

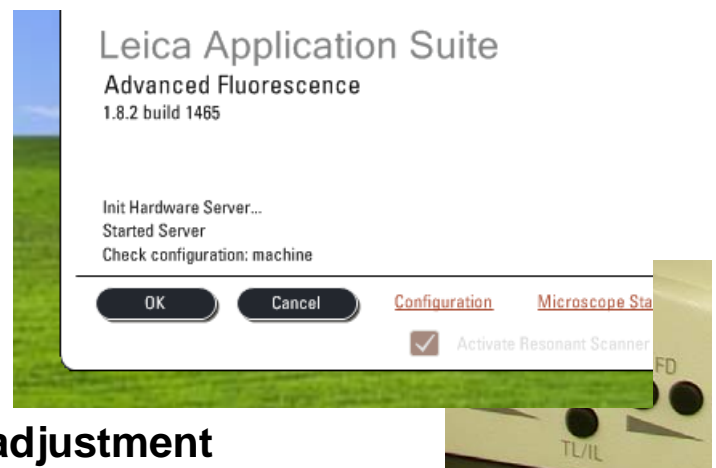
Observing Life As It Happens

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

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## Startup procedure:

- switch on fluorescence lamp (separate white box, on the right 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)
- start LEICA Application Suite (in the startup-window check whether configuration is set to "machine", else click on "Configuration" and choose "machine" from the upcoming menu)



## 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***

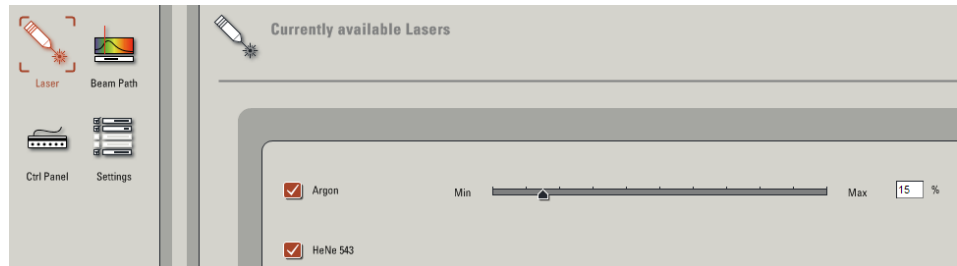
- Press the TL/IL button to switch to fluorescence (FLUO shows in display)
- Choose the desired filter cubes with the buttons in front of the microscope
- If necessary change the illumination brightness with the INT buttons (*same as for brightfield*)



- 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

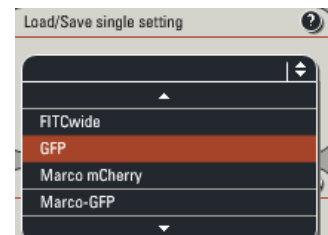
## Software / Image Acquisition

- Start the computer with your username and password
- Start the Leica Confocal Acquisition Software (see Startup Procedure)
- In the software, go to the CONFIGURATION tab, LASER and turn on the lasers you need

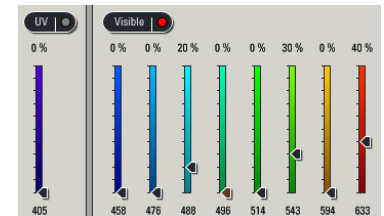


- Set the percent power on the Argon laser:
  - leave it on 15% for normal imaging, it's plenty and increases the laser lifetime
  - for bleaching turn the power higher up

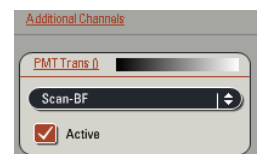
- go to the ACQUIRE tab
- choose the desired acquisition mode
- if you have already saved your settings previously or use presets load it from the scrolling list



- 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 excitation curve can be loaded 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) (→ if the brightfield image has stripes, click on AOBs and uncheck ENHANCED DYNAMICS for all lasers)




- start preview scanning clicking on LIVE 

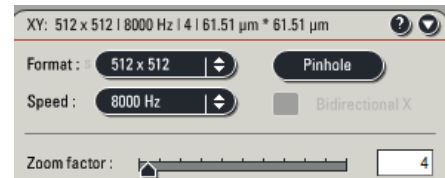
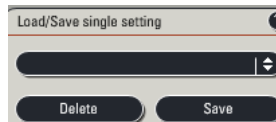
- in the image window on the right-hand side activate the MULTI-PANEL VIEW 

- 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 
- 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
- save settings



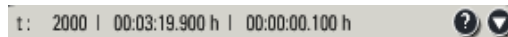
- in the XY panel set the required IMAGE FORMAT and ZOOM FACTOR

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



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

- if doing LIVE IMAGING (xyt or xyzt acquisition mode), expand the time panel and adjust TIME INTERVAL and DURATION



- ACQUIRE IMAGE clicking the CAPTURE (single image) or START button (series)



## 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

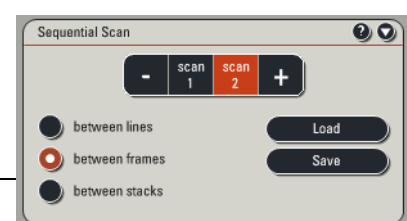
- to reset the same settings as used in a previous image, right-click on the image name in the file list or the image window itself and open PROPERTIES
- click on the APPLY button at the bottom of the upcoming window.

!!! Cave: there is a bug in the software, so that normally not all of your settings are applied, always cross-check!!!

## 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 one can 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
- open the SEQUENTIAL SCAN field
- with 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 (not recommended), 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

## **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