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Startup procedure:
- on the main control panel switch on – left to right:
  - PC / microscope, wait 15 sec
  - switch on SCANNER
  - LASER and turn the LASER KEY to ON
- login and start “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 (NB. if using the multiphoton - 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.**

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 !!

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 large silver wheel (1)
• Move the field iris to the centre with the Köhler screws (2) (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 shows in display)
• Choose the desired filter cubes with the buttons in front of the microscope
• open the shutter (always close it when you are not using the eyepiece)
• turn on (and adjust the intensity if required) with the on/off button on the LED box
• 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
• if needed change objective in software

**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:
  o 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
- 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 fluorophore
- 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 xyt 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.

- press the SEQ-button in the ACQUIRE tab to get an additional SEQUENTIAL SCAN panel
- expand the SEQUENTIAL SCAN panel
- 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 off lasers in software
- 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
- 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