Confocal Microscope usage manual
ZEISS LSM 510 Meta

Detailed Summary

1 - Powering up the confocal microscope Zeiss LSM 510 Meta

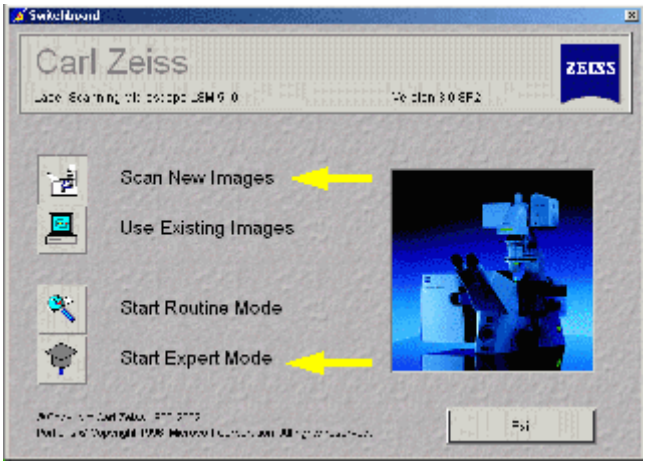
- Switch on the mercury arc lamp (HBO) – before turning on "Remote Control" switch.
- If conventional fluorescence is not required there is no need to turn on the HBO Lamp.
- Let the lamp strike (about 15-30 seconds) and ensure light is not flickering.
- If HBO hours are greater than 200 hours please inform facility staff and put note in log book.
- The computer should be already on.

- If necessary switch it on.

2 - Starting the LSM 510 Software

- Logon with user name, password and domain name set to « CIF »
- Double click on the "LSM 510 META" icon.

- Choose "Scan New Images".

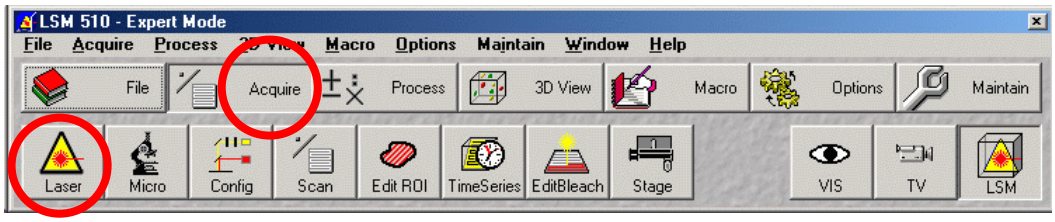


Then "Start Expert Mode".

The software is now started. You have now to configure the system for your own acquisitions.

3- Powering up the lasers

- The « expert mode » window appears - Choose "Acquire".



- Press the "Laser" button – Start only the laser(s) required for the fluorophores used in your sample.

- If the Argon laser 458, 488, 514 nm (blue excitation for FITC, GFP, YFP, Bodipy, CY2, etc.) is to be started :

  - Select "Laser unit Argon/2 "
  - First go to standby and wait until the text says "on line"
  - Then click on "on"
  - Adjust the Output level (%) between 40% and 50%
  - Running higher Output (%) is generally not necessary
  - Check that the cuurent does not go beyond 8.1 Amps

- If the HeNe1 laser 543 nm (green excitation for Rhodamine, Texas Red, CY3, Alexa 543/568, etc.) is to be started:

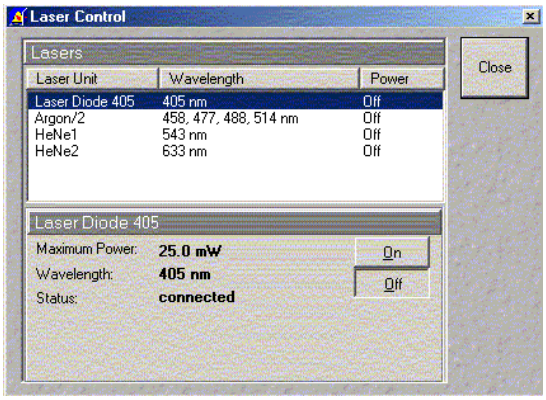
  - Select "Laser unit HeNe1 543 nm"
  - click on On

- If the HeNe2 laser 633 nm (red excitation for CY5, Alexa 633, etc.) is to be started:

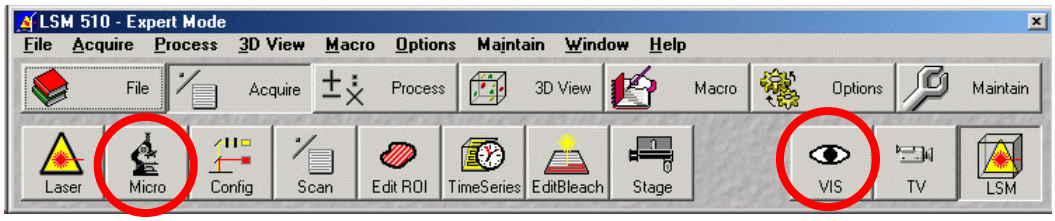
  - Select "Laser unit HeNe2 633 nm"
  - click on On

- If the Diod laser 405 nm (DAPI, Hoechst, etc.) is to be started:

  - Select "Laser unit Diode 405 nm"
  - click on On

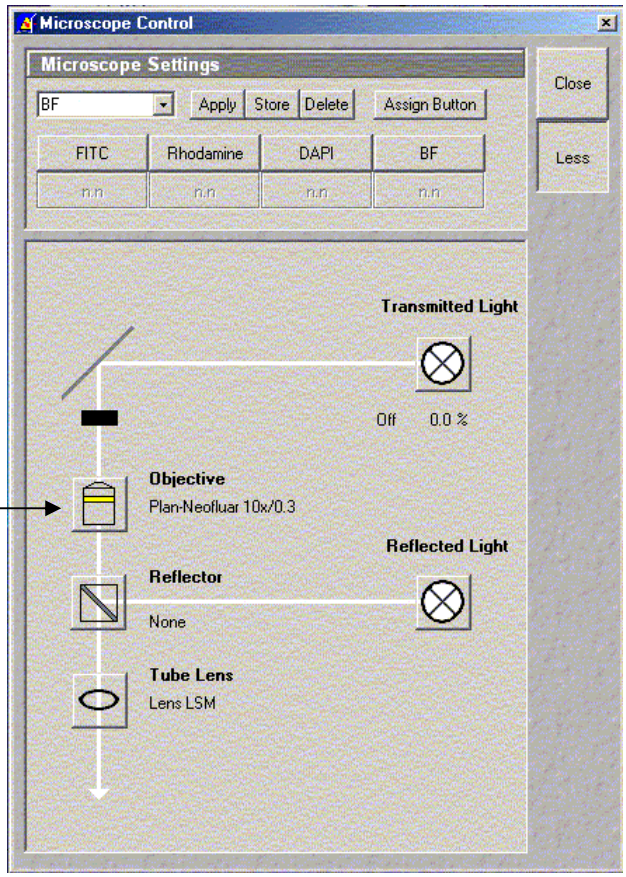


4 - Getting ready for viewing through eye pieces



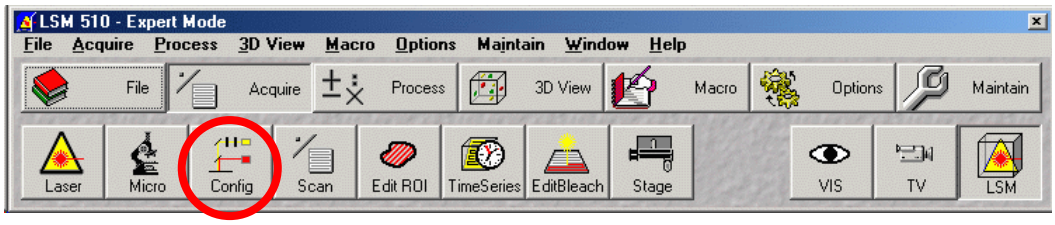
- Press the "Micro" button and then the "Vis" button
- Select the viewing mode : FITC, Rhodamine, DAPI, or BF (Bright Field)
- (N.B. If these buttons are blank please contact the CIF staff)
- Objectives may also be changed.
- Focus on sample and check fluorescence

If your sample has been viewed with an oil immersion objective , do not forget to clean the slice BEFORE switching to a dry objective !

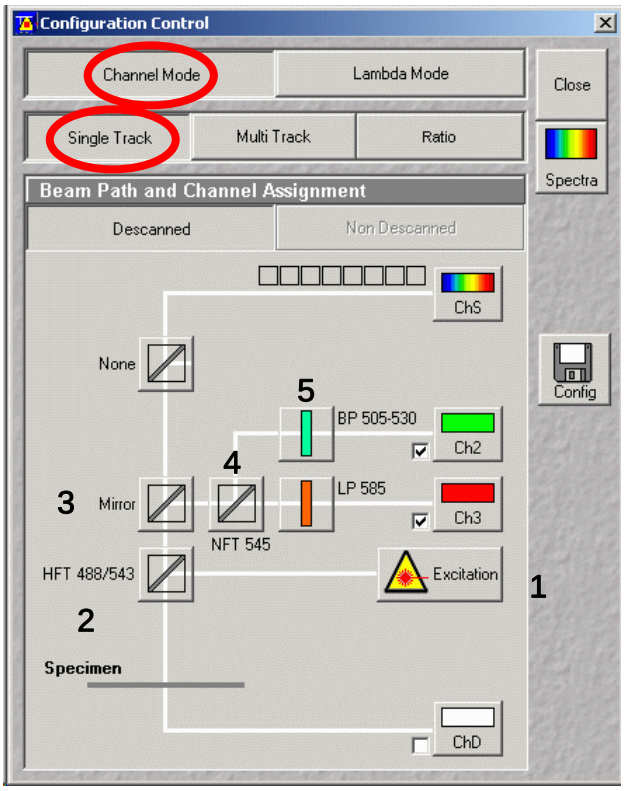


5- Setup of the scanning configuration – Choosing filters for fluorophores in sample

- Press the "Config" button (see picture below)

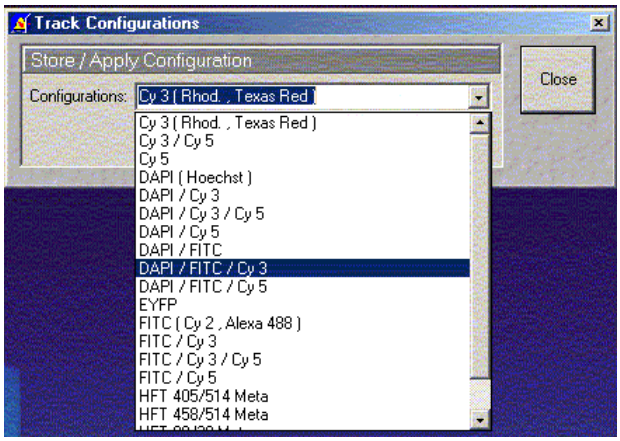


- For standard PMT detection select the "Channel Mode" button
- For single fulorescence channel detection or simultaneous double channel detection choose the "Single Track" button

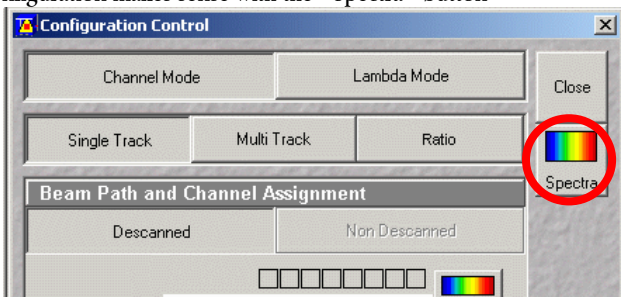


- At this point you can choose an existing configuration, or create your own one. To use an existing configuration :
  - Press the « Config » button

  - Then choose the appropriate configuration in the pull-down menu

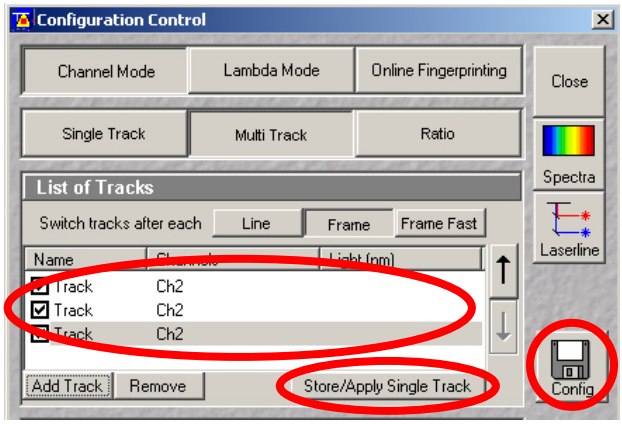


- To create your own configuration :
  - Select the different object you want to put in the light path (filters, dichroïc mirrors, see labels on the screenshot)
  - Check that your configuration makes sense with the « Spectra » button



- For a multi-channel detection :
  - Select the "Multi Track" button (NB: good choice for colocalisation trials to avoid crosstalk artefacts)

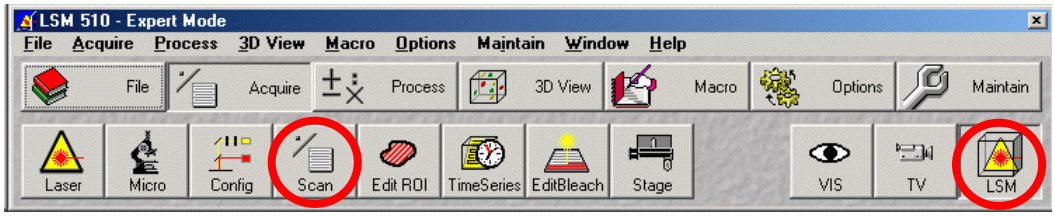
  - Create ONE configuration oer channel and give them an appropriate name in the box designed for this purpose :



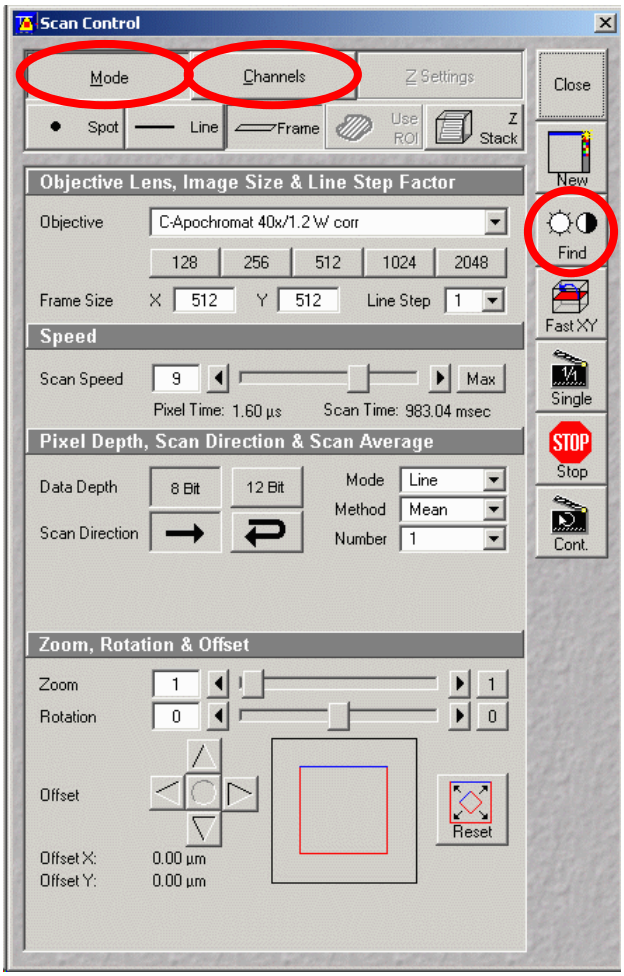
You can choose to apply a « single track » configuration to each track by clicking the button « Store/Apply Single Track », or use an existing multitrack configuration by clicking the « Config » button.

- NB : Many other scanning options are available such as :
    - Spectral detection (called Meta by Zeiss)
    - Sequencial detection with the Meta channel and PMT channels

6 - Scanning and detection tuning



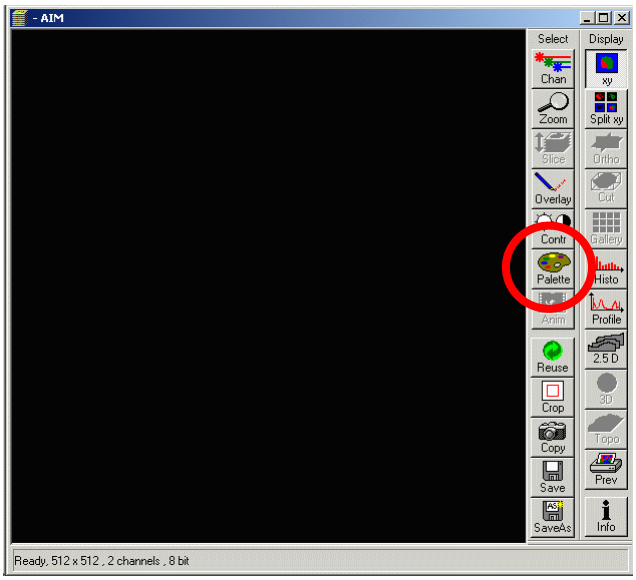
- Switch to the LSM mode by clicking on the « LSM » button
- Open the Scan Mode window by clicking on the « Scan » button
- Press the "Mode" button
- Tune the following parameters :
    - Select "Frame" (from spot - line - frame)
    - Choose the image size (size in pixels, e.g : 512x512 ou 1024x1024), begin with 512x512
    - "Scan speed" : leave the default value as it is this time
    - "Pixel depth" (8 bit) : leave this value to 8 bit you do not plan to do quantitative analysis.
    - Mode: choose "Line", or "Frame" for noise reduction in the picture
    - Method (Mean) : Leave the default value.
    - Number (tipically 1, 2 or 4, depending on noise amount and signal intensity in the picture)



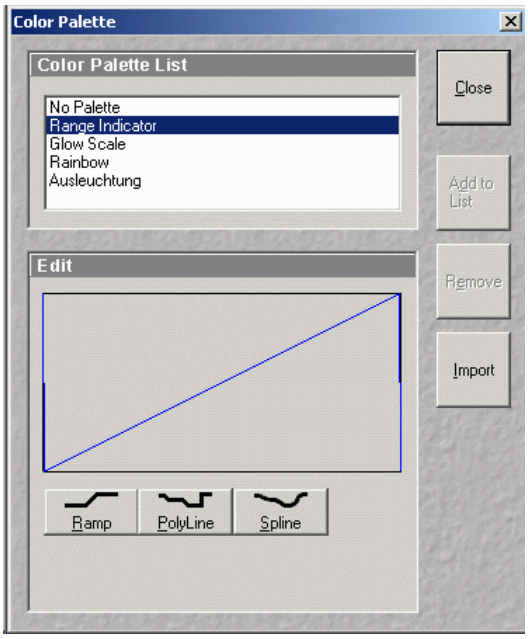
- Select the "Channels" buttons
- Tune the following parameters :
  - Select a Pinhole of 1 (for now)
  - Set the 488 nm excitation laser slider to ~ 0.05% - 5% de transmission (typically 2%)
  - Set the 543 nm ou 633 nm laser sliders to 30-40%
  - Set the 405 nm laser slider to 1-5%

7 - Acquisition Image optimisation

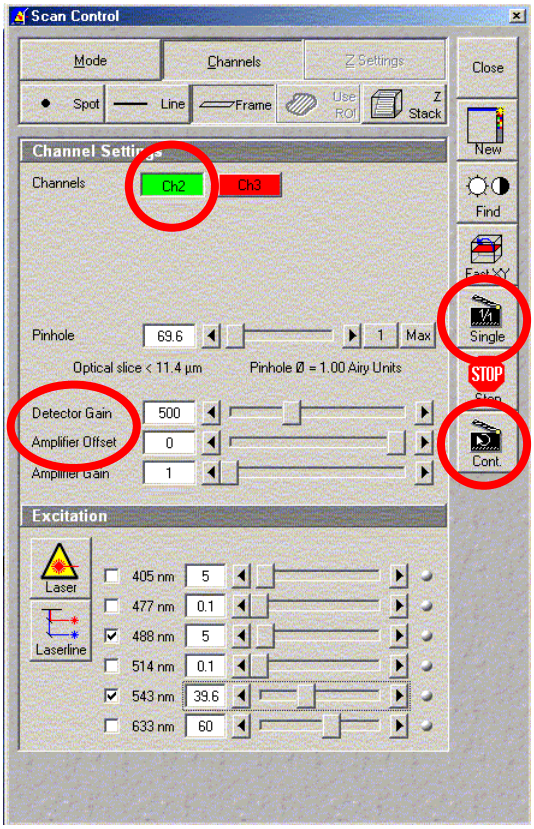
- Use the "Find" button to automatically find gain and offset values for the detector for each channel individually. These settings will then have to be optimized.



- Press the "Palette" button in the image window
- Select "Range Indicator" - red pixels = saturated - blue pixels = zero value

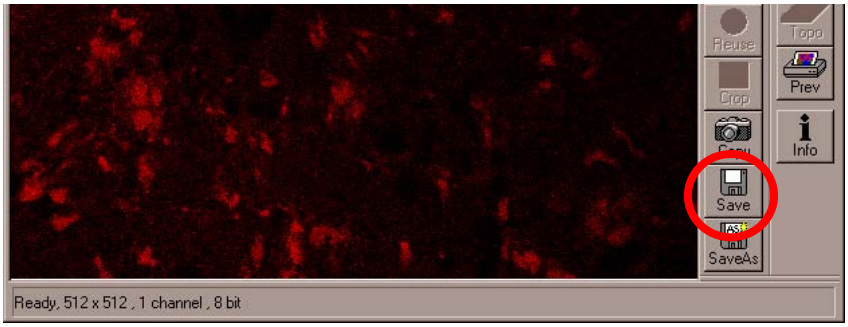


- Press "Cont." for continuous scanning
- Choose a "Channels" button (that's Ch2 or Ch3)
- Set the "Amplifier Offset" slider to a value where blue pixels become rare ("black clipping").
- Set the "Detector Gain" slider to a value where red pixels become rare too ("saturation").
- Click on the "Single" button for a full resolution image with meaning

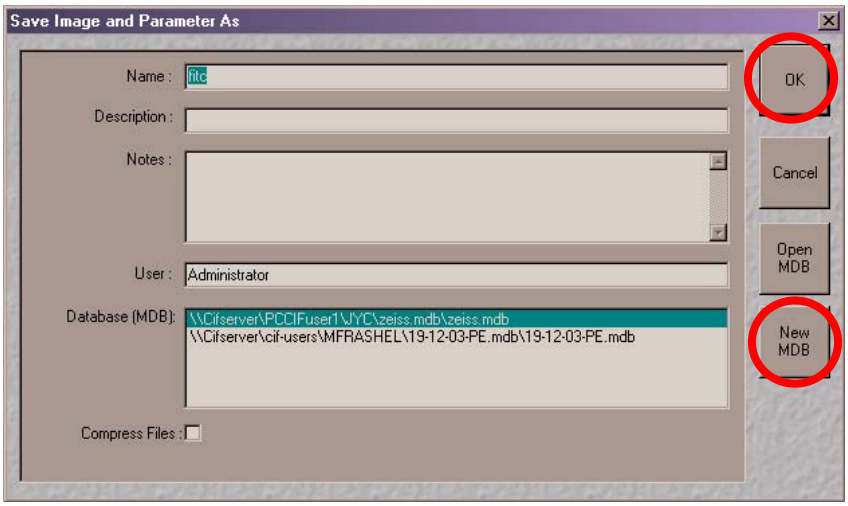


8 – Saving images

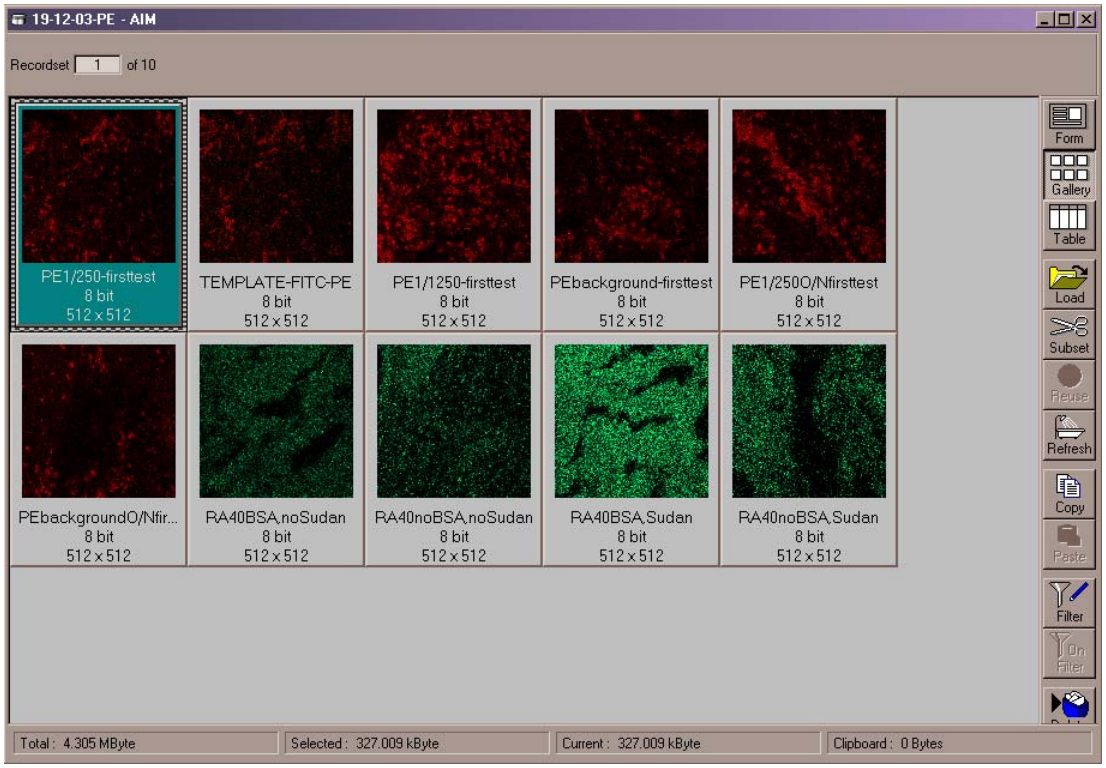
- Click on the « save » button on the image window



- If you haven't created any database yet, create one by clicking on the « MDB » button – The MDB MUST be created in your personnal directory (\\Cifserver\CIF-USERS*yourlogin*\)

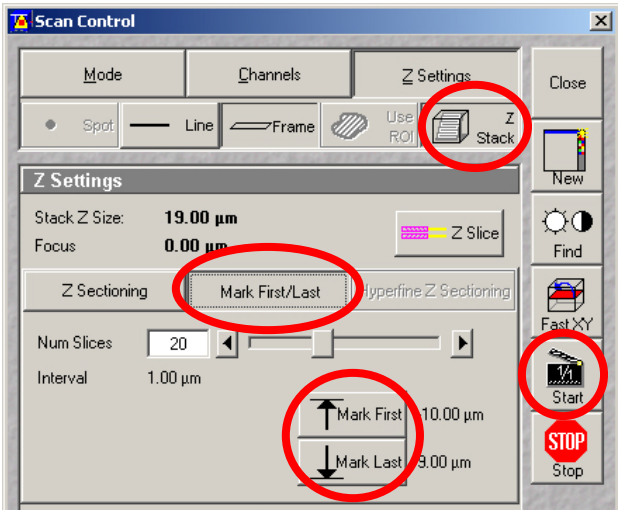


- Then save your image by filling the fields and choosing your database, then click «OK »
- Your images are then stored and visible in the database pannel :



9 – Acquire a Z-stack

- After having chosen a field of interest and fine tuned your image, choose Z-Stack then «Mark First/Last » :



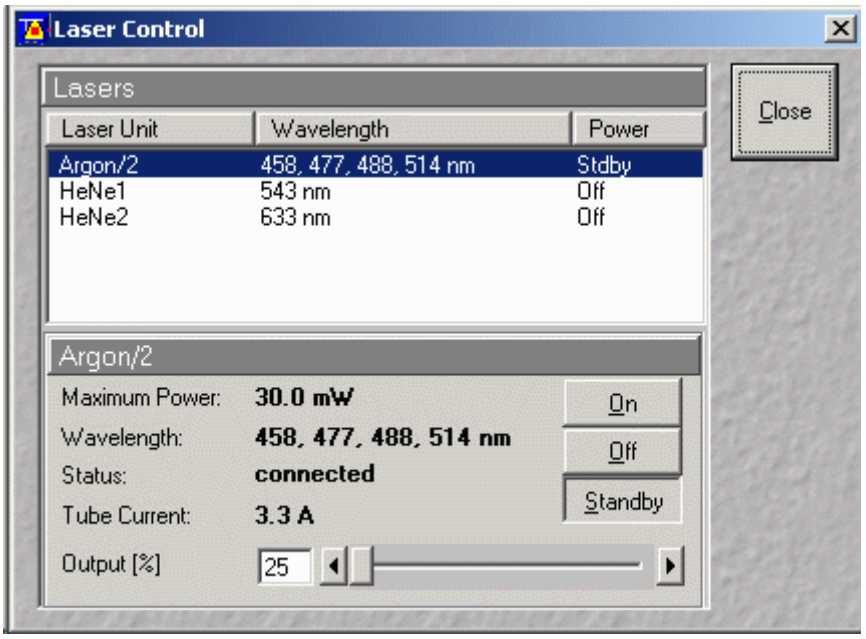
En mode Z Stack le bouton « Single » devient le bouton « Start ».

- Go to « Fast XY » mode and move the focus in one direction until there is no signal anymore, and clcik on « Mark First ».
- Move the focus in the reverse direction until there is no signal anymore and click on « Mark Last ».
- Select the number of slices you want to do in this interval and click on the « Start » button.

NB : Do not forget to click on Z Stack one more time when you have finished to leave the Z Stack mode.

10 - Closing your session / Shutting down the system

- If another user is going to use the confocal in the next hour, (See RESERVATIONS) the lasers can be left ON
- If this is not the case, the switch all lasers to OFF, and the HBO lamp too.



- Leave the Remote Control on ON (sur 1)
- The cooling fans will still work during 5 minutes and then shut down automatically (difference will be audible)
- Clean the oil immersion objectives with 2 coton-swabs (Q-Tips) briefly dipped in ether by sweeping first the glass lens then the metal barrel
- Clean oil and fluids from microscope stage
- Quit the Zeiss software
- Transfer your data (images) on e.g. \\CIF-SERVER\CIF-USERS or burn them on a CD
- Shut down the computer
- Replace the dust cover on the microscope
- Put all disposable paper products , cans, wrappers into the trash can
- Once the fans has become quiet switch off the Remote Control
- The last user is responsible for ensuring that the system was correctly shut down. If you have cancelled your session the same day then :
    - Call the CIF to ensure the system has been shut down
    - Or come over to the facility to ensure the system is shut down yourself.