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Information microscope: <http://www.lcam-fnwi.nl/facilities/olympuspicoquant-flccs-microscope/>

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

Startup procedure (general)

1. Start up PC-Olympus (shelf at the wall) and, if required, PC-Picoquant (besides the sink) for FCS, FLIM or confocal imaging using 405, 440, 485 & 560 nm lasers.
2. PC-Olympus: User: **Administrator**, Password: **F=m*a**
3. PC-Picoquant: *no login required*
4. Since both computers are connected to our SILS-S0-dataserver, the program Input Director will start, allowing that the mouse and keyboard of PC-Olympus can be used to control BOTH computers.

Startup of the Olympus FV-1000 confocal microscope

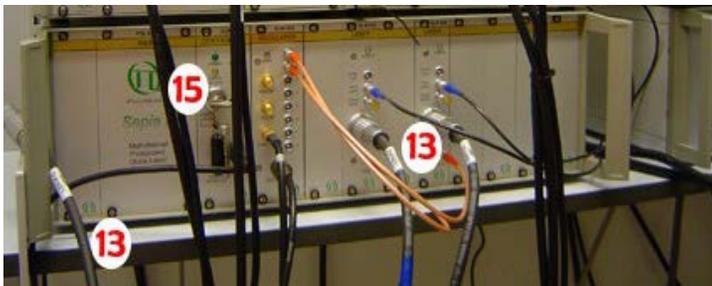
5. Turn on the main switch of the Praxis multicable unit that **powers** the Olympus confocal unit.
6. If needed switch on the power button for the mercury lamp (shelf within the frame).



7. If 488 nm (cw, not pulsed) is needed connect the powercable with the Obis powerunit.
8. For 514 nm light turn the key of the laser unit (next to the Obis 488).

8. If 561 nm (cw, not pulsed) is needed, turn the key of the Melles Griot laserunit to '1' and activate it by pressing the LASER ON button.
9. If 633 nm is needed turn the key of the Uniphase laserunit.
10. If 405, 440, 485 or 560 nm pulsed light is needed follow "Startup of Picoquant system"
11. Startup the **Fluoview software (FV10-ASW)** using the login name **Administrator** and password **Administrator**.

Startup of Picoquant system (when needed)

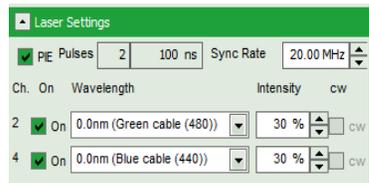
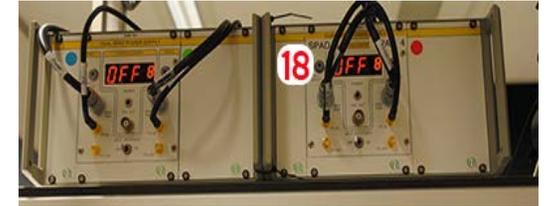


13. Check the SepiaII laser unit if the proper laser cables are connected to the SLM828 boards, exchange them when needed. **White tape indicates the 405 nm diode, Blue the 440 nm, Green the 480 nm and Orange the 560 nm diodes.**

14. Turn on the main power switch of the multicable unit using the red button located behind the monitor, all Picoquant units will be activated.



15. Turn the *laser locked* key on the SepiaII unit such that the *laser locked* **red** light is turned off.
16. Start the *SymPhoTime 64-bit (SPT)* software on PC-Picoquant.
17. Adjust manually the dichroics and emission filters in the *Detection Unit*, such that no laser light will be detected directly by one of the active APDs.
18. Activate one or more APD detectors by pressing the grey *CHX select* buttons on the *Dual Spad Power Supply* units. Burning or blinking lights indicate the active APDs. The detected intensity (in Hertz) of the non-blinking channel will be displayed. Note that these detectors are very expensive. They should never be exposed to lightintensities higher than 1.0E6 Hz. For safety reasons: Turn them off if you will not use them for longer than 30 min.

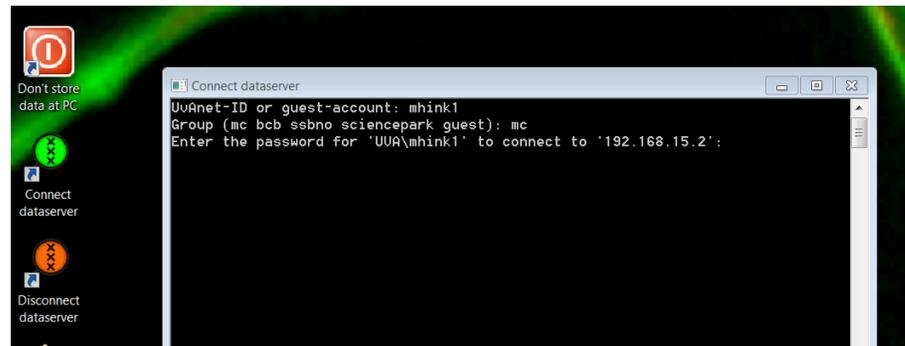


19. Adjust laser color, frequency and pulsing order to be used in the experiment.

20. For CO₂ and temperature control an OKO lab control unit is connected and placed at the right of the top shelf.
The system will require a few hours to stabilize at 37°C.

Main operation (general)

1. 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.
2. 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.



Main operation of the Olympus FV-1000 confocal microscope

1. Main microscope settings can be found in the *Acquisition settings* and *Image Acquisition Control* windows.
2. When using one of the PQ lasers (405, 440 or 485 nm) the 405 laser tab should be activated and ALWAYS set to 100% output



3. For adjusting the 561 nm (cw) laser: Check the filterwheel manual below.



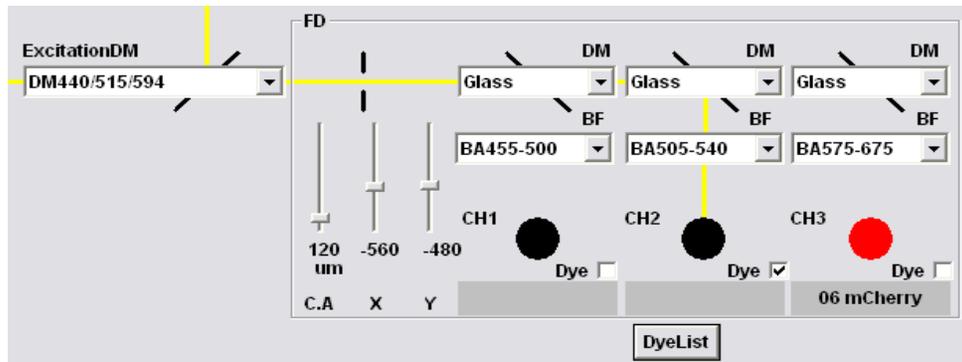
4. Manually adjust the lightpath options under the *Light path and dyes* button



5. Check if the objective is clean and what kind of immersion liquid is required
6. Transmission & Mercury lamp can be set at manual controller, left from the microscope or via the software

Main operation of the Picoquant system

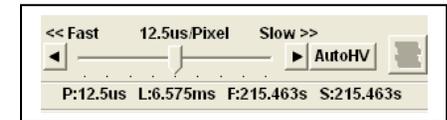
6. Create a new datafolder using Windows Explorer on the datasever SILS-S0 (see above).
7. Create in the SymPhoTime software a new Workspace, located in this new datafolder (see 6.)
8. Adjust the measurement settings like Point/Image, the size of the image and especially the Laser Settings. Select which laser is connected to the SepiaII unit, the output power of the diode (at least 23% in order to lase) and indicate if the Pulse Interleaved Excitation method should be active. In addition the pulse frequency of the diodes should be set typically at 20 MHz.
9. Adjust the layout of the online results, indicate which results should be displayed and change the options like which detector signals should be plotted, the total time of the intensity to be displayed (fe 120 sec) and the minimal correlation time for FCS (typical 0.001 ms)



11. For FCS and FLIM one uses the APDs and not the microscope internal PMTs for detection. Make sure that in the light path off the microscope all secondary dichroics are set to *Glass* (not *None*).

12. For FLIM: First press *START* in the SPT software and then start imaging using the *XY repeat* or *XYt* mode. Stop scanning when sufficient images have been collected.

13. For FCS: Take one *XY* image. Select the single-point measurement option  and draw in previous image your favorite single-point location (preferably around the centre). Set both the pixel-time P , in the scanspeed window, as repetitions t in the *TimeScan* window at a large value such that the sample acquisition time S for the Olympus (see *Acquisition settings* window) is larger than your preferred FCS data collection time, as set in the remote software. Start *Point-t* imaging and push only then *START* in the SPT software. Stop the datacollection yourself in the SPT software and stop the imaging of the Olympus scanner.



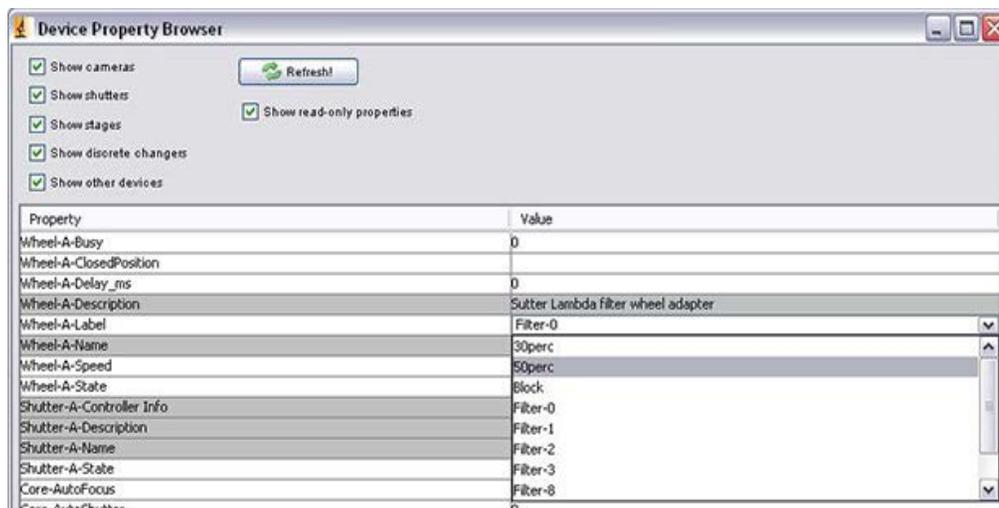
Main operation of the filterwheel to control the 561 nm cw laser power

1. After starting up the Olympus computer and logging into Windows open Micromanager (shortcut at desktop)
2. The settings are stored in a Configuration file called “Filter_shutter.cfg” that will start by default.
3. In the main screen the **Automatic shutter** option should be disabled that allows to Open and Close the shutter.



manually

4. To change density filter go to the “Tools” menu and select “Device Property Browser”.



5. Select the item “Wheel-A-Label” and select your favorite “#perc” density filter in the dropdown menu. **Block** will completely block the excitation light and empty places **Filter0-Filter3** will allow to use all the light.

Switching off (general)

0. Disconnect the links to your folder at the SILS-S0 dataserer using the red **Disconnect dataserer** icon at the computer desktop.

Switching off the Picoquant system

1. Deactivate all APDs by pressing the *CHX select* buttons on the *Dual Spad Power Supply* units. No lights should be burning or blinking on these units anymore.
2. Turn the *laser locked* key on the *SepiaII* unit such that the *laser locked* red light is burning
3. Turn off the main power switch, behind the monitor, in order to turn all Picoquant units down
4. Exit the *SymPhoTime 64bit* software and make sure no data is left at the local computer discs (move the data to the data server SILS-S0).
5. Turn off PC-Picoquant

Switching off the Olympus FV-1000 confocal microscope

6. Press the *ESC* button at the microscope body in order to move the objective to it's lowest, safe position.
7. Exit the Fluoview software
8. Turn down all laser units, don't forget when cw lasers have been used to deactivate them in room A2.38 (For this system none of the lasers needs a cooling delay time)
9. Switch off the mercury lamp
10. Turn off the main switch of the Olympus switch box.
11. Turn off PC-Olympus.
12. Clean the objective with special lens paper and leave the benches clean, f.e. remove sample holders, flasks, tubes with sample solutions and ice buckets.
13. Cover the objective by placing the black shielding plate on top of the stage to prevent collection of dust when the incubator box around the microscope is open.