User Procedure for Fluoromax2 Fluorimeter

1. Before starting to use the fluorimeter, *obtain a UV/Vis absorption spectrum* of the sample to find the absorption properties and the optimum excitation wavelength. The optical absorption of a solution should not be higher than 0.2 at the excitation wavelength and at higher wavelength. Too high absorption will lead to uneven light absorption and internal filter effects. For strongly emitting compounds an absorbance of 0.15 is optimum.

2. Now it's time to use the fluorimeter. Log into the logbook! *NEVER Quit the program and NEVER shut down computer!* If you do so, the monochromators need to be recalibrated. To change from the fluorimeter software to the main Windows surface use the "ALT" + "TAB" keys.

3. Change the directory where your data files will be automatically saved by clicking on:
Main (F4)
Manage Files
Change Directory
\Data\Name\Subfolder hit enter
NB: When inputting information into data fields, leave mouse off of window and toggle between fields using the arrow keys. Always remember to hit enter after inputting a value. To return to main screen, click outside the information field.

4. Insert sample after taking a UV/Vis spectra. Use a clean cell for your sample solution or you will produce useless data.

5. *Emission Scan*
Main (F4)
Define Experiment
Emission Scan
Data Acquisition Parameters
Set Excitation Wavelength from UV/Vis spectra (e.g. Last Absorption Peak)
Set Scan Start approx. 10 nm higher than the excitation wavelength
Leave Experiment, Acquisition Mode, and Auto Zero fields alone

6. Slit widths
ACCS'Y (F7)

For Emission: start with Emiss = 0.2, Excit = 1. Adjust as needed to increase or decrease intensity. For example, if signal is weak, try Emiss = 0.5, Excit = 2

7. Start Experiment
Main (F4)
Run Experiment
Input Data File name and Data Title, include slit widths in the title for your record
Leave Experiment File and Experiment Title alone

7. Be sure not to saturate the detector. The counts (Y-axis) should not be higher than 4×10^6 in "s" mode.
For a high quality scan, set integration time to 1 sec, or 0.2 for a quick scan

8. *Excitation Scan*
Main (F4)
Define Experiment
Excitation Scan
Data Acquisition Parameters
Set Emission Wavelength from last peak in Emission Scan
Set scan start at a reasonable wavelength (higher than 200 nm) and stop the scan approx. 10 nm BELOW the emission wavelength.
Set acquisition mode to "s/r" to correct for the emission spectrum of the Xe-lamp.

9. Slit widths
ACCS'Y (F7)
For the slit width values, use the reverse of what was used for the emission scan. Typically, Emiss = 1, Exc = 0.5.

10. All spectra are automatically saved, if you give a new file name for each new scan. To generate ASCII files to import the data into a graphics program (e.g. IgorPro or EXCEL):
Main (F4)
Other Activities
Output to ASCII

11. **NEVER Quit the program and NEVER shut down computer!** If you do so, the monochromators need to be recalibrated. To change from the fluorimeter software to the main Windows surface use the "ALT" + "TAB" keys. In the Windows surface you can copy your ASCII files to a ZipDisk (100MB) or Floppy Disk for data transfer.