

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

2.3. Guilbault, G. G. Practical Fluorescence. Modern Monographs in Analytical Chemistry.

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.**

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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

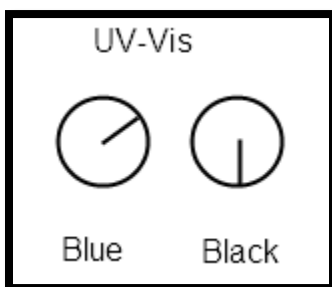
- 8.1. Turn power cord(s) switch to 'ON'
- 8.2. Turn lamp 'ON' (allow min of 15 min for warm up)
- 8.3. Check water level in the recirculatory tank (add if required)
- 8.4. Check slits.

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

9. Initial Standard Fluorescence Set-up

9.1. Monochromator Grating Selection:



9.2. Modules Initialized

- Turn on the ASOC -10
- Turn on the Motor Stirrer
- IF DOING TEMPERATURE MEASUREMENTS, 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

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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)

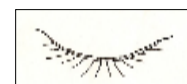
11. Evaluation Fluorescence Data

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.**