

Operating Instructions for Fluoromax-2

Part I. Starting Instrument and Checking Calibration

Part II. Finding the Excitation Wavelength

Part III. Obtaining an Emission Spectrum

Before proceeding, make sure that the computer and monitor for the instrument are turned off. This protects them while you turn on the lamp, which causes a sharp AC pulse.

If the instrument is on and has already been calibrated, skip to Part II.

Part I: Starting Instrument and Checking Calibration

1. On the Fluoromax-2, turn on Lamp.
 2. On the Fluoromax-2, turn on Power. Wait until the green light on the floppy drive goes out.
 3. Turn on computer and monitor. Printer will come on when computer is turned on.
 4. On the Windows desktop, double click to open **Instrument Control Center**.
 5. A box will appear and ask which layout you want. Click on **Generic Fluoromax-2**.
 6. Wait for the instrument to set itself up. It will ask if you want the hardware in the same configuration as it was previously. Click **No**.
 7. A small window with four buttons will appear on the screen. Click the first one on the left, the **Run Experiment** button.
 8. Check the **lamp output** by collecting an output spectrum as follows:
 - Under the **Collect** menu, pick **Experiment**.
 - Click **Experiment Type** and pick **Excitation Acquisition**. Click **OK**.
 - Click **Experiment** and load the file c:\datamax\calib\lamp.exp.Verify these settings:
 - Scan Start: 250nm Scan End: 600 nm
 - Increment: 1 nm Integration: 0.1
 - Emission wavelength: 650 nm
 - Click **Signals**; set signals in the first line to **R**. Click **OK**.
 - Click **Slits**; set as follows: Excitation: 1.2 **nm** Emission: 0.0 **mm**.
 - Click **OK**
 - Click **DataFile** and enter a file name for the **data** file, ending with **.spc**. Direct the file to your your own folder or floppy disk. Click **OK**.
 - Click **Run**.
 9. After the run, click the pointer at the top of the highest peak; note wavelength and output. Maximum lamp output should be above 0.04 mV at 467±1 nm.
- If the calibration results are not as expected, consult your instructor before you proceed.
10. Check the **detector** by collecting a Raman spectrum of water. First place a cuvette of **deionized water** in the sample holder. Collect an emission spectrum with these settings:

Under the **Collect** menu, pick **Experiment**.

Click **Experiment Type** and pick **Emission Acquisition**. Click **OK**.

Click **Experiment** and load the file c:\datamax/calib/water.exp.

Verify these settings:

Wavelengths Start: 365nm End: 450 nm

Increment: 0.5 nm Integration: 0.1

Excitation Wavelength: 350 nm

Click **Signals**; set signals in the first line to **S**. Click **OK**.

Click **Slits**; set as follows: Excitation: 5 **nm** (or 2**mm**) Emission: 5 **nm** (or 2**mm**).

Click **OK**.

Click **DataFile** and enter a file name for the **data** file, with ending **.spc**. Direct the file to your own folder. Click **OK**.

Click **Run**.

After the run, click the pointer at the highest peak; note wavelength and intensity.

Maximum absorbance should be above 250,000 at 397±1 nm.

If the calibration results are not as expected, consult your instructor before you proceed.

Part II. Finding the Excitation Wavelength

If you already know the excitation wavelength for your sample, Skip to Part III.

The first step in obtaining an emission spectrum is to determine the *excitation wavelength*: the best wavelength for stimulating fluorescence by the sample. This entails two steps: a) guessing an excitation wavelength and using it to find the wavelength of a strong emission peak, and then b) finding the precise excitation wavelength that makes this emission the strongest. Steps 1-3 guide you in this process.

1. Under the **Collect** menu, pick **Experiment**.

Make these settings:

Experiment Type: Emission Acquisition (You will take scan emission wavelengths at a fixed excitation wavelength.)

Scan Start: 315 nm Scan End: 550 nm

Increment: 2 nm Integration: 0.1

Excitation Wavelength: 300 nm (or your first guess at the excitation wavelength)

Click **Signals**; set signals in the first line to **S**. Click **OK**.

Click **Slits**; set as follows: Excitation: 5 **nm** Emission: 5 **nm**.

Click **OK**.

Click **DataFile** and enter a file name for the **data** file, ending with **.spc**; direct the file to your own folder or floppy disk.

Click **Run**.

2. Find the peak of greatest intensity. If it exceeds 4.0×10^6 cps, repeat step 1, but decrease slit widths by 50%. Once you obtain a spectrum of peak intensity less than 4.0×10^6 cps, record the wavelength of the peak intensity (and remember the slit widths). This is the emission peak that you will use to optimize the excitation wavelength.

3. Under the **Collect** menu, pick **Experiment**.

Make these settings:

Experiment Type: Excitation Acquisition (You will scan excitation wavelengths while recording emission at the wavelength determined in 2.)

Scan Start: 250 Scan End: Peak emission wavelength (step 2) **MINUS** 15 nm

Increment: 2 nm Integration: 0.1

Emission Wavelength: As determined in step 2.

Click **Signals**; set signals in the first line to **S/R**. Click **OK**.

Click **Slits**; set to same widths as used for final run in Step 2.

Click **OK**.

Click **DataFile** and enter a file name for the **data** file, ending with .spc; direct the file to your own folder or floppy disk.

Click **Run**.

4. Find the wavelength of greatest intensity. This is the excitation wavelength that best excites the emission discovered in step 2.

Part III. Obtaining an Emission Spectrum

1. Under the **Collect** menu, pick **Experiment**.

Make these settings:

Experiment Type: Emission Acquisition (You will scan emission wavelengths while exciting at the wavelength discovered in 4.)

Scan Start: Excitation wavelength **PLUS** 15 nm Scan End: 650 nm

Increment: 1.0 nm Integration: 0.1

Excitation Wavelength: As provided, or as determined in Part II.

Click **Signals**; set signals in the first line to **S**. Click **OK**.

Click **Slits**; set as follows: Excitation: 5 **nm** Emission: 5 **nm**.

Click **OK**.

Click **DataFile** and enter a file name for the **data** file; direct the file to your own folder.

Click **Run**.

2. Print your spectrum as follows:

Under the **File** menu, pick **Printer Setup**. Set **Orientation** to **Landscape**. Click **OK**.

Under the **File** menu, pick **Print**. Set **Color Mode** to **Black and White**. Click **OK**.

3. For data analysis, you can use **Edit: Copy Screen As: Data** and paste data into Excel.