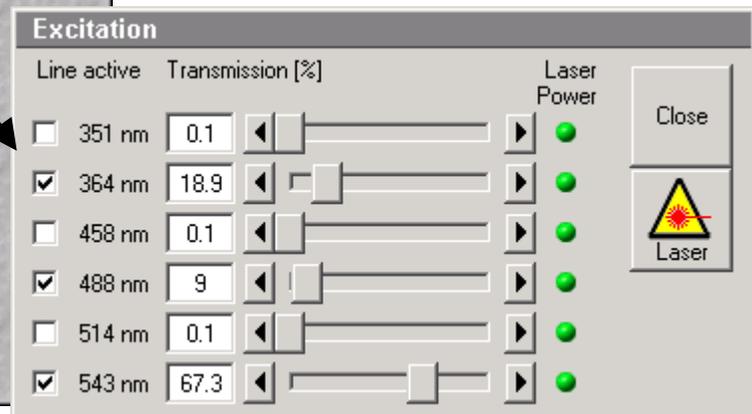
2) Select *Single Track*

3) Select the appropriate filters and activate the Channels

Transmitted light image can also be generated. Transmission channel is usually set to white colour.

The main Configuration Control window. It features a top bar with 'Channel Mode', 'Lambda Mode', and 'Online Fingerprinting'. Below this are buttons for 'Single Track', 'Multi Track', and 'Ratio'. The 'Beam Path and Channel Assignment' section contains a schematic diagram of the optical setup. It shows a 'Specimen' at the bottom, with light passing through a 'None' filter, an 'HFT v/488/543/633' filter, a 'Mirror', an 'NFT 545' filter, a 'BP 435-485' filter, and an 'LP 560' filter. The light then passes through a 'Plate' and is directed to a 'Fiber Out'. There are also 'Excitation' and 'ChM' components. On the right side of the window, there are 'Spectra' and 'Laserline' buttons, and a 'Config' button with a floppy disk icon.

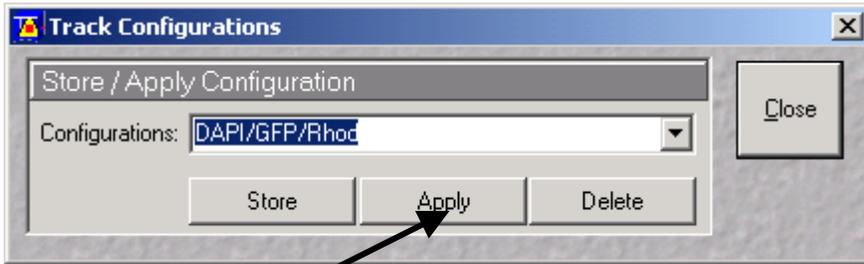
4) The *Config* button opens the pull down menu to load/store Track configurations

The Track Configurations window, which is a pull-down menu. It has a title bar 'Track Configurations' and a section 'Store / Apply Configuration'. A list of configurations is shown, with 'DAPI/GFP/Rhod' selected. Other configurations include 'CFP', 'CFP/YFP FRET', 'Cy2', 'Cy5', 'FITC/Rhod', 'GFP LP', 'Lucifer Yellow', 'Rhod/Cy5', and 'YFP'.The Excitation window, which controls the laser lines. It has a title bar 'Excitation' and a table of laser lines. Each line has a checkbox for 'Line active', a 'Transmission [%]' field, a slider for attenuation, and a 'Laser Power' indicator (a green light). A 'Close' button and a 'Laser' warning icon are also present.

Line active	Transmission [%]	Laser Power
<input type="checkbox"/>	351 nm 0.1	●
<input checked="" type="checkbox"/>	364 nm 18.9	●
<input type="checkbox"/>	458 nm 0.1	●
<input checked="" type="checkbox"/>	488 nm 9	●
<input type="checkbox"/>	514 nm 0.1	●
<input checked="" type="checkbox"/>	543 nm 67.3	●

3) Click *Excitation* to select the laser and attenuation

Applying a stored configuration and checking the settings

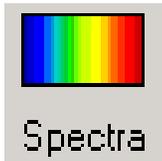


If you select *Store* by mistake, software will ask you, if you want to overwrite the configuration. **ANSWER NO!**

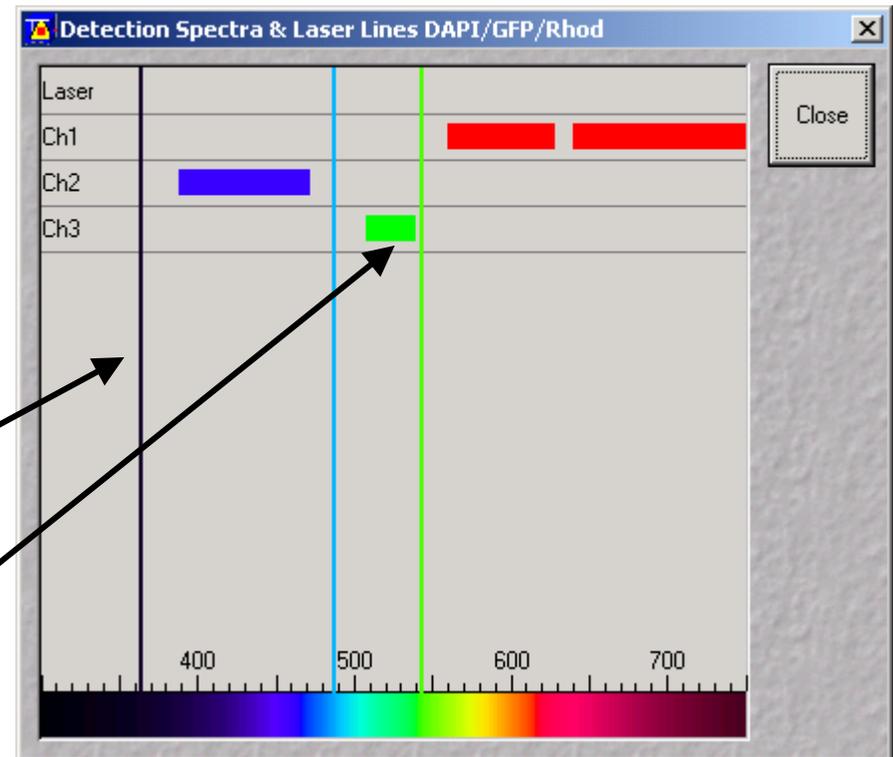
Each new login loads a predefined set of correct configurations.

5) Chose a configuration in the *Track Configuration* menu. Select *Apply*

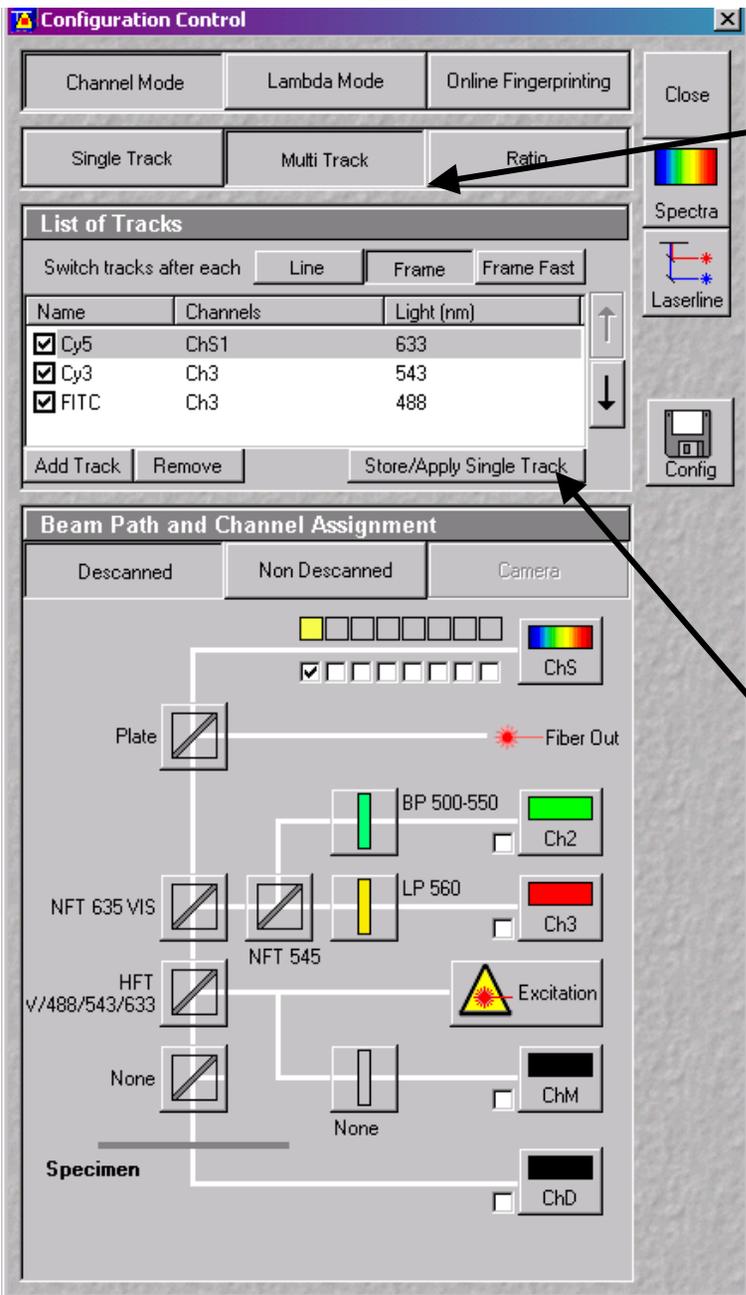
6) To check for correct settings, click the *Spectra* button



The *Spectra* button opens a window to display the activated laser lines for excitation (colored vertical lines) and channels (colored horizontal bars)



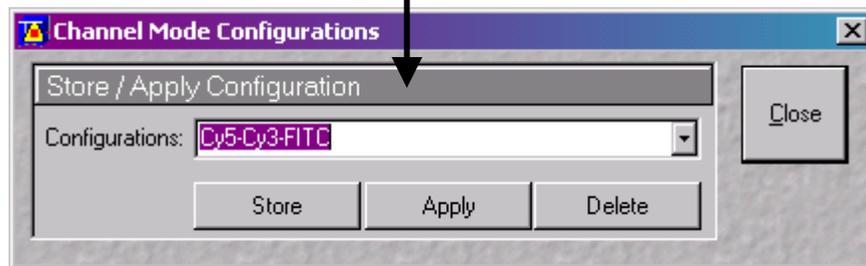
Multi Track Configuration



1) Select *Multi Track* for sequential scanning

2) Select *Config*

3) Select a stored track from the pull down menu, click on *Apply*



This button stores only the highlighted single track or applies a single track.

Cy5-Cy3-FITC Multi Track

Three laser lines and channels activated sequentially

Excitation

Excitation

Line active	Transmission [%]	Laser Power
<input type="checkbox"/> 458 nm	0.1	<input type="checkbox"/>
<input type="checkbox"/> 488 nm	16.9	<input type="checkbox"/>
<input type="checkbox"/> 514 nm	0.1	<input type="checkbox"/>
<input type="checkbox"/> 543 nm	0.1	<input type="checkbox"/>
<input checked="" type="checkbox"/> 633 nm	61.4	<input checked="" type="checkbox"/>
<input type="checkbox"/> 880 nm	0.1	<input type="checkbox"/>

Close 

Excitation

Line active	Transmission [%]	Laser Power
<input type="checkbox"/> 458 nm	0.1	<input type="checkbox"/>
<input type="checkbox"/> 488 nm	0.1	<input type="checkbox"/>
<input type="checkbox"/> 514 nm	0.1	<input type="checkbox"/>
<input checked="" type="checkbox"/> 543 nm	43.6	<input checked="" type="checkbox"/>
<input type="checkbox"/> 633 nm	0.1	<input type="checkbox"/>
<input type="checkbox"/> 880 nm	0.1	<input type="checkbox"/>

Close 

Excitation

Line active	Transmission [%]	Laser Power
<input type="checkbox"/> 458 nm	0.1	<input type="checkbox"/>
<input checked="" type="checkbox"/> 488 nm	3	<input checked="" type="checkbox"/>
<input type="checkbox"/> 514 nm	0.1	<input type="checkbox"/>
<input type="checkbox"/> 543 nm	0.1	<input type="checkbox"/>
<input type="checkbox"/> 633 nm	0.1	<input type="checkbox"/>
<input type="checkbox"/> 880 nm	0.1	<input type="checkbox"/>

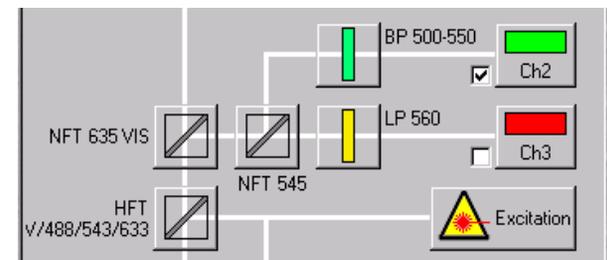
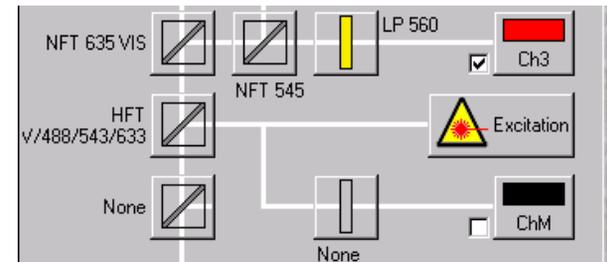
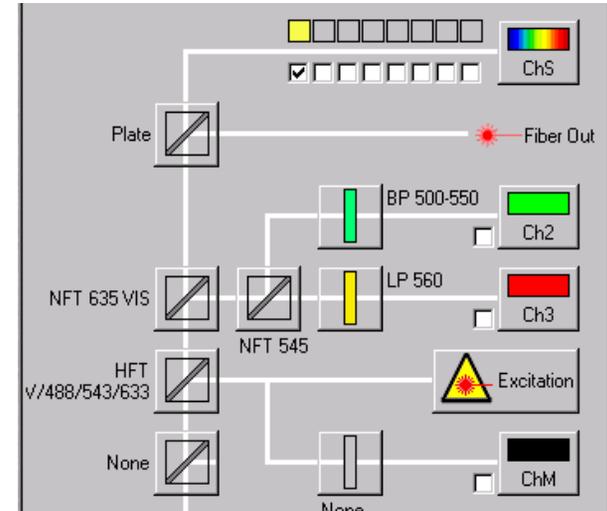
Close 

633 nm, using
the META
detector in
Channel mode

543 nm

488 nm

Detection



Setting the parameters for scanning

1) Select *Scan*

2) Select *Mode*

3) Select the *Frame Size* as predefined number of pixels or enter your own values (e.g 300 x 600). Use *Optimal* for calculation of appropriate number of pixels depending on N.A. and λ .

The number of pixels influences the image resolution!

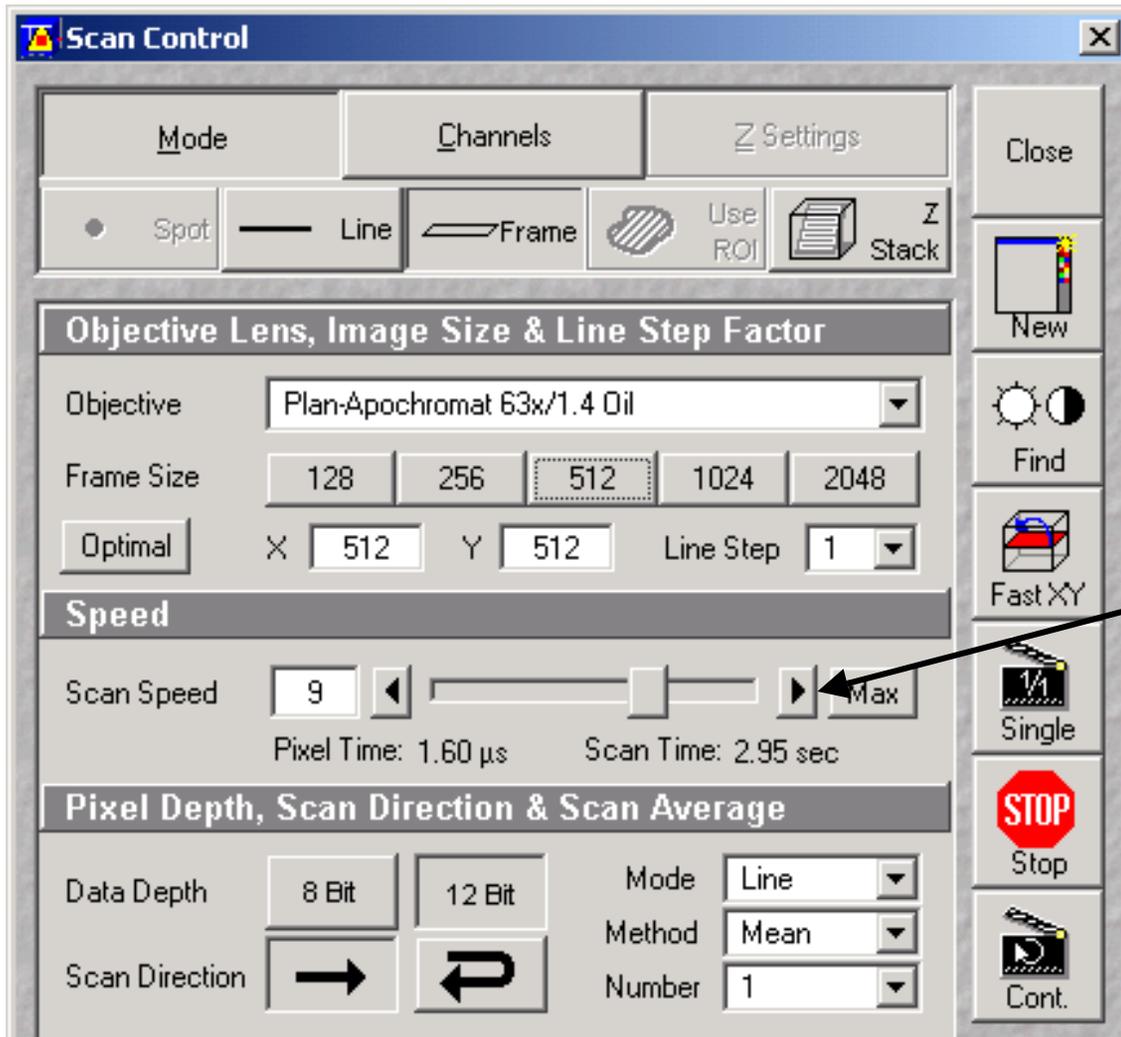
Setting the parameters for scanning

The screenshot shows the LSM 510 Expert Mode software interface. The main window is titled "LSM 510 - Expert Mode" and has a menu bar with options: File, Acquire, Process, 3D View, Macro, Options, Maintain, Window, Edit UI, Help. Below the menu bar is a toolbar with icons for File, Acquire, Laser, Micro, Config, and Scan. The Scan Control window is open, showing the following parameters:

- Mode: Spot (selected), Line, Frame
- Channels: Use ROI, Z Stack
- Objective Lens, Image Size & Line Step Factor:
 - Objective: Plan-Apochromat 63x/1.4 Oil
 - Frame Size: 128, 256, 512 (selected), 1024, 2048
 - Optimal: X 512, Y 512, Line Step 1
- Speed:
 - Scan Speed: 9 (selected), Max
 - Pixel Time: 1.60 μ s, Scan Time: 2.95 sec
- Pixel Depth, Scan Direction & Scan Average:
 - Data Depth: 8 Bit, 12 Bit
 - Scan Direction: \rightarrow , \curvearrowright
 - Mode: Line
 - Method: Mean
 - Number: 1

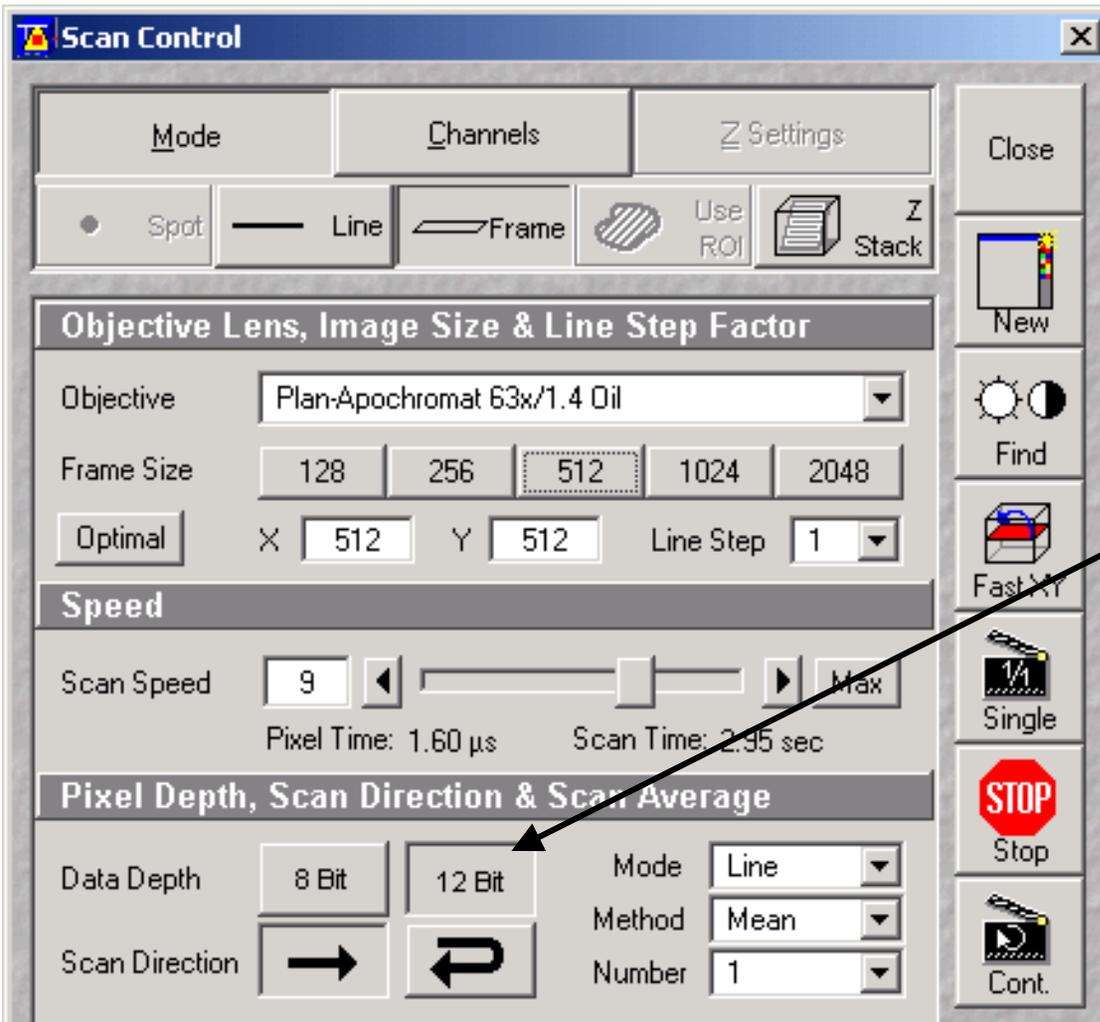
A note on the left side of the image states: "Note: When using a Axioskop 2 FS, indicate the Objective that is in use in the Scan Control window. This ensures correct calculation of pinhole, z-stack optimization etc." An arrow points from this note to the Objective field in the Scan Control window.

Adjusting the scan speed



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 6 or 7 for superior images.

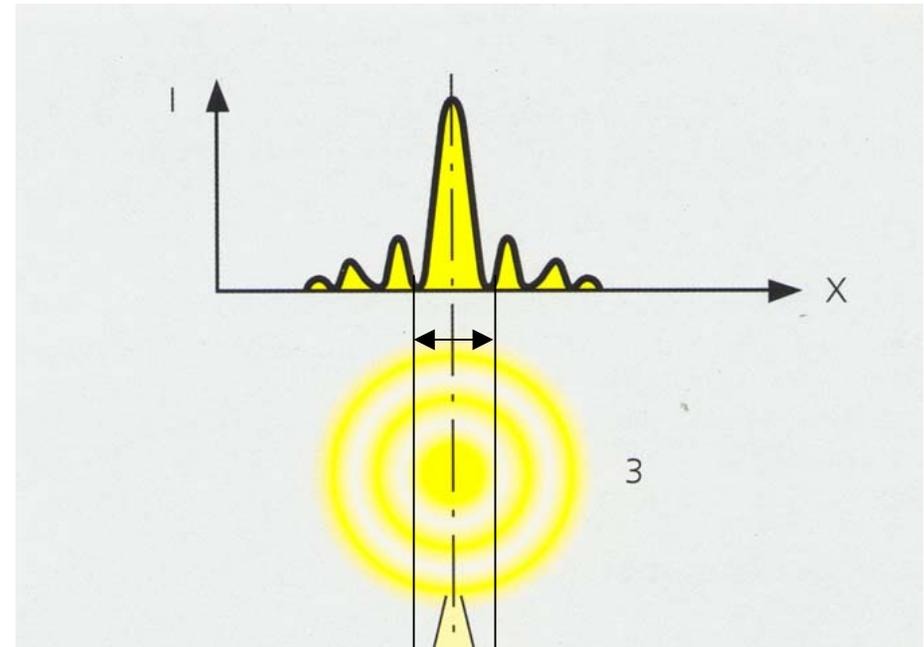
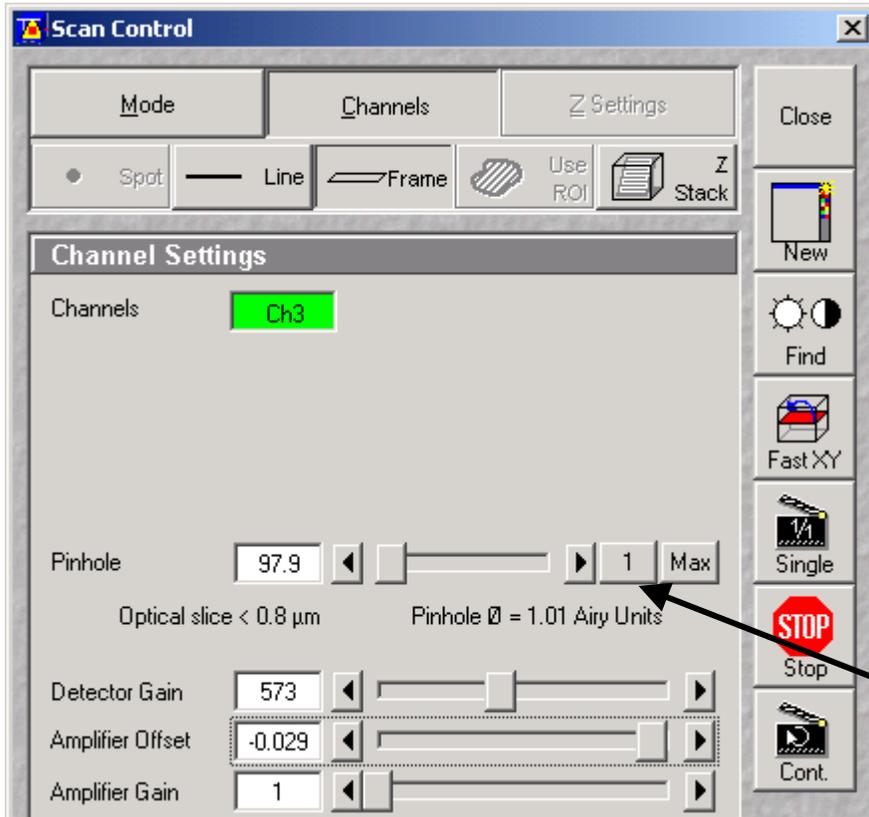
Choosing the Dynamic Range (8/12 Bit per pixel)



Select the dynamic range - 8 bit will give 256 gray levels, 12 Bit will give 4096 levels. Photoshop 5 will import 12 and 16 Bit images.

Publication quality images should be acquired using 12 Bit.

Channel Settings - Adjusting the Pinhole



Pinhole size
= 1 Airy unit

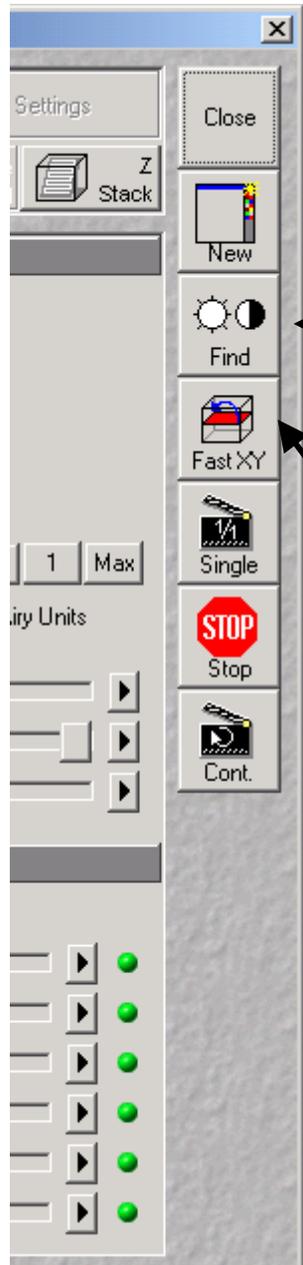
0.8 "Airy units" produces the best signal : noise ratio

Pinhole adjustment changes the "Optical slice".

When collecting multi channel images, adjust the pinholes so that each channel has the same "Optical Slice".

This is important for colocalization studies.

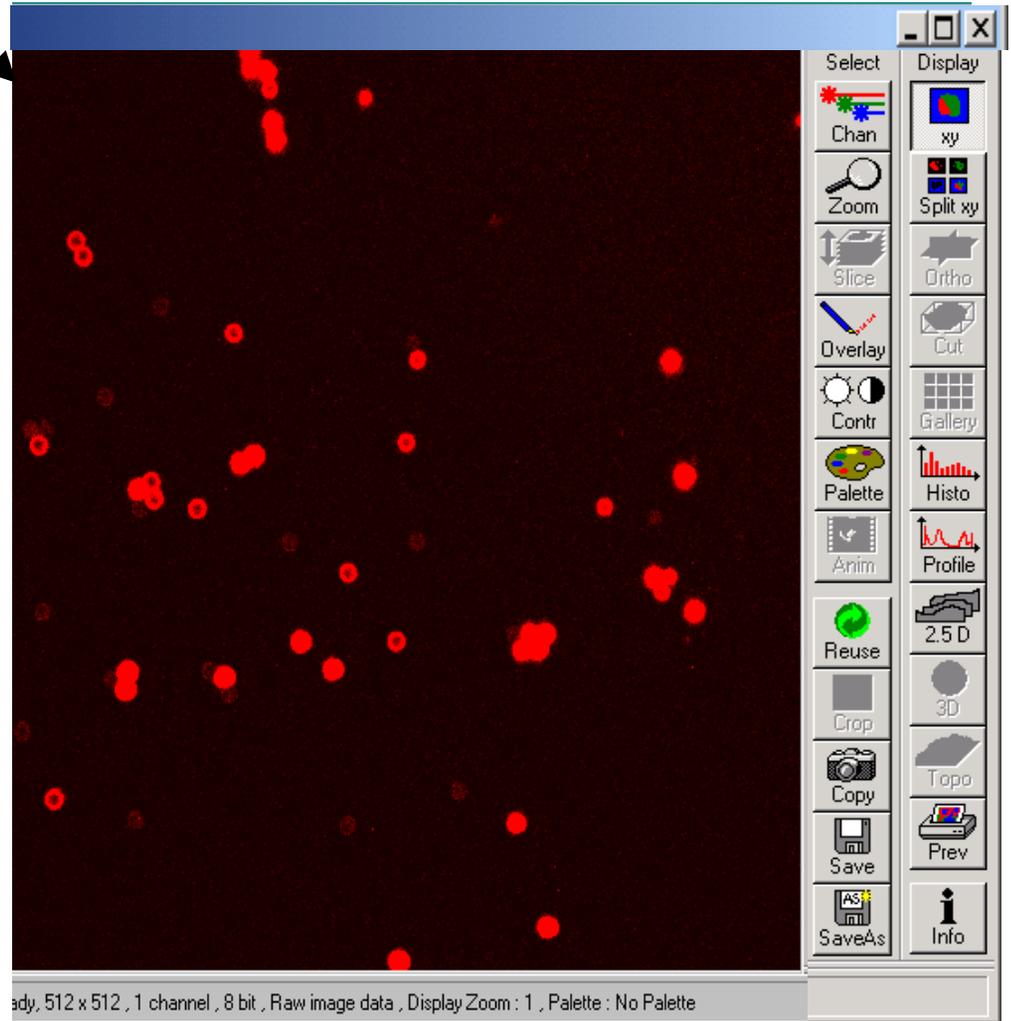
Image Acquisition



1) *Find* automatically pre-adjusts detector sensitivity

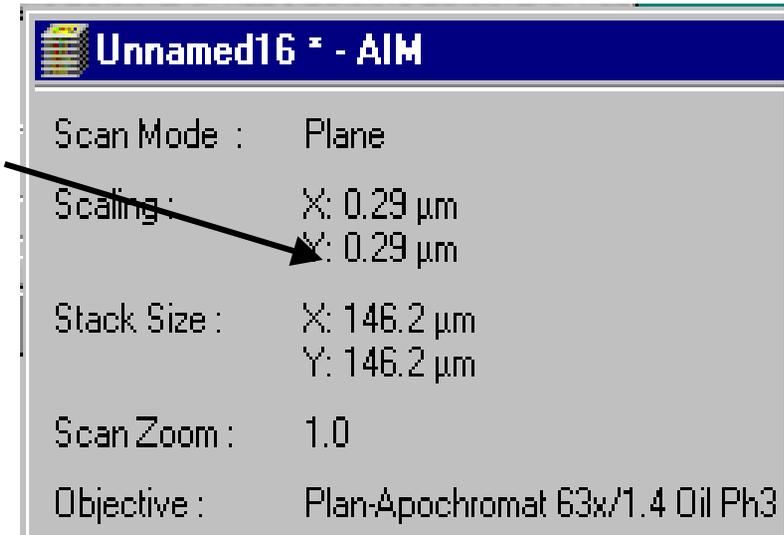
2) Select *Fast XY* for continuous fast scanning - useful for finding and changing the focus

3) *Stop* blanks the laser beam and stops the scanning mirrors



Minimal Pixel Size determined by Nyquist Sampling

×	NA	PIXEL SIZE
5	0.15	1.03 μm
10	0.3	0.51 μm
20	0.5	0.31 μm
40	1.3 (oil)	0.12 μm
63	1.4 (oil)	0.11 μm
100	1.4 (oil)	0.11 μm



Values are for scan zoom = 1.0

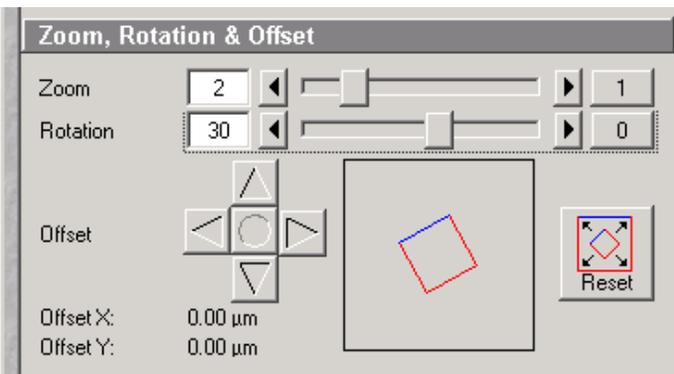
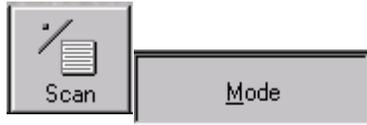
Adjusting the field size ("XY") to 56 μm with the 63 \times lens, would produce a pixel size of 0.1 μm

Brightness of image =
Magnification²/NA²

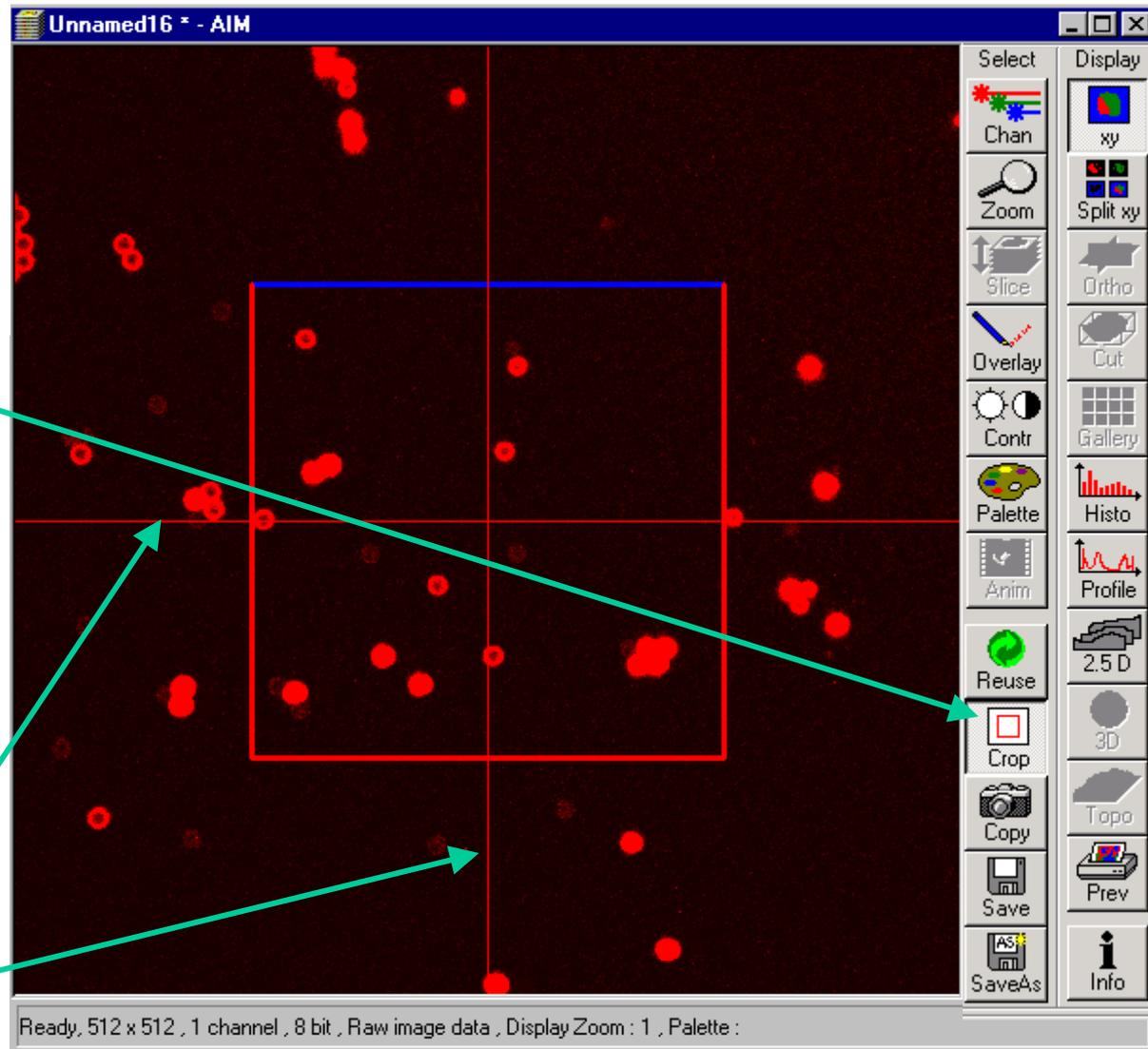
Field size can be adjusted by changing the objective magnification, or by optical zooming. Changing from 63 \times to 100 \times will reduce the field size, but will also reduce the amount of light available.

Optical Zooming

The level of zoom can be changed either by using the *Zoom, Rotation & Offset* control in *Mode* menu of the *Scan Control*, or by selecting *Crop* in the image menu.



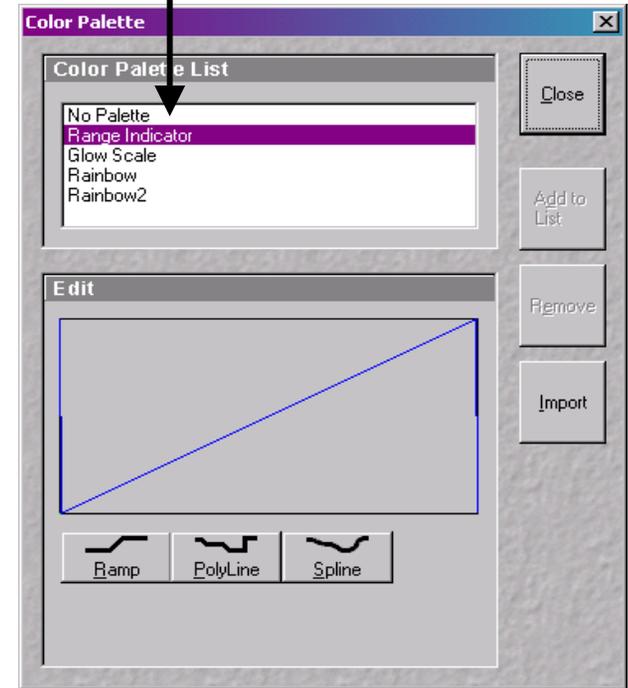
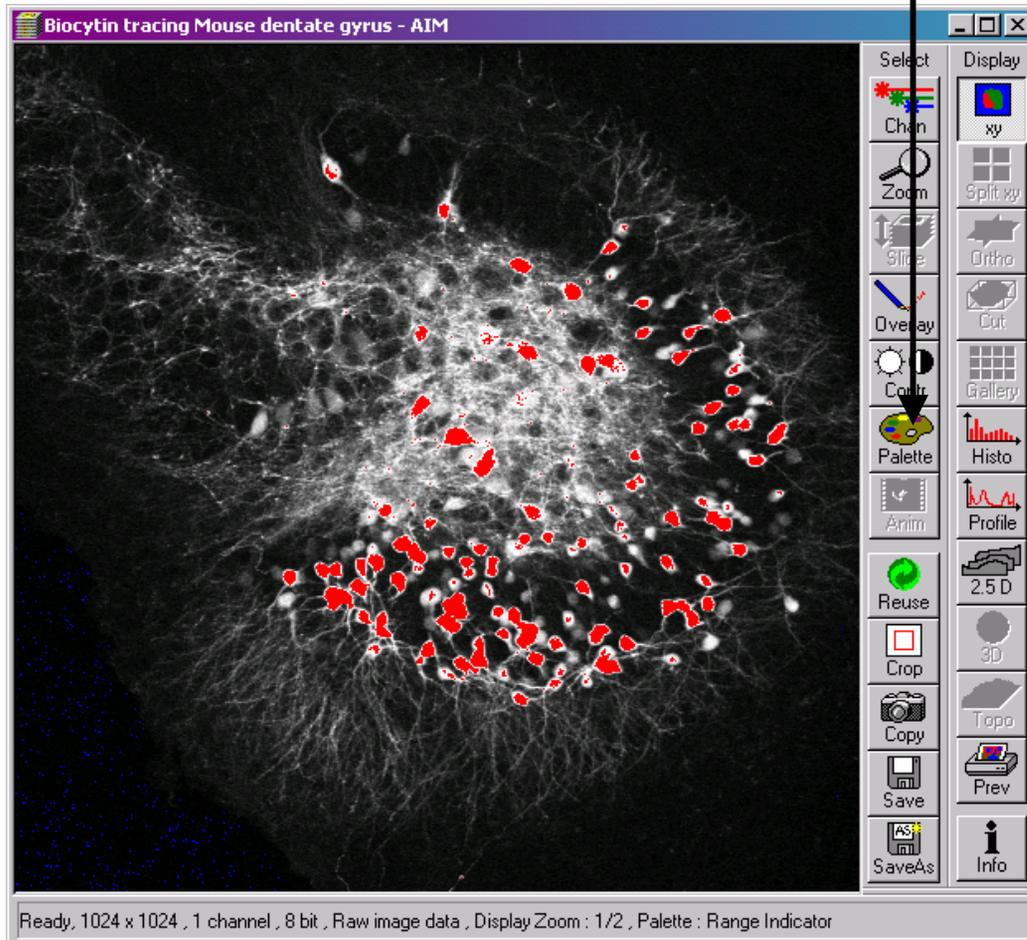
The image can also be rotated by selecting and dragging the bars



Selecting gain and offset – Choosing a lookup table

1) Select *Palette*

2) Select Range Indicator



Red = Saturation (maximum)

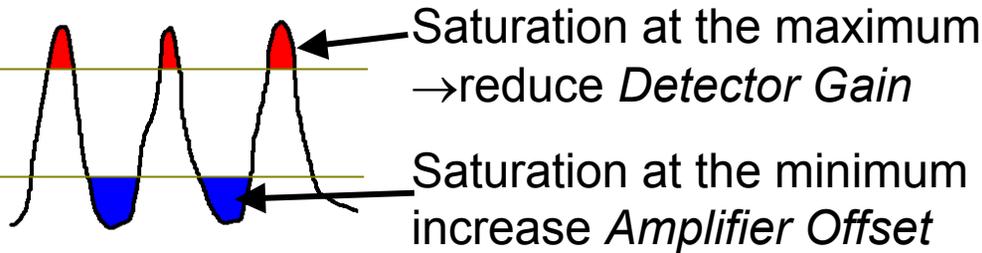
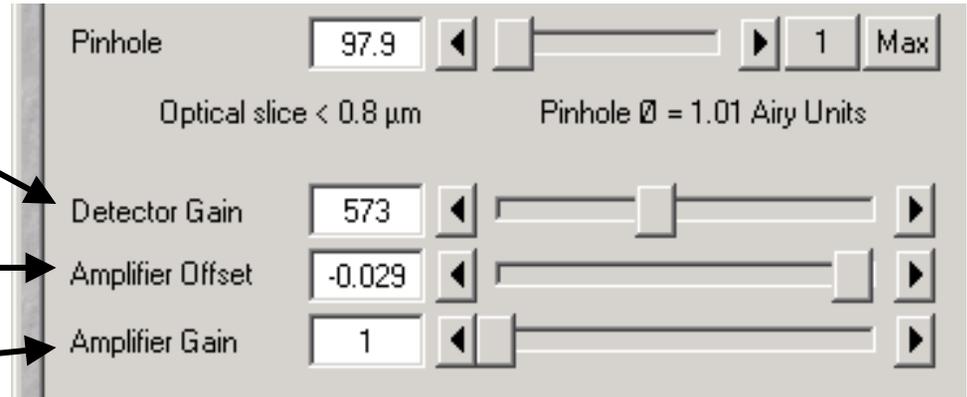
Blue = Zero (minimum)

Scan Control – Setting Gain and Offset

Detector gain determines the sensitivity of the detector by setting the maximum limit

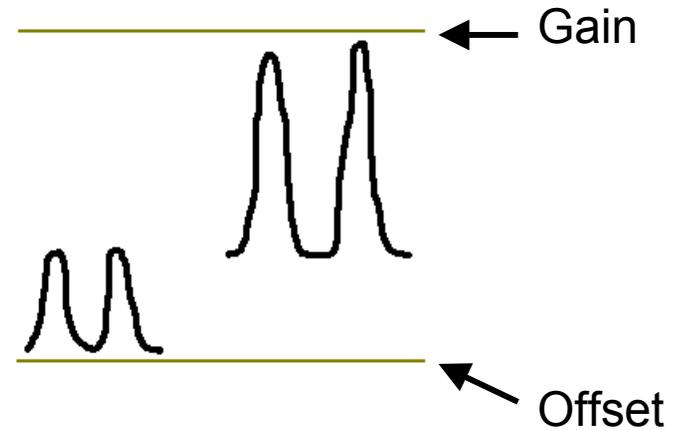
Amplifier Offset determines the minimum intensity limit

Amplifier Gain determines signal amplification



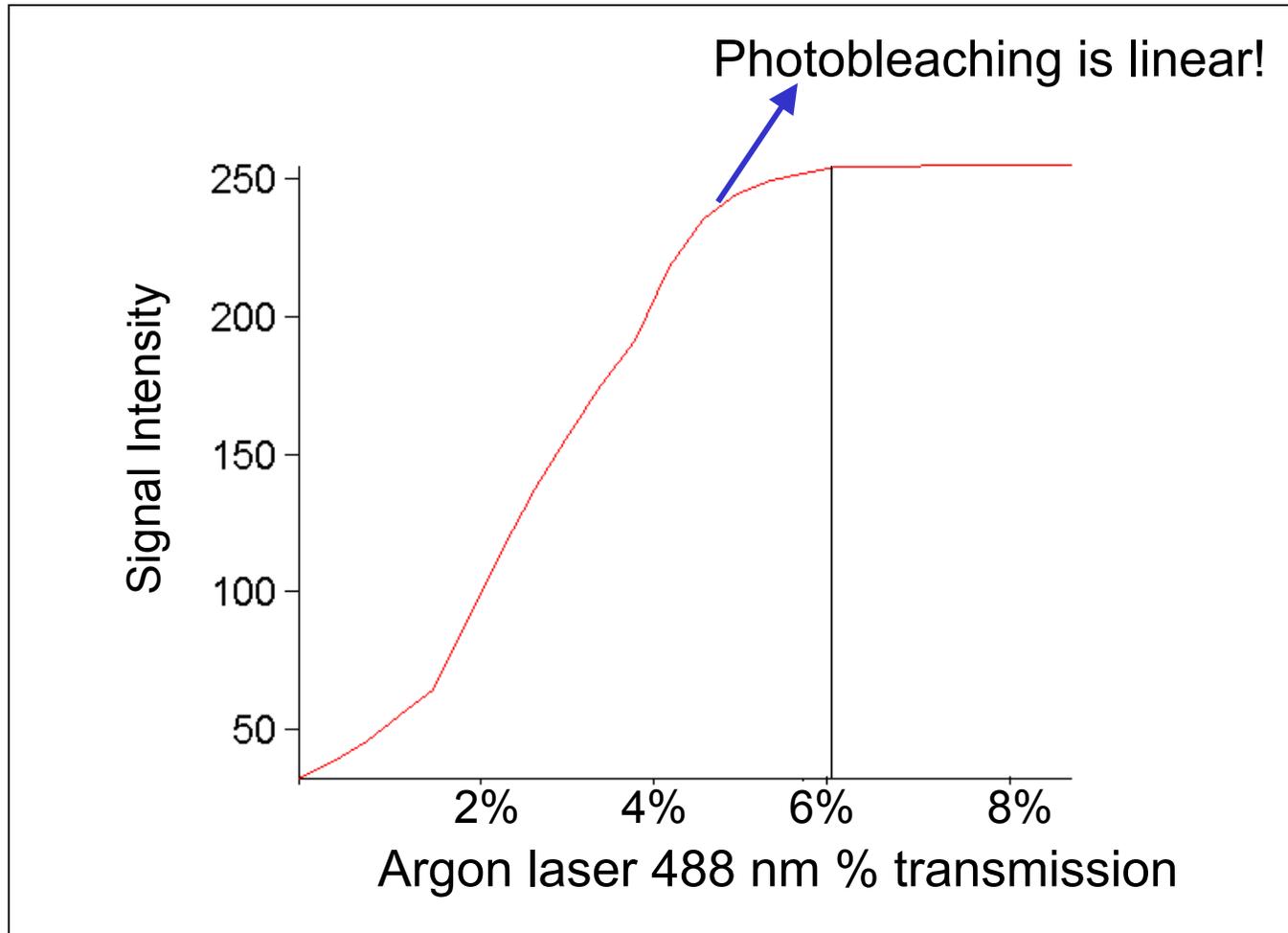
Gain set correctly

Offset set correctly



Amplifier Gain increases the whole signal, and the *Amplifier Offset* will need to be decreased.

Saturation of Signal Intensity with Laser Power

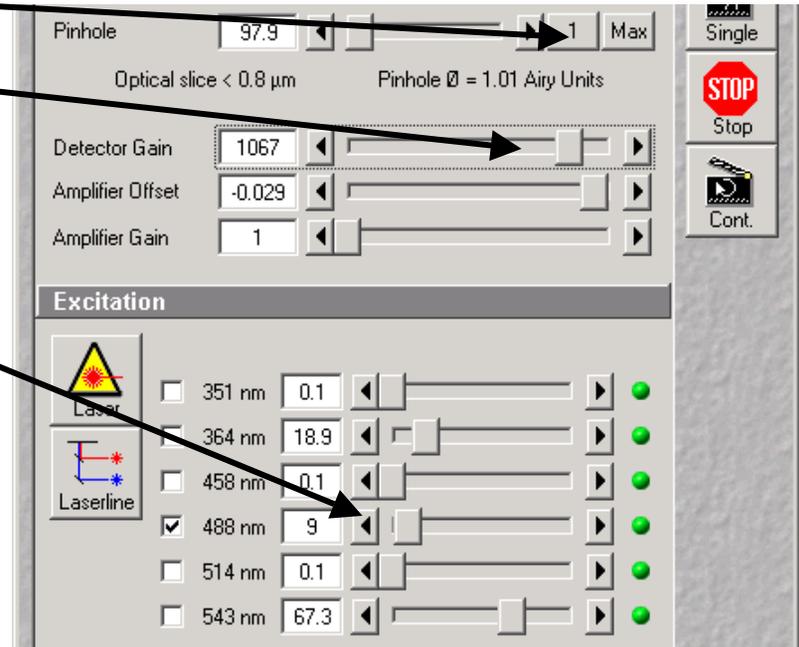
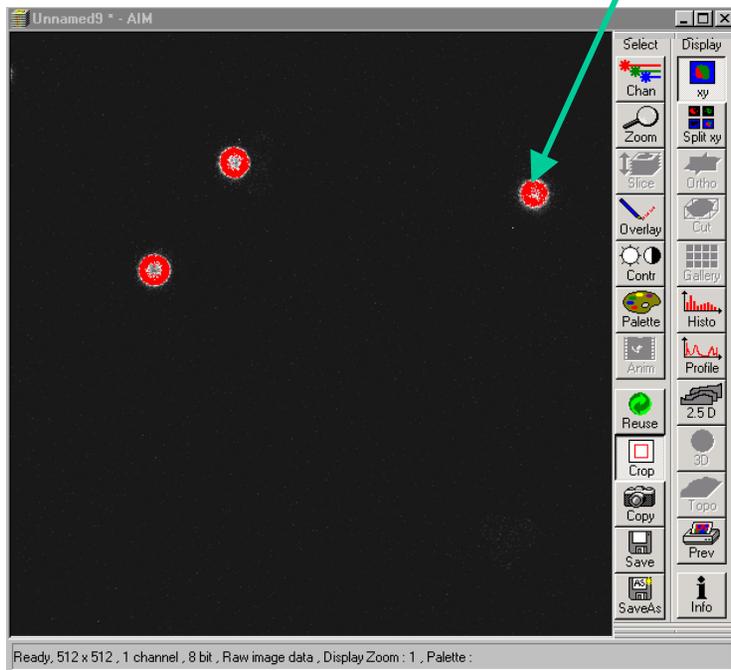


- Fluorophore saturates at 6% laser transmission
- Photobleaching is linear

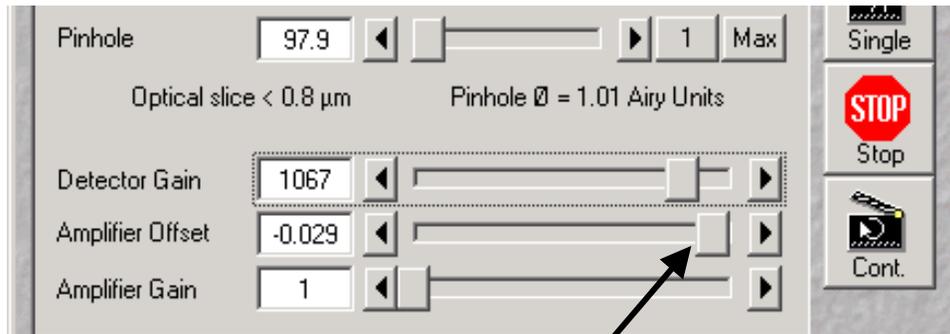
Laser transmission should not be set higher than the saturation level.

Adjusting the Laser Intensity

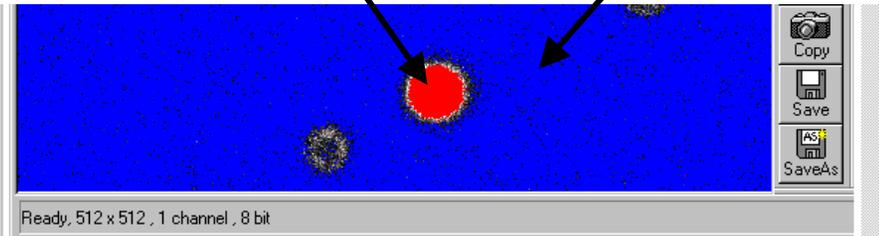
- 1) Set *Pinhole* to 1 Airy unit
- 2) Set *Detector Gain* high
- 3) When the image is saturated, reduce AOTF transmission in the *Excitation* panel to reduce the intensity of the laser light at the specimen



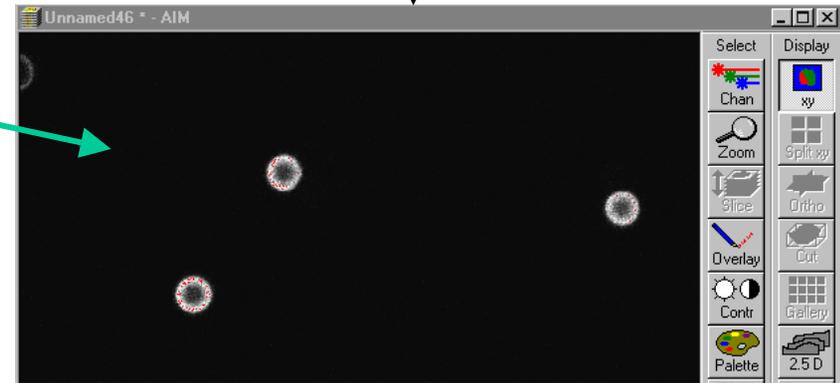
Adjusting Gain and Offset



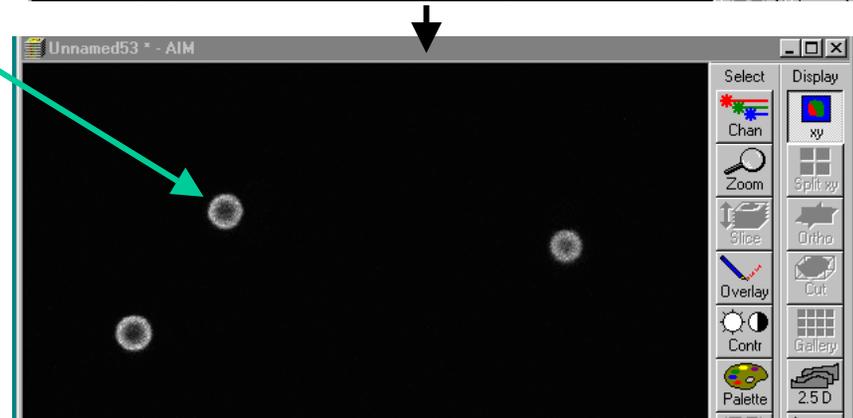
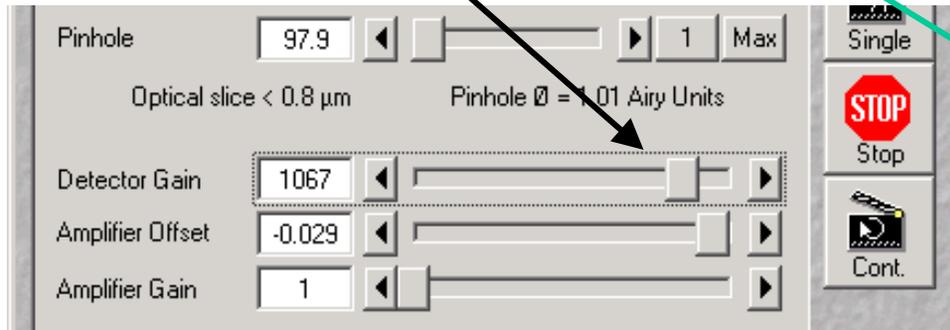
Both *Detector Gain* and *Amplifier Offset* saturated



1) Increase the *Amplifier Offset* until all blue pixels disappear, and then make it slightly positive.



2) Reduce the *Detector Gain* until the red pixels only just disappear.

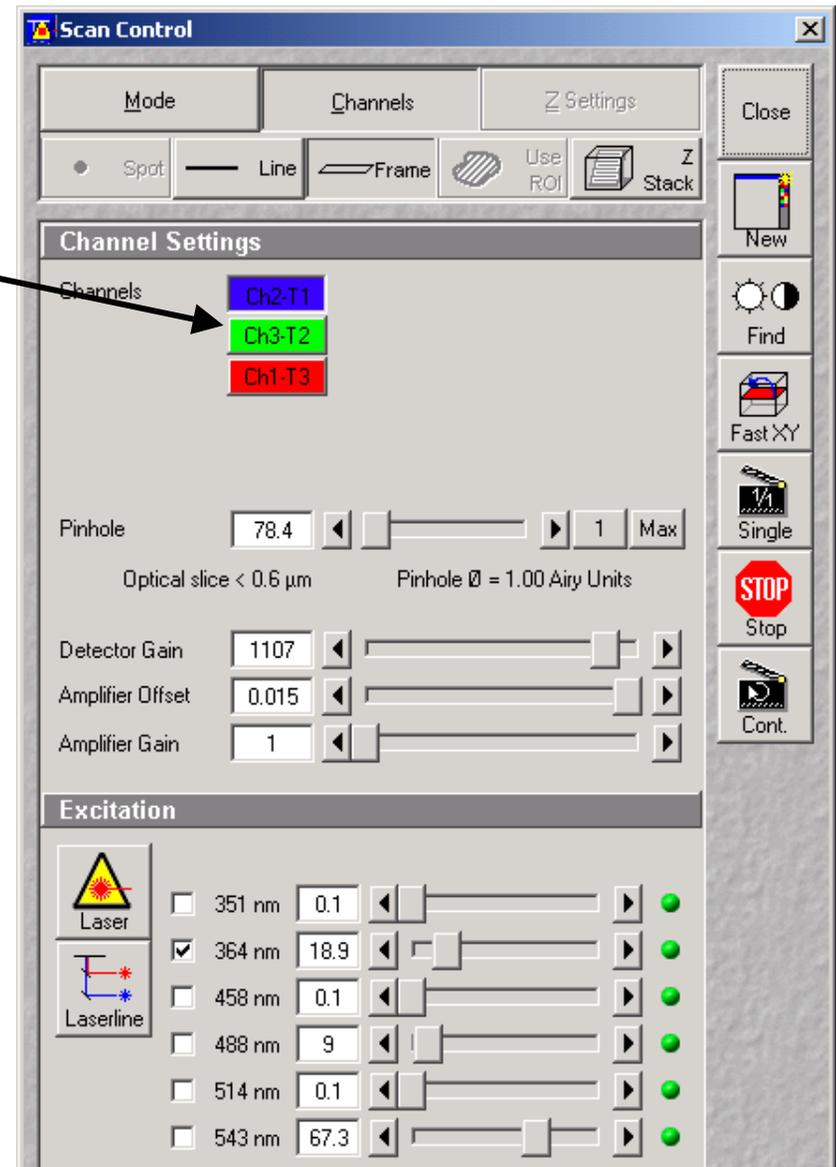


Adjusting the Laser, Gain and Offset using a Multi Track Configuration

Each channel is selected independently by clicking on the colour button indicating the channel i.e. *Ch2-T1* (Channel 2, Track 1). The laser power and all other parameters are optimised as described in the previous slides for each selected channel.

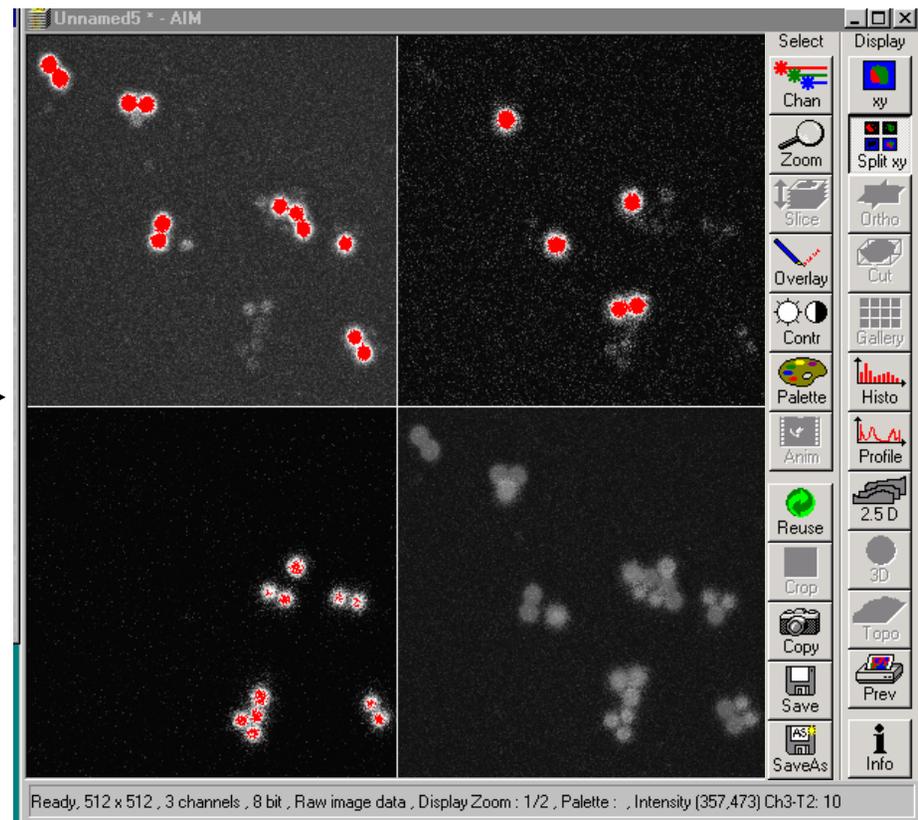
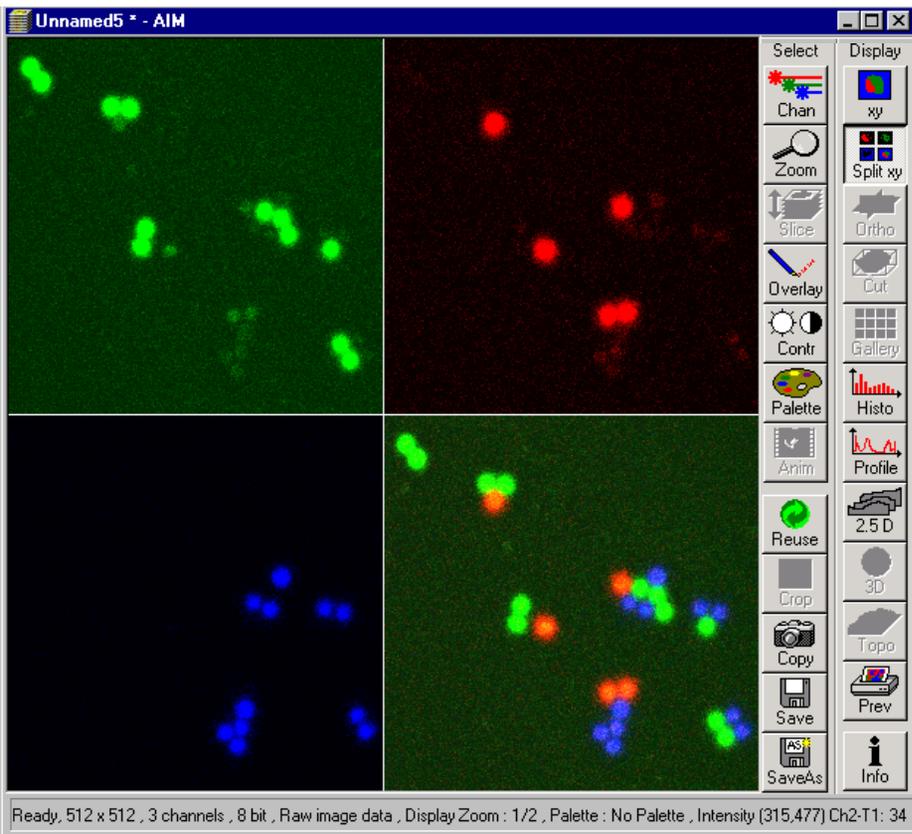
For accurate colocalisation, adjust each *Pinhole* so that each channel has the same *Optical Slice*

0.8 *Airy units* gives the best signal:noise ratio



Setting up Gain and Offset - Multi Track

- 1) Select *Split XY* in the Image window
- 2) In *Palette*, select *Range indicator*
- 3) Select each channel separately under *Channels* in the *Scan control* window and adjust the *Laser intensity*, *Detector Gain*, and *Amplifier Offset* as described previously.



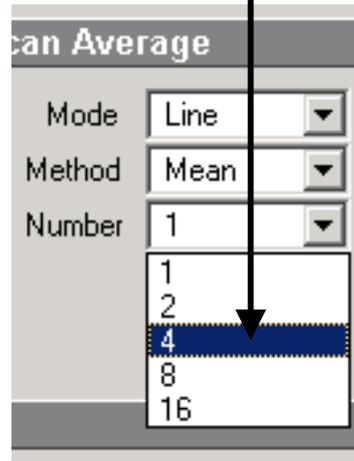
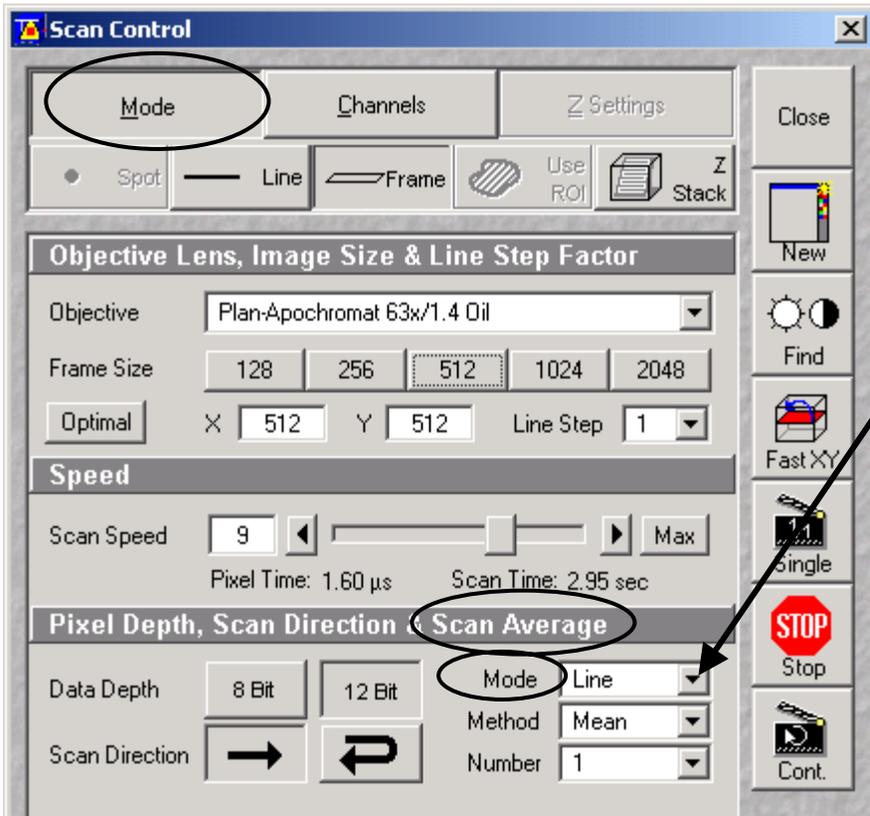
Line Averaging

Averaging improves the image by increasing the signal : noise ratio

Averaging can be achieved line by line, or frame by frame

1) Select *Line* or *Frame* under *Mode* in *Scan Average* within the *Mode* panel of the *Scan Control* window

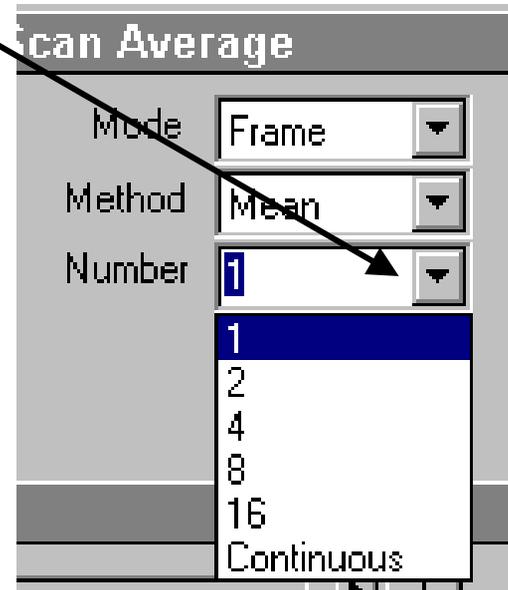
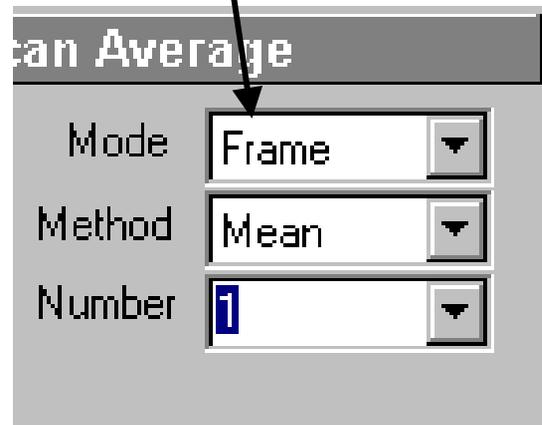
2) Select *Number* for averaging. The more the better for the signal to noise ratio (max 16) in this case, each line will be scanned 4 times. But: Averaging increases the exposure time of the sample!!



Frame Averaging

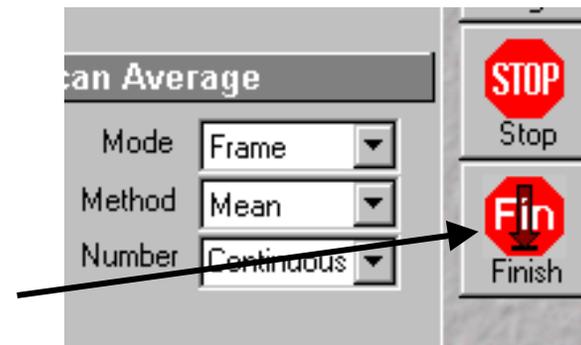
1) Select *Frame*

2) Select the *Number* for averaging - The more the better for signal to noise ratio (max 16). Continuous averaging is possible in this mode.



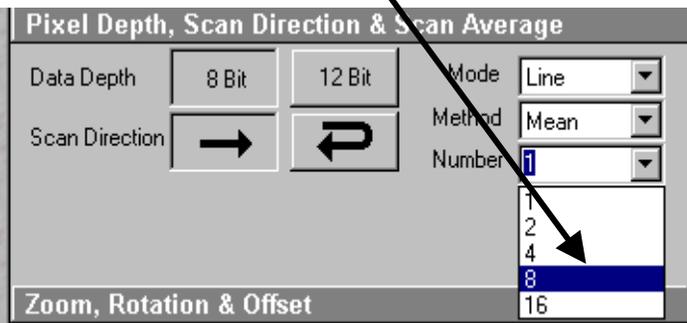
Frame averaging helps reduce photobleaching, but does not give quite such a smooth image. There is also a longer delay between each track when using “Multi Track”.

Continuous averaging has a *Finish* button which allows the scan currently in progress to be completed before stopping

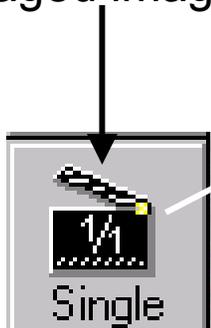


Collecting an Averaged Image

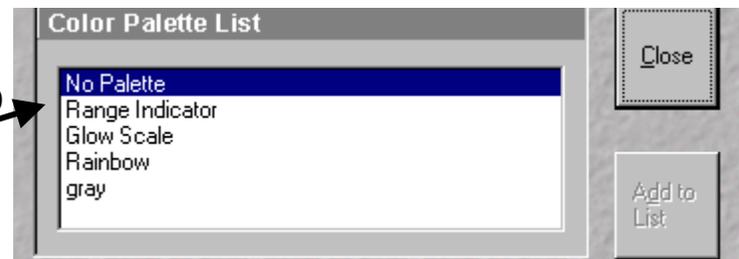
1) Under *Scan Average* select the *Number* for the average.



In the *Channels* panel of the *Scan Control* window select *Single*. An averaged image will be collected.



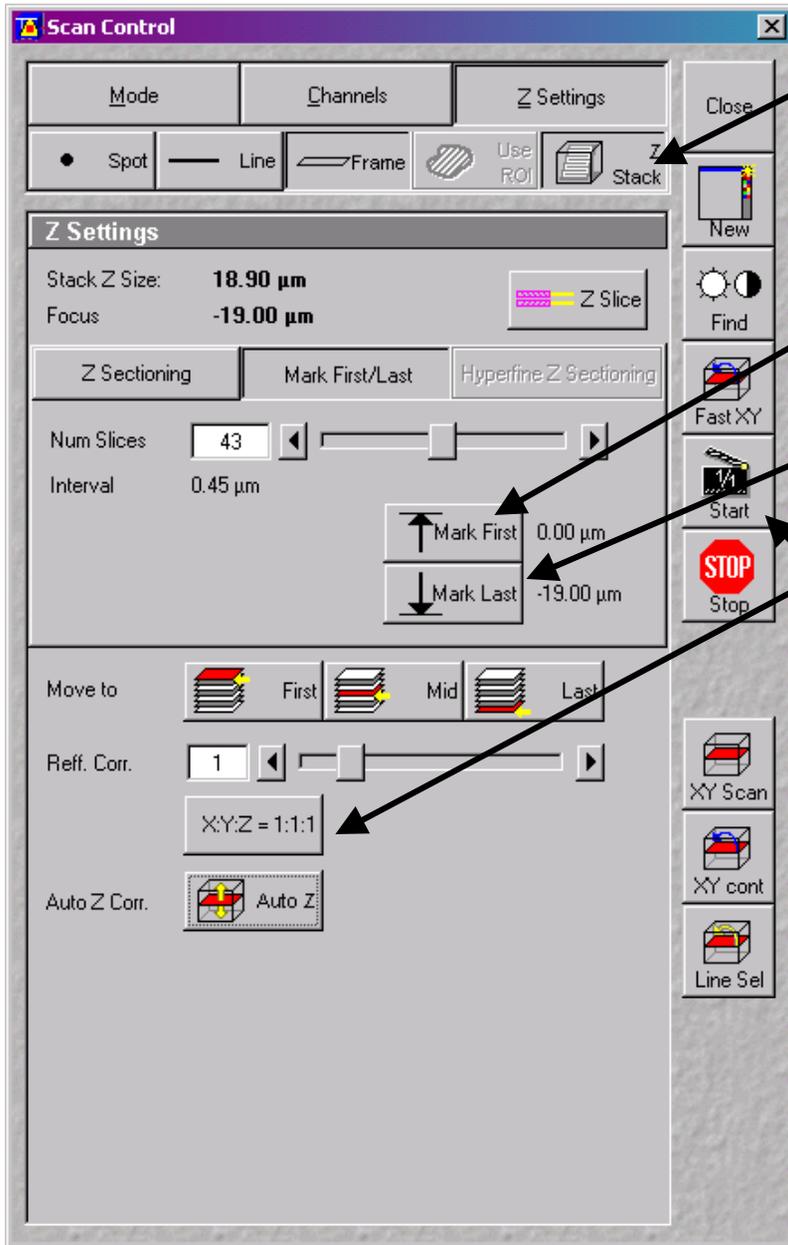
Range indicator set to *No Palette*



Contents

- Starting the Zeiss LSM 510 microscope, software and laser
- Selecting an objective and focusing the microscope
- Configuring the laser scanning and detection for confocal image acquisition
- **Acquiring a Z- and Time - Series**
- Data storage

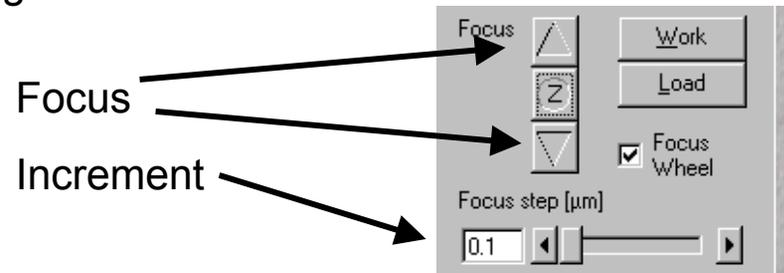
Scanning a Z-Series using *Mark First/Last*



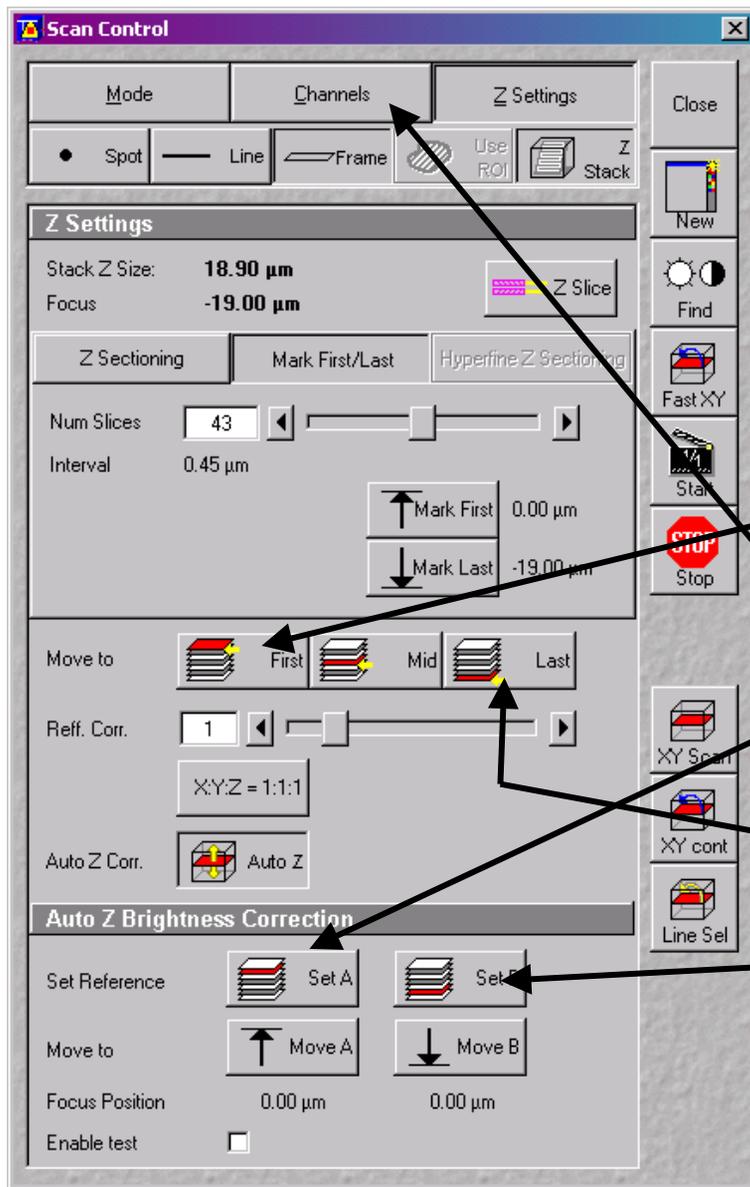
- 1) Select *Z Stack*
- 2) Start scanning using *Fast XY* or *XY cont*
- 3) Keep your eye on the image and move the focus to the beginning of the Z-Series, then select *Mark First*
- 4) Move the focus back in the opposite direction to the end of the Z-Series, then select *Mark Last*
- 5) $X:Y:Z = 1:1:1$ sets the Z-interval so that the voxel has identical dimensions in X, Y, and Z.
- 6) *Start* will initiate the acquisition of the Z-Stack. The acquisition can be stopped at any time.

NOTE

Focusing can be achieved manually (preferred), or using *Stage* in the LSM menu if there is a motorized scanning table.



Using Auto Z Brightness Correction



Auto Z provides an automatic gradual adjustment of *Detector Gain*, *Amplifier Offset*, *Amplifier Gain*, and *Laser intensity* setting between the first and last optical slice of a *Z Stack*.

1) After defining the *Z* position of the first and last optical slice activate *Auto Z*.

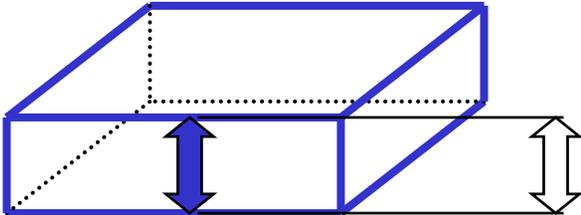
2) Move to the *First* Slice and adjust the parameter for the image acquisition in the *Channels* panel for each used channel as described in the previous slides. Then click on *Set A* to store the values.

3) Repeat the procedure after moving to the *Last* Slice. Click on *Set B* to store the parameters for the last slice.

4) The parameters for image acquisition will be gradually and linearly adjusted between the first and last slice of the *Z Stack*. Thus signal intensity and image quality is comparable throughout the *Z Stack*.

Confocal Z Sectioning

Number of Sections for correct sampling

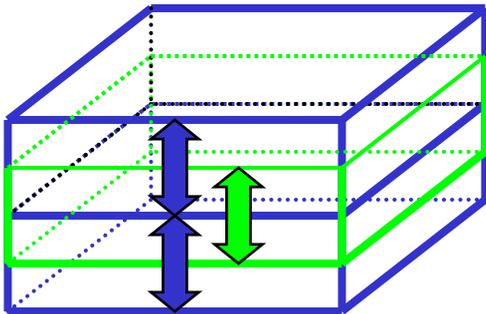


Optical thickness d depends on:

- Wavelength λ
- Objective lens, $N.A.$
- Refractive index n
- Pinhole diameter P

$$d \sim P n \lambda / (N.A.)^2$$

$$\sim 0.5 \mu\text{m} @ 63\times 1.4$$

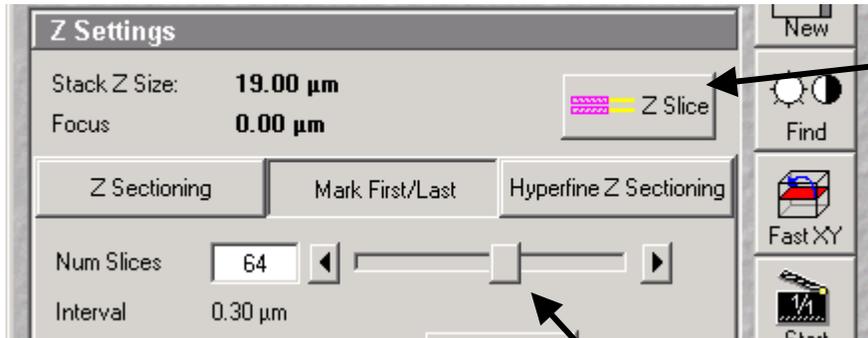


Optimal: (no missing information @ minimal number of sections)

Slices overlap by the half of their thickness

„Nyquist-“ or Sampling- Theorem

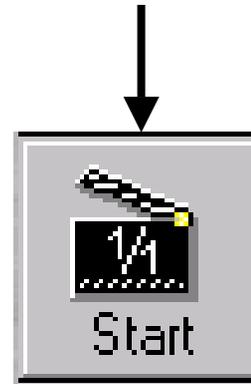
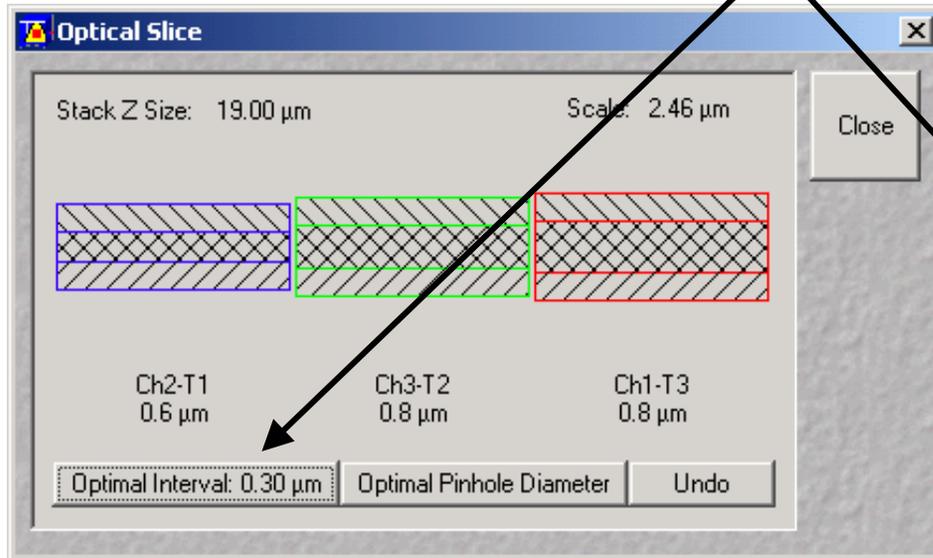
Z Stack – Number of Slices and Increment



1) Select *Z slice* - the window *Optical Slice* will appear

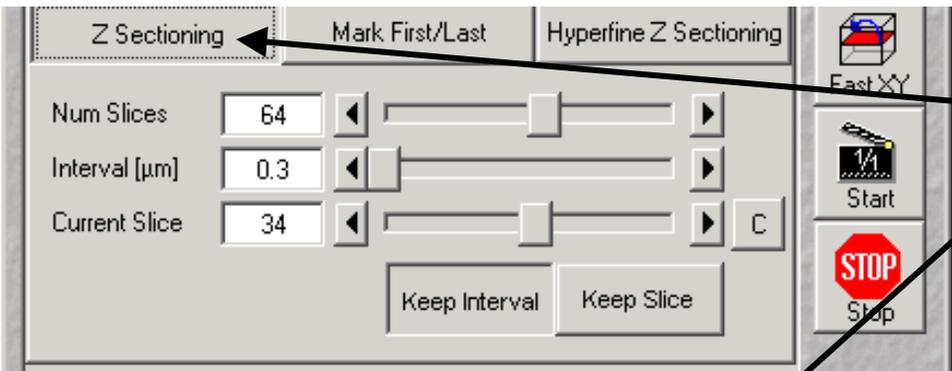
2) Select *Optimal interval* the computer will calculate the optimum number of sections

3) Select *Start*

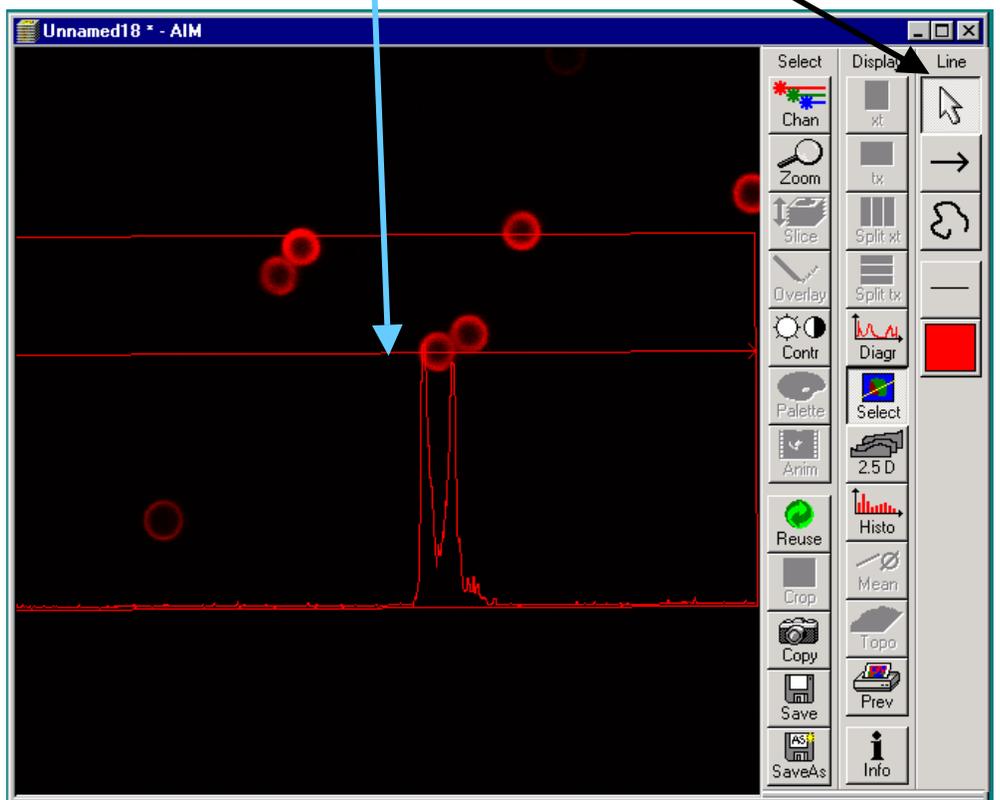


For more or less sections - adjust *Num Slices*

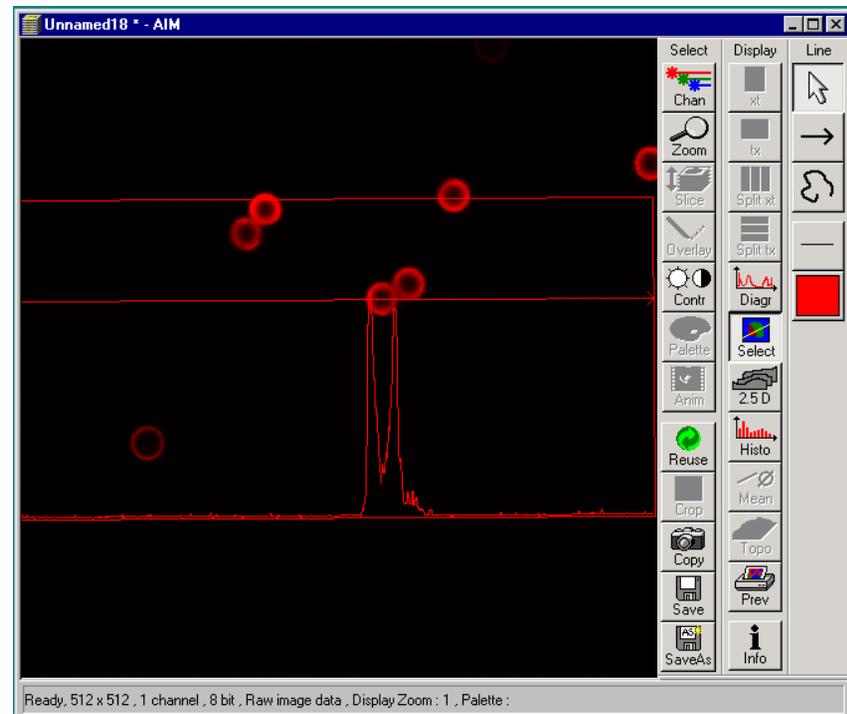
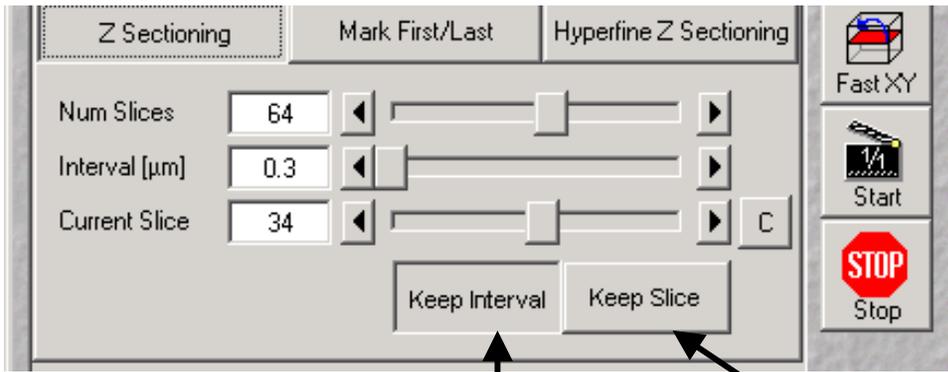
Z - Series using Z Sectioning



- 1) Select *Z Stack*
- 2) Select *Z Sectioning*
- 3) Select *Line Sel*
- 4) Select the large arrow button and position the XZ cut line



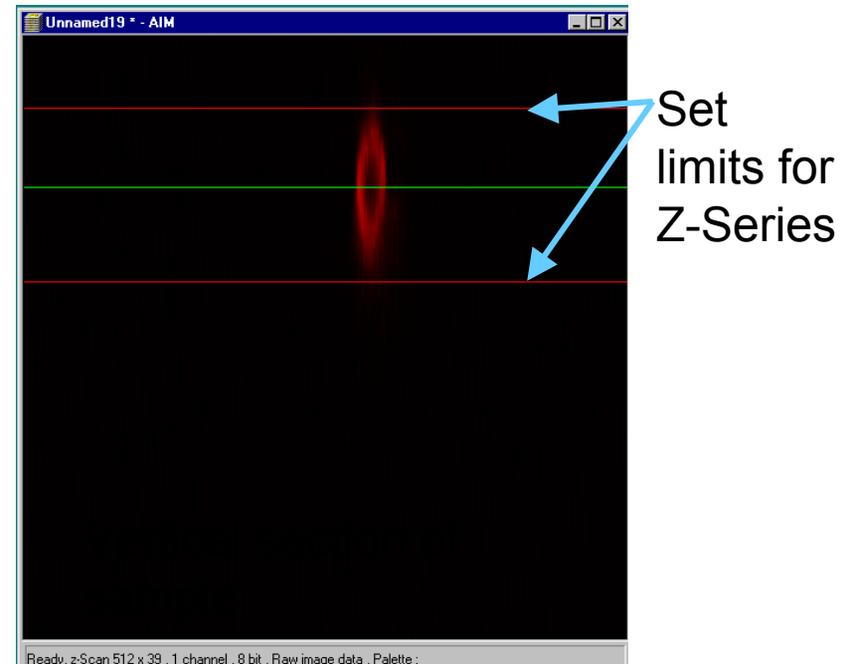
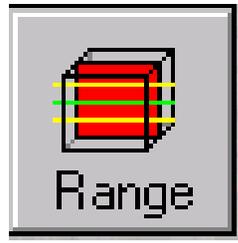
Z Sectioning – Setting Range



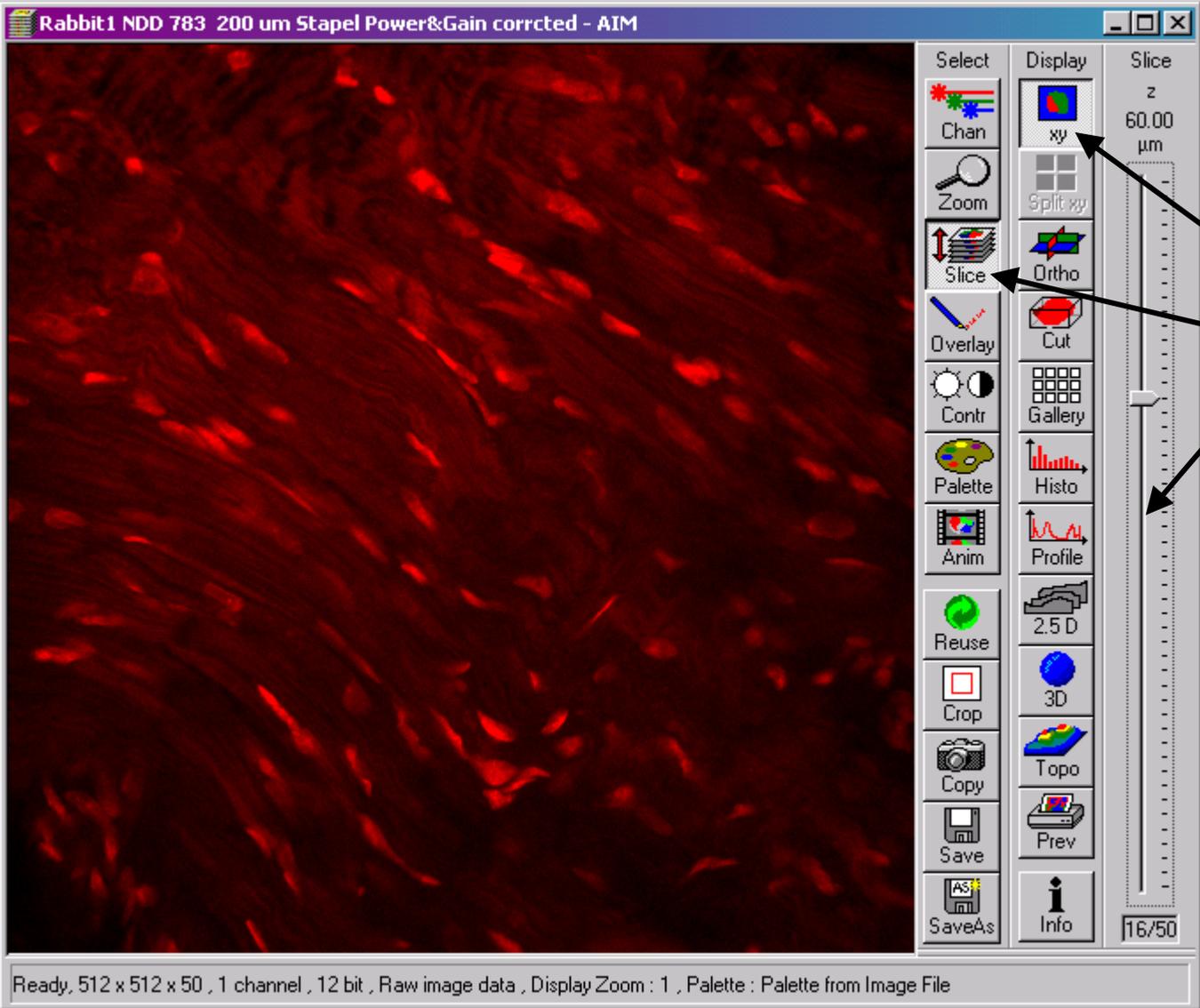
1) Decide whether to *Keep Interval* (number of slices will change) or *Keep Slice* (Interval between slices will be adjusted)

2) Select *Range* and position bars to decide where the Z - Series begins and ends

3) Select *Start* for image acquisition



Viewing a Z - Series



In the image window

1) Select xy

2) Select *Slice*

3) Use scroll bar to view individual sections

Viewing a Z - Series using *Gallery*

Rabbit1 NDD 783 200 um Stapel Power&Gain corrcted - AIM

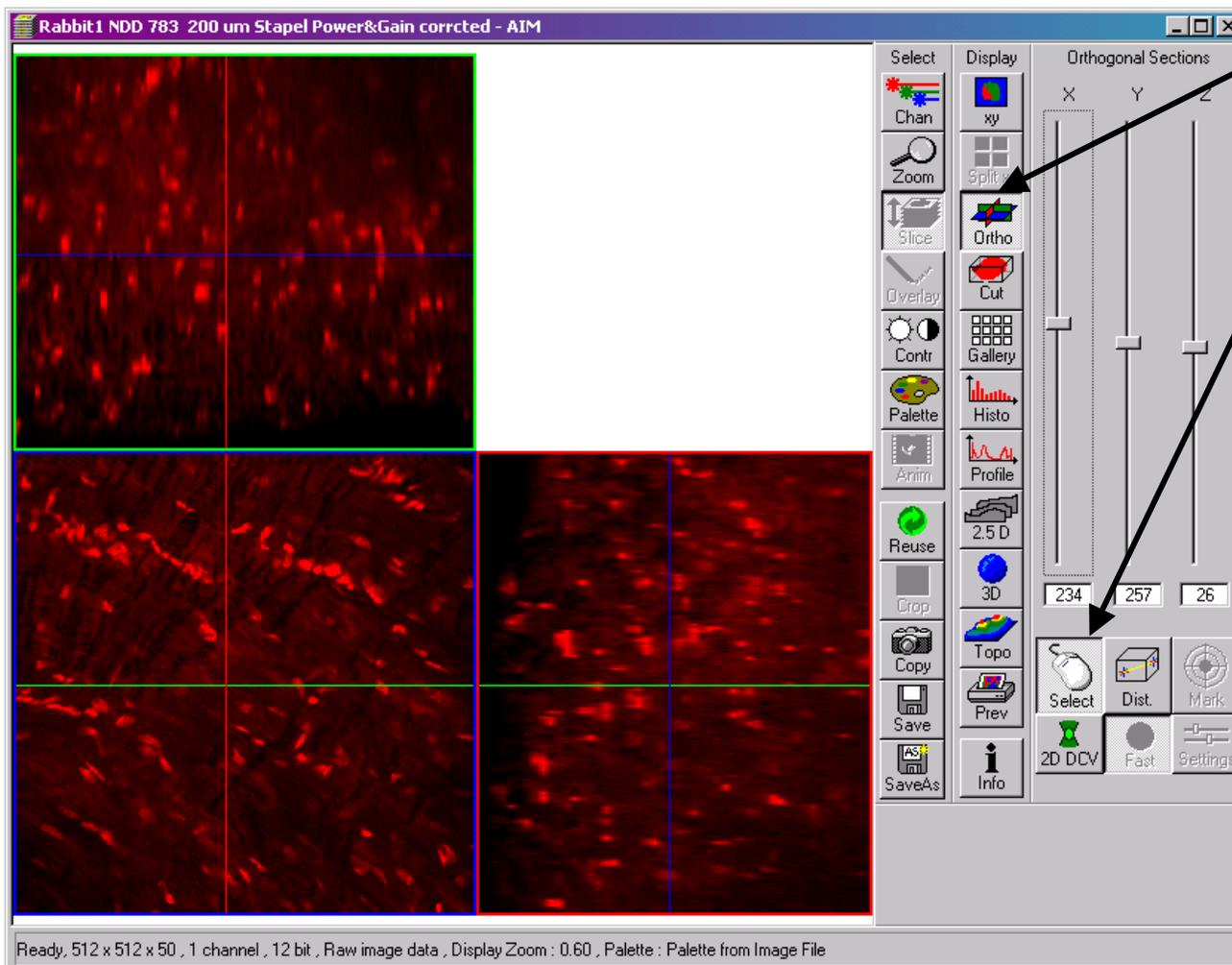
0.0 μm	4.0 μm	8.0 μm	12.0 μm	16.0 μm	20.0 μm	24.0 μm	28.0 μm	32.0 μm	
36.0 μm	40.0 μm	44.0 μm	48.0 μm	52.0 μm	56.0 μm	60.0 μm	64.0 μm	68.0 μm	
72.0 μm	76.0 μm	80.0 μm	84.0 μm	88.0 μm	92.0 μm	96.0 μm	100.0 μm	104.0 μm	
108.0 μm	112.0 μm	116.0 μm	120.0 μm	124.0 μm	128.0 μm	132.0 μm	136.0 μm	140.0 μm	
144.0 μm	148.0 μm	152.0 μm	156.0 μm	160.0 μm	164.0 μm	168.0 μm	172.0 μm	176.0 μm	
180.0 μm	184.0 μm	188.0 μm	192.0 μm	196.0 μm					

Ready, 512 x 512 x 50, 1 channel, 12 bit, Raw image data, Display Zoom: 1/8, Palette: Palette from Image File

- 1) Select *Gallery*
- 2) Select *Data* for scale

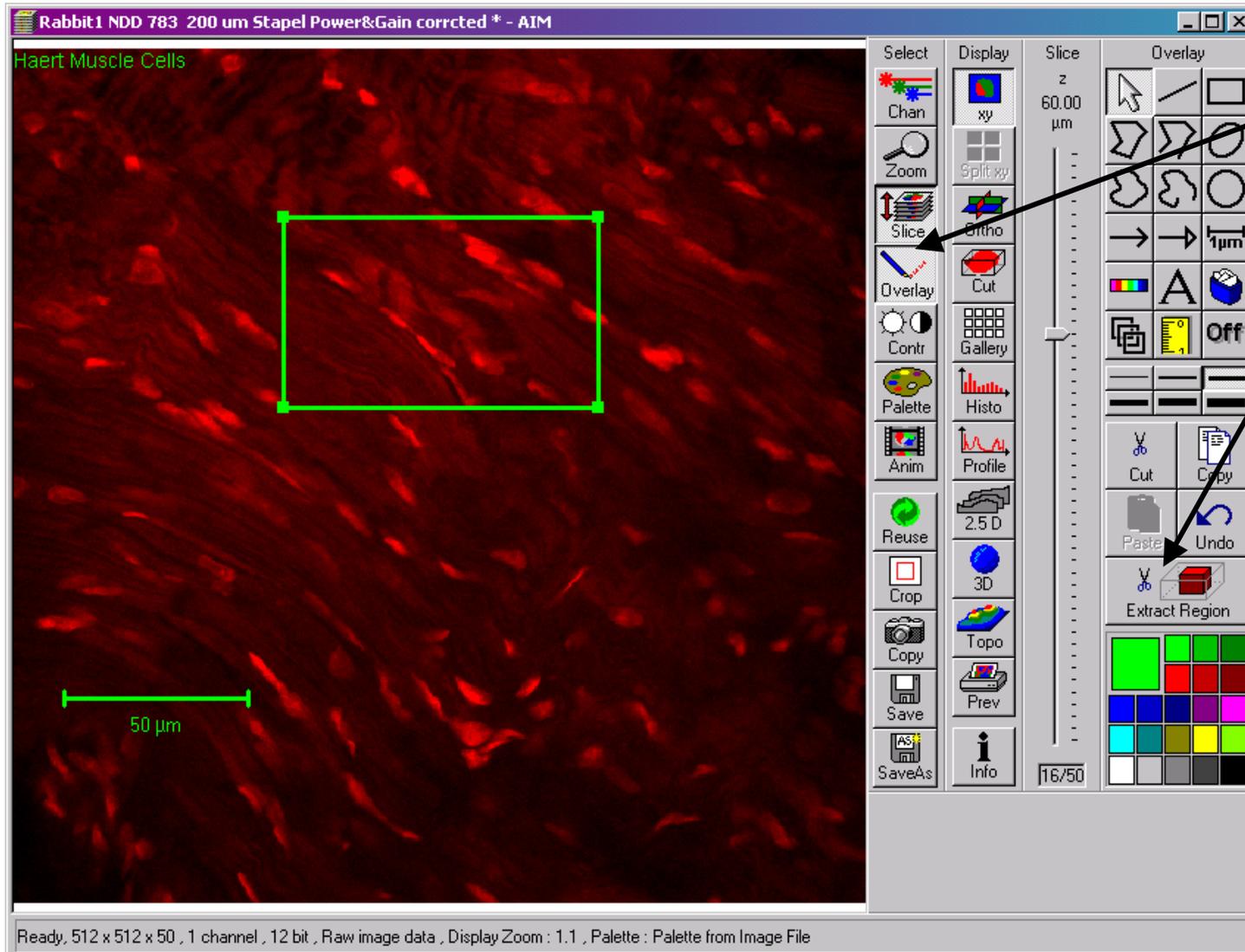
Use *Subset* to extract sections

Viewing a Z- Series using Orthogonal Sections



- 1) Select *Ortho*
 - 2) Select mouse (*Select*)
 - 3) Using the mouse, position the cut lines.
- To save orthogonal sections, select *Export* and save as *contents of image window*.

Selecting and Saving a Region of Interest (ROI)



- 1) Select *Overlay* and define shape of ROI
- 2) *Extract region* creates a Z-Stack from the ROI
- 3) Save data

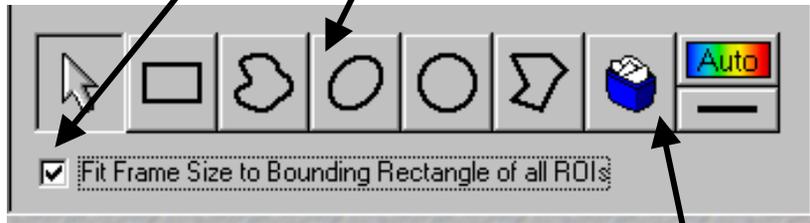
Using a ROI for faster image acquisition and data saving



1) Select *EditROI* from the LSM menu bar

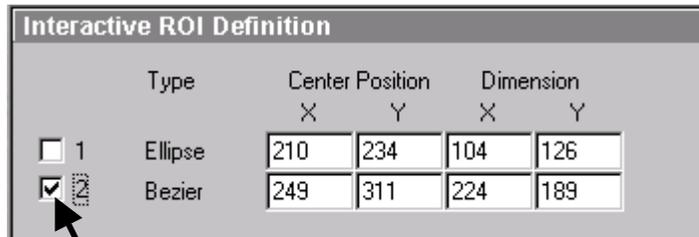
2) Select *Fit Frame Size to bounding Rectangle*

3) Choose shape of ROI

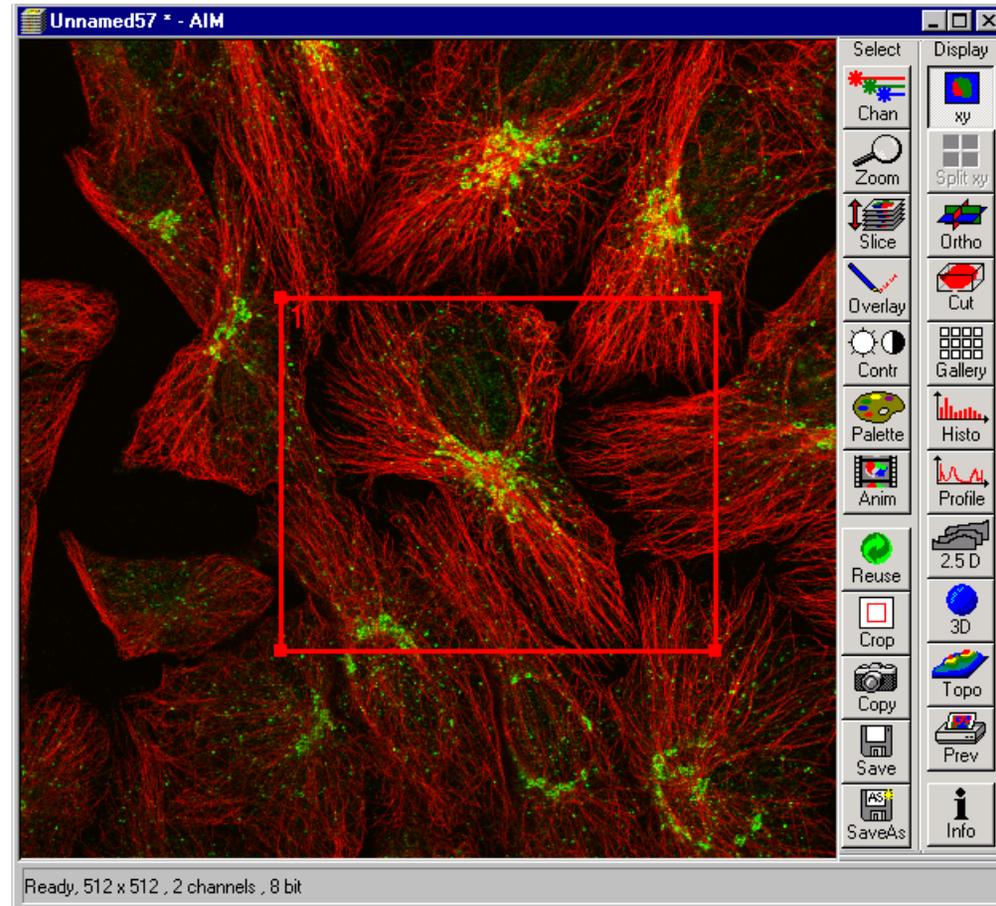


4) Position and size the ROI in the image with the mouse

5) Start Scan



To remove ROI and overlay select blue bin or deactivate ROI. Closing the window only removes overlay, ROI is still active. Deactivate *Use ROI* in the LSM menu.



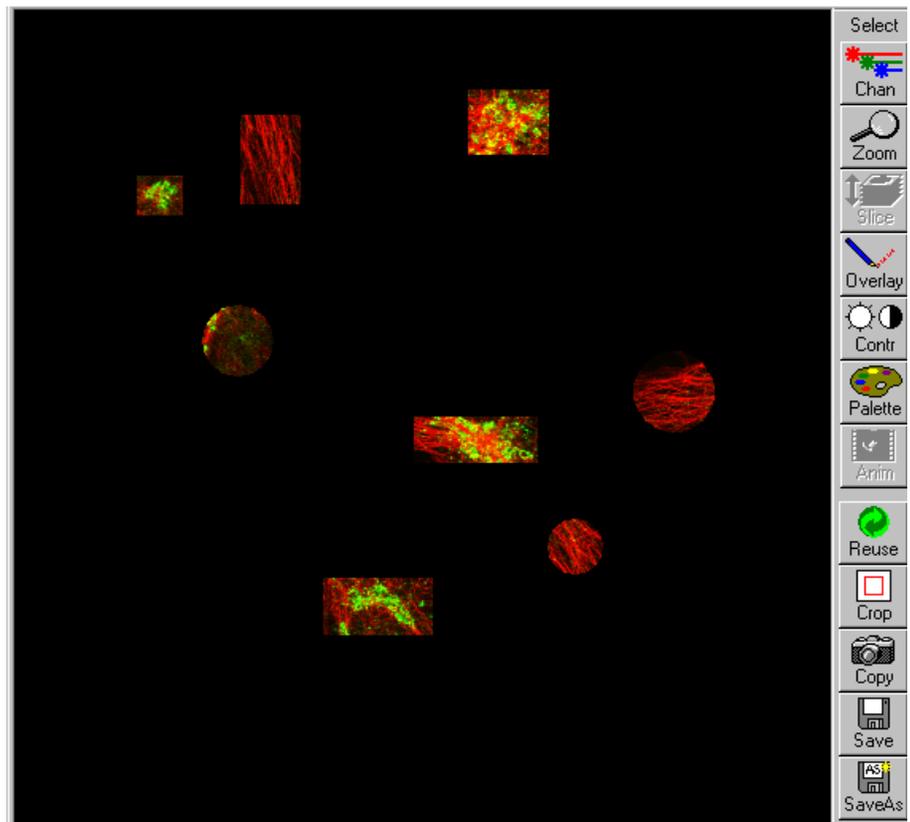
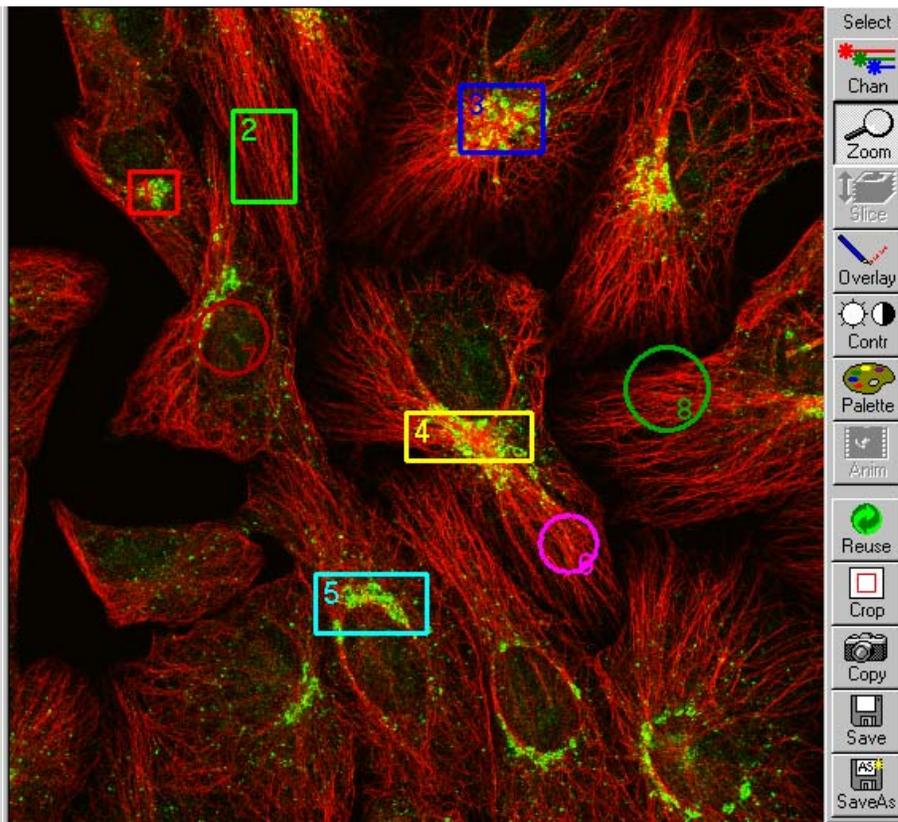
Multiple Regions of Interest

1) Un-select *Fit Frame Size to bounding Rectangle*, Choose shapes of ROIs

4) Position and size the ROIs with mouse

5) Start Scan

To remove ROIs and overlay select blue bin or deactivate ROIs. Closing the window only removes overlay, ROIs are still active. Deactivate *Use ROI* in the LSM menu.



Time Series

1) Set up scanning parameters for image acquisition as described in previous slides

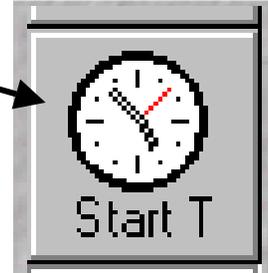
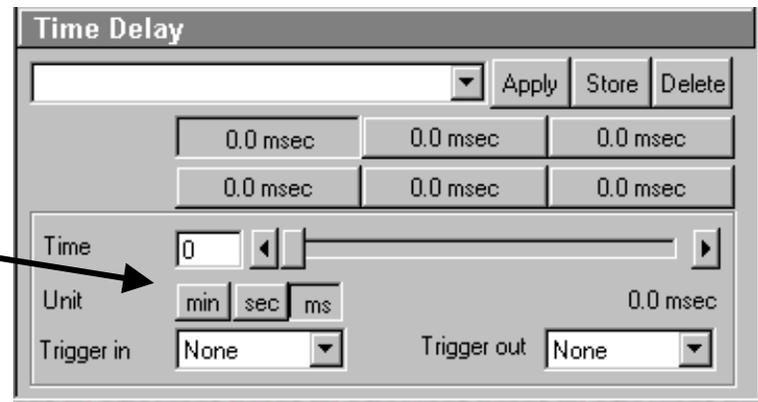
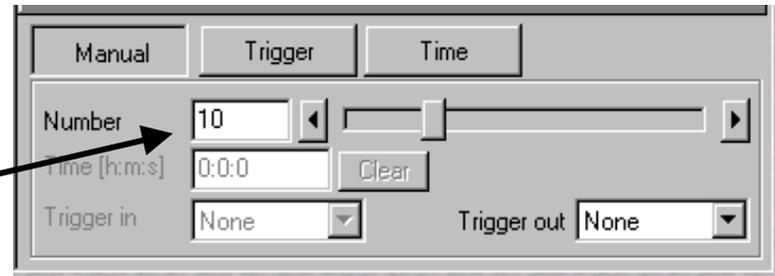
2) Select *TimeSeries* from the LSM menu

3) Enter the *Number* of cycles

4) For a Time Delay between image acquisition select *min*, *sec* or *ms* and set time with the slider

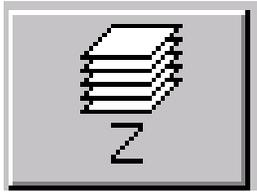
5) Select *Start T* to start image acquisition

Start and Stop of the Time Series using *Time* as trigger uses the system time!

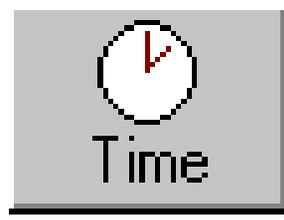


Viewing a Time Series of a Z Stack

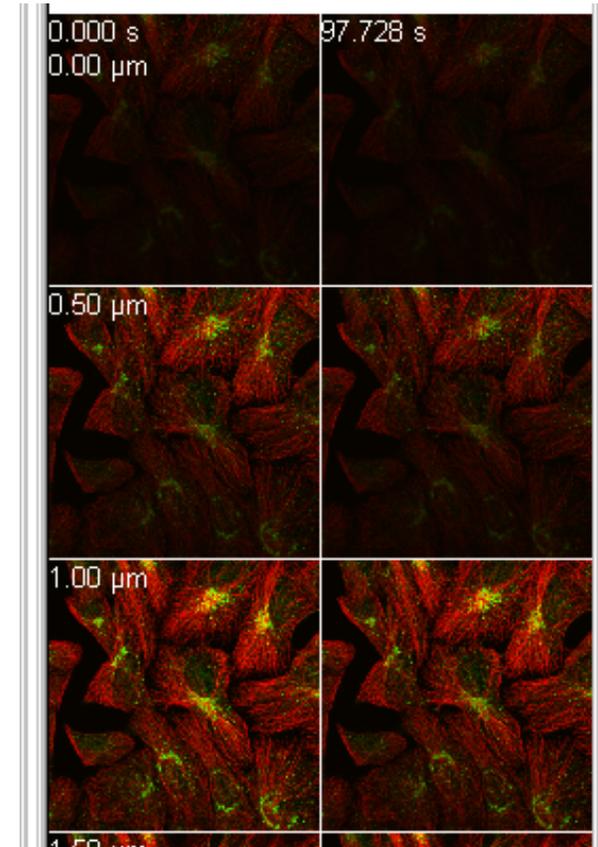
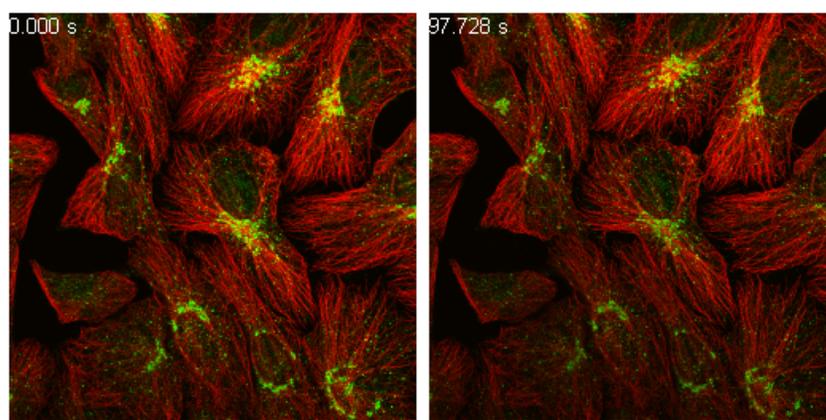
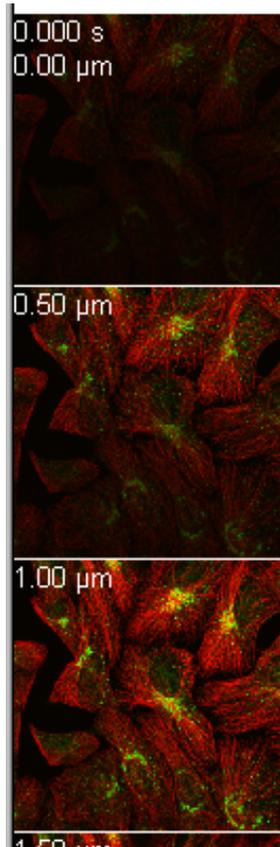
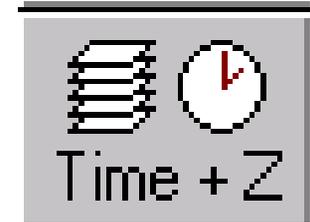
Z Sections
for any time



Time points for
any Z Section



Both Z sections
and time series

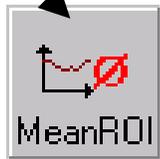


Time Series – Physiology Experiments

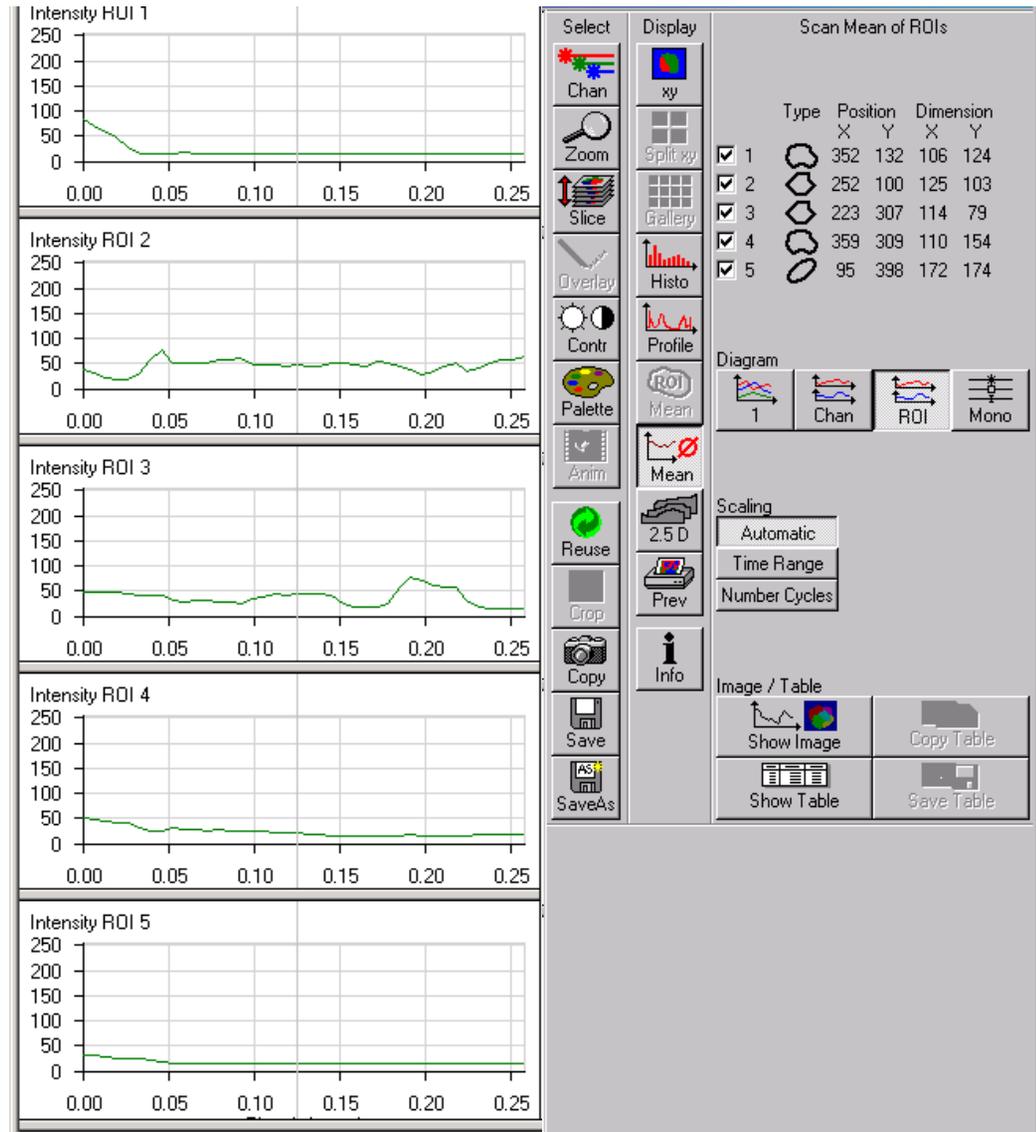
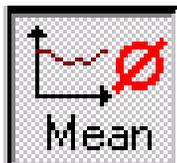
1) If required, use multiple regions of interest

2) Set up Time Series as before

3) Instead of using StartT select MeanROI to start scanning

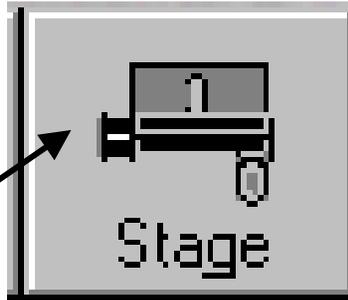


View and save data by selecting *Mean* in the image window



Imaging a large area using *Tile Scan*

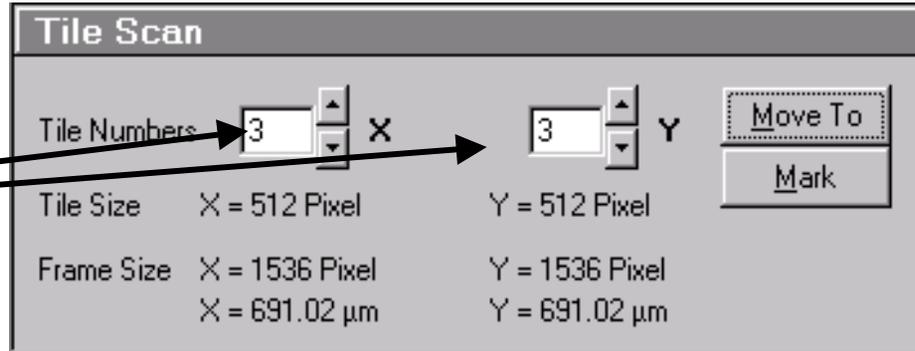
This function is only available with a motorized stage



1) Select *Stage* on LSM menu

2) Enter the *Tile Numbers*

3) Select *Start*



The maximum size is 4096 x4096 pixels

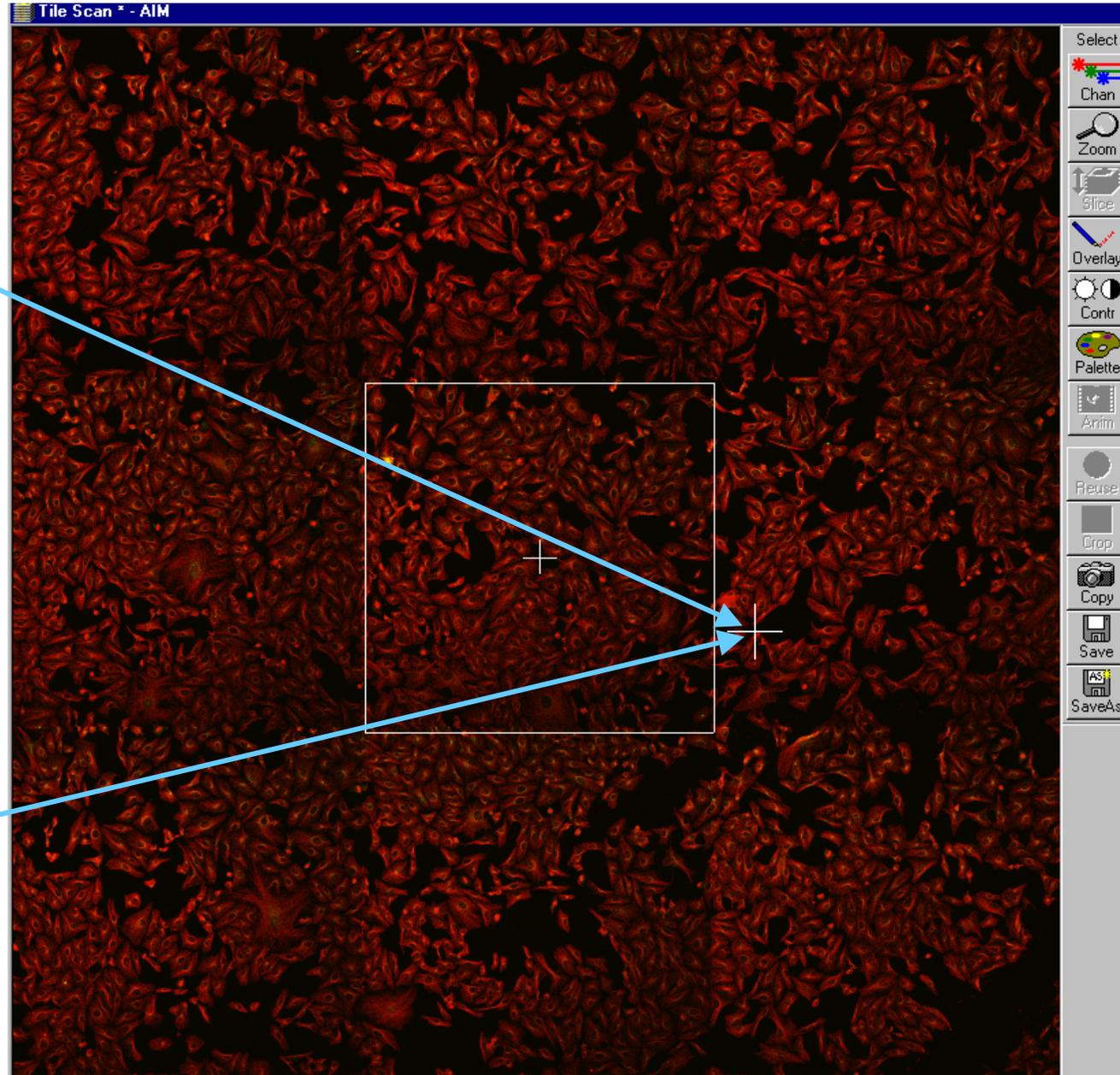
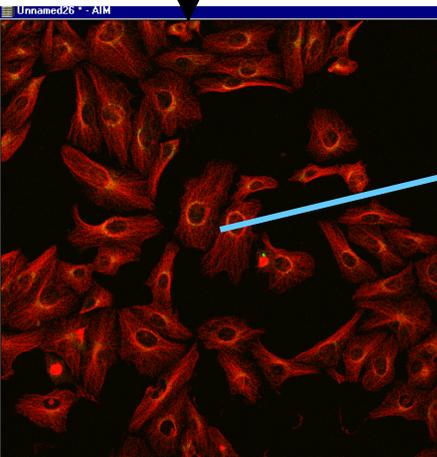
Tiled Image

Any position can then be marked and a single image acquired by selecting *Move to* and then single

Mark

Move To

Single



Contents

- Starting the Zeiss LSM 510 microscope, software and laser
- Selecting an objective and focusing the microscope
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- Acquiring a Z- and Time - Series
- **Data storage**

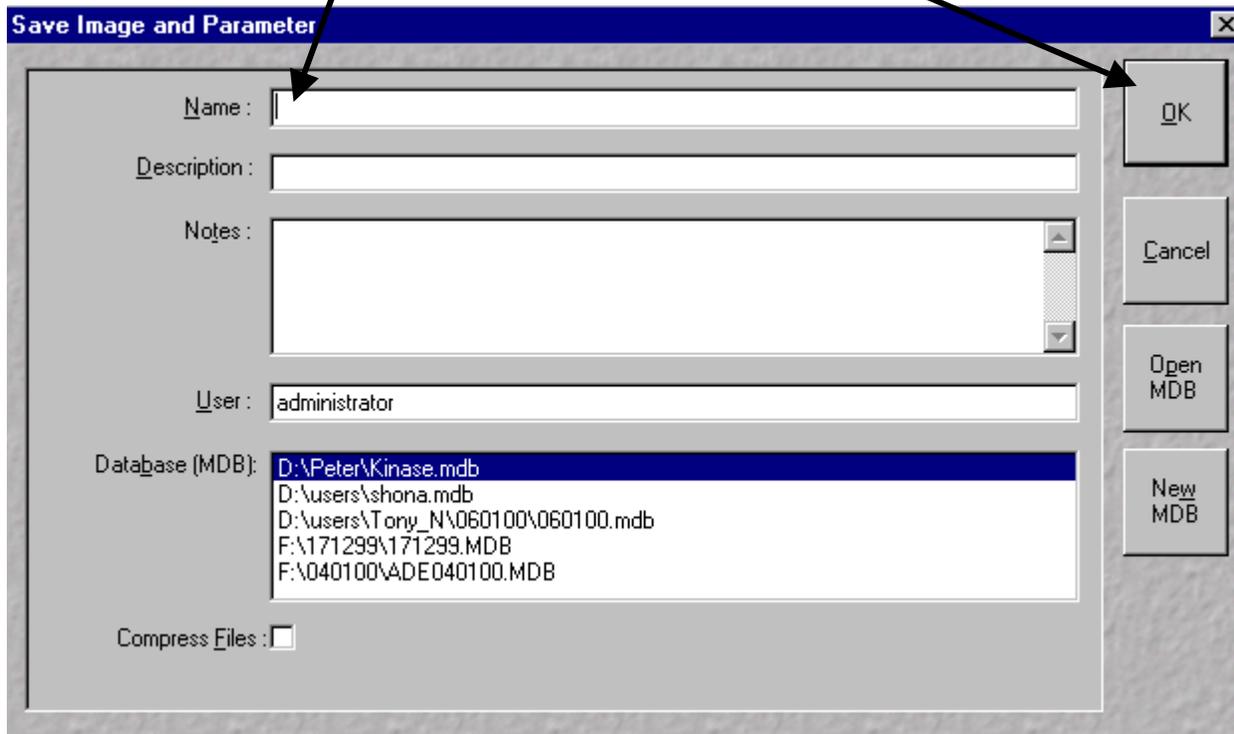
Saving Data - Using Database



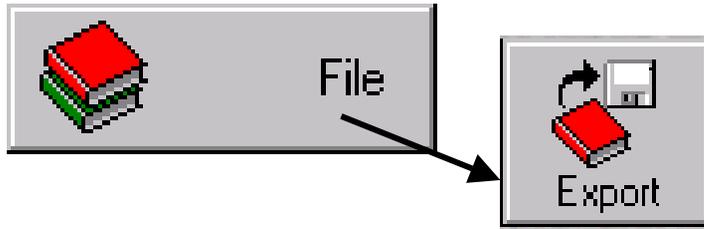
1) Select *Save* or *Save as* on image window or LSM menu bar

2) Enter file name and notes if required

3) Select *OK*

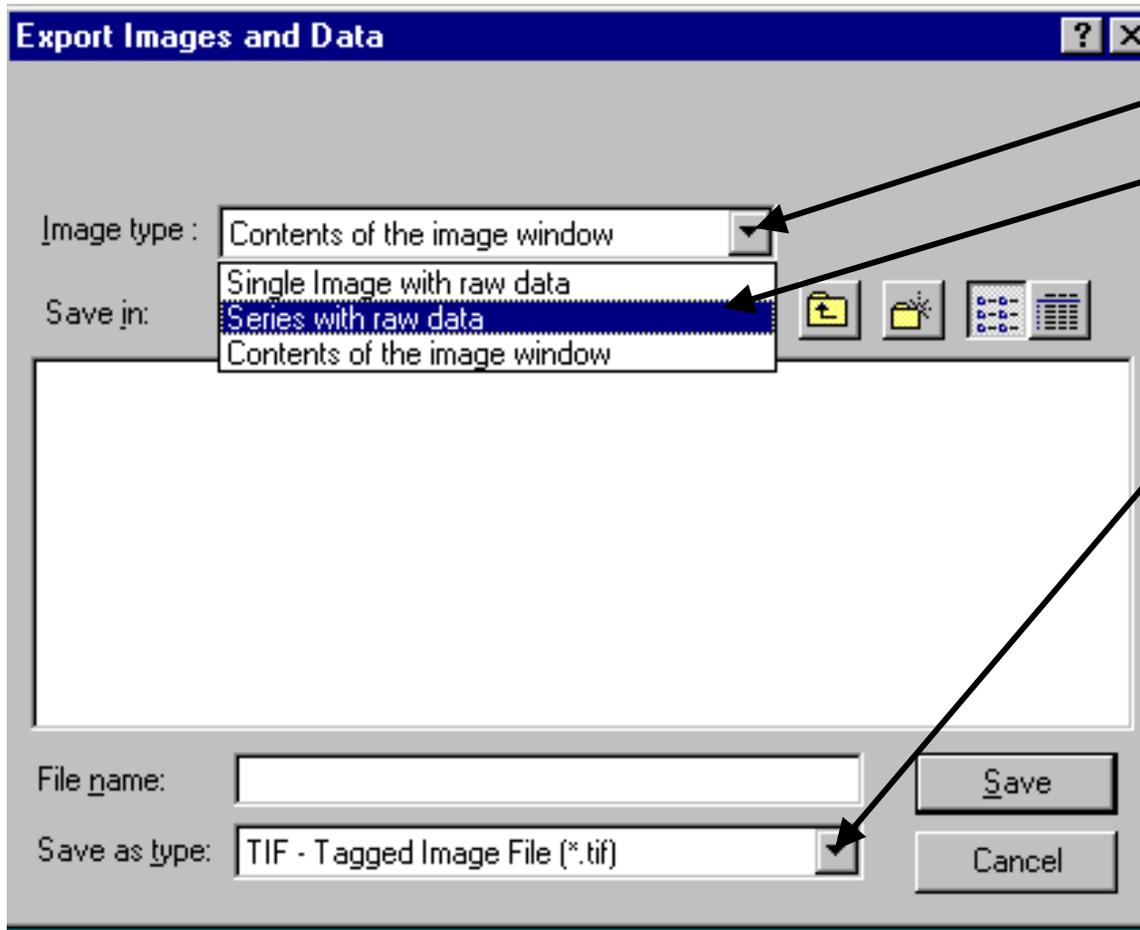


Saving Data – Using *Export*



1) Select *File* from LSM menu

2) Select *Export*



3) Select *Image type*

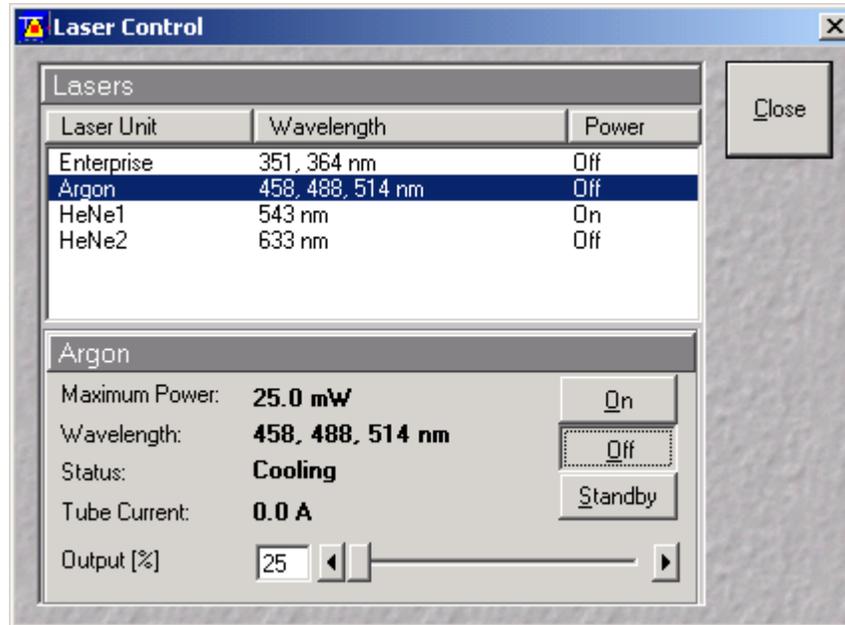
4) Select *Single image with raw data* (No overlay or Look up table etc. is saved), *Series with raw data*, or *Contents of the image window* (Saves the image as shown on the screen)

5) Select *Save as type*

“Tif - Tagged image File” is OK for 8 bit - use “Tiff -16 bit” for 12 bit acquired images (Most other software will not recognize 12 bit)

Shut Down Procedure

1. Go to: *Acquire* in the LSM menu - *Laser* – and deactivate all Lasers by clicking *Off* to switch off Lasers

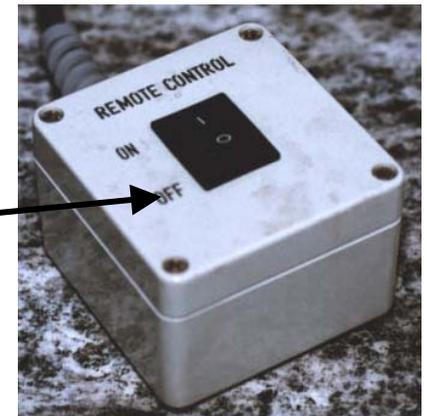


2. Go to *File* - *Exit* to leave LSM 510 program

3. Shut down the computer

4. Turn off the remote control box

5. Switch off the mercury vapour lamp.



**This guided tour is based on
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