FL1
Operating Instructions for Spectrofluorimeter

You will need:
Samples on ice
Cuvettes (if not using the ones already there)
Waste container for used tips, gloves, tubes etc.
P1000 pipette and tips
Fine tipped pasteur pipette to recover peptide solution
Distilled water for washing
Cuvette cleaning solution
Gloves

Proceedure

1) Login to Novel (as usual), then into Pguest
2) Turn on the lamp and fan, switches between machine and wall.
3) If not already running, turn on Spectracto machine under desk, and wait until it has booted from floppy.
4) On the PC, open "Fluralog" from shortcut on desktop.
5) The stored configurations appear in a dialogue box. At present there is only one option. Select it.
6) Fill cuvette and place it in sample holder 1 (2 has been custom modified), putting the long axis of the cuvette along the line of illumination, such that the thin wall faces the lamp.
7) The FF/RA knob on top can be used to either collect fluorescence at 90° angle, going directly through the hole in the side of the detector box, or using mirrors to collect at 22°, with the beam redirected using mirrors. This allows collection from the front of the cuvette with and may be better for turbid samples, since there will be less scattering in the bulk of the sample. RA: 90°, FF: 22°. Usually use RA.
8) On the PC, there should be a tool bar with 4 buttons. Select the left hand one – Run Experiment.
9) Log into the Gram library.
10) Go to collect → experiment.
11) A dialogue box will appear, where the several settings must be adjusted. Alternatively, select an experiment setup used earlier. These options are accessed through the following buttons:

Experiment Type
Select the required type of experiment

- Excitation Acquisition: Choose one wavelength to measure emission and the machine will measure the emission intensity throughout the range of wavelengths specified.
- Emission Acquisition: Choose one wavelength to excite, and the machine will measure the emission intensity through the specified wavelength range.

It is a good idea to measure excitation first, and then measure emission when exciting at the peak excitation wavelength.

Signals
Under data channels, select S for a simple spectrum output, and correct the units to cps (type it in). Sc would automatically correct for blank, dark spectrum and an instrument correction file. However, if this is desired, it is easier to see what has happened by taking these files separately and correcting in Excel later.

Slits
Set the slit width to determine (1) the amount of sample illumination and (2) the amount of fluorescence collected. Start at 5nm, but adjust so that peak detection is less than 100 000cps, since above this detection is non-linear. Alternatively, dilute the sample.

Shutter
Leave on Auto

12) Back in the main dialogue box, specify the range for excitation/measurement and the specific value for excitation/measurement, depending on whether an excitation or emission spectrum is collected.
13) Specify the integration time. This gives the time taken for each measurement. It will not change the actual intensity measurement, since it is measured in cps. However, longer times give smoother spectra, since the measurement was taken for longer. Each scan also takes longer (total time = integration time x data points to be acquired)
14) Specify a filename for the experimental settings and for the data file to be collected. Ideally, use the same, so that they can be correlated. If the autosave box is checked, then until the file names are changed, it will overwrite the previous file and save the latest version with each run. Then, the final version for each experiment will be saved. If it is not, then the Save button must be clicked each time a spectrum should be saved. All files should initially be saved into personal folder on computer D-drive.
15) Click Run to collect the data, and the spectrum will appear on the screen.
16) Repeat, adjusting the settings until the final spectrum is obtained. Close each preliminary file, and close the view to make it less confusing.
17) Save as an ASCII type N (file extention *,prn); a format that can be read by Excel.
18) Transfer this file to your networked drive.
19) Once finished, log out of PC, and turn off the lamp of the fluorimeter (top switch). DO NOT TURN OFF THE FAN at this stage. Clear up, then after about 10min turn off the fan, so that the lamp does not overheat.