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Measuring and analysis of multifrequency FLIM data

A) Switching on

1) Disconnect (if connected) the BNC cable (bajonet) from the AOM amplifier and connect to the MDL300 unit to the BNC connecter (HF input)

2) Switch on FLIM system in regular order but change the following:

-don't switch on the AOM amplifier or the bath to control the AOM temperature

-For switching on the 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 toggle to "ext"

-Make sure the flipper mirror is turned upward to guide the 442 laser into the microscope

3) Set equal frequencies on both signal generators (a red error message appears but ignore) in the FLIM software.

4) If you measure at 75.1 MHz you can use gain 200 bias 90 on the intesifier, if you want to do multifrequency FLIM then use gain 200 bias 110.

B) switching off

1) use same order as standard FLIM switch off procedure

2) switching off the laser diode:

-Turn modulation toggle to "off"

-Turn key to "stand by"

-wait 10 s

-Switch off MDL 300 unit

C) Measurement

1) for measuring multifrequency data use the YFP filter cube

2) Always use the same height: focus cells and then type:

get_stage

for the next sample you can set the height by:

set_stage(number)

3) Use the green button to select a decent ROI to avoid memory problems

4) Perform also a calibration with EB

5) Store the multifrequency image stacks

D) multifrequency analysis

1) Use the multifrequency analysis tool in the FLIM software

-Optionally you can subtract background from areas outside cells

-by selecting a rectangle you can get the multifrequency data for an ROI

2) Copy/paste the multifrequency data in the multifrequency excel file

3) Copy/paste the multifrequency data of EB reference into the multifrequency excel file

4) The excel file automatically corrects the sample data with the EB data

5) Copy paste thhe corrected data in a standard CFS (ASCI text) input file below the "CLOSE" line

6) save this file with a file name with not more then 8 characters: xxxxxxxx.txt

7) repeat steps 1-6 for all your data
8) copy all CFS input files to the CFS folder
9) start CFS_ls
10) follow instructions: function, data etc
-you can select hetanl-2 or hetanl-1 for 2 and 1 lifetime component respectively
11) upon display of the fit result press “print”
12) if the plot of the fit looks fine press “save” and choose a eps format. Save as xxxxxxxx.ps
13) quit cfs_ls (type q)
14) sort the folder cfs_ls by data. The most recent file is the print file. Move this file to”notepad” and save it as a result file (ie: xxxxxxxxres.txt
15) double click on the ps file and it will be converted to pdf
16) print both the result file and pdf
17) repeat steps 9-16 for all data
18) at the end move all your data files back from the cfs folder to your data folder.