Switch on / Switch off procedure FLIM-system

Content

Switch on

- Switch on the Olympus FluoView 3000
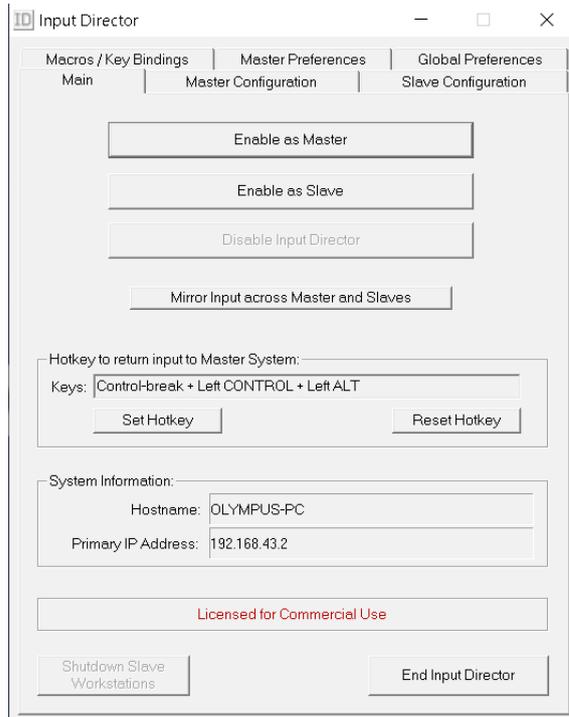
- Start the FluoView software: use monodirectional scan, do not use resonant scanner, no sequential scan, averaging off.

- check that the emission is NOT directed to the fiber port in the confocal beam path

- turn on the left power strip with FLIM PC and laser unit control

- switch on FLIM-PC, wait till it has booted

- In the FluoView PC, open the Ïnput Director Software

- Click „Enable as Master" to allow mouse/keyboard sharing between the Olympus and FLIM PCs
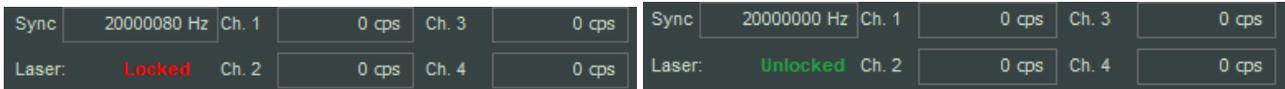

- turn on the switch on the back of the PDL828 „SEPIA" laser controller unit. (the key should be in Standby position)

- turn on all other FLIM hardware on the right power strip

- check that the lever on the exit port is in the right position for your measurements (IN: FLIM, FCS, OUT: Anisotropy). See the image. When OUT chosen light goes in the position one. When IN: light is split between the tower positions 1 and 3 of the detection unit.

- Check that the correct filter cubes for your experiment are inserted into the detection unit. Remember: the 3 positions for the cubes are labelled with a number, and the cubes must be inserted into the position corresponding to the label.
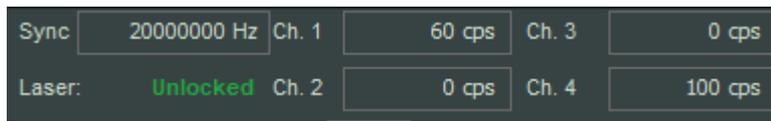
- Start the SymPhoTime software

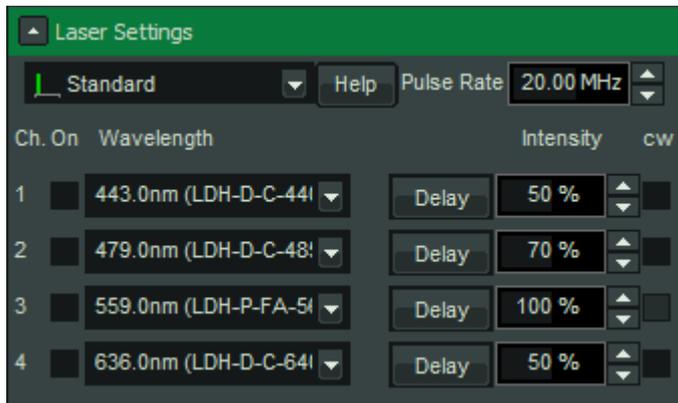- Create a workspace for your data or open an existing one.

- Turn the key from the laser unit from „Laser locked" (horizontal) to „Laser Active"(Vertical)
  - in the SymPhoTime software, the red „locked" changes into a green „unlocked". The **laser repetition rate** set („SYNC") should be displayed above. Adjust it if needed: 20MHz, 40MHz etc depending on the lifetime you want to measure

| Sync | 20000080 Hz | Ch. 1 | 0 cps | Ch. 3 | 0 cps |
| Laser: | Locked | Ch. 2 | 0 cps | Ch. 4 | 0 cps |

| Sync | 20000000 Hz | Ch. 1 | 0 cps | Ch. 3 | 0 cps |
| Laser: | Unlocked | Ch. 2 | 0 cps | Ch. 4 | 0 cps |

- Switch on the FLIM Detection Unit

- if needed, switch on the SPAD detectors on the DSN 102 unit (in the Olympus cabinet). This is not necessary, if you only want to work with detector 1 (or with det. 1 and 4).

- SPAD detectors are used for FCS and single molecule imaging.

- you should now see in the SymPhoTime software the dark counts of the detectors, which you have switched on:

| Sync | 20000000 Hz | Ch. 1 | 60 cps | Ch. 3 | 0 cps |
| Laser: | Unlocked | Ch. 2 | 0 cps | Ch. 4 | 100 cps |

- When measuring with the SymPhoTime, you will have to check the laser(s) you want to use: either PicoQuant or Olympus. **Note that it is important to** uncheck the laser if you want to use the Olympus lasers, otherwise you may have additional pulsed excitation on your measurements
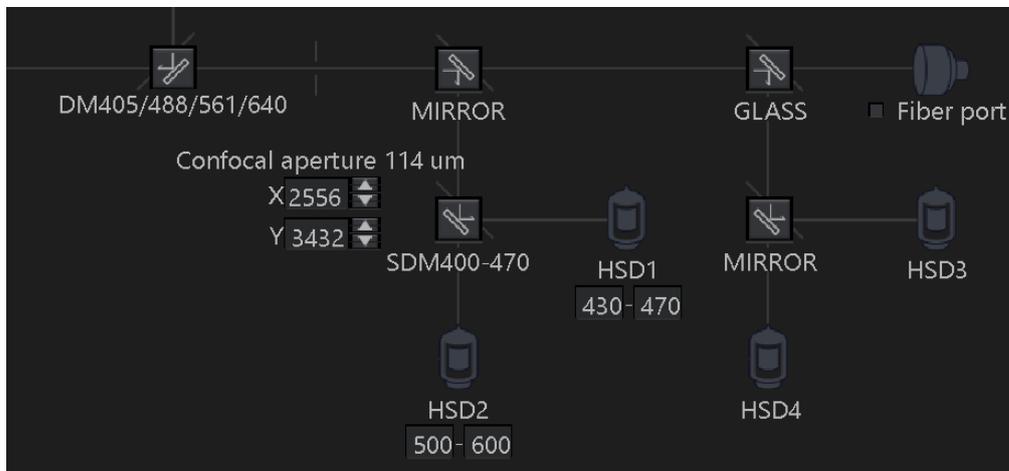

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- Adjust laser power with the attenuation controls

- Check that pixel size and number of pixel match with Olympus settings

- Check your image in FV software (Note that the FluoView detectors are only comparable in sensitivity, if the voltage is set high, otherwise, better attenuate the laser a bit more, before starting the imaging via "Test")

- Check the box for the "fiber port" in the confocal beam path in FV software

- You are ready to go: press Start in Symphotime and then Live in FV software.

- To stop imaging: stop live in FV software and then stop scan in symphotime.

- Go to analysis (read more https://www.tcspc.com/doku.php/howto:lifetime-fitting_using_the_flim_script)
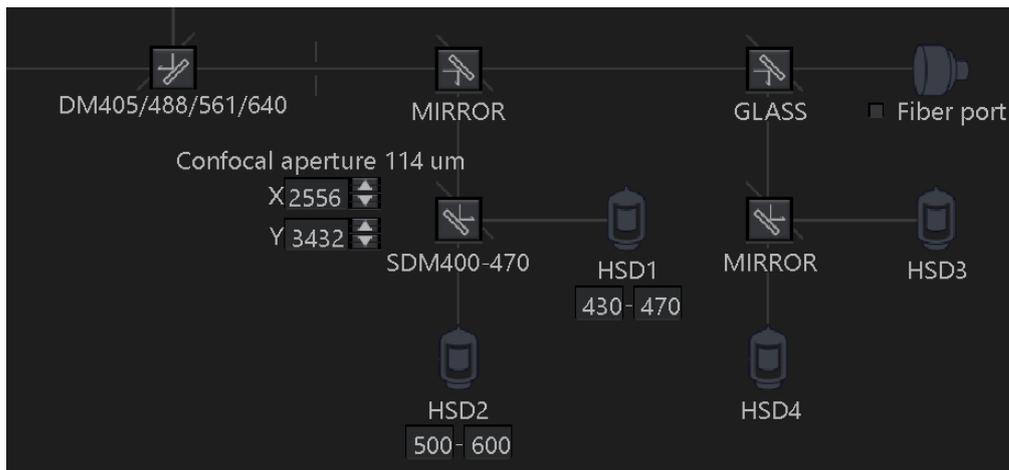
Switch off

- In the FluoView software, uncheck the "Fiber port" option and make sure that a mirror is inserted:

- In the SymPhoTime, save all analysis data you want and quit the software

- turn the key at the PDL828 „SEPIA"laser controller to standby („locked", horizontal position.

- Switch off the PDL828 „SEPIA"laser controller

- if they had been switched on, turn off the SPAD detectors on the DSN102 unit in the Olympus cabinet.

- Turn off the FLIM-detector unit

- set the lever of the exit port to the FLIM-position (lever in)

- switch off the FLIM-components on the right main power strip

- shut down the FLIM PC

… if leaving for a longer break.

- In the FluoView software, uncheck the "Fiber port" option and make sure that a mirror is inserted:

- turn the key at the PDL828 „SEPIA"laser controller to standby („locked", horizontal position.

- if they had been switched on, turn off the SPAD detectors on the DSN102 unit in the Olympus cabinet.

- Turn off the FLIM-detector unit

- when returning from the break, reverse the procedure

...if you just want to analyze data

- Turn on the FLIM-PC

- grab the mouse keyboard from the FLIM PC.
  - Alternatively, also turn on the Olympus PC and activate the mous/keyboad-sharing via the Input-director-program.

...what if…

- If the SPAD-detectors are overilluminated, they will automatically switch off. Remove the light exposure of the detectors and switch them on again on the DSN102-unit in the Olympus cabinet

- If the Hybrid detectors are overilluminated, they produce a loud beep and close a shutter for several seconds (the shutter reopens automatically after a few seconds). Immediately reduce the light exposure (laser off or uncheck the „fiber port"on the Fluoview software and insert a mirror in the FluoView beam path.

- o If the overillumination has been massive, the detectors may show an increase level of dark counts. It usually recovers, but this can take several minutes or even1-2 hours.

- Computer crash?

  - o The systems settings are stored in the factory configuration file „settings.pfs" (in the program folder) and the user configuration file „standard.pus" (you may define various additional ones. There are copies of these files in the documentation\backup-folder on the desktop as well as on the black USB-stick.

- Anything else: drop an email here:

  - o https://www.picoquant.com/contact/support

  - o important: give the system's serial number: 1042684 (this way we know, which configuration you have)

… to keep in mind when measuring

- No line-averaging, no sequential should be used.

- If the image size is larger than 1024x1024, the image preview is switched off, however the system is measuring and the data can be analyzed later, they are just not displayed during the recording

- The peak intensities „max. intensities"of the images should be max. 10% of the excitation rate for the SPADs and 15-20Mcps for the HPDs (you can also go higher, if necessary, however, you will need to switch the fitting model to "rapid Reconvolution" then:

Max Photons	560 Cnts
Max Countrate	5948.91 kcps
Avg Photons	2 Cnts
Avg Countrate	18.11 kcps
Frames	47

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  - ∘ for the Hybrid detectors (Det. 1 & 4): best max. 15-20Mcps

  - ∘ for the SPADs: (Det. 2&3) best stay below 1/10 of the laser repetition rate (below the red line) [e.g. Laser repetition rate: 25MHz => max. Det. Count rate: 2.5Mcps»], if you want to analyse the data in these channels.