LCAM startup manual, FLIM-1 microscope (A2.42)

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Information microscope: http://www.lcam-fnwi.nl/facilities/fd-flim/

Before using the FLIM-1 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

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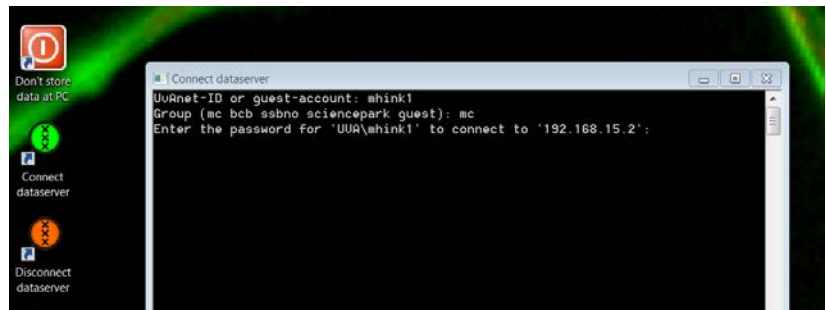
2. Login using the **green Connect dataserver** icon at the desktop: Type your userID (UvAnetID), group and password. After succesful login a network drive U:\ will be visible where you should store your data.

Switch on procedure FLIM with AOM and lasers 488, 514, 532 and 561 nm. For 440 laser switch on procedure, see other side and omit steps 1-6 below, continue at step 7.

1) Check on top frequency synthesizer if it is set at 37.55 MHz and 500 mV. If this is the case goto step 3. If not, disconnect BNC cable labeled "MDL300" and connect the BNC cable labeled "AOM". Press the "RCL" button (low right to display) of top frequency synthesizer until the menu changes to "recall" and then select presetting 002 (rotate wheel and press). It should now be set at 37.55 MHz and 500 mV.

2) In laser room flip the mirror down that is in line with the orange fiber coupling (just right of diaphragm).

3) In laser room (also steps 4-6) switch on water bath for AOM. Fill water level if needed (use demi water). Press power switch and the ↑ button to start. Temperature should be 40˚C.

4) Turn on AOM (red button should be locked inward).

5) -Switch on 488 (flip toggle switch labeled 488 & FLIM (left) on grey box on the floor).
   -Or switch on 514 (flip toggle switch labeled 514 & FLIM (middle) on grey box on the floor).

-Or switch on 532 (see instructions printed on top of 532 nm laser control box (under table)).
-Or switch on 561 (press power switch, wait till bottom red led switches off and yellow top led switches on (15 s), turn key, wait till top 2 leds light up then turn wheel to 200).
Check that modulation is ok (multiple spots projected onto diaphragm) and central spot goes through the diaphragm. If not, call Dorus or Ronald to adjust light path in laser room.

6) Wait half an hour to have the AOM and laser stabilize (otherwise phase and modulation are unstable).

7) Laser room: switch on fiber shaker (toggle switch on light grey box with orange fibre) underneath table.

8) In FLIM room, (if needed) switch on mercury arc lamp.

9) Startup computer (if it is not started yet).

10) Turn on CoolSnap CCD camera (black power supply).

11) Turn Lambert Instruments intensifier on (green button on LI module, switch should light up). First make sure that manual slider in front of intensifier is closed (left port of microscope).

12) Turn Zeiss microscope on (red switch on right rear end, should light up). Turn down transmitted light (toggle switch on front side of microscope).

13) Start Matlab by using the 'MATLAB 6.1' icon on the desktop.

14) Type 'flim' + [enter] in the command window. The flim/spim program. Enter your user name and select 'enable flim' from the FLIM menu. Choose the excitation laser wavelength you use (step 5). Press 'OK'.

15) From the settings menu choose 'microscope', 'intensifier', 'signal generator'. From the acquisition menu select 'Acquire FLIM stack', from the processing menu select 'Blur' and 'Background ROI subtraction (regular FLIM stack)' and from the analysis menu select 'calculate lifetimes' and 'histogram'. Position the control panels so that they can be viewed and used.

16) Set the microscope to the correct filter position, objective (and for arc lamp) the correct excitation filter. Check that the microscope selects 'laser' as excitation source.

17) Set MCP gain to 200 and cathode DC to 70 (or 80, 90) by typing numbers and pressing [enter].

18) Switch cube to 'reference' on 'microscope' control panel. Turn on fluorescence shutter (press 'FL on/off' button on the right side of microscope) and check that you see a full field of (dimmed) excitation light. If only top or bottom half of the field is illuminated ask Dorus to correct the reference filter cube mounting.

19) Move manual shutter in front of image intensifier to 'open' position. Make a FLIM stack of 12 phase images of reference laser light and use an exposure time of 100 ms. Press acquire on 'Acquire FLIM stack' control panel. Check the calculated reference phase and modulation (given in the command window). Check that laser modulation is high (e.g. > 70%, preferably ~100%). If lower contact Dorus.

20) Mount your sample. Select 'middle' output and change to the appropriate cube on 'microscope' control panel. Open the fluorescence shutter ('FL on/off' button on right side microscope) focus and put desired cell in the middle. Close the fluorescence shutter.

21) Select 'Acquire FLIM stack' from the FLIM 'acquire' menu (if control panel is not open yet). Set exposure time between 10 and 1000 ms (depending on brightness of the sample). Select 12 phase steps and press 'acquire'.

22) After acquisition, a FLIM image stack is displayed. By pressing 'n' and 'p' you can browse through the stack. Ideally the maximum pixel value should be between 2500 and 3000, but is should not exceed 3900. This max value is shown in the command window. Adjust the image

exposure time to get the correct intensity. For very bright specimen, move the ND filter in the rear of the microscope to ND1.5. Repeat step 21, but remove the ND filter just prior to recording of the reference phase/modulation.

23) For processing data, one can 'blur' the image (3x3) or 'subtract background' (use ROI near the centre of the image outside cells). Then one can calculate the lifetime image from phase and from modulation. With the 'histogram' control panel a histogram of the lifetimes can be displayed (don't forget to draw an ROI in the lifetime image and refer to the correct input image for the histogram).

24) Save the FLIM stack by selecting 'save image' from the FLIM 'file' pulldown menu. Type filename and press save, and select correct input image and press again save.

Standard image processing steps 25-28:

25) For exporting to image J color output: type 'edit' + [enter] in command window.

26) Edit the 'export_to_ij.m' macro. Just change the filename and directory, omit the extention .ics. Save the changed macro (ctrl-s) and run it by typing 'export_to_ij' + [enter] in the command window. It will display a new image in image buffer C with the calculated DC, tau(phi) and tau(mod) images. These can be displayed in image J. The macro automatically saves an image with an extension '_ij.ics'

27) Start imageJ and import the lifetimes10c or lifetimes14 macro (they are present on the desktop).

28) Run the FLIM macro and use 'open file' pointing to the file created in step 26. Alternatively, import the image created in step 26 into image J (drag it in the ImageJ command window). You can crop the image to select the cells that you want in image J. Then run the lifetimes macro using 'work on current image'. Image J automatically saves a file with extension '_ij.tif'.

Alternative switch on procedure for 440 directly modulated laser

1) Check on top frequency synthesizer if it is set at 75.1 MHz and 100 mV. If this is the case go to step 3. If not, disconnect BNC cable labeled "AOM" and connect the BNC cable labeled "MDL300". Press the "RCL" button (low right to display) of top frequency synthesizer until the menu changes to "recall" and then select presetting 001 (rotate wheel and press). It should now be set at 75.1 MHz and 100 mV

2) In laser room flip the mirror up that is in line with the orange fiber coupling (just right of diaphragm).

3) In laser room: switch on the 440 diode laser. First turn on the power switch at the back of the MDL300 driver. Then turn the key to "on" on the MDL300. Wait 10 s. Turn modulation on toggle to "ext". This should start the blue light emission from the laser.

4) In laser room: check that blue light correctly moves through diaphragm. If not contact Dorus or Ronald to adjust mirrors in the laser room.

5) Go to step 7 on other side.

Switch off procedure FLIM:

1) Type 'exit'+ [enter] in FLIM command window. Disconnect the links to your folder at the SILS-S0 data-server using the **red** Disconnect dataserver icon at the computer desktop.

2) <mark>Close manual shutter in front of intensifier, left side of microscope</mark>

3) Switch off intensifier (green switch on Lambert instruments control unit)

4) Switch off Photometrics CCD camera.

5) Switch off microscope.

6) Switch off mercury lamp (if it was on).

7) In Laser room: switch off AOM.

8) In laser room: switch off laser (440: switch toggle from 'ext' to 'off' on MDL unit and switch MDL300 off), (488 and 514: toggle key to off), (532: follow instructions in reverse order of switch on) (561: turn key to off, wait 10 s, switch off).

9) In laser room: switch off water bath.

10) In laser room: switch off fiber shaker.

    440: steps 7) and 9) are omitted in the switch off procedure if the 440 diode laser was used.