Imperial College London

Facility for Imaging by Light Microscopy

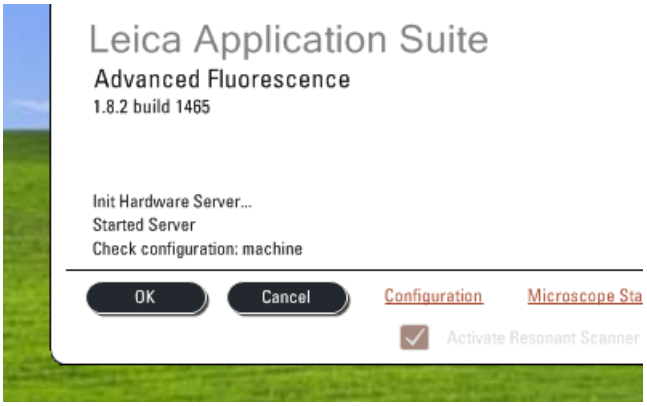
Observing Life As It Happens

QUICKSTART GUIDE:
CONFOCAL 2:
LEICA SP5 RESONANT
inverted
(SAFB 530)

Startup procedure:

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

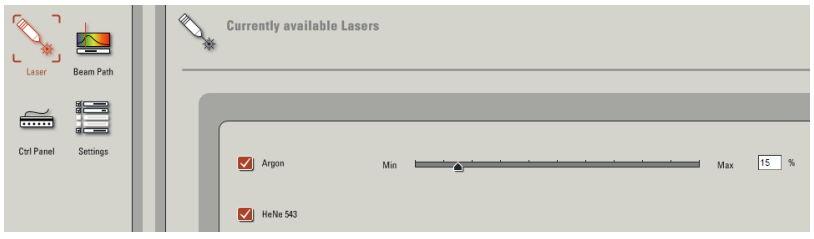
- PC Microscope,
- wait 20 sec
- Scanner Power
- Laser Power and turn the Laser Emission Key to ON
- login (IC network account)
- start LEICA Application Suite “LAS AF”
- In the startup-window check whether configuration is set to "machine", else click on "Configuration" and choose "machine" from the upcoming menu



In the software window, go to the CONFIGURATION tab:

Turn on Lasers:

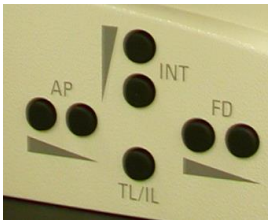
- Select LASER and turn on the lasers you need
- Set the percent power on the Argon laser:
  - 15% for normal imaging, it’s plenty and increases the laser lifetime
  - for bleaching turn the power higher up



Select SETTINGS:

- Check the box to set LINE AVERAGING to apply to LIVE image
- Set Bit Depth to 12 bit if required

Finding your cells / brightfield adjustment



Köhler illumination

- push the button TL/IL (left side of the microscope) until the display on the microscope shows ‘TL’ (not FLUO)
- look through the eyepiece, if necessary adjust the eyepieces to your eyes
- adjust the brightness with the INT buttons
- focus on your sample
- fully open the condenser iris (aperture iris, AP buttons)
- fully close the field iris
- Looking down the eyepiece, focus the black edges of the field iris with the silver wheel (**1**)
- Move the illumination iris to the centre with the Köhler screws (**2**)
- Reopen the field iris so that the black edge just disappears from your field of view

Fluorescence

Guide to using the new LED Systems

In addition to the microscope controls

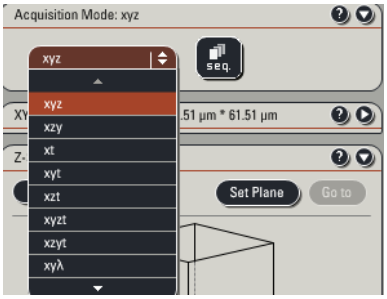
- Select all or individual channels using the select buttons
- Use the on/off button to switch on/off
- Use the +/- buttons to change intensity

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

On the front panel

- Choose the desired filter cubes with the buttons in front of the microscope
- Open 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

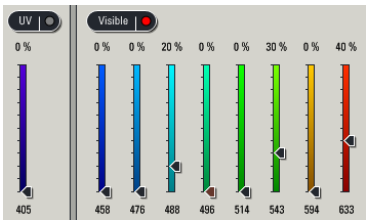
Software / Image Acquisition



ACQUIRE tab

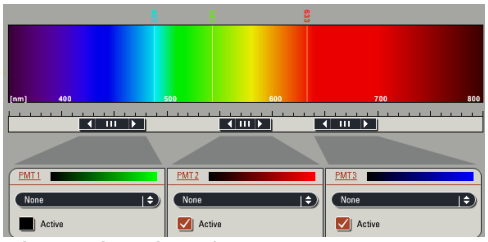
- Choose the desired acquisition mode eg XYZ

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)



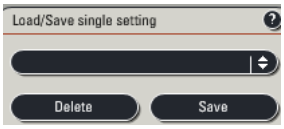
  - Select colour LUT (doesn't affect image data, so can be changed any time later)
- To turn on a brightfield image, click on ADDITIONAL CHANNELS and select SCAN-BF

Start preview scanning clicking on LIVE 

- Zoom is set to 4 by default – zoom out if required (1.7 is lowest)
- 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 clicking the CAPTURE Image (single image) or START button (series or process)

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)

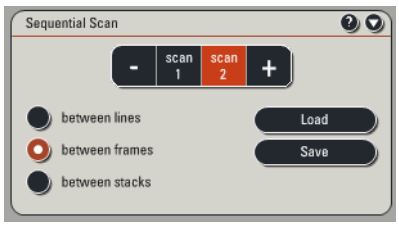
Sequential scanning

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