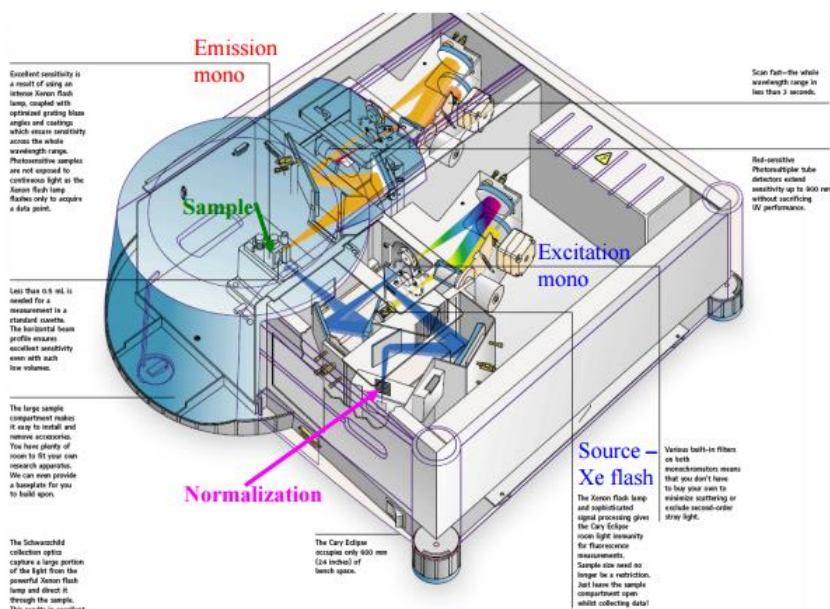


Cary Eclipse Fluorimeter General Procedure

Materials Research Laboratory
University of California, Santa Barbara



Facilities Billing System (FBS) & MRL Recharge Information



The instrument time is billed through FBS. Your FBS time = your recharged time. Recharge is calculated at an hourly rate with a minimum charge of 1 hour. For current recharge rates go to the MRL website, http://www.mrl.ucsb.edu/sites/default/files/mrl_docs/recharge.pdf

- ❖ To begin an instrument session, you must log onto FBS first and either click on **Start Timer** if a reservation has already been made or select **Walk-Up**.

To do so use the FBS designated computer in the lab or any internet connected device, and navigate to <http://ucsb.fbs.io>

- ❖ Now you can log onto your account on the instrument computer. Remember to log off your computer account when finished.
- ❖ Once the session is finished you must log into your FBS account and click **Stop Timer**. This will stop your FBS time. If you do not do this you could incur extra charges.
- ❖ The paper log sheet by the instrument is used as back up for the FBS system. Remember to mark your time, recharge number, and any notes or problems you would like to convey, feel free to use as many lines on the page as needed to be clear.

- ⚠ No shows will be charged **75%** of the scheduled time. If you cannot use your reserved time cancel it.
- ⚠ FBS records the billable time as the longer time between the scheduled time and the time used. So if your scheduled time is longer than the actual time on the instrument then the scheduled time will be charged.

1.) Getting Started

- (a) Log into FBS and start your timer.
- (b) Sign in on the Log Sheet. Record your full name, advisor's name, department, start time, and your recharge number.
- (c) Log onto the computer with your username and password.

2.) Sample Preparation

Fill a cuvette to half-full and make sure the bottom half of the cuvette is clean and free of any residue such as fingerprints, etc. Note: cuvettes must be transparent on all sides. Sample absorbance at the excitation wavelength should be below 0.1 Au to minimize inner filtering effects. Absorbance can be measured using the Simple Reads application in the Cary Eclipse software (see Cary Help).

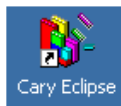
3.) Instrument Setup

- (a) Install a proper sampling accessory, either a single-cell holder or a Multi-cell holder (the Multi-cell is the DEFAULT accessory) into the sample compartment.
If the wellplate reader accessory will be used, please follow the procedure found in the procedure notebook



(b) Turn the fluorimeter ON by flipping the switch on the lower right front corner to the right (line side down).

(c) Open Cary Eclipse by double clicking the icon:



(d) Double click on the *Scan** icon. Click “OK” on the box that appears.

* Scan is the most common application for the instrument. For more information on other applications available please refer to [page 5](#) of this procedure

4.) Acquiring Excitation or Emission Spectra

Zeroing and Running a Blank Scan

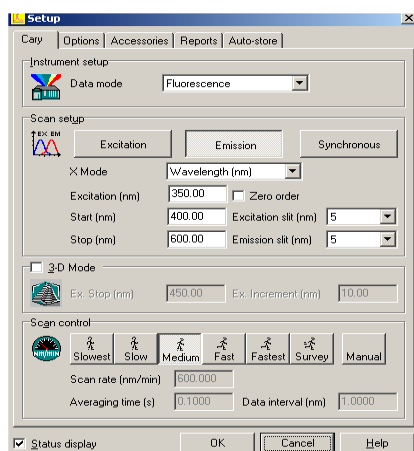
To remove the effects of buffer/sample matrix place a cuvette containing only the blank solution (this can be done with an empty cuvette as well) and click the **Zero** button. When finished the word “zeroed” will appear in the Y display box. Next run a full scan of the blank to check for irregularities in the baseline. This blank scan can be subtracted from the sample scan later by saving it as a .CSV file.

Prescan Option

This option is useful to determine the optimum parameters for your sample. It will determine the excitation and emission λ and the PMT voltage that should be used/needed. Once the prescan is finished, the new parameters will automatically be saved to the method.

(a) Click on **Setup...** in the left hand column. Go to the **Accessories** tab; check the **Multicell Holder** and check all boxes in which you have sample in. Click **OK**.

(b) Click on the **Prescan** button in the left hand column, choose which scatter you wish to search for (all 3 can be chosen at the same time). Click **OK**; the scan will start automatically.



Create Scan

Click on the *Setup...* button on the upper left panel

(a) **Data Mode**

Set to *Fluorescence*, *Bio/Chemluminescence*, or *Phosphorescence*

(depends on what experiment you want to run)

If *Luminescence* or *Phosphorescence* is chosen, then an *Options* tab will appear to the right where additional experiment parameters can be set.

(b) **Scan Setup**

Choose *Emission* or *Excitation*, then enter the appropriate λ for your experiment (if the prescan was done, they should already be set)

A *Synchronous* scan can also be selected – this scans both the excitation and emission monochromators at the same time with a fixed delta (λ) between them.

Excitation and *Emission slit widths* are sample dependent: 5 nm is a good starting value.

Increasing the slit width increases the intensity of the respective light.

(c) **3-D Mode** (refer to Software Help section for more information)

3-D data set can also be created by checking the box and setting the necessary parameters. 3-D graphs enable you to quickly produce distinct peaks of samples that may be emitting at a number of different wavelengths. Using the 3-D graph, you will be able to determine the most suitable excitation and emission wavelengths for a sample.

(d) **Scan Control**

How fast the instrument scans. Medium is a good starting point, depending on your sample.

(e) **Options Tab**

In this tab you can set

- *Overlay Traces* – Spectra will be overlaid in one window. If you do not check the box, then each spectrum taken will appear in its own window.
- *Excitation* or *Emission Filters* - These help decrease interference from second harmonics and the 3% T Attenuator allows only 3% of the total emission to be collected; it's great for strongly emitting samples, but a larger slit width should be used.
- *Cycle Mode* – Allows you to collect scans as a function of time to see if the signal is changing with time.
- *PMT Detector Voltage* - A Prescan will determine this value, but Medium is a good starting point.

If using the Multicell Holder:

Go to the *Accessories* tab in the Setup window => check the ***Multicell Holder*** box and the ***respective positions*** that your samples are located (Locations 1 – 4)

** This is also the **Temperature Stage** as well. Check the Temperature Control box to set the desired temp and turn ON the TC Unit located to the right of the instrument (has 2 hoses coming out of it).

If using the Wellplate Reader:

Follow procedure found further in the notebook

If using the Single Cell Holder:

- Remove the Multi-cell Holder by lifting up on the 2 black tabs and turning the silver knob counter-clockwise.
- Partially remove the accessory and then remove the computer connection.
- Remove entire accessory and place on top of the temperature control unit.
- Proceed to Step (3). Also, REMEMBER to put the multi-cell accessory back into place when you are finished.

Click “OK” to exit the Setup window.

Click on the stoplight *Start* button at the middle top and enter a file name when you are prompted; the instrument will start scanning after the file name has been entered.

When the instrument is finished with the scan, the spectrum will appear in the window. You can move the cursor along the spectrum and read its coordinates in the lower right corner.

5.) Saving Data

(a) Go to *File => Save as...* and save in your own folder. If you do not have your own folder then create one in **C:/Data**. The data can also be saved in text format for importing it into Excel: Save it as the “.CSV” extension.

It is always a good idea to back up all of your data on your own USB flash drive, CD, etc; we are not responsible for any lost data.

(b) To remove all spectra from the window choose *Graph => Remove all Traces* or to just remove one graph choose *Graph => Remove Graph* or just click the *Trash Can icon*. If you have more samples then repeat the above procedures.

6.) When You Are Finished

(a) Make sure the instrument is put back into DEFAULT MODE – The Multi-cell Holder accessory needs to be installed if it was removed for another sample accessory. Please refer to the end of the “Wellplate Accessory Procedure” for any help needed for reinstallation of the Multi-cell Holder.

** Turn OFF the Temperature Control Unit if temperature experiments were performed.

(b) Exit out of the software. **Log off** of the computer.

(c) Turn off the instrument.

(d) **REMOVE** your samples from the sample compartment.

- (e) Finish filling out the RECHARGE SHEET.
- (f) In FBS click “Stop Timer”, and make sure to log out of your FBS account.
- (d) Clean up and take your samples with you.

*****Other Applications for the Cary Eclipse Fluorimeter*****

For more information on how to run these applications, please refer to the Cary Eclipse Help section.

- **Kinetics** – Measures the increase or decrease of emission intensity as a function of time.
- **Simple/Advanced Reads** – Measures absorbance readings at a single wavelength for one sample/multiple samples, respectively.
- **Concentration** – Used when quantitative data is desired.
- **Lifetimes** – Measures phosphorescence lifetimes
- **Thermal** – performs thermal analysis on samples
- **Ratio** – Measures a change in the intensity at two different wavelengths. Used to determine concentration values.