

Photoluminescence Spectrometer (FLS980)

Instrument Summary:

The Edinburgh Instruments' FLS980 photoluminescence spectrometer offers both steady state and time resolved (lifetime) fluorescence spectroscopy measurements of solid and liquid samples. The instrument is equipped with four tunable, pulsed repetition rate lasers (wavelengths including: 315, 375, 635, and 980 nm), a flashlamp, and a xenon arc lamp. The FLS980 incorporates two silicon detectors (200-1100 nm) and an InGaAs detector. By combining time correlated single photon counting (TCSPC) and multi-channel scaling (MCS) the FLS980 excels in fluorescence lifetime measurements which span 12 orders of magnitude, ranging from picoseconds to seconds.

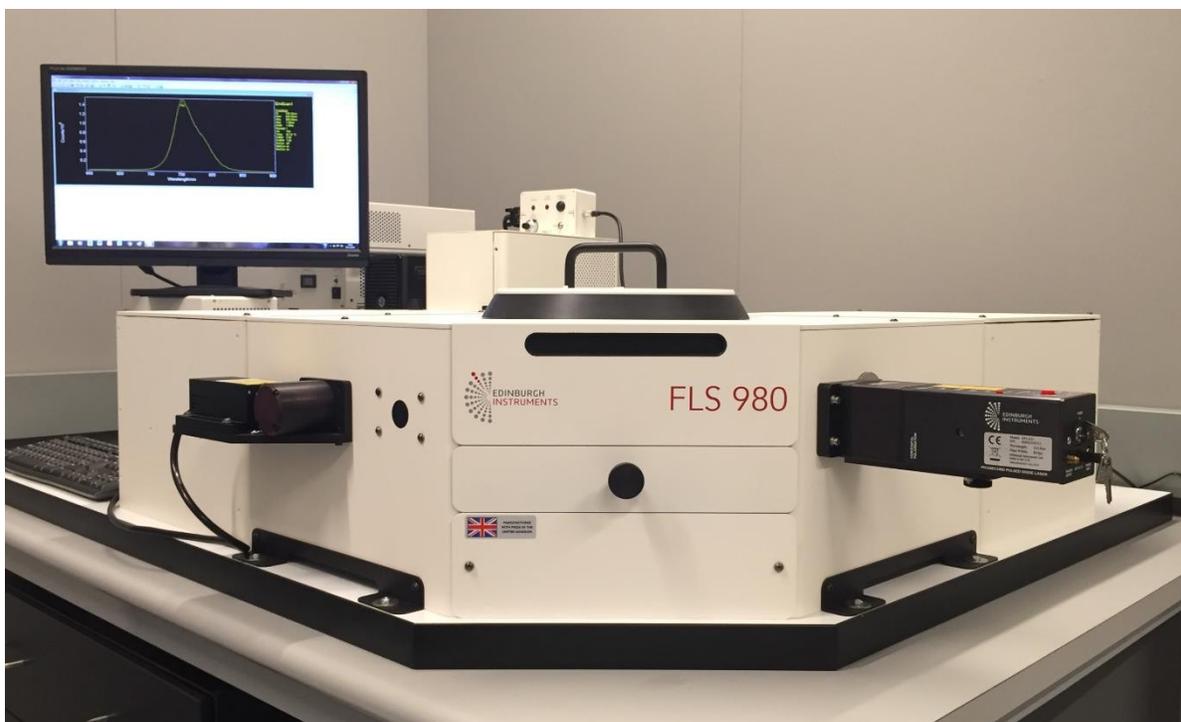


Figure 1. The Edinburgh Instruments FLS980, photoluminescence spectrometer.

Safety and Precautions:

- **Startup:** If you are the first user of the day, turn on the FLS980 and let it run for 20 min.
- **Shutdown:** Turn off the lamp's and instrument if you are the last user for the day.
- **Warning:** Avoid running a steady-state excitation or emission scan in which $\lambda_{Ex} = \lambda_{Em}$.
- **Warning:** Check the compatibility of liquid samples with your cuvettes. Plastic cuvettes (Ex: Polystyrene) can absorb organic liquids (Ex: Toluene) and can leak in the instrument.
- **Check:** If you do not get signal, check the beam dump to ensure the instrument is configured properly. Additionally, check to make sure there are no optical filters.

I. Experiment Planning:

1. **Determine the Approximate Fluorescence Profiles of your Sample** – It is useful to have a basic understanding of the excitation and emission wavelengths of your sample before starting an experiment. If the sample has unknown fluorescence properties you can run a signal probing experiment to determine approximate values for excitation and emission. See the “**Analysis of Unknown Samples**” section below.
2. **Steady-State Measurement** – After determining the approximate excitation and emission values you will want to run a “**Steady-State Excitation Scan**” and a “**Steady-State Emission Scan**” to more accurately determine the fluorescence properties of your sample.
3. **Lifetime Measurement** – After determining the steady-state properties of your sample, you are ready to measure the “**Fluorescence Lifetime**” using either a pulsed LED laser or the flash lamp.

II. Start the FLS980:

1. **Turn on the Instrument** – Flip the “PH1” toggle switch behind the computer monitor.
2. **Turn on the Fan** – Flip the “CO1” toggle switch located behind the monitor.
3. **Turn on Xe Lamp** – Flip the lower toggle switch located on the back of the Xe lamp.
4. **Instrument Stabilization** – Allow the instrument to stabilize for 20 minutes.
5. **Ignite the Xe Lamp** – Press and hold the silver button located on top of the instrument.
6. **Open the Software** – Open the Desktop (**Password: RandLab**). Click the “F980” Icon.

III. Insert a Sample:

1. **Open the Chamber** – Lift the circular lid off the top of the FLS980.
2. **Check Instrument Configuration** – Ensure that the beam dump is oriented in the correct position and that no glass filters are inserted in the path of the beam.
3. **Insert the Sample in the Holder** – The FLS980 can be configured to accommodate cuvettes and films. The mounts are kept in the sample chamber or in the drawers beneath the instrument.
***Cuvette Note 1:** For liquid samples it is best to utilize dilute concentrations. Samples with high concentrations can limit penetration of the beam through the cuvette. This “**inner filter effect**” will hurt accuracy of excitation scans. Check to make sure the beam fully penetrates through the liquid.
***Cuvette Note 2:** Check the compatibility of liquid samples with your cuvettes. Plastic cuvettes (Ex: Polystyrene) can absorb organic liquids and could leak in the instrument.
4. **Close the Chamber** – Replace the lid on the FLS980, this compresses the interlock.
5. **Position the Sample to Maximize Signal** – The sample can be oriented back and forth to maximize signal by turning the black knob located on the front of the instrument. Check the counts detected in the “Signal Rate Window”.

IV. Analysis of Unknown Samples:

***Note:** For unknown samples it is useful to do a manual excitation and emission scan using the signal rate window. This will provide approximate excitation and emission values prior to running more accurate steady-state excitation and emission scans.

1. **Open the Signal Rate Window** – The “Signal Rate Window” lists live counts as recorded within the instrument and can be used to optimize signal parameters (See **Figure 2**).
2. **Set the Bandwidth ($\Delta\lambda$) to ~ 0.1** – The bandwidth governs the amount variation in wavelength. By setting a low value for unknown samples we prevent detector saturation.
3. **Set the Step Sizes to ~ 5 nm** – The step size controls the step size taken when scanning the spectrum. By setting a large step size we can quickly assess the sample.
4. **Set the Excitation and Emission Wavelengths** – Set λ_{Ex} to ~ 300 nm and λ_{Em} to ~ 400 nm.
5. **Manually Increase the Excitation λ** – Press the arrow key to iteratively increase the excitation λ by 5 nm. Monitor the emission signal; look for an increase in CPS. If no signal is detected in this range, increase λ_{Ex} to ~ 400 nm and λ_{Em} to ~ 500 nm and continue.
6. **Scan the Emission λ Range** – Once the excitation peak is determined, enter this value as the excitation wavelength. Next iteratively step through the emission spectrum to find the maximum emission λ .
7. **Record Approximate Excitation and Emission Wavelengths** – These values will help design a steady-state fluorescence procedure.
8. **Adjust Sample Position** – Use the translator knob to adjust sample position and maximize signal.

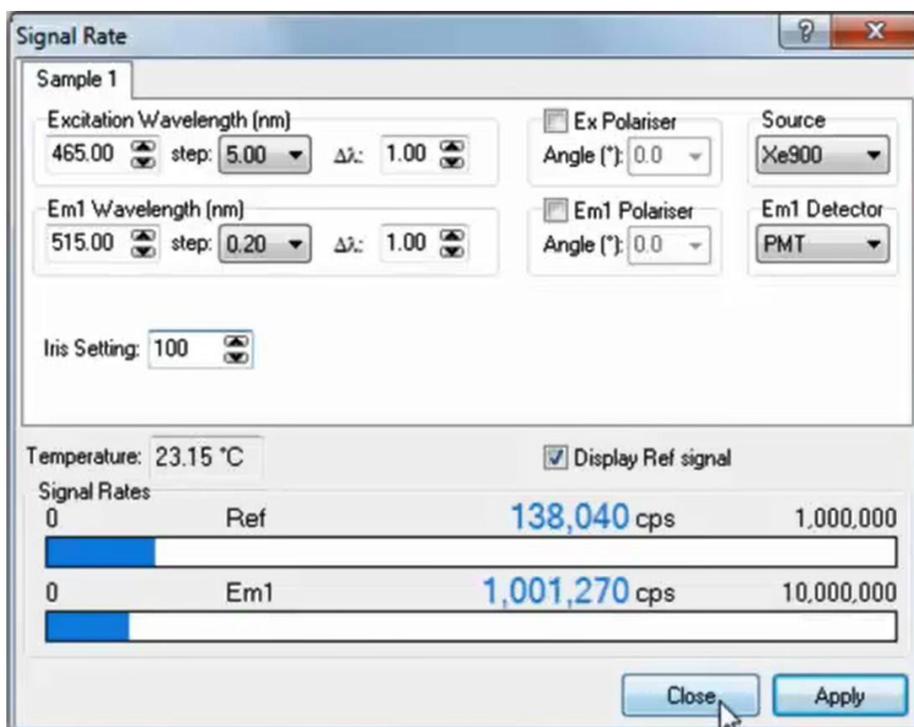


Figure 2. Screenshot of the signal rate window.

V. Steady-State Emission Scan:

***Note:** During a steady-state emission scan, the excitation λ is held constant and the detector is used to incrementally scan the light emitted by the sample to determine the emission profile.

- 1. Setup the Emission Scan Parameters** – Open the signal rate window and set values.
 - **Excitation Wavelength** – Enter an excitation wavelength (λ_{EX}), this wavelength (constant) should be at a λ which excites the sample and at least 15 nm shorter than the onset of the emission profile.
 - **Emission Wavelength** – Enter the emission wavelength (λ_{EM}), this wavelength should correspond approximately with the emission peak.
 - **Enter Step Size and Band Width** – A step size of 0.2 nm and a $\Delta\lambda$ of 1.0 should work for most samples.
 - **Select a Light Source and Detector** – The Xe900 lamp and the PMT detector should work for most samples.
- 2. Click the “Apply” Button** – This stores the settings in the software.
- 3. Check the Signal** – The sample should produce a detectable signal that is between 0 and 2 million CPS. If the signal is >2 million CPS, reduce the Iris setting from 100% until the signal drops below 2 million CPS.
- 4. Open the Steady-State Window** – Press the λ icon to open the steady-state scan options.
 - **Check the General Tab** – For emission scans select the: EmCorr Scan option.
 - **Check the Excitation Tab** – The excitation settings should reflect the settings selected in the signal rate window.
- 5. Update the Emission Tab** – Update the “Emission Scan Parameters” options at the bottom of the window.
 - **Scan Range** – The scan should begin at a wavelength that is at least 15 nm greater than the λ_{EX} value (*i.e.* $\lambda_{EM}^{Start} = \lambda_{EX} + 15$ nm) and end at a final value that is ~200 nm greater than the starting wavelength (*i.e.* $\lambda_{EM}^{STOP} = \lambda_{EM}^{Start} + 200$ nm).
 - **Step** – 1.00 nm is a standard step size.
 - **Dwell Time** – 0.2 Seconds.
 - **Number of Scans** – 5 Scans (this will average data collection over 5 runs).
- 6. Insert a Filter** – Add an appropriate *high-pass filter* that is between λ_{EX} and λ_{EM}^{Start} .
 - Check for and remove any previous filters.
- 7. Press Start to begin the Scan** – The experiment can be aborted by hitting the *stop sign*.

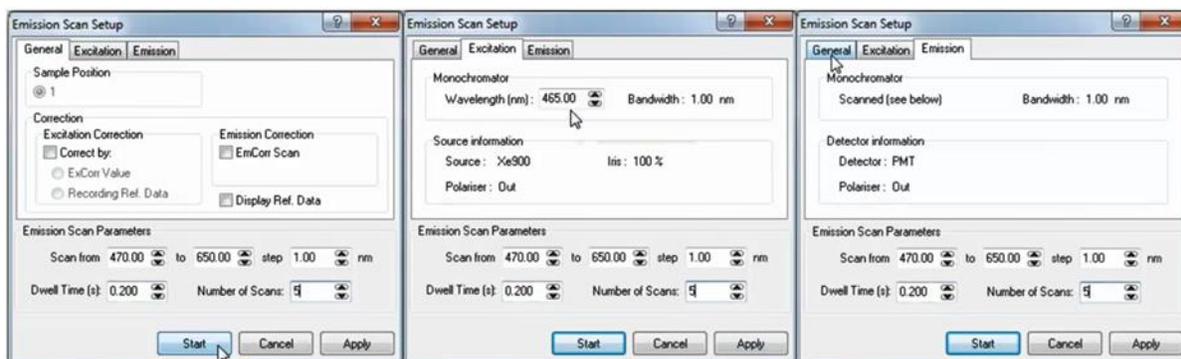


Figure 3. Emission Scan Setup Window.

VI. Steady-State Excitation Scan:

***Note:** During a steady-state excitation scan, a constant emission wavelength is monitored to determine CPS produced as the excitation wavelength is varied (See **Figure 4**).

- 1. Setup the Excitation Scan Parameters** – Open the signal rate window and set values.
 - **Excitation Wavelength** – Enter an excitation wavelength (λ_{Ex}), this wavelength should correspond approximately with the excitation peak.
 - **Emission Wavelength** – Enter the emission wavelength (λ_{Em}), this λ should correspond to a value that is longer than the upper limit of the excitation range.
 - **Enter Step Size and Band Width** – A step size of 0.2 nm and a $\Delta\lambda$ of 1.0 should work for most samples.
 - **Select a Light Source and Detector** – The Xe900 lamp and the PMT detector should work for most samples.
- 2. Click the “Apply” Button** – This stores the settings in the software.
- 3. Check the Signal** – The sample should produce a detectable signal that is between 0 and 2 million CPS. If the signal is >2 million CPS, reduce the Iris setting from 100% until the signal drops below 2 million CPS.
- 4. Open the Steady-State Window** – Press the λ icon to open the steady-state scan options.
 - **Check the General Tab** – For excitation scans select: Recording Ref Data.
 - **Check the Emission Tab** – The emission settings should reflect the settings selected in the signal rate window.
- 5. Update the Excitation Tab** – Update the “Excitation Scan Parameters” options at the bottom of the window.
 - **Scan Range** – The scan should end at a wavelength that is at least ~15 nm less than the emission wavelength value (*i.e.* $\lambda_{Ex}^{Stop} = \lambda_{Em} - 15 \text{ nm}$) and start at a value that is ~200 nm less than the emission wavelength (*i.e.* $\lambda_{Ex}^{Start} = \lambda_{Em} - 200 \text{ nm}$).
 - **Step** – 1.00 nm is a standard step size.
 - **Dwell Time** – 0.2 Seconds.
 - **Number of Scans** – 5 Scans (this will average data collection over 5 runs).
- 6. Insert a Filter** – Add an appropriate *high-pass filter* that is between λ_{Em} and λ_{Ex}^{Stop} .
 - Check for and remove any previous filters.
- 7. Press Start to begin the Scan** – The experiment can be aborted by hitting the **stop sign**.

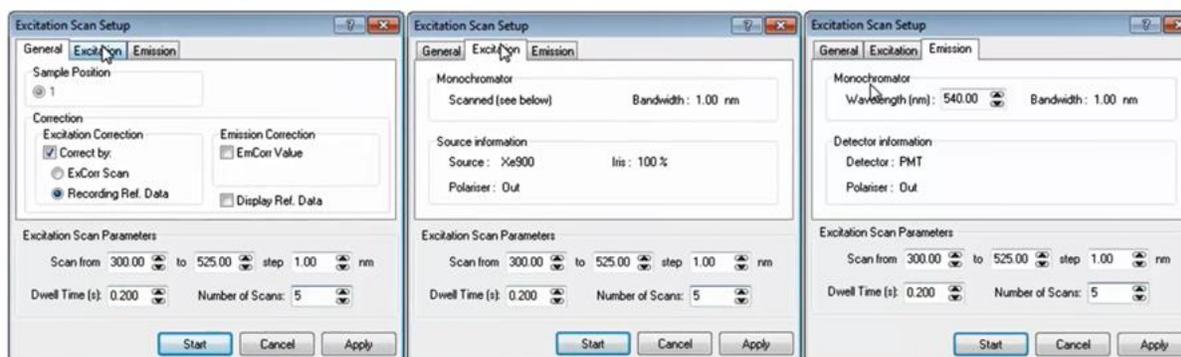


Figure 4. Excitation Scan Setup Window.

VII. Lifetime Scan:

***Note:** A lifetime scan can be performed after obtaining the steady-state excitation and emission profile of a sample.

1. **Configure the Instrument for Lifetime Measurements** – Flip the beam dump over to block the Xe lamp. Rotate the sample holder 90 ° to face the LED laser source.
2. **Choose an Appropriate Light Source** – Review the excitation and emission data, select a laser closest to the excitation peak of your sample. A range of pulsed LED lasers are available with wavelengths including: 315, 375, 635, and 980 nm. Additionally, a flashlamp is available for samples with long lifetimes.
3. **Mount the Light Source** – Loosen the knob under the current LED source and slide the previous LED off of the mount. Cap the LED using a red LED end cap. Unplug the red and black cords from the previous LED and connect with the appropriate LED laser. Slide the new LED on the mount and retighten the knob under the LED.
4. **Turn the LED On** – Turn the key on the back of the LED. Wait for the blinking light to stop blinking and stay lit. Then press the red button.
5. **Select a Repetition Rate** – Turn the dial on the LED to a desired repetition rate. This rate should be long enough to capture the full lifetime of the sample, yet short enough to efficiently collect and bin the data.
6. **Open the Signal Rate Window** – Change the source to the TCSPC Laser. Increase $\Delta\lambda_{Em}$ to ~5.00 nm. Ensure that the Emission signal is < 2% of the TCSPC's excitation value. Rotate the optical power knob on the LED to reduce the excitation signal if necessary.
7. **Open the Lifetime Window** – Press the τ icon and select “New Lifetime”.
8. **Edit Lifetime Settings:**
 - **Time Range** – This setting should roughly match value selected on the LED dial.
 - **Channels** – Set the number of channels (~2048) that you would like to bin data in.
 - Higher values give better resolution but divides counts across more bins.
 - **Stop Condition** – Set the Peak counts to value ~2000 counts.
9. **Press Start** – The experiment can take several minutes to hours.
10. **Check Data** – The data should present a peak with a gradually sloping tail. If the data is binned in such that a series of horizontal lines begin to form, then it is likely that the repetition rate is too high. If this is the case, adjust the knob on the LED and update the Time range setting in the software.

***Note:** For samples which have extremely fast lifetimes, it may be necessary to deconvolve the **instrument's response function (IRF)** from the data. If this is the case, select the “IRF” setting and first run an IRF scan before analyzing your sample.

VIII. Data Analysis:

1. **Combining Spectra** – Click the “Join Visible” button at the top of the software. This will create a new window that plots multiple spectra on the same graph for comparison.
2. **Normalizing Data** – Data is often normalized for presentation. To normalize data, click “Data”, “Normalize”, select the desired file, and enter a normalized value (Ex: 1.0).
3. **Data Fitting** – Select “Analysis” → “Ex. Tail Fit” → estimate a value for τ_1 and select the “Time Resolved Emission Scan (TRES)” option. Alter the τ_1 value if it fits the data poorly.
4. **Saving Data** – Export data by selecting “File” and “Export Data to ASCII”.

IX. Instrument Shutdown:

1. **Remove Samples from the Instrument** – Remove samples and clean up the instrument.
2. **Remove Filters from the Instrument** – This helps the next user.
3. **Turn off the Lamp** – If you are the last user of the day, turn off the Xe lamp. Allow ~15 minutes for the lamp to cool down before powering off the cooling unit.
4. **Turn off the Fan and Main Power Switch** – After the lamp has cooled sufficiently, turn off the remaining toggle switches in the reverse order as that used to turn on the instrument (turn off CO1 followed by PH1).
5. **Sign the Log Book.**
6. **Log Off of the Instrument.**

Emergency Information:

Medical Emergencies: **Contact 911 and Public Safety (609) 258-1000**

Room / facility emergencies: **Contact Public Safety (609) 258-1000**

Issues related to the instrument:

1. **Contact IAC Staff.**
2. **If unsure, leave system as is.**
3. **Try to power down the system.**

Audible/Siren Emergency Alerts:

Follow previous steps 2 & 3 and leave the building.

Emergency Contact Information:

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