Leica SP5 operation

Start-up protocol for image acquisition

Switch on

- Switch on all three green power switches (PC/Microscope, Scanner Power and Laser Power) and turn the key (Laser Emission).
- Allow computer to boot up
- Log on as 'TCS User'
- Double click LAS AF to start the acquisition software
- Check configuration – Machine (default)
- Next question is about the motorised stage – if you would like to acquire tile scans or use the mark and find option (both useful for tissue sections) then select YES, otherwise select NO
- Connection should be successful and program will now complete loading and initialise the hardware
- If required, switch on the LED light source

Set configuration for your experiment

Go to Configuration at top left corner

Lasers

- Select Lasers and switch on all lasers that you require – please check if you are using after someone else that the right lasers for your experiment have been switched on or switch off the lasers that have been left on and you do not require
- The argon laser should be set to 30% and the fans should start up as well
- The lasers take about 30 minutes to warm up completely and should be left on for at least 30 minutes

- Please note that it is more harmful for them to be switched on and off between users than left on for a 1-hour gap at lunchtime. The Argon laser should be switched to ‘stand-by’, if it is not used.

Control Panel

- The second circle on the control panel should be highlighted and changed to 1% per turn to allow for fine adjustment of your smart offset.
- There will be no immediate need to alter any of the other settings at this point. However, if you wish to change the function of any of the control panel knobs, activate it by clicking on the appropriate circle and then choose the function from the menu below
- If you wish to take higher resolution images then you can adjust the settings to 12-bit or 16-bit from the 8-bit default
- The other icons allow you to find out more information about the microscope objectives and common fluorochrome spectra.

*Return to the Acquire option***.**

This is where you will set up the laser paths you require

This section will be covered in the introductory training session and repeated during your first assisted session

Run-down of system

- The laser emission key and the mercury lamp should be switched off once you have acquired your data
- However, please check the booking system before you do so in case someone else is about to use it. If in doubt, leave the lasers and mercury lamp switched on
- Once the laser emission key is turned/switched off, the system starts powering down all lasers and eventually the fans
- The number of hours on the mercury lamp together with the switch off time should be logged on the sheet

- Remove your last slide and clean off any oil residues left on the objectives. Check the stage for oil spills and remove if necessary
- Save images and export data as required, otherwise you will loose all the data not saved or exported
- Go to file and exit program
- Copy data to memory stick, CD, DVD or server
- Shut down computer
- Switch of PC/Microscope and Scanner Power buttons
- The Laser Power button should only be switched off when the fans have gone off
- You should now complete the log in sheet and add any problems to the query sheet.
- Switch off all room lights and lamps

To export or analyse data

- Switch on PC/Microscope button
- Allow computer to boot up
- Log on as TCS User
- Double click LAS AF
- Choose configuration – Simulator SP5
- Wait for program to finish loading
- Go to File menu and open previous experiment as required
- To save the original lif files, find them in your user folder and copy them to the destination storage medium (DVD, memory stick or server)
- To export data, right click on individual images or on experiment folder and choose required format: tiff, jpeg or avi
- Choose whether you want overlay (merged image) or individual channels
- Can do this twice so that you have both options
- File formats: Tiff – holds some Meta data and is a valid interchangeable format; jpeg – purely contains the image data, which are compressed, and

should be avoided; avi – movie format, which is severely compressed and purely for display purposes

- The actual .lif file, which is generated is the best file to take to the analysis computer (see notice board for what packages are available) as all Meta data is stored. Please note that Image J requires a plugin to open a .lif. Details can be found in this folder
- After you have checked your data, please delete them from the hard drive of the microscope PC asap. This hard drive is not for long-term storage of data and it will be cleared from time to time. This also guarantees that there is enough storage space for the next user on the system