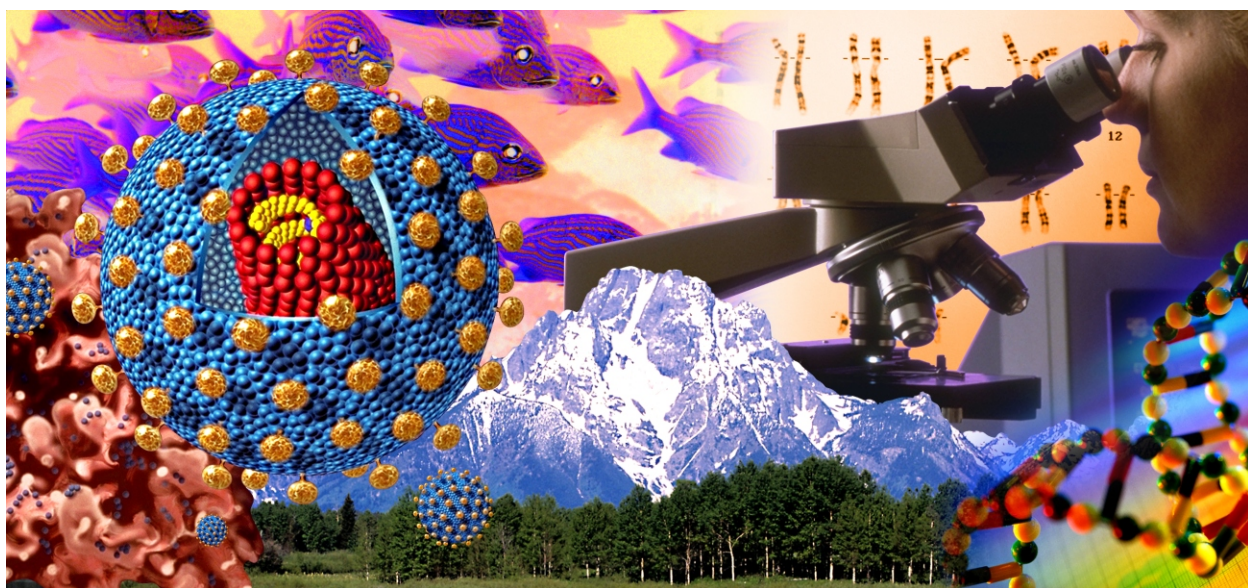


FL WINLAB SOFTWARE



User's Guide

Release History

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Contents

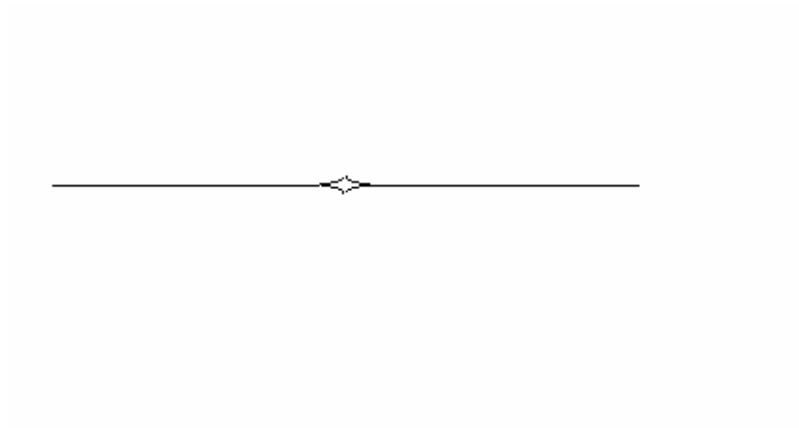
Contents	3
Introduction	9
Conventions used in this Manual	11
About This Manual	12
Use of FL WinLab with the LS 45 and LS55	14
Help Functions	15
Installing FL WinLab Software	17
Working with FL WinLab	18
Starting FL WinLab	18
The FL WinLab Software	18
Exiting FL WinLab	22
Macro Commands	23
Available macro commands	24
Application Quick Summary	26
Configuring FL WinLab	29
Configuring the software	31
Creating a configuration	31
Customizing the Toolbar	34
Instrument Status	37
Status Application	39
Starting the Application	39
Selecting Instrument Settings	41
Source options	42
Excitation and Emission Monochromators	47
Excitation and Emission Filter Wheels	49
Sampling Accessories	54
Detector Parameters	61
Using Application Methods	63
Application Methods	65
GLP/Expert mode	65
Working with Application Methods	67
Opening an application method	67
Printing an application method	67
Exiting an application	67
Saving an application method	68
Starting/Stopping Data Collection	69
Start/stop options available when setting up application methods	70
Ways of Viewing Data	72
Viewing data on-screen within an application	72
Viewing data off-line within the FL WinLab Software	72

Viewing data on-screen within the FL WinLab Software	72
Generic Viewing Features	74
Application Toolbars	74
Displaying graphs	75
Selecting curves on a graph	75
Formatting the graph	76
Adding text to a graph	77
Using Cursors	78
Labelling Peaks	78
Viewing multiple curves.....	79
Zooming into a Graph	79
Viewing Dataset Status Information.....	80
Copying to Report Builder	81
Viewing options available when setting up application methods.....	82
Saving Results and Curves	83
Results Save As Dialog	83
Curve Save As Dialog	83
Arithmetic Application.....	85
Arithmetic	87
Area	89
Arithmetic Spectrum	91
Arithmetic Value	93
Average and Standard Deviation Spectrum.....	96
Average and Standard Deviation Value	98
Build Spectra	100
Calculate Height.....	103
Convert X	105
Convert Y	106
Derivative	109
Interpolate.....	111
Merge.....	113
Normalize	115
Reflection Correction	117
Selection	119
Slope.....	122
Smooth.....	124
Film Thickness	126
Result Template.....	129
Single Read Application	135
Introduction	137
Using the Application.....	138
Setting the Parameters	139

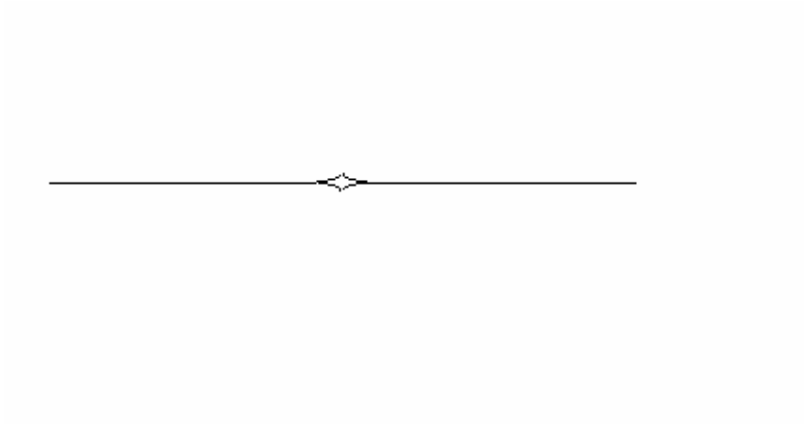
Setup Parameters tab	139
User Info tab	145
Scan Application	147
Introduction	149
Single scan mode	149
3-D scan mode	150
Kinetic scan mode	150
Accumulation scan mode	151
Performing a Pre-Scan	152
Background	152
Functional description	153
Using the Application	155
Setting the Parameters	156
Setup parameters tab	156
Realtime options tab	165
User info tab	166
View results tab	168
TimeDrive Application	169
Introduction	171
Using the Application	172
Setting the Parameters	173
Setup Parameters tab	173
Realtime options tab	175
User info tab	177
View results tab	178
Wavelength Program Application	179
Introduction	181
Using the Application	182
Setting the Parameters	183
Setup parameters tab	183
Realtime options tab	186
User info tab	187
View results tab	188
The ICBC Calibration Application	189
Introduction	191
Using the Application	192
Setting the Parameters	194
Autofluorescence tab	194
Calibration tab	196
Concentration Application	205
Introduction	207
Using the Application	208

Setting the Parameters	210
Setup parameters tab.....	210
References tab	213
Samples tab.....	216
User info tab	217
View results tab	219
TLC Scan Application.....	221
Introduction	223
Using the Application.....	224
Setting the Parameters	225
Setup parameters tab.....	225
Realtime options tab.....	227
User info tab	228
View results tab	229
Plate Reader Application	231
Introduction	233
Using the Application.....	234
Viewing the intensity in the well before starting a run.....	234
Setting the Parameters	236
Setup parameters tab.....	236
Setup plate tab	239
User info tab	243
View results tab	245
Defining a Kinetic Run.....	246
External Device Control.....	247
Defining the Read Pattern.....	249
Random Pattern	249
Filled Block Read Pattern.....	250
Aligning a Plate Format.....	251
Fast Filter Data Collection Application	253
Introduction	255
Using the Application.....	257
Setting the Parameters	258
Setup parameters tab.....	258
Realtime options tab.....	263
User info tab	265
View results tab	266
Ratio Data Application.....	267
Introduction	269
True Ratio Mode	270
Quick Ratio Mode	271
Determining the Isobestic point.....	272

Using the Application	273
Setting the Parameters	274
Setup parameters tab.....	274
Realtime options tab	277
User info tab	278
View results tab	279
The Validation Application.....	281
Introduction	283
Using the Application	284
Functional description	286
Sensitivity	286
Wavelength accuracy.....	287
Acceptance criteria	287



Introduction **1**



Conventions used in this Manual

Normal text is used to provide information and instructions.

Bold text refers to text that is displayed on the screen.

UPPERCASE text, for example ENTER or ALT, refers to keys on the PC keyboard. '+' is used to show that you have to press two keys at the same time, for example, ALT+F.

All eight digit numbers are PerkinElmer part numbers unless stated otherwise.

NOTE: *A note indicates additional, significant information that is provided with some procedures.*

About This Manual

The FL WinLab Software is an extensive and easy to operate software package for luminescence spectroscopy (fluorescence, phosphorescence and bioluminescence).

FL WinLab is operated under the MS Windows™ environment. In order to work with FL WinLab a basic knowledge of Windows is necessary. When working with Windows for the first time, you should first review the Windows documentation.

This FL WinLab Software User's Guide describes the installation of FL WinLab and gives an overview of FL WinLab Software and details on how to use the different application programs available. The first four chapters give general information about using FL WinLab Software to setup up your instrument and work with applications:

- Introduction - Installing and starting FL WinLab Software, and an overview of the software.
- Configuring FL WinLab - Setting up multi-user directory structures and configuring the Function keys and the Toolbar.
- Instrument Status - How to set up your instrument with non-application specific parameters.
- Using Application Methods - General details on working with application methods (saving, opening, printing, starting/stopping), a description of the GLP and Expert modes, and details on viewing the data collected.

The rest of this user's guide is dedicated to the individual application packages:

- The Arithmetic Application - Data manipulation tools for spectral manipulation or data conversion, comparison and reduction.
- The Single Read Application - A simple data collection package for intensity, concentration, polarization, or anisotropy measurements at fixed wavelengths.
- The Scan Application - A data collection package that enables you to collect single scans, 3D scans, kinetic scans and accumulation scans of luminescence measurements (fluorescence, phosphorescence and bioluminescence).

- The TimeDrive Application - A data collection package that enables you to collect time-dependent luminescence measurements (fluorescence, phosphorescence and bioluminescence) at fixed wavelengths with defined intervals over a specified period of time.
- The Wavelength Program Application - A data collection package enabling you to collect time-dependent multiple-channel measurements, which can be programmed for the 4-cellchanger or single position cuvette holders with multiple wavelengths.
- The ICBC Calibration module - A calibration module for converting raw data from a variety of time-dependent data collection applications (Ratio Data Collection, Fast Filter, TimeDrive, Wavelength Program) into [ion] vs. Time.
- The Concentration Application - A method for creating a linear graph from reference standards and then measuring the concentration of unknown samples, for use with standard cuvette holders, the sipper accessory or an external autosampler.
- The TLC Scan Application - A data collection package enabling you to scan continuously over a flat sample using the Plate Reader accessory to produce 2D and 3D datasets.
- The Plate Reader Application - A data collection package enabling you to collect data from a variety of microplate formats, using the Plate Reader Accessory. Data can be collected in end-point or kinetic modes, using a user-defined wavelength program.
- The Fast Filter Data Collection Application - A data collection package enabling you to collect multiple (up to 4) wavelength data channels rapidly using the Fast Filter Accessory.
- The Ratio Data Collection Application - A method for measuring changes in intracellular concentration of free ions in living cells using ion-specific fluorescent probes.
- The Validation Application - Tests the sensitivity (signal-to-noise ratio) and wavelength accuracy of the instrument, and produces a validation report, which can be printed or saved.

Use of FL WinLab with the LS 45 and LS55

FL WinLab can be used to control the LS 45, LS-50B and LS 55 Luminescence Spectrometers. The LS 45 differs in several areas to the LS 50B and LS 55, these being:

Feature	LS 45	LS 50B/55
Excitation and emission slits	Fixed (10nm)	Variable
Emission filter wheel	Not applicable	Standard
Excitation filter wheel	Not applicable	Standard
Polarizers	Not applicable	Optional
Fast Filter accessory	Not applicable	Optional
Plate Reader accessory	Not applicable	Optional
4-position cellchanger	Not applicable	Optional
Biokinetics accessory	Not applicable	Optional
Low Temperature accessory	Not applicable	Optional
Stirring	Not applicable	Optional

Because of this there are aspects of the software that either do not apply, or behave differently when used with an LS 45. The following points must be noted when using an LS 45:

- Wherever a slit width appears in an FL WinLab application, set the parameter entry to **10**.
- The cuvette position column in the Wavelength program application can only be set to **1**.
- The emission filter selection in the concentration application can only be set to **Open**.

NOTE: *Differences that occur within the Status application are described in that chapter, starting on page 39.*

Help Functions

In addition to this manual, FL WinLab contains extensive on-screen help functions.

On-screen Help

Whenever you need on-screen help, simply press the F1 function key on your PC keyboard or use the commands in the Help menu.



Please note that images within the on-screen help contain hotspots: wherever the mouse pointer changes to a hand, simply click with the mouse to obtain additional help on the corresponding parameter or button.

All screen shots displayed in the on-screen help and this manual were recorded from a Windows 95 system at 800x600 SVGA screen resolution, and may look different on other systems.

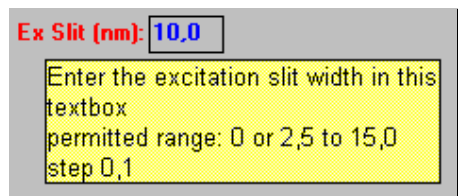
Quick-help

Quick-help is an on-screen help system which is intended to help the first-time user to become familiar with the functions of the screen objects without having to click on them first.

The Quick-help function is activated when an application is started the first time. However, you may switch off the function.

- To do switch off Quick-help, click the Quick-help button, . The Quick-help function is now deactivated and the button changes to show a cross, .
- To switch on the Quick-help function again, click the button again. When the application is closed the current status of the Quick-help button is stored. The next time the application is started this status is restored.
- To view quick-help for a particular parameter, leave the mouse indicator over the object of interest. A yellow textbox will appear containing information on the selected object.

For example, if you move the mouse indicator to the excitation slit textbox the following information will appear:



The first line contains a brief description of what the entry field is used for. The second line describes which values are valid for the entry field.

Installing FL WinLab Software

NOTE: *This procedure MUST be carried out by a Windows Administrator.*

1. Insert your FL WinLab CD.
2. From the Windows Start menu, select **Run**.
The Run dialog is displayed
3. Enter **d:\fl.exe**, where d:\ is the drive letter for your CD and then click **OK**.
4. Follow the instructions on the screen to install the FL WinLab software.
5. When the software is installed, enter **d:\FLInstall2000XP.vbe** in the Run dialog and then click **OK**.

Replace d:\ with the drive letter for your CD.

You should only enter this text if the installation directory is c:\flwinlab. If you selected a different installation directory you must specify this directory as an argument, for example

d:\FLInstall2000XP.vbe f:\FLPrograms

This step sets the permissions on the FLWINLAB folder so that Windows users other than Administrators can use the software.

Working with FL WinLab

Starting FL WinLab

1. Turn on your instrument.

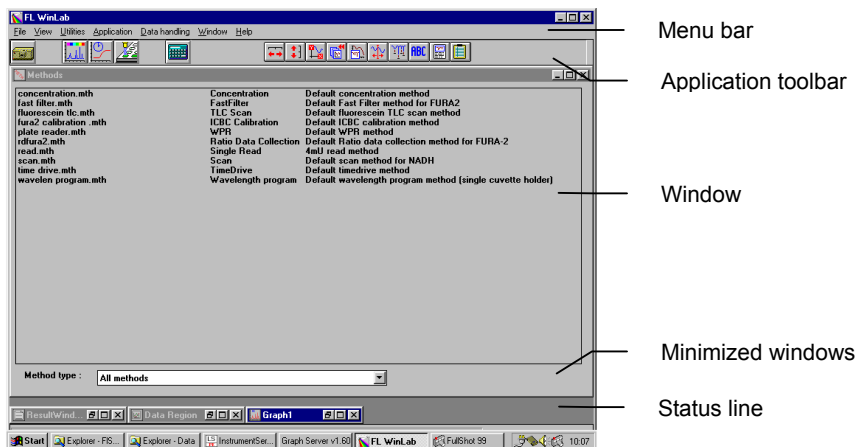
NOTE: Before starting data collection, it is necessary for the instrument to warm up for 15 minutes after switching on.

2. Turn on the PC.
3. From the Windows Start menu, select **FL WinLab** in the **PerkinElmer Applications** group.

The FL WinLab Software screen is displayed.

The FL WinLab Software

From the FL WinLab Software, application programs and specific application methods can be started, data can be edited and data treatment performed.



The Menu Bar

The menu bar contains the menus of FL WinLab. Click on a menu title to open the menu and select a command from the list.

Menu	Contains commands for ...
File	Opening, saving, and printing files and exiting FL WinLab
View	Viewing data
Utilities	Configuring FL WinLab
Application	Starting applications
Data handling	Data manipulation
Window	Handling the windows of the software
Help	Starting the on-screen help

Application Toolbar

The application toolbar contains icons as shortcuts for applications. Click on an icon to start the corresponding application. The application toolbar can be customized, see page 34.

Status Line

Shows the instrument status and a short information text for the selected icon, command or parameter.

Windows

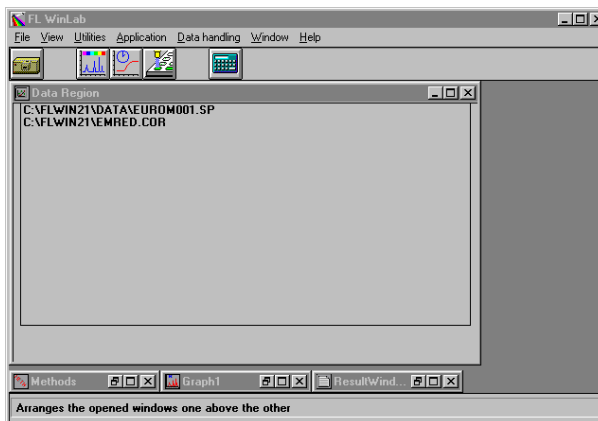
The FL WinLab Software contains several windows: Method window, Data Region window, Graph window and Results window.

NOTE: *These windows can be minimized and re-opened by clicking on the minimized window at the bottom of the software.*

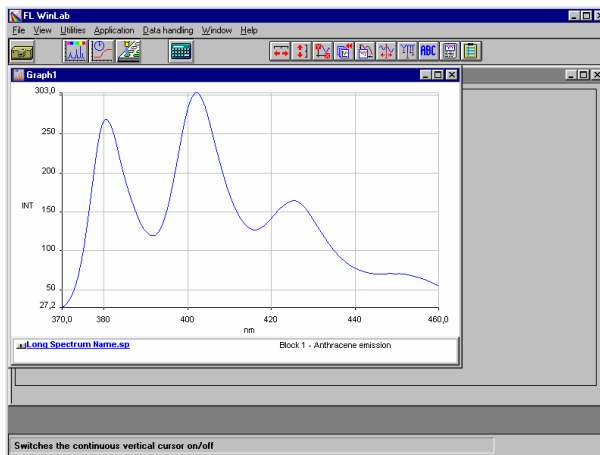
- The Method Window shows a list of all methods of the desired type in the current methods directory on the hard disk. If **All methods** is selected for the **Method type**, the Methods window displays the method name, the method type and the method comment, otherwise only the method name and the method comment are shown. To start a method, double-click on a method filename. Working with application methods is described in detail in *Working with Application Methods* starting on page 67.



- The Data Region Window shows a list of all the files in the data region. The data region is the temporary memory area for spectra, results tables, etc.



- The Graph Window is used to show and edit spectral curves using the View menu commands. The graph window Graph1 appears automatically when the software is started. To display another graph window, select **New Graph Window** from the View menu. Viewing data is described in detail starting on page 72.



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

500.00	0.7860	0.7820
500.50	0.7820	0.7790
501.00	0.7790	0.7750
501.50	0.7750	0.7690
502.00	0.7690	0.7620
502.50	0.7620	0.7570
503.00	0.7570	0.7570
503.50	0.7570	0.7510
504.00	0.7510	0.7650
504.50	0.7650	0.7640
505.00	0.7640	0.7630
505.50	0.7630	0.7610
506.00	0.7610	0.7540
506.50	0.7540	0.7390
507.00	0.7390	0.7250
507.50	0.7250	0.7240
508.00	0.7240	0.7380
508.50	0.7380	0.7520
509.00	0.7520	0.7550
509.50	0.7550	

Exiting FL WinLab

- From the File menu, select **Exit**.

NOTE: *Applications programs are not automatically shut down when you exit FL WinLab Software.*

Macro Commands

The FL WinLab applications support a set of macro commands. The macro commands can be defined in a corresponding ini file (for example, Scan: PEBFScan.ini). All applications support the following macro hooks:

OnAppStart	OnMethodStart
OnAppEnd	OnMethodEnd

To define a macro, the commands must be entered in the relevant ini file (for example PEBFScan.ini) under the appropriate topic. For example, the following macro:

```
[OnAppStart]
Name=Calculator
SHELL:Calc.exe
```

automatically starts the Windows calculator each time the Scan is started. Any **Name** can be used for the macro, but it must be unique for the application (for example, you could not also use Calculator as **Name** for the OnAppEnd macro).

It is also possible to use key words within macros for example:

```
[OnAppStart]
Name=Start Calculator
SHELL:Uvappla.exe
WAIT:2000

[OnMethodEnd]
Name=Calc Area
DDE:uvappla.exe|System|0;1;<ResultFilename>;NO;NO;
OFF;NO;OFF;NO;NO;350;400
```

In this example the arithmetic calculator is started when the scan is started. When a method is finished the calculator is invoked to calculate the area of the dataset. The keyword <ResultFilename> is replaced by the name of the current dataset.

Available macro commands

The following macro commands are currently supported:

Spectrometer

INST:<DIPCommand>

<DIPCommand>=Any valid DIP-command for the spectrometer. To obtain the list of valid DIP commands, open the instrument server by double-clicking on its icon in the task bar. Then select **Print available commands**. Enter the name of the file you want to store the command list in (for example **LamdaCmd.txt**), in the appearing textbox and click **OK**. All available commands are now printed to the given file. The file is an ASCII file and can be opened using any text editor.

Windows Shell

SHELL:<Command>

<Command>=Any valid command for the Windows shell. Typically it has the structure "Exe-Filename CommandLine", for example "c:\Notepad c:\Test.txt" or "Excel text.xls". The second example automatically starts Excel and loads test.xls.

Miscellaneous

MSGBOX:<Text>

Displays the given text in a message box. The execution of the program is interrupted until you click **OK**.

WAIT:<Time>

Waits until the given time has passed, then continues execution of program. <Time> is the time to wait in seconds for example (5 or 3.5).

DDE**DDE:<Server>|<Topic>|<Cmd>**

Sends the command to the given server. For available topics and commands please refer to the manual of the DDE-server. Note that you need to start the DDE server using the shell program, before you can send DDE commands.

All applications

The following key words are currently supported by all applications:

<MethodName>

Contains the name of the currently used method.

<ResultFilename>

Contains the full name of the current result file/dataset.

Application Quick Summary

The following table gives an overview of which accessories and software modules are necessary for several typical applications:

User's application	Application	Calibration module	Accessory	Accy/config.
Kinetics, single cuvette	TimeDrive	Slope by Data Calculator, Enzyme activity*	<i>Biokinetics</i>	<i>Stirrer</i>
Kinetics, stopped-flow	TimeDrive	Slope by Data Calculator	Stopped flow device	
Kinetics, 4 cuvettes simultaneously	Wavelength Program	Slope by Data Calculator, Enzyme activity*	<i>4-cellchanger</i>	<i>Stirrer</i> , Calibration for sensitivity differences
Kinetics, multiple wavelengths, single cuvette	Wavelength Program	Slope by Data Calculator, Enzyme activity*	<i>Biokinetics</i>	<i>Stirrer</i>
Kinetics with 2 wavelengths & 4 cuvettes	Wavelength program	Data calculator, ICBC Calibration	<i>4-cellchanger</i>	<i>Stirrer</i> , Calibration for sensitivity differences
Luminescence kinetics	TimeDrive	Slope/peak area by Data Calculator	<i>Biokinetics</i> , TEM	Luminescence mode, <i>stirrer</i>
Emission correction with tungsten lamp	Scan	Data Calculator	any	Luminescence mode

User's application	Application	Calibration module	Accessory	Accy/config.
Intracellular ion concentration (Medium speed)	Ratio data collection	ICBC calibration	<i>Biokinetics, coverslip accy</i>	<i>Stirrer</i>
Intracellular ion concentration (Fast)	Ratio data collection	ICBC calibration	<i>Biokinetics, coverslip accy</i>	<i>Stirrer</i>
Intracellular ion concentration (Fast filter)	Fast Filter application	ICBC calibration	<i>Biokinetics, coverslip accy</i>	<i>Stirrer</i>
Static polarization measurements	Read		<i>polarizers, biokinetic</i>	<i>Stirrer</i>
Rapid polarization measurements	Fast Filter application		<i>polarizers, biokinetic</i>	<i>Stirrer</i>
Automatic polarization/ anisotropy vs. temperature	Autopole*		<i>polarizers, single stirrer</i>	n/a
Kinetics, plate reader	Well Plate Reader	Kinetic module*	<i>Plate Reader</i>	n/a
3D spectra (EEM)	Scan	3D View	any	
3D kinetic spectra	Scan	3D View	any	
3D assorted spectra	Scan	3D View	any	

User's application	Application	Calibration module	Accessory	Accy/config.
Simple intensity measurement	Read		any cellholder	
Simple quantitation	Concentration		any cellholder, sipper	
3D Scanning TLC plates/gels/flat samples	TLC scan	3D View	<i>Plate reader</i>	n/a
Routine testing of the instrument's performance	Validate instrument		Sealed water cuvette	n/a
Protein unfolding	TimeDrive; Autopole*		<i>Single stirrer</i>	<i>Stirrer</i>
Microsphere measurement	WPRScan*		<i>Plate reader</i>	
Multiple dye D.N.A. quantitation	WPRScan*		<i>Plate reader</i>	

* These applications are available from BioLight Ltd. (www.biolight.com).

NOTE: *Items in italics are not compatible with the LS 45.*

Configuring FL WinLab **2**

Configuring the software

FL WinLab can be configured for different users or different projects.

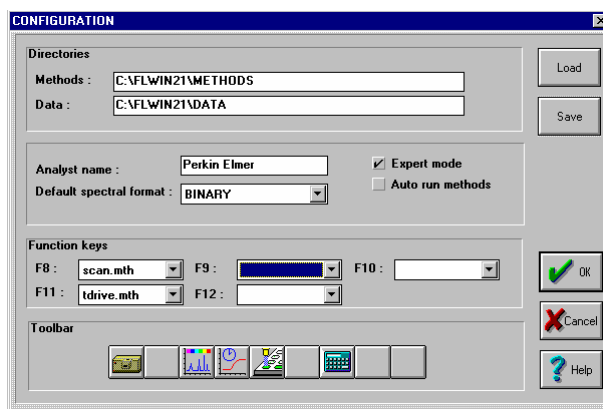
You can set or modify the following parameters: Data and methods directories, analyst name, default data save format, Expert/GLP mode, autorun methods option, the assignment of the function keys F8-F12 and the application toolbar.

NOTE: For each configuration a separate FL WinLab icon can be created by copying the shortcut and changing the command line to include the path to the configuration file.

Creating a configuration

1. On the Utilities menu, select **Configuration**.

The Configuration dialog is displayed.



2. Enter the desired configuration parameters.

Parameter	Description
Methods Directory	<p>The software uses this directory as the default directory to store and retrieve methods.</p> <p>NOTE: <i>The directory must already exist, it will not be created automatically.</i></p>
Data Directory	<p>The software uses this directory as the default directory to store and retrieve data and results.</p> <p>NOTE: <i>The directory must already exist, it will not be created automatically.</i></p>
Analyst name	<p>This name will be used by all software modules (Scan, TimeDrive) and stored in data sets.</p>
Default spectral format	<p>Defines the format of the data files. All data acquisition modules (like Scan or TimeDrive) will save the collected data in this format. Three formats are available:</p> <p>Binary: This is the standard PerkinElmer format. It contains the complete dataset header information set like creation time, analyst and instrument parameters. Since it needs the least disk space, and guarantees storage of collection parameters, we recommend you use this format, especially for acquisition modules with high data throughput like FFA.</p> <p>ASCII: This format is convenient for exporting data to generic programs like Microsoft Excel. The ASCII format contains the complete dataset header information set. The main drawback is the large amount of disk space required.</p> <p>Data Manager: This is the old PerkinElmer FLDM format.</p>

Parameter	Description
Expert mode	<p>If this option is selected all application programs are started in Expert mode. Otherwise they are started in GLP Mode. For more information, see <i>GLP/Expert mode</i> on page 65.</p> <p>NOTE: <i>Application programs do not automatically detect a change in the mode. That is, an application program started in expert mode stays in expert mode until it is terminated and restarted.</i></p>
Auto run methods	<p>If this option is selected, double-clicking on a method name in the method window loads the method into the corresponding application program and then automatically starts the data acquisition. If the option is unselected the method is only loaded, allowing for modifications before the data acquisition is started.</p>
Function Keys	<p>Here methods can be assigned to the function keys F8 to F12, so that when the function key is pressed, the corresponding application program is started. Then the method is loaded and, if Auto run methods is selected, the method is started.</p> <p>NOTE: <i>Starting an application program from the applications toolbar or applications menu does not start data acquisition, independent of the setting of this option.</i></p>
Toolbar	<p>Here the application toolbar can be customized. Click on one of the nine available buttons to start the Toolbar Configuration dialog, as described on page 34.</p>
Load	<p>Click on this button to load a personal configuration from a configuration file.</p>
Save	<p>Click on this button to save the current configuration to a configuration file. These configuration files can be used to start FL WinLab with a personal configuration.</p>

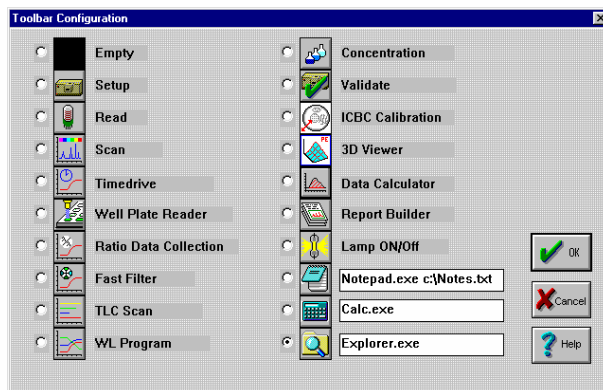
3. Click **Save**.
The Save dialog is displayed.

4. Enter a filename with a **.cfg** file extension and click **OK**.
The configuration is saved as a **.cfg** file.

Customizing the Toolbar

You can customize the Application Toolbar for each user. Proceed as follows:

1. From the Utilities menu, select **Configuration**.
2. Click on one of the **Toolbar** positions.
The Toolbar Configuration dialog is displayed.



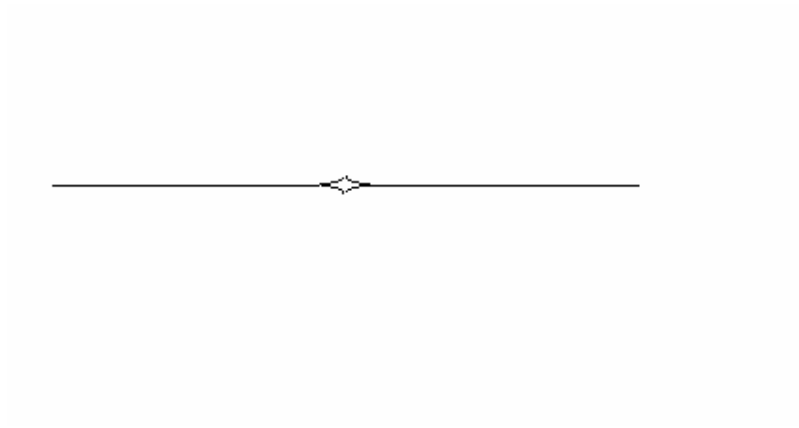
3. Make the changes required:
 - Select **Empty** to clear the selected position of the toolbar.
 - Select one of the standard application icons to assign a standard application to the selected position of the toolbar.
 - Select one of the three icons to assign a user defined application to the selected position of the toolbar.
 - Enter the command line to invoke the application. The first part of the command is interpreted as the name of the application to start. (It may be necessary to add the complete path to the exe file name). The second part (delimited by a blank) is passed as command line to the called application.

For example, the text **c:\programs\notepad.exe DNAdat1.xls** will call Notepad and automatically load the Plate Reader application results file DNAdat1.wpr.

4. Click **OK**.

The Configuration dialog is displayed and shows the required change in the **Toolbar** section.

5. Click **Save** to save the configuration.
6. Click **OK** to leave the Configuration Dialog.



***Instrument
Status*** **3**

Status Application

The instrument Status Application supplements the other application programs, for example Scan and TimeDrive and is used to specify non- application specific instrument parameters, for example the luminescence mode (fluorescence, phosphorescence, bioluminescence), filter, photomultiplier voltage, etc.

The instrument Status Application displays a schematic of the instrument optical system with icons representing the individual components and the current setup of the instrument. This allows for a quick overview of the instrument status.

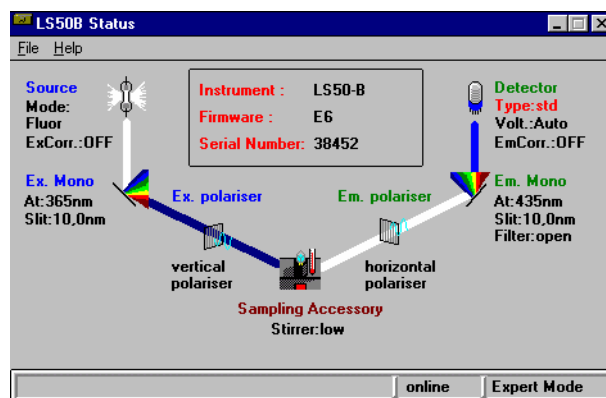
If you are operating your system in Expert mode it is possible to set up instrument parameters by clicking on the icon relating to that part of the optical system. In GLP mode, the instrument Status application is locked, so no parameters can be changed (this is done for method security).

Parameters specified in the instrument Status Application are saved in the method files of the other applications as a configuration section.

Starting the Application

- In the Application menu select **Instrument Status**.

The instrument Status graphic is displayed:



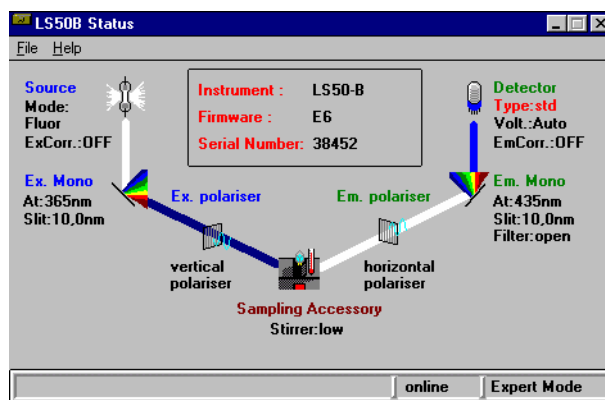
The page displays a schematic of the instrument optical system with icons representing the individual components. The current status of the instrument is described by the text next to the icons. The instrument type, the firmware revision and the serial number of the instrument are also displayed. The 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. Changing the instrument parameters is described in detail in the following chapters.

Selecting Instrument Settings

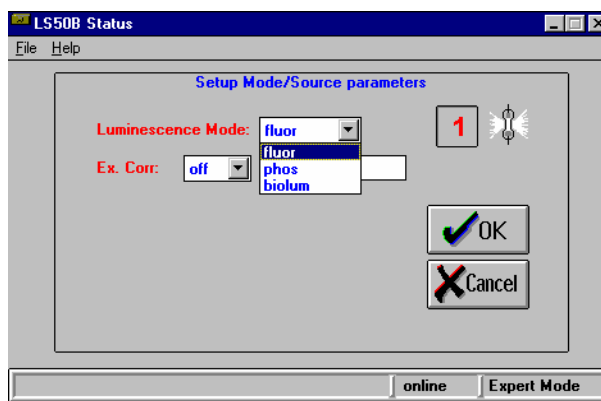
If you are operating your system in Expert Mode, you can use the Status page for setting the instrument parameters, but in GLP mode this option is not available.

1. From the Application menu, select **Instrument Status**.
The Status page is displayed.



2. Click on the part of the optical system you want to change settings for.
A specific configuration dialog is displayed.
3. Make the required changes and then click **OK**.

Source options



Fluorescence

Fluorescence emission is a short-lived process that usually occurs within 10^{-9} to 10^{-7} seconds of light being absorbed by the sample. When operating in the fluorescence mode, two gating periods occur. During the first gating the instrument integrates the excitation and emission photomultiplier signals at the instant of the flash of light. This is followed by a second gating period, which occurs shortly before the next flash and integrates the dark current signal (the signal produced when no light is falling on the photomultiplier). The value obtained from the second gating is subtracted from that obtained from the first gating to produce a number that represents the signal free from dark current contribution and any long-lived luminescence emission.

Phosphorescence

Phosphorescence emission has a longer decay time than fluorescence emission, having a decay time between 10^{-6} s to several seconds after excitation depending on the sample.

During a phosphorescence measurement, the integration time of the photomultiplier signal (Gate Time) begins after a user-defined Delay Time, so that the emission being measured does not coincide with the flash of the source. This short delay (use at least 0.03 ms) means that short-lived fluorescence, scattered light and background fluorescence are ignored.

Each cycle can have more than one flash. This is used to increase the efficiency of excitation if long cycle times are used. If multiple flashes (Flash Count>1) are used, then the time between the flashes is always 1 mains cycle (20 ms for 50 Hz mains supply).

For example, using a cycle time of 200 ms with Flash Count=5 means that the lamp will be pulsed five times very rapidly, followed by 100 ms of measurement window for long-lived phosphorescence species, for example. Note that scanning of spectra is not allowed if the cycle time exceeds 200 ms.

In phosphorescence mode the dark current signal is measured once and subtracted from all of the following sample and reference signals. Whenever the gate time or photomultiplier voltage are changed a new dark current signal should be measured.

Bioluminescence

When the instrument is operating in bioluminescence mode the source is switched off and the signal is measured directly from the sample photomultiplier. There is no ratioing against the reference detector. Since light is emitted from a bioluminescent sample continuously, the setting of the gate time has a direct effect on signal size. For a 200 ms cycle time, a gate time of 180 ms would be used to obtain the best sensitivity. Using the default conditions of cycle time 20 ms and gate time 1 ms will lead to the integration of only 1/20th of light emitted by the sample.

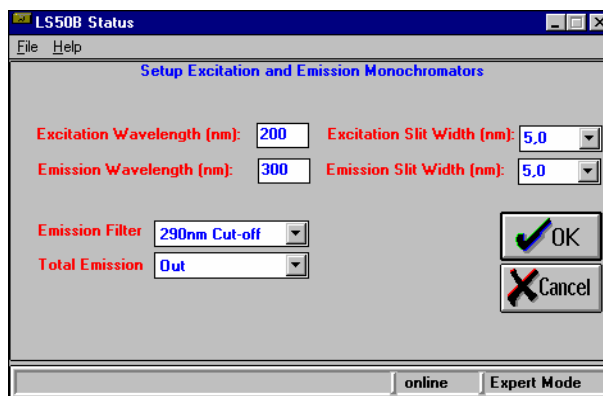
In bioluminescence mode the dark current signal is measured once and subtracted from all of the following sample and reference signals. Whenever the gate time or photomultiplier voltage are changed a new dark current signal should be measured.

Option	Description
Luminescence Mode	<p>The three options are: fluor (fluorescence), phos (phosphorescence) and biolum (bioluminescence), as discussed above.</p> <p>NOTE: <i>The lamp and luminescence mode are set immediately when the related button is clicked. They are not reset when the dialog is exited by clicking Cancel. If phosphorescence or bioluminescence mode is selected, other options (delay time, gate time, flash count, cycle time, and dark current subtraction) become visible.</i></p>
Delay Time	<p>The Delay Time is the time from the beginning of the flash to the beginning of the integration time of the photomultiplier signal. Using a delay time of more than 0.03 ms will ensure that all short-lived fluorescence, background and scattered light will be ignored.</p>
Gate Time	<p>The Gate Time is the time over which the signals from the sample and reference photomultipliers are integrated, and is equivalent to the measurement time window. The width of this window directly controls the signal size.</p>
Flash Count	<p>The Flash Count is the number of flashes in a cycle. To optimize the sample excitation and data collection, it is possible to select up to 10 excitation pulses at the start of a run. The delay and integration times then relate to the start of the last excitation flash. If, for example, a Flash Count of three is entered, with a cycle time of 100 ms, then every 0.1 seconds there will be 3 pulses, 20 ms apart.</p>

Option	Description
Cycle Time	<p>The Cycle Time sets the time between flash cycles and combines the flash count, delay time and integration time. It must be a multiple of 1/Mains frequency (20 ms at 50 Hz, 16.66 ms at 60 Hz) and it must follow the following equation:</p> $cycle\ time \geq (flash\ count \cdot 20\ ms) + delay\ time + integr.\ time - 12.99\ ms$ <p>If the sum of the delay and integration times is greater than 12.99 ms, then the cycle time must be greater than 20 ms to make a longer data collection time possible.</p>
Measure and Set Dark Current	<p>The dark current is the signal produced when no light falls on the photomultiplier. In fluorescence mode the dark current signal is measured automatically for every flash of the lamp. In phosphorescence and bioluminescence modes the dark current signal is measured only once and then subtracted from all subsequent sample and reference signals.</p> <p>Varying the gate time or the photomultiplier voltage will vary the size of the signal that is measured. Whenever the gate time or the photomultiplier voltage are changed a new dark current signal should be measured. Please note that clicking on the Measure and set dark current button automatically sets the delay time, gate time, flash count and cycle time to the values given in the dialog.</p>
Source	<p>Click on this button to turn the source on or off. By turning off the source during long periods of time between runs, the sample is protected from any possible photochemical degradation. The life of the source is also increased.</p>

Option	Description
Excitation Correction	<p>Excitation correction is used to remove instrument artifacts (for example wavelength dependency of the intensity of the source) from an excitation spectrum. This ensures that the shape of an excitation spectrum closely matches that of the absorption spectrum of a sample. FL WinLab offers three options:</p> <p>Off -No excitation correction is performed.</p> <p>On - The instrument automatically produces corrected excitation spectra using an internally stored correction curve generated from a rhodamine 101-quantum counter.</p> <p>File - A corrected excitation spectrum is recorded by the user and applied via software, not applied on-board as in the On option above. Enter the name of the curve to be used for excitation correction in the textbox or double-click on the textbox to select it directly from the FL WinLab directory.</p> <p>NOTE: <i>The spectrum must cover the whole excitation range (200 nm-800 nm), it must be located in the FL WinLab directory (not in the data directory), and that the file extension must be .cor.</i></p> <p>FL WinLab provides an example spectrum, ex.cor. If the excitation correction spectrum was generated using a quantum counter with narrower spectral range than the required full range, then the spectrum must be extrapolated to cover the entire region.</p>

Excitation and Emission Monochromators



Option	Description
Excitation wavelength	Enter the required excitation wavelength, in nm.
Emission wavelength	Enter the required emission wavelength, in nm.
Excitation slit	The excitation slit width is the spectral bandwidth of the excitation monochromator. On an LS-50B or LS 55, you can select the required slit width for the excitation monochromator. On the LS 45, this is fixed.
Emission slit	The emission slit width is the spectral bandwidth of the emission monochromator. On an LS-50B or LS 55, you can select the required slit width for the emission monochromator. On the LS 45, this is fixed
Emission filter wheel	If the instrument has a filter wheel fitted in the emission monochromator, the wheel has five cut-off filters (these transmit light above the stated wavelength and block off light below), a 1% transmission attenuator, and an open and a closed position. The emission filter wheel is not fitted to the LS 45.

Option	Description
Total Emission	<p>This option is only visible if the total emission mirror (TEM) is fitted inside the instrument. The total emission accessory is a plane mirror that can be moved in place of the emission grating and is used to collect the entire spectrum of light from the sample. This increases the sensitivity by up to 20 times and is especially recommended for bioluminescence measurements. You can select the required position of the total emission mirror.</p> <p>The Total Emission accessory is used in combination with the emission filter wheel and large slit widths for optimal sensitivity. When the mirror is in the beam the emission grating is automatically moved out of the way.</p>

<p>CAUTION</p>	<p style="text-align: center;">Danger of damaging the sample photomultiplier</p> <p><i>If you set the total emission mirror into the beam when the emission filter wheel is in the open position, the high intensity of light produced can damage the sample photomultiplier. The software will display a warning message.</i></p> <p><i>For the LS 45, a 50 mm² cut-off filter must be manually inserted into the right-hand sample area filter bracket.</i></p>
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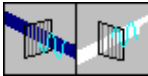

Excitation and Emission Filter Wheels

NOTE: *Not applicable to the LS 45*

Clicking on the icons for the excitation and emission filter wheels enables you to set the parameters for the Ex. Polarizers / Ex. Filter wheels, Fast Filter Accessory and Em. Polarizers / Em. Filter wheels, depending on which are fitted.

The filter wheels are situated in the excitation beam between the excitation monochromator and sample and in the emission beam between the sample and emission monochromator.

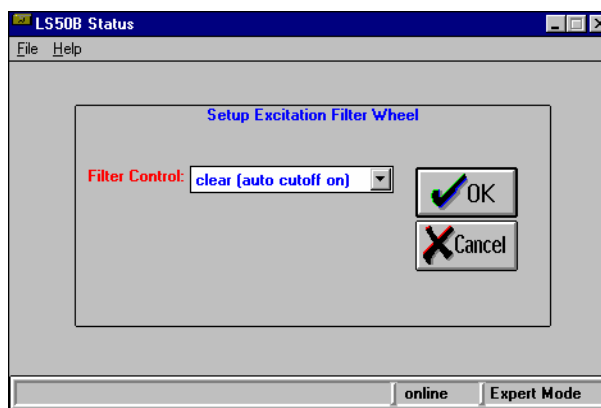
The type of excitation and emission filter wheel icons in the schematic of the instrument optical system depend on which accessory is fitted in the current configuration file/ instrument. There are three possibilities:

 <p>Excitation filter wheel only</p>	<p>The excitation filter wheel has an open and a closed position, two positions for vertical and horizontal polarization filters, three positions for the addition of custom filters, and a reserved position for an automatic 350 nm cut-off filter.</p>
 <p>Excitation and emission polarizers</p>	<p>Fluorescence polarization is used to study the rotational movement of small molecules in solution or suspension. For example the technique is used to measure the binding of coenzymes to proteins, in the study of antigen-antibody reactions, in determining low molecular weight haptens, and to measure cell membrane fluidity.</p>
 <p>Fast filter accessory</p>	<p>The Fast Filter accessory is used for the rapid dynamic measurement of intracellular ion concentrations. Specific fluorescent dyes are used and these chelate with, for example, Ca^{2+} or H^+, to give characteristic excitation and emission spectral shifts.</p> <p>Filters of appropriate wavelength are rapidly rotated in the excitation or emission beam, synchronized with the lamp pulsing, and at 50 Hz ratio measurements can be made every 40 ms.</p>

Fast polarization changes can also be measured with the fast filter accessory by using two filter pairs with vertical and horizontal polarization filters in each of the beams. It is also possible to use the standard emission filter wheel in conjunction with the accessory. The Fast Filter can also be used for normal analytical runs.

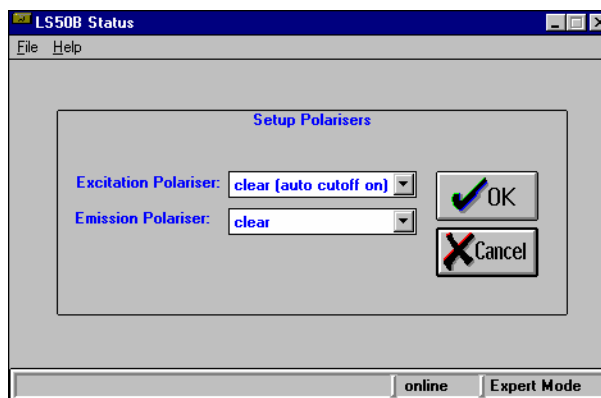
NOTE: *The excitation filter wheel must be physically removed when the excitation fast filter is fitted.*

Excitation Filter Wheel Parameter



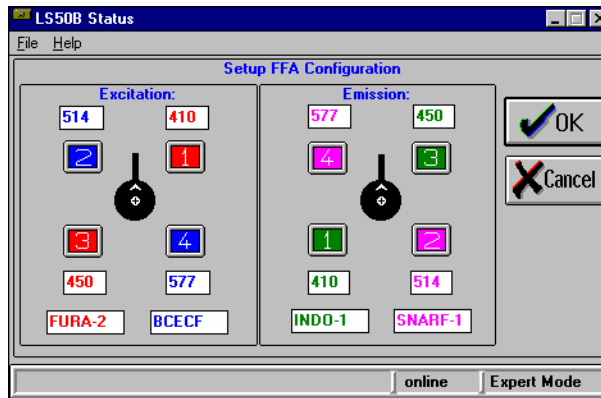
Option	Description
Filter Control	<p>The excitation filter wheel has an open and a closed position, two positions for vertical and horizontal polarization filters, three positions for the addition of custom filters and a reserved position for an automatic 350 nm cut-off filter.</p> <p>The 350 nm cut-off filter is automatically brought into the excitation beam when the excitation monochromator exceeds a wavelength of 410 nm. This eliminates second order light reaching the sample when exciting at long wavelengths with narrow slit widths. For example when exciting at 500 nm, sharp peaks from the source spectrum at around 250 nm are also present as second order artifacts. These can affect the shape of the excitation spectrum, particularly when the second order light at 250 nm is detected by the reference detector but not absorbed by the sample. The names of the filters are determined in the .ini file in the FL WinLab directory.</p>

Polarizer Parameters



Option	Description
Excitation Polarizer	Select one of the filter wheel positions in the list. The names of the filters are determined in the .ini file in the FL WinLab directory.
Emission Polarizer	The filter wheel for the emission polarizer has a vertical and a horizontal polarizer, an open and a closed position and four positions for the addition of custom filters. The names of the filters are determined in the .ini file in the FL WinLab directory.

Fast Filter Accessory (FFA) Parameters



The Setup FFA Configuration dialog is divided into two sections. The left section contains a diagram representing the front view of the excitation fast filter wheel and the right section contains a diagram representing the front view of the emission fast filter wheel.

For each filter pair the wavelengths, positions of the installed filters and name of the probe used in the application can be saved here.

Define the wavelengths for excitation and emission filters in these textboxes. Each fast filter consists of two filter pairs. The filter pairs are color-coded and numbered. The numbers 1,3 and 2,4 correspond to each of the two filter pairs in the excitation and emission beams, respectively. For each filter pair there is a text box in which the name of the appropriate probe can be indicated (for example probe FURA-2 uses filter pair 1,3 in excitation beam with filters of 410 nm and 450 nm).

It is also possible to use the fast filter accessory with vertical and horizontal polarization filters in each of the beams. In this case, enter **V** and **H** in the appropriate boxes, instead of wavelengths.

NOTE: *The entries are used for documentation by other applications. Since they cannot be checked automatically, wrong entries will lead to wrong information in datasets or the use of the wrong filters for data collection.*

NOTE: *The positions of the filters are predefined: for ratio measurements, the top intensity for the ratio (for example, the 410 nm filter for FURA-2 analysis) must be located in position 1 or in position 2. Similarly, the vertical polarizer must be located in position 1 or position 2. Positions 1 and 2 for the excitation fast filter wheel are the two top positions: for the emission fast filter wheel these are the bottom two positions.*

The definitions are stored when you click **OK**.

- To set the Fast Filter to a static position, double-click on one of the position numbers 1-4.
The background of the appropriate wavelength textbox becomes yellow.
- To set the Fast Filter to the clear beam position, that is remove any static filters from the beam and stop a currently running filter wheel, double-click on the circular hub image in the middle.
The backgrounds of all wavelength textboxes become white.







NOTE: *The position of the excitation and emission filter can be set independently.*

NOTE: *The filter positions are actually set when **OK** is clicked.*

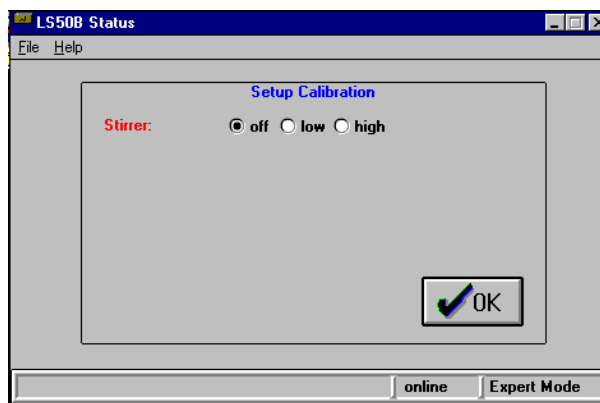
Sampling Accessories

NOTE: *Not applicable to the LS 45.*

The icon displayed in the optical schematic depends on the currently installed accessory. The options are:

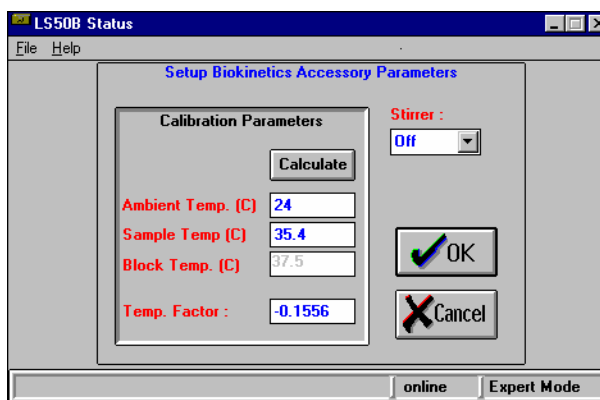
Option	Description
	Single position cell holder (no control functions).
	Single position stirred cell holder.
	Biokinetics accessory.
	4-position stirred cell changer.
	Sipper.
	Well plate reader.

Single Position Stirrer Parameter



Option	Description
Stirrer	Select one of the three stirrer speeds. Select the low stirrer speed for keeping cells in suspension and for biochemical reactions, and high stirrer speed for rapid mixing. NOTE: <i>Selecting a speed immediately sets the stirrer speed in the instrument.</i>

Biokinetics Accessory Parameters



This dialog can be used to determine the temperature calibration factor for the biokinetics accessory.

The temperature sensor can be calibrated so that during a run the temperature of the sample itself is displayed rather than the block temperature. The block temperature is the temperature of the cellholder block in the biokinetics accessory (the maximum readable temperature is 100 °C). The temperature of the sample may be different from the block temperature, but it can be calculated from the block temperature using the temperature factor:

1. Enter the ambient temperature around the cell holder in °C, **Ambient Temp. (C)**.
2. Use the water bath to heat the cuvette(s) to the highest temperature you intend to be used. Then, measure the temperature in the cuvette using a temperature measuring device with small thermal mass.
3. Enter the temperature within the cuvette in °C, **Sample Temp. (C)**.
4. Click **Calculate**.

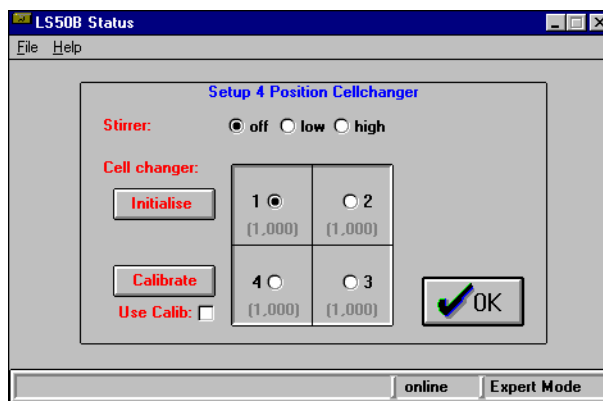
The instrument now measures the block temperature, **Block Temp. (C)**, and uses it to calculate a value for the **Temp. Factor** using the following equation:

$$F = \frac{T_s - T_B}{T_B - T_A}$$

Where: F = Temp. Factor, T_s = Sample Temp., T_B = Block Temp.,
 T_A = Ambient Temperature

NOTE: *If a temperature sensor has already been calibrated, then the previously determined temperature correction factor can be used. Simply enter the **Temp. Factor** determined for your measurement.*

4-Position Stirred Cell Changer Parameters



Option	Description
Stirrer	Select one of the three stirrer speeds. Select the low stirrer speed for cell suspensions and biochemical reactions and high stirrer speed for rapid mixing. NOTE: <i>Selecting a speed immediately sets the stirrer speed in the instrument.</i>
Initialize	Click this button to initialize and set the 4-position cell changer to Position 1.
1, 2, 3, 4	Use to select a cuvette position.
Use Calib	If this checkbox is selected, the calibration factors are automatically used in all other applications where the 4-position cellchanger is used. To indicate that the use of the factors has been activated, the color of the factors changes from gray to black.
Calibrate	Starts a calibration as detailed below

Calibrate is used to start a calibration. This is used to correct for variations in sensitivity between the positions of the cell changer, which can occur if one or more positions become scratched or corroded.

	Intensity	Factor
Pos 1:	<input type="text"/>	<input type="text"/>
Pos 2:	<input type="text"/>	<input type="text"/>
Pos 3:	<input type="text"/>	<input type="text"/>
Pos 4:	<input type="text"/>	<input type="text"/>

Standardize on position: 1

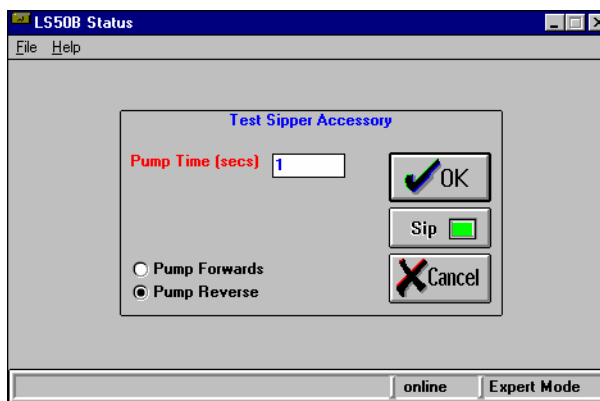
Read position 1

Cancel

1. Place a standard sample in the first cuvette position.
2. Click **Read position 1**.
The **Intensity** is reported for the first cuvette.
3. Repeat this for cuvette positions **2 to 4**.
Each time inserting the standard in exactly the same orientation to the excitation and emission light beams (this is particularly important when using solid standards which may not be homogeneous with respect to distribution of fluorescence within the solid block).
4. When all four cuvette positions have been measured, one cuvette position can be used for standardization. Select the required number in **Standardize on position**.
The factor for each cuvette position is then calculated using the following equation:

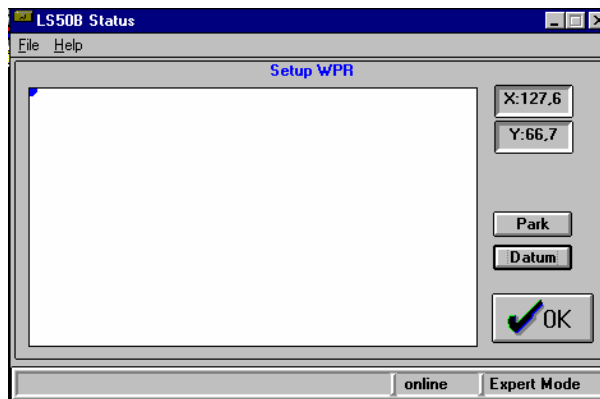
$$Factor(cuvette) = Intensity(cuvette) / Intensity(standard\ cuvette)$$
5. Click **OK**.
The factors are saved to a file and will be used when **Use Calib** is selected.

Sipper Parameters



Option	Description
Pump Time	The pump time is the time (in seconds) that the system pumps to fill the flowcell with the sample.
Pump Forwards	Select the forwards pump direction to pump the sample to the waste.
Pump Reverse	Select the reverse pump direction to return the sample to the tube.
Sip	Click to operate the sipper for the time that was specified for Pump Time . To stop the sipper before the end of the indicated pump time, click Stop .

Well Plate Reader Parameters

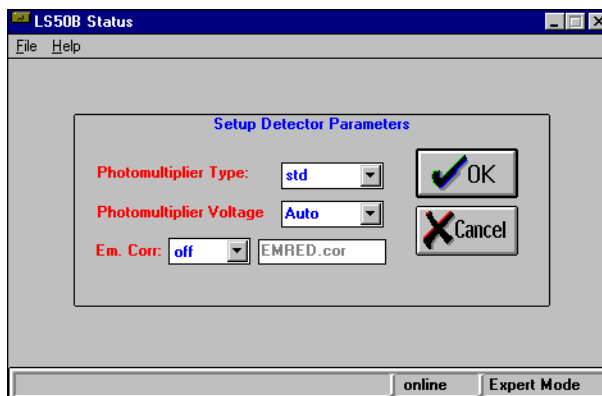


This dialog enables you to set the position of the well plate reader (WPR) to any X,Y position inside the allowed physical range, to send it to **Park** position, or to reset it by clicking **Datum**. It does not support plate formats. To use plate formats use the Well Plate Reader application. However, this function is useful for driving the Plate reader to a specified point on a flat sample where a spectrum or other data type can be collected.

- To move the WPR to a specific position, click on the image of the plate in the required position.
The current **X** and **Y** coordinates of the WPR are displayed in mm.
- Click **Park** to send the plate reader accessory to the park position.
It is recommended that plates are inserted or removed only with the accessory in this position to avoid spillage of plate contents.
- Click **Datum** to reset the plate reader accessory and send it to the 0,0 position.
This should correspond to the extreme corner of the plate nearest the A1 position.

Detector Parameters

Use the Detector icon to select the parameters for the installed detector. Different photomultiplier types are used depending on the spectral range. For long wavelength emissions, that is for wavelengths longer than 650 nm, a red-sensitive photomultiplier should be used, for example, the R928.



Option	Description
Photomultiplier Type	<p>Select the detector to be used. The selected detector model is recognizable by the base color of the photomultiplier icon. A standard photomultiplier has a blue base, a red-sensitive photomultiplier has a green base.</p> <p>NOTE: <i>The photomultiplier must be physically changed. This function is only a graphical comment to remind the user of which detector is fitted.</i></p>
Photomultiplier Voltage	<p>The photomultiplier voltage determines the sensitivity of the measurement.</p> <p>If Auto is selected, the photomultiplier voltage is automatically selected by the instrument and is a function of the slit width of the excitation monochromator.</p>

Option	Description
<p>Emission Correction</p>	<p>Emission correction is used to remove instrument artifacts (for example wavelength dependency of the detector) from an emission spectrum. The emission correction can be used to normalize the spectral characteristics of different instruments or to compare emission spectra from the instrument with different types of instrument, or with spectra in the literature. FL WinLab offers two options:</p> <p>Off - No emission correction is performed</p> <p>File - All emission data are corrected using a user defined correction curve. Enter the name of the curve to be used for emission correction in the textbox or double-click on the textbox to select it directly from the FL WinLab directory.</p> <p>NOTE: <i>The spectrum must cover the whole emission range (200 nm-900 nm), it must be located in the FL WinLab directory (not in the data directory), and the file extension must be .cor.</i></p> <p>FL WinLab provides two example spectra: em.cor for a standard detector and emred.cor for a red-sensitive detector.</p>

***Using Application
Methods*** **4**

Application Methods

Measurements in FL WinLab are made with application methods. A method is a set of parameters that completely define the measurement conditions.

A method is a set of parameters for an application. Since applications like TimeDrive only control part of the instrument parameters (wavelengths, slits, etc.), it can be desirable to send an initial setup (for example, a filter wheel position) to the spectrometer before any measurement is started. Therefore each method contains a separate section with the parameters for the initial setup, besides the section for method specific parameters (for example, the data interval).

FL WinLab can be operated in two modes, which differ mainly in the way this initial setup is handled: GLP mode and Expert mode.

GLP/Expert mode

NOTE: *GLP/Expert mode is selected on the Configuration dialog, see Configuring the software starting on page 31.*

In Expert mode, the parameters of the initial setup section of a method are sent to the instrument each time a method is loaded. Now the Setup program can be used to modify the instrument configuration. When a measurement is started only the method specific parameters are sent to the instrument. This allows the expert user to repeat the measurement by toggling to the Setup program and manually changing the instrument configuration. When the method is eventually saved, the setup section of the method is updated with the current instrument configuration.

In contrast to the Expert mode, it is not possible to modify the initial setup parameters in GLP mode. The Setup program only displays the current instrument configuration, all dialogs are disabled. The instrument is set to the parameters of the initial setup section every time a measurement is started, before the method specific parameters are sent. Since all applications lock the instrument during a data acquisition, so that no other application can send a command to the instrument, it is guaranteed that all measurements are performed with exactly the same instrument setup. If a method is modified and saved under a different name the initial setup section of the original method is copied.

In Expert mode it is possible to lock or unlock methods. If a method is locked, all entry fields and the command **method save** are disabled. The method parameters cannot be modified. Since it is not possible to unlock the method in GLP mode this guarantees a defined measurement.

It is recommended that you generally work in GLP mode and change to Expert mode only if new methods are to be optimized or generated. The example methods, distributed with FL WinLab, contain default setup sections.

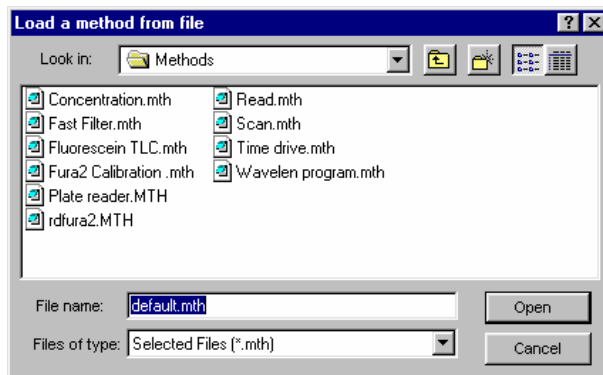
The mode can be set in the instrument Configuration dialog, and can be different for each user. The current mode is displayed in the status bar along the bottom of each FL WinLab application.

NOTE: *The initial setup section does not contain the settings of the monochromators and slits. This avoids unnecessary overhead since these parameters are controlled directly by every application. Furthermore, the setting of certain accessories, like the cuvette changer position and the position of the well plate reader accessory, are not saved since these settings depend on the sample or sampling sequence.*

Working with Application Methods

Opening an application method

- To open a method, select **Method Open** from the File menu.
The method open dialog appears:



- Select the required method, and then click **Open**.
In Expert mode loading a method sets up the parameters for the application (immediately, and only once) and data collection for this method can be started immediately. In GLP mode parameters are not sent immediately to the instrument. Instead, they are sent every time that the method is run.

Printing an application method

- To print out the current method, select **Method Print** from the File menu.
The application will use the default Windows printer settings.

Exiting an application

- To close an application, select **Exit** from the File menu.
If changes have been made to the method and not saved, a warning will be displayed, enabling you to save the changes.

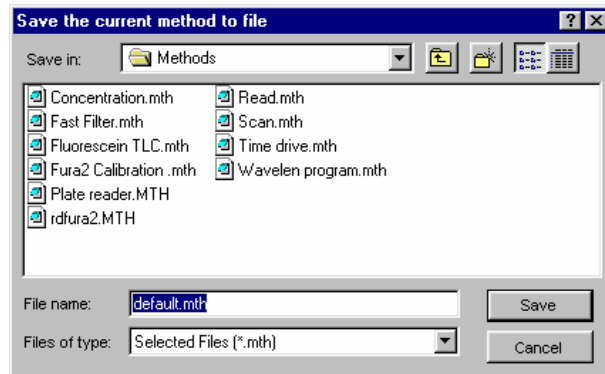
NOTE: You cannot close an application while it is collecting data (traffic light is red) or while it is busy (mouse pointer has the shape of an hourglass). Stop the data acquisition, wait until the traffic light becomes green and then try again.

Saving an application method

When an application method has been created or changed, it must be saved.

1. Select **Method Save** from the File menu.

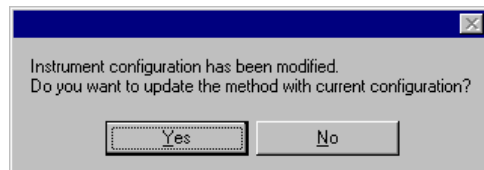
A save dialog is displayed.



2. Enter a new **File name** or select an existing method to over-write it.
The file extension of .mth will be automatically added.

NOTE: *We recommend that you save the method in the standard FL WinLab method directory.*

In Expert mode the application checks if the current instrument setup is the same as the instrument setup stored in the method file. If the instrument setup has changed the following message will appear:






Click **Yes** to update the method with the current instrument setup parameters, click **No** to save the method with the original instrument setup parameters.

In GLP mode the instrument setup parameters cannot be changed.

Starting/Stopping Data Collection

The Start/Stop button has the appearance of a traffic light, which can be red, yellow or green.

Traffic Light	Meaning
 Yellow	<p>This is the status at the start of the application. It indicates that the application is currently initializing. If the instrument is offline, the traffic light stays yellow until the instrument is switched on. When the application is ready to start the data acquisition, the traffic light changes to green.</p>
 Green	<p>When the button is clicked the instrument is setup for the method, the run is started and the acquired data is displayed in the graph window as it is collected. In Expert mode, only the application specific parameters are sent to the instrument (for example slit widths), the non-application specific instrument parameters (like position of emission filter) remain unchanged. In GLP mode all instrument parameters are set up. After data acquisition has been started the traffic light changes to red.</p>
 Red	<p>If the button is clicked the run is stopped and the traffic light goes back to green. When the data collection is stopped, the data obtained up to this point is not lost but saved in the current FL WinLab data directory.</p>

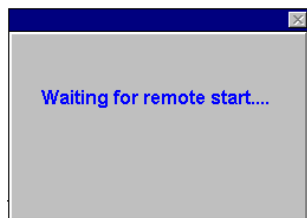
Start/stop options available when setting up application methods

The following options may be available when you set up the application method.

Remote start

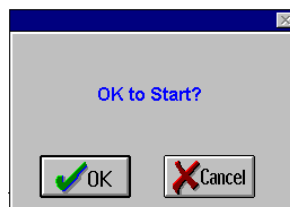
If this option was selected when the method was set up, data collection is not started until a contact closure is sensed between the OVA and Remote Start connections on the rear panel of the instrument.

When you click on the green traffic light and the instrument is set up, the following dialog is displayed until the remote start signal is sent.



Keyboard start

If this option was selected when the method was set up, after clicking on the green traffic light, the dialog below is displayed and data collection will not start until you press ENTER on the keyboard, or click **OK** on the dialog.



Immediate start

If this option was selected when the method was set up, when you click on the green traffic light the instrument is set up and then data collection begins immediately.

Show timed events

If this option was selected when a TimeDrive method was set up, you may, for example mark the addition of a reagent to the sample. A timed event can be marked either by using the PerkinElmer Biokinetics accessory, which has an integrated Event Button, or by contact closure between the OVA and Event Mark connections on the rear panel of the instrument. Once the data acquisition has been started, the data run file with the extension *.TD plus a second file using the same name but with the file extension *.TDE will be displayed in the View results page. This has a constant ordinate value of -5 except for the marked events, which result in a spike for each event.

Ways of Viewing Data

Viewing data on-screen within an application

All applications which display graphical data have a toolbar containing several graphics control buttons. These are all active during data collection (with the exception of the dataset delete button). During data collection, the user can for example rescale axes, zoom, and autoexpand for specific datasets.

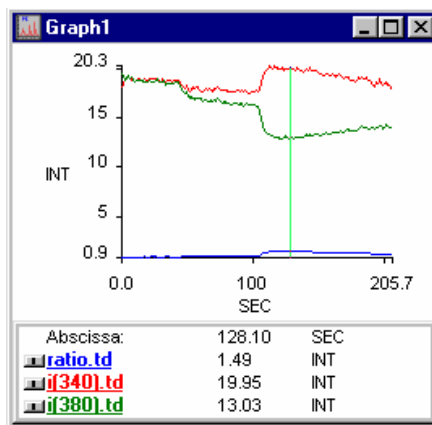
Viewing data off-line within the FL WinLab Software

FL WinLab can be used to view data off-line, for example for the evaluation of previously collected data while an application is collecting new data in the background. There is a set of toolbar buttons that provide rapid access to a range of graphics rescaling and handling functions.

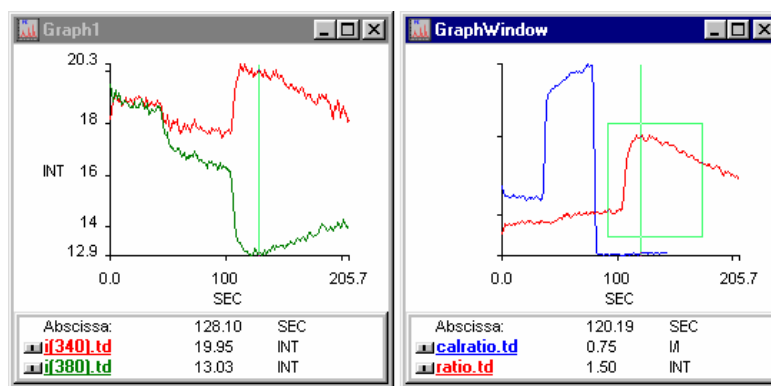
Viewing data on-screen within the FL WinLab Software

In addition to the off-line viewing capabilities of FL WinLab, there is another very useful feature: the user can load copies of data currently being collected into one or more view windows. The significance of this is that current data can be compared to existing data or displayed in separate windows for clarity.

An example of this is intracellular ion analysis, where the application collects two intensity TimeDrives and the ratio of these two in real time. It is important to view all 3 datasets, to ensure that neither intensity is off-scale, and to view the ratio in real-time. With only one view window, it is often not possible to see all datasets clearly, since they have very different ordinate levels, compressing the ratio in comparison to the intensities:



By opening two view windows, the user can view the intensities clearly, and also view the ratio data simultaneously against a previously stored calibration dataset, for example:



This greatly clarifies the presentation of graphical data, and assists in real-time diagnosis of the experiment.







Applications where this feature is particularly significant include:




- Wavelength Program
- Ratio Data Collection
- Fast Filter

Generic Viewing Features

Application Toolbars

The following tools are available for changing views of graphs.

Tool	Description	Use
	Auto-expansion of X-axis	Displays the entire abscissa range of all selected data files. If for example you have selected two TimeDrives of 0-50 and 20-200 second ranges respectively, clicking this tool will set the abscissa range to 0 to 200.
	Auto-expansion of Y-axis	Displays the entire ordinate range of all selected data files. If for example you have selected two TimeDrives of 0-50 and 20-200 ordinate ranges, clicking this tool will set the ordinate range to 0 to 200.
	Format graph ranges	Displays a window for the manual formatting of abscissa and ordinate ranges, as described in <i>Formatting the graph</i> on page 76.
	Select default Y-range	Set the ordinate range to the values defined in ordinate minimum and ordinate maximum of the real-time options page.
	Radar Window	Displays the radar window, which can be used to show any required sections of data in an enlarged format in the window, with the selected range shown in the green section. A new area can be selected by either moving the green section or altering its size by dragging the edges with mouse.
	Vertical cursor	Activates the vertical cursor, which can be continuously moved with the mouse to show abscissa and ordinate values along with the file name in a panel at the bottom of the window. To deactivate the cursor, click on the button again.

Tool	Description	Use
	Delete Curves	Deletes selected curves. This button is deactivated during data collection.
	Print	Prints the content of the current window using the default Windows printer settings.
	Copy to clipboard	Transfers a bitmap of the graph window into the windows clipboard for copying into word processors, graphics packages or spreadsheets for report generation.

Displaying graphs

1. Select **New Graph Window** from the View menu
A new graph window is displayed.
2. Select **Add Curve** from the View menu.
A file selector is displayed, which you can use to add curves from hard disk, floppy disk or data region to the active graph window.
To add a curve from the data region select the curve by clicking on the name.
You can select more than one file from a list by holding the SHIFT or CTRL key, while clicking on filenames.


Selecting curves on a graph

Each curve on the graph is listed in the pane at the bottom of the graph.



Curves with their names bold and underlined are currently selected.

1. To select or deselect a curve, click on it with the left mouse button.
To select more than one curve either hold down the CTRL key and click on each unselected curve you want to select, or hold down the SHIFT key and click on the first and the last curve of a range of curves to be selected.

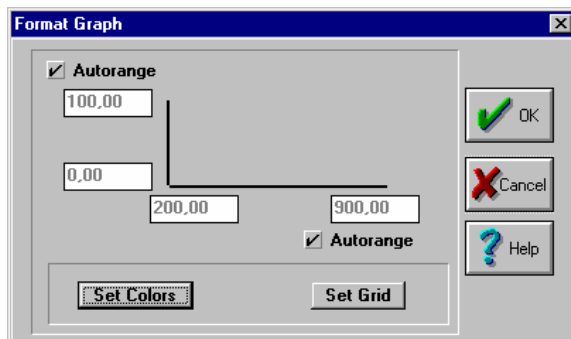
2. To then remove the selected curves from the graph, click .
If all the curves are not on view in the pane, you can increase the size of the pane by clicking on the upper border and holding down the left mouse button while dragging the mouse.
The mouse pointer changes to a two-headed arrow when it is in the correct position for dragging.



Formatting the graph

NOTE: During data collection manual formatting is deactivated.

1. Select **Format Graph** from the View dialog.
The Format Graph dialog is displayed.

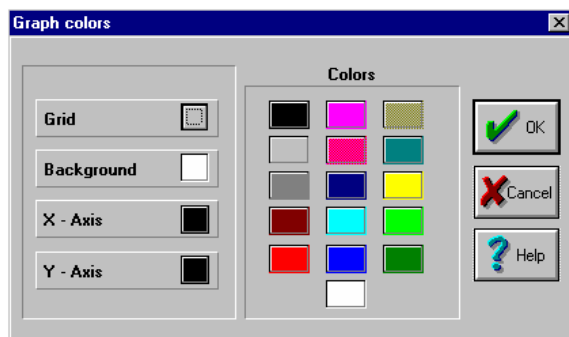


2. Select **Autorange** on either axis to automatically set that axis to the maximum range of the displayed curves when a curve is loaded,

OR

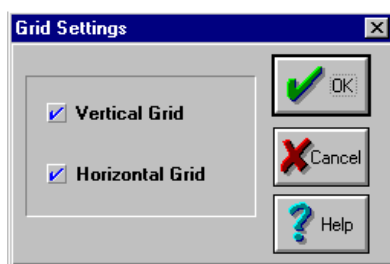
Define the ranges you want displayed by typing in the boxes.

- Click **Set Colors** to select the colors for grid, background and axis from the Graph colors dialog.



For example, to select the color for the grid, click on **Grid** then click on the color you require.

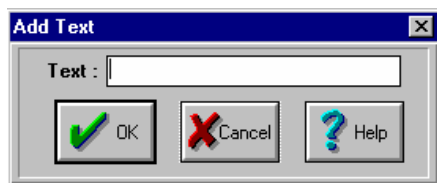
- Click **Set Grid** to switch on the vertical and horizontal grids from the Grid Settings dialog.



- Click **OK** on the Format Graph dialog.
The graph is re-formatted as set.

Adding text to a graph

- Select **Add Text** from the View menu.
The Add Text dialog is displayed.



2. Type the text required and then click **OK**.
The new text will appear in the middle of the active graph window.
3. Move the text to the desired position by clicking on it and then holding down the left mouse button as you drag it to the required position.
Releasing the mouse button sets the position of the text.

NOTE: To remove the text, click on it to select it and then select **Remove Text** from the View menu.

Using Cursors

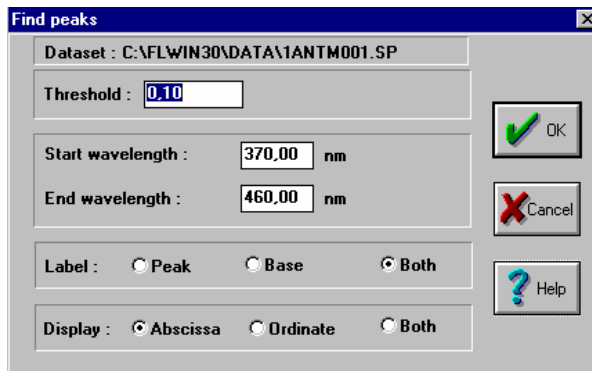
The View menu commands **Vertical Cursor Continuous**, **Vertical Cursor Peak** and **Horizontal Cursor** toggle cursors on the graph that enable you to read off abscissa and ordinate values from the panel at the bottom of the window.

- To move a cursor, move the mouse indicator onto the cursor until it takes the form of a double arrow, click with the left mouse button and drag the cursor to the desired spot.
The continuous cursor can be positioned anywhere on the graph, while the peak cursor will automatically snap to the nearest peak.

Labelling Peaks

You can display peak labels on the maxima and minima of the selected curves in the active Graph window.

1. Select **Label Peaks** from the View menu.
The Find peaks dialog is displayed.



2. Select the required **Threshold**.
This defines the sensitivity of peak detection. If a peak height is less than the threshold, it is not identified as a peak and not labelled.
The threshold is the difference in ordinate units between the peak and the bases on either side of it.
3. Set the required **Start wavelength** and **End wavelength**.
Defines the start and end of the abscissa range inside which peaks are to be identified and labelled.
4. Choose whether to **Label** only peaks, only bases or both.
5. Choose whether to **Display** the abscissa value, the ordinate value or both.
6. Click **OK**.
The peak labels are displayed.

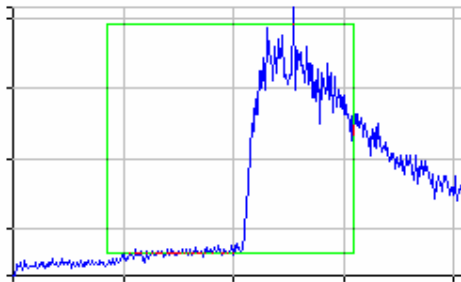
NOTE: *To remove peak labels, select **Clear Peak Labels** from the View menu.*

Viewing multiple curves

- For a clearer view of multiple curves on a single graph, select **Split** from the View menu.
The curves are displayed one above the other in the active Graph window.
On deactivation, curves are again superimposed on one another.

Zooming into a Graph

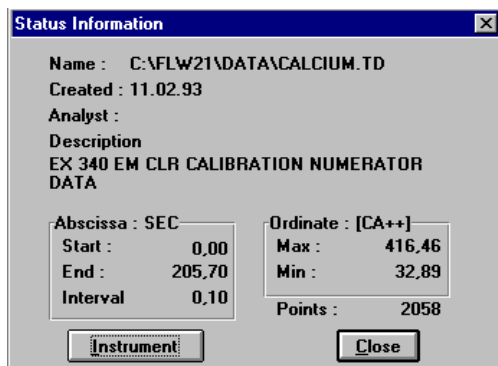
1. To select the zoom area, click with the left mouse button on the upper left point and move the mouse to the lower right point, keeping the left mouse button pressed.
A green rectangle appears.



2. To reposition or resize the green rectangle, drag the borders with the mouse while holding down the left mouse button.
3. When the green rectangle is correctly positioned, double-click the left mouse button within the rectangle.
The area in the rectangle is displayed filling the window.

Viewing Dataset Status Information

1. To view the status information related to a curve, click on the gray button next to the name of the curve in the pane in the bottom of the window.
The Status Information dialog is displayed.



2. To display the stored information about the instrument the spectrum was collected with, click **Instrument**.
3. When finished, click **Close**.

Copying to Report Builder

- Selecting **Copy to Report Builder** from the View menu copies the selected curve from the active Graph window to the Report Builder.
Report Builder starts if it is not currently running.

NOTE: *For more information on Report Builder, see the on-screen help file supplied with Report Builder, which you start by selecting **Contents** from the Help menu in Report Builder.*

Viewing options available when setting up application methods

The following options may be available when you set up the application method.

Ordinate label

Enables you to decide how you want the ordinate label to read. The label is stored in the dataset and is displayed on the ordinate axis of the graph.

Ordinate Max and Min

These ordinate maximum and minimum values are used as defaults for the ordinate range displayed in the graph when a measurement is started. If you leave the boxes empty the graph's ordinate range does not change when a measurement is started.

Auto-clear curves

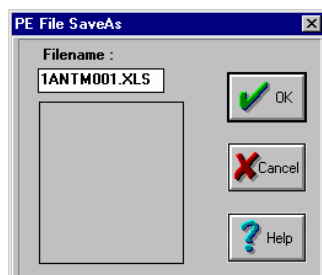
This option enables you to define whether previous curves will be left in the graph window or will be automatically deleted on starting a run.

Saving Results and Curves

When you select **Save As** from the File menu, the format of the Save As dialog depends on whether the current window is a results window or a graph window.

Results Save As Dialog

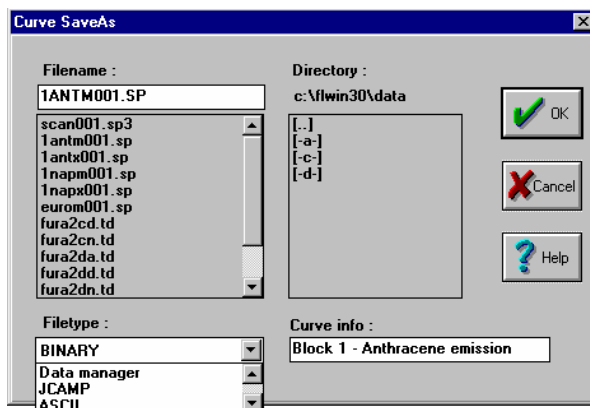
This dialog is used to save results from a Results window in a file.



- Enter a **Filename** and click **OK**.
The results file is saved.

Curve Save As Dialog

This dialog is used to save curves from a Graph window in a file, or to convert spectral curves into another file format.



1. Enter a **Filename** or select one to overwrite.

2. Select the required **Filetype**.

Defines the format in which the data file is stored. Four formats are available:

- **Binary** - This is the standard PerkinElmer format. It contains the complete dataset header information set like creation time, analyst and instrument parameters. Since it uses the least disk space it is the recommended format, especially for acquisition modules with high data throughput like FFA.
- **ASCII** - This format is especially convenient when you want to export data to generic programs like Microsoft Excel. The ASCII format contains the complete dataset header information set. The main drawback is the large amount of disk space required.
- **Data Manager** - This is the old PerkinElmer format.
- **JCAMP** - The data is stored in JCAMP-DX 4.24 format. Use this format to transfer the data to other data processing programs. However, in this format the header information like instrument settings are not stored and the data server can recognize the JCAMP format only if the file extension is **.dx**. To re-import JCAMP data files it is therefore necessary to rename them with this extension.

3. Enter any **Curve info** required.

4. Click **OK**.

The curve is saved in the file format specified.

Arithmetic **5**
Application

Arithmetic

- Select **Arithmetic** from the Data Handling menu.
The Arithmetic application is displayed.

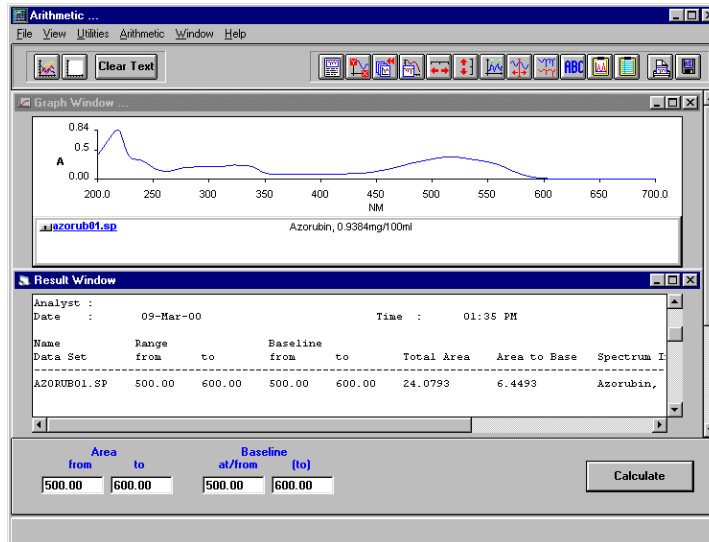
The Arithmetic application is used for spectral manipulation, data conversion and comparison, and data reduction. One Graph Window displays the original, unaltered spectrum whilst a second window displays the spectral or tabular results of the calculations.


Menu Item	Application
Area	To calculate the area beneath the spectral curve.
Arithmetic Spectrum	To perform mathematical operations on spectral data, e.g. add two spectra or multiply a spectrum by a factor.
Arithmetic Value	To perform mathematical operations on spectral data, e.g. Ratio, Difference or multiply an ordinate value with a factor.
Average and Standard Deviation Spectrum	To calculate the average and standard deviation from spectra.
Average and Standard Deviation Value	To calculate average, standard deviation, and rms at a specified wavelength range.
Build Spectra	Build a spectrum via input values.
Calculate Height	To calculate the total height and the height to a specified base.
Convert X	To convert the abscissa unit.

Convert Y	To convert the ordinate unit.
Derivative	To calculate the first, second, third or fourth derivative of a spectral curve.
Interpolate	To change the data interval and to copy part of a spectral curve.
Merge	To merge two spectral curves.
Normalize	To normalize spectral curves to a given ordinate value.
Reflection Correction	To correct a reflectance spectrum for dark and white values.
Selection	To display ordinate values at defined abscissa values.
Slope	To calculate the slope and the standard deviation of a spectral curve within a selected abscissa range. For TimeDrive curves, also use to calculate the enzyme activity.
Smooth	To smooth spectral data.
Film Thickness	To calculate the film thickness of single layers.

Area

Use the **Area** command to calculate the area beneath the spectral curve.



1. From the File menu select **Open**, or click .

If no spectra have previously been selected since opening the module, an Open Spectra from disk file selector is displayed.

If at least one other spectrum has previously been opened, an Add Spectra from dialog is displayed listing the previously opened spectra. Click on the name of the required spectrum in the list or, if the spectrum is not listed, click **Add spectra from disk**, to display a file selector.

2. Select the required spectrum or spectra that you wish to perform the calculations on.

The spectra are displayed in the Graph Window.

3. Enter the **from** and **to** values for the range over which you wish to calculate the area.

OR

You may use the vertical cursor to determine the exact values. Place the mouse in the field whose value you want to determine e.g. **from**. Select **Vertical Cursor Cont** from the View menu. Use the mouse to position the vertical cursor at the required location. The value in the field updates to the position of the vertical cursor. Click the mouse outside the field to accept the value.

4. If you wish to additionally calculate an area with a Baseline correction enter the **at/from** and **(to)** values.

To correct for a sloping baseline enter a value in both the **at/from** and **(to)** fields. Either manually enter the required values or use the vertical cursor as described in step 3 to determine the values.

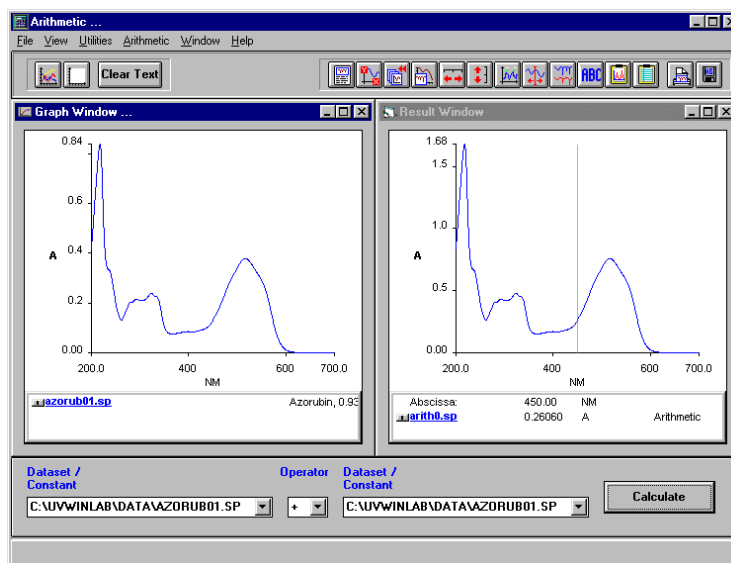
To correct an offset baseline, enter a value in the **at/from** field and leave the **(to)** field empty. Either manually enter the required value or use the vertical cursor as described in step 1 to determine the value.

5. Click **Calculate**.

The calculation is performed on the spectrum/spectra displayed in the Graph Window and the results are displayed in the Results Window.

Arithmetic Spectrum

Use the **Arithmetic Spectrum** command to perform mathematical operations on spectral data, for example, add two spectra or multiply a spectrum by a factor.



1. Click on the left hand drop-down **Dataset/Constant** list and select **<disk>**.
An Open spectrum from disk, file selector is displayed.
2. Select the required spectrum.
The spectrum is displayed in the Graph Window and the name of the spectrum is displayed in the Dataset/Constant field.

NOTE: *If spectra have previously been selected, these are listed in the drop-down list and may be selected.*

3. Select the required **Operator** from the drop-down list.
4. Click on the right hand drop-down **Dataset/Constant** list and select **<disk>**.
An Open spectrum from disk, file selector is displayed.

5. Select the required spectrum.

OR

Instead of selecting a spectrum in step 1 or step 4 you may enter a constant that you wish to apply to the spectrum listed in the other **Dataset/Constant** field.

NOTE: *At least one of the Dataset/Constant fields must contain the name of a spectrum. If two spectra are displayed, they must have the same abscissa units. If the spectra have different abscissa ranges, the calculation is only performed over the common range.*

6. Click **Calculate**.

The calculation is performed and the resultant spectrum is displayed in the Result Window.

7. To save the resultant spectrum, ensure that the Result Window is selected and choose **Save As** from the File menu.

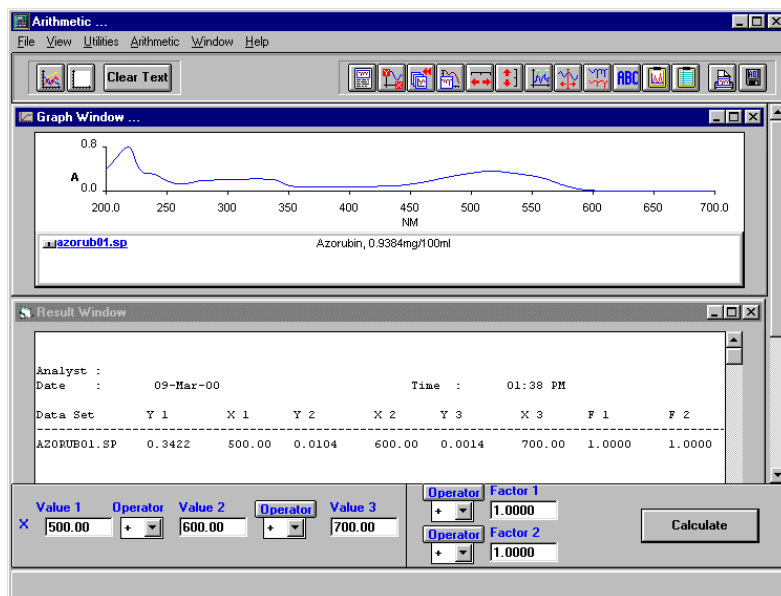
The Spectra Save As dialog is displayed. The default filename of the result spectrum is arith**.yy, where ** is an autoincremented number and yy is the same file extension as the spectrum displayed in the Graph Window. The results are saved to the ../data directory. The ordinate unit of the resulting curve depends on the ordinate unit of the source data in the Graph Window.


NOTE: *You may alter the automatic file name. Any text entered in the **InfoLine** field is saved with the spectrum and is displayed in the information line when the spectrum is displayed.*

Arithmetic Value

Use the **Arithmetic Value** command to perform mathematical operations on spectral data, for example, Ratio, Differences or multiply an ordinate value with a factor.

Up to three abscissa values and two factors, or two abscissa values and three factors can be used in the calculations.



1. From the File menu select **Open**, or click .

If no spectra have previously been selected since opening the module, an Open Spectra from disk file selector is displayed.

If at least one other spectrum has previously been opened, an Add Spectra from dialog is displayed listing the previously opened spectra. Click on the name of the required spectrum in the list or, if the spectrum is not listed, click **Add spectra from disk**, to display a file selector.

2. Select the required spectrum or spectra that you wish to perform the calculations on.

The spectra are displayed in the Graph Window.

3. Enter the starting wavelength for the calculation in the **Value 1** field.

OR

You may use the vertical cursor to determine the exact value. Place the mouse in the **Value 1** field. Select **Vertical Cursor Cont** from the View menu. Use the mouse to position the vertical cursor at the required location. The value in the field updates to the position of the vertical cursor. Click the mouse outside the field to accept the value.

4. Select the required **Operator** from the drop-down list.
5. Enter the end wavelength for the calculation in the **Value 2** field.

OR

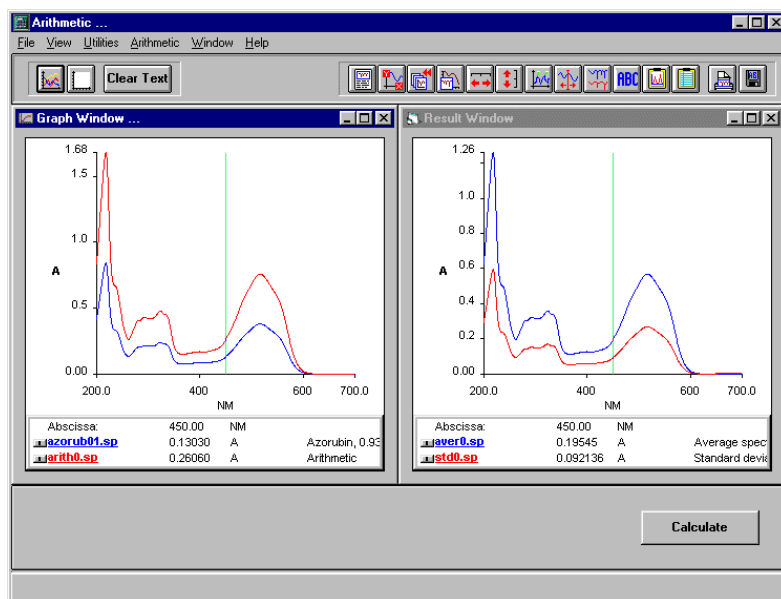
You may use the vertical cursor to determine the exact value. Place the mouse in the **Value 2** field. Select **Vertical Cursor Cont** from the View menu. Use the mouse to position the vertical cursor at the required location. The value in the field updates to the position of the vertical cursor. Click the mouse outside the field to accept the value.


6. If you require a third abscissa value or a factor for the calculation, click **Operator**.
Value 3 field is displayed.
7. Select the required **Operator** from the drop-down list.
This is the drop-down list between Value 2 and Value 3.
8. Enter the required wavelength for the calculation, or enter the required **Factor** in the **Value 3** field.
9. If you require a Factor (or an additional factor if you have already entered one in Value 3 field), click **Operator**.
This is the operator button directly beneath the Result window.
A Factor 1 field is displayed.
10. Select the required **Operator** from the drop-down list.
11. Enter the required Factor in the **Factor 1** field.

12. If you require another Factor, click **Operator**.
This is the second operator button beneath the Result window.
A Factor 2 field is displayed.
13. Select the required **Operator** from the drop-down list.
14. Enter the required Factor in the **Factor 2** field.
15. Click **Calculate**.
The calculation is performed and the results are displayed in the Result Window.

Average and Standard Deviation Spectrum

Use the Average and Standard Deviation command to calculate the average spectrum and standard deviation spectrum. A minimum of two spectra are required, and a maximum of 16 spectra may be used for the calculations.



- From the File menu select **Open**, or click .

If no spectra have previously been selected since opening the module, an Open Spectra from disk file selector is displayed.

If at least one other spectrum has previously been opened, an Add Spectra from dialog is displayed listing the previously opened spectra. Click on the name of the required spectrum in the list or, if the spectrum is not listed, click **Add spectra from disk**, to display a file selector.
- Select the required spectra that you wish to perform the calculations on.

The spectra are displayed in the Graph Window.

NOTE: *The spectra must have the same ordinate type and the same abscissa units. If the spectra have different abscissa ranges, the calculation is only performed over the common range.*

3. Click **Calculate**.

The resultant average spectrum and standard deviation spectrum are displayed in the Results Window.

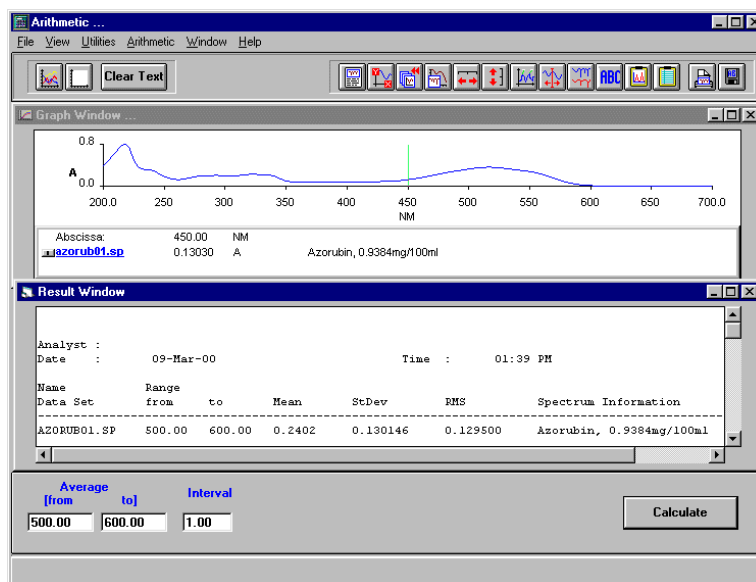
4. To save the resultant spectra, ensure that the Result Window is selected and choose **Save As** from the File menu.


The Spectra Save As dialog is displayed, enabling you to save the average spectrum and then the standard deviation spectrum. The default filename of the average spectrum is aver**.yy, where ** is an autoincremented number and yy is the same file extension as the spectrum displayed in the Graph Window. The default filename of the standard deviation spectrum is std**.yy. The results are saved to the ../data directory. The ordinate unit of the resulting curves depends on the ordinate unit of the source data in the Graph Window.

NOTE: *You may alter the automatic file names. Any text entered in the **Infoline** field is saved with the spectrum and is displayed in the information line when the spectrum is displayed.*

Average and Standard Deviation Value

Use the **Average and Standard Deviation Value** command to calculate the average, standard deviation, and rms (root mean square) over a specified wavelength range.



1. From the File menu select **Open**, or click .

If no spectra have previously been selected since opening the module, an Open Spectra from disk file selector is displayed.

If at least one other spectrum has previously been opened, an Add Spectra from dialog is displayed listing the previously opened spectra. Click on the name of the required spectra in the list or, if the spectrum/spectra are not listed, click **Add spectra from disk**, to display a file selector.

2. Select the required spectra that you wish to perform the calculations on. The spectra are displayed in the Graph Window.

3. Enter the **from** and **to** values for the range over which you wish to calculate the average, standard deviation and rms.

OR

You may use the vertical cursor to determine the exact values. Place the mouse in the field whose value you want to determine e.g. **from**. Select **Vertical Cursor Cont** from the View menu. Use the mouse to position the vertical cursor at the required location. The value in the field updates to the position of the vertical cursor. Click the mouse outside the field to accept the value.

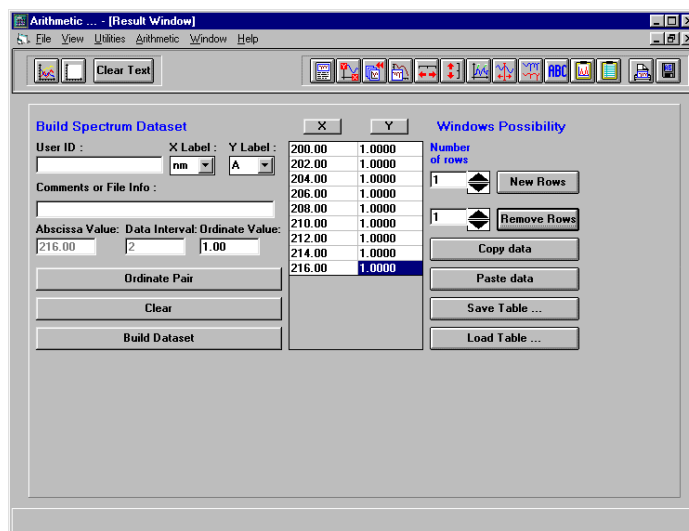
4. Enter the required **Interval**.

5. Click **Calculate**.

The calculations are performed for all the selected spectra and the results displayed in the Result Window.

Build Spectra

Use the **Build Spectra** command to generate a synthetic spectrum.



1. Enter the **User ID** to be stored with the spectrum.
2. Select the required **X Label** (Abscissa label) from the drop-down list.
3. Select the required **Y Label** (Ordinate label) from the drop-down list.
4. Enter the required **Abscissa Start** value.
This value is incremented based on the starting wavelength and the data interval.
5. Enter the required **Data Interval**.
6. Enter the required **Ordinate Value** for the **Abscissa Start** value.
7. Click **Ordinate Pair**.

The Abscissa and Ordinate values are displayed in the XY Table. The Abscissa Start value field is now titled Abscissa value and is automatically incremented. Both the **Abscissa Value** and **Data Interval** fields become greyed. The Data Interval is fixed at the selected value.

8. Enter the **Ordinate Value** for the given auto-incremented **Abscissa Value**.
9. Click **Ordinate Pair**.
The table of values is updated.
10. Repeat steps 8 and 9 until all the required values have been entered.

NOTE: *If an incorrect **Ordinate Value** has been entered, double-click on the incorrect value in column Y of the table; a Change Data dialog is displayed. Enter the correct value and click **OK**. The value in the table is updated.*

11. Click **Save**.
A Save Text File dialog is displayed.
12. Enter the name of the spectrum.
The data is saved as a .rbs file (build spectrum).
13. Click **Build Dataset**.
The spectrum is displayed.
The Spectra Save As dialog is displayed, enabling you to save the spectrum. The default filename of the spectrum is newset**.sp, where ** is an autoincremented number. The spectrum is saved to the ../data directory.

Clicking on **X** or **Y** above the table column highlights whole column.

Clear

Clear removes all values in the table and re-enables the abscissa and data interval.

New rows

Click **New Rows** to add new blank rows to the table. Use the **Number of rows** selector to choose the number of rows to be added. One row contains the values for one data point.

Remove rows

Click **Remove Rows** to remove rows from the table. Use the **Number of rows** selector to choose the number of rows to be removed.

NOTE: *The rows are removed incrementally from the bottom of the table upwards.*

Copy data

Copy data copies selected data in the grid table to the clipboard.

Paste data

Paste data copies data from the clipboard into the table.

NOTE: *If you wish to paste data into the table, the number of new (blank) rows must be at least the same as the number of rows to be pasted. If you try to paste more rows than there are blank rows available, the blank rows will be filled and the remaining data will not be pasted into the table.*

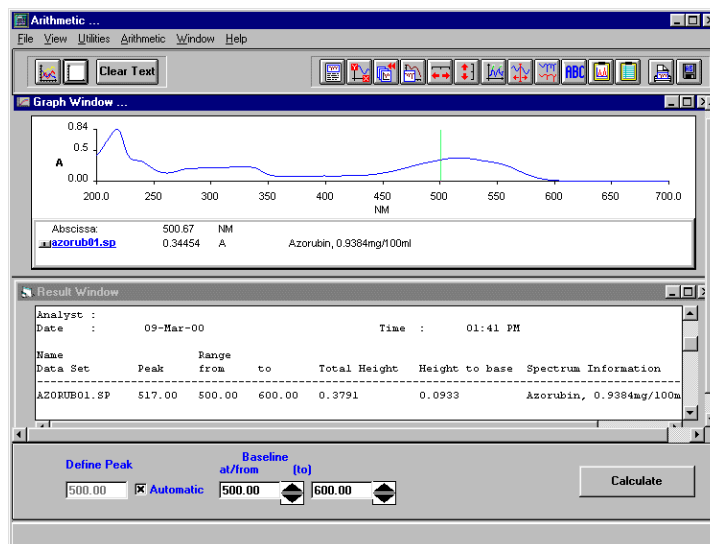
Load


Load allows you to open a previously saved method file (*.rbs). An excel spread sheet can be opened if it has the formatting (Tab delimited):

Number of Rows	[Tab]	Start Abscissa	[Enter]
Abscissa values	[Tab]	Ordinate values	[Enter]

Calculate Height

Use the **Calculate Height** command to calculate the total height and the height to a specified baseline.



- From the File menu select **Open**, or click .

If no spectra have previously been selected since opening the module, an Open Spectra from disk file selector is displayed.

If at least one other spectrum has previously been opened, an Add Spectra from dialog is displayed listing the previously opened spectra. Click on the name of the required spectrum in the list or, if the spectrum is not listed, click **Add spectra from disk**, to display a file selector.
- Select the required spectrum that you wish to perform the calculations on. The spectrum is displayed in the Graph Window.

3. Enter the **Define Peak** wavelength.
This is the value of the peak maximum.

OR

You may use the vertical cursor to determine the exact value. Place the mouse in the **Define Peak** field. Select **Vertical Cursor Cont** from the View menu. Use the mouse to position the vertical cursor at the required location. The value in the field updates to the position of the vertical cursor. Click the mouse outside the field to accept the value.

4. Enter the **at/from** and **(to)** values for the abscissa range over which to calculate the total height and the height to base.

OR

You may use the vertical cursor to determine the exact values. Place the mouse in the field whose value you want to determine e.g. **at/from**. Select **Vertical Cursor Cont** from the View menu. Use the mouse to position the vertical cursor at the required location. The value in the field updates to the position of the vertical cursor. Click the mouse outside the field to accept the value.

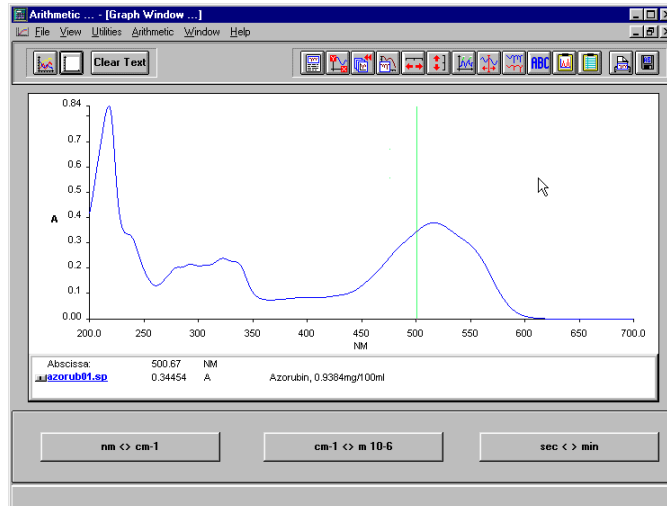
A horizontal marker appears on the graph to show the range defined and the baseline that will be used to calculate the Height to Base.


NOTE: *Instead of entering a value in the **DefinePeak** field you can select **Automatic**. If you select **Automatic**, the software automatically searches for a peak in the specified baseline range.*

5. Click **Calculate**.
The calculation is performed and the results are displayed in the Result Window.

Convert X

Use the **Convert X** command to convert the abscissa unit.

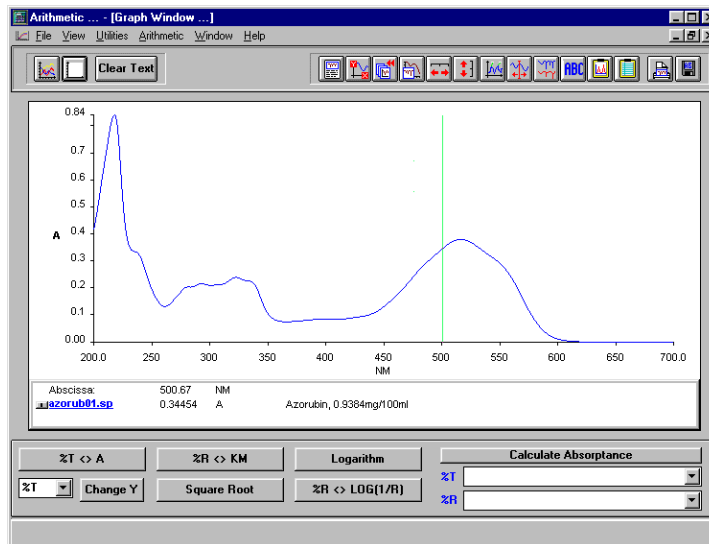



- From the File menu select **Open**, or click . If no spectra have previously been selected since opening the module, an Open Spectra from disk file selector is displayed. If at least one other spectrum has previously been opened, an Add Spectra from dialog is displayed listing the previously opened spectra. Click on the name of the required spectrum in the list or, if the spectrum is not listed, click **Add spectra from disk**, to display a file selector.
- Select the required spectrum or spectra that you wish to perform the calculations on. The spectra are displayed in the Graph Window.
- Click the appropriate button to convert the abscissa values.

nm <> cm⁻¹	Converts the abscissa unit from nm to cm ⁻¹ and vice versa.
cm⁻¹ <> m 10-6	Converts the abscissa unit from cm ⁻¹ to μm and vice versa.
sec <> min	Converts the abscissa unit from sec to min and vice versa.

Convert Y

Use the **Convert Y** command to convert the ordinate unit, for example, from % transmittance to absorbance, or to calculate absorbance ($AP = 1 - T - R$).



1. From the File menu select **Open**, or click .

If no spectra have previously been selected since opening the module, an Open Spectra from disk file selector is displayed.

If at least one other spectrum has previously been opened, an Add Spectra from dialog is displayed listing the previously opened spectra. Click on the name of the required spectrum in the list or, if the spectrum is not listed, click **Add spectra from disk**, to display a file selector.

2. Select the required spectrum or spectra that you wish to perform the calculations on.
The spectra are displayed in the Graph Window.
3. Click the required button to perform the **Convert Y** calculation.

%T <> A

Converts spectral data from transmittance to absorbance (optical density) and vice versa.

%R <> KM

Converts spectral data from reflectance to Kubelka-Munk and vice versa.

NOTE: *The source data must have the ordinate unit %R or %T.*

Logarithm

Calculates the logarithm of all ordinate values. The logarithms of zero and negative values are set to very small negative values.

Change Y

Changes the ordinate label with the specified label.

- Select the required ordinate label from the drop-down list and click **Change Y**.
The ordinate label is changed.

NOTE: *Only ordinate labels with the same scale can be changed. For example, it is possible to change %T to %R but not to A.*

Square Root

Calculates the square root of all ordinate values.

%R <> Log(1/R)

Converts spectral data from reflectance to log(1/R) and vice versa.

Calculate Absorptance

Absorptance spectrum = 1 – Transmittance – Reflectance

Transmittance = Transmission / 100 Reflectance = Reflection / 100

1. Click on the **%T** drop-down list and select **<disk>**.
An Open spectrum from disk, file selector is displayed.
2. Select the required transmission spectrum.
The spectrum is displayed in the Graph Window and the name of the spectrum is displayed in the **%T** field.

NOTE: *If spectra have previously been selected, these are listed in the drop-down list and may be selected.*

3. Click on the **%R** drop-down list and select **<disk>**.
An Open spectrum from disk, file selector is displayed.
4. Select the required reflection spectrum.
The spectrum is displayed in the Graph Window and the name of the spectrum is displayed in the Select Dataset field.

NOTE: *If spectra have previously been selected, these are listed in the drop-down list and may be selected.*

5. Click **Calculate Absorptance**.
The absorptance spectrum is displayed.
6. To save the resultant spectrum, ensure that the Result Window is selected and choose **Save As** from the File menu.
The Spectra Save As dialog is displayed. The default filename of the result spectrum is abs**.yy, where ** is an autoincremented number and yy is the same file extension as the spectrum displayed in the Graph Window. The results are saved to the ../data directory.

NOTE: *You may alter the automatic file name. Any text entered in the **Infoline** field is saved with the spectrum and is displayed in the information line when the spectrum is displayed.*

Derivative

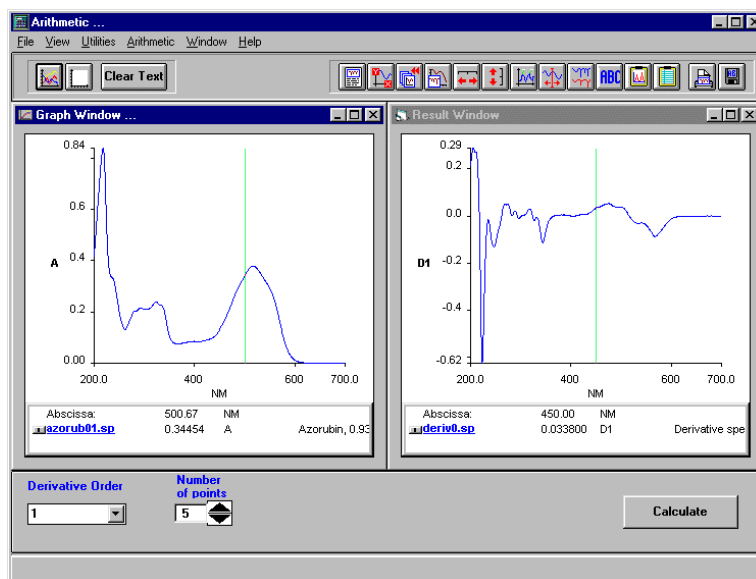
Use the **Derivative** command to calculate the first, second, third or fourth derivative of a spectral curve.


Derivative spectra usually have sharper features than the original spectra. You can use the amplitudes of features in derivative spectra in the same way as peak absorbances. The elimination of some baseline effects in derivative spectra can be useful in quantitative methods.

You can use the Derivative command to take the first, second, third or fourth order derivative of a spectrum.

The first derivative removes any baseline offset and the second derivative removes any linear slope also.

Second-derivative spectra have sharp minima where there are maxima in the original spectrum and so can be used to identify band positions in complex regions.



1. From the File menu select **Open**, or click .
If no spectra have previously been selected since opening the module, an Open Spectra from disk file selector is displayed.
If at least one other spectrum has previously been opened, an Add Spectra from dialog is displayed listing the previously opened spectra. Click on the name of the required spectrum in the list or, if the spectrum is not listed, click **Add spectra from disk**, to display a file selector.
2. Select the required spectrum or spectra that you wish to perform the calculations on.
The spectra are displayed in the Graph Window.
3. Select the **Derivative Order** to be applied to the spectrum/spectra from the drop-down list.
4. Select the **Number of points**.
The **Number of points** sets the number of data points used to calculate the derivative. As you increase the number of data points used in the calculation, the contributions of broader features increases relative to narrow features.
5. Click **Calculate**.
The calculation is performed and the derivative spectra are displayed in the Result Window.

NOTE: *To help you see the details of the result spectra, they are multiplied by a scaling factor depending on the order of the derivative. First order spectra are not scaled, second order are scaled by a factor of 10, third order by a factor of a hundred, and fourth order by a factor of a thousand.*

6. To save the resultant spectrum, ensure that the Result Window is selected and choose **Save As** from the File menu.
The Spectra Save As dialog is displayed with a default filename (deriv**.yy, where ** is an autoincremented number and yy is the same file extension as the spectrum displayed in the Graph Window).

NOTE: *You may alter the automatic file name. Any text entered in the **Infoline** field is saved with the spectrum and is displayed in the information line when the spectrum is displayed.*

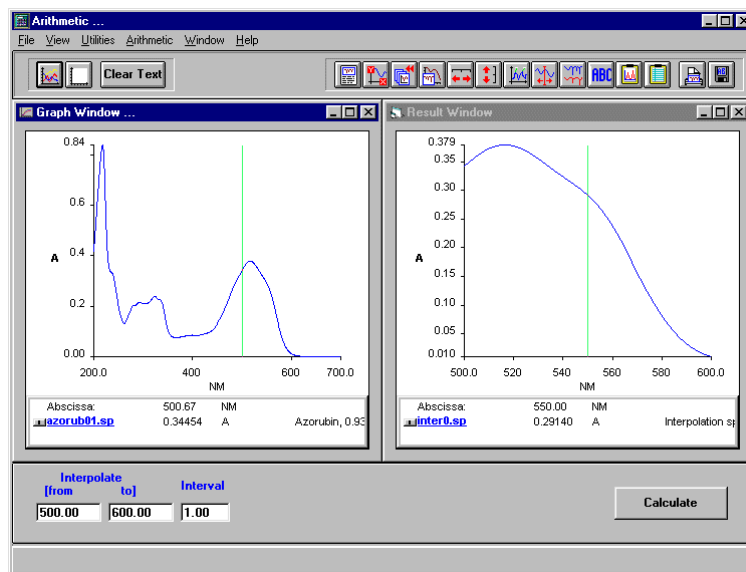
Interpolate


Use the **Interpolate** command to change the data interval (the number of points that make up the spectrum) and copy all or part of a spectrum.

Interpolation adds points to a spectrum by adding new points between the points that already exist. Interpolation removes points from a spectrum by selecting every *n*th point of a spectrum and removing it.

The number of data points needed in a spectrum depends on the width of the features in the spectrum. If the separation of the data points is smaller than that needed to define the features in the spectrum, the number of points can be reduced without reducing the amount of information in the spectrum.

Increasing the number of data points in a spectrum cannot increase the amount of information in the spectrum.



1. From the File menu select **Open**, or click .
If no spectra have previously been selected since opening the module, an Open Spectra from disk file selector is displayed.
If at least one other spectrum has previously been opened, an Add Spectra from dialog is displayed listing the previously opened spectra. Click on the name of the required spectrum in the list or, if the spectrum is not listed, click **Add spectra from disk**, to display a file selector.
2. Select the required spectrum or spectra that you wish to perform the calculations on.
The spectra are displayed in the Graph Window.
3. Enter the **from** and **to** values for the interpolation range.

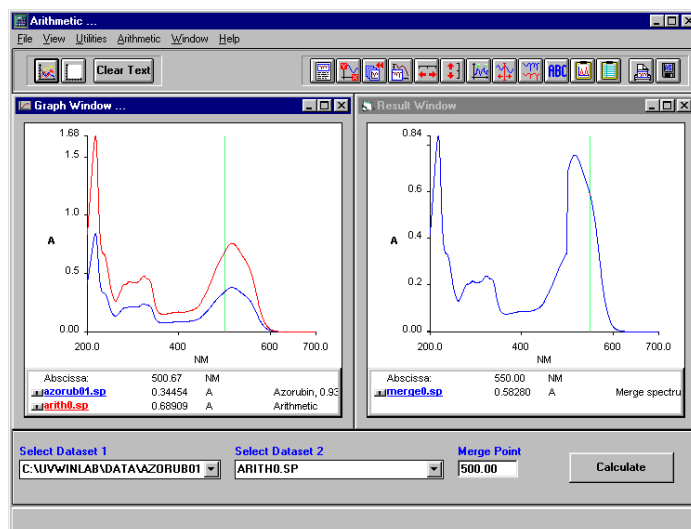
OR

- You may use the vertical cursor to determine the exact values. Place the mouse in the field whose value you want to determine e.g. **from**. Select **Vertical Cursor Cont** from the View menu. Use the mouse to position the vertical cursor at the required location. The value in the field updates to the position of the vertical cursor. Click the mouse outside the field to accept the value.
4. Enter the required new data **Interval**.
 5. Click **Calculate**.
The resultant spectrum or part of a spectrum is displayed in the Result Window.
 6. To save the resultant spectrum, ensure that the Result Window is selected and choose **Save As** from the File menu.
The Spectra Save As dialog is displayed. The default filename of the result spectrum is inter**.yy, where ** is an autoincremented number and yy is the same file extension as the spectrum displayed in the Graph Window.
The results are saved to the ../data directory.

NOTE: *You may alter the automatic file name. Any text entered in the **Infoline** field is saved with the spectrum and is displayed in the information line when the spectrum is displayed.*

Merge

Use the **Merge** command to merge two spectral curves.



1. Click on the drop-down **Select Dataset 1** list and select **<disk>**.
An Open spectrum from disk, file selector is displayed.
2. Select the required spectrum.
The spectrum is displayed in the Graph Window and the name of the spectrum is displayed in the Select Dataset 1 field.

NOTE: *If spectra have previously been selected, these are listed in the drop-down list and may be selected.*

3. Click on the drop-down **Select Dataset 2** list and select **<disk>**.
An Open spectrum from disk, file selector is displayed.
4. Select the required spectrum.
The spectrum is displayed in the Graph Window and the name of the spectrum is displayed in the Select Dataset 2 field.

NOTE: *The spectra must have the same abscissa and ordinate units.*

5. Enter the **Merge Point**.

The value will be in the same units as the two spectra displayed in the Graph Window.

OR

You may use the vertical cursor to determine the exact value. Place the mouse in the Merge Point field. Select **Vertical Cursor Cont** from the View menu. Use the mouse to position the vertical cursor at the required location. The value in the field updates to the position of the vertical cursor. Click the mouse outside the merge Point field to accept the value.

6. Click **Calculate**.

The merged spectrum is displayed in the Result Window.

7. To save the resultant spectrum, ensure that the Result Window is selected and choose **Save As** from the File menu.

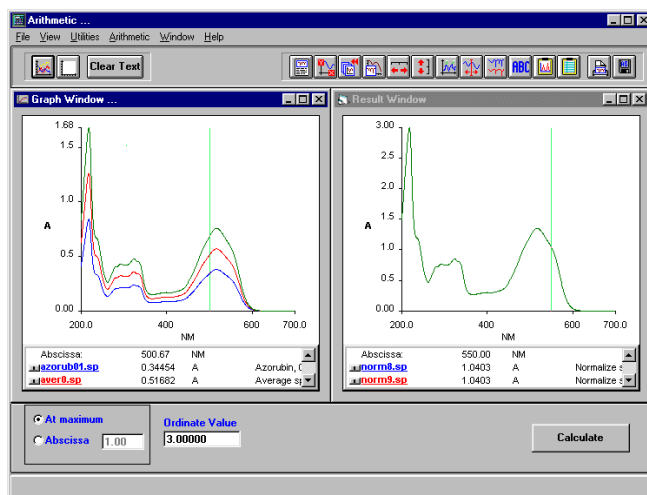
The Spectra Save As dialog is displayed. The default filename of the result spectrum is merge**.yy, where ** is an autoincremented number and yy is the same file extension as the spectrum displayed in the Graph Window.


The results are saved to the ../data directory.

NOTE: *You may alter the automatic file name. Any text entered in the **Infoline** field is saved with the spectrum and is displayed in the information line when the spectrum is displayed.*

Normalize

Use the **Normalize** command to normalize spectral curves to a given ordinate value. Normalize can be used to set a common peak in several spectra to the same ordinate limit. The source data are multiplied by a factor to match the ordinate value at the selected abscissa position.



- From the File menu select **Open**, or click .

If no spectra have previously been selected since opening the module, an Open Spectra from disk file selector is displayed.

If at least one other spectrum has previously been opened, an Add Spectra from dialog is displayed listing the previously opened spectra. Click on the name of the required spectrum in the list or, if the spectrum is not listed, click **Add spectra from disk**, to display a file selector.
- Select the required spectrum that you wish to perform the calculations on. The spectrum is displayed in the Graph Window.
- Select **At maximum** or **Abscissa**.

At maximum uses the abscissa position of the highest peak.

4. If you select **Abscissa**, enter the required value.

OR

You may use the vertical cursor to determine the exact value. Place the mouse in the Abscissa field. Select **Vertical Cursor Cont** from the View menu. Use the mouse to position the vertical cursor at the required location. The value in the field updates to the position of the vertical cursor. Click the mouse outside the field to accept the value.

5. If you select **Abscissa**, enter the required **Ordinate Value**.

NOTE: *All Normalize calculations are performed in absorbance. The **Ordinate Value** is in absorbance.*

6. Click **Calculate**.

The resultant spectrum is displayed in the Result Window.

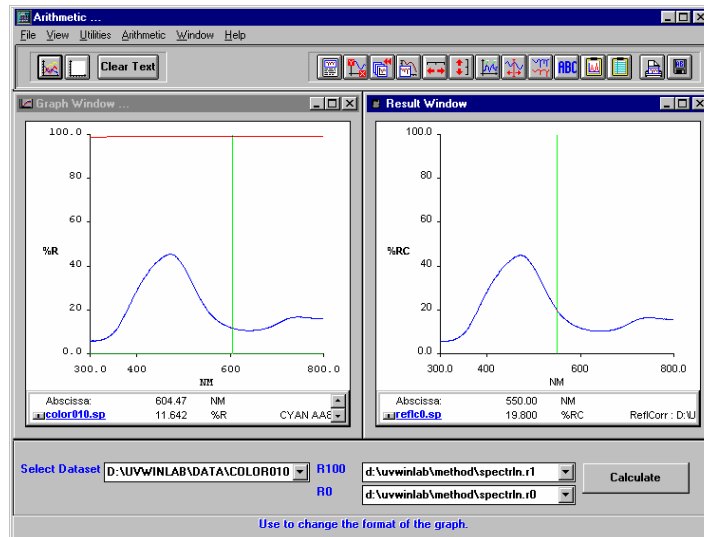
7. To save the resultant spectrum, ensure that the Result Window is selected and choose **Save As** from the File menu.

The Spectra Save As dialog is displayed. The default filename of the result spectrum is norm**.yy, where ** is an autoincremented number and yy is the same file extension as the spectrum displayed in the Graph Window. The results are saved to the ../data directory.

NOTE: *You may alter the automatic file name. Any text entered in the **Infoline** field is saved with the spectrum and is displayed in the information line when the spectrum is displayed.*

Reflection Correction

Use the **Reflection Correction** command to correct a reflectance spectrum for dark and white values.



1. Click on the drop-down **Select Dataset** list and select **<disk>**.
An Open spectrum from disk, file selector is displayed.
2. Select the required spectrum.
The spectrum is displayed in the Graph Window and the name of the spectrum is displayed in the Select Dataset field.

NOTE: *If spectra have previously been selected, these are listed in the drop-down list and may be selected.*

3. Click on the drop-down **R100** list and select **<disk>**.
An Open spectrum from disk, file selector is displayed.
4. Select the required spectrum.
The file extension of the R100 spectrum is .r1. The spectrum is displayed in the Graph Window and the name of the spectrum is displayed in the R100 field.

NOTE: *The R100 spectrum (white reference material) must cover the spectral range of the spectrum to be corrected.*

5. Click on the drop-down **R0** list and select **<disk>**.

An Open spectrum from disk, file selector is displayed.

6. Select the required spectrum.

The file extension of the R0 spectrum is .r0. The spectrum is displayed in the Graph Window and the name of the spectrum is displayed in the R0 field.

NOTE: *The R0 spectrum (spectral curve of a dark measurement) must cover the spectral range of the spectrum to be corrected.*

7. Click **Calculate**.

The resultant spectrum is displayed in the Result Window.

8. To save the resultant spectrum, ensure that the Result Window is selected and choose **Save As** from the File menu.

The Spectra Save As dialog is displayed. The default filename of the result spectrum is reflc**.yy, where ** is an autoincremented number and yy is the same file extension as the spectrum displayed in the Graph Window. The results are saved to the ../data directory.

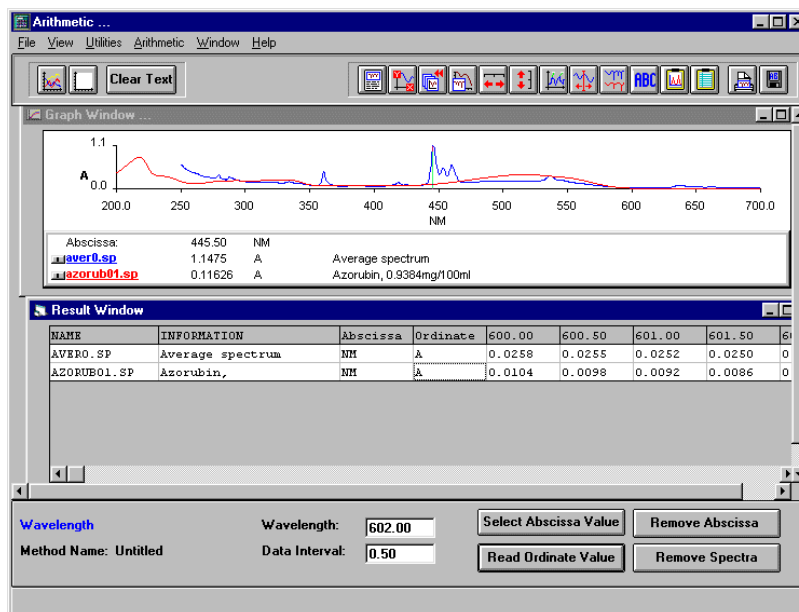
NOTE: *You may alter the automatic file name. Any text entered in the **Infoline** field is saved with the spectrum and is displayed in the information line when the spectrum is displayed.*


The correction is automatically performed using the following formula:

$$R_{\text{actual}} = \frac{R_{\text{measured}} - (R0)}{100 - (R0)} (R100)$$

Selection

Use the **Selection** command to determine ordinate values at defined abscissa values.



- From the File menu select **Open**, or click .

If no spectra have previously been selected since opening the module, an Open Spectra from disk file selector is displayed.

If at least one other spectrum has previously been opened, an Add Spectra from dialog is displayed listing the previously opened spectra. Click on the name of the required spectrum in the list or, if the spectrum is not listed, click **Add spectra from disk**, to display a file selector.
- Select the required spectrum that you wish to perform the calculations on. The spectrum is displayed in the Graph Window.

3. Enter the **Wavelength** at which you wish to calculate the Ordinate value.

OR

You may use the vertical cursor to determine the exact value. Place the mouse in the Wavelength field. Select **Vertical Cursor Cont** from the View menu. Use the mouse to position the vertical cursor at the required location. The value in the field updates to the position of the vertical cursor. Click the mouse outside the field to accept the value.

4. If required, enter the **Data Interval** of the spectrum.
If the **Data Interval** is entered, the Wavelength value is automatically updated each time **Select Abscissa Value** is clicked, enabling a series of incremented wavelengths to be added to the table. If the Data Interval is not entered, only the current value in the Wavelength field is added.
5. Click **Select Abscissa Value**
The value in the Wavelength field is added to the table in the Result Window.
6. Click **Read Ordinate Value**.
The Ordinate value for the selected wavelength is added to the table, together with the spectrum name and any information saved with the spectrum.
7. If the Data Interval was entered, repeat steps 5 and 6 until all the required values have been entered in the table.

NOTE: *If the Data Interval was entered, it is possible to enter all the abscissa values first. Repeatedly click **Select Abscissa Value** until all the required abscissa values have been entered in the table, then click **Read Ordinate Value**. All the ordinate values are then added to the table.*

8. If you wish to save the data, click **Save As** from the File menu.
A Save As dialog is displayed.

Remove Abscissa

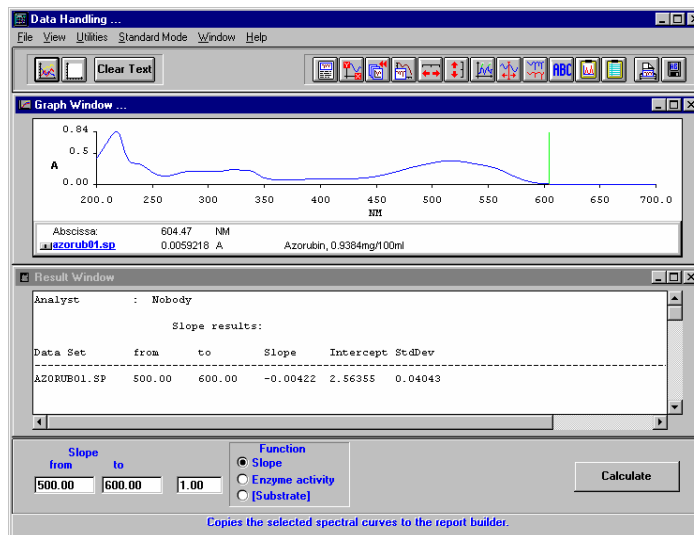
Removes all the abscissa values and any associated ordinate values from the table. The spectrum name and information are retained.


Remove Spectra

Removes the spectrum name, information and any ordinate values from the table. The abscissa values entered are retained.

Slope

Use the **Slope** command to calculate the slope and standard deviation of a spectral curve within a selected abscissa range. It can also be used to calculate Enzyme activity and Substrate.



1. From the File menu select **Open**, or click .

If no spectra have previously been selected since opening the module, an Open Spectra from disk file selector is displayed.

If at least one other spectrum has previously been opened, an Add Spectra from dialog is displayed listing the previously opened spectra. Click on the name of the required spectrum in the list or, if the spectrum is not listed, click **Add spectra from disk**, to display a file selector.
2. Select the required spectrum or spectra that you wish to perform the calculations on.

The spectrum is displayed in the Graph Window.

3. Enter the Slope **from** and **to** values.

OR

You may use the vertical cursor to determine the exact value. Place the mouse in the required field. Select **Vertical Cursor Cont** from the View menu. Use the mouse to position the vertical cursor at the required location. The value in the field updates to the position of the vertical cursor. Click the mouse outside the field to accept the value.

4. Choose the **Function**.
5. If Enzyme activity or Substrate is selected, enter the required **Factor**.

NOTE: *No factor is required when calculating the slope. A factor, other than 1, is needed when calculating Enzyme activity or Substrate. When calculating Enzyme activity, the factor must correspond to the unit absorbance per minute.*

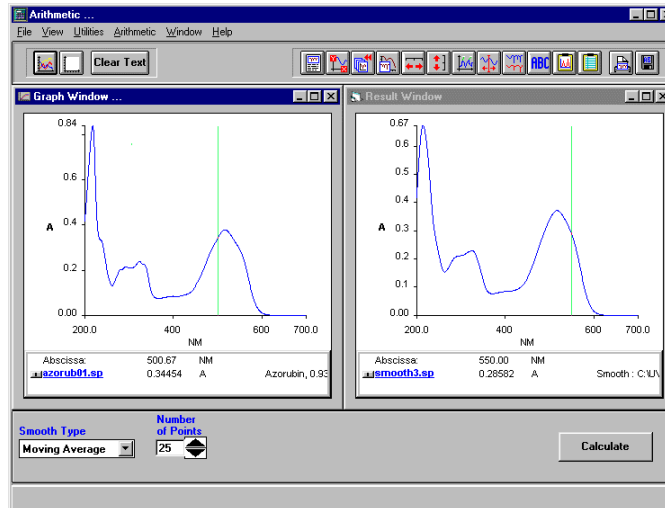
The enzyme activity is calculated using the following equation:


$$\text{Enzyme activity} = \text{slope} * \text{factor} * 60$$

6. Click **Calculate**.
The results are displayed in the Result Window.

Smooth

Use the **Smooth** command to smooth spectral data.



1. From the File menu select **Open**, or click .

If no spectra have previously been selected since opening the module, an Open Spectra from disk file selector is displayed.

If at least one other spectrum has previously been opened, an Add Spectra from dialog is displayed listing the previously opened spectra. Click on the name of the required spectrum in the list or, if the spectrum is not listed, click **Add spectra from disk**, to display a file selector.
2. Select the required spectrum or spectra that you wish to perform the calculations on.

The spectra are displayed in the Graph Window.

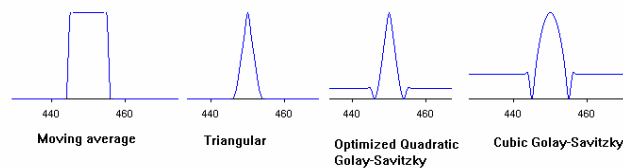
3. Select the **Smooth Type** from the drop-down list.

Cubic Golay-Savitzky: Golay-Savitzky smoothing, using a cubic polynomial.

Moving Average: Smoothing, using the statistical mean of the selected number of data points.

Quadratic Golay-S: Golay-Savitzky smoothing, using a quadratic polynomial.

Triangular: Smoothing, using a weighted moving average.



4. Select the **Number of Points**.

The Number of Points defines the degree of smoothing applied to the spectrum; the more points, the more smoothing that is applied.

5. Click **Calculate**.

The smooth calculation is performed and the resultant spectrum/spectra are displayed in the Result Window.

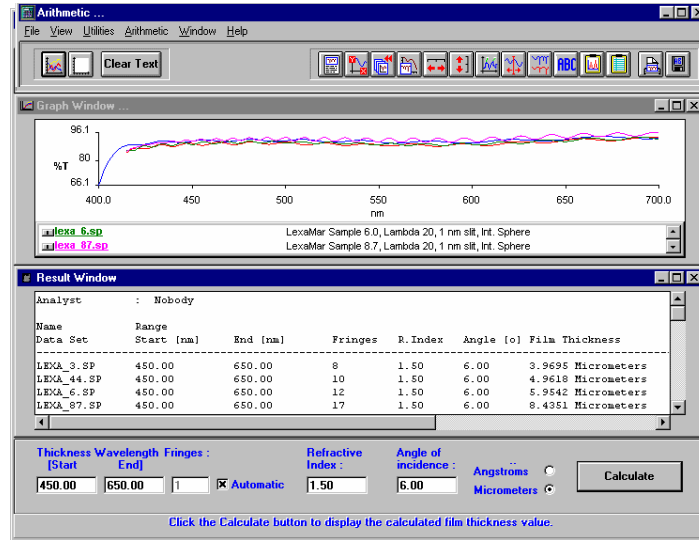
6. To save the resultant spectra, ensure that the Result Window is selected and choose **Save As** from the File menu.


The Spectra Save As dialog is displayed, enabling you to save the smoothed spectrum. The default filename of the smoothed spectrum is smooth**.yy, where ** is an autoincremented number and yy is the same file extension as the spectrum displayed in the Graph Window. The results are saved to the ../data directory. The ordinate unit of the resulting curves depends on the ordinate unit of the source data in the Graph Window.

NOTE: You may alter the automatic file names. The **InfoLine** contains the path and filename of the spectrum that was smoothed. Any text entered in the **Infoline** field is saved with the spectrum and is displayed in the information line when the spectrum is displayed.

Film Thickness

Use the **Film Thickness** command to calculate the film thickness of single layers.



- From the File menu select **Open**, or click .

If no spectra have previously been selected since opening the module, an Open Spectra from disk file selector is displayed.

If at least one other spectrum has previously been opened, an Add Spectra from dialog is displayed listing the previously opened spectra. Click on the name of the required spectrum in the list or, if the spectrum is not listed, click **Add spectra from disk**, to display a file selector.
- Select the required spectrum or spectra that you wish to perform the calculations on.

The spectra are displayed in the Graph Window.

3. Enter the **Start** and **End** values for the Thickness Wavelength Fringes.

OR

You may use the vertical cursor to determine the exact values. Place the mouse in the field whose value you want to determine e.g. **Start**. Select **Vertical Cursor Cont** from the View menu. Use the mouse to position the vertical cursor at the required location. The value in the field updates to the position of the vertical cursor. Click the mouse outside the field to accept the value.

4. Enter the number of **Fringes** in the specified wavelength range.

OR

Select **Automatic**.

The software automatically searches for fringes in the specified wavelength range.

5. Enter the **Refractive Index** value of the material.

6. Enter the **Angle of incidence**.

This is the angle of light incident on the sample.

7. Select **Angströms** or **Micrometers**.

The film thickness will be calculated using the chosen units.

8. Click **Calculate**.

The film thickness is calculated and the results displayed in the Result Window.

The calculation is performed using the following formula:

$$d = \frac{N * \lambda_1 * \lambda_2}{2(\lambda_2 - \lambda_1) * (n^2 - \sin^2 \alpha)^{1/2}} \text{ [nm]}$$

d = Thickness

N = Fringes (max. between the specified wavelength)

λ_1 = Wavelength start

λ_2 = Wavelength end

n = Refractive index

α = Angle of incidence

Result Template

Numerical results, for example, a Slope calculation, are presented and stored in tabular form. The format and contents of each table is governed by a results template.

A results template is a text file consisting solely of standard ASCII characters. It holds a frame of texts - e.g., titles, comments, headings, etc. - together with special keywords, characterized by a leading \$ sign. The keywords serve as tokens for values that are read from the system - e.g., date, time, instrument parameters, ordinate and abscissa readings, etc.

Default results templates are included with the software. If required, you can generate your own results templates for customized reports. A text editor such as Notepad, that generates ASCII files, can be used to generate a results template. Every line in the results template can contain up to 200 characters. The PC screen display actual is limited to 80 characters. Take care when generating a result template that any text you wish to have on the screen is actually visible.

Naming and Saving a Results Template

A results template should be saved in the directory `.\uvwinlab`. It must have the extension `.txt`, and the name should reflect the type of calculation. For example, the default template for the area calculation has the filename `dh_area.txt`.

Example Results Template

Good examples of customized results templates are the default templates (`dh_area.txt`, `dh_aritv.txt`, `dh_avsv.txt`, `dh_hh.txt`, `dh_enzac.txt`, `dh_slope.txt`, `dh_subs.txt`, or `dh_film.txt`). These results templates governs the format of the output for the numeric values of the Advanced Spectroscopy Software module Arithmetic.

e.g. Area Template

\$HEADER

Analyst: \$ANALYST

Date: \$DATE

Time: \$TIME

Name	Range	Baseline	Total Area	Area to Base
Data Set	from to	from to		

\$SI \$AREAC01[04.02] \$AREAC02[04.02] \$AREAB01[04.02] \$AREAB02[04.02] \$AREAT \$AREAA
\$REPEAT

Selecting the calculations to be listed in the Arithmetic menu and changing the default templates for calculations

1. From the Utilities menu select **Option**.
The Option dialog is displayed.
2. Click **Selected Calculation**.
The Selected Calculation dialog is displayed.
3. Select the required calculations **On**.
Any calculations that are switched **Off** will not appear in the Arithmetic menu.
The following calculations have a result template defined when selected **On**:
area, arithmetic value, average value, calculate height, slope, film thickness.
4. To change a result template, double-click on the template name.
A file selector is displayed.
5. Select the required template.
6. Click **OK**.
The file selector closes and the calculation result template is updated.

Template Keywords

The keywords are placeholders for the current value of a variable, for example, \$DATE represents the current date.

	Keyword
General	
Header information	\$HEADER
Analyst name	\$ANALYST
PC Date	\$DATE
PC Time	\$TIME
Spectrum name	\$SI
Spectrum information	\$SC
Format of numerical values e.g. \$AREAT[02.02] The total area value is output with two digits before and two digits after the decimal sign.	[XX.XX]
If a line in a results template should be used from sample to sample, it must end with the keyword \$REPEAT. If \$REPEAT is not present, the screen display is presented and cleared after each sample.	\$REPEAT
Area	
Abscissa range start for the area calculation	\$AREAC01
Abscissa range end for the area calculation	\$AREAC02
Baseline abscissa range start for the area calculation	\$AREAB01

Baseline abscissa range end for the area calculation	\$AREAB02
Total area	\$AREAT
Area to base	\$AREAA
Arithmetic Value	
Ordinate value first wavelength read out	\$ARIV01
First wavelength	\$ARIW01
Ordinate value second wavelength read out	\$ARIV02
Second wavelength	\$ARIW02
Ordinate value third wavelength read out	\$ARIV03
Third wavelength	\$ARIW03
First factor	\$ARIF01
Second factor	\$ARIF02
Arithmetic result	\$ARIR
Average, Standard Deviation, Root Mean Square	
Abscissa range start for the calculation	\$AVR01
Abscissa range end for the calculation	\$AVR02
Average value	\$AVA01
Standard deviation value	\$AVS01

Root mean square value	\$AVRMS01
Calculate Height	
Peak wavelength	\$CHHP01
Abscissa range start for the calculation	\$CHHR01
Abscissa range end for the calculation	\$CHHR02
Total height	\$CHHT01
Height to base	\$CHHB01
Slope	
Abscissa range start for the calculation	\$SLOPER01
Abscissa range end for the calculation	\$SLOPER02
Slope	\$SLOPES01
Intercept	\$SLOPEI01
Standard Deviation	\$SLOPESTD01
Factor	\$SLOPEF01
dA/min	\$SLOPEDA01
Enzyme Activity EA	\$SLOPEEA01
Delta A	\$SLOPEAD01
Substrate	\$SLOPEUS01

Film Thickness	
Abscissa range start for the calculation	\$FILMR01
Abscissa range end for the calculation	\$FILMR02
Fringes	\$FILMF01
Refractive Index	\$FILMRI01
Angle of Incidence	\$FILMA01
Film Thickness	\$FILMT01

Single Read **6**
Application

Introduction

NOTE: *For general information on using Applications, see Using Application Methods starting on page 65.*

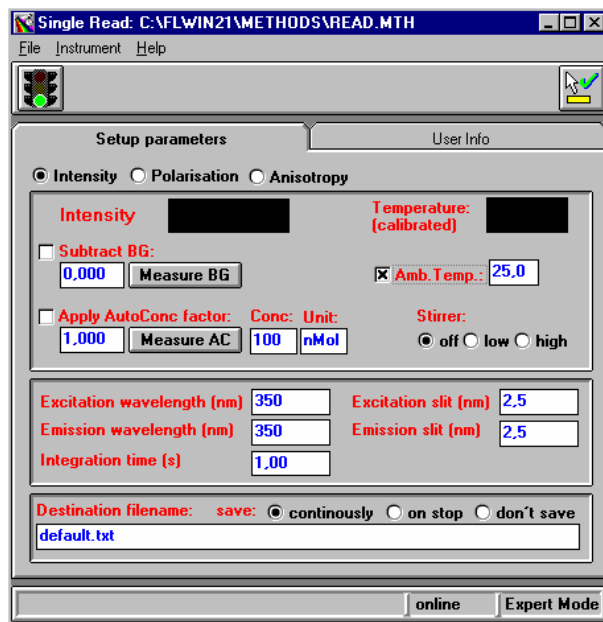
The Single Read application enables measurements (intensity, concentration, polarization, anisotropy) to be made at fixed wavelengths. The data are saved on the hard disk in an Excel compatible file format.

NOTE: *Polarization and anisotropy cannot be measured with the LS 45.*

Using the Application

1. Select **Read** from the Application menu.

The Read application is opened.



2. Select the required reading.
The options available will depend on the accessories fitted to your instrument.
3. Enter the required Setup parameters.
The parameters available will depend on which type of reading you selected. The parameters are discussed below.
4. Click on the green traffic light (Start / Stop button) in the toolbar to start the method.
The intensity/value then scrolls (is automatically repeated) until you click on the red traffic light.
5. To exit the application, select **Exit** from the File menu.

Setting the Parameters

Setup Parameters tab

Intensity Parameters

Parameter	Description
Intensity / Concentration	The intensity or concentration is displayed here. The concentration is displayed if the Apply AutoConc. factor option is selected.
Subtract BG	<p>Use the background (BG) subtraction option to automatically subtract the given background from the signal. The background intensity is stored in the results file. Enter the required intensity or click Measure BG to measure the background intensity. The instrument is setup with the method parameters (wavelengths, slit widths) before the measurement is done.</p> <p>NOTE: <i>Four different background intensities are used if the cell changer accessory is fitted. To (re)measure the background for a cuvette first click on the desired cuvette position in the cell changer accessory box and then click Measure BG.</i></p>

Parameter	Description
Apply AutoConc Factor	<p>Select this option if you want to display the signal in terms of concentration instead of intensity, assuming the signal intensity is directly proportional to concentration. The concentration is calculated as $Conc = ACFactor \times (Intensity - Background)$. The factor is stored in the results file.</p> <p>Enter the required factor or to determine the factor automatically, place a cuvette containing a sample of known concentration (reference sample) in the sample compartment, enter the concentration value (Conc) and the concentration unit (Unit) and then click Measure AC. The factor is determined using the given wavelengths, slitwidths and integration time and calculated as $ACFactor = Conc / (Intensity - Background)$.</p> <p>NOTE: <i>Only one factor is available, even if the 4 cell changer accessory is fitted.</i></p>

Polarization Parameters

Parameter	Description
Polarization	<p>The polarization or value is displayed here. The polarization is measured using the following equation:</p> $Polarisation = \frac{I_{vv} - (GF \cdot I_{vh})}{I_{vv} + (GF \cdot I_{vh})}$ <p>where I_{vv} is the intensity with the polarizers vertical and vertical (excitation and emission), I_{vh} is the intensity with the polarizers vertical and horizontal (excitation and emission) and GF is the Grating Factor.</p>

Parameter	Description
GF	<p>The Grating Factor GF corrects for instrumental polarization. It can be entered manually or to calculate it automatically, insert a depolarizing sample into the cuvette holder and click Calc. GF to calculate the grating factor. The grating factor is calculated using the following equation:</p> $GF = \frac{I_{hv}}{I_{hh}}$ <p>where I_{hv} is the intensity with the polarizers horizontal and vertical (excitation and emission), and I_{hh} is the intensity with the polarizers horizontal and horizontal (excitation and emission).</p>

Anisotropy Parameters

Parameter	Description
Anisotropy	<p>The anisotropy value is displayed here. The anisotropy is measured using the following equation:</p> $Anisotropy = \frac{I_{vv} - (GF \cdot I_{vh})}{I_{vv} + (2 \cdot GF \cdot I_{vh})}$ <p>where I_{vv} is the intensity with the polarizers vertical and vertical (excitation and emission), I_{vh} is the intensity with the polarizers vertical and horizontal (excitation and emission) and GF is the Grating Factor.</p>

Parameter	Description
GF	<p>The Grating Factor GF corrects for instrumental polarization. It can be entered manually or to calculate it automatically, insert a depolarizing sample into the cuvette holder and click Calc. GF to calculate the grating factor. The grating factor is calculated using the following equation:</p> $GF = \frac{I_{hv}}{I_{hh}}$ <p>where I_{hv} is the intensity with the polarizers horizontal and vertical (excitation and emission), and I_{hh} is the intensity with the polarizers horizontal and horizontal (excitation and emission).</p>

Biokinetics Accessory Parameters

Parameter	Description
Temperature	<p>Here the temperature from the biokinetics accessory is displayed. The displayed sample temperature TSample is (depending on the status of the Calibrate temperature option) either the uncorrected block temperature Tblock or the corrected block temperature, calculated from the block temperature Tblock using the calculated temperature calibration factor and the ambient temperature Tamb:</p> $T_{Sample} = factor * (T_{Block} - T_{Amb}) + T_{Block}$ <p>NOTE: The Calibrate temperature option is only available if a temperature calibration factor was determined and a value is entered for Ambient Temperature. A calibration factor is determined using the <i>Instrument Setup Application</i> (see <i>Biokinetics Accessory Parameters</i> starting on page 55).</p>

Other Accessories

- If a Stirrer is fitted, you can select one of the three stirrer speeds. Select the low stirrer speed for cell suspensions and biochemical reactions and high stirrer speed for very rapid mixing.
- If a Cell Changer is fitted, to select a cuvette position, click on the corresponding radio button (only visible if the 4 cellchanger accessory is fitted).

Excitation / Emission Parameters

Parameter	Description
Excitation wavelength	Enter the excitation wavelength in nm.
Emission wavelength	Enter the emission wavelength in nm.
Excitation slit	The excitation slit width is the spectral bandwidth of the excitation monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.
Emission slit	The emission slit width is the spectral bandwidth of the emission monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.
Integration time	Enter the required integration time in seconds or minutes depending on the time unit selected. The optimal signal-to-noise ratio is obtained by selecting a long integration time. However for fast kinetics a short integration time should be used. Typical values range from 0.1 to 1.0 seconds.

Save Parameters

Parameter	Description
Save	<p>Select one of the following options to determine how the data are saved (the data are saved in an Excel compatible format using tabs as delimiter):</p> <p>continuously - Every time a new result is measured it is saved to the results file with its collection time. Use this option to measure intensity changes against time. The data interval is determined by the integration time. However, the timing is less accurate than in the TimeDrive application.</p> <p>on stop - The current result is saved to the results file with the collection time when data collection is stopped. This enables you to wait for the intensity to equilibrate, for example when the measurement is based on a reaction or chelation.</p> <p>don't save - The data are not saved. Use this option if you for example want to use the read application to optimize the instrument setup for other applications.</p>
Destination filename	<p>Enter the filename for saving the data or double-click in the textbox and a file selector will appear to enable you to overwrite a file. The result files are always stored in the default FL WinLab data directory.</p>

User Info tab

On this tab, information about the sample and analyst are entered.

Parameter	Description
Analyst	The name is automatically taken from the configuration dialog, but can be altered for documentation purposes. The name is saved in the header of any collected datasets and, if a method is saved, in the header of the method.
Sample info	This information will be 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' field of the dataset, and can be viewed using Report Builder.
last modified	This shows the date the current method was last saved.
by	This shows the name of the analyst who last saved the method.

Parameter	Description
locked	This option enables the locking of all entries in a method. The option can be altered in Expert mode only. If the option is selected all entry fields and the Method save command are disabled.
Comments	The comment in this textbox is saved in the method header and is also displayed in the method window of the software, but is not saved in the dataset (use Sample info for comments to be saved in datasets).

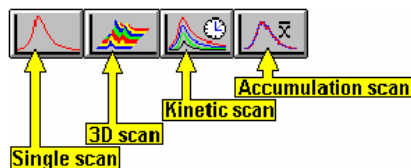
Scan Application **7**

Introduction

NOTE: For general information on using Applications, see Using Application Methods starting on page 65.

The Scan application is used for collecting various types of spectral data in a variety of modes (fluorescence, phosphorescence and bioluminescence).

There are four scan modes, selected by clicking on the icons:



Single scan mode

There are five types of scan available from the Single scan mode:

- An excitation spectrum recorded using a fixed emission wavelength. The start and final wavelengths refer to the excitation monochromator.
- An emission spectrum recorded using a fixed excitation wavelength. The start and final wavelengths refer to the emission monochromator.
- A synchronous scan, where both monochromators are scanned simultaneously with a constant wavelength difference between the excitation and emission monochromators. This technique is used for rapid screening in environmental analysis, for example for differentiating between various types of crude oil (oil fingerprinting), since the technique greatly simplifies the spectra of complex mixtures with overlapping spectral components. In a synchronous scan, the start and final wavelengths always refer to the excitation monochromator and the emission monochromator always starts at a higher wavelength than the excitation monochromator.

- A synchronous scan, where both monochromators are scanned simultaneously with a constant energy difference between excitation and emission monochromators. The emission monochromator accelerates relative to the excitation monochromator. In a synchronous energy scan, a synchronous spectrum is recorded at a constant energy difference (wavenumber) between excitation and emission monochromator. This technique can be used for the investigation of very complex mixtures where the spectral sensitivity at constant wavelength difference is too low. Scanning at constant energy difference between the monochromators has an advantage over the recording of spectra at constant wavelength difference by having a higher spectral resolution and lower background fluorescence.
- A pre-scan using either one or both monochromators can be used to determine the optimum excitation and emission wavelength for unknown samples.

3-D scan mode

In 3-D scan mode, four scan types - excitation scan, emission scan, synchronous scan at constant wavelength and synchronous scan at constant energy difference are available. In each case, a scan is repeated automatically over the range parameters entered, however between each scan the fixed parameter is incremented. For an emission 3D scan, an emission scan is repeated, but each time the excitation wavelength is incremented. This results in the collection of excitation and emission data in a single graphic.

When a 3D Scan method is run, data is automatically saved as a 3D dataset with the same name as the first file in the run, with the extension SP3. The 3D graphics can be viewed and edited with the 3D View application.

Kinetic scan mode

In the Kinetic scan mode, four scan types - excitation scan, emission scan, synchronous scan at constant wavelength and synchronous scan at constant energy difference, are available. Spectra are collected with respect to time; after an external contact closure; or after a keyboard entry.

When a Kinetic method is run, the data is automatically saved as a 3D dataset with the same name as the first file in the run, with the extension SP3. The 3D graphics can be viewed and edited with the 3D View application.

Accumulation scan mode

In Accumulation scan mode, four scan types - excitation scan, emission scan, synchronous scan at constant wavelength and synchronous scan at constant energy, are available. After all the spectra have been scanned they are averaged in order to eliminate any random noise and therefore increase the signal-to-noise ratio. The parameters for the individual scans are identical to the parameters for the scan types in single scan mode.

Subsequent to clicking on the green traffic light, the individual spectra will be scanned and superimposed in real time. Once the selected number of accumulation scans has been carried out, all the spectra will be averaged onto a single spectrum and displayed. The individual raw spectra, that is the spectra from which the average spectrum was determined, will not be saved.

Performing a Pre-Scan

Background

There are three ways of recording a pre-scan:

An Excitation monochromator pre-scan (with the emission monochromator at constant wavelength).

Select the start and end wavelengths for the excitation monochromator by clicking on the excitation from and to boxes and entering the wavelengths. The starting wavelength must be smaller than the final wavelength. The emission monochromator from and to boxes should be set to the same wavelength.

The excitation slit width should be set between 2.5 nm and 5 nm and the emission slit width set between 10 nm and 15 nm. In order to obtain the optimal signal-to-noise ratio enter a slow scan speed such as 150 nm/min. For photochemically sensitive samples select a high scan speed.

At the end of the pre-scan the maximum intensity (*) and the corresponding excitation wavelength is displayed. Following the pre-scan, the excitation monochromator will be set to the wavelength of maximum intensity and the value inserted in the excitation box.

An Emission monochromator pre-scan (with the excitation monochromator at constant wavelength).

Select the start and end wavelengths for the emission monochromator by clicking on the emission from and to boxes and entering the wavelengths. The starting wavelength must be smaller than the final wavelength. The excitation monochromator from and to boxes should be set to the same wavelength. Typically this should correspond to the wavelength of maximum absorption, if known.

The excitation slit width should be set between 10 nm and 15 nm and the emission slit width between 2.5 nm and 5 nm. In order to obtain the optimal signal-to-noise ratio enter a slow scan speed. For photochemically sensitive samples select a high scan speed.

At the end of the pre-scan the maximum intensity (*) and the corresponding emission wavelength is displayed.

Following the pre-scan, the emission monochromator will be set to the wavelength of maximum intensity and the value is inserted in the emission box.

A combined Excitation/emission pre-scan (automatic sequence of the above pre-scans).

Select the start and end wavelengths for the excitation monochromator by clicking on the excitation from and to boxes and entering the wavelengths. The starting wavelength must be smaller than the final wavelength. The range should cover the wavelength of maximum absorption, if known. Select the start and end wavelengths for the emission monochromator by clicking on the emission from and to boxes and entering the wavelengths. The starting wavelength must be smaller than the final wavelength.

Both the excitation and emission slit width should be set between 2.5 nm and 5 nm. In order to obtain the optimal signal-to-noise ratio enter a slow scan speed. For photochemically sensitive samples select a high scan speed.

- To perform a pre-scan over the entire wavelength range of the excitation and emission monochromators, click **Full range**.
The minimum and maximum wavelengths for the excitation and emission monochromator will then be automatically inserted in the corresponding boxes.

Functional description

On starting the pre-scan, the excitation and emission scans will automatically be performed in the following manner:

Excitation Pre-Scan

- The emission monochromator will be set to 'zero order' and an excitation spectrum will be recorded over the entire wavelength range.
- On completion of the excitation scan, the excitation monochromator will be set to the wavelength that was found to produce maximum intensity.

Emission Pre-Scan

- The excitation monochromator will be set to a fixed wavelength (either user-input or that obtained from the excitation Pre-Scan) and an emission spectrum will be recorded over the entire wavelength range.
- On completion of the emission scan, the emission monochromator will be set to the wavelength that was found to produce maximum intensity.

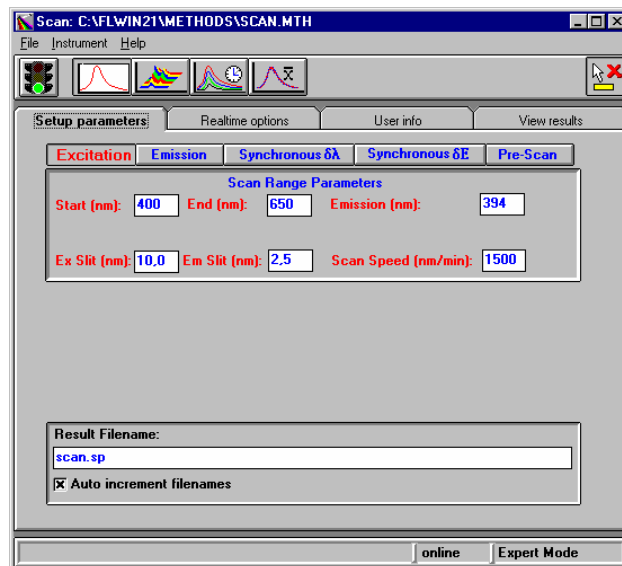
Following the pre-scan, the excitation and emission monochromators will be set to the wavelengths producing maximum intensity and the values are entered in the excitation and emission boxes. However, the Pre-Scan function is not intended to supply photophysically exact data: it should be used only as a starting point for unknown samples. If the intensity exceeds 999.999 (the maximum signal for the instrument), then no sensible peak information can be derived from the data. If the sample is highly light-scattering, then harmonic order peaks can obscure the fluorescence peaks. In addition it is also possible to automatically mark the Rayleigh and Raman scatter bands together with any second order peaks.

NOTE: *The pre-scan button appears only in the Single scan mode.*

Using the Application

1. Select **Scan** from the Application menu.

The Scan dialog is displayed.



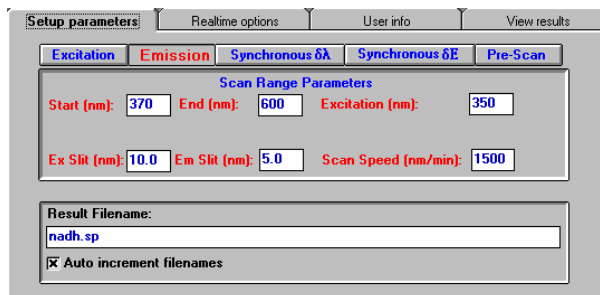
2. Select the required scan mode (single, 3D, kinetic, or accumulation) from the large toolbar icons next to the traffic lights icon.
3. Select the scan type (**Excitation**, **Emission**, **Synchronous** or **Pre-scan**) by clicking the relevant button.
4. Enter the required parameters on each tab of the software. Use the tabs to move between pages.
5. Click on the green traffic light to start the application.
6. Use the icons in the toolbar to format the graphic display, as discussed in *Application Toolbars* on page 74.
7. To exit the application, select **Exit** from the File menu.

Setting the Parameters

Setup parameters tab

The appearance of the Setup parameters page depends on the scan mode and type.

Single Scan Range Parameters



Parameter	Description
Start	Scan start wavelength. This must be smaller than the end wavelength.
End	Scan end wavelength. This must be greater than the start wavelength.
Excitation or Emission	Fixed monochromator's wavelength in nm. For excitation scans this is the position of the emission monochromator, and should be at a wavelength greater than the end of the excitation scan. For emission scans this is the position of the excitation monochromator, which should be at a wavelength shorter than the start of the emission scan.

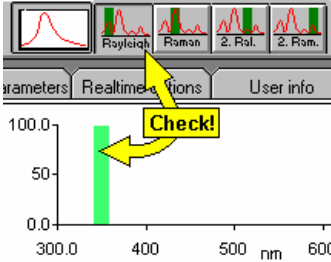
Parameter	Description
Ex slit	The excitation slit width is the spectral bandwidth of the excitation monochromator. In order to obtain the best resolution for excitation spectra with narrow bands, select a narrow slit width, for example 2.5 or 5 nm. To record excitation spectra with broad bands, you may select a wide excitation slit width. The best signal-to-noise ratio is obtained by selecting a large slit width.
Em slit	The emission slit width is the spectral bandwidth of the emission monochromator. For good resolution of spectra with narrow bands, select a narrow width for the emission slit. To record emission spectra with broad bands, select a large emission slit width. The best signal-to-noise ratio is obtained by selecting a large slit width.
Scan speed	Since the data interval for scans is always 0.5 nm, the scan speed determines the integration time of data acquisition. For example a scan speed of 300 nm/min (5 nm/sec) is equivalent to an integration time of 0.1 sec. (Integration time = data interval / scan speed). The optimal signal-to-noise ratio is obtained by selecting a slow scanning speed. However, for photochemically sensitive samples a fast scan speed should be used.
Delta lambda	Only available for Synchronous $\delta\lambda$ scans. The wavelength difference between the monochromators is typically set between 10 nm and 100 nm, which should correspond to the Stoke shift between the excitation and emission maxima of the compound of interest. Much sharper bands are obtained compared to excitation or emission spectra. The synchronous spectrum of a complex mixture shows simpler spectral structure and thus achieves a higher degree of sensitivity and selectivity.

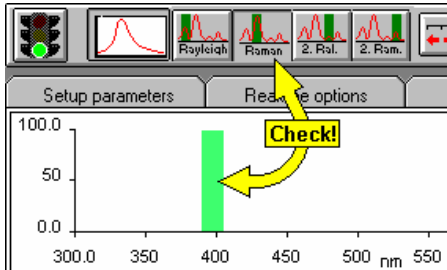
Parameter	Description
Delta energy	Only available for Synchronous δE scans. Here the energy difference between both monochromators is entered and are typically within the range -1000 to -4000 cm^{-1} (the energy difference has a negative sign as the emission monochromator always starts at a higher wavelength than the excitation monochromator. Synchronous energy scans provide better selectivity across the spectral range, particularly for complex mixtures.

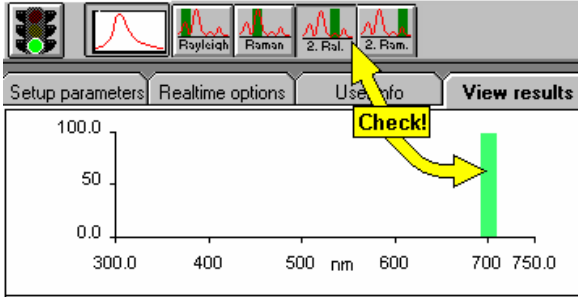
Pre-Scan Parameters

Parameter	Description
Full Range	Clicking Full Range sets the Excitation Range and Emission Range to the maximum ranges of both monochromators.
Excitation Range	Select start and end wavelengths for the excitation monochromator. The starting wavelength must be smaller than the end wavelength. Set the From and To boxes to be the same to perform an emission pre-scan only.
Emission Range	Select the start and end wavelengths for the emission monochromator. Starting wavelength must be smaller than the end wavelength. Set the From and To boxes to the same wavelength to perform an excitation pre-scan only.

Parameter	Description
Ex slit	The excitation slit width is the spectral bandwidth of the excitation monochromator. In order to obtain the best resolution for spectra with narrow bands, select a narrow slit width, for example 2.5 or 5 nm. To record spectra with broad bands, select a wide excitation slit width. The best signal-to-noise ratio is obtained by selecting a large slit width.
Em slit	The emission slit width is the spectral bandwidth of the emission monochromator. For a good resolution of spectra with narrow bands, select a narrow width for the emission slit. To record spectra with broad bands, select a large emission slit width. The best signal-to-noise ratio is obtained by selecting a large slit width.
Scan speed	Since the data interval for scans is always 0.5 nm, the scan speed determines the integration time of the data acquisition. For example a scan speed of 300 nm/min (5 nm/sec) is equivalent to an integration time of 0.1 sec. (Integration time = data interval / scan speed). The optimal signal-to-noise ratio is obtained by selecting a slow scanning speed. However for photochemically sensitive samples a fast scan speed should be used.
Auto Peak Find	To exclude Rayleigh, Raman and second order scatter peaks from the Automatic Peak Find . A series of check buttons appear in the toolbar during the PreScan data collection, and clicking on one of these superimposes a green area on the scan graphic indicating the expected region for that scatter peak. If a peak is seen inside the green area, then the user can be suspicious of its validity.
Solvent	Lists the most frequently used solvents - Water, Ethanol, Cyclohexane, Chloroform, and Carbon tetrachloride . The wavenumber difference between excitation wavelength and the Raman band will automatically be entered as the Raman Wavenumber .

Parameter	Description
<p>Raman Wavenumber</p>	<p>To enter the Raman wavenumber for a solvent not in the Solvent list, select Other as the Solvent and enter the Raman energy for the solvent to be used, taking into account that the Raman energy has a negative sign as Raman scatter is of longer wavelength and thus possesses less energy than the excitation radiation.</p>
<p>Rayleigh scatter check</p>	<p>Light scattered by the sample at the excitation wavelength is called Rayleigh scatter. Selecting the option will exclude the area where this scatter is expected from the peak find:</p> $\Delta Wl = \frac{ExSlitWidth + EmSlitWidth}{2}$ <p>excluded area = $ExWl - \Delta Wl$ to $ExWl + \Delta Wl$</p> <p>The excluded area can be displayed graphically by using the Display Rayleigh peak area button on the View Results page:</p>  <p>The screenshot shows a software interface with a menu bar containing 'Parameters', 'Realtime', 'Conditions', and 'User info'. Below the menu bar is a row of five small plot thumbnails labeled 'Rayleigh', 'Raman', '2. Pol.', and '2. Pol.'. The main plot area shows a graph with a y-axis from 0.0 to 100.0 and an x-axis from 300.0 to 600 nm. A green vertical bar is highlighted on the graph at approximately 350 nm, with a yellow arrow pointing to it from a yellow box labeled 'Check!'.</p>

Parameter	Description
Raman scatter check	<p>Photons can be scattered by the solvent, resulting in an emission at a predictable longer wavelength than the excitation. This is Raman scatter. The position of the Raman scatter peak depends on solvent-solvent interactions (hydrogen bonding, Van Der Waal's interactions). Select the Raman Wavenumber as discussed above, the area where the Raman peak of the selected solvent is expected will now be excluded from the automatic peak find:</p> $RamanWl = \frac{ExWl - \frac{1}{RamanWNR}}{ExWl \cdot \frac{1}{RamanWNR}}$ $\Delta Wl = \frac{ExSlitWidth + EmSlitWidth}{2}$ <p>excluded area = $RamanWl - \Delta Wl$ to $RamanWl + \Delta Wl$</p> <p>The excluded area can be displayed graphically by using the Display Raman peak area button on the View Results page:</p> 

Parameter	Description
<p>2nd order check</p>	<p>When the emission monochromator is scanned through a wavelength equal to 2 or 3 times the excitation wavelength, symmetrical peaks are observed, due to excitation light being transmitted to the detector. These peaks are second order and third order scatter respectively and are caused by characteristics of all gratings, which transmit the fundamental, selected wavelength but additionally harmonics of this wavelength.</p> <p>Selecting 2nd order check will automatically exclude any second order peaks in the emission spectrum from the automatic peak find. The excluded area can be displayed graphically by using the two Display second order peak area buttons on the View Results page:</p> 

3D Scan Range Parameters

3D Scan Range Parameters

Number of scans: Excitation increment (nm):

Parameter	Description
<p>Number of scans</p>	<p>The number of required scans must be greater than 1.</p>

Parameter	Description
increment	Enter the increments per scan. A typical parameter set for a 3D scan would be: Emission scan type, range 400-650 nm. Excitation wavelength 250 nm, increment 5 nm, number of scans 30. Slits 5/5 nm (Ex/Em). This set would produce 30 emission scans over the range 400-650 nm, covering the excitation range 250-400 nm.

Kinetic Scan Range Parameters

Kinetic Scan Range Parameters

Number of scans: Repeat with time

Delay (secs): Repeat on User Prompt

Repeat on External Contact

Repeat on Sipper Contact

Auto lamp off Delay before meas.[s]

Parameter	Description
Number of scans	The number of scans must be greater than 1.
Delay	Enter the delay time between successive kinetic scan cycles.
Repeat with time	Select this option if you wish to start each kinetic scan after a defined delay, including the first cycle.
Repeat on User Prompt	Select this option if you wish to start each kinetic scan manually, so that the sample can be removed/replaced between scans. A dialog box will appear before each scan, click OK or press ENTER on the keyboard to start the next cycle.
Repeat on External Contact	Select this option if you wish to start each kinetic scan from the external remote contact closure, to allow automation when coupled to an external device, for example an autosampler.
Repeat on Sipper Contact	Select this option to start each kinetic cycle from the external event contact closure, or the sipper button.

Parameter	Description
Auto lamp off	Select this option to automatically switch off the source lamp between measurements. This is useful to preserve the source lifetime and to avoid photobleaching.
Delay before meas.	Enter the delay between turning the source on and starting measurement (the instrument needs no delay, the source stabilizes instantly) to allow for sample stabilization. If used together with the kinetic cycle delay, both delays are added together.

Accumulation Scan Parameters



Parameter	Description
Number of Accumulation scans	The number of accumulated scans must be greater than 1.

Datafile Saving Parameters

Parameter	Description
Result Filename	Enter the name of the file for saving the data or double-click in the textbox and a file selector will be displayed to enable you to overwrite a previous file. NOTE: <i>Result files are always stored in the default FL WinLab data directory.</i>

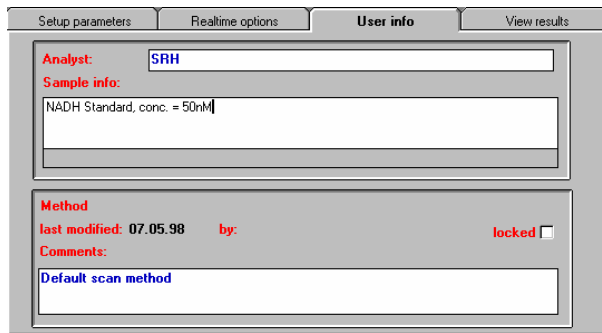
Parameter	Description
Auto increment filenames	If this option is selected the application generates a new numbered filename for each run. The base filename is given as the Result Filename , and is extended with a number starting from #001. If a file with this filename already exists on the hard disk in the current FL WinLab data directory, the number is incremented by one, until the filename is unique. The numbering process can be forced to start from a different number by adding a number to the base filename, for example Test#5.sp would make the first generated filename Test#005.sp and the second Test#006.sp and so on.

Realtime options tab

Parameter	Description
Response width	The response width must be at least three times the data interval (which is 0.5 nm). It cannot be larger than 99 times the data interval and it must be an uneven multiple of the data interval. Valid values for the width are therefore 1.5, 2.5, 3.5, up to 44.5. The program inserts the nearest valid response if an invalid value is entered, for example a response of 10 becomes 9.5. Selecting Auto automatically sets the response width according to the scanning slit width

Parameter	Description
Auto Background Subtract	Select this option to automatically subtract the chosen Background Spectrum from each spectrum in real-time. If the data type or wavelength range of the background spectrum is incompatible with the scan range, or the specified background spectrum cannot be found, an appropriate error message will appear on starting the scan. The name of the background spectrum is stored in the header of the dataset in the FL memo field. When selecting the background spectrum, double-click on the Background Spectrum box to display a file selector.
Ordinate label	The label will be displayed on the ordinate axis of the graph and will be stored in the dataset.
Ordinate Max	Sets the default ordinate maximum value. Leave empty to retain the same ordinate value as last time.
Ordinate Min	Sets the default ordinate minimum value. Leave empty to retain the same ordinate value as last time.
Auto-clear curves	If this option is selected all curves are deleted from the graphic before a measurement starts, rather than each successive scan being superimposed on the previous scans. The curves are not deleted from the hard disk.

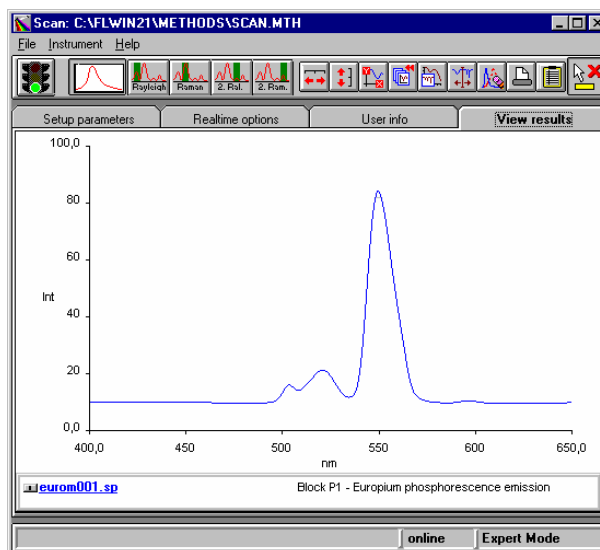
User info tab



Parameter	Description
Analyst	The name is automatically taken from the configuration dialog, but can be altered for documentation purposes. The name is saved in the header of any collected datasets and, if a method is saved, in the header of the method.
Sample info	This information will be 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' field of the dataset, and can be viewed using Report Builder.
last modified	This shows the date the current method was last saved.
by	This shows the name of the analyst who last saved the method.
locked	This option enables the locking of all entries in a method. The option can be altered in Expert mode only. If the option is selected all entry fields and the Method save command are disabled.
Comments	The comment in this textbox is saved in the method header and is also displayed in the method window of the software, but is not saved in the dataset (use Sample info for comments to be saved in datasets).

View results tab

This page contains a toolbar in which graphics icons appear, depending on the selected scan mode. For further details on viewing data in real-time see *Generic Viewing Features* starting on page 74.



TimeDrive **8**
Application

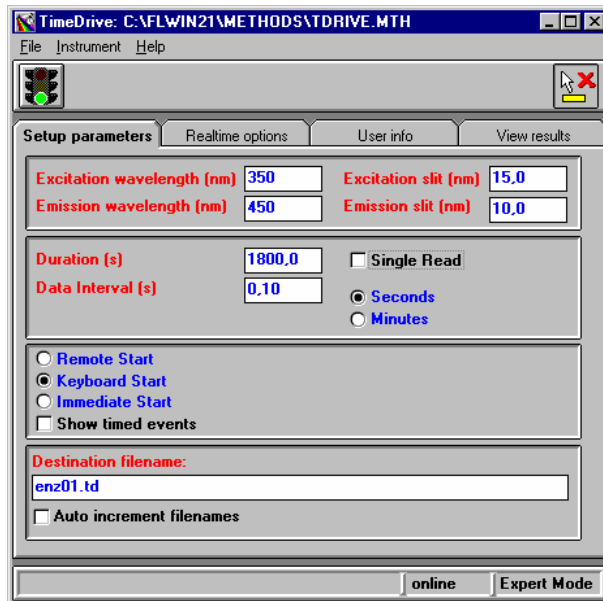
Introduction

NOTE: *For general information on using Applications, see Using Application Methods starting on page 65.*

The TimeDrive application enables time-dependent luminescence measurements (fluorescence, phosphorescence and bioluminescence) to be made at fixed wavelengths, with defined intervals over a specified period of time. The data are recorded and saved on the hard disk in a file with the file extension .TD.

Using the Application

1. Select **TimeDrive** from the Application menu.
The TimeDrive application is opened.



2. Enter the required parameters on each tab of the application.
The parameters are discussed below.
3. Click on the green traffic light in the toolbar to start the method.
The View Results tab is displayed automatically, use the icons in the toolbar to format the graphic display.
4. To exit the application, select **Exit** from the File menu.

Setting the Parameters

Setup Parameters tab

Parameter	Description
Excitation wavelength	Enter the excitation wavelength in nm.
Emission wavelength	Enter the emission wavelength in nm.
Excitation slit	The excitation slit width is the spectral bandwidth of the excitation monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.
Emission slit	The emission slit width is the spectral bandwidth of the emission monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.
Duration	Enter the total time for data collection for the TimeDrive in seconds or minutes, depending on the time unit selected.
Data interval	Enter the interval between two measuring points in seconds or minutes depending on the time unit selected. In TimeDrive mode the data interval is equivalent to the integration time.
Single Read	In single read mode the timing of the measurement is controlled by the PC rather than by the instrument. This has the drawback that the timing is less accurate, but it allows for more flexibility. In single read mode the integration time can be different from the data interval, furthermore it is possible to monitor the temperature of the sample, if the biokinetics accessory is fitted. The longest data interval is 1000 seconds (10 seconds in true TimeDrive mode). The following * options only become available when Single Read is selected.

Parameter	Description
* Integration time	Enter the required integration time in seconds or minutes, depending on the time unit selected. The optimal signal-to-noise ratio is obtained by selecting a long integration time, however for fast kinetics a short integration time should be used. The integration time must be at least 1 second shorter than the data interval since the PC needs some time to receive and display the measured data and issue new commands.
* Show temperature	This option, only available if the Biokinetics Accessory is fitted, enables you to monitor the temperature of the sample during data collection. Once data collection starts, the data file with the extension .TD plus a second file using the same name but with the file extension .TDT (containing the uncorrected temperature of the block) will be displayed on the View results tab.
* Auto lamp off	If this option is selected the lamp is switched off automatically between measurements. This avoids photobleaching of the sample and preserves the source lifetime for long TimeDrives.
* Delay before measurement	This determines the delay between turning the source on and starting measurement (the instrument needs no delay, the source stabilizes instantly). This function allows for sample stabilization.
* Background subtraction	This automatically subtracts the background from the signal. The background intensity is stored in the header of the dataset in the FL memo field.
Start options	The options of Remote Start , Keyboard Start , Immediate Start , and Show timed events are described in <i>Start/stop options available when setting up application methods</i> on page 70.

Parameter	Description
Result Filename	Enter the name of the file for saving the data or double-click in the textbox and a file selector will be displayed to enable you to overwrite a previous file. NOTE: <i>Result files are always stored in the default FL WinLab data directory.</i>
Auto increment filenames	If this option is selected the application generates a new numbered filename for each run. The base filename is given as the Result Filename , and is extended with a number starting from #001. If a file with this filename already exists on the hard disk in the current FL WinLab data directory, the number is incremented by one, until the filename is unique. The numbering process can be forced to start from a different number by adding a number to the base filename, for example Test#5.sp would make the first generated filename Test#005.sp and the second Test#006.sp and so on.

Realtime options tab

Setup parameters	Realtime options	User info	View results
Response			
width (s): <input type="text" value="3.00"/>			
Background <input checked="" type="checkbox"/> Subtract			
Intensity <input type="text" value="0.000"/> <input type="button" value="Measure background"/>			
Graph			
Ordinate label <input type="text" value="Int"/> <input checked="" type="checkbox"/> Auto-clear curves			
Ordinate Max <input type="text"/>			
Ordinate Min <input type="text"/>			

Parameter	Description
Response width	The response width must be at least three times the data interval. It cannot be larger than 99 times the data interval and it must be an uneven multiple of the data interval, for example for a data interval of 1 sec valid values for the filter width are 3,5,7, up to 99 sec. The program automatically calculates the next (smaller) valid response if an invalid value is entered, for the above example a response of 101 becomes 99, and 1 becomes 3.
Background Subtract	Select this option to automatically subtract the given Intensity from the signal. The Intensity can either be entered manually or automatically by clicking Measure background .
Ordinate label	The label will be displayed on the ordinate axis of the graph and will be stored in the dataset.
Ordinate Max	Sets the default ordinate maximum value. Leave empty to retain the same ordinate value as last time.
Ordinate Min	Sets the default ordinate minimum value. Leave empty to retain the same ordinate value as last time.
Auto-clear curves	If this option is selected all curves are deleted from the graphic before a measurement starts, rather than each successive scan being superimposed on the previous scans. The curves are not deleted from the hard disk.

User info tab

The screenshot shows the 'User info' tab with the following content:

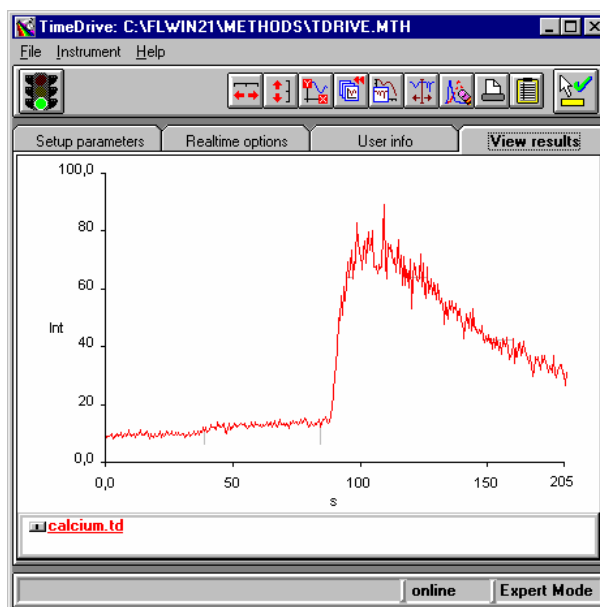
- Analyst:** srh
- Sample info:** single wavelength calcium
- Method:** last modified: date by: Analyst: locked
- Comments:** loading conc. dye 2.0.10-6M
500000 cells/ml

Parameter	Description
Analyst	The name is automatically taken from the configuration dialog, but can be altered for documentation purposes. The name is saved in the header of any collected datasets and, if a method is saved, in the header of the method.
Sample info	This information will be 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' field of the dataset, and can be viewed using Report Builder.
last modified	This shows the date the current method was last saved.
by	This shows the name of the analyst who last saved the method.
locked	This option enables the locking of all entries in a method. The option can be altered in Expert mode only. If the option is selected all entry fields and the Method save command are disabled.

Parameter	Description
Comments	The comment in this textbox is saved in the method header and is also displayed in the method window of the software, but is not saved in the dataset (use Sample info for comments to be saved in datasets).

View results tab

This page contains a toolbar in which graphics icons appear. For further details on viewing data in real-time see *Generic Viewing Features* starting on page 74.



***Wavelength
Program
Application*** **9**

Introduction

NOTE: *For general information on using Applications, see Using Application Methods starting on page 65.*

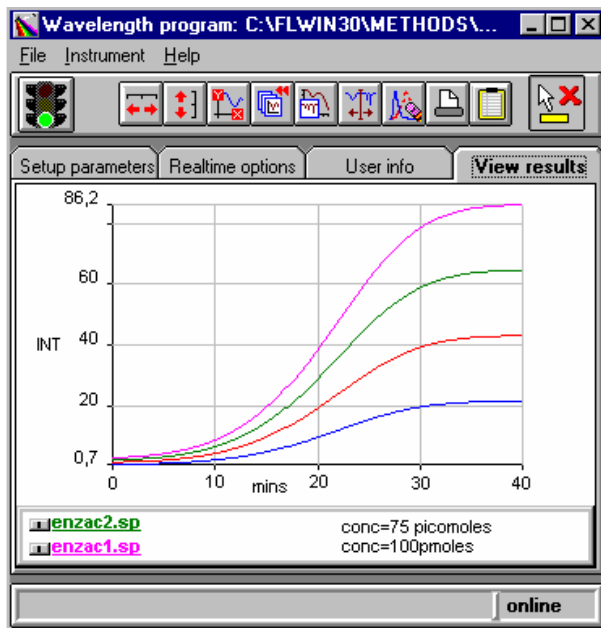
The Wavelength Program application enables multiple-channel time-dependent luminescence measurements (fluorescence, phosphorescence and bioluminescence) to be made at fixed wavelengths, with defined intervals over a specified period of time. Each channel can independently control the cuvette position (using the 4-position cellchanger if applicable) or wavelengths, slits etc., for each channel a separate graphical TimeDrive file will be displayed and saved automatically.

You can freely mix the channels, collecting for example three wavelength sets from the first cuvette, one from the second, two from the third, etc. Alternatively, the Wavelength Program application can be used to collect multiple channels of data from a single position cellchanger.

NOTE: *Only this last mode is possible with the LS 45.*

Using the Application

1. Select **Wavelength Program** from the Application menu.
The Wavelength program application is opened.



2. Enter the required parameters on each tab of the application.
The parameters are discussed below.
3. Click on the green traffic light in the toolbar to start the method.
The View Results tab is displayed automatically, use the icons in the toolbar to format the graphic display.
4. To exit the application, select **Exit** from the File menu.

Setting the Parameters

Setup parameters tab

Setup parameters							
Realtime options		User info		View results			
Insert WL set		Add WL set		Delete WL set		Fill down	
	Destination	Ex wl	Em wl	Ex slit	Em slit	Cuv.	Comment
1	WLPR001.td	350	450	10.0	10.0	1	conc=1
2	WLPR002.td	350	450	10.0	10.0	2	conc=2
3	WLPR003.td	350	450	10.0	10.0	3	conc=5
4	WLPR004.td	350	450	10.0	10.0	4	conc=10

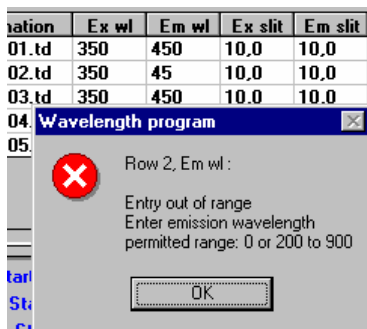
<input type="radio"/> Remote Start	Duration (s)	<input type="text" value="1800"/>	<input checked="" type="radio"/> Seconds <input type="radio"/> Minutes
<input checked="" type="radio"/> Keyboard Start	Data Interval (s)	<input type="text" value="30"/>	<input checked="" type="checkbox"/> Set shortest interval
<input type="radio"/> Immediate Start	Integration time (s)	<input type="text" value="0.10"/>	
<input type="checkbox"/> Auto lamp off	Delay before meas.(s)	<input type="text" value="0"/>	

Parameter	Description
Insert WL set	Inserts a new line (wavelength set) into the wavelength program grid above the currently selected line.
Add WL set	Adds a new line (wavelength set) to the bottom of the wavelength program grid.
Delete WL set	Deletes the selected line (wavelength set) from the wavelength program grid.
Fill down	Select a cell on the required line then click Fill down to copy the contents of the cell vertically downwards to the bottom of the grid. <i>NOTE: If used on the Destination column, the selected filename will be automatically incremented down the grid.</i>
Destination	This is the name of the file that will be created for the current wavelength program data channel.
Ex wl	Enter the excitation wavelength in nm.


Parameter	Description
Em wl	Enter the emission wavelength in nm.
Ex slit	The excitation slit width is the spectral bandwidth of the excitation monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.
Em slit	The emission slit width is the spectral bandwidth of the emission monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.
Cuv	Enter the desired cuvette position for this data channel. When using a single position cellholder (or with the LS 45), enter 1 as position.
Comment	The contents of this cell will be copied into the comments section of the dataset header.
Start options	The options of Remote Start , Keyboard Start , and Immediate Start are described in <i>Start/stop options available when setting up application methods</i> on page 70.
Duration	Enter the total time for data collection in seconds or minutes depending on the time unit selected.
Data interval	Enter the required time interval between measurements, in seconds or minutes depending on the time unit selected, or select Set shortest interval to automatically sets the shortest data interval given the currently selected cuvette and wavelength changes.
Integration time	Enter the period of time over which the intensity will be measured, in seconds or minutes depending on the time unit selected.

Parameter	Description
Auto lamp off	If this option is selected the lamp is switched off automatically between measurements. This avoids photobleaching of the sample and preserves the source lifetime for long measurements.
Delay before meas	This determines the delay between turning the source on and starting measurement (the instrument needs no delay, the source stabilizes instantly), which allows for sample stabilization.

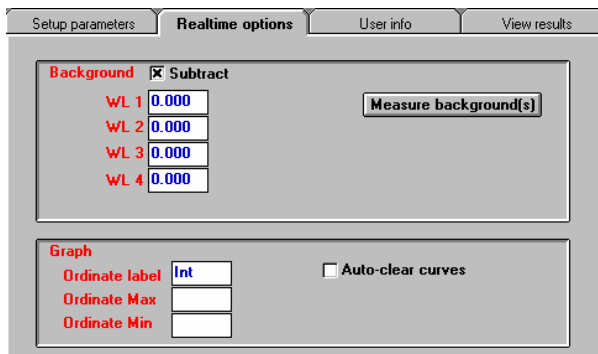
NOTE: Range/error checking of the grid parameters is carried out immediately you move on from a cell in the grid. Unacceptable parameters will result in a dialog that specifies the row of the grid, which parameter is out of range, and what the permitted range for this parameter is:



Customizing the Wavelength Program Grid

- To change the width of a column, move the mouse over the left or right border of the header of the column to be modified.
The mouse pointer will change to .
- Hold down the left mouse button and drag the border to the desired position.
The column width will be resized accordingly.
The size of the grid and the columns is stored when the application is left and will be restored when the application is restarted.

Realtime options tab



Parameter	Description
Background Subtract	This option enables you to obtain automatic background subtraction of each data channel in real-time. Each data channel (there may be up to 8) has its own specific background value for real-time subtraction. These values can be entered manually, or obtained automatically (before the run is started) by clicking Measure Background(s) . For the 4-cellchanger, prepare one cuvette containing a background sample for each data channel and insert the series into the cellchanger.
Ordinate label	The label will be displayed on the ordinate axis of the graph and will be stored in the dataset.
Ordinate Max	Sets the default ordinate maximum value. Leave empty to retain the same ordinate value as last time.
Ordinate Min	Sets the default ordinate minimum value. Leave empty to retain the same ordinate value as last time.
Auto-clear curves	If this option is selected all curves are deleted from the graphic before a measurement starts, rather than each successive scan being superimposed on the previous scans. The curves are not deleted from the hard disk.

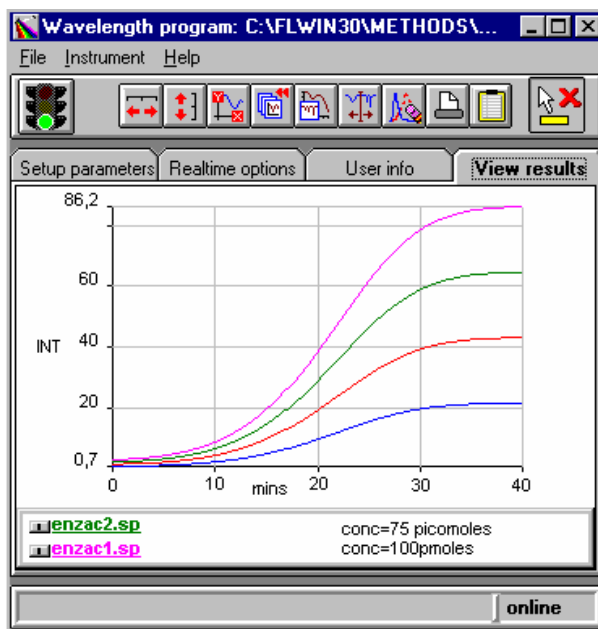
User info tab

Parameter	Description
Analyst	The name is automatically taken from the configuration dialog, but can be altered for documentation purposes. The name is saved in the header of any collected datasets and, if a method is saved, in the header of the method.
last modified	This shows the date the current method was last saved.
by	This shows the name of the analyst who last saved the method.
locked	This option enables the locking of all entries in a method. The option can be altered in Expert mode only. If the option is selected all entry fields and the Method save command are disabled.
Comments	The comment in this textbox is saved in the method header and is also displayed in the method window of the software, but is not saved in the dataset (use Sample info for comments to be saved in datasets).

Parameter	Description
Save Spreadsheet	<p>This option enables you to obtain a single spreadsheet containing all of the data for all of the channels. The format is an Excel™ compatible tab-delimited ASCII file. Enter the name of the spreadsheet you want to create.</p> <p>NOTE: <i>This is in addition to the graphical plots that are automatically saved.</i></p>

View results tab

This page contains a toolbar in which graphics icons appear. For further details on viewing data in real-time see *Generic Viewing Features* starting on page 74.



***The ICBC
Calibration
Application*** **10**

Introduction

NOTE: *For general information on using Applications, see Using Application Methods starting on page 65.*

The ICBC Calibration application is used for converting raw data into intracellular [ion]. The application accepts single wavelength raw data from TimeDrive and Wavelength Program applications, and ratio raw data from Ratio Data Collection, Fast Filter and Wavelength Program applications.

For ratio data, the data for each run consists of three TimeDrive files with an extension of .TD. The file name ending N, D or A is automatically added for identification of the data type contained within that file:

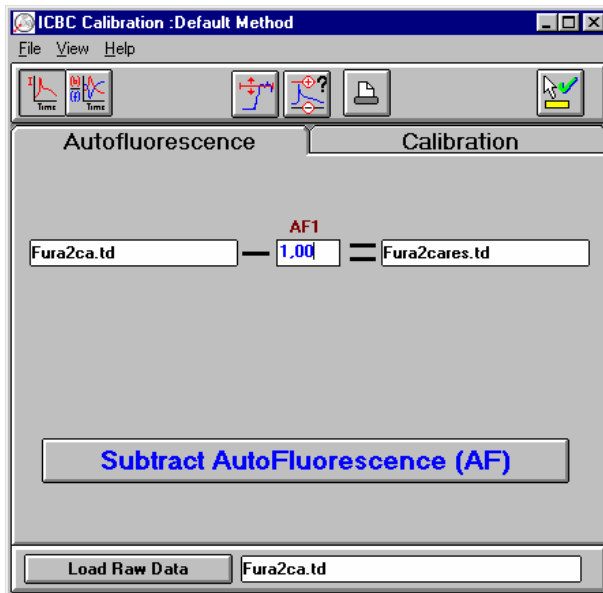
*N.TD = numerator for ratioing the measured data

*D.TD = denominator for ratioing the measured data

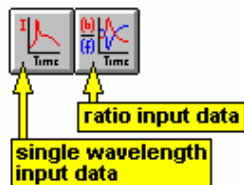
*A.TD = ratio of the intensity values of the above files

Using the Application

1. Select **ICBC Calibration** from the Application menu.
The ICBC Calibration application is opened.

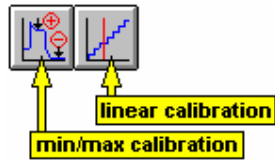


2. Use the toolbar buttons to determine whether single wavelength or ratio raw data is to be calibrated.



- Single wavelength input data - If the raw data to be converted to [ion] consists only of a single wavelength (for example, FLUO-3), then click on the first button. The calibration module will then show single wavelength structure for autofluorescence subtraction and calibration.

- Ratio input data - If the raw data to be converted to [ion] consists of a ratio of 2 wavelengths (for example, FURA-2), then click on the second button. The calibration module will then show wavelength ratio structure for autofluorescence subtraction and calibration.
3. Use the toolbar buttons to determine whether min/max or linear calibration will be applied.



- Min/Max calibration mode - If the conversion to [ion] algorithm involves min/max calibration (for example, Fmin/Fmax for FLUO-3 or Rmin/Rmax for FURA-2) then click on the first button.
 - Linear calibration mode - If the conversion to [ion] algorithm involves linear calibration (for example, for BCECF) then click on the second button.
4. Select the raw data set(s) to be calibrated using **Load Raw Data** at the bottom of the application window.



5. Set up autofluorescence options as required (see page 194) and click **Subtract Autofluorescence (AF)** on the **Autofluorescence** tab.
6. Set up calibration options as described for the various options starting on page 196.
7. Convert the raw data to [ion] by clicking on the **Convert to [ion]** on the **Calibration** tab.
8. To exit the application, select **Exit** from the File menu.

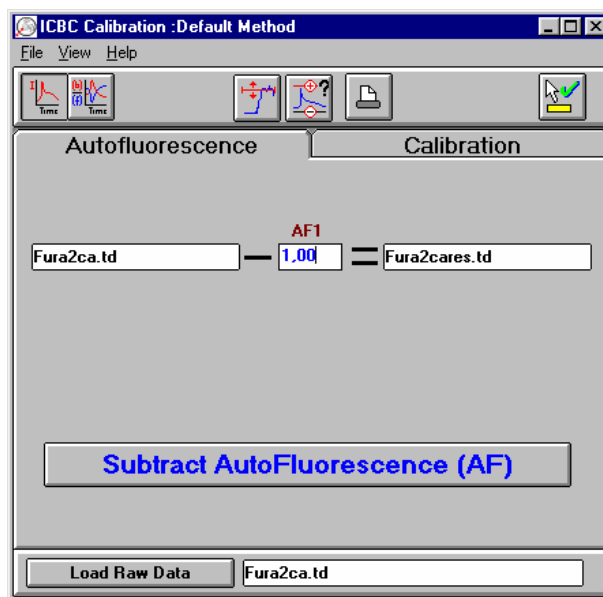
Setting the Parameters

Autofluorescence tab

The appearance of the Autofluorescence tab depends on the setting of the single/ratio buttons.

Single wavelength mode

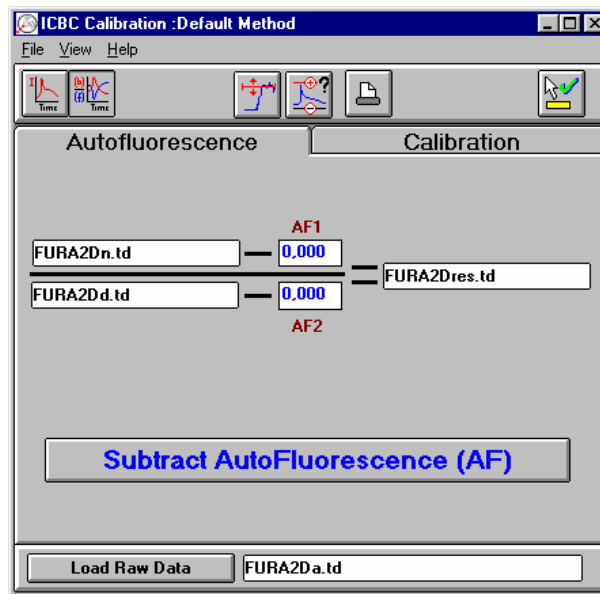
In this mode the page appears as follows:



1. Enter the autofluorescence value, the intensity of a cell without any fluorescent dye, as **AF1**.
You can use the calibration cursor to read in this data from a dataset, as described on page 201.
2. Click on **Subtract AutoFluorescence** to subtract the autofluorescence value from the raw dataset.
The autofluorescence subtracted data are stored in a new result dataset. For convenience the AF value is stored in the header of the source dataset, but the raw data of the source dataset are not modified.

Ratio mode

In **ratio mode**, the tab appears as follows:

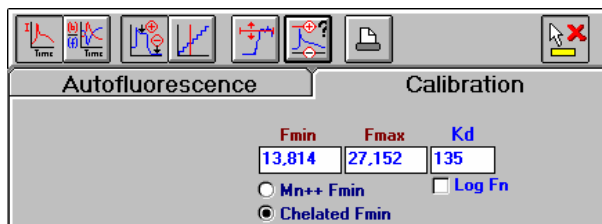


1. Enter the autofluorescence values, the intensity of a cell without any fluorescent dye, as **AF1** and **AF2**.
You can use the calibration cursor to read in this data from a dataset, as described on page 201.
2. Click on **Subtract AutoFluorescence** to subtract the autofluorescence value from the raw intensity datasets and re-generate the ratio dataset.
The autofluorescence subtracted data are only stored in the ratio dataset. For convenience the AF values are stored in the headers of the source datasets, but the raw data of the source datasets are not modified.

Calibration tab

The calibration tab consists of two distinct parts: the top part, which contains the user-interaction for calibration values, and the bottom part, which contains the user interaction for the formatting of the result data. The appearance of the top part of the tab depends on the setting of the single /ratio and the calibration type (min/max or linear) buttons.

Single wavelength, min/max mode

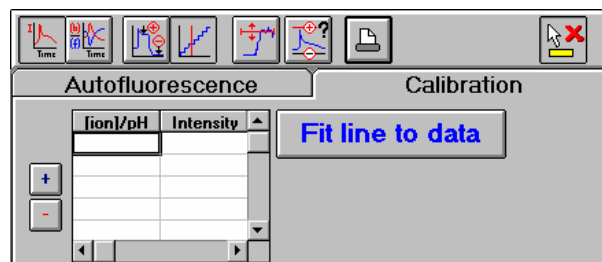


NOTE: The on-screen help contains practical information on how to measure AF, Fmax and IC. Select **Contents** from the Help menu, and then scroll to the relevant topic(s).

Parameter	Description
Fmin	Minimum fluorescence level, obtained during the calibration experiment when [ion]=0. Enter manually, or you can use the calibration cursor to read in this data from a dataset, as described on page 201.
Fmax	Maximum fluorescence level, obtained during the calibration experiment when [ion] is saturating. Enter manually, or you can use the calibration cursor to read in this data from a dataset, as described on page 201.
Kd	Dissociation constant, as applies to your particular experiment, and relevant to the temperature of the experiment.

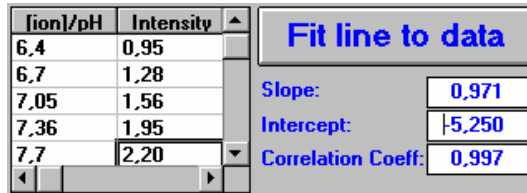
Parameter	Description
Log Fn	Select this to perform calibration using a \log_{10} function. This is useful for calibration at high [ion], where min/max calibration has poorest linearity.
Mn++ Fmin	Select this for calibration using the equations: $F_{min} = AF + (F_{max} - AF) / IC$ $[Ca^{++}] = Kd \cdot (F - F_{min}) / (F_{max} - F)$
Chelated Fmin	Select this for calibration using the equation: $[Ca^{++}] = Kd \cdot (F - F_{min}) / (F_{max} - F)$

Single wavelength, linear mode

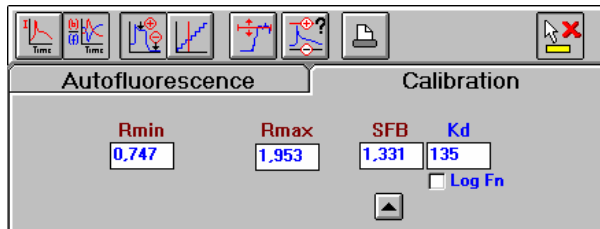


1. Fill in the grid of **[ion]/pH** versus **Intensity**.
You can use the calibration cursor to read in **Intensity** data from a dataset, as described on page 201.
2. To add a calibration point, click **+**.
Use this to set the grid to the number of pH values used in the generation of the calibration data.
3. To remove a calibration point, click **-**.
This removes the bottom calibration point from the grid.

- When the grid is complete, click **Fit line to data**.
Fits a linear least squares fit to the data points in the grid and displays the results of the fit under the button.




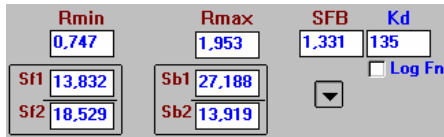
Wavelength ratio, min/max mode



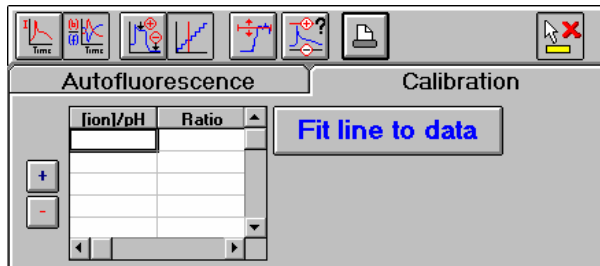
This mode uses the calibration equation:

$$[Ca^{++}] = Kd \cdot (R - Rmin) / (Rmax - R) \cdot SFB$$

Parameter	Description
Rmin	Minimum ratio value, obtained during the calibration experiment when [ion]=0. Enter manually, or you can use the calibration cursor to read in this data from a dataset, as described on page 201.
Rmax	Maximum ratio value, obtained during the calibration experiment when [ion] is saturating. Enter manually, or you can use the calibration cursor to read in this data from a dataset, as described on page 201.

Parameter	Description
SFB	Ratio of the denominator intensity components of Rmin and Rmax respectively. <i>NOTE: This value is automatically recalculated each time values are transferred back from cursor-derived calibration values, but if Rmin and Rmax are entered manually, then SFB must also be entered manually.</i>
Kd	Dissociation constant, as applies to your particular experiment, and relevant to the temperature of the experiment.
Log Fn	Select this to perform calibration using a log ₁₀ function. This is most useful for calibration of data near the calibration value limits, where the calibration process has poorest linearity.
	This button expands the display of values to include the intensity components, and is intended for clarity only. When Rmin and Rmax values are obtained from calibration data using a cursor, the intensity components are also transferred automatically. When activated, the calibration section appears like this: 

Wavelength ratio, linear mode




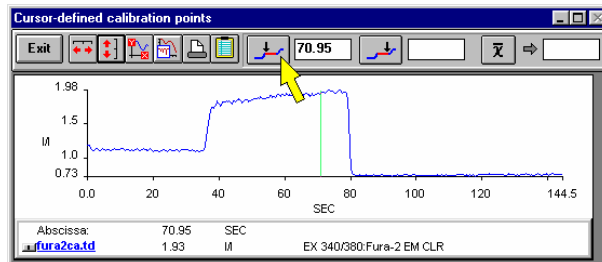
1. Fill in the grid of **[ion]/pH** versus **Intensity**.
You can use the calibration cursor to read in **Intensity** data from a dataset, as described on page 201.
2. To add a calibration point, click **+**.
Use this to set the grid to the number of pH values used in the generation of the calibration data.
3. To remove a calibration point, click **-**.
This removes the bottom calibration point from the grid.
4. When the grid is complete, click **Fit line to data**.
Fits a linear least squares fit to the data points in the grid and displays the results of the fit under the button.

[ion]/pH	Ratio	Fit line to data	
6.4	0.51	Slope:	0.751
6.8	0.84	Intercept:	-4.314
7.3	1.05	Correlation Coeff:	0.993
7.75	1.56		
8.36	1.98		

Using the Calibration Cursor

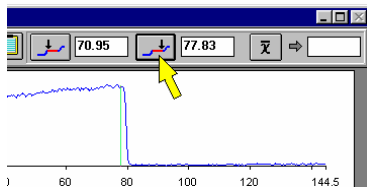
Many of the parameters set on the calibration tab are best done by displaying the dataset and using a cursor.

1. To display a dataset and cursor, click .
A file selector is displayed.
2. Select a calibration dataset.
The dataset is loaded into a graphic window where a cursor is displayed.
3. Drag the cursor to the start of the Rmax range, then click on the calibrant start button



The cursor value is loaded into the box to the right of the button.

4. Drag the cursor to the end of the Rmax range then click on the calibrant end button:



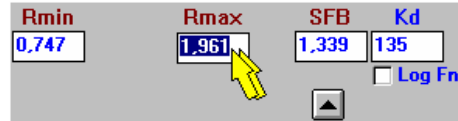
The cursor value is loaded into the box to the right of the button.

5. Click on the average button:




The result is loaded into the box to the right of the button.

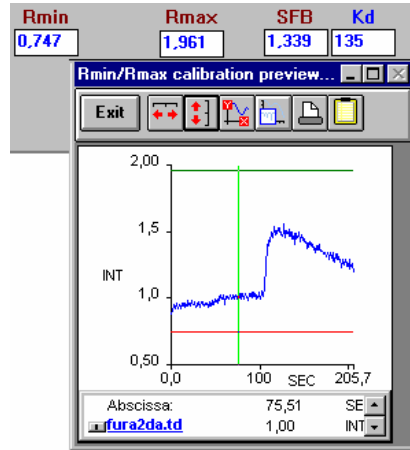
6. To transfer data back to the application, double-click on relevant box on the Calibration tab of the ICBC Calibration application.



The value is loaded into the box.

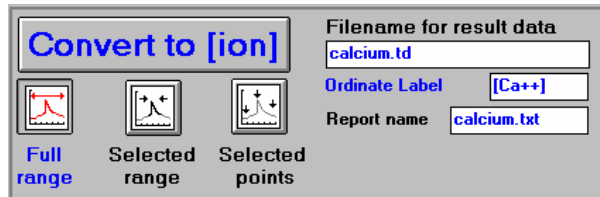
Previewing autofluorescence and calibration

- Click  to compare the current raw dataset against the current levels of min and max, autofluorescence etc., for diagnostic purposes.

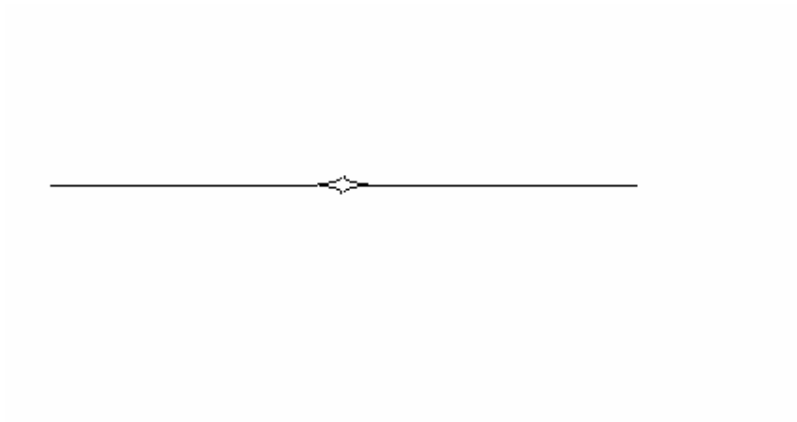


Calibration Result format

The bottom part of the calibration page is used to define how the results file will be generated:



Parameter	Description
Convert to [ion]	Convert the raw dataset (the filename in the textbox at the bottom of the window) to [ion] using the calibration values in the section at the top of the calibration page.
Full range	Sets the conversion to be over the full time limits of the raw dataset. Generates a graphic (*.td) result file.
Selected range	Sets the conversion to be over time limits specified by you, using a cursor. Generates a graphic (*.td) result file.
Selected points	Sets the conversion to be at several points (each point specified using a cursor). This option generates a text result file.
Filename for result data	Enter a filename for the results file.
Ordinate Label	Enter the ordinate label to be displayed in the results file.
Report name	Enter a filename for the calibration report, which will contain a full set of information describing how the calibration was performed.



Concentration **11**
Application

Introduction

NOTE: *For general information on using Applications, see Using Application Methods starting on page 65.*

The Concentration application enables you to perform routine quantitation of unknown samples.

You can construct separate reference sample and unknown sample grids and measure these in any sequence: as reference samples are collected a linear calibration graph is constructed and updated.

Unknown samples can be measured separately (where you select a sample for measurement from anywhere in the sample grid) or automatically (where you are prompted for each unknown sample).

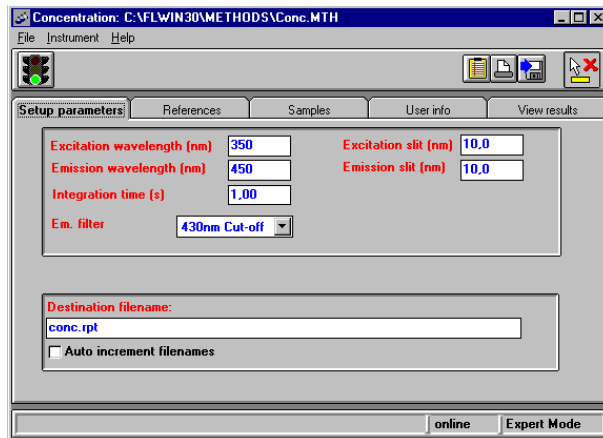
All measurement conditions, reference sample data, calibration fit data and unknown sample results are presented on a single page for convenience. These results can be printed or saved in a spreadsheet-compatible file for further work.

For quality control work, a permitted calculated concentration range can be applied to the unknown samples: unknown results are then identified as 'in range' or 'out of range' accordingly.

Reference sample data is stored in the application method for instantaneous recall and immediate unknown sample calculation. Unknown sample data can be stored on disk for subsequent recall (for example for checking against several reference calibration sets).

Using the Application

1. Select **Concentration** from the Application menu.
The Concentration application is opened.



2. Enter the required parameters on the **Setup parameters** tab (see page 210) and the **User info** tab (see page 217).
3. Set up a calibration graph as detailed on page 214.
4. Click on the green traffic light in the toolbar to start the method.
The way the method progresses is determined by the measurement mode (as set on the **User info** tab):

- If **Automatic run** is switched off, when the measurement is started the Sample tab appears with three additional buttons:



Click **Measure** to measure the sample and automatically go to the next sample.

OR

Click **Skip** to skip the measurement of the sample.

OR

Click **Redo** to go back to the previous sample.

- If **Automatic run** is switched on and **Keyboard Start** is selected, you need to click **Measure** to perform each measurement, but the options to **Skip** or **Redo** are not available. After the last sample has been measured the button caption changes to **Save**. Click **Save** to automatically save the results report to the destination file name.
- If **Automatic run** is switched on and **Remote Start** is selected, each measurement is started via the external remote contact closure. After all measurements have been performed, the results report is saved automatically. This mode allows for automatic data collection when coupled to an external device, for example an auto-sampler.
- If **Automatic run** is switched on and **Sipper Start** is selected, each measurement is started via the external event contact closure, or the sipper button. After all measurements have been performed, the results report is saved automatically. This mode allows for automatic data collection when coupled to an external device, such as the Sipper accessory.

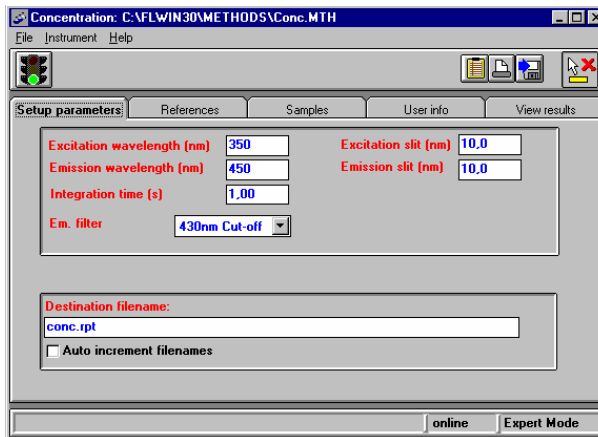
When a measurement is taken, the sample intensity is measured and the background subtracted value is written into the sample grid. Then the concentration is calculated, using the calibration graph.

NOTE: *The measure background button is enabled through the run, allowing you to redefine the background at any time.*

5. To exit the application, select **Exit** from the File menu.

Setting the Parameters

Setup parameters tab



Parameter	Description
Excitation wavelength	Enter the excitation wavelength in nm.
Emission wavelength	Enter the emission wavelength in nm.
Excitation slit	The excitation slit width is the spectral bandwidth of the excitation monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.
Emission slit	The emission slit width is the spectral bandwidth of the emission monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.

Parameter	Description
Integration time	Enter the required integration time in seconds. The optimal signal-to-noise ratio is obtained by selecting a long integration time. NOTE: <i>Changing the integration time will invalidate an existing set of references.</i>
Em filter	Select the required emission filter (with the LS 45, only Open can be selected). NOTE: <i>Changing the emission filter will invalidate an existing set of references.</i>
Result Filename	Enter the name of the file for saving the data or double-click in the textbox and a file selector will be displayed to enable you to overwrite a previous file. NOTE: <i>Result files are always stored in the default FL WinLab data directory.</i>
Auto increment filenames	If this option is selected the application generates a new numbered filename for each run. The base filename is given as the Result Filename , and is extended with a number starting from #001. If a file with this filename already exists on the hard disk in the current FL WinLab data directory, the number is incremented by one, until the filename is unique. The numbering process can be forced to start from a different number by adding a number to the base filename, for example Test#5.sp would make the first generated filename Test#005.sp and the second Test#006.sp and so on.

Total Emission Mirror (TEM) Accessory

The total emission mirror accessory is a plane mirror that can be moved in place of the emission grating and is used to collect the entire spectrum of light from the sample. This increases the sensitivity by up to 20 times and is especially recommended for bioluminescence measurements.

TEM is only available if the TEM is fitted inside the instrument, and should always be used in conjunction with an emission cut-off filter. When the mirror is in the beam the emission grating is automatically moved out of the way.

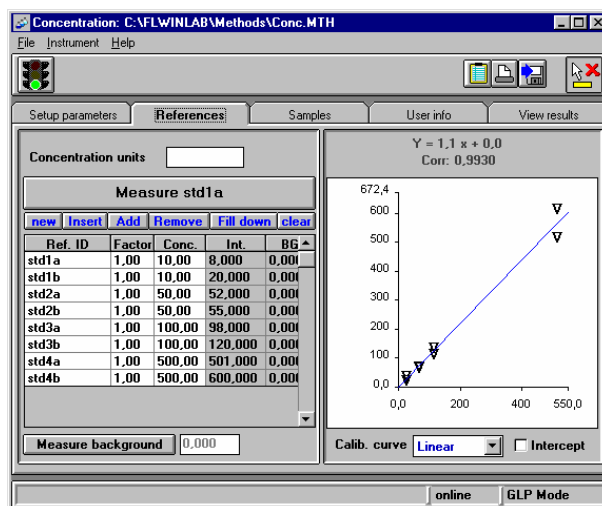
Sipper Accessory

If the sipper accessory is fitted to the instrument, sipper parameters are available.

Sipper on <input checked="" type="checkbox"/>	Pump time (s)	<input type="text" value="5.0"/>	<input type="radio"/> purge forwards
	Delay time (s)	<input type="text" value="1.0"/>	<input checked="" type="radio"/> purge backwards
	Purge time (s)	<input type="text" value="10"/>	

Parameter	Description
Sipper on	If selected, the sipper will be run before each measurement.
Pump time	Enter the length of time that the system will pump to fill the flowcell with the sample.
Delay time	Set the amount of time between the pump stopping and the measurement being taken, for instance to wait for any bubbles to leave the sample.
Purge time	Set the amount of time that the system purges the flowcell.
Purge forwards	Select the forwards pump direction to pump the sample to the waste.
Purge reverse	Select the reverse pump direction to return the sample to the tube.

References tab




Parameter	Description
Concentration units	Enter the concentration units to be used for standards and unknowns.
Measure (Ref. ID)	Measures the intensity of the selected reference standard. The Ref. ID of the selected standard appears on the button.
new	<p>Clears the grid and creates a new set of references. On the dialog displayed, enter a number and click OK to create the new grid. The minimum number of references is 2, the maximum number is 40.</p> <p>NOTE: All reference information will be lost unless it has been saved to a method.</p>
Insert	Inserts a new reference in the grid, above the currently selected row.
Add	Adds a new reference at the bottom of the grid.
Remove	Removes the currently selected reference from the grid.

Parameter	Description
Fill down	In the Ref. ID column, this will give all of the cells below the selected one the same name except with the number increased sequentially by 1. In the Factor and Conc. columns, this will give all of the cells below the selected one the same value as the selected cell.
clear	Clears all intensities (Int.) and background (BG) values from the references grid. The concentration (Conc.) values of the sample grid are cleared as well.
Measure background	Measures the current background intensity. The value is displayed next to the button and used for all subsequent reference measurements.
Calib. curve	Select the required type of graph: Linear , Cubic , Quadratic or Automatic . Automatic will use a statistical F-test with 90% confidence limits to determine which type of graph to use.
Intercept	Select whether to allow an intercept on the graph, that is do not force the graph through 0,0.

Creating a Calibration Graph

1. Enter the required **Concentration units**.
2. Enter the information about the reference standards (**Ref. ID**, **Factor** and **Conc.**) into the grid.

The contents of this grid is saved in the method and in the results report. It is restored every time a method is loaded.

You can change the width of the columns in the grid by moving the mouse pointer over the left or right border of the header of the column, so the mouse pointer changes to , and then hold down the left mouse button and drag the mouse to change the column width.

3. Select the type of graph you want to see by selecting the **Calib. curve** and deciding whether to allow an **Intercept**.

The parameters **Calib. curve** and **Intercept** define the mathematical function to use for the calibration curve and the minimum number of reference measurements (data points):

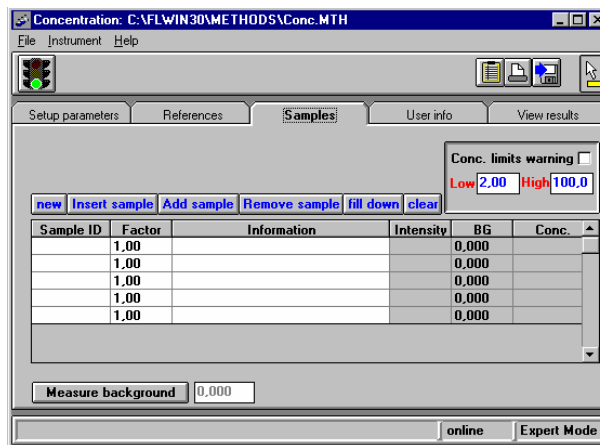
Curve	Intercept	Mathematical function	Minimum number of standards
Linear	No	$y = bx$	1
Linear	Yes	$y = bx + a$	2
Quadratic	No	$y = cx^2 + bx$	2
Quadratic	Yes	$y = cx^2 + bx + a$	3
Cubic	No	$y = dx^3 + bx$	2
Cubic	Yes	$y = dx^3 + bx + a$	3

NOTE: *You need at least one more reference than the minimum number required by the function, to obtain a reasonable correlation correction as a measure of the quality of the calibration.*

4. Prepare the first standard and insert it in the instrument.
5. Select the correct row in the grid.
The caption of the **Measure** button changes to reflect the standard selected.
6. Click **Measure**.
The instrument is setup with parameters on the setup page and the intensity measured. The reference background value (**BG**) and background subtracted intensity (**Int.**) are copied into the appropriate boxes in the grid and a data point is added to the graph, which displays the concentration of the standard against the measured intensity, draws the best fit line and displays the parameters of the best fit.
7. Repeat steps 4, 5 and 6 for the rest of the standards.

NOTE: At least two references with different concentrations and different intensities are necessary to calculate a sensible fit. If a fit was calculated successfully the samples grid and the results page are updated accordingly.

Samples tab



Parameter	Description
Conc. Limits warning	This option is used for rapid quality control screening. Enter the minimum (Low) and maximum (High) permissible concentration values. Concentrations of the unknown samples will now be color coded: green for under-range, red for over-range and black for in-range.
new	Clears the grid and creates a new set of samples. On the dialog displayed, enter a number and click OK to create the new grid. The minimum number of references is 2, the maximum number is 40. NOTE: All reference information will be lost unless it has been saved to a method.
Insert sample	Inserts a new sample in the grid, above the currently selected row.
Add sample	Adds a new sample at the bottom of the grid.

Parameter	Description
Remove sample	Removes the currently selected sample from the grid.
fill down	In the Sample ID column, this will give all of the cells below the selected one the same name except with the number increased sequentially by 1. In the Factor and Information columns, this will give all of the cells below the selected one the same value as the selected cell.
clear	Clears all Intensity , background (BG), and concentration (Conc.) values from the grid and the report.
Measure background	Measures the current background intensity. The value is displayed next to the button and used for all subsequent reference measurements.

User info tab

Analyst:

Method
last modified: 07.05.98 **by:** **locked**

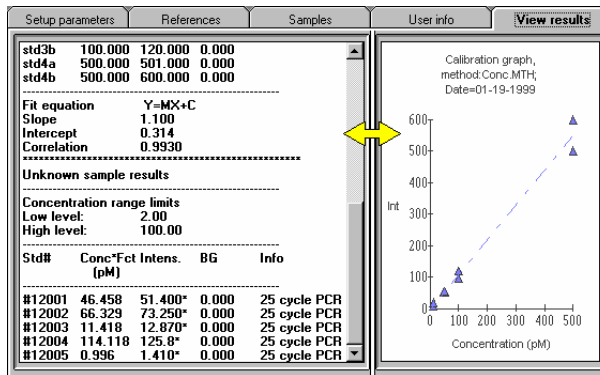
Comments:

Options: **Automatic run** **allow intensity edit**

Keyboard Start
 Remote Start
 SipperStart

Parameter	Description
Analyst	The name is automatically taken from the configuration dialog, but can be altered for documentation purposes. The name is saved in the header of any collected datasets and, if a method is saved, in the header of the method.
last modified	This shows the date the current method was last saved.
by	This shows the name of the analyst who last saved the method.
locked	This option enables the locking of all entries in a method. The option can be altered in Expert mode only. If the option is selected all entry fields and the Method save command are disabled.
Comments	The comment in this textbox is saved in the method header and is also displayed in the method window of the software, but is not saved in the dataset (use Sample info for comments to be saved in datasets).
Automatic run	Select this option to perform the data acquisition in automatic run mode, using one of the three triggers offered. If the option is not selected, measurements are done in Sequential Measurement Mode.
Allow intensity edit	Selecting this option enables you to edit the sample and reference backgrounds, as well as the intensities in the reference and sample grid. Modified intensities are color coded in blue and marked in the results file by a leading “*”, whereas the backgrounds stay unmarked.

View results tab



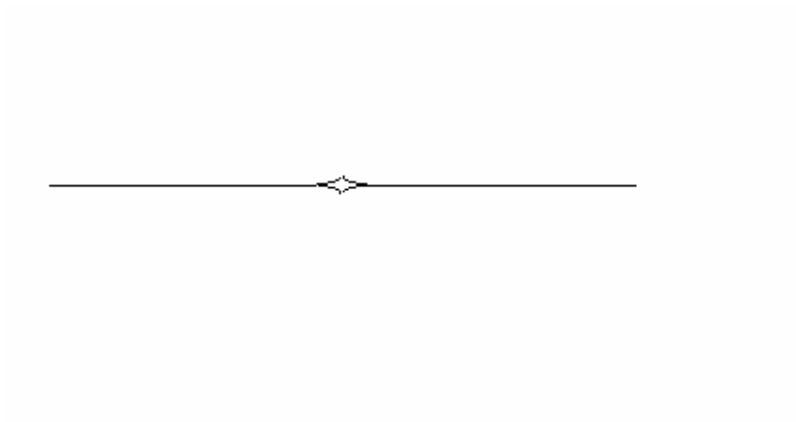
This tab displays the results report and the calibration graph. The bar down the middle of the window can be dragged left or right by placing the mouse pointer on it, holding down the left mouse button and dragging the mouse.

Results Report

The results report shows the measurement conditions, the reference sample results, line fit values and unknown results. If the concentration range option has been setup, results will also indicate whether samples are in or out of range. The results report can be resized, by dragging the split bar between the results report and the calibration graph (as shown by the yellow arrow above).

Calibration Graph

This graph displays the concentration of the references against the measured intensity, the best fit line, the used method and the current date. This graph can be printed together with the results report.



TLC Scan **12**
Application

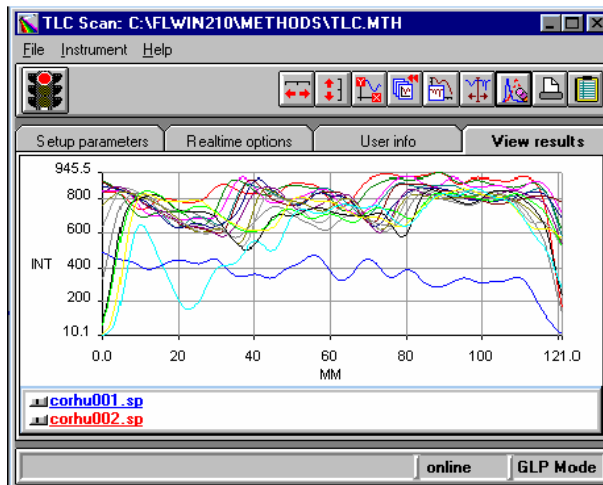
Introduction

NOTE: *For general information on using Applications, see Using Application Methods starting on page 65.*

The TLC Scan application enables luminescence measurements to be made at fixed wavelengths for an individual position, a single scan or a multiple scan over the surface of any flat sample, TLC plate or gel. Multiple scans are performed with a selected data collection interval, resulting in a 3D plot, which can be viewed in the 3D View application.

Using the Application

1. Select **TLC Scan** from the Application menu.
The Wavelength program application is opened.

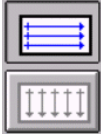
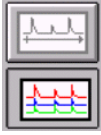


2. Enter the required parameters on each tab of the application.
The parameters are discussed below.
3. Click on the green traffic light in the toolbar to start the method.
Open the View Results tab to look at the data, and use the icons in the toolbar to format the graphic display.
4. To exit the application, select **Exit** from the File menu.

Setting the Parameters

Setup parameters tab

Parameter	Description
X, Y and Int	This shows the current X and Y position of the cursor over the plate image, and the last measured intensity. To get the intensity at the current probe head position right mouse click. This is also useful in determining the positional parameters for a 3D scan.

Parameter	Description
Left, Right, Top, Bottom and Delta	<p>To align the plate, specify the distance from the edges of the plate to the scan start and stop points. For Left enter the distance from the left edge of the plate to the scan start point. For Right enter the distance from the left edge of the plate to the scan end point. For Top enter the distance from the top edge of the plate to the scan start point. For Bottom enter the distance from the top edge of the plate to the scan end point. For Delta enter the distance between successive traces during a multiple run. When finished, click Redraw to update the picture of the plate with these values.</p> <p>Alternatively, on the picture of the plate, click with the left mouse button and drag to draw the scan trace(s). The Left, Right, Top and Bottom values are updated accordingly.</p>
	<p>Click on the top button to scan the plate from left to right in the Plate Reader Accessory.</p> <p>Click on the bottom button to scan the plate from back to front in the Plate Reader Accessory</p>
	<p>Click on the top button to scan a single trace of the plate/gel.</p> <p>Click on the bottom button to scan multiple traces and create a 3D plot of the plate/gel.</p>
Excitation wavelength	Enter the excitation wavelength in nm.
Emission wavelength	Enter the emission wavelength in nm.
Plate scan speed	The plate scan speed controls the time for the fiber optic to scan across the plate/gel.
Excitation slit	The excitation slit width is the spectral bandwidth of the excitation monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.

Parameter	Description
Emission slit	The emission slit width is the spectral bandwidth of the emission monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.
Data interval	Enter the required data interval (interval between two measuring points) in millimeters.

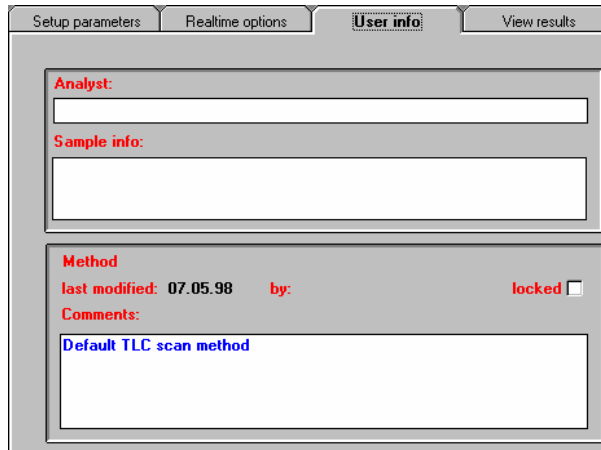
Realtime options tab

The screenshot shows a software interface with four tabs: 'Setup parameters', 'Realtime options', 'User info', and 'View results'. The 'Realtime options' tab is active. It contains a 'Graph' section with three input fields labeled 'Ordinate label', 'Ordinate Max', and 'Ordinate Min'. To the right of these fields is a checkbox labeled 'Auto-clear curves'. Below the graph section are three radio buttons: 'Remote Start', 'Keyboard Start', and 'Immediate Start', with 'Immediate Start' selected.

Parameter	Description
Ordinate label	The label will be displayed on the ordinate axis of the graph and will be stored in the dataset.
Ordinate Max	Sets the default ordinate maximum value. Leave empty to retain the same ordinate value as last time.
Ordinate Min	Sets the default ordinate minimum value. Leave empty to retain the same ordinate value as last time.
Auto-clear curves	If this option is selected all curves are deleted from the graphic before a measurement starts, rather than each successive scan being superimposed on the previous scans. The curves are not deleted from the hard disk.

Parameter	Description
Start options	The options of Remote Start , Keyboard Start , and Immediate Start are described in <i>Start/stop options available when setting up application methods</i> on page 70.

User info tab

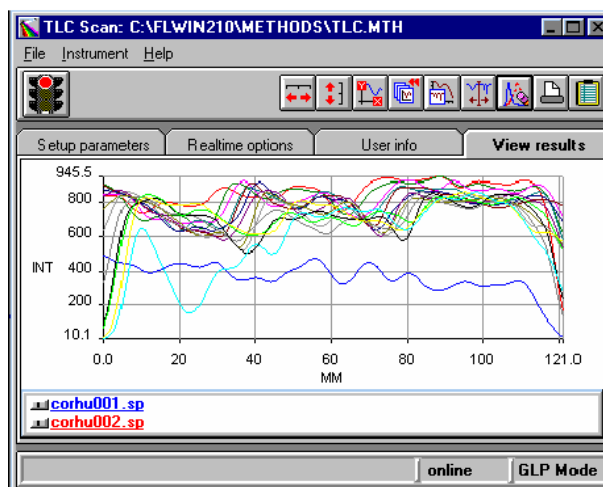


Parameter	Description
Analyst	The name is automatically taken from the configuration dialog, but can be altered for documentation purposes. The name is saved in the header of any collected datasets and, if a method is saved, in the header of the method.
Sample info	This information will be stored in 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' field of the dataset, and can be viewed using Report Builder.
last modified	This shows the date the current method was last saved.
by	This shows the name of the analyst who last saved the method.

Parameter	Description
locked	This option enables the locking of all entries in a method. The option can be altered in Expert mode only. If the option is selected all entry fields and the Method save command are disabled.
Comments	The comment in this textbox is saved in the method header and is also displayed in the method window of the software, but is not saved in the dataset (use Sample info for comments to be saved in datasets).

View results tab

This page contains a toolbar in which graphics icons appear. For further details on viewing data in real-time see *Generic Viewing Features* starting on page 74.



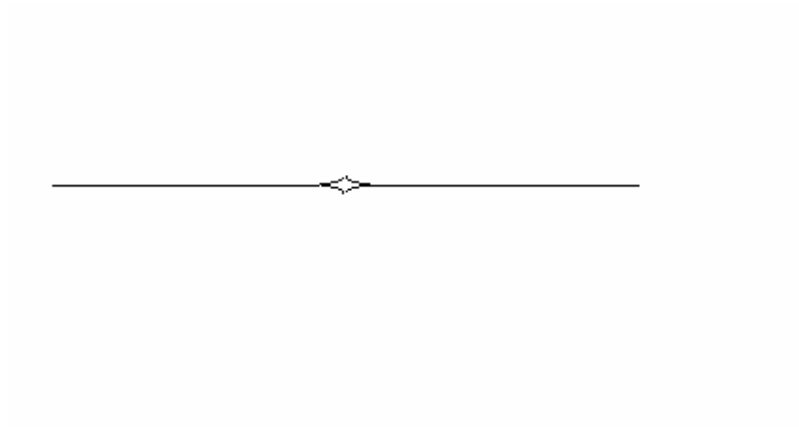


Plate Reader **13**
Application

Introduction

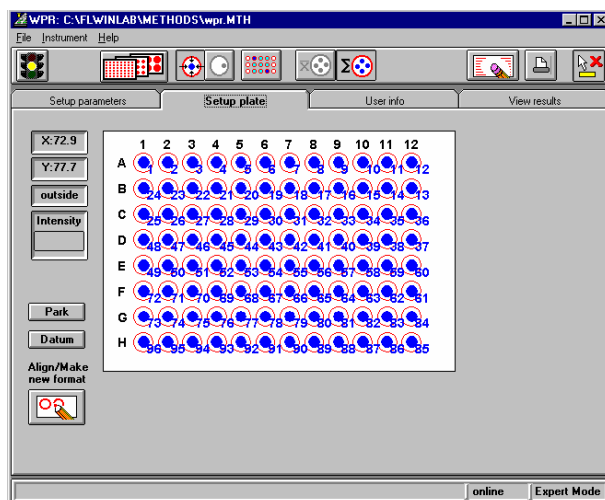
NOTE: *For general information on using Applications, see Using Application Methods starting on page 65.*

The Plate Reader application enables you to perform measurements using the Well Plate Reader (WPR) accessory. You can:

- Measure samples in any plate format, for example 6, 12, 24, 48, 96 wells
- Create new plate formats graphically and interactively
- Define measurement points with a point and click
- Randomly define plate measurement sequences, for example A1, B4, H12, A3, D5, etc.
- Automatically select measurement blocks, for example whole plate, or whole plate but excluding the outer wells, etc.
- Automatically center a reading in the middle of the well.
- Define a measurement point anywhere in the well, typically for large wells as in 6 well plates
- For multiple measurement points per well, automatically repeat the pattern in well A1 throughout the entire plate
- User-programmable wavelength program (up to 20 sets)
- Measure kinetic data from the plate for single wavelength or wavelength program
- Free-format sample information spreadsheet
- Save data in a single Microsoft Excel compatible file, on disk.

Using the Application

1. Select **Plate Reader** from the Application menu.
The Wavelength program application is opened.



2. Enter the required parameters on each tab of the application.
The parameters are discussed below.
3. Click on the green traffic light in the toolbar to start the method.
Open the View Results tab to look at the data, and use the icons in the toolbar to format the graphic display.
4. To exit the application, select **Exit** from the File menu.

Viewing the intensity in the well before starting a run

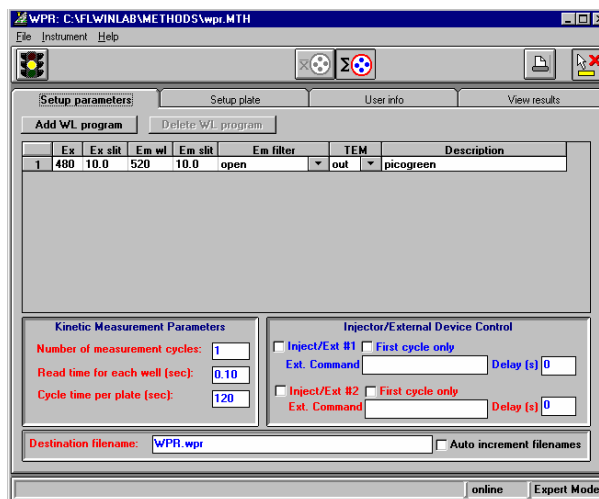
It is sometimes desirable to be able to observe the intensity in one or more wells before starting a potentially time-consuming kinetic run.

1. Open the View results tab
2. Select the required wavelength program from the list.
You can obtain the detailed parameters for the measurement from the wavelength program grid on the Setup parameters tab.

3. Open the Setup plate tab.
The current plate format is display.
4. Click on the well to be measured using the right mouse button.
The Plate Reader moves to that well, sets up the parameters for the selected wavelength program, measures the intensity and displays the result in the **Intensity** box.

Setting the Parameters

Setup parameters tab



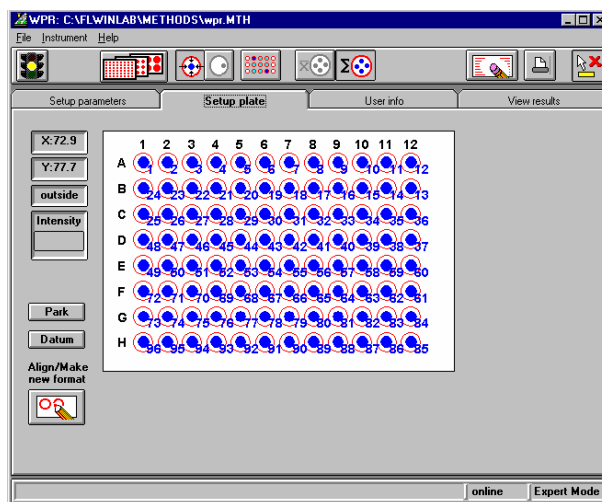
Parameter	Description
Add WL program	Click to add a new wavelength program measurement to the bottom of the grid. The maximum number of wavelength programs is restricted to 20.
Delete WL program	Click to delete the bottom wavelength program measurement set.

Parameter	Description
Wavelength program parameters	<p>A wavelength program is a series of measurements, each with its own excitation and emission wavelengths and slits, emission filter position and Total Emission accessory (when fitted) status. The wavelength program enables you to collect multiple wavelength data, for example for FURA2 measurement, cell viability, diagnostic DNA assays, etc.</p> <p>To set up a wavelength program, enter in each line of the grid the parameters for a measurement. In the Ex wl and Em wl columns enter the excitation and emission wavelengths, respectively. In the Ex slit and Em slit columns enter the excitation and emission slit widths, respectively. Select the desired emission filter from the Em filter list, and the TEM position from the TEM list. Enter a Description as required.</p>
Number of measurement cycles	This is the number of times that the measurement sequence for the selected measurement points in the plate is repeated. Enter a number greater than 1 to define a kinetic run.
Read time for each well	This is the integration time for each wavelength program measurement for each defined measure point.
Cycle time per plate	This is the time taken from the start of the first measurement of a well to the start of the next measurement of the same well for each successive cycle. This value is only used for kinetic runs, that is, if the Number of measurement cycles is greater than 1.
Inject/Ext	This enables you to drive up to two external devices. This can be used to inject samples into the wells or to drive any other device that has a Windows driver (or a DOS driver if the driver has a Windows PIF file - see Windows help for details of setting up PIF files).

Parameter	Description
First cycle only	When selected, the injector will only be driven during the first kinetic cycle. Otherwise the injector will be driven EVERY cycle of the kinetic run. The two devices driver commands can be sent asynchronously, so that one of the devices is only activated during the first kinetic cycle but the other is activated for every cycle.
Ext. Command	Define the driver program and its associated command line parameters. Enter the file name of the driver program with its full path or double-click on the textbox to use a file selector. You can also append any command line parameters which the program may need (this depends of course on the driver), for example for an injector driver to dispense 200 microlitres from injector number 1, the command line may appear: “c:\extdrive.exe 1 200”.
Delay	The two delays which can be added during the run are primarily intended for use with the external device drivers, for example to allow for temperature equilibration after addition of reagent then to allow for reaction after addition of analyte. However, they can also be used to control the time between wells for long kinetic measurements. Any delay is inserted after driving to the next well, and before the wavelength program is measured.
Destination filename	Enter the name of the file for saving the data or double-click in the textbox and a file selector will be displayed to enable you to overwrite a previous file. NOTE: <i>Result files are always stored in the default FL WinLab data directory.</i>









Parameter	Description
Auto increment filenames	If this option is selected the application generates a new numbered filename for each run. The base filename is given as the Result Filename , and is extended with a number starting from #001. If a file with this filename already exists on the hard disk in the current FL WinLab data directory, the number is incremented by one, until the filename is unique. The numbering process can be forced to start from a different number by adding a number to the base filename, for example Test#5.sp would make the first generated filename Test#005.sp and the second Test#006.sp and so on.



Setup plate tab



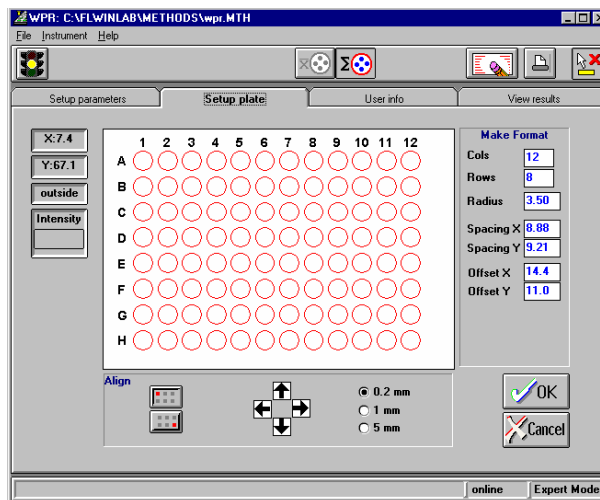
Displays an image of the currently selected plate and the current set of measurement points. The numbers beside the measurement points indicate the order of the measurement.

- To add a measurement point, left click on the desired plate position,
- To delete a read point, hold down ALT and left click on the read point.
- To obtain an intensity, right click on the desired position.



Parameter	Description
Plate position	X and Y show the plate position, while the box underneath shows the corresponding well of the current mouse position.
Intensity	Displays the intensity for the current wavelength program, when you right mouse click on the desired position.
	Will send the Plate Reader accessory to the park position. It is recommended that plates are inserted or removed only with the accessory in the parked position.
	Resets the Plate Reader accessory and sends it to the datum (0,0) position. This should correspond to the extreme corner of the plate nearest the A1 position.
	Click to load a previously saved plate image (*.wpc) from file. NOTE: <i>Loading a new plate image automatically clears all measurement points.</i>
	Selecting this means that whenever you click on a well to set a measurement point, the measurement point is automatically set to the center of the well.
	Selecting this means that whenever you click on a well to set a measurement point, the measurement point is set exactly where you clicked, rather than being automatically centered on the well. This is primarily for multiple measurements per well on 6 or 12 well plates.
	If selected, multiple reads for a given well are averaged.
	If selected, multiple reads for a given well are summed (integrated). The summed values are updated in the results spreadsheet each time a read is performed.
	Used to automatically fill a pattern of measurement points.

Parameter	Description
	Click to clear all measurement points from the plate image.
	Use to either align an existing plate or to generate a new plate format file. Information about the Align plate dialog is given below.

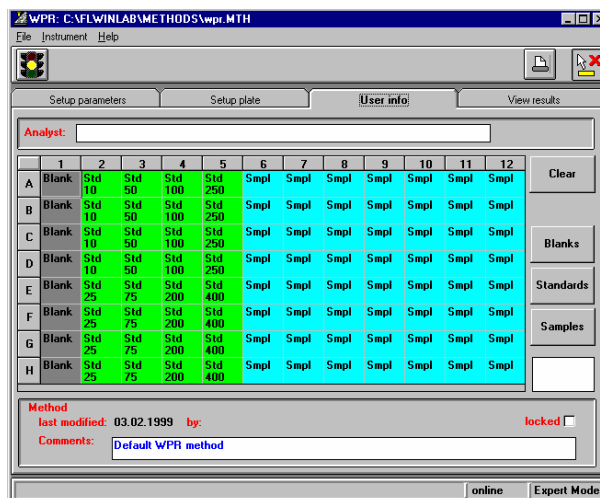
Align plate dialog



Parameter	Description
Plate Image	Displays an image of the currently defined plate. If no alignment button is selected right clicking on a position in the image will move the plate reader probe to the corresponding position over the plate.

Parameter	Description
Make format	Here the complete set of the plate format parameters are displayed. To define the format of a new plate, enter the values for the number of columns (Cols) and Rows and the Radius of the wells in the plate. Then use the Spacing X and Spacing Y to define the distance between the centers of two neighboring wells in the x and y directions. Finally, set the x and y distance of the A1 well to the upper right corner of the plate as Offset X and Offset Y . The values for the spacing and the offsets can be given approximately, since they are recalculated during the alignment process.
	Press this button to select the well in upper left corner of the plate for alignment. The program will automatically move the probe head over the appropriate well. The upper left well on the plate image will be filled in red to indicate that that well is being aligned. Align the probe head using the arrows. To unselect the well click on this button again.
	Press this button to select the well in lower right corner of the plate for alignment. The program will automatically move the probe head over the appropriate well. The upper left well on the plate image will be filled in red to indicate that that well is being aligned. Align the probe head using the arrows. To unselect the well click on this button again.
Alignment arrows	Use the arrows to align the probe. Each time you click on one of the arrows the probe is moved in the indicated direction. The distance the probe is moved is determined by the alignment distance. There will be a short delay while the program drives the probe head, during which time the cursor shape changes to an hourglass.
Alignment distance	Use these settings to define the distance the probe is moved head, each time an alignment arrow is pressed. Use 5 mm for the coarse tuning and 0.2 mm for the fine tuning.

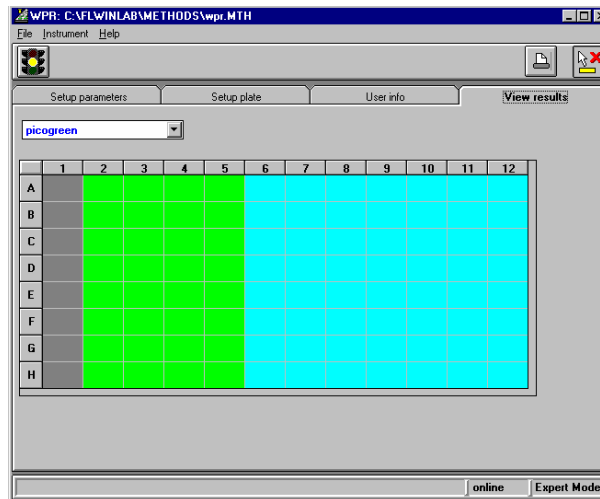
User info tab



Parameter	Description
Analyst	The name is automatically taken from the configuration dialog, but can be altered for documentation purposes. The name is saved in the header of any collected datasets and, if a method is saved, in the header of the method.
Sample info grid	Displays the sample information for the plate. Note that the grid cannot be edited directly. Use the buttons next to the grid together with the comments text box below the buttons to edit the fields. To enter information into the grid, enter text in the comments text box, select a region on the sample info grid (drag the cursor over the desired region), then click the appropriate button (Blanks , Standards , or Samples).
Clear	Click to clear the selected area on the grid.

Parameter	Description
Blanks	Blank wells can be used to subtract the background signal from sample intensities. Click to define the selected area as blanks, all cells in the area will then be color coded in gray and will contain the keyword blank . If the sample info comments textbox (right below the buttons) contains any text, this text is added.
Standards	Standards wells can be used to calibrate sample intensities. Click to define the selected area as standards, all cells in the area will then be color coded in green and will contain the keyword Std . For standards a concentration value is required, which must be entered into the textbox (right below the buttons).
Samples	Click to define the selected area as samples, all cells in the area will then be color coded in blue and will contain the keyword Sample and if the sample info comments textbox (right below the buttons) contains any text, this text is added.
last modified	This shows the date the current method was last saved.
by	This shows the name of the analyst who last saved the method.
locked	This option enables the locking of all entries in a method. The option can be altered in Expert mode only. If the option is selected all entry fields and the Method save command are disabled.
Comments	The comment in this textbox is saved in the method header and is also displayed in the method window of the software, but is not saved in the dataset.

View results tab



- Select the desired wavelength program set from the list.

If intensities are measured before the run is started, the parameters of the selected wavelength program are sent to the instrument before the measurement is started.

During a run the results of the selected wavelength program are displayed in the results grid with the same logical format as the plate. Cells are color coded depending on sample type information: white = empty, gray = blank, green = standard, blue = sample.

If you have set up a wavelength program (that is more than one wavelength set per well), then the data in the grid can be switched in real-time to toggle through the wavelength program sets. Signals are automatically summed or averaged if more than one measurement position per well has been selected.

The grid cannot be edited.

Defining a Kinetic Run

Kinetic runs are used to collect time-dependent data by repeating the measurement sequence for the selected wells in the plate.

1. To define a kinetic run and its parameters, select the Setup Parameters tab.
2. Enter a value of two or more for the **Number of measurement cycles**.
3. Then enter the **Cycle time per plate**.

This time is taken from the start of the first measurement of a well to the start of the next measurement of the same well for each successive cycle.

NOTE: *There are many events in Windows that may interfere with the cycle time. The Plate Reader application monitors the cycle time for every cycle, stores these times in the data file, and then inserts the longest cycle time into the cycle time textbox at the end of the run.*

In order to obtain an accurate measure of the cycle time, it is best to enter a very short time for the cycle time (for example 1 second) and carry out a 'dry run' without any reagents. This will result in the real minimum guaranteed cycle time being displayed and this value will be stored with the method.

During the run, the number of cycles selected and the current cycle will be displayed on the status bar. After a cycle is completed the remaining time until the next cycle is started is displayed.

External Device Control

The FL WinLab Plate Reader application has facilities for driving two external devices. This can be used to inject samples into the wells or to drive any other device that has a Windows driver (or a DOS driver if the driver has a Windows PIF file - see Windows help for details of setting up PIF files).

The devices are always activated just before measurement is made, so the Plate Reader will drive to a new well position, then activate the devices, add delays for each and then perform the wavelength program measurement.

The two devices driver commands can be sent asynchronously, so that one of the devices is only activated during the first kinetic cycle but the other is activated for every cycle, or one device can be activated during the first kinetic cycle and no devices are activated subsequently. Applications for these examples would include:

- A standard addition method, where the reagent is added first then successive sample aliquots are added and measured individually.
- A kinetic intracellular calcium method, where the user wishes to add FURA2-labelled cells to a protein-bound plate at time=0, then observe the change in intracellular calcium with time.
- Bioluminescence assay for ATP, where the user wishes to add luciferase reagent to each well, then delay to allow for temperature equilibration, then add ATP and measure.

To specify the external devices, go to the Setup Parameters tab, then click on each **Inject/Ext** checkbox required.

Then define the driver program and its associated command line parameters. To define the driver program, enter the file name of the driver program with its full path in the driver textbox or double-click on the driver textbox to use a file selector. Then append any command line parameters which the program may need (this depends of course on the driver), for example for an injector driver to dispense 200 microlitres from injector number 1, the command line may appear: "c:\extdrive.exe 1 200".

If the First cycle only checkbox is checked then the injector will only be driven during the first kinetic cycle. Otherwise the injector will be driven EVERY cycle of the kinetic run.

So for example if the user has selected a 2 wavelength program:

10 kinetics cycles

injector 1 active with First cycle only checked

10 second delay

the program will do the following on starting the run:

For the first cycle:

1. Drive to the well
2. Activate the driver #1
3. Delay for 10 seconds
4. Measure the wavelength program

For every subsequent cycle (for every well selected):



1. Drive to the well
2. Measure the wavelength program

Defining the Read Pattern

A read pattern is the sequence of locations at which measurements will be taken, for example A1, A2, A3, A4, etc. The read pattern can be made randomly, for example A1, B7, H3, etc., or the application can automatically fill in a user-defined block for simplicity.

Random Pattern

To define a random pattern:

1. Open the Setup plate tab.
2. To automatically place the measurement point in the center of the well (this is very convenient) click , or click  to locate the measurement point anywhere in the well.

The latter mode is intended primarily for use with plates with large wells, for example 6 well plates. These plates are frequently used for cell culturing, cell confluence, etc. where it is important to measure the whole volume of the well.

3. Enter the measurement points by clicking in the well.
The application will draw a blue circle to indicate a measurement point. The number associated with the blue circle indicates where in the measurement sequence that measurement point will occur. The sequence of measurement points can be made in any pattern throughout the plate, however only one intensity is calculated per well. If more than one measurement point is located in a well the intensities are either averaged or summed, depending on whether the average or sum button is selected.

Filled Block Read Pattern

A filled block read pattern enables you to: quickly define measurement of the entire plate, to ignore the outermost wells, to measure one or several columns or rows, or to define a smaller block inside the plate. To define a filled block read pattern:

1. Open the Setup plate tab.
2. Select the top left well of the block by clicking on it.
3. Select the bottom right well of the block by clicking on it.

4. Click .

The application then fills in the wells between these two points for convenience.

5. To add measurement points to the pattern, click on the well with the left mouse key.
6. To delete a measurement point from the pattern, hold down the ALT key and click on the unwanted well with the left mouse key.

NOTE: *Only one intensity is calculated per well. If more than one measurement point is located in a well, the intensities are either averaged or summed, depending whether the Average or Sum button is selected.*

Aligning a Plate Format

Optical alignment of the Plate Reader must be carried out as specified in the installation. Additionally, for every different plate type that is to be used, the Plate Reader application alignment procedure must be used to specify where the wells are in relation to the Plate Reader accessory.

There are two issues in aligning any plate, these are:

- The absolute dimensions of the plate
- the well locations, the mechanical offset of the Plate Reader accessory compared to the datum.


When you perform an alignment, the system records the position for well A1 and also calculates the well-to-well spacing that applies to the plate using the number of columns and rows and the position of well H12. With these co-ordinates, the program can reproducibly position the probe head over any well in the plate.

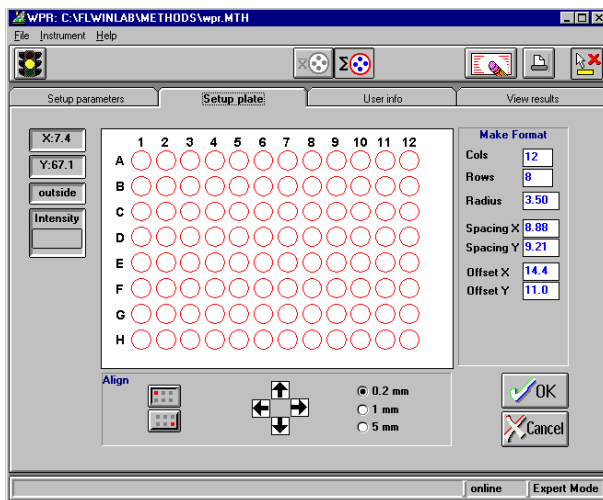
NOTE: *It is not necessary to enter accurate values for the X,Y spacing and X,Y offsets during the alignment, these values will be accurately calculated during the alignment.*



Align the plate reader as follows:

1. Insert the plate for alignment into the Plate Reader accessory.



2. Go to the Setup plate tab and click . The Align Plate dialog is displayed.



3. Click  to move the Plate Reader to well A1.
Well A1 on the plate image turns red, to indicate that that well is being aligned.
4. Observe the probe head in the Plate Reader accessory. If the probe head is not directly over the center of well A1, then alter the position by clicking on the arrows.
Use the 0.2 mm, 1 mm and 5 mm step size for coarse and fine tuning.
When you click on an arrow, there will be a short delay while the program drives the probe head and then returns the new position.
5. Click  to move the Plate Reader to lowest rightmost well of the plate (for example, H12 for the 96 well plate).
The appropriate well the plate image turns red.
6. Use the arrow buttons to refine the position again.
7. Click **OK**.
8. Enter a file name and click **OK**.
The plate format is saved as a file on disk.

***Fast Filter Data
Collection
Application*** **14**

Introduction

NOTE: For general information on using Applications, see Using Application Methods starting on page 65.

The Fast Filter Data Collection application is used to specify the data collection conditions when using the Fast Filter accessory.

The Fast Filter accessory enables rapid measurement of intracellular concentrations, and consists of one or two filter wheels, which are installed in either or both of the excitation or emission monochromators. Up to two pairs of filters or polarizers can be fitted on each filter wheel: the filter wheels rotate rapidly and enable each filter to be in the beam coincident with the flash of the lamp. A data point is then measured.

For example, if calcium changes are being monitored using FURA-2, one excitation filter wheel (with a pair of filters, 340 and 380 nm) is fitted in the instrument in place of the standard excitation filter wheel and the emission is monitored at 510 nm using the emission monochromator. The data is recorded and saved on the hard disk in a file with the extension .TD.

The polarizer filter set can be fitted to calculate the polarization or anisotropy of a sample. Fluorescence polarization is a very powerful technique for studying the rotational movement of molecules dynamically. This technique can be used for a wide variety of applications, including:

- the measurement of the binding of coenzymes to proteins,
- membrane structure and function research,
- biopolymer structure research,
- the study of antigen-antibody reactions in determining low molecular weight haptens (Immunoassays).

Polarization is calculated using the following equation:

$$Polarisation = \frac{I_{vv} - (GF \cdot I_{vh})}{I_{vv} + (GF \cdot I_{vh})}$$

Anisotropy is calculated using the following equation:

$$Anisotropy = \frac{I_{vv} - (GF \cdot I_{vh})}{I_{vv} + (2 \cdot GF \cdot I_{vh})}$$

where:

Polarization = Corrected polarization,

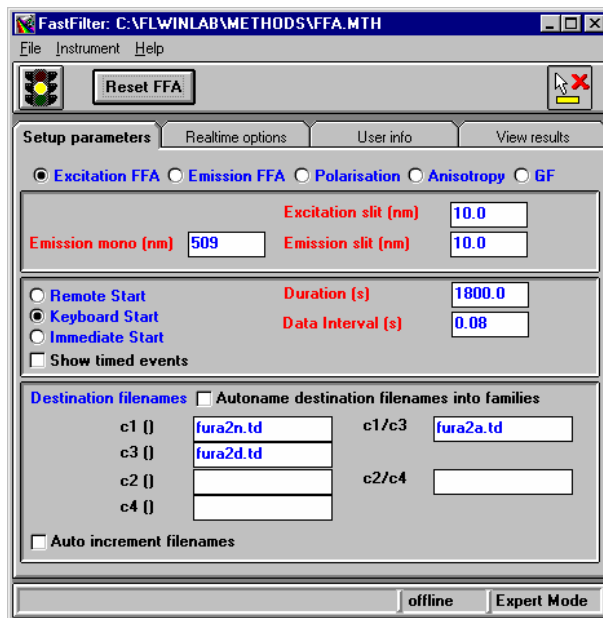
I_{VV} = Emission intensity with both excitation and emission polarizers vertical.

I_{VH} = Emission intensity with excitation polarizer vertical and emission polarizer horizontal.

GF = Grating factor

Using the Application

1. Select **Fast Filter Data Collection** from the Application menu.
The Fast Filter Data Collection application is opened.



2. Enter the required parameters on each tab of the application.
The parameters are discussed below.
3. Click on the green traffic light in the toolbar to start the method.
Open the View Results tab to look at the data, and use the icons in the toolbar to format the graphic display.
4. To exit the application, select **Exit** from the File menu.

Setting the Parameters

Setup parameters tab

The Setup parameters tab has three distinct sections:

- Mode selection
- Mode specific settings
- General settings

Selecting the mode



Parameter	Description
Excitation FFA	Select this option to run the excitation fast filter wheel.
Emission FFA	Select this option to run the emission fast filter wheel.
Polarization	Select this option to calculate the polarization of a sample. Your instrument must have an excitation and an emission fast filter wheel fitted, and the positions of the horizontal and vertical polarizer for the excitation and the emission fast filter wheel must have been defined in the Status application (see page 52).
Anisotropy	Select this option to calculate the anisotropy of a sample. Your instrument must have an excitation and an emission fast filter wheel fitted, and the positions of the horizontal and vertical polarizer for the excitation and the emission fast filter wheel must have been defined in the Status application (see page 52).

Parameter	Description
GF	Select this option to collect grating factor data using spinning Fast Filters. Your instrument must have an excitation and an emission fast filter wheel fitted, and the positions of the horizontal and vertical polarizer for the excitation and the emission fast filter wheel must have been defined in the Status application (see page 52).

Excitation FFA specific parameters

The screenshot shows a software interface with four tabs: 'Setup parameters', 'Realtime options', 'User info', and 'View results'. The 'Setup parameters' tab is active. It contains five radio buttons: 'Excitation FFA' (selected), 'Emission FFA', 'Polarisation', 'Anisotropy', and 'GF'. Below the radio buttons are four input fields: 'Emission mono (nm)' with the value 509, 'Excitation slit (nm)' with the value 10.0, and 'Emission slit (nm)' with the value 10.0.

NOTE: When the Excitation Fast Filter is selected, the excitation wavelength is fixed at zero order to allow the excitation wavelength to be defined solely by the Fast Filter, so the excitation wavelength is not entered here.

Parameter	Description
Emission mono	Enter the fixed emission wavelength in nm.
Excitation slit	The excitation slit width is the spectral bandwidth of the excitation monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.
Emission slit	The emission slit width is the spectral bandwidth of the emission monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.

Emission FFA specific parameters

The screenshot shows a software interface with four tabs: 'Setup parameters', 'Realtime options', 'User info', and 'View results'. Under 'Setup parameters', there are five radio buttons: 'Excitation FFA', 'Emission FFA' (selected), 'Polarisation', 'Anisotropy', and 'GF'. Below the radio buttons are three input fields: 'Excitation mono (nm)' with the value 350, 'Excitation slit (nm)' with the value 10.0, and 'Emission slit (nm)' with the value 10.0.

NOTE: When the Emission Fast Filter is selected, the emission wavelength is fixed at zero order to allow the emission wavelength to be defined solely by the Fast Filter, so the emission wavelength is not entered here.

Parameter	Description
Excitation mono	Enter the fixed excitation wavelength in nm.
Excitation slit	The excitation slit width is the spectral bandwidth of the excitation monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.
Emission slit	The emission slit width is the spectral bandwidth of the emission monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.

Polarization, Anisotropy, GF specific parameters

The screenshot shows the same software interface as above, but with the 'Polarisation' radio button selected. The input fields are: 'Excitation mono (nm)' (350), 'Excitation slit (nm)' (10.0), 'Emission mono (nm)' (509), and 'Emission slit (nm)' (10.0).

Parameter	Description
Excitation mono	Enter the fixed excitation wavelength in nm.

Parameter	Description
Emission mono	Enter the fixed emission wavelength in nm.
Excitation slit	The excitation slit width is the spectral bandwidth of the excitation monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.
Emission slit	The emission slit width is the spectral bandwidth of the emission monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.

Generic parameters

Parameter	Description
Start options	The options of Remote Start , Keyboard Start , and Immediate Start are described in <i>Start/stop options available when setting up application methods</i> on page 70.

Parameter	Description
Duration	Enter the total time for data collection in seconds.
Data interval	Enter the required time interval between measurements, in seconds. In excitation and emission modes the data interval is equivalent to the integration time.
Autoname destination filenames into families	<p>When this option is selected, destination filenames for all channels are automatically edited when any one filename is changed from the keyboard. If Excitation FFA or Emission FFA is selected, the endings n, d, and a are automatically added to the filenames in Channels 1/2, 3/4, and 5/6, respectively. n and d refer to the numerator and denominator datasets respectively, a refers to the ratio dataset generated as n/d. If Polarization, Anisotropy or GF is selected, the endings v, h, and p are automatically added to the filenames in Channels 1/2, 3/4, and 5/6, respectively.</p>
Result filenames	<p>The result files are grouped in two sets of three files: the leftmost two files correspond to the FFA positions channel 1 and channel 3, and then channel 2 and channel 4. The rightmost file contains the result of the ratio calculation.</p> <p>The comments in brackets are the wavelengths (or polarization) of the filters fitted in the appropriate FFA positions. This information must have been previously defined by the user in the FFA-dialog of the Status application.</p> <p>It is possible to leave a complete group empty, in this case no data is stored. If one textbox of a group contains a file name the other two textboxes of the group must contain file names as well. In polarization, anisotropy or GF mode only the first or the second set of files is measured, not both.</p> <p>NOTE: <i>The files are always stored in the default FL WinLab data directory.</i></p>

Parameter	Description
Auto increment filenames	If this option is selected the application generates a new numbered filename for each run. The base filename is given as the Result Filename , and is extended with a number starting from #001. If a file with this filename already exists on the hard disk in the current FL WinLab data directory, the number is incremented by one, until the filename is unique. The numbering process can be forced to start from a different number by adding a number to the base filename, for example Test#5.sp would make the first generated filename Test#005.sp and the second Test#006.sp and so on.

Realtime options tab

The Realtime options tab has two distinct sections:

- Mode specific settings
- General settings

Excitation/Emission FFA specific options

Parameter	Description
Background Subtract	This option enables you to obtain automatic background subtraction of each data channel in real-time. Each of the four channels has its own specific background value for real-time subtraction. These values can be entered manually, or obtained automatically (before the run is started) by clicking Measure background .

Polarization/Anisotropy specific options

Parameter	Description
Grating factor	Enter the grating factor manually or calculate it by inserting the sample to be measured into the cuvette holder and clicking Auto GF calculation .

Generic options

Parameter	Description
Ordinate label	The label will be displayed on the ordinate axis of the graph and will be stored in the dataset, except for the intensity datasets (c1-c4), in which case the ordinate labels are always set to Int .
Ordinate Max	Sets the default ordinate maximum value. Leave empty to retain the same ordinate value as last time.
Ordinate Min	Sets the default ordinate minimum value. Leave empty to retain the same ordinate value as last time.
Auto-clear curves	If this option is selected all curves are deleted from the graphic before a measurement starts, rather than each successive scan being superimposed on the previous scans. The curves are not deleted from the hard disk.
Show all data	If this option is selected, all generated data sets are displayed in real-time. Otherwise only the result dataset(s) (ratio or polarization/anisotropy) are displayed. However, in both cases all data sets are stored.

User info tab

The screenshot shows a window with four tabs: 'Setup parameters', 'Realtime options', 'User info' (selected), and 'View results'. The 'User info' tab contains the following fields:

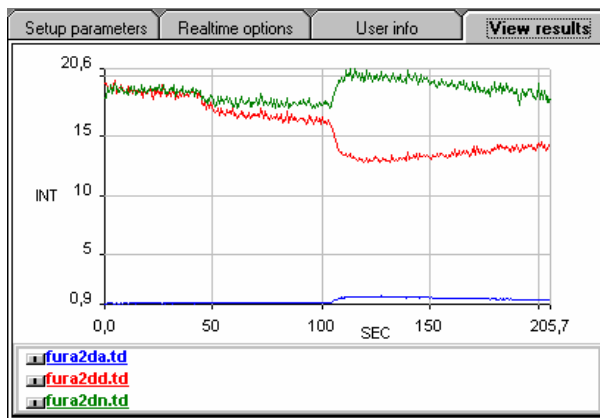
- Analyst:** A text input field.
- Sample info:** A text input field.
- Method:** A section header.
- last modified:** 07.05.98
- by:** A text input field.
- locked:** A checkbox that is currently unchecked.
- Comments:** A text input field containing the text 'Default Fast Filter method'.

Parameter	Description
Analyst	The name is automatically taken from the configuration dialog, but can be altered for documentation purposes. The name is saved in the header of any collected datasets and, if a method is saved, in the header of the method.
Sample info	This information will be 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' field of the dataset, and can be viewed using Report Builder.
last modified	This shows the date the current method was last saved.
by	This shows the name of the analyst who last saved the method.
locked	This option enables the locking of all entries in a method. The option can be altered in Expert mode only. If the option is selected all entry fields and the Method save command are disabled.

Parameter	Description
Comments	The comment in this textbox is saved in the method header and is also displayed in the method window of the software, but is not saved in the dataset (use Sample info for comments to be saved in datasets).

View results tab

This page contains a toolbar in which graphics icons appear. For further details on viewing data in real-time see *Generic Viewing Features* starting on page 74.



NOTE: *There is a difference in ordinate scale between the intensities and the ratio. Select the ratio dataset and then on the expand ordinate button in the toolbar. The ratio dataset will then be expanded to fill the ordinate scale for clarity.*

Ratio Data **15**
Application

Introduction

NOTE: *For general information on using Applications, see Using Application Methods starting on page 65.*

The Ratio Data Collection application is used for measuring changes in the intracellular concentration of free ions such as Ca^{++} and H^+ in living cells using ion-specific fluorescent probes. The fluorescent characteristics of probes such as FURA-2, INDO-1 and BCECF change depending on the intracellular concentration of the free ion. The binding of an intracellular ion to a probe results in a shift in the excitation or emission wavelength and thus the overall spectrum will contain contributions from both bound and free probe. The ratio of the fluorescence intensities at the maxima for bound and free forms of the probe is proportional to the concentration of the metabolite. The ratioing corrects for artifacts such as cell count, probe concentration and instrument specific factors.

Data is collected by driving either the excitation or the emission monochromator between the two wavelengths of interest for the duration of the analysis. To minimize time discrepancies, due to the time interval between measuring each intensity, interpolation is carried out before the ratio is generated from the intensities.

The data for each run is saved in three TimeDrive files with an extension of .TD. The file name ending N, D or A is automatically added for identification of the data type contained within that file:

*N.TD = numerator for ratioing the measured data

*D.TD = denominator for ratioing the measured data

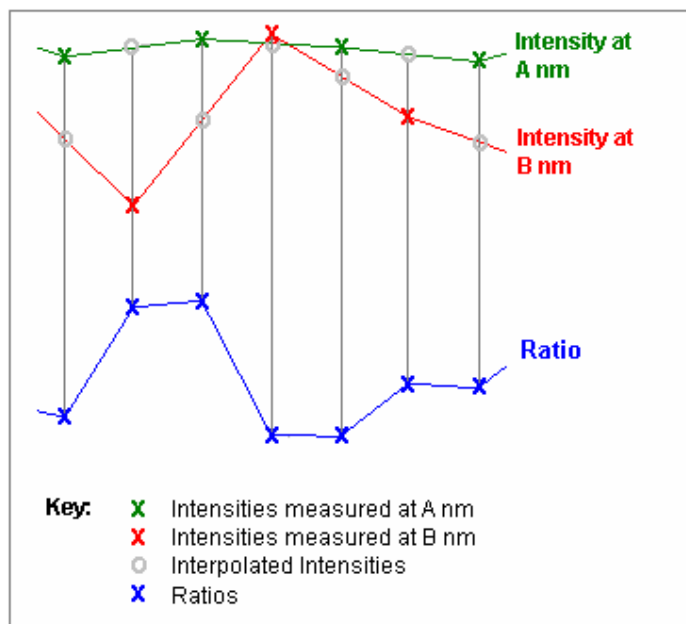
*A.TD = ratio of the intensity values of the above files

NOTE: *Using the ICBC Calibration application, as described in section 10 starting on page 191, raw data from the Ratio Data Collection application can be converted to [ion] or pH.*

True Ratio Mode

When the true ratio mode is selected, the data is collected by rapidly driving the monochromator backwards and forwards between the wavelengths of interest for the duration of the analysis. The intensities are recorded at each wavelength.

To minimize time discrepancies, due to the time interval between measuring each intensity, interpolation is carried out before the data is ratioed. The following scheme shows as how the ratio is calculated from two data sets collected at A nm and B nm, where A and B stand for a pair of excitation and emission wavelengths:

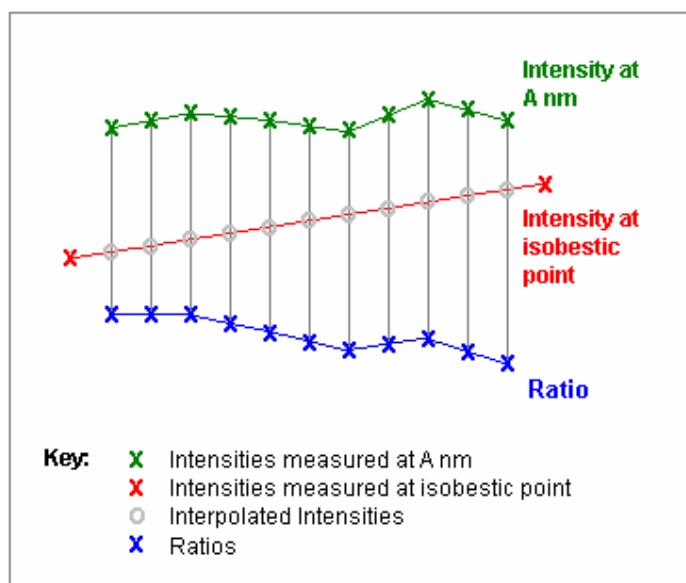


Here the second ratio is calculated by interpolating between two A nm measurements to find the intensity at the time that the B nm measurement was made. The value obtained from the interpolation is ratioed with the value of the B nm measurement. The next ratio is calculated from the second measured A nm value and an interpolated B nm value.

Quick Ratio Mode

In order to increase the rate of data collection when the quick ratio mode is selected the data is ratioed against the isobestic point. The isobestic point is the wavelength at which the fluorescence intensity is independent of metabolic concentration. The intensity of the isobestic point changes very slowly and this removes the need to continually monitor the intensity at the wavelength.

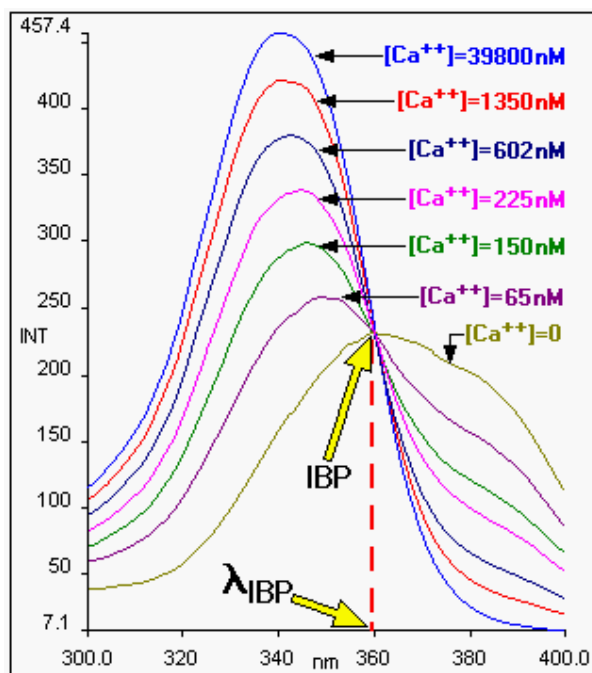
The data is collected by measuring the isobestic point intensity followed by performing a single wavelength TimeDrive. At the end of the TimeDrive the intensity of the isobestic point is measured again. During the TimeDrive the data displayed is on the screen is the ratio of the TimeDrive and the first isobestic point intensity. When the second isobestic point has been measured the experimental ratios are re-calculated using linear interpolation between the two isobestic points as the denominator:



Since the metabolite concentration data is still determined from ratio measurements the data are independent of cell path length, probe concentration and other instrumental factors.

Determining the Isobestic point

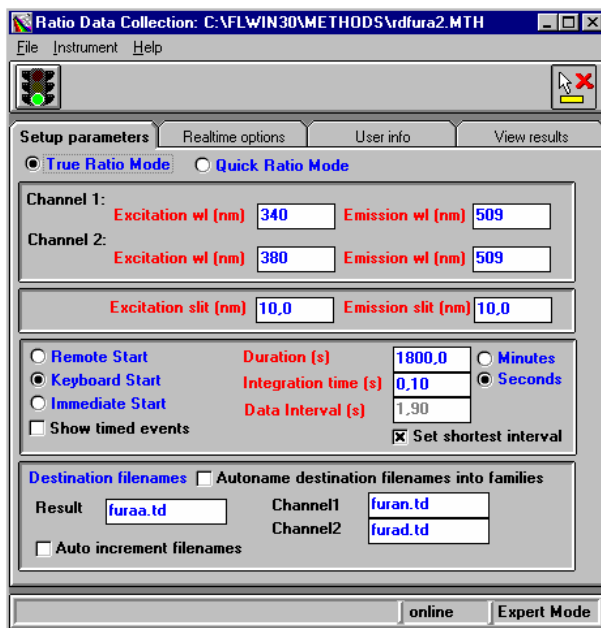
To determine the isobestic point, for example for FURA-2, perform excitation scans (excitation wavelength 300-400 nm, emission wavelength 509 nm, emission slit width and excitation slit width 5nm) for various calcium concentrations. The Isobestic point excitation wavelength can then be determined from the crossing point of the curves as shown below. The Isobestic point emission wavelength is 509 nm.



For other wavelength shifting fluorescent dyes, the isobestic point is always the spectral wavelength that is independent of ion-concentration. For specific details refer to “Handbook of Fluorescent probes and Research Chemicals” by Richard P.Haugland.

Using the Application

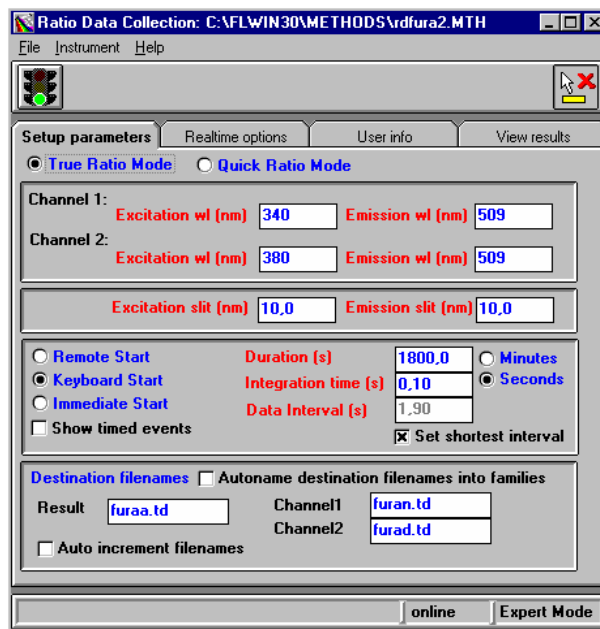
1. Select **Ratio Data Collection** from the Application menu.
The Ratio Data Collection application is opened.



2. Select the required mode.
True Ratio Mode means that the application collects a ratio of two wavelengths continuously, whereas **Quick Ratio Mode** uses the isobestic point once before and once after the collection of a single wavelength TimeDrive. This mode therefore does not collect true ratio data: it is assumed that the isobestic point intensity is either static or changes linearly.
3. Enter the required parameters on each tab of the application.
The parameters are discussed below.
4. Click on the green traffic light in the toolbar to start the method.
Open the View Results tab to look at the data, and use the icons in the toolbar to format the graphic display.
5. To exit the application, select **Exit** from the File menu.

Setting the Parameters

Setup parameters tab



Parameter	Description
Excitation wl	Enter the excitation wavelength for the relevant channel in nm.
Emission wl	Enter the emission wavelength for the relevant channel in nm.
Channel 2 (True Ratio mode only)	For emission ratioing, enter the same excitation wavelength for both channels and for excitation ratioing, enter the same emission wavelength for both channels.
Isobestic point (Quick Ratio mode only)	Enter the emission and excitation wavelengths for the isobestic point in nm.

Parameter	Description
Excitation slit	The excitation slit width is the spectral bandwidth of the excitation monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.
Emission slit	The emission slit width is the spectral bandwidth of the emission monochromator. In order to obtain the best spectral resolution, select a narrow slit width, for example 2.5 or 5 nm. The best signal-to-noise ratio is obtained by selecting a large slit width.
Start options	The options of Remote Start , Keyboard Start , Immediate Start , and Show timed events are described in <i>Start/stop options available when setting up application methods</i> on page 70.
Duration	Enter the total time for data collection in seconds or minutes depending on the time unit selected.
Integration time	<p>Enter the required integration time in seconds. The optimal signal-to-noise ratio is obtained by selecting a long integration time. However for fast kinetics a short integration time should be used.</p> <p>NOTE: For <i>Quick Ratio Mode</i>, the integration time is automatically set to the Data Interval.</p>
Data Interval	<p>Enter the required time interval between measurements in seconds or click Set shortest interval to automatically set the shortest data interval. The minimum data interval for True Ratio Mode is determined by the sum of integration time and the time the monochromators need to move between the desired wavelengths for channel1 and channel2.</p> <p>NOTE: For <i>Quick Ratio Mode</i>, Set shortest interval is not available.</p>

Parameter	Description
<p>Autoname destination filenames into families</p>	<p>When this option is selected, destination filenames for all channels are automatically edited when any one filename is changed from the keyboard. The endings denote the data type within each file:</p> <p>*N.TD: Channel 1 TimeDrive = numerator for the ratioing of the measured data</p> <p>*D.TD: Channel 2 TimeDrive = denominator for the ratioing of the measured data</p> <p>*A.TD: Channel 1 TimeDrive/Channel 2 TimeDrive = ratio of the intensity values</p>
<p>Filenames</p>	<p>Three result files are generated and saved:</p> <p>Channel 1 TimeDrive = numerator for the ratioing of the measured data</p> <p>Channel 2 TimeDrive = denominator for the ratioing of the measured data</p> <p>Channel 1 TimeDrive/Channel 2 TimeDrive = ratio of the intensity values.</p> <p>The filenames can either be entered manually or by double-clicking on a field to display the file selector.</p> <p>NOTE: <i>The files are always stored in the default FL WinLab data directory.</i></p>

Parameter	Description
Auto increment filenames	If this option is selected the application generates a new numbered filename for each run. The base filename is given as the Result Filename , and is extended with a number starting from #001. If a file with this filename already exists on the hard disk in the current FL WinLab data directory, the number is incremented by one, until the filename is unique. The numbering process can be forced to start from a different number by adding a number to the base filename, for example Test#5.sp would make the first generated filename Test#005.sp and the second Test#006.sp and so on.

Realtime options tab

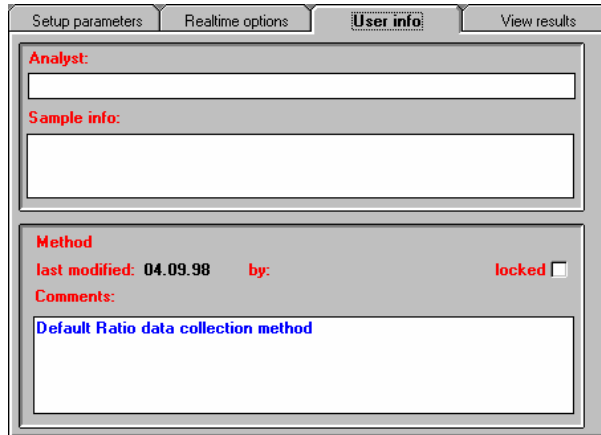
The screenshot shows the 'Realtime options' tab with the following settings:

- Background**: Subtract
- Channel 1: 0.000
- Isobestic point: 0.000
- Measure background button
- Graph**:
 - Ordinate label: R
 - Ordinate Max: (empty)
 - Ordinate Min: (empty)
 - Auto-clear curves
 - Show all data

Parameter	Description
Background Subtract	Select this option to automatically subtract the given Intensity from the signal. The Intensity can either be entered manually or automatically by clicking Measure background .
Ordinate label	The label will be displayed on the ordinate axis of the graph and will be stored in the dataset.
Ordinate Max	Sets the default ordinate maximum value. Leave empty to retain the same ordinate value as last time.
Ordinate Min	Sets the default ordinate minimum value. Leave empty to retain the same ordinate value as last time.

Parameter	Description
Auto-clear curves	If this option is selected all curves are deleted from the graphic before a measurement starts, rather than each successive scan being superimposed on the previous scans. The curves are not deleted from the hard disk.
Show all data	If this option is selected all generated data sets are displayed in real-time, otherwise only the ratio dataset is displayed. However, in both cases all data sets are stored.

User info tab

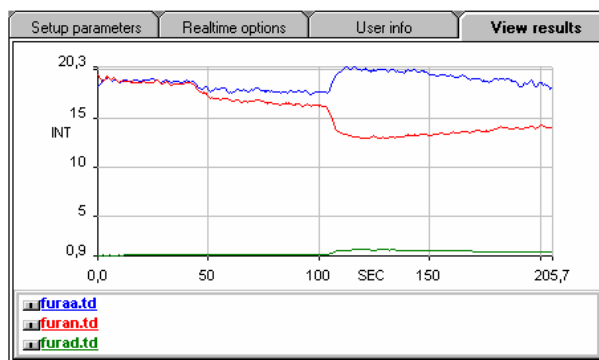


Parameter	Description
Analyst	The name is automatically taken from the configuration dialog, but can be altered for documentation purposes. The name is saved in the header of any collected datasets and, if a method is saved, in the header of the method.
Sample info	This information will be stored in 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' field of the dataset, and can be viewed using Report Builder.

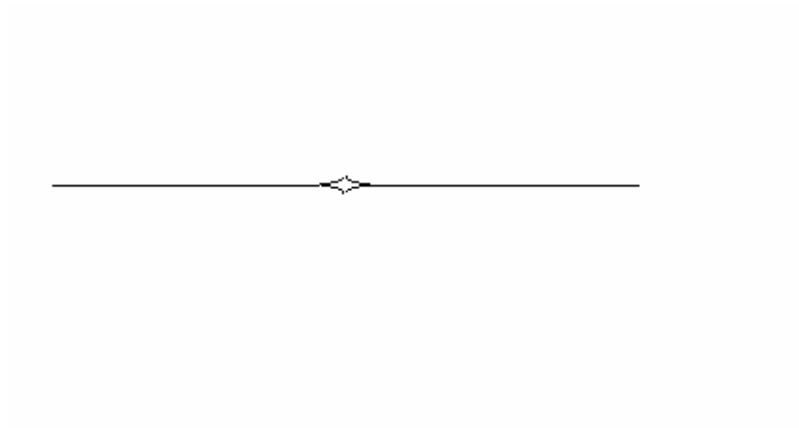
Parameter	Description
last modified	This shows the date the current method was last saved.
by	This shows the name of the analyst who last saved the method.
locked	This option enables the locking of all entries in a method. The option can be altered in Expert mode only. If the option is selected all entry fields and the Method save command are disabled.
Comments	The comment in this textbox is saved in the method header and is also displayed in the method window of the software, but is not saved in the dataset (use Sample info for comments to be saved in datasets).

View results tab

This page contains a toolbar in which graphics icons appear. For further details on viewing data in real-time see *Generic Viewing Features* starting on page 74.



NOTE: *There is a difference in ordinate scale between the intensities and the ratio. Select the ratio dataset and then on the expand ordinate button in the toolbar. The ratio dataset will then be expanded to fill the ordinate scale for clarity.*



The Validation **16**
Application

Introduction

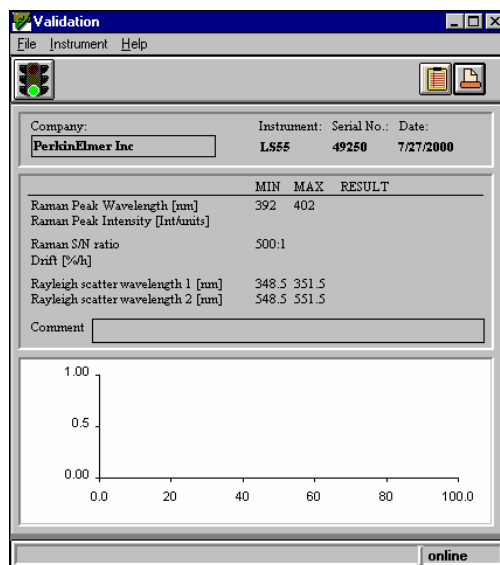
NOTE: *For general information on using Applications, see Using Application Methods starting on page 65.*

The Validation application is used for quantitatively measuring performance characteristics of the instrument. Sensitivity (by Raman band signal-to-noise) and wavelength accuracy are tested automatically.

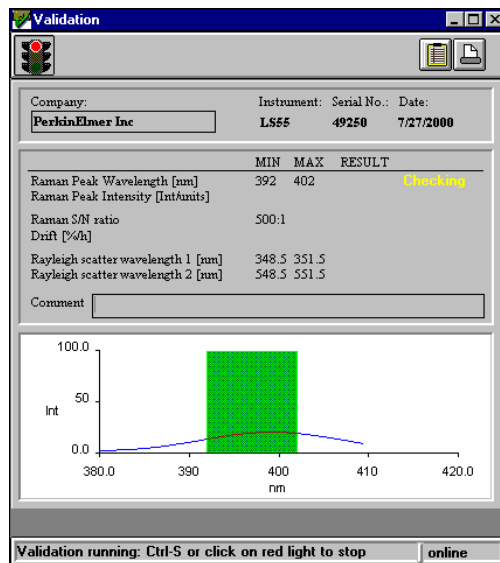
Using the Application

1. Select **Validate** from the Application menu.

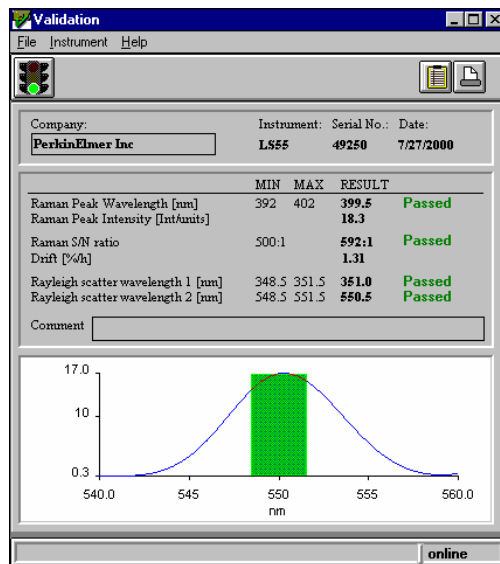
The Validation application is opened.



2. Insert the sealed water cell.
3. Click on the green traffic light in the toolbar to start the validation.
Validation begins.



- When validation is complete the final report is displayed and you can print the report.



- To exit the application, select **Exit** from the File menu.

Functional description

Sensitivity

Sensitivity of the instrument is determined by determination of the signal to noise value for the Raman band of water. Parameters are as follows.

Raman band scan (for signal measurement)

- Excitation wavelength 350 nm
- Emission scan range 380 nm-420 nm
- Slits 10/10 nm (Ex/Em)
- Scan speed 120 nm/min

Raman band TimeDrive (for noise measurement)

- Excitation wavelength 350 nm
- Emission wavelength ~397 nm (determined from scan)
- Slits 10/10 nm (Ex/Em)
- Response 4 seconds

Signal-to-noise testing

- The Raman band peak positions and intensity are derived from the scan. Peak height is automatically baseline subtracted.
- Noise values (R.M.S. and peak-to-peak) are derived from the TimeDrive (at the peak wavelength, not on the baseline) and the signal-to-noise value and Raman peak positions generated and written into the report.
- If the signal to noise passes the minimum specified value (500:1 R.M.S.), then the report is marked accordingly. If the value is below this, then a **Failed** comment is added to the sensitivity result section.

Wavelength accuracy

The positions of the Raman band peak is also tested. If this falls within the acceptance limits, then the report is marked accordingly. If the wavelength is outside the acceptance limits, then a **Failed** comment is added to the sensitivity result section.

The wavelength accuracy is tested more stringently by recording Rayleigh scatter peaks at 350 nm and 550 nm. The excitation wavelength is set to the nominal wavelength, and then the emission monochromator is scanned through the excitation wavelength. The position of the Rayleigh scatter peak, which is expected to be at the excitation wavelength, is then verified. Acceptance criteria for these scatter peaks depend on the wavelength position and the slit width used to record the scans.

Acceptance criteria

The acceptance criteria are displayed on the dialog:

	MIN	MAX	RESULT
Raman Peak Wavelength [nm]	392	402	
Raman Peak Intensity [Int/units]			
Raman S/N ratio		500:1	
Drift [%/h]			
Rayleigh scatter wavelength 1 [nm]	348.5	351.5	
Rayleigh scatter wavelength 2 [nm]	548.5	551.5	
Comment	<input type="text"/>		

NOTE: During data collection, the expected region of the Raman band and of the two wavelength accuracy peaks is indicated by a green range box, for quick reference of the current data against the acceptance criteria.

