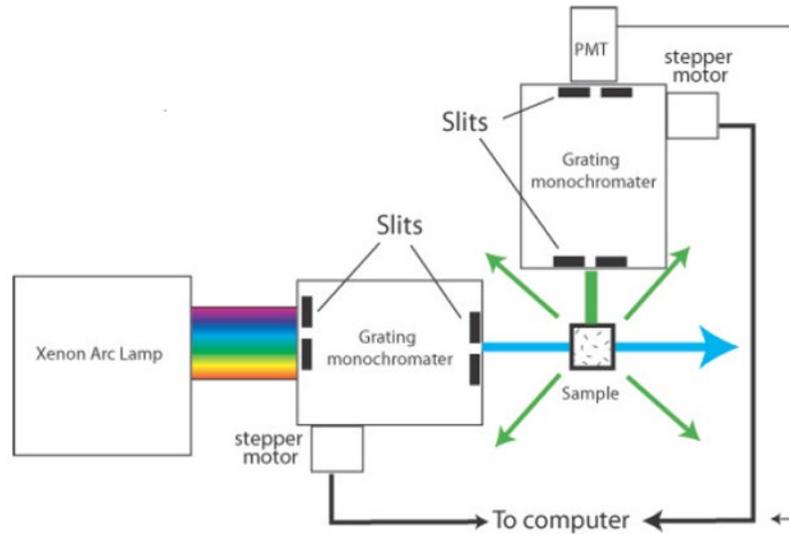


# PTI Fluorometer (with FelixGX Software) User Guide



*Schematic of a Typical Fluorescence Spectrometer*

## Turn on Sequence

1. On the Lamp Power Supply, press the power button (it is illuminated when powered).
2. Press and hold Ignite button. You will hear a click as the lamp ignites.
3. When the LCD display shows the wattage reading (ˆ65 Watts), the lamp has ignited.
4. Record time in Log Book.
5. Wait 10 seconds, then turn on
  - a. HORIBA Stirrer
  - b. HORIBA Box below
6. Allow the lamp to warm up for 15 minutes.
7. Set slit width. There are 4 slits and 3 shutters: at lamp, behind sample compartment, before Em monochromator, after EM monochromator
  - a. Set slits\* (1 full turn = 2 nm)
  - b. Open 3 shutters or keep ex shutter closed until ready
8. Turn on computer (if not on)
  - a. Login Windows with net id and password

9. Start FelixGX (ver 4) Software
  - a. Choose *Qm* at upper right (should be default)
10. Setup > Acquisition Type | Acq settings | Real Time Correction | Backgrounds | etc.
  - a. Choose Acquisition Type
  - b. Acq settings: Excitation and Emission wavelengths (must be > 10 nm apart)
  - c. Slits just for reference (they are manual)
  - d. Typical integration time ~0.3 s
11. Real Time Correction (see explanation below)
  - a. Enable both Ex and Em
  - b. Excitation: choose Excorr
  - c. Type in the excitation wavelength and click 'Go To'
  - d. Adjust the bias to obtain a signal of about 1.0 V (use scroll wheel on mouse)
  - e. Emission: use default
12. Background
  - a. Detector 1 is your PMT data
  - b. RCQC is detector for excitation correction data
13. *Accept* when done
14. *Run*.
  - a. If you want to run a buffer first , just run it like another sample and *subtract it manually*
  - b. Insert sample
  - c. Start
15. Datasets appear on left, RGCG is the correction detector, Detector 1 is your PMT
  - a. D1 is raw data
  - b. ExCorr is the correction factor for lamp (close eye for this)
  - c. D1 name [Cor] is your data corrected for the lamp fluctuations
  - d. Right click to rename, etc.
  - e. Click on eye to view or not
16. View on left of trace window, can view data, 2 graphs, etc.
17. Math
  - a. Math > Trace Math > Peak Finder
  - b. Integrate, drag bars to choose end points
  - c. Normalize, Smooth, etc.
18. Save / Export data
  - a. Highlight trace name
  - b. Right click, copy, paste into Excel

### Note on adjusting the slits:

- How far the slits are opened will depend on the resolution you want to have and on the intensity of your signal.
- If you open the slits too far, you will have problems with light from the excitation hitting the emission detector. If you open the slits too narrow, 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.

## Turn off Sequence

1. Exit Felix software. Turn off all boxes (power supply, stirrer, etc.) in reverse order.
2. Logoff Windows
3. Record Lamp hours in logbook

## Corrections to Data

- During setup, use *Both* the Ex and Em corrections.
- The Ex correction uses the RCQC detector to correct for lamp fluctuations and ratios the data.
- We suggest adjusting Bias to get a Signal Voltage of 1.0 to make your corrected data have the same magnitude as the raw data.
- **Don't change bias once you start** or you cannot compare your data.
- The Em correction is necessary because the detector is not sensitive to all wavelengths equally. This does not change, so you can use the default.
- **D1** data is raw, uncorrected data.
- **ExCorr** is the Ex correction.
- **D1 name [Cor]** is corrected by ratio of D1/ExCorr. This is the best data and the only data worth saving.

## Measurement Options

- Excitation spectrum
- Emission spectrum
- Time-based measurements
- Polarization Optics
- Light Scatter
- Max data rate (noisiest)

## Hardware Options

- Stirring
- Heating, Cooling
- Polarizers