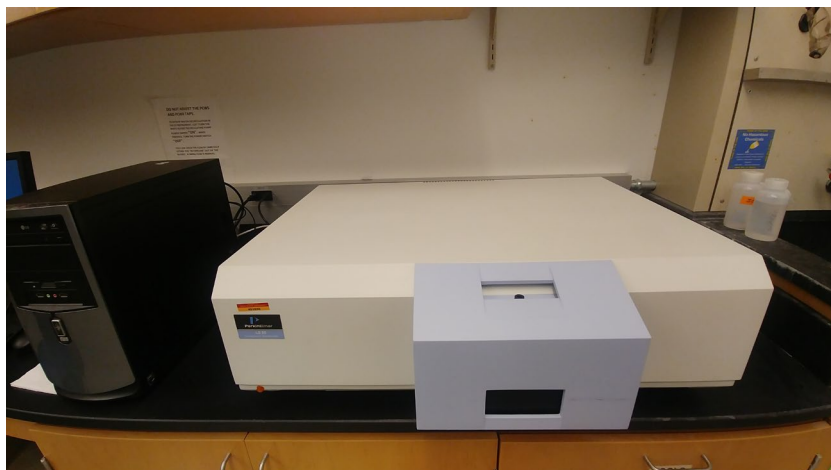


Perkin Elmer LS 55 Fluorescence Spectrometer



Location: 1240 Hach Hall
Contact: Brett Boote, 1234 Hach Hall

The LS 55 is a dual monochromator fluorescence spectrometer, capable of a wide variety of high-sensitivity luminescence measurements. Standard methods include the following:

1. Excitation/Emission/synchronous spectra collection
2. Concentration determinations
3. Kinetics
4. Additional capabilities can be discussed with CIF staff

Getting Started

Log in to the *LockScreen* window using your unique password to unlock the computer. In the taskbar launch VMWare, which will open a console window for virtual machines. The LS 55 spectrometer interfaces with a Windows XP virtual machine named XPro, which can be started from the VMware console. While the XP virtual machine boots, press Ctrl+Alt+Enter to make the virtual machine full screen.

Once XP has loaded, power on the mains for the fluorometer. You will hear the buzz of the pulsed Xe lamp, this is a normal operating sound for the instrument. After the instrument is on, start FL Winlab from the desktop shortcut or quick launch bar. This will start several server

windows in the background as well as the main interface. Under *Applications*, select *Status* to see the optical path status of the instrument. This view will take a minute or so to initialize, but it is nice to see what is going on in the instrument.

Loading Samples

Suprasil quartz cuvettes with Teflon stoppers are located in the TCSPC desk drawer, one of which likely has a scattering solution in it. The other will be stored with methanol to preserve the interior cuvette surfaces from dry residues. For routine samples, use the second standard cuvette. Half-full cuvettes will have sufficient sample volume for analysis.

In general, the samples should be dilute as the detector is very sensitive.

For limited-volume samples there is also a microcell (3x3 mm) and adapter for the cuvette holder.

A solid sample holder is also available for analyzing the luminescence spectra of solid samples and films, just remove the standard cuvette holder and install the solids holder. The current sample holder is set up for turbid liquid samples and thin films on thin substrates (i.e. glass coverslips). Glass does show a background due to scattering at the back of the slide, but it can be subtracted easily by collecting a clean glass spectrum.

Setting Instrument Parameters

To collect a routine luminescence spectrum, select *Scan* under *Applications*. This will load a new window with the method, with several parameters to set up. You can select the type of spectrum to acquire and set the parameters here. The slit widths are adjustable between 5.0 and 15.0 nm in 0.1 nm increments.

For both excitation and emission spectra, there should be at least a 10 nm offset between the monochromators (*i.e. excite at 300 nm and start the collection at 310 nm, or similar*). In addition, there will always be some signal at the second order diffraction peak (*i.e. if you collect emission at 600 nm with 300 nm excitation, the second order diffraction of the grating will show an intense band due to the excitation*).

This instrument has a limited dynamic range (0-1000 counts), so there are three optical filters in the emission monochromator filter wheel to allow for tuning the total light throughput: 50%T, 10%T and 1%T filters. These are selectable by clicking on the emission monochromator icon in the *Status* window. *Note: many method files will overwrite this value when they are called, so pay attention to what is shown in the status display.*

Synchronous spectra can probe samples for specific energy differences or Stokes shifts by scanning both monochromators together, which can be helpful for separating species with overlapping emission bands but different absorbance properties.

The data file name can be specified, but is restricted to 5 characters since there is an 8 character maximum in the software and a number is assigned to each run by adding #01 to the name. By default, all data is stored in Z:\XP_Share\DATA\RAW. When your measurements are complete, please transfer them into a directory in DATA with your university username (e.g. DATA\bboote).

To begin data collection, press the stoplight button. The new data will be drawn on the Results tab.

Sample Cleanup

When finished with measurements, place all generated waste in the appropriate container (based on solvent) in the back lab of 1238 Hach. In the event the sample or solvent should be in a special waste container, please retain and dispose of that waste in your research lab where there is likely a waste disposal strategy in place.

Thoroughly rinse the sample cuvette with DI water (if applicable), methanol, and then refill and cap with methanol for storage.

Instrument Shut Down

Close all instance windows of FLWinlab, then power off the spectrometer. Then the VM can be shut down. Once the VM is off, the VMWare parent window can be closed. Next, please run the *backup.bat* file on the desktop to copy all of the shared data to the PE Data folder on the desktop. Log off the *LockScreen* program using the lower right *Logoff* button, then turn off the monitor. Return all loose components, tools, and cuvettes to the cabinet above the LS 55 system, or to the TCSPC drawer.

Maintenance:

No user maintenance is necessary for this optical system. Contact CIF Staff if anything seems disconnected or out of the ordinary.

System alignment:

Any alignment adjustments will be made by CIF staff.