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Edinburgh FLS1000 Lifetime Spectrometer Standard Operating Procedure

The Edinburgh FLS1000 Lifetime Spectrometer is designed for fluorescence measurements of liquid and solid samples and excititation and emission wavelengths of 250-900 nm. Variable bandwidth excitation and emission monochromators determine the wavelengths measured. A photon counting detector is used across the entire energy range. Fluorescence lifetime measurements can be made using fixed wavelength pulsed light sources.

Start-up

The power for the system is controlled by a single switch, on the topmost unit labeled 'MAIN POWER SUPPLY'. With the exception of the external pulsed light sources, this should be the only switch that needs to be flipped. After turning on the power, look at the screen on the lamp housing near the lamp power button. It should say "Lamp off. Fans on. Ready to start", or just "Ready to start". Assuming that you want to use the continuous source, an open free Xenon arc lamp, press the lamp power button. "Lamp starting", then switch to show the lamp power, current, voltage and total operating time. Please wait 10 minutes for the lamp to stabilize. If you know that you want to make lifetime measurements and you know what excitation wavelength you want to use (presently 280, 405, and 450 nm available), mount the appropriate source (described below) and switch on using the key. Now would be a good time to make sure you have all of your samples prepared.

To summarize:

1. Turn on main power.
2. Press lamp power button.
3. Wait 10 minutes for lamp to stabilize.

Sample mounting

There are three sample mounts, but we will primarily use 2; one for liquids and one for solids. Please read about both mounts if you need to switch between them.

Liquids

The sample mount for cuvettes includes integrated thermo-electric heater, thermocouple, and drive for a mini magnetic stirrer bar (should not be needed in most experiments). A cuvette sits fully within the housing. To remove a cuvette, pull gently pull the the clear plastic peg that

extends above the top of the house; this will lift the cuvette so it can be grasped easily. Cuvettes should only be filled a little more than half-way (aim for 2/3 full). Since the light is focused at the sample, only the middle portion is illuminated and overly full cuvettes lead to spills.

Solids

The solid sample mount holds something of similar dimensions of a cuvette tilted off vertical and rotated so that the a similar view would be seen along either the incident of exit light beam path, but specular reflect won't propagate along the exit path. A holder with a spring-loaded bracket can hold a small frame and slide into the sample mount.

Changing mounts

Connections for water are made through quick connect fittings below the housing, which must be accessed through the lowest panel (with the Manufactured in the United Kingdom sticker) on the on the angled side. It is held in place with magnets and pins, so grip from the side and pull out. If you need to disconnect the water lines, first make sure that there is no water flowing. Open the lowest drawer beneath the instrument, where a pump sits in a clear plastic tub. The water return tub passes through the lid an should end a few centimeters above the surface of the water. If water is flowing out of that tube and you want to disconnect the water tubes from the sample mount, go into Fluoracle on the control computer and select Setup->Temperature Device Setup->Temp. control=Off. Before removing the liquid sample mount, disconnect the gray DB15 cable beneath the holder.

Controlling the spectrometer and taking measurements

Log on to Coral and enable the instrument by selecting Spectroscopy and Electronic and Magnetic Measurements->Optical Spectroscopy->Edinburgh-Lifetime-Spectrometer and choosing Equipment Actions->Enable. On the computer to the right of the instrument, open Fluoracle. Open the Signal Rate window. Displayed are the intensity on the reference detector (before the sample) and on the emittance detector. The intensity on the reference should stay under 4,000,000, and the detector intensity should stay below 1,000,000. Above these levels the detector response is non-linear, and could potentially be damaging to the detector. These raw intensity measurements are after the emission or emittance monochromator, so they can be decreased by decreasing the appropriate bandwidth in the Signal Rate window. This window also has options for attenuating the beam. The scale runs from 0-100, but is very non-linear. The Signal Rate window also has options for inserting excitation and emission polarizers, and setting them to 0, 35, 55, or 90 degrees. This window is also used to switch from continuous source to external pulsed source.

Continuous illumination measurements

A typical series of measurements might proceed as follows.

1. Open the Signal Rate window and set reasonable bandwidths for excitation and emission

2. Set the excitation wavelength to the shortest value (highest energy per photon) within the instrument's range that you might expect a peak. Make sure that the reference intensity is well below 4,000,000. Close the Signal Rate window.
3. Open an emission scan window by selecting New Spectral->Emission Scan. Sweep the emission wavelength from the lowest reasonable wavelength, which must be much larger than either monochromator bandwidth. During the sweep, when near peaks watch the intensity displayed in the lower right hand corner of the screen. This is the raw count rate on the detector, and should stay below 1,000,000. The plotted count rate is corrected based on variation in the monitor intensity and the instrument's efficiency as a function of wavelength.
4. Set the emission wavelength higher than the emission peak (+10 nm, for example). Select New Spectral->Excitation Scan. Scan the excitation wavelength from a low value up to the emission peak.
5. With the excitation and emission plots open, click the merge plots button along the top to make a combined graph that has both pieces of information

Excitation-emission maps can be made, where both wavelengths are scanned. This is obviously more time consuming than individual plots, and is mostly useful when there are multiple fluorescent compounds present in a sample. The use specifies a width that the emission measurement stays away from the excitation wavelength, so the end result is not a rectangular grid of data.

Lifetime measurements

The Edinburgh FLS lifetime spectrometer can readily measure decay times from 1 ns to 50 us. Faster decay times, down to 5 ps, can in principle be measured, but great care has to be taken to characterize the pulse shape and fit the decay. Decay measurements are made by recording the time between an excitation pulse and a single emitted photon being detected. Because of the instrument design, only one photon can be measured per pulse. In order to ensure that the all emitted photons have an equal probability of being observed, the count rate on the detector must be <= 0.03 times the pulse rate.

A typical procedure follows:

1. Determine which available wavelength source -- 280 nm LED, 405 nm laser diode laser, or 450 nm diode laser -- is most appropriate for the experiment. This is based on prior experience, literature information, or based on an excitation scan as described above.
2. Install the pulsed the side shelf outside the spectrometer. Check that the repetition time is 500 ns or longer. If in doubt, set it to a longer time. Turn on the laser.
3. Wait 5 minutes.
4. In Fluoracle, go to Setup->EPL Lasers Setup and choose the appropriate wavelength source.
5. Open the Signal Rate window, and change the Source Light Path. The top Detector Signal should have changed from Reference to arbitrary units to EPL in Hz.
6. Press the red Laser On/Off button on the laser.

7. With the Emission wavelength at the desired value, check that the emission count rate <0.03xEPL_rate. For example, using the 1 us setting on the laser, the EPL rate should be very close to 1 MHz, and the emission rate should be below 30 kHz. If this is not the case, decrease the emission bandwidth or change the Intensity (attenuate the incident beam). Close the Signal Rate window.
8. Select New Lifetime->Manual. Configure the measurement:
    1. Time range - this should be much longer than your decay time (~10x) and much shorter than your repetition time (~0.1x).
    2. Chanels - 512 is usually reasonable.
    3. Peak counts - this determines the statistical quality of the data an how long the measurement takes. The software builds up a histogram of photons vs delay time with a bin width determined by time range/channels, and ends when any single channel gets to 'peak counts'.
    4. Click new. The histogram of events is plotted on a logarithmic plot. Within ten seconds of measuring, you should have an idea of whether the time range chosen was suitable.
    5. Press the laser of/off button when done with the scan. It does have a finite lifetime.
9. Zoom in on the portion of the scan which appears to have a linear decay on the log plot. This should start a few ns after the peak.
10. Select Analyze-Exponential Tail fit. Put in an initial guess for the decay time tau, and run the fit. Fluoracle generates plots of fit data and measured data vs time and fit error vs time and displays the decay time.

Cleanup

1. Turning off sources.
    1. If there is no user coming after you during the day, and the Xenon arc lamp is on, press the lamp on/off button. The lamp fans stay on for a time (potentially a few minutes), and the display near the button says that the lamp is off and the fans are on. The main power to the unit should not be shut off while the instrument is in this start. Once the fans are off, the display says that the fans are off, and says please wait with a timer counting down for roughly 15 minutes. This is how long one should wait before restarting the lamp. The main power supply can be switched off during the wait period.
    2. If a pulsed source was used, it should be switched off with the key.
2. All samples should be removed and cleaned away.