Standard **O**perating **P**rocedure

Zeiss LSM710/Elyra

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.

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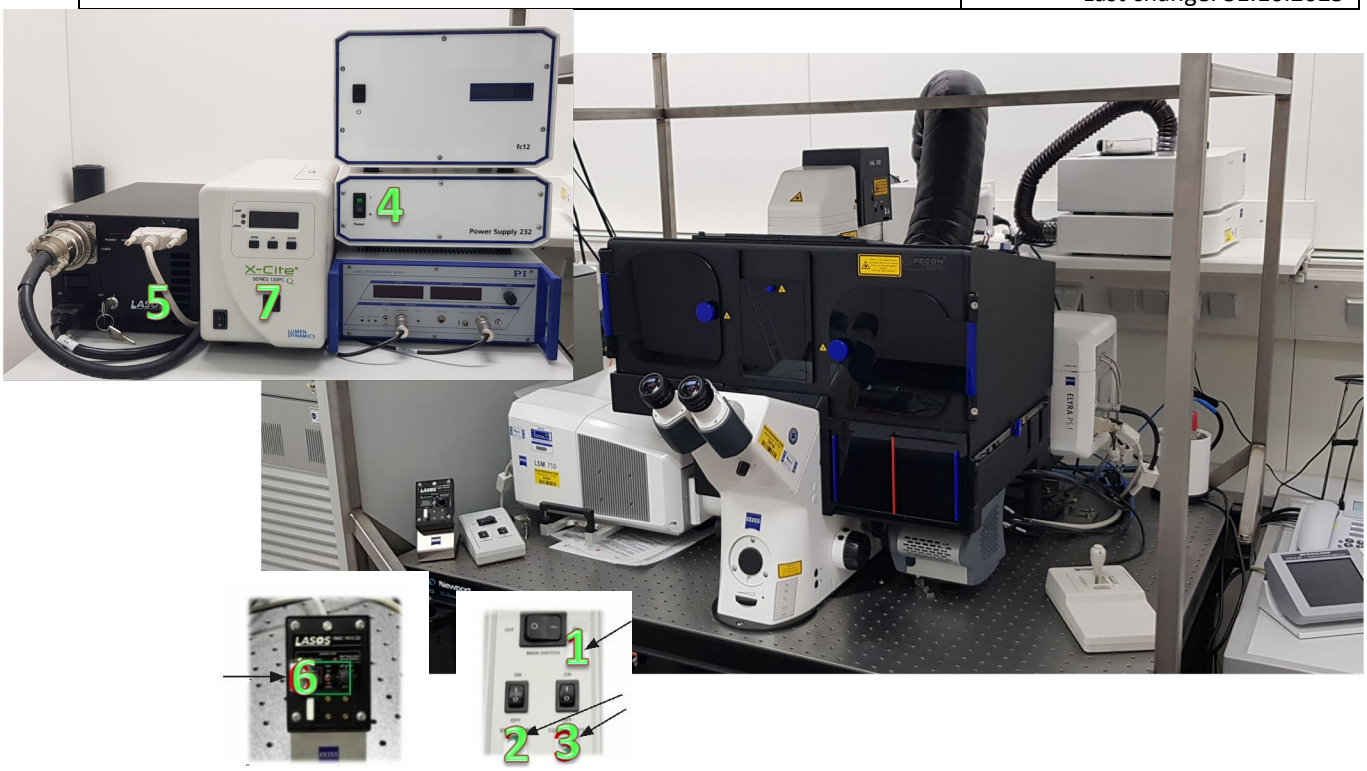


Starting the System

- Turn on the main power switch **<1>**
- Switch on the “system/PC” **<2>** and the “Components” **<3>**.
- Make sure the power supply is turned on (should never be switched off) **<4>**
- Turn on the X-Cite. **<7>** for locating your sample
- If you need the Argon (for LSM) turn the key to the “on” position **<5>** and switch the little toggle switch to the run position **<6>** and wait approximately 5-10 minutes to stabilize the laser. Wait until the power stabilizer light becomes green before you start your measurement.
- Start the computer and open the ZEN black software.

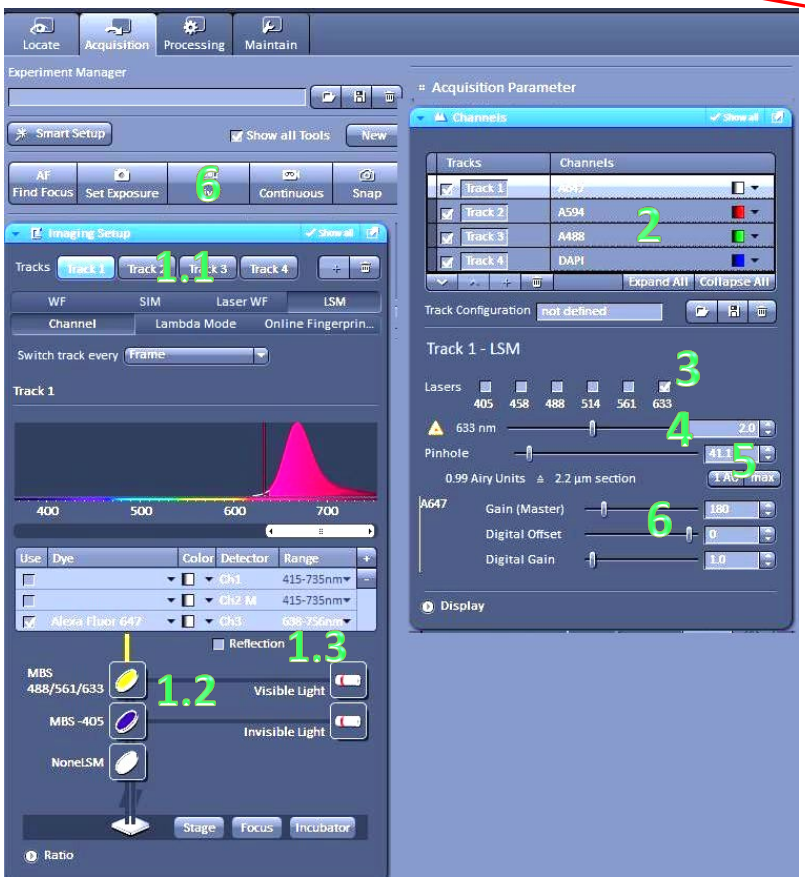
Load and focus the specimen

- Bring the objective into the “Load Position” by pressing the corresponding button on the touch screen **<8>**
- Select the “Locate” tab in the Zen software **<1>**
- Choose a suitable objective for your experiment **<2>**
- Select the filter “FSet77 HE” **<3>**
- And activate the fluorescent lamp by clicking the button and raising the power to ca. 12%. **<4>**
- Make sure the shutter is not closed **<4>**
- Depending on the sample, the transmitted light **<5>** can be more convenient
- If you need immersion medium place a droplet of the correct ZEISS Immersion liquid fitting to the objective on the coverslip
- Place your sample into the sample holder with the coverslip pointing down towards the objective and bring the objective into contact with the immersion medium using the coarse focus wheel

Confocal Setting

Configuration

- Switch to the „Acquisition“ tab and
- press „Smart Setup“
- choose the fluorochromes applied to your sample from the Dye drop down list in the appearing menu
- select your fluorochromes always in the order from red to blue fluorescence
- select the “best signal” option
- and click “Apply” to setup the system automatically

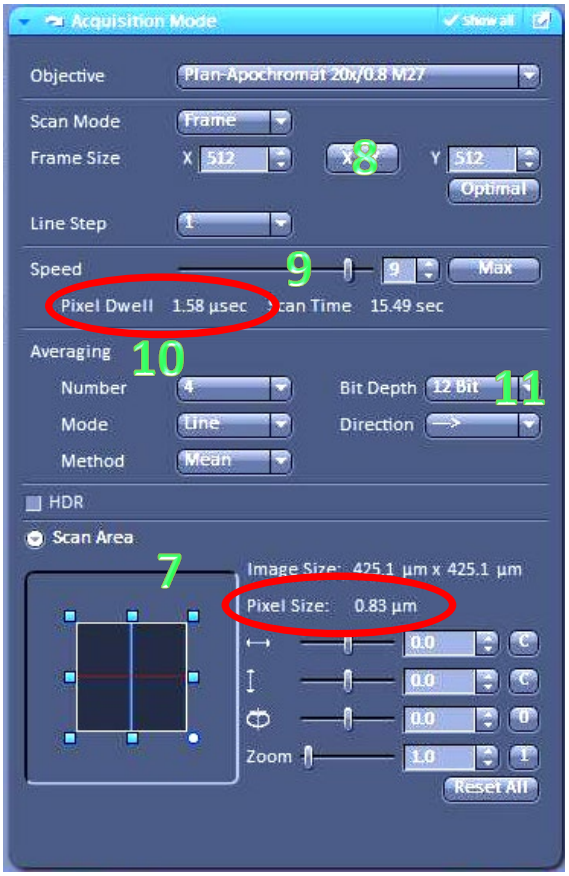


Acquisition setup

- Set the filter combination **< 1.2>** to minimize filter changes between tracks **<1.1>**
- Go to the “channels” dialogue and tick and highlight only one single track **<2>**
- Set the detection window **<1.3>** to be at least 10 nm away from the closest active laser line **<3>**
- Apply some laser power (typically 2~5%) **<4>**
- Press the “1AU” button **<5>**
- Apply some “Gain (Master)” **<6>**
- Activate the “Live” button **<7>** and select the “range indicator” color table for the resultant image; red regions indicate saturated detection
- Adjust the laser power and gain until you obtain bright but not saturated

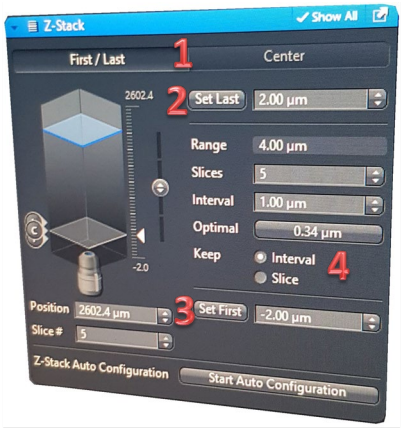
Image Acquisition

- If you already did some measurements on this system you can reload your old experiment with the “Reuse” function to import all previous settings
- For proper image acquisition always think about the pixel size (should be at least half the size of the structure you want to resolve) and pixel dwell time (changes image intensity)
- Adjust the pixel size either with the scan area **<7>** (is changed by the crop function) or with the Frame size **<8>**
- If needed, apply averaging to improve signal to noise in the acquired image **<10>**
- For quantitative analysis set the bit depth to 12 bit by default **<11>**
- For acquiring a multichannel image tick multiple tracks in the channels dialogue
- Press the ”Snap” button **<12>**
- For taking another image don´t forget to press “New” **<13>**. Otherwise your previously acquired image will be overwritten



Z-Stack

- First activate the Z-Stack option
- Decide if you want to define only the center position of the stack, or if you want to define the first and last stack position **<1>**
- If you choose the first/last option move with the focus to your lowest position of interest in the "Live" mode and push the "Set Last" button **<2>**
- Then move the focus to the highest position and press "Set First" **<3>**
- Take care to choose a proper stepping size **<4>** (*e.g.,* at least half the size of the structure of interest in axial direction) and choose interval (or the number of slices) accordingly. You can also choose the "Optimal" option to get the full axial resolution offered by the objective
- Make sure you select the "Z-stack" option in the drop down menu **<5>** in the imaging setup tab
- Activate (by ticking) as many pre-adjusted channels as needed and press "Start Experiment" to acquire



Shutdown Procedure

- Go to laser configuration in the Zen software and turn off the lasers
- Bring the argon laser toggle switch into the idle position <6>
- Turn the key at the argon laser power supply <5> into the off position. Wait for the cooling fan to stop cooling (approx. 10 minutes before you can turn off the laser)
- Move the objective into the load position and remove the sample
- Clean the objective from immersion medium
- Change to the smallest magnification (normally 5x Apo Calibration)
- Save the images you did not already save
- Close the Zen 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)
- Shutdown the computer
- Turn off the buttons on the power module <3>, <2> and <1>
- And the HBx-Lamp on the tower on the left side of the instrument <7>

Shutdown Policy

- You MUST look in the scheduler at the END of your imaging session, to confirm the arrival time of the next user, since the next user could have changed or cancelled their booking while you were at the instrument.
- If the next user arrives more than an hour after you, or if you are the last person in the scheduler then you MUST switch off the system.
- If you have a scheduler reservation and cannot make it then you MUST cancel your reservation in the scheduler, so that the user before you can decide upon the appropriate switch down procedure.
- If your reservation cancellation occurs more than an hour after the previous reservation then it becomes your responsibility to ENSURE that the instrument is switch down.
- If you are unable to cancel, because you are within the 2 hours cancellation time limit or do not have internet access, then it is STILL your responsibility to ENSURE that the instrument is switched down.
- If you cannot switch down the instrument yourself (because you are somewhere else indisposed) then arrange for a colleague or an IMCES staff member to switch down the system for you.

Inform the person DIRECTLY in person or by phone - do NOT assume they will read their emails in time!