Imperial College London

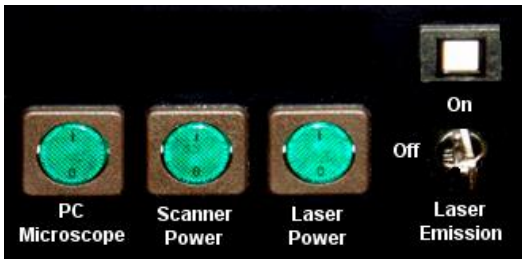
Facility for Imaging by Light Microscopy

Observing Life As It Happens

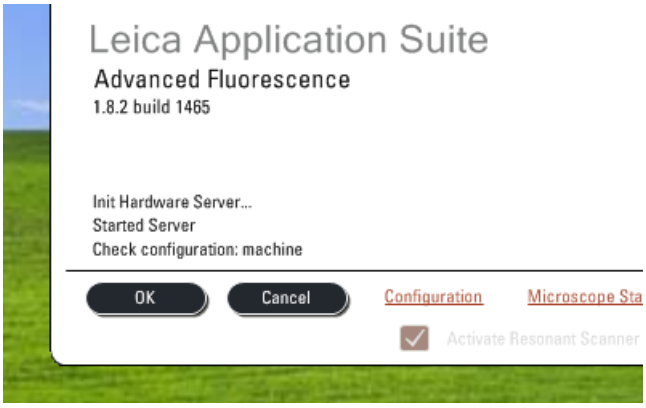
QUICKSTART GUIDE:
CONFOCAL 3:
LEICA SP5 MP / FLIM
upright
(SAFB 408)

Startup procedure:

On the main control panel Switch on (left to right):



- PC Microscope
- wait 15-20 seconds
- Scanner Power
- Laser Power and turn the Laser Emission Key to ON
- Login (IC network account)
- Start the Leica Acquisition Software “LAS-AF”
- In the startup-window make sure that you defined your right configuration from the drop-down-menu of the same name: SP5 NO MP if you do not need the multiphoton (for multiphoton use see separate instructions)

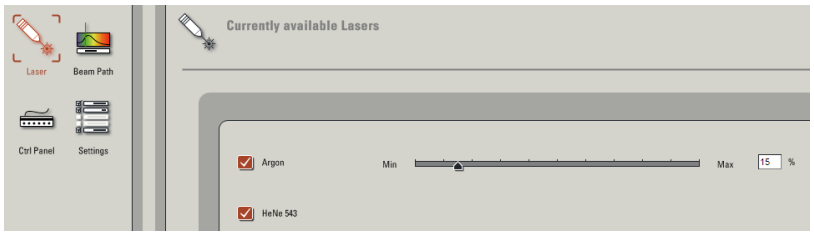


- You will get a message window asking you, if you want to calibrate the stage, just click on YES or NO. The calibration is required for multipoint/tiling scanning. **If you choose YES, the stage will move to all extreme of its range, so make sure the objective turret is in its lowest position.**

In the software window, go to the CONFIGURATION tab:

Turn on Lasers: (for MP-laser see separate Quick Start Guide)

- Select LASER and turn on the lasers you need
- Set the percent power on the Argon laser:
  - 15% for normal imaging, it’s plenty and increases the laser lifetime
  - for bleaching turn the power higher up

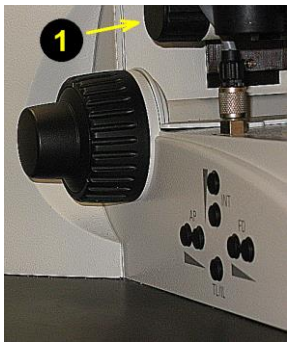


Select SETTINGS:

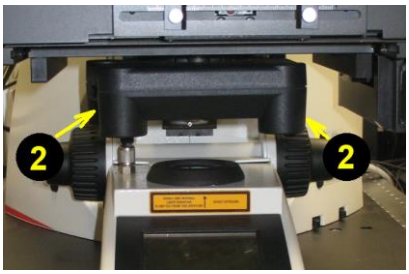
- Set Bit Depth to 12/16 bit if required

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

Guide to using the new LED Systems

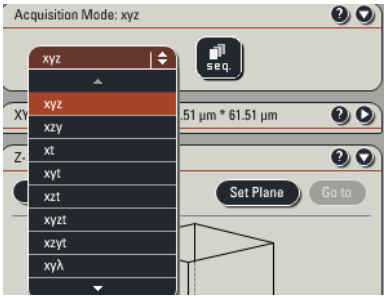
In addition to the microscope controls

- Select all or individual channels using the select buttons
- Use the on/off button to switch on/off
- Use the +/- buttons to change intensity

!! Please remember to switch off after viewing and at the end of your session !!

- Use the touchscreen to select Fluorescence
- Choose the desired filter cubes with the buttons in front of the microscope
- Open the shutter
- Turn on (and adjust) the LED box
- If necessary change the shape and size of the illuminated area on the sample with FD buttons

Software / Image Acquisition

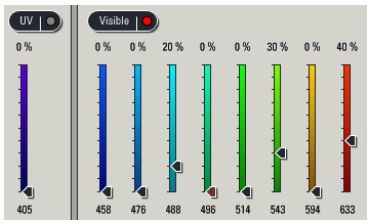


ACQUIRE tab

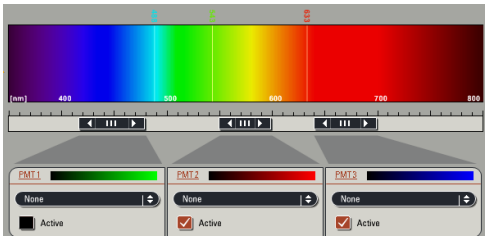
- Choose the desired acquisition mode eg XYZ

Either load previously saved settings or use the pre-sets load it from the scrolling list

Or setup your own settings by:



- Activating and adjusting 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 excitation curve can be loaded from the fluorophore list (it`s just a curve displayed, it doesn`t make any change to your settings)
  - Select colour LUT (doesn't affect image data, so can be changed any time later)

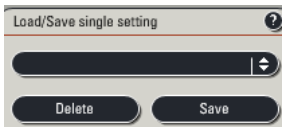


- To turn on a brightfield image, click on ADDITIONAL CHANNELS and select SCAN-BF

Start preview scanning clicking on LIVE

- Click on the BF image and adjust BF intensity using the SMART GAIN and SMART OFFSET controls on the control panel
- Fine-focus using the Z POSITION control

- Change the screen colour to the "Range Indicator"
- Click on the panel of the first fluorophore
- Increase SMART GAIN until a few single blue dots appear (saturated pixels)
- Decrease SMART OFFSET until a few green dots appear (black pixels)



- Repeat for all channels
- Save settings

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:
  - line averaging for live imaging
  - line or frame averaging for fixed cells

For 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)

Saving Data

- Images are stored in a library
- Every time Capture or Start is pressed the image is added (NOT SAVED) to the Library in the EXPERIMENT tab.
- Right clicking on the individual images allows renaming or deleting.
- To save the images click the “Save All” button at the bottom of the window.
- The first time it will prompt for a location and the Library name.
- Then every time you capture an image press the “Save All” to update the library or you could lose your data.

Re-appling 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, load the image library and right-click on the image name in the file list in the EXPERIMENT window and open PROPERTIES
- Click on the APPLY button at the bottom of the upcoming window.

!!! Cave: there is a bug in the software, so that normally not all of your settings are applied, always cross-check!!! (particularly the pixel number, bit depth and zoom)

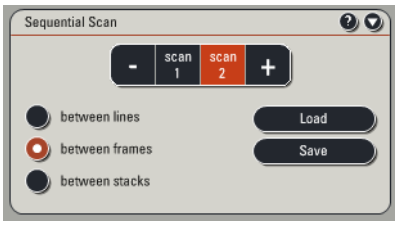
Sequential scanning

For most combinations of fluorophores you have a certain amount of crosstalk between the emitted signals. To get a better separation of the fluorescent signals acquire the channels sequentially. This is highly recommended in any kind of colocalisation study.

Press the SEQ-button in the ACQUIRE tab to get an additional SEQUENTIAL SCAN field

Either load settings or:

- Open the SEQUENTIAL SCAN field
- Use the + and - buttons adjust the number of scan settings that should be used sequentially
- Press SCAN 1 and set up/ load a setting, then press SCAN 2 and set up/ load the next setting, etc.



- Choose when settings should be switched: after each line, frame or stack
- Switch between settings with the SCAN 1/2/etc. buttons to check whether all settings are correctly reapplied, if not please ask for help.
- Save settings

Shutdown procedure

- Check if anyone is booked after you within 2 hours
- Update booking if necessary.

If nobody is booked within two hours:

- Turn off lasers in software
- Turn LASER KEY to off (**IMPORTANT leave the LASER cooling button on the main control panel ON for cooling for 5-10min!**)
- Remove your samples and make sure the LEDs are off.
- Clean objective lenses with fresh lens tissue and close incubation chamber
- Save files onto the server
- Clean up the desk
- Shut down computer and switch off SCANNER and PC STAND on the Main Control Panel
- 5-10 minutes after turning the LASER KEY, switch off LASER cooling button

If someone is booked within two hours:

- Update booking if necessary.
- Remove your samples & clean objective lenses with fresh lens tissue and close incubation chamber
- Clear up the desk
- Save files onto the server
- Log off