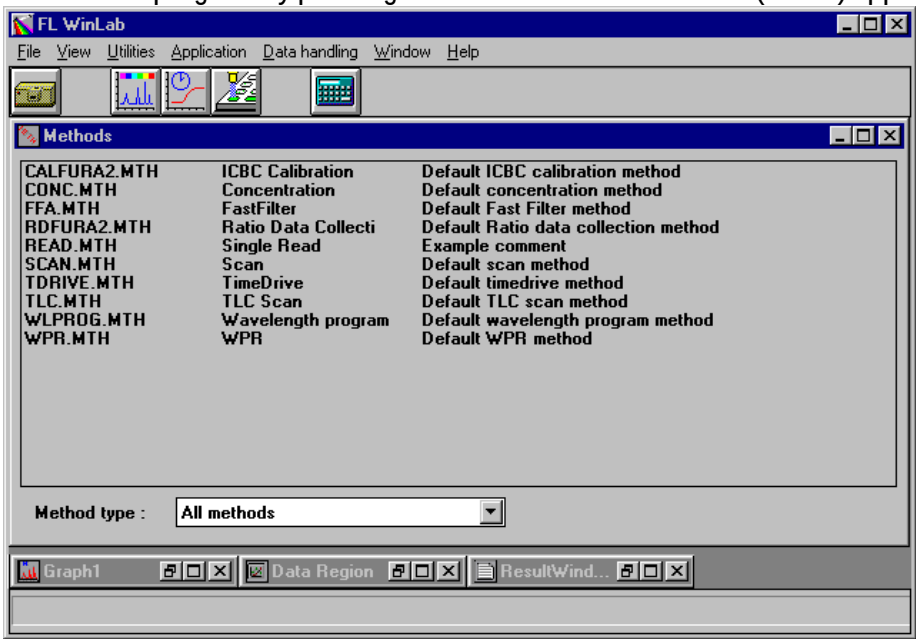
SOP for Perkin Elmer Luminescence Spectrometer LS45

Turning ON the Instrument:
The LS45 luminescence spectrometer has a toggle button on the left side towards the back of the instrument. Turn on before starting software.

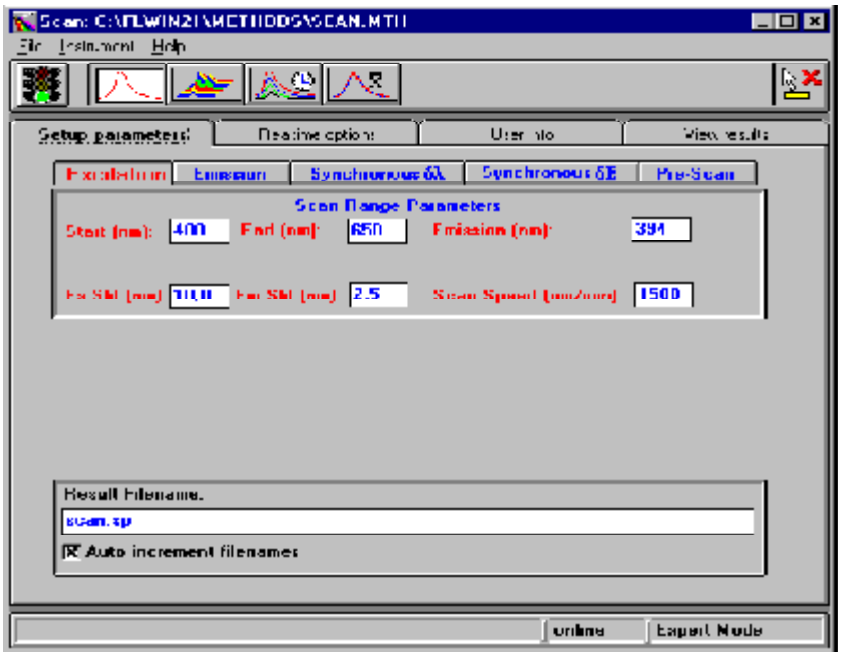
Running the Instrument:

Open the FL-WinLab program by pressing  . The methods window (below) appears:



Data Acquisition: Obtaining the excitation and emission spectra

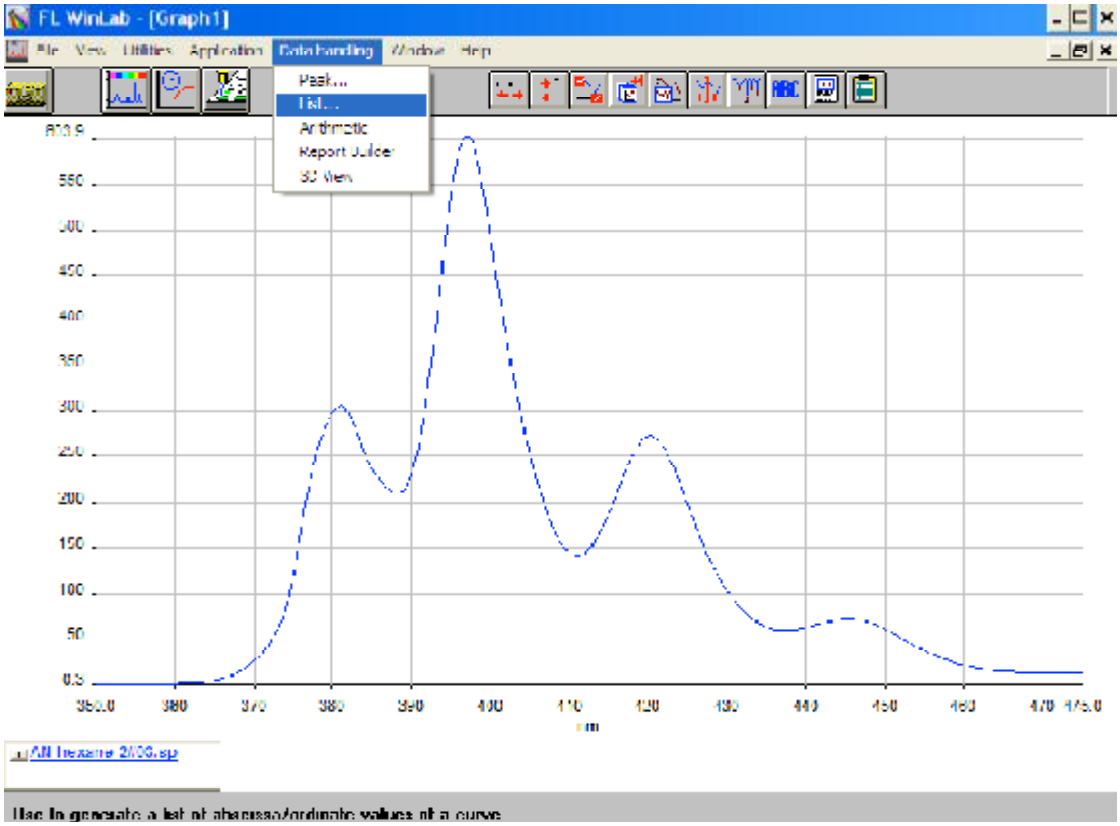
1. In the menu of the "Methods" window select [Application] then [Scan]. A new "Scan" dialogue box opens:



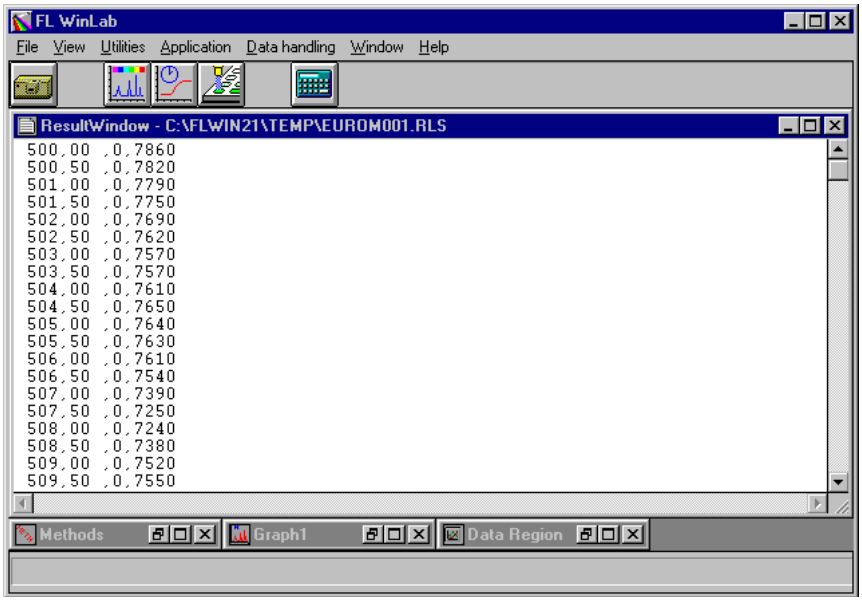
2. Select the scan type (Emission), insert emission start and end wavelength and use the absorption wavelength of your compound (from the spectrum obtained using a UV-Vis spectrophotometer) as your initial excitation wavelength.
3. Name your file in the result filename section.
4. Press the start/stop button  .
5. The instrument will now collect the emission spectrum. After the collection of the spectrum, the start/stop button lights green again and a graph is displayed.
6. Use the icons  to format the graph as per your needs.

Data Handling of Graphs:

1. Go to the graph options at the bottom of the results page showing the graph. Enlarge the graph icon and from file recall your saved file:



2. Go to data handling and select list. A window or two will appear to define the start and end wavelength, insert appropriate values as per your needs, then a list window is shown:



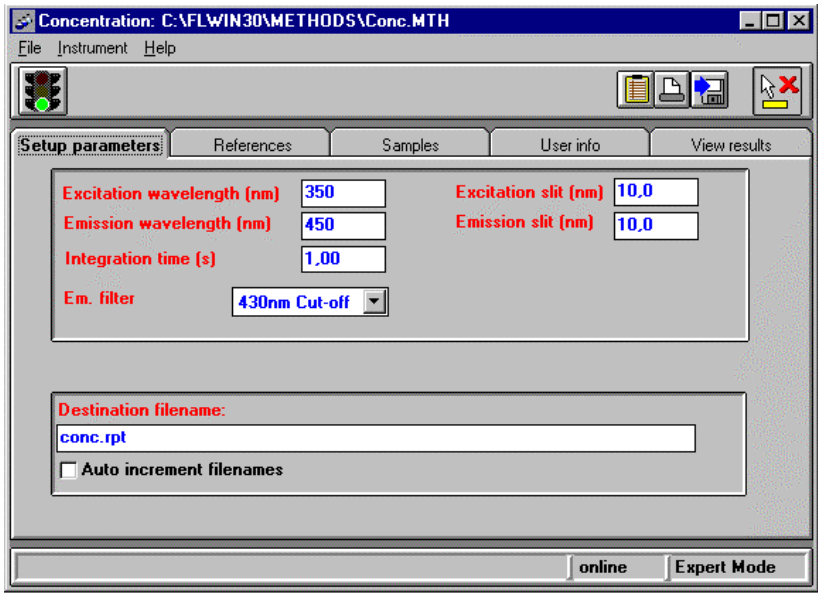
3. Select the data and copy it, then open an excel file and paste it. Now save your data as an excel file for further manipulation.

Measuring the Excitation Spectrum:

Now you know your emission wavelength so repeat all steps used for determination of the emission spectrum as described above and do the same for the excitation spectrum.

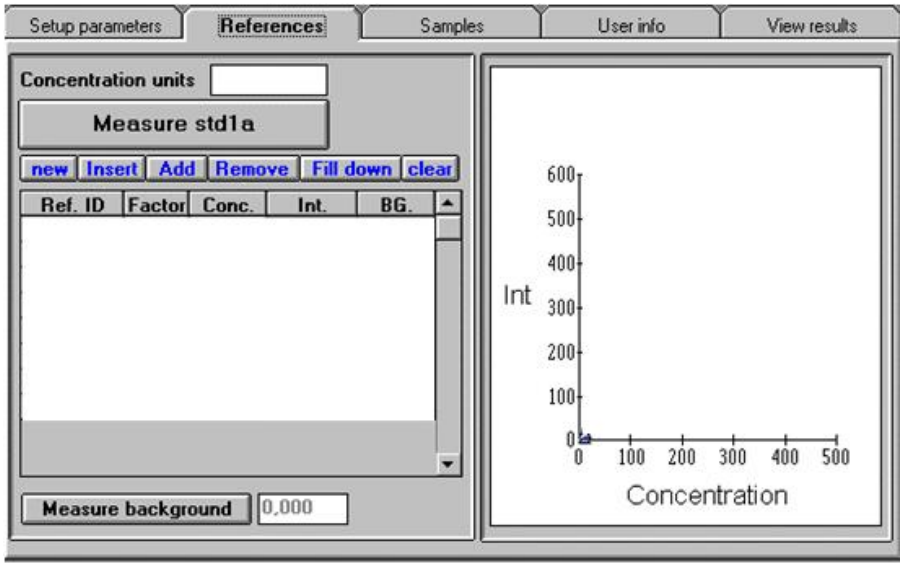
Calibration Curves and Determination of Unknowns:

1. From the main menu click on applications then CONCENTRATION, a window like this will appear:



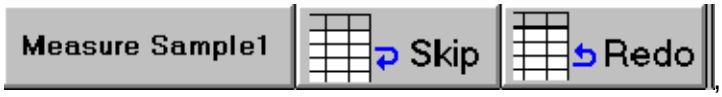
2. Follow the icons in sequence, first setup parameters allow you to insert the excitation and emission wavelength, slit widths, integration time, as will as emission filter. Do not use a cutoff filter. Finally name your file.
3. Now go to the second section and insert your references (standards) that will be used to construct the calibration curve. A window like the following will appear:

In the figure region select zero intercept if you are performing simple calibration, or intercept if you are using the method of standard addition.



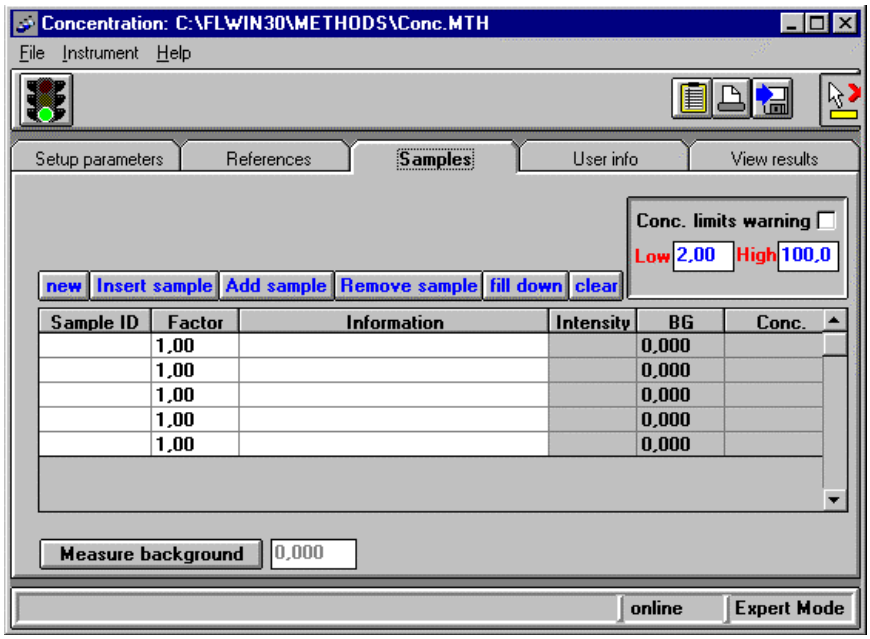
4. Use the add icon to add a reference, you name the reference and can add as many references (standards) as you like. Also in the concentration field insert the concentration of each reference.
5. Make sure to use your blank as your first reference, with concentration equals zero.
6. Now place the blank in the sample cell and click measure background. This value will be subtracted from all reference measurements.
7. Start your measurement sequence by placing your first reference in the sample cell and the cursor at the box of the first standard (assume a name std1a). You will see an icon as in the above figure saying measure std1a. click this icon, the instrument will measure the fluorescence of this first solution and place a point on the graph to the write.
8. Place the cursor in the box showing the name of the second reference, an icon reading measure (standard name) will appear. Do the same as in previous step.
9. Repeat for all standards. You will get the values of fluorescence intensity and a right graph with the linear least squares equation and the correlation coefficient.

Measuring your Unknowns:
Now you have your calibration curve ready. In order to measure your unknowns, do the following:

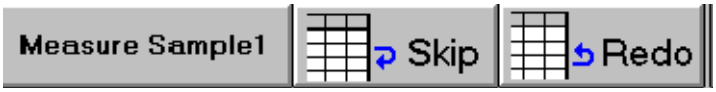
1. In the same window, click on SAMPLES, a window will be displayed:
2. Insert the concentration limits as per your standards in the calibration plot.
3. Add as many samples as you like, and name your samples under sample ID.
4. Place the blank in the sample cell and click measure background. This value will be subtracted from all sample measurements
5. Place the first sample in the sample cell and place the cursor in the first box under sample ID, then hit the start/stop button  . The instrument will give you the following icons 

, press

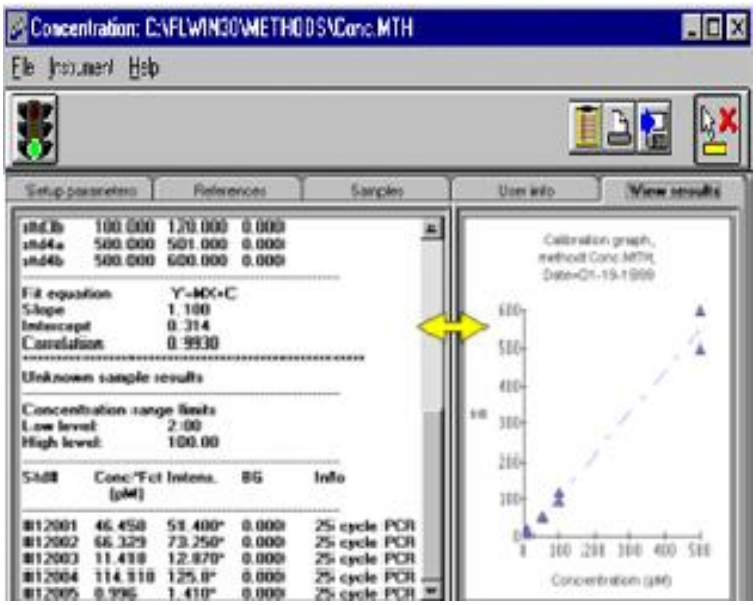
measure sample 1 to measure the fluorescence intensity of the first sample and also use the calibration plot to calculate its concentration.



6. Now the instrument prompts you that it is ready to measure sample number 2.
7. If you want to skip a sample or redo another you just press the appropriate icon



8. Repeat step 5 for all samples.
9. Now go to the user info icon and insert your info, etc.
10. The final step is to go to the result icon and click it to get a summary of all your work, including results for references, results for your samples, the calibration plot, the linear regression equation, the correlation coefficient, as well as general info. Now if the icon  is hit, the report will be copied to clipboard and can thus be pasted into a MS word document.


Concentration: C:\FL\WIN30\METHODS\Conc.MTH
File Instrument Help
Setup parameters
References
Samples
User info
View results
std.3b 100.000 120.000 0.000
std4a 500.000 501.000 0.000
std4b 500.000 600.000 0.000
Fit equation Y=MX+C
Slope 1.100
Intercept 0.314
Correlation 0.9930
Unknown sample results
Concentration range limits
Low level: 2.00
High level: 100.00
Stdll Conc.*Fct (pM) Intens. BG Info
#12001 46.450 51.400* 0.000 25 cycle PCR
#12002 66.329 73.250* 0.000 25 cycle PCR
#12003 11.410 12.870* 0.000 25 cycle PCR
#12004 114.910 125.0* 0.000 25 cycle PCR
#12005 0.996 1.410* 0.000 25 cycle PCR
Calibration graph,
method Conc.MTH,
Date=01-19-1999
Intens
Concentration (pM)