LSM 510 Meta
Training Notes

LSM 510 Meta Training Notes

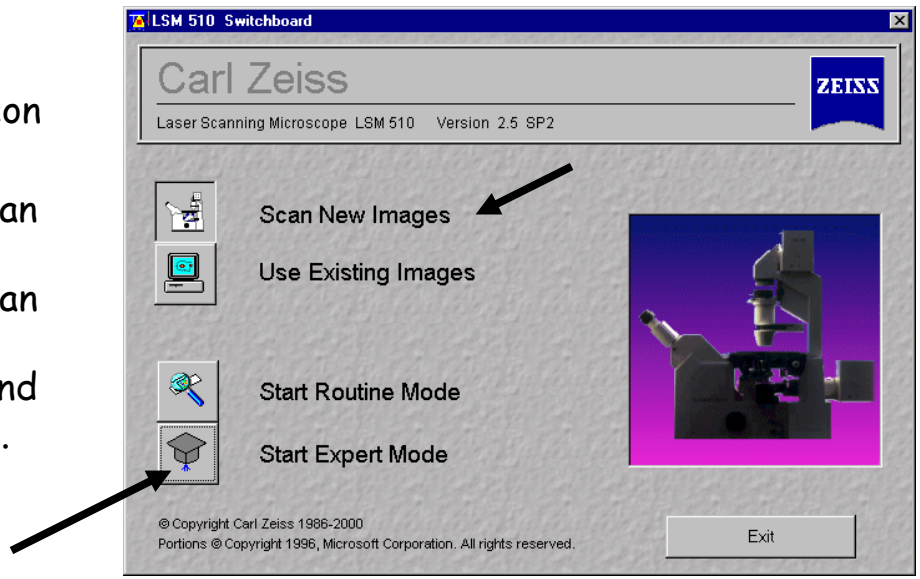
Turning on the system

- Turn on X-Cite power supply. This supplies light for epifluorescence for viewing your samples through the microscope.
- Turn on the remote control switch. This powers up the LSM system.
- Turn on the computer. The power button is located on the upper right hand corner of the tower.
- When prompted, press Ctrl +Alt + Del to login. Enter lab user name and password. Be sure the domain is CCAM. You will be logging on to \\fs3.vcell.uchc.edu\ccam\data\ your_lab

LSM 510 Meta Training Notes

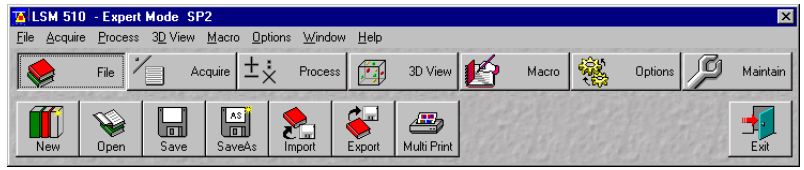
Initializing the software

- Double click on the LSM 510 Meta icon located on the desktop.
- In the LSM Switchboard, select "Scan New Images" and "Start Expert Mode". Note: if you do not select Scan New Images, the software will start but the hardware will not initialize and you will not be able to collect images.



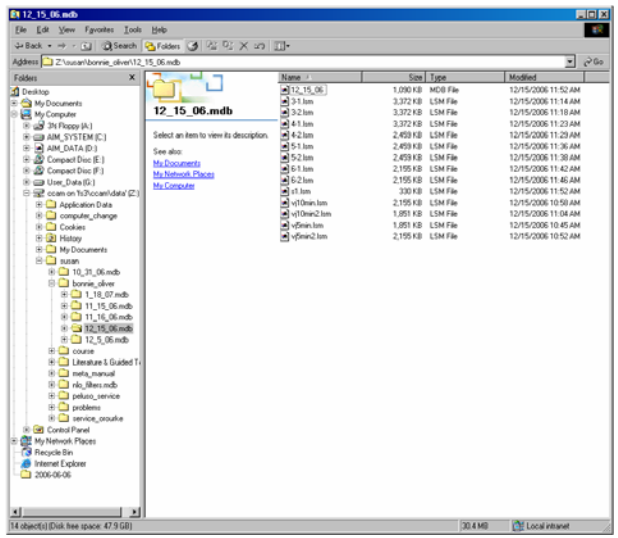
LSM 510 Meta Training Notes

LSM 510 Meta Toolbar



You can easily navigate from the left to right on the toolbar. Selections from the top row will change options available on the bottom row.

Creating/Accessing your database



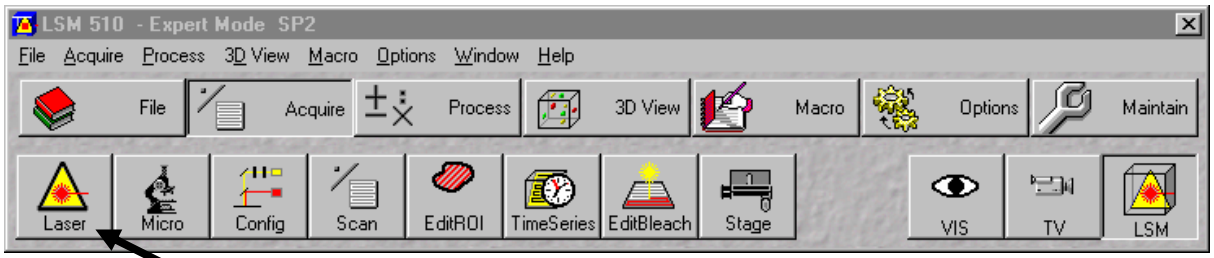
- Select "File", then select "New" to create a new database or select "Open" for an existing database.
- Store all your data on the z partition (//fs3/ccam/data/your_lab) that has been set up for your lab. DO NOT store data on the desktop or in My Documents. Each lab is allowed 50 Gb of storage space. It is up to each lab to manage that space and to be responsible for their own backups.

LSM 510 Meta Training Notes

Database Structure and Accessing Files

- When you create a new database, the software automatically creates a new folder with the database name. In that folder, you will find the database file and your .lsm image files. These files are tiff files with Zeiss acquisition information stored in the header.
- If you want to delete files from your database, do so using the Zeiss software rather than manually deleting the files directly from the folder. Deleting the files manually has created problems with the database in the past.
- You can open the files via the database with the free Zeiss LSM Image Browser, available from their web site, http://www.zeiss.com/micro, or with an ImageJ plugin, the Zeiss LSM Reader, http://rsb.info.nih.gov/ij/index.html. The files themselves can also be directly opened with Photoshop however you must use the "Open As" function and specify that it is a tiff file.
- Be aware that you can have two files with the same name in the database. The software will not prompt you to overwrite the existing file.

LSM 510 Meta Training Notes



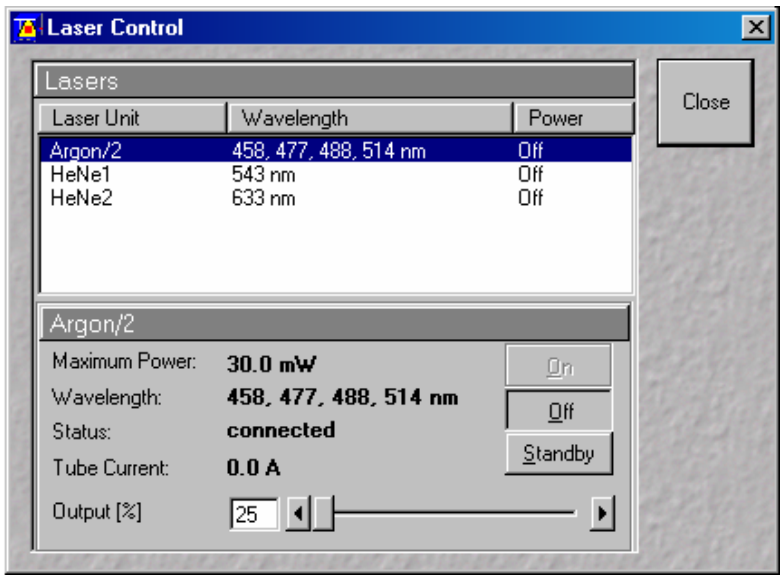
The lasers

- Go to the Acquire tab and select Laser to open the Laser Control dialog that gives you access to the three lasers on the Meta:
  - Argon (30mW) 458,488, and 514 nm
  - HeNe 1 (1.0 mW) 543 nm
  - HeNe 1 (5.0 mW) 633 nm
- Select the laser(s) that would provide the correct wavelength(s) for exciting your specimen.
  - Argon – This laser is fan cooled and has a specific start up and shut down procedure. Select Standby, when Status indicates Ready, click On. Set Tube Current to ~6.1 -6.5 amps. This should correspond to ~ 50% output.

LSM 510 Meta Training Notes

The lasers (cont.)

- HeNe 1 and HeNe 2 – The helium-neon lasers are much weaker lasers than the argon laser and do not require fan cooling. Simply select the laser line required and press On to start the laser.
- Press Close in the Laser Control dialog once you have turned on the required laser(s).

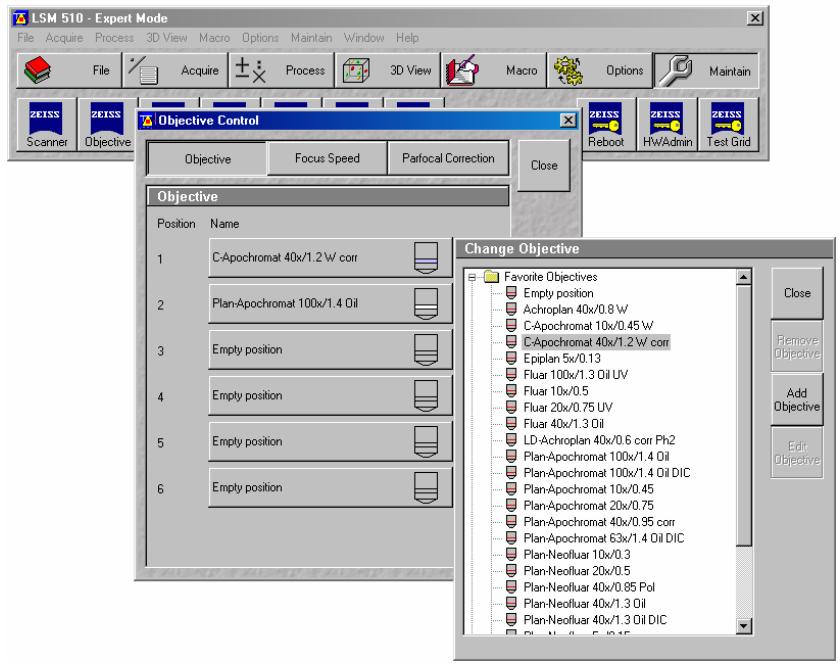


LSM 510 Meta Training Notes

Selecting Objectives

It is easiest to define the objective(s) you will be using in the software, and then use the Microscope Control dialog to select the correct turret position.

- Press Maintain>Objective to open the Objective Control dialog. Click once to select a turret position, 1-6.
- In the Change Objective dialog, find the objective you are using and double click your selection. Repeat this procedure for any other objectives you may have.
- Close both dialogs once you have made your selections and return to the Acquire tab.



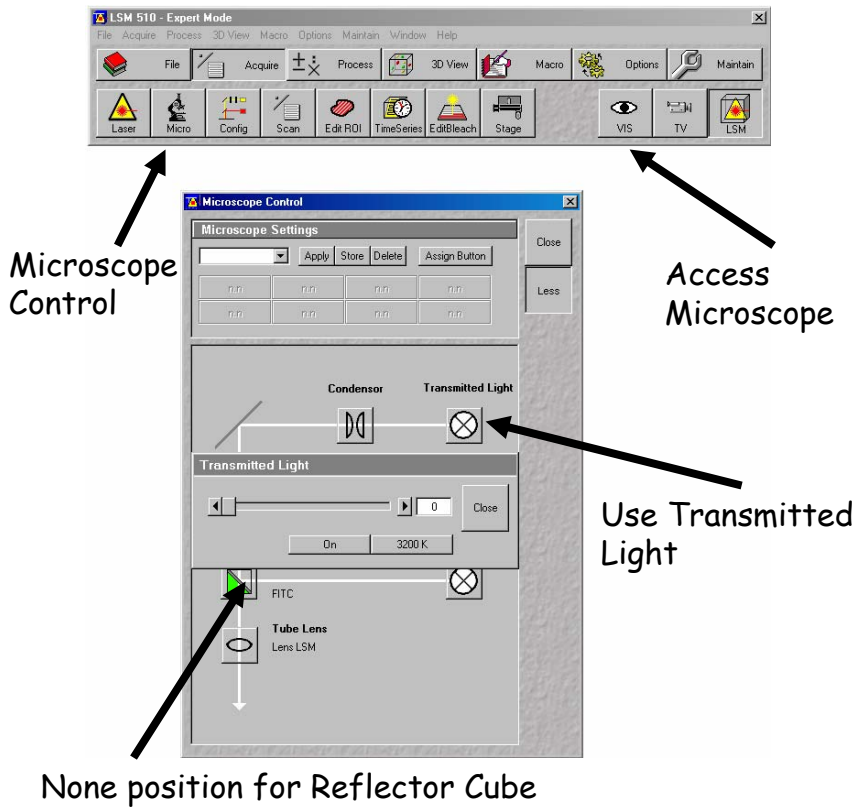
LSM 510 Meta Training Notes

Microscope Control

Transmitted Light Control

On the Acquire tab, press VIS to allow you to look through the microscope, and then press Micro to open the Microscope Control dialog.

- Press the Objective icon, and select your objective from the pull down menu.
- Make sure the Reflector Cube is in the None position.
- Press the Transmitted Light icon to access the control dialog. Press On and move the slider to adjust the intensity of the bulb. BE CAREFUL WITH THE INTENSITY SLIDER. Deselect On to turn the bulb off.



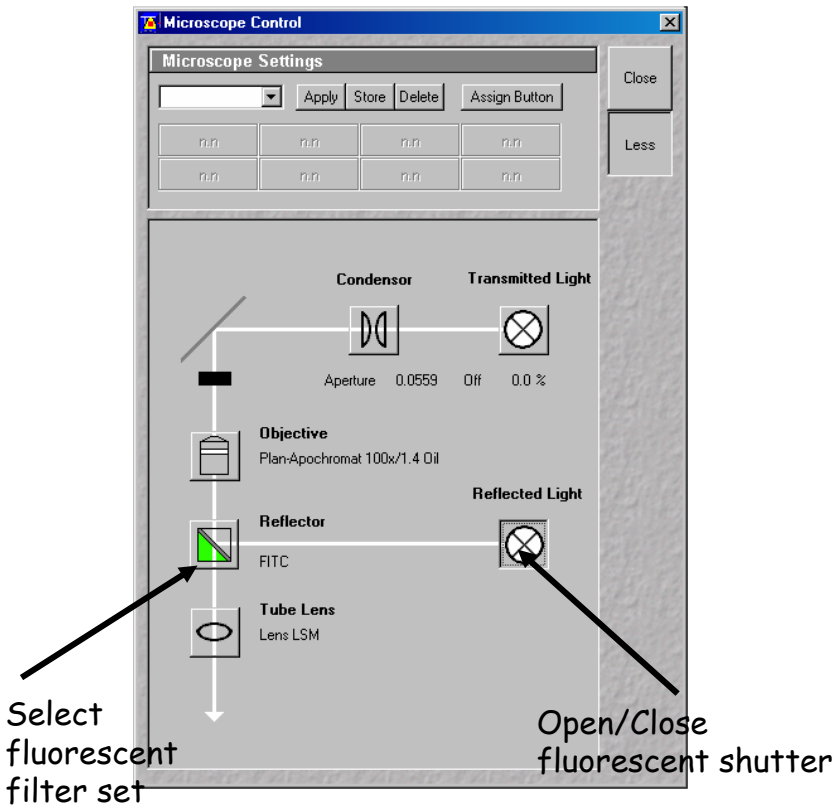
LSM 510 Meta Training Notes

Microscope Control (cont.)

Fluorescent Light Control

You should still be in the VIS position on the Acquire tab, and have the Microscope Control dialog open.

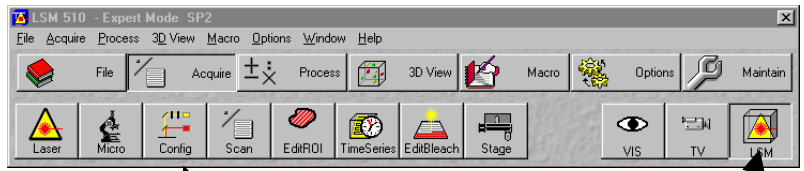
- Press the Objective icon, and select your objective from the pull down menu.
- Press Reflector to select the appropriate fluorescent filter.
- Press the Reflected Light icon to open the fluorescent shutter on the microscope. Deselect the Reflected Light icon once you have finished viewing your sample to close the shutter.



LSM 510 Meta Training Notes

Using the Confocal

- On the Acquire tab, press LSM to access the confocal.
- Press Config to open the Configuration Control dialog. Here you will choose between Multi and Single Track configurations and make your laser, filter and detector selections.
- All configurations can be stored for future use.

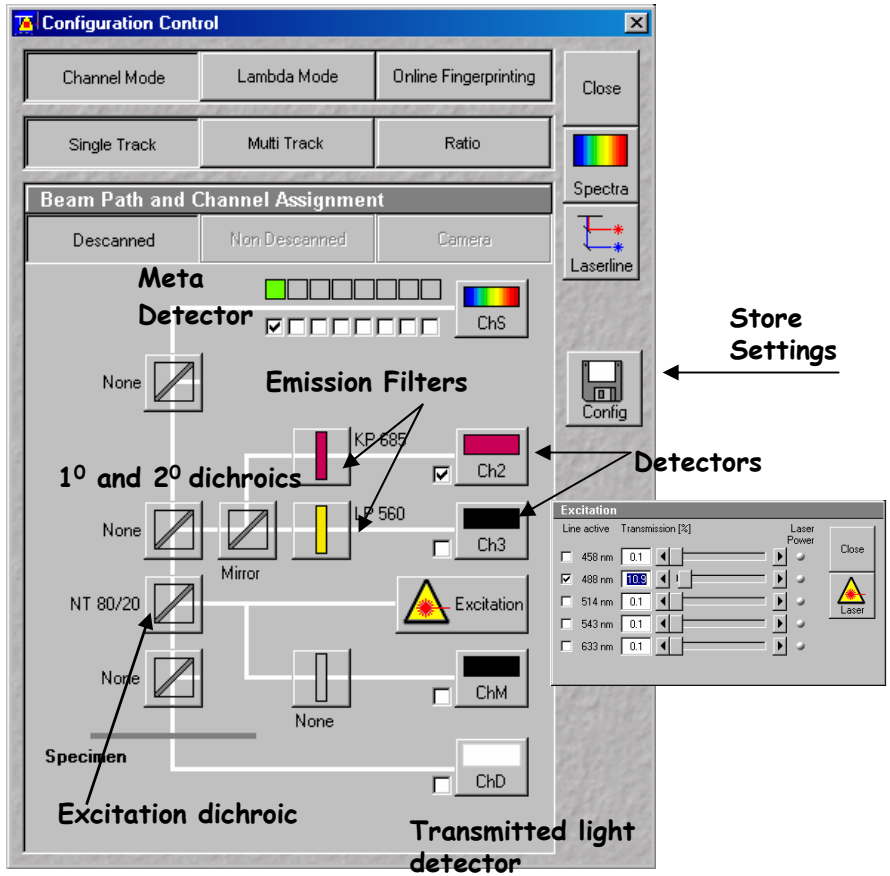


Single Track	Multi Track
Collects one probe or multiple probes simultaneously.	Collects multiple probes sequentially; by line or by frame.
Does not prevent bleed through between channels.	Avoids bleed through between channels
Better for sample because of reduced exposure to laser.	Exposes sample to increased laser light due to multiple scans.
Faster acquisition	Slower acquisition

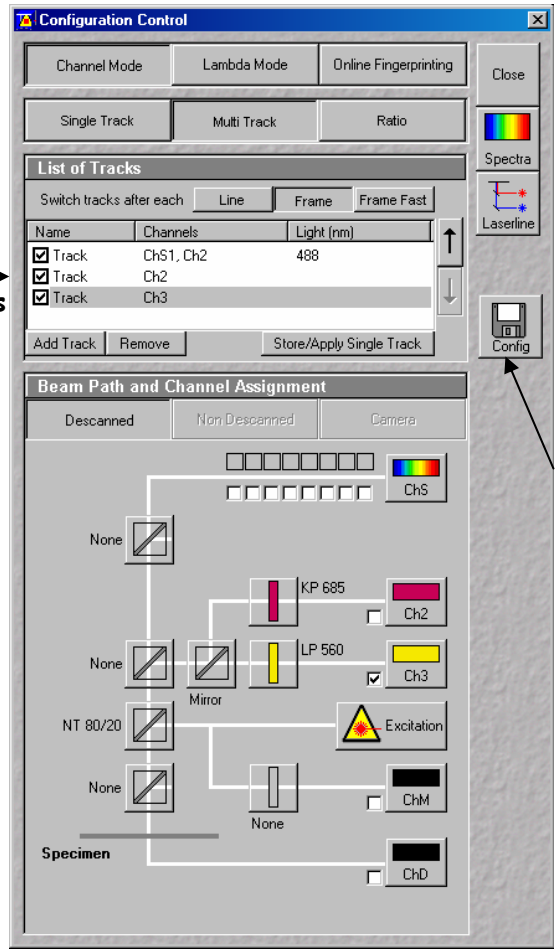
LSM 510 Meta Training Notes

Single Track

- Select Channel Mode for normal scanning and Single Track. Follow the excitation/emission pathway in the image to define your configuration.
- Press Excitation to select laser line and % transmission.
- Press and select an Excitation dichroic; this selection depends on the laser lines being used to excite the sample.
- Select 1^0 and 2^0 dichroics; selection is based upon wavelengths you want to capture. Dichroics reflect short wavelengths and pass long wavelengths.
- Activate detectors; ChS, Ch2 or Ch3 and select psuedocolor for display. ChD is for transmitted light.



LSM 510 Meta Training Notes



Activate tracks

Store settings

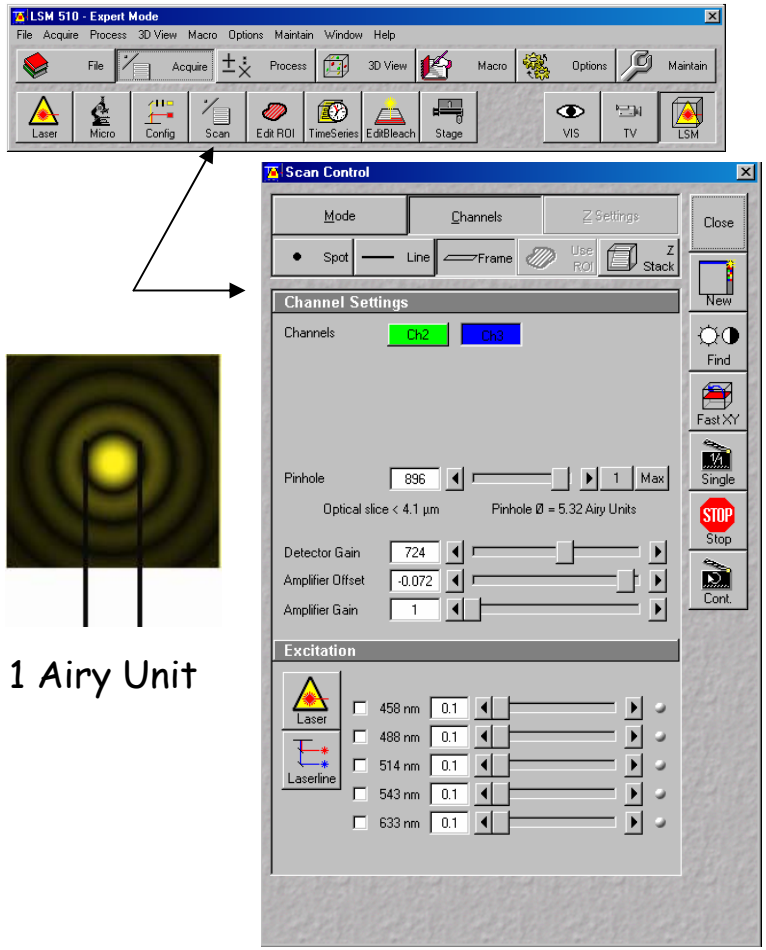
Multi Track

- Select Channel Mode and Multi Track.
- Press Add Track for each fluorescent probe.
- Select excitation line, dichroics, emission filters and detectors as explained for Single Track configurations.
- Activate the tracks by selecting a check box.

LSM 510 Meta Training Notes

Scanning Parameters (Channels)

- Under the Acquire tab, select Scan to access the Scan Control Dialogue.
- Select the Channels tab, set pinhole for each channel. Start at 1 Airy Unit for best resolution. Make sure multiple channels have the same Optical Slice; adjust pinhole as necessary.
- Adjust the Detector Gain, maximum sensitivity, and the Amplifier Offset, minimum intensity, for image settings.

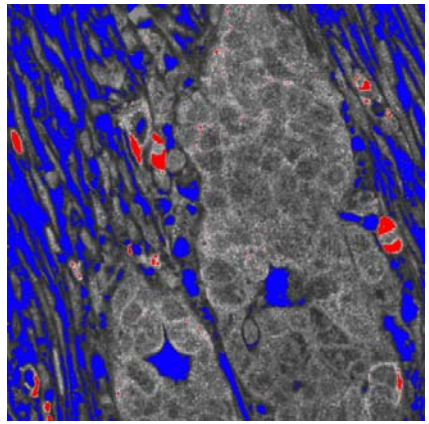


1 Airy Unit

LSM 510 Meta Training Notes

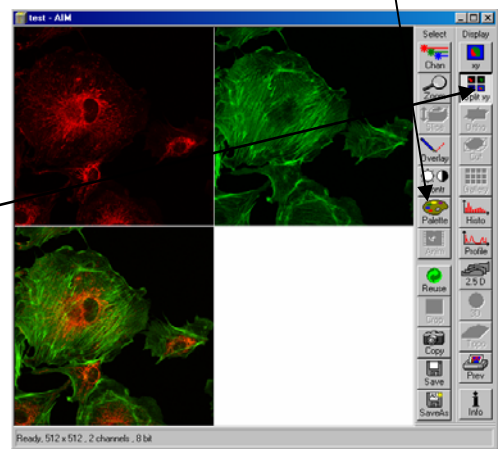
Scanning Parameters (Channels cont.)

- Choose Range Indicator from Palette, on the image display window, to adjust the gain and offset. Blue= minimum, Red= maximum (0 and 255 for 8 bit image, 0 and 4095 for 12 image.)
- Adjust Detector Gain and Amplifier Offset to remove red and blue pixels. Minimum should be ~ 10-20 gray levels above 0 and Maximum should be 10-20 gray levels below 255.
- Use Split x,y to view channels separately in single track. In Multi Track you can deselect a channel in Configuration Control to scan them one at a time when adjusting the settings.

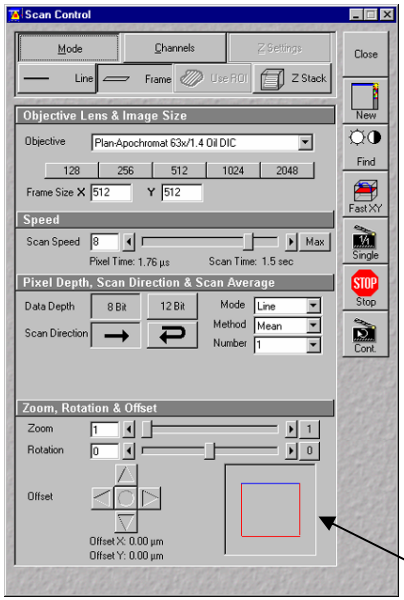


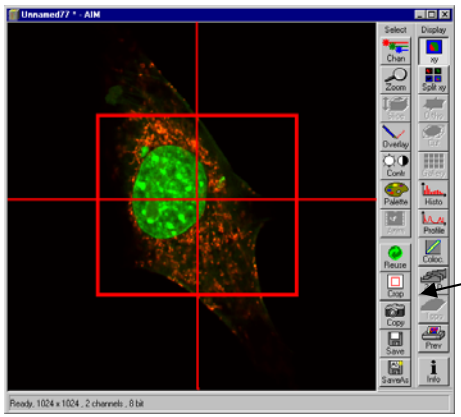
Palette->Range Indicator

Split x,y



LSM 510 Meta Training Notes





Scanning Parameters (Mode cont.)

- Switch to the Mode tab to gain access to:
  - Scan Speed – slower scan speed will improve image quality
  - Pixel Depth – choose from 8 (256 gray levels) or 12 (4096 gray levels) bit data depth. 12 images are recommended for publication and for analysis.
  - Scan Averaging – select from Line or Frame mode, Mean or Sum method and Number of Line or Frames.
- Optical Zoom and Rotation – Use the Crop tool on the image display window to set zoom and rotate image.

LSM 510 Meta Training Notes

Nyquist Sampling

- In order to preserve the spatial resolution in an image the sampling rate should be twice that of the maximum frequency.
- Adjust the optical zoom in order to achieve the optimal x,y pixel size.

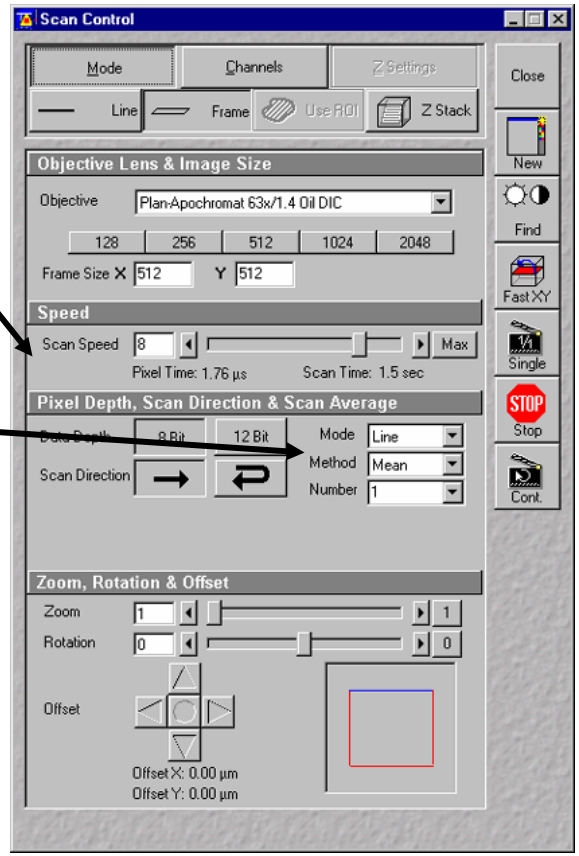
Objective	Numerical Aperture	Immersion	Coverslip Thickness (mm)	Working Distance (mm)	Z Resolution (μm) (1 Airy Unit)	*Optimal Z-Step (μm)	**XY Resolution (μm)	*Optimal XY pixel size	***Optimal Zoom
40x C-Apochromat	1.2	Water	.14-.18	.22	.7	.35	.25	.13	3.5
40x Plan-Apochromat	.95	Air	.31 - .21	.16	.8	.41	.31	.16	2.9
63x Plan-Apochromat	1.4	Oil	.17	.18	.6	.29	.21	.11	2.5
100x Plan-Apochromat	1.4	Oil	.17	.09	.6	.29	.21	.11	1.6
10x Plan-Neofluar	.3	Air	.17	5.6	9.0	4.48	1.0	.5	3.6
20x Plan-Neofluar	.5	Air	.17	2.0	3.2	1.59	.60	.3	3.0
63x Plan-Neofluar	1.25	Oil	.17	.10	.7	.37	.24	.12	2.3
10x Fluar	.5	Air	.17	2.0	3.2	1.59	.60	.3	5.9
40x Fluar	1.3	Oil	.17	.14	.7	.34	.23	.12	3.7

LSM 510 Meta Training Notes

- Improve image quality
  - Slow down the scan speed to allow for longer pixel dwell time.
  - Averaging to improve signal to noise ratio
    - Line or Frame average
      - Select Method: mean or sum
      - Select number for averaging

Decreased scan speed and line averaging result in increase laser exposure.

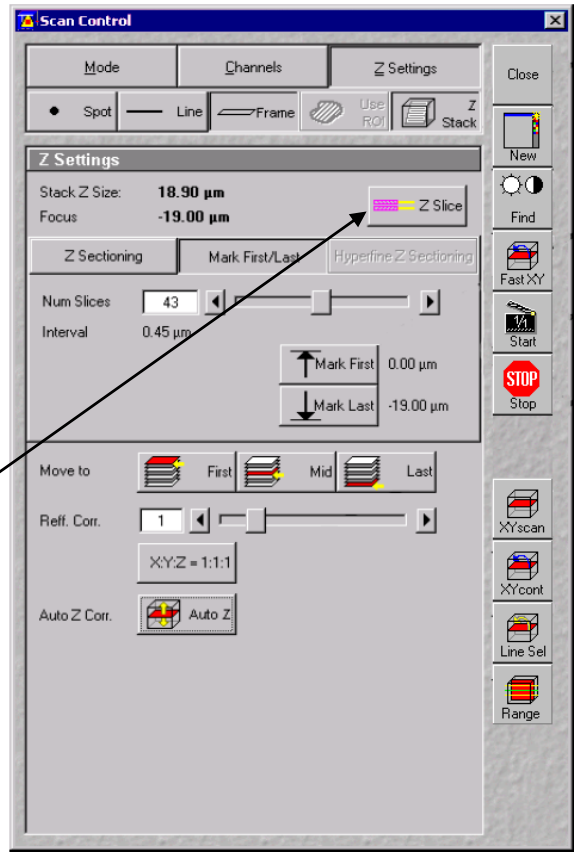
Frame averaging reduces photobleaching but does not produce such a clean image.



LSM 510 Meta Training Notes

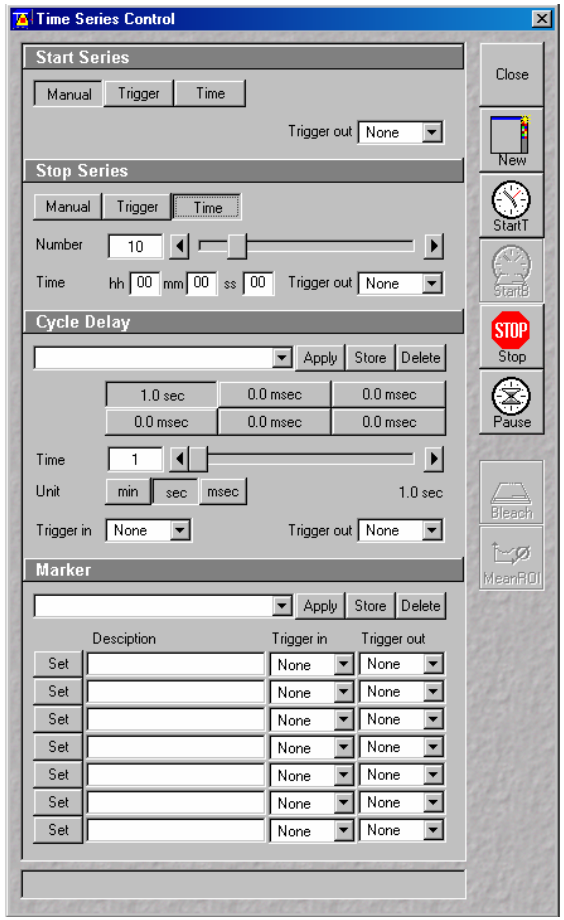
Z-Stack Settings

- Adjust settings at optimal plane of focus.
- Select Mark First/Last tab
- Use the fast scan setting to focus down through image until image fades; press Mark First.
- Use the fast scan setting to focus up through image until image fades; press Mark Last.
- Select Z-Slice and then press Optimal Interval to ensure correct sampling. The Z-slice is calculated to be ½ of the Z-resolution (Optical Slice on Channels tab.).
- Press Start to begin collecting the stack. The entire stack of images will be saved as one file.



Note scan buttons are different under Z-settings

LSM 510 Meta Training Notes



Time Series

- Start Series – Select manual start, press the StartT icon.
- Stop Series – Select either Manual or Time.
  - Manual – specify number of cycles you wish to capture
  - Time – calculate duration of time series in addition to the number of cycles you wish to capture. Remember to include scan time when calculating the duration.
- Cycle Delay – Enter time of delay between each image capture. Select minutes, seconds or milliseconds (optional).
- Marker – selection for fixed stage positions (optional).

Files are as saved as a single time series file.

The time series may be used in conjunction with the z-series. This should be initiated with the Time Series and not the Zseries.

LSM 510 Meta Training Notes

Shutdown Procedure (Final User)

- Open the Laser Control Dialog.
  - Argon – Lower power, select Standby, select Off. Wait until cooling cycle ends, ~ 3 minutes, Status will be reported as Connected.
  - HeNe 1 and HeNe 2 – Select Off.
  - Close Laser Dialog, close all LSM windows and exit software.
  - Shutdown computer
  - Turn off Remote Switch
  - Turn off Arc lamp.

Shutdown Procedure (Between users, verify if next user is coming if possible. If in doubt, shut it down.)

- Argon – Lower power, select Standby.
- HeNe 1 and HeNe 2 – Select Off.
- Close all LSM windows, exit software.
- Log off from computer.