

SP5 Startup and Shutdown Procedure

Startup

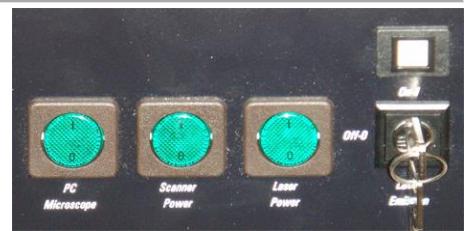
note: normally the system will be switched on for you. Please do not start up the system yourself (steps 1-4 below) unless you have been authorised to do so.

- (1) Switch on the 2 wall sockets labelled *SP5* and the wall socket labelled *Hg lamp*.



- (2) Switch on the 3 green switches on the main console (under RHS of desk) - the order of switch on isn't important but do them one at a time to prevent a power surge.

Turn the laser key 90 degrees clockwise to start the lasers (the square indicator above the key will light up yellow).



- (3) Check that the room door is wedged open with the wooden block / that the cooling hose isn't crushed.



(4) Make sure that the microscope control box (on floor under microscope) is switched on at its front switch (green status light above switch is illuminated). The control box should permanently be left on and so powers up when the PC-Microscope switch on the console is switched on.

The information panel on the microscope stand will illuminate and display a start up message. The microscope stage will initialise.



(5) Switch on the mercury vapour lamp. The bottom 2 indicator lights (*temp* and *safety*) will show green.

After a few seconds, the arc will strike and the top indicator light (*lamp*) will show green.



The lamp is ready to use immediately the arc strikes but takes a few minutes to stabilise fully and warm up to maximum intensity.

(6) Switch on the PC and log onto the local machine (not SOTON domain) as user *TCS-user* with password *SP5*.

(7) Start the Leica Advanced Fluorescence Application using the LAS AF desktop icon at top right of the LH monitor screen.



(8) On the startup screen, check that the configuration is set to *machine*, that the microscope stand is set to *DMI6000* and that the resonant scanner option is unchecked..

note: if you want to use the software to analyse previously captured data, rather than to acquire new data, use the *Simulator S5* configuration mode as it will load up faster.

Click on *OK* to continue startup.

As the microscope initialises, the text in the bottom LH corner of the startup screen will update.



DefaultDynamicWidefieldTree
✓ machine
SimulatorAF6000
SimulatorSP5
SimulatorSPE

DM Manual-6
DM5000
DM6000
✓ DM16000

OK

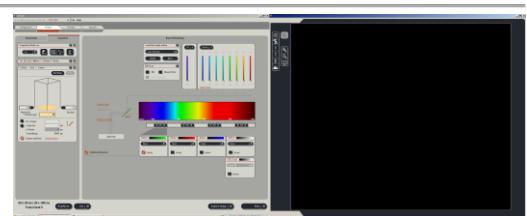
- (9) When the *Microscope Stand* initialisation box comes up, check that there is nothing that will prevent stage movement, and that there is nothing on the stage that will catch on the condenser (e.g. gas feed needle / tubing in microplate for live cell imaging)

Click on Yes to initialise the stage.

note: stage initialisation is essential for using the *tile scan* and *mark and find* functions

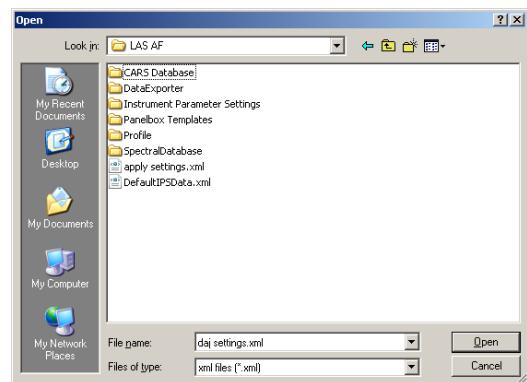
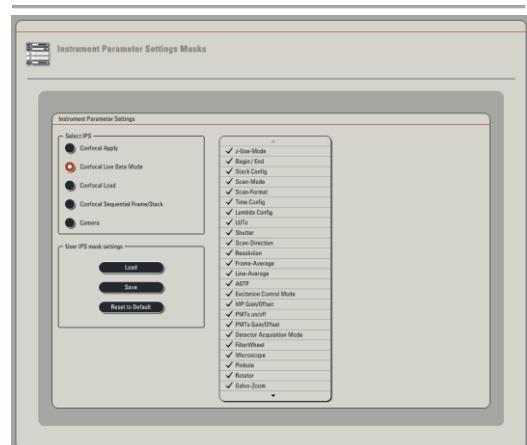


- (10) Once initialisation is complete you will see the LAS user interface across the 2 screens



- (11) Under the Configuration tab

(1) Select the IPS Masks icon: click on the Load button and select the *mask settings.xml* file.



(2) Select the Control Panel icon: load the BIU settings option.

(3) Select the Lasers icon: turn on the lasers needed for the session - if using the Argon laser, turn laser power to 30%.

You are now ready to configure the microscope and software for image acquisition

default settings:

Format: 1024 x 1024

Speed: 600 (via *more...*)

Zoom: 1

Line Averaging: 4

Rotation: +90

Shutdown

Incorrect shutdown procedure can severely damage the lasers

Normally the system will be shut down for you at the end of the day

Do not shut down the system if other users are booked onto the system later in the day

At the end of your session:

- save your data and close your experiment file
- remove specimens from the microscope stage and clean immersion objectives with lens tissue – please take care to:
 - clean the sides of the objectives as well as the lens face (as it is an inverted stand, immersion medium tends to run down the sides of the objectives and can gum up objective screw threads, correction collars etc.).
 - not cross contaminate the oil and glycerol lenses.
- leave the system with the x10 objective in position (it is the cheapest objective if anything gets dropped onto the nosepiece!).

If other users are booked onto the system later in the day and you are taking your data away on a USB drive:

- go to the *Configuration* Tab, *Laser* sub tab and simply turn off all lasers in the software interface.
- leave the software and electronics running, ready for the next user.
- copy your data to your USB drive.

If other users are booked onto the system later in the day and you need to access a university server / filerstore to transfer your data to:

- go to the *Configuration* Tab, *Laser* sub tab and simply turn off all lasers in the software interface.
- leave the software and electronics running, ready for the next user.
- minimise the LAS-AF software.
- connect to the BIUfilestore using shortcut at RHS of LHS monitor as user *SOTONlyourUoSusername* with your UoS password. You can then transfer your data across to the filestore in the normal manner.
- Leave everything else running.

If you are the last user of the day and you have been asked to shut down the system by BIU staff, follow the instructions below:

Shut down the LAS-AF software. Note: periodically the software performs and logs system checks on shutdown, including auto powering each active laser to 100% strength. Therefore it may take up to a minute to fully shut down.

Shut down the mercury lamp starter box and switch it off at the wall socket.





On the console, turn the laser key 90 degrees anticlockwise to power down the laser.

Do not switch off the green laser power button.

The lasers need to cool down by active cooling and the cooling fans are powered by this switch.

Cooling takes about 5 minutes. When you can no longer feel air movement from the outlet of the cooling duct, cooling is complete.



Whilst you are waiting for the system to cool is a good time to log off as SP5-user, log onto SOTON domain with your UoS credentials and copy your data onto whatever UoS server / filestore / swap space you are storing your data on. We recommend that you use the BIU's filestore space on the UoS server at Highfield.

Once your data is copied, shut down the PC.



Once the system has cooled (the external fan has stopped blowing):

- switch off the *PC / Microscope* switch
- switch off the *Scanner Power* switch
- switch off the *Laser Power* switch



Switch off the 2 wall sockets labelled SP5.



Turn off the air conditioning and room lights.