  R·I·T (RIT logo, 1829)	Chemistry Department Bldg. 8 Research Group	SOP #	1
		Revision #	1
Prepared by	Chris J. Collison	Implementation Date	
Page Number	1 of 4	Last Reviewed Date	
SOP Owner	Christopher J. Collison	Approval	

1. Purpose

To promote the effective use of the Horiba Fluoromax to make simple emission and excitation scan measurements, such that wear and tear is minimized. To educate students in the basic technique of fluorescence measurement.

2. Scope

This SOP is intended for in-group use by trained and certified personnel in the Chemistry Department.

3. Prerequisites

The experimenter must complete the associated certification exam before using this SOP.

4. Responsibilities

The responsibility for this instrument lies with Dr. Christopher J. Collison.

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5. Procedures and information

- The Lamp is a consumable item on this spectrofluorometer but is nonetheless expensive. Furthermore an overused lamp will give poor results. The lamp lasts for only 1200 hours. Please switch the instrument off if it will not be used for a period in excess of 90 minutes!
- Switch on the instrument
- Press M in the toolbar to obtain the main experimental menu
- Click the "Spectra" box and then select the experiment that you want to run – Emission or Excitation. Click "next"
- There are options on the left hand side of the screen that allow you to set up the parameters for your experiment. You can save experimental parameters as an experiment file at the top left of the "Monos" screen.
- The SLITS run in a range from 0.5nm-30nm in "bandpass" mode.
- Slit Widths and Wavelength range parameters: It is suggested that the smallest slit widths are used initially so as to protect the instrument and detector.
- Emission wavelength range – select your excitation wavelength that coincides with the lambda-max of your absorbance spectrum. Add up the slit widths and add half of the sum to get a wavelength value, n. (i.e. with both slits set at 2nm, n=[2nm + 2nm +(2+2)/2]=5nm). Your wavelength range must not START until [Excitation wavelength + n]nm. So for slits of 2nm,2nm and an excitation of 450nm, you must start your emission scan at 455nm or higher.
- Excitation wavelength range – select your emission wavelength that coincides with the lambda-max of your emission spectrum. Add up the slit widths and add half of the sum to get a wavelength value, n. (i.e. with both slits set at 5nm, n=[5nm + 5nm +(5+5)/2]=15nm). Your wavelength range must not FINISH beyond [Emission wavelength + n]nm. So for slits of 5nm,5nm and an emission wavelength of 600nm, you must finish your excitation scan at 585nm or lower.
- S1 is the signal on the Photomultiplier tube – the main detector for this instrument
- R1 is the signal on a photodiode that specifically measures the intensity of the lamp, after the excitation monochromator. R1 is important when measuring an excitation spectrum since the intensity of the lamp does change as a function of wavelength and we must account for this.

- The scan speed is determined by a combination of "Increment" on the "Monos" menu and "integration time", found on the "Detectors" menu. The integration time is the time that the
- instrument dwells at a particular wavelength. The signal to noise will scale with the reciprocal
- of the square root fo the integration time. Hence we might expect in increase of signal to noise of exactly 2 if we quadruple the integration time for a spectral measurement.
- There are correction factor files (CFF) for the two detectors. These CFFs represent and allow correction for the variation in sensitivity of the detectors as the wavelength is varied.
- The correction checkboxes must be selected in the "Detectors" menu and the appropriate function must be selected.
- To read the data, click on the "Data Reader" box (looks like a square with a target in it)
- The box under "Formulas" in the detectors box determines what data is actually recorded and transferred to the Origin graphing software (FluorEssence).
- So for the case when an excitation spectrum is being recorded it is important to click both correction factor checkboxes and also it is important to actually measure "S1c/R1c"
- For the case when an emission spectrum is being recorded it is important to click the S1 correction factor checkbox and to actually measure "S1c"
- Make sure you click out of the parameter entry box to ensure that your settings have been retained in the "Method". Sometimes the software reverts back to the default settings and this can be a source of frustration. Saving your experimental file makes a lot of sense.
- Dark Offset measures the dark signal and corrects data to lower the baseline by 200-500 CPS
- Units are always CPS.
- Data saving " Please enter a project name" – all data will then be saved to that project.
- RTC is the real time display – it can be used to obtain some real-time control of the detectors. Activate the "Continuous" checkbox and then click the "Run" button to modify the signal by adjusting the desired instrumental parameters as necessary.
- The count rate must stay below 4×10^6 Counts per Second (CPS). It is recommended that the count rate stay below 2×10^6 CPS because the detector will be NON-LINEAR above this range. This can severely impact the quality of data collected.
- Data can always be analyzed after collecting data if the appropriate correction factor files were not used but it makes sense and saves a huge amount of time to use the appropriate corrections at the time of measurement.

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Page Number	4 of 4	Last Reviewed Date	
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6. Certification test

There will be a test that you must complete before becoming certified.

- How long will the lamp last for?
- What can you do to preserve the life of the lamp?
- What colors are the detectors more sensitive to?
- How can you ensure longevity of the detectors by
  - Using real-time measurement
  - Setting appropriate scanning parameters based on the slits settings?
- What is the difference between S1c and S1?
- Why is it important to use "S1c/R1c" for an excitation spectrum measurement?