

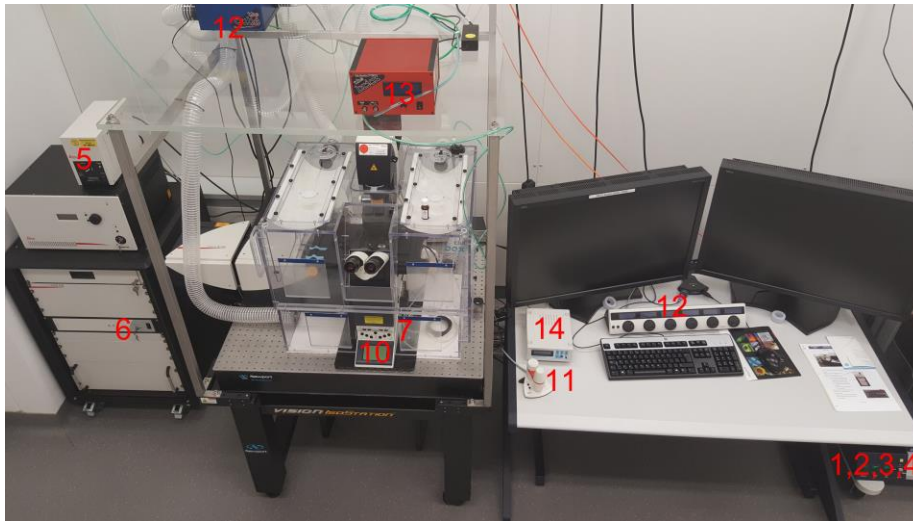
Standard Operating Procedure
Leica SP8

Page 1 von 5

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: 08.07.2019



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

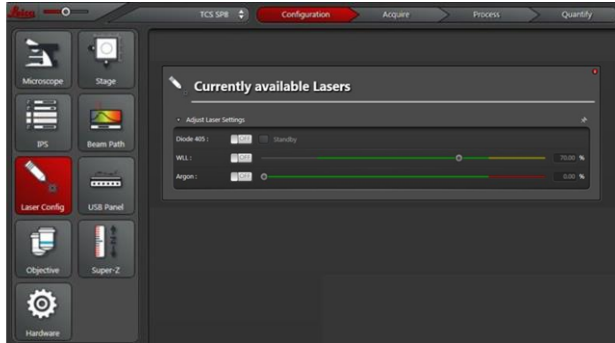
Standard Operating Procedure
Leica SP8

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: 08.07.2019

Starting the laser



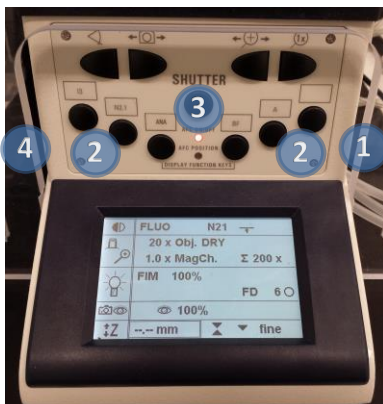
- Go to the configuration tab and activate the laser you need in the „Laser Config“
 - Argon and WLL lasers have a 60-70% preset increase only if really needed
- Update the bit depth to 12 bit in the “Hardware” setting
- If you want you can adjust personal configurations in the “IPS” settings
- Switch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

Confocal Setting



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

Load the specimen



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

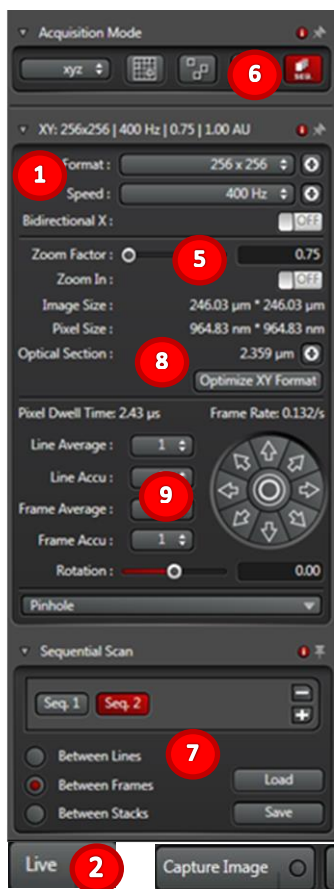
Standard Operating Procedure
Leica SP8

Page 3 von 5

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: 08.07.2019



- Set format to 512x512 and speed to 400Hz **1**
- Activate the imaging with the “Live” button **2**
- Adjust PMT/HyD gain and/or Laser **3** for good contrast
Use the saturation indicator mode for adjustment
- Adjust the focus for the best signal **4** with the USB-Panel:



- Zoom in if needed **5**
- 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
by adjusting the scan speed
- Note, format settings are global but Average, Accumulation
and Pinhole-settings are to be set for each channel individually
- Use the “Start” button to create a multilayer image with all
adjusted image settings

Standard Operating Procedure
Leica SP8

Page 4 von 5

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: 08.07.2019

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**

**Standard Operating Procedure
Leica SP8**

Page 5 von 5

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: 08.07.2019

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 1h 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