

# Basic protocol for PTI - Fluorimeter Room D381

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This protocol gives step-by-step instructions for basic measurements; it **does not replace the training**.

All **new** users **must** undergo a short training session with Dr.Erbse (see contact info above).

## Signing Up for Instrument Time

1. After your training is complete you will be invited to the Google Calendar for the fluorimeter. You can reserve time for your experiments using the calendar. Please be careful not to book times overlapping with other users.
2. Try to sign up as early as possible so that other users can plan their instrument time.
3. If your plans change, please change your signup time. Other users might want to use the time instead.
4. If your plans change, go to the fluorimeter at your appointed time and check that the fluorimeter was not left on for you. If it was, switch it off (if nobody else is signed up in the next 5 hours).
5. Please be considerate to the needs of other users. Talk to each other if you need instrument time urgently. You can find the email addresses of other users at the Google calendar site.

## Good Practice and Required Procedure

### Switching the Instrument On:

1. Make sure the computer is switched off!! Power spikes from switching on the lamp can damage the computer, if it is on!
2. Switch all parts of the instrument on by switching on the power strip.
3. Sign the logbook. Never switch on the fluorimeter without immediately signing in.
4. Switch on the water bath (if it is connected and you want to control the temperature in the cuvette turret). Please check the fill level of the water bath and refill if necessary.
5. After the lamp has ignited switch on the computer.
6. Warm up the lamp for at least 20 min.
7. Check the lamp power supply. Make sure it is set on Watts. If it is not at 75 Watts after the lamp is warmed up, regulate it to 75. Be gentle, the regulator is sensitive.
8. Record the Volts of the Lamp at 75 Watts in the logbook.



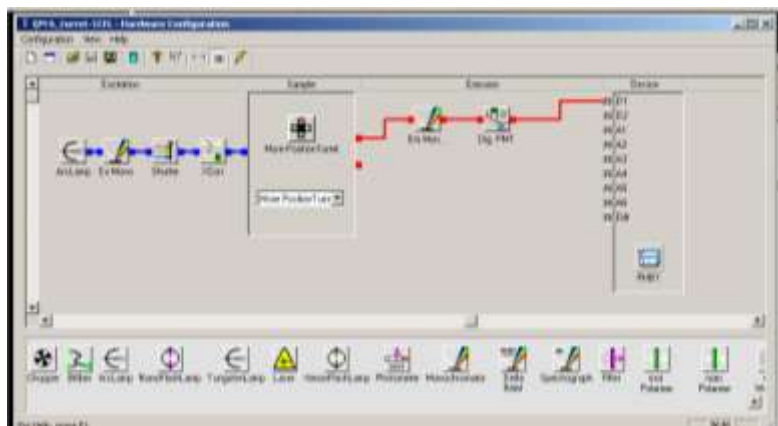
## Things To Watch Out For:

1. **Never measure the emission at the excitation wavelength or twice the excitation wavelength. This will damage the detector!!!**
2. Try to have your starting emission wavelength at least 5 to 10 nm higher than your excitation wavelength plus the slit width you are planning to use for excitation and emission. If your excitation and emission maxima are close, minimize the slit width and/or excite/detect a little of maxima.  
Example: Excitation at 340, slit width 2 nm for excitation and emission.  $340 + 2 + 2 = 344$ . Start emission wavelength should be between 354 and 349. The bigger the gap the better to avoid excitation light bleed through.
3. **The mechanical shutters at the exits from the sample compartment to the emission monochromators should close if you open the lid of the sample compartment. But they can get stuck occasionally in the open position. To protect the PMTs please close the manual sliders every time before opening the lid.**
4. Measure an absorbance UV/Vis spectrum from your sample before starting the fluorescence experiments. Avoid highly concentrated samples. High optical density will not allow even excitation in your sample and inner filter effects can influence your results. An  $OD \leq 0.1$  is recommended.

***You are encouraged to read the manual. It is kept next to the instrument. Do not remove it!***

## Setting the Instrument Up

1. Follow the instructions under "Switching the Instrument On".
2. Switch on the computer **after** the lamp has ignited.
3. The password is : emission
4. Open Felix32. The PTI password is: pti
5. Click "Configuration" → "Hardware Conf."  
→select "QM6\_turret\_1CH" (if the 4 cuvette turret is installed). In the new window you will see the configuration you have chosen.
6. Click "Configuration" → "Initialize" or click the lightning bolt and wait for the monochromators to go to the programmed position.
7. Read settings of excitation monochromator and emission monochromators 1 (and 2 if using both) from the hardware and enter the correct values in the box of the program if necessary.



8. Click save.
9. Set the slits (all 4) to the desired value by turning the micrometer screw. For this fluorimeter 0.25 mm equal 1 nm. For example if you want to open the slits to 2 nm you set the screws to **0.5 mm**.
10. Important:  
NEVER close the slits tight. You will damage the Micrometer screw.



### **Note to adjusting the slits:**

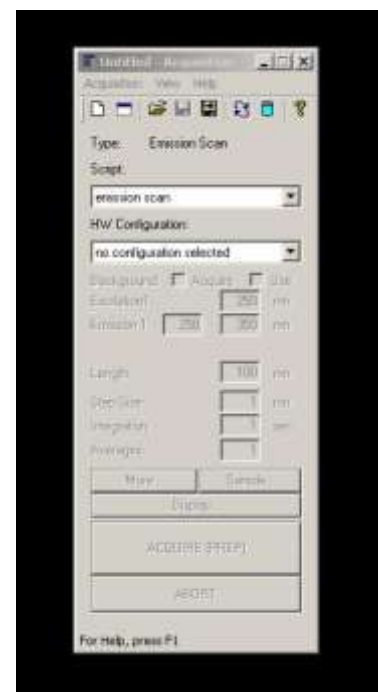
How far you open the slits will depend on the resolution you want to have and on the intensity of your signal. If you open them too far you will have problems with light from the excitation hitting the emission detector. If you open them too little, you can get interference effects. Most organic compounds and labels have a broad maximum. Therefore in many cases 1 nm or more will be fine. If the excitation and emission maxima are very close try to minimize the slit to protect the detector or/and excite/detect a little of maxima if your excitation/emission peaks are broad enough.

11. Set voltage on the PMT(s).

**Note:** The PMTs are linear between 900 and 1100. Never set them above 1100. If you set them below 900 your signal intensity will not be linear with the concentration anymore.

### **Acquiring Data**

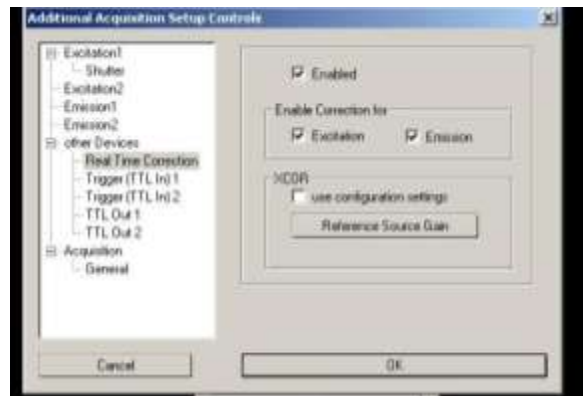
1. Go to acquisition and load an acquisition method or make a new one.
2. If you are loading an existing method continue with 17.
3. If your making a new one :
4. Choose an acquisition method (for this example Emission Scan).
5. Choose HW Configuration : QM6\_turret\_1CH
6. Set Excitation and Emission range (try to stay in the limit  $\lambda_{max} > 2\lambda_{min}$  for Emission range)



**Never measure the emission at the excitation wavelength or twice the excitation wavelength. This will damage the detector!!!**

Try to have at least 15 nm between excitation and emission.

7. Set Step Size, Integration Time and # of Averages. You will have to optimize them according to the resolution you need, the signal to noise you have and the stability of your sample.
8. Click "More"
9. In the new window click "Real Time Correction" if you want to use it. Real Time Correction allows you to correct for fluctuations in lamp output, differences in monochromators and differences in sensitivity of the PMTs to light of different wavelengths.



**Note:**

The software will show raw data and the corrected data if you choose to use corrections. You will not lose the raw data by selecting Real Time Correction.

10. If you want to use corrections click "Enable" and choose Excitation and/or Emission.
11. Unclick use configuration settings.
12. Click "Reference Source Gain".
13. Set wavelength of Ex Mono to your excitation wavelength and click "Go To".
14. Click "shutter" to set it to open. Remember that in addition to the automatic shutter there is also a manual slider before the excitation monochromator. Don't forget to open that slider.
15. Adjust gain to get as close to a signal reading of 1 Volt as possible.

**Note:**

If you change the slit width you might have to re-adjust the gain.

16. Click to close the excitation shutter you can leave the manual slider in the open position.
17. Click ok and close the reference gain window.
18. Click "Sample" to choose sample position.
19. Choose the sample positions you want to use and in what sequence they should be measured.

**Note:**

For scans choose sequential. This way one spectrum per sample will be measure before the next one, otherwise it will measure each sample at each step. Slight errors in repositioning might affect the quality of your data.

20. Close Sample window.
21. You are now ready to collect your scan.
22. Click "Acquire"; wait for the instrument to get ready.
23. The emission detectors are protected by manual sliders and two mechanical shutters. Make sure you have the manual sliders in the open position before you click start.

**Note:**

The mechanical shutters should close if you open the lit of the sample compartment. But they can get stuck occasionally in the open position. To protect the PMTs please close the sliders every time before opening the lit.

24. Click "Start".

25. Observe the **raw** data trace (D traces). Should the intensity become **higher than  $2 \cdot 10^6$  close the emission shutter immediately**. Your signal is high enough that it will start to saturate the detector. If it gets any higher it will damage the PMTs.
26. Adjust the signal intensity by lowering the voltage on the PMT's and/or by narrowing the slit width. If both is not an option dilute your sample.
27. Measure again.
28. Save your data by clicking the export button (don't use save, you will not be able to choose the directory this way). Save you file as .ana file. This is the format of the felix software.
29. To export your data make sure that all traces you want to export are toggled on! Click the grit button next to the 3D button. Your data should now be displayed as data columns. Select everything, copy and paste into Notepad. Save from Notepad as text file.
30. The computer is not connected to the internet. Use a flash drive to transfer data.

**Important:**

**Check the flash drive for PC and MAC viruses before you use it on this computer. Since Windows XP is not supported anymore by Microsoft this computer has no up to date protection! MAC-users make sure that your anti-virus software checks for both. Many MAC anti-virus programs do only check for MAC viruses. If this is the case you might be spreading PC viruses!**

**Switching the Instrument Off:**

1. Save your data (I recommend as .ana (felix) AND text files).
2. Double check if someone is signed up after you in the next 5 hours. If that is the case, leave everything on **and write the next user an email**, telling him/her that you left everything on. It is important that the next user knows about it and does switch off the lamp should her/his plans change and he/she should not need the fluorimeter.
3. Shut down the computer.
4. Switch off the power strip (which switches off all other parts if the instrument).
5. Switch off the water bath.
6. Check the fill level of the water bath and refill if necessary.
7. Record lamp hours in the logbook. Record your individual hours and add them and any time the lamp was left on by a previous user to the total lamp hours.
8. If something did not work right, please write it down in the logbook and inform Dr.Erbse