

User Guide for the PTI – Fluorimeter

Clark Lab

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1. Make sure that every instrument, including the computer and monitor attached to the fluorimeter, is turned off (the computer should be turned off at the back).
2. Turn on the power for the lamp and wait a few seconds. Then ignite the lamp by pushing the ignite button until the display for the watts shows a number around 65 watts.
3. After this, all the other instruments including the computer can be turned on.
4. Wait till the display for the lamp reaches 75 watts (might take a few minutes). If 75 watts is not reached or if the number is >75 W, change it to 75 watts.
5. The voltage for the two photomultipliers should be at 1100.
6. The water bath has to be set to 20 °C. Use the “Limit” button to change the temperature and push “Start/Finish” to start cooling.
7. Start the software with the felix 32 icon; password is “pti”.
8. Choose “New Acquisition” from the menu and select your experiment.
9. When you want to measure the emission over a range of wavelengths, start an “Emission Scan”. Set HW configuration to digital, fill in your excitation wavelength and your emission wavelength. Emission 1 corresponds to the right detector and will be represented by the red raw data in the graph (corrected data is green). Emission 2 corresponds to the left detector and will be represented by the orange raw data in the graph (corrected data is blue). To use the excitation correction, you have to click on the button “More...”, go to “other devices” – “Real Time Correction”. Activate the “Enabled” button and enable excitation, disable emission; never use both corrections in one experiment. In the “XCOR” part of this window, disable “use configuration settings” and then check on “Reference Source Gain”. First thing to do will be to change the excitation wavelength to your desired value, push “Goto” and change the gain so that the voltage reaches the optimal value of 4 V. If the excitation slit width is too narrow, 4 V cannot be reached, so use gain 5 and push OK and then again OK. When you want to correct for the background as well, check “Background Acquire” and close the excitation shutters. After this push “Acquire (Prep)” and then “Start”. After this the box “Background” should change from “acquire” to “use”. Make sure the box use is activated. Push again the “Acquire (Prep)” button and start your measurement.
10. When you want to make a kinetic experiment, select “Timebased” from the new acquisition window. Choose digital as the HW configuration and setup your excitation and emission wavelength. It is possible to use different wavelength for the two detectors. Use the same correction and background procedure as for emission scans.

11. To save a file you have basically two options: save it as an “ana” or “ang” file, which can be read by this software, but not by Excel, or as a “text” file which can be read by Excel. To save it as a text file, do not use the save buttons from the menu; instead go to the left part of the window to the list:

Timebased ...

New 1

D1...

D2...

XCOR...

COR D1...

COR D2...

Click on ‘New 1’ with the right mouse button, rename file without using any “dot” in the name, click outside box to set it, then right button, choose EXPORT AS GROUP, and check name, save as “.txt” file format in folder on hard drive.

12. Turn off all instruments, including the computer and monitor (shut down the computer and then turn it off at the back).
13. Lastly, turn off the power supply of the lamp.

Settings for measurements with P69 Pertactin:

Excitation slit width: 0.8 nm*

Emission slit width: 20 nm*

Excitation wavelength: 280 nm

Emission wavelength: 300-400 nm

Integration: 1 sec

*For manual slits: 1 nm = 1/2 turn of setting screw