

Observing Life As It Happens

QUICKSTART GUIDE:  
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**LEICA SP2 upright**  
(ROOM 530)

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## Startup procedure:

- turn on fluorescence lamp (separate white box)
- on the black control unit at the right-hand side of the keyboard, switch on LASER, SCANNER and PC STAND
- turn on microscope and brightfield lamp (both black switches on right-hand side of the microscope, bottom-back: microscope main switch on the right, brightfield on the left)
- turn on the lasers as required: Arg, HeNe, 561 diode and UV:
  - > for Arg/lon: turn key all the way clockwise and let it flip back and set power button to the “9 o'clock” position
  - > for HeNe 633: turn key in "ON" position
  - > for 561nm diode (small box between screens): turn on key, then press LASER ON button for 2 sec
  - > for UV: turn on the “Main Power”, turn key to “on”-position, laser power should adjusted to one turn clockwise from zero-position
- turn on the computer and log in as user
- start LEICA ACQUISITION SOFTWARE



## Finding your cells / brightfield adjustment

### *Köhler illumination*

- push in the main light path switch (eyepiece position)
- move the brightfield light path switch to the right (lamp position)
- move the filter wheel (top) to position '4'
- move the condenser wheel (bottom) to position 'D'
- turn silver wheel on the top-right of the microscope to “1x” position
- on the left-hand side in the back / bottom, turn the condenser iris (*upper black wheel*) to position 4



- focus on your sample (*if you can't find it, try to fully close the condenser iris or focussing on the edge of the coverslip*):

- move stage up close to the lens using the black UP button behind the focus wheel
- set the focus speed to '3' using the Z STEP button above the focus wheel (*display at the front of the microscope*)
- focus on your cells or coverslip (*with oil objectives it is easier to observe when the lens touches the oil from outside*)

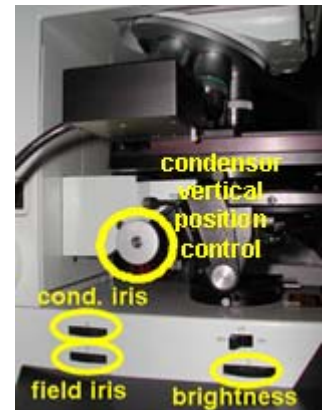


- fully close the field iris (*black wheel underneath condenser iris*)
- look through the eyepiece

→ make sure the light path switch is in eyepiece (right) position

→ make sure the light intensity is high enough to give you a bright image (it can be increased using the black wheel on the left / front) of the microscope

- using the condenser vertical position control, move the condenser up and down until the edges of the visible diaphragm are as sharp as possible



- bring the aperture to the centre of the visible field using the 2 silver Köhler screws underneath the stage
- re-open the field iris (black wheel left / back) until the diaphragm is just not visible anymore



→ if you have problems seeing your sample in transmitted light, try closing the condenser iris fully: this gives you an extreme depth of focus and contrasts so helps focussing, but you can see every dust anywhere in the light path and artefacts around edges, so should be reopened (~two thirds) after focussing

## **Differential Interference Contrast (DIC)**

- focus on your sample:
  - set the focus speed to '1-2' (see above)
  - focus on your cells
- insert the top polariser (*black slider above the objectives on the left, labelled 'P'*)
- insert bottom polariser (*black round holder underneath the condenser, above the lamp*)
- turn bottom polariser until your cells are clearly visible, without exaggerating the contrast



## Fluorescence

- turn off brightfield lamp
  - turn on the excitation light moving the 3<sup>rd</sup> lever at the side of the microscope down (position "0") (the three others can stay up)
- turn the excitation off as soon as possible, avoiding bleaching
- to further reduce bleaching, lever 4 can be moved down as well
- change filters / colours as required (top silver filter wheel)

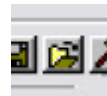


## Software / Image Acquisition

- turn off brightfield lamp (if not already done)
- pull the main light path switch all the way out (silver lever, scanning position)
- move brightfield light path switch to the left (black slider, scanning position)
- turn silver wheel on the top-right of the microscope from "1x" to 'UV/SCAN' position
- turn filter wheel to position 4
- Start the **Leica LCS** (LAS) software in "PERSONAL" mode



- to assign the hardware control to the control bar click on the opening icon on the bottom-right of the screen (recommended choice is "Hardware Control ")

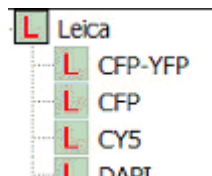


- check if the right

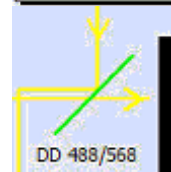
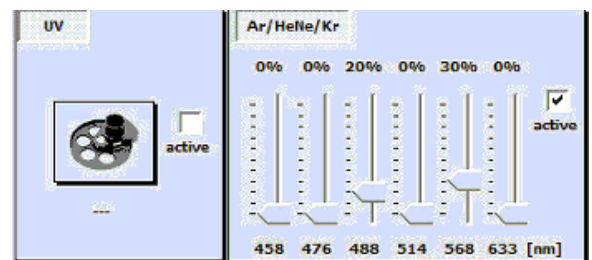


objective lens is selected

- click on the BEAM icon
- load the required setting from the preset list
- if necessary, adjust the following (top to bottom):



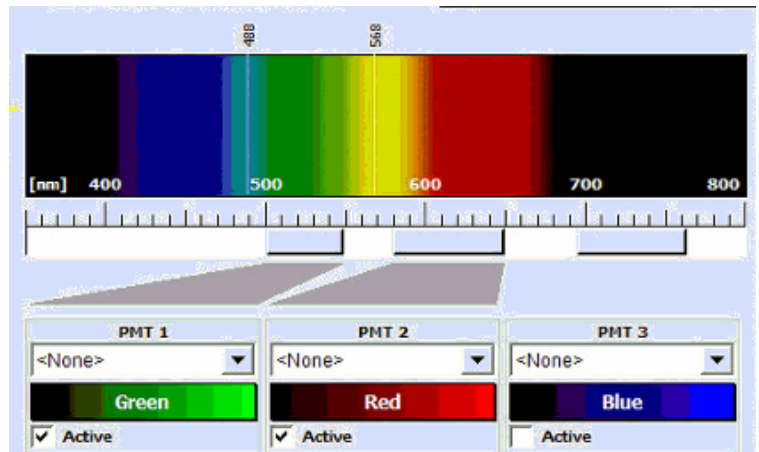
- laser lines and intensity (excitation)
- beam splitter (dichroic mirror):
  - > the wavelengths indicate the light that is reflected, they must correspond to the excitation wavelengths (the laser light is reflected onto the sample, on the way back the emission light goes through the dichroic
  - > for UV laser use "Substrat"



mirror)

- emission light settings for each PMT:

- > make sure none of the PMT covers any of the active laser lines – stay at least 10nm away from the laser lines



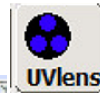
- screen colour for each channel

- > you can get the emission curves for most fluorophores from the dropdown menu; the selection does not change any settings (except screen colour), it's purely for your information!

- > don't forget to assign all necessary parameters to each required PMT

- turn on PMT TRANS to image transmitted light

- if exciting with UV, select the right UV lens

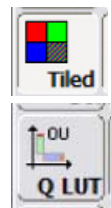


- in main toolbar click SIGNAL

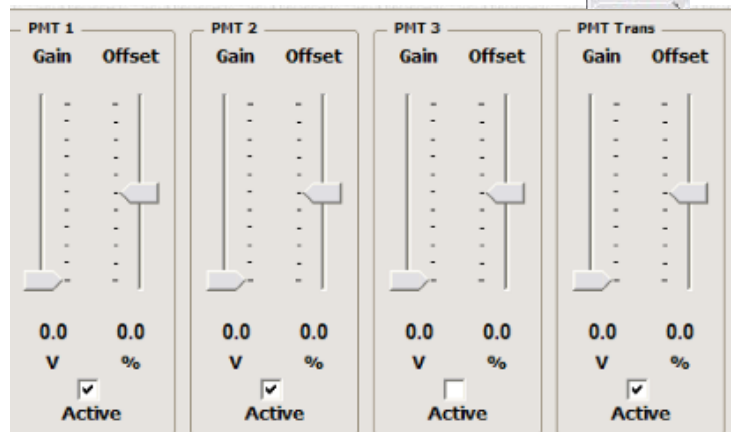


- start fast preview with CONTINUOUS

- in IMAGE WINDOW click TILED icon (if using more than 1 colour channel) and Q LUT (pseudocolour lookup table for adjustments converting the grayscale image into GREEN('black/empty/0')-RED-scale-BLUE('white/saturated/255'))



- adjust each PMT in the SIGNAL window, starting with the transmitted light channel:



- click on the image you want to adjust

- using the quick-access buttons, for each fluorophore adjust:

- > adjust GAIN so that only a few BLUE pixels are visible

- > adjust OFFSET so that only a few GREEN pixels are visible

- > → if necessary refocus after adjusting the transmitted light using the Z POSITION button on the 6-button control bar

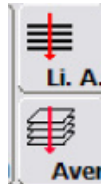


- stop fast preview clicking on CONTINUOUS again (to avoid bleaching of the sample)

## Settings refinements

- Averaging:

- Li. A. (Line Averaging): rescans each line several times, averages the intensity
- Aver: same as line averaging, but repetition of the scan starts after the whole frame rather than the line
- → *in general average at least 4 times (ideal: 8-16, depending on the zoom factor)*
- PINHOLE:
  - '1 Airy unit' is a good starting value (theoretical ideal value for the objective)
  - if brightness is limiting, an increase up to '1.5' has a moderate effect on the image quality
- SPEED: can generally stay at 400 Hz (*unless you have very special requirements*)



## Scan

- to take a single image, click SCAN
- to zoom in, click on Z IN, then draw a rectangle around the desired area anywhere on the image window
- click CONTINUOUS
- clicking on the ZOOM button ( → *usually it is advisable to adjust the zoom to the same level for all images; this helps comparing many images, otherwise you might miss effects of your treatment on the size of cells or structures!*)
- stop fast preview (clicking CONTINUOUS again)
- click SCAN to get your image
- save the image with SAVE ALL (*save data on drive ' D: ' only*)



## Sequential Scan of fluorophores with overlapping spectra

- adjust settings for each fluorophor separately, making sure that there is no excitation / detection bleedthrough and save each setting
- activate SEQUENTIAL scanning
- ADD each required setting to the list of scans (or drag / drop them into the left panel)
- set MODE to BETWEEN FRAMES
- start scan with SERIES
- → *if set to BETWEEN LINES, all mechanical settings (dichroic mirror, PMT wavelengths etc.) must be identical, otherwise the switch doesn't work, but the software doesn't tell you! (all lasers stay on for all channels!)*



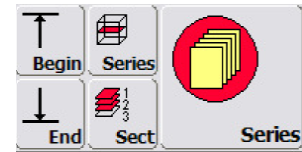
## **Reapply settings from previous images**

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

→ unfortunately, not all settings are reliably reapplied; partially they can be defined in the user preferences, but some don't work; to make sure your settings are identical check the settings frame.

## **Taking image stacks**

- focus to the top of the sample using the focus wheel on the control bar
- click BEGIN
- focus to the bottom of the sample
- click END
- check your stack settings by clicking the small SERIES button
- set the number of stacks with SECT (the number of stacks for maximal ("Optimal") Z resolution is set by default)
- start scanning by clicking the big SERIES button



## **Shutdown procedure**

### ***If you are the last user of the day:***

- check the booking for a last-minute change
- turn off UV laser by turning the key into OFF position, then switching off Main Power
- with keys on control panel turn off Arg and HeNe lasers
- turn off diode laser by pressing the LASER OFF button, then turning the key
- turn off fluorescence
- remove sample and clean objective lenses with fresh lens tissue
- save files onto portable device or the imaging server
- shut down computer & lamps
- turn off microscope, then turn off PC STAND and SCANNER on the black control unit
- wait at least 10 minutes from turning LASER off at the black control unit, because some lasers need subsequent cooling
- cover microscope and clear up the desk

***If someone's booked after you (or in the next 2 hours):***

- check the booking for a last-minute change
- clean objective lenses with fresh lens tissue and clear up the desk
- save files onto portable device or the imaging server
- log off