

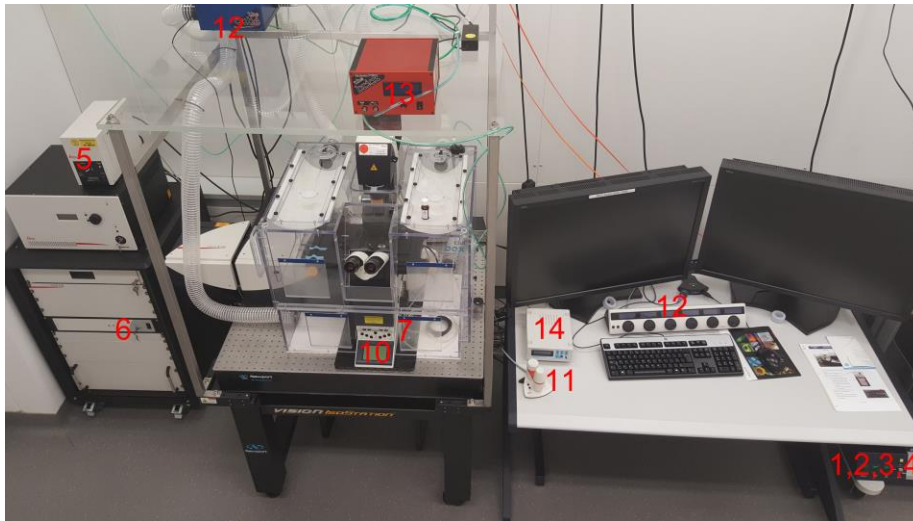
Standard Operating Procedure
Leica SP8

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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.

Last change: 21.06.2019



Starting the System



- Activate the HBX lamp (up on the left instrument tower) <5>
- Turn on the switches and the interlock <1> <2> <3> <4>
- If you intend to work with the STED Laser <6> : activate the power button for the 592nm laser (wait until it turns from orange to green) and then turn the key-switch to the “ON” position
 - Don't forget to insert the phase plate for STED measurement

Starting the Software



- Start the LASx Software
- Use the resonant scanner e.g. for live cell imaging
- Use the STED option if you want to measure a STED sample
- “Load settings” if you want to work with a personalized setup
- You have to initialize the stage if you want to perform multi position, or tiling. Take care there are no obstacles for the stage to hit

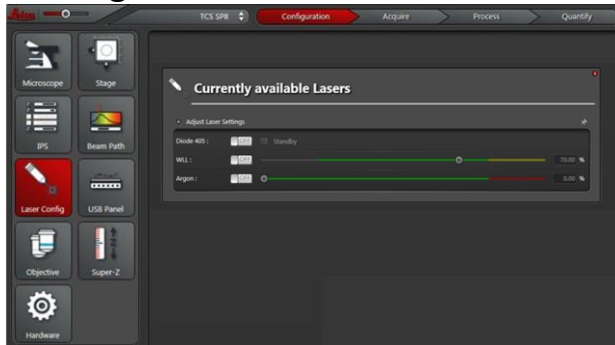
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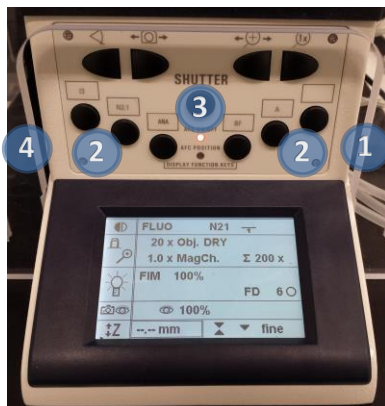
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Starting the laser



- Go to the configuration tab and activate the laser you need in the „Laser Config“
 - Argon and WLL lasers have a 60-70% preset increase only if really needed
- Update the bit depth to 12 bit in the “Hardware” setting
- If you want you can adjust personal configurations in the “IPS” settings

Load specimen



- Bring the Objectives into the load position ①
- Insert your sample
- Bring the objectives into touch with the immersion liquid if applied
- Activate a channel and ②
- Press the shutter- button ③
- Or activate the TL on the left side of the stand ④
- Now find the focus with the coarse focus wheel

Confocal setting



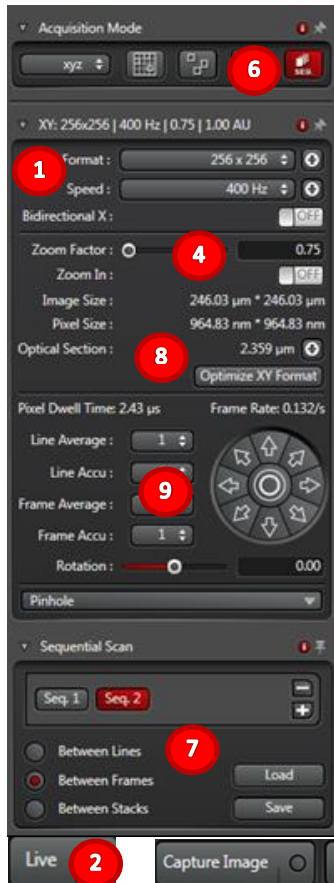
- ① • Choose an appropriate objective take care when changing immersion media
- ② • Activate a preset argon laser line or
- ② • Tick a WLL line and move to desired wavelength (add new with a “+”)
- Activate the shutter
- ③ • Apply some % power to the laser line
- Insert your specimen
- ④ • Set detector slider to the emission maximum of your fluorochrome

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- Set format to 512x512 and speed to 400Hz 1
- Activate the imaging with the “Live” button 2
- Adjust the focus for the best signal 3 with the USB-Panel:



- Zoom in if needed 4
- Adjust PMT/HyD gain and/or Laser power for good contrast 5
Use the saturation indicator mode for adjustment
- For sequential acquisition of several fluorochromes
 - 6 ○ activate the sequence tab
 - 7 ○ choose “between frames”
 - and add sequences with the “+” button
 - look at the new sequences in the live mode and adjust Excitation light as well as detector settings
- Read pixel size and dwell time 8
- For better signal to noise performance apply some averaging or accumulation if signal intensity is very poor 9
- Adjust pixel size with “Format” or the “best pixel size” button 1
- For the same image brightness keep Pixel dwell time the same ab adjusting the scan speed
- Note, format settings are global but Average and Accumulation are to be set for each channel individually
- Use the “Start” button to create a multilayer image with all adjusted image settings

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Z-Stack settings



- Find the starting point of your z-stack with the USB-Panel to set this as starting point for you scanning push „Begin“ **1**
- Now move to the end of you z-stack with the USB-Panel and confirm with “End” **2**
- Control the Nr. of steps and Z-Step Size **3** and adjust if “System optimized” is not suitable.
- To create a new z-stack or to delete the z-stack option press the discard button **4**

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Shutdown Procedure

- Go to laser configuration and turn off the laser
- Clean the objective you used
- Change to the smallest magnification
- Save the images you did not save
- Shutdown the software
- Check for the next user in the scheduler (if the next person is registered within the next 2h you can leave the system switched on. Only perform a Windows logout and turn the Interlock)
- Shutdown the computer
- Wait for the Argon-fan to stop cooling (if used)
- Turn off the buttons right below the table
- And the HBx-Lamp on the tower on the left side of the instrument