Standard Operating Procedure

PTi QuantMaster 40 Spectrofluorometer
Standard Fluorescence Emission (Source: 190-900nm)

1. Introduction

1.1. Purpose
The purpose of this document is to familiarize the user with the mode of function of the PTi-QuantMaster 40 spectrofluorometer (FLD) available in TRACES, and to describe the sampling procedures available for emission (em), excitation (ex), and time-based fluorescence studies.

1.2. Scope
This instrument is used to characterize the relationship between absorbed and emitted photons at specified wavelengths. Fluorescence occurs when a molecule absorbs photons from the u.v.-visible light spectrum (190-900nm), causing transition to a high-energy electronic state and then emits photons as it returns to its initial state, in less than 10-9 sec. This procedure is applicable to undergraduate and graduate students enrolled in courses within the Department of Physical and Environmental Sciences. This document may also be used as a template for research users within the Department of Physical and Environmental Sciences.

1.3. Responsibility
User

1.4. Accountability
Principal Investigator/Course Instructor/Teaching Assistant

1.5. Emergency Contacts
- Emergency Fire/Police/Ambulance:911
- UofT Police:416-978-2222

2. Referenced Documents

2.1. FelixGX 4.1.2 Software User’s Manual Revision A
2.2. PTi Technical Notes

3. Chemicals & Supplies

3.1. PTi QuantMaster 40 Fluorospectrometer
3.2. Kimwipes
3.3. Course-provided or Course-produced samples ONLY
   - Use of this instrument for purposes other than laid out by the course instructor will result in severe penalty and academic offense.
4. Personal Protective Equipment
   4.1. Nitrile/Nylon Gloves
   4.2. Laboratory Coat/Jacket
   4.3. Safety Glasses

5. COVID-19 Related Safety Precaution
   5.1. Do not enter if you have one of the following symptoms:
       • cough
       • fever
       • difficulty breathing
       • pneumonia in both lungs
       • travelled outside the country in the last 14 days
   5.2. Cleaning and Sanitizing Hands
   5.3. General Laboratory Practice during COVID-19
       • You MUST work >2m from others. The use of adjacent instruments less than 2m is suspended at this time. Please schedule your analysis appropriately.

6. Safety and Electronic Equipment Concern
   6.1. Chemical Safety
       • Read and become acquainted with the SDS of all the chemicals you will be using and/or exposed to during the lab period - including the alcohols for cleaning.
       • Dispose of the chemical waste and chemicals-soaked paper in the designated containers.
   6.2. Electronic Safety
       • Please refer to the manufacturer’s recommendations and warning label.
   6.3. Before Commencing work
       • Obtain a bottle of alcohol and several paper towels. Kimwipes should also be available near the FLD.
       • Clean the keyboard, mouse, GLOVES, and any area you will be exposed to (monitor excluded) with the solvent-soaked paper towels. DO NOT spray directly onto surfaces.
       • Discard ALL the paper towels (whether they were used or not) into the designated waste container.
       • Wait 5 minutes before commencing work.

7. Operational Instrument Parameters
   7.1. PMT: 190-900nm UV-Vis
   7.2. InGaS: 500-1700nm NIR
   7.3. Temperature Controller: -20°C-105°C

8. Initial Instrument Set-up
   8.1. Turn power cord(s) switch to ‘ON’
   8.2. Turn lamp ‘ON’ (allow min of 15 min for warm up)
   8.3. Check water level in the recirculatory tank (add if required)
   8.4. Check slits.
9. Initial Standard Fluorescence Set-up

9.1. Monochromator Grating Selection:

- Assure that ex slits match & em slits are matched
- Please be advised that the slit width of 0.25mm=1nm optical slit width

9.2. Modules Initialized
- Turn on the ASOC -10
- Turn on the Motor Stirrer
- IF DOING TEMPERATURE MEASURMENTS, turn on the TC125

9.3. Computer Software
- Turn on the computer
- Select the FelixApp icon
  - Wait for the software to initialize with the instrument before proceeding

9.4. Mode Selection
- dig-vis: for routine FLD experiment (190-900nm)
- dig-vis-temp: for routine FLD experiment (190-900nm) with temperature control

9.5. FLD Experimental Parameter Setup
- Select Setup
  - Tabs will appear once the FLD experiment has been selected
- For Emission Scan
  - Select EM Scan
  - Temperature Controller and Stirrer can be enabled
  - Click on traces to view different parameters (i.e., temp, detector): Leave as default
  - Real-time correction can be selected to view either em or ex correction
- Acquisition Settings
  - Select the ex wavelength of choice
  - Select em wavelength range (ensure there is a 20nm separation)
  - Select step size and integration level (experiment dependent)
  - Slits for em and ex should be matched and manually set into software
10. Acquiring Fluorescence Spectra

10.1. Background

- Close the lamp slit
- Acquire background for 5-10 sec scan
- Accept background (will be timestamped)
- OPEN THE LAMP SLIT

10.2. Start Acquisition

- Select the Start button to commence acquisition of fluorescence spectra
- If running a temperature experiment, the run will start after it has reached the desired temp
- Wait till the experiment is completed

10.3. Time-based Acquisition

- Select the Time-Base tab and enable this acquisition
- Enable the Stirrer (if available)
- Static Experiment
  - One temperature selected for entire run
- Time-Base Experiment
  - Specific temperature and duration (in seconds) is selected
- Temperature Ramp Experiment
  - Start to End Temperatures are selected with a given rate ramp (°C/min)

11. Evaluation Fluorescence Data

11.1. Select Data for Evaluation

- Data is selected for viewing:

- Data is closed and not viewable:

- Manipulate Cursor
  - Default interaction doesn’t allow free movement of cursor
  - Panning and zooming selection available
  - Cursor option available

11.2. Data Evaluation: Math

- Peak Finder
  - Select the Low X (wavelength)
  - Select the High X (wavelength)
  - Execute
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• Normalize
  • To the highest reference peak
  • Select Min-Max computation

• Export Data
  • Session: includes entire experiment run
  • Trace: relates to a single specific experimental run

12. Shutdown
• In reverse order of start-up

13. Waste Disposal
• All waste generated during the lab experiment are to be disposed of in the appropriate waste container

*The TRACES Manager will provide further details during hands-on training.