Starting the system

- Turn on the green switches from left to right
- Switch on the key-switch on the table to the on position
- Turn on the fluorescent lamp
  - If you want to do STED turn on the switch of the 592nm depletion Laser wait until the lamp turns from green to orange and back to green and then
  - Turn the key-switch to the “on” position
- When the PC is running log in and start the red “Leica LAS AF” software icon.
- Decide if you need the STED and/or the resonant scanner (only for live cell imaging)

Please note:
If images acquired on IMCES instruments are used in publications we would be grateful if you could mention the facility in the Acknowledgements.
• Make sure there is no obstacle in the range of the moving stage if you are initializing

• Go to the task bar choose “configuration” and switch on the laser you need
  • For the WLL and the Argon use 70% power
  • The 592nm STED 70%-100% power
Acquiring an image

- Choose the objective in the optical path
  - take care when switching between different types of immersion
- You have to switch on the laser with the On/OFF switch in the „laser settings“
- In the WLL you can define any excitation Wavelength (WL) from 470-670nm
- You can also define up to 8 laser lines excitation wavelength per Sequence
  - for this you have to highlight as many numbers as wavelength you need
- If you need several sequences you have to tick the Sequences button,
  Add sequences with the “+” button
- If you need several Sequences tick the „between frames“ button

Hit the WLL bar to switch to the WLL settings
Tick the little Square to activate the excitation WL; enter a WL-value and raise the power button.
• Decide if you want to detect your emission with a PMT or with a HyD

• Tick the “On/Off” button of the detector you need and choose the corresponding detector range

• For a quick setup check: choose a small frame and a fast detector speed
  - E.g. 512x512; 400Hz

• Hit the Live-button at the lower left corner of the screen.
  o If you choose the PMT set up the Gain and Offset with the USB control panel (use the range indicator button)
  o If you choose the HyD make sure there is not too much light reaching the HyD (it will shut down immediately)
    o Don´t use gain or offset with the HyD; only “average” and “accumulation” are effectively changing your image quality

Choose different colors for different detectors/sequences (double click on the color bar)!
• If you want to Zoom into your image:
  - Activate the “zoom in” function
  - draw a square into the image
  - The Zoom may be set to the values 0.75 to 20
  - Activate the “Live” button shortly to take over your settings

If you now want to acquire a high resolution image you might optimize for “Nyquist sampling”. But for getting the same signal intensity as your set up: You can increase the frame size and reduce the detector speed. But keep the **Pixel dwell time** the same.
Creating a Z-Stack

- If you want to acquire a Z-Stack you can choose the lowest position of your sample-stack as “End” scroll through to the upper position and call it “Begin”
- Or you can define the middle of your sample and after hitting the “Z Around Current” button and type in the size of the stack or how many steps you want.
- The Software automatically gives you the “Nyquist” Step-Size. But you could also alter it by hitting the “Nr. of Steps” option
- Hit the “Start” button to acquire the Z-stack

Creating a Tiling

- Hit the tiling button in the Acquisition mode
- During the “Live” observation hit the select button at each of the 4 corners of your specimen
- Start your measurement with the “Start” button
• If you are done with your measurements
• Go to the task bar choose “configuration” and switch OFF all lasers you used
• Choose the smallest magnification objective
• Move down the stage to the lowest z-position
• Switch off the software
• Shut down the computer

• (If you used the Argon let it cool down for about 15 minutes) Switch off the green switches and the key