

Leica TCS SP5 confocal user manual

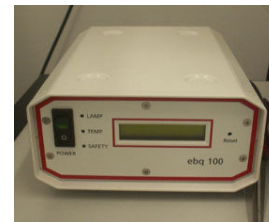
Only people who have had an introduction from Kees Straatman are allowed to operate the system. An introduction from your boss/supervisor is not enough!!!

The system has 5 detectors. Two of these detectors are very sensitive HyD SP GaAsP-detectors. They are at position 2 and 4. Use these detectors only for weak signals, so for example NOT for DAPI. If you don't use these detectors please make the sliders for these two detectors as narrow as possible.

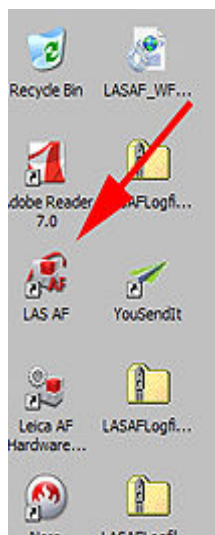
The new detectors are sensitive to interference of mobile phones so switch of your phone when using these detectors.

Starting the system:

- Write your name, grant code, date and starting time in the log book.
- Switch on the air-conditioning. This is necessary to cool and protect the lasers.
- Use account TCS-USER. For password contact a member of the AIF.
- When you need the fluorescent mercury bulb turn on the unit left (see image right) of the computer screens.



- On the right hand site under the table are three buttons marked 'PC Microscope', 'Scanner Power' and 'Laser Power'. Switch these on in this order and turn the 'Laser Emission' key 90 degrees clockwise to 'on'.

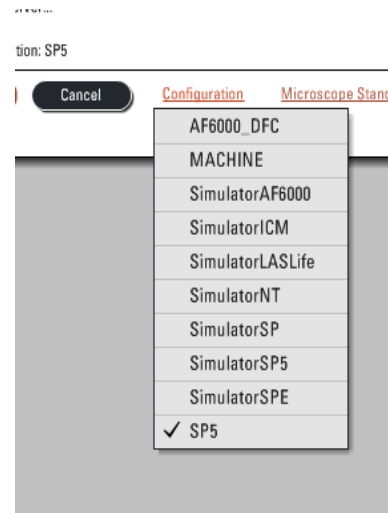


- The system will now start. Wait till you get the login screen on the computer monitor.
- Login as TCS-USER. Obtain Password via facility manager.
- Click on the icon **LAS AF**. This will start the confocal software

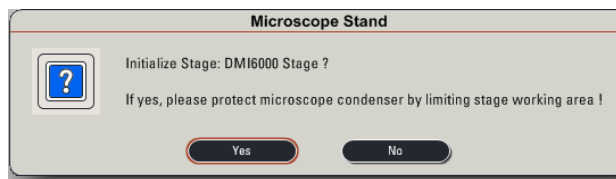
- After a short moment you will get the ‘Leica Application Suite’ start screen. Check under configuration that **SP5** is selected



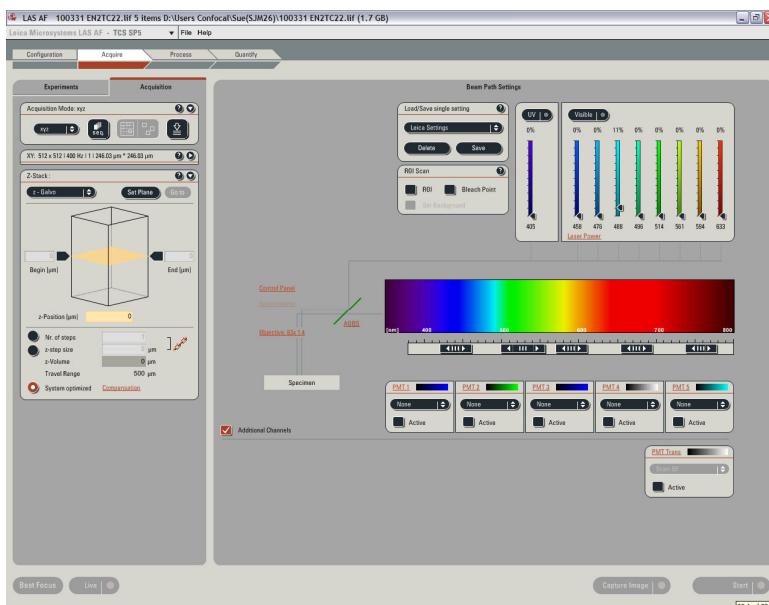
If NOT go to ‘**Configuration**’ and change this to **SP5**



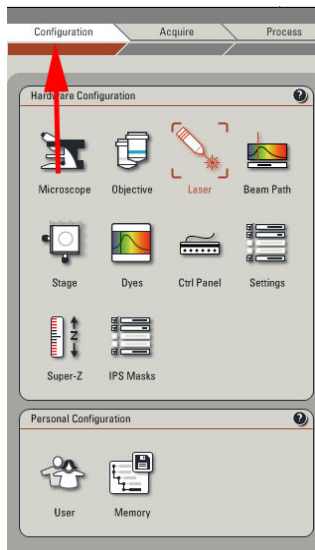
- Press ‘**OK**’
- The confocal software will load now. Be patient!
At the end it will ask you if you want to initialize the stage. Click ‘**Yes**’ if you want to do multi-position imaging, mosaic imaging or want to save or use saved stage positions for imaging.



- You should now get a left screen that looks like:

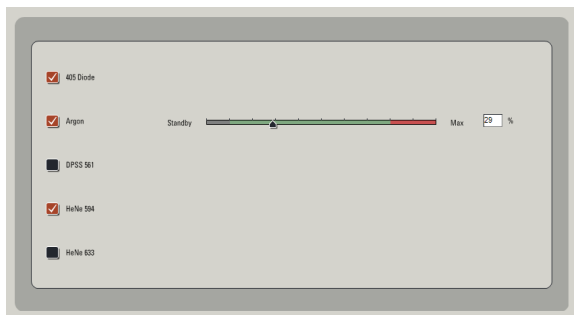


If NOT, most likely the configuration was NOT set to SP5



- To switch on a laser go to '**Configuration**' and select '**Laser**'.

- This should give you the list of lasers available. Flag the laser you need.



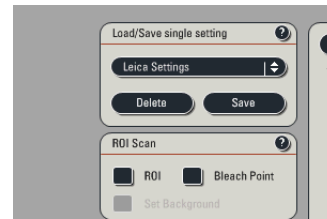
- If you need the Argon laser (458, 476, 488, 496 or 514 nm laser line), you will hear the cooling fan starting when activated. Set the slider at 30%.

- If you do quantitative imaging wait 15 minutes before using the argon laser, so the laser gets up to temperature. If you just need nice pictures you can start imaging. Over time you might need to re-adjust the laser output and/or detector settings
- The system is now ready to use. Read the 'Leica TCS SP5 quick guide' if you need to refresh your memory or contact Kees Straatman (krs5, 3/45) if you encounter problems.
- If the temperature in the room gets too high switch on the air conditioning using the panel at the right site of the door.

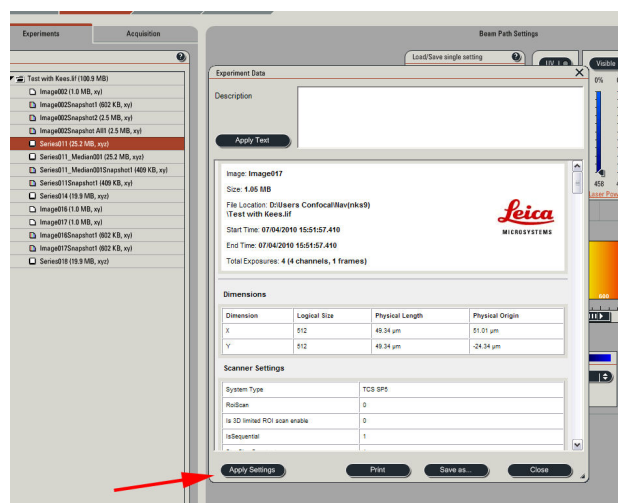
If during imaging you experience problems with the system write this problem in the Error Log. Give date, description of the problem ('system crash' does not give much information. Tell what you were doing when it happened. What lasers, timeseries, z-scan, seq, etc).

Some basics to set up the system:

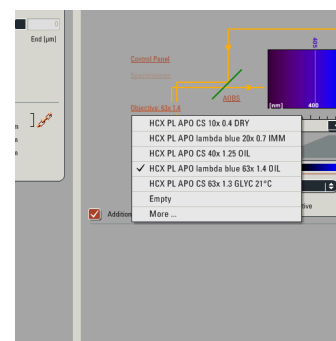
- To use imaging settings you have saved you can select them from the dropdown menu.



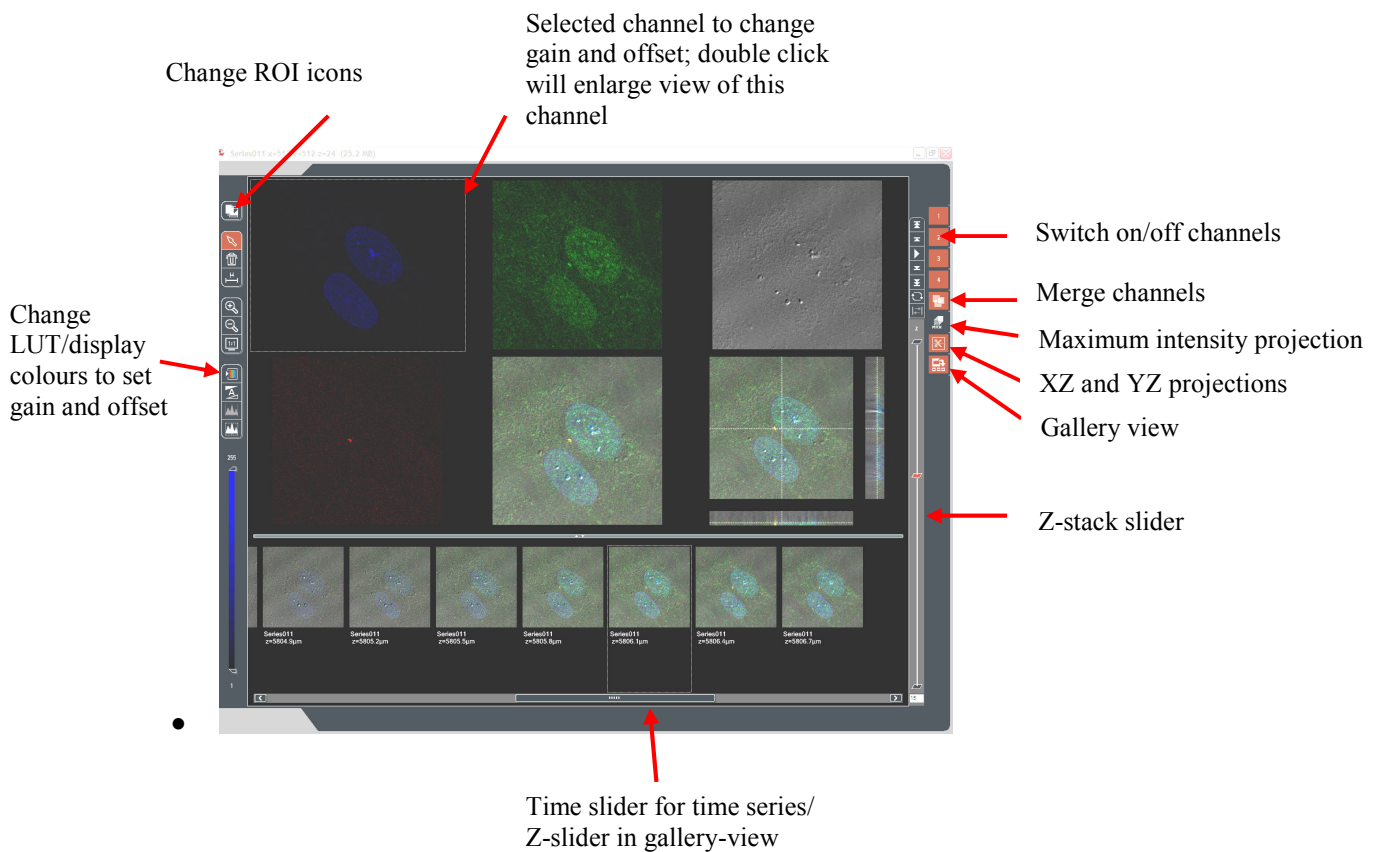
- To use settings used before you can open a database and right mouse click on a dataset in the database which settings you want to re-use and select 'Properties of.....'. This will open a menu with all Meta-data of this file (see left). At the bottom is a option to select 'Apply settings'.



- If you cannot use saved or used settings you have to setup the system manually by selecting the lasers and detectors you need.
- There are 5 objectives on the system: 10x dry, 20x immersion (water, glycerol or oil; don't forget to change the adjustment collar on the objective!), 40 and 63x oil and a 63x glycerol. To change objectives select 'Objective' in the main window and the objective you need.



View panel



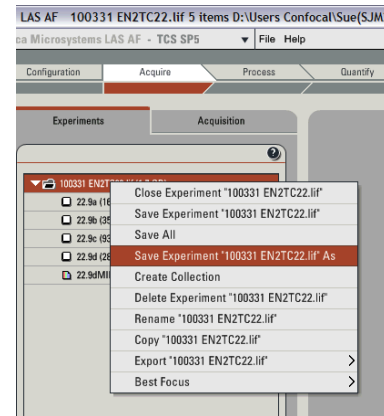
To optimize your image, select the channel you want to change in the view panel by mouse click and a border will show that the channel is selected. Change the LUT to the orange kind of colour (not grey and not the colour you have given to the channel). Bleu means overexposed, green underexposed. You can now adjust the imaging settings by changing the laser output, gain or offset for this channel.

Closing down the system:

Check if somebody has booked the system after you. If this is the case, contact this person to tell that you are ready and ask if you have to leave the system on or switch it off. As last user YOU are responsible for the system!!

To switch off:

- Go to '**Configuration**' and select '**Laser**' and switch of the lasers that are active.
- Turn '**Laser Emission**' key anti clockwise to **off**. If you have used the argon laser (458, 476, 488, 496 Or 514 nm) wait till the cooling fan stops before totally closing down the system (see below).
- Remove sample and clean **all** objectives you have used with lens tissue.
- Save files. Go to the '**Experiments**' tab and right mouse click on the database you made, select the appropriate item from the menu and save it on the D-drive in your subdirectory.
- Close program and shut down windows.
- (If you have used the argon laser wait till cooling fan stops!) Switch off '**Laser Power**', '**Scanner Power**' and '**PC Microscope**' in this order
- Sign out in the log book (time and hours of mercury bulb).
- Switch off the fluorescence mercury bulb.
- Clean the desk.
- Turn off air conditioning if used.
- Switch off light in room.



On your way out, if it is the end of the day and nobody is in the data analysis area, switch off any computer/mac still running (except for the server) and switch off the light in this room when you leave.

Thank you!