Perkin Elmer Luminescence Spectrometer LS50B FL-WinLab Instruction Manual V3 is saved on desktop in .pdf format as well as this SOP

Computer: Username: rafail Password: magnit

## **Turning ON the Instrument:**

The LS50B luminescence spectrometer has a toggle button on the left side towards the back of the instrument. Turn on before starting software.

The Username and PW for the spectrometer computer will be given on a need to know basis by asking the Chemistry dept. secretary.

## **Running the Instrument:**

Login to the computer and open the FL-WinLab program

The LS-50B spectrometer has a 4-position stirred cell changer and requires 1.0 cm path length cells. Unlike conventional UV-VIS instruments, the LS50B requires that cells be transparent on all 4 sides.

To view the Instrument status and also access the sampling tray, in the task bar, choose [Application] and then click [Status]. A new window will show that status of the current system.

The page displays a schematic of the LS-50B optical system with icons representing the individual components. The current status of the instrument is described by the text next to the icons. The instrument type, the firmware revision and the serial number of the instrument are also displayed. The colors of the beams between each icon in the optical schematic are an approximate indication of the wavelength. In Expert Mode the setup of the instrument can be modified by clicking on the related icon.





Sample Tray is accessed by choosing the "Sampling accessory" feature under XXX

## **Data Acquisition:**

In the menu of the "Methods" window select [Application]  $\rightarrow$  [Scan]. A new "Scan" dialogue box opens

1. In the FL WinLab window open the **Application** menu: and click on scan:

2. Select the scan mode (single, 3D, kinetic, accumulation) from the large toolbar icons.

3. Select the scan type (Excitation, Emission, Synchronous or Prescan), enter the parameters on each page of the application. Use the tabs to move between pages.

	CLECAN NTH		
File Instrument Help	JUST CAN BE THE		
Setup parameters Rea	altime options	User info	View results
Excitation Emission	Synchronous δλ	Synchronous &E	Pre-Scan
Start (nm): 400 End (	Scan Range Param nm): 650 Emi	eters ission (nm):	394
Ex Slit (nm): 10,0 Em Sli	it (nm): <mark>2,5 Sca</mark>	an Speed (nm/min):	1500
Result Filename:			
scan.sp			
🗙 Auto increment filename	<b>3</b> 5		
•			
		online	Expert Mode

The Scan dialogue box allows the user to define the parameters (by clicking "Setup Parameters" tab) of the scan as well as view the results (by clicking "View Results" tab) of a scan.

For an emission scan click on the "Emission" button and enter the following

parameters:

Start: 340 End: 480 Emission: 388 Ex Slit: 5 Em Slit: 2.5 Scan Speed: 50 Filename:



4. Click on the green traffic light (**Start / Stop** button) to start.

5. Use the icons in the toolbar to format the graphic display.

6. To exit the application, open the **File** menu and click on **Exit**.

Upon completion of the scan make note of the name assigned to the spectrum, found at the bottom of the "View Results" tab.



Dataset Status Information; Click on the grey button next to a dataset's name:

Status Information	×
Name : C:\FLW21\DA1	AVCALCIUM.TD
Created : 11.02.93	
Analyst :	
Description EX 340 EM CLR CALIBR/ DATA	ATION NUMERATOR
Abscissa : SEC	Ordinate : [CA++]
Start : 0,00	Max : 416,46
End : 205,70	Min : 32,89
Interval 0,10	Points : 2058
Instrument	<u>C</u> lose

Background Spectrum: Double click on the Background spectrum box in Realtime Options:

Response Auto width (nm): 7.5		
X Auto Background Subtract		
R Auto Background Subtract Background Spectrum : subtract.sp	P	
X Auto Background Subtract Background Spectrum : subtract.sp Graph Oxforate label	Auto-clear ourves	
R Auto Background Subtract Background Spectrum : subtract.sp Graph Ordinate label	Auto-clear ourves	]

a window will appear for selection of the background spectrum. Double click on the required File name in the file name box or enter the required file name and click the OK button. If the data type or wavelength range of the file in the background spectrum textbox is incompatible with the desired scan range an appropriate error message will appear on **starting** the scan. The name of the background spectrum is stored in the header of the Data et in the "FL MEMO" field

Data et in the "FL MEMO" field.

#### **Realtime Options:**

**Analyst**-This textbox contains the name of the current analyst. The name is automatically taken from the benchtop's configuration dialog, but can be altered for documentation purposes. The name is saved in the dataset header.

**Sample info**-The information entered in this textbox is stored in the dataset header. The first line of the information is stored in the "comment" field of the header, which is displayed in all graph windows. The complete information is stored in the "FL memo.

setup parameters	Realtime options	User info	View results
Analyst: S	RH		
Sample info:			
NADH Standard, conv	c. = 50nM		
Method			
Method last modified: 07.05	5.90 by:		locked 🗖
Method last modified: 07.05 Comments:	5.90 by:		locked 🗖
Method last modified: 07.05 Connents: Default scan netho	5.90 by:		locked (***
Nethod last modified: 07.05 Comments: Default scan metho	5.90 by: od		locked [

### **Processing data:**

Used to show and edit spectral curves via the View menu commands. The graph window Graph1 appears automatically when the benchtop is started. To display another graph window, use the New Graph window command in the View menu.



In order to process fluorescence spectra in Excel format, click on the FL WinLab [Graph] tab and in the task bar choose [Data handling] then [List...]. A list of wavelengths verses intensity is given. These can be copied and pasted in Excel to make your own spectra.

## **Plotting Options:**

Auto-expansion of X-axis 🕶	Auto-expansion of Y-axis
<b>Format graph ranges</b> Click on this bu abscissa and ordinate ranges:	tton to open a window for the manual formatting of

TLC Scan	X
100	
0	100
€ОК	Cancel

**Copy to clipboard** Click on the button to transfer a bitmap of the graph window into the windows clipboard for copying into word processors, graphics packages or spreadsheets for report generation.

*Label Peaks* Use to label the maxima and minima of the selected curves in the active Graph window. While zooming repeatedly, the labels may shift but always show the correct values.

Find peaks				×
Dataset :	C:\FLWIN30\	DATA\1ANTMO	01.SP	
Threshold	i : 0.10			
Start way	relength :	370.00 nm		🗸 ок
End wave	elength :	460,00 nm		Cancel
Label :	C Peak	C Base	@ Both	7 Help
Display :	· Abscissa	C Ordinate	CBoth	
Display :	Abscissa	C Ordinate	C Both	Y Help

**Threshold:** Defines the sensitivity of peak detection: If a peak height is less than the threshold, it is not identified. The threshold is the difference in ordinate units between the peak and the bases on either side of it.

**Results window** is a part of the application window. It appears automatically when you start the List or Peak command. The window displays numerical results in a table. You can copy these results to other programs via the MS Windows clipboard: Select the text you want to copy and in the File menu, select Copy to Clipboard.

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File	⊻iew	<u>U</u> tilities	Application	<u>D</u> ata handling	<u>W</u> indow	<u>H</u> elp		
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🗎 R	esult\	√indow	- C:\FLWIN	21\TEMP\EU	ROM001	.RLS		_ 🗆 🗡
501 502 502 502 502 502 502 502 502 502 502	0,00 0,50 1,50 2,50 2,50 3,50 4,00 5,50 6,00 6,50 7,50 6,50 7,50 8,50 8,50 9,50	,0,78 ,0,78 ,0,77 ,0,76 ,0,76 ,0,76 ,0,76 ,0,76 ,0,76 ,0,76 ,0,76 ,0,76 ,0,76 ,0,76 ,0,76 ,0,76 ,0,76 ,0,76 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77 ,0,77,	60 20 90 50 20 20 70 10 50 40 30 40 90 50 40 20 50 40 50 50					
				7				
<b>*</b> 4 H	ethod	s		Graph1	80	X Data Region	BOX	

# **Procedure for Saving Data:**

In the "FL WinLab" window select [File]  $\rightarrow$  [open], and then select your spectra to be saved and click "OK".

This opens the spectra in a new window titled "Graph 1" and now the data can be saved as a text file:

- a) Click File $\rightarrow$ Save As $\rightarrow$ select file to be saved.
- b) Change the file extension to ".txt".
- c) Change file type to "ASCII".
- d) Select "OK".

ve SaveAs		
Filename :	Directory :	
1ANTM001.SP	c:\flwin30'	\data 🥢
scan001.sp3 1antm001.sp 1antx001.sp 1napm001.sp 1napx001.sp eurom001.sp fura2cd.td	▲ [] [-a-] [-c-] [-d-]	
fura2da.td fura2dd.td fura2dd.td fura2dn.td	•	
Filetype :	Curve info	:
BINARY	Block 1 - A	Anthracene emission
Data manager JCAMP ASCII	▲ ▼	

The files can then be found in c:  $\rightarrow$  FL WinLab  $\rightarrow$  Data.