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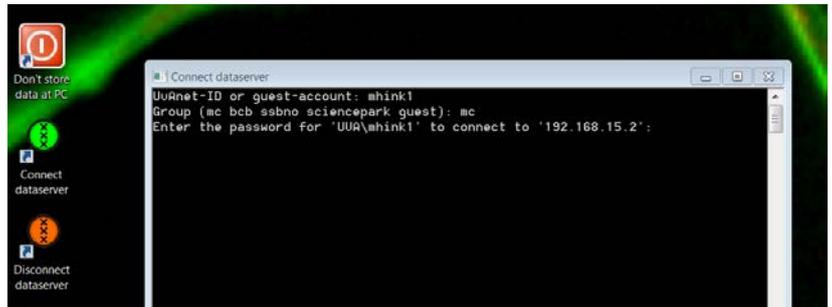
2nd contact person: Marten Postma, C2.267 (+5189)

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

Before using the FLIM-scope 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 FLIM-1 microscope

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 server 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.



Switch on procedure

- Switch computer on and wait until Windows is started, or restart Windows.
- Switch on microscope (rear, right side) and switch to port selector "EYE" (front).
NB Don't touch optosplit so walk around the table.
Wait until microscope is fully initialized before moving to step 3.
- Switch on Prior Proscan III (wait for sound initializing the filter wheel)
- Switch on Lambert Instruments module.
- Startup LI-FLIM 1.2.23 software.
- Check settings in Lambert software (*all Left monitor left side of window*).
 - In top left acquisition menu:
 - Take 18 phase images.
 - Reference lifetime 0.086 ns.
 - Mod frequency 40.000 MHz (=40 MHz).
 - Uncheck Multi-frequency, z-stack, and averaging.
 - On Lifa10007 menu:
 - Set MCP gain to 650 V.
In expert mode otherwise invisible:
Cath DC +1V
Cath AC -12 dBm
LED DC 150 mA. (for 470 LED also 100 mA works)
LED AC 3.0 V
 - Check if correct LED is selected (wavelength indicated).
If not: -uncheck "LED always on".

-select correct LED.

-check "LED always on" wait till LED is ready.

C) In left Nikon menu:

- Check if dichroic filter block matches LED: Filter (Position-Name).
- Select correct objective (e.g. 40x air MRD00405)

D) In left Prior Scientific menu:

- Go all the way down (do not use the mouse scroll wheel)

and select correct Excitation Filter.

NB Pos 4=for 446 LED (448/20) optimal for CFP.

Pos 5=for 470 LED (472/30) optimal for GFP.

Pos 6=for 507 LED (500/24) optimal for YFP.

Pos 7=for 532 LED (534/20) optimal for OFP/RFP.

- 7 Uncheck expert mode in top "view" pull down menu (in case it was on).
- 8 Check that switch under excitation light port selector (underneath filter wheel at right rear end of microscope) is at 45 degrees and not parallel to the front end of the optical table.
- 9 Set PRIOR shutter on filterwheel open. For this press on menu button (=top left) of PRIOR console (=device with joystick). Press menu until you see SH1 displayed. Then press second button to open/close. Open =o closed =•.

Now the FLIM is ready for use (see other manual for taking reference & sample stacks).

Switch off procedure

1. Close manual (orange) shutter in front of LI camera (left port microscope).
2. Switch microscope port selector to "EYE" (front).
3. Quit the Lambert Instruments LI-FLIM 1.2.23 software.
4. Turn off Lambert Instruments module.
5. Turn off Nikon Microscope.
6. Turn off Prior Proscan III.
7. Disconnect the links to your folder at the SILS-S0 data-server using the red Disconnect datasever icon at the computer desktop.
8. Turn off Computer.
9. Optional: Reset ND filters at rear microscope to open (pulled out) if used.
10. Clean up desk and microscope.