

LCAM startup manual, LSM510 confocal microscope (A2.40)

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Information microscope: <http://www.lcam-fnwi.nl/facilities/zeiss-lsm-510/>



Before using the Zeiss LSM510 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 LSM510.

Start-up procedure LSM510

1. Switch on the microscope using the grey Main switch (“Remote Control”) box and check if the computer is still switched on, if not switch on computer.
2. If wished switch on the mercury HBO lamp at the power unit at the upper shelf (right image).
3. Start the **LSM510** software (called AIM) and select the **Scan New Images** and **Expert mode**. Ignore the warning about the non-calibrated 405 nm laser.
4. The AIM software has four main buttons that you are going to use from left to right.



5. When you plan to use the visible diode lasers (445, 488, 514 or 561 nm lasers) goto the Laser window and start the Argon laser (Standby modus is sufficient). Now start the Coherent Connection software, present as a shortcut at the desktop, and start the laserline(s) you'll need. Note that the 561 nm laser is called 568 in the AIM software and the 445 nm laser is called 458.



6. The HeNe lasers (543 & 633 nm) can be switched on in the main AIM software ->Laser window->Change the switches to ON.

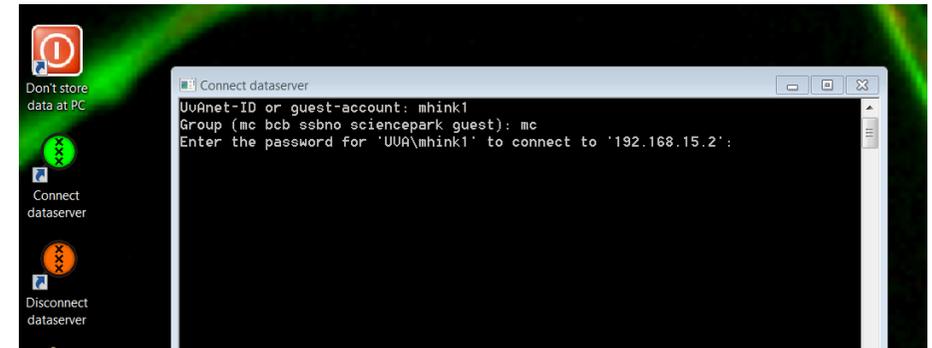
7. When the 355 nm UV-laser is needed (e.g. DAPI imaging) switch on the UV-laser (called 405 laser in AIM) in the Laser window. Then turn the key at the Coherent powersupply at the upper shelf. Wait a little and then turn up the power to 40mW by turning the power knob



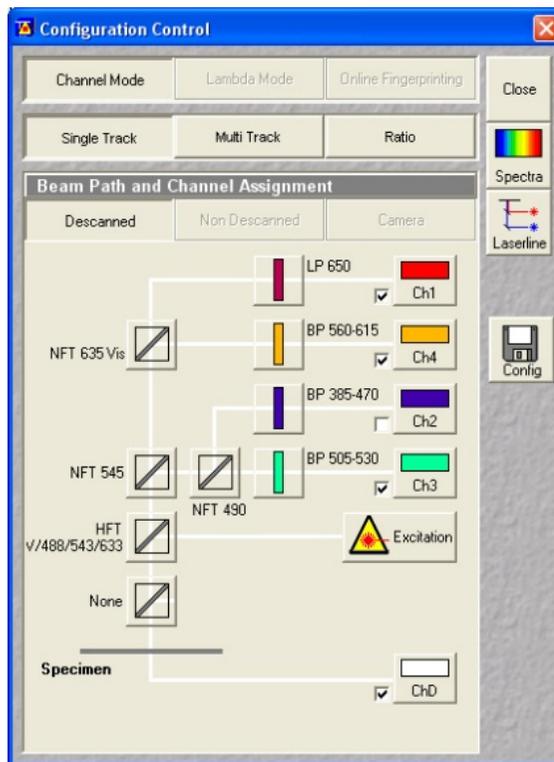
Data storage

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 **Micro** button in the main software. Here you can activate transmission light or mercury lamp light and for the latter one you select the appropriate filterblock (None, **DAPI**, **FITC** or **TRITC**).
3. In the **Config** menu you define the lasers and filters of your experiment. Since the AIM software can only handle 5 of the available 7 laser lines simultaneously check if your favourite lines have been activated by clicking on the **Laserline** button. In this opened Wavelength Switch Control window there are dropdown menus where you can change the active laser lines and **Store** these after your modifications.
4. In the **Config** menu you can manually design your filter combinations and laser settings. If you wanna use pre-defined settings there is a **Config** button. When opening this in the **Single Track** modus you get a dropdown menu with all the filter combinations for simultaneous detection. Select your favourite track and **Apply**. If you want to prevent cross-talk in multi-color experiments you can go to the **Multi Track** modus and design/load individual multiple tracks that will allow sequential scanning.

5. In the Scan menu you will find all basic scan parameters like number of pixels, speed of the scanner and a crop option to zoom into a subregion.

Special features of the LSM510 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. 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 (Multiphoton microscopy)** 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.
3. 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 LSM510 confocal microscope

1. Deactivate all lasers in the AIM Laser menu and the Coherent Connection software.
2. Switch off the HBO lamp power supply.
3. Disconnect the link to your folder at the datasever using the **red Disconnect datasever** icon at the desktop.
4. Close the AIM software.
5. Switch off the microscope using the grey Main switch ("Remote Control") box.
6. Clean the objective with special lens tissue (and ethanol if required).
7. If the Multiphoton laser has been used: In room A2.38 turn the key to the **Standby** 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.