Imperial College London

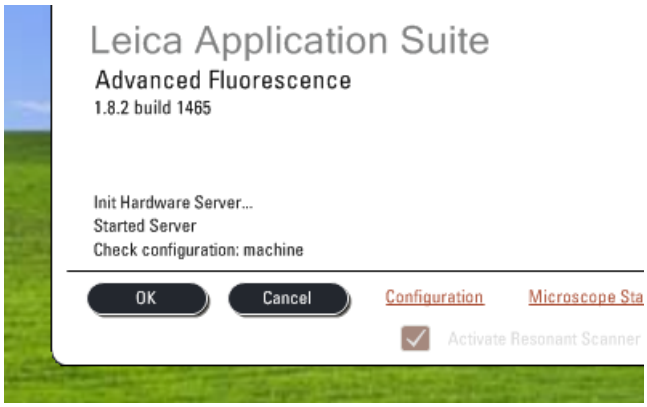
Facility for Imaging by Light Microscopy

Observing Life As It Happens

QUICKSTART GUIDE:
CONFOCAL 3:
LEICA SP5 MP / FLIM
upright
(SAFB 408)

Startup procedure:

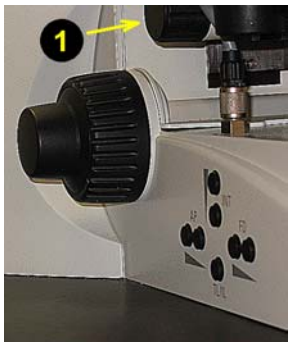
- switch on fluorescence lamp (separate white box, on the left of the microscope)
- on the main control panel switch on – left to right:
  - > PC / microscope
  - > switch on SCANNER, wait 20 sec
  - > LASER and turn the LASER KEY to ON
- login with your local account (don't use IC network account)
- in the startup-window make sure that you defined your right configuration from the drop-down-menu of the same name: SP5 MP if you do not need the multiphoton (for multiphoton use see separate instructions)



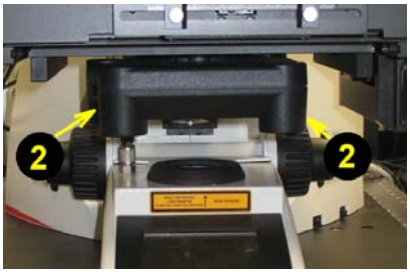
- you will have a message window asking you, if you want to calibrate the stage, just click on YES or NO. The calibration is required for multipoint scanning. **If you choose YES, the stage will move to all extreme of its range, so make sure the objective turret is in its lowest position.**

Finding your cells / brightfield adjustment

Köhler illumination



- push the button TL/IL (left side of the microscope) until the touchscreen at the bottom of the microscope shows 'TL' (not FLUO)
- look through the eyepiece, if necessary adjust the eyepieces to your eyes
- if there is no light visible: make sure that the brightfield light path switch is in the correct position
- adjust the brightness with the INT buttons
- focus on your sample
- fully open the condenser iris (aperture iris, AP buttons)
- fully close the illumination iris (field iris, FD buttons)
- looking down the eyepiece, focus the black edges of the illumination iris with ❶



- move the illumination iris to the centre with the 2 Köhler screws ❷ (you need an Allen key to adjust them) (> a set of Allen keys is supplied for CF3 and 4)

- reopen the illumination iris so that the black ring just disappears from your field of view

Fluorescence

- press the TL/IL button to switch to fluorescence (FLUO is displayed on touchscreen)
- choose the desired filter cubes over the touchscreen
- if necessary change the illumination brightness with the INT buttons *(same as for brightfield)*
- if necessary change the shape and size of the illuminated area on the sample with FD buttons
- select a bright, but not unphysiologically bright fluorescent cell / area

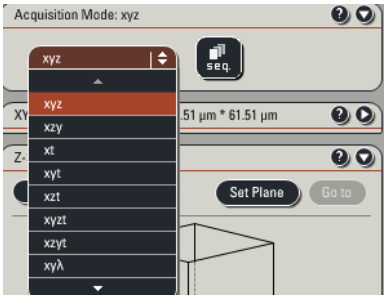
Software / Image Acquisition

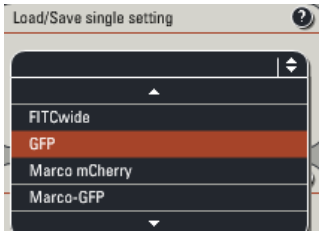
if not already done, start the Leica Confocal Software (see Startup Procedure)

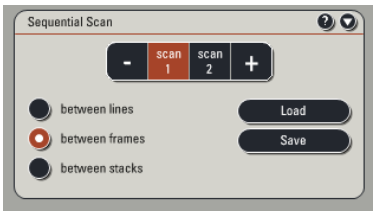
- In the software window, go to the CONFIGURATION tab, LASER and turn on the lasers you need (for MP-laser see separate Quick Start Guide)

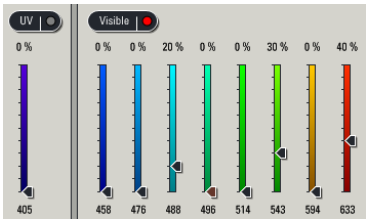
CAUTION : Verify the shutter of the Multiphoton laser is CLOSED if you do not need it!

- set the percent power on the Argon laser:
  - leave it on 15% for normal imaging, it's plenty and increases the laser lifetime
- go to the ACQUIRE tab
- choose the desired acquisition mode
- if you have already saved your settings previously or want to use presets, load it ...
  - ...form the single settings drop-down menu if you are working with 1 fluorophore only
  - ...by clicking on the LOAD button in the sequential scan panel
- otherwise activate and adjust the required laser lines

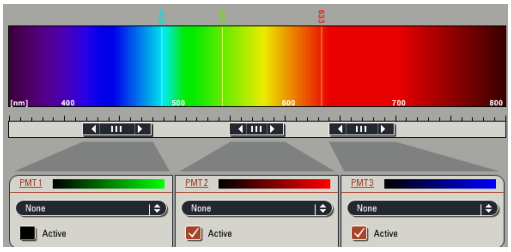




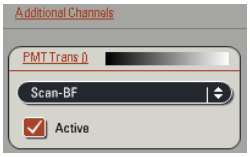


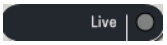


- activate and adjust the required PMT's



  - make sure the detection wavelengths don't cover any laser line
  - if needed, an emission curve can be loaded from the database from the fluorophore list (it`s just a curve displayed, it doesn`t make any change to your settings)
  - a screen colour can be selected (doesn't affect image data, so can be changed any time later)



- to turn on a brightfield image, click on ADDITIONAL CHANNELS and select SCAN-BF (first check if the brightfield light path switch is in the correct position)
- start preview scanning clicking on LIVE 

- in the image window on the right-hand side activate the MULTI-PANEL VIEW 
- click on the BF image and adjust BF intensity using the SMART GAIN and SMART OFFSET buttons on the control panel

- fine-focus using the Z POSITION button
- change the screen colour to SATURATION PSEUDOCOLOURS 
- in the image window click on the panel of the first fluorophor
- using the control bar, increase SMART GAIN until a few single blue dots appear (saturated pixels)
- decrease SMART OFFSET until a few green dots appear (black pixels)

- repeat method for all channels in your current scan setting and save it
- in the XY panel set the required IMAGE FORMAT and ZOOM FACTOR

- if required, move the ZOOM AREA using the arrow icons
- adjust the AVERAGING required to give you sufficient image quality

- if doing LIVE IMAGING (xyt or xyzt acquisition mode), expand the time panel 

and adjust TIME INTERVAL and DURATION
- ACQUIRE IMAGE by clicking the CAPTURE (single image, as defined with your x, y and λ) or START button (series, as defined by your x, y, z, t and λ settings)

Reapply settings from previous images

→ it is highly recommended to acquire images throughout an experiment – and, if possible, in similar experiments - with identical settings, which allows images to be compared and quantified

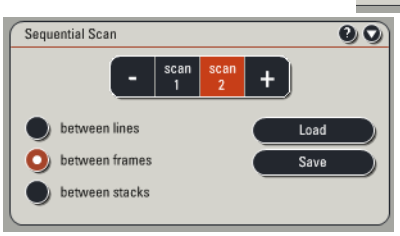
- to reset the same settings as used in a previous image, right-click on the image name in the file list or the image window itself and open PROPERTIES
- click on the APPLY button at the bottom of the upcoming window.

Reapplying settings does not work for sequential scanning

Sequential scanning

For many combinations of fluorophores you have a certain amount of crosstalk between the emitted signals. In this case, separate fluorophores must be imaged sequentially.

o press the SEQ-button in the ACQUIRE tab to get an additional SEQUENTIAL SCAN panel



o expand the SEQUENTIAL SCAN panel

o with the + and - buttons adjust the number of scan settings that should be used sequentially

o press SCAN 1 and set up/ load a setting, then press SCAN 2 and set up/ load the next setting, etc.

o choose when settings should be switched: after each line (not recommended), frame or stack

o switch between settings with the SCAN 1/2/etc. buttons to check whether all settings are correctly reapplied, if not please ask for help

Shutdown procedure

- check if anyone is booked after you within 2 hours

If nobody is booked within two hours:

- Turn LASER KEY off (**leave the LASER button on the main control panel ON for cooling for 5-10min!**)
- remove your samples and switch off fluorescence mercury lamp
- clean objective lenses with fresh lens tissue and close incubation chamber
- save files onto the server or on a mobile harddrive
- fill in user log excel sheet
- clear up the desk
- shut down computer and switch OFF SCANNER and PC STAND on the Main Control Panel
- 10 minutes after turning the LASER KEY, switch all 3 main buttons OFF

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