Imperial College London

Facility for Imaging by Light Microscopy

FILM

Observing Life As It Happens

QuickStart Guide:
Confocal 5:
Zeiss LSM 510 Inverted
(SAFB 408)

# Start-up procedure

Turn on in this order

1. Main power switch (high voltage switch on the left wall if you are standing in front of the microscope)
2. Fluorescent lamp (X-cite, right side of microscope)

on the panel at the right side of the microscope:

3. Components
4. System/ PC

All parts of the microscope can be controlled by the software, so after logging in and filling in the user data in the excel sheet the <u>LSM 510 software should be started</u>:

o double click on "LSM 510" icon

o in the upcoming window select "Scan New image" and "Start Expert Mode"

Switching on the lasers

o In the software: select "Acquire"

o Select "Laser" and switch on the required lasers in the "Laser Control" dialogue:

o HeNe543 and HeNe633: click "ON"

o Diode 405-430: click on "Standby", after "Warming up" changes to "Ready" (usually very fast) click "ON"

o Argon laser: click on "Standby", after "Warming up" changes to "Ready" click "ON" and adjust the "Output %" so that you get a tube current of 6.1A
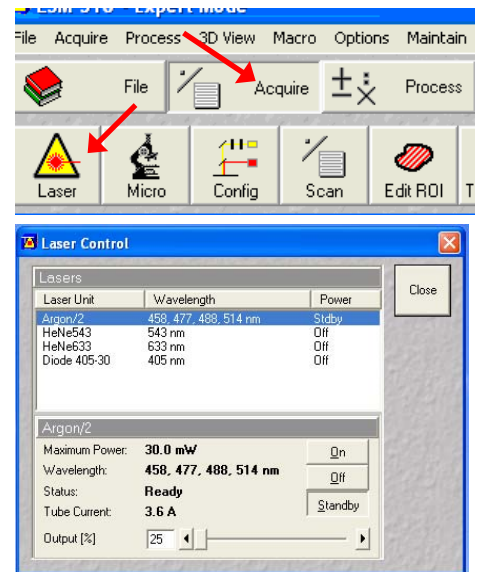
Adjusting brightfield Köhler illumination

always recommended, <u>essential</u> for transmitted light confocal mode

o in the software switch to VIS mode

o switch to 10x objective

o adjust brightness with large black button on front / bottom of microscope)

o make sure condenser iris is not completely closed (two buttons on top of illumination filter wheel)

o focus on your sample

o completely close field iris (top silver lever) (if the image turns completely black, reopen until you see some light, procede to next step and close again after you have focussed the iris)

o focus iris (black condenser focus wheel)

o centre iris (2 silver Köhler screws

o reopen field iris until the edge is just not visible anymore

o readjust every time you switch to a different objective
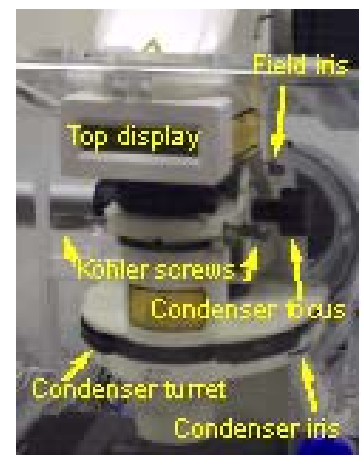
Observing your specimen through the eyepiece

o   in the software switch to VIS mode

o   select "Acquire" - "Micro"

o   in the upcoming dialogue you can choose the desired objective, filter cube (reflector), and light source for observation through the eyepiece; for faster access, presets can be stored, loaded and assigned to the buttons in the upper third of the dialogue box
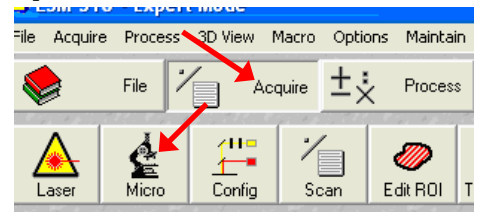
Acquiring images with the Zeiss LSM 510 software

After starting the software you can either open an old database and add new images or create a new one with "New" from the "File" menu. Please use only drive "D:" for your data!

## *Scanning*

There are 2 scanning modes:

Single Track	**Multi Track**
o use for single, double and triple labelling o simultaneous excitation/ emission of all fluorophores	o use for multiple labelling with fluorophore crosstalk o sequential scanning (line by line or frame by frame)
Advantages	Advantages
faster image acquisition	selective laser/ detection channels for each fluorophore, one at a time, avoids crosstalk
Disadvantages	Disadvantages
possible crosstalk between channels	slower image acquisition

o   in the software switch to LSM mode

o   select "Acquire" - "Config"

o   in the upcoming "Configuration Control" dialogue

o   choose track mode

o   when using Multi Track

   ▪   choose track mode ("Frame" is recommended)

o   click on the icons to get your desired dichroics and filters

   ▪   HFT: reflects the indicated wavelengths, all other wavelengths pass

   ▪   NFT: reflects the indicated and all shorter wavelengths, longer wavelengths pass

   ▪   BP: wavelengths of the indicated range pass, others are blocked

   ▪   LP: the indicated and longer wavelengths pass, shorter wavelengths are blocked

o   tick the check box of the appropriate detectors

o   open the "Excitation" dialogue
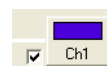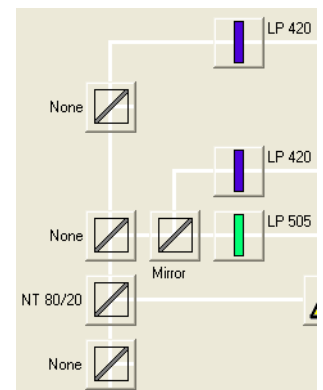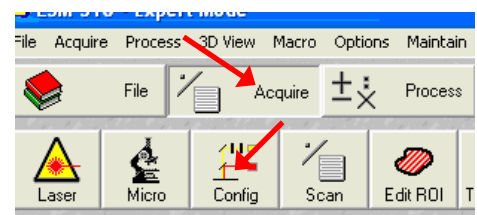
- open the shutters for the desired lasers
- adjust the excitation power for the desired lasers

o repeat these steps in Multi Track mode for each channel setting, it is recommended (In Line/ Frame Fast mode: essential) to keep the settings for dichroics and filters constant (each change needs additional time during acquisition)

o store the configuration setup by clicking on "Config" (with the same button you can apply formerly stored settings)

o select "Acquire" - "Scan"

o in the upcoming "Scan Control" dialogue:

  o choose the type of scan you desire (point, line, frame, stack)

  o the right button panel gives you these useful options:

    - New: opens new image display window

    - Find: automatic optimization of image brightness and contrast (use carefully: can affect the comparability of your data)

    - Fast XY/ XY cont.: fast scanning in the actual focus plane without averaging and with 512x512 resolution

    - Single/ Start: starts a scan with all configured scan control options

    - XY Scan: starts a scan with all configured scan control options but only in the actual focus plane

    - Line Sel/ Range: configure/ start a XZ-scan

    - during acquisition there is a Stop/ Finish button displayed to interrupt the ongoing scan
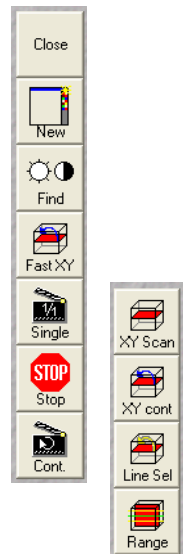
  o in the "Mode" tab:

    - choose the frame size, line step should be 1 and the objective is recognized automatically

    - choose bit depth (8 or 12 bit), scan direction (→ is recommended) and average mode (higher averaging gives you a better image quality but increases sample bleaching)

  o in the "Channel" tab:

    - select each of the channels and adjust the settings separately during a Fast XY scan

    - for the Pinhole pressing "1" (for 1 airy) gives you a good starting point

    - to adjust gain and offset open the "Palette" dialogue from the "Image Display" window and choose "Range Indicator"

    - adjust the "Detector Gain" so that just a few saturated pixels (shown in red) are visible

    - adjust the "Ampl.Offset" so that just a few black pixels (shown in blue) are visible

    - if you cannot obtain a bright enough image with adjusting the above parameters, turn up the "Ampl. Gain", otherwise leave it on 1
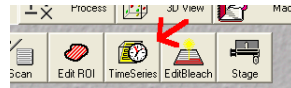
Z-Scan

Go to the "Z-Settings" tab and adjust the following during a Fast XY scan:

  - select "Mark First/ Last", select "Keep Interval"

  - enter the desired interval (z-distance between xy-planes)

  - focus to the upper and lower limit of your stack and press the suitable "Mark" button
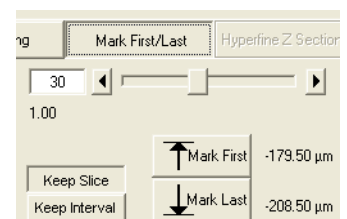
Time scan

o   select "Acquire" - "Time Series"

o   adjust the settings in the upcoming dialogue

Saving/ Exporting data

Shutdown procedure

- check if anyone is booked after you within 2 hours

If nobody is booked within two hours:

o   go to "Acquire" - "Laser"

o   select lasers and switch them off

o   **for the argon laser**:

  o   switch off

  o   wait until "connected" is displayed instead of "cooling"

  o   now you can procede with shutting down the software

o   go to "File" - "Exit"

o   update the time in Sharepoint

o   shut down computer

o   turn off everything in the reverse order of the Startup Procedure

o   remove specimen

o   if oil was used, clean objective with fresh lens tissue

If someone's booked within two hours:

- update usage in Sharepoint

- remove your samples

- clean objective lenses with fresh lens tissue and close incubation chamber

- clear up the desk

- save files onto the server or on a mobile harddrive

- log off

Additional information

Control buttons on the left side of the microscope:

bottom button: cycles between frontport (empty) <> eyepiece <> baseport (camera)

middle button: switches between sideport 100% (empty) <> 50/50 <> eye/ front/ base 100%

top: Optovar in / out

File formats (selection):

LSM4: original Zeiss format, TIFF Planar or TIFF Chunky (both *.tif), contains all imaging information, all images in 1 file as real layers

TIFF: raw (original) data with original bit depth, looses all enhancements and metadata

TIFF for publication: 8-bit screen version of your displayed dataset

- o SAVE AS: all selected layers in 1 file as TIFF layers
- o SAVE AS MULTIPLE: all selected layers in separate files

Movie format: AVI

Offline image viewer for Zeiss LSM files

freely available, see link on FILM homepage