

SPEX Fluorolog 2 – Instructions

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Turning on the instrument.

1. Turn on the main switch on the power supply for the Xenon lamp. It is located at the far left side of the instrument.
2. Wait for at least one minute, and then press the lamp start button in order to ignite the Xenon lamp. Allow it to warm up for approximately one-half hour before attempting to make measurements with the instrument.
3. Turn on the peripherals of the spectrofluorometer by turning on the main switch on the white power strip located immediately adjacent to the right side of the controller unit.
4. Double click on the “Instrument Control Center” icon on the desktop.
5. A layout selection dialog box appears. Highlight “FL111 Generic Layout” and click OK. The Initialization process may take one or more minutes.
6. The program will now prompt you to enter the wavelength values that appear in the small windows on the fronts of the excitation monochromator and the emission monochromator. It is most important that you enter the exact values of these readings within 0.1 nm. Select “OK”.
7. Click yes in the hardware system dialog box.
8. Select OK for all of the Excitation and Emission slits and other parameters, if they appear. (These will normally be the default values unless specified otherwise.)
9. When the calibration has been finished, the “Instrument Control Center” will appear on the screen.
10. Click on the “Run Experiment” box and the datamax graph window will appear.

Acquiring an Uncorrected Emission Spectrum

In an emission spectrum, the excitation wavelength is fixed and the emission monochromator scans over a range of wavelengths, effectively collecting the wavelength spectrum of radiation coming out of the sample.

1. Click on “Collect” and then click on “Experiment.”

2. An Acquisition dialog screen will appear. Click on the “Experiment Type” button, select “Emission Acquisition,” and then click on the OK button.
4. When the Acquisition dialog box reappears, enter appropriate values for the different parameters required to take an emission spectrum. For example:

Scan Start (nm) = 400 Scan End (nm) = 800
Increment (nm) = 1.0 Integration Time (s) = 1.0
Excitation (nm) = 436
Number of Scans = 1

- a. Click on the datafile button, then enter the name of your particular data file (For example: “C:\data” directory and enter the file name. (There is no need to enter a file extension, since the instrument will automatically assign an *.spc extension for you.)
 - b. Click on the “Correction” button. In the dialogue box, leave the S and R fields blank.
 - c. Click on the “Blank” button. In the dialogue box, leave the S and R fields blank.
 - d. Check the “Dark Offset” box when you are acquiring the spectrum of a blank solution (For example: solvent, buffer system, etc.)
 - e. Click on the “Signals” button, then click on the “Clear All” button. Enter S/R, and click OK.
 - f. Click on the slit button. You may enter any values here, but it is recommended that you enter the actual size of the slits used in mm. These values in no way affect the collected data and are merely stored as extra information in the data file.
 - g. There is no need to change the Setup file and the Exp files, since the following default settings are fine: Setup file = C:\datamax\isa_ini\al.set and Exp. file = C:\datamax\dfilt0.exp.
5. Place your fluorescence cuvette containing a blank solution (for example: solvent, buffer system, etc.) into the sample compartment and close the lid. You will hear a click (as a switch closes) indicating that the cover is in the proper operating position.
 6. Click on the “Run” button.
 7. The instrument will measure the dark offset for 10 seconds, then prepare itself to make a scan. You will notice the acquisition of the spectrum (counts per second versus wavelength) on the screen as it takes place.
 8. When the instrument has completed acquiring the spectrum, it will store the data in the file that you selected previously in Step 4-a (example: ABC.spc).

9. Remove the cuvette containing the blank solution (solvent, buffer system, etc.) from the sample compartment chamber and replace it with your sample solution and close the lid.
10. Click on "Collect" and then click on "Experiment."
11. Click on data file and enter a new name (example: XYZ), then click on OK.
13. Do not change any of the other parameters, since they should remain the same. You can, however, verify them by clicking on the appropriate boxes.
14. Click on "Run," and the scan will begin after a brief delay. When the scan has been completed, the instrument will automatically save the data in the file you previously selected (step 11, example: XYZ.spc). Note: If you change any parameters except the data file and dark offset box, you will be asked to click on OK in order to overwrite the dflt0.exp file (this is not a data file). Say Yes, and click on OK.
15. If you have additional samples to run in the same solvent, repeat steps 9-14, listed above, by designating different file names for the data files.

Acquiring an Uncorrected Excitation Spectrum

In an excitation spectrum, the emission wavelength is fixed and the excitation monochromator scans over a range of wavelengths. The resulting data reveal which wavelengths of excitation are responsible for producing the emission you have selected.

The steps for acquiring an excitation spectrum are almost identical to those of the emission spectrum, except "Excitation Acquisition" is selected in step 2, and "Excitation" parameter in step 4 will be replaced by an "Emission" parameter.

Turning off the Instrument

1. Exit from the control software back to Windows.
2. Turn off the main switch on the white power strip (located to the immediate right of the controller) in order to shut off power to the instrument's peripherals.
4. Push the lamp stop button (which shuts off only the Xenon lamp itself), and allow the blower to continue operating for a minimum of five minutes to facilitate cooling the lamp and its housing.
5. When five minutes have elapsed, turn off the main switch on the lamp's power supply. This completes the shut-down procedure.