LCAM startup manual, Meta confocal microscope (A2.40)

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Information microscope: http://www.lcam-fnwi.nl/facilities/zeiss-meta/

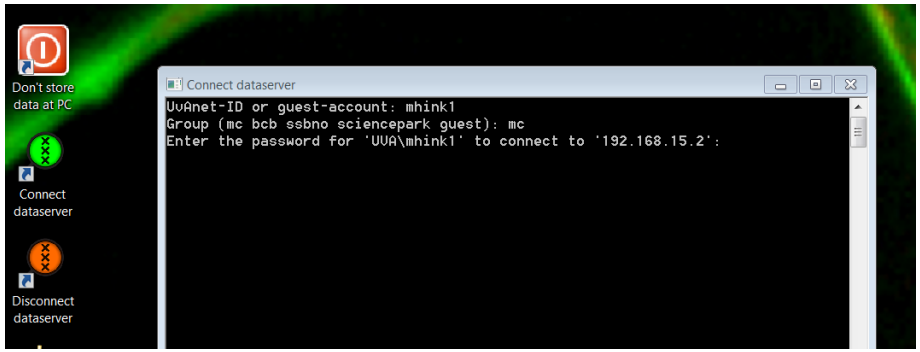
Before using the Zeiss Meta microscope, the user (and co-workers) should have had the official intake discussion with the LCAM-staff, succeeded the LCAM-confocal training course & exam and had an individual training at the Zeiss Meta.

Start-up procedure Meta

1. Switch on the microscope using the grey Main switch ("Remote Control") box and startup the computer.
2. If wished switch on the mercury HBO lamp at the power unit (right image).
3. Start the ***Zen2009*** software and push the ***Start System*** button.
4. When using the visible lasers (445, 488, 514 or 545 nm lasers) activate the ***Coherent Connection*** software, present as a shortcut at the desktop. Activate the wished lasers by pressing ***START***. However before activating lasers via Coherent Connection, activate lasers in the Zen2009 software!
5. The HeNe lasers (543 & 633) can be switched on in the main ZEN software *SetupManager window>Laser submenu>* Change the switches to ***ON***.
6. When the multiphoton laser should be used goto the MP startup section in this manual below.

Data storage

Never store data on the local harddisk, all data should be stored at the dataserver. Data present on the local PC will be deleted without further notice. Be aware that the storage of data on the sever will be your own responsibility as well. Although there is a regular backup of the server we will not take any responsibility for lost or damaged data, so make backups yourself. Contact Mark Hink in order to get access to the data-server from your office computer.

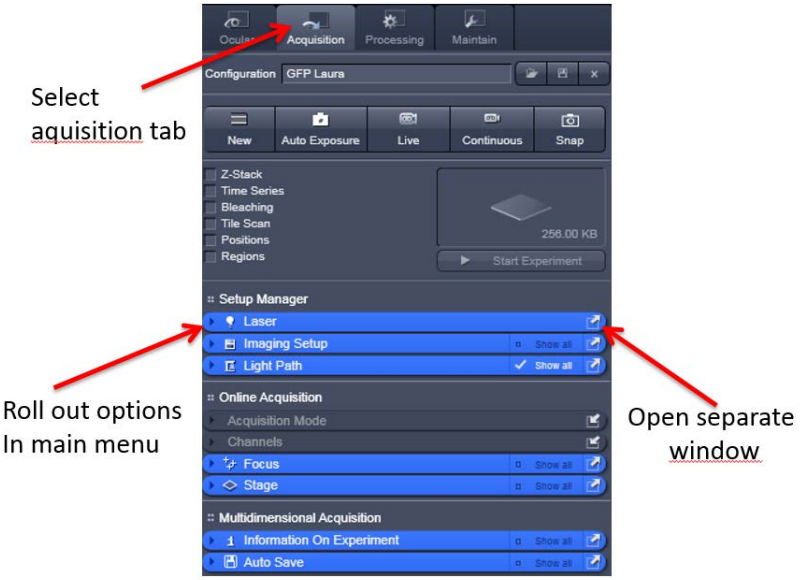


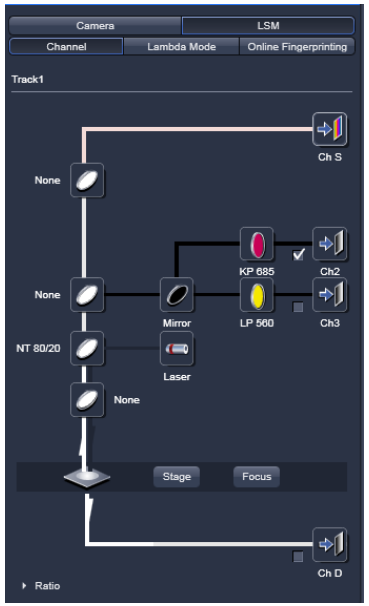
Login using the **green** ***Connect dataserver*** icon at the desktop**:** Type your userID (UvAnetID), group and password. After succesful login a network drive U:\ will be visible where you should store your data.

Basic handling of the confocal microscope

1. Put a droplet of the correct immersion liquid on top of the objective (air, water or oil). A small bottle of MilliQ water can be found nearby as well as a small **blue**-labeled bottle of Zeiss oil. In case the oil bottle is empty: **NEVER** use the immersion oil from other brands (Leica/Nikon/Olympus) but contact Anna or Ronald for a refill.

2. For a visual inspection of your sample you can use the ***Ocular*** tab. Press ***Online*** to activate. Switch on the Halogen Lamp (typical output 10%) for transmission images using white light. To inspect fluorescence by eye open the shutter of the mercury lamp (Switch to ***Open***) and select the appropriate filterblock (None, **DAPI**, **FITC**, **TRITC**). When finished with the visual inspection press ***Offline*** in case you want to make a pictures.

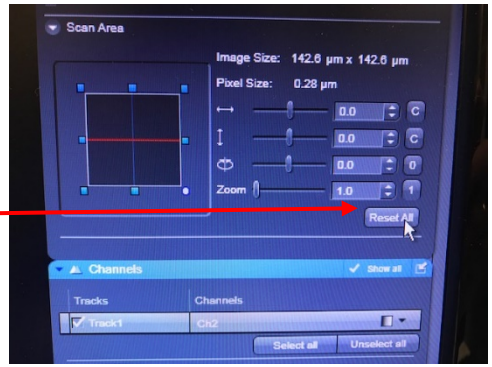




3. To setup your confocal experiment go to the ***Acquisition*** tab and roll out the menus wished. In the ***Light Path*** submenu one can adjust the dichroic mirrors, emission filters and activate one of the two standard PMT detectors or the (less sensitive) spectral detector (Ch S) (***>LSM>Channel***).

4. In the ***Imaging Setup*** submenu one can add multiple tracks for simultaneous or sequential scanning.

5. The ***Acquisition Mode*** submenu allows to control imaging parameters like nrs of pixels, speed, averaging zoom, pinhole size, laser power (AOTF) control and detector sensitivity (typical, ***Gain: 700***, ***Offset: 0.10*** and always keep ***Digital Gain at 1***).



6. Below the first acquired image a ***Crop*** button is present that allows to zoom in at a place in the image that you define. You can reverse this in the submenu Scan Area.

7. Pushing the ***Live*** button will scan at the fastest possible speed, ***Continous*** and ***Snap*** buttons will use the speed as defined by the user. Note that this speed might change when more pixels are selected.

Special features of the Meta confocal microscope

1. Camera based detection with the microscope. In order to detect widefield images in combination with the mercury lamp and the CCD camera go to the ***LightPath*** submenu and activate the camera TV1. As a good alternative one can use the ***Micromanager (1.4.22)*** program, present as a shortcut at the desktop. The software will directly start in the camera acquisition screen. Don't forget to open (and close) the mercury lamp shutter.

2. Since the Meta contains a spectral detector one can acquire the emission spectrum of the sample by Activating the Lambda Mode (***Light Path submenu>LSM>Lambda Mode***) and define the Start- & End-wavelengths and Step sizes of the acquisition. Now a number of images will be produced each specific for a small spectral region.

3. By using infrared light (IR) one can image deeper inside scattering tissues because of the lower light scattering. In order to use the pulsed multiphoton IR laser go to the ***Lasers*** submenu to activate it and the ***Channels*** submenu to fill in the correct output wavelength of the infrared laser. For details how to operate the laser contact Ronald Breedijk.

4. For Fluorescence Correlation Spectroscopy (FCS) measurements using the second bottom scanhead contact Mark Hink (+7860). A special FCS submenu is present to detect fluorescence using two external Avalanche Photodiodes.

Switching off procedure of the Meta confocal microscope

1. Deactivate all lasers in the Zen software (Laser submenu>***Off***) and/or the Optical software.
2. Switch off the HBO lamp.
3. Disconnect the link to your folder at the dataserver using the **red** **Disconnect dataserver** icon at the desktop.
5. Close the ZEN software and shut down the computer.
6. Switch off the microscope using the grey Main switch ("Remote Control") box.
7. If the Multiphoton laser has been used: In room A2.38 turn the key to the ***Standbye*** mode and Close the shutter (***Shutter Open*** button) at the Coherent Verdi power box. Switch of the water chiller in A2.38 and switch off the Coherent Mira 900 controller in A2.40. When used, switch off the Ocean Optics software and turn down the laptop.