DataMax version 2.20

Operation Manual

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0: Introduction

DataMax for Windows™ is a powerful data-acquisition and data-manipulation program, accessed through point-and-click operations. Measurements of fluorescence, phosphorescence, lifetimes, single-point analyses, experiments requiring temperature-control, and real-time display of incoming data are all possible. DataMax monitors and controls all Spex® spectrofluorometers.

The software is a collaborative effort between Jobin Yvon® and Thermo Galactic Corporation. Therefore, several different operation manuals come with the instrument and accessories purchased.

This manual covers DataMax. For information concerning programming in Visual BASIC, which is automatically included with DataMax, see the *GRAMS/32® User's Guide*.

Manuals supplied

- Windows™, which covers the computer's operating system, and its commands and utilities. Windows™ is the environment in which DataMax runs.
- *GRAMS/32® User's Guide*, which covers data-manipulation, configuring screens, and special arithmetic data functions.
- *GRAMS/32® Array Basic User's Guide*, which covers a programming language, similar to BASIC, developed by Thermo Galactic, and useful for customization.
- *DataMax 2.20 Operation Manual*, (this book) which contains information about data-acquisition using your Spex® instrument and accessories.
- Various hardware *Operation Manuals*, which describe installation, calibration, troubleshooting, and operation of the specific instruments and accessories.

Manual overview

Part I: Steady-state data-acquisition (for all users)

Part II: Lifetime data-acquisition (for users of Tau-3 Lifetime systems)

Part III: Data display functions (for all users)

Part IV: Appendices

Symbols used in this manual

Certain symbols are used throughout the text for special conditions when operating the instruments:

Warning:

A hazardous condition exists, or danger exists that could damage the equipment. Jobin Yvon®, Inc., is not responsible for damage arising out of improper use of the equipment.

Note:

General information is given concerning operation of the equipment.

1: Getting Acquainted

Systems controlled by DataMax:

- FluoroMax®-3, the economical, self-contained Spex® spectrofluorometer
- Fluorolog®-3, the modular Spex® spectrofluorometer for analyses from the UV through IR wavelengths
- Fluorolog®-Tau-3 (Lifetime), a modular steady-state and lifetime Spex® spectrofluorometer

Conventions used in this manual

This manual uses some of the same conventions as Microsoft Windows™:

Convention	indicates a(n)	Example
"Quotes"	Label	Tab to the "File Slot Number" text box.
	Something to be typed	Type "`exper.one`" at the prompt.
Italic	Menu or button choice	Choose *View* from the main menu.
BOLD SMALL CAPS	Keyboard key	Use the **PAGE UP** key to see a new spectrum view.
Keyboard keys joined with +	Set of keys to be pressed simultaneously	Press **CTRL+ENTER**.
Bold Italic	Commonly used dialog box	Retrieve the ***Emission Acquisition*** dialog box.
`Courier New`	File name	The file name is `layout1.lay`
1 Number	Itemized instruction	1 Click *Collect*. 2 Select *Real Time Display*.

Data-acquisition terminology

Current experiment — A set of parameters retained in the computer's memory. Data are always acquired using the current experiment's parameters.

Data entry fields — *See* **Parameters**.

Data file — A file containing the actual data collected during the experiment, along with information related to setting up the instrument and specific conditions active during the experiment.

Default experiment — The set of parameters that appears when an experiment type is first activated. Each scan type has a default set of parameters associated with it.

Define — To specify experiment parameters.

Experiment — For this manual, all instrument and data-acquisition parameters used to collect data.

Experiment file — All experimental parameters needed to set up an experiment, but without data.

Parameter — Setting for the instrument that determines how an experiment will be performed and where to store the resultant data. Examples: High-voltage, slits, monochromator position.

Setup file — Information concerning settings for a particular experiment.

Installation

DataMax is delivered on a CD-ROM, plus a 3½" floppy disk. The 3½" floppy disk is the DataMax Instrument Disk (or DataMax INI disk), which contains the spectrofluorometer's specific hardware configuration.

1 Know the spectrofluorometer COM port.

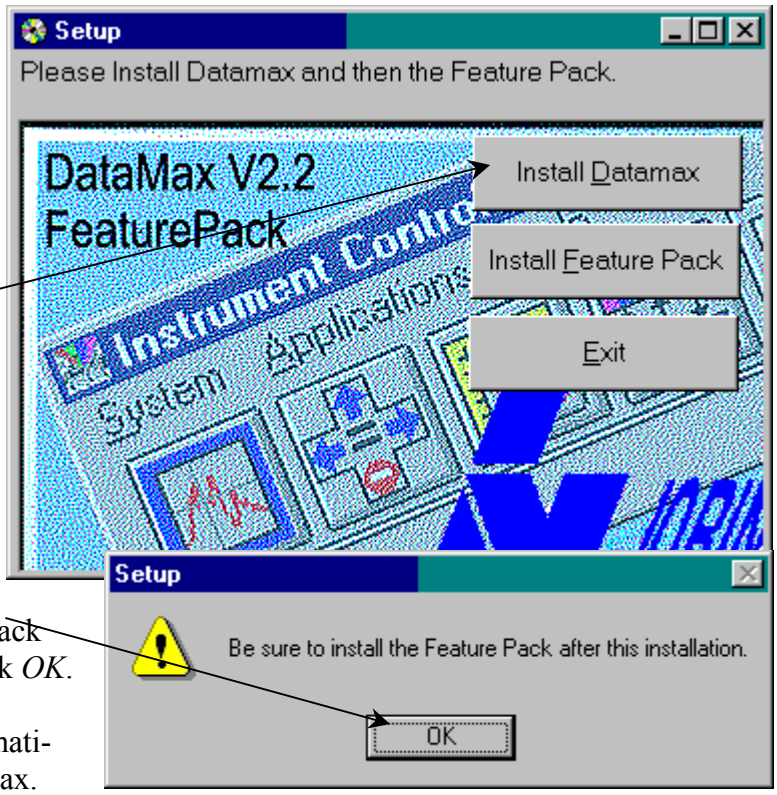
2 Know in which directory to install DataMax.

Default is C:\DataMax.

If Autorun is enabled:

1 Turn on the computer, and insert DataMax CD-ROM.

The set-up window to install DataMax appears:



2 Click *Install Datamax*.

A warning appears, reminding you to install the Feature Pack after DataMax. Click *OK*.

The computer automatically installs DataMax.

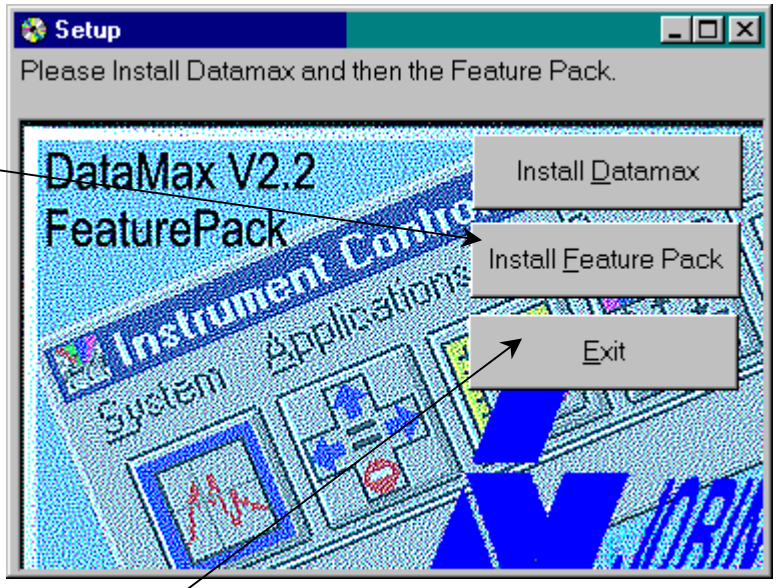
3 The computer asks for the instrument (INI) floppy disk.

Insert the floppy disk into drive A:.

4 Use the default options.

The computer installs the options, then returns to the set-up window:

5 Click *Install Feature Pack*.



Note: *The Feature Pack is also on the DataMax CD-ROM.*

6 Accept the default options.

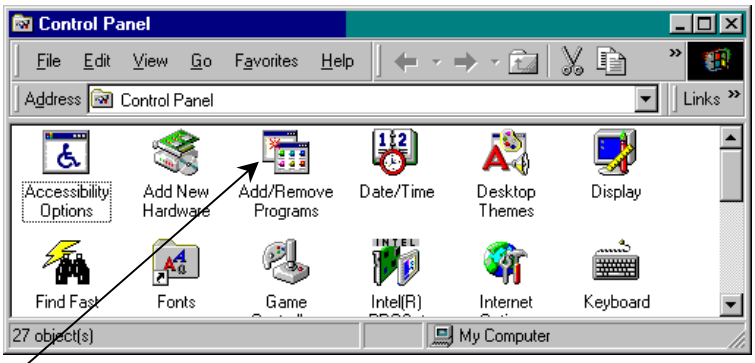
After installation, the computer returns to the set-up window.

7 Click *Exit*.

If Autorun is disabled, use the following—or other appropriate—method:

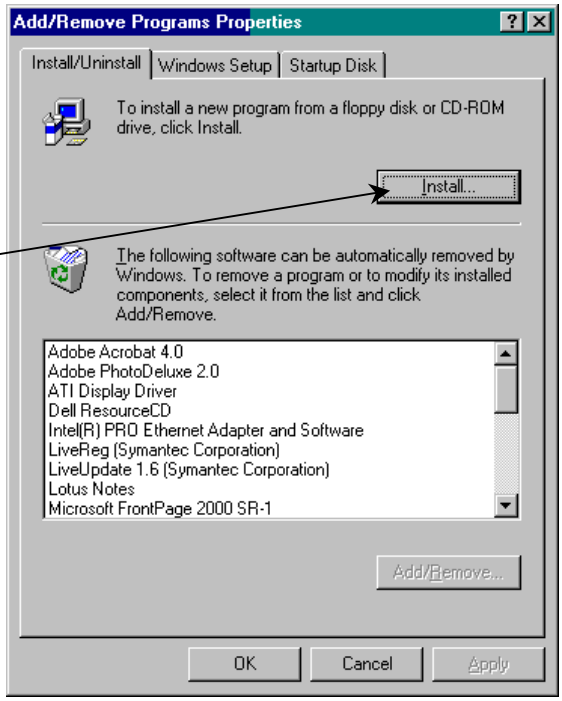
1 Insert the DataMax CD-ROM into the drive.

2 Go into Windows™ ***Control Panel***.



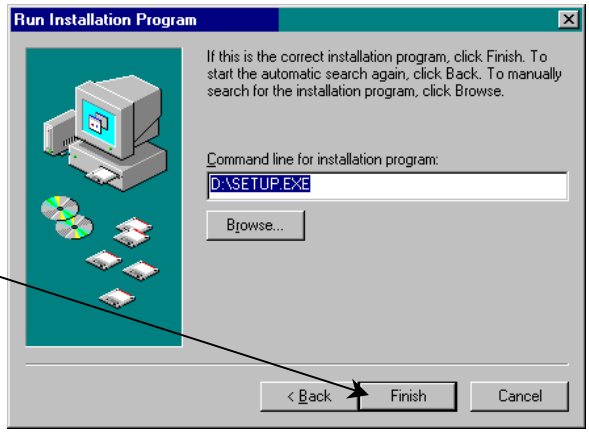
3 Click *Add/Remove Programs*.

This opens the ***Add/Remove Programs Properties*** dialog box:



4 Click *Install…*

This opens the ***Run Installation Program*** window. The computer should find the `SETUP.EXE` file on the CD-ROM drive.



5 Click *Finish* to finish the installation.

Users outside of the USA:

Users outside of the USA receive a softkey device that connects to the printer port of the host computer for software security. The softkey should be left in place on the host computer at all times.

Basic operation of DataMax 2.20

Mouse versus keyboard

DataMax responds to both mouse point-and-click operation, and keyboard commands. This manual emphasizes use of the mouse. For corresponding keyboard commands, see *Chapter 12: Appendix, "Data-acquisition speed keys"*.

Modes of operation

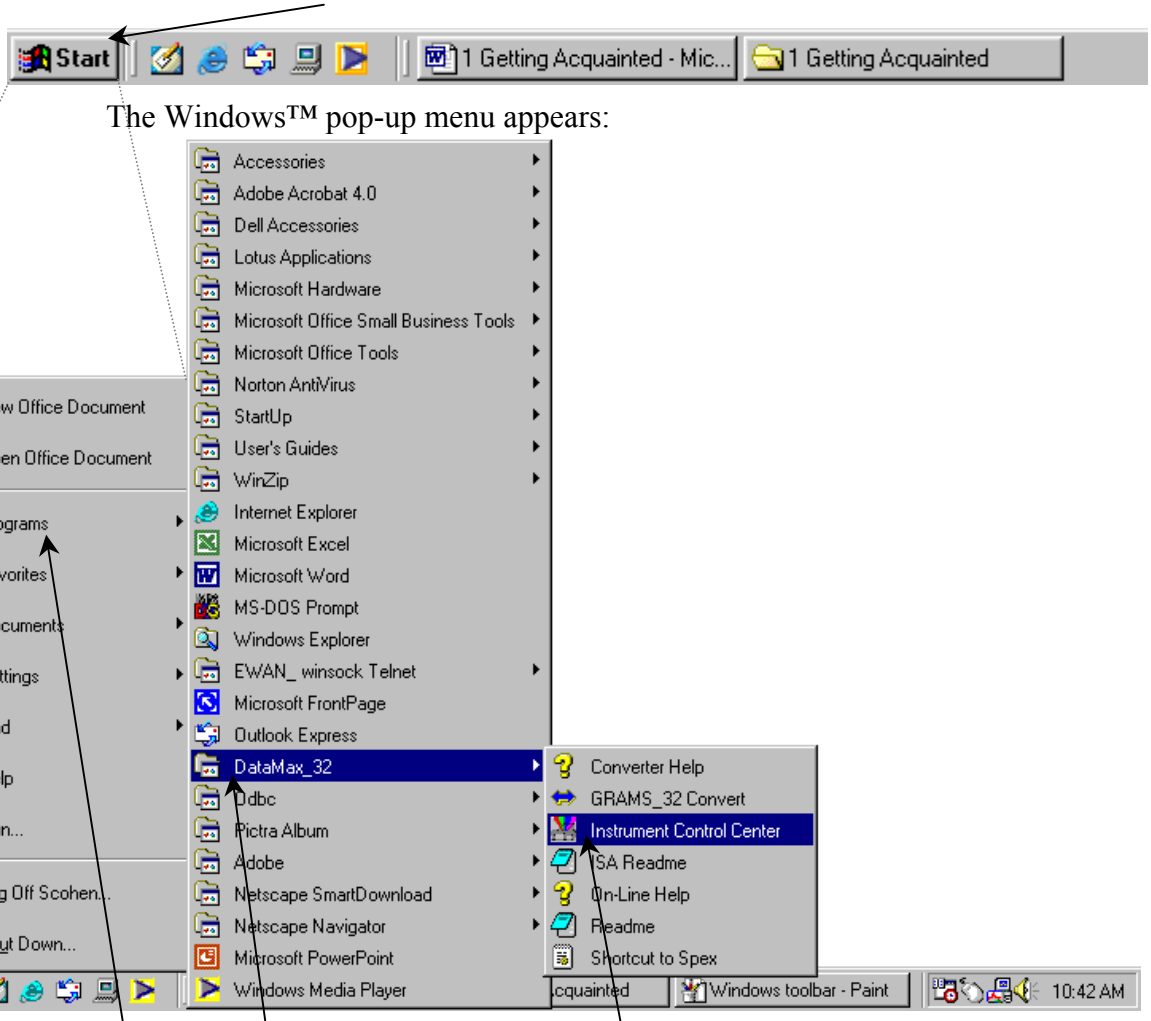
There are two modes of operation within DataMax:

- *Emulation mode*, in which the hardware is disabled. This is useful for learning to run the system without distractions of setting up the accessories and components.
- *Hardware mode*, in which the hardware is active. All specified accessories and components operate in hardware mode.

The default mode is Hardware mode, in which the software searches for the instrument and accessories. If the software fails to find some or all of the hardware specified, you have the chance to enter Emulation mode.

Starting DataMax

1 Click the *Start* button in the Windows™ toolbar.

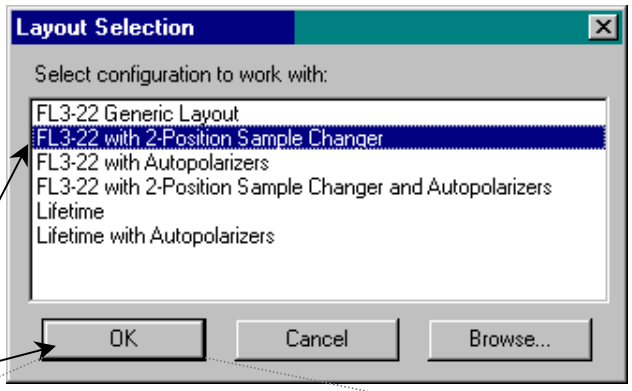


2 Click *Programs* to open a menu of available programs.

3 Click *DataMax_32* to open the ***DataMax*** menu.

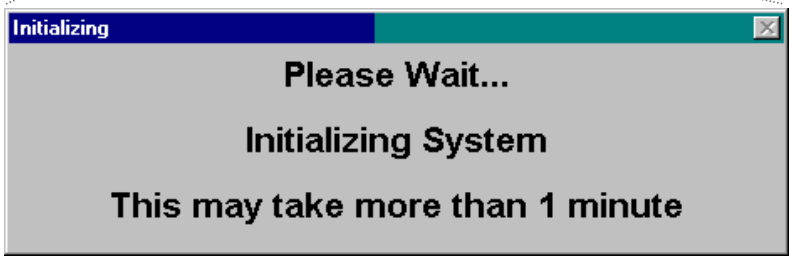
4 Click *Instrument Control Center*.

The ***Layout Selection*** dialog box opens. Each configuration available for the instrument is called a *layout*, and includes all accessories chosen for a particular type of experiment.

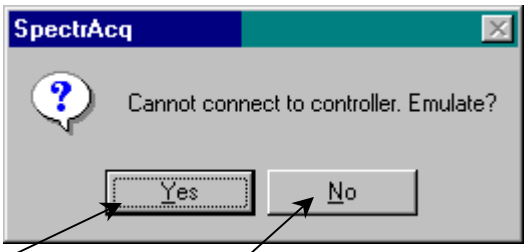


5 Click on a desired layout.

Click *OK*. An initialization screen appears. Let the system initialize the monochromator drives and slit motors.



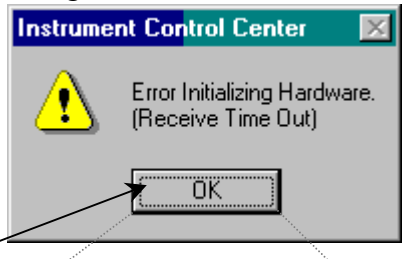
6 If DataMax cannot connect properly to the hardware, a dialog box appears.



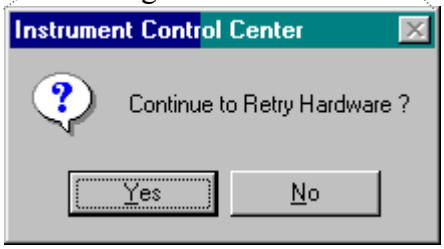
The title bar identifies the hardware causing the problem. In this example, the SpectrAcq shows difficulty in connecting to the instrument. Be sure that all cables are connected and the appropriate accessories are switched on.

a Choose *Yes* to enter Emulation mode. Parameter settings are stored in the computer's memory, but DataMax does not connect to the hardware. All functions are accessible in Emulation mode, but the "data" taken are invalid. Use Emulation mode to learn DataMax.

b Choose *No* to open the ***Error Initializing Hardware*** dialog box:



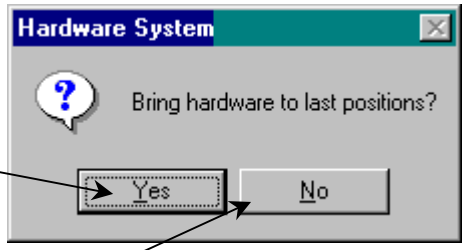
Click *OK* to continue. This opens the ***Retry Hardware*** dialog box:



Click *Yes* to try to establish contact with the hardware. Continuing problems cause the cycle of dialog boxes to re-appear.

Click *No* to quit the DataMax program.

7 The ***Hardware System*** dialog box appears:

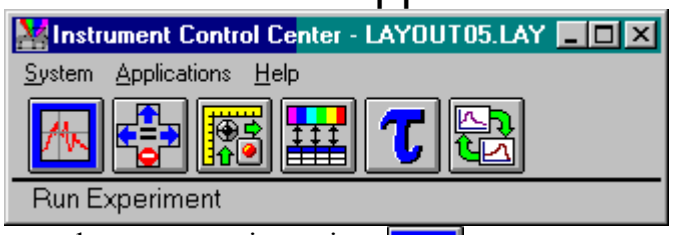


a Choose *Yes* to return to the most recent settings for DataMax's last use.

b Choose *No* to leave the instrument settings as they are now.

8 The ***Instrument Control Center*** appears.

The six basic applications are run from ***Instrument Control Center***. They are:



Run Experiment, in which a steady-state experiment is set up and run.

Real Time Display, in which the instrument settings are set.

Visual Instrument Setup, in which the accessories and components are set up.

Constant Wavelength Analysis, in which batches of samples may be scanned at a particular wavelength for concentration determination.

Lifetime Acquisition, in which a fluorescence lifetime experiment is set up and run.

Modeling, in which data may be fit.

When the mouse is passed across the ***Instrument Control Center***'s buttons, the status bar at the bottom shows the name of the button.

9 Click on a button to start an application.

Main menus and toolbars

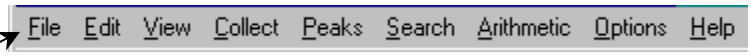
With DataMax, the following functions can be controlled:

- Set experiment parameters
- Acquire, display, and process data
- Manage files
- Specify hardware components and accessories
- Control monochromators
- Supply the detectors' high-voltage

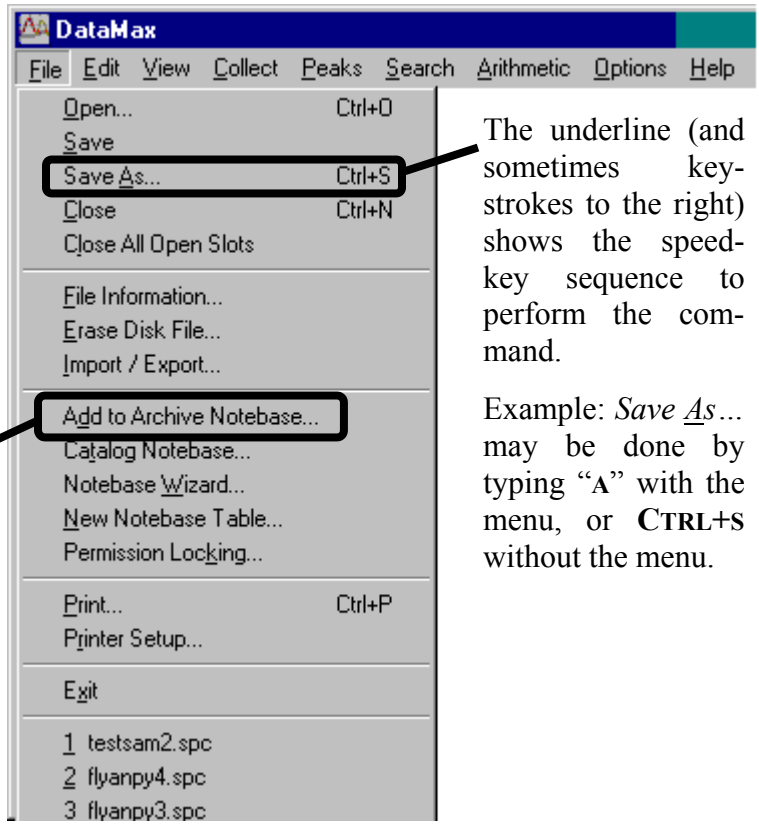
To perform these functions, use the *main menu* or *toolbar* in each of the six applications.

Main menu

In most applications, as with other Windows™-compatible software, the main menu is at the top of the window. Here is an example of a main menu.



Click on a menu command to reveal a drop-down menu:



The choice *File* opens a drop-down menu. This example is the *File* menu, containing commands associated with manipulating files.

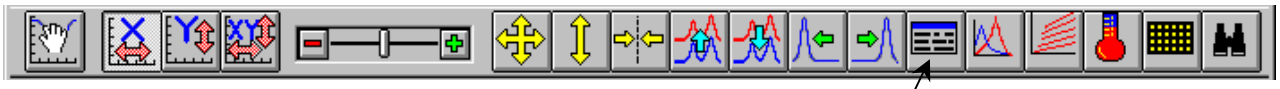
An ellipsis (…) indicates a cascade menu of subsequent options.

The underline (and sometimes key-strokes to the right) shows the speed-key sequence to perform the command.

Example: *Save As*… may be done by typing "**A**" with the menu, or **CTRL+S** without the menu.

Toolbar

A *toolbar* is often found directly underneath the main menu. The toolbar contains buttons to execute many commands, including some from a main menu. Here is an example of a toolbar, from the ***Run Experiment*** window.



As an example, clicking on this button activates the ***Experiment Definition*** window.

File names

Name

To store data, experimental setups, or other information requires a file. In DataMax, file names may be up to eight characters long (unlike Windows™ 95 or above, which allow many more characters). Spaces and most punctuation are not allowed. Examples of valid file names are `sample01`, `newfile`, and `quinine`.

Extension

Each file name may have an extension containing up to three characters. Each DataMax application provides a default extension automatically recognized by the software. Each extension indicates the type of information that the file stores. For example, `.SPC` is a general DataMax data file, and `.SET` is a DataMax setup file.

Extension	Type of file
`.CWA`	***Constant Wavelength Analysis*** data file
`.DAT`	***Lifetime*** data file
`.EXP`	***Run Experiment*** experiment file
`.LAY`	Layout file indicating the instrumental configuration
`.SET`	Setup file
`.SLY`	***Real-Time Display*** screen layout file
`.SPC`	***Run Experiment*** data file
`.TXT`	Text file
`.XLS`	Microsoft® Excel™ spreadsheet file

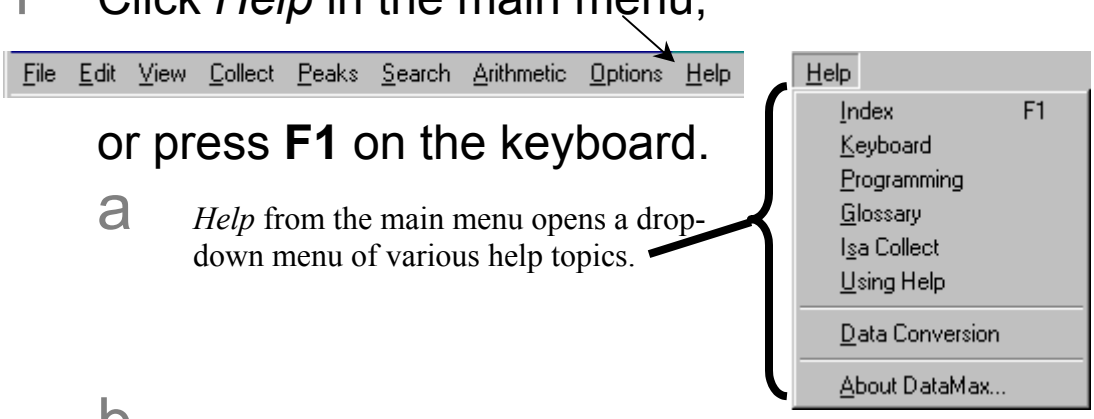
Examples of valid file names with extensions are `test01.spc`, `layout05.lay`, and `expt6b.exp`.

Instead of using default extensions, other extensions may be substituted.

Help

General help

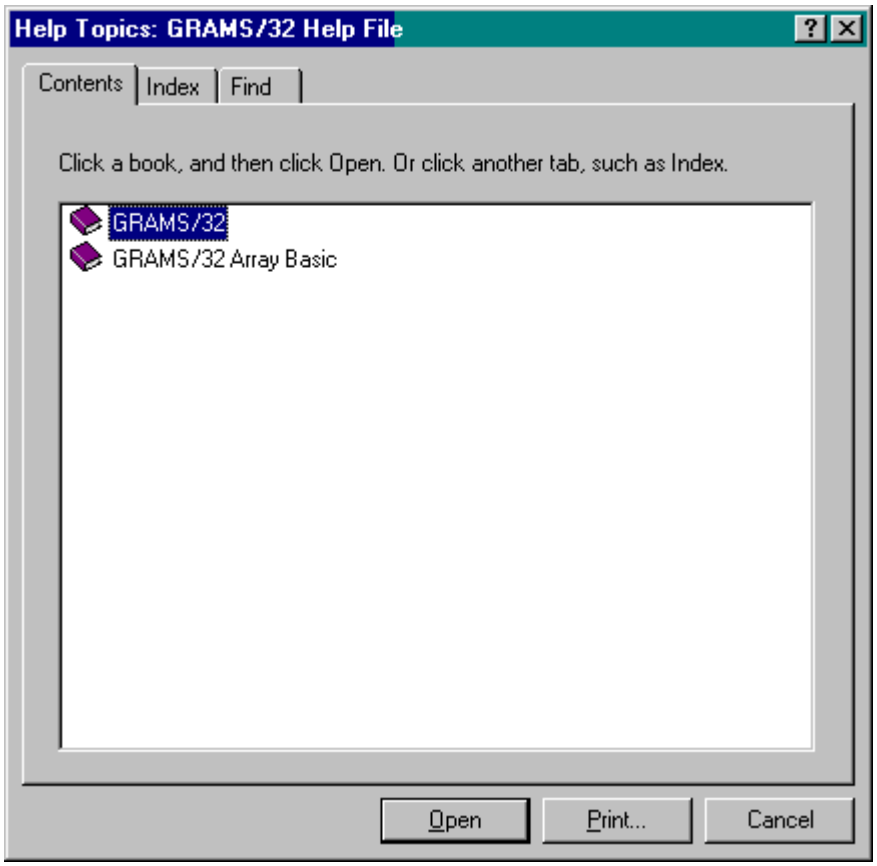
1 Click *Help* in the main menu,



or press **F1** on the keyboard.

a *Help* from the main menu opens a drop-down menu of various help topics.

b *Help* from **F1** opens a ***Help Topics*** window:



Help for a particular topic

The context-sensitive help in most applications lets you **TAB** through various fields, or place the mouse on a particular field and press **F1**. If help is available for that field, it appears when **F1** is released.

Leaving Help

1 Press **ESC**.

or

1 Move the mouse outside the ***Help*** menu.

2 Click the left mouse-button.

Error messages

When the software encounters a problem, an error message may appear. The message identifies the difficulty. To remove the error message,

Click *OK*.

Or

Double-click the ☒ at the upper-right corner of the dialog box.

Acquiring a scan

From the ***Instrument Control Center***, one or multiple applications may be opened. To conduct complete experiments, use the ***Run Experiment*** routine for steady-state acquisitions, and the ***Lifetime Acquisition*** routine for lifetime experiments.

As an illustration, the following procedure takes a scan of the 450-W xenon lamp source in the Fluorolog®, FluoroMax®, and Tau systems. The xenon lamp produces a characteristic peak at 467 nm, which is used to indicate whether the excitation spectrometer is properly calibrated. Thus, this procedure is called a *lamp scan*.

During the lamp scan, the system monitors the reference detector. From the peak position and appearance of the resultant scan, the condition of the xenon lamp and the calibration accuracy of the excitation spectrometer can be assessed.

1 Be sure the instrument and accessories are switched on and operating properly.

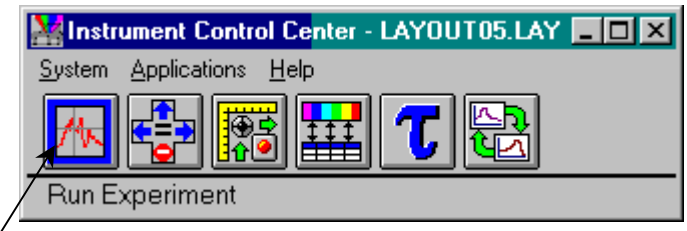
(See the appropriate hardware manual for instructions on switching on the equipment.)

2 Remove any samples from the sample chamber.

3 Close the sample-compartment lid.

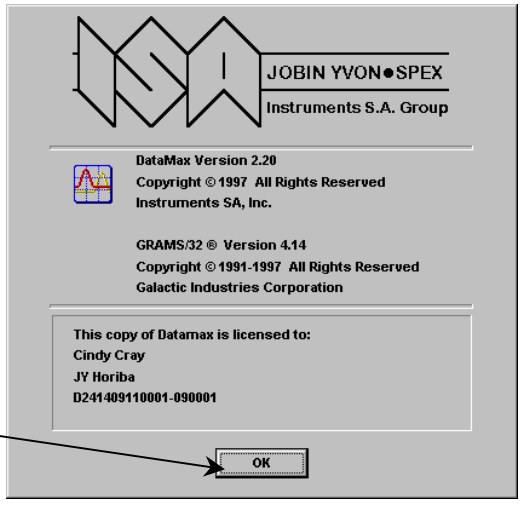
4 Start DataMax.

Note the ***Instrument Control Center*** when the start-up sequence is complete.



5 Click the *Run Experiment* button.

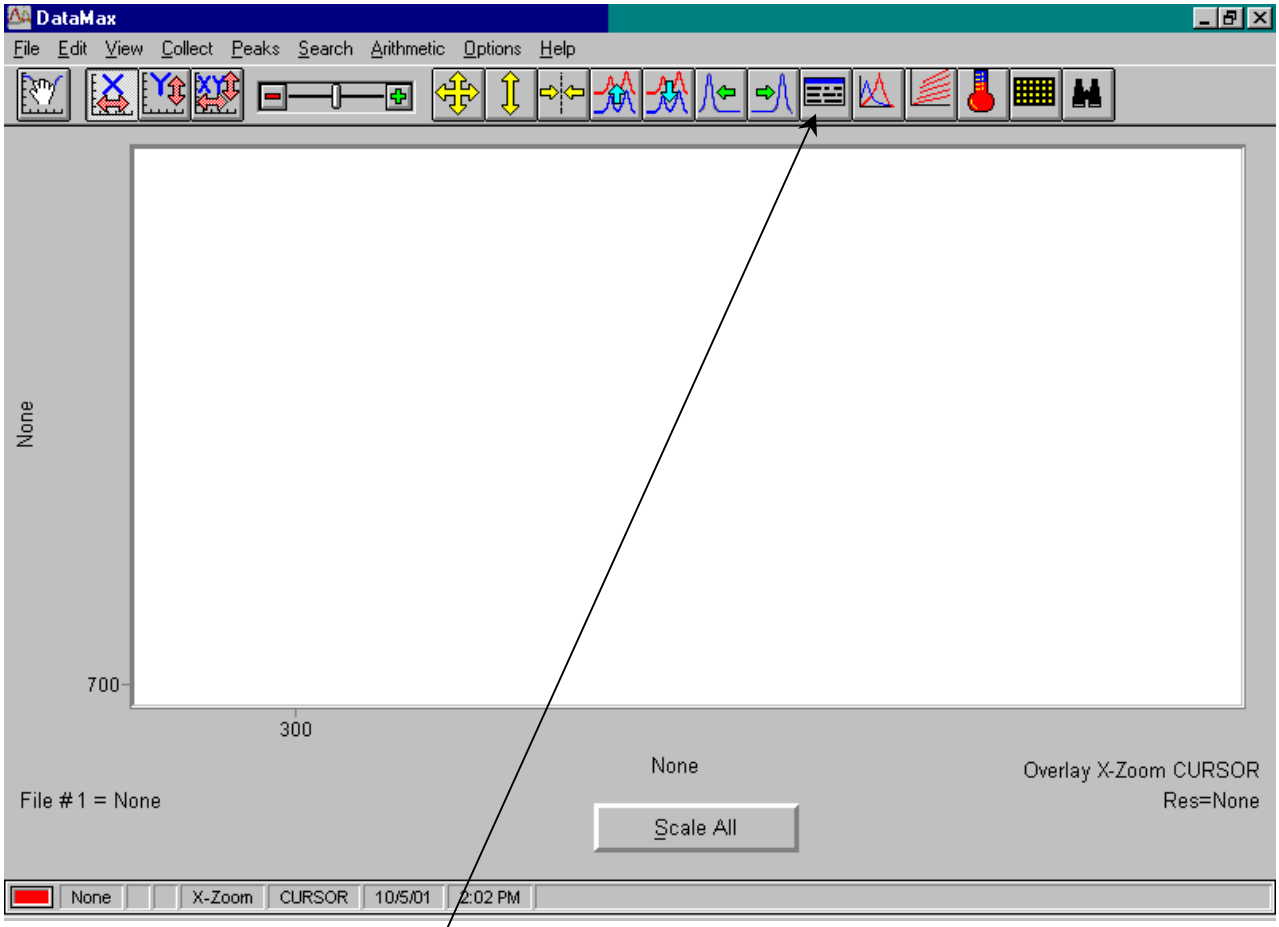
A dialog box displaying information about the program, version number, and license agreement appears briefly.



6 Click *OK*

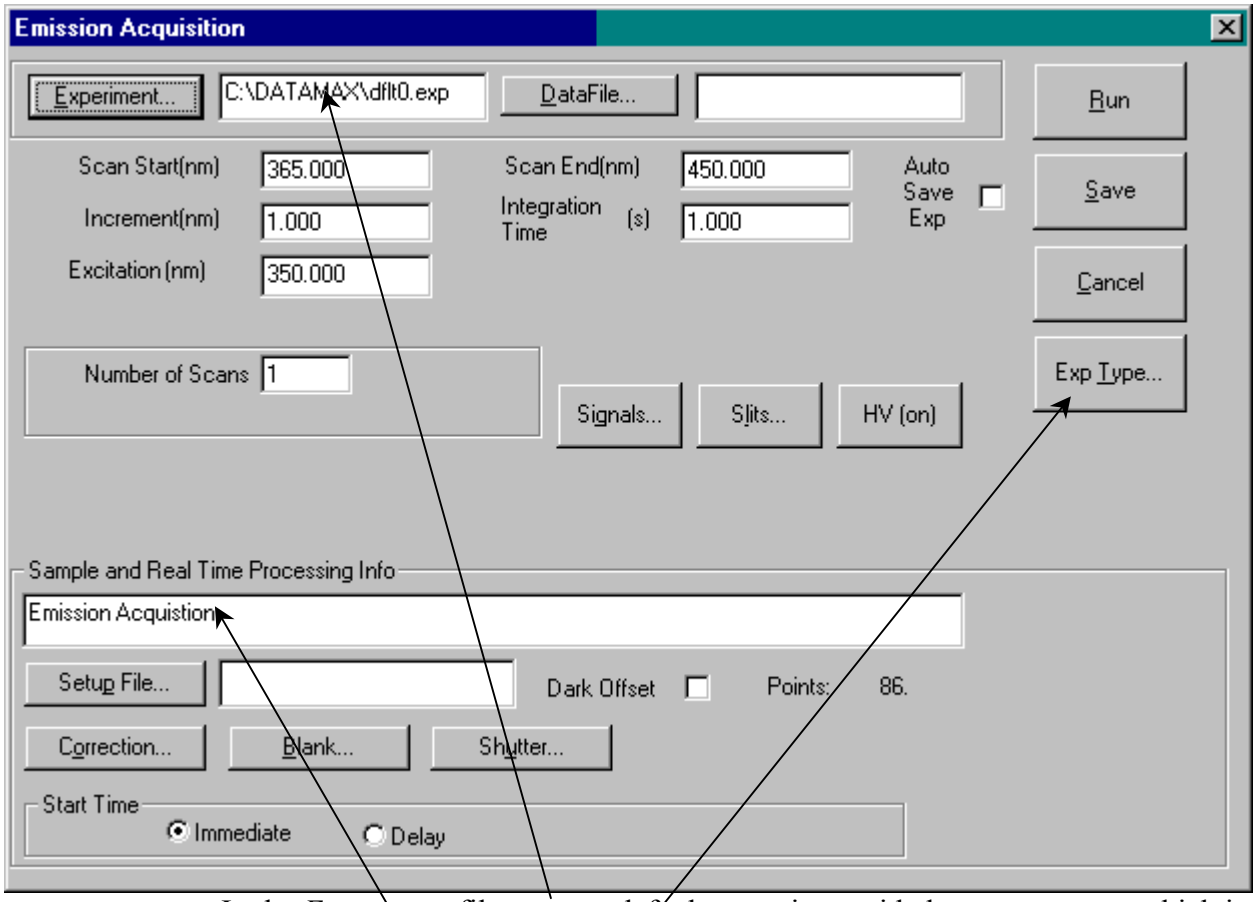
or let the window disappear automatically.

The default main ***Run Experiment*** window appears:



7 Click the *Run Experiment* button.

This opens the ***Emission Acquisition Experiment*** dialog box (or ***Lifetime Acquisition***, for a Tau system):



In the *Experiment* file name, a default name is provided, `dflt0.exp`, which is considered an emission acquisition experiment. Enter new names or parameters as desired, or leave them as is.

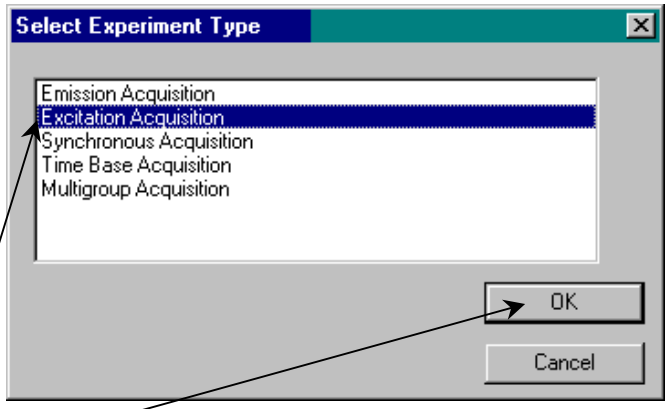
- If you change any of the parameters, and save the experiment under a new name, the standard default experiment is saved with the new parameters.
- If no parameters are changed, it is not necessary to rename the experiment file.

For a lamp scan, the experiment type ought to be an excitation scan.

8 Click Exp Type….

The ***Select Experiment Type*** dialog box appears. This box

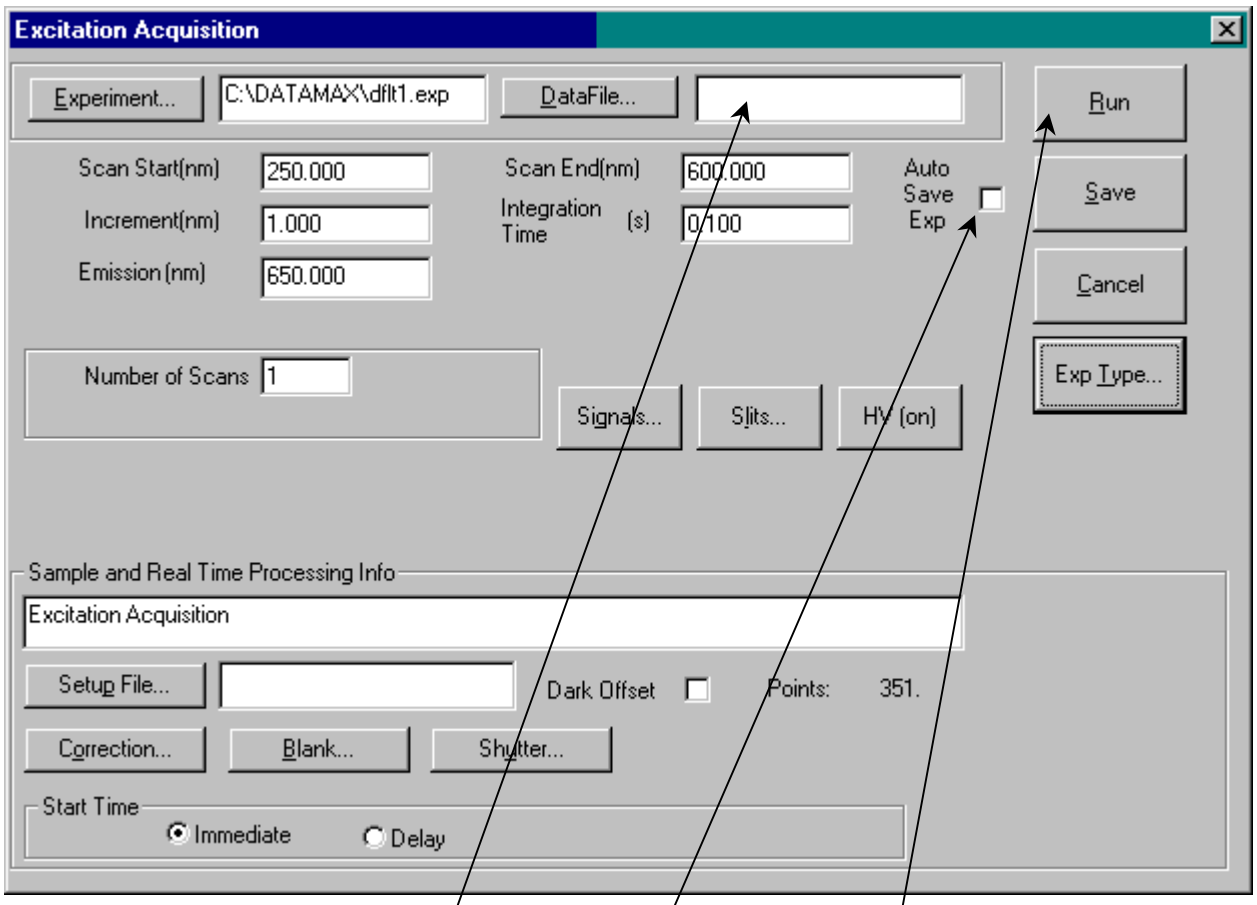
- Lists all available types of scans, and
- Allows changes in the type of scan.



9 Click *Excitation Acquisition*.

Click *OK*.

The ***Emission Acquisition Experiment*** window is replaced by an ***Excitation Acquisition Experiment*** window:



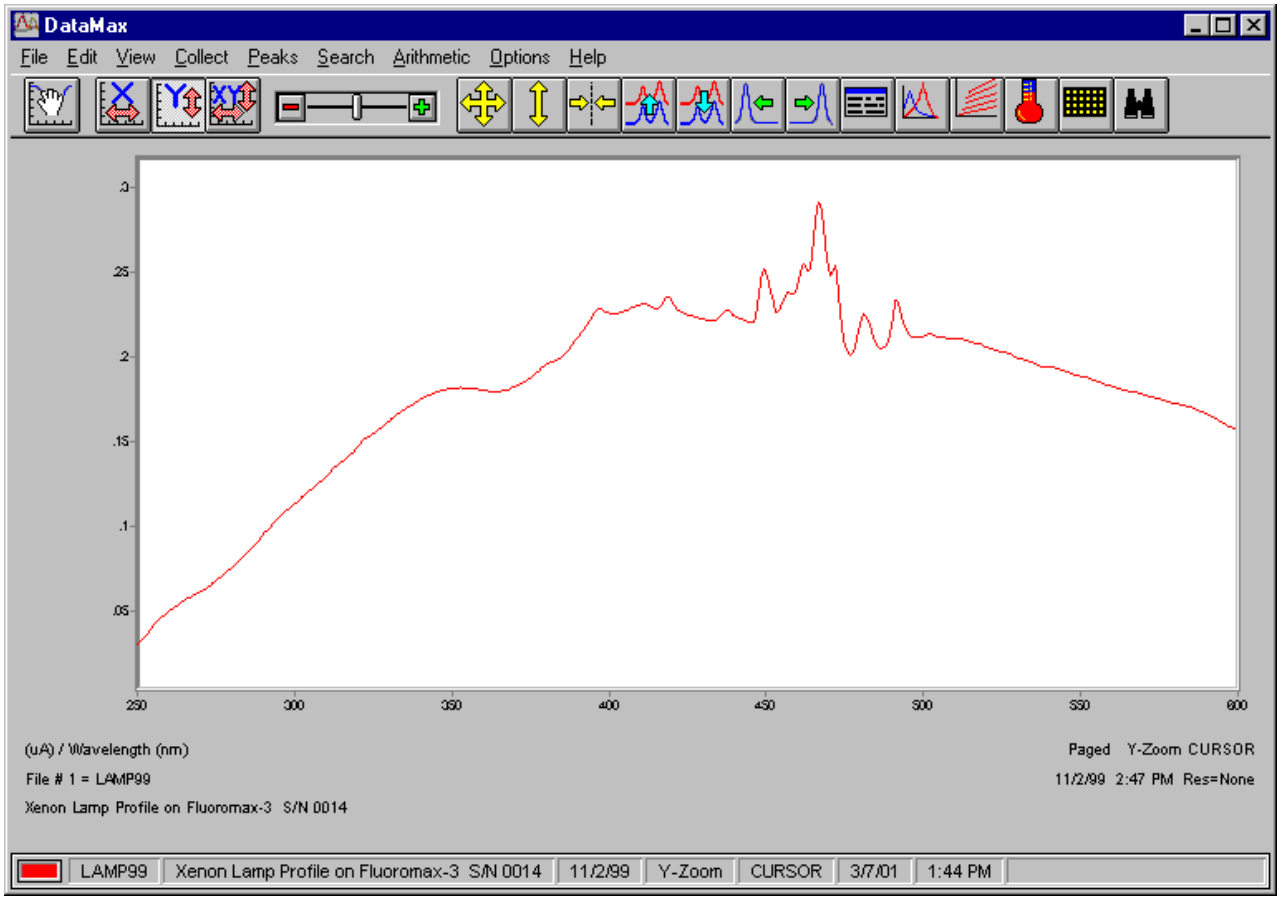
Appropriate parameters and experiment file name are different. The parameters indicated above are the standard xenon-lamp scan parameters.

10 Make sure the *Auto Save Exp* checkbox is empty.

11 Place the mouse cursor within the *DataFile…* field, and type “xenondat”.

12 Click *Run*.

DataMax adjusts the hardware to the settings specified, and the scan is displayed as the data are collected. The final spectrum should resemble the scan below:

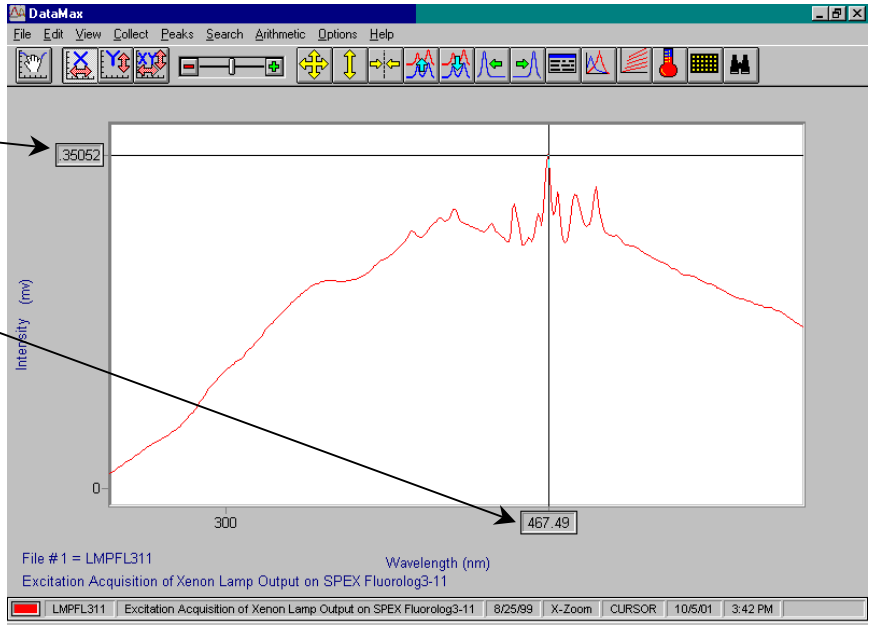


Xenon-lamp scan taken with a single-monochromator Fluorolog®-3.

The actual intensity of each peak depends upon the system configuration and slits widths.

13 Click on the maximum peak.

The peak intensity appears on the *y*-axis, and the precise wavelength appears on the *x*-axis.



a If the maximum peak is at 467 ± 5 nm, the system has passed the performance test.

b If the maximum peak is not within 462–472 nm, follow the procedure in the hardware manual to recalibrate the monochromator gratings.

14 The procedure is complete.

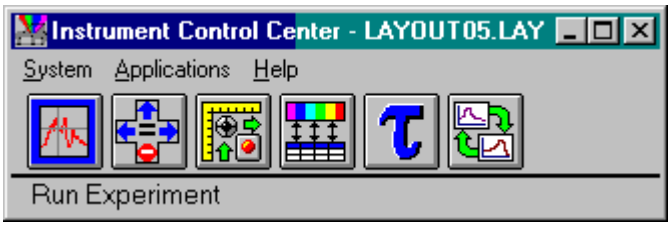
2: Exploring the Applications

Introduction

DataMax is a collaborative effort between Thermo Galactic (GRAMS/32® software) and Jobin Yvon® (manufacturer of Spex® spectrofluorometers). The software is a fully integrated program with all the tools to:

- Define and conduct experiments
- Establish system units and settings
- Tweak hardware parameters and settings while viewing the results in real time
- Perform single-wavelength analysis of a sample
- Determine sample concentration
- Evaluate data after processing.
- Take lifetime data and model them in real time or later (with a Fluorolog®-Tau system)
- Operate optional accessories

Instrument Control Center



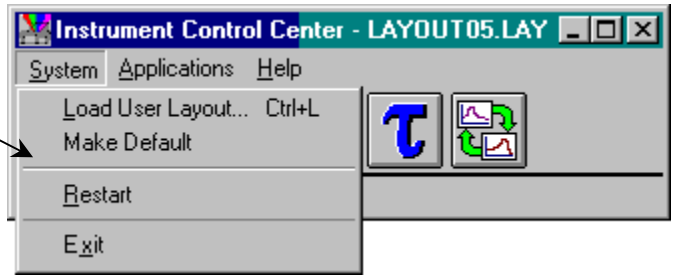
DataMax is composed of several sub-programs, or *applications*. All applications are launched from the ***Instrument Control Center***. Each application is a separate program, and is self-sufficient. More than one application may be opened at the same time, and each can use another application's files.

Each button on the ***Instrument Control Center*** represents an application:

- ***Run Experiment***
- ***Real Time Display***
- ***Visual Instrument Setup***
- ***Constant Wavelength Analysis***
- ***Lifetime Acquisition*** (available only with a Tau lifetime system)
- ***Modeling***

System menu

Within the ***Instrument Control Center*** is the *System* menu. The *System* menu offers selection of different hardware configurations, default files, and exiting the program.



Selecting accessories and layouts

Each Spex® spectrofluorometer is available with a variety of accessories. These accessories may be added or removed to accommodate many experiment types. To choose a broad category of accessories, the user specifies an instrumental *layout* in ***Instrument Control Center***. A layout is defined as a particular configuration of the spectrofluorometer. DataMax is supplied with several layout files custom generated for your system and its accessories. The layout files were loaded during the installation of DataMax, and are retrieved in the ***Instrument Control Center***. Because DataMax controls all accessories, the software must be told which accessories the system shall use. By selecting one of the layouts, DataMax can control or ignore polarizers, temperature bath, autotitrator, etc. Loading a new layout automatically replaces the existing layout. The software then re-initializes the system and its accessories.

A variety of layouts exists. The available layouts for the system depend upon the system and its accessories. Not all layouts are available for all systems. Some common layouts available are listed below:

Note: *This list is not exhaustive; many layouts exist based on the combinations of accessories.*

FluoroMax®-3	Fluorolog®-3	Fluorolog®-Tau-3
Standard (no accessories) With polarizers	*L-configuration* Standard (no accessories) With phosphorimeter With polarizers With temperature bath With autotitrator With MicroMax With sample changer Assorted combination of above *T-configuration* Standard (no accessories) With phosphorimeter With polarizers With temperature bath With autotitrator With MicroMax With sample changer Assorted combination of above	*L-configuration* Fluorolog®-3 (no accessories) Lifetime system Lifetime system with polarizers *T-configuration* Fluorolog®-3 (no accessories) Lifetime system

To change layout

1 Exit DataMax.

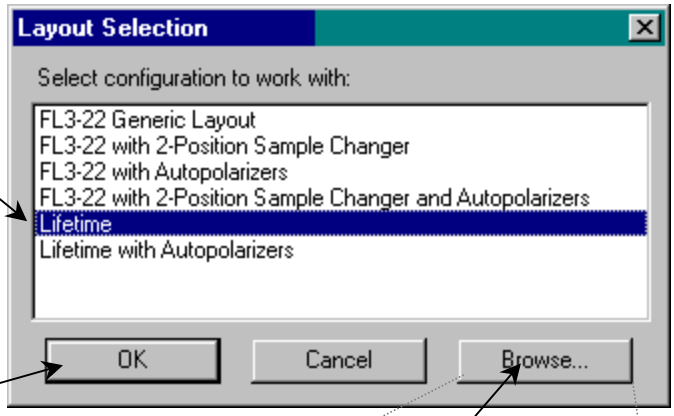
2 Turn off the instrument.

3 Change the instrument and accessories to the desired configuration.

4 Switch on the instrument and host computer.

5 Start DataMax.

The ***Layout Selection*** dialog box appears:

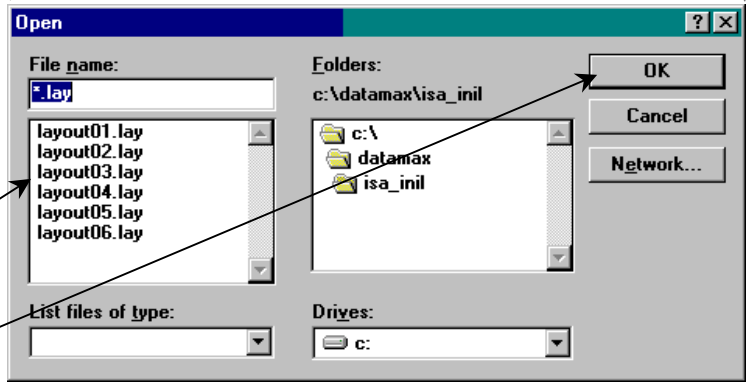


6 Choose the desired layout (containing the appropriate accessories) from the menu.

Click *OK*.

a If the desired configuration is elsewhere, click *Browse*... to search for the layout in other folders.

b The ***Open*** window appears:



c Search the drives and folders for the layout.

d Click on the file name in the list.

e Click *OK*.

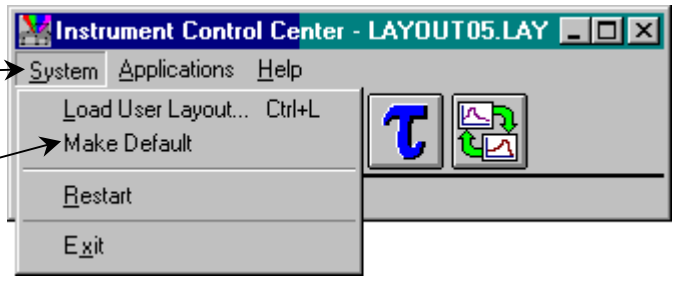
7 DataMax initializes the system.

Forced default layouts

A forced default file is any default file other than the last one used. To force a default layout, with the present configuration as the desired default layout,

1 Choose the *System* menu.

2 Choose *Make Default*.

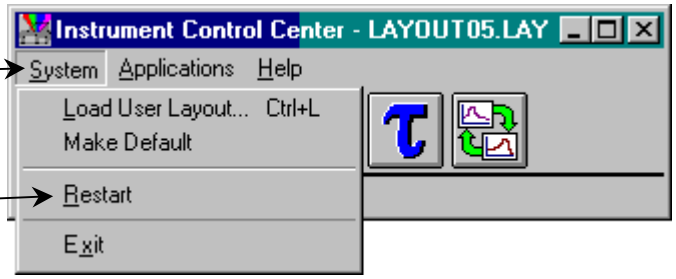


DataMax saves the current layout as the default file. When DataMax is restarted, this is now the expected instrument configuration.

Restarting DataMax

1 Choose the *System* menu.

2 Choose *Restart*.

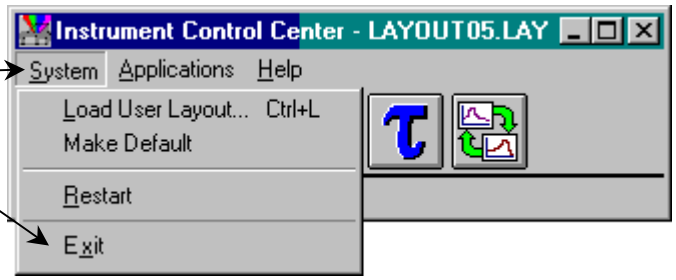


DataMax begins the initialization procedure.

To exit DataMax

1 Choose the *System* menu.

2 Choose *Exit*.

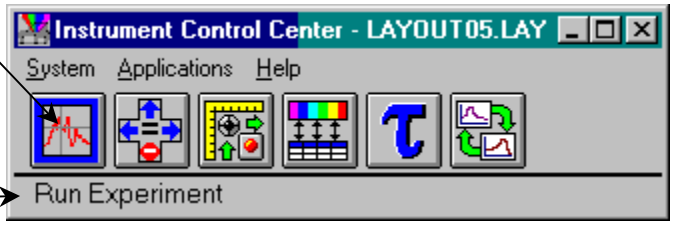


Starting an application

Start an application in the ***Instrument Control Center***.

1 Click the desired button in the ***Instrument Control Center***.

As the mouse moves over each button, its name appears in the status bar.

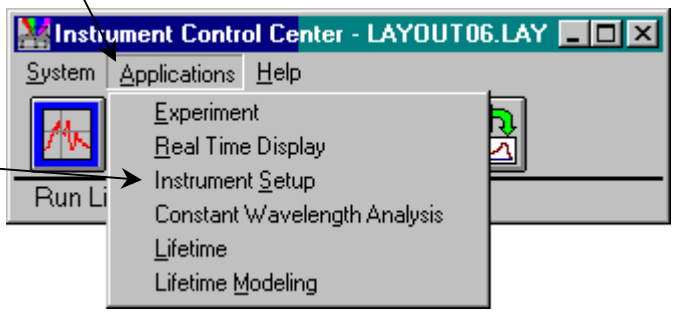


Or

1 Choose the *Applications* menu.

A drop-down menu of available applications appears.

2 Click on the name of the desired application.



Once an application is chosen, the default view of that application appears.

Run Experiment

Run Experiment is the application through which steady-state experiments are defined and conducted. Either pre-defined experiments may be conducted, or the parameters may be varied to customize the procedure. After taking the data, arithmetic functions may be used to manipulate the data. Plots of the data may be adjusted into three-dimensional perspectives, tables, or contour plots. Multiple files may be opened simultaneously.

Note: *This section of the manual only discusses features specific to data-collection* ***not mentioned*** *in the GRAMS/32® manual. The* GRAMS/32® User's Guide *contains comprehensive instructions for GRAMS/32® features.*

Starting ***Run Experiment***

In ***Instrument Control Center***,

1 Click the *Run Experiment* Button.

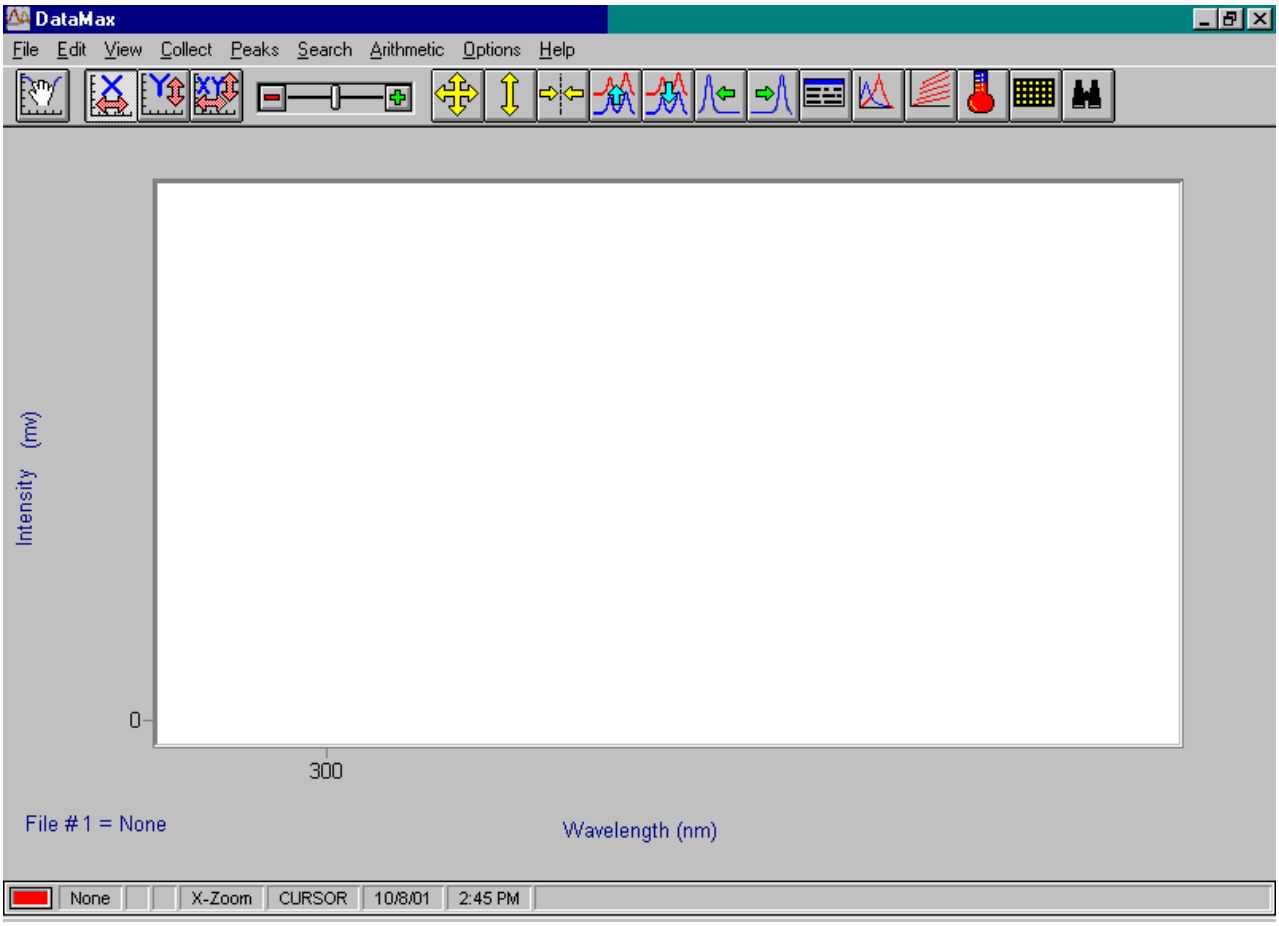
The title screen appears.

2 Click *OK*.

Or

Wait for the title screen to disappear.

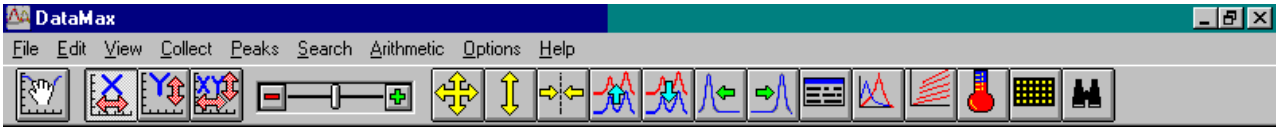
The default view of ***Run Experiment*** appears:



After data are taken or a spectral file is opened, the spectrum appears in the central rectangular blank area. Surrounding this area is information that describes the spectrum, its associated parameters, and user comments.

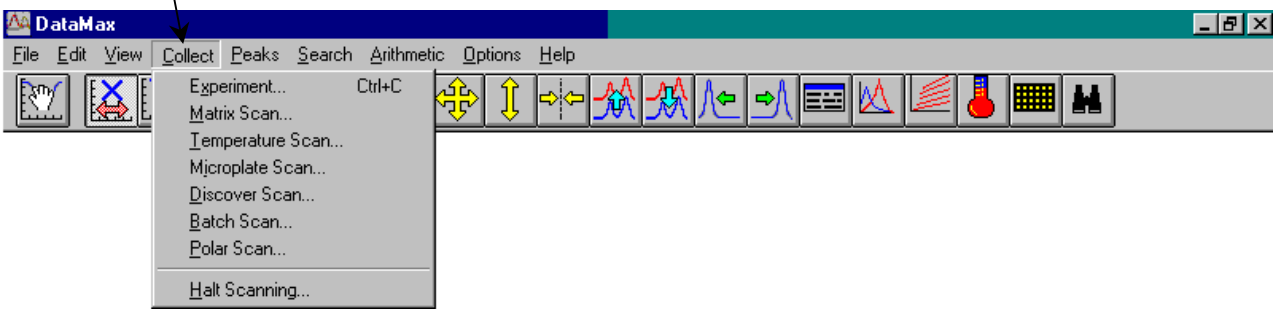
Note: *The* GRAMS/32® User's Guide *contains a complete description of this area, and describes the portions of the screen that reveal information about data collected.*

Main menu



Certain features not mentioned in the *GRAMS/32® User's Guide* are as follows:

Collect



In the *Collect* drop-down menu are a number of special features.

Experiment…

Experiment… sets up parameters and runs steady-state acquisitions. This command opens an ***Experiment Acquisition*** or ***Data Acquisition*** dialog box. Default is an ***Emission Acquisition***. The experiment type may be changed, and appropriate parameters automatically are modified to reflect the experiment type. After setup, click the *Run* button to start the experiment. When the experiment is finished, the ***Experiment Acquisition*** dialog box closes, and the spectrum appears in the ***Run Experiment*** central rectangle. Complete instructions are covered in Chapters 4 and 5.

Steady-state scan types available in *Experiment…*:

Standard scans (for all systems)	**Phosphorimeter scans (for Fluorolog systems)**
Emission scan	Phosphorimeter emission scan
Excitation scan	Phosphorimeter excitation scan
Synchronous scan	Phosphorimeter synchronous scan
Time-base scan	Phosphorimeter delay by decay
Multigroup scan (*not* available with polarizers)	Phosphorimeter delay by window (Multigroup is *not* available here)

Lifetime scans (for Tau lifetime systems)	**Lifetime scans *not* available in this window (see Chapter 9 instead)**
Lifetime-resolved acquisition	Lifetime acquisition Time-resolved Anisotropy-decay (only with polarizers)

Matrix Scan…

Sometimes, when setting up the experimental parameters, the fixed wavelength is an estimate. *Matrix Scan…* runs an experiment once. Then, using the experimental parameters, the original fixed wavelength may be varied incrementally over a range. The resultant spectra may be simultaneously viewed.

Temperature Scan…

With an optional temperature-bath accessory, *Temperature Scan…* opens a window to control the temperature bath. Use this function to maintain a sample at a particular temperature while a scan is run. Whether a probe is used to measure a sample also may be specified in *Temperature Scan…*. Like *Matrix Scan…*, a saved experiment may be recalled so that a new temperature may be set, and then the scan may be re-executed. All scans appear in a single view, with temperature on the *z*-axis.

Note: *An optional temperature bath must be initialized in the layout before using* Temperature Scan….

Microplate Scan…

An optional MicroMax microwell plate reader allows scanning of dozens of samples in less than one minute. Choose *Microplate Scan…* to run high-speed data-acquisition using the MicroMax. See the *MicroMax Operation Manual* for details on this function.

Note: *An optional MicroMax must be initialized in the layout before using* Microplate Scan….

Discover Scan…

Especially designed for previewing, *Discover Scan…* runs an array of emission scans at increasing excitation wavelengths. The scan stores the highest six peaks that it finds with their corresponding excitation and emission wavelength sets. Peaks found around the Rayleigh band are ignored. Some predefined parameters can be overwritten.

Batch Scan…

Choose *Batch Scan…* to set up a series of scans and sample positions. Select any type of scans and in any order. The set of scans can be saved and retrieved later. This can be useful for repeated operations and for long sequences of scans that can be run unattended.

Note: *The optional sample changer is required for a batch scan.*

Polar Scan…

This is also called quick polarization. Rather than running polarization scans that move the polarizers at each wavelength, *Polar Scan…*sets the polarizer, runs a scan, moves the polarizer, runs a scan, etc.

Note: *Optional polarizers are needed for a polar scan.*

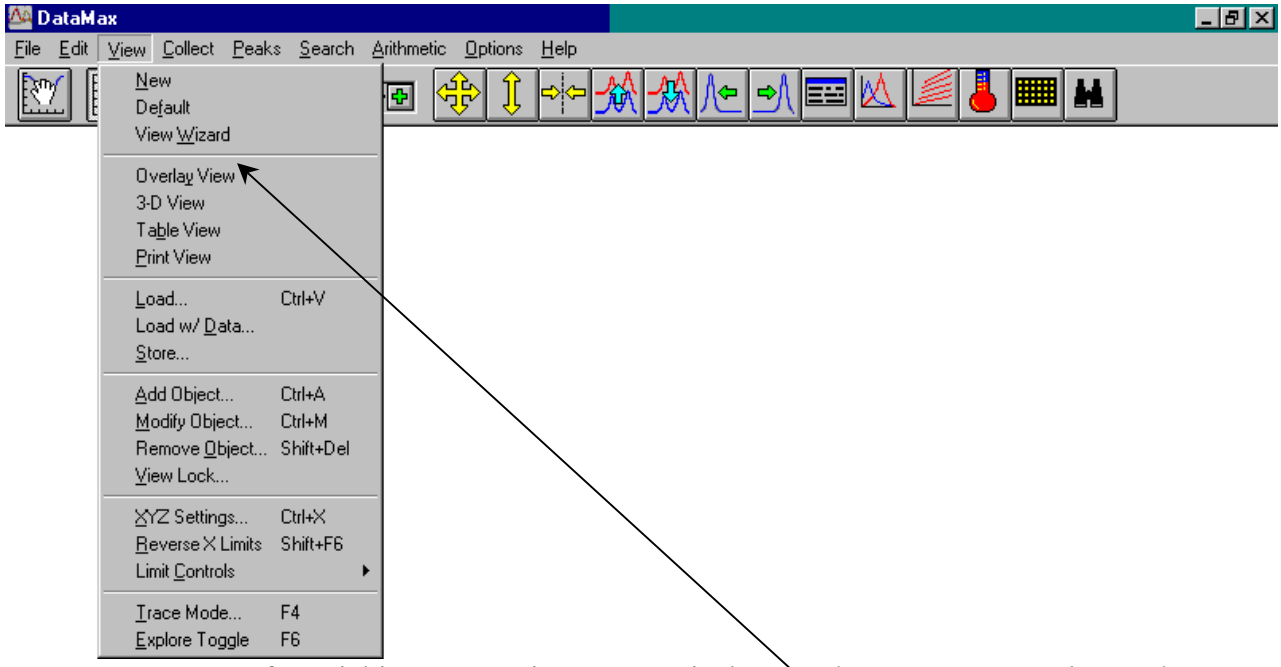
The polarization and anisotropy values may be calculated, and the raw components may be saved. This is sometimes the only available method for measuring polarization with certain polarizer configurations (e.g., manual slits in certain positions).

Halt Scanning…

The *Halt Scanning…* command immediately stops a scan in progress. The **ESC** key also stops a scan in progress.

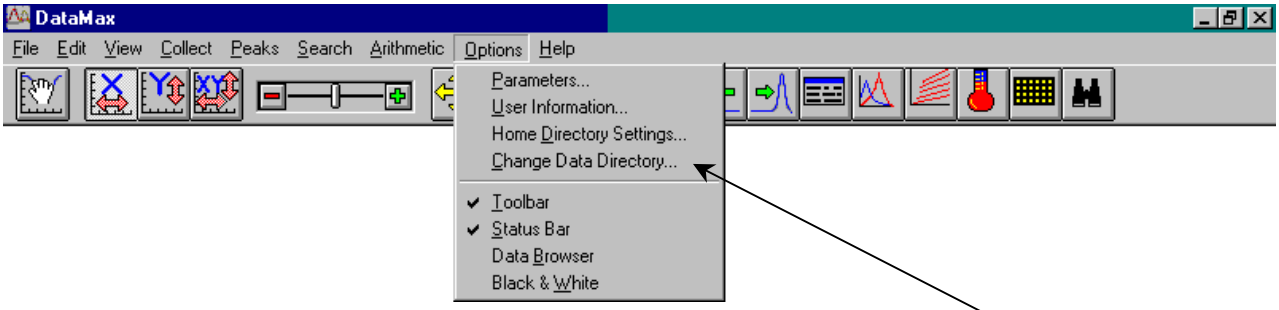
Note: *When* Halt Scanning… *or* **ESC** *is executed, only fully completed scan information is retained, either in memory or on disk. Any partially completed information is discarded.*

View



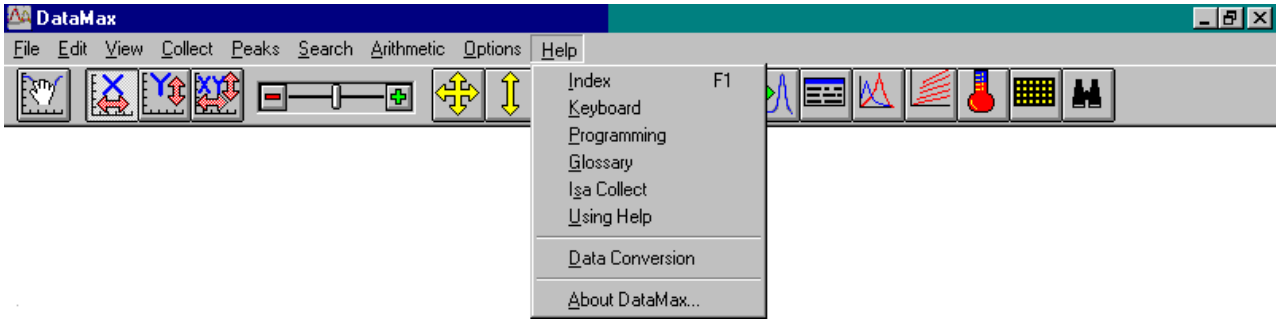
Of special importance in DataMax is the *Overlay View* command. *Overlay View* allows several spectral files to be open simultaneously on the same screen. Use **PAGE UP** and **PAGE DOWN** to activate a specific open file. Click the *Scale All* button to scale all open spectra to the active trace. The name of the active file appears at the bottom of the window. More information is in Chapter 8 and the *GRAMS/32® User's Guide*.

Options



In DataMax, the *Options* menu contains the command *Change Data Directory*.... When DataMax is installed, the default directory to store data is the `Data` subdirectory. To save data in any other directory, use this command. Specify the new directory each time an experiment is executed, or permanently change the default data directory.

Help



The *Help* menu is divided into logical sections so locating a specific topic is easier. The topics pertain to

- Experiments
- Post-processing of data
- Programming
- General features

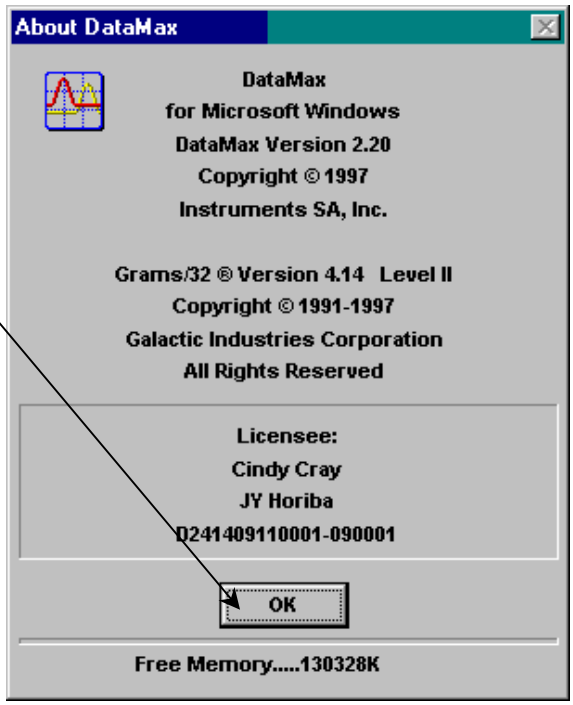
The general features, programming, and post-processing are discussed in detail in the *GRAMS/32® User's Guide*.

Isa Collect

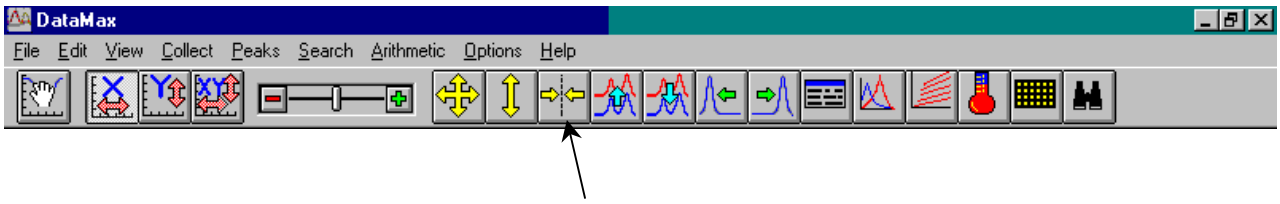
Help for data collection is found in *Isa Collect*. Essentially, *Isa Collect* is a sub-topic purely dedicated to data acquisition.

About DataMax…

About DataMax… opens a dialog box with information about the version of DataMax, level of the software, the serial number, and the computer's free memory. Click *OK* to exit this window.



Tool bar



Just below the main menu is the *toolbar* (or *button bar*). Each button shown corresponds to a function. Click on a button to activate that function.

Buttons not mentioned in the *GRAMS/32® User's Guide* include:

Run Experiment

Run Experiment sets up parameters and runs steady-state acquisitions. This button opens an ***Experiment Acquisition*** or ***Data Acquisition*** dialog box. Default is an ***Emission Acquisition***. The experiment type may be changed, and appropriate parameters automatically are modified to reflect the experiment type. After setup, click the *Run* button to start the experiment. When the experiment is finished, the ***Experiment Acquisition*** dialog box closes, and the spectrum appears in the ***Run Experiment*** central rectangle. More information is found earlier in this chapter under the *Experiment*... command, and complete instructions are covered in Chapters 4 and 5.

Overlay View

Overlay View allows several spectral files to be open simultaneously on the same screen. Use **PAGE UP** and **PAGE DOWN** to activate a specific open file. Click the *Scale All* button to scale all open spectra to the active trace. The name of the active file appears at the bottom of the window. More information is in Chapter 8 and the *GRAMS/32® User's Guide*.

Matrix Scan

Sometimes, when setting up the experimental parameters, the fixed wavelength is an estimate. *Matrix Scan* runs an experiment once. Then, using the experimental parameters, the original fixed wavelength may be varied incrementally over a range. The resultant spectra may be simultaneously viewed. More about *Matrix Scan* is given in Chapter 8.

Temperature Scan

With an optional temperature-bath accessory, *Temperature Scan* opens a window to control the temperature bath. Use this function to maintain a sample at a particular temperature while a scan is run. Whether a probe is used to measure a sample also may be specified in *Temperature Scan*. Like *Matrix Scan*, a saved experiment may be recalled so that a new

Note: *An optional temperature bath must be initialized in the layout before using* Temperature Scan.

temperature may be set, and then the scan may be re-executed. All scans appear in a single view, with temperature on the *z*-axis.

Microplate Scan

An optional MicroMax microwell plate reader allows scanning of dozens of samples in less than one minute. Choose *Microplate Scan* to run high-speed data-acquisition using the MicroMax. See the *MicroMax Operation Manual* for details on this function.

Note: *An optional MicroMax must be initialized in the layout before using* Microplate Scan….

Discover Scan

Especially designed for previewing, *Discover Scan* runs an array of emission scans at increasing excitation wavelengths. The scan stores the highest six peaks that it finds with their corresponding excitation and emission wavelength sets. Peaks found around the Rayleigh band are ignored. Some predefined parameters can be overwritten.

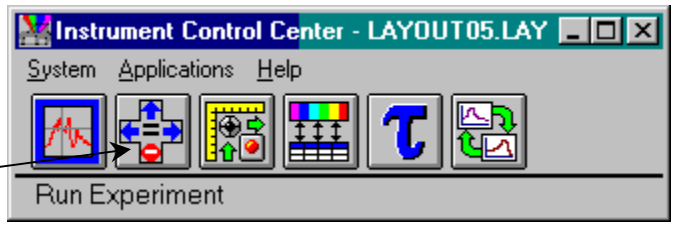
Real Time Display

The ***Real Time Display*** application exists to help the research establish experimental parameters, as well as try out parameters and immediately see the results. ***Real Time Display*** is useful for checking unknowns and for determining the best settings and parameters for a particular sample.

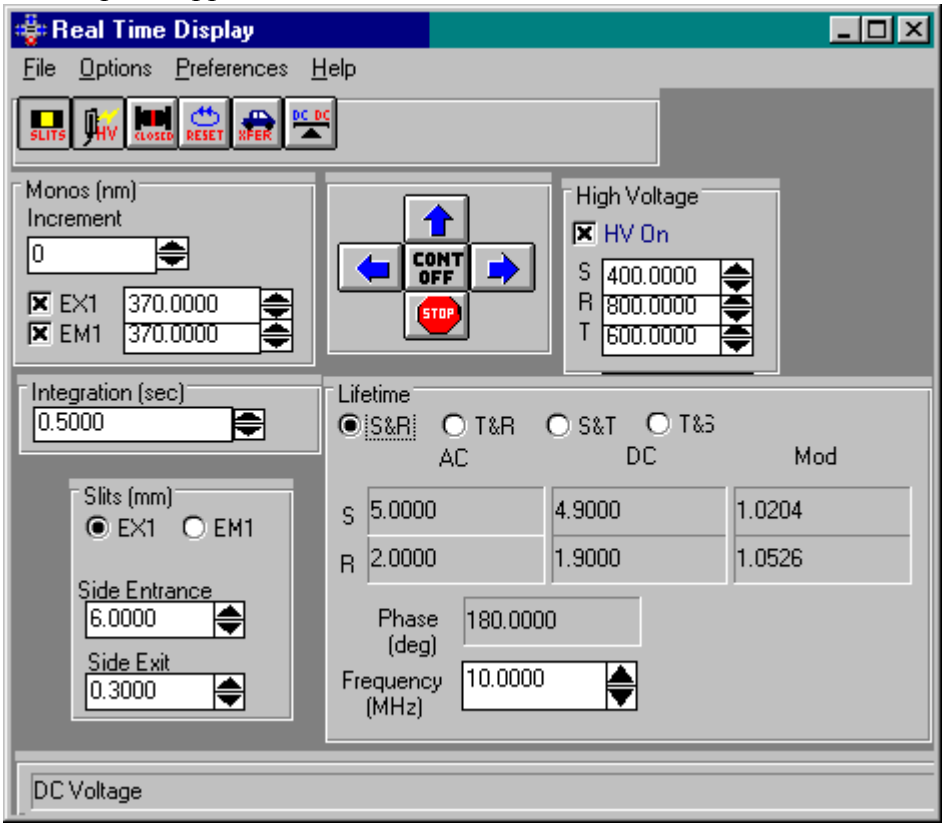
Starting ***Real Time Display***

In ***Instrument Control Center***,

1 Click the *Real Time Display* button.



A control panel appears:



Note: *The internal layout and dialog boxes open within the control panel depend upon the system's configuration.*

Changing settings

1. Click on the desired field.
2. Type the new value.
3. Click **ENTER**.

Watching effects with ***Real Time Display***

Opening the ***Real Time Display*** and the ***Run Experiment*** window simultaneously lets the user view how changes affect a spectrum. The format of the spectral screen depends upon the number of active detectors. The output of each active detector is presented as a single trace in a window by itself on the screen. All traces for all active detectors appear simultaneously, so the more detectors made active, the narrower each window is.

In ***Real Time Display***, the trace is plotted as data point versus intensity.

For more information about ***Real Time Display***, see Chapter 6.

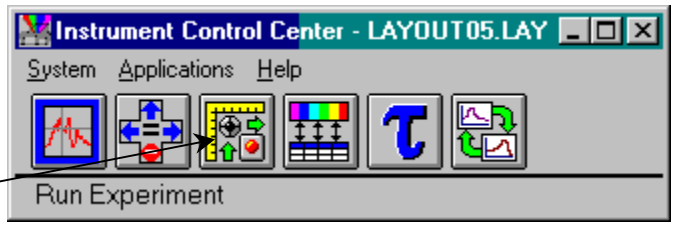
Visual Instrument Setup

In order to adjust defaults for system-wide units, slit widths, and so forth, use the ***Visual Instrument Setup*** application.

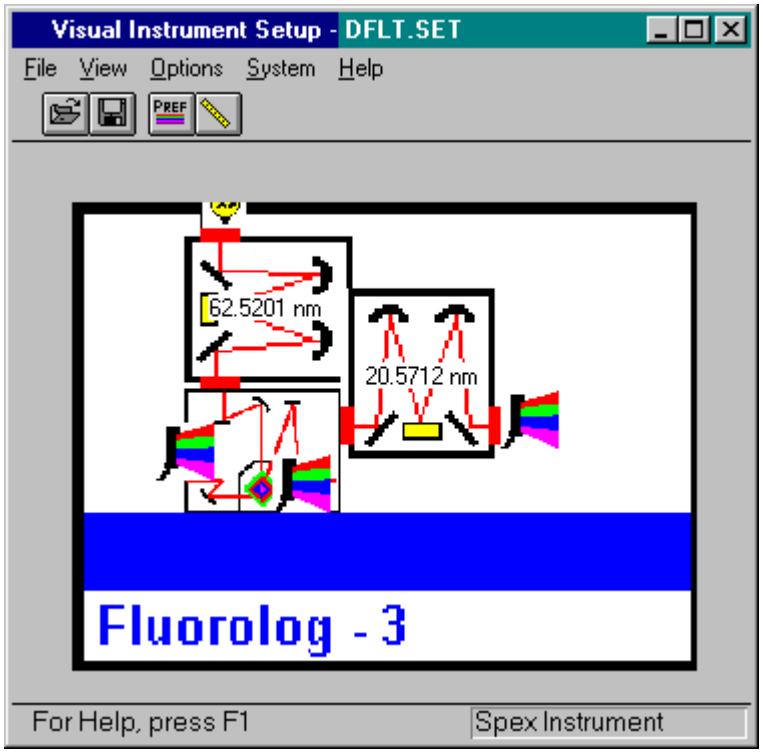
Starting ***Visual Instrument Setup***

In ***Instrument Control Center***,

1 Click the *Visual Instrument Setup* button.



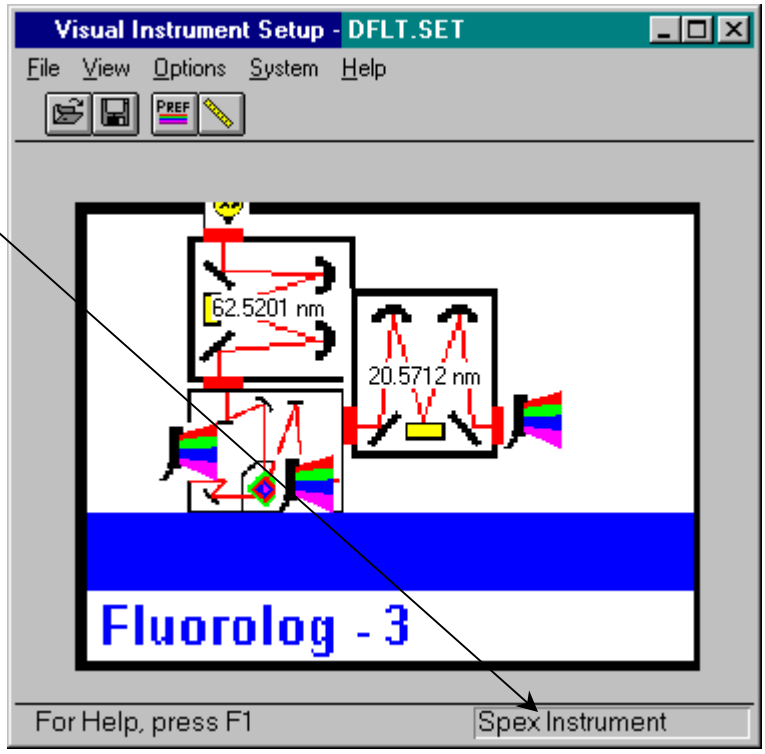
A schematic block diagram of the instrument appears:



Using *Visual Instrument Setup*

Introduction

As the mouse is passed over an adjustable component, its name appears in the status bar at the bottom of the window.



Click on a named component to adjust its settings.

Examples of adjustable components include

- Xenon lamp
- Grating turret
- Monochromator
- Sample chamber
- Detector
- Entrance slit
- Exit slit

The menu and toolbar provide additional features:

- Storage of a new setup file
- Recalling a previously-saved setup file
- Set the user's level of operation of the software

Setup files

Setup files may be saved to and retrieved from the hard disk. There is no limit to the number of setup files that may be saved, except for hard-disk space. These setup files are used in the ***Run Experiment*** and ***Real Time Display*** applications.

For more information about ***Visual Instrument Setup***, see Chapter 3.

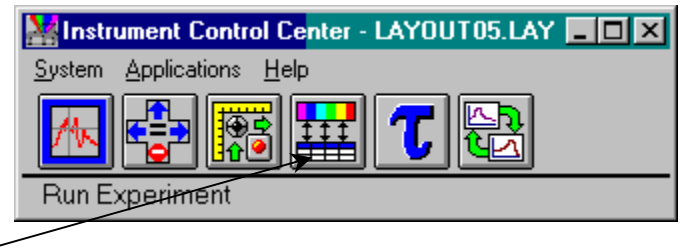
Constant Wavelength Analysis

Constant Wavelength Analysis is useful to view fluorescent emission from multiple samples at a single wavelength, for example, quantitative analysis and determination of unknown concentration. The user determines the number of times and the wavelength to be scanned. The software averages the emissions from the readings and calculates the standard deviation. With a set of standards to establish a concentration curve, DataMax can determine the concentration of unknowns by fitting a fluorescence-emission curve to the fluorescence-emission curve of the standard.

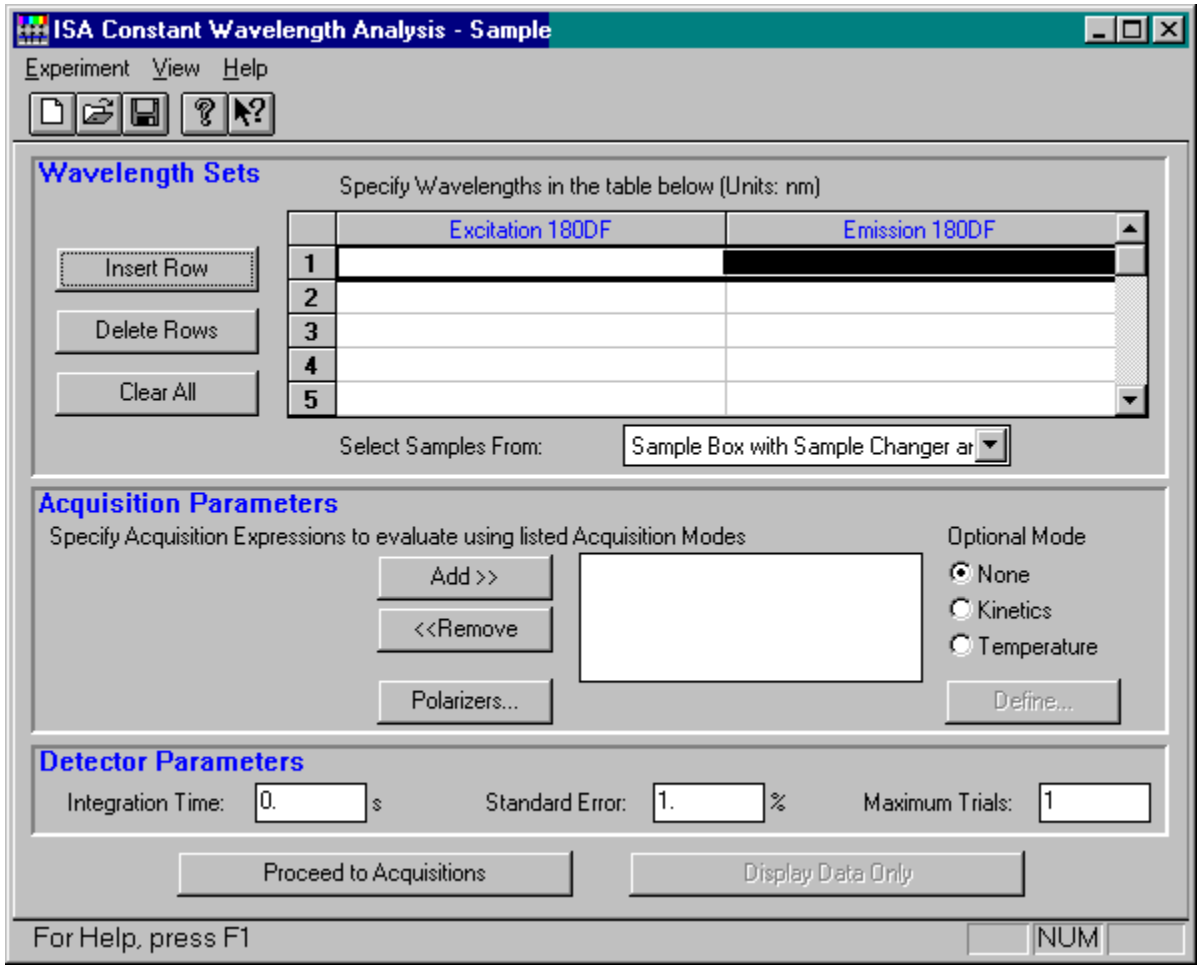
Starting ***Constant Wavelength Analysis***

In ***Instrument Control Center***,

2 Click the *Constant Wavelength Analysis* button.



The ***Constant Wavelength Analysis*** dialog box appears:



Using ***Constant Wavelength Analysis***

Wavelength pairs, acquisition modes, detector parameters, and more can be specified in the initial ***Constant Wavelength Analysis*** window. After clicking the *Proceed to Acquisitions* button, a second dialog box allows entry of sample identification information.

DataMax looks for specification of any standards to determine the type of run. If standards are entered, DataMax automatically calculates the unknowns' concentrations. Without standards, DataMax scans each sample, monitors the emission at the specified wavelength, and provides single-point data for each sample.

The resultant data are displayed in spreadsheet format. They can be saved and later manipulated using popular spreadsheet programs such as Microsoft® Excel™ and Lotus 1-2-3™.

See Chapter 8 for more details about ***Constant Wavelength Analysis***.

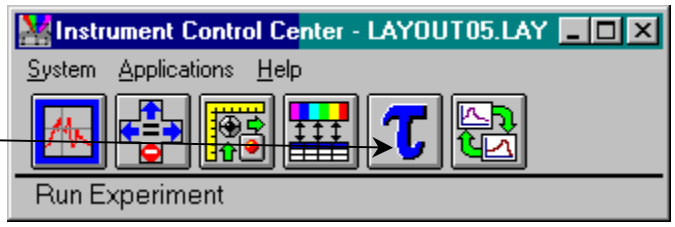
Lifetime

Only available with Fluorolog®-Tau-3 systems, the ***Lifetime*** application measures fluorescence lifetimes for samples. A fluorescence lifetime is the mean time between absorption and emission of light by a population of fluorescent molecules. Lifetime analysis is used to study protein conformations, energy transfer to determine intra- and intermolecular distances, carrier concentrations in semiconductors, etc.

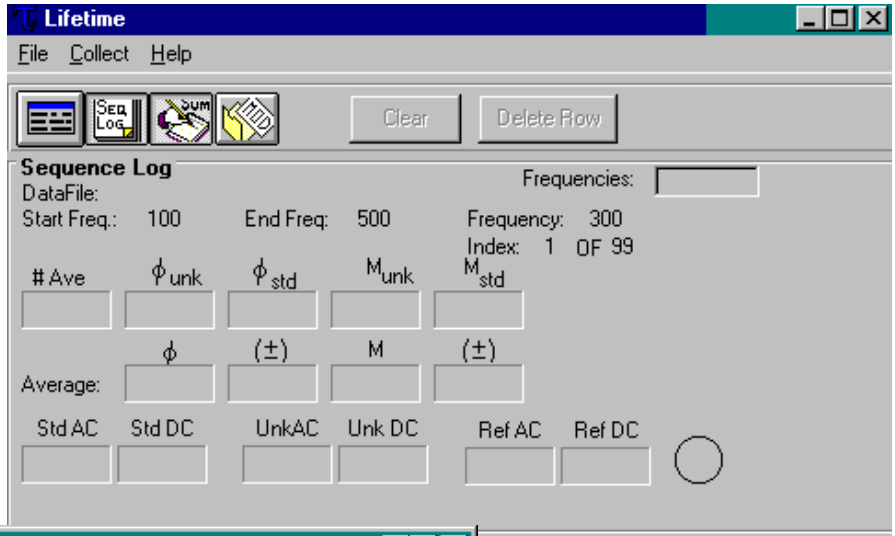
Starting ***Lifetime***

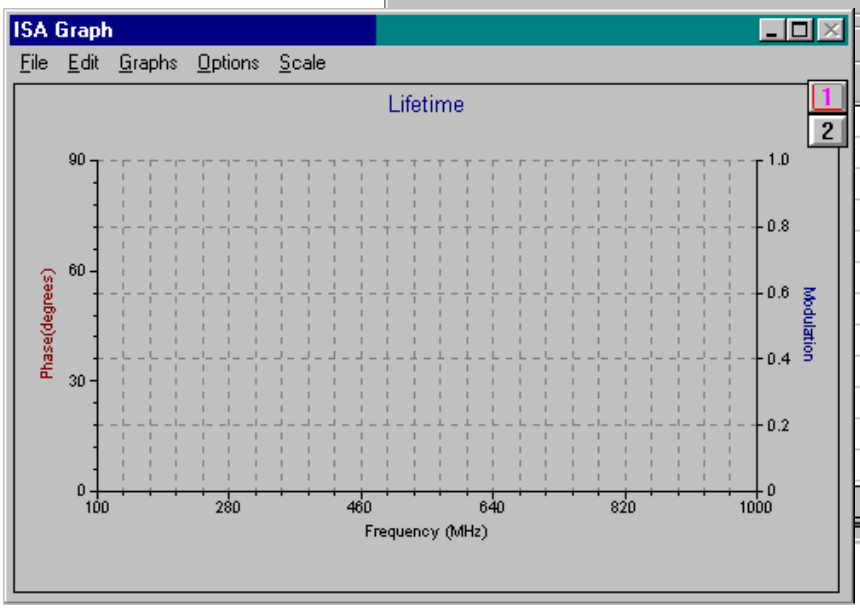
In ***Instrument Control Center***,

1 Click the *Lifetime* button.



The ***Lifetime*** dialog box appears along with the ***ISA Graph*** window:





Lifetime features

- Under *Collect*, a number of different lifetime experiments are available
- Real-time analysis data analysis with built-in ***Real-Time Modeling***
- Plot the data
- Print the data

To fit the data after a lifetime experiment, see ***Modeling*** (the next section of this chapter).

For detailed information on the ***Lifetime*** application, refer to Chapter 9. For more about plotting data, see Chapter 11.

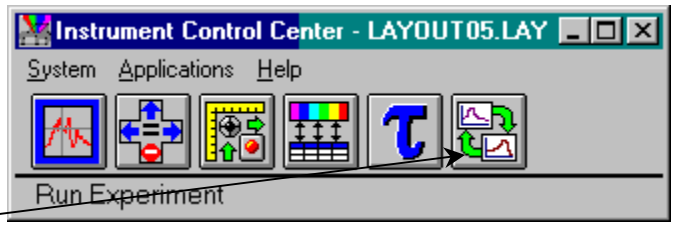
Lifetime Modeling

After the acquisition is saved onto a disk, recall it in ***Lifetime Modeling***, and analyze it. Use one of the modeling definitions to set up a variety of "what if" scenarios. Instantaneous results provide answers at the click of a button. Experiment and save each file, or discard those files that are not helpful.

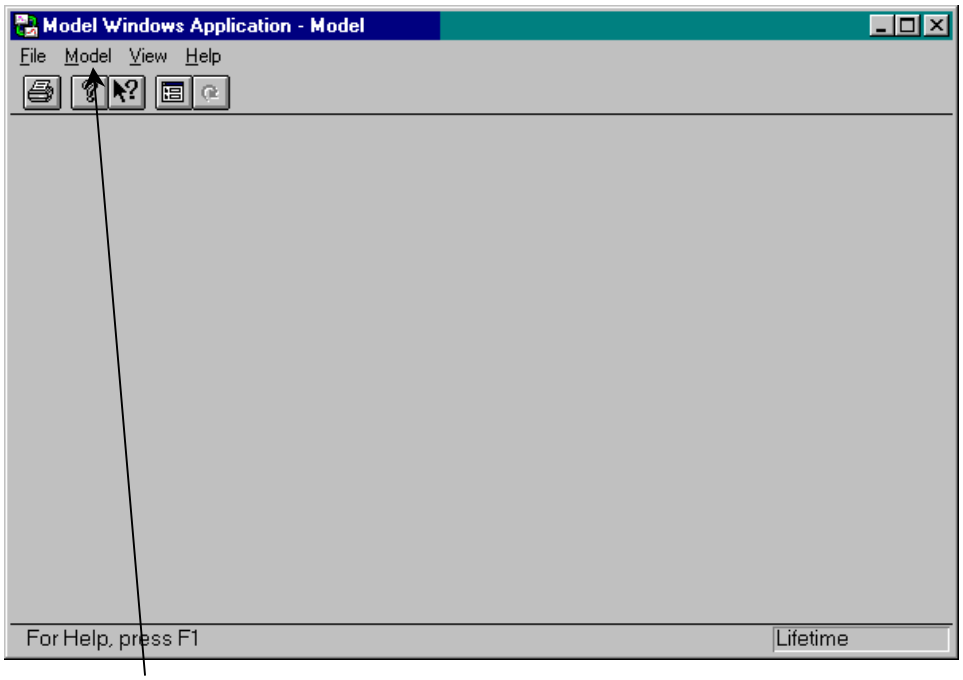
Starting ***Lifetime Modeling***

In ***Instrument Control Center***,

1 Click the *Lifetime Modeling* button.

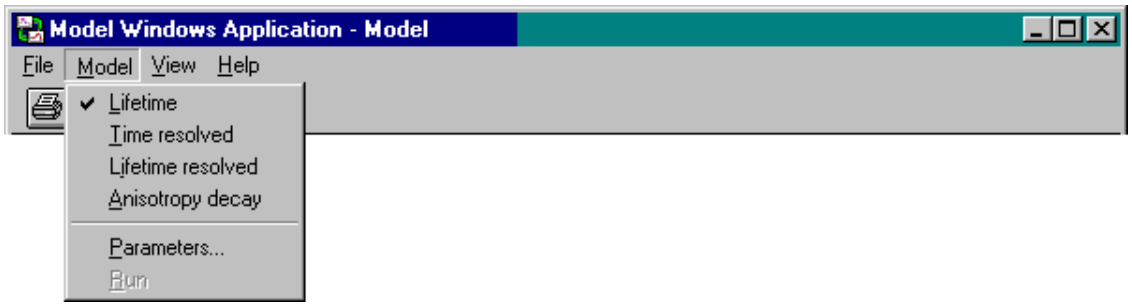


The ***Model Windows Application – Model*** dialog box appears:



2 Click *Model*.

A drop-down menu of experiment types appears:



3 Click on the desired experiment type.

Immediately, a modeling screen appears.

To learn more about post-experiment modeling, refer to Chapter 10. For an in-depth examination of plotting and graphing options, see Chapter 11.

3: Customizing DataMax

Introduction

DataMax lets each user create and save personal sets of parameters and hardware information. Use the ***Visual Instrument Setup*** application to design and save these files.

The ***Visual Instrument Setup*** controls system-wide units, hardware settings, and start-up instructions. When DataMax is started, the software refers to the ***Visual Instrument Setup***, and follows the instructions there. When any application within DataMax is launched, the application assumes the specified units and settings from ***Visual Instrument Setup***.

Although DataMax reads specifications from ***Visual Instrument Setup*** into an application, these automatic settings may be overridden. For example, recall an experiment in the ***Run Experiment*** application. Say the slits were set in micrometers, but the current setup specifies millimeters. The system assumes millimeters—the default unit of the current experiment. The default, or system, units, however, are retained in the ***Visual Instrument Setup***, and are still considered the default values.

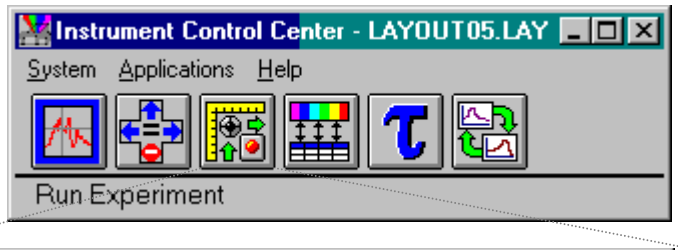
Setup files also are automatically saved in ***Run Experiment*** and ***Real Time Display***, and may be recalled within these applications.

Visual Instrument Setup

Starting *Visual Instrument Setup*

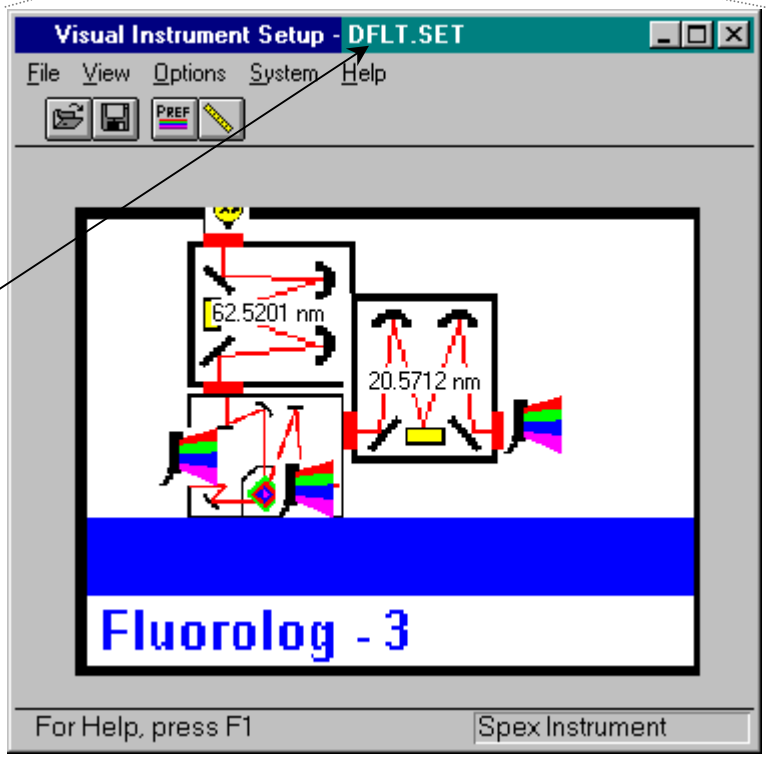
In ***Instrument Control Center***,

1 Click the *Visual Instrument Setup* button.



A schematic diagram of the system appears:

At the top of the dialog box is the name of the setup file. Here it is `dflt.set`, a default setup file.



Visual Instrument Setup automatically opens with the default parameters active. The original contents of the default file are defined by Jobin Yvon®, but can be changed by saving the current file as the default file.

Click on various components in the schematic to gain access to their setup, units, and settings.

By using the main menu, toolbar, the schematic diagram, or a combination of all three, the system units and setup files may be modified.

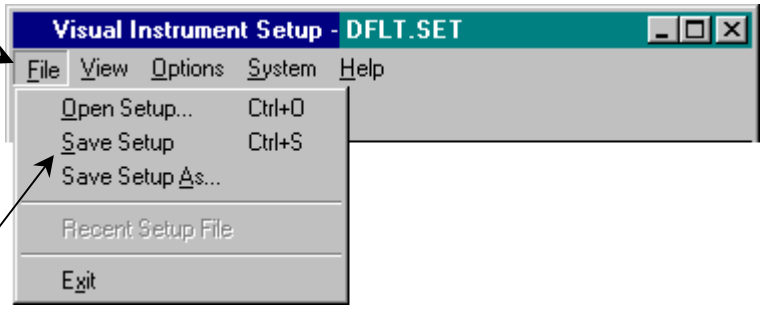
Main menu

Below are listed the commands in the main menu.

File

A setup file is a collection of information associated with a particular setup, and saved to a floppy disk or hard drive.

1 Click *File* to open the *File* drop-down menu.

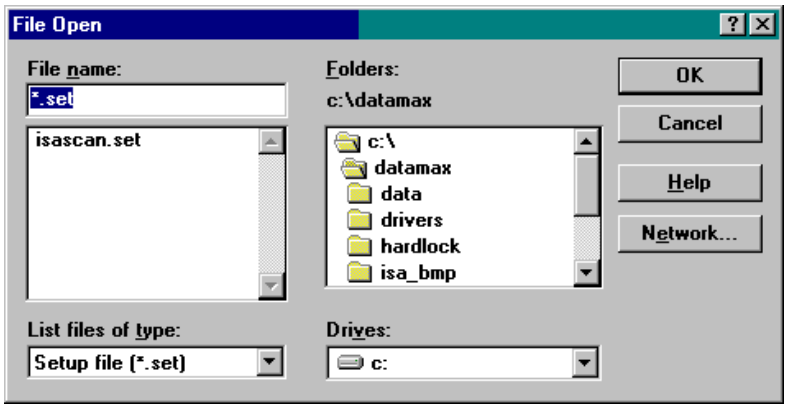


2 Click on the desired command:

Open Setup…
Save Setup
Save Setup As…
Exit

Open Setup…
Use *Open Setup…* to recall a stored setup file.

a Click *Open Setup…*.
The ***File Open*** dialog box appears.



b Select the desired setup file.

c Click *OK*.
The ***Visual Instrument Setup*** window, and, where applicable, system units and hardware settings, change to reflect the new setup file. The top of the ***Visual Instrument Setup*** dialog box changes to the new setup file's name.

Save Setup
The number of setup files is limited only by the amount of space on the hard disk.

Once a setup file is designed,

a Click *Save Setup*
The current configuration is saved under the current file name, even if the current file is the default file.

Warning: *Use caution with Save Setup, for no warning appears before overwriting the set up.*

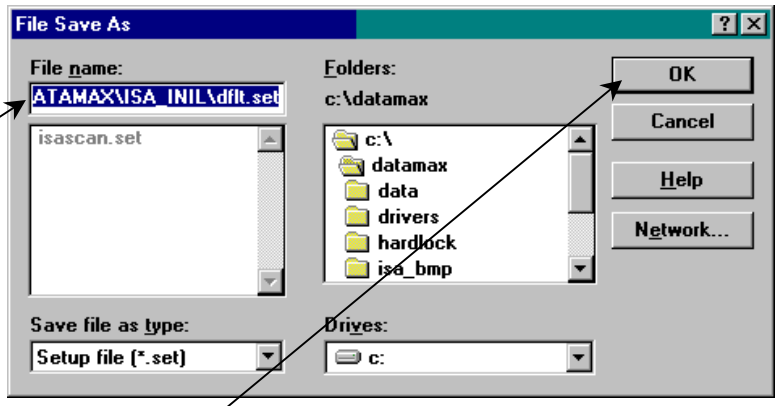
Save Setup As…
Use *Save Setup As…* when multiple setup files are required, but they have several common parameters.

b Use *Open Setup…* to recall a saved setup file.

c Modify the setup file.

d Click *File*.

e Click *Save Setup As….*
The ***File Save As*** dialog box appears.



f Replace the name displayed in the text box with the new name for the file.
Specifying an extension for the file name is unnecessary. By default, setup files are saved with a `.SET` extension. Any three-character extension may be assigned to a setup file, but DataMax does not display this file automatically when browsing in *Open Setup….*

g Click *OK*.
The first file was unchanged, and the new file is now saved in the specified directory.

Exit
To close ***Visual Instrument Setup***, click *Exit*. The active setup is automatically saved as the default file; it will be active when ***Visual Instrument Setup*** is re-opened later. After quitting ***Visual Instrument Setup***, any DataMax applications active before opening ***Visual Instrument Setup*** are still active.

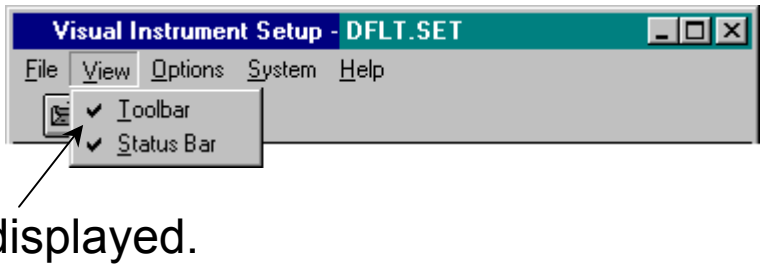
View

This menu displays or hides the toolbar and status bar. A check mark (tick) next to the item means it is displayed.

To remove or display an item,

1 Click *View*.

2 Click on the item to be removed or displayed.

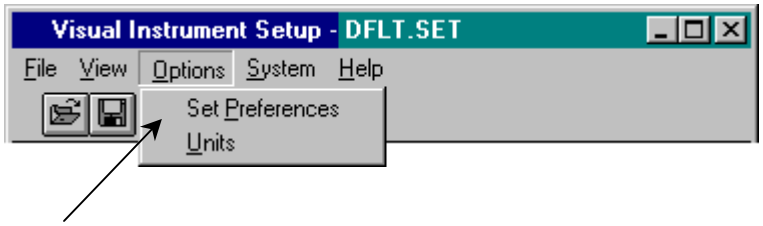


Options

Many powerful customization tools exist in the *Options* menu. Use *Options* to establish system-wide units, hardware settings, and the user level.

1 Click Options.

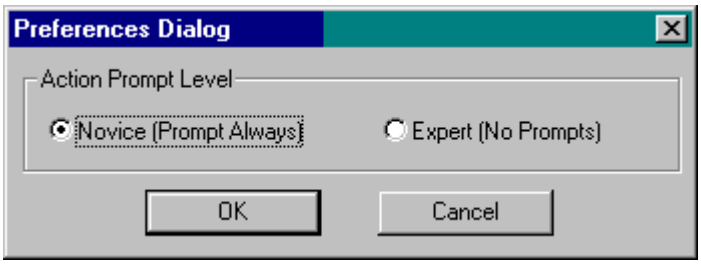
A drop-down menu appears.



2 Choose the item to adjust.

Set Preferences

The ***Preferences Dialog*** box appears.

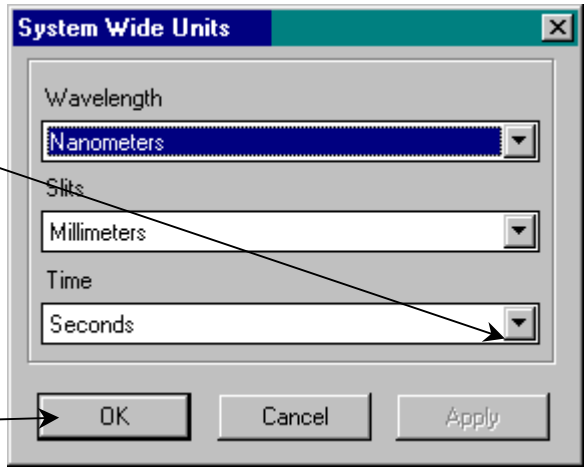


Action Prompt Level tells DataMax how experienced the user is. There are two levels of experience:

Novice	The user is prompted often to adjust slits, close the sample-chamber lid, etc.
Expert	No prompts are displayed. DataMax assumes the user does everything carefully, correctly, and in the proper order.

Units

The default instrument units—or, more accurately, *system-wide units*—are controlled within *Options*. The ***System Wide Units*** dialog box appears:



To set units,

a Click the down arrow next to the measurement.

b Select the desired unit for that measurement.

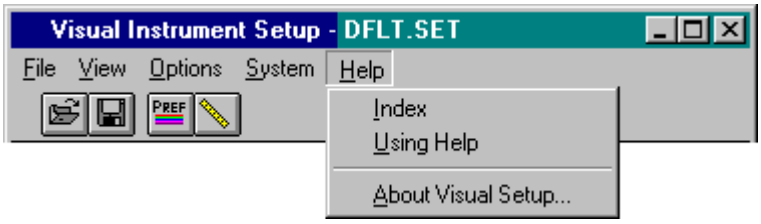
c Repeat steps a and b for the next measurement.

d Click *OK*.

Available choices are:

Wavelength:	Nanometers Angstroms Electron-volts Wavenumbers	Slits:	Millimeters Bandpass Micrometers	Time:	Milliseconds Seconds

Help

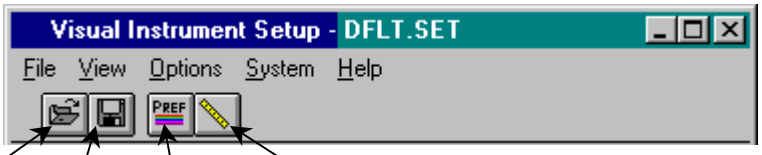


The *Help* menu is divided into three topics:

- *Index*
- *Using Help*, which describes how to use the Help routine
- *About Visual Setup…*, which includes the program's version number and copyright date.

Toolbar

The toolbar contains buttons that ease use of several *Options* and *File* menu items. Click on the desired button to activate the command.



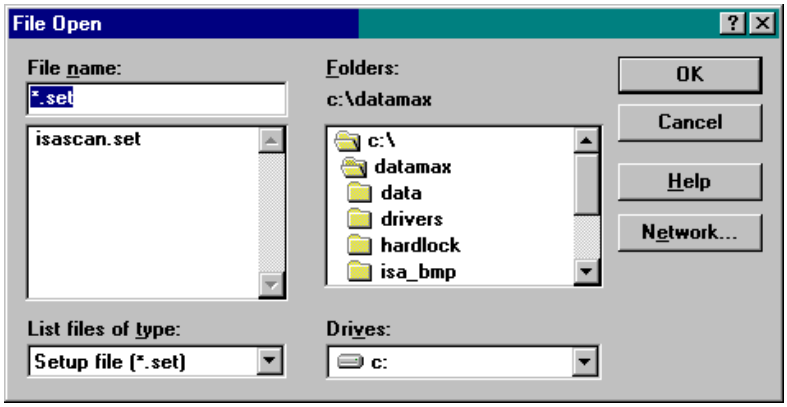
Open Setup — Save As — Set Preferences — Units

Open Setup

Use *Open Setup* to recall a stored setup file.

a Click *Open Setup*.

The ***File Open*** dialog box appears.



b Select the desired setup file.

c Click *OK*.

The ***Visual Instrument Setup*** window, and, where applicable, system units and hardware settings, change to reflect the new setup file. The top of the ***Visual Instrument Setup*** dialog box changes to the new setup file's name.

Save As

Use *Save As* when multiple setup files are required, but they have several common parameters.

a Use *Open Setup* to recall a saved setup file.

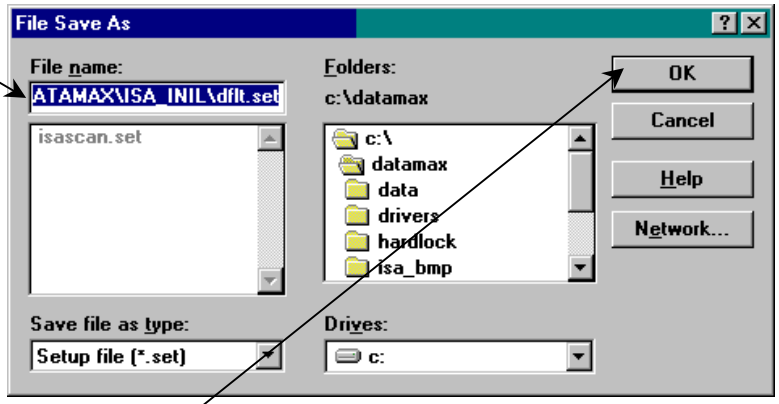
b Modify the setup file.

c Click *File*.

d Click *Save As*.

The ***File Save As*** dialog box appears.

e Replace the name displayed in the text box with the new name for the file.



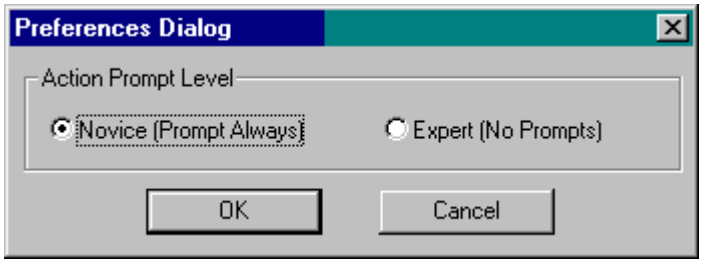
Specifying an extension for the file name is unnecessary. By default, setup files are saved with a `.SET` extension. Any three-character extension may be assigned to a setup file, but DataMax does not display this file automatically when browsing in *Open Setup*.

f Click *OK*.

The first file was unchanged, and the new file is now saved in the specified directory.

Set Preferences

The ***Preferences Dialog*** box appears.

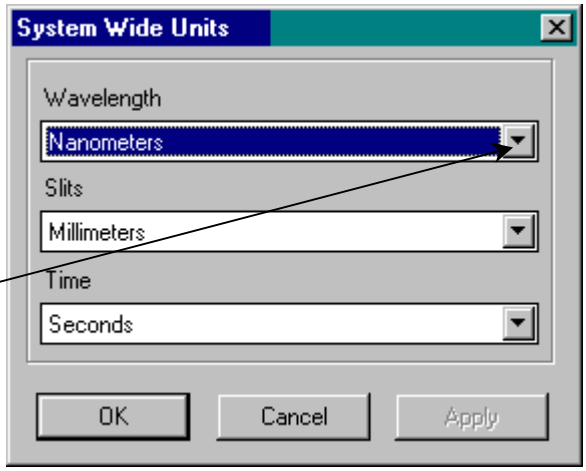


Action Prompt Level tells DataMax how experienced the user is. There are two levels of experience:

Novice	The user is prompted often to adjust slits, close the sample-chamber lid, etc.
Expert	No prompts are displayed. DataMax assumes the user does everything carefully, correctly, and in the proper order.

Units

Set the default system-wide units here. The ***System Wide Units*** dialog box appears:



To set units,

a Click the down arrow next to the measurement.

b Select the desired unit for that measurement.

c Repeat steps a and b for the next measurement.

d Click *OK*.

Available choices are:

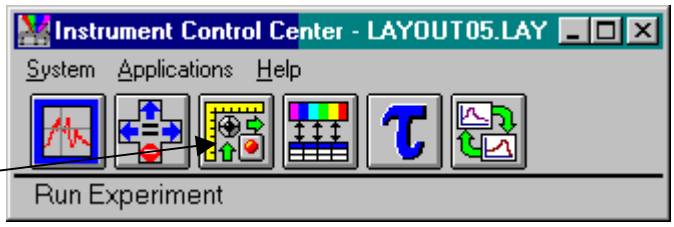
Wavelength:	Nanometers Angstroms Electron-volts Wavenumbers	Slits:	Millimeters Bandpass Micrometers	Time:	Milliseconds Seconds

Changing hardware settings

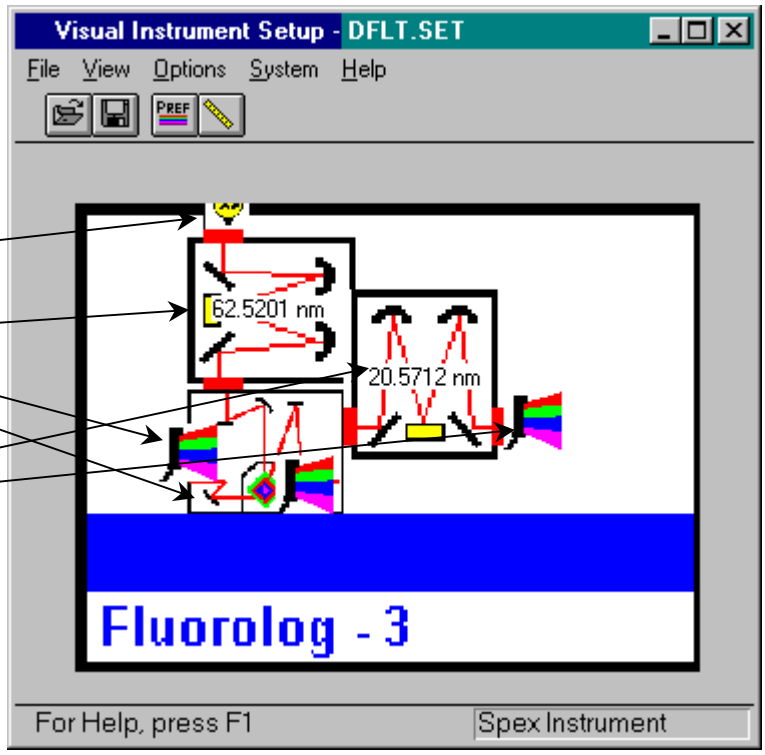
To change settings for various components,

In ***Instrument Control Center***,

1 Click the *Visual Instrument Setup* button.



A schematic diagram of the system appears:



A spectrofluorometer consists of the following parts:

- Light source
- Excitation spectrometer
- Reference detector
- Sample compartment
- Emission spectrometer
- Emission detector

A complete discussion of your system's precise optical layout is in the system manual. The following section considers those components solely within ***Visual Instrument Setup***.

To adjust a component,

1 Place the mouse cursor over that component.

The status bar on the lower right corner provides the name of the component.

2 Click on the component.

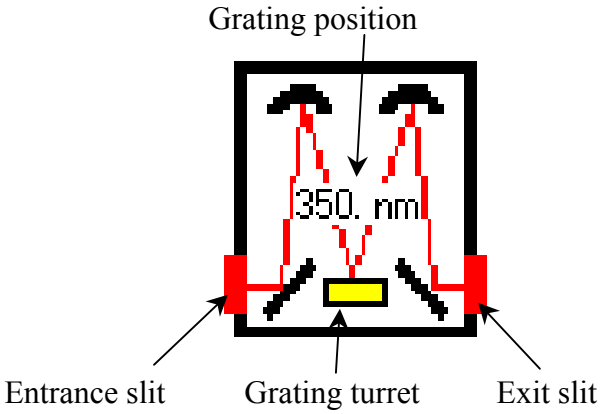
A dialog box opens to adjust settings.

Light source

A standard illumination source for exciting the sample is a 450-W ozone-free xenon lamp. Turn on all types of light sources before starting the spectrofluorometer. No other control of the light source is available.

Emission or excitation spectrometer

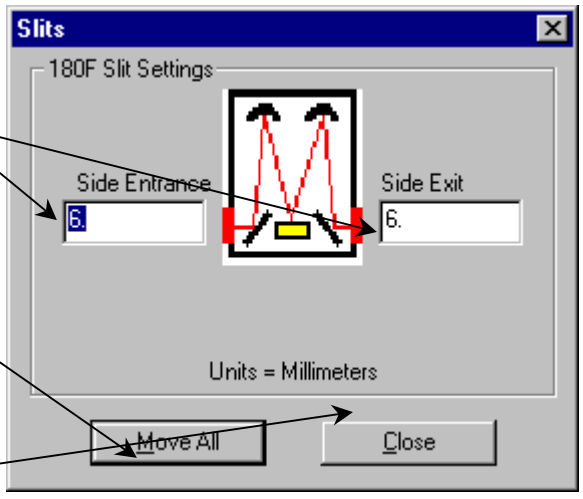
Spex® spectrometers are modified Czerny-Turner spectrometers.



Slits

Click on a slit to open the ***Slits*** dialog box:

1. Enter a slit width for the entrance or exit slit.
2. Click *Move All* to activate the change.
3. Click *Close* when finished.

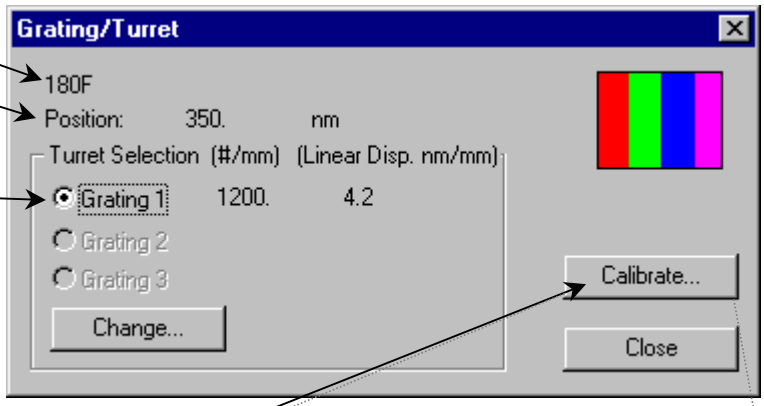


Gratings

Standard gratings are blazed at 330 nm for the excitation spectrometer, and at 500 nm for the emission spectrometer. The standard groove density is 1200 grooves/mm. Within the ***Grating/Turret*** dialog box, the current position can be viewed, change to a different groove-density, or reposition the grating.

Click on a turret icon to open the ***Grating/Turret*** dialog box:

Spectrometer type
Grating position

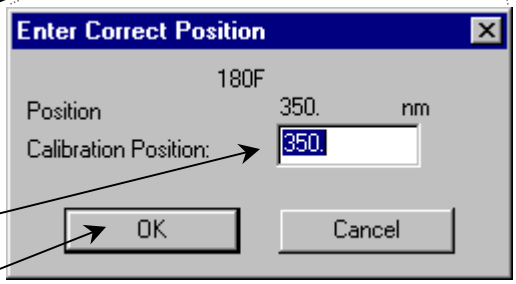


To calibrate a grating,

1 Click the radio button for the desired grating.

2 Click *Calibrate...*.

This opens the ***Enter Correct Position*** dialog box:

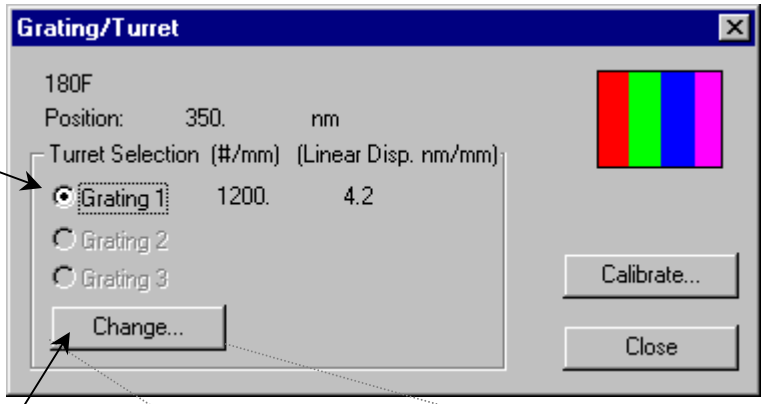


3 Enter the correct position.

4 Click *OK*.

The gratings move to the new position.

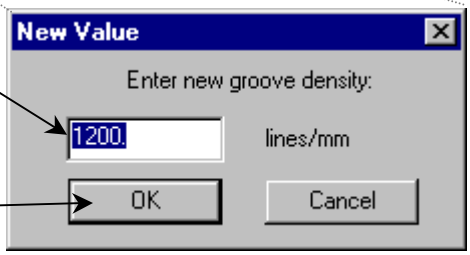
To change a grating's groove density,



1 Click the radio button for the desired grating.

2 Click the *Change...* button.

The ***New Value*** dialog box opens.



3 Enter the correct grating density.

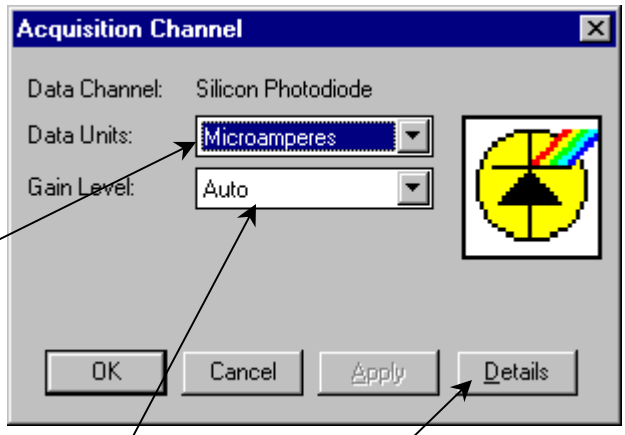
4 Click *OK*.

When finished with adjusting the grating(s), click the *Close* button.

Reference detector

To compensate for small changes in lamp intensity during a scan, a reference detector is used. A small amount of the excitation beam is diverted to the reference detector before it reaches the sample. A standard Fluorolog®-3, FluoroMax®-3, or Fluorolog®-Tau-3 uses a silicon photodiode as a reference detector.

Click on the reference detector in the schematic to open an ***Acquisition Channel*** dialog box.



To adjust data units,

1. Click on the down arrow next to the *Data Units* text box.
2. Click on the desired units.

To change the gain level,

3. Click on the down arrow next to the *Gain Level* text box.
4. Click on the desired gain.

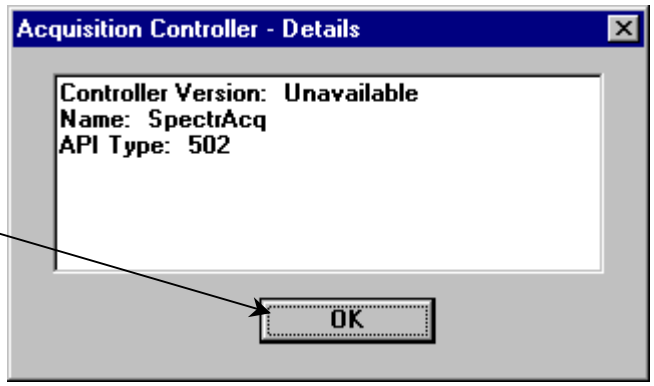
To view details about the reference detector,

5. Click *Details*.

   The ***Acquisition Controller – Details*** window opens.

6. Click *OK*.

   The window closes.



When finished with the reference detector, click *OK* in the ***Acquisition Channel*** dialog box.

Signal detector

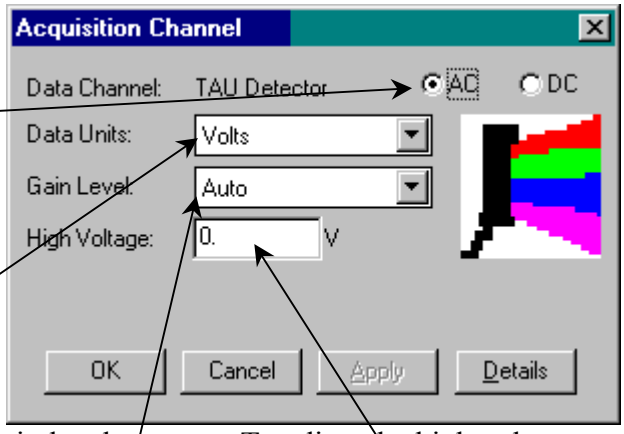
The signal detector monitors light leaving the emission spectrometer. The standard signal detector for Spex® systems is an R928P photomultiplier tube operating in photon-counting mode. The system may contain more than one signal detector, depending on the system's configuration. With an optional phosphorimeter, the signal detector settings include data units, gain level, and high voltage for the phosphorimeter. For more information on phosphorimeters, see the hardware manuals and software-control section of this manual.

Click on a signal detector to open the ***Acquisition Channel*** dialog box:

To adjust the AC or DC component of the signal,

1 Click on desired radio button.

2 Adjust the following settings.



To adjust data units,

1 Click on the down arrow by the *Data Units* text box.

2 Click on the desired units.

To change the gain level,

1 Click on the down arrow by the *Gain Level* text box.

2 Click on the desired gain.

To adjust the high voltage,

1 Enter the desired high voltage in the *High Voltage* field.

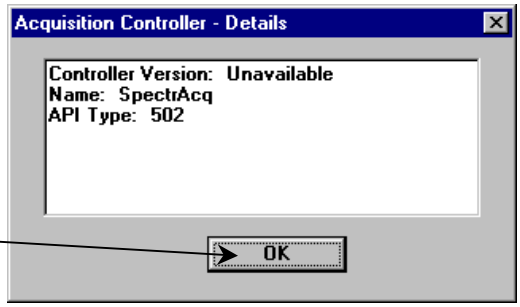
To see details on the reference detector,

1 Click *Details*.

The ***Acquisition Controller – Details*** window opens.

2 Click *OK*.

The window closes.



When finished with the reference detector, click *OK* in the ***Acquisition Channel*** dialog box.

Sample compartment & accessories

The sample compartment contains any samples being tested. Many accessories are either within or directly attached to the sample compartment, such as:

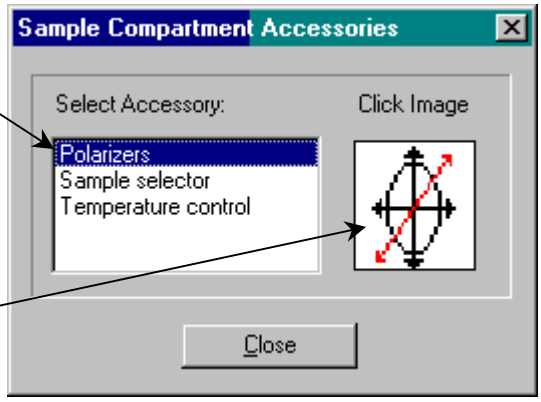
- Sample changer
- Polarizers
- Temperature bath
- Autotitrator
- MicroMax

To adjust accessories within the sample compartment, click on the sample-compartment icon. This opens the ***Sample Compartment Accessories*** dialog box:

1 Highlight the desired accessory in the list on the left.

Only those accessories specified in the instrument layout are listed.

2 Click the accessory's image on the right.



The appropriate dialog box to adjust that accessory appears.

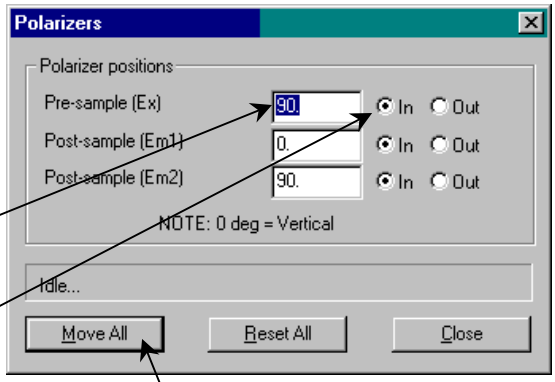
Polarizers

When polarizers are installed in Spex® spectrofluorometers, all applications allow access to polarizer parameters. The default polarizer parameters can be specified in ***Visual Instrument Setup***.

a Click on *Polarizers* in the list on the left side of the ***Sample Compartment Accessories*** dialog box.

b Click on the *Polarizers* image on the right of the dialog box.

The ***Polarizers*** dialog box appears. The available polarizers are shown, depending on the polarizers installed (dual or also third polarizer unit) in the active layout.



c Replace the current value in the field with the desired value.

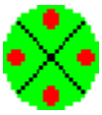
d Click *In* to move the polarizer into the optical path, or *Out* to move the polarizer out of the optical path.

e If all the polarizers have been adjusted, click *Move All* to reset them all to the new values entered.

f Click *Close*.

This closes the ***Polarizers*** window. The software repositions the polarizers.

g Click *Close* in the ***Sample Compartment Accessories*** window.



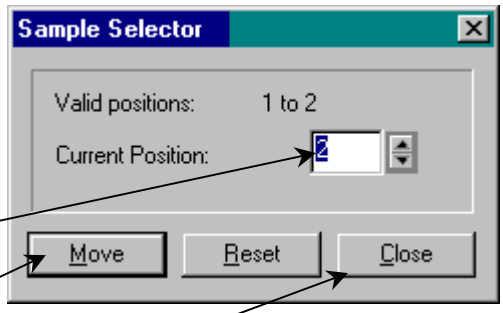
Sample changer

An automatic sample changer is an accessory available in Spex® spectrofluorometers. Two types of sample changers are possible: a 2-position and a 4-position. A sample changer allows multiple samples to be inserted automatically into the light path.

a Click on *Sample Selector* in the list on the left side of the ***Sample Compartment Accessories*** dialog box.

b Click on the *Sample changer* image on the right of the dialog box.

The ***Sample Selector*** dialog box appears. The available sample positions are shown, depending on the sample changer installed (2 or 4 positions) in the active layout.



c Enter the current position in the *Current Position* field, or change it with the up and down arrows.

d Click *Move*.

The sample changer rotates to the correct position.

e Click *Close*.

This closes the ***Sample Selector*** dialog box.

f Click *Close* in the ***Sample Compartment Accessories*** window.

Temperature bath or Peltier device

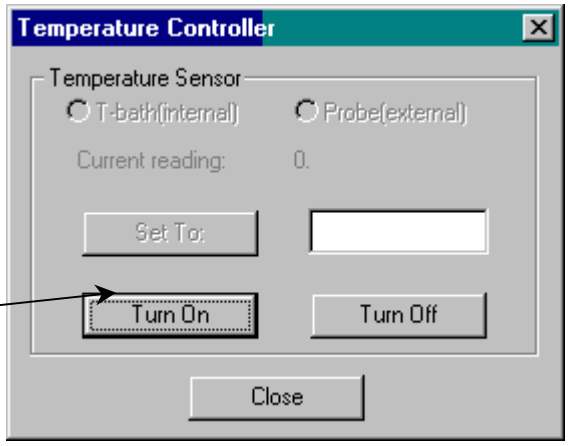
To hold the temperature of the sample constant, a temperature bath or a Peltier Sample Heater/Cooler is a useful accessory. The temperature of the bath or Peltier device may be monitored and set within ***Visual Instrument Setup***.

a Click on *Temperature control* in the list on the left side of the ***Sample Compartment Accessories*** dialog box.

b Click on the *Thermometer* image on the right of the dialog box. Depending on the type of temperature control, either the ***Temperature Controller*** or ***Peltier Setup*** dialog box appears.

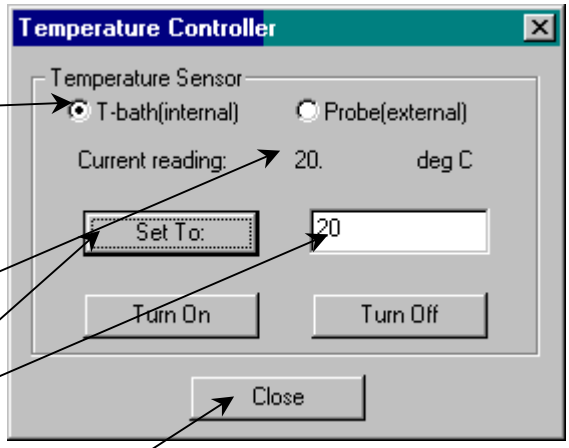
Temperature Bath Accessory

If the *Current reading* is gray and the *Set To:* button is inaccessible, as shown here, the temperature bath is switched off. To correct this,



c Click the *Turn On* button to switch on the temperature bath.

The dialog box changes into:



d Choose to adjust the bath's temperature or an optional external probe's temperature.

e View the current reading here.

f Enter the present value in the *Set To:* field.

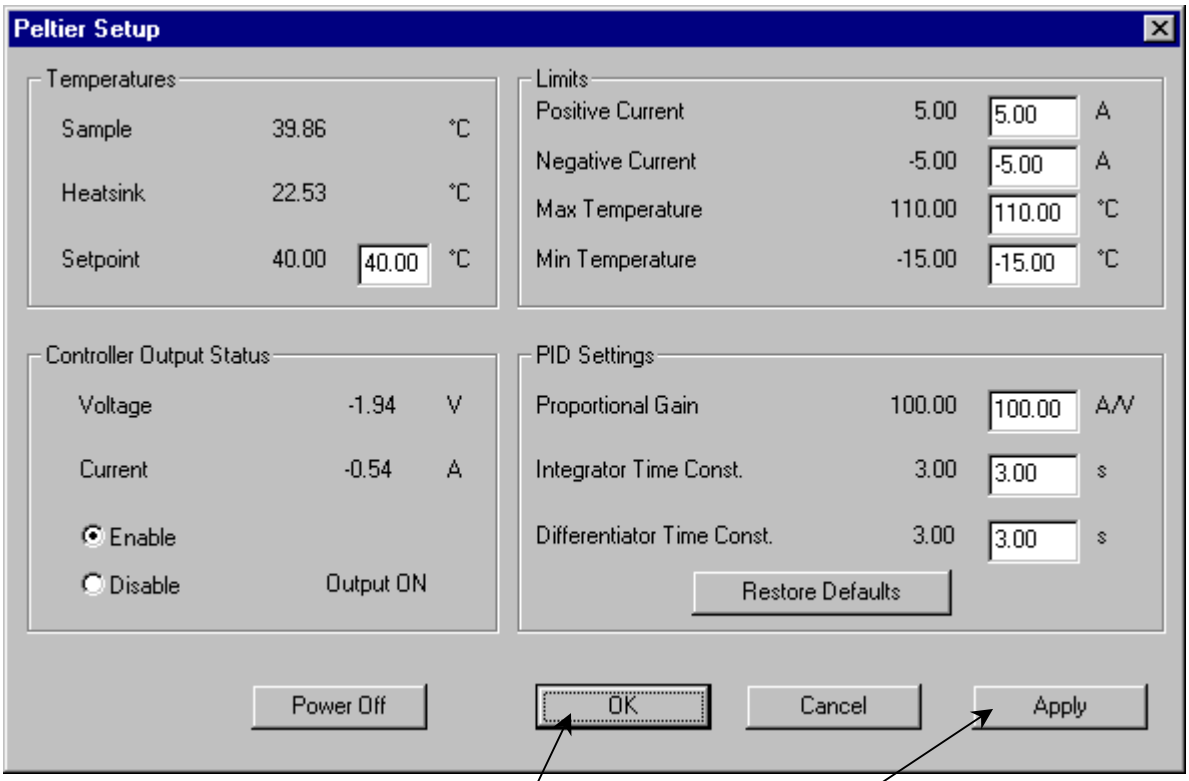
g Click *Set To:*

h Click *Close* when finished.

This closes the ***Temperature Controller*** dialog box.

i Click *Close* in the ***Sample Compartment Accessories*** window.

Peltier Sample Heater/Cooler Accessory



Basic operations for the Peltier device are displayed here, including:

- Current and setpoint temperatures
- Peltier device thermal and electrical limits
- Controller status
- *PID* parameters for the Peltier device

a Change settings as desired.

b Click *Apply*.
This activates the new parameters.

c Click *OK*.
This closes the ***Peltier Setup*** dialog box.

d Click *Close* in the ***Sample Compartment Accessories*** window.

For more information on operation of the Peltier Sample Heater/Cooler, see the *F-3004 Peltier Sample Cooler Operation Manual*.

AutoTitrator

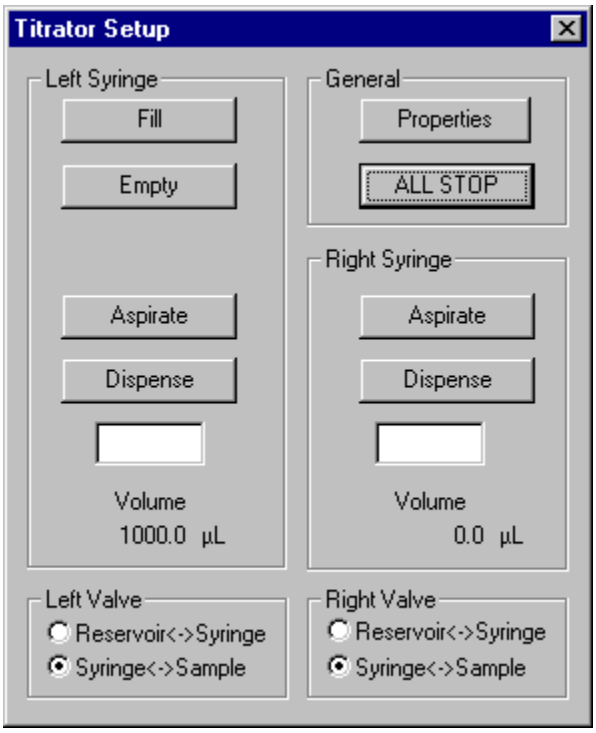
An AutoTitrator optional accessory may be used to remotely inject aliquots of solution into a sample cuvette, to monitor fluorescence effects over a period of time.

a Click on *Pump or Syringe* in the list on the left side of the ***Sample Compartment Accessories*** dialog box.

b Click on the beaker-cuvette image on the right of the dialog box. The ***Titrator Setup*** dialog box appears.

Basic titrator operations are accessible:

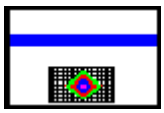
- Fill the left syringe
- Empty the left syringe
- Aspirate into left or right syringe
- Dispense from left or right syringe
- Switch directions of valves



For more details on the AutoTitrator's operation, see the *F-3005/6 AutoTitrator Injector Operation Manual*.

c When finished adjusting the syringe, click the *Exit* box ☒ in the upper right.

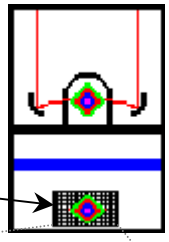
d Click *Close* in the ***Sample Compartment Accessories*** window.



MicroMax

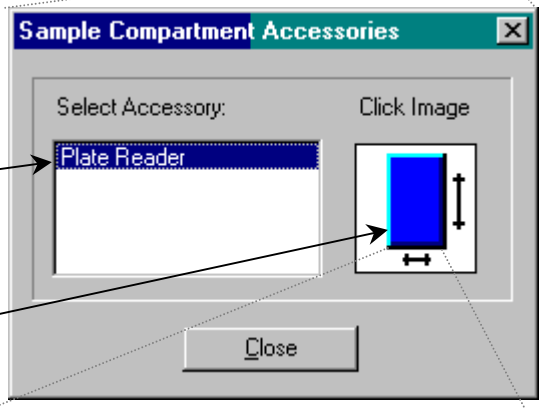
The MicroMax microwell plate reader is used to example many samples rapidly, especially when they are all examined at the same wavelength. Access to this optional accessory is slightly different from other accessories. Only the position of the plate reader may be adjusted within ***Visual Instrument Setup***.

In the ***Visual Instrument Setup*** schematic of the instrument,



1 Click the symbol for the MicroMax next to the sample compartment:

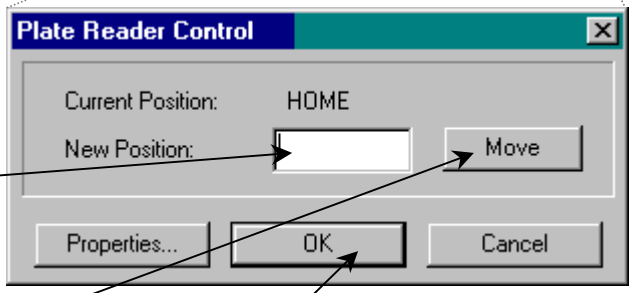
The ***Sample Compartment Accessories*** dialog box opens.



2 Click *Plate Reader* in list on the left side.

3 Click on the microwell-plate image on the right side.

The ***Plate Reader Control*** dialog box opens:



4 Enter the new position for the microwell plate.

For example, enter “A12”, or “home”.

5 Click *Move*.

The MicroMax moves the plate to the desired position.

6 Click *OK* when finished.

The ***Plate Reader Control*** dialog box closes.

7 Click *Close* in the ***Sample Compartment Accessories*** window.

This closes the ***Sample Compartment Accessories*** dialog box.

Phosphorimeter

The phosphorimeter measures phosphorescence via a programmable pulsed light source and selectable gating on the reference detector. This icon is only visible when the optional phosphorimeter accessory is attached and selected with a phosphorimeter layout.

In the ***Visual Instrument Setup*** schematic of the instrument,

1 Click the symbol for the phosphorimeter.

The ***Acquisition Channel*** dialog box opens.

2 Choose the *Data Units* from the drop-down menu.

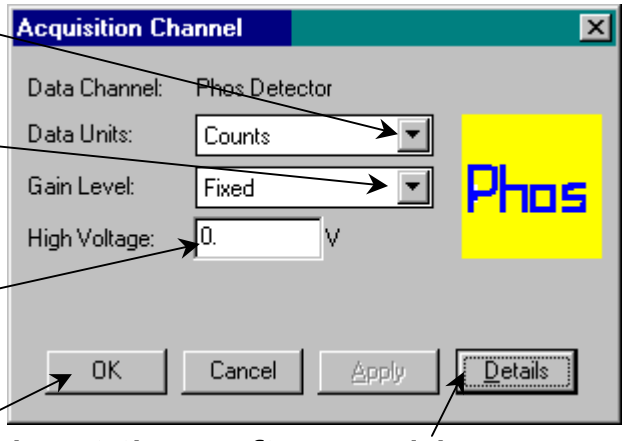
3 Choose the *Gain Level* from the drop-down menu.

4 Enter the desired *High Voltage*.

5 Click *Details* to obtain information about the software driver.

6 Click *OK* when finished.

The ***Acquisition Channel*** dialog box closes.

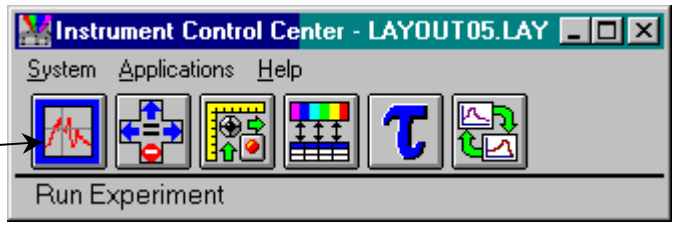


4: Conducting Experiments

Basic steps

To define a steady-state experiment and acquire data in DataMax, enter the ***Run Experiment*** application.
In ***Instrument Control Center***,

1 Click the *Run Experiment* button.



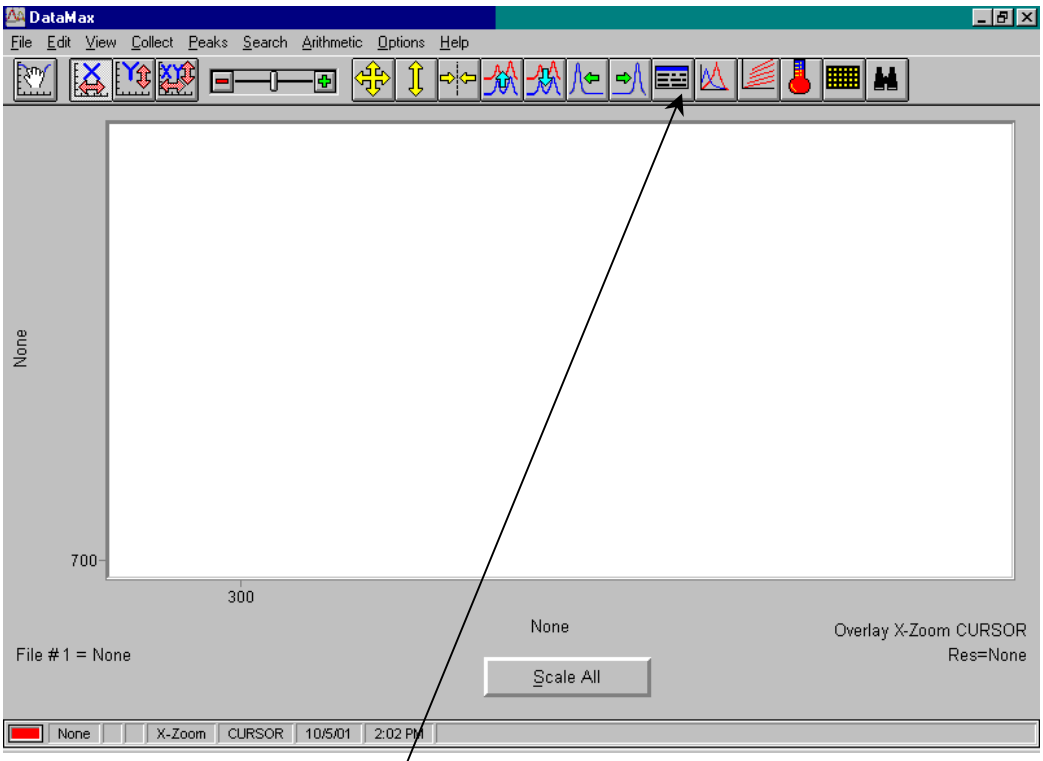
The title screen appears.

2 Click *OK*.

Or

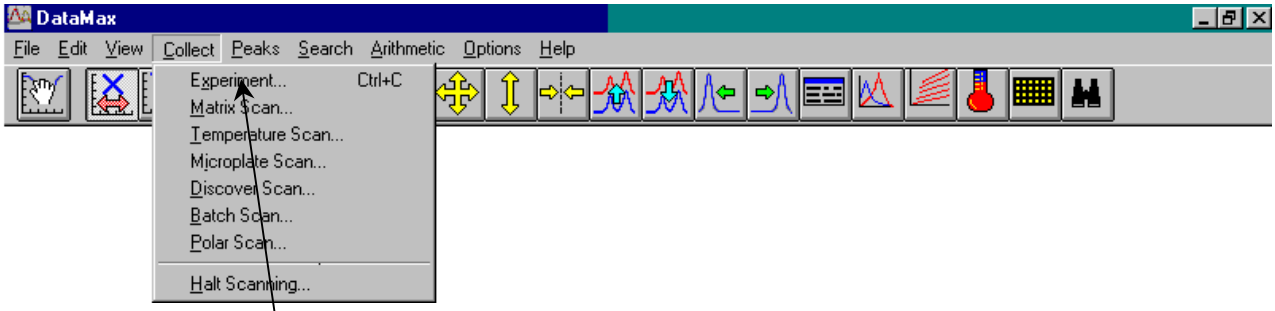
Wait for the title screen to disappear.

The default view of ***Run Experiment*** appears.



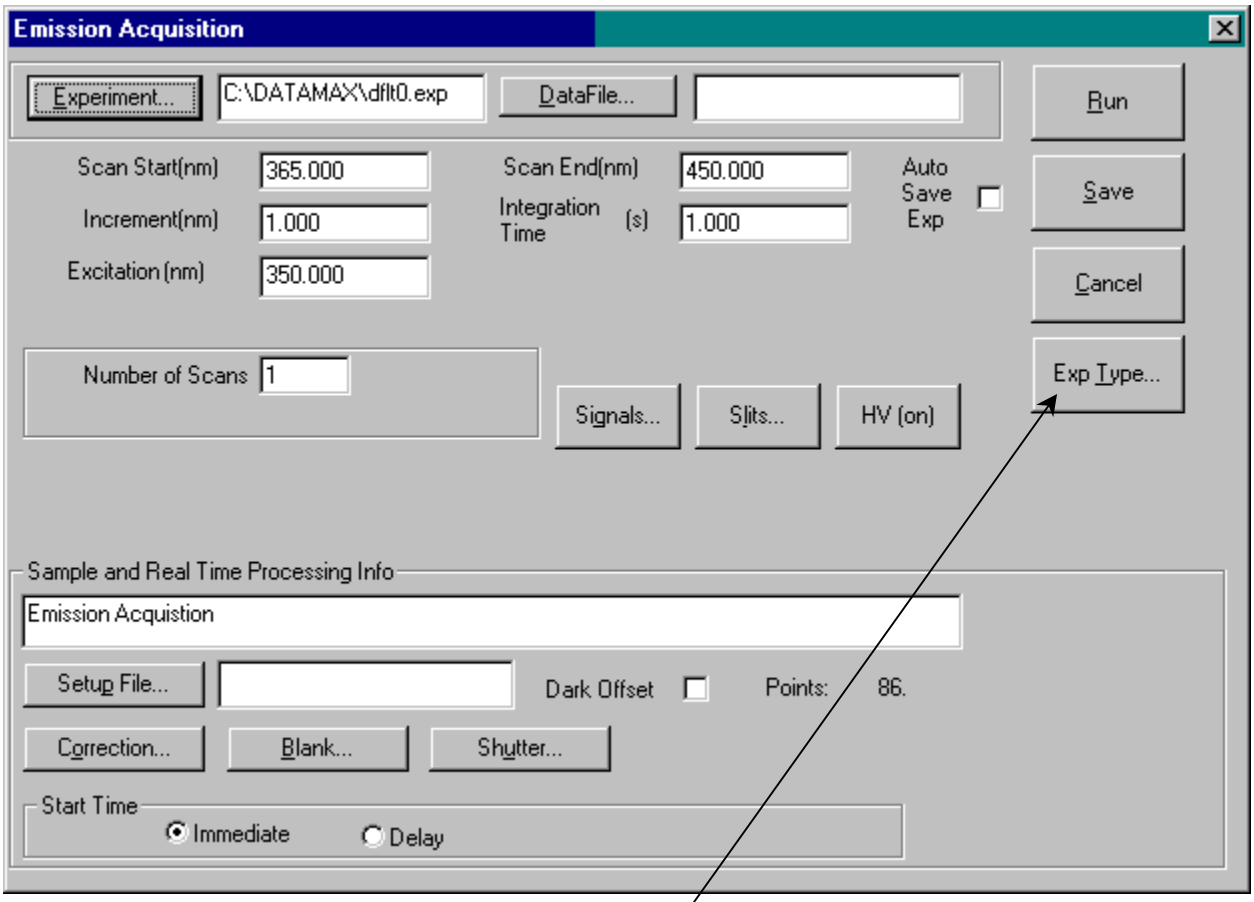
3 Click the *Experiment* button on the toolbar.

0r



Choose *Collect* in the main menu, then *Experiment…* from *Collect*'s drop-down menu.

An ***Experiment Acquisition*** dialog box appears.



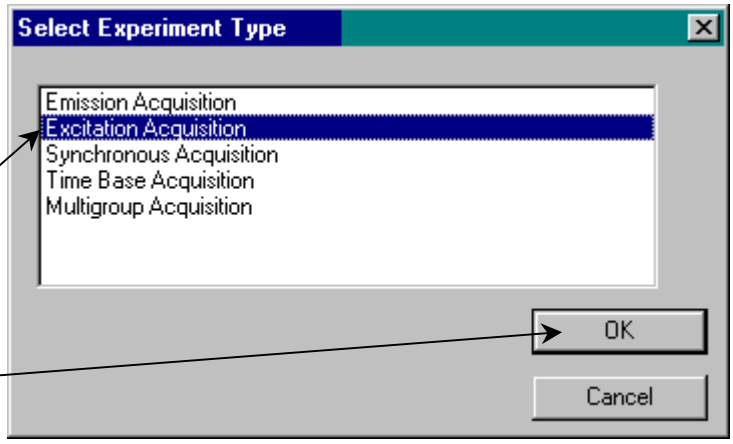
4 If available, click *Exp Type…*.

The ***Select Experiment Type*** dialog box appears.

5 Choose accessories.

6 Choose type of experiment.

7 Click *OK*.



This closes the ***Select Experiment Type*** dialog box.

8 Enter experiment parameters and file names in the ***Experiment Acquisition*** dialog box.

9 Click *Run*.

About performing experiments

File types

For easy information retrieval,

- Experiment parameters
- Data
- System units and hardware settings

are saved in several files—experiment, data, and setup. Each of these files can be retrieved to review or modify experiments.

Ways to perform an experiment

Recall and execute an existing experiment

Any existing experiment or data file can be recalled and rerun at any time. Each type of experiment has a default set of parameters, so this default may be used also.

Recall, modify, and execute an existing experiment

Recall a stored experiment or data file, modify its parameters, rename the file, and run the modified version.

Define a new experiment

Select a scan type, and enter appropriate parameters.

Note: *Setup files must be selected. They cannot be created in this application.*

Experiment types

DataMax allows several standard types of scans to be run. Depending on the layout of the spectrofluorometer system with accessories, the ***Select Experiment Type*** dialog box presents a list of scans available:

Standard scans (for all systems)
Emission scan
Excitation scan
Synchronous scan
Time-base scan
Multigroup scan (*not* available with polarizers)

Phosphorimeter scans (for Fluorolog systems)
Phosphorimeter emission scan
Phosphorimeter excitation scan
Phosphorimeter synchronous scan
Phosphorimeter delay by decay
Phosphorimeter delay by window
(Multigroup is *not* available here)

Lifetime scans (for Tau lifetime systems)
Lifetime-resolved acquisition

Lifetime scans *not* available in this window (see Chapter 9 instead)
Lifetime acquisition
Time-resolved
Anisotropy-decay (only with polarizers)

Emission scan and phosphorimeter scan

Excite the sample with one wavelength of light, while the emission monochromator scans a defined spectral range. (For systems with more than one emission monochromator, the emission monochromator also must be specified.) The resulting emission scan reveals wavelengths at which emission occurs. The data are plotted as intensity versus emission wavelength.

Note: *Default parameters for this scan are for a water Raman scan.*

Excitation scan and phosphorimeter excitation

Excite the sample scanning the excitation monochromator across a defined spectral region, while the emission monochromator stays at a fixed wavelength. From the resulting excitation spectrum, the wavelength that produces the maximum excitation of the sample may be found. The data are plotted as intensity versus excitation wavelength.

Note: *Default parameters for this scan are for a xenon lamp scan.*

Synchronous scan and phosphorimeter synchronous

Scan a both excitation and emission monochromators with a constant offset (either wavelength or energy) between them. The resulting spectrum shows the overlapping region of the excitation and emission spectrum. This technique can improve a spectrum's resolution; it is especially useful for separation of components of a mixture.

Note: *Default values for this scan are practical values, a good starting point for most fluorescent samples.*

Time-based scan

Monitor the emission intensity at a fixed excitation and emission wavelengths for an adjustable length of time. The data are plotted as intensity versus time. This technique is useful for kinetics studies.

Note: *Default parameters are for a time-based water Raman scan.*

Phosphorimeter decay by delay

The sample is exposed to flashes from a xenon lamp for a predetermined length of time. Information is then collect, starting at a specified time. Use this scan type to obtain information about a sample's phosphorescent properties. A phosphorimeter optional accessory is necessary for running this scan.

Note: *Default values for this scan are practical values, a good starting point for many samples.*

Phosphorimeter decay by window

The sample is exposed to flashes from a xenon lamp repeatedly, while monitoring emission intensity. Use this scan type to obtain information about a sample's phosphorescence lifetime. A phosphorimeter optional accessory is necessary for running this scan.

Note: *Default values for this scan are practical values, a good starting point for many samples.*

Multigroup scan

Sequentially excite a sample with different wavelengths of radiation, then plot the emission data on one view. The data are plotted as intensity versus time (a user-definable parameter). This scan is useful for energy-transfer studies, and dual-wavelength experiments with fluorescent probes to examine ion-transport (e.g., Ca^{2+}, Mg^{2+}, K^{+}, and H^{+}) in cells. Fast data-acquisition of up to eight wavelength-pairs (i.e., one excitation plus one emission) allows use of more than one probe. For example, Fura-2 and BCECF can be used to measure Ca^{2+} and determine pH during the same experiment. Additional accessories may be necessary for a multigroup scan:

Note: *Default values for this scan are practical values, a good starting point for many samples.*

- **Injection port:** To make additions to the sample using a pipette or other injection device without removing the sample-chamber lid.
- **Thermostatted single-cell holder with magnetic stirrer:** Maintains proper temperature for cell suspensions in a cuvette. The magnetic stirrer prevents cells from settling to the bottom, and mixes the sample during additions. The stirrer's speed is user-adjustable.
- **Trigger accessory:** To start data-acquisition of a time-based scan without using the host computer's keyboard.

Lifetime resolved acquisition

This scan requires a Tau-3 Lifetime system with polarizers. Lifetime-resolved acquisitions can separate overlapping spectra based on the differences in fluorescence lifetimes. Up to four components can be resolved completely. More complex systems run with this scan type may give improved resolution of one or more spectra, but complete resolution requires extra manipulation of parameters such as excitation wavelength.

Note: *See Chapter 9 for more information about this scan type.*

Other lifetime scan types

The following lifetime scan types are available only with Tau-3 Lifetime systems, and only through the *Lifetime* button in ***Instrument Control Center***:

- **Lifetime acquisition:** Records phase-shift and modulation at specified frequencies for an unknown relative to a reference material, determines lifetimes from single- and multi-component systems.

- **Time-resolved acquisition:** Examines the change in spectral characteristics of a sample during the lifetime of the excited state.
- **Anisotropy-decay acquisition:** As a fluorophore rotates, a change occurs in its polarization. Studies the rotational properties of fluorescent molecules and probes. Useful for energy-transfer, re-absorption, re-emission, and light-scattering.

Note: *See Chapter 9 for more information about these scan types.*

Overview of *Run Experiment*

Introduction

Run Experiment contains controls and choices for processing of data after running an experiment. This manual covers only data collection; therefore, see the *GRAMS/32® User's Guide* for information about post-processing in DataMax.

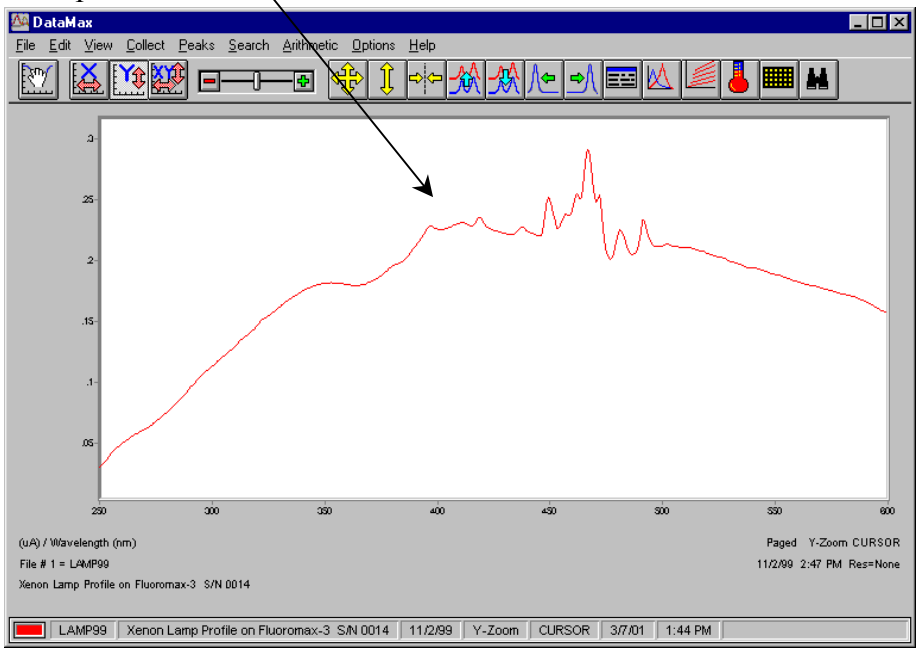
Performing an experiment consists of

- Selecting a scan type: depends on the system and its active layout
- Defining a scan type: the same for all experiment types—enter the experiment parameters in the ***Experiment Acquisition*** dialog box, or recall them via an existing experiment.
- Running the scan

The software checks the hardware configuration and makes any adjustments. For example, if the software detects multiple monochromators, the ***Experiment Acquisition*** dialog box contains data-entry fields for all detected monochromators. If a recalled experiment was conducted using accessories no longer extant, an error occurs. Thus, only information pertaining to the choices selected at the ***Select Experiment Type*** dialog box are presented on the associated ***Experiment Acquisition*** dialog box. For example, *all* scan types require an acquisition mode and an integration time. In contrast, an excitation acquisition requires starting and ending positions for the excitation monochromator. A time-based acquisition needs the length of time data are collected and at which wavelengths the monochromators are fixed.

Screen view

After an experiment is defined and run, the spectrum is displayed on the central area of the screen, called the spectral screen.



The spectral screen has a standard default view, plus other views as well. DataMax always displays separate traces for each acquisition mode (signal) on the same screen view. The screen splits horizontally to

display each acquisition mode. The more acquisition modes specified, the narrower the traces become.

Each acquisition mode is identified by the overall file name, plus a letter of the alphabet is automatically appended to the file name, to distinguish one detector's signal from another. For example, to view a signal from the S channel, the R channel, and the ratio S/R, the file names might be `signalA.spc`, `signalB.spc`, and `signalC.spc`, respectively. Each of these files can be accessed and manipulated individually.

Note: *See the* GRAMS/32® User's Guide *for more information about available views and screen notes.*

File types and acquisition modes

There are three types of files:

Experiment

An experiment file stores instrument settings and acquisition parameters about a specific experiment, but no actual data.

When an ***Experiment Acquisition*** dialog box is opened, the default experiment or last experiment file used in the current session appears. When saving a file, you are asked if it is okay to overwrite the existing file, even if it is a default file.

Warning: *Jobin Yvon® does not recommend overwriting or replacing the original default file.*

The user names the experiment file. By default, the file is given an `.EXP` extension. Any three-character extension is valid, but DataMax assumes that `.EXP` extensions represent experiment files. Any other extensions are not automatically listed in the ***Define Exp File*** dialog box as experiment files.

To save an experiment, an experiment file must be specified. If a data file is recalled from disk using the *Experiment...* button, the experimental parameters and associated setup information are called up also.

Data

A data file stores data taken during an experiment, plus instrument settings and acquisition parameters. A data file is automatically saved with an `.SPC` (spectrum) extension. Any three-character extension is valid, but DataMax assumes that `.SPC` extensions represent data files.

Setup

The setup file retains information about the system, e.g., hardware settings and preferred units for each experiment. Multiple setup files may be saved and recalled as needed. If a setup file is not selected or recalled for a particular experiment, the system

uses the current parameters. A setup file is automatically saved with an `.SET` extension. Any three-character extension is valid, but DataMax assumes that `.SET` extensions represent setup files.

The table summarizes what kind of information is stored in each file type:

File type	Kind of information stored: Setup	Experiment	Data
Data	✓	✓	✓
Experiment	✓	✓	
Setup	✓		

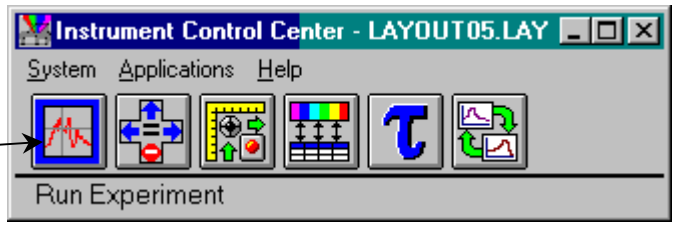
Under many conditions, specifying an experiment or setup file is unnecessary. Failure to specify a setup file causes the system to use the current setup file (the last one or default); if no experiment file is given, no experiment information is loaded, and the current experiment is not saved to an `.EXP` file.

DataMax makes a distinction between each acquisition mode's data collected during an experiment. When experiments with multiple acquisition modes are defined, DataMax saves and displays each acquisition mode separately. Each acquisition mode is identified by the overall file name, plus a letter of the alphabet is automatically appended to the file name, to distinguish one detector's signal from another. For example, to view a signal from the S channel, the R channel, and the ratio S/R, the file names might be `signalA.spc`, `signalB.spc`, and `signalC.spc`, respectively. Maximum length of the file name is eight characters (as with all DOS files). Therefore, with multiple acquisition modes specified simultaneously, an eight-character file name is truncated to seven characters, and a letter of the alphabet is appended to the file name. For example, suppose the overall data are called `glycogen`, and three acquisition modes are chosen. The individual data files are then `glycogeA.spc`, `glycogeB.spc`, and `glycogeC.spc`.

Recalling and executing an existing steady-state experiment

In ***Instrument Control Center***,

1 Click the *Run Experiment* button.



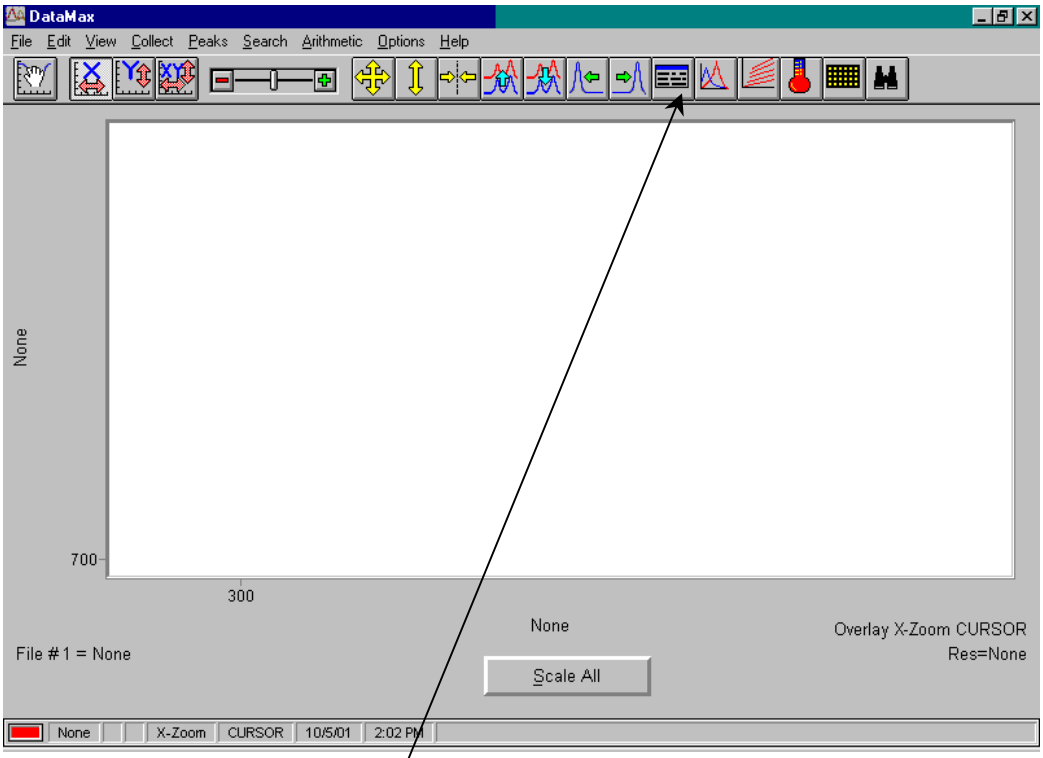
The title screen appears.

2 Click *OK*.

Or

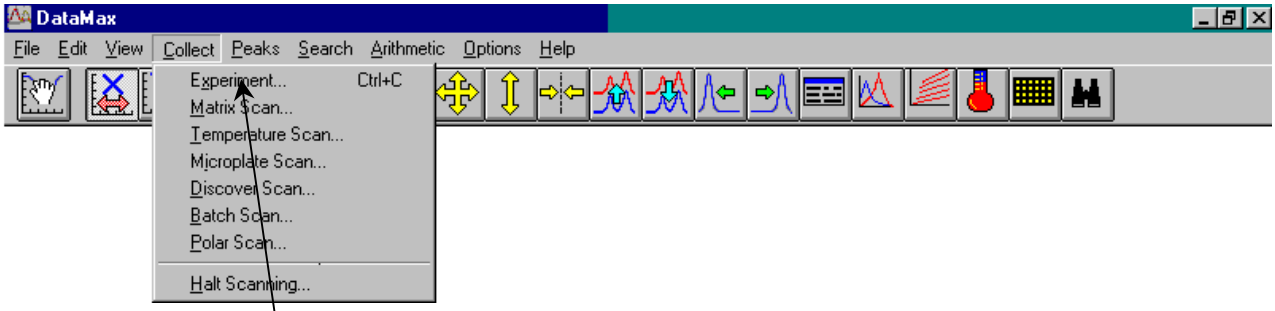
Wait for the title screen to disappear.

The default view of ***Run Experiment*** appears.



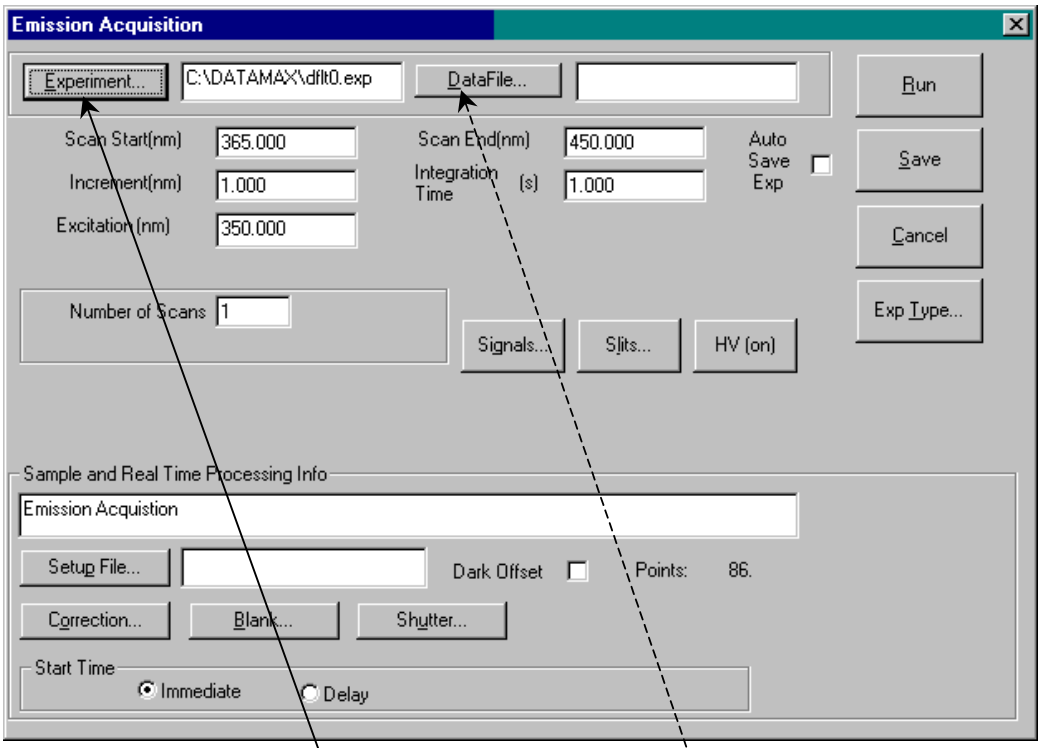
3 Click the *Experiment* button on the toolbar.

Or



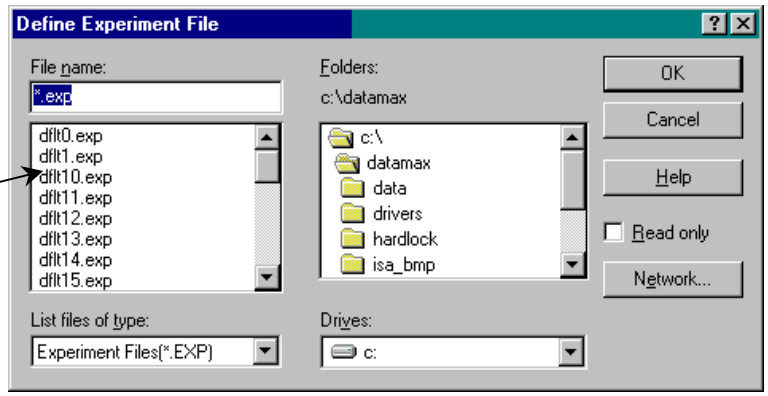
Choose *Collect* in the main menu, then *Experiment…* from *Collect*'s drop-down menu.

An ***Experiment Acquisition*** dialog box appears.



4 Click the *Experiment…* (or *DataFile…*) button.

The ***Define Experiment File*** (or ***Define Data File***) dialog box opens:



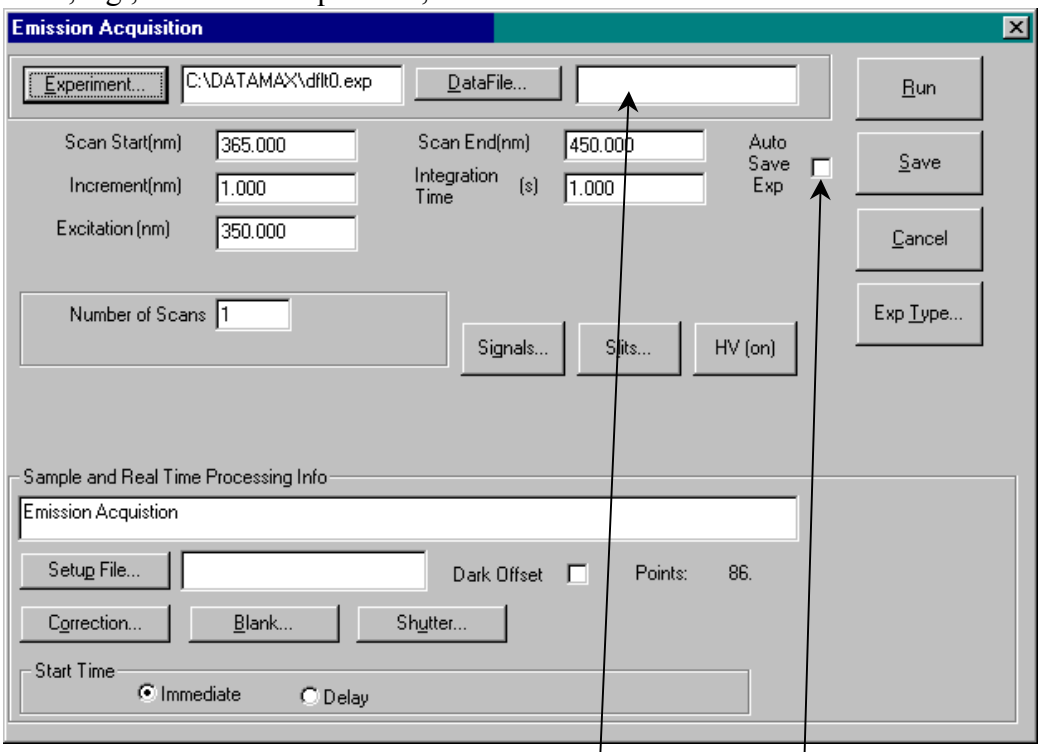
5 Select an experiment (or data) file.

If an `.EXP` file is chosen and no setup file is specified, the setup information from the recalled experiment is used. (If an `.SPC` file is chosen, the data, experimental parameters, and instrumental setup from the `.SPC` file all appear. Running and saving this experiment causes the original data to be replaced by the new data. To prevent overwriting data, change the name in the *DataFile* text area.)

6 Click *OK*.

Note: *If the experiment contains a layout using accessories no longer available, an error occurs.*

The ***Define Experiment File*** (or ***Define Data File***) dialog box closes. Note how all information relative to the experiment and instrument (and possibly data) are displayed in the appropriate fields. The type of experiment, e.g., excitation acquisition, is shown in the title bar.



7 Enter a name for the data file, if none is given.

8 Decide whether or not to use *Auto Save Exp*.

To save the experiment and data automatically during the run, check the *Auto Save Exp* checkbox. Data are saved automatically to the specified data file name.

If the *Auto Save Exp* checkbox is not checked, the experiment is not automatically saved. The data file saved, however, does contain the same information as

the experiment file, so the experiment's parameters can be recalled from within the data file.

To modify the present experiment, change any parameter.

Note: *To prevent overwriting the experiment or data file, change the experiment or the data file name(s).*

9 Click *Run*

The experiment begins. The window reverts to the default ***Run Experiment*** window, with the spectrum plotted while it is collected.

10 Click the *Experiment* button or *Experiment…* in the *Collect* drop-down menu, to rerun the experiment or review the parameters.

Before rerunning the experiment, the software requests permission to overwrite old information.

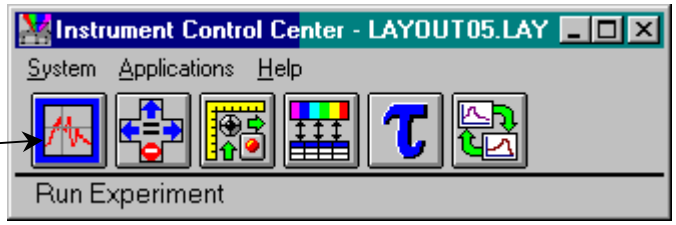
a Click *Yes* to proceed.

b Click *No* to cancel the run before it begins.

Running a new steady-state experiment

In ***Instrument Control Center***,

1 Click the *Run Experiment* button.



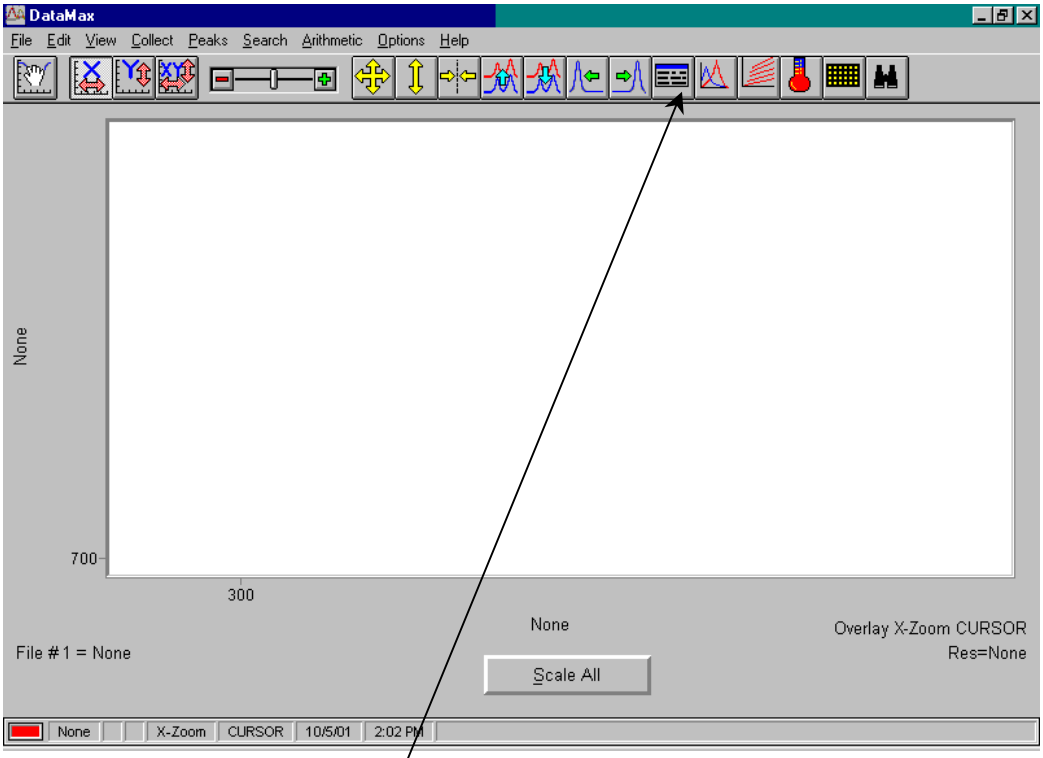
The title screen appears.

2 Click *OK*.

Or

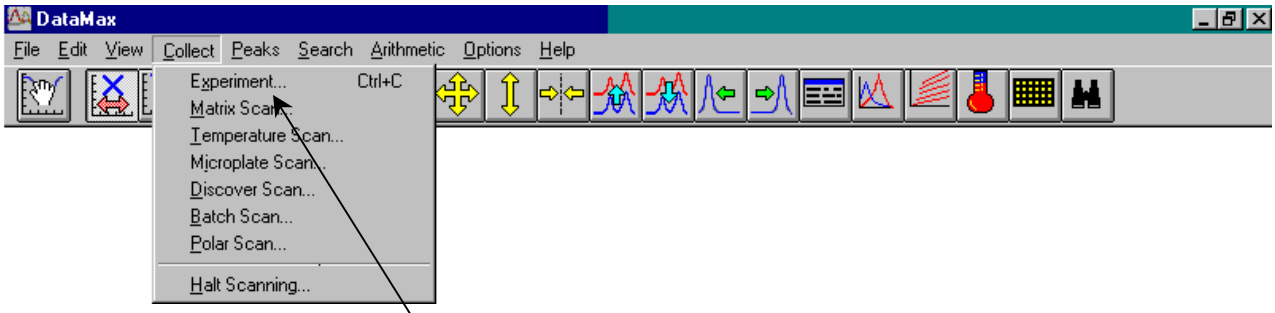
Wait for the title screen to disappear.

The default view of ***Run Experiment*** appears.



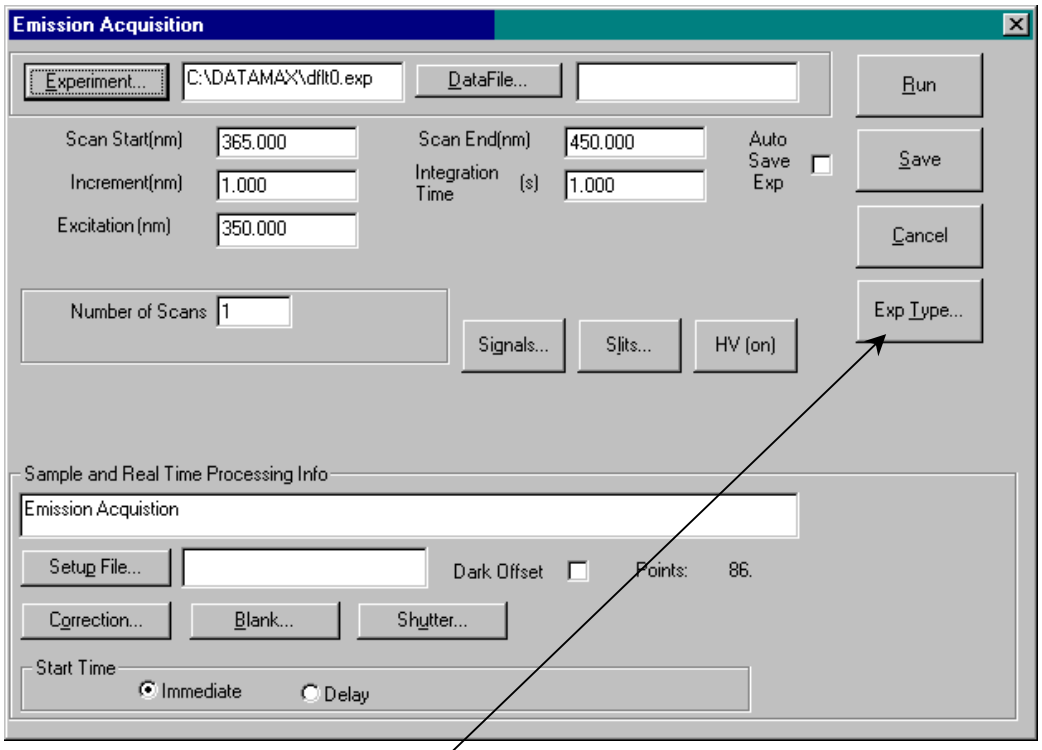
3 Click the *Experiment* button on the toolbar.

Or



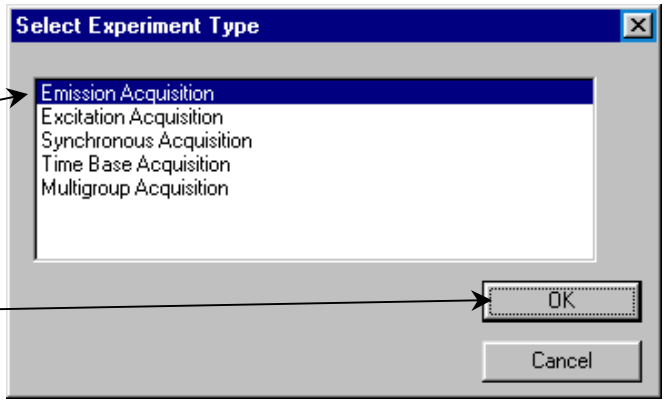
Choose *Collect* in the main menu, then *Experiment…* from *Collect*'s drop-down menu.

An ***Experiment Acquisition*** dialog box appears.



4 Click the *Exp Type…* button.

The ***Select Experiment Type*** window opens.



5 Choose the type of experiment.

6 Click *OK*.

The ***Select Experiment***

Type dialog box disappears. The ***Experiment Acquisition*** dialog box changes into the appropriate acquisition dialog box.

7 Replace the current default values with correct parameters.

See Chapter 5 for explanation of all data-entry fields.

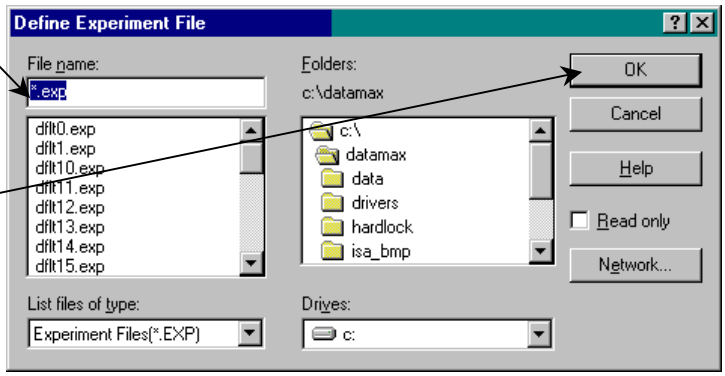
8 Decide whether or not to use *Auto Save Exp*.

To save the experiment and data automatically during the run, check the *Auto Save Exp* checkbox. Data are saved automatically to the specified data file name.

a

Replace the *Experiment…* file name with a new name.
Or
Click the *Experiment…* button.
The ***Define Experiment File*** window opens.

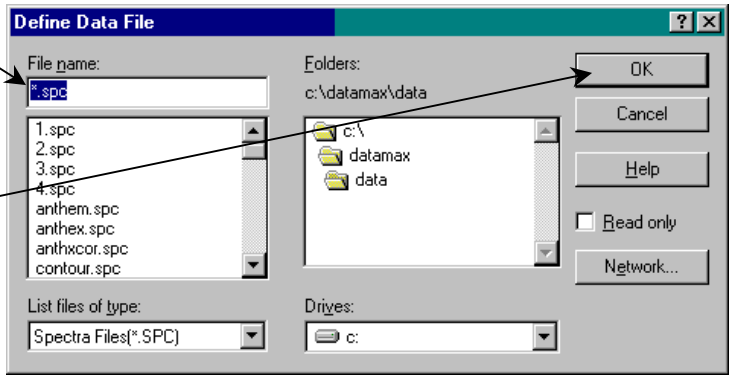
- Enter an 8-character DOS-style experiment file name.
- Click *OK*.
- The dialog box closes.



b

Replace the *DataFile…* file name with a new name.
Or
Click the *DataFile…* button.
The ***Define Data File*** window opens.

- Enter an 8-character DOS-style data file name.
- Click *OK*.
- The dialog box closes.



If the *Auto Save Exp* checkbox is not checked, the experiment is not automatically saved. The data file saved, however,

Note: *To prevent overwriting the experiment or data file, change the experiment or the data file name(s).*

does contain the same information as the experiment file, so the experiment's parameters can be recalled from within the data file.

To modify the present experiment, change any parameter.

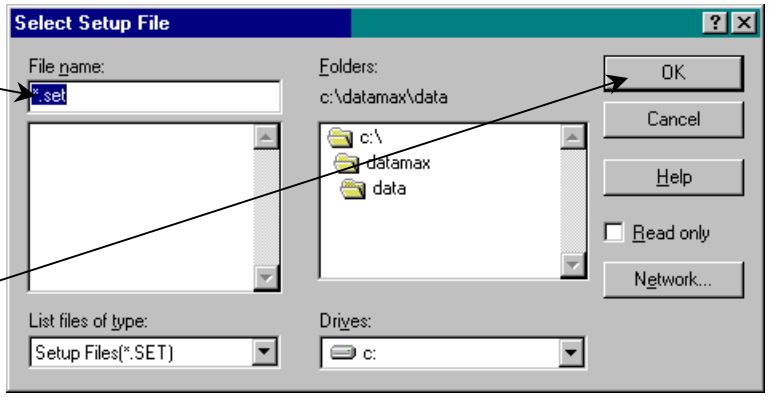
9 If a setup file is desired, click the *Setup File…* button.

The ***Select Setup File*** dialog box opens.

a Select an existing setup file from the appropriate folder.

b Click *OK*.

The ***Select Setup File*** dialog box closes.



10 Enter information in the remaining fields of the ***Experiment Acquisition*** box.

See *Chapter 5: Setting Parameters* for details about these data-entry fields.

11 Choose one of the following options:

Click *Run* to start the experiment.

Click *Save* to store the experiment.

Click *Cancel* to return to the main window, without running or saving the experiment.

The main screen returns. If *Run* was chosen, the spectrum is plotted in the central area as the data are taken.

Note: *Using one of the file types (experiment, setup, and data) is not dependent on using others. For example, to use an existing experiment, but not to overwrite existing data, rename the data file.*

12 Exit the ***Run Experiment*** application when finished.

a Click *File*.

The drop-down menu appears.

b Choose *Exit*.

5: Setting Parameters

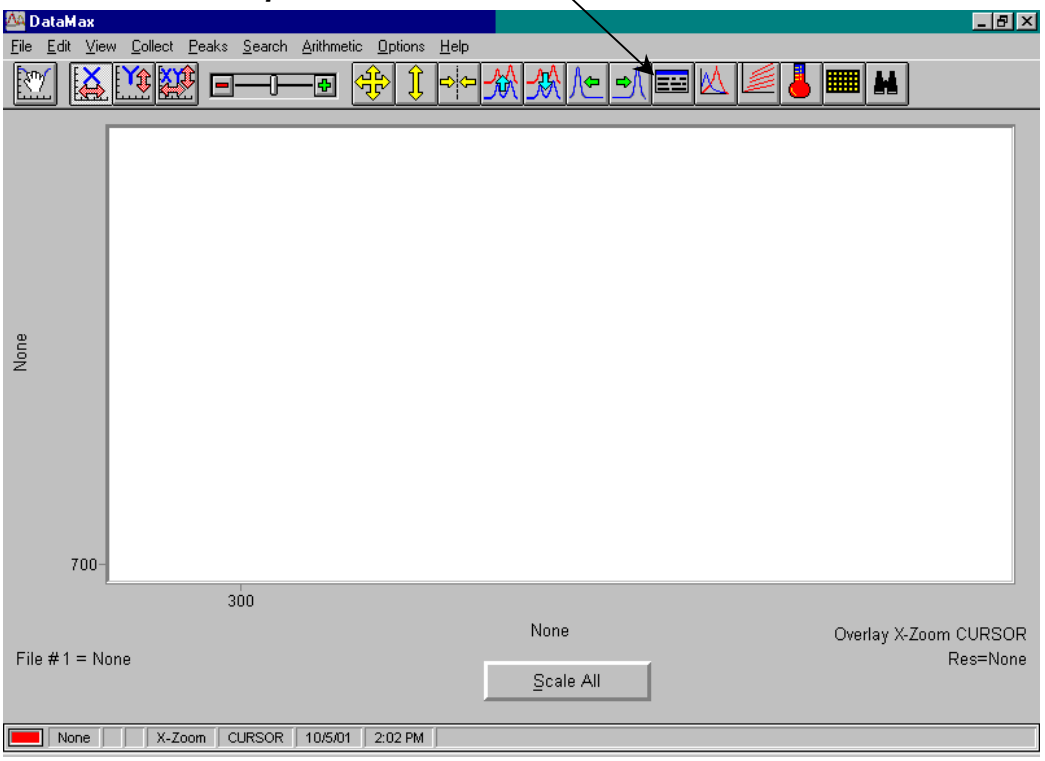
Acquisition screen

To establish experimental parameters, the appropriate scan type already must be selected along with appropriate accessories (e.g., polarizers).

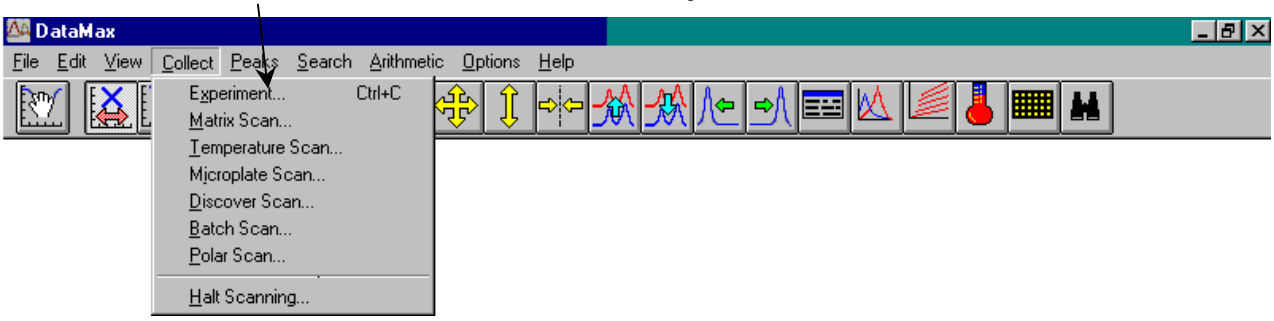
In ***Run Experiment***,

1 Click the *Experiment* button on the toolbar.

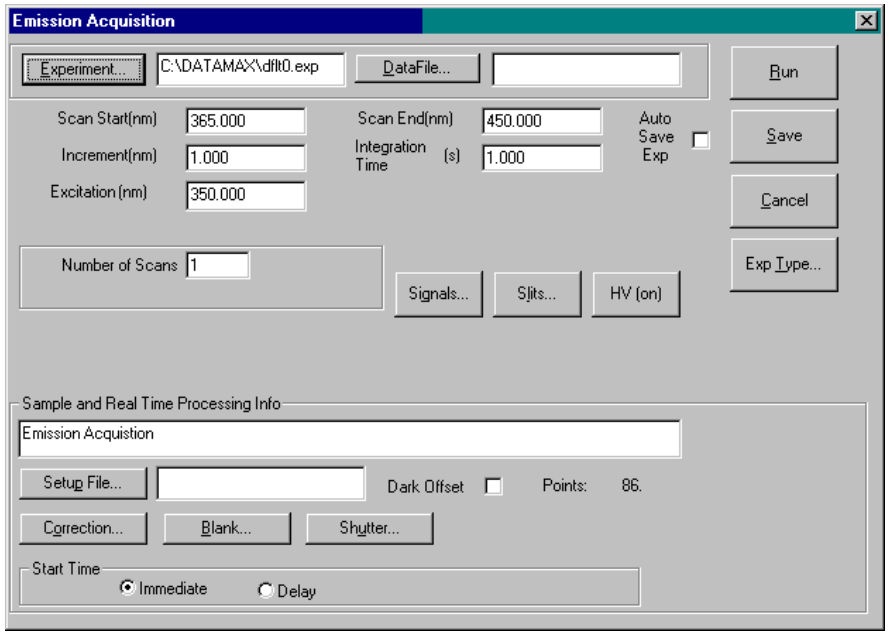
Or



Choose *Collect* in the main menu, then *Experiment…* from *Collect*'s drop-down menu.



An ***Experiment Acquisition*** dialog box appears.



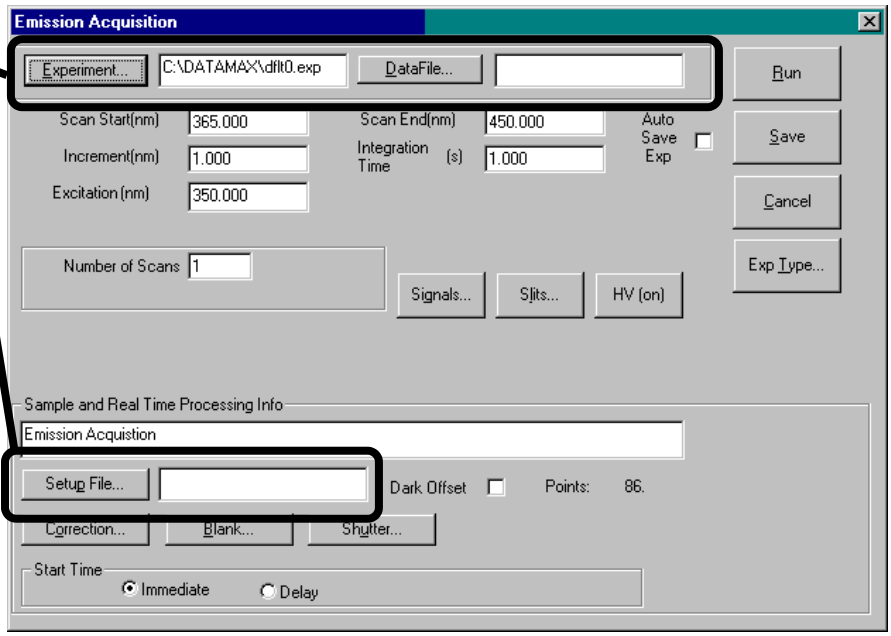
Different scan types require different parameters, so the acquisition screens appear different. For example, all scan types require an acquisition mode, but an emission acquisition does not use starting and ending wavelengths for the excitation monochromator: this monochromator is fixed.

There are three general kinds of information in the ***Experiment Acquisition*** dialog box:

- Information about files
- Information about actual data collection
- Information about experiment times and correction

Information about files on the acquisition screen

Information about files is found in these areas:



To specify file information,

1 Load existing files.

Load the existing experiment, data, or setup files for the experiment.

0r

Perform a new experiment.

a Assign file names to the experiment or data files.

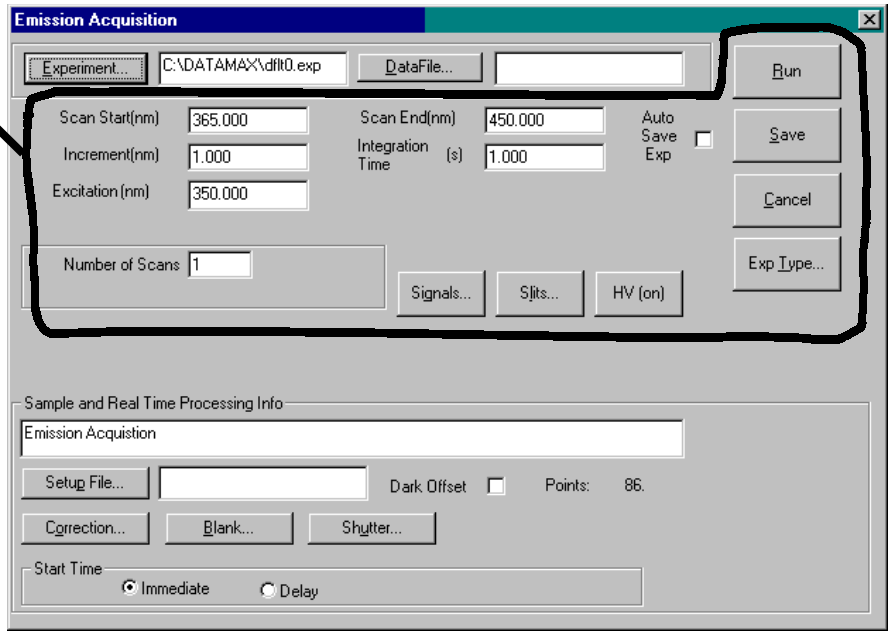
b Select a setup file (optional).

c Complete data-field entry for the remaining parameters.

For more information about loading files, see Chapter 4.

Information about data collection

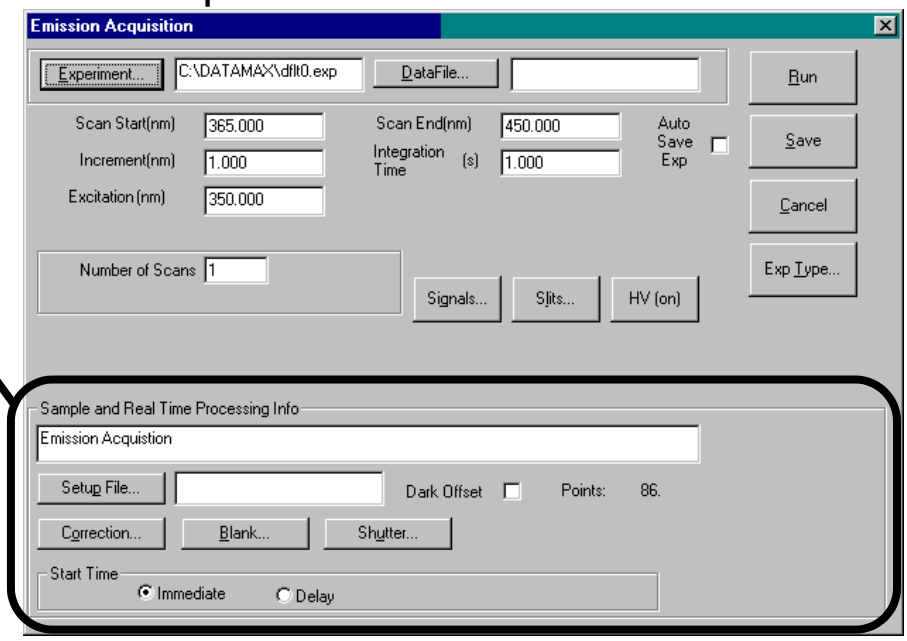
Information about data collection is found in this area:



Selections found in this area depend on the type of experiment and the accessories specified. For example, in *Signals…*, certain acquisition modes are found with polarizers.

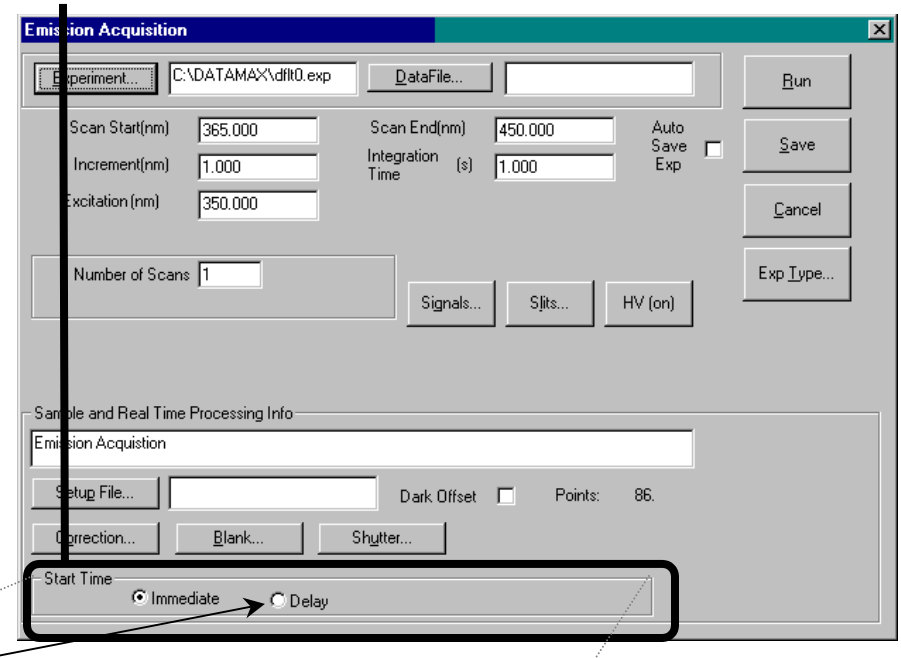
Information about sample times and correction

This information is grouped in *Sample and Real Time Processing Info*. This area specifies delay time, spectral corrections, dark-count offsets, etc.



Hidden fields

The ***Experiment Acquisition*** window contains hidden fields. That is, occasionally a selection of one of the standard choices causes another field to appear. For example, note the radio buttons for *Start Time*:



When *Delay* is chosen, an addition field for delay time appears:

With this hidden field, the user can specify how long to wait before starting an experiment.

List of adjustable parameters

Following is an alphabetical list of data-entry fields that are user-adjustable. For information on actually running a steady-state experiment, see Chapter 4.

Auto Save Exp

The *Auto Save Exp* checkbox is found on every Experiment Acquisition screen. Checking the box causes the host computer to save the experiment file automatically. Each time an experiment is run, the user is prompted to enter an experiment file name along with a data file name.

If the *Auto Save Exp* checkbox is not enabled, the user manually must save the experiment file to the hard disk.

Blank

Raw data incorporate the optical properties of the sample, plus the sample holder, light source, gratings, detector, solvent, and so forth. To reduce or eliminate these extraneous effects, a dark-offset scan (without the light source) and a blank scan are run along with the sample. A blank file is run of the solvent without the sample. Then, to remove the instrument- and solvent-dependent effects of the scan, the blank's data and dark counts are subtracted from the sample's data.

A blank scan is

Blank scan = (Solution scan) – (Dark Offset)

and a blank-subtracted scan is

Blank-subtracted scan = (Sample scan) – (Blank scan)

When a blank file is indicated in DataMax, the software automatically scans the dark counts, and subtracts this spectrum from the blank scan.

Create a blank file

1. Turn on spectrofluorometer system.
2. Place a blank solution (contains no sample) in a cuvette identical to the cuvette used for the sample.
3. Place the blank cuvette in the sample holder in the sample compartment.
4. Close the sample-compartment lid.
5. Open the appropriate *Experiment Acquisition* dialog box, with all appropriate accessories.
6. Fill in all parameters identically to those used for the sample.
7. Name the experiment and data files.
8. Save the experiment and data files.

Open a previously saved blank file

1 Open the appropriate *Experiment Acquisition* dialog box.

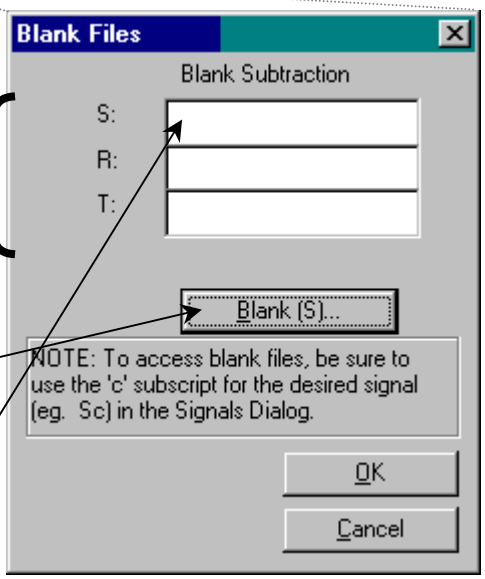
2 Enter parameters identical to those used for the sample.

Or

Recall the experiment used to create the blank file.

a Click the *Blank…* button.

b This opens the ***Blank Files*** dialog box:



Each detector can have an associated blank file. The number of detectors shown in the list depends on the system configuration. Here, a T-format system with S channel, R channel, and T channel are shown.

The *Blank (*x*)…* button indicates which detector's file is selected.

3 Select specific detector's blank file.

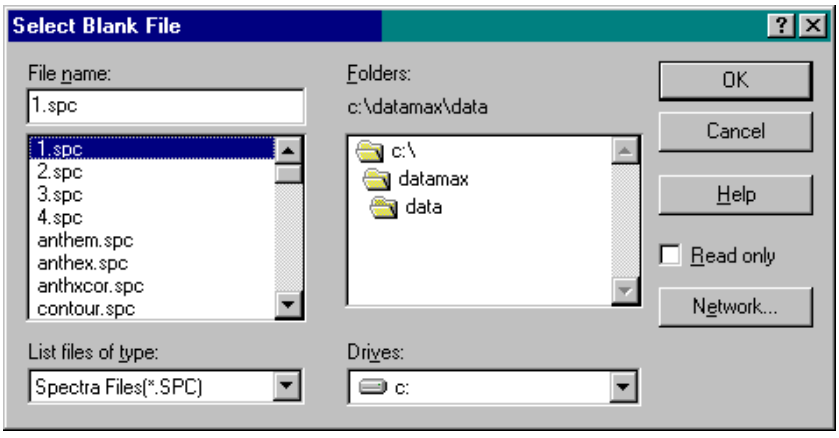
For the signal detector's blank file:

a Click on the *S:* text box.

The name of the button changes to *Blank (S)….*

b Click the *Blank (S)…* button.

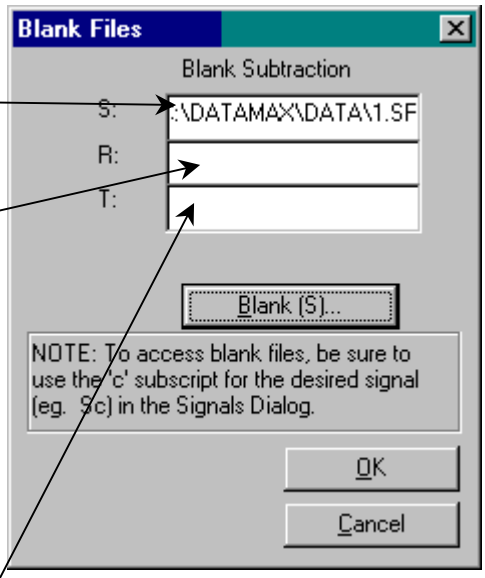
The ***Select Blank File*** dialog box appears.



c Choose the appropriate blank file.

d Click *OK*.

The ***Select Blank File*** dialog box closes. The chosen blank file appears in the *S:* text field:



For the reference detector's blank file:

a Click on the *R:* text box.

The name of the button changes to *Blank (R)*….

b Click the *Blank (R)*… button.

The ***Select Blank File*** dialog box appears.

c Choose the appropriate blank file.

d Click *OK*.

The ***Select Blank File*** dialog box closes. The chosen blank file appears in the *R:* text field.

For the optional third detector's blank file (if the system has a T-format):

a Click on the *T:* text box.

The name of the button changes to *Blank (T)*….

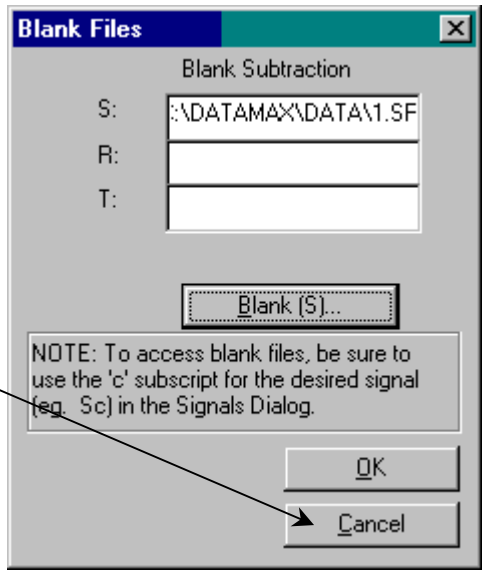
b Click the *Blank (T)*… button.

The ***Select Blank File*** dialog box appears.

c Choose the appropriate blank file.

d Click *OK*.

The ***Select Blank File*** dialog box closes. The chosen blank file appears in the *T:* text field.



4 Click *OK* in the ***Blank Files*** dialog box.

The dialog box closes.

When the experiment is started, each chosen blank file is opened and subtracted from the spectrum of the appropriate detector, along with the dark

counts. The traces that appear on the main screen are the blank-subtracted spectra.

To abort the operation without indicating a blank file, click *Cancel*.

Note: *In an experiment using a blank, do not enable* Dark Offset*. Checking* Dark Offset *will cause the software to subtract dark counts twice from the same spectrum.*

Cancel

Most dialog boxes contain a *Cancel* button. Clicking on *Cancel* nullifies any information entered. In an ***Acquisition*** screen, clicking on cancel removes the entered information and returns to the default view.

To abort an experiment without saving or executing it,

1 Click *Cancel*.

The screen returns to the view that was active before the acquisition dialog box was accessed.

Comment

The *Comment* field (labeled *Sample and Real Time Processing Info*) allows the user to enter any desired information. When the spectrum is shown, the information is displayed on the screen. There is no limit to the length of the comment, but only the first 80 characters can be displayed on the spectral screen.

To place text below the spectral file name on the bottom of the screen,

1 Position the mouse cursor within the *Comment* field.

2 Begin to type.

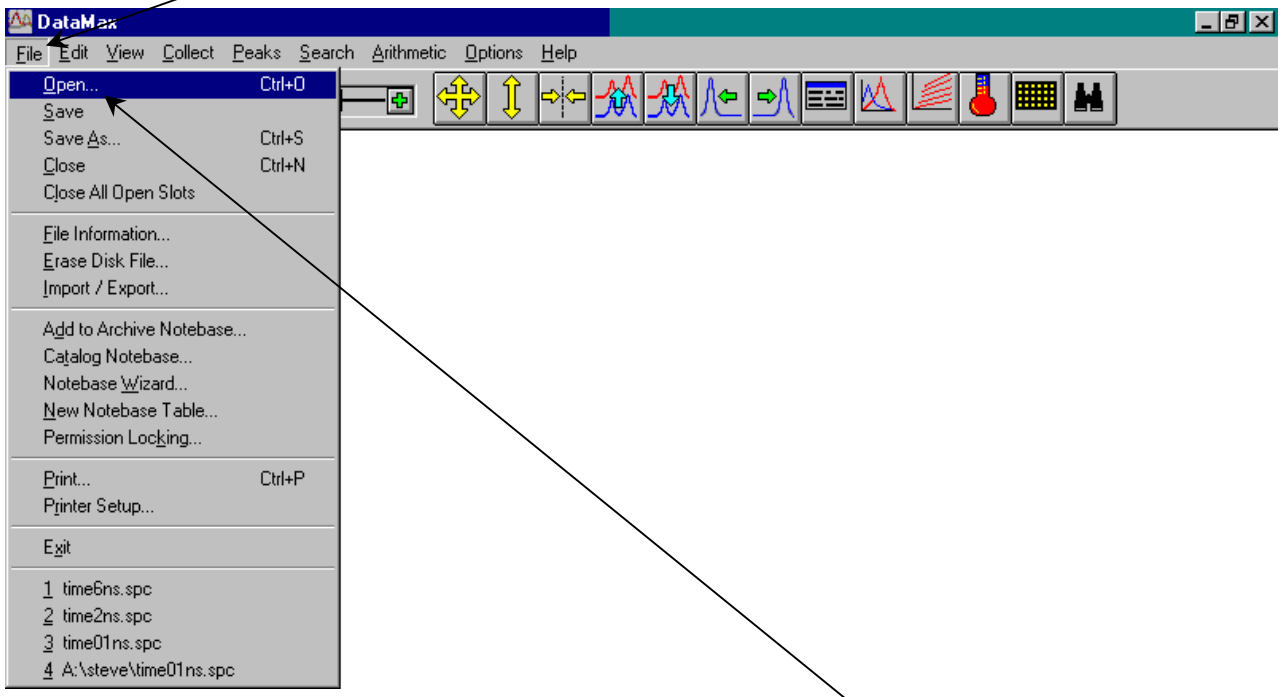
To edit text, use the standard editing keys.

Note: *Special characters cannot be entered in this field.*

To view a comment in a saved file,

1 Open the ***Run Experiment*** application.

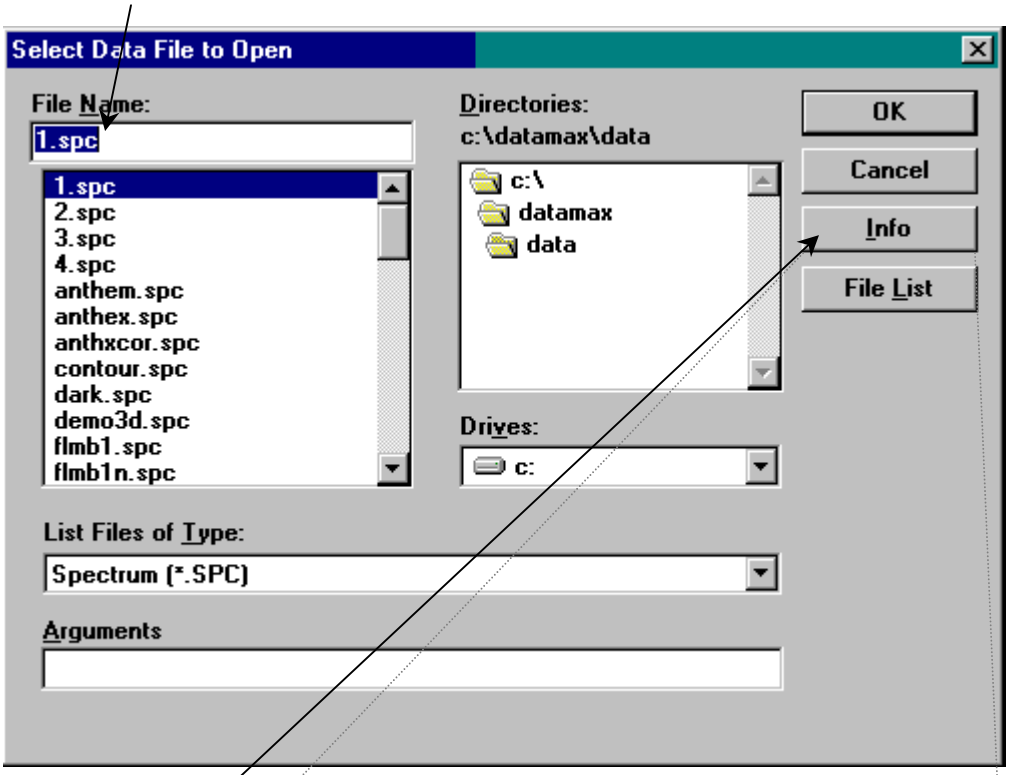
2 Click *File* in the main menu.



3 In the drop-down menu, click *Open…*.

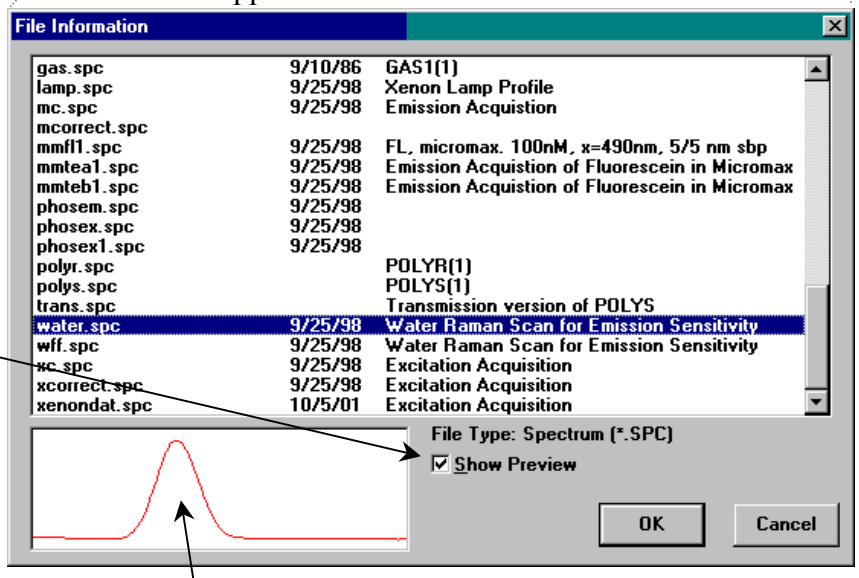
The ***Select Data File to Open*** dialog box appears:

4 Choose the desired file.



5 Click the *Info* button.

The ***File Information*** window appears: File names, last saved date, and first 80 characters of the comment appear.



6 Check the *Show Preview* box to view a preview of the spectrum.

Correction

The *Correction…* button selects correction factors for spectra. These correction factors compensate for the wavelength-dependent components of the system, such as:

- Light source
- Gratings
- Signal detector

When a correction-factor file is selected, the spectrum is blank-subtracted, and then multiplied by the correction factors before display. Mathematically,

Corrected spectrum = (Sample scan – blank scan) × (Correction file)

where the blank scan is defined as

Blank scan = (Solution scan – Dark offset)

The wavelength range of the correction-factor file must be the same as the wavelength-range of the current experiment. The increment of the correction-factor file and the acquired data do not have to be the same; interpolation is performed as necessary.

Excitation and emission correction-factor files (`xcorrect.spc` and `mcorrect.spc`, respectively) are provided with DataMax. To learn more about correction-factor files and how to generate them, see the spectrofluorometer operation manual.

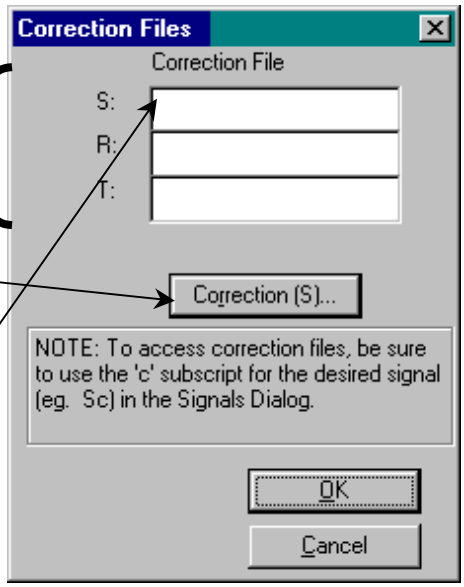
Note: *In an experiment using a correction factor, do not enable* Dark Offset*. Checking* Dark Offset *will cause the software to subtract dark counts twice from the same spectrum.*

To select a correction-factor file,

1. Enter the parameters for an experiment.
2. Click the *Correction…* button.

   The ***Correction Files*** dialog box appears:

Each detector can have an associated correction file. The number of detectors shown in the list depends on the system configuration. Here, a T-format system with S channel, R channel, and T channel are shown.



The *Correction (x)…* button indicates which detector's file is selected.

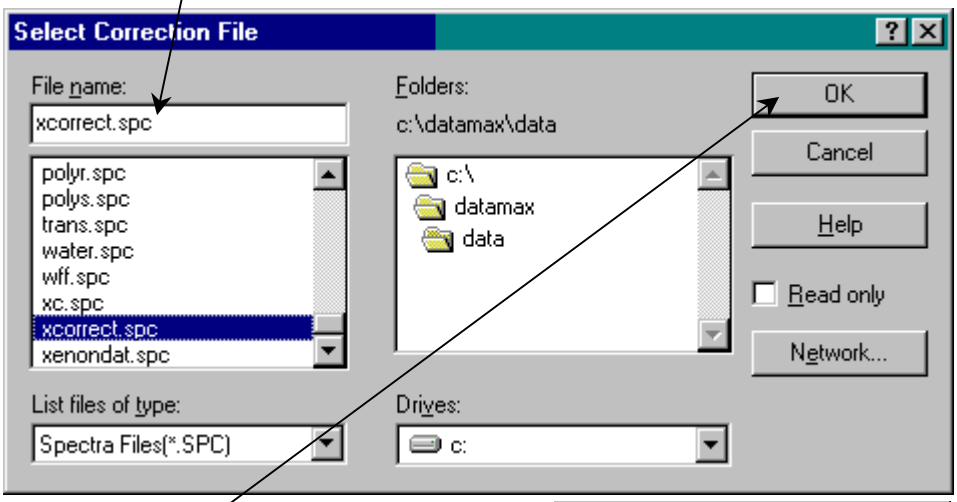
3 Select the specific detector's correction file.

For the signal detector's correction file:

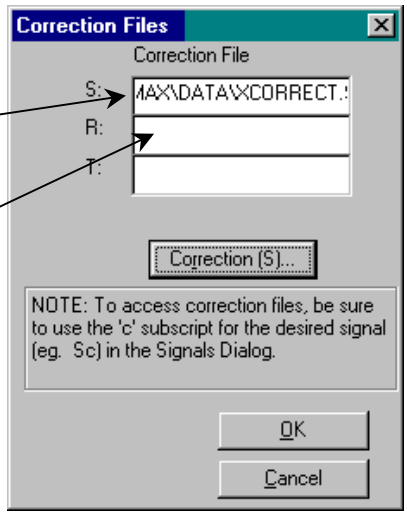
a Click on the *S:* text box.
The name of the button changes to *Correction (S)….*

b Click the *Correction (S)…* button.
The ***Select Correction File*** dialog box appears.

c Choose the appropriate correction file.



d Click *OK*.
The ***Select Correction File*** dialog box closes. The chosen correction file appears in the *S:* text field:



For the reference detector's correction file:

a Click on the *R:* text box.
The name of the button changes to *Correction (R)….*

b Click the *Correction (R)…* button.
The ***Select Correction File*** dialog box appears.

c Choose the appropriate correction file.

d Click *OK*.
The ***Select Correction File*** dialog box closes. The chosen correction file appears in the *R:* text field.

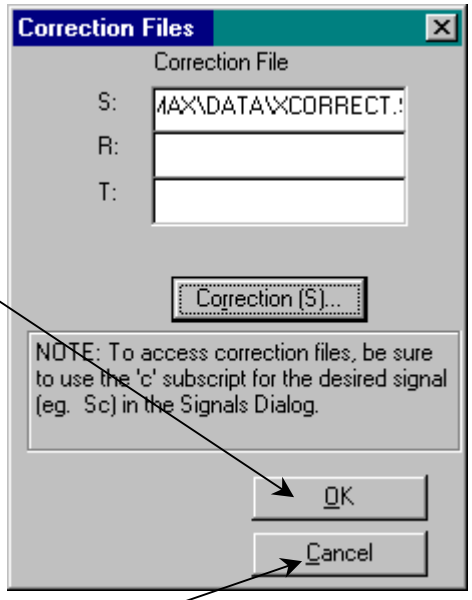
For the optional third detector's correction file (if the system has a T-format):

a Click on the *T:* text box.
The name of the button changes to *Correction (T)….*

b Click the *Correction (T)…* button.
The ***Select Correction File*** dialog box appears.

c Choose the appropriate correction file.

d Click *OK*.
The ***Select Correction File*** dialog box closes. The chosen correction file appears in the *T:* text field.



4 Click *OK* in the ***Correction Files*** dialog box.

The dialog box closes.

When the experiment is run, the correction file(s) selected are opened. Each detector's signal scan is corrected using the chosen correction files. The trace on the main screen and saved to disk is the corrected spectrum.

To abort the operation without indicating a correction file,

1 Click *Cancel*.

Dark Offset

Dark Offset compensates for the inherent background signal of a photomultiplier tube. When *Dark Offset* is enabled, DataMax instructs the spectrofluorometer to close the shutter for 10 s. During this time, the background noise is collected. The resulting value, the dark counts, is subtracted automatically from the collected data.

Note: *In an experiment using a blank or correction factor, do not enable* Dark Offset*. Checking* Dark Offset *will cause the software to subtract dark counts twice from the same spectrum.*

If *Dark Offset* is not checked, the background is not subtracted automatically.

Delay After Flash

Delay After Flash (only available with phosphorimeter scans) sets the time, in ms, between the start of the lamp flash and the beginning of data acquisition, when the *Sample Window* opens. This delay can range from 0.01 ms–10 000 ms, in increments of 0.001 ms. The delay from one flash is accurate to within ± 0.001 ms.

To delay data acquisition,

1. Click on the data field.
2. Enter the length of time after the flash, before the *Sample Window* opens.

Note: Delay After Flash *should be long enough to allow fluorescence emission and lamp decay to occur.*

Delay Incr

Delay Incr (only available with phosphorimeter scans) specifies the time, in ms, to add to the *Delay After Flash* for each subsequent measurement.

To adjust the delay increment,

1 Click in the data field.

2 Enter the amount of time by which to delay the increment.

Emission

Emission represents the wavelength, in nm, at which the emission monochromator is set. Often the wavelength for which the sample fluoresces with maximum intensity is used.

To set the emission monochromator's position,

1. Click on the data field.
2. Enter the desired position of the emission monochromator.

Excitation

Excitation represents the wavelength, in nm, at which the excitation monochromator is set.

To set the excitation monochromator's position,

1 Click on the data field.

2 Enter the desired position of the emission monochromator.

Exp Type

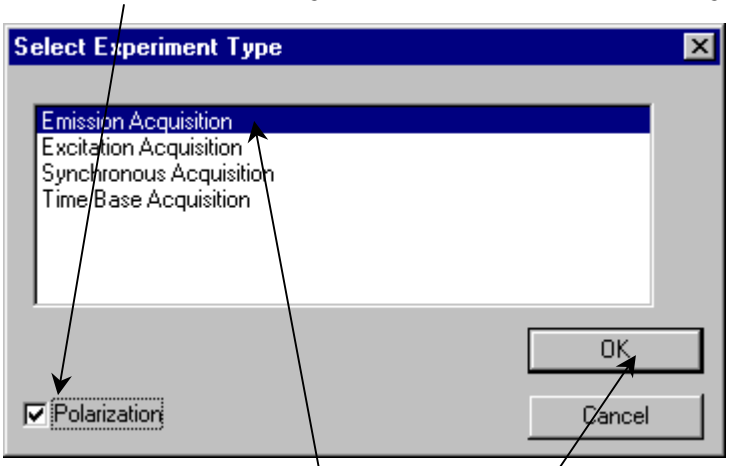
Exp Type... Use *Exp Type...* to choose the type of experimental scan to run, along with accessories for that particular type of experiment.

To change the type of experiment,

1 Click *Exp Type...*.

The ***Select Experiment Type*** dialog box appears.

2 Check any desired accessory.



Note: *The scan types depend on the accessories, so choose the accessory **first**, and then the scan type.*

3 Choose the acquisition type.

a Double-click the acquisition type.

Or

a Click the acquisition type.

b Click *OK*.

The ***Select Experiment Type*** dialog box closes. The ***Experiment Acquisition*** dialog box changes into the scan type chosen.

Note: *When a Tau lifetime system is initialized, the* **Lifetime Resolved Acquisition** *dialog box opens, without an* Exp Type... *button. Other lifetime scan types are available from the "Tau" (lifetime) button in* the **Instrument Control Center**. *To use steady-state scans with a Tau system, load a non-lifetime layout.*

Frequency

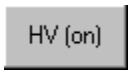
Frequency is a parameter only available with Tau lifetime instruments in lifetime mode. *Frequency* sets the frequency to be monitored in a lifetime-resolved acquisition. The frequency must be from 0.1–330 MHz.

To change the frequency to be monitored,

1 Click on the data field.

2 Enter the desired flash rate.

HV (high voltage)

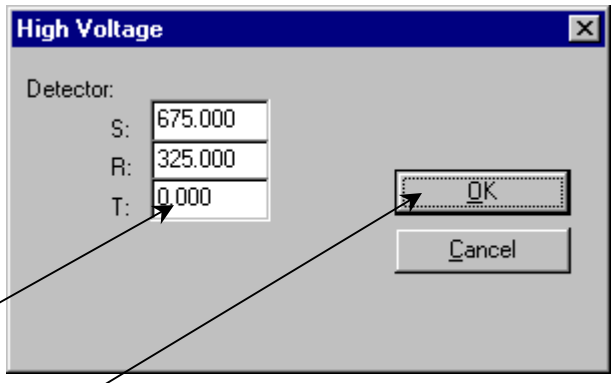


The *HV* button sets high voltage for the detector(s). The face of the button shows the current condition of detector's voltage (*on* or *off*).

To change voltage to a detector,

1 Click HV.

The ***High Voltage*** dialog box opens. The available detectors depend on the instrument and layout. Here a T-format is shown, with S channel, R channel, and T (third) channel. Units are volts.



2 Click in the data field next to the desired detector.

3 Replace the current voltage with a new voltage.

4 Click OK.

The ***High Voltage*** dialog box closes.

Increment

The distance between data points in a scan is the *Increment*. The lower limit of the increment is constrained by hardware, and the upper limit is the difference between the starting and ending points in the scan. The smaller the increment, the higher the resolution is.

To change the increment,

1 Click on the data field.

2 Enter the desired increment.

Initial Delay

Initial Delay sets the time, in ms, that the sample is exposed to light before data-collection starts.

To change the initial delay,

1 Click on the data field.

2 Enter the desired delay.

Integration Time

The *Integration Time* sets the length of time that data are collected for each data point. The minimum integration time is 1 ms; the maximum is as large as is necessary to collect data.

To change the integration time,

1 Click on the data field.

2 Enter the integration time.

Max Delay

Max Delay appears only on the ***Phosphorimeter Decay by Delay*** acquisition screen. *Max Delay* sets the greatest amount of time before another flash begins.

To change maximum delay time,

1 Click on the data field.

2 Enter the maximum delay time.

Max Window

Max Window appears only on the ***Phosphorimeter Decay by Window*** acquisition screen. The time unit is ms. Roughly, *Max Window* sets the greatest number of flashes allowed to capture decay.

To change the maximum window,

1 Click on the data field.

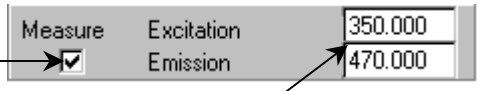
2 Enter the maximum window, in ms.

Measure

To run a lifetime-resolved acquisition, a reference (or standard) phase and modulation are required. The system can measure a reference phase and modulation, or the phase and modulation can be entered manually.

For a reference whose phase and modulation are unknown,

1 Check the *Measure* checkbox.



The data fields for the excitation and emission monochromators appear:

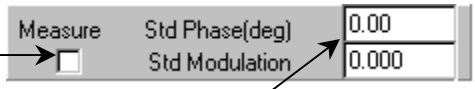
2 Click within each data-entry field.

3 Enter the desired position for each monochromator.

When the scan is executed, DataMax moves the monochromators to the indicated positions, and measures the phase and modulation for the standard. These values will be used in the lifetime calculations.

For a reference whose phase and modulation are known,

1 Clear the *Measure* checkbox.



The data fields for the standard's phase and modulation appear:

2 Click within each data-entry field.

3 Enter the desired phase and modulation for the standard.

When the scan is executed, these values will be used in the lifetime calculations.

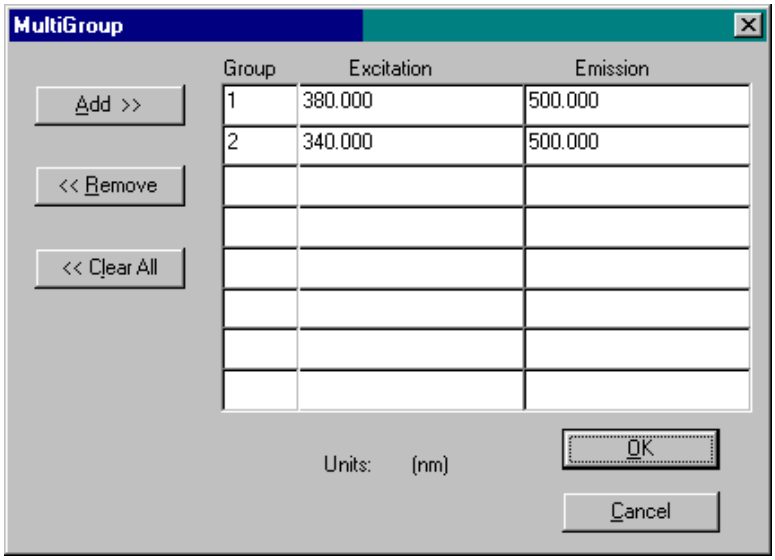
Multigroup

Multigroup... To excite a sample sequentially with different wavelengths, and have the data plotted in a single view, use *Multigroup*…. The data are plotted as intensity versus time. Uses for multigroup scans include energy-transfer and dual-wavelength studies with fluorescent probes for cellular ion-transport (Ca^{2+}, Mg^{2+}, K^{+}, and H^{+}). *Multigroup*... is only available in the ***Multigroup Acquisition*** dialog box.

To specify wavelengths and numbers in a multigroup scan,

1 Click *Multigroup*….

The ***MultiGroup*** dialog box appears:
Default values are provided automatically. A group consists of an excitation wavelength and an emission wavelength. Complete groups must be specified. The maximum number of groups is 8.



To specify a group,

a Click on an empty group.

b Click *Add»*.
A number identifying the group appears.

c Click on the cell in the *Excitation* column.

d Enter the desired excitation wavelength.

e Click on the cell in the *Emission* column.

f Enter the desired emission wavelength.

g Repeat steps a through f to enter more groups.

To remove an existing group,

a Place the cursor on an occupied field in the group.

b Click *«Remove*.

All groups below the removed group move up one row.

To remove all groups in the ***MultiGroup*** window,

a Click *«Clear All*.

To exit the dialog box without changing anything,

a Click *Cancel*.

2 Click *OK*.

The ***MultiGroup*** dialog box closes.

Num Flashes

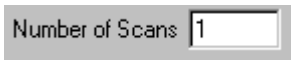
Num Flashes sets the number of lamp flashes used per data point. The signal is collected during the sample time, and integrated over the number of lamp pulses, before the data are analyzed by DataMax. *Num Flashes* can range from 1–999 flashes. This field is only available for phosphorimeter scans.

To specify the number of flashes per data point,

1 Click on the data field.

2 Enter the number of flashes.

Number of Scans



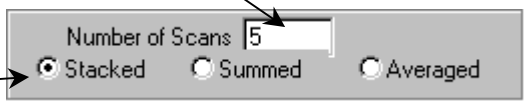
Number of Scans determines the number of times that the appropriate monochromator scans the defined wavelength range. In an emission scan, the emission monochromator scans during the experiment, while in a synchronous scan, both emission and excitation monochromators scan during the experiment. The default value for *Number of Scans* is 1.

To change the number of scans,

1 Click on the data field.

2 Enter the number of scans.

If a number > 1 is entered, hidden fields appear:



3 Choose the appropriate radio button for multiple-scan mode options:

Stacked Displays separate traces when *3D View* is used.
Summed Displays the graphical sum of all traces.
Averaged Averages the traces and displays the result.

Only one option is available at one time. The option is used until another option is chosen.

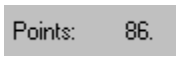
Offset from Excitation

Offset from Excitation sets the number of scan units by which the emission monochromator is separated (offset) from the excitation monochromator. This field is available only in a synchronous acquisition.

To adjust the monochromator offset,

1. Click on the data field.
2. Enter the offset.

Points



Points shows the number of data points that DataMax obtains during the experiment. *Points* cannot be changed directly; it is calculated automatically. Once the appropriate data fields in an experiment acquisition are completed, DataMax calculates and displays the number of data points to be obtained.

Run

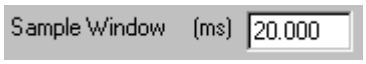
Run confirms that all experiment settings are correct, and the experiment is to be started. Before *Run* can be activated, a data file name must be entered. If *Auto Save Exp* is enabled, an experiment file name must be entered.

To execute an experiment,

1 Click *Run*.

The experiment starts, and the spectrum is displayed as it is acquired.

Sample Window



Available only for phosphorimeter scans, the *Sample Window* sets the duration of signal acquisition. The sample window has a range of 0.01–10 000 ms. The window opens when *Delay After Flash* ends. While open, the signal enters the control module, is counted, and integrated. The sample window closes, the integrated signal is passed to the software, and incoming signal is ignored.

To set the length of time for an open sample window,

1 Click on the data field.

2 Enter the time for an open window.

Save

To store experiment-related information on the default drive, use *Save*. When *Save* is executed, the information is saved to a user-named file. The experiment and data can be recalled later.

To save experiment parameters,

1 Enter a valid experiment file name in the *Experiment…* text box.

2 Click *Save*.

The view does not change. If a file with the same name already exists, a warning appears. Either abort the procedure or overwrite the existing file with new parameters.

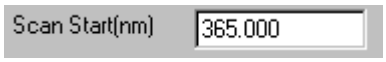
Scan End

In general, *Scan End* sets the wavelength at which the active monochromator stops scanning. For synchronous scans, however, *Scan End* sets the end point for the excitation monochromator. The emission monochromator's end-point in a synchronous scan is the *Scan End* plus the *Offset from Excitation*.

To set an end point for scanning,

1. Click on the data field.
2. Enter the end-point for scanning.

Scan Start



In general, *Scan Start* sets the wavelength at which the active monochromator stops scanning. For synchronous scans, however, *Scan Start* sets the start point for the excitation monochromator. The emission monochromator's start-point in a synchronous scan is the *Scan Start* plus the *Offset from Excitation*.

To set a starting point for scanning,

1 Click on the data field.

2 Enter the starting point for scanning.

Signals

To specify the appropriate kind of signal information to gather and store, use the *Signals…* button.

Detector signals

Spex® spectrofluorometers have two kinds of detectors: a signal detector and a reference detector. The fluorescence from the sample is detected by the signal detector, denoted S. To monitor fluctuations in the light source and compensate for variations in spectrometer response, a reference detector, denoted R, is used. For systems with more than one emission monochromator, a third detector on the extra monochromator is denoted T. For systems with additional user-defined accessories, a detector denoted A may be included.

Other signals

Use algebraic functions (addition, subtraction, multiplication, division, exponentiation, and trigonometric) to create new kinds of signals. With optional polarizers, more signal types (vertical, horizontal, magic-angle, etc.) are available. Corrected signals are denoted c after the signal symbol.

Storing and displaying signals

Depending on the acquisition mode, data files may be stored and displayed as separate files. For instance, define an experiment and name the associated file `quinine` to retrieve its data later. Specify the signals as S and R, and DataMax stores these signal traces as `quininea` and `quinineb`, where `a` indicates S signal, and `b` indicates R signal. With more signal types, a letter (in alphabetical order) is appended to the data file's name.

The display shows all traces separately. Each is identified by its assigned data file name plus the appended letter (in alphabetical order). The more signal types requested, the narrow the spectrum appears on the screen.

To change signal types,

1 Click *Signals….*

The ***Signals*** dialog box appears.

There are four important areas:

- Data Channel
- Function
- Selected Signal
- Units

Data Channel

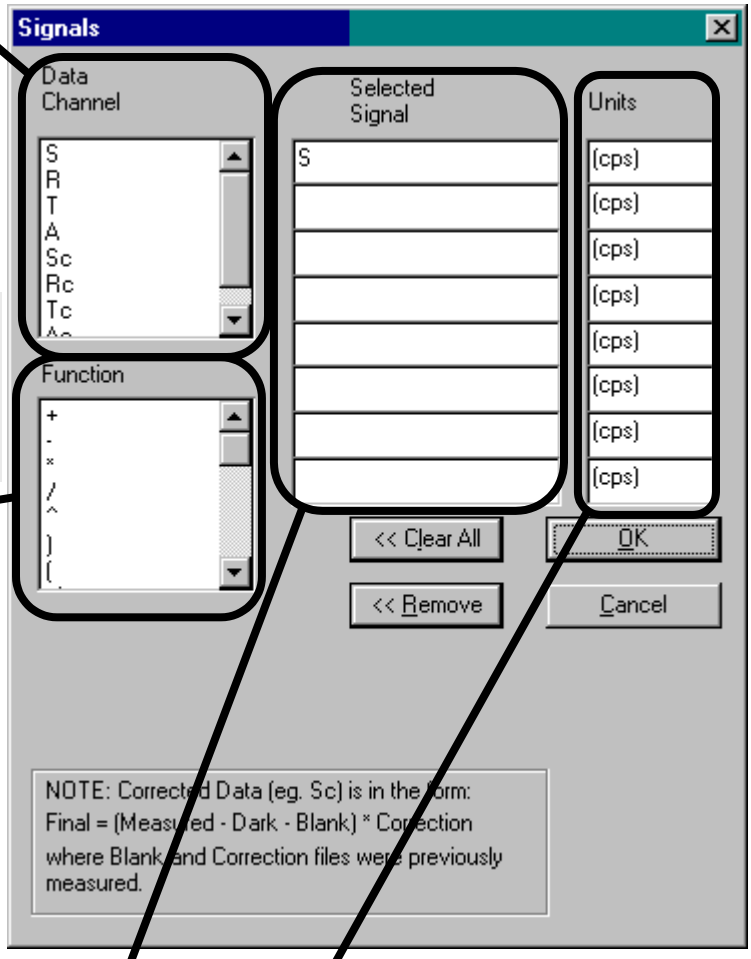
S, R, T, and A without a subscript represent raw signals. These have not been corrected. Any letter combination followed by a "c" is a corrected signal. A corrected signal needs a specified blank file and a correction file, and the *Dark Offset* should be disabled. Mathematically,

Corrected signal = (Sample scan – Blank) × (Correction-factor file)

where the blank has been dark-subtracted.

Scroll down the list to view all available signals.

Note: *The signal types listed depend on the system and accessories.*



Function

The *Function* area lists mathematical operators to create one or more complex signal types. Scroll down the list to see all the available mathematical operators.

Selected Signal

This area shows all the acquisition modes to be recorded and displayed. Up to 8 signal types may be recorded simultaneously during a scan.

Units

For each selected signal, there is an associated unit. The default unit is "cps" (counts per second).

2 Specify a signal.

- For a simple signal type,

a Place the cursor within a *Selected Signal* data field.

b Double-click a signal type displayed in the *Data Channel* list.

The signal appears in the field where the cursor is.

- To specify a complex signal type,

a Place the cursor within a *Selected Signal* data field.

b Double-click a signal type displayed in the *Data Channel* list.

c Double-click a function or operator within the *Function* list.

d Enter a numerical constant as needed in the *Selected Signal* data field.

e Continue adding signals, functions, or constants until complete.

3 To remove all selected signals, click *«Clear All*.

4 To remove a single signal, place the cursor on the unwanted signal, and click *«Remove*.

Any signals below the removed signal in the list automatically move upward.

5 Click *OK* when finished.

The ***Signals*** dialog box closes.

Slits

Slits… sets the width of all slits in the optical path. Slit-width affects the amount of light reaching the detectors. Bandpass (wavelength range) is directly proportional to slit width. Bandpass also affects the spectral resolution. Too broad a bandpass may not resolve narrow peaks. Therefore, by adjusting slit widths, the intensity and bandpass of the light may be controlled.

The slits of the excitation spectrometer determine the amount of light that passes through the excitation spectrometer and reaches the sample. The slits of the emission spectrometer control the amount of fluorescence that the signal detector sees. Mathematically,

Bandpass = Slit width × Dispersion

where slit width is measured in mm and dispersion is measured in nm/mm. The dispersion can be found in the hardware operation manual.

To adjust the slits using slit width,

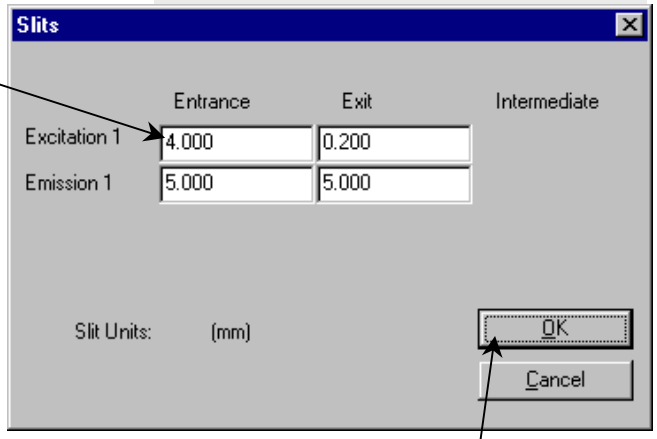
1 Click *Slits…*.

The ***Slits*** dialog box appears. The units for the slit width are in the lower left corner. Either mm or µm are possible units.

Note: *The dialog box's appearance depends on the system's configuration.*

2 Click on the data field for the entrance or exit slit for the desired monochromator.

3 Enter the slit width.



4 Complete steps 2 and 3 for all slits.

5 Click *OK* to confirm the new slit widths.

To adjust the slits using bandpass,

1 Click *Slits*….

The ***Slits*** dialog box appears. The units for the slit width are in the lower left corner.

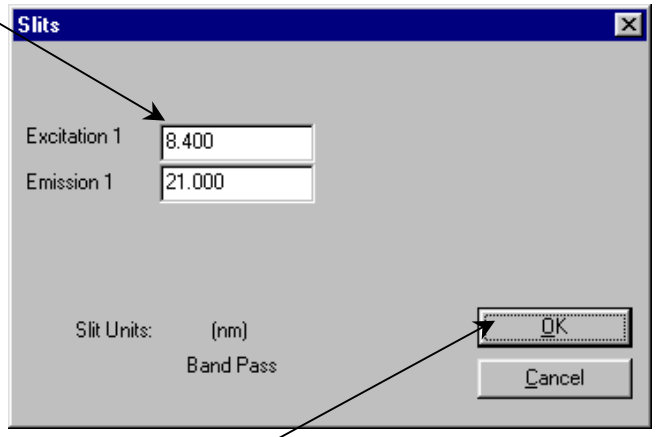
Note: *Each data-entry field adjusts the slit-width for all slits in the chosen monochromator.*

2 Click on the data field for the slits for the desired monochromator.

3 Enter the slits' width.

4 Complete steps 2 and 3 for all slits.

5 Click *OK* to confirm the new slit widths.



Note: *To change slit units, use the* **Visual Instrument Setup**.

Standard

Standard specifies the position of the reference in the sample changer. This field is only available with Tau lifetime layouts, in the lifetime-resolved acquisition. For DataMax to scan the correct standard and unknown at the proper time, the software needs to know their positions in the sample changer.

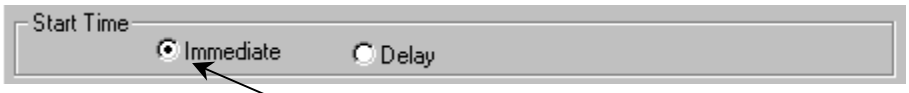
To specify the position of the standard in the sample changer,

1 Click the down arrow next to *Standard*.

2 Select the correct position for the standard.

As the scan is executed, DataMax rotates the turret at the correct time to place the standard into the optical path.

Start Time



The *Start Time* sets the exact time that an experiment begins. Using *Start Time*, experiments may be run any time of the day. Scans requiring minimal intervention can begin after normal working hours; the results are retained until the user is ready.

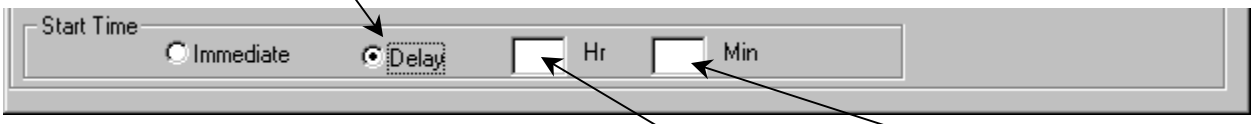
To start an experiment immediately,

1 Click the radio button next to *Immediate*.

To delay an experiment,

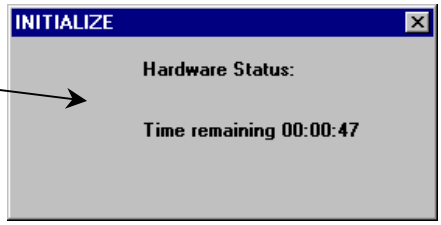
1 Click the radio button next to *Delay*.

A hidden field appears, displaying a timer.



2 Enter the number of hours and minutes to delay the start of the experiment.

Complete both fields. A reminder timer remains on the screen and counts down to the delay time.



Notes:

1 Once the delay time is set,

a The system and host computer must remain on.

b The ***Run Experiment*** application must remain open.

c Do not run any other experiment.

Note: *Violating any of rules a to c immediately aborts the original experiment.*

2 All other DataMax applications are accessible during the countdown.

3 When the delayed experiment starts, all unsaved information in other applications is lost.

Time Incr

Time Incr, only found on time-based acquisition screens, sets the time increment between data points collected. When two excitation monochromators are used, the time increment must be at least 3 times the integration time.

To set the time increment,

1. Click on the data field.
2. Enter the time increment.

Time Per Flash

Time Per Flash sets the amount of time, in ms, for a full cycle of a xenon-lamp flash, including on time, shut-off, and dead time between one pulse of light and the next. The *Time Per Flash* is the reciprocal of the repetition rate. This field is only found in phosphorimeter acquisitions.

To set the time per flash,

1. Click on the data field.
2. Enter the time per full cycle of a flash.

Total Time

Total Time, only for time-based acquisitions, sets the total time during which data are collected.

To set the total time,

1 Click on the data field.

2 Enter the total time.

Unknown

Unknown specifies the position of the unknown in the sample changer. This field is only available with Tau lifetime layouts, in the lifetime-resolved acquisition. For DataMax to scan the correct standard and unknown at the proper time, the software needs to know their positions in the sample changer.

To specify the position of the unknown in the sample changer,

1 Click the down arrow next to *Unknown*.

2 Select the correct position for the unknown.

As the scan is executed, DataMax rotates the turret at the correct time to place the unknown into the optical path.

Window Incr

Window Incr sets the time increment, in ms, for the sample window in a phosphorimeter scan.

To set the window increment,

1 Click on the data field.

2 Enter the window increment.

6: Real Time Display

Introduction

With the ***Real Time Display*** application, hardware parameters and settings can be changed with results immediately visible on the acquisition screen. Once an acceptable trace is obtained, the hardware settings can be transferred to the ***Run Experiment*** application.

Real Time Display works with GRAMS/32® in two modes:

Continuous acquisition	Data points are acquired continuously according to the specified settings.
Prompt step	Data are collected stepwise, i.e., only when an arrow—indicating the direction of travel (if any) of the active monochromator—is clicked.

Real Time Display is started from ***Instrument Control Center***. ***Real Time Display*** is a virtual control panel for the instrument. All commands are executed by adjusting one of the virtual controls displayed.

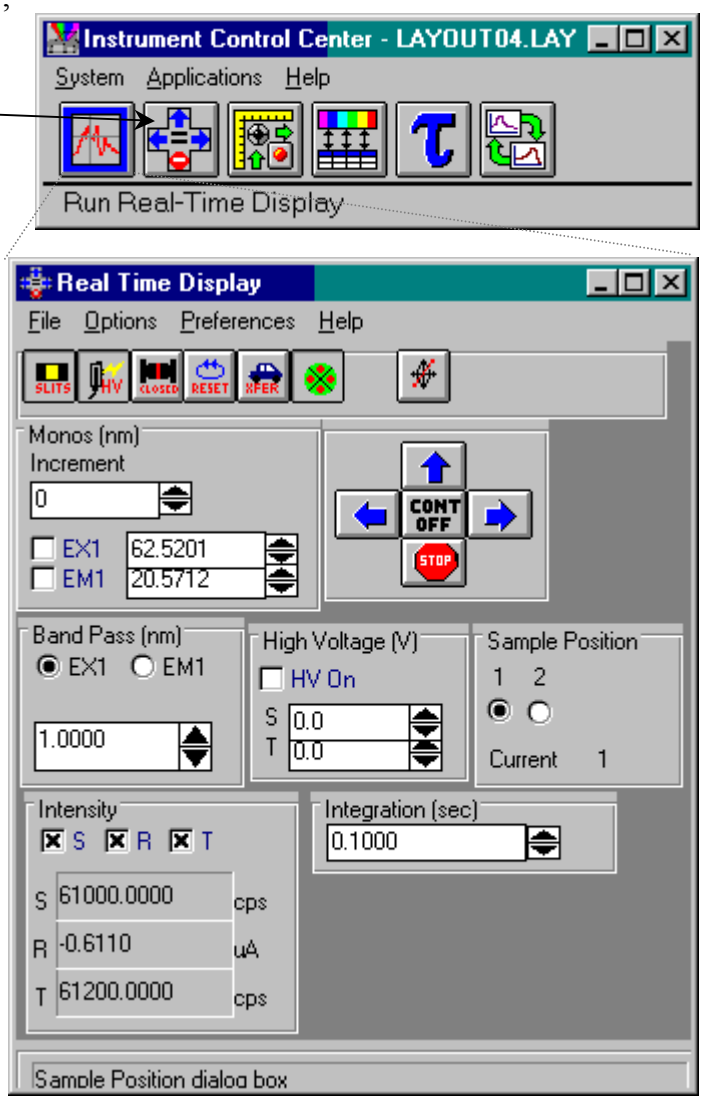
Basic operation

Starting ***Real Time Display***

From ***Instrument Control Center***,

1 Click the *Real Time Display* button.

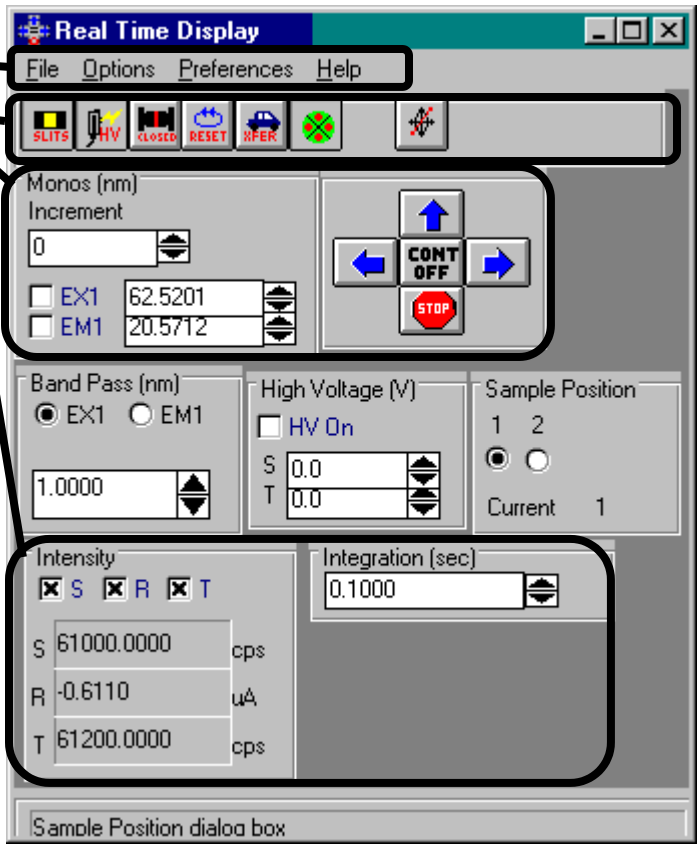
The ***Real Time Display*** opens. The screen is the default view, or the last view used:



Real Time Display control panel

When the ***Real Time Display*** opens, the control panel appears. The control panel contains several items:

- Main menu
- Toolbar
- Stationary control devices

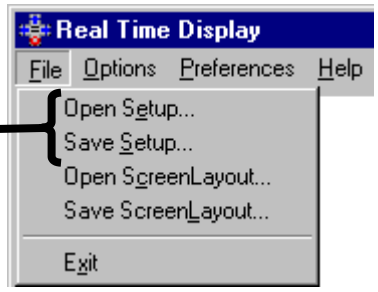


Real Time Display controls hardware settings and parameters through all of the above items. Immediate results can be seen on the acquisition screen. Use this responsiveness to determine the best parameters before running an experiment.

Main menu

File

Under *File* are various choices for setup and layout files, and the means to exit ***Real Time Display***.

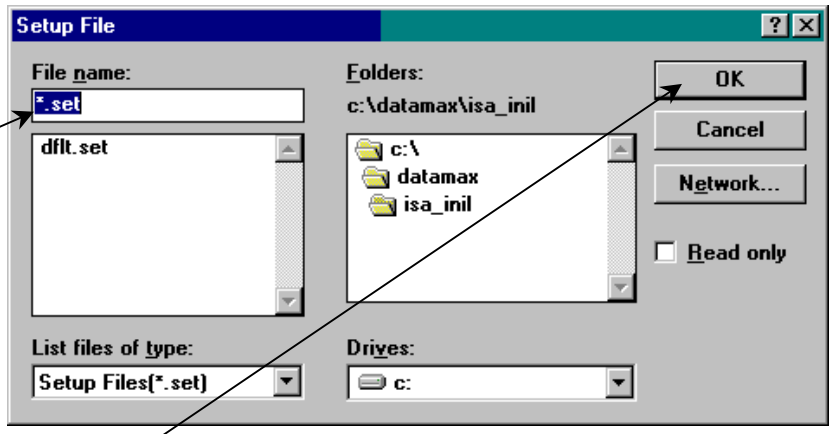


Open Setup… and *Save Setup…* concern system units and hardware settings. Screen layouts (files with extension `.SET`) can be customized with regard to placement of items on the screen. Customized settings and screen layouts can be designed, saved, and recalled. These setup files are the same types of files accessed with the *Setup* button in ***Experiment Acquisition*** dialog boxes. Setup files contain information about units for wavelengths, slits, time, detectors, gratings, and accessories.

To open a previously saved setup,

1 Click *File*.
The drop-down menu appears.

2 Click *Open Setup…*.
The ***Setup File*** dialog box appears.



3 Choose the setup file in the correct folder and drive.

4 Click *OK*.
The ***Setup File*** dialog box closes.

To save a setup,

1 Click *File*.
The drop-down menu appears.

2 Click *Save Setup…*.
The ***Setup File*** dialog box appears.

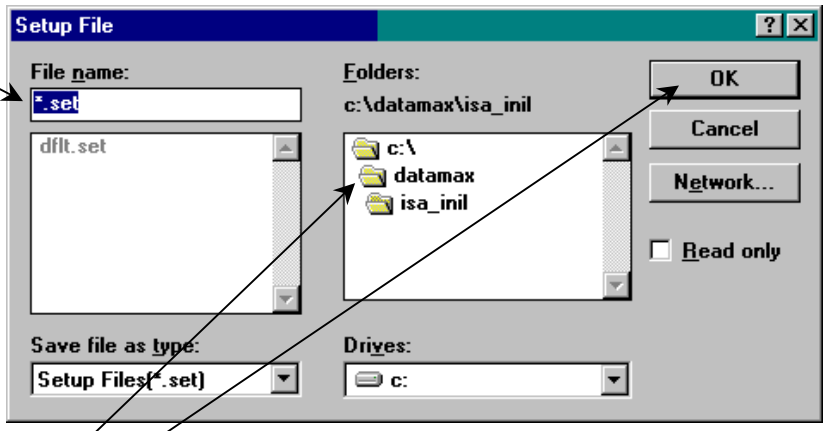
Note: *DataMax issues no overwrite warning. Exercise caution when saving.*

3 Enter the name of new setup file.

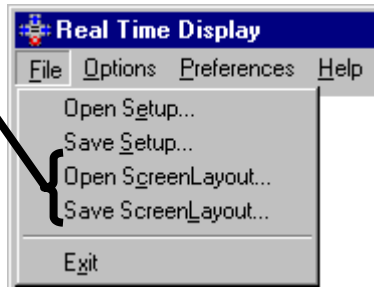
4 Select the correct folder and drive.

5 Click *OK*.

The ***Setup File*** dialog box closes.



Open ScreenLayout… and *Save ScreenLayout…* adjust the ***Real Time Display***'s appearance, using screen layouts. A screen layout uses a file extension `.SLY`. Items on the screen may be moved, rearranged, and hidden from view.



To open a screen layout,

1 Click *File*.

The drop-down menu appears.

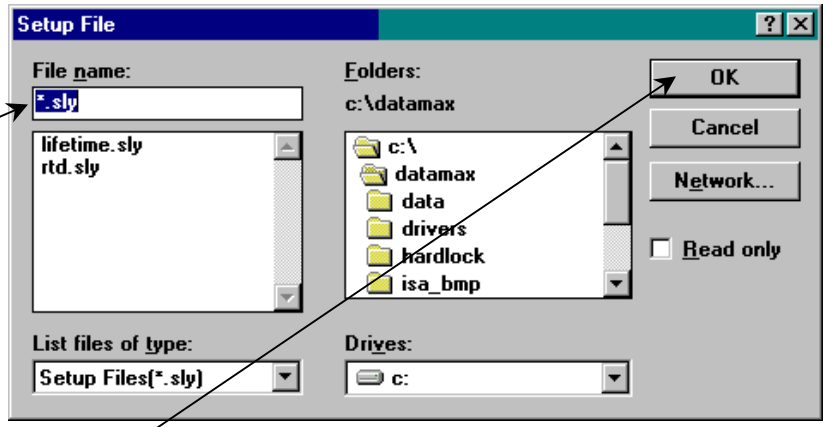
2 Click *Open ScreenLayout…*.

The ***Setup File*** dialog box appears.

3 Choose the screen layout file in the correct folder and drive.

4 Click *OK*.

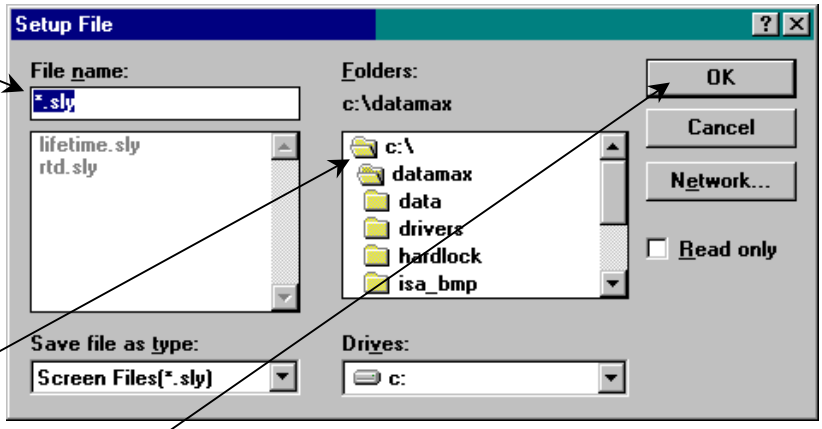
The ***Setup File*** dialog box closes.



To save a screen layout,

1 Click *File*.
The drop-down menu appears.

2 Click *Save ScreenLayout….*
The ***Setup File*** dialog box appears.



3 Enter the name of new setup file.

4 Select the correct folder and drive.

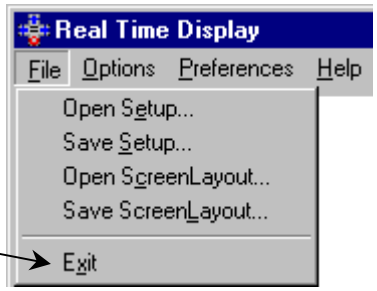
5 Click *OK*.
The ***Setup File*** dialog box closes.

Note: *DataMax issues no overwrite warning. Exercise caution when saving.*

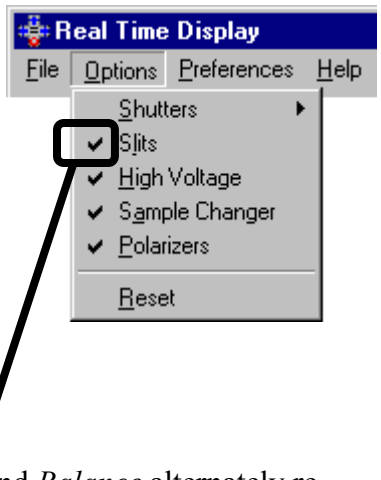
To exit ***Real Time Display***,

1 Click *File*.
The drop-down menu appears.

2 Click *Exit*.
Real Time Display disappears.



Options



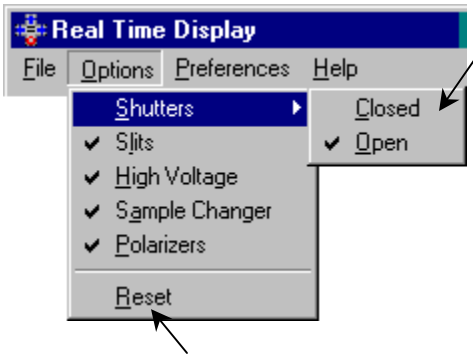
The drop-down menu under *Options* contains the same functions as the toolbar:

- Shutters
- Slits
- High Voltage
- Reset

Optional accessories in the drop-down menu include:

- Polarizers
- Sample Changer
- Balance (for Tau lifetime systems)

Clicking on *Shutters*, *Slits*, *Polarizers*, *Sample Changer*, and *Balance* alternately removes and displays a dialog box—concerning that part of the system—on the ***Real Time Display***. A check (✓, tick) on the drop-down menu indicates that the dialog box is displayed.



Clicking on *Shutters* opens a cascade menu, in which shutters can be opened or closed. The status of the shutters is shown on the *Shutter* button on the toolbar:

Open

Closed

Clicking on *Reset* changes the current values back to those when the ***Real Time Display*** was first opened.

To execute the changes entered into the ***Real Time Display*** dialog boxes,

1 Press **ENTER**.

Or

1 Press the **TAB** key.

Or

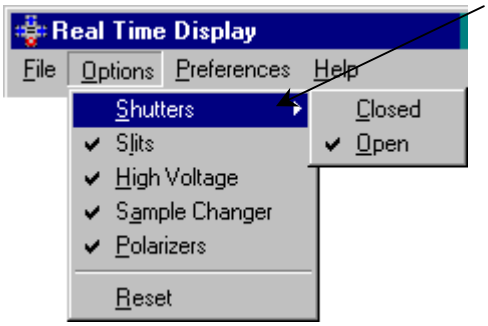
1 Move the mouse cursor to a different field.

2 Left-click the mouse.

The hardware responds to the changes. While the hardware moves, the values in the data-entry fields turn gray and inaccessible. When the change is complete, the field returns to black. If an error occurs, the value turns red, and a dialog box appears. Change the value to an acceptable one.

Note: *Some hardware components move faster than others. Thus, some fields remain gray longer than others, and some change too fast to be noticeable.*

DataMax includes built-in upper and lower limits for hardware. If these limits are violated, an error dialog box appears. Click *OK* to remove the error dialog box, and reset the erroneous value. DataMax ignores the value until it is within hardware limits.



Shutters

The *Shutters* option opens or closes the shutters. This overrides the automatic shutter control.

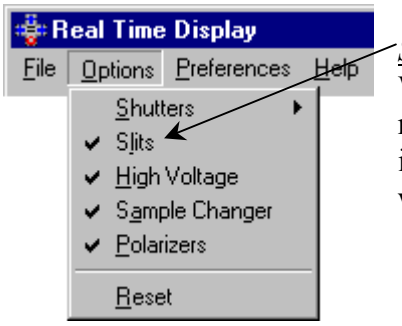
When DataMax starts, the shutters are closed. The shutters open when data acquisition begins; they are close when data acquisition ends. In *Cont On* (continuous) mode, the shutter opens at the beginning, and closes when continuous mode is stopped or paused.

To adjust the shutter,

a Click *Options*.
A drop-down menu appears.

b Click *Shutters*.
A cascade menu appears.

c Choose *Closed* or *Open*.
A check (✓) appears next to the new state.



Slits

With *Slits*, set the slit width or bandpass for each valid monochromator. Depending on the layout and information in ***Visual Instrument Setup***, either a slit-width or bandpass window appears in the control panel.

To adjust slits via slit widths,

a Click *Options*.

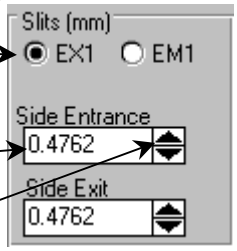
b Click *Slits* (if *Slits* is not already checked).

The ***Slits*** dialog box appears (if *Slits* was not already checked).

Note: *For manual slits, a reminder to change the slits appears.*

c Click on the radio buttons to view the *EX*citation or *EM*ission monochromator.

d Enter the new slit width in the data-entry field.
Or
Use the up and down arrows to set the slit width.

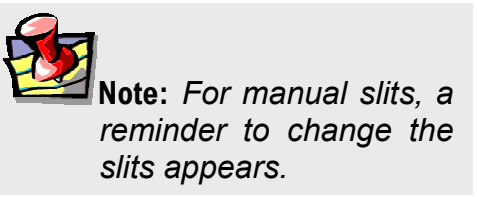


To adjust slits via bandpass,

a Click *Options*.

b Click *Slits* (if *Slits* is not already checked).

The ***Band Pass*** dialog box appears (if *Slits* was not already checked).



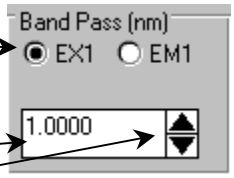
Note: *For manual slits, a reminder to change the slits appears.*

c Click on the radio buttons to view the *EX*citation or *EM*ission monochromator.

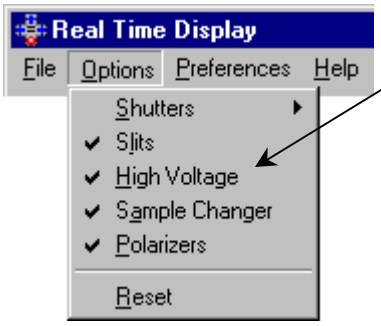
d Enter the new bandpass in the data-entry field.

Or

Use the up and down arrows to set the slit width.



Note: *The value represents a bandpass for all slits in that monochromator.*



High Voltage

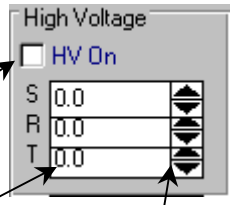
The ***High Voltage*** dialog box sets the high voltage for the detectors. Depending on the system and configuration, the high voltage may not be adjustable, and no ***High Voltage*** dialog box may appear.

To set the detectors' high voltage,

a Click *Options*.

b Click *High Voltage* (if *High Voltage* is not already checked).

The ***High Voltage*** dialog box appears (if *High Voltage* was not already checked).

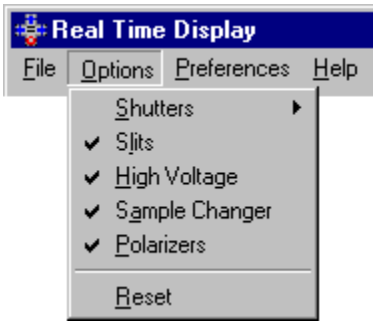


Each available detector (S, R, and optionally T) has its own data-entry field. When the high voltage is disabled to the detectors, the voltage to each detector is shown as 0. If the high voltage is switched on, the last voltage to that detector appears in the field. If a voltage is entered into a field, DataMax does not activate this voltage until the *HV On* box is checked.

c Click the box next to *HV On* to switch on or off voltage to all detectors.

d Adjust a detector's voltage.

- Click inside a field.
- Enter a voltage in the field, or click on the up and down arrows.
- To switch only one detector on, enter 0 in the other detectors' fields.



Sample Changer

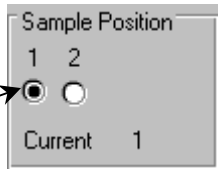
If an optional sample changer is included in the layout, then *Sample Changer* appears as a choice in the drop-down menu. *Sample Changer* sets the current position of the sample changer in the optical path.

To set the current position of the sample changer,

a Click *Options*.

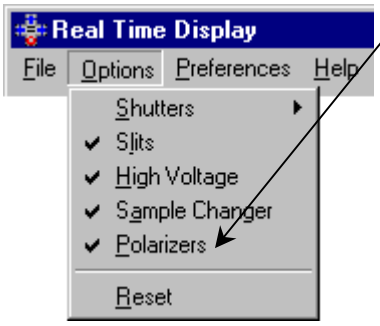
b Click *Sample Changer* (if *Sample Changer* is not already checked).

The ***Sample Position*** dialog box appears in the control panel. The current position in the optical path is labeled at the bottom of the window.



c Click a radio button to choose a new sample position.

Note: *The* **Sample Position** *dialog box varies according to the number of positions that the sample changer accommodates.*



Polarizers

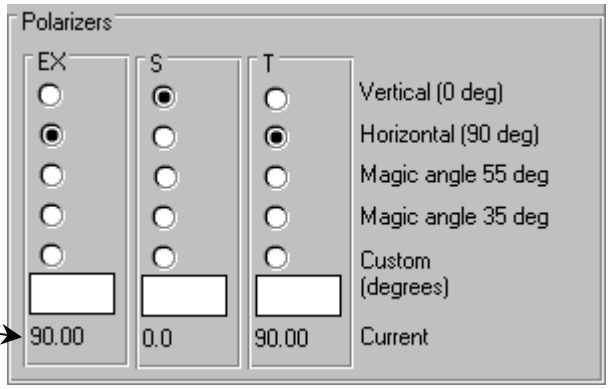
The ***Polarizers*** dialog box, available when polarizers are included in the layout, sets the rotational positions for all polarizers (Excitation, R, and optional T). Any angle is possible, but special settings are shown for vertical, horizontal, and magic angles.

To rotate a polarizer to a new angle,

a Click *Options*.

b Click *Polarizers* (if *Polarizers* is not already checked).

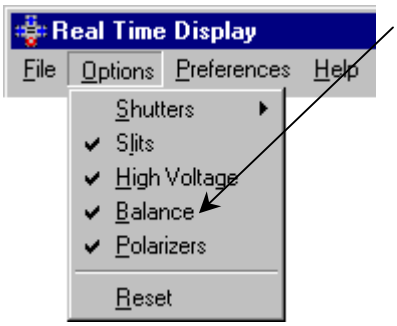
c The ***Polarizers*** dialog box appears. Current settings are shown at the bottom of the window.



d Enter a new angle for a polarizer.

Click a preset angle (vertical, horizontal, magic angle), or enter a custom angle in the field near the bottom.

Note: *See the* Polarizers Operation Manual *for more information about using polarizers.*



Balance

Only available with Tau lifetime layouts, *Balance* sets the standard and unknown positions in a sample changer, set monochromator position(s), and view detector signal in the ***Lifetime*** dialog box.

To balance the standard and unknown samples,

a Click *Options*.

b Click *Balance* (if *Balance* is not already checked).

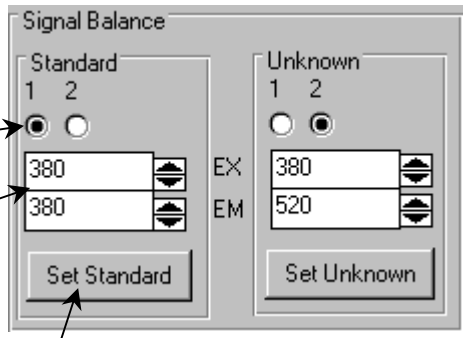
The ***Signal Balance*** dialog box appears on the control panel.

c Position the desired sample in the optical path.

d Click on the radio button next to the appropriate position.

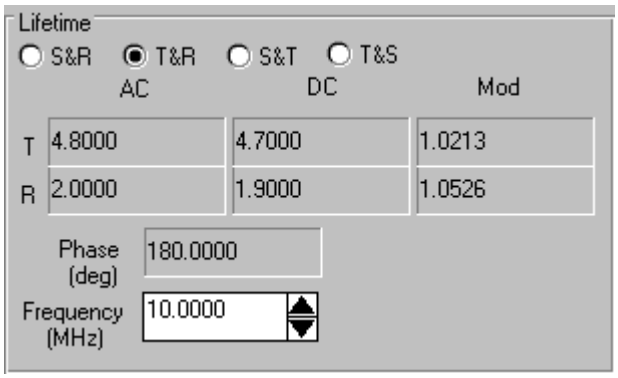
e Set monochromator positions.

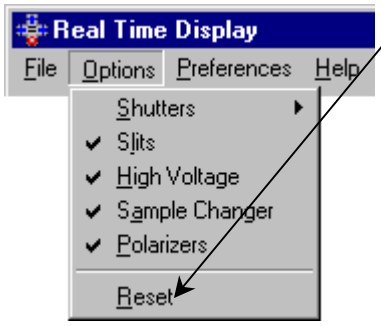
Enter the desired value in the standard or unknown's field for the *EXcitation* or *EMission* monochromator.



f Click *Set Standard* or *Set Unknown* to confirm the change.

g View the effect in the ***Lifetime*** dialog box on the control panel.





Reset

Reset restores the setup information and values for integration time, high voltage, slit width or bandpass, and increment in effect when ***Real Time Display*** was first opened. Use *Reset* to experiment with different settings, and return to the original settings easily.

To reset the ***Real Time Display***,

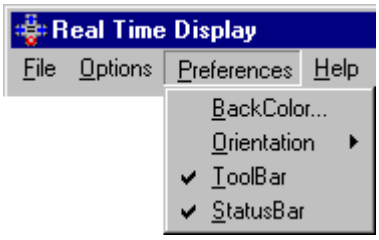
a Click *Options*.

b Click *Reset*.

Preferences

Preferences controls general appearance of the ***Real Time Display***, such as

- Screen colors
- Window orientation
- Position of the toolbar
- Position of the status bar



BackColor…

To change the background color of the control panel,

a Click *Preferences*.

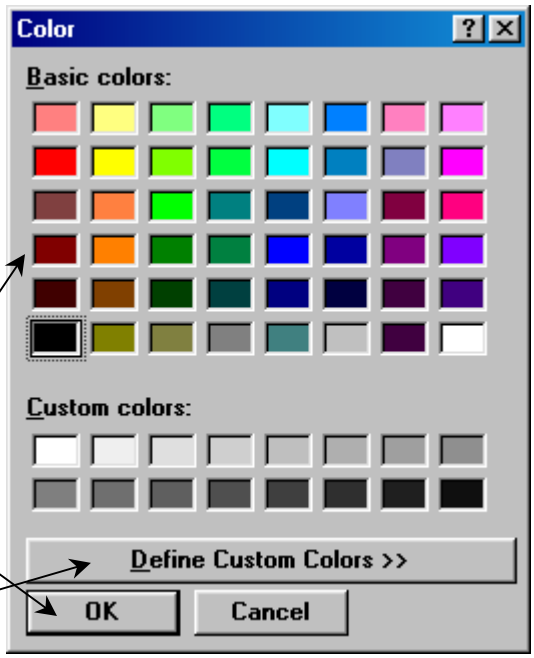
b Click *BackColor…*.

The ***Color*** dialog box opens.

c Choose a color.

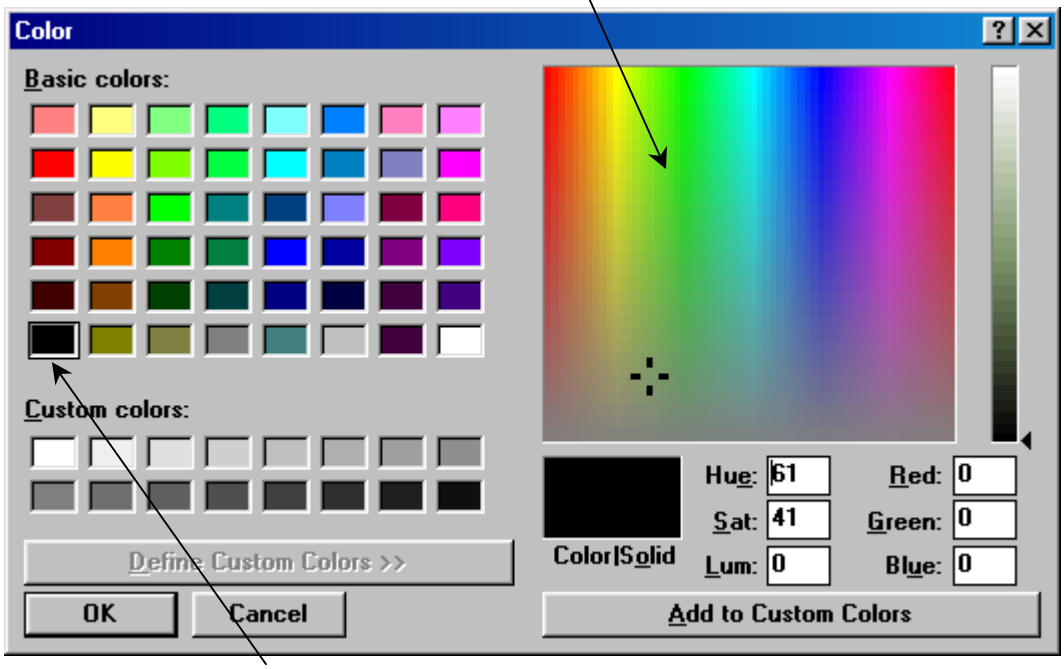
For a basic color,

- Click on the desired color swatch.
- Click *OK* to confirm.



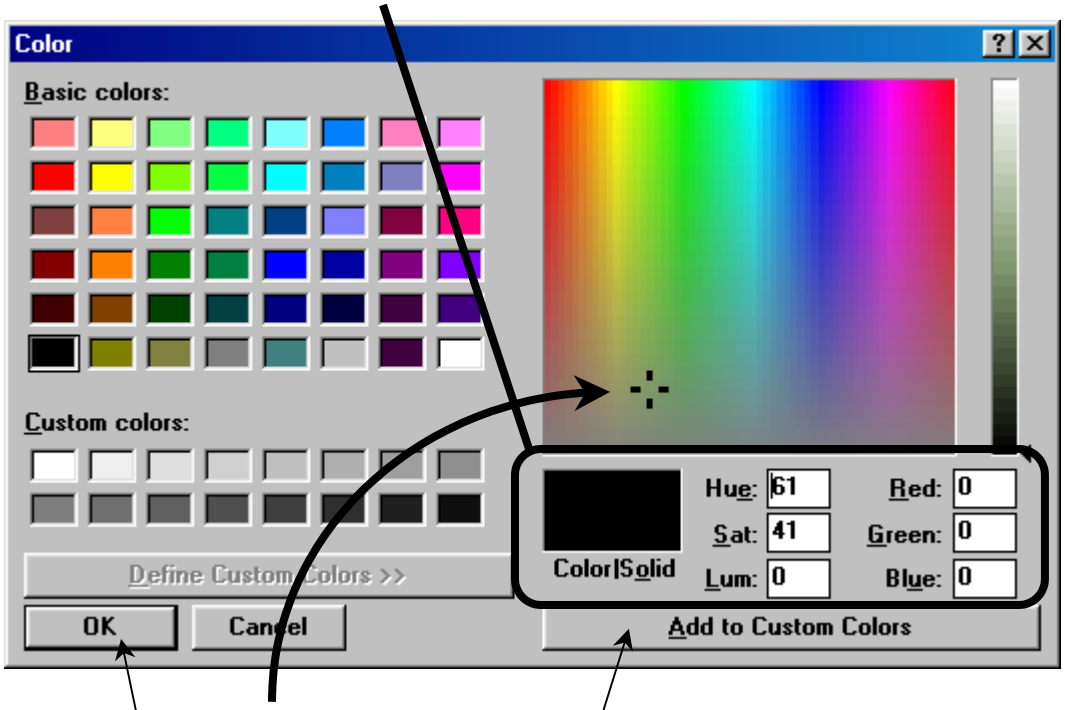
For a custom color,

- Click *Define Custom Colors»*.
- A scrolling color pallet appears:



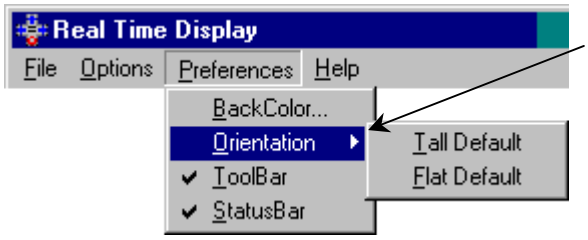
- Click on a basic color closest to that desired.

- The basic color appears in the *Color|Solid* box, and is numerically described by *Hue*, *Sat*(uration), *Lum*(inance), *Red*, *Green*, and *Blue*.



- Place the mouse cursor in the scrolling color pallet.
- Drag the cursor toward the desired color mixture.
- The *Color|Solid* box and numerical attributes of the color all change.
- At the correct color, release the mouse button.
- Click *Add to Custom Colors*.
- The new color appears in the set of custom colors.
- Click *OK*.

d The control panel's background is the desired color.



Orientation
Orientation sets the way that the stationary control devices are displayed on the control panel.

The two choices are:

- *Tall Default* portrait style, with toolbar on top
- *Flat Default* landscape style, with toolbar on left

Orientation provides a convenient rescue point. If a dialog box is missing, choose an orientation; all enabled dialog boxes then appear in a default view.

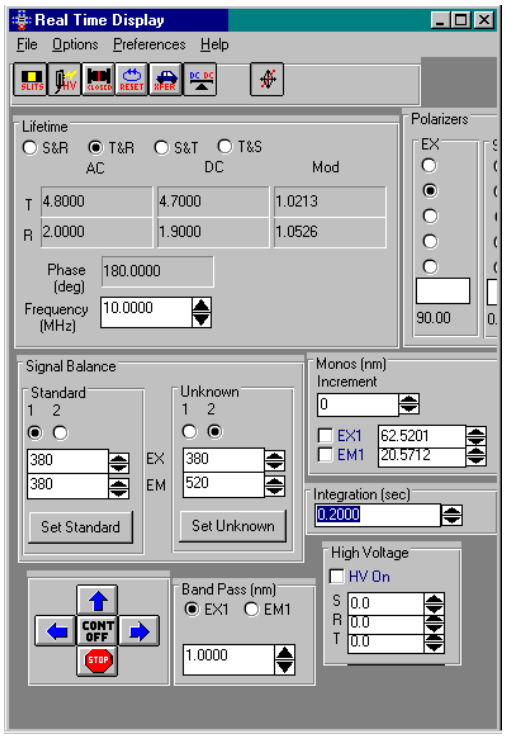
To change orientation,

a Click *Preferences*.

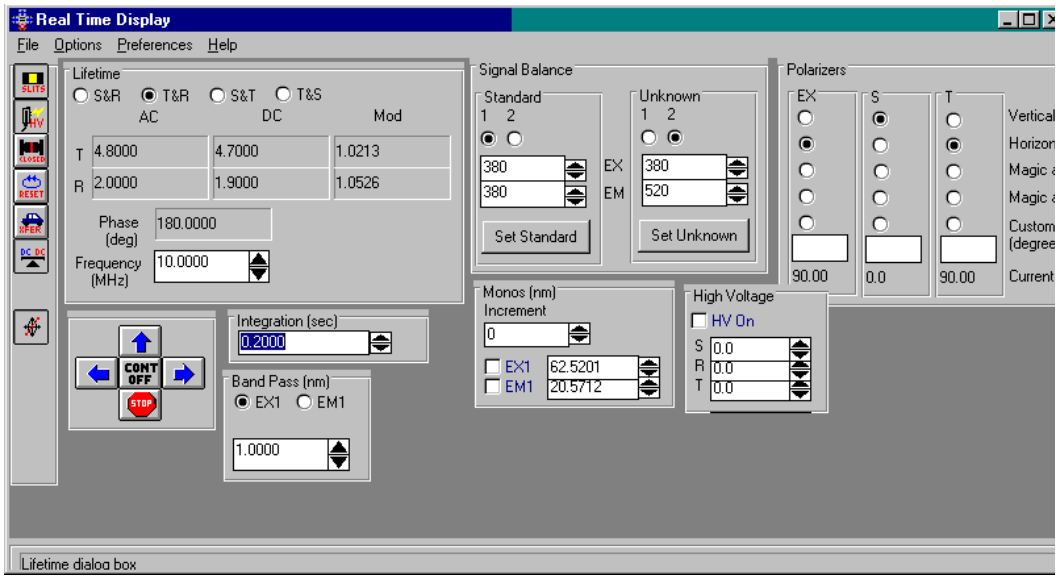
b Click *Orientation*.

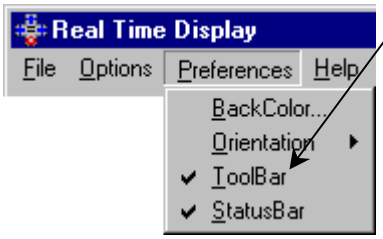
c Click on the desired orientation. The ***Real Time Display*** changes to the desired view.

Tall Default view



Flat Default view

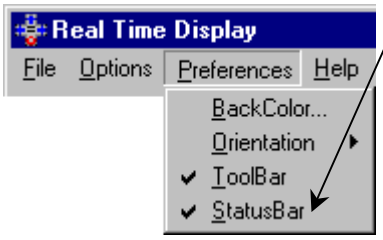




ToolBar

ToolBar displays or hides the toolbar. When the toolbar is displayed, a check (✓) is displayed next to *ToolBar*. The toolbar can be dragged to a new location.

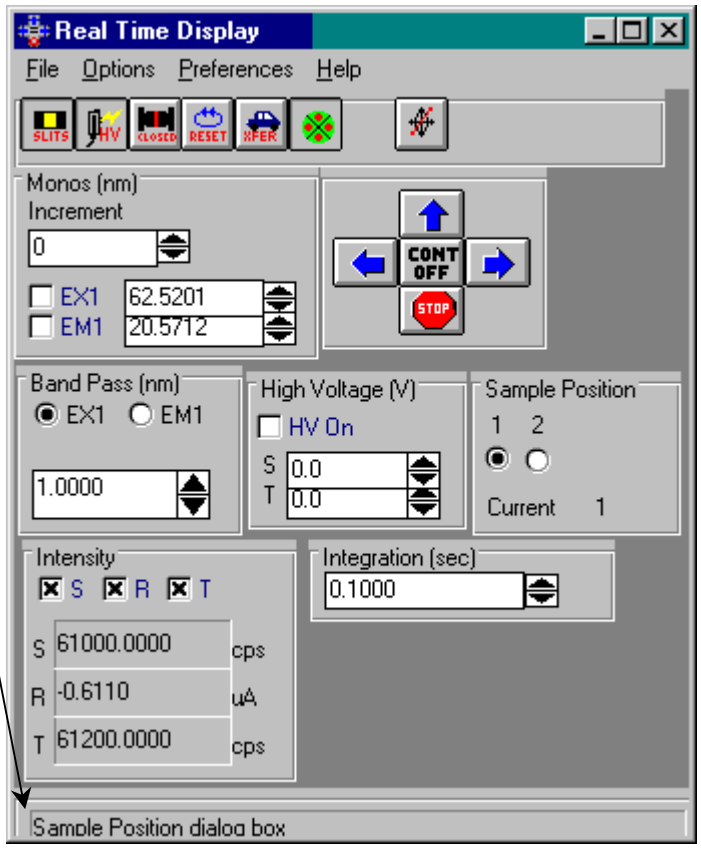
a Click *ToolBar* to display or hide the toolbar.



StatusBar

StatusBar displays or hides the status bar at the bottom of ***Real Time Display***. The status bar contains text concerning the current position of the cursor. As the cursor moves on the screen, the information in the status bar changes. When the status bar is displayed, a check (✓) is displayed next to *StatusBar*.

a Click *StatusBar* to hide or display the status bar.



Help

Help provides assistance and information about ***Real Time Display***.

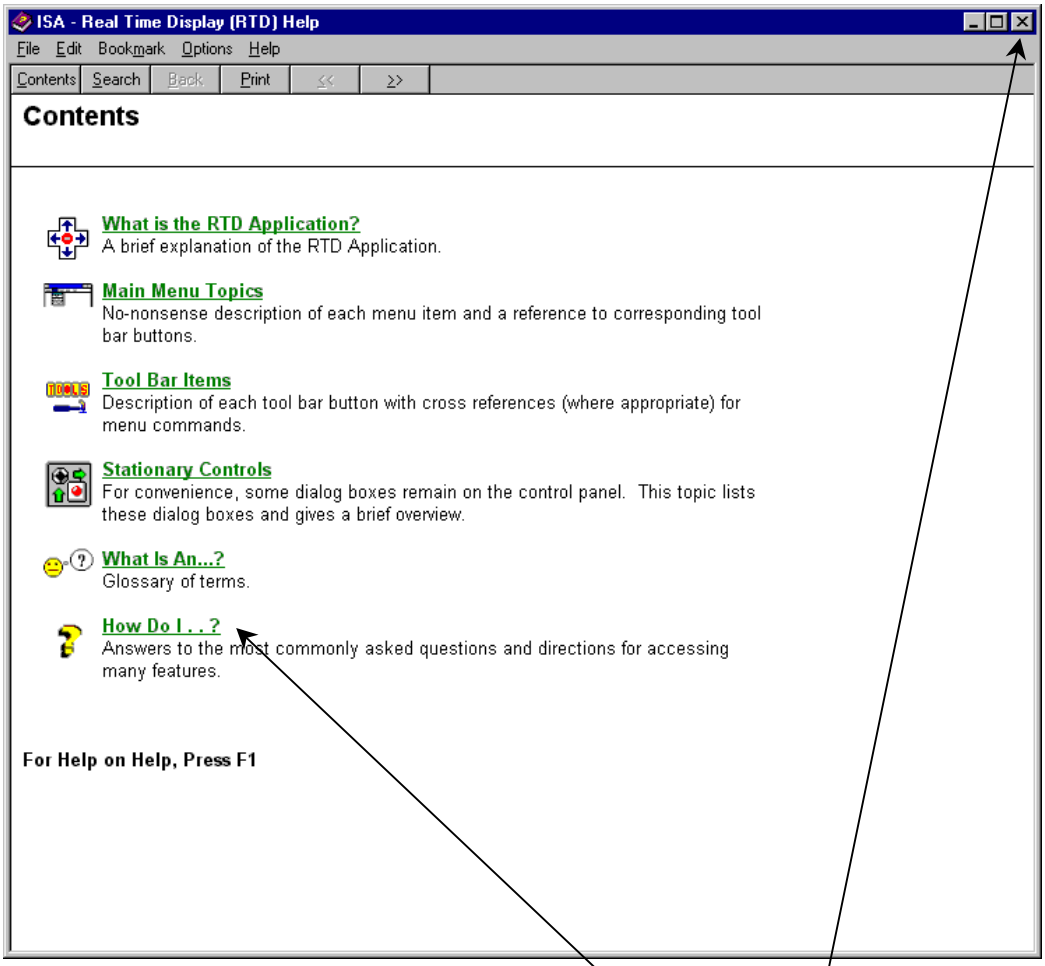
Contents...

For help on ***Real Time Display*** commands, choose *Contents...*.

a Click *Help*.

b Click *Contents...*.

c The ***ISA – Real Time Display (RTD) Help*** dialog box appears.



d Click on the appropriate topic in this *Help* window.

e To close this window, click the ☒ box in the upper right.

About...

For information on the version of ***Real Time Display***, copyright, and free memory, choose *About...*.

a Click *Help*.

b Click *About...*.

The ***About Real Time Display*** dialog box opens.

c Click *OK*.

The ***About Real Time Display*** window disappears.

Note: *When contacting the Service Department, be sure to have the information in* **About Real Time Display** *available.*

Stationary control devices

Certain dialog boxes on the control panel cannot be hidden, though they can be moved. These windows, *stationary control devices*, are the following:

- Monos
- Integration
- Spectrometer Step Control
- Intensity
- Phosphorimeter (optional accessory)

To move a stationary control device,

1 Place the cursor on an area of the device that does not cause action.
For example, to move the toolbar, place the cursor on the very top or left or right edges of the toolbar.

2 Click and hold the mouse button.
When the hold is successful, a hollow highlight appears. As the mouse is moved, the highlight moves to show the proposed spot for the device.

3 Drag the device to the new location.

4 Release the mouse button.

Note: *To avoid the dialog boxes from being obscured by other dialog boxes, the smaller ones usually take priority. This means that, even if a larger box is placed over a smaller one, the smaller one remains on top.*

To change a value in a stationary control device,

1 Press **ENTER**.
Or

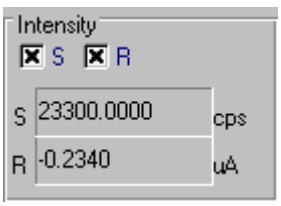
1 Press **TAB**.
Or

1 Move the cursor to a different data-entry field.

2 Left-click the mouse.

Intensity (or Lifetime)

The ***Intensity*** (or ***Lifetime***) dialog box's appearance depends upon whether the layout is set for a steady-state Fluorolog® or Tau lifetime instrument.



Intensity
The ***Intensity*** dialog box reports the intensity of the light recorded by the selected detector (S, R, or optional T or A). The

intensity cannot be directly controlled. Varying other parameters or the sample may change the displayed value.

To view or hide an intensity for a detector,

a Click the option box next to the desired detector.

Lifetime

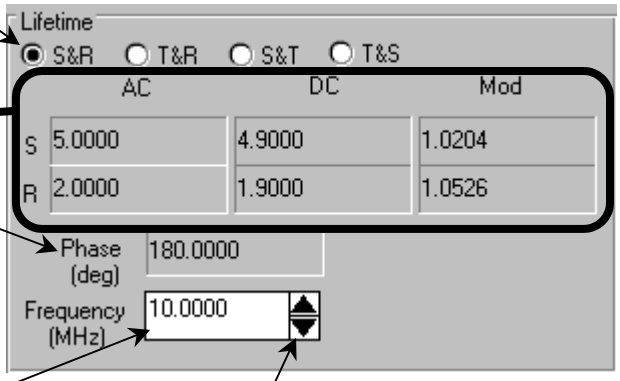
Lifetime is a modified ***Intensity*** dialog box for the Tau lifetime layout. Detector intensities and frequencies are reported.

To change the detector combination,

a Click the radio button next to the desired combination.

View *AC*, *DC* components, and *Mod* (modulation) for desired detectors.

Phase shows the real-time phase-shift.



To set the frequency,

a Click on the *Frequency* text box.

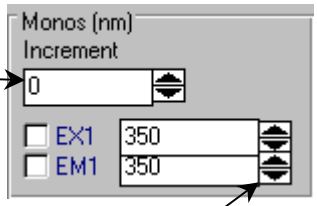
b Enter the desired value.
Or
Click on the up and down arrows to set the frequency.

Monos

Monos sets and controls the spectral position of all monochromators. The appearance of ***Monos*** depends on the system and layout.

Increment

The *Increment* sets the distance that the selected monochromator moves before each data reading. If the increment is less than a monochromator step size, no motion occurs. To set the increment,

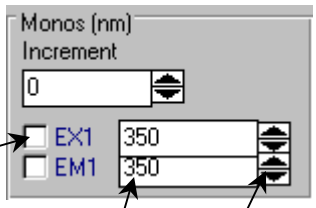


c Click on the text box.

d Enter an increment.
Or
Click the up and down arrows to set a value.

Checkbox (monochromator status)

The checkbox next to a monochromator switches it on and off. When on, the monochromator moves according to the *Increment*, in the direction given by the ***Spectrometer Step Control***. When off, the monochromator is fixed unless physically moved.



a Click in the checkbox for the appropriate *EM*ission or *EX*citation monochromator.

- If an × appears, the monochromator is now on.
- If the × disappears, the monochromator is now off.

Text field (monochromator position)

To set the position of a monochromator,

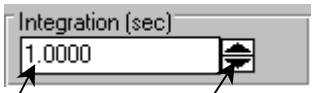
a Click in the data-entry field for that monochromator.

b Enter the new position.
Or
Click on the up and down arrows to change the position.

Note: *If a value is out of range, it flashes red, and a warning message appears. Change the value to within limits.*

Integration

Integration sets the time to collect data at each data point. Units are set in ***Visual Instrument Setup***.



To set integration time,

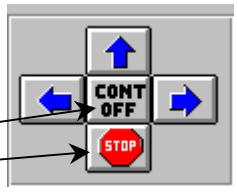
a Click in the data-entry field for that monochromator.

b Enter the new position.
Or
Click on the up and down arrows to change the position.

Spectrometer Step Control

Spectrometer Step Control operates the method of data collection. The five regions in the ***Spectrometer Step Control*** are:

- Left arrow
- Right arrow
- Up arrow
- Cont On/Cont Off
- Stop



Each button has two functions and two positions. The *Cont On/Cont Off* button affects the operation of the other buttons.

Cont Off

When the ***Spectrometer Step Control*** displays *Cont Off*, the system operates in *prompt-step mode*. The arrows, when clicked, cause the monochromator to acquire one set of data in the direction of the activated arrow.

	Left arrow	Takes a reading after moving the active monochromator backward by the specified increment
	Up arrow	Takes a reading at the monochromator's current position
	Right arrow	Takes a reading after moving the active monochromator forward by the specified increment
	Stop	Halts all motion, monitoring, and data collection
	Cont Off	Begins continuous-acquisition mode

Cont On

When the ***Spectrometer Step Control*** displays *Cont On*, the system is in *continuous-acquisition mode*. The up arrow is automatically activated. The monochromators begin to take readings at their current positions. Pressing any other arrow activates that arrow instead. Data are acquired as follows:

	Left arrow	Moves the active monochromator backward by the specified increment before each reading
	Up arrow	Takes readings at the current monochromator position
	Right arrow	Moves the active monochromator forward by the specified increment before each reading
	Stop	Halts continuous-acquisition mode and all motion, monitoring, and data collection
	Cont On	Pauses continuous-acquisition mode

Phosphorimeter

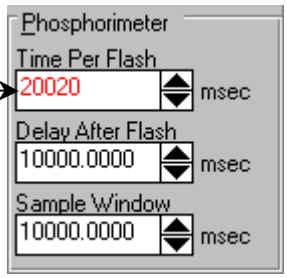
Phosphorimeter sets several parameters for the optional phosphorimeter accessory.

Time Per Flash

The *Time Per Flash* is the length of time, in ms, that the xenon lamp remains on for one flash. To set the time for each flash,

a Click on the text box.

b Enter the time.
Or
Click the up and down arrows to set a value.

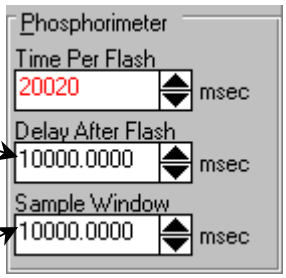


Delay After Flash

The *Delay After Flash* controls how long the system waits, in ms, after a flash before starting to collect data. To determine the waiting time after a flash,

a Click on the text box.

b Enter the time.
Or
Click the up and down arrows to set a value.



Sample Window

The *Sample Window* sets how long the detector collects phosphorescence signal after the *Delay After* Flash ends. The unit is ms. To set the length of the sample window,

a Click on the text box.

b Enter the time.
Or
Click the up and down arrows to set a value.

Toolbar

The toolbar uses buttons to activate system hardware. The buttons on the toolbar are based on the layout in use.

Shutter

Shutter opens and closes the spectrofluorometer's shutters. When DataMax starts, the shutters usually are closed, and the *Shutter* button displays "closed". Whenever data acquisition commences, the shutters automatically open, and the *Shutter* button displays "open". When data acquisition ceases, the shutters automatically close. In continuous-acquisition mode, the shutters open when continuous-acquisition mode starts, and they close when continuous-acquisition mode stops or pauses.

The *Shutter* button overrides the automatic open or closed state of the shutter. The face of the button shows the current state of the shutter.

To open or close the shutter,

a Click the *Shutter* button.

Slits

Slits sets the slit width or bandpass for each valid monochromator. Depending on the layout and information in ***Visual Instrument Setup***, either a slit-width or bandpass window appears in the control panel.

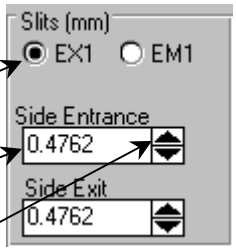
Note: *For manual slits, a reminder to change the slits appears.*

To adjust slits via slit widths,

a Click *Slits* (if the ***Slits*** dialog box is not already visible).
The ***Slits*** dialog box appears.

b Click on the radio buttons to view the *EX*citation or *EM*ission monochromator.

c Enter the new slit width in the data-entry field.
Or
Use the up and down arrows to set the slit width.

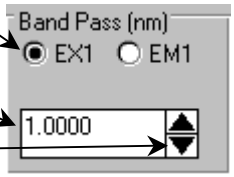


To adjust slits via bandpass,

a Click *Slits* (if the ***Slits*** dialog box is not already visible).
The ***Band Pass*** dialog box appears.

b Click on the radio buttons to view the *EX*citation or *EM*ission monochromator.

c Enter the new bandpass in the data-entry field.
Or
Use the up and down arrows to set the slit width.



Note: *The bandpass is for all slits in that monochromator.*

HV

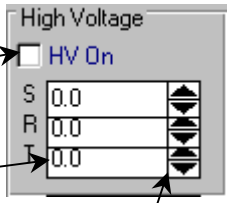
HV sets the high voltage for the detectors. Depending on the system and configuration, the high voltage may not be adjustable, and no ***High Voltage*** dialog box may appear.

To set the detectors' high voltage,

a Click *HV* (if ***High Voltage*** is not already visible).

The ***High Voltage*** dialog box appears.

Each available detector (S, R, and optionally T) has its own data-entry field. When the high voltage is disabled to the detectors, the voltage to each detector is shown as 0. If the high voltage is switched on, the last voltage to that detector appears in the field. If a voltage is entered into a field, DataMax does not activate this voltage until the *HV On* box is checked.



b Click the box next to *HV On* to switch on or off voltage to all detectors.

c Adjust a detector's voltage.

- Click inside a field.
- Enter a voltage in the field, or click on the up and down arrows.

Note: *To switch only one detector on, enter 0 in the other detectors' fields.*

Reset

Reset restores the setup information and values for integration time, high voltage, slit width or bandpass, and increment in effect when ***Real Time Display*** was first opened. Use *Reset* to experiment with different settings, and return to the original settings easily.

*To reset the **Real Time Display**,*

a Click *Options*.

b Click *Reset*.

XFER

XFER transfers information from the ***Real Time Display*** to ***Run Experiment***. This button saves time and reduces errors, because a single click transfers optimized slit widths, high voltages, positions of monochromators, and so on, into an ***Experiment Acquisition*** dialog box.

To transfer settings,

a In ***Run Experiment***, open an ***Experiment Acquisition*** dialog box.

b Choose the desired experiment type.

c In ***Real Time Display***, choose the correct hardware settings.

d Click *XFER*.

All settings are transferred to the ***Experiment Acquisition*** dialog box.

Balance

Only available with Tau lifetime layouts, *Balance* sets the standard and unknown positions in a sample changer, sets monochromator position(s), and views the detector signal in the ***Lifetime*** dialog box.

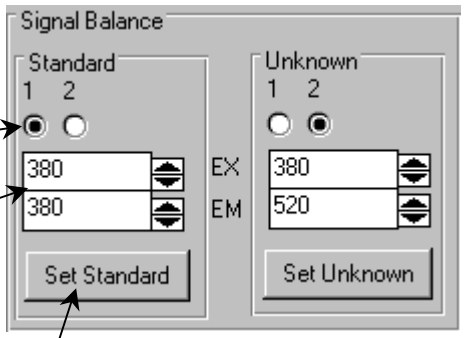
To balance the standard and unknown samples,

a Click *Balance* (if the ***Signal Balance*** dialog box is not already visible). The ***Signal Balance*** dialog box appears on the control panel.

b Position the desired sample in the optical path.

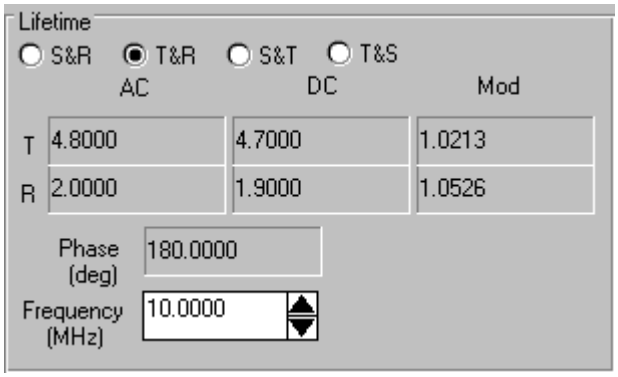
c Click on the radio button next to the appropriate position.

d Set monochromator positions. Enter the desired value in the standard or unknown's field for the *EXcitation* or *EMission* monochromator.



e Click *Set Standard* or *Set Unknown* to confirm the change.

f View the effect in the ***Lifetime*** dialog box on the control panel.

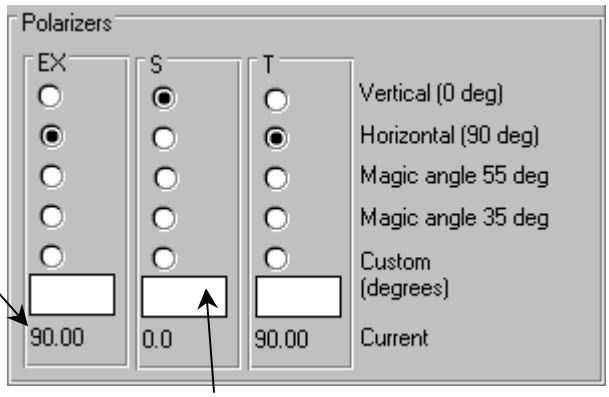


Polarizers

The *Polarizers* button, available when polarizers are included in the layout, sets the rotational positions for all polarizers (Excitation, R, and optional T). Any angle is possible, but special settings are shown for vertical, horizontal, and magic angles.

To rotate a polarizer to a new angle,

a Click *Polarizers* (if the ***Polarizers*** dialog box is not already visible). The ***Polarizers*** dialog box appears. Current settings are shown at the bottom of the window.



b Enter a new angle for a polarizer.

Click a preset angle (vertical, horizontal, magic angle), or enter a custom angle in the field near the bottom.

Note: *See the* Polarizers Operation Manual *for more information about using polarizers.*

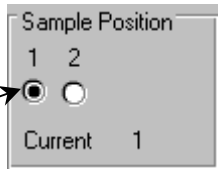
Sample Changer

If an optional sample changer is included in the layout, then a *Sample Changer* button appears. *Sample Changer* sets the current position of the sample changer in the optical path.

To set the current position of the sample changer,

a Click *Sample Changer* (if the ***Sample Position*** dialog box is not already visible).

The ***Sample Position*** dialog box appears in the control panel. The current position in the optical path is labeled at the bottom of the window.



b Click a radio button to choose a new sample position.

Note: *The* **Sample Position** *dialog box varies according to the number of positions that the sample changer accommodates.*

Screen views

Introduction

The ***Real Time Display***'s screen may be customized, and the resulting screen may be saved and recalled. As many screen layout files as is necessary may be created, as long as storage space on the disk can accommodate them. Among the benefits of customizing the view are:

- Allow multiple users
- Resize the control panel

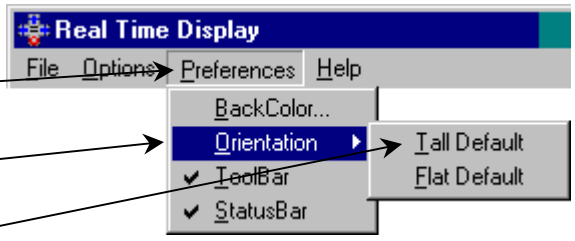
Tutorial

When the ***Real Time Display*** is opened, the screen layout and hardware settings are the same as those that were active when the program was last used.

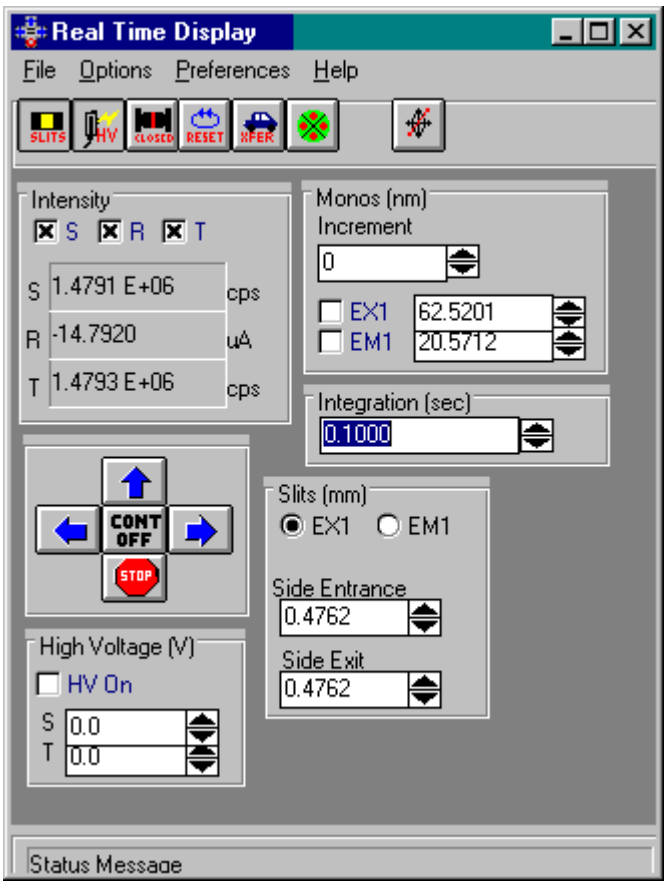
1 Choose *Preferences*.

2 Choose *Orientation*.

3 Choose *Tall Default*.



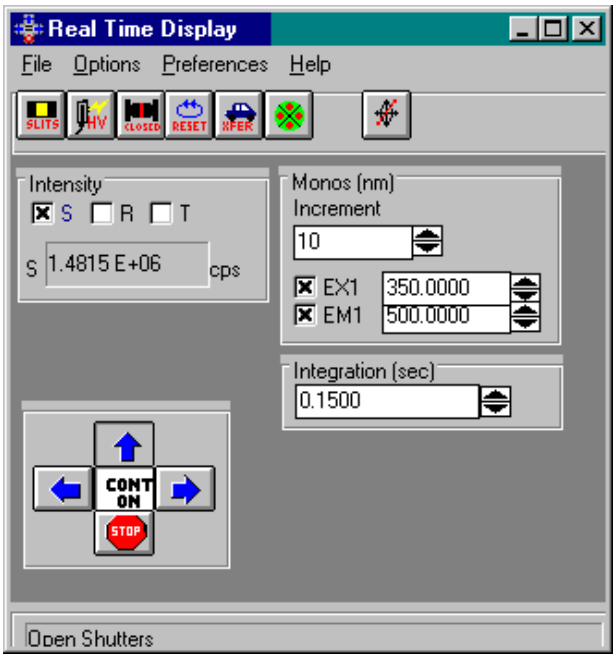
The ***Real Time Display*** should appear something like this:



Suppose that, for a new experiment, a slit width giving maximum emission is needed. The general procedure is: Set the items that shall not change, and place the ***Slits*** dialog box in easy reach. Resize the control panel to remove excess blank area, and hide those dialog boxes that shall not change.

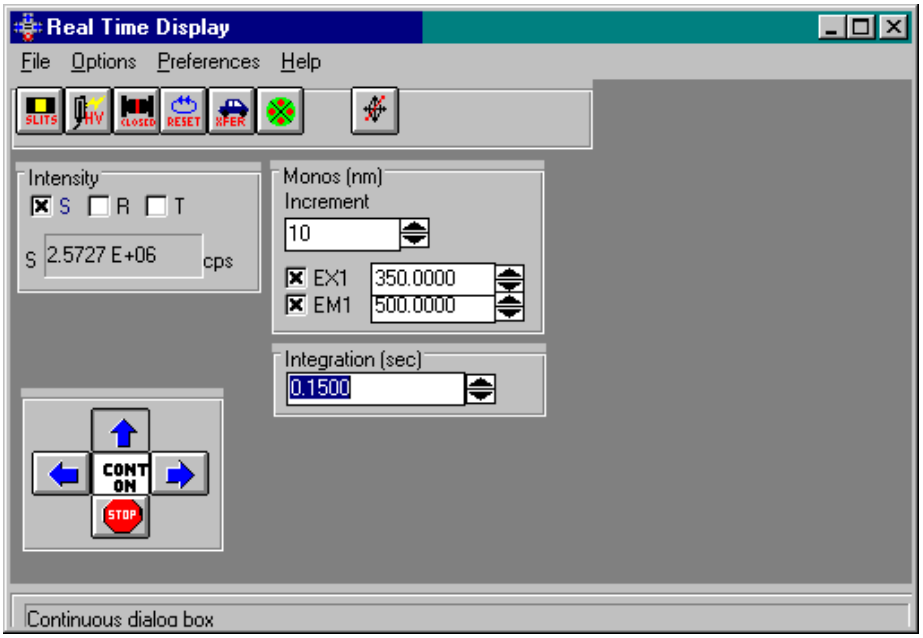
The settings in the following diagram cause the excitation and emission monochromators (now at 350 nm and 500 nm, respectively) to move automatically at 10-nm increments in the forward direction, while collecting data for 0.150 s at each data point.

For the purposes of this tutorial, the monochromator settings and integration time will not change. Therefore, we can hide the associated dialog boxes beyond the border of the control panel, and leave the ***Slits*** dialog box in view, for it is the only setting to change.



4 Place the mouse cursor on the right border.

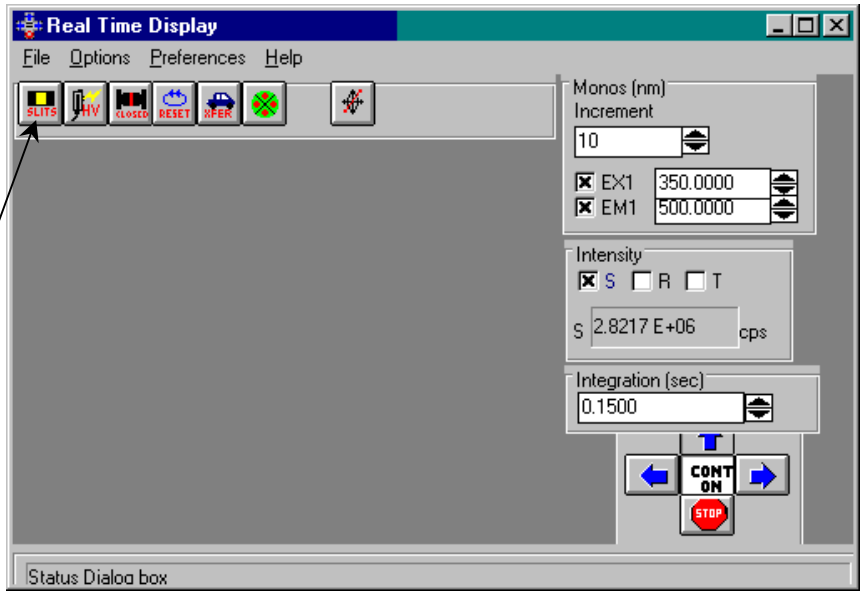
5 When the ↔ appears, drag the side of the control panel to the right:



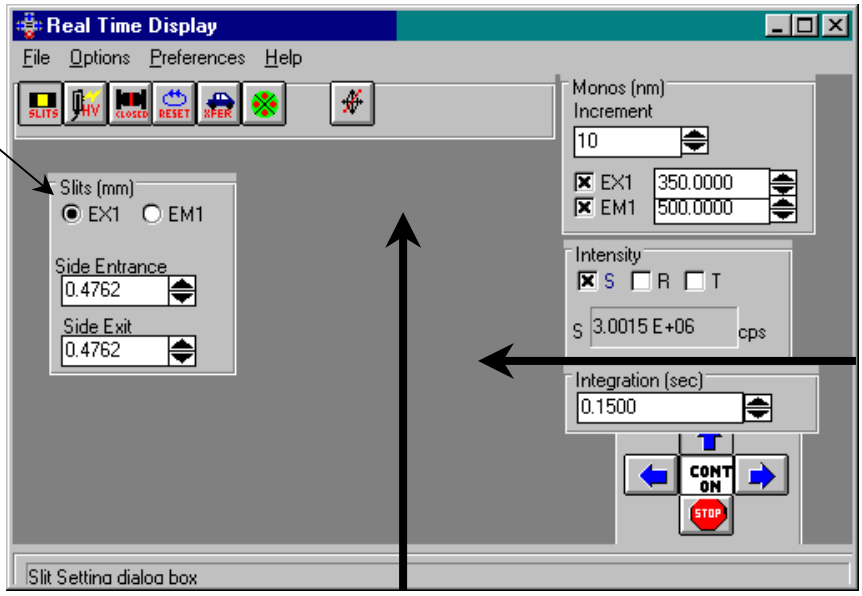
6 Move the unnecessary items to the right.

Drag the ***Monos***, ***Intensity***, and ***Integration*** windows:

7 Click the *Slits* button.

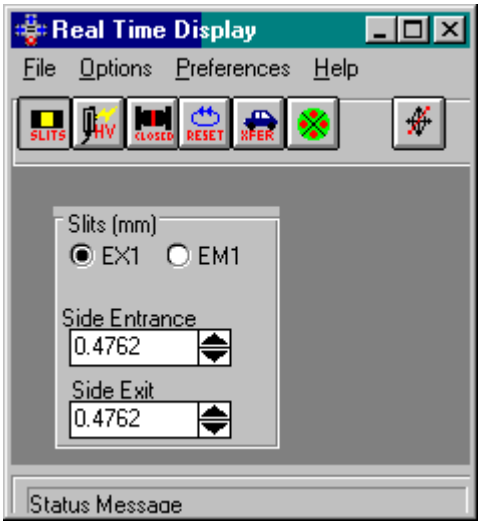


The ***Slits*** dialog box appears:



8 Resize the control panel.

Move the right and bottom sides toward the center. This hides the unnecessary dialog boxes:



Customizing the control panel gives a much neater view, and displays only those items necessary. This screen view can be saved and recalled later.

Note: *Even if a dialog box is hidden, an error can occur, and an error message appears.*

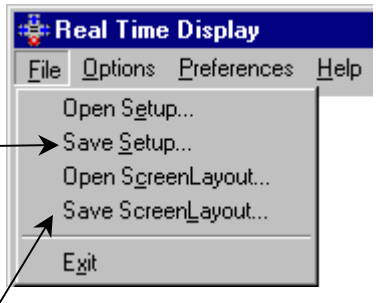
Multiple setup and screen layout files

Once hardware settings and parameters are set, they can be saved so they do not need to be set again. Setup files (saved with extension `.SET`) contain information about hardware and parameters. Screen layout files (saved with extension `.SLY`) contain information about placement of screen items.

To save settings,

1 Choose *File*.

2 Choose *Save Setup…*.



To save a screen layout,

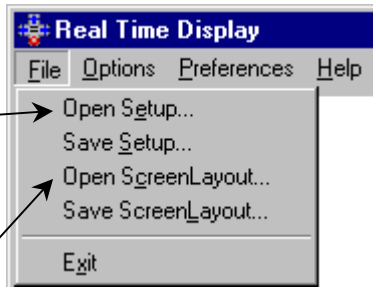
1 Choose *File*.

2 Choose *Save ScreenLayout…*.

To recall a setting,

1 Choose *File*.

2 Choose *Open Setup…*.



To recall a screen layout,

1 Choose *File*.

2 Choose *Open ScreenLayout…*.

When the ***Real Time Display*** is exited, the current setup file is saved automatically as a temporary file called `RDT.SET`. When ***Real Time Display*** is restarted, `RDT.SET` is opened, and the last settings in use are restored.

7: Advanced Scanning and Displaying

Introduction

The ***Experiment Acquisition*** dialog box is used for basic data-acquisition, that is, acquiring a single spectrum. DataMax also offers a number of advanced scanning features, including varying the sample's temperature during an acquisition, incrementally varying an originally fixed wavelength and plotting the results three-dimensionally, scanning with an optional microwell-plate reader, batch scanning, and more. This chapter discusses these advanced acquisitions.

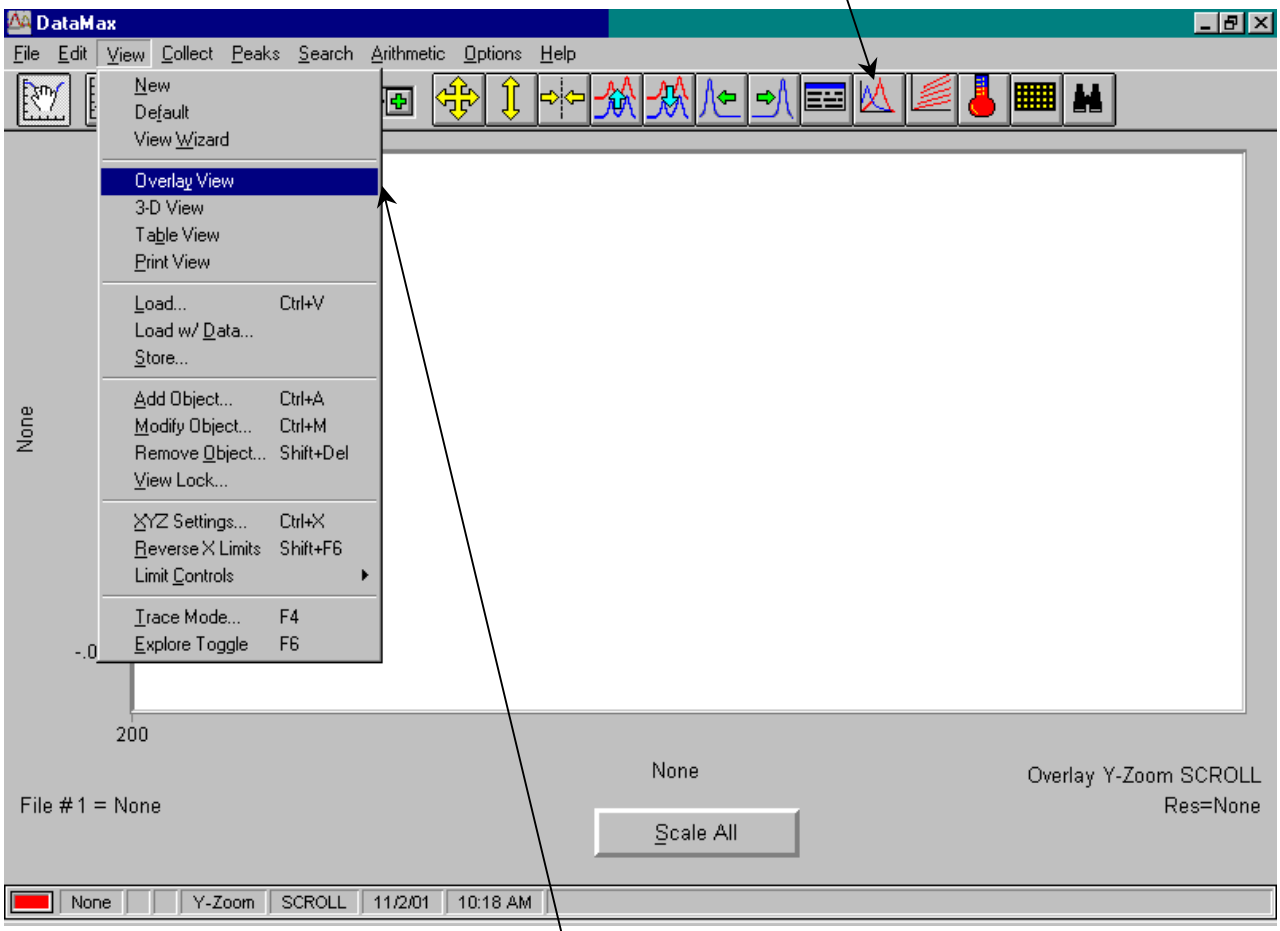
Overlay view

When working with multiple files, the ability to view them all simultaneously is important. Use *Overlay View* to display several traces at the same time and rescale them. For more information about *Overlay View*, see the *GRAMS/32® User's Guide*.

To view several spectra simultaneously,
In ***Run Experiment***,

1 Click the *Overlay View* button.

2 Open as many files as desired.



Or

1 Click *View*.
A drop-down menu appears.

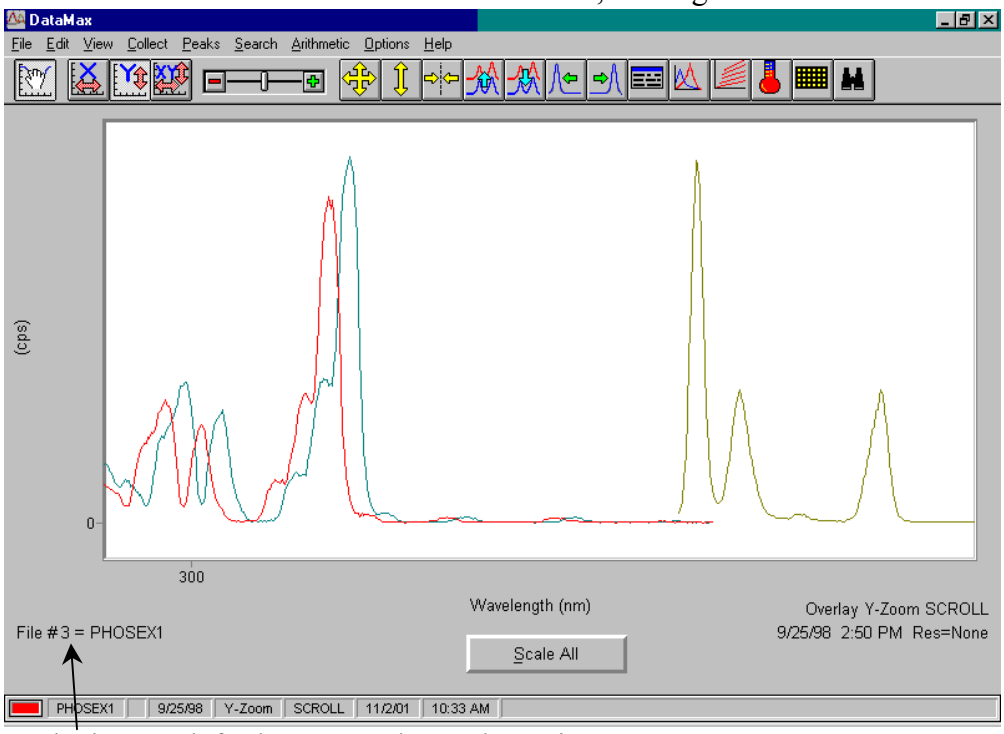
2 Click *Overlay View*.

3 Open as many files as desired.

To choose an active spectrum from several opened traces,

1 Click **PGUP** or **PGDN**.

This moves the cursor from trace to trace, making one trace active at a time.

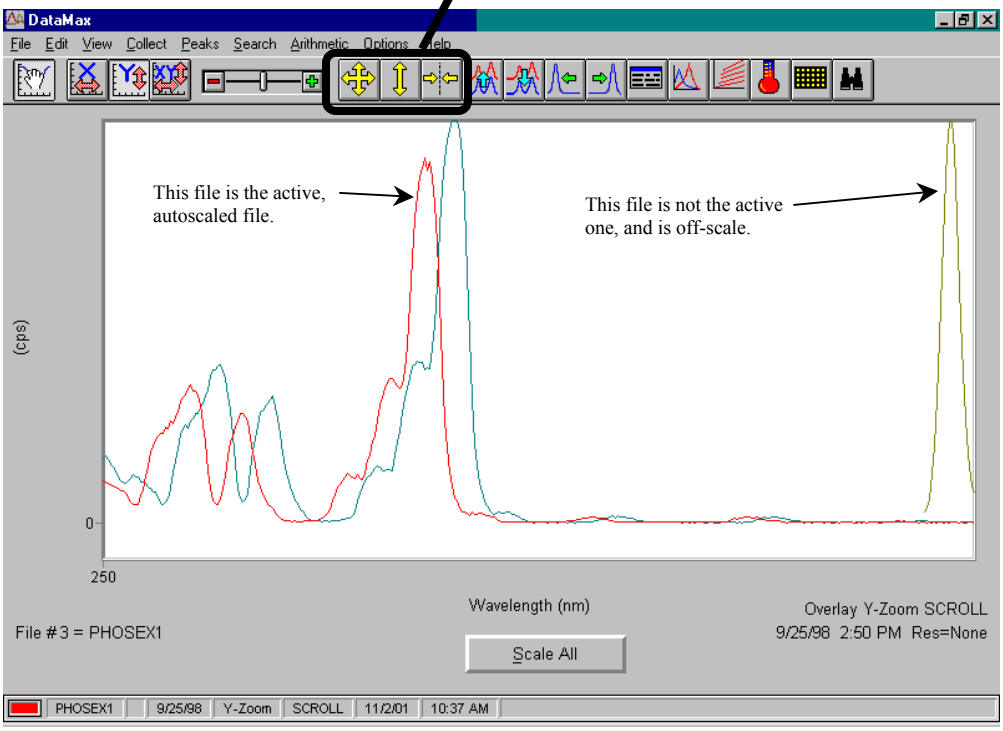


Near the bottom left, the screen shows the active spectrum's name.

To scale one spectrum out of a group,

1 Make the desired trace active.

2 Use the autoscale buttons.



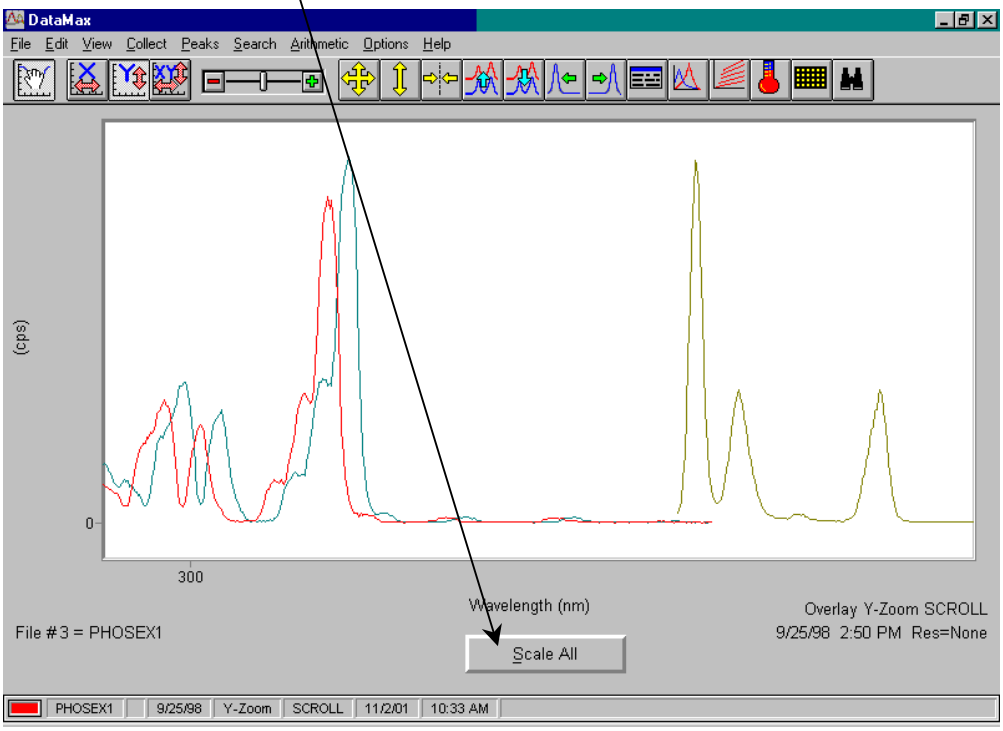
To scale all traces simultaneously,

1 Select one trace as the active trace.

This trace is only used as a reference.

2 Click *Scale All*.

All spectra on the screen are scaled to the same axes.



Advanced scan types

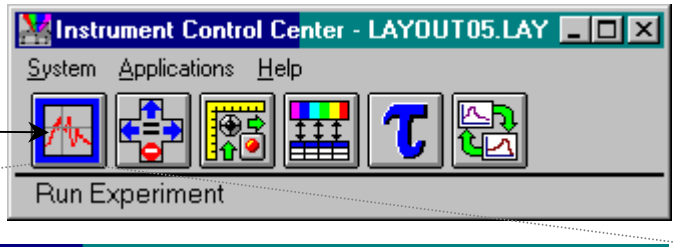
Introduction

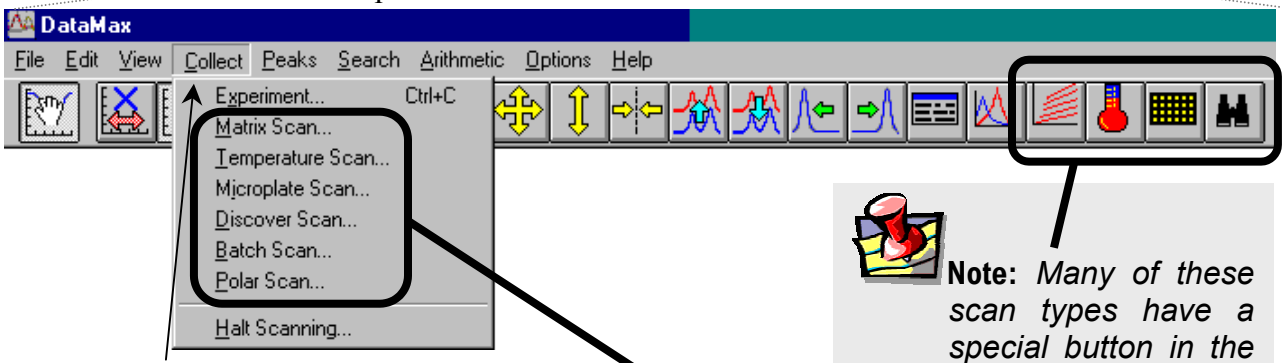
Beyond the basic acquisitions available (emission, excitation, phosphorimeter, lifetime, etc.) in the ***Run Experiment*** application in DataMax, are advanced kinds of scans for special purposes:

- Matrix scan — Vary one monochromator incrementally, and scan the other
- Temperature scan — Vary temperature during an acquisition
- Microplate scan — Examine a series of samples rapidly, usually all at the same wavelength pair, with the MicroMax
- Discover scan — Preview a spectrum by taking a series of acquisitions, to determine the region of interest
- Batch scan — Run a variety of lengthy experiments automatically
- Polar scan — Quick scan using polarizers

To gain access to these scans,
In ***Instrument Control Center***,

1 Click the *Run Experiment* button.
The ***Run Experiment*** window opens.





Note: *Many of these scan types have a special button in the toolbar.*

2 Choose *Collect*.
A drop-down menu appears.

3 Choose the desired scan type.
A dialog box corresponding to that scan type appears.

Matrix scan

Introduction

What happens when one wavelength is varied by a specified increment, and the other wavelength is scanned across a certain region? Use *Matrix Scan…* to run this type of experiment. For example, use a previously saved excitation-acquisition experiment, specify a range and increment for the emission monochromator, and run a matrix scan. The experiment is performed for each increment, and the spectra appear together on the screen.

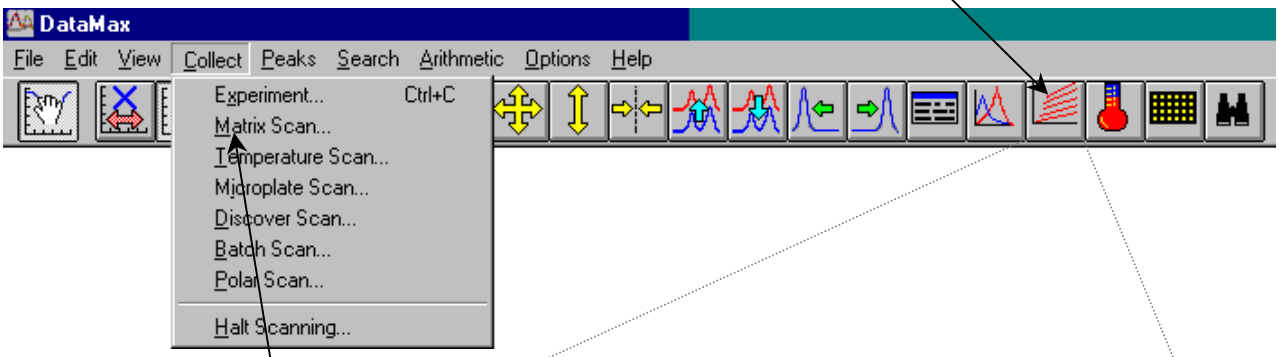
To start a matrix scan,

In ***Run Experiment***,

1 Click the *Matrix Scan* button.

The ***Matrix Scan*** dialog box appears.

Or

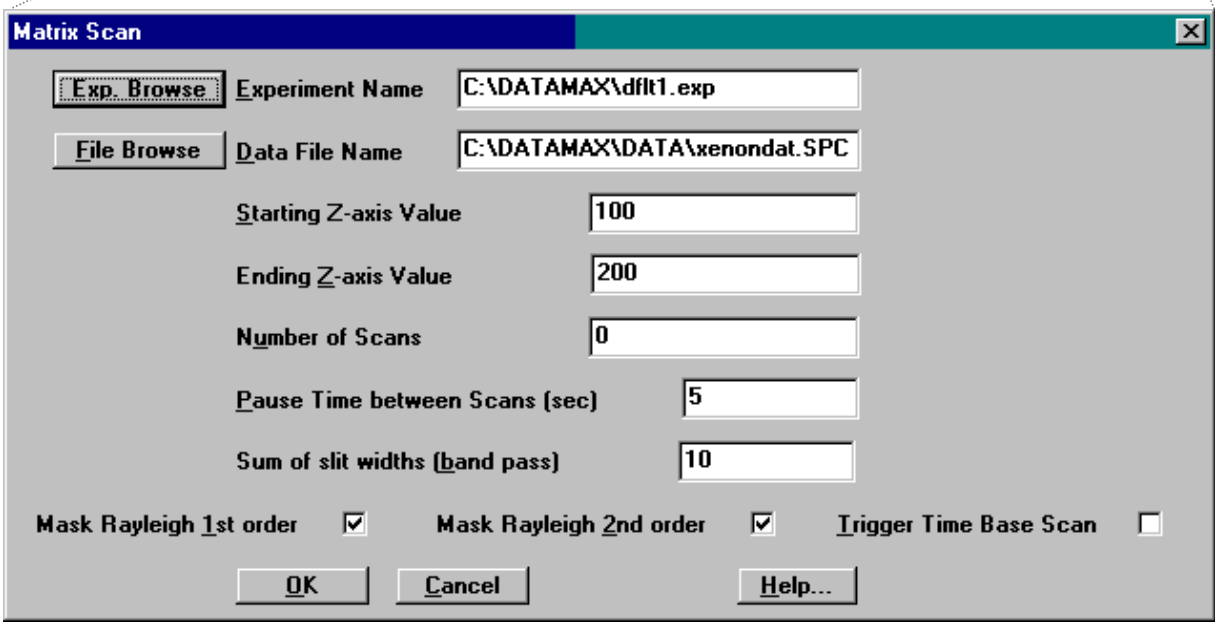


1 Choose *Collect*.

A drop-down menu appears.

2 Choose *Matrix Scan…*.

The ***Matrix Scan*** dialog box appears:



A matrix scan can be performed with any type of acquisition, and the parameters do not vary. The wavelength that varies is the monochromator that was fixed in the original acquisition type. For example, in an excitation acquisition, the emission monochromator is fixed. In a matrix excitation acquisition, the emission monochromator is varied by a specified increment, and an excitation scan is taken at each increment.

3 Enter the appropriate settings.

4 Click *Run*.

The matrix scan starts.

Temperature scan

Introduction

What happens when the temperature is varied during an acquisition? Use *Temperature Scan…* to run this type of experiment. For example, use a previously saved excitation-acquisition experiment, specify a range and increment for the optional temperature bath or Peltier device, and run an acquisition. A scan is taken at each desired temperature and the results are displayed together on the main screen.

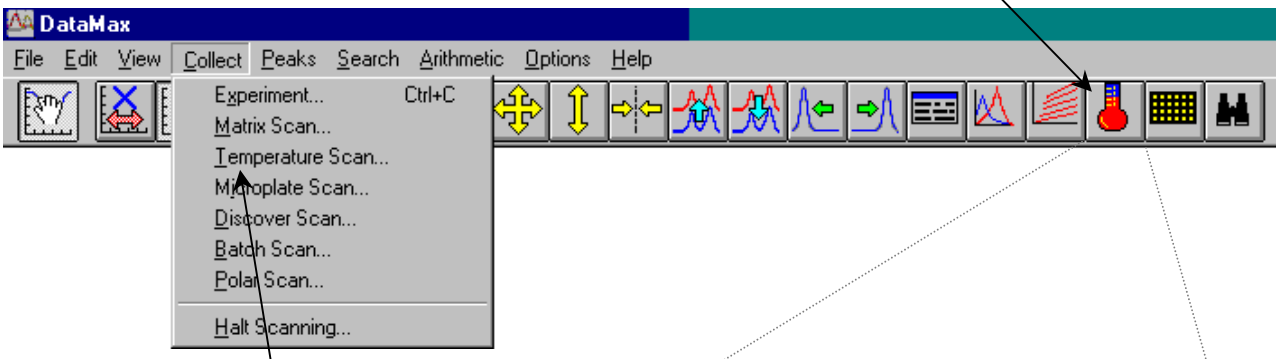
To start a temperature scan,

In ***Run Experiment***,

1 Click the *Temperature Scan* button.

The ***Temperature Scan*** dialog box appears.

Or

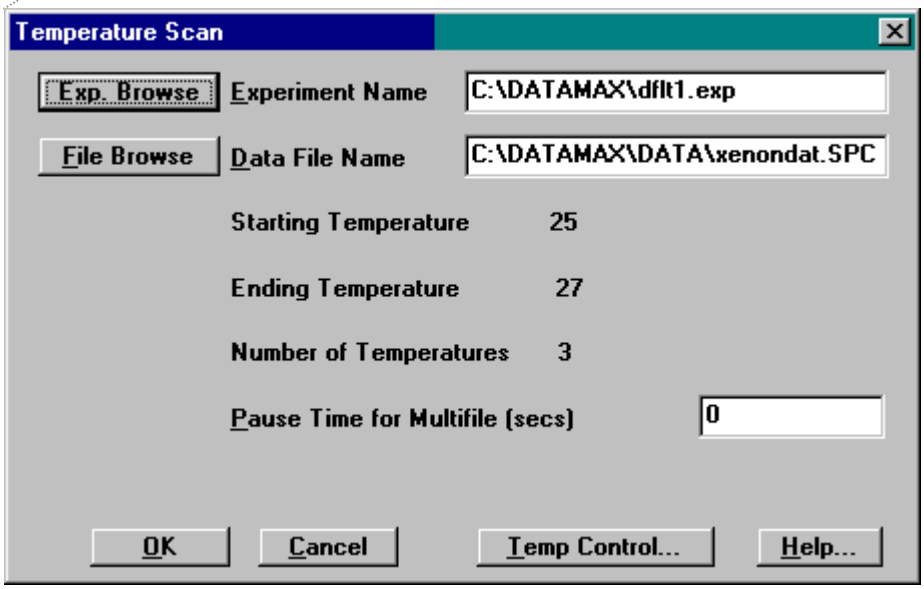


1 Choose *Collect*.

A drop-down menu appears.

2 Choose *Temperature Scan…*.

The ***Temperature Scan*** dialog box appears:



3 Enter the appropriate settings.

4 Click *OK*.

The scan runs automatically.

Microplate scan

Introduction

To examine a series of samples rapidly, especially all at the same excitation and emission wavelength-pair, use *Microplate Scan*…. The samples are placed in a small tray containing an array of tiny wells. The tray, called a microwell plate, is inserted into the optional MicroMax plate reader, and scanned rapidly at the desired wavelengths. This section briefly discusses microwell-plate scans; see the *MicroMax Operation Manual* for more details.

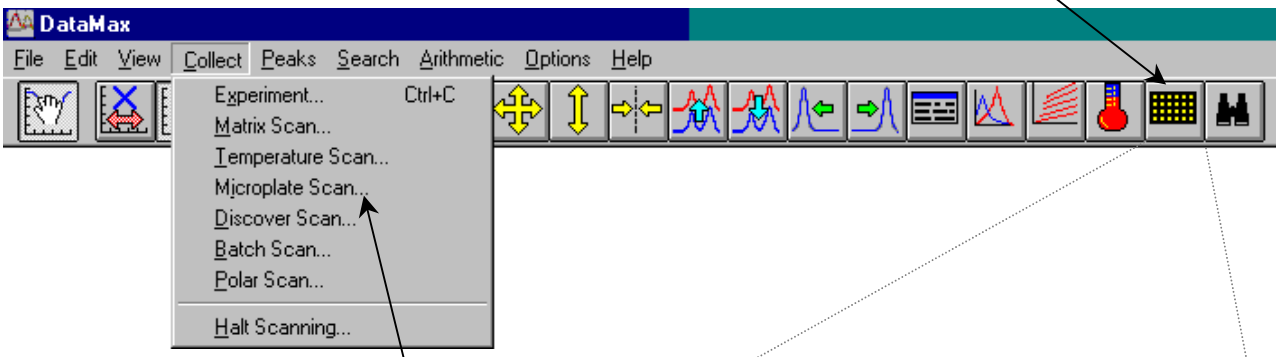
To start a microwell plate scan,

In ***Run Experiment***,

1 Click the *Microplate Scan* button.

The ***MicroPlate Matrix Scan*** dialog box appears.

Or



1 Choose *Collect*.

A drop-down menu appears.

2 Choose *Microplate Scan*….

The ***MicroPlate Matrix Scan*** dialog box appears:

3 Enter all parameters.

4 Click *OK*.

The microplate scan begins.

Discover scan

Introduction

To preview a spectrum, in order to decide where the region of interest is, choose a discover scan. A discover scan runs an array of emission scans at increasing excitation wavelengths. It stores the highest six peaks that it finds, with their corresponding excitation- and emission-wavelength sets. Peaks near the Rayleigh line are ignored. Preset parameters are:

Monochromator	Starting point (nm)	Ending point (nm)	Increment (nm)
Excitation	260	500	20
Emission	280	850	10

Slits' bandpass = 1 nm
Integration time = 0.05 s
S detector's high voltage = 950 V

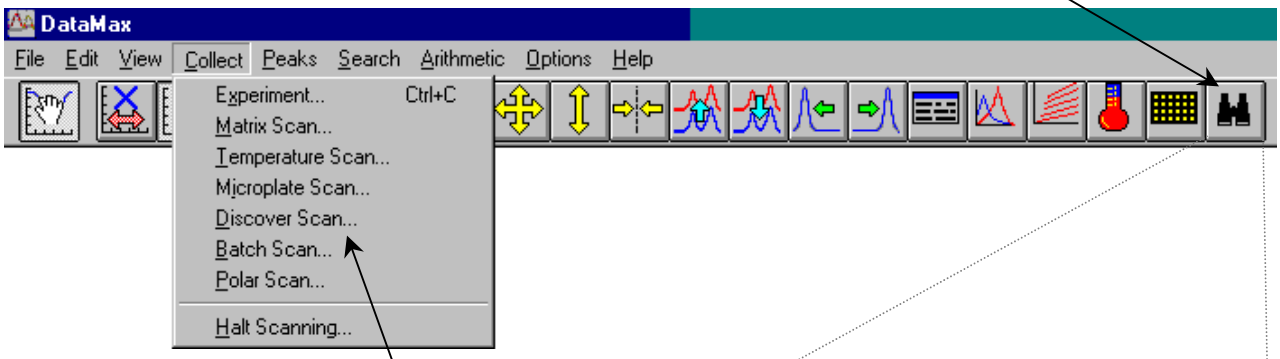
To start a discover scan,

In ***Run Experiment***,

1 Click the *Discover Scan* button.

The ***Discover Scan*** dialog box appears.

Or

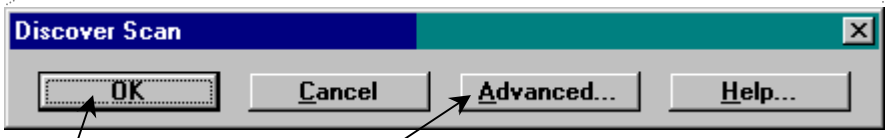


1 Choose *Collect*.

A drop-down menu appears.

2 Choose *Discover Scan…*.

The ***Discover Scan*** dialog box appears:



3 Adjust any parameters.

4 Click *OK*.

The discover scan starts.

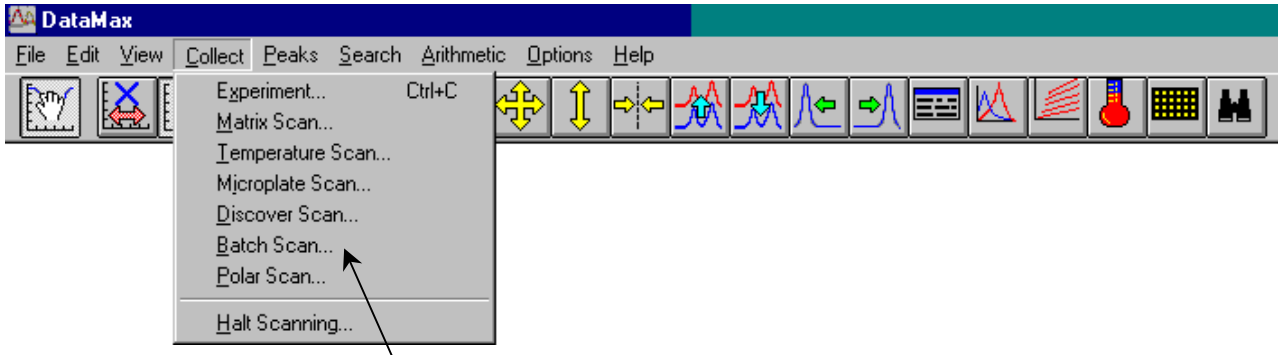
Batch scan

Introduction

To run an automated series of different and lengthy experiments, use *Batch Scan…*. With an optional sample changer, different sample positions also may be used. Any type of scan in any order can be programmed.

To start a batch scan,

In ***Run Experiment***,

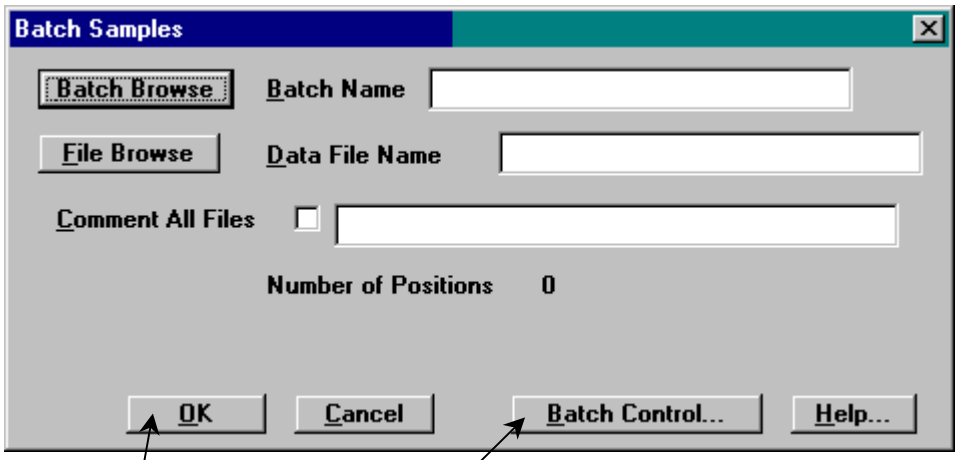


1 Choose *Collect*.

A drop-down menu appears.

2 Choose *Batch Scan…*.

The ***Batch Samples*** dialog box appears:



3 Fill in appropriate parameters.

Use *Batch Control…* as necessary.

4 Click *OK*.

The batch scan starts.

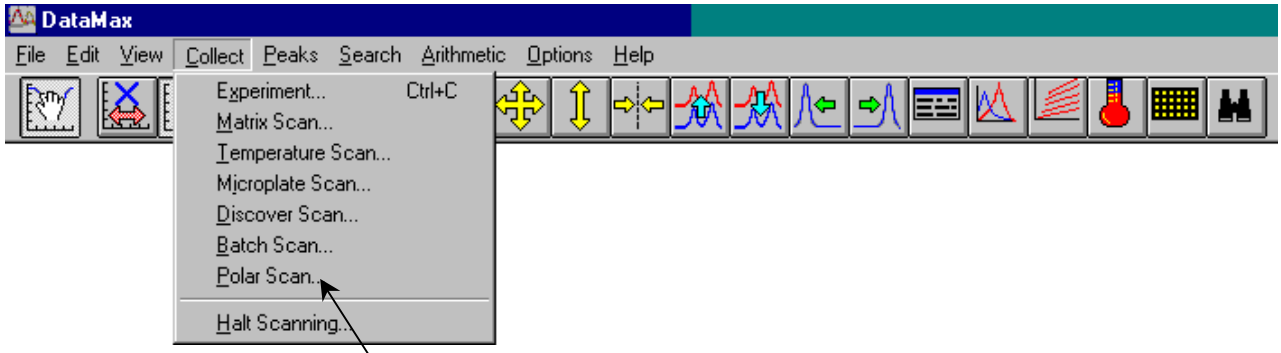
Polar scan

Introduction

Polar Scan (or quick polarization) sets up and runs an experiment using optional polarizers. Instead of running polarization scans that move the polarizers at each wavelength, this scan sets the polarizer, runs a scan, moves the polarizer, runs a scan, etc. After all necessary components of the polarization are taken, the polarization and anisotropy values may be calculated. *Polar Scan* is sometimes the only method for measuring polarization with certain polarizer configurations (e.g., manual slits in certain positions).

To start a polar scan,

In ***Run Experiment***,

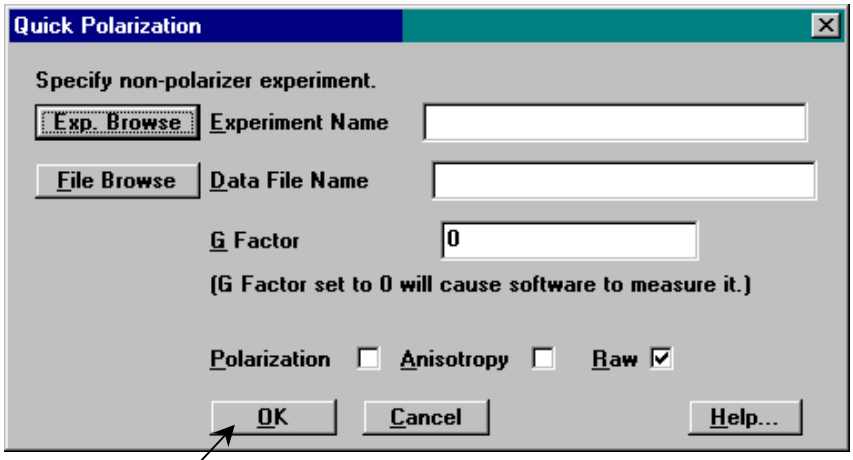


1 Choose *Collect*.

A drop-down menu appears.

2 Choose *Polar Scan….*

The ***Quick Polarization*** dialog box appears:



3 Choose an existing non-polarization experiment.

4 Fill in appropriate parameters.

5 Click *OK*.

The quick polarization scan starts.

Settings for advanced scans

Following are a list of all fields and settings in the various advanced-scan dialog boxes, in alphabetical order.

Advanced… (Advanced...) Advanced…

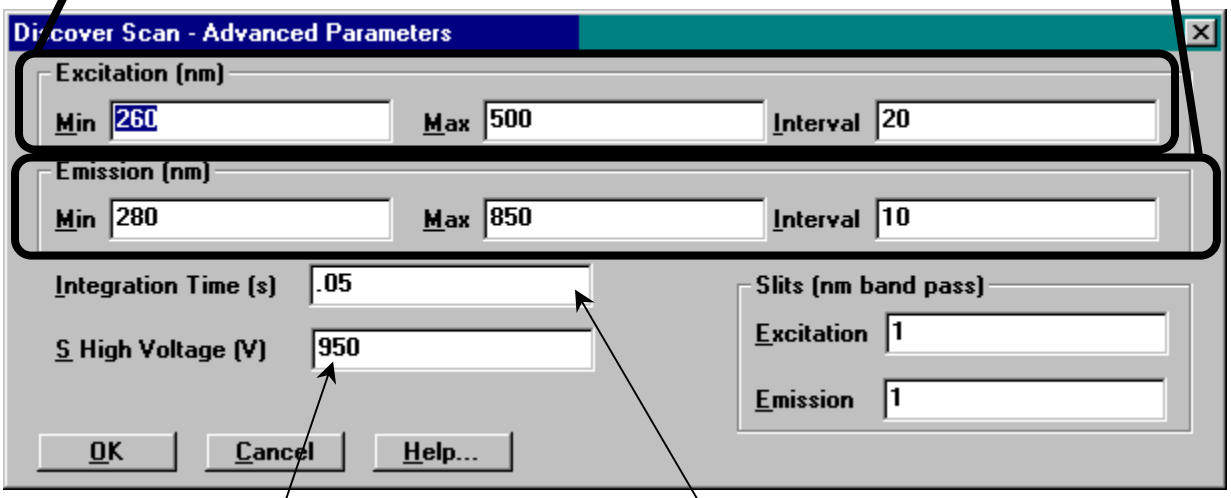
To change the preset values for a discover scan, use the *Advanced…* button.

1 Click *Advanced….*

The ***Discover Scan – Advanced Parameters*** dialog box opens:

a To adjust the excitation monochromator,
- Place the cursor on the *Min* field, and enter a minimum wavelength.
- Place the cursor on the *Max* field, and enter a maximum wavelength.
- Place the cursor on the *Interval* field, and enter a new increment.

b To adjust the emission monochromator,
- Place the cursor on the *Min* field, and enter a minimum wavelength.
- Place the cursor on the *Max* field, and enter a maximum wavelength.
- Place the cursor on the *Interval* field, and enter a new increment.



c To adjust the integration time per data point,
- Place the cursor on the *Integration Time* field, and enter a time.

d To adjust the S detector's high voltage,
- Place the cursor on the *S High Voltage* field, and enter a voltage.

e To change the slits,
- Place the cursor on the *Excitation* field, and enter a new excitation-monochromator slit-width.
- Place the cursor on the *Emission* field, and enter a new emission-monochromator slit-width.

f For help,

- Click the *Help*... button.

2 Click *OK*.

The ***Discover Scan – Advanced Parameters*** dialog box closes.

Anisotropy

When the *Anisotropy* checkbox is enabled, the system records the anisotropy values during a polar scan.

To record the anisotropy component of the signal,

1 Click the checkbox next to *Anisotropy*.

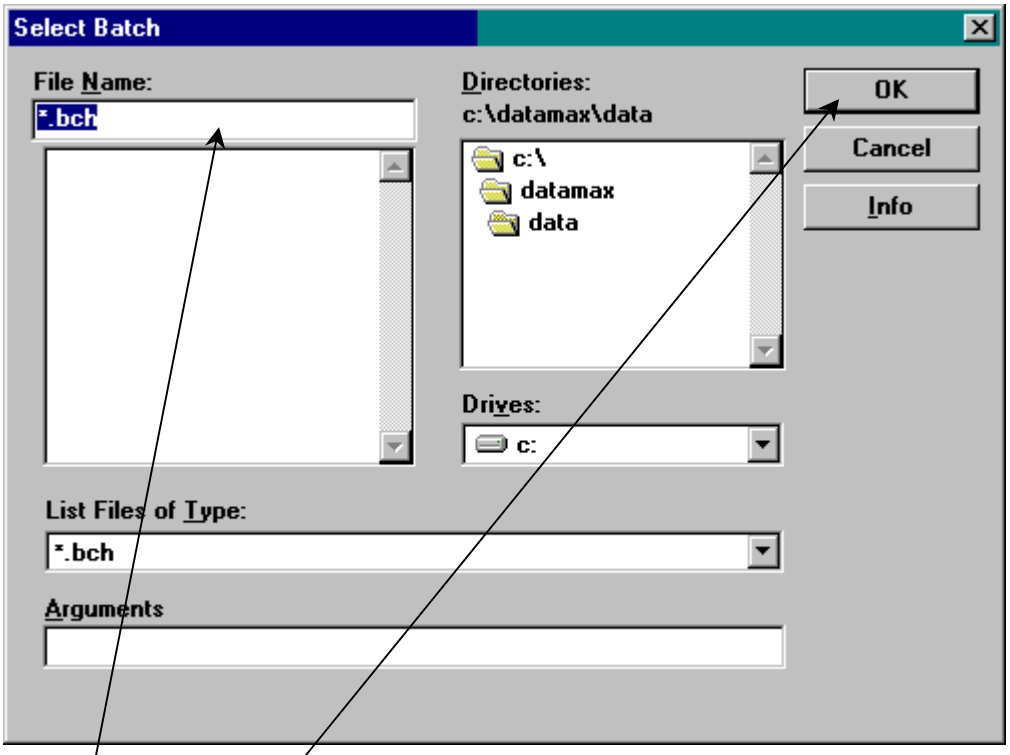
Batch Browse

Batch Browse selects a saved batch file—with batch parameters—upon which to perform a batch scan. DataMax uses the batch file's batch parameters for the batch scan. A batch file has the extension `.BCH`.

To select a batch file for a batch scan,

1 Click *Batch Browse*.

The ***Select Batch*** dialog box appears:



2 Choose the desired batch file.

3 Click *OK*.

The ***Select Batch*** dialog box closes, and the batch's name appears in the *Batch Name* field in the ***Batch Samples*** window.

Batch Control… Batch Control…

The *Batch Control…* button sets the order and types of experiments to do in the batch process.

To set up a batch process,

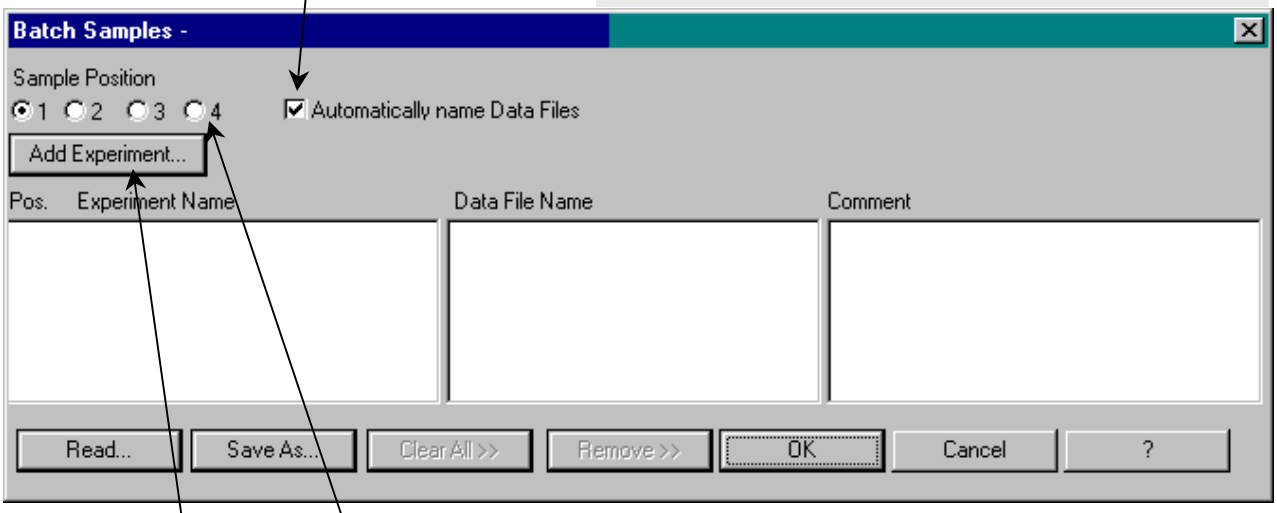
1 Click *Batch Control….*

The ***Batch Samples*** – dialog box opens.

2 If desired, click the *Automatically name Data Files* checkbox.

This names all data files sequentially.

Note: *If the* Automatically name Data Files *checkbox is disabled, all data file names must be entered manually.*



3 Click the appropriate *Sample Position* radio button.

This chooses the position of the sample in the optional sample changer for the first experiment.

Note: *The* Sample Position *must be 1 if there is no optional sample changer available.*

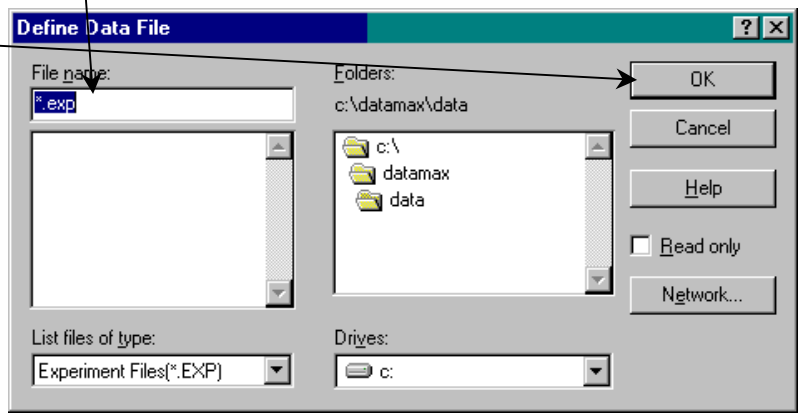
4 Click *Add Experiment….*

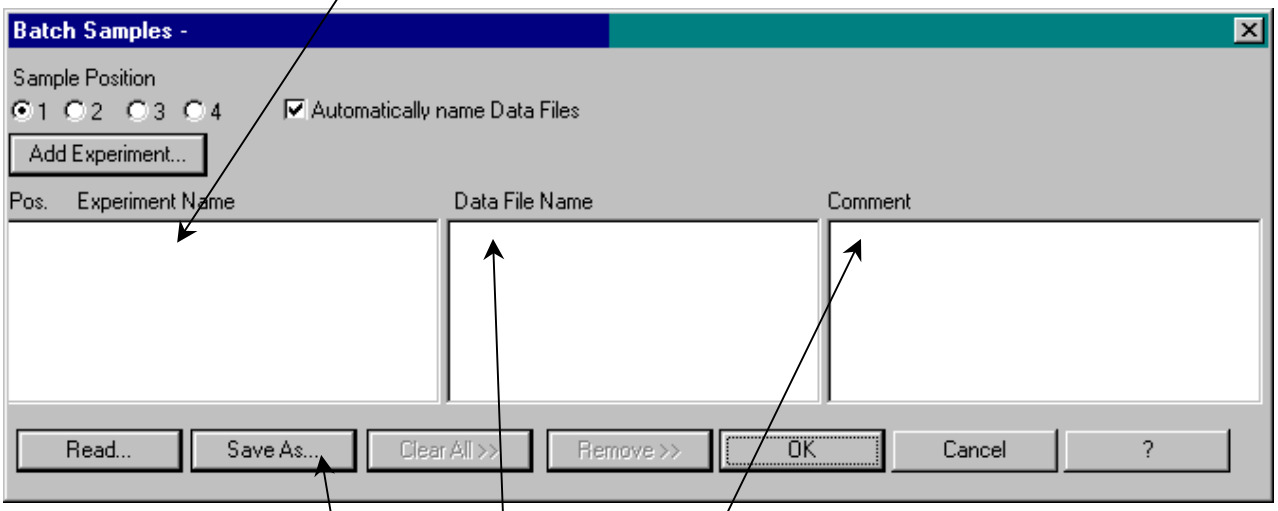
The ***Define Data File*** dialog box appears.

5 Choose an existing experiment file (`.EXP`) for the batch process.

6 Click *OK*.

The ***Define Data File*** window closes, and the experiment file name appears in the *Experiment Name* column.





7 Enter a data file name (if not done automatically with the checkbox).

8 Enter a comment.

9 Repeat the process from step 3 through 8.

10 To remove an experiment, click *Remove»*.

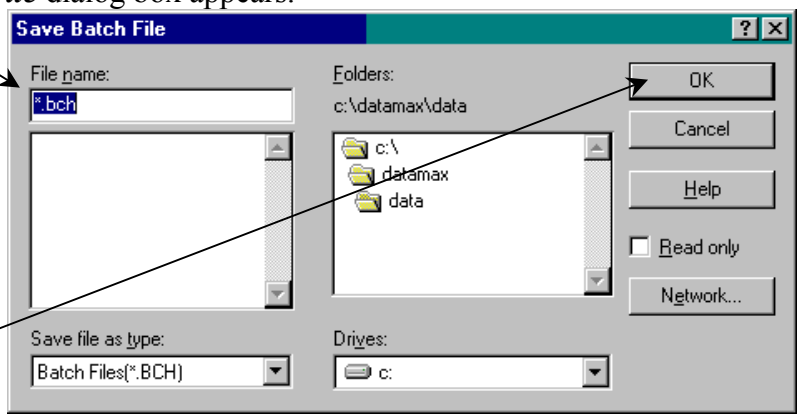
11 To remove all experiments from the table, click *Clear All»*.

To save a batch process,

1 Create a batch process as listed above.

2 Click *Save As…*.

The ***Save Batch File*** dialog box appears.



3 Enter the desired name for the batch file.

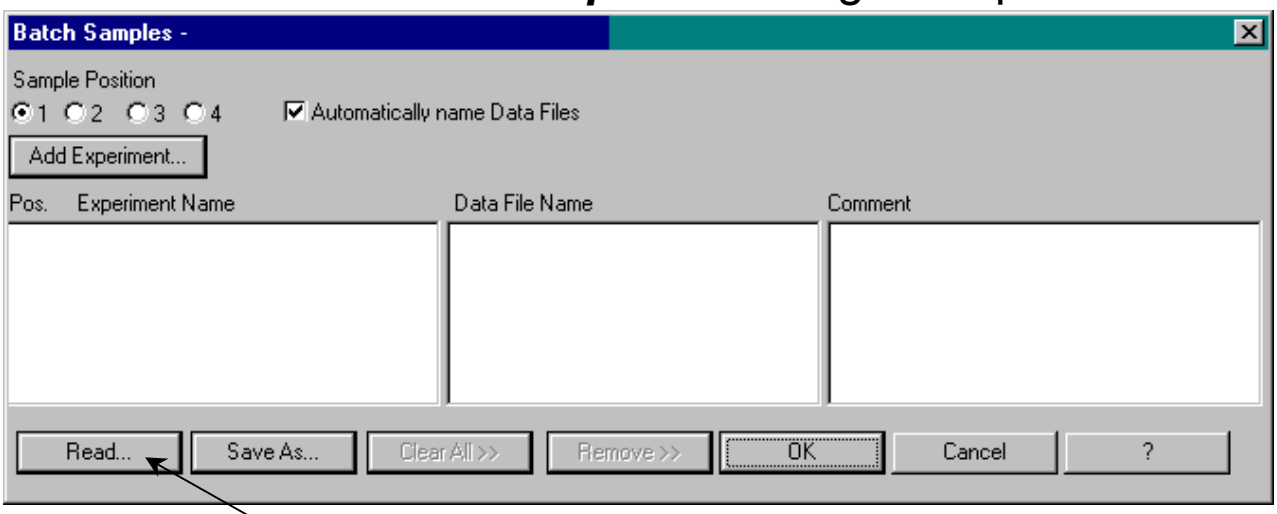
4 Click *OK*.
The batch file is saved, and the ***Save Batch File*** dialog box closes.

5 Use or modify the open batch process as required.

To open an existing batch file,

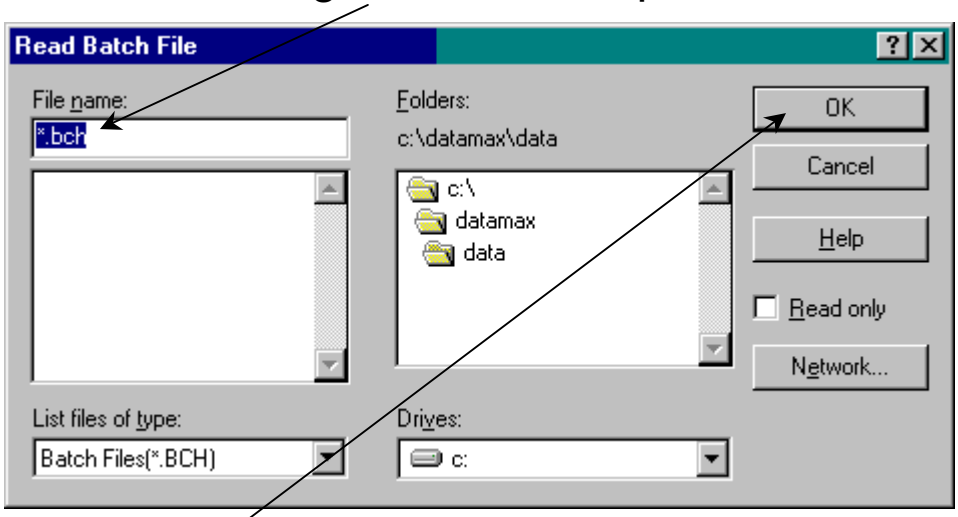
1 Click *Batch Control….*

2 The ***Batch Samples –*** dialog box opens:



3 Click *Read….*
The ***Read Batch File*** dialog box opens:

4 Choose an existing batch file to open.



5 Click *OK*.

The ***Read Batch File*** dialog box closes; DataMax reads the batch file and displays the parameters in the ***Batch Samples –*** dialog box.

Batch Name

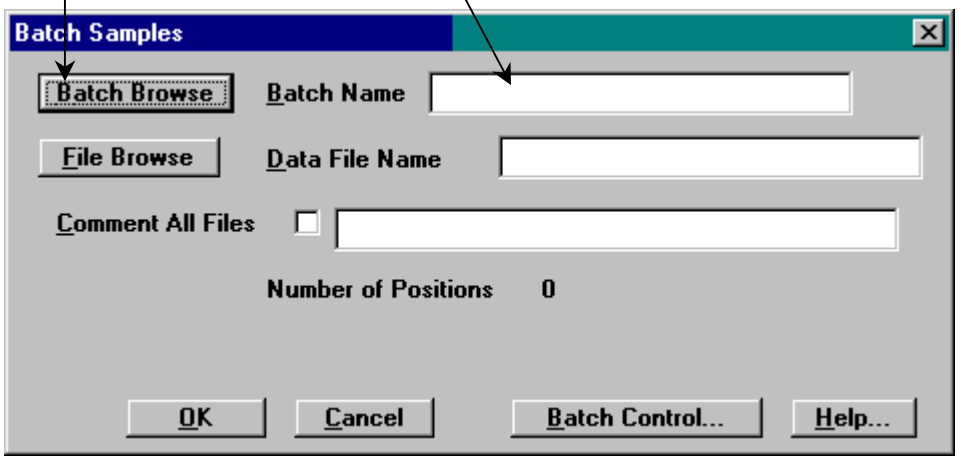
The *Batch Name* data-entry field displays the name of the batch file upon which the batch scan is performed. The file name can be directly entered, or selected via the *Batch Browse* button. The file name chosen here must be an existing file.

To select an existing batch for a batch scan,

1 Click *Batch Browse*.

Or

1 Type the exact name, including the drive and directory, if necessary.



Comment All Files

To add the same comment to each file in the batch scan,

1 Click the *Comment All Files* checkbox.

Comment field

Visible in the batch scan's dialog box, the comment field is unlabeled, but has a *Comment All Files* checkbox next to it. To add a remark or comment about a batch scan,

1. Click in the comment field.
2. Enter the comment.

Data File Name

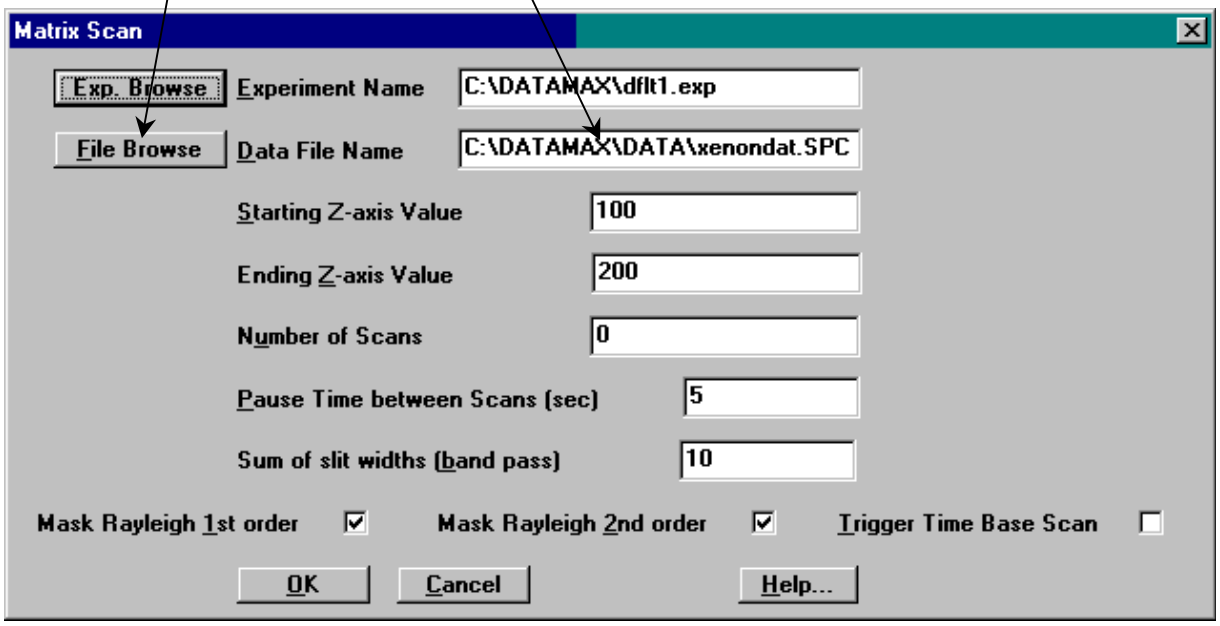
Found on most advanced-scan dialog boxes, the *Data File Name* data-entry field displays the name of the file to which the scan data are saved. The name can be directly entered, or selected via the *File Browse* button.

To name the data file for an advanced scan,

1 Click *File Browse*.

Or

1 Enter the name in the *Data File Name* field.



Ending Temperature 27

Ending Temperature

Not accessible in the temperature scan dialog box, the *Ending Temperature* displays the temperature at which a temperature scan experiment must end. The temperature units are specified in the original experiment. The temperature controller adjusts to this temperature before the final scan.

Set the *Ending Temperature* using the *Temp Control*... button.

Ending Z-axis Value

The *Ending Z-axis Value* sets the ending wavelength (in the original experiment's units) for a matrix scan. The originally fixed monochromator stops here at the conclusion of the matrix scan.

To set the ending point,

1. Click on the *Ending Z-axis Value* field.
2. Enter the ending value.

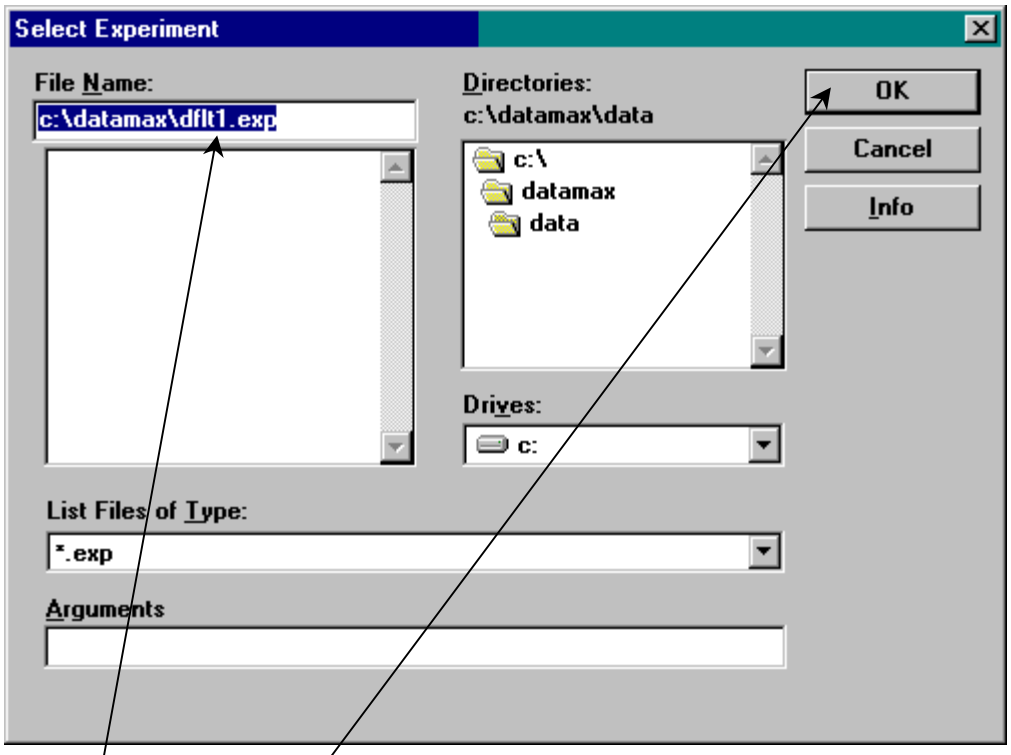
Exp. Browse Exp. Browse

Exp. Browse selects a saved experiment upon which to perform an advanced scan. DataMax uses the chosen experiment's parameters for the scan.

To select an experiment for a matrix scan,

1 Click *Exp. Browse*.

The ***Select Experiment*** dialog box appears:



2 Choose the desired experiment.

3 Click *OK*.

The ***Select Experiment*** dialog box closes, and the experiment's name appears in the *Experiment Name* field in the advanced scan's window.

Note: *For a polar scan, choose only non-polarizer experiments.*

Experiment Name

Found on most advanced-scan dialog boxes, the *Experiment Name* data-entry field displays the name of the file upon which the advanced scan is performed. The name can be directly entered, or selected via the *Exp. Browse* button. The file name chosen here must be an existing file.

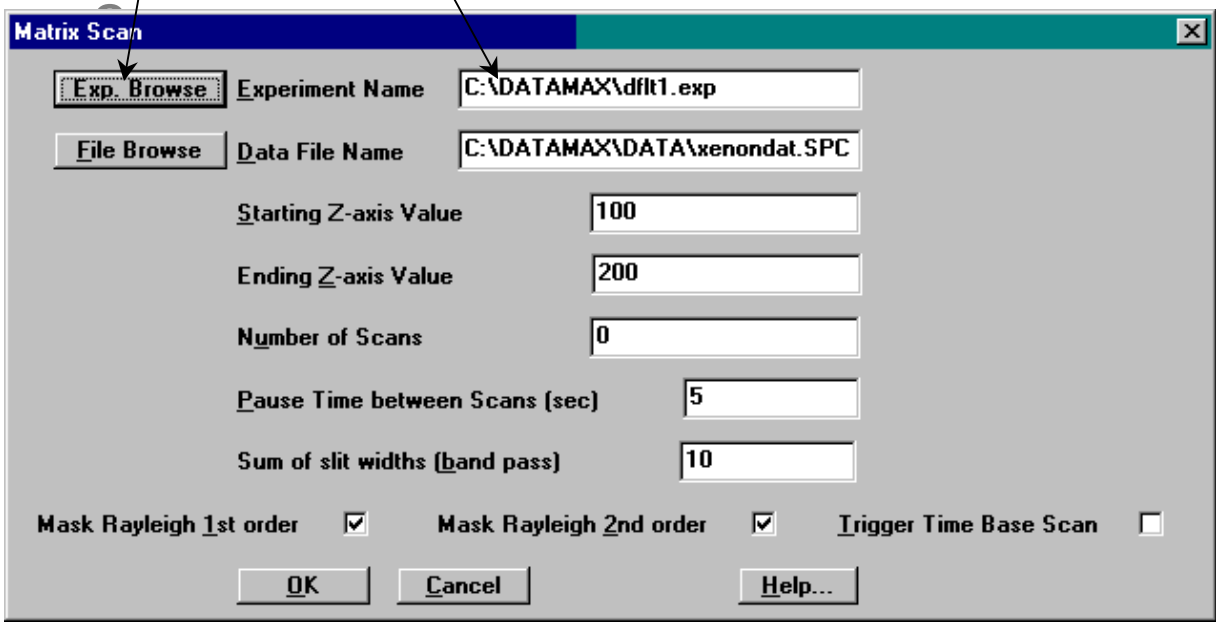
To select an existing experiment for an advanced scan,

1 Click *Exp. Browse*.

Or

1 Type the exact name, including the drive and directory, if necessary.

Note: *For a polar scan, choose only non-polarizer experiments.*



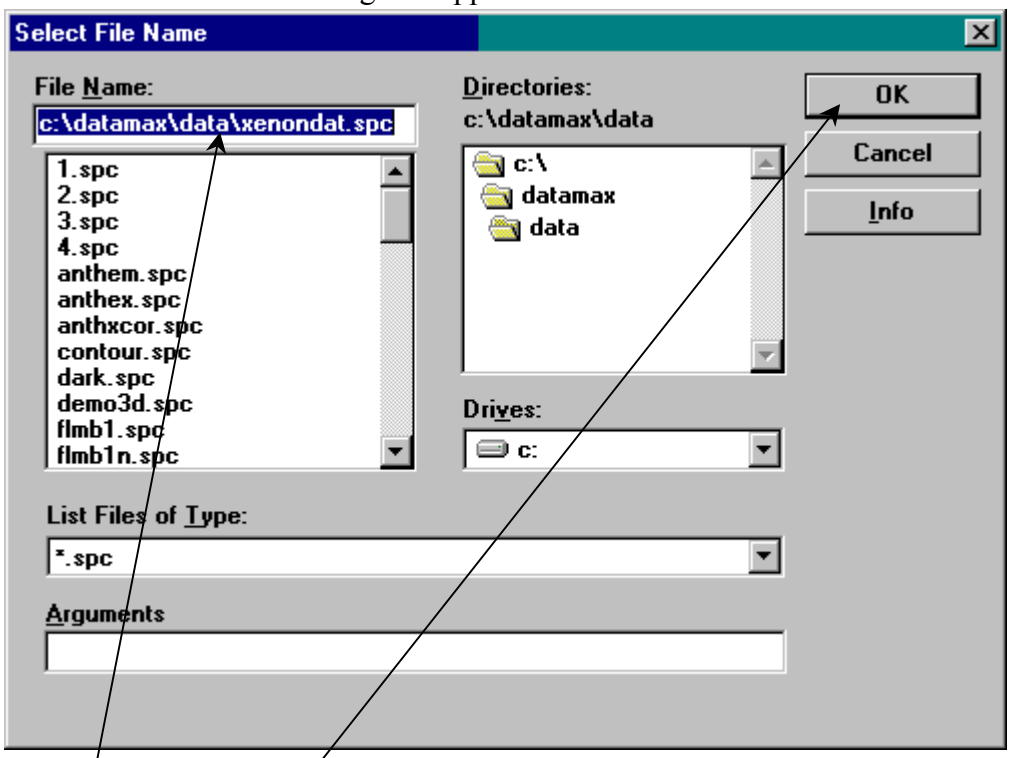
File Browse | File Browse

File Browse selects a saved data file—with experimental parameters—upon which to perform an advanced scan. DataMax uses the data file's experiment parameters for the scan.

To select a data file's experiment parameters for an advanced scan,

1 Click *File Browse*.

The ***Select File Name*** dialog box appears:



2 Choose the desired data file.

3 Click *OK*.

The ***Select File Name*** dialog box closes, and the experiment's name appears in the *Data File Name* field in the advanced scan's window.

Note: *For a polar scan, choose only non-polarizer experiments.*

Warning: *The original data are overwritten when the experiment is run.*

G Factor

The *G* factor, or grating factor, is a correction for the wavelength response to polarization of the emission optics and detectors. See the *Polarizers for Spex® Spectrofluorometer Systems Operation Manual* for more detail about the *G* factor. Found on the polar scan window, *G Factor* sets or measures the *G* factor for the system.

To set a predetermined G factor,

1. Click on the *G Factor* field.
2. Enter the *G* factor.

To let the system measure the G factor,

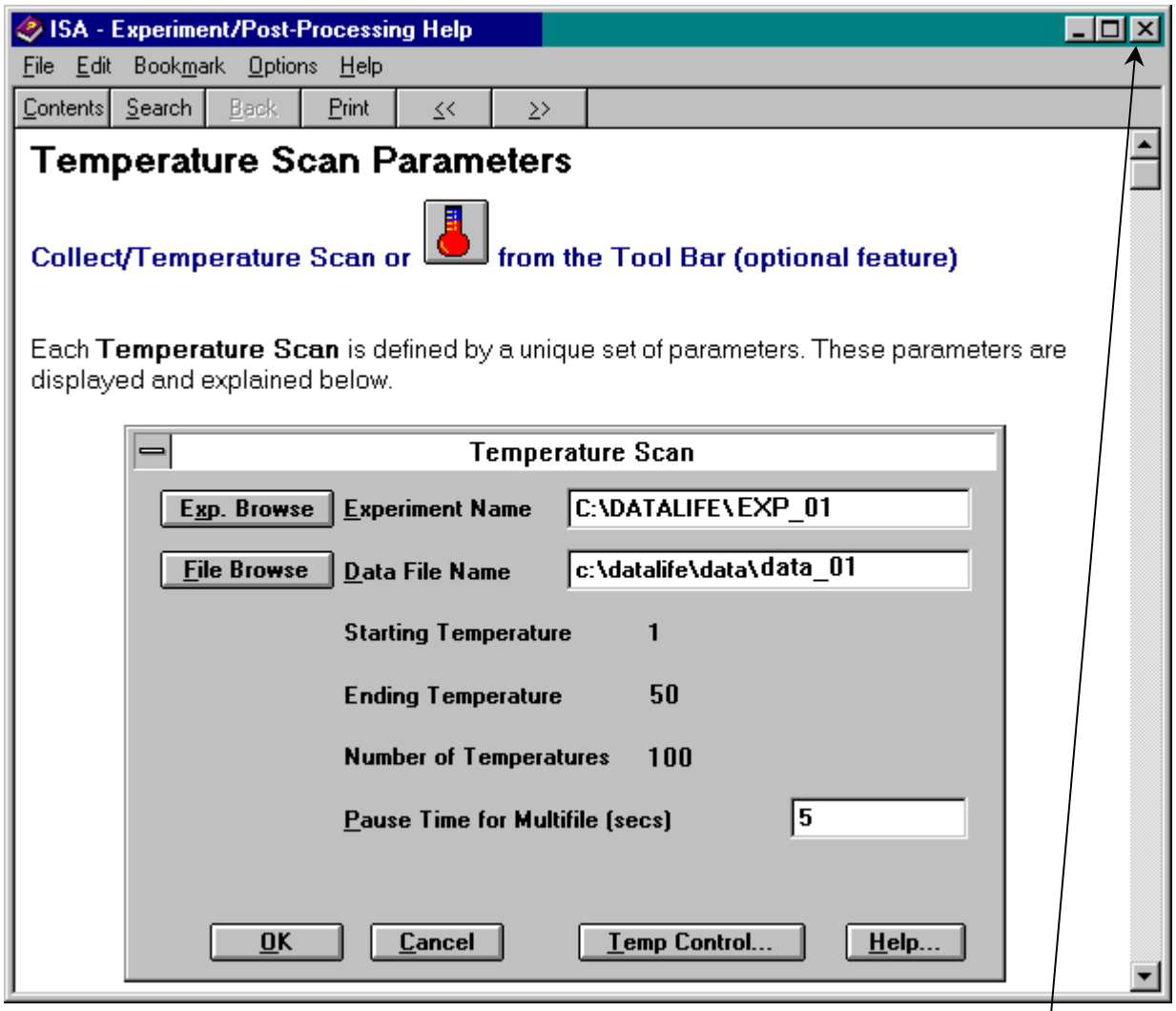
1. Click on the *G Factor* field.
2. Enter 0 (zero).

Help... **Help…**

Most advanced scan dialog boxes contain a help button. For additional on-line help,

1 Click *Help….*

A ***Help*** window appears:



2 Click on the feature of interest.

A window containing an explanation of the feature appears.

3 Click the ☒ box in the upper right corner.

The ***Help*** window closes.

Mask Rayleigh

When light travels through transparent media (e.g., solutions, air), a small fraction is scattered. Rayleigh scattering is related to the inhomogeneities in the molecular structure, or caused by particles smaller than the wavelength of the incoming light. Glass is an example in which small fluctuations in the refractive index induced by its amorphous nature scatter light in all directions, without changing the frequency of the scattered light, because the inhomogeneities are locked into the glass's microscopic structure. DataMax has the ability to mask these strong Rayleigh-scattering peaks in a spectrum, in order to observe nearby Raman bands. The *Mask Rayleigh* checkboxes are found on the matrix scan dialog box. The *Mask Rayleigh 1st order* and *Mask Rayleigh 2nd order* checkboxes tell DataMax to block the first-order and second-order Rayleigh lines, respectively.

To mask the first-order Rayleigh scatter,

1 Check the checkbox next to *Mask Rayleigh 1st order*.

To mask the second-order Rayleigh scatter,

1 Check the checkbox next to *Mask Rayleigh 2nd order*.

Number of Positions

Number of Positions is found on the microplate and polar scan windows; it displays the total number of wells that the MicroMax is to scan. This field is not directly adjustable in this window. Instead, DataMax calculates it from parameters set using *Plate Control*….

Number of Scans

Found on the matrix scan dialog box, *Number of Scans* sets the number of scans that the system runs, including the one at the *Starting Z-axis Value* and the *Ending Z-axis Value*.

To choose the number of scans in the matrix scan,

1. Click on the *Number of Scans* field.
2. Enter the number of scans.

Number of Temperatures

On the temperature scan window, the *Number of Temperatures* displays the total number of different temperatures at which the system will acquire data. This value is not accessible using this window. Instead, DataMax calculates the *Number of Temperatures* from the specified temperatures, using the *Temp Control*... button.

Pause Time between Scans

Only found on the matrix scan window, *Pause Time between Scans* sets the time, in seconds, that each spectrum remains on the screen before the next scan is run.

To set the pause time,

1. Click on the *Pause Time between Scans* field.
2. Enter the length of time.

Pause Time for Multifile

Pause Time for Multifile sets the time, in seconds, that each spectrum remains on the screen before the next scan is run. Enter 0 for no pause time.

To set the pause time,

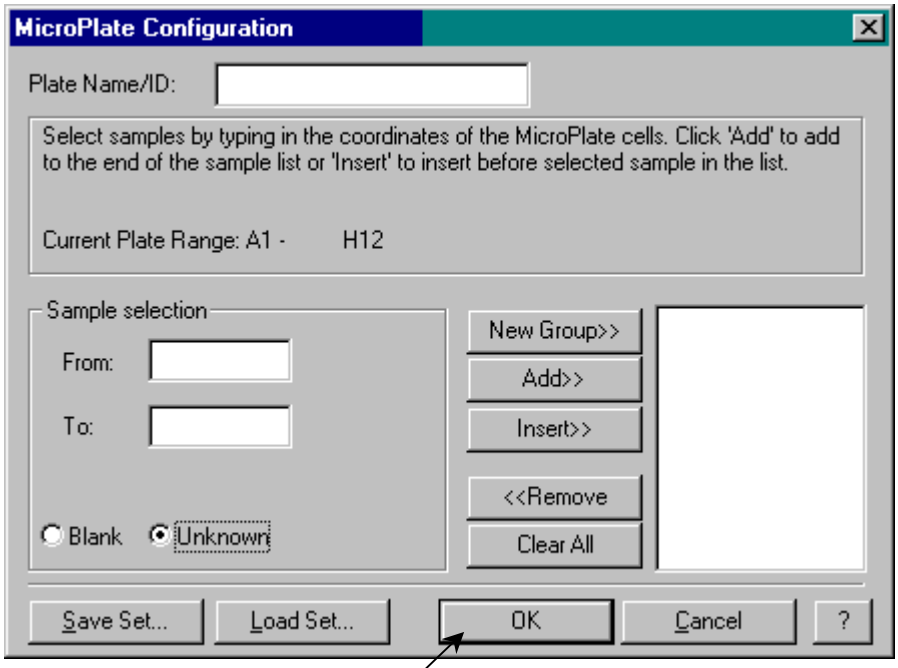
1. Click on the *Pause Time for Multifile* field.
2. Enter the length of time.

Plate Control… Plate Control…

Plate Control… selects the particular wells to scan in a microplate scan.

1 Click *Plate Control…*.

This opens the ***MicroPlate Configuration*** dialog box:



2 Enter the correct parameters:

- Plate Name
- Groups of blanks or unknowns

3 Click *OK*.

Note: *For more information on running microplate scans and selecting wells, see the* MicroMax Operation Manual.

Polarization

Polarization is available only with polar scans. When the *Polarization* checkbox is enabled, the system records the polarization values during a polar scan.

To record the polarization component of the signal,

1 Click the checkbox next to *Polarization*.

Raw

Raw is only available in polar scans. When the *Raw* checkbox is enabled, the system records the four raw values (VV, VH, HV, HH) during a polar scan. Four files are created: one for each set of polarizer orientations.

To record the raw components of the signal,

1 Click the checkbox next to *Raw*.

Starting Temperature 25

Starting Temperature

Not accessible through the temperature scan dialog box, the *Starting Temperature* displays the temperature at which the experiment must start. The temperature unit is specified in the original experiment. The temperature controller adjusts to this temperature before starting the scan.

Note: *Set the* Starting Temperature *using the* Temp Control… *button.*

Starting Z-axis Value

Available with a matrix scan, the *Starting Z-axis Value* sets the starting wavelength (in the original experiment's units) for the matrix scan. The originally fixed monochromator begins here at the start of the matrix scan.

To set the starting point,

1. Click on the *Starting Z-axis Value* field.
2. Enter the starting value.

Sum of slit widths

Sum of slit widths is visible on the matrix scan window. The *Sum of slit widths* sets the total bandpass of the system during a matrix scan.

To set the sum of slit widths,

1. Click on the *Sum of slit widths* field.
2. Enter the bandpass.

Temp Control...

In a temperature scan, use *Temp Control...* to set starting and ending temperatures, the number of temperatures, temperature tolerance, and equilibration time.

1 Click *Temp Control...*.

The ***Temperature Control*** dialog box opens.

To set the starting temperature for the experiment,

a Click in the field under *Start Temp*.

b Enter the desired temperature.

To set the ending temperature for the experiment,

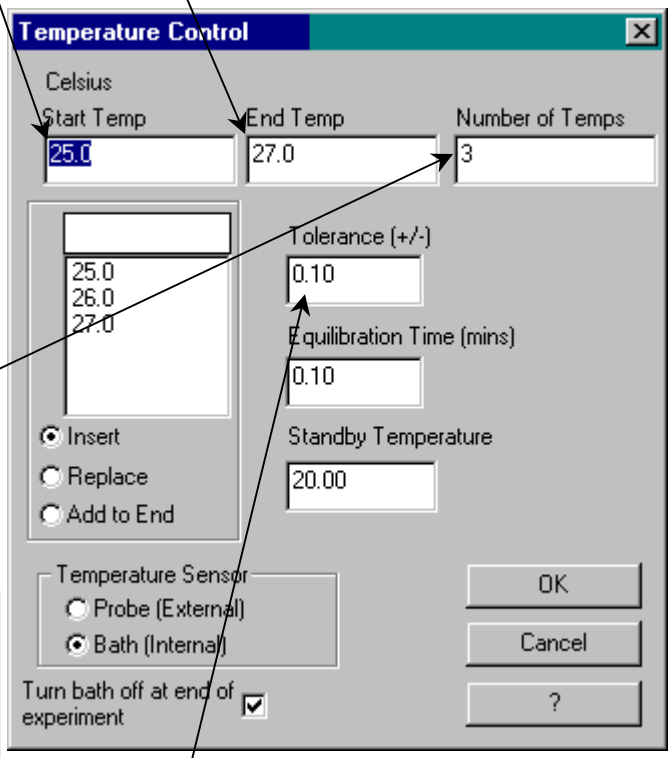
a Click in the field under *End Temp*.

b Enter the desired temperature.

To set the total number of temperatures during the run,

a Click in the field under *Number of Temps*.

b Enter the number of temperatures.



Note: *The number of temperatures also can be calculated from the specific temperatures to be used in the experiment.*

To set the tolerance of a temperature reading,

a Click in the field under *Tolerance*.

b Enter the tolerance.

This value is the specified accuracy of a temperature reading. The temperature of the sample will be as shown, plus or minus the tolerance, within the length of time designated in *Equilibration Time*.

To set the equilibration time,

a Click in the field under *Equilibration Time*.

b Enter the equilibration time.

The *Equilibration Time* is the time that the temperature controller holds the temperature within the *Tolerance*. The equilibration time starts as soon as the temperature is within the tolerance. If a temperature spike occurs, the timer resets, and the equilibration time restarts.

To set the standby temperature,

a Click in the field under *Standby Temperature*.

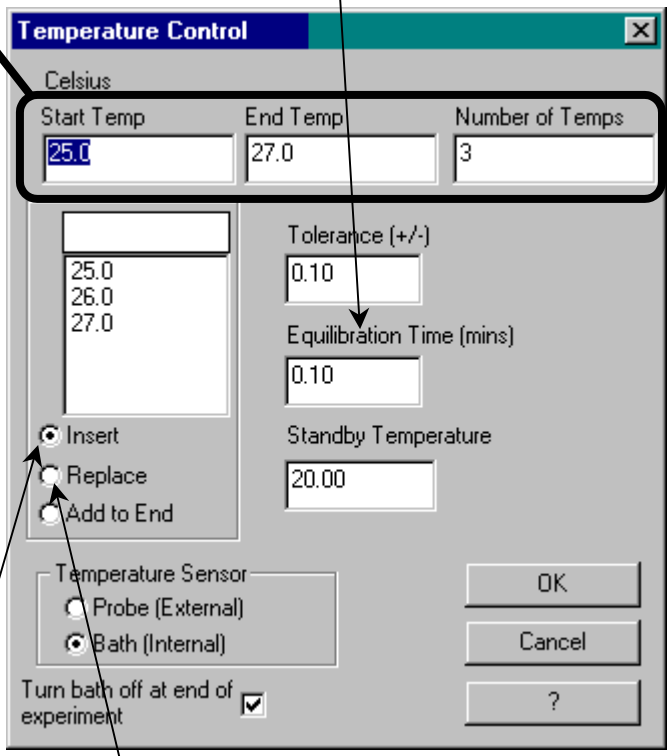
b Enter the standby temperature.

The *Standby Temperature* is the temperature to which the temperature controller sets itself after the final measurement. If the *Turn bath off at end of experiment checkbox* is active, the controller shuts off after it reaches the standby temperature. If the checkbox is not checked, the controller maintains the standby temperature indefinitely.

To specify a list of temperature values automatically,

a Complete *Start Temp*, *End Temp*, and *Number of Temps* fields.

DataMax automatically calculates temperature values and displays them in the list, in ascending order, using a linear scale.



To specify a list of temperature values manually,

a To insert a new temperature in the list,

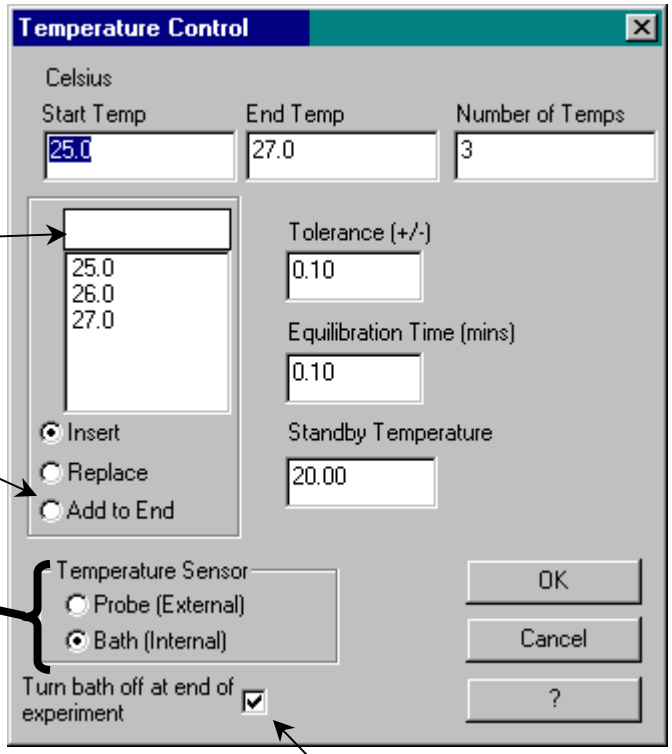
- Place the mouse cursor where the new value should appear.
- Enter the new value.
- Click the *Insert* radio button.

b To replace an existing temperature with a different value,

- Place the mouse cursor where the alternate value should appear.
- Enter the alternate value.
- Click the *Replace* radio button.

c To add a new temperature to the end of the list,

- Place the mouse cursor in the text area above the list.
- Enter the new value.
- Click the *Add to End* radio button.



To choose the type of temperature sensor,

a Click the radio button for external *Probe* or internal *Bath*.

To turn off the temperature controller at the end of the experiment,

a Click the checkbox *Turn off bath at end of experiment*.

2 Click *OK*.

The ***Temperature Control*** dialog box disappears, and the starting and ending temperatures are displayed.

Trigger Time Base Scan

For a matrix scan, the *Trigger Time Base Scan* checkbox tells DataMax to trigger a time-based scan.

To trigger a time-based scan,

1 Click the checkbox.

8: Constant-Wavelength Analysis

Introduction

The ***Constant Wavelength Analysis*** application has two functions:

- Batch collection of single-wavelength emission data from a large number of samples. A record of the fluorescence with respect to all specified acquisition modes is made.
- Quantitative analysis, i.e., determine the concentration of analyte in samples. Two or more standards are used as a reference first, then the unknowns are scanned.

The samples can be in either the system's sample compartment, or the optional Micro-Max microwell-plate reader (connected to the sample compartment with optional fiber-optic cables). After data-acquisition, the data may be exported into a spreadsheet, and analyzed using a spreadsheet program such as Microsoft® Excel™.

In ***Constant Wavelength Analysis***, adjustable parameters include:

- Number of times to scan a sample
- Wavelength at which a sample is scanned
- Statistical analysis
- Locations of sample(s) and blank(s)

Quick guide for constant-wavelength analysis

To start ***Constant Wavelength Analysis***,

In ***Instrument Control Center***,

1. Click the *Constant Wavelength Analysis* button.
   The ***Constant Wavelength Analysis*** main screen appears.
2. Specify the *Wavelength Sets* (the excitation and emission wavelengths for the monochromators).
3. Set *Detector Parameters*:
   a. Accept the default parameters.
   Or
   Enter new *Integration Time*, *Standard Error*, and *Maximum Trials*.
4. Choose to use the sample compartment, or optional MicroMax with microwell plate.
5. Define acquisition modes.
   a. Choose and select optional polarization modes, if available.
   b. Select acquisition modes from the available ones in the list.
   Or
   Define and select custom acquisition modes.
6. Choose optional kinetics or temperature mode, if available.
7. Define optional kinetics or temperature mode, if chosen.
8. Click *Proceed to Acquisitions*.
   This activates the parameters and opens the ***Data Display*** window.

For batch processing without the MicroMax,

In the ***Data Display*** window,

1. Enter an optional comment.
2. Choose *Options….*
3. Choose to display acquisition mode or raw data.
4. Click *Start Acq*.
   The ***New Sample*** dialog box appears.
5. Choose the sample type as unknown.
6. Select the position of the sample (with optional sample changer).
7. Enter a name for the sample, if desired.
8. Place the sample in the correct position in the sample chamber.
9. Choose dark correction, if desired.
   a. Check *Dark correction enabled*.
   b. Click *Dark Values….*

   The ***Dark Values*** dialog box opens,

   a. Enter dark values.
      Or
      Acquire Now.
   b. Click *OK*.
10. Click *Run Sample*.
    Data collection begins.
11. Repeat steps 5 through 10 for the rest of the samples.
    After the last sample is scanned,
12. Click *Cancel*.
    One index card is created per wavelength set. The tab of each index card identifies the wavelength set. To view each index card,

    a. Click on the index card's tab.

The index card moves to the front.

13 Click *Save Data* to save the data on that index card.
0r
Click *Done* to return to the main screen.
0r
Click *Start Acq* to start another run.
0r
Click *Print…* to print a table of the data and parameters.
0r
Click *Append…* or *Delete…* to edit the data.

For batch processing using the MicroMax,

In the ***Data Display*** window,

1. Enter an optional comment.
2. Click *Start Acquisition*.
3. Click *OK* when the ***Concentration Reference*** dialog box appears.
4. Click *OK* when the ***Concentration Fit*** dialog box appears.
   The ***Samples From...*** dialog box appears.
5. Choose *Titer Plate Box*.
6. Click *OK*.
   The ***Titer Plate Configuration*** dialog box appears.
7. Enter a name for the sample, if desired.
8. Choose the type of sample (unknown, standard, blank).
9. Place the samples correctly in the MicroMax.
10. Click *OK*.
    Data collection begins. When done,

    a. Continue data collection.
    - Change the configuration of samples to scan.
    - Click *OK*.
    - The new samples are scanned; the data are appended to the existing data.

    b. Save the titer-plate configuration model.
    - Choose *Save Set...*.
    - This stores the microwell-plate's configuration in a file.

    c. Load a new titer-plate configuration model.
    - Click *Load Set...*.
    - This recalls a previously saved microwell-plate configuration.

    d. End data collection.
    - Click *Cancel*.

The collected information appears on the ***Data Display*** screen. One index card is created per wavelength set. The tab of each index card identifies the wavelength set. To view each index card,

11 Click on the index card's tab.

The index card moves to the front.

12 Click *Save Data* to save the data on that index card.

Or

Click *Done* to return to the main screen.

Or

Click *Start Acquisition* to start another run.

Or

Click *Save Data* to save the parameters and data.

Or

Click *Append*, *Delete*, or *Calc* to edit the data.

Or

Click *Print* to print a table of the parameters and data.

For concentration determination without the MicroMax,

In the ***Data Display*** window,

1. Enter an optional comment.
2. Choose *Options….*
   The ***Data Display Settings*** dialog box opens.
3. Enable fit.
4. Choose type of fit.
5. Choose to display acquisition mode or raw data.
6. Click *OK*.
   The ***Data Display Settings*** dialog box closes.
7. Click *Start Acq*.
   The ***New Sample*** dialog box appears.
8. Choose the sample type (unknown, standard, blank, empty).
9. If standard, enter concentration.
10. Select the position of the sample (with optional sample changer).
11. Enter a name for the sample, if desired.
12. Place the sample in the correct position in the sample chamber.
13. Choose dark correction, if desired.
    a. Check *Dark correction enabled*.
    b. Click *Dark Values….*
    c. The ***Dark Values*** dialog box opens,
    d. Enter dark values.
       Or
       Acquire Now.
    e. Click *OK*.

14 Click *Run Sample*.
Data collection begins.

15 Repeat steps 7 through 14 for the rest of the samples.
After the last sample is scanned,

16 Click *Cancel*.
One index card is created per wavelength set. The tab of each index card identifies the wavelength set. To view each index card,

a Click on the index card's tab.
The index card moves to the front.

17 Click *Save Data* to save the data on that index card.
0r
Click *Done* to return to the main screen.
0r
Click *Start Acq* to start another run.
0r
Click *Print…* to print a table of the data and parameters.
0r
Click *Append…* or *Delete…* to edit the data.

For concentration determination using the MicroMax,

In the ***Data Display*** window,

1. Enter an optional comment.
2. Click *Start Acquisition*.
   The ***Concentration Reference*** dialog box appears.
3. Choose a reference signal.
   The ***Concentration Fit*** dialog box appears.
4. Choose the type of fit.
   The ***Samples From...*** dialog box appears.
5. Choose *Titer Plate Box*.
6. Click *OK*.
   The ***Titer Plate Configuration*** dialog box appears.
7. Choose the type of sample (unknown, standard, blank).
8. Enter a concentration (if the sample is a standard).
9. Enter a name for the sample, if desired.
10. Place the samples correctly in the MicroMax.
11. Click *OK*.
    Data collection begins. When done,

    a. Continue data collection.
    - Change the configuration of samples to scan.
    - Click *OK*.
    - The new samples are scanned; the data are appended to the existing data.

    b. Save the titer-plate configuration model.
    - Choose *Save Set...*.
    - This stores the microwell-plate's configuration in a file.

    c. Load a new titer-plate configuration model.
    - Click *Load Set...*.
    - This recalls a previously saved microwell-plate configuration.

    d. End data collection.

- Click *Cancel*.

The collected information appears on the ***Data Display*** screen. One index card is created per wavelength set. The tab of each index card identifies the wavelength set. To view each index card,

12 Click on the index card's tab.

The index card moves to the front.

13 Click *Save Data* to save the data on that index card.

Or

Click *Done* to return to the main screen.

Or

Click *Start Acquisition* to start another run.

Or

Click *Save Data* to save the parameters and data.

Or

Click *Append*, *Delete*, or *Calc* to edit the data.

Or

Click *Print* to print a table of the parameters and data.

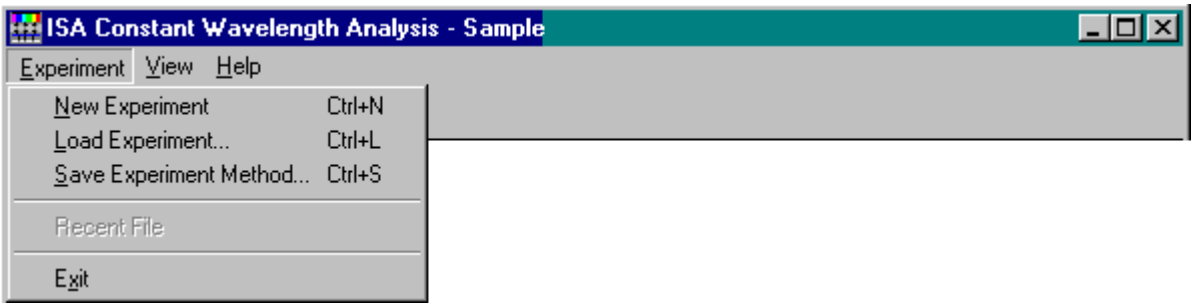
Main menu

The main menu of ***Constant Wavelength Analysis*** contains three items:

- Experiment
- View
- Help

Clicking on any of the above three items reveals a drop-down menu with choices. This section describes those choices in detail.

Experiment



The *Experiment* command deals with creating, loading, and saving constant-wavelength analysis experiments. There is a provision for quitting ***Constant Wavelength Analysis***.

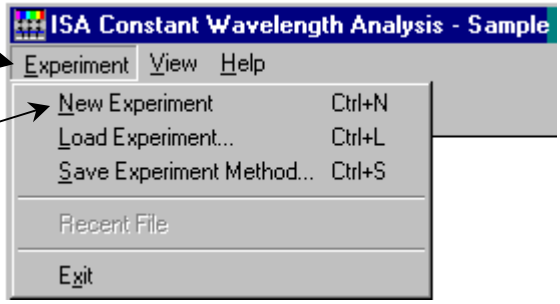
New Experiment

The *New Experiment* command has two functions. The first function is to clear existing settings and entries in the ***Constant Wavelength Analysis*** application. The second function is to update the various units, if they were changed in other applications (e.g., ***Visual Instrument Setup***) open at the same time.

To clear settings or update units in ***Constant Wavelength Analysis***,

1 Click *Experiment*.
A drop-down menu appears.

2 Choose *New Experiment*.
All settings disappear, and the units are updated.



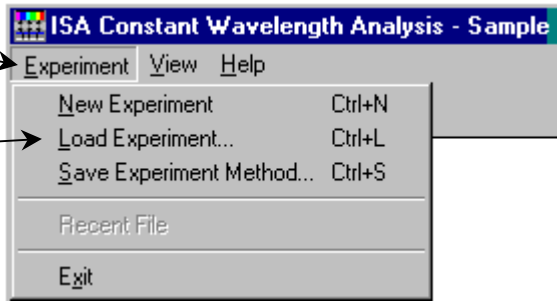
Load Experiment...

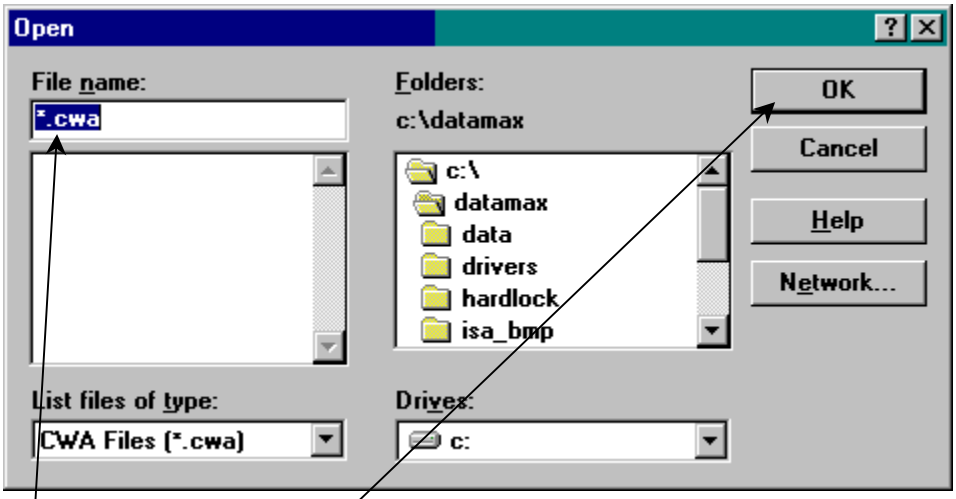
Load Experiment... opens a previously saved experiment (with the `.CWA` extension), to use those experimental parameters.

To open an existing constant-wavelength analysis experiment,

1 Click *Experiment*.
A drop-down menu appears.

2 Choose *Load Experiment...*.
The ***Open*** dialog box appears.





3 Choose an existing constant-wavelength analysis experiment to load.

4 Click *OK*.

The ***Open*** dialog box closes, the desired file opens, and the settings appear in the ***Constant Wavelength Analysis*** window.

Save Experiment Method...

Save Experiment Method... saves the settings and parameters for the constant-wavelength experiment, without the saving any data. The experiment is saved with a `.CWA` extension.

To save a constant-wavelength analysis experimental setup,

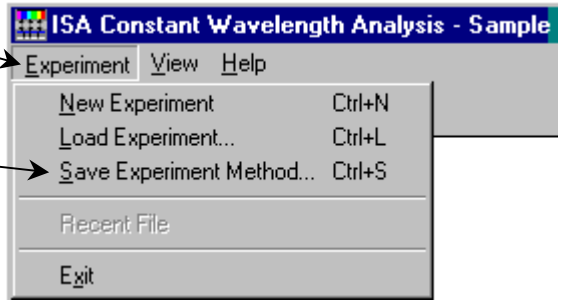
1 Set the experimental parameters.

2 Click *Experiment*.

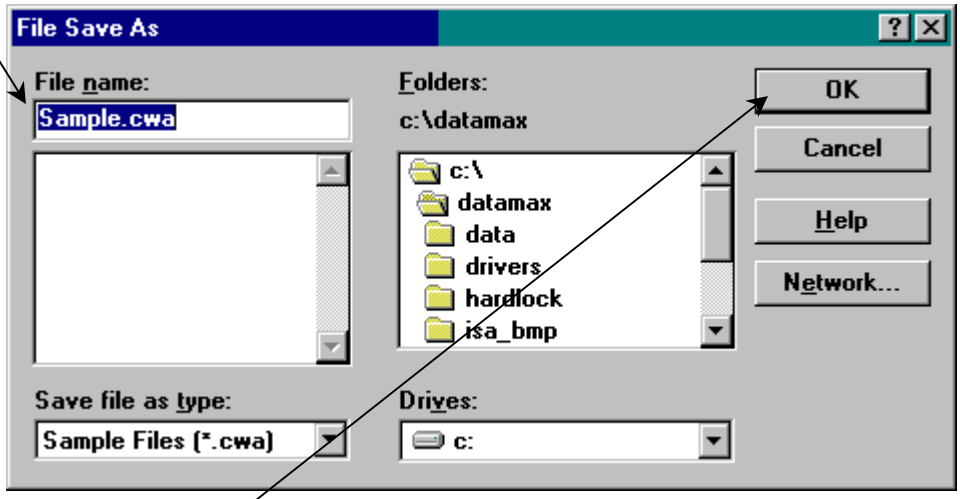
A drop-down menu appears.

3 Choose *Save Experiment Method...*.

The ***File Save As*** dialog box appears.



4 Enter a name for the experiment.



5 Click *OK*.

The ***File Save As*** dialog box closes, and the file is given a `.CWA` extension automatically.

Exit

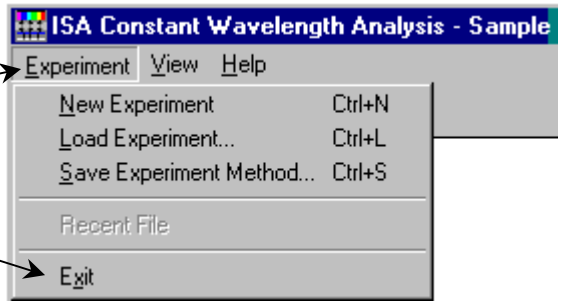
To leave ***Constant Wavelength Analysis***,

1 Click *Experiment*.

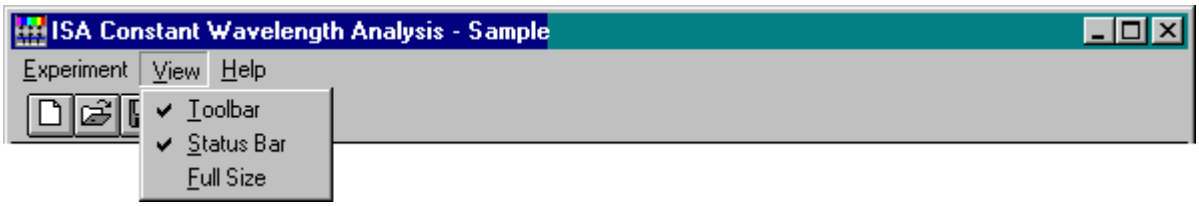
A drop-down menu appears.

2 Choose *Exit*.

The ***Constant Wavelength Analysis*** application closes.



View



The *View* command controls how the ***Constant Wavelength Analysis*** screen appears, affecting the toolbar, the status bar, and the size of the window.

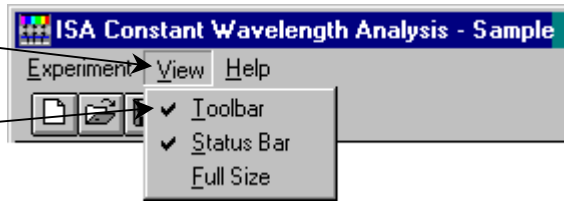
Toolbar

The *Toolbar* option chooses whether or not the toolbar appears on the screen. If a check appears next to *Toolbar*, then the toolbar appears on the screen.

To remove or add the toolbar to the ***Constant Wavelength Analysis*** window,

1 Click *View*.
A drop-down menu appears.

2 Click *Toolbar*.
The toolbar appears or disappears, and a check appears or disappears next to the command.



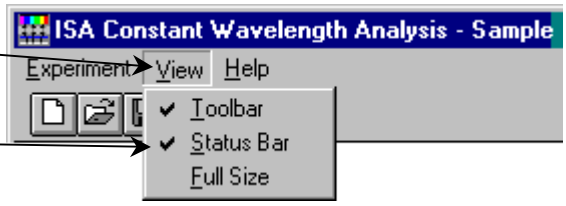
Status Bar

The status bar is the description, on the bottom of the window, of where the mouse cursor is. The *Status Bar* option chooses whether the status bar appears on the screen. If a check appears next to *Status Bar*, then the status bar appears on the screen.

To remove or add the status bar to the ***Constant Wavelength Analysis*** window,

1 Click *View*.
A drop-down menu appears.

2 Click *Status Bar*.
The status bar appears or disappears, and a check appears or disappears next to the command.



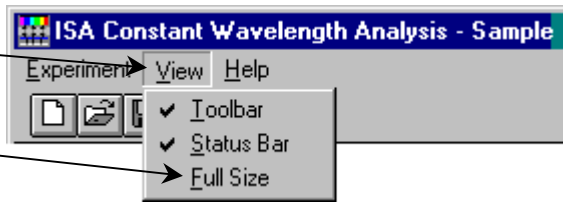
Full Size

The *Full Size* option resizes the ***Constant Wavelength Analysis*** screen. When *Full Size* is activated, the screen reverts to the default size.

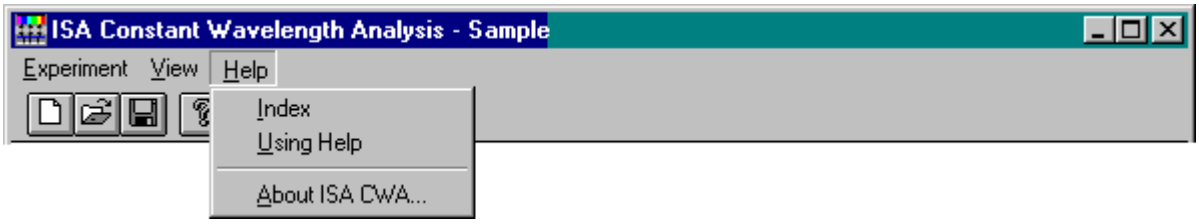
To return the ***Constant Wavelength Analysis*** window to its default size,

1 Click *View*.
A drop-down menu appears.

2 Click *Full Size*.
The ***Constant Wavelength Analysis*** window shrinks or expands back to the default size.



Help



Help provides assistance and information about ***Constant Wavelength Analysis***, such as an index of commands, how to use the *Help* function, and the version level of ***Constant Wavelength Analysis***.

Index

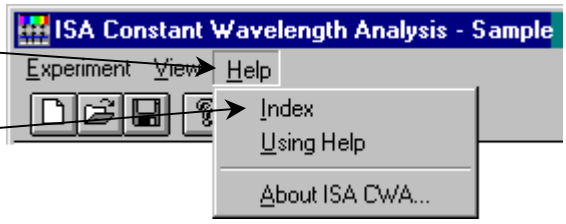
The *Index* command displays detailed information about how to use ***Constant Wavelength Analysis***, toolbar buttons, and commands.

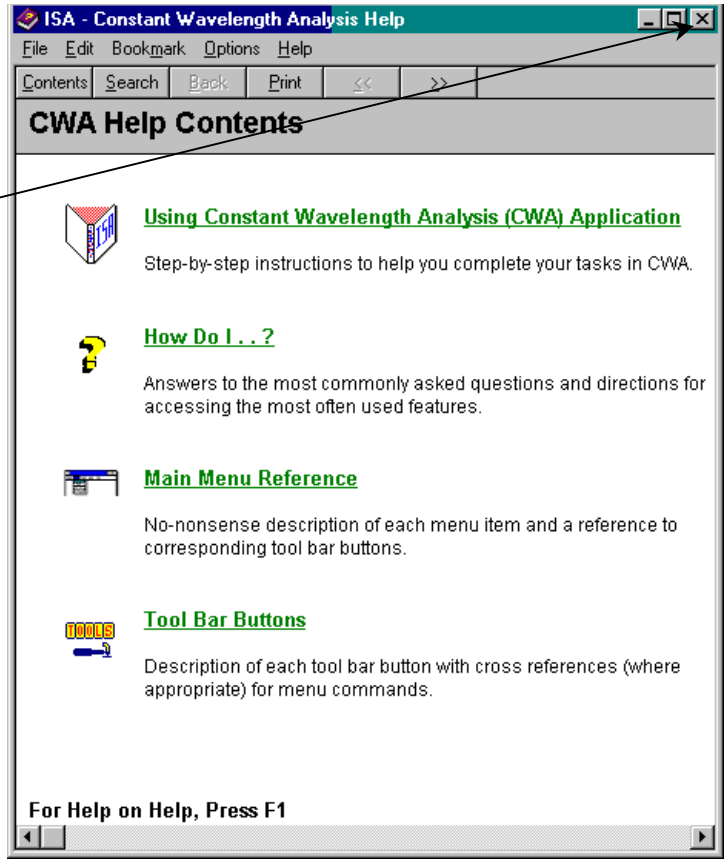
To view help about ***Constant Wavelength Analysis***,

1 Click *Help*.
A drop-down menu appears.

2 Click *Index*.
The ***CWA Help Contents*** window opens.

3 Click the ☒ box in the upper right corner.
The ***CWA Help Contents*** dialog box closes.





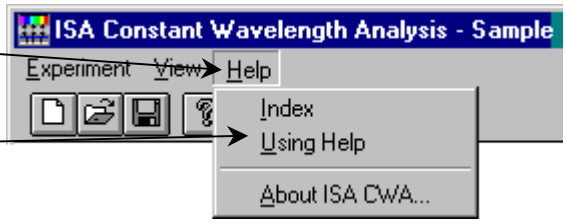
Using Help

The *Using Help* command shows information about how to use Windows™ help functions.

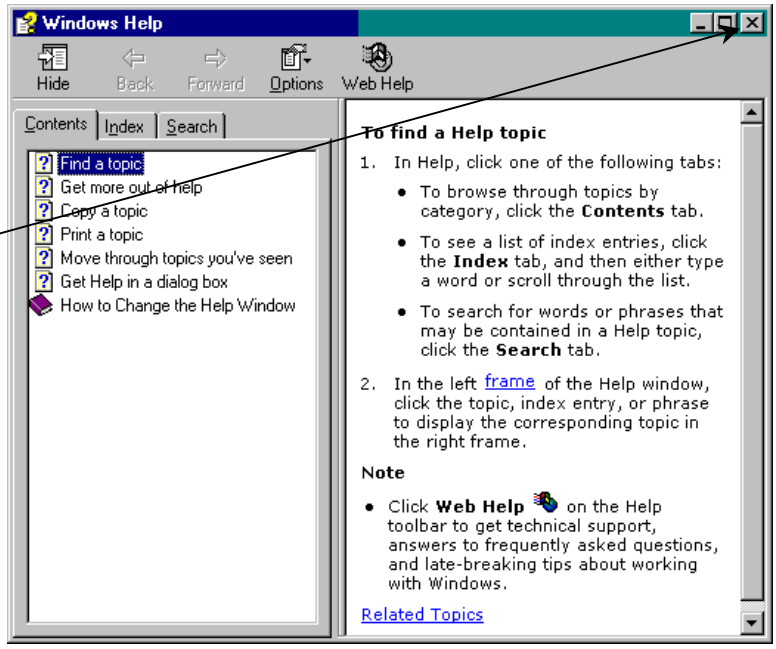
To view information about Windows™ help,

1 Click *Help*.
A drop-down menu appears.

2 Click *Using Help*.
The ***Windows Help*** dialog box appears.



3 Click the ☒ box in the upper right corner.
The ***Windows Help*** dialog box closes.



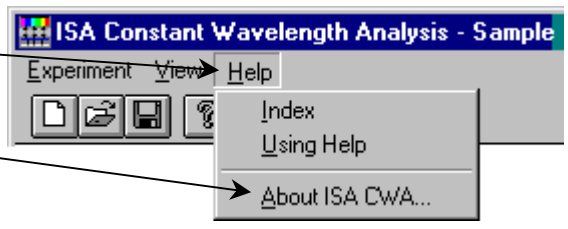
About ISA CWA...

About ISA CWA... provides the version number and copyright date for the ***Constant Wavelength Analysis*** application.

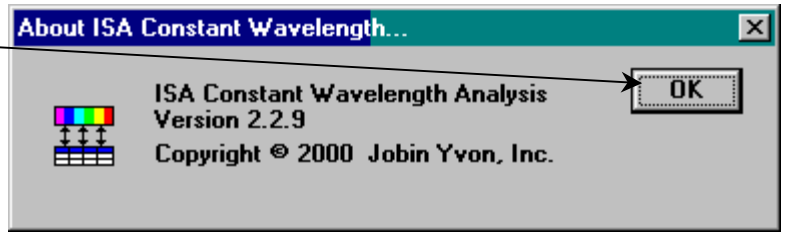
To view the version number and copyright,

4 Click *Help*.
A drop-down menu appears.

5 Click *About ISA CWA...*.
The ***About ISA Constant Wavelength...*** dialog box opens.



6 Click *OK*.
The dialog box closes.



Toolbar 

The toolbar provides shortcut buttons for commonly used commands in ***Constant Wavelength Analysis***. The buttons available are:

 New Experiment

 Load Experiment

 Save Experiment Method

 About ISA CWA

 Help

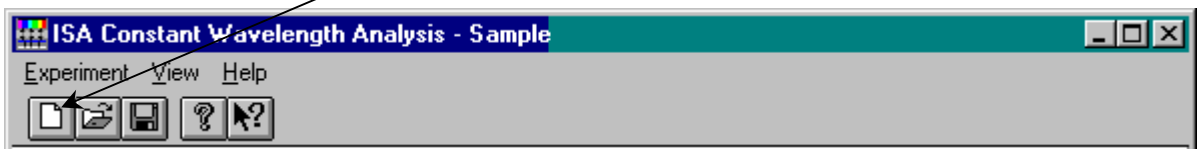
Clicking on any of these buttons activates the corresponding command. The following section describes these buttons in detail.

New Experiment

The *New Experiment* command has two functions. The first function is to clear existing settings and entries in the ***Constant Wavelength Analysis*** application. The second function is to update the various units, if they were changed in other applications (e.g., ***Visual Instrument Setup***) open at the same time.

To clear settings or update units in *Constant Wavelength Analysis*,

1 Click *New Experiment*.



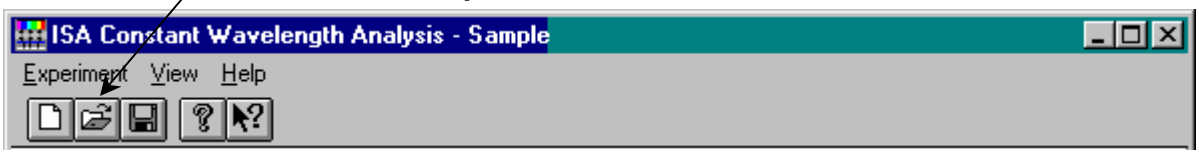
All settings disappear, and the units are updated.

Load Experiment

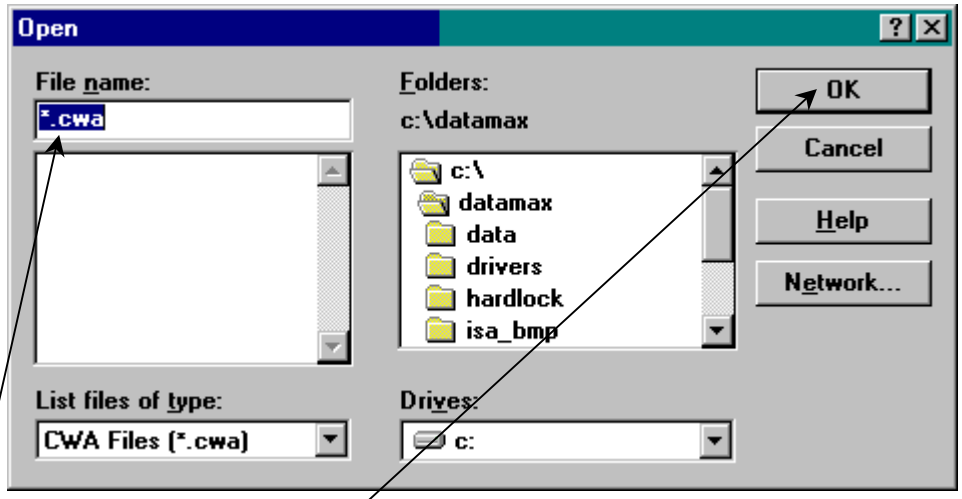
Load Experiment opens a previously saved experiment (with the `.CWA` extension), to use those experimental parameters.

To open an existing constant-wavelength analysis experiment,

1 Click *Load Experiment*.



The ***Open*** dialog box appears.



2 Choose an existing constant-wavelength analysis experiment to load.

3 Click *OK*.

The ***Open*** dialog box closes, the desired file opens, and the settings appear in the ***Constant Wavelength Analysis*** window.

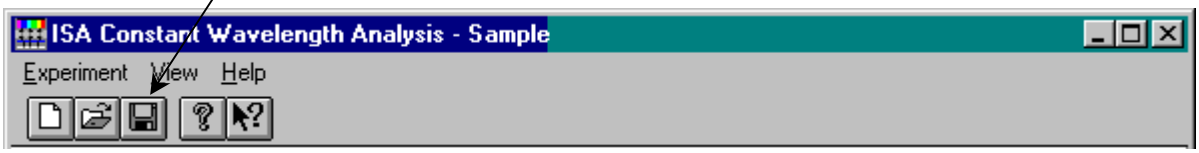
Save Experiment Method...

Save Experiment Method... saves the settings and parameters for the constant-wavelength experiment, without the saving any data. The experiment is saved with a `.CWA` extension.

To save a constant-wavelength analysis experimental setup,

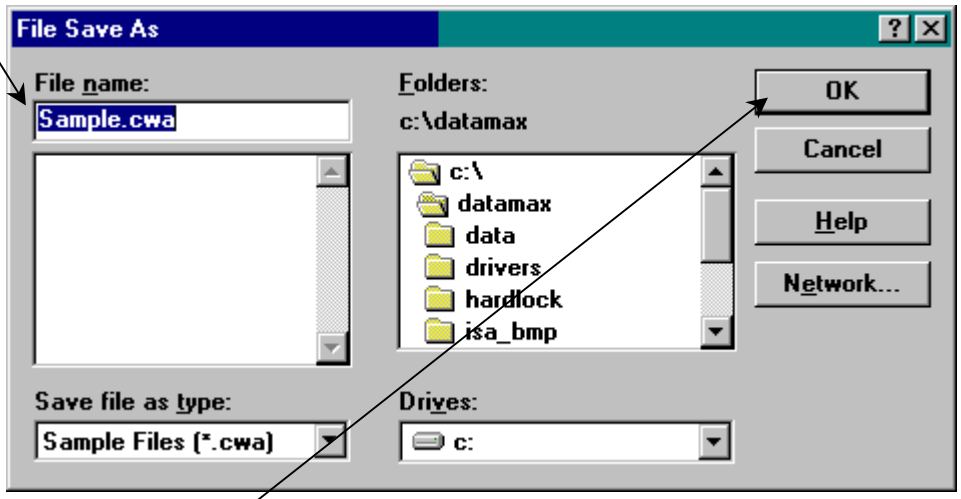
1 Set the experimental parameters.

2 Click *Save Experiment Method*.



The ***File Save As*** dialog box appears.

3 Enter a name for the experiment.



4 Click *OK*.

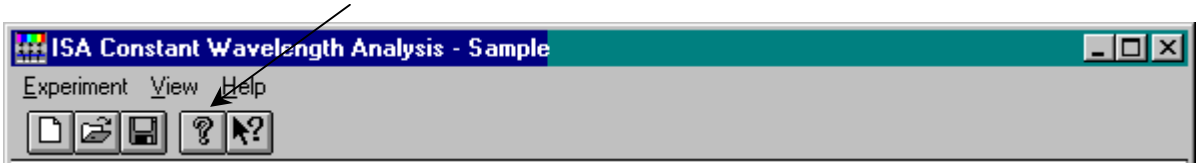
The ***File Save As*** dialog box closes, and the file is given a `.CWA` extension automatically.

About ISA CWA...

About ISA CWA... provides the version number and copyright date for the ***Constant Wavelength Analysis*** application.

To view the version number and copyright,

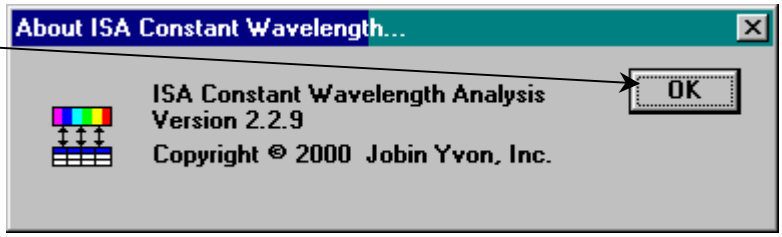
1 Click *About ISA CWA....*



The ***About ISA Constant Wavelength...*** dialog box opens.

2 Click *OK*.

The dialog box closes.

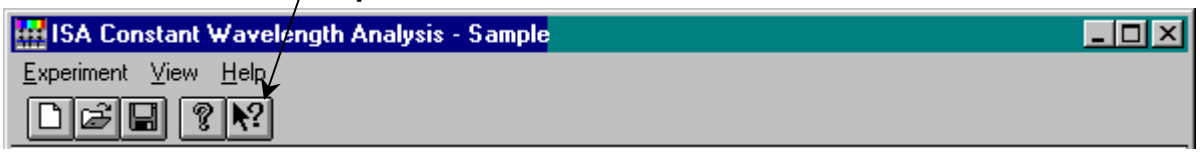


Help

The *Help* button provides information about functions and settings in the ***Constant Wavelength Analysis*** window.

To get help in the *Constant Wavelength Analysis* window,

1 Click *Help*.



The cursor—normally shaped like an arrow—becomes an arrow with a question mark, a floating-help cursor.

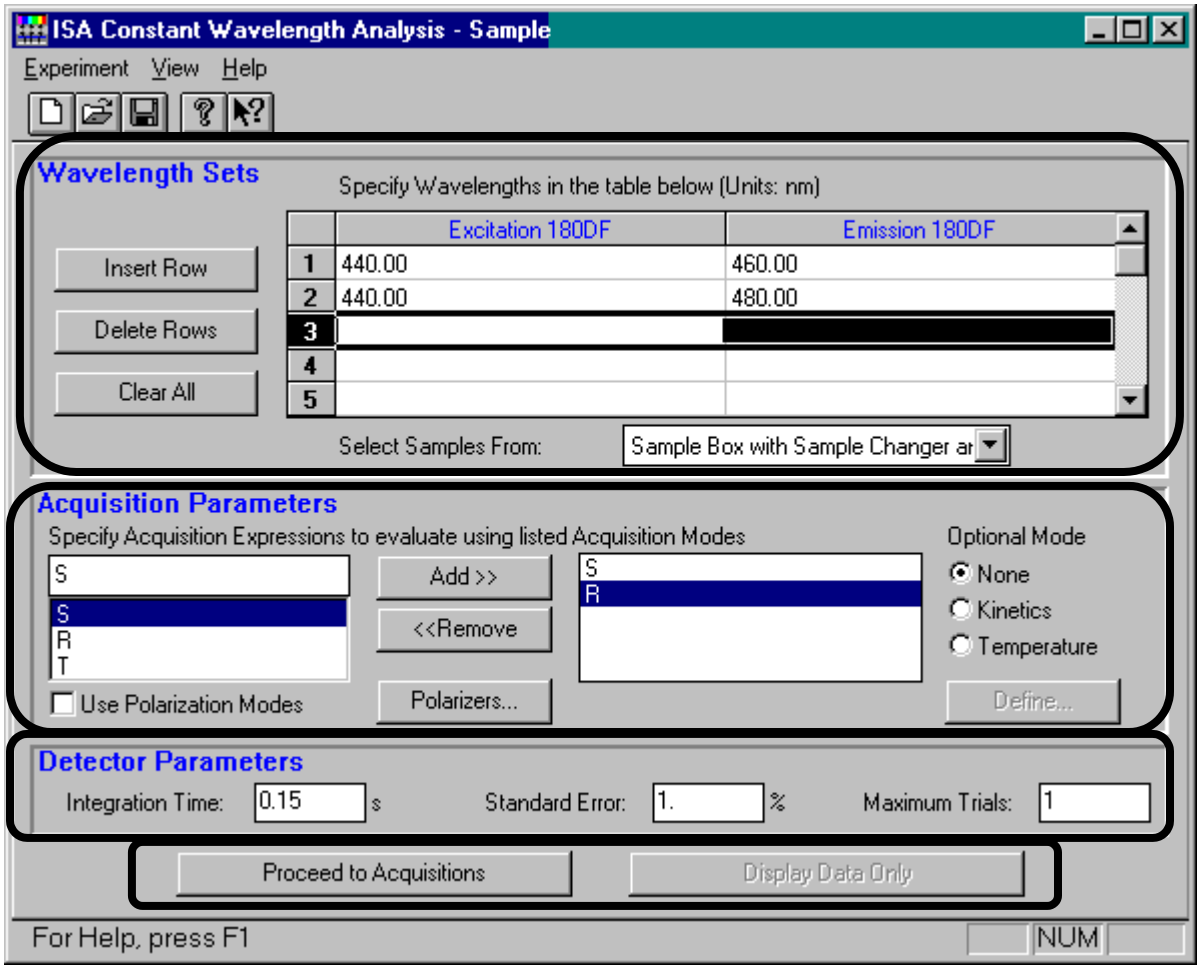
2 Move the *Help* cursor onto the desired field or button.

3 Click on the setting.

If available, a ***Help*** window appears concerning that field or button. If no help is available on that topic, the general ***Help*** contents appear.

Description and operation

Constant Wavelength Analysis main screen

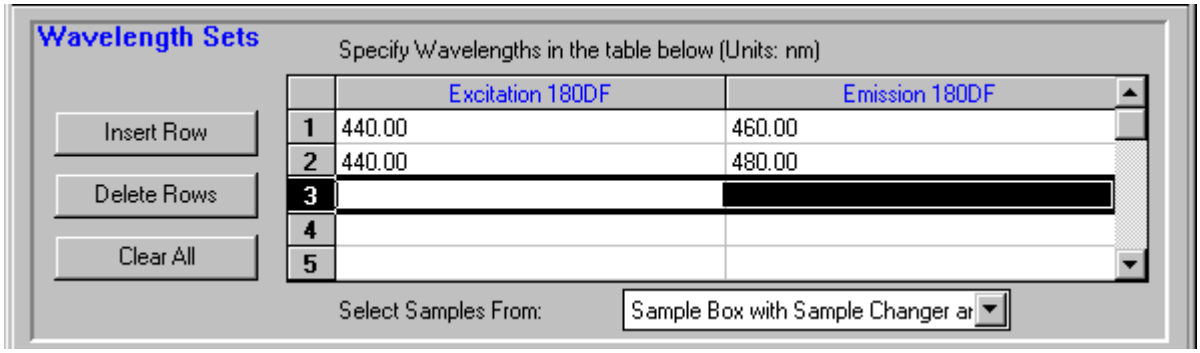


There are several areas on the main entry screen in ***Constant Wavelength Analysis***:

- Wavelength Sets
- Acquisition Parameters
- Detector Parameters
- Buttons

These areas specify the parameters required to run a constant-wavelength analysis acquisition. This section discusses in detail the commands and fields in each area.

Wavelength Sets



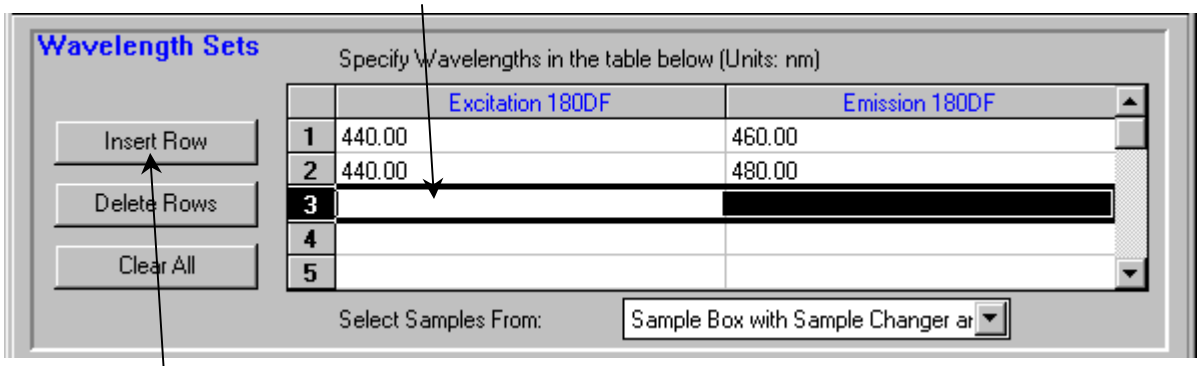
Scanning wavelengths for each monochromator are set in this area. One column for each monochromator appears in the table: one excitation monochromator, and one or two emission monochromators (depending on the system configuration).

Up to 27 sets of wavelengths can be monitored. For example, with the sets 300 nm and 500 nm, 350 nm and 550 nm, and 400 nm and 600 nm, the following procedure occurs:

> In a system with one excitation and one emission monochromator, the excitation monochromator irradiates a sample at 300, 350, and 400 nm, while the emission monochromator monitors fluorescence at 500, 550, and 600 nm.

To specify the monochromators' wavelengths,

1 Enter the wavelength in the field underneath each monochromator.



2 Repeat step 1 for each new set of wavelengths.

To insert a row,

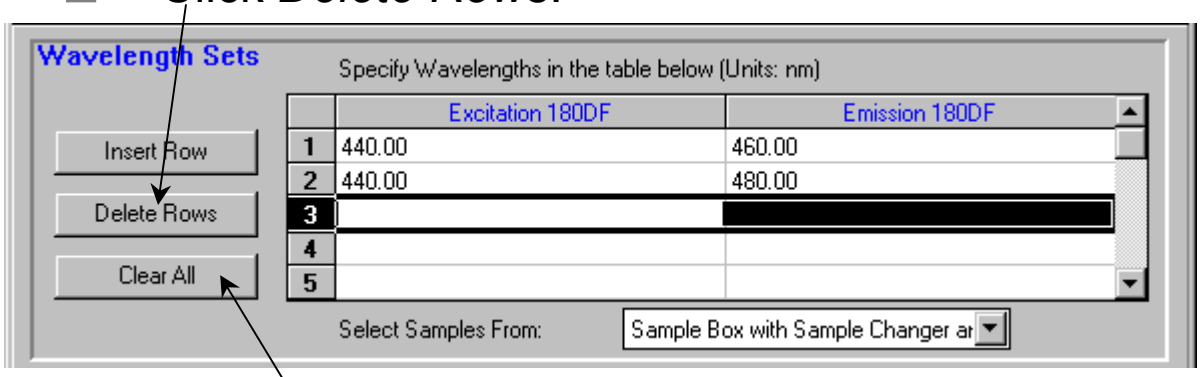
1 Click on the set, above which a new row should appear.

2 Click *Insert Row*.
The existing set moves down one row.

To remove a set of wavelengths,

1 Click on the set to eliminate.

2 Click *Delete Rows*.

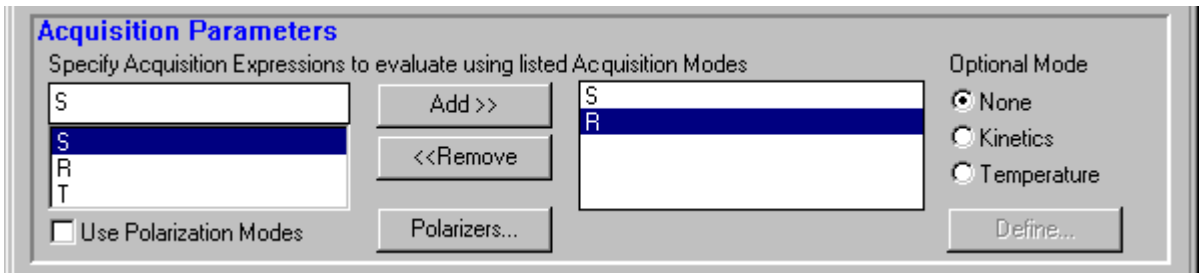


The sets underneath move upward.

To clear all sets from the table,

1 Click *Clear All*.

Acquisition Parameters



The *Acquisition Parameters* area sets the detector modes to record, and what type of options to use, such as:

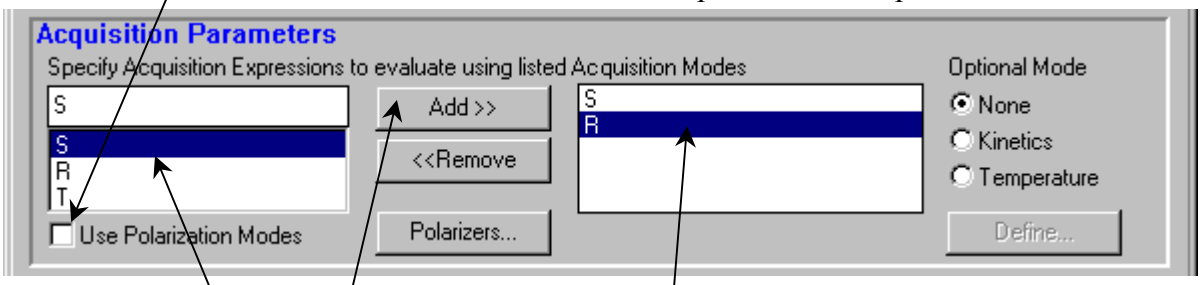
- Polarizers and polarizer setup
- Kinetics acquisition
- Temperature acquisition

An acquisition mode specifies what happens to the raw data channel before it is recorded. The raw data may be recorded unchanged (e.g., S, R, or T), a combination of data channels (e.g., S/R, (S+T)/R, or T*3), or even blank-subtracted or corrected data. Polarization records special modes (VV, HH, etc.); see the *Polarizers Operation Manual* for more information.

To add an acquisition mode to record,

1 Click the optional checkbox to *Use Polarization Modes*, if desired.

The list of data channels on the left side updates to show polarization modes.



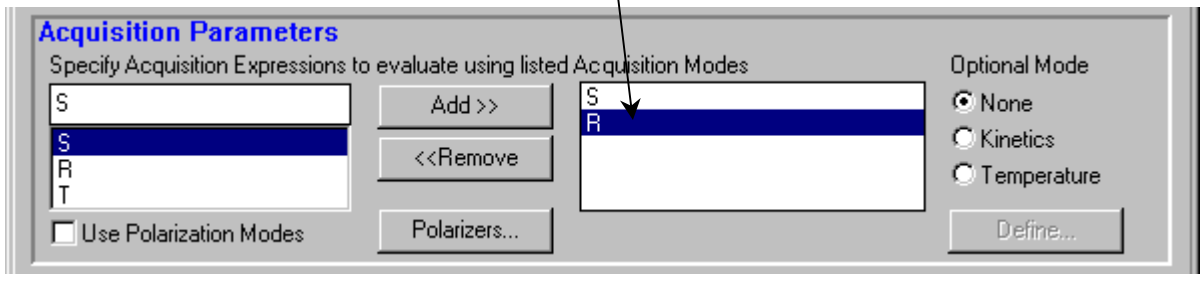
2 Click on the desired available mode.

3 Click *Add»*.

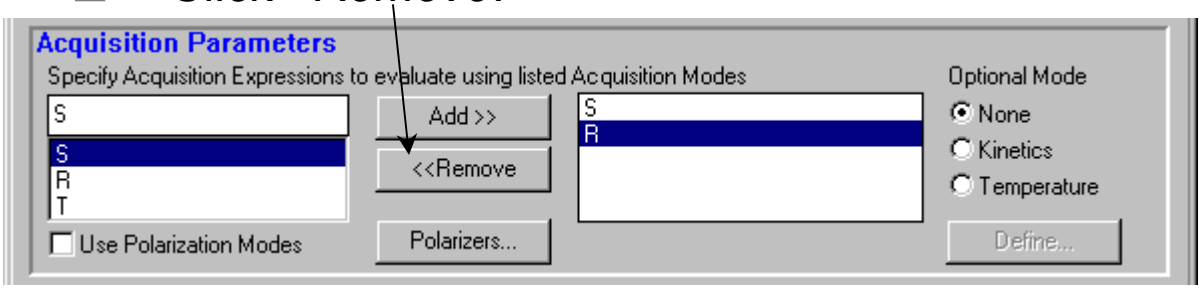
The mode appears in the current acquisition-mode list.

To remove an acquisition mode from the current list to record,

1 Click on the mode to remove.



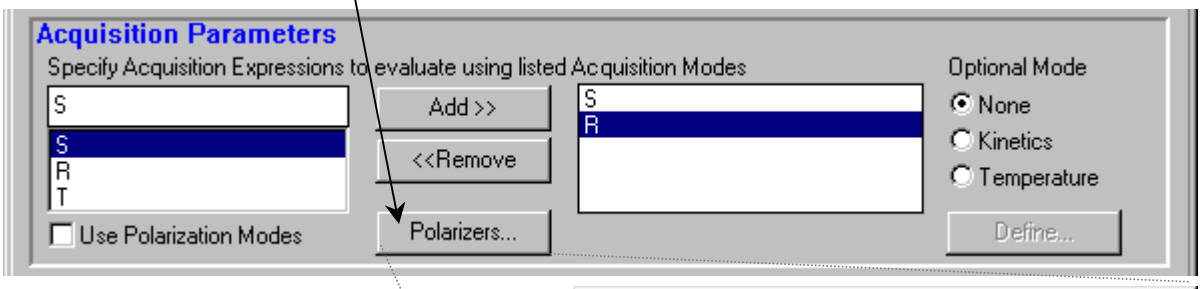
2 Click *«Remove*.



The mode disappears, and any modes underneath move upward.

To specify L-format or T-format for polarizers,

1 Click *Polarizers…*.

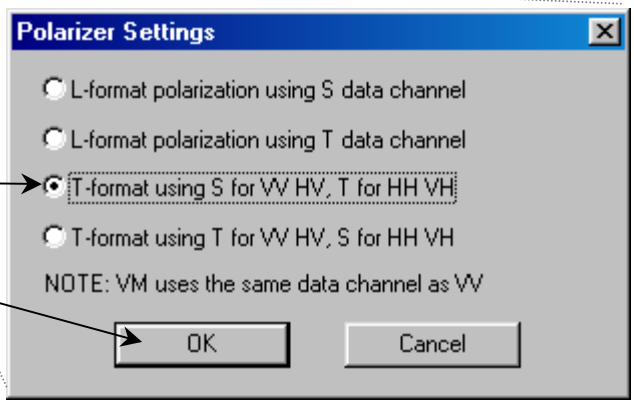


The ***Polarizer Settings*** dialog box appears.

2 Click the desired radio button.

3 Click *OK*.

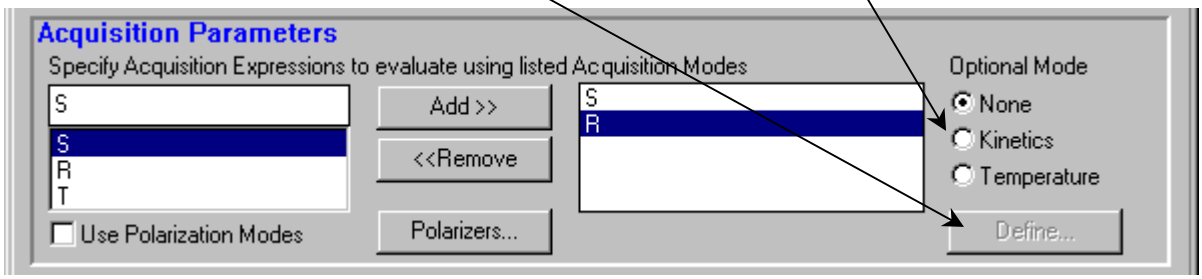
The ***Polarizer Settings*** dialog box closes.



To run a kinetics constant-wavelength analysis experiment,

1 Click the *Kinetics* radio button.

2 Click *Define…*.



The ***Time-based Acquisition Parameters*** dialog box appears.

3 Enter a numerical value and choose units (*s* or *min*) for *Repeat Acquisition Every*.
This tells DataMax the time interval between repetitions.

4 Enter a number value and choose units (*s* or *min*) in *for a total of*.
This tells DataMax the time for the whole experiment.

5 For a variable sampling interval, fill in the *Time Sequence List* table.

a Enter a *Start At* time.

b Enter a *Time Increment* between acquisitions.

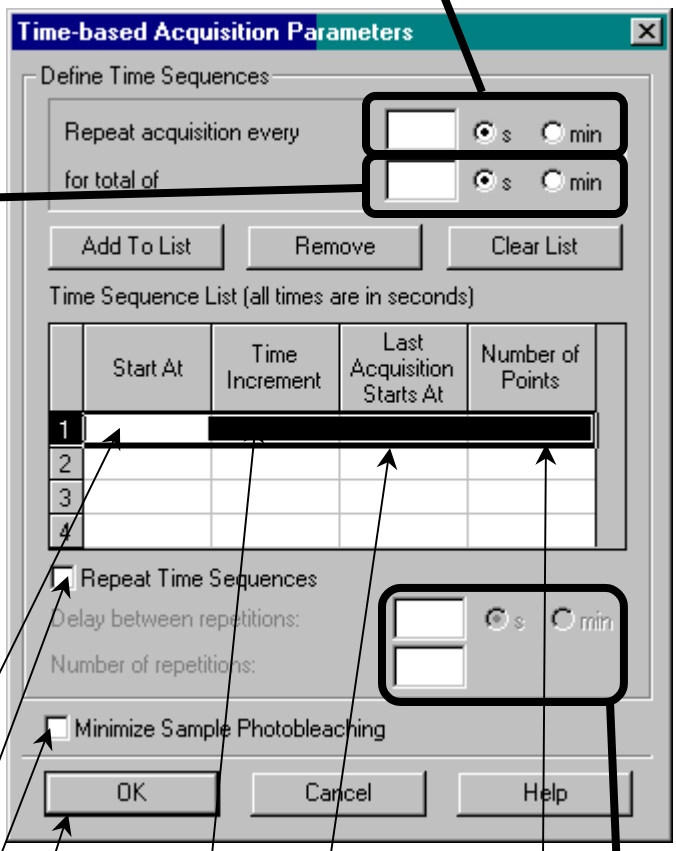
c Enter a time for the *Last Acquisition Starts At*.

d Enter the number of data points to acquire per section in *Number of Points*.

e Check the *Repeat Time Sequences* checkbox, if desired.

f If the *Repeat Time Sequences* checkbox is enabled, fill in the *Delay between repetitions* time and units, and the *Number of repetitions*.

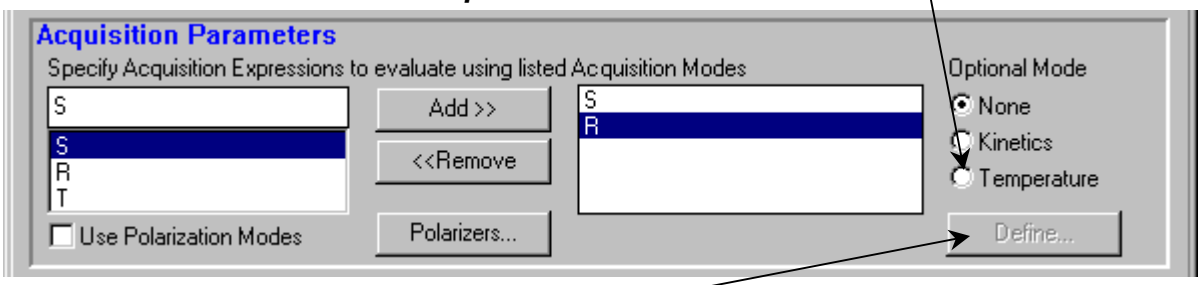
g Check the optional *Minimize Sample Photobleaching* checkbox.
This closes the shutter between data points for intervals > 4 s between acquisitions.



6 Click *OK*.

To run a temperature-based constant-wavelength analysis acquisition,

1 Click the *Temperature* radio button.



2 Click *Define….*

The ***Temperature Setup*** dialog box opens.

a Enter a *Start* Temperature.

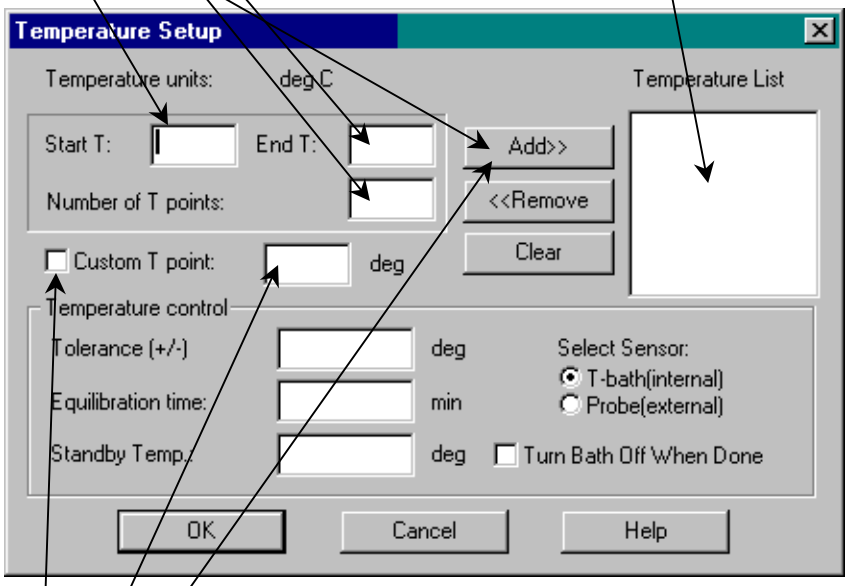
b Enter an *End* Temperature.

c Enter the total *Number of T points* between the start and end.

d Click *Add»*.

Steps a through d set up a linear sequence of temperatures, which appears in the *Temperature List* on the right.

To add a custom temperature,



e Check the *Custom T point* checkbox.

f Enter the temperature (in °C) in the *deg* field.

g Click *Add»*.

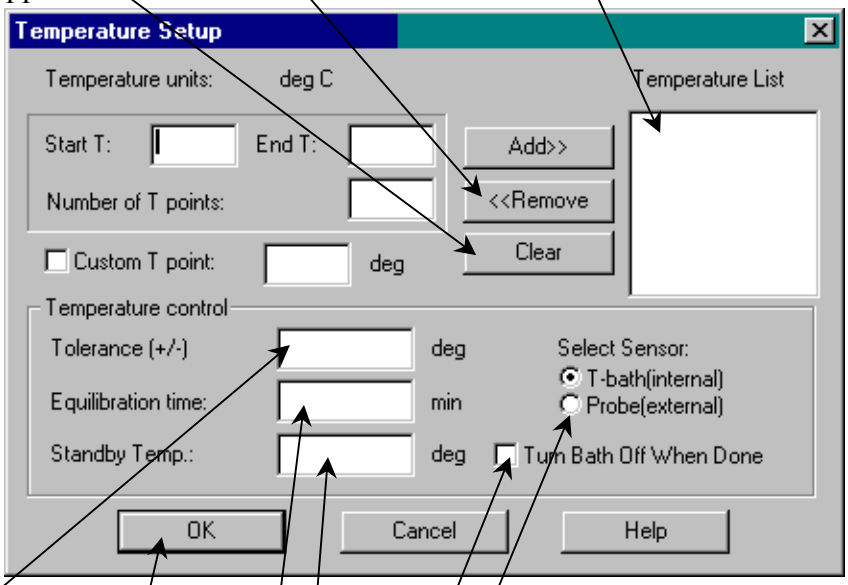
To remove a temperature from the *Temperature List*,

h Click on the desired temperature in the list.

i Click *«Remove*.
The temperature disappears, and any temperature beneath it moves up.

To clear all temperatures from the *Temperature List*,

j Click *Clear*.
All values disappear from the list.



k Enter the *Tolerance* (in °C).
This sets the precision of the temperature controller.

l Enter the *Equilibration Time* (in min).
This sets the length of time for the temperature controller to reach the temperature, to within the *Tolerance*.

m Set the *Standby Temp* (in °C).
This sets the temperature of the bath when the experiment ends.

n Check the *Turn Bath Off When Done* checkbox, if desired.
This shuts off the temperature control at the end of the experiment.

o Choose the desired sensor.
Choose a probe for the temperature bath or external probe, if available.

3 Click *OK*.
The ***Temperature Settings*** dialog box closes.

Detector Parameters

In this area, the integration time, allowable standard error, and maximum number of trials are set.

The *Integration Time* is the amount of time data are collected per data point.

The *Standard Error* sets the standard error limit at which to stop averaging readings. If the average reading has a standard error outside this limit, the acquisition may be stopped before *Maximum Trials* is reached. At least two readings will be taken, however. Mathematically, *Standard Error* is defined as

$$Standard\ Error = \frac{\text{standard deviation}}{\sqrt{\text{number of trials}}}$$

The *Maximum Trials* sets the maximum number of readings to average for each sample.

To set the integration time,

1. Click on the field next to *Integration Time*.
2. Enter the integration time.

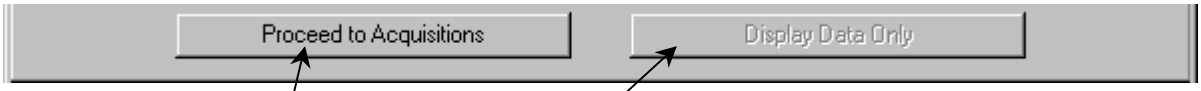
To set the standard error,

1. Click on the field next to *Standard Error*.
2. Enter the standard error.

To set the maximum number of trials,

1. Click on the field next to *Maximum Trials*.
2. Enter the maximum number of trials.

Buttons

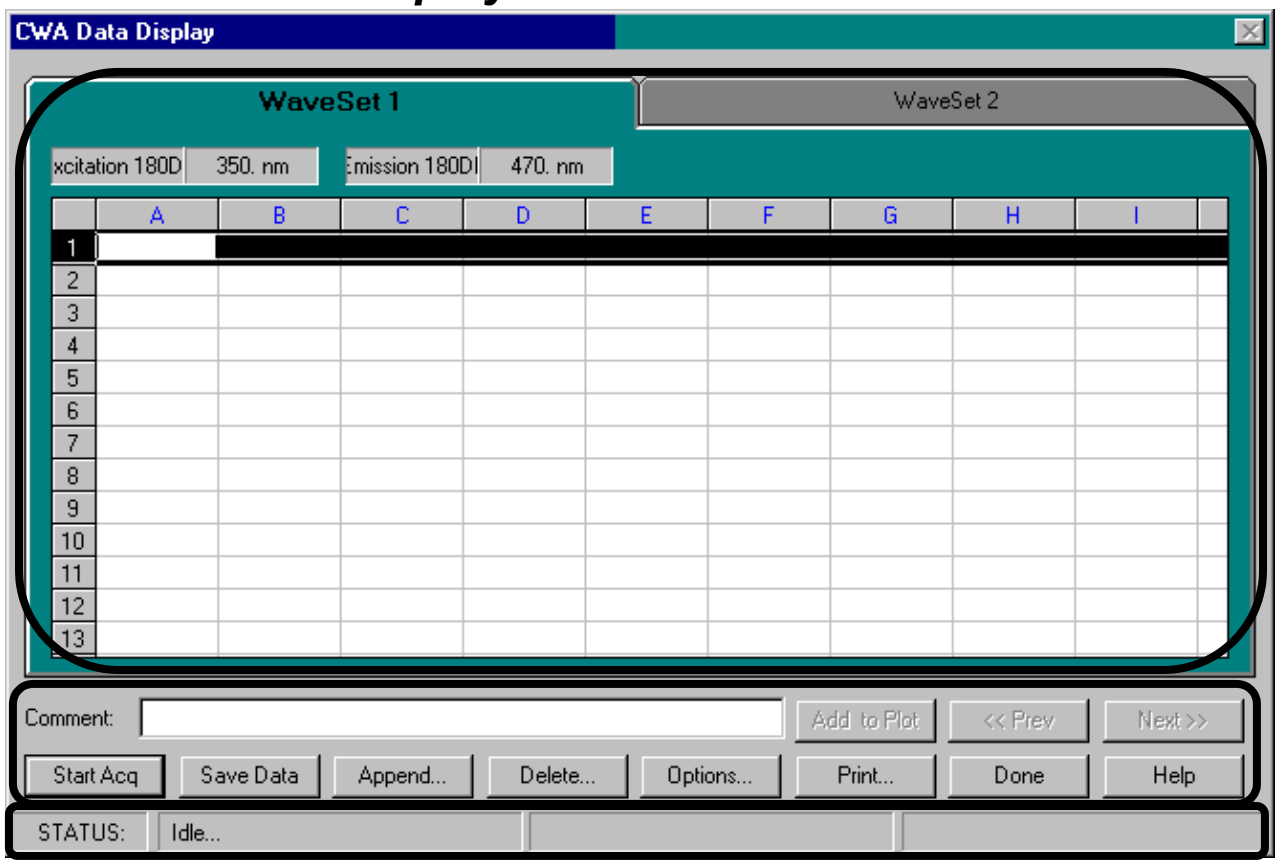


The two buttons in this section execute commands after all constant-wavelength parameters have been set up.

Proceed to Acquisitions
The *Proceed to Acquisitions* button leads to the ***CWA Data Display*** window, in which data are displayed as they are acquired.

Display Data Only
The *Display Data Only* button recalls saved data for display. The data must be in a `.CWA` file, and have been loaded already with the *Load Experiment* command in the ***Run Experiment*** application. If the button is gray and inaccessible, no data are loaded.

CWA Data Display screen



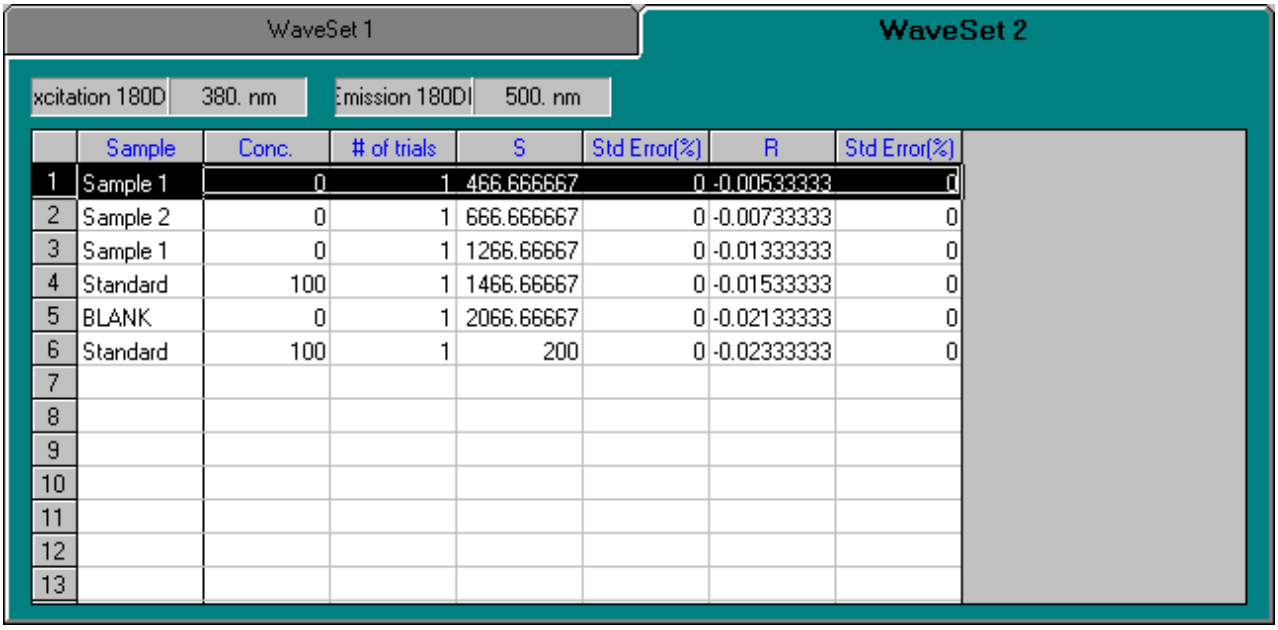
There are three areas on the ***CWA Data Display*** window:

- Index cards
- Function buttons
- Status bar

These areas display the data as they are recorded, and after the scan. They also allow manipulation and saving of the data after an experiment.

Shown above is how the ***CWA Data Display*** window appears before the start of an experiment.

Index cards



	Sample	Conc.	# of trials	S	Std Error(%)	R	Std Error(%)
1	Sample 1	0	1	466.666667	0	-0.00533333	0
2	Sample 2	0	1	666.666667	0	-0.00733333	0
3	Sample 1	0	1	1266.66667	0	-0.01333333	0
4	Standard	100	1	1466.66667	0	-0.01533333	0
5	BLANK	0	1	2066.66667	0	-0.02133333	0
6	Standard	100	1	200	0	-0.02333333	0
7							
8							
9							
10							
11							
12							
13							

Each index card on the ***CWA Data Display*** represents a wavelength set. Each index card is ruled with columns for *Sample* (sample identification), *Conc.* (concentration), acquisition-mode data, and optional statistics [*Std Error(%)*, *# of trials*]. Each row on an index card represents an experimental run. In the example shown above, two wavelength-pairs were scanned; the second pair (380 nm excitation, 500 nm emission) is displayed. For this wavelength-pair, 3 samples, 2 standards, and one blank were scanned using acquisition modes S and R.

To switch to a new wavelength-pair,

1 Click the desired index-card tab.

Above the table are listed the types and wavelengths of the system's spectrometers.

Constant Wavelength Analysis is designed to scan a large number of samples, or to perform quantitative analysis of unknowns. Therefore, if samples are not scanned, DataMax assumes that the run is not quantitative analysis, and assigns 0 (zero) to the cells in the concentration column.

When polarizers are enabled, certain options and features are available and others are not. These differences are discussed later in this section.

Function buttons

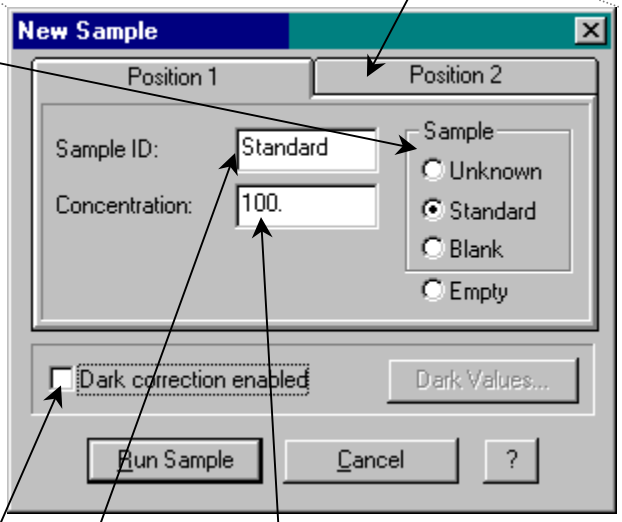
The function buttons change what data is displayed on the table, manipulate the data files, save the data, and print them.

To start an acquisition after all parameters are set,

1 Click *Start Acq*.
The ***New Sample*** dialog box appears.

2 Click on the index-card tab (if displayed).
This chooses the sample position in the optional sample changer.

3 Click the radio button for the type of sample:



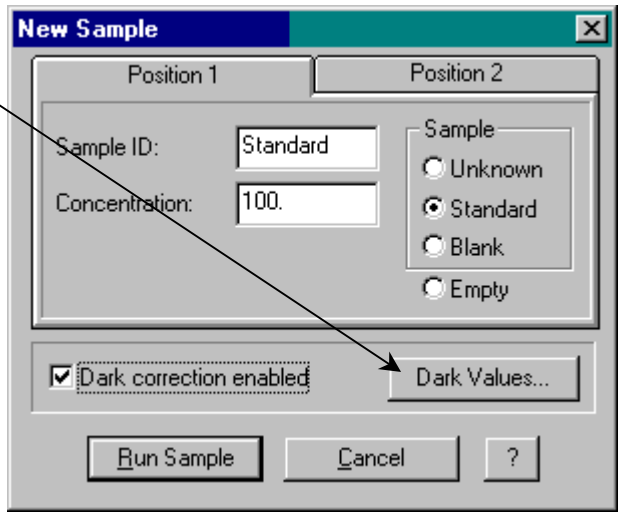
- Unknown: DataMax records the emission data for each data-acquisition mode, and calculates the standard error.
- Standard: DataMax requires a concentration to be entered for quantitative analysis.
- Blank: The active substance is missing from the solution or container. The blank value then is subtracted from the sample to give only the active substance's fluorescence.
- Empty (no sample in the slot)

4 Enter a label in the *Sample ID* field to identify the sample.

5 Enter a *Concentration* (if a standard).

6 Check the *Dark correction enabled* box, if desired.
The *Dark Values…* button becomes accessible.

a Click *Dark Values*….

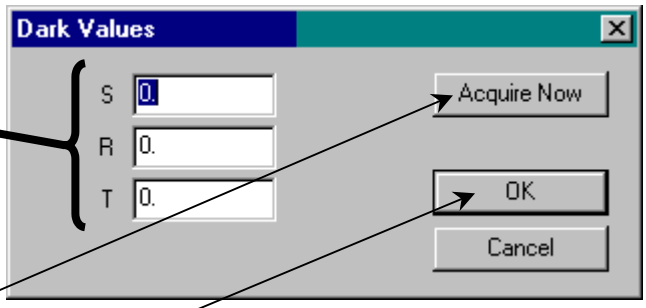


The ***Dark Values*** dialog box appears:

b Enter the dark counts for each detector.

Or

Click *Acquire Now*, to let the instrument measure the dark counts automatically.



c Click *OK* when done.

The ***Dark Values*** dialog box closes.

7 Click *Run Sample*.

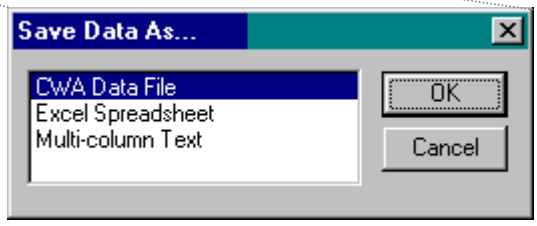
This begins the data acquisition.

To save data and parameters after a run,

1 Click *Save Data*.

The ***Save Data As…*** dialog box opens.

2 Choose the type of file in which to save the data:



- CWA Data File: Parameters plus all data in all wavelength-pairs are saved. DataMax saves the file with the `.CWA` extension automatically.
- Excel Spreadsheet: Only data in the displayed wavelength-pair index card are saved. DataMax saves the file with the `.XLS` extension automatically.

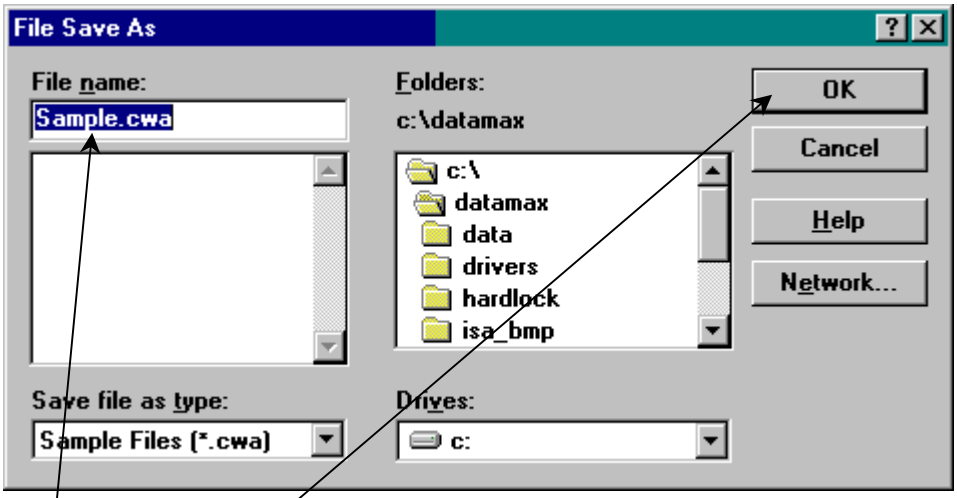
To save another index card, click that index-card tab, and save the data under another name.

- Multi-column Text: Only data in the displayed wavelength-pair index card are saved. Data are saved as a text file with the extension `.TXT`. To save another index card, click that index-card tab, and save the data under another name.

Note: *Excel™ and Text files cannot be opened in* **Constant Wavelength Analysis**.

3 Click *OK*.

The ***Save Data As…*** dialog box disappears, and is replaced by the ***File Save As*** (or ***Save WavelengthSet* x *As…***) dialog box:



a Choose or enter the name of the file.

b Click *OK*.

The ***File Save As*** (or ***Save WavelengthSet* x *As…***) window disappears.

4 If saving as an Excel™ or text file, choose another index card, and repeat steps 1–3.

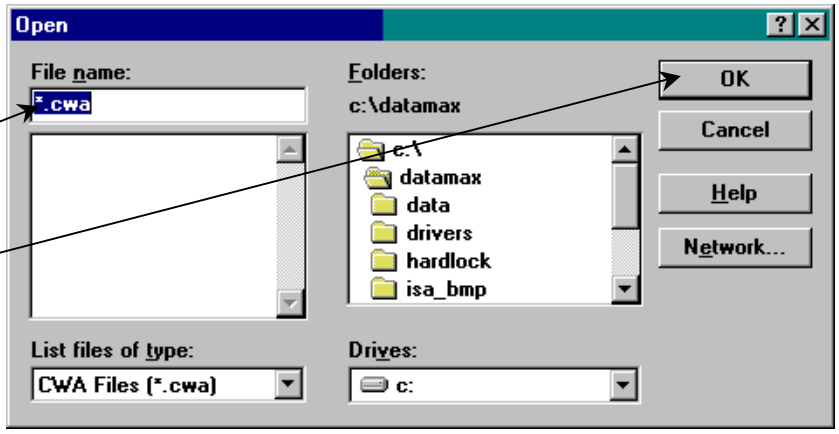
To append an existing data file to a file on screen,

1 Click *Append…*.

The ***Open*** dialog box appears:

2 Select a file to open.

3 Click *OK*.



The ***Open*** dialog box disappears. The data from the newly opened file are appended to those appearing on the ***CWA Data Display*** window.

An advantage of using *Append…* is that standards and unknowns can be run independently of one another. Thus, the file containing unknown data can be pulled into the file containing data about the standards and renamed, while the original standards file remains intact. The original standards file can be used whenever necessary for quantitative analysis of a new group of unknowns.

Note: *The file to which data are to be added must be in the* **CWA Display Data** *window. The two sets of data must have identical parameters, including accessories, wavelength pairs, monochromator positions, etc. If they do not have identical parameters, an error message appears, and the append function cannot continue. Choose a different file with correct parameters.*

To delete one or more runs from the table of data,

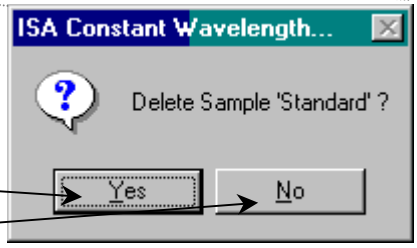
1 Click the row.

This highlights the undesired row of data. For multiple rows, hold the **SHIFT** button while clicking each undesired row.

Note: *Only complete rows can be deleted.*

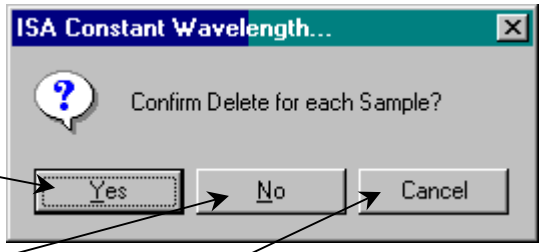
2 Click *Delete…*.

A confirmation dialog box, whose appearance depends on how many rows to be deleted, appears:



a For one row highlighted,
- Click *Yes* to delete.
- Click *No* to leave the data intact.

b For multiple rows highlighted,

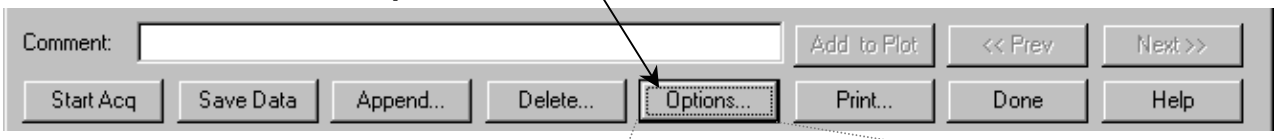


- Click *Yes* to confirm deletion of each separate row.
- Click *No* to delete all rows simultaneously.
- Click *Cancel* to leave the data intact.

The rows below the deleted row move up in the table. *Sample ID* does not change, however. If runs are deleted, concentrations are recalculated automatically.

To change aspects of the display, including statistics, curve-fits, detectors versus acquisition modes, etc.,

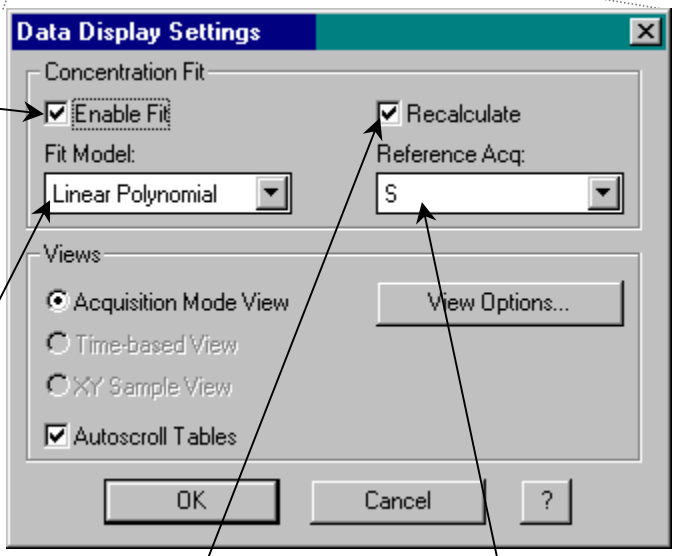
1 Click *Options*….



The ***Data Display Settings*** window appears:

2 Click the *Enable Fit* checkbox, to fit a quantitative analysis curve.

If unchecked, other *Concentration Fit* parameters are inaccessible.



a Choose the *Fit Model* from the drop-down menu:

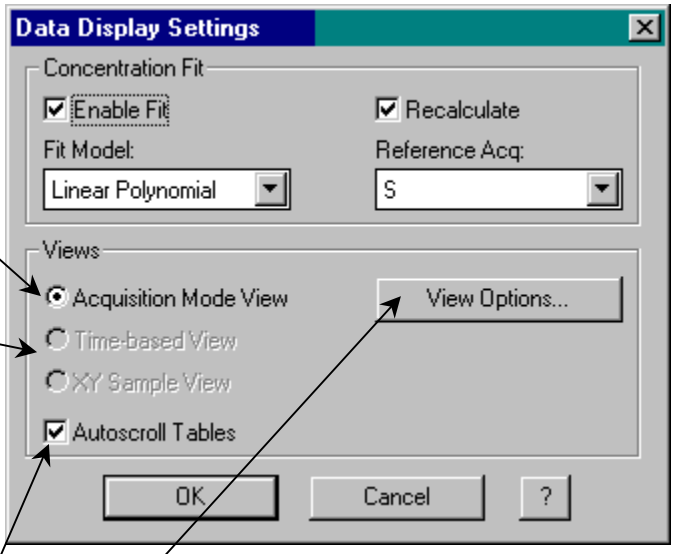
- Linear Polynomial: 2 standards are required to fit the curve
- Quadratic Polynomial: 3 standards are required to fit the curve
- Cubic Polynomial: 4 standards are required to fit the curve

If insufficient standards are supplied, an error message appears and the fit is aborted.

b Click the *Recalculate* checkbox, to refit using a new model when the ***Data Display Settings*** window is closed.

c In the *Reference Acq* drop-down menu, choose the signal that corresponds to the concentration.

3 Adjust the view of the data.



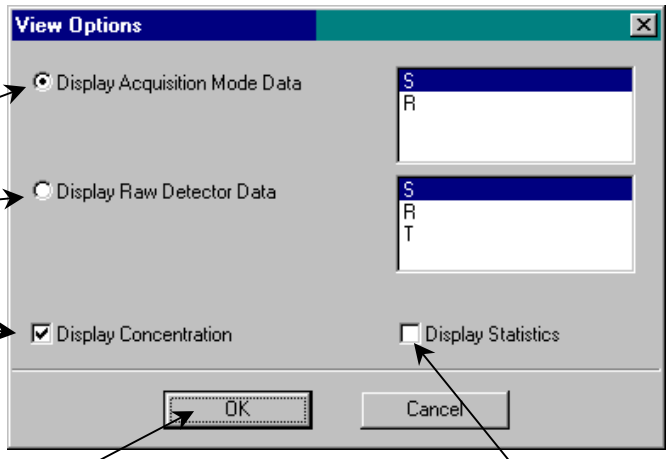
a *Acquisition Mode View* is always active.

b An additional choice *of Time-based View* (for Kinetics) or *XY Sample View* (for MicroMax scans) may be accessible.

c Click the *Autoscroll Tables* checkbox to automatically scroll the table as data are added.

d Click *View Options…*.

The ***View Options*** dialog box appears:



- Choose the type of data to display:
  Acquisition mode: may include algebraic operators
  Raw detector: signals exactly as received from the detectors
- Click the *Display Concentration* check-box, to display a concentration column in the table for quantitative analysis.
- Click the *Display Statistics* checkbox, to display columns for the number of trials and standard error in the table.
- Click *OK*.

The ***View Options*** dialog box closes.

4 Click *OK* in the ***Data Display Settings*** window.

The ***Data Display Settings*** window closes, and the changed settings take effect.

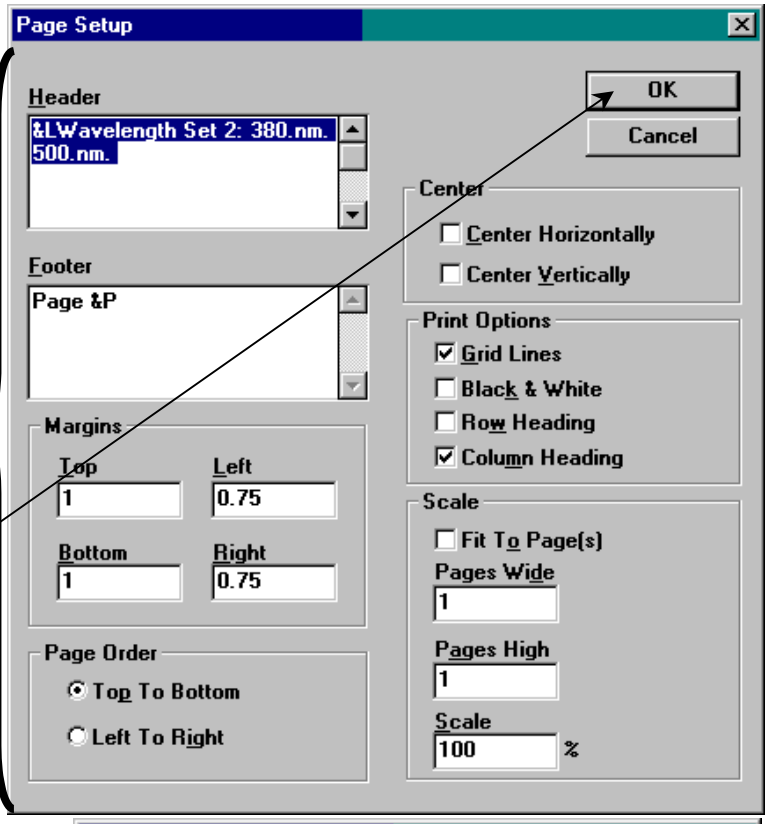
To print the data,

1 Choose the desired wavelength-pair index card.

2 Click *Print…*.

The ***Page Setup*** dialog box opens:

Details on ***Page Setup*** are found at the end of the chapter.

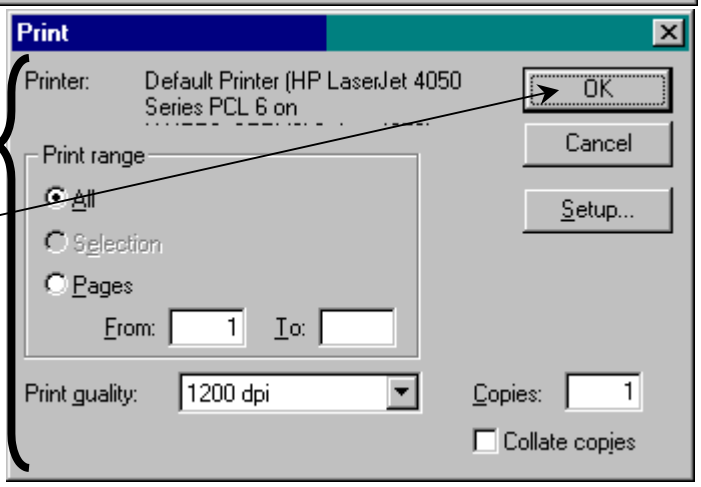


3 Adjust the page-setup parameters as desired.

4 Click *OK*.

The ***Page Setup*** dialog box closes.

The ***Print*** dialog box appears:



5 Choose the parameters.

6 Click *OK*.

The data are printed, and the ***Print*** dialog box closes.

Data appear in the printout as shown below:

Wavelength Set 2: 380.nm 500.nm.

Sample	Conc.	# of trials	S	Std Error(%)	R	Std Error(%)
Sample 1	0	1	466.6666667	0	-0.005333333	0
Sample 2	0	1	666.6666667	0	-0.007333333	0
BLANK	0	1	2066.6666667	0	-0.021333333	0
Standard	100	1	200	0	-0.023333333	0
Standard	100	1	800	0	-0.029333333	0
Standard	100	1	1000	0	-0.031333333	0

To add a comment to the data file,

1 Click in the *Comment* field.

2 Enter text of any length.

To move between data sets,

1 Click *«Prev* or *Next»*.

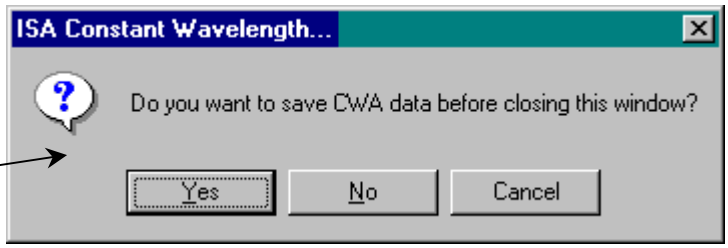
These buttons are only accessible when multiple data sets exist. Multiple data sets are collections of data displayed on different pages, and appear when

- More than 400 samples are scanned, for the spreadsheet handles up to 400 samples (or 200 for kinetics experiments)
- Previous data are appended, and the two files are not merged
- Multiple microwell plates are scanned, for each plate is considered one data set.

When all scans are complete,

1 Click *Done*.

The ***CWA Data Display*** window closes. If the data have not been saved, a prompt appears.

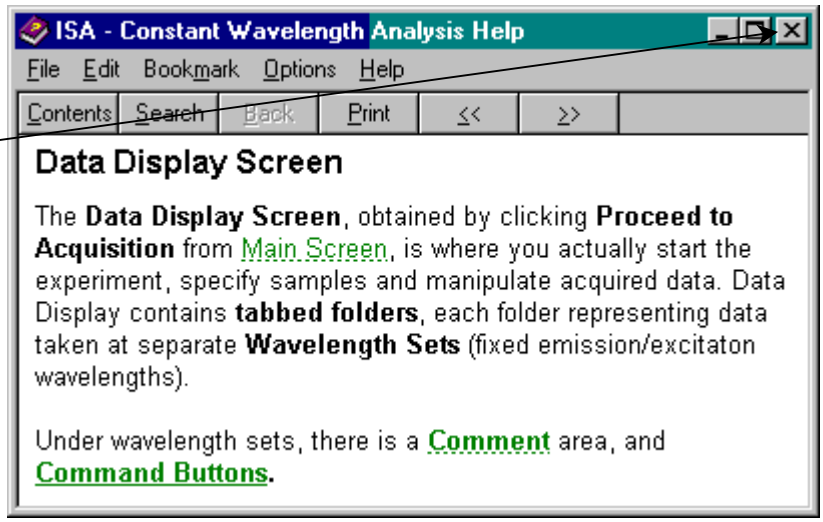


For help in the **CWA Display Data** *dialog box,*

1 Click *Help*.

The ***ISA – Constant Wavelength Analysis Help*** window appears:

2 Click the ☒ box to exit.



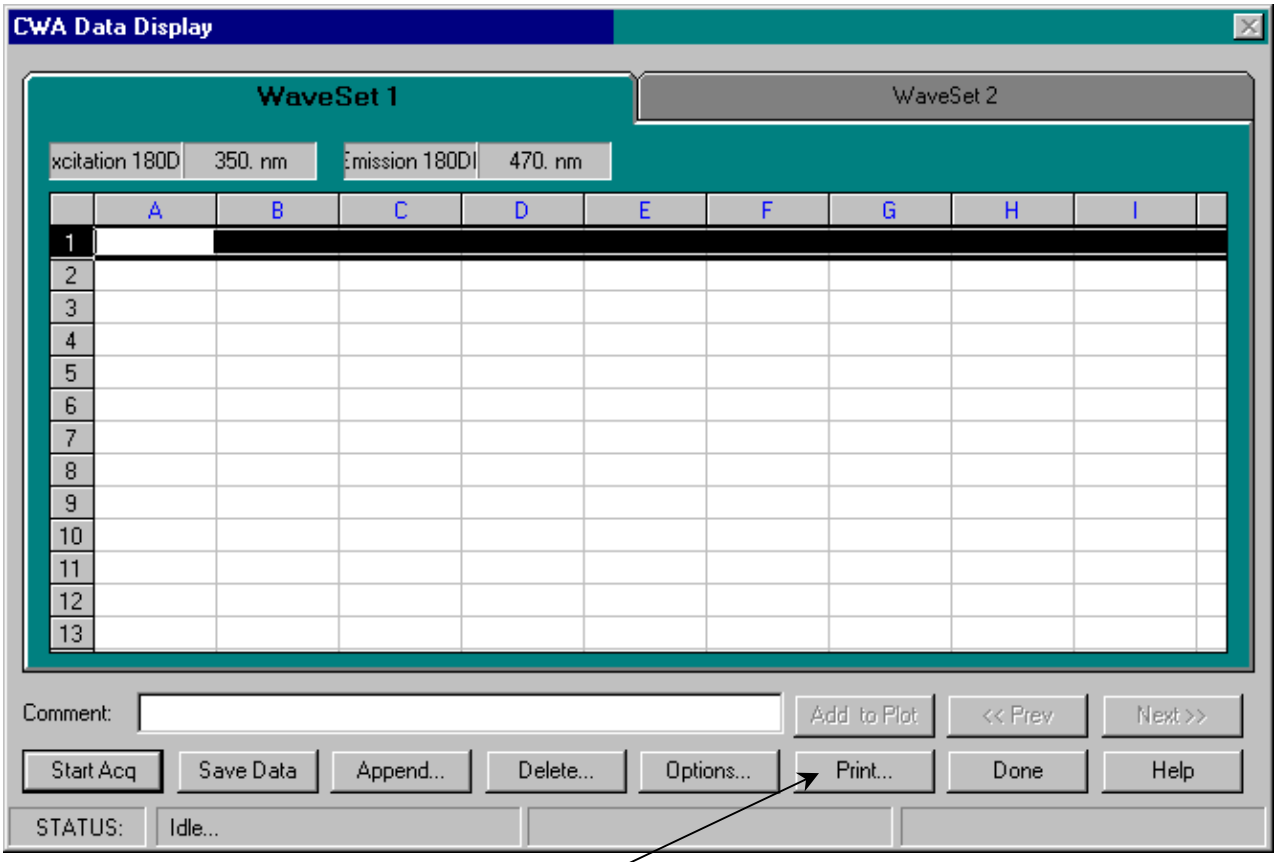
Status bar

The status bar shows the present activity of the system. Above is an example of a status bar waiting for a command. There are no user-adjustable fields.

Printing specifications in ***Constant Wavelength Analysis***

Two dialog boxes are responsible for page-setup and print specifications: ***Page Setup*** and ***Print Parameters***. To change the settings in these dialog boxes,

1 Enter the ***CWA Data Display*** dialog box:



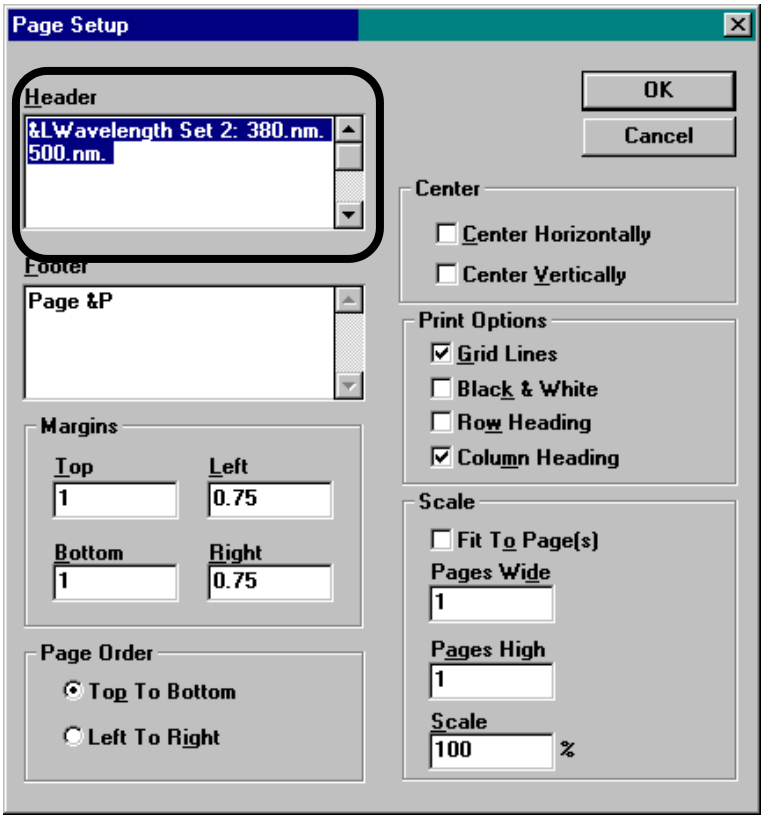
2 Click *Print...*.

The ***Page Setup*** dialog box opens.

Page Setup dialog box

Header

The header is always printed near the top of the page. The adjustable parts of the header are the *text* and its *justification*. The first character in the header field is &, a non-printing character. Immediately after & is a non-printing control character indicating the position of the text.



To edit the text in the header,

3 Click in the *Header* field.

4 After the "&*x*" pair of characters, type the desired text.

To left-justify the text,

5 Click in the *Header* field.

6 Replace the first two characters in the field with "&L".

To center the text,

7 Click in the *Header* field.

8 Replace the first two characters in the field with "&C".

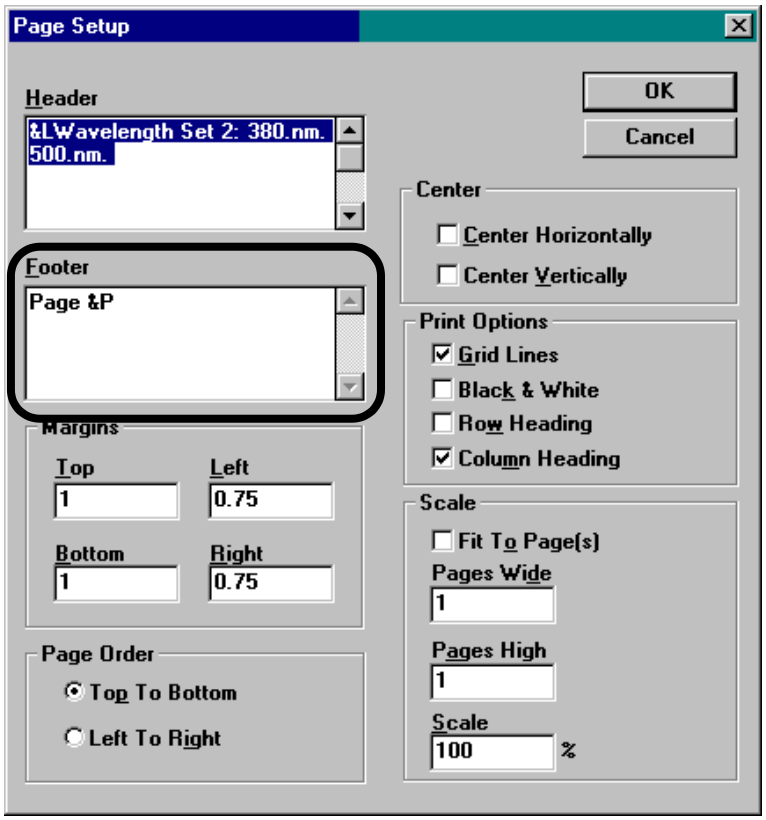
To right-justify the text,

9 Click in the *Header* field.

10 Replace the first two characters in the field with "&R".

Footer

The footer is always printed near the bottom of the page. The adjustable parts of the footer are the *text*, its *justification*, *page numbers*, and inclusion of a *time* or *date stamp*. The first character in the footer field is &, a non-printing character. Immediately after & is a non-printing control character indicating the position of the text.



To edit the text in the footer,

1. Click in the *Footer* field.
2. After the "&*x*" pair of characters, type the desired text.

To left-justify the text,

1. Click in the *Footer* field.
2. Enter "&L" before the text.

To center the text,

1. Click in the *Footer* field.
2. Enter "&C" before the text.

To right-justify the text,

1. Click in the *Footer* field.
2. Enter "&R" before the text.

To print automatic page numbers,

1. Click in the *Footer* field.

2 Enter “&P”.

To print a date stamp on each page,

1 Click in the *Footer* field.

2 Enter “&D”.

To print a time stamp on each page,

1 Click in the *Footer* field.

2 Enter “&T”.

To underline all following text,

1 Click in the *Footer* field.

2 Enter “&U” before all text to be underlined.

To print a sheet number,

1 Click in the *Footer* field.

2 Enter “&F”.

This is useful when printing a table that extends over several sheets of paper.

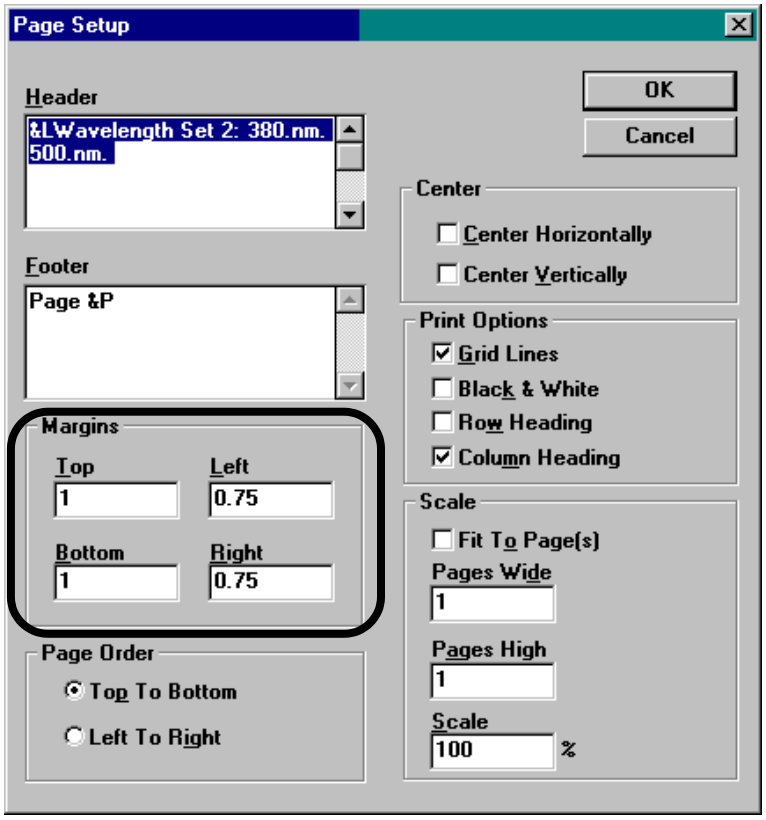
Margins

Default values for the paper's margins are:

Top	1" (2.5 cm)
Bottom	1" (2.5 cm)
Left	0.75" (1.8 cm)
Right	0.75" (1.8 cm)

To change a margin,

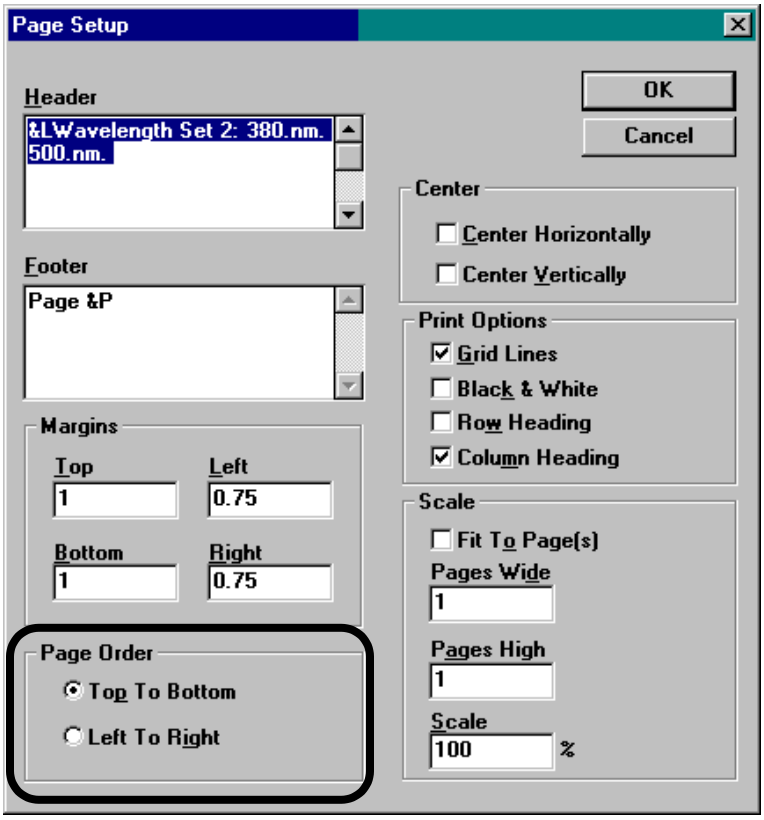
1. Click in the desired margin field.
2. Enter a new value.



Page Order

With a very large table, including many rows and columns, several sheets of paper may be necessary to print the entire table of data. The *Page Order* function tells DataMax in what order to print parts of the table. A useful additional parameter is the "&F" command in the *Footer* field. (See the *Footer* section.)

The following example explains how *Page Order* sets up the print job.

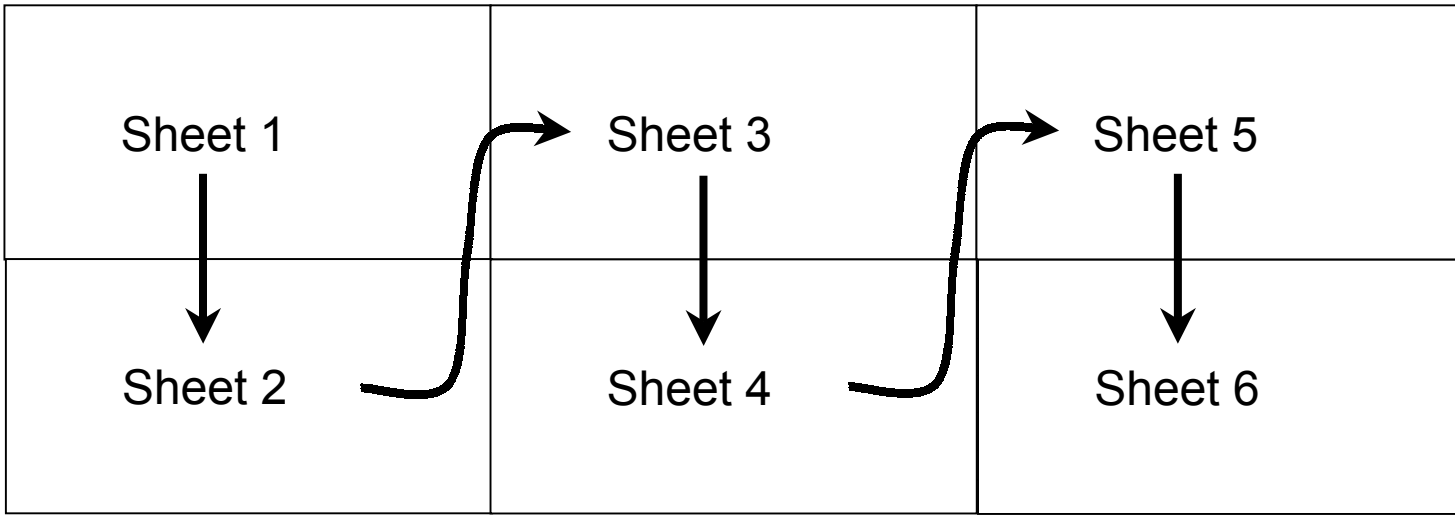


Suppose the experiment generates the following table of data:

Sheet a	Sheet b	Sheet c
Sheet d	Sheet e	Sheet f

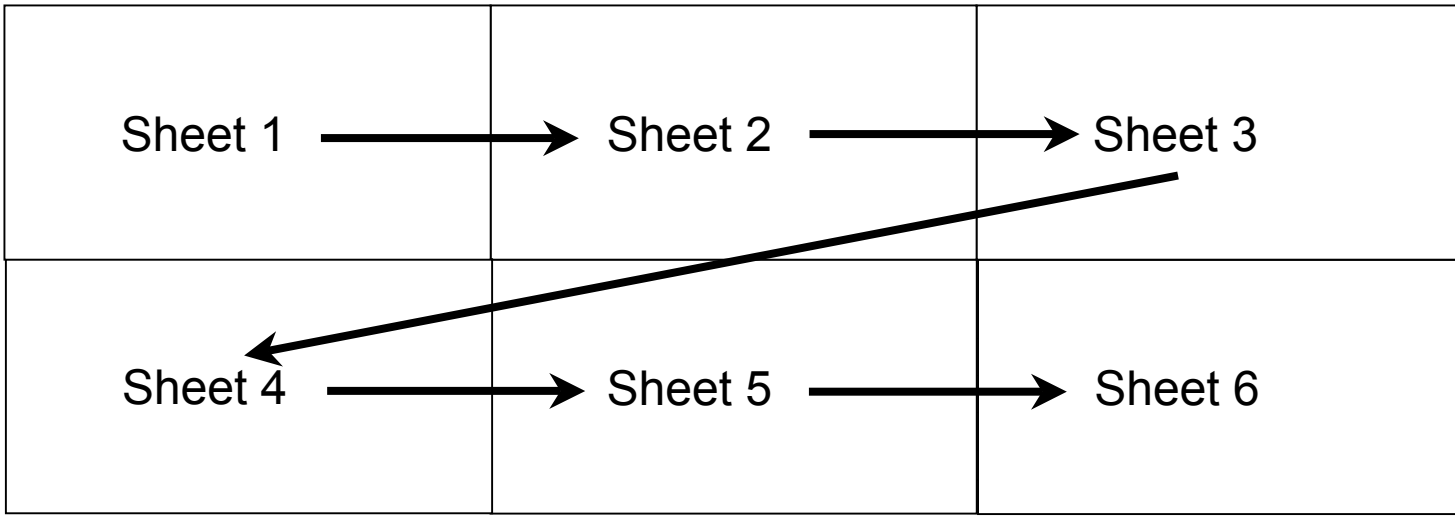
Notice how six sheets of paper are required to print the entire table.

To print Top to Bottom*, as shown below,*



1 Click the *Top to Bottom* radio button.

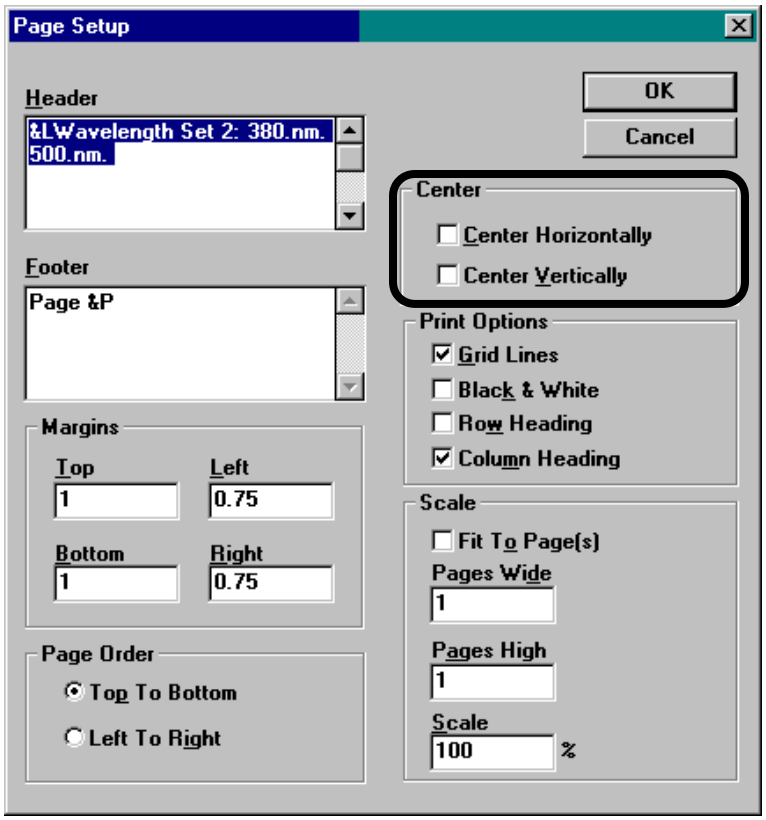
To print Left to Right, as shown below,



1 Click the *Left to Right* radio button.

Center

The *Center* area controls placement of the table on the page. The header and footer are unaffected by these checkboxes.



To center the table between the left and right margins,

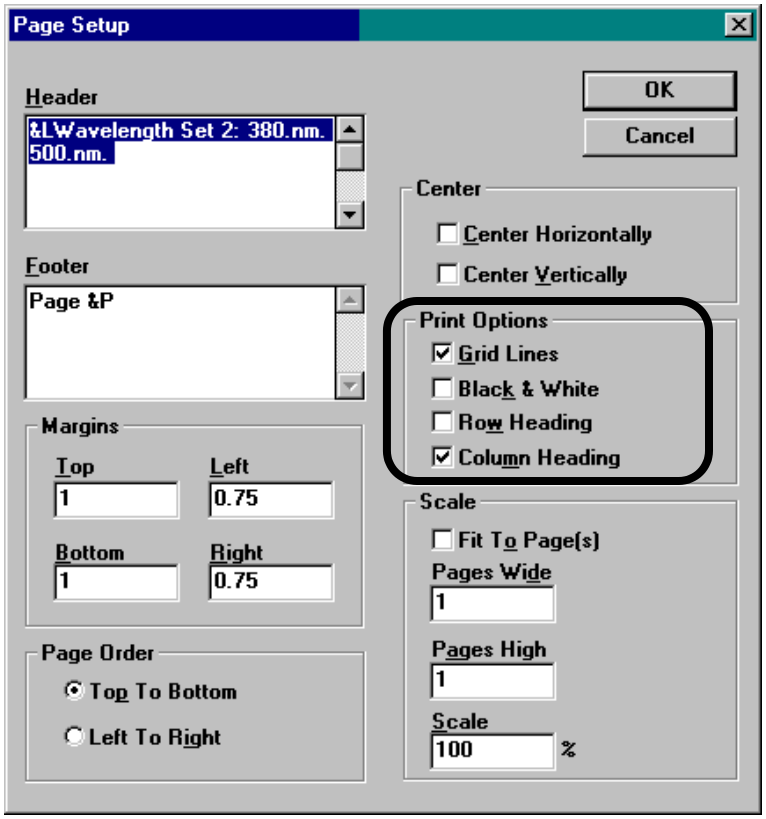
1 Check the *Center Horizontally* checkbox.

To center the table between the top and bottom margins,

1 Check the *Center Vertically* checkbox.

Print Options

Print Options controls the appearance of the table itself.



To add grid lines between the table's rows and columns,

1 Check the *Grid Lines* checkbox.

To print the table only in black and white,

1 Check the *Black & White* checkbox.

Note: Black & White *only affects color printers and plotters. It does not affect black-and-white printers or plotters.*

To print a row number,

1 Check the *Row Heading* checkbox.

The row number is printed to the left of the first column.

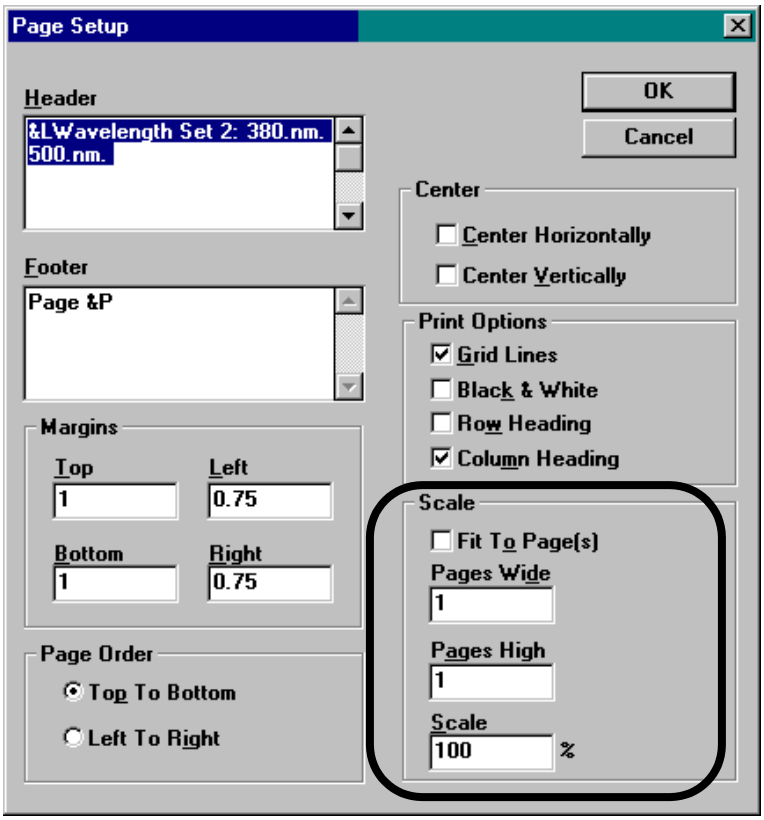
To print a title for each column,

1 Check the *Column Heading* checkbox.

Note: *Disabling* Column Heading *removes the row of titles at the top of the table, e.g.,* Sample, Conc., # of trials, *etc.*

Scale

The *Scale* area chooses the size of the printout. Forcing the printout to fit within a smaller size shrinks the size of the font.



To force the printout to fit onto a specific number of pages,

1. Check the *Fit to Page(s)* checkbox.
2. Click in the *Pages Wide* field.
3. Enter the number of pages wide the printout should be.
4. Click in the *Pages High* checkbox.
5. Enter the number of pages high the printout should be.

To shrink or enlarge the printout a certain amount,

1. Click in the *Scale* field.
2. Enter the percentage to compress or enlarge the printout.

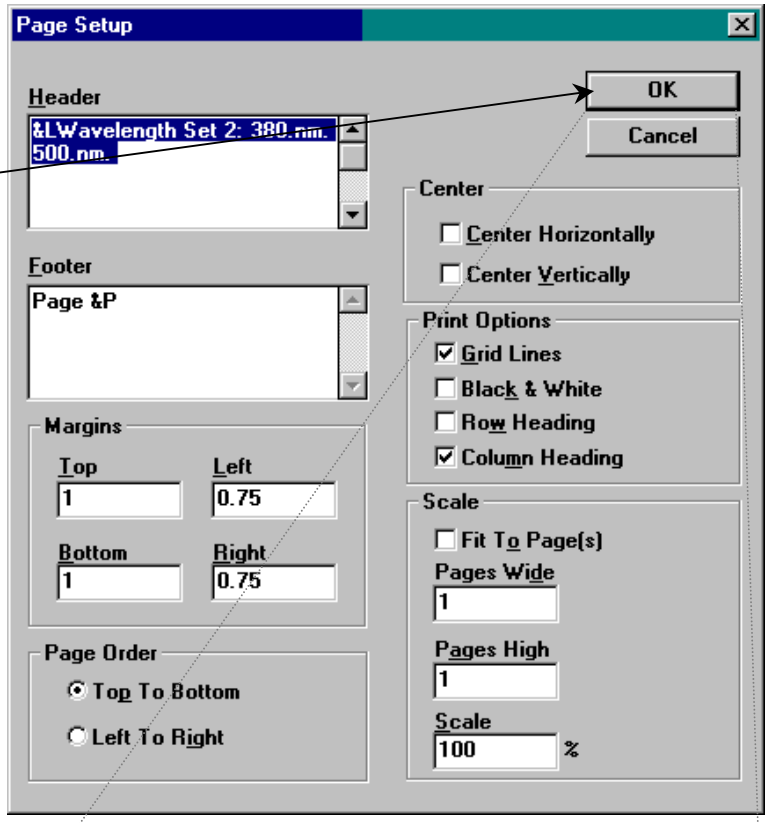
   A value < 100 shrinks the printout; a value > 100 enlarges the printout.

OK

When all parameters for page setup are completed,

1 Click *OK*.

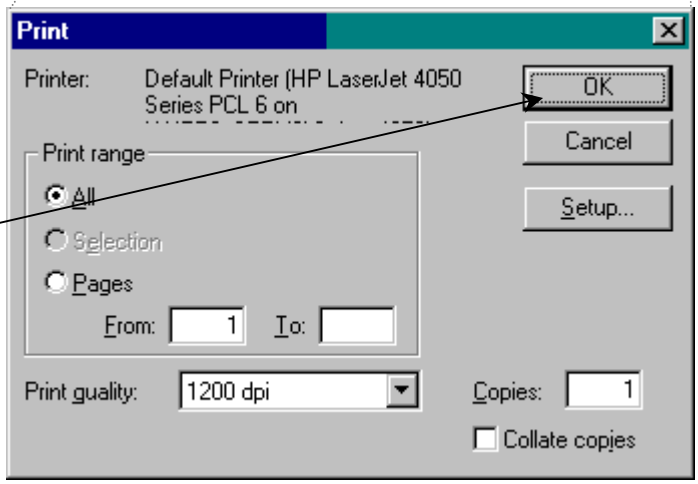
The ***Page Setup*** dialog box closes, and the Windows™ ***Print*** dialog box appears.



2 Complete the ***Print*** dialog box:

3 Click *OK*.

The printer creates a printout, and the ***Print*** dialog box closes.



9: Lifetime Measurements

Introduction

Lifetime measurements are possible only with the Fluorolog®-Tau-3 instrument. The Tau-3 operates in both steady-state and lifetime modes. In steady-state mode, the Tau-3 mimics the Fluorolog® system, and all steady-state features are accessible in the ***Run Experiment*** application. In lifetime mode, the Tau-3 can use extra features in the ***Run Experiment*** and ***Lifetime*** applications. The extra experiment type in ***Run Experiment*** is the *Lifetime Acquisition* scan, whose description is in Chapter 4. The other extra features in lifetime mode, using the ***Lifetime*** application, are discussed in this chapter.

Like the ***Real Time Display***, the ***Lifetime*** application is actually a control panel. Tabular data are presented on mini-panels, while graphical data and real-time models are presented on pop-up screens. Although the ***Lifetime*** application is self-contained, it can operate concurrently with any other DataMax application.

Note: *The* **Lifetime** *application is accessible only when the Tau-3 is in lifetime mode.*

Quick guide for lifetime measurements

1 Make sure the Fluorolog®-Tau-3 is in lifetime mode.

See the *Fluorolog®-Tau-3 Lifetime Operation Manual* for setup details.

2 Place the sample(s) in the sample compartment.

3 Start DataMax.

4 Load the correct lifetime layout.

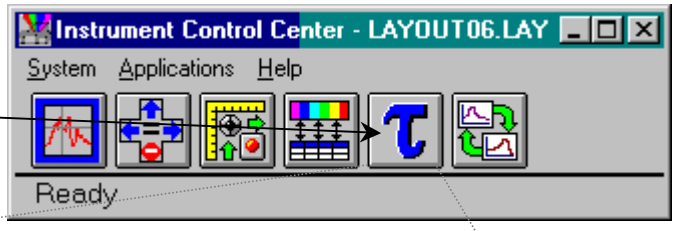
Include all appropriate accessories, including polarizers (which must be initialized), if necessary.

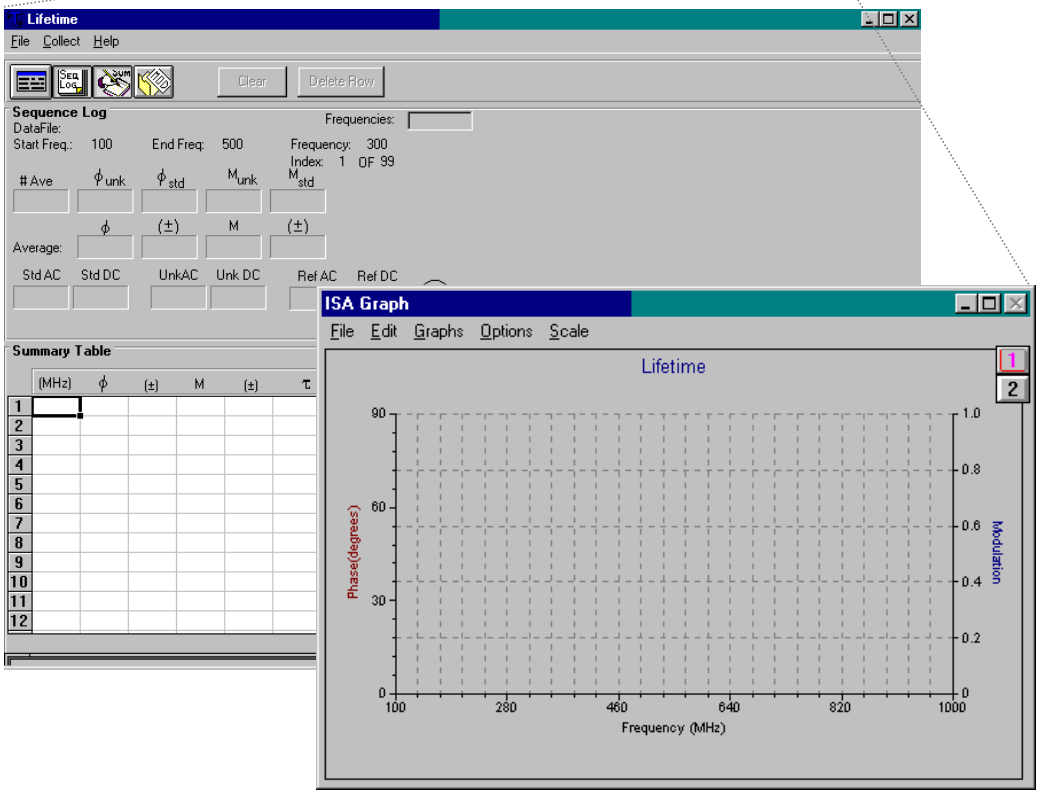
5 Make sure DataMax knows which sample corresponds to which position in the sample changer.

In ***Visual Instrument Setup***, use the sample-changer control panel, if necessary.

6 Click on the *Tau* button.

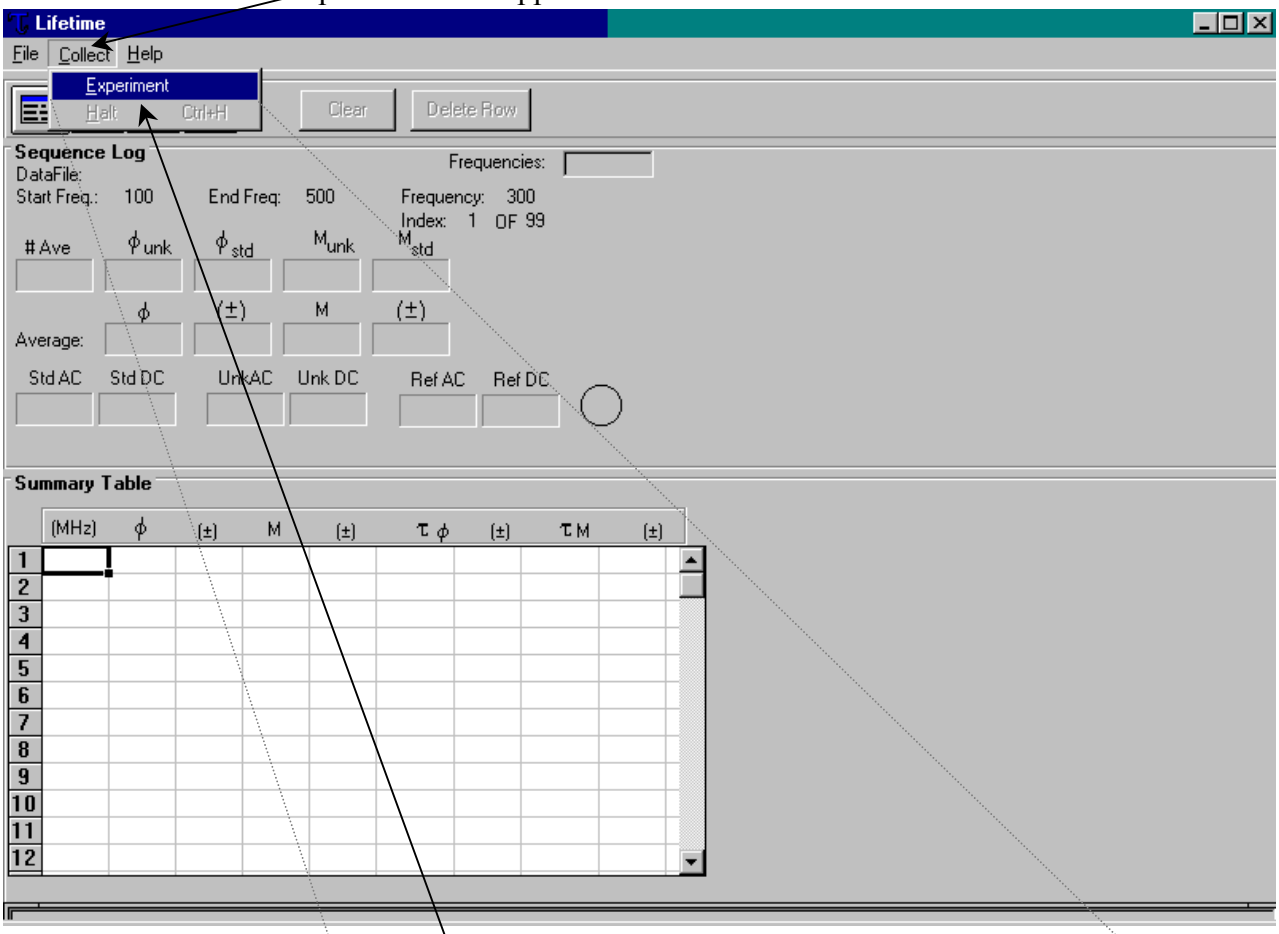
The ***Lifetime*** and the ***ISA Graph*** dialog boxes appear.





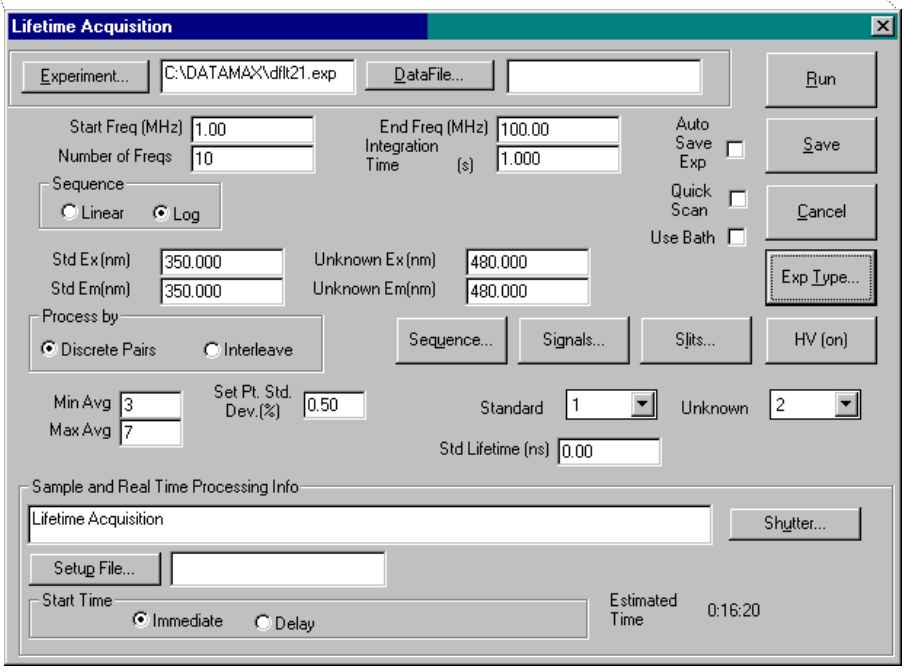
7 Choose *Collect* from the menu.

A drop-down menu appears.



8 Choose *Experiment*.

The ***Lifetime Acquisition*** dialog box appears. This is the default experiment type.



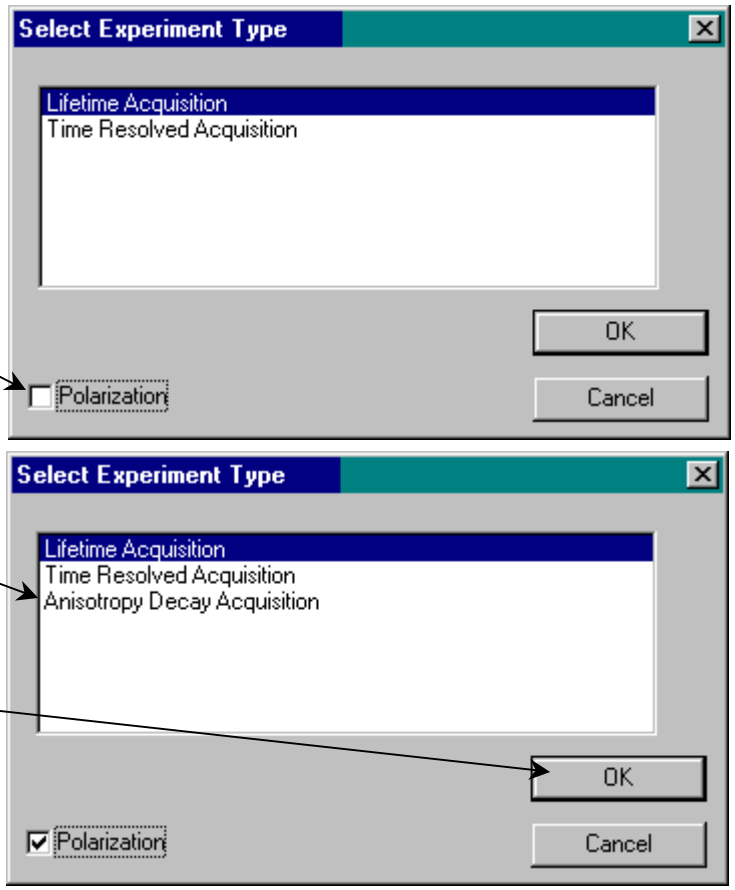
9 Change the experiment type, if desired.

a Click *Exp Type*….
The ***Select Experiment Type*** dialog box appears.

b For a polarization experiment, click the *Polarization* checkbox.
The *Anisotropy Decay Acquisition* choice appears in the list.

c Choose the desired experiment type.

d Click *OK*.
The ***Select Experiment Type*** dialog box closes.



10 Complete the data-entry parameters in the ***Acquisition*** dialog box.

11 Click *Run*.

The scan begins. The present data are shown in the *Sequence Log* area. All data taken so far are given in the *Summary Table*. A graph and real-time model of the data are plotted in the ***ISA Graph*** window.

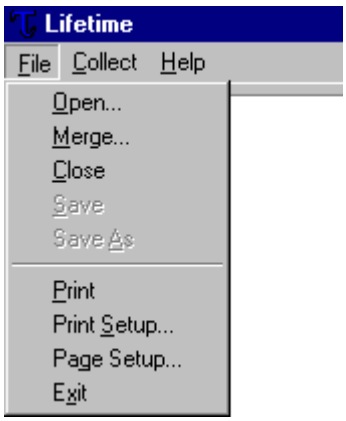
Main menu

The main menu of ***Lifetime*** contains three items:

- File
- Collect
- Help

Clicking on any of the above three items reveals a drop-down menu with choices. This section describes the choices in detail.

File

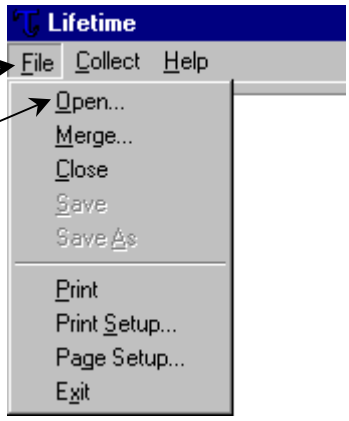


The *File* command deals with creating, loading, saving, and printing files. There is a provision for quitting the ***Lifetime*** application.

Open…

Open… opens an existing lifetime data file (with the `.DAT` extension).

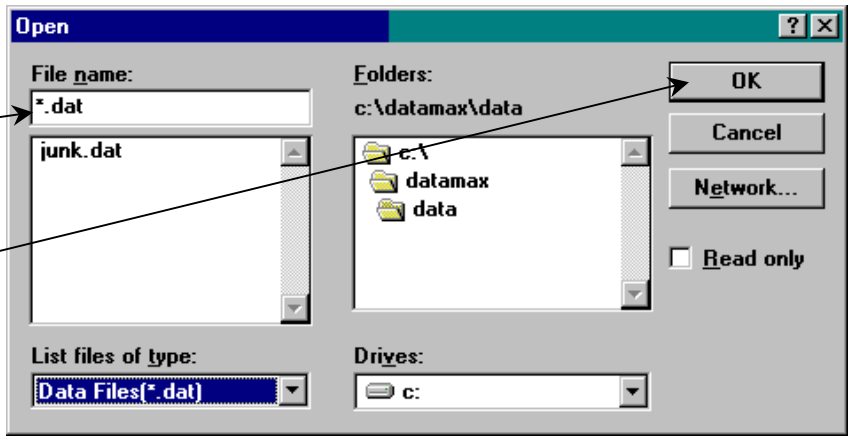
To open an existing file of lifetime data,



1 Click *File*.
A drop-down menu appears.

2 Choose *Open…*.
The ***Open*** dialog box appears.

3 Choose an existing lifetime data file to load.



4 Click *OK*.
The ***Open*** dialog box closes, the desired file opens, and the data appear in the *Summary Table*.

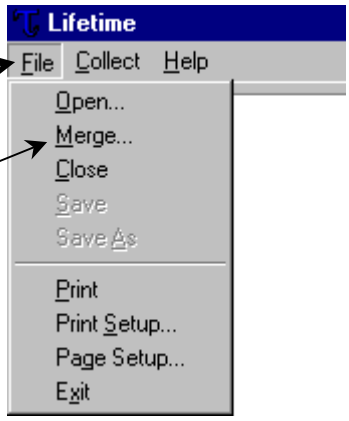
Merge…

Merge… takes two existing lifetime data files, and combines them together under a new file name. Two previously saved files may be merged, or one existing file may be merged with the current data in the *Summary Table*.

To merge two data files,

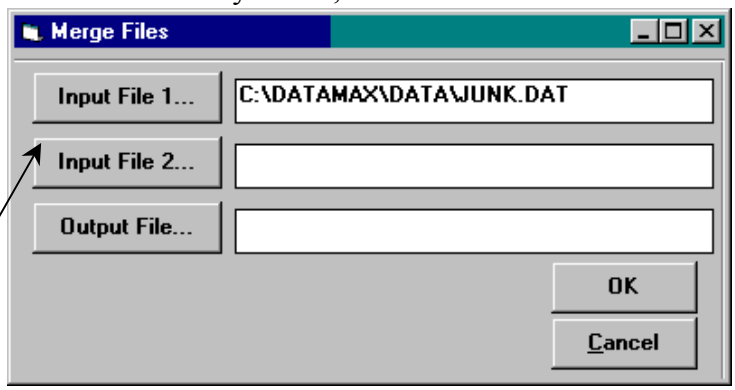
1 Click *File*.

A drop-down menu appears.



2 Choose *Merge…*.

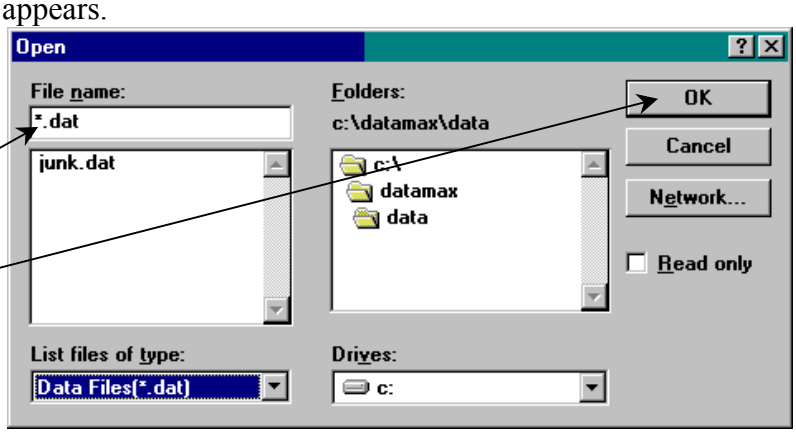
The ***Merge Files*** dialog box appears. If a data set is currently active, its name appears in the field next to *Input File 1…*. If no data set is currently active, no data file names appear.



3 Choose the first file to merge.

a Click Input File 1….

b The ***Open*** dialog box appears.



c Choose the first file's name.

d Click *OK*.

The ***Open*** dialog box closes, and the first file's name appears next to *Input File 1…*.

4 Choose the second file to merge.

a Click *Input File 2…*.

The ***Open*** dialog box appears again.

b Choose the second file's name.

c Click *OK*.

The ***Open*** dialog box closes, and the second file's name appears next to *Input File 2…*.

5 Choose the name of the merged file.

a Enter a new name in the field next to *Output File…*.

Or

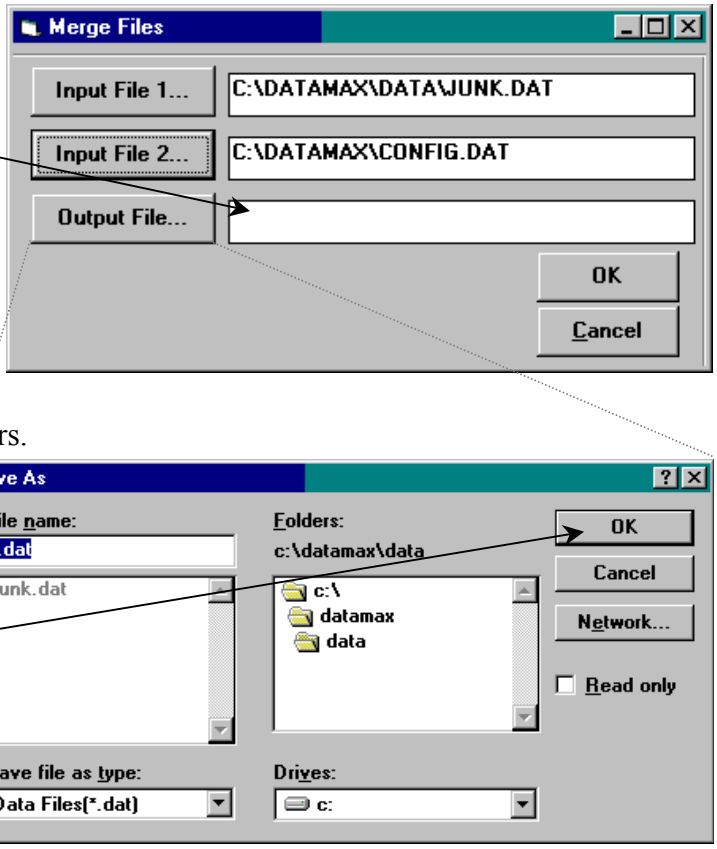
a Click *Output File…*.

b The ***Save As*** dialog box appears.

c Choose the name of the merged file.

d Click *OK*.

The ***Save As*** window closes.

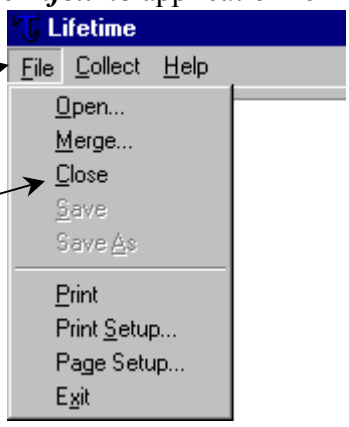


6 Click *OK* in the ***Merge Files*** window.

The merge occurs. DataMax checks for duplicate frequencies. If a duplicate frequency is found, a dialog box appears, asking which file's data point to use, or average the two. At the end of the merge, the active file is the merged file. The ***ISA Graph*** window displays the merged data.

Close

Close closes the active file and clears the *Summary Table*. The ***Lifetime*** application remains open, however.



To close the active file,

1 Click *File*.

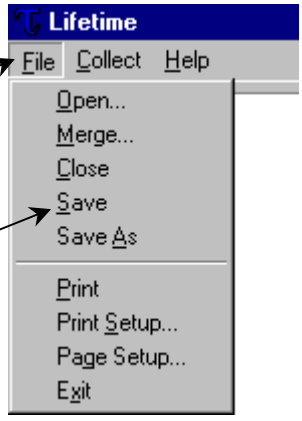
A drop-down menu appears.

2 Choose *Close*.

The *Summary Table* is cleared.

Save

The *Save* command stores the active data using the current file name. When more than one file is open, the *Save* command affects the active (last opened) file.



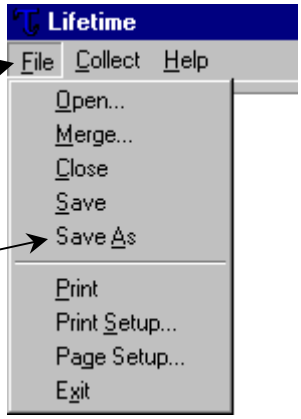
To save data into a file,

1 Click *File*.
A drop-down menu appears.

2 Choose *Save*.
The file is saved under the current file name in the current directory. The current file is still the active file.

Save As

Save As stores the active data under a different file name. The original file remains unchanged.

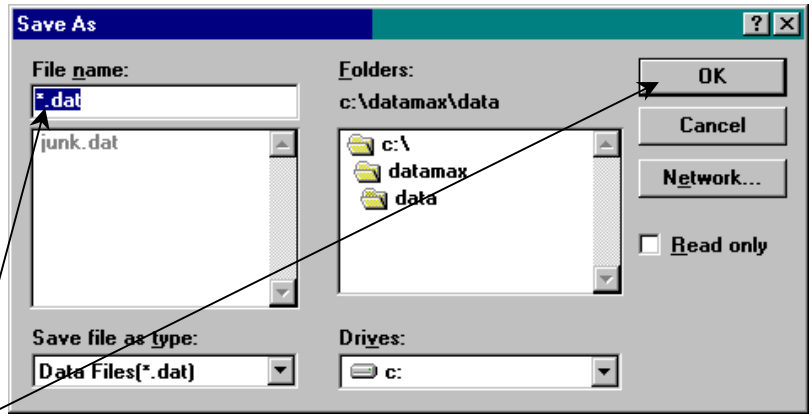


To save data under a different file name,

1 Click *File*.
A drop-down menu appears.

2 Choose *Save As*.
The ***Save As*** dialog box appears.

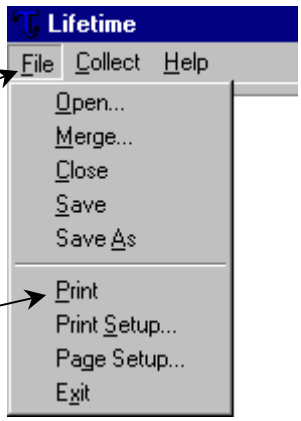
3 Enter or choose the new file name and directory.



4 Click *OK*.
The ***Save As*** dialog box disappears, and the file is saved under the new name.

Print

To print the *Summary Table*, use the *Print* command. When the job is sent to the printer, the data are printed according to the parameters established in the *Print Setup…* and *Page Setup…* commands.



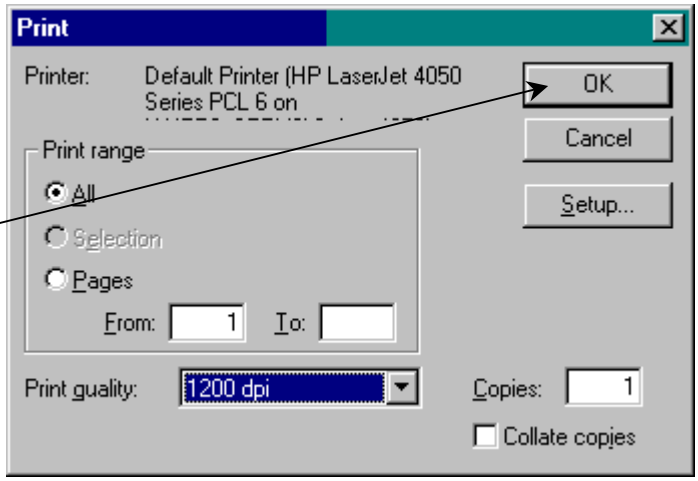
To print the active data,

1 Click *File*.
A drop-down menu appears.

2 Choose *Print*.
The ***Print*** dialog box appears.

3 Choose the appropriate parameters.

4 Click *OK*.
The ***Print*** dialog box closes, and the job is sent to the printer.



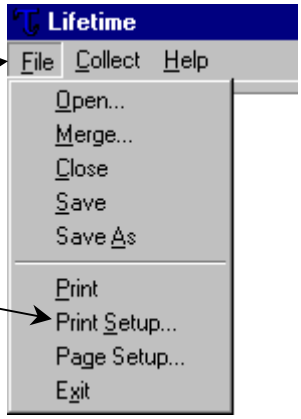
Print Setup

Print Setup controls which printer to use, and the paper orientation and size. See the instruction manual for the printer to determine the printer's precise capabilities.

To adjust the printer's setup,

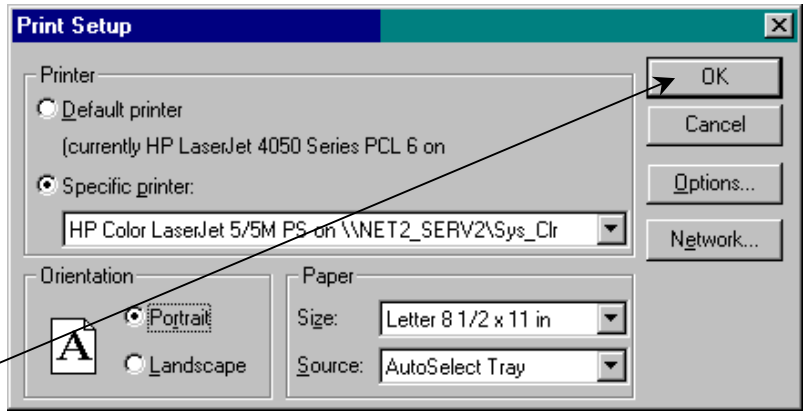
1 Click *File*.
A drop-down menu appears.

2 Click *Print Setup…*.
The ***Print Setup*** dialog box appears.



3 Adjust the printer's parameters.

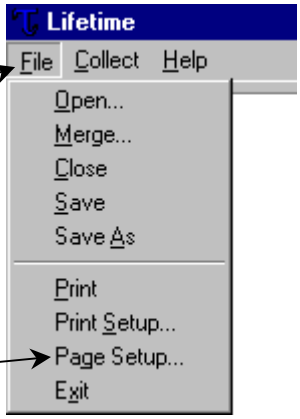
4 Click *OK*.



The printer parameters are changed, and the ***Print Setup*** dialog box closes.

Page Setup

Page Setup controls the appearance, size, and placement of the data table on the printout page(s). For details on use of *Page Setup*, see Chapter 8.



To change the setup of the page,

1 Click *File*.

A drop-down menu appears.

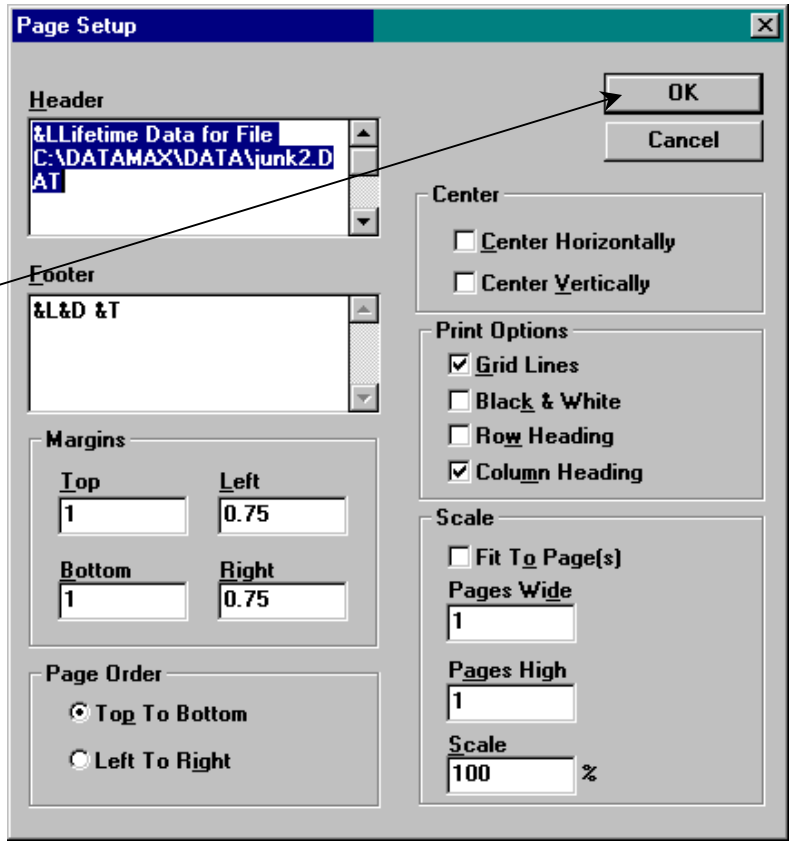
2 Click *Page Setup…*.

The *Page Setup* dialog box appears.

3 Adjust the page parameters.

4 Click *OK*.

The ***Page Setup*** dialog box closes, and the printout's appearance is set.



Exit

To quit the ***Lifetime*** application, use the *Exit* command.

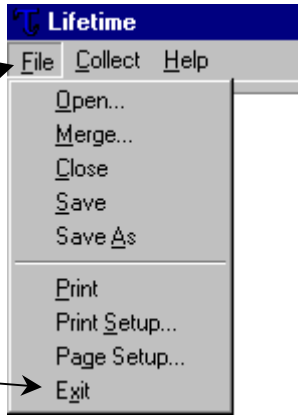
To leave the ***Lifetime*** application,

1 Click *File*.

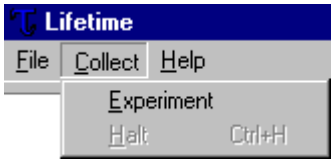
A drop-down menu appears.

2 Choose *Exit*.

The ***Lifetime*** application shuts down. Both ***Lifetime*** and ***ISA Graph*** windows close.



Collect



The *Collect* command is concerned with choosing an experiment type and halting an experiment in progress. Either the *Experiment* or the *Halt* option is available at a particular time.

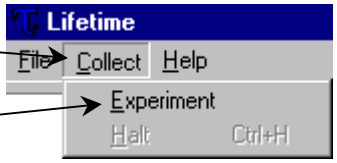
Experiment

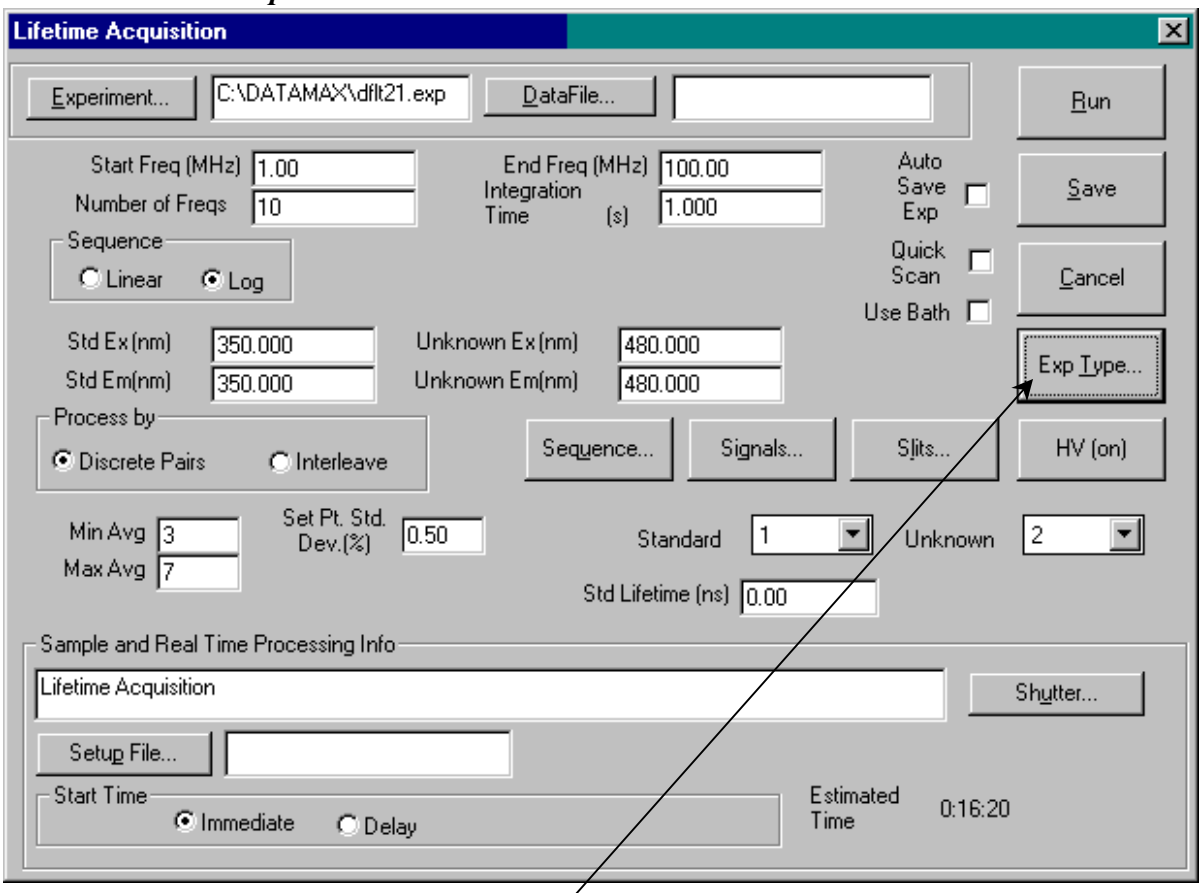
Use *Experiment* to choose the type of lifetime experiment. The *Experiment* choice appears only when the system is idle between experiments. During a scan, the *Experiment* option is gray and inaccessible.

To choose the type of lifetime experiment,

1 Click *Collect*.
A drop-down menu appears.

2 Choose *Experiment*.
An experimental acquisition screen appears. The default dialog box is the ***Lifetime Acquisition*** window.

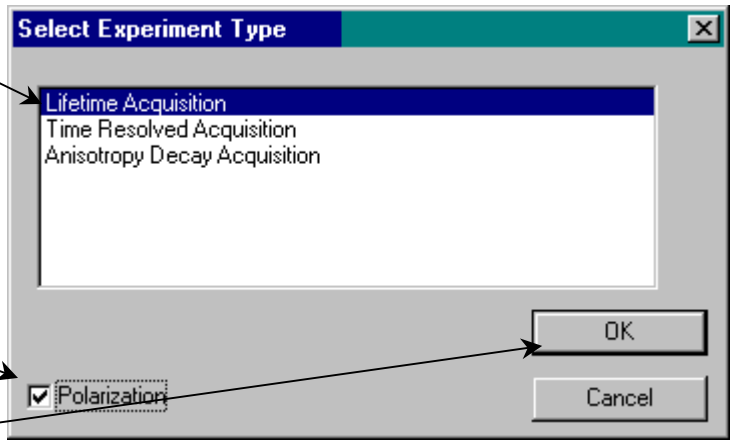




3 Click *Exp Type…*.
The ***Select Experiment Type*** dialog box appears.

4 Choose the experiment type.

Be sure the *Polarizers* box is enabled, to show the *Anisotropy Decay Acquisition* choice.



5 Click *OK*.

The ***Select Experiment Type*** dialog box closes. The experimental acquisition window changes into the desired experimental acquisition type.

Halt

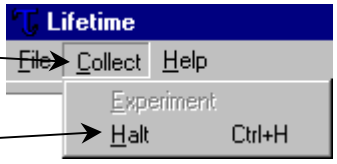
Halt ends a scan before the experiment is complete. The *Halt* choice appears only when the system is actually running a scan. Between scans, the *Halt* option is gray and inaccessible.

To stop a scan before completion,

1 Click *Collect*.

A drop-down menu appears.

2 Choose *Halt*.



The scan stops. All data collected so far are saved, and calculations are performed using the existing data.

Help

The *Help* command provides information about functions and settings in the ***Lifetime*** application.

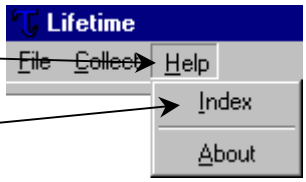
Index

Index describes the various functions and data-entry fields in the ***Lifetime*** application.

To get help about the functions and data-entry fields in the ***Lifetime*** application,

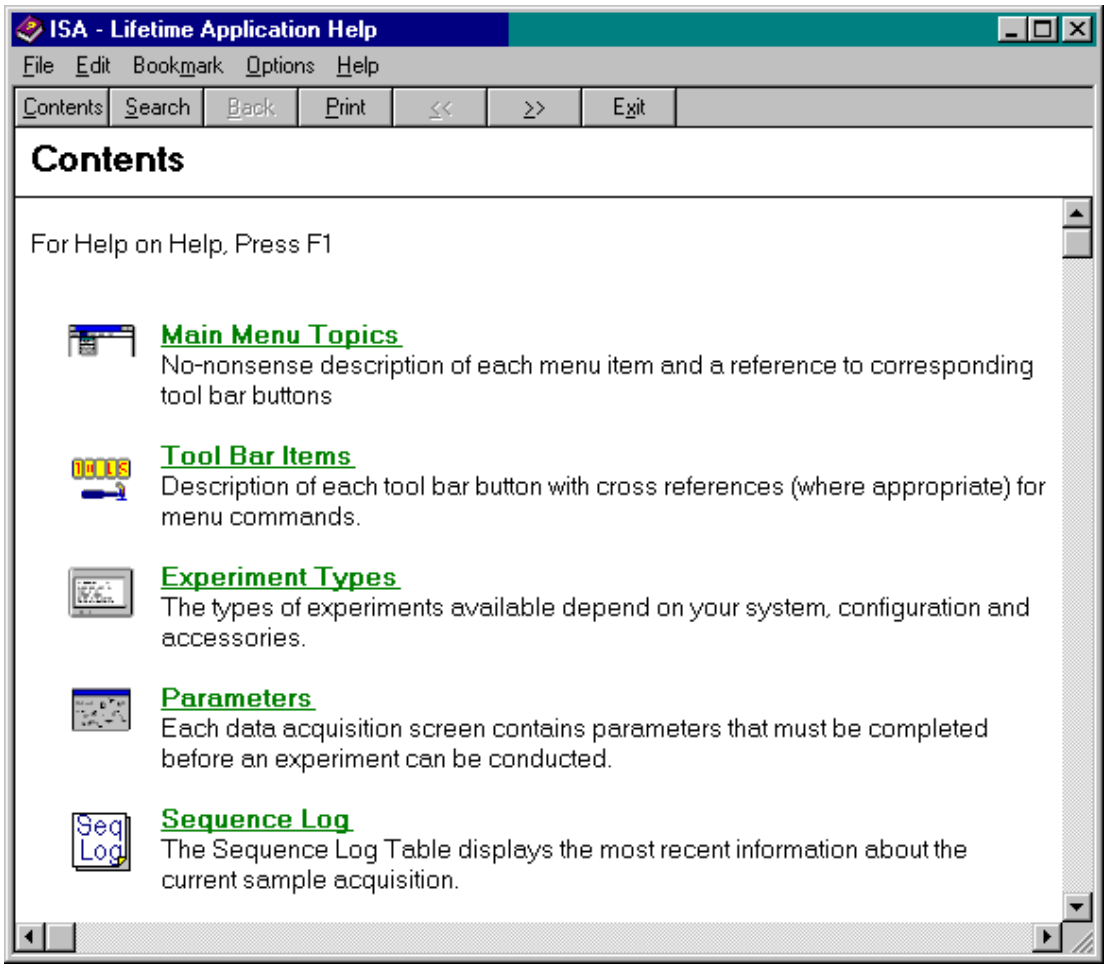
1 Click *Help*.

A drop-down menu appears.



2 Click *Index*.

The ***ISA – Lifetime Application Help*** window appears.



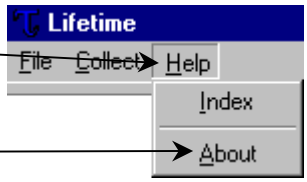
3 Click ☒ to close the ***Help*** window.

About

About displays the serial number, version number, copyright date, and other settings of the hardware and ***Lifetime*** software. When calling Spex® Fluorescence Service, have this information handy.

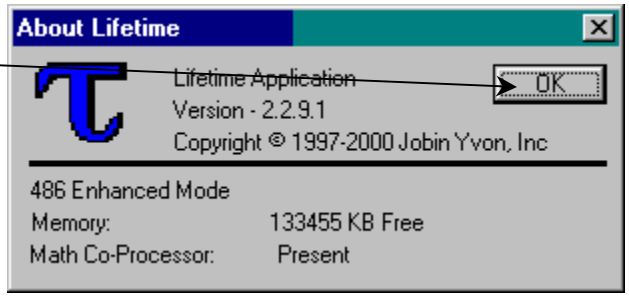
To obtain information about the ***Lifetime*** program,

1 Click *Help*.
A drop-down menu appears.



2 Click *About*.
The ***About Lifetime*** window opens.

3 Click *OK*.
The ***About Lifetime*** window closes.



Toolbar

The toolbar provides shortcut buttons for commonly used commands in ***Lifetime***. The buttons available are:

 Experiment

 Sequence Log

 Summary Table

 Real-time modeling

 Clear

 Delete Row

Clicking on any of these buttons activates or opens the corresponding function or window. The following section describes these buttons in detail:

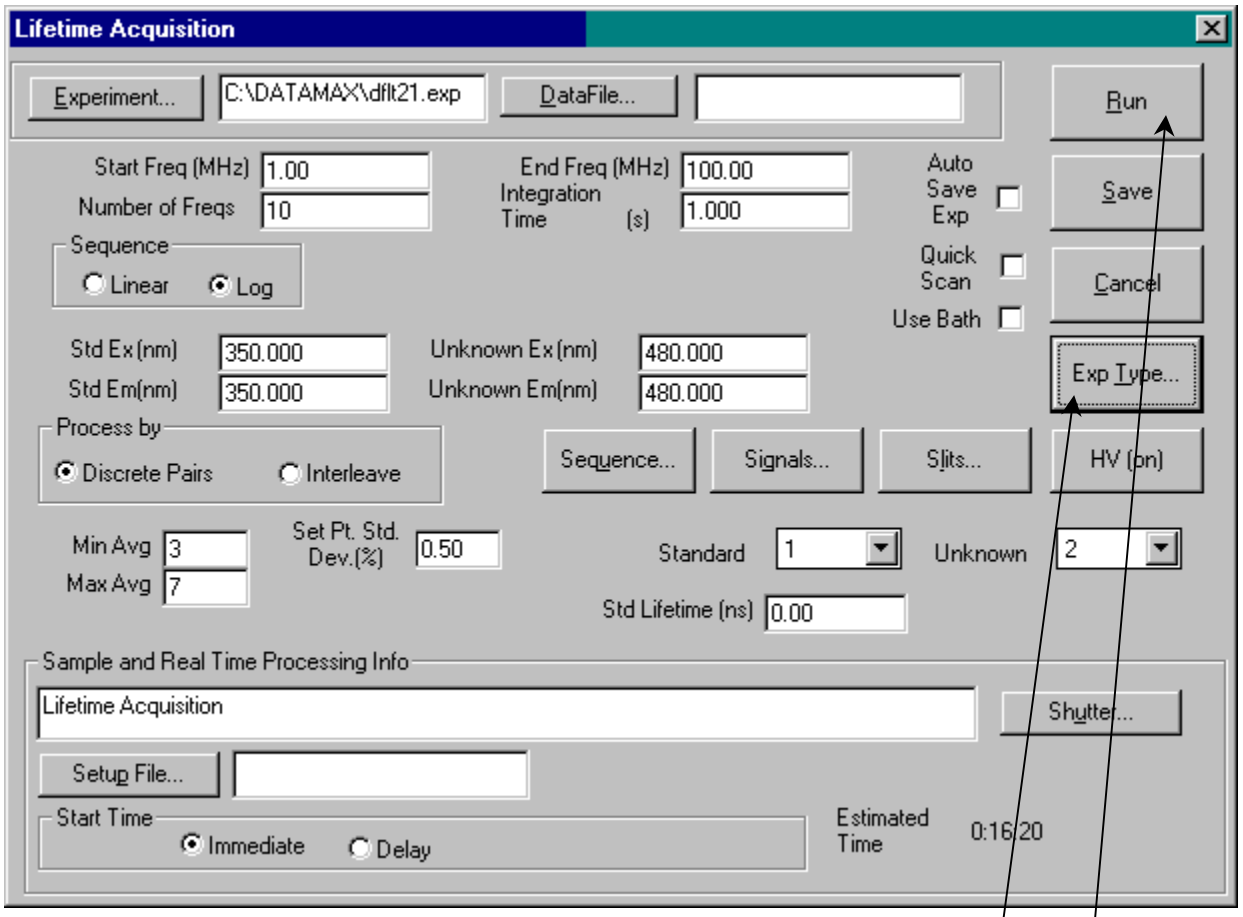
Experiment

The *Experiment* button opens an acquisition window. To run a lifetime experiment, adjust experimental parameters, or to choose a new type of lifetime experiment, the acquisition window must be open.

To run an experiment or modify parameters,

1 Click the *Experiment* button.

The default experiment acquisition dialog box appears:



2 To change experiment type, click *Exp Type…*.

3 To run the experiment as shown, click *Run*.

Sequence Log

The *Sequence Log* button reveals and displays the *Sequence Log*, an information table containing the most recent data from the current experiment. Each time a new data point is recorded, the *Sequence Log* is updated. The button itself shows the status of the *Sequence Log*: if the button is depressed, the *Sequence Log* is displayed; if the button is not depressed, the *Sequence Log* is not visible.

To remove or add the *Sequence Log* to the window,

1 Click the *Sequence Log* button.

The *Sequence Log* appears or disappears, and the button is depressed or not depressed, respectively.

Summary Table

The *Summary Table* button displays or hides the *Summary Table*, a list of data and parameters for the current experiment. The button itself shows the status of the *Summary Table*: if the button is depressed, the *Summary Table* is displayed; if the button is not depressed, the *Summary Table* is not visible.

To view or hide the *Summary Table*,

1 Click the *Summary Table* button.

The *Summary Table* appears or disappears, and the *Summary Table* button is depressed or not depressed, respectively.

Real-Time Modeling

The *Real-Time Modeling* button opens or closes the ***ISA Graph*** window, a plot of collected data and a theoretical model to which to compare the data. The button itself shows the status of the *Real-Time Modeling*: if the button is depressed, the ***ISA Graph*** is displayed; if the button is not depressed, the ***ISA Graph*** is not visible.

To display a graph of the data with a model,

1 Click the *Real-Time Modeling* button.

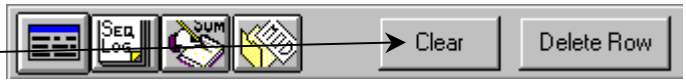
The ***ISA Graph*** plot appears or disappears, and the *Real-Time Modeling* button is depressed or not depressed, respectively.

Clear

The *Clear* button deletes one or more highlighted cells from the *Summary Table* of data. Any calculations or models are redone with the remaining data. If no data are yet displayed in the *Summary Table*, the *Clear* button is gray and inaccessible.

To delete one or more cells from the *Summary Table*,

1. Scroll up and down in the *Summary Table*, as necessary.
2. Highlight the undesired cell(s).
3. Click *Clear*.



The information is deleted from the cell(s), and any calculations are redone.

Delete Row

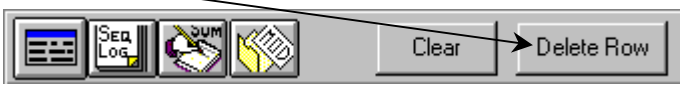
The *Delete Row* button deletes entire undesired rows from the *Summary Table*. Any calculations or models are redone with the remaining data. If no data are yet displayed in the *Summary Table*, the *Delete Row* button is gray and inaccessible.

To delete a row from the Summary Table,

1 Scroll up and down in the *Summary Table*, as necessary.

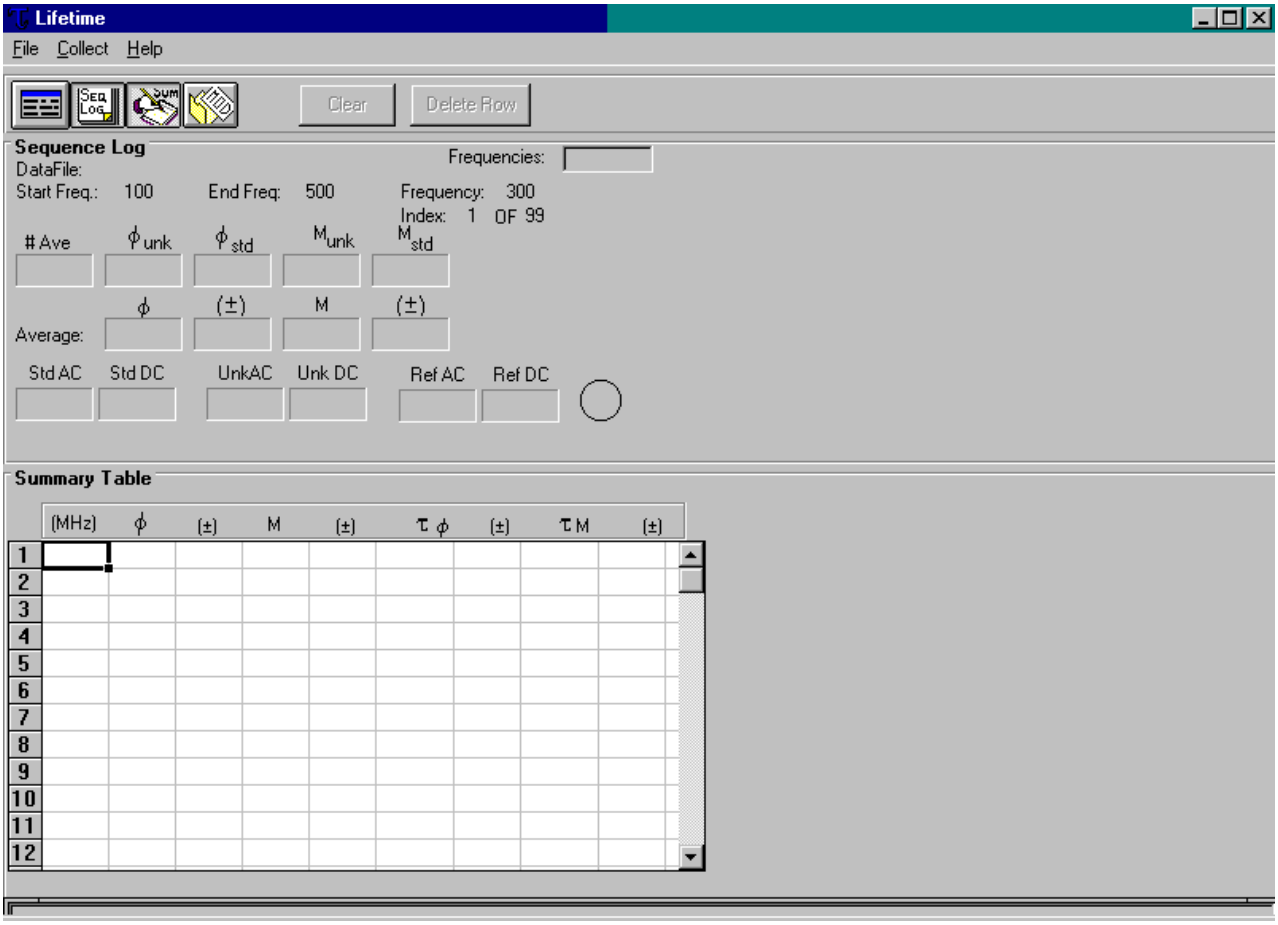
2 Click anywhere within the undesired row.
The row becomes highlighted automatically.

3 Click *Delete Row*.
The row disappears, and remaining rows underneath move upward to fill the gap. All calculations and modeling are redone.



Description and operation

Lifetime main screen



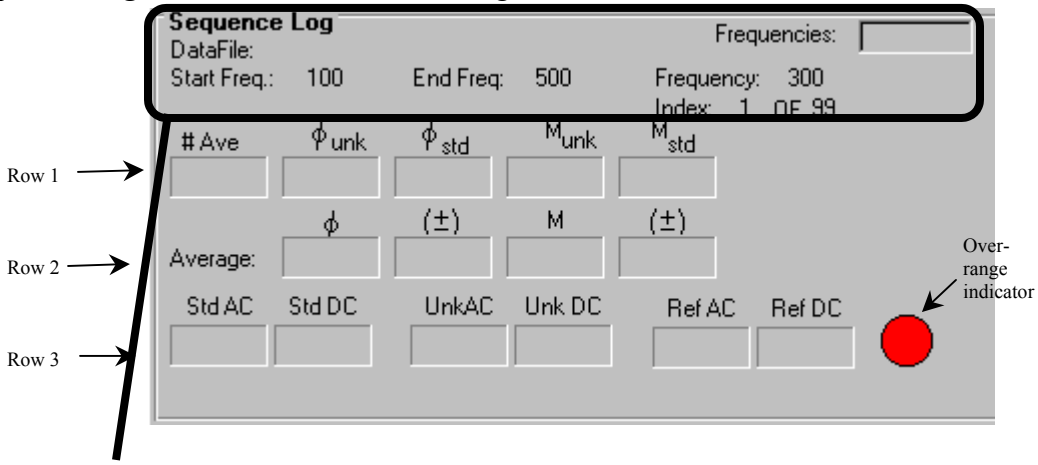
There are two important areas on the main screen in the ***Lifetime*** application:

- Sequence Log
- Summary Table

Because the ***Lifetime*** main screen is really a control panel, these areas are meant as a display of data. This section discusses each area in detail.

Sequence Log

The *Sequence Log* is an information table that contains the most recent data about the current experiment. The data in the *Sequence Log* is updated as each data point is recorded. There are no user-controllable fields; the information is displayed, and the *Sequence Log* is removed or inserted using the toolbar.



The uppermost area contains information about the data file:

DataFile	Name of the file
Frequencies	Number of frequencies the file displays
Start Freq	Starting frequency (in MHz)
End Freq	Ending frequency (in MHz)
Frequency	Current frequency (in MHz)
Index: *x* OF *y*	Current data point's number *x*, out of the total number of points *y*

Row 1: Current data

# Ave	Number of points contributing to the average
ϕ_{unk}	Measured phase-shift of the unknown at this frequency
ϕ_{std}	Measured phase-shift of the standard at this frequency
M_{unk}	Modulation of the unknown at this frequency
M_{std}	Modulation of the standard at this frequency

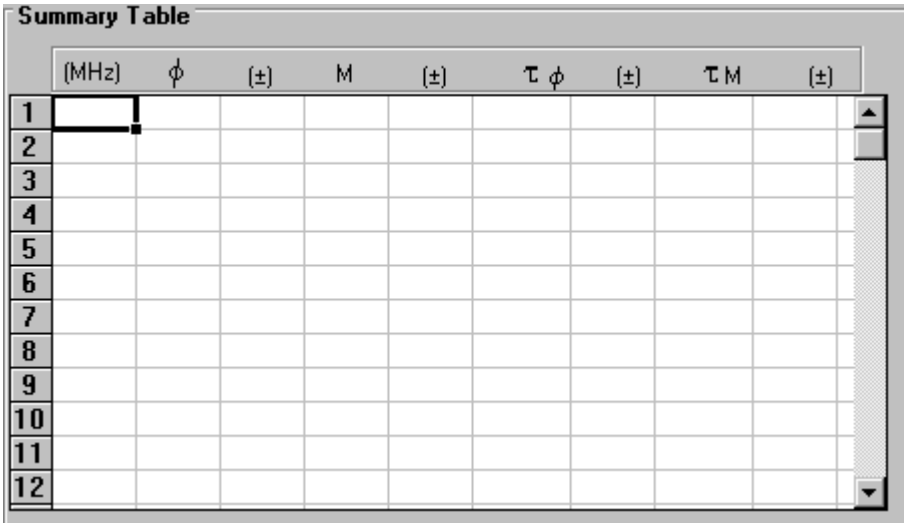
Row 2: Averaged data so far

ϕ	Average phase-shift for the unknown
(±)	Standard deviation for the unknown's average phase-shift
M	Average modulation for the unknown
(±)	Standard deviation for the unknown's average modulation

Row 3: Current values of the AC and DC signals

Std AC	AC component of the standard at the current frequency
Std DC	DC component of the standard at the current frequency
Unk AC	AC component of the unknown at the current frequency
Unk DC	DC component of the unknown at the current frequency
Ref AC	AC component at the reference detector
Ref DC	DC component at the reference detector
Circle	Over-range indicator (turns red when out of range)

Summary Table



The *Summary Table* contains all information collected so far. The data can be edited using the *Clear* button, the *Delete Row* button, or by double-clicking within a cell. The *Summary Table* is removed or inserted using the toolbar. For large data sets, use the scroll bars to move the table up and down to view all data points.

Column headings, from left to right:

(MHz)	Frequency at which measurements and calculations were made
ϕ	Average phase-shift of the unknown at this frequency
(±)	Standard deviation of the average phase-shift
M	Modulation of the unknown
(±)	Standard deviation of the average modulation
τ_ϕ	Calculated lifetime with respect to the average phase-shift at this frequency
(±)	Standard deviation of the average lifetime, using this average phase-shift
τ_M	Calculated lifetime with respect to the average modulation at this frequency
(±)	Standard deviation of the average lifetime, using this average modulation

Editing cells in the *Summary Table*

Contents of the cells in the ***Summary Table*** can be edited or deleted. Use *Clear* to delete the contents of one or more cells. Use *Delete Row* to remove an entire row.

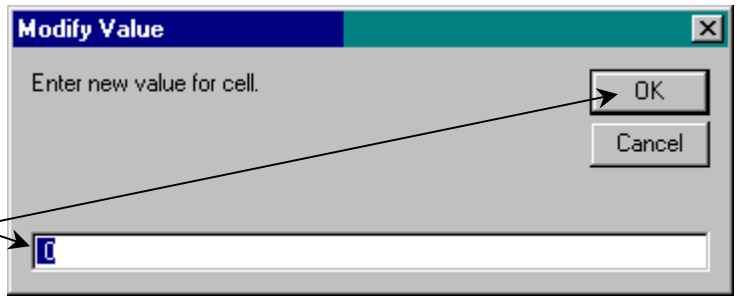
To change a value in a cell,

1 Double-click on the desired cell.

The ***Modify Value*** dialog box appears.

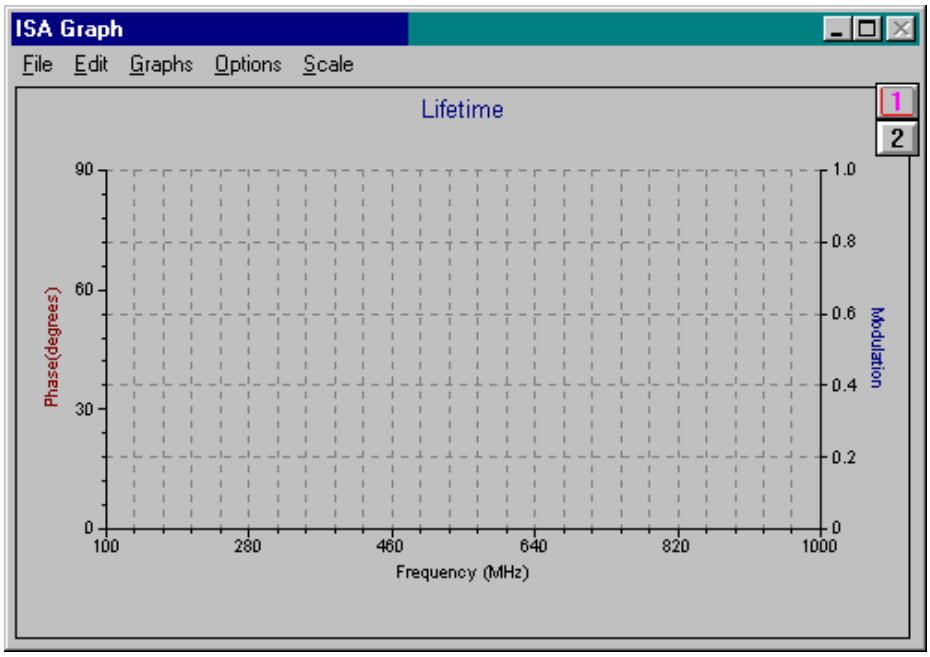
2 Enter a new value for the cell.

3 Click *OK*.



The ***Modify Value*** dialog box closes, and the cell's contents show the new value.

ISA Graph screen

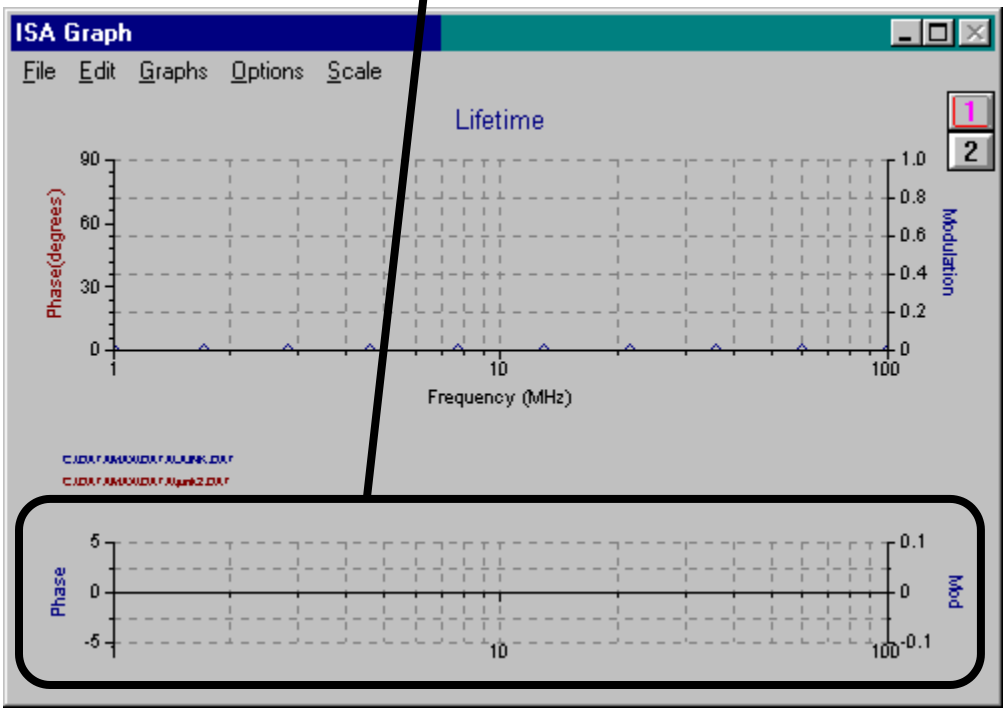


The ***ISA Graph*** window displays the data during or after collection, and displays a curve fitted to the data. Plot appearance can be adjusted, and the scale automatically changes to present the best view. The ***ISA Graph*** is removed or inserted using the toolbar on the ***Lifetime*** window.

To view the model (curve fit),

1 Click the *Modeling* button in the ***Lifetime*** tool-bar.

The ***ISA Graph*** window expands downward, and the model appears below the data plot:

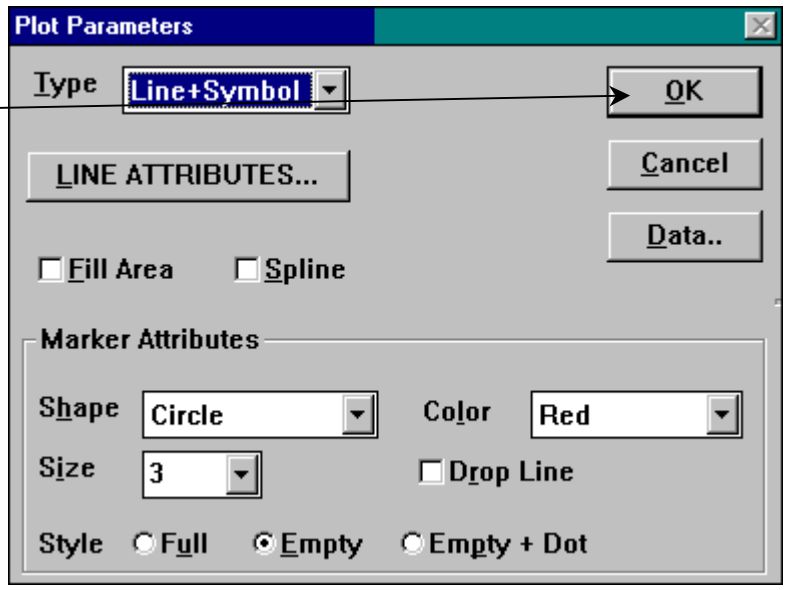


To edit, copy, or reformat data in *ISA Graph*

1 Double-click anywhere along the data curve to be edited.

The ***Plot Parameters*** dialog box appears.

2 Click *Data…*



A list of data appears.

To edit the values,

a Double-click on the point to be changed.

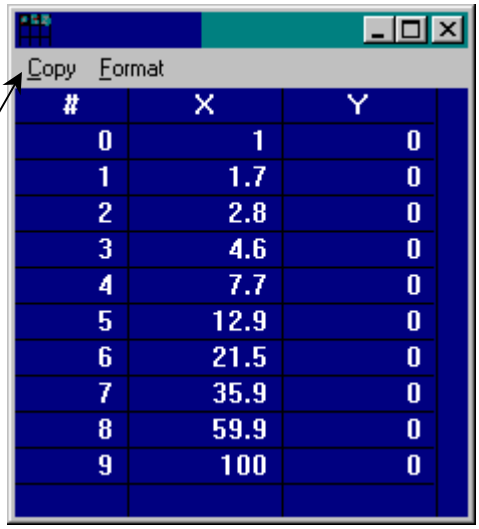
b Delete the contents of the cell.

c Enter a new value.

d Press **ENTER**.

e The graph changes immediately.

f Click on any other field in the list of data.



To copy the data to another program,

a Click *Copy*.

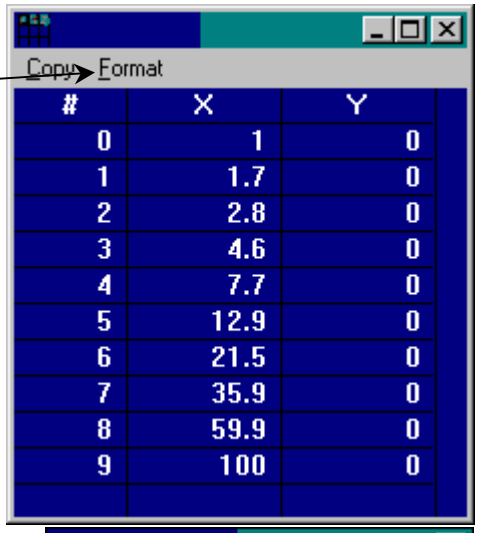
b The data are copied to a clipboard.

c Paste the information into the desired program.

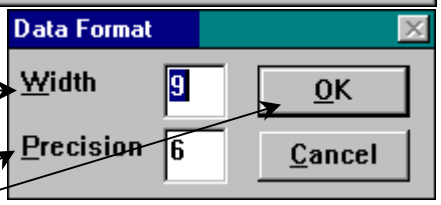
The data now can be manipulated and edited.

To format the data in the list,

a Click *Format*.



The ***Data Format*** window appears.



b Adjust the column width in the *Width* field.

c Adjust numerical precision in the *Precision* field.

d Click *OK*.

The ***Data Format*** window closes; the list of data changes to the new formatting.

To change the format of the plot,

See Chapter 11.

To change the real-time model (curve fit)

The model is calculated using parameters entered in the ***Lifetime Model*** dialog box. Whenever the ***ISA Graph*** window shows the curve fit, the ***Lifetime Model*** dialog box is also open.

1 Click the *Modeling* button in the ***Lifetime*** toolbar.

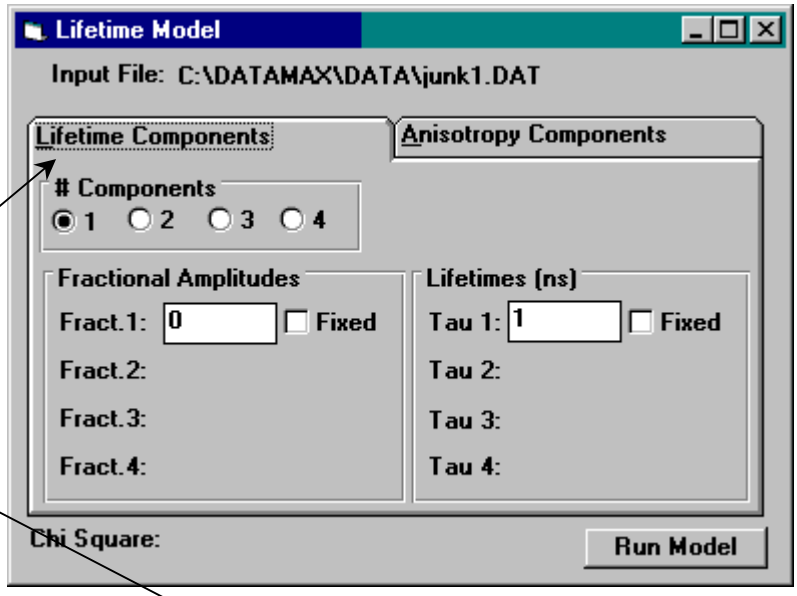
This toggles the ***ISA Graph*** between showing only data, and showing data plus curve fit. Simultaneously, the *Modeling* button reveals and closes the ***Lifetime Model*** dialog box, respectively. (The ***ISA Graph*** window may have to be moved to see the ***Lifetime Model*** dialog box.)

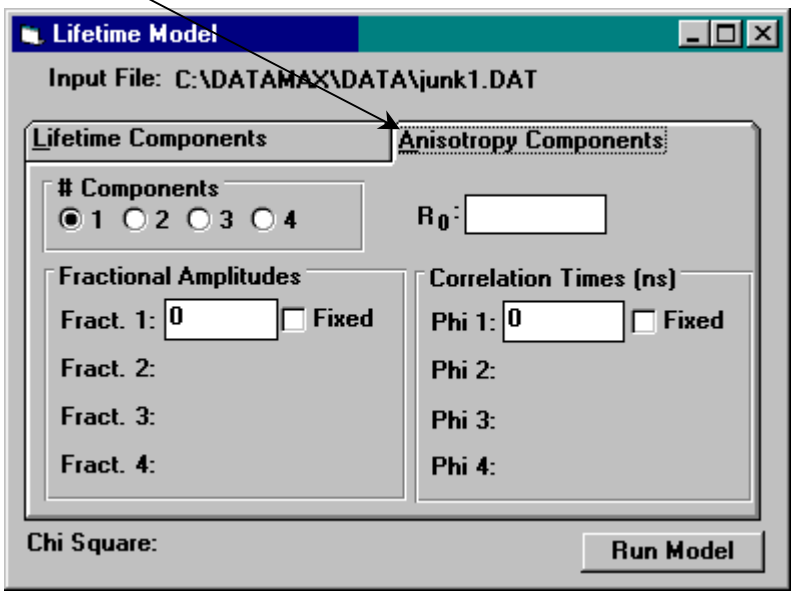
Modeling can be performed with no, some, or all variables fixed.

The ***Lifetime Model*** displays two index cards:

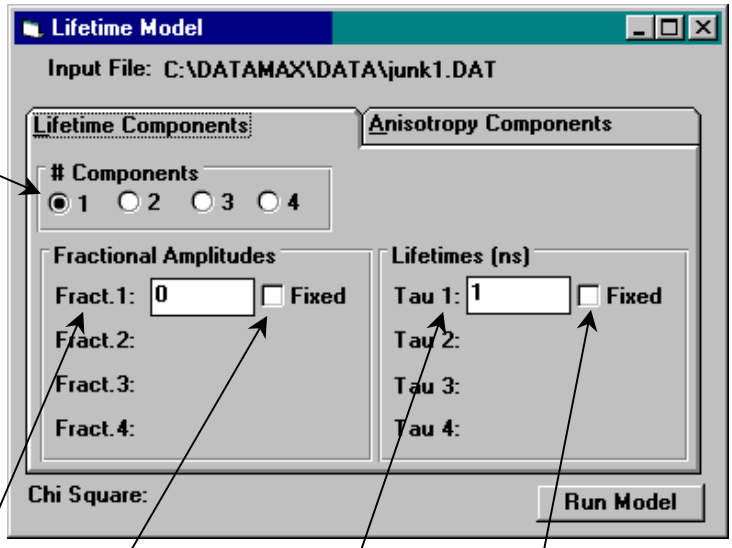
- *Lifetime Components*, which fits the lifetimes
- *Anisotropy Components*, which fits the anisotropies.

Click on the appropriate index-card tab to change the desired parameters.





2 Choose the *Lifetime Components* parameters



a Choose the desired # *Components* radio button.
The number of components is the number that the model uses to fit the total fluorescence response. Anywhere from 1 to 4 components may be chosen.

b Enter the *Fractional Amplitudes* for each component.
A fractional amplitude is the amount of the total lifetime that each component contributes. The sum of all fractional amplitudes must be exactly 1. Therefore, for a 1-component fit, the Fract.1 must be 1; for a 2-component fit, Fract.1 + Fract.2 = 1; and so on.

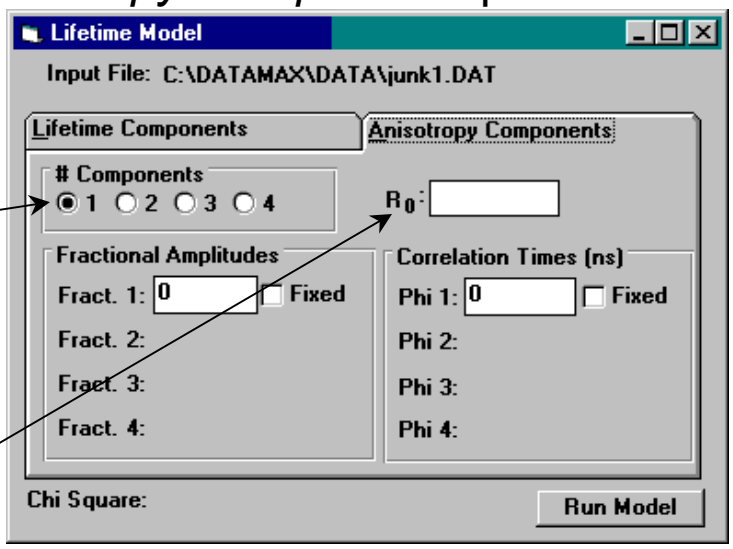
c Check the *Fixed* box for each fractional amplitude not allowed to float.

d Enter a *Lifetime* (in ns) for each fractional amplitude.

e Check the *Fixed* box for each *Tau* (lifetime) not allowed to float.

f Click the *Anisotropy Components* index-card tab.
This switches to the *Anisotropy Components* index card.

3 Choose the *Anisotropy Components* parameters.

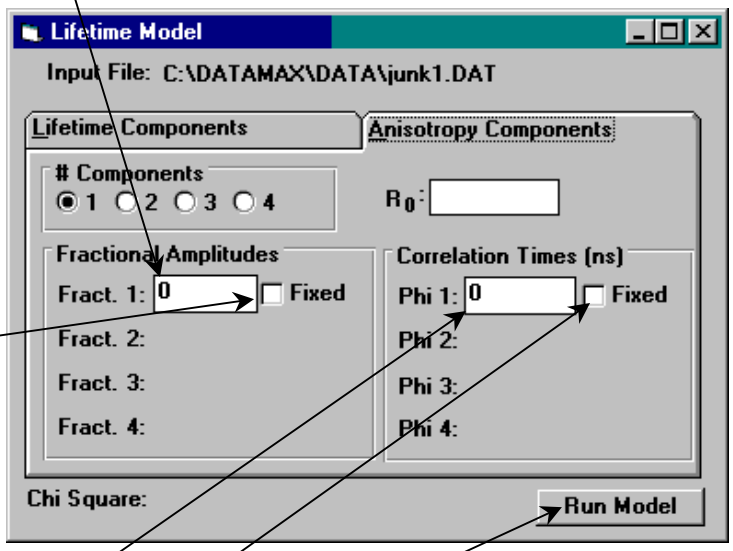


a Choose the desired # *Components* radio button.
Anywhere from 1 to 4 components may be chosen.

b Enter the R_0 (limiting anisotropy).

c Enter the

Fractional Amplitudes for each component. The sum of all fractional amplitudes must be exactly 1.



d Check the *Fixed* box for each fractional amplitude not allowed to float.

e Enter a *Correlation Time* (in ns) for each fractional amplitude.

f Check the *Fixed* box for each *Phi* (correlation time) not allowed to float.

4 Click *Run Model*.

The fit appears on the ***ISA Graph*** window, floating parameters are replaced in the ***Lifetime Model*** dialog box, and the reduced χ^2 appears in the *Chi Square* field at the bottom of the ***Lifetime Model*** dialog box.

5 Repeat steps 2–4 until a satisfactory model is found.

6 Save the model using the ***ISA Graph*** window.

Choosing and defining experiment types

Experiment types

Of the four lifetime types of experiments available, one—*Lifetime Resolved Acquisition*—is accessible from the ***Run Experiment*** application, and is described therein. The other three are available in the ***Lifetime*** application. They are:

- Lifetime Acquisition
- Time Resolved Acquisition
- Anisotropy Decay Acquisition

This section describes these three types of lifetime experiments.

Lifetime Acquisition

The *Lifetime Acquisition* experiment—the default experiment—determines accurate lifetimes from single-component and multi-lifetime systems. This experiment records a phase-shift and modulation at specified frequencies for an unknown, with respect to a standard. The *Lifetime Acquisition* data file's name automatically receives the `.DAT` extension, and is stored on the default drive in the default directory, unless otherwise specified. Data can be modeled during or after acquisition.

Time Resolved Acquisition

Use a *Time Resolved Acquisition* to examine a sample's change in spectral characteristics during its excited state's lifetime. This experiment determines the frequency response of the unknown over a specified emission range. Applications include solvent-relaxation of the excited state, and excimer formation. Specify the number of scans to run before starting the experiment. Each scan is executed sequentially. The name of the data file may be truncated, if necessary, in order to append the code 01, 02, 03, …, etc. The data file's name automatically receives the `.DAT` extension, and is stored on the default drive and directory, unless otherwise specified.

Following is an example of a series of *Time Resolved Acquisition* data files. The parent data file is `TIME`. The experiment is run six times. The resulting data file names are:

```
TIME01.DAT
TIME02.DAT
TIME03.DAT
TIME04.DAT
TIME05.DAT
TIME06.DAT
```

Anisotropy Decay Acquisition

Examine the rotational properties of fluorescent molecules and probes via an *Anisotropy Decay Acquisition*. As the fluorophore rotates, its polarization changes, yielding information about the excited state of the sample. The anisotropy is affected by Brownian motion, energy-transfer, re-absorption, re-emission, and light-scattering. Applications of the *Anisotropy Decay Acquisition* include: studying asymmetric complex molecules, environmental perturbations on molecules, binding, phase-transitions involving hindered rotation, and internal viscosities of bilayers. The data recorded from an *Anisotropy Decay Acquisition* provide no lifetime, so they cannot be modeled. Executing an *Anisotropy Decay Acquisition* creates one data file, with default extension `.DAT`, saved to the default directory and drive, unless otherwise specified.

The *Anisotropy Decay Acquisition* resolves overlapping spectra based on differences in their fluorescence lifetimes. Up to three components can be resolved completely. More complex systems may give improved resolution of one or more spectra, but complete resolution requires additional data manipulation of the data-acquisition parameters, such as excitation wavelength. An application using lifetime-resolved scans could resolve tyrosine and tryptophan emission spectra from a protein containing both compounds. To improve resolution, measure the lifetime and spectral characteristics first. Generally, the *Anisotropy Decay Acquisition* works best for a difference of at least 1.5 between the lifetimes to be resolved.

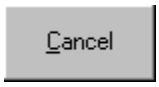
Data-entry parameters for the ***Lifetime*** application

Auto Save Exp

The *Auto Save Exp* checkbox provides the choice of automatically saving the experiment files, or saving them by request only. If the *Auto Save Exp* checkbox is enabled, then a prompt appears for the *Experiment File Name* and *Data File Name* each time an experiment is run.

To enter an experiment's file name and data file name each time an experiment is run,

1 Click the *Auto Save Exp* checkbox.



Cancel

The *Cancel* button terminates the initiated action or command. The screen view then returns to the view visible before opening this dialog box.

To abort an operation without saving or executing,

1 Click *Cancel*.

Comment

The *Comment* field inserts any desired text on the spectral screen below the file name. The amount of text that may be entered has no limit, but only the first 80 characters are displayed on the spectral screen. Standard editing keys are used to correct or insert text.

Note: *Special characters are not allowed in comments.*

To place text below the file name on the spectral screen,

1. Place the mouse cursor in the *Comment* field.
2. Enter the desired text.

To view the comment field of a saved file,

1. Enter the ***Run Experiment*** application.
2. Choose *File*.
   A drop-down menu appears.
3. Choose *Open…*.
   The ***Open File*** dialog box appears.
4. Click *Info*.
   The ***File Information*** dialog box appears, showing the first 80 characters of the comment.

DataFile…

DataFile… stores experimental data and information about the instrument and acquisition. These files are saved automatically with the `.DAT` extension, unless otherwise requested. To execute a scan, a data file name must be entered in the *DataFile…* field.

To enter a data file name,

1 Place the mouse cursor in the *DataFile…* data-entry field.

2 Enter the name of the file to which the data will be saved.

No extension is required. By default, a `.DAT` extension is added.

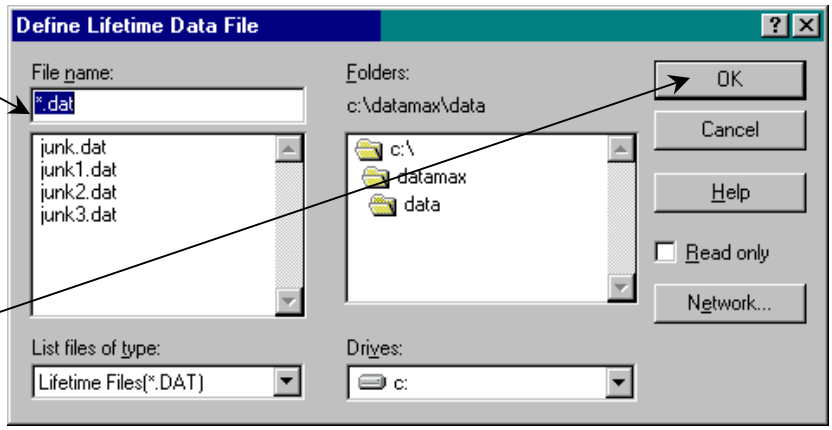
To open a saved data file, and execute its parameters,

1 Click *DataFile….*

The ***Define Lifetime Data File*** dialog box appears.

2 Select the desired file.

3 Click *OK*.



The ***Define Lifetime Data File*** dialog box closes, and the parameters automatically fill the acquisition screen.

Emission

The *Emission* field, found only on the ***Anisotropy Decay Acquisition*** dialog box, represents the position of the emission monochromator. Usually this position is the wavelength at which the sample fluoresces with maximum intensity.

To enter the position of the emission monochromator,

1. Click in the *Emission* field.
2. Enter the desired position of the emission monochromator.

End Freq

The *End Freq* field sets the final frequency, in MHz, at which samples are scanned.

To set the final scanning frequency,

1. Click in the *End Freq* field.
2. Enter the final scanning frequency, in MHz.
3. Hit **TAB**, or click on another field.

Estimated Time

The *Estimated Time* field shows the estimated amount of time for the scan, using the parameters entered. The field is not user-adjustable, but calculated based on the existing parameters.

Excitation

The *Excitation* field sets the position for the excitation monochromator. This field is found only on the ***Anisotropy Decay Acquisition*** dialog box.

To set the position of the excitation monochromator,

1. Click in the *Excitation* field.
2. Enter the desired position for the excitation monochromator.

Experiment…

An experiment file stores instrument and acquisition parameters, but no actual data. The default extension to the file name is `.EXP`. Experiment files must be provided in order to save an experiment. The *Experiment...* button saves and accesses experiment files. If a data file (`.DAT`) is opened using the *Experiment...* button, the experimental parameters are recalled also.

When an acquisition dialog box is first opened, it appears with the name of the default experiment. The *Comment* field identifies the type of experiment having these default parameters.

To enter an experiment file name (to save an experiment file),

1 Place the mouse cursor in the *Experiment…* field.

2 Replace the default file name with the name under which the experiment is to be saved.

The `.EXP` extension is not required; it is supplied automatically.

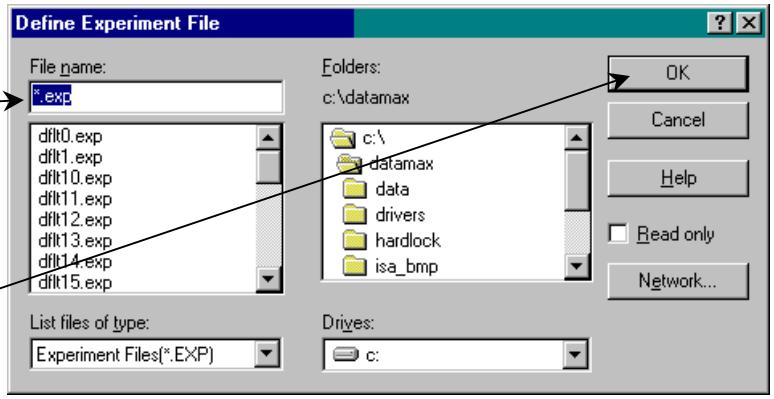
To open an existing experiment file,

1 Click the *Experiment…* button.

The ***Define Experiment File*** dialog box appears.

2 Select the desired experiment file.

3 Click *OK*.



The ***Define Experiment File*** dialog box closes, and the parameters fill the screen.

HV

The *HV* button sets the high voltage for the detectors. Detectors must be included in the layout in order to appear in the ***High Voltage*** dialog box. The *HV* button itself shows whether high voltage is operating:

- If at least one detector has high voltage, the *HV* button displays "on".
- If no detectors receive high voltage, the *HV* button displays "off".

To change a detector's high voltage,

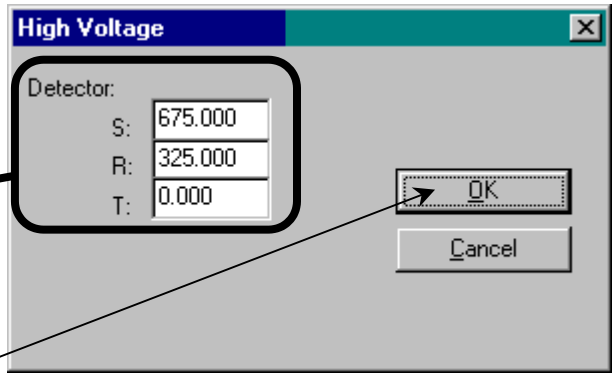
1 Click *HV*.

The ***High Voltage*** dialog box appears. All available detectors are listed.

2 Click on the appropriate detector's data-entry field.

3 Enter the desired high voltage.

4 Click *OK*.



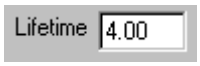
The high voltage is reset to the new value, and the ***High Voltage*** dialog box disappears.

Integration Time

The *Integration Time* sets the length of time that data are collected for each data point. The minimum integration time is 1 ms.

To set the integration time,

1. Click in the *Integration Time* data-entry field.
2. Enter the desired integration time.
3. Hit **TAB**, or click on another field.



Lifetime

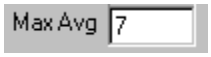
The *Lifetime* field sets the lifetime of the sample, in ns. The *Lifetime* field is found only on the ***Anisotropy Decay Acquisition*** dialog box.

To set the sample's lifetime,

1 Click in the *Lifetime* field.

2 Enter the lifetime, in ns.

3 Hit **TAB**, or click on another field.

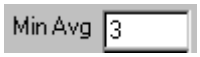


Max Avg

The *Max Avg* field sets the maximum average to be acquired per frequency. The more data points averaged, the better the data.

To set the maximum average per frequency,

1. Click in the *Max Avg* data-entry field.
2. Enter the maximum average.
3. Hit **TAB**, or click in another field.



Min Avg

The *Min Avg* field sets the minimum average to be acquired per frequency. The more data points averaged, the better the data.

To set the minimum average per frequency,

1. Click in the *Min Avg* data-entry field.
2. Enter the minimum average.
3. Hit **TAB**, or click in another field.

Mono Positions...

Mono Positions…

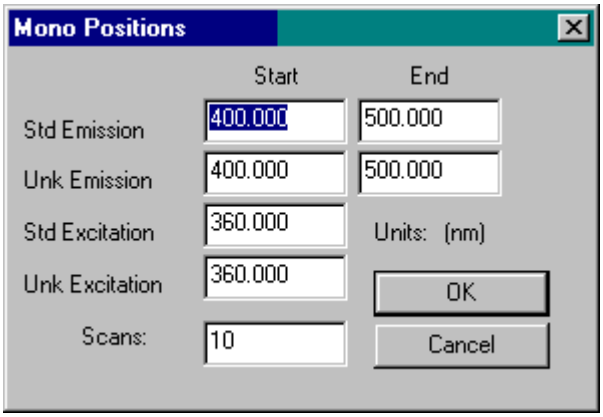
To specify the wavelengths for the monochromators in a *Time Resolved Acquisition* scan, use the *Mono Positions…* button. Each scan is saved to a file, and can be displayed separately, or as a multifile group.

To change the monochromators' positions,

1 Click *Mono Positions….*

The ***Mono Positions*** dialog box opens.

2 Set the positions for the emission and excitation monochromators:



Std Emission Start	Where the emission monochromator begins a scan of the standard
Std Emission End	Where the emission monochromator ends a scan of the standard
Unk Emission Start	Where the emission monochromator begins a scan of the unknown
Unk Emission End	Where the emission monochromator ends a scan of the unknown
Std Excitation Start	Where the excitation monochromator remains fixed during a scan of the standard
Unk Excitation Start	Where the excitation monochromator remains fixed during a scan of the unknown
Scans	The number of scans to be conducted.

3 Click *OK*.

The information is saved, the monochromators are repositioned, and the ***Mono Positions*** dialog box is closed.

Number of Freqs

The *Number of Freqs* sets the number of frequencies to be measured. The possible range is 1–99 frequencies. The more frequencies measured, the longer the scan takes. Use the *Number of Freqs* field in conjunction with the *Sequence* field and radio buttons, to determine the distribution and actual values of the frequencies.

To set the number of frequencies,

1 Click in the *Number of Freqs* data-entry field.

2 Enter the number of frequencies.

3 Hit **TAB**, or click in another field.

Process by

The *Process by* area specifies how the data are processed. See the *Fluorolog®-Tau-3 Operation Manual* for details on the two methods: discrete pairs, or interleaving.

To choose the method of processing,

1 Click the appropriate radio button:

- Discrete Pairs
- Interleave

Quick Scan

To measure lifetimes quickly, enable the *Quick Scan* checkbox. Using *Quick Scan*, the unknown is positioned in the optical path, and entire frequency range is measured. After the unknown scan is complete, the standard is moved into the optical path, and its entire frequency range is measured. With an automatic sample changer, the samples can be moved quickly.

To run the scans more quickly,

1 Check the *Quick Scan* checkbox.

Run

The *Run* button is used to start the experiment, after all settings are correct. Be sure a data file name is entered. If *Auto Save Exp* is enabled, be sure an experiment file name is entered also.

To execute an experiment,

1 Click *Run*.

Save

To store experiment information to the hard drive, use the *Save* button. When *Save* is activated, the information is stored to a user-named or data file. Be sure a valid experiment file name and data file name are entered.

To save a file,

1 Click *Save*.

If a file of the same name already exists, a warning appears. The file can be overwritten, or the procedure stopped.

Sequence (radio buttons)

The *Sequence* radio buttons choose the distribution of frequencies to use in the experiment. Either a linear or a logarithmic sequence is possible. To specify the precise frequencies or enter custom frequencies, see the *Sequence*... button.

To choose the distribution of frequencies,

1 Click either *Linear* or *Log*.

Sequence...

Sequence… (button)

The *Sequence…* button customizes the precise frequencies to use during the experiment. The starting, ending, distribution, and number of frequencies all can be adjusted.

To select a set of standard frequencies,

1 Click *Sequence….*
The ***Frequencies*** dialog box opens.

2 Click in the *Start* field.

3 Enter the starting frequency.

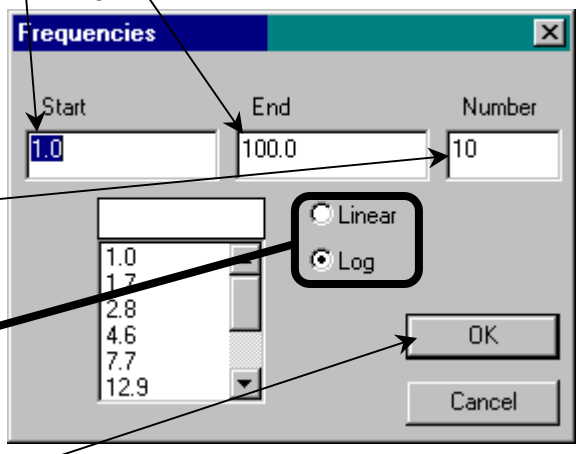
4 Click in the *End* field.

5 Enter the ending frequency.

6 Click in the *Number* field.

7 Enter the number of frequencies to use.

8 Click either *Log* or *Linear*.
This chooses either a logarithmic or linear sequence of frequencies.

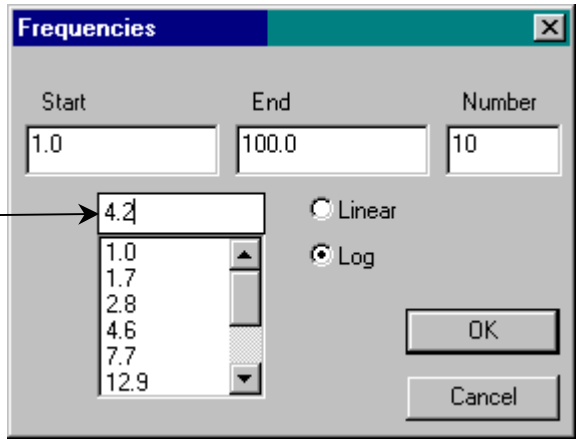


9 Click *OK*.
The ***Frequencies*** dialog box closes, and the distribution of frequencies is set.

To enter a set of custom frequencies,

1 Click *Sequence….*
The ***Frequencies*** dialog box opens.

2 Place the mouse cursor in the frequencies data-entry field.



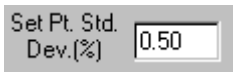
3 Enter the desired frequency.

4 Click on a different data-entry field, or hit **TAB**.

5 Repeat steps 2 to 4 for each custom frequency.

6 Click *OK*.

The ***Frequencies*** dialog box closes, and the distribution of frequencies is set.



Set Pt. Std. Dev.(%)

The *Set Pt. Std. Dev.(%)* sets the acceptable tolerance for the Tau-3's measurements. The higher the percentage is, the shorter the acquisition time is.

To set the standard deviation,

1. Click on the data-entry field.
2. Enter the desired value.
3. Hit **TAB**, or click on another field.

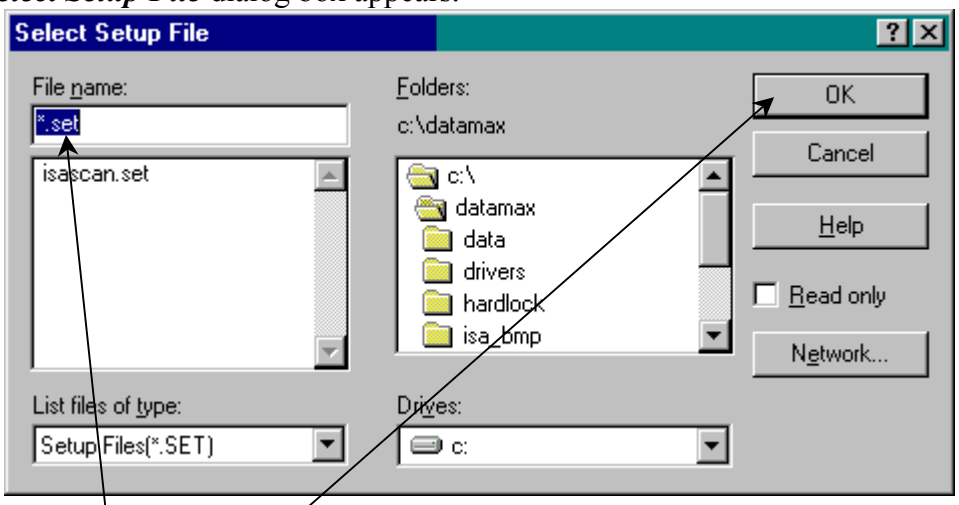
Setup File... **Setup File…**

Setup File… is used to store or retrieve setup information. A setup file (with extension `.SET`) contains information about setting up the instrument, such as hardware settings and system units. Selecting a setup file is optional; if no setup file is selected, the system uses current or default values.

To open an existing setup file,

1 Click *Setup File….*

The ***Select Setup File*** dialog box appears.



2 Choose the desired setup file.

3 Click *OK*.

The ***Select Setup File*** dialog box closes, and the parameters associated with that setup file fill the acquisition screen.

Signals...

Signals…

The *Signals…* button chooses which detectors to use for the acquisition. To calculate the phase data, the following automatic method is used:

a Collect phase data from the first detector for the standard.

b Collect phase data from the second detector for the standard.

c Calculate the difference between a and b.

d Collect phase data from the first detector for the unknown.

e Collect phase data from the second detector for the unknown.

f Calculate the difference between d and e.

g Calculate the difference between c and f.

h Save g to a data file (`.DAT`).

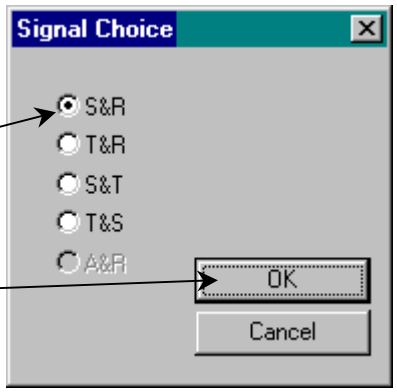
The accessible detector choices depend on the system's layout and configuration.

To choose the unknown and standard detectors,

1 Click *Signals…*.
The ***Signal Choice*** dialog box appears.

2 Click the radio button next to the desired acquisition mode.

3 Click *OK*.
The ***Signal Choice*** dialog box closes.



Slits...

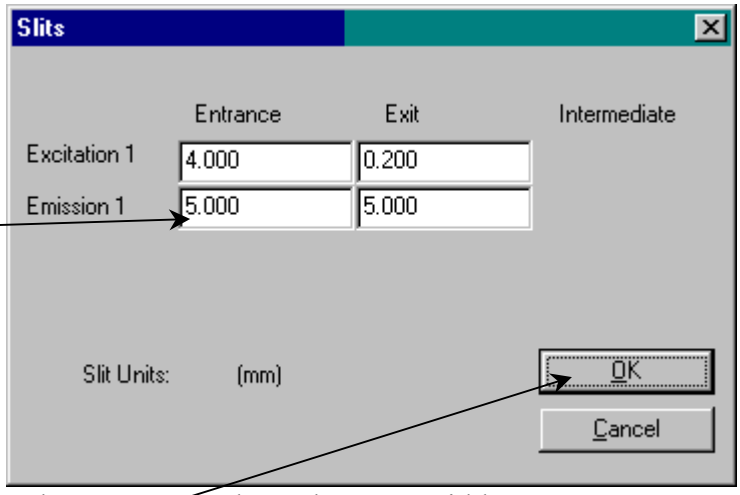
Slits…

To adjust the slit width of each available monochromator, use *Slits*…. The units for the slits (mm, µm, bandpass) are changed in ***Visual Instrument Setup***.

To change a slit width,

1 Click *Slits*….

The ***Slits*** dialog box opens.



2 Click on the desired slit's data-entry field.

3 Enter the desired value.

In bandpass mode, all slits for a given monochromator must have the same width.

4 Click *OK*.

The ***Slits*** dialog box closes.

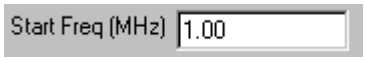
Standard

Standard specifies the position of the standard in the sample changer. DataMax must know the position of the standard and unknown in the sample changer, in order to scan and calculate results correctly.

To set the position of the standard in the sample changer,

1 Click the down arrow next to the data-entry field.

2 Select the correct position from the list.
Often, 2 is used for the standard's position.



Start Freq

The *Start Freq* sets the starting frequency for the experiment. The allowed range of frequencies is 0.1–330 MHz.

To set the starting frequency,

1. Click in the data-entry field.
2. Hit **TAB**, or click on another data-entry field.

Start Time

To tell DataMax to start the experiment immediately, or wait a user-specified delay, use the *Start Time* area. This feature lets experiments needing minimum intervention or supervision to start after normal working hours, and the results are retained. When the *Delay* radio button is selected, a hidden field appears with a timer. The experiment begins after the length of time set in the timer, or at the onset of a trigger.

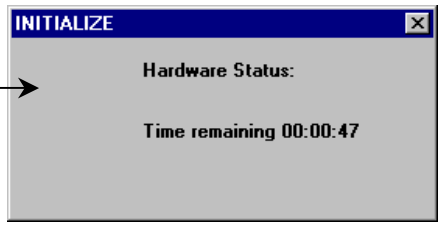
To start the experiment immediately after clicking *Run*,

1 Click the *Immediate* radio button.

To start the experiment after a delay,

1 Click the *Delay* radio button.
A hidden-field timer appears.

2 Enter the hours to delay in the *Hr* field, and the minutes to delay in the *Min* field.
The range is 0–99 h, and 0–59 min.
Complete both fields. A reminder timer remains on the screen, and counts down.



Notes:

1 Once the delay time is set,

a The system and host computer must remain on.

b The ***Run Experiment*** application must remain open.

c Do not run any other experiment.

Note: *Violating any of rules a to c immediately aborts the original experiment.*

2 All other DataMax applications are accessible during the countdown.

3 When the delayed experiment starts, all unsaved information in other applications is lost.

Std Em

There are two uses for *Std Em*:

- For a lifetime acquisition, *Std Em* sets the position at which the emission monochromator remains fixed during a scan.
- For a time-resolved acquisition, *Std Em* sets the beginning wavelength for the emission monochromator scan.

To set the standard-emission field,

1 Click in the data-entry field.

2 Enter the new value.

Std Ex

Std Ex sets the position at which the excitation monochromator remains fixed during a scan. For a time-resolved acquisition, the *Std Ex* field is found in the ***Mono Positions*** dialog box.

To set the standard-excitation field,

1 Click in the data-entry field.

2 Enter the new value.

Std Lifetime

Std Lifetime sets the value for the lifetime of the standard, in ns.

To set the standard's lifetime, in ns,

1. Click in the data-entry field.
2. Enter the standard's lifetime.
3. Hit **TAB**, or click in another data-entry field.

Steady State G Factor

The *Steady State G Factor* data-entry field, found on the ***Anisotropy Decay Acquisition*** dialog box, is the correction for instrumental response to polarized light at a particular wavelength. For mathematical details about the *G* factor, see the *Spex® Polarizers Operation Manual*.

To enter the *G* factor,

1. Click in the *Steady State G Factor* data-entry field.
2. Enter the *G* factor.
3. Hit **TAB**, or click on another field.

Unknown

Unknown sets the position of the unknown sample in the sample changer. For DataMax to scan the correct standard and unknowns in the proper sequence, it must know the position of the unknown. Usually the unknown is placed in position 1 of the sample changer.

To set the position of the unknown in the sample changer,

1 Click the down arrow next to the data-entry field.

2 Select the correct position.

Unknown Em

The *Unknown Em* data-entry field has two purposes:

- In a lifetime acquisition, *Unknown Em* sets the position at which the emission monochromator is fixed during the scan.
- In a time-resolved acquisition, *Unknown Em* sets the starting position for the emission monochromator scan.

To set the starting or fixed position of the emission monochromator,

1 Click in the data-entry field.

2 Enter the desired position.

Unknown Ex

Unknown Ex sets the position at which the excitation monochromator remains fixed during a scan. For a time-resolved acquisition, the *Unknown Ex* field is found in the ***Mono Positions*** dialog box.

To set the unknown-excitation field,

1 Click in the data-entry field.

2 Enter the new value.

10: Post-Experiment Modeling

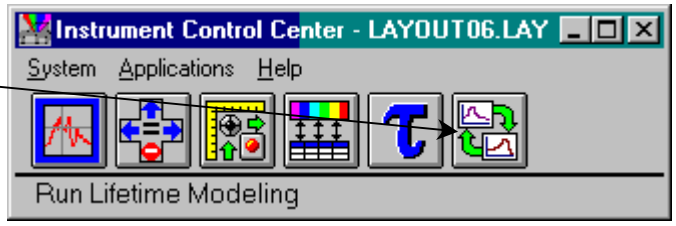
Introduction

The ***Post-Experiment Modeling*** application, used only with the Fluorolog®-Tau-3 system, provides models of lifetime acquisition data previously stored on disk. (For modeling while a lifetime experiment is in progress, see Chapter 9.) The ***Post-Experiment Modeling*** application is stand-alone, that is, it can analyze data obtained through DataMax or elsewhere. Initialization of the hardware is not required to run ***Post-Experiment Modeling***.

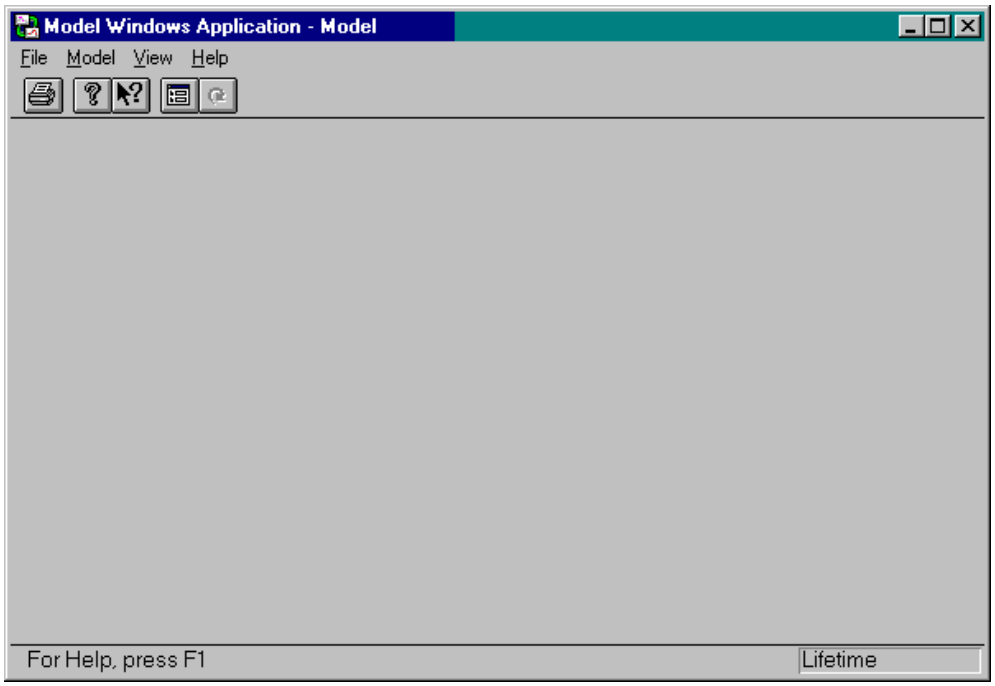
As with the ***Real Time Display*** and ***Lifetime*** applications, the ***Post-Experiment Modeling*** application is a control panel, upon which the plot of the model and data appear.

Quick guide to ***Post-Experiment Modeling***

1 Click the *Modeling* icon in the ***Instrument Control Center***.

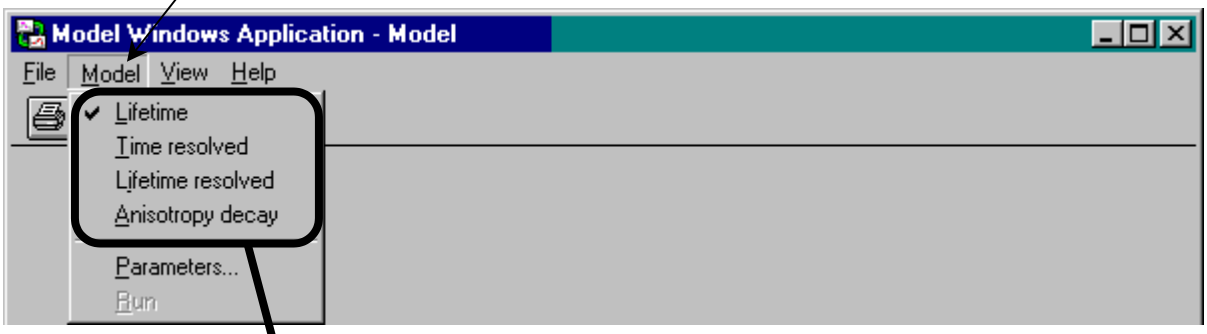


The ***Model Windows Application – Model*** window opens:



2 Click *Model*.

A drop-down menu appears.



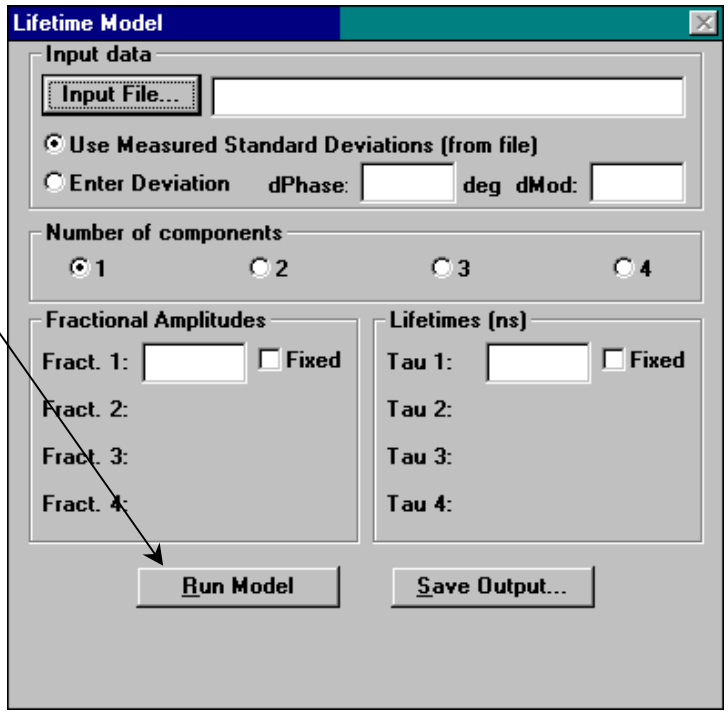
3 Click on the type of model.

The appropriate Model window opens:

4 Complete the parameters.

5 Click *Run Model*.

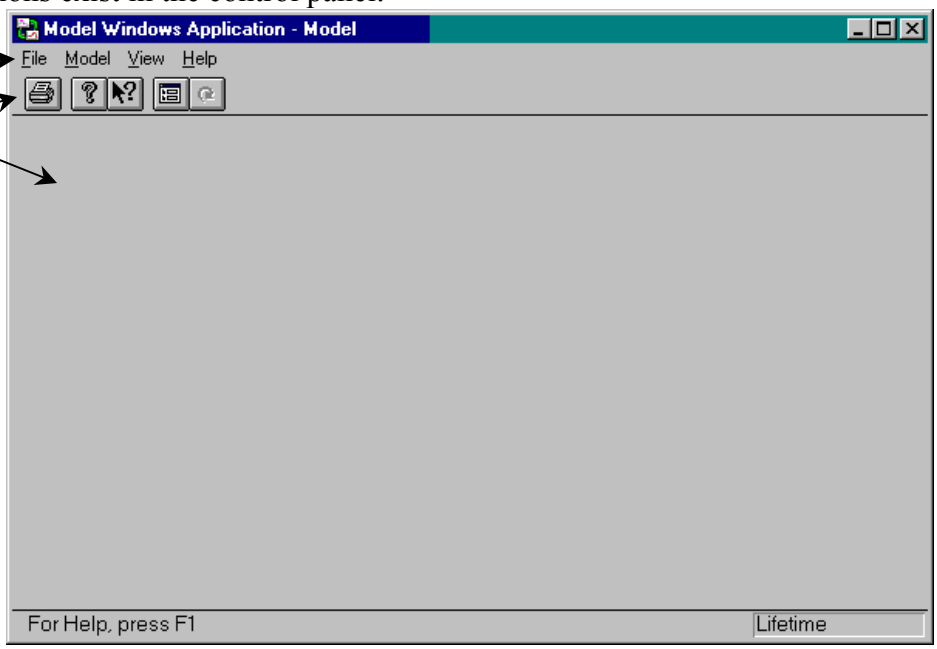
The model with desired parameters is run. The associated graph appears on the ***Model Windows Application*** dialog box.



Control panel

When the ***Post-Experiment Modeling*** application starts, the control panel appears. Three main regions exist in the control panel:

- Main menu
- Toolbar
- Plot area



Many commands are executed from the main menu and toolbar. Those commands specific to the graphs and data are run from the plot area, by pointing and clicking with the mouse. See Chapter 11 for details about the choices for plotting.

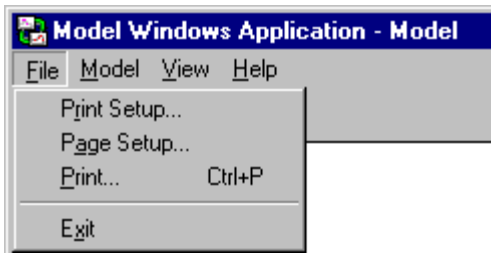
Main menu

The main menu of ***Post-Experiment Modeling*** contains four items:

- File
- Model
- View
- Help

Clicking on any of the above items reveals a drop-down menu with choices. This section describes the choices in detail.

File



The *File* command deals with printing options, such as page specifications and printer choices. An option to quit the ***Post-Experiment Modeling*** application is included also.

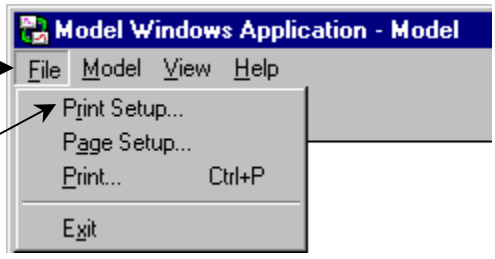
Print Setup…

Print Setup… controls the various printer options, such as which printer, and page orientation and size.

To adjust the printer options,

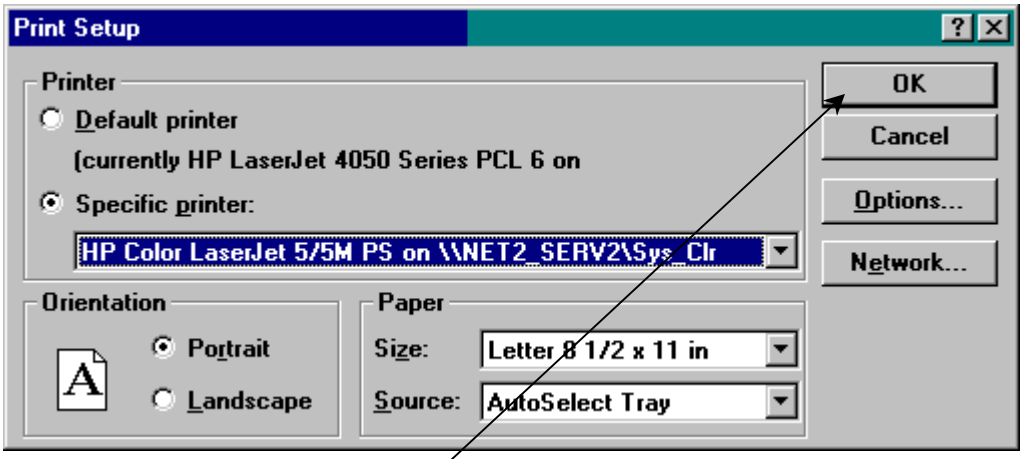
1 Click *File*.

A drop-down menu appears.



2 Click *Print Setup*….

The ***Print Setup*** dialog box appears.



3 Choose the appropriate parameters.

Consult the printer manual for the full range of options available.

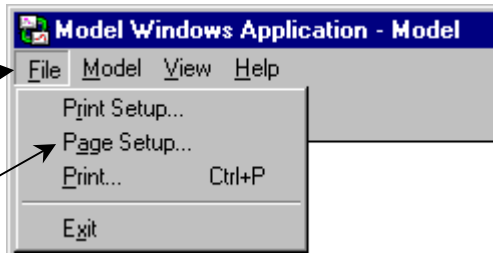
4 Click *OK*.

The ***Print Setup*** dialog box closes, and the printer options are set.

Page Setup…

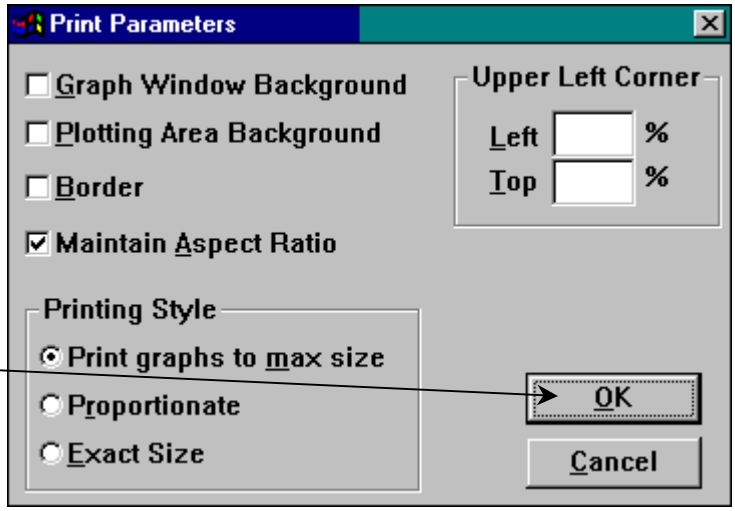
Page Setup… controls how the information appears on the page.

To specify the page setup,



1 Click *File*.
A drop-down menu appears.

2 Click *Page Setup…*.
The ***Print Parameters*** dialog box appears.



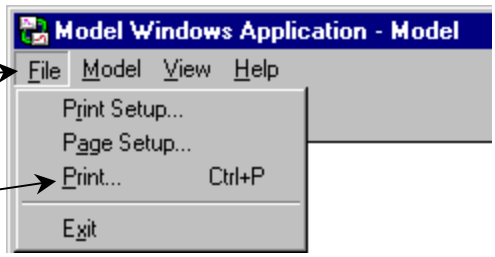
3 Choose the desired parameters.
See Chapter 11 for full details on the data-entry fields.

4 Click *OK*.
The page setup is set, and the ***Print Parameters*** dialog box closes.

Print…

The *Print*... command sends causes the printer to print the graph(s) displayed in the control panel, as specified by the *Print Setup*... and *Page Setup*... options.

To print the graph(s),



1 Click *File*.

A drop-down menu appears.

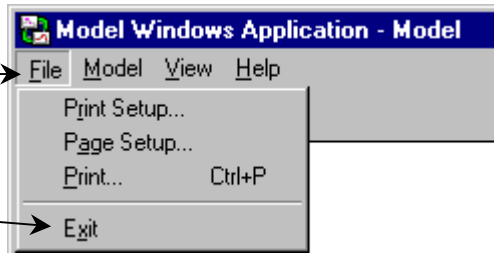
2 Click *Print*….

The information is sent to the printer.

Exit

The *Exit* command quits the ***Post-Experiment Modeling*** application.

To quit Post-Experiment Modeling,

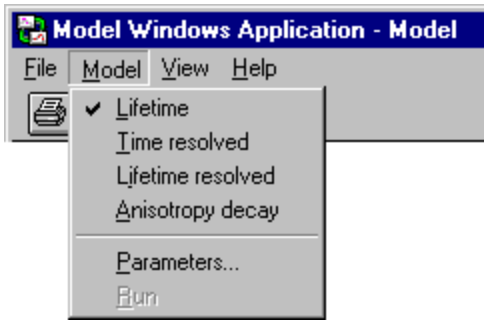


1 Click *File*.
A drop-down menu appears.

2 Click *Exit*.
The ***Post-Experiment Modeling*** application closes.

Note: *Make sure that all files needed later have been saved before clicking* Exit.

Model



The *Model* command is chooses the type of experiment to model, and display or hide the ***Model*** dialog box.

Lifetime

Lifetime modeling provides two graphs: *Lifetime* and *Residuals*. The graphs are displayed on the control panel; their appearance and data can be edited. See Chapter 11 for functions and features related to the plot's appearance. To run a lifetime model, an input file is needed. An input file is the basic data, generated by an application such as DataMax.

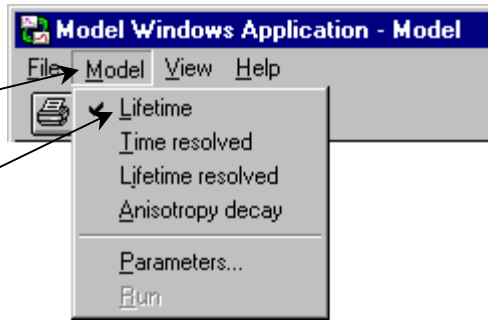
To run a lifetime model,

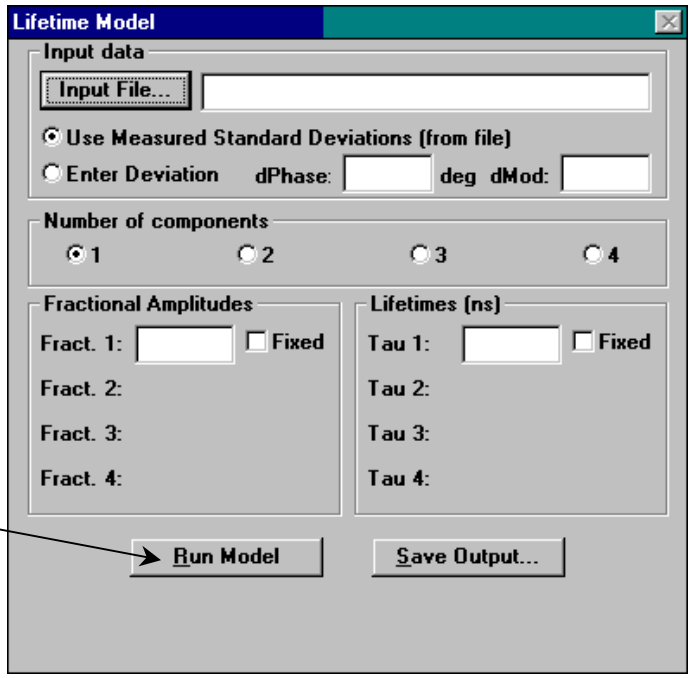
1 Click *Model*.
A drop-down menu appears.

2 Click *Lifetime*.
The ***Lifetime Model*** dialog box opens.

3 Complete the data-entry fields.
The data-entry fields are discussed later in this chapter.

4 Click *Run Model*.
The model is executed, and the graphs are displayed on the control panel. To adjust the plot's appearance, see Chapter 11.



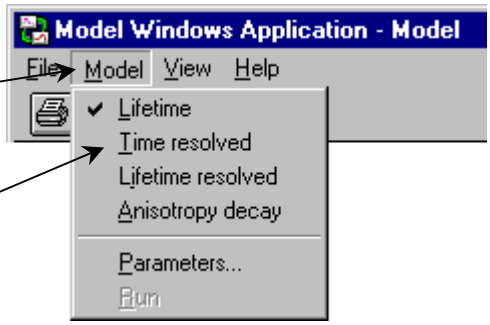


Time Resolved

Time-resolved modeling provides two graphs: *Time Resolved* (for wavelength and intensity) and a second graph (for time, spectral width, and center of gravity). The graphs are displayed on the control panel; their appearance and data can be edited. See Chapter 11 for functions and features related to the plot's appearance. To run a time-resolved model, two input files are needed:

- *Spectral file*, an emission scan executed on the sample with the same parameters as the lifetime file. This file normalizes the time-resolved data file.
- *Time Resolved file*, a group of files specified by the user, and generated by an application such as DataMax. This group of files resolves a substance into its components with respect to time, and derives a lifetime for each.

To run a time-resolved model,

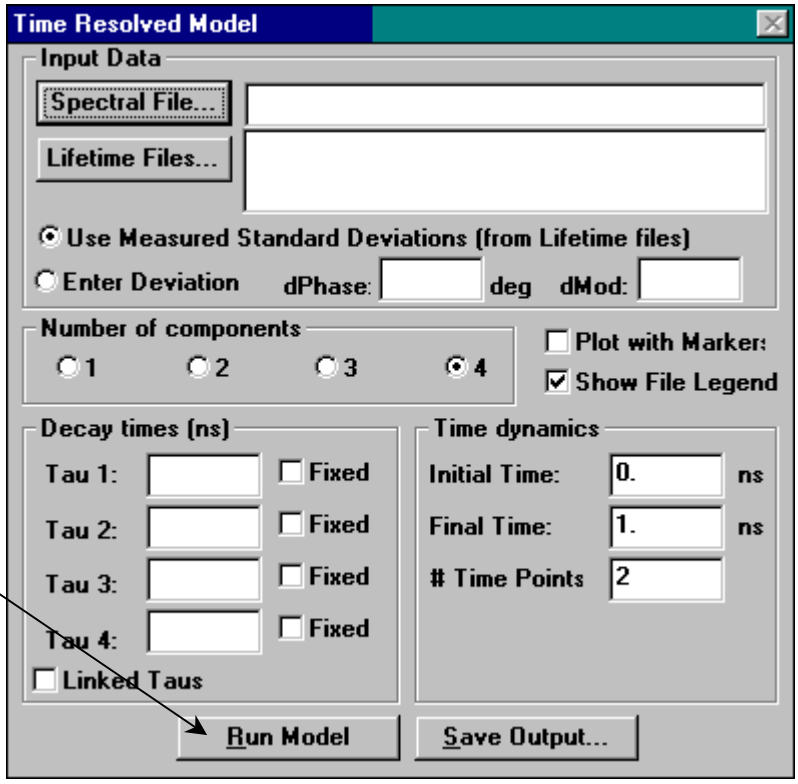


1 Click *Model*.

A drop-down menu appears.

2 Click *Time resolved*.

The ***Time Resolved Model*** dialog box opens.



3 Complete the data-entry fields.

The data-entry fields are discussed later in this chapter.

4 Click *Run Model*.

The model is executed, and the graphs are displayed on the control panel. To adjust the plot's appearance, see Chapter 11.

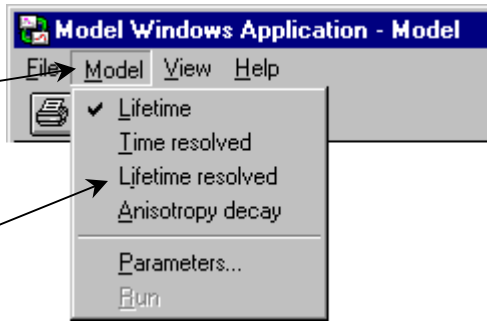
Lifetime Resolved

Lifetime-resolved modeling provides a *Spectral Deconvolution* graph, displayed on the control panel; the appearance and data can be edited. See Chapter 11 for functions and features related to the plot's appearance. To run a lifetime-resolved model, three input files are needed:

- *Spectral file*, a file generated by an application such as DataMax. This file contains information from two or three components, in order to deconvolve the spectra.
- *Phase file*, a file generated by an application such as DataMax.
- *Modulation file*, a file generated by an application such as DataMax.

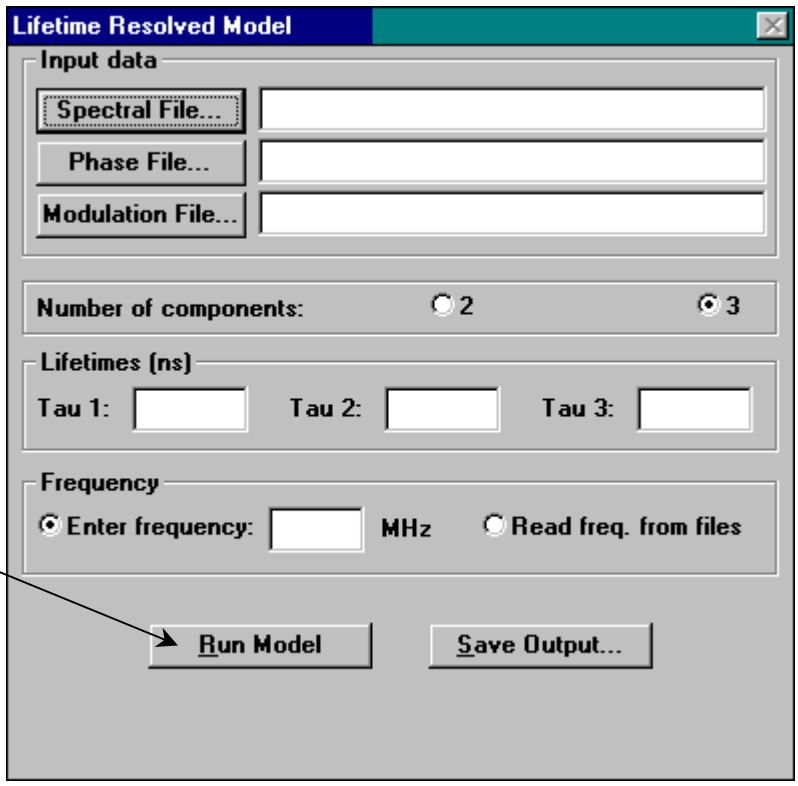
With this model, lifetimes (Taus) must be known already.

To run a time-resolved model,



1 Click *Model*.
A drop-down menu appears.

2 Click *Lifetime resolved*.
The ***Lifetime Resolved Model*** dialog box opens.



3 Complete the data-entry fields.
The data-entry fields are discussed later in this chapter.

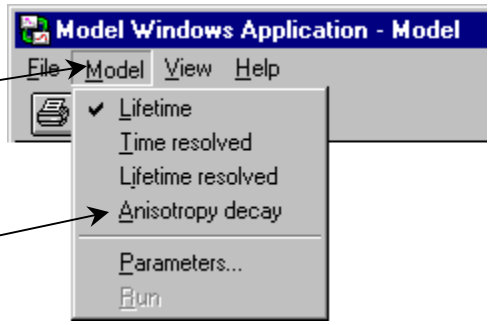
4 Click *Run Model*.
The model is executed, and the graphs are displayed on the control panel. To adjust the plot's appearance, see Chapter 11.

Anisotropy decay

Anisotropy-decay modeling provides two graphs, *Anisotropy Decay* and *Residuals*. They are displayed on the control panel; their appearance and data can be edited. See Chapter 11 for functions and features related to the plot's appearance. To run an anisotropy-decay model, an input file is needed. The input file is a file generated by an application such as DataMax.

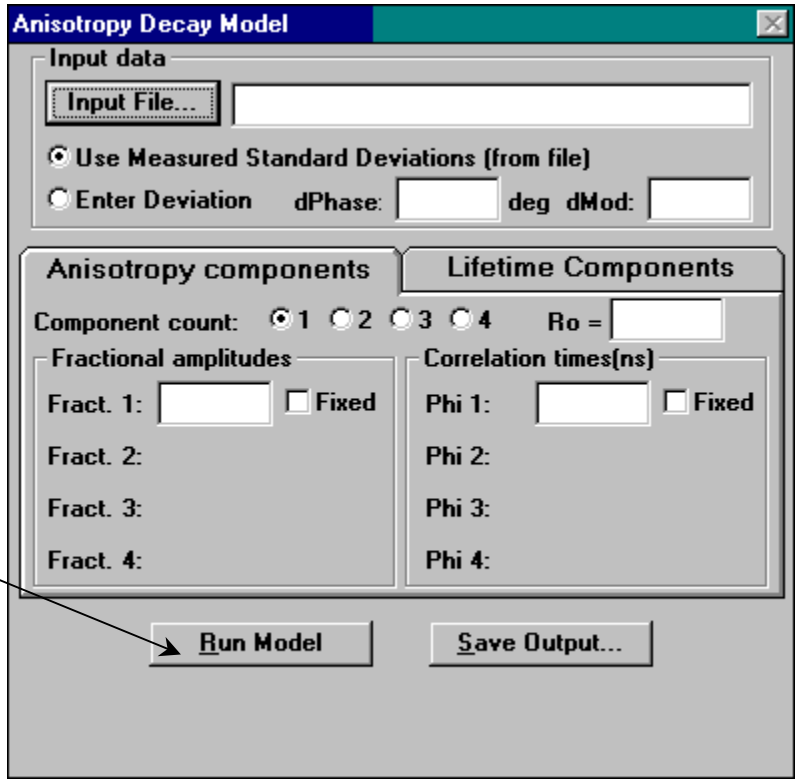
The ***Anisotropy Decay Model*** dialog box has two index cards, *Anisotropy Decay Components*, and *Lifetime Components*. Both must be completed to run the model. Click on the index-card tabs to switch between index cards.

To run an anisotropy-decay model,



1 Click *Model*.
A drop-down menu appears.

2 Click *Anisotropy decay*.
The ***Anisotropy Decay Model*** dialog box opens.



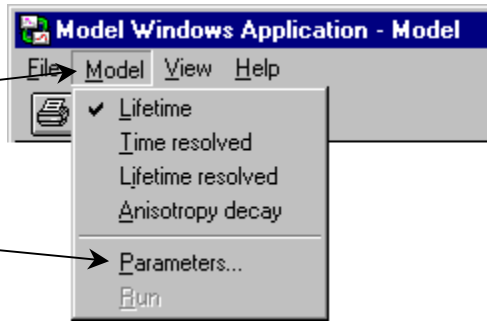
3 Complete the data-entry fields.
The data-entry fields are discussed later in this chapter.

4 Click *Run Model*.
The model is executed, and the graphs are displayed on the control panel. To adjust the plot's appearance, see Chapter 11.

Parameters…

Parameters… hides or displays the current ***Model*** dialog box.

To hide or display the model dialog box,

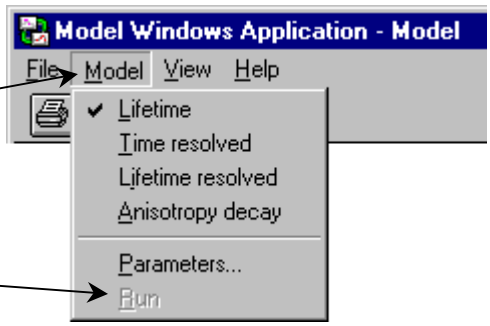


1 Click *Model*.
A drop-down menu appears.

2 Click *Parameters…*.
The ***Model*** dialog box appears or disappears.

Run

One of several methods to execute a model is to choose *Run* from the main menu. *Run* is accessible only if the parameters all have been entered. If the parameters are incomplete, *Run* is gray and inaccessible.



To run a model,

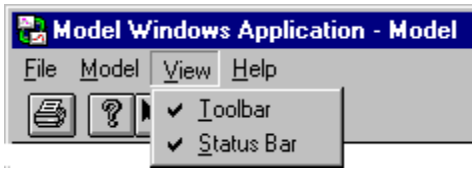
1 Click *Model*.

A drop-down menu appears.

2 Click *Run*.

The current model is executed.

View



The *View* command displays or hides the toolbar and status bar. The status of each icon is listed in the drop-down menu: a check appears next to the toolbar or status bar if it is visible on the control panel.

To display or hide the toolbar,

1 Click *View*.
A drop-down menu appears.

2 Click *Toolbar*.
The toolbar appears or disappears, and a check appears or disappears next to the *Toolbar* choice, respectively.

To display or hide the status bar,

1 Click *View*.
A drop-down menu appears.

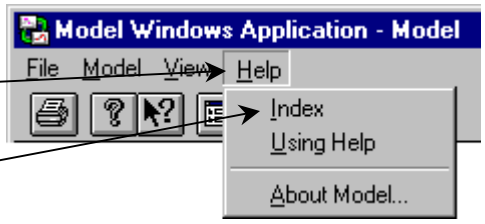
2 Click *Status Bar*.
The status bar appears or disappears, and a check appears or disappears next to the *Status Bar* choice, respectively.

Help

Help offers two categories:

- *Index* and *Using Help*, the contents of *Help*
- *About Model...*, a window that displays information about the ***Post-Experiment Modeling*** software version. If technical support is necessary, be prepared to supply this information.

To search the Help *index,*

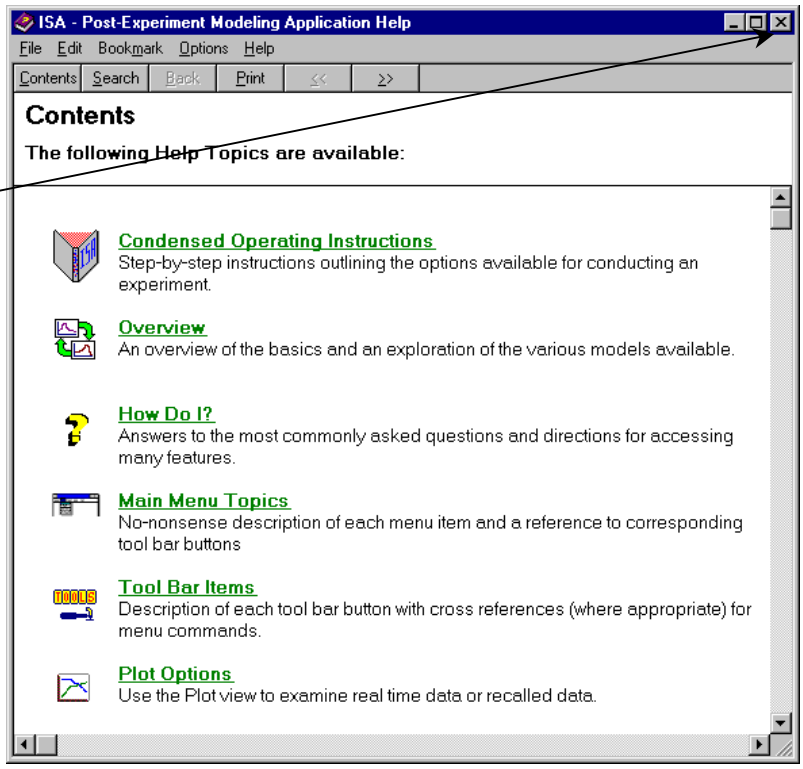


1 Click *Help*.
A drop down menu appears.

2 Click *Index*.
The ***ISA – Post-Experiment Modeling Application Help*** window opens.

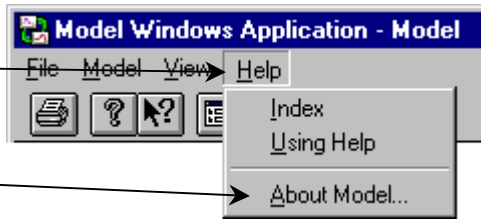
3 Choose a topic.

4 Click the ☒ when done.
The ***ISA – Post-Experiment Modeling Application Help*** window closes.

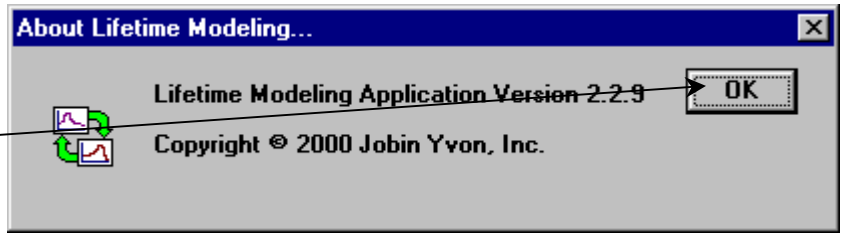


To get information about the **Post-Experiment Modeling** *application,*

1 Click *Help*.
A drop-down menu appears.



2 Click *About Model....*
The ***About Lifetime Modeling...*** window appears.



3 Click *OK*.
The ***About Lifetime Modeling...*** window closes.

Toolbar 

The toolbar provides shortcuts for commonly used commands in ***Post-Experiment Modeling***. The buttons available are:

 Print

 About

 Context-sensitive help

 Parameters

 Run

Clicking on any of these buttons activates or opens the corresponding function or window. The following section describes these buttons in detail.

Print

The *Print* button sends causes the printer to print the graph(s) displayed in the control panel, as specified by the *Print Setup*... and *Page Setup*... options.

To print the graph(s),

1 Click *Print*. 

The information is sent to the printer.

About

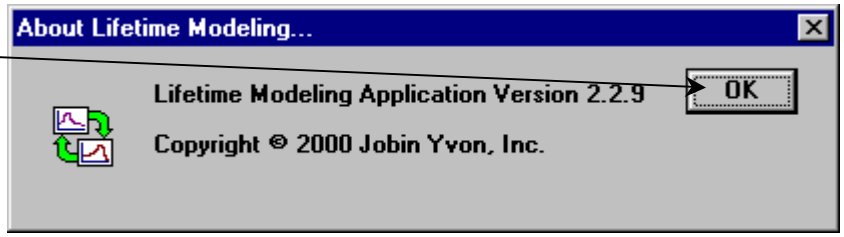
The *About* button provides information about the version of ***Post-Experiment Modeling***.

To see the version of the software,

1 Click *About*.

The ***About Lifetime Modeling...*** window appears.

2 Click *OK*.



The ***About Lifetime Modeling...*** window closes.

Context-sensitive help

Context-sensitive help provides information about the current function or command. If help exists for the desired field, that subject's help file appears.

To get context-sensitive help,

1 Click *Context-sensitive help*.

The cursor changes into a pointer with a question mark.

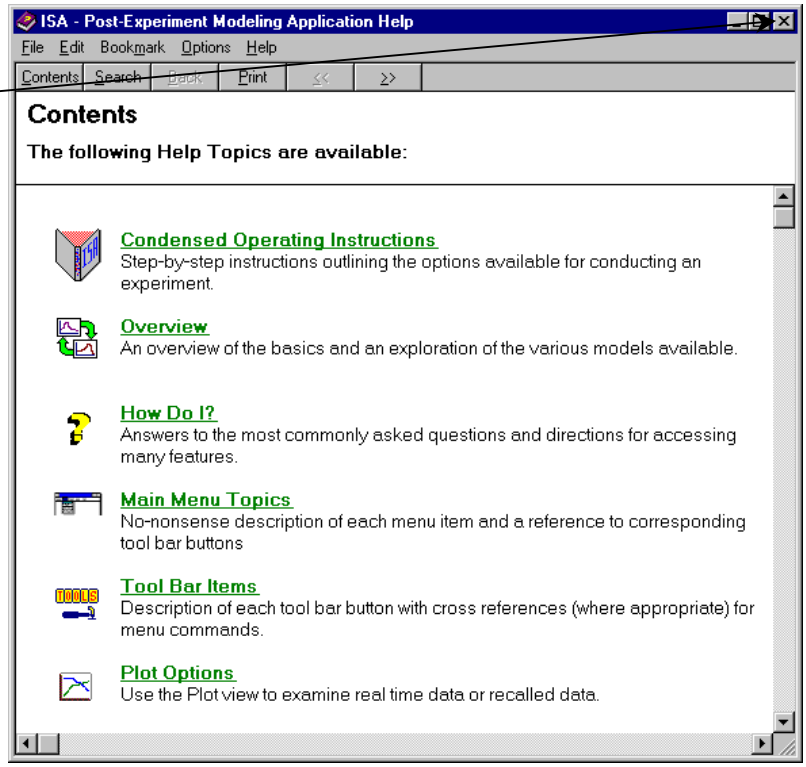
2 Click the field for which help is desired.

A ***Help*** window appears:

a If help is available, that subject's help file appears.

b If no help is available, the ***ISA – Post Experiment Modeling Application Help*** appears.

3 Click ☒ to exit.



Parameters

Parameters hides or displays the current ***Model*** dialog box.

To hide or display the model dialog box,

1 Click *Parameters*.

The ***Model*** dialog box appears or disappears.

Run

One of several methods to execute a model is to click the *Run* button. *Run* is accessible only if the parameters all have been entered. If the parameters are incomplete, *Run* is gray and inaccessible.

To run a model,

1 Click *Run*.

The current model is executed.

Modeling parameters

The following parameters appear in some or all of the ***Model*** dialog boxes.

Time Points

 # Time Points sets the number of points between the *Initial Time* and *Final Time*. This field is found in the ***Time Resolved Model*** dialog box.

To set the number of times to scan,

1 Click in the data-entry field.

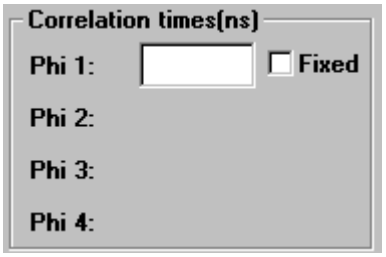
2 Enter the number of times.

Component count

Component count sets the number of components to model for an anisotropy decay. For each component, a *Fractional Amplitude* and *Lifetime* are necessary. Without these fields completed, the ***Anisotropy Decay Model*** cannot run.

To set the number of components,

1 Click the correct radio button.

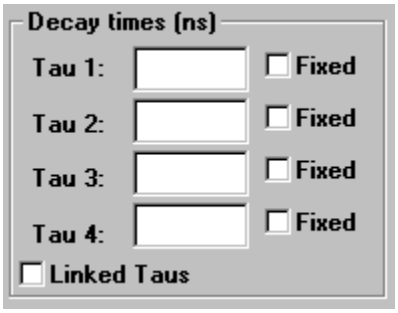


Correlation times

Correlation times sets a correlation time (in ns) for each phase component in an anisotropy decay. Up to four phase components are possible.

To set the correlation time(s),

1. Click in the data-entry field next to each *Phi* (phase).
2. Enter the correlation time, in ns.



Decay times

Decay times sets the decay time (in ns) for up to four components in a time-resolved acquisition. For models with < 4 components, enter a zero for the remaining *Tau*s.

To set the decay time(s),

1. Click in each data-entry field.
2. Enter the decay time, in ns.
   For unwanted components, enter 0.

Final Time

Final Time sets the end time, in ns, for the time-resolved model. This field, with the *Initial Time* and # *Time Points*, determines the total number of scans.

To set the final time,

1. Click in the data-entry field.
2. Enter the desired ending time, in ns.

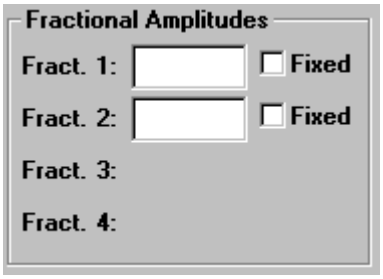
☐ Fixed Fixed

The *Fixed* checkbox determines whether the associated parameter is allowed to float during modeling, or must remain fixed.

To fix or let float a parameter during modeling,

1 Click the *Fixed* checkbox next to that parameter.

An "×" in the box means the parameter cannot change during a modeling run.
No "×" in the box means the parameter can change.



Fractional Amplitudes

The *Fractional Amplitudes* area sets the "percentage" of the total value that each component occupies. Up to four fractional amplitudes, labeled *Fract. 1*, *Fract. 2*, *Fract. 3*, and *Fract. 4*, may be set.

To set a fractional amplitude,

1. Click next to a *Fract. x* data-entry field corresponding to a component.
2. Enter the fractional amplitude.

Note: *For every component chosen, a fractional amplitude must be assigned before a model is run.*

Initial Time

Initial Time sets the starting time, in ns, within the model. Along with *Final Time* and # *Time Points*, *Initial Time* determines the number of scans to be executed.

To set the initial time in the model,

1 Click in the data-entry field.

2 Enter the desired initial time, in ns.

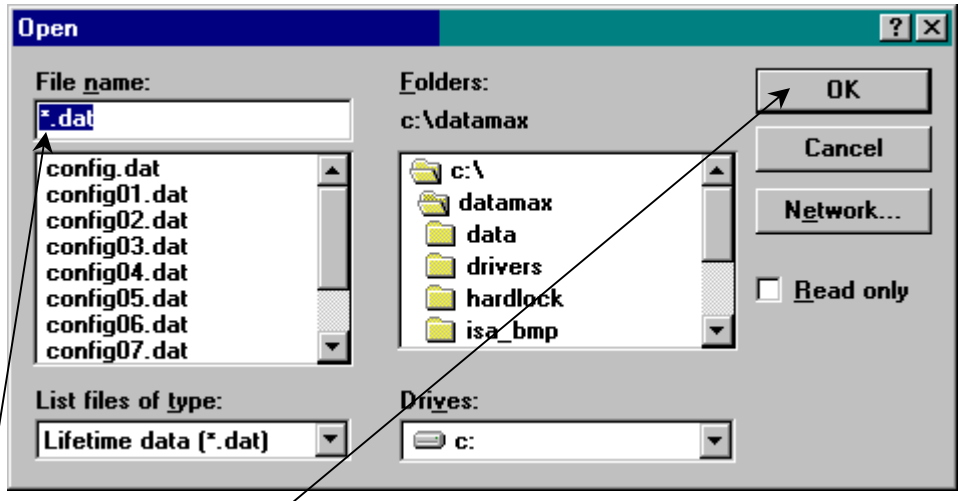
Input File…

The *Input File…* data-entry field specifies the data used for the lifetime or anisotropy-decay models. The input file may be generated from DataMax or another program.

To specify the input file,

1 Click *Input File…*.

The ***Open*** dialog box appears:



2 Choose the desired input file to model.

3 Click *OK*.

The ***Open*** dialog box disappears, and the desired file's name appears in the field next to the *Input File…* button.

Lifetime Files…

Lifetime Files… specifies the set of lifetime files needed to model a time-resolved acquisition. When lifetime files are created in DataMax, usually they are numbered sequentially to form a complete set. *Lifetime Files…* lets the user choose particular files, or the entire set.

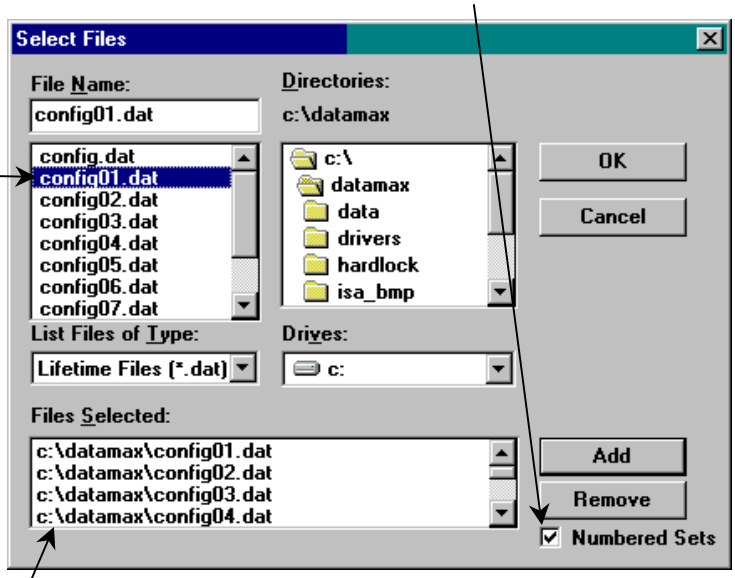
To select the lifetime files,

1 Click the *Lifetime Files…* button.

The ***Select Files*** dialog box opens.

a Click the *Numbered Sets* checkbox to select an entire set of files.

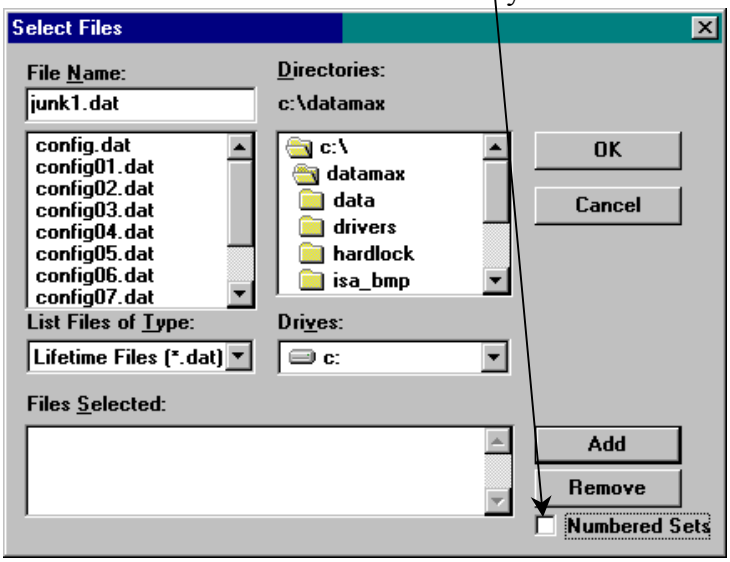
- Click the first file of the numbered series.
- All files with the same basic name, followed by increasing numbers, appear in the *Files Selected* area.



- Click *Remove* to remove an undesired file.
- Click *Add* to add another file.

b Deactivate *Numbered Sets* to select each file individually.

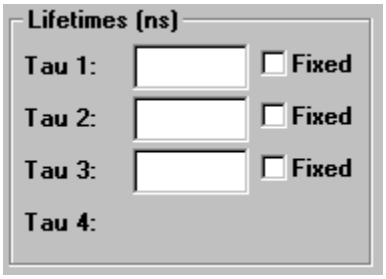
- Highlight the desired file.
- Double-click the desired file.
- The selected file appears in the *Selected File* area.



- Click *Remove* to remove an undesired file.
- Click *Add* to add another file.

2 Click *OK*.

The ***Select Files*** dialog box closes, and the names of the desired files appear in the field next to *Lifetime Files…*.



Lifetimes

The *Lifetimes* area sets each component's lifetime. The component lifetimes are labeled *Tau 1*, *Tau 2*, *Tau 3*, and *Tau 4*. Up to four lifetimes may be modeled.

To set the lifetime for a component,

1 Click in the desired lifetime's data-entry field.

2 Enter the known or estimated lifetime.
Give an estimate if a lifetime is not known.

Linked Taus

The *Linked Taus* checkbox specifies whether the lifetimes (*Tau*s) in the model are wavelength-independent or not. Checking the box tells DataMax that the lifetimes are wavelength-independent. Pre-exponential amplitudes, however, remain wavelength-dependent.

To link the lifetimes,

1 Check the *Linked Taus* checkbox.

To keep the lifetimes wavelength-dependent,

1 Uncheck the *Linked Taus* checkbox.

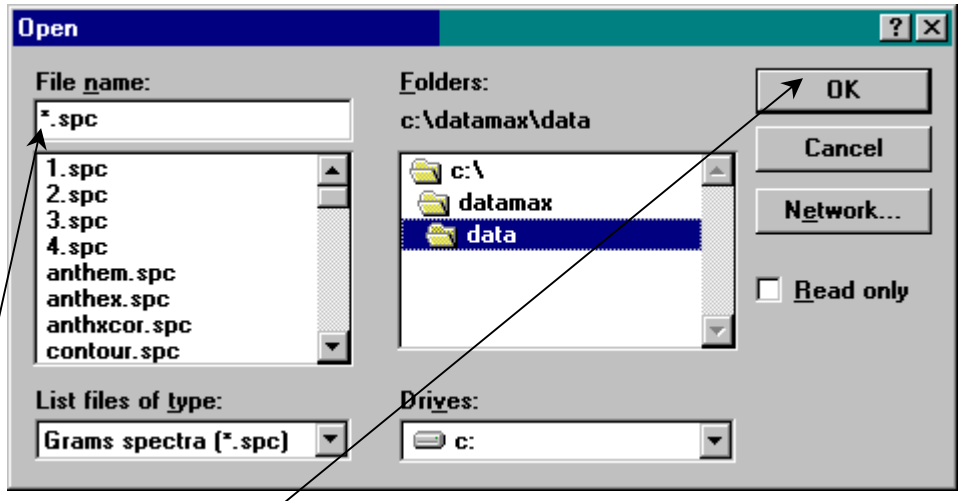
Modulation File…

Modulation File… specifies the modulation file used to model a lifetime-resolved acquisition.

To specify the modulation file,

1 Click *Modulation File….*

The ***Open*** dialog box appears.



2 Choose the desired modulation file.

3 Click *OK*.

The ***Open*** dialog box closes. The name of the file appears in the field next to *Modulation File….*

Number of components

The *Number of components* sets the number of components for a lifetime or lifetime-resolved model. Up to four components may be chosen for a lifetime model; up to three may be selected for a lifetime-resolved model. For each component, a fractional amplitude must be entered also.

To specify the number of components in a system,

1 Click the desired radio button.

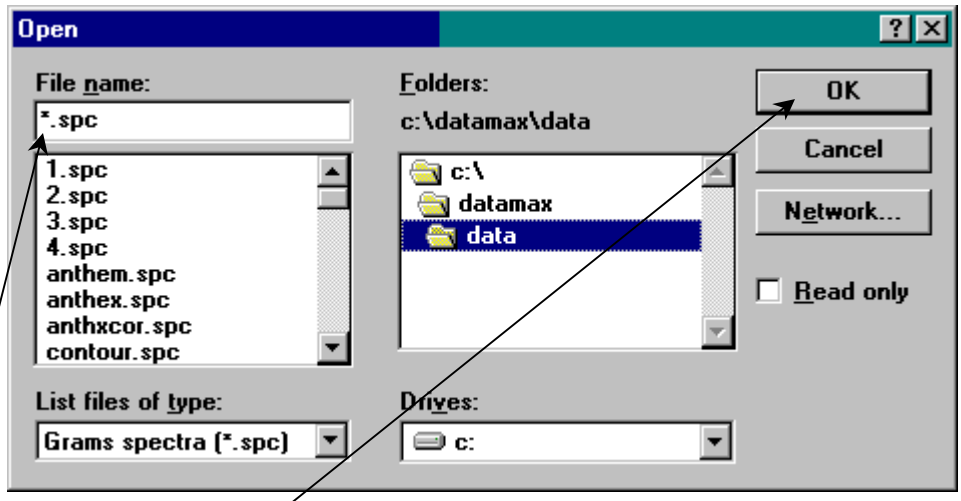
Phase File…

Phase File… specifies the phase file used to model a lifetime-resolved acquisition.

To specify the phase file,

1 Click *Phase File….*

The ***Open*** dialog box appears.



2 Choose the desired phase file.

3 Click *OK*.

The ***Open*** dialog box closes. The name of the file appears in the field next to the *Phase File…* button.

Ro

Ro sets R_0, the limiting anisotropy in the absence of diffusion, for an anisotropy-decay acquisition.

To set the limiting anisotropy,

1. Click in the data-entry field.
2. Enter the desired value.

Run Model

The *Run Model* button executes the model, after all data-entry fields are complete. The analysis begins immediately. Plots are displayed on the control panel.

To run a model,

1 Click *Run Model*.

If parameters are incomplete or invalid, an error message appears. Correct the problem and click *Run Model* again.

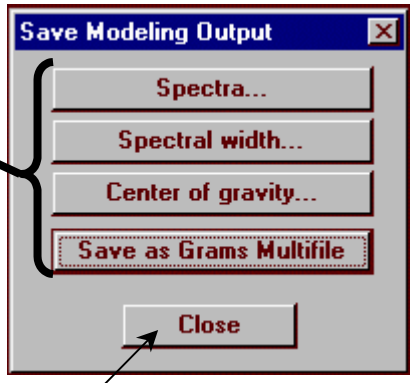
Save Output… Save Output…

Save Output… saves the result of modeling in various data formats. The spectra, spectral width, or center of gravity may be saved.

To save the results of a model,

1 Click *Save Output…*.

2 If applicable, the ***Save Modeling Output*** dialog box opens.



3 Choose the type of file:

- Spectra…
- Spectral width…
- Center of gravity…
- Save as Grams Multifile

The ***Save As*** dialog box opens.

4 Enter the name for the new file.

The results are saved in the desired format under the new name, and the ***Save As*** dialog box closes. If applicable, the ***Save Modeling Output*** dialog box returns.

5 If applicable, click *Close*.

If applicable, the ***Save Modeling Output*** dialog box closes.

Spectral File…

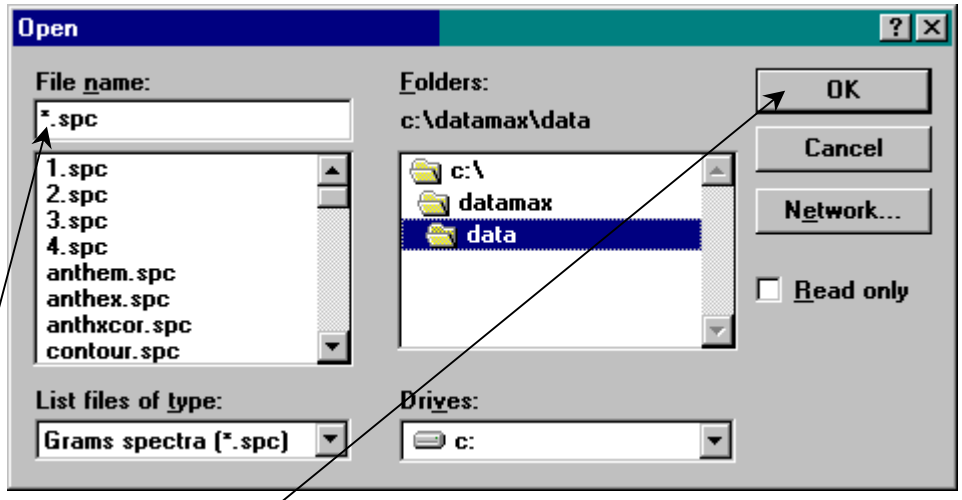
Spectral File… specifies the spectral file (emission data) from an emission scan to use for a lifetime-resolved or time-resolved acquisition is run. For the time-resolved acquisition, a spectral file is used to normalize the lifetime files. For the lifetime-resolved acquisition, the spectral file contains data for two or three components to deconvolve the spectra.

Note: *The wavelength range for this file must be identical to that of the lifetime scan.*

To specify the spectral file,

1 Click *Spectral File…*.

The ***Open*** dialog box appears.



2 Choose the spectral file.

3 Click *OK*.

The name of the file appears in the field next to the *Spectral File…* button, and the ***Open*** dialog box disappears.

Use Measured Standard Deviations

The *Use Measured Standard Deviations* radio button forces DataMax to measure the standard deviation. If not enabled, DataMax uses the default values for phase, 0.5°, and modulation ratio, 0.005.

To measure the standard deviation for modeling,

1 Click the *Use Measured Standard Deviations* radio button.

To use the default standard deviation for modeling,

1 Disable the radio button.

11: Graphs and Plots

Introduction

DataMax displays several types of graphs and plots. In ***Run Experiment*** and ***Real Time Display***, DataMax relies on GRAMS/32® to create scans and plots. In the ***Lifetime*** and ***Post-Experiment Modeling*** applications, the displays are not scans, but graphs and plots. Therefore, in ***Lifetime*** and ***Post-Experiment Modeling***, DataMax does not use GRAMS/32® to display data.

Use the *GRAMS/32® User's Manual* to learn about the ways to create and modify a GRAMS/32® scan. This chapter describes creation and modification of non-GRAMS/32® graphs and plots.

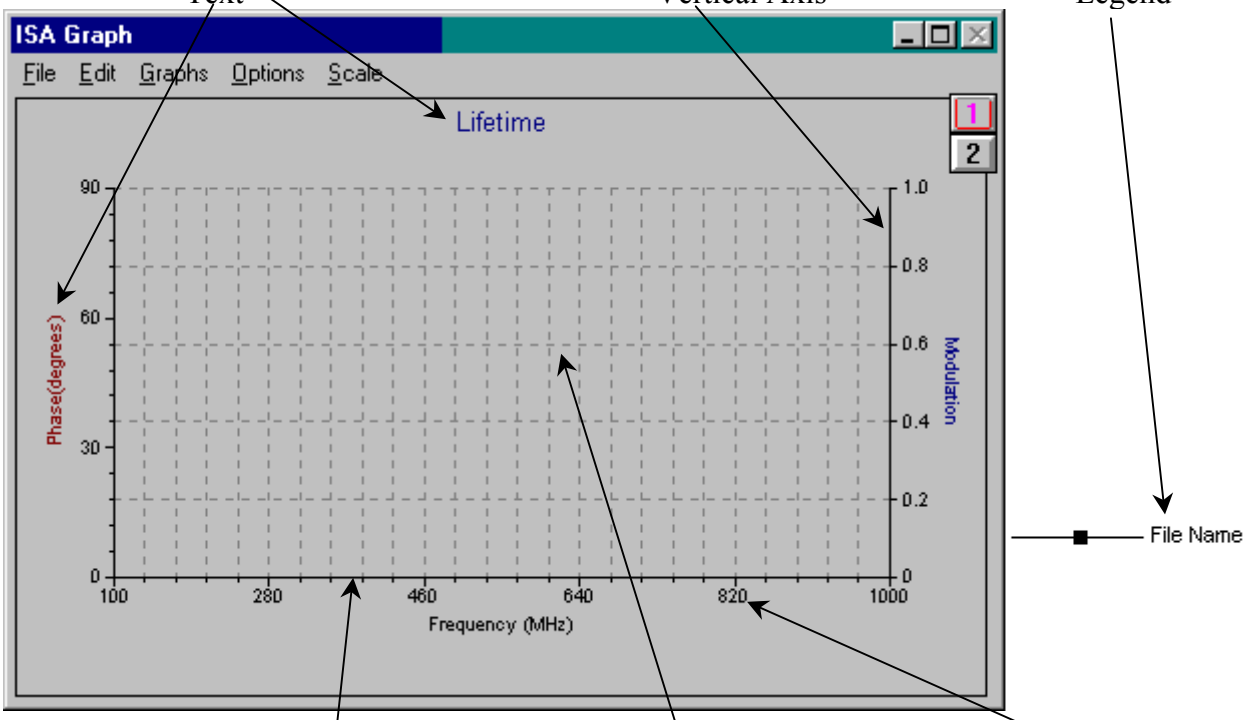
Point-and-click modification

Introduction

Parts of a graph

Each graph is composed of up to six parts that can be edited via pointing and clicking:

- Text
- Vertical Axis
- Legend



- Horizontal axis
- Plot area
- Axis labels

Changing a part of a graph

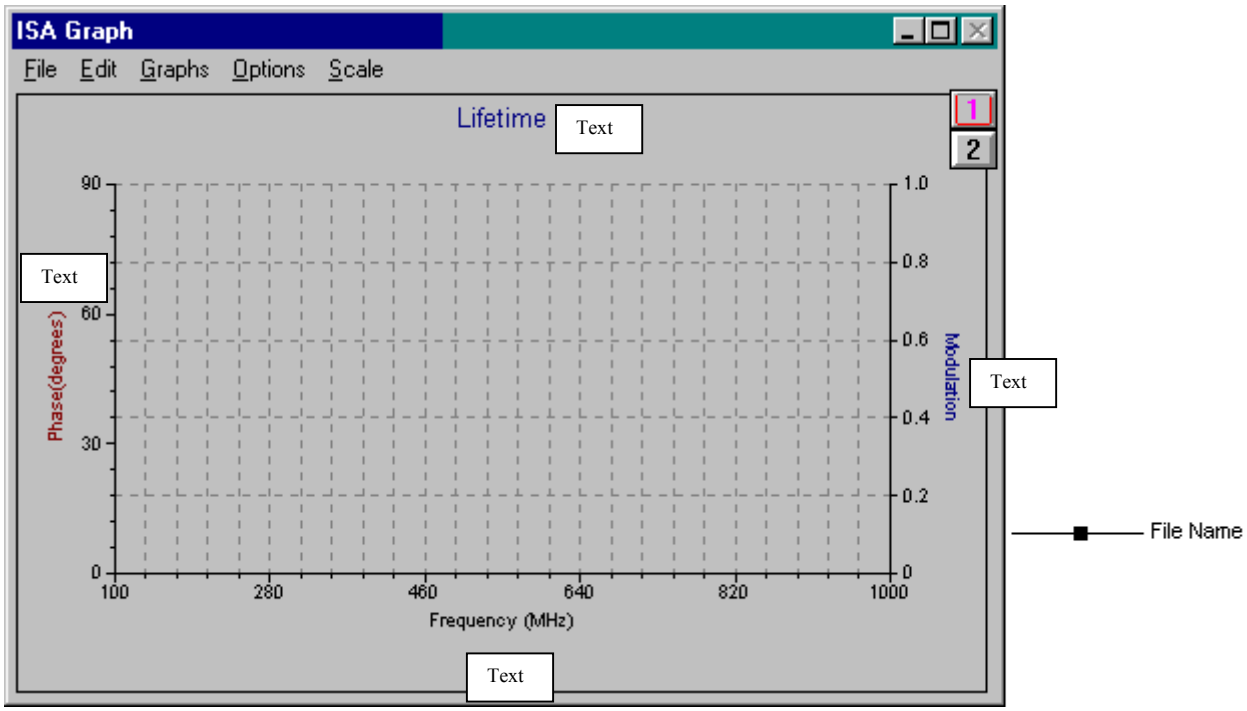
1. Place the mouse cursor on the part to be changed.
2. Double-click the left mouse-button.

   The part flashes momentarily, and a dialog box appears. If the cursor is placed incorrectly, a flashing question mark appears. Reposition the cursor and double-click again.
3. Change the parameters in the dialog box.
4. Click *OK*.

   The changes are permanent, usually until the application is closed. When the application is re-opened, the parameters generally are reset back to the default view.

Text

Text surrounds the graph; any text may be entered. Below all areas of text are shown.



To change text or its appearance,

1 Double-click on the text to be changed.

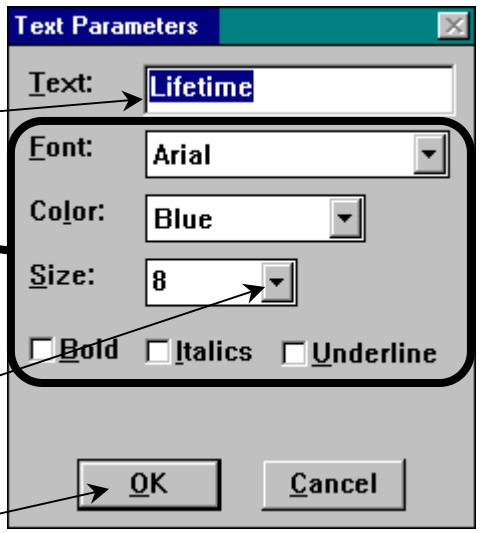
The ***Text Parameters*** dialog box appears.

2 Enter the new text in the *Text* field.

3 Change font, font style, font size, or font color as desired.

a Click on the down-arrow or checkbox next to the option.

b Select the option.

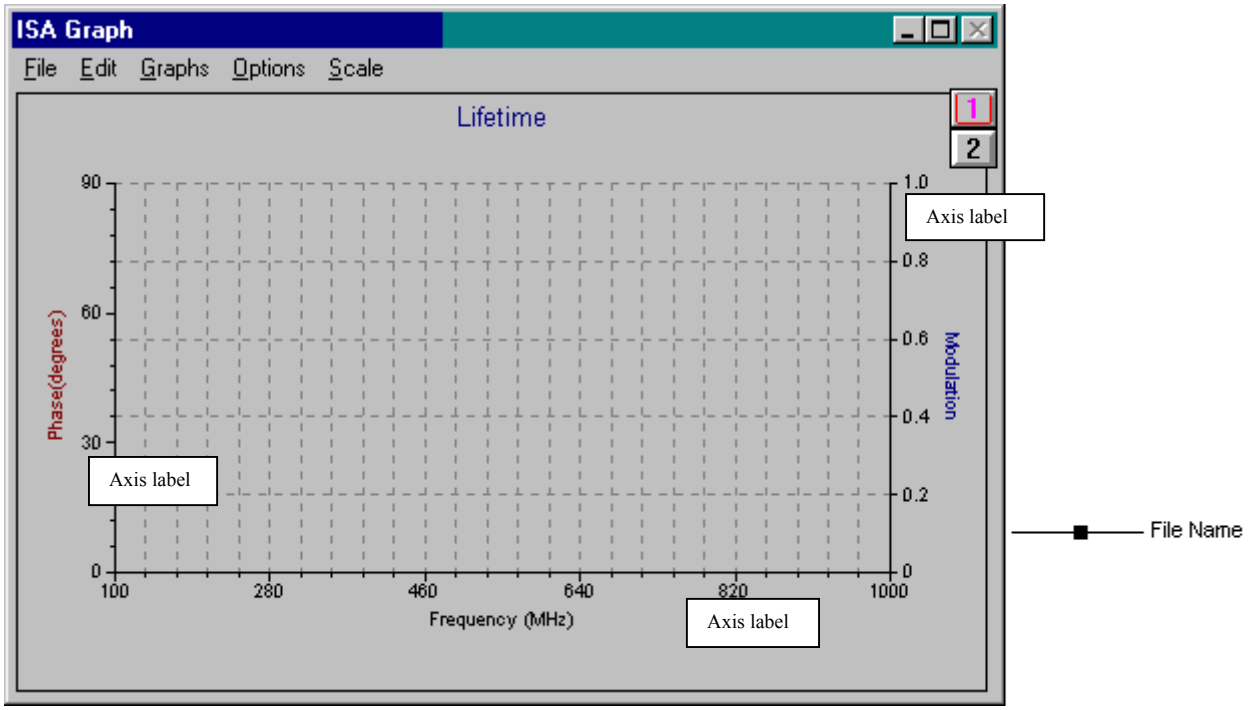


4 Click *OK*.

The ***Text Parameters*** dialog box closes, and the text is changed on the graph. The change is temporary. If the application is re-opened, the text appears with default options.

Axis labels

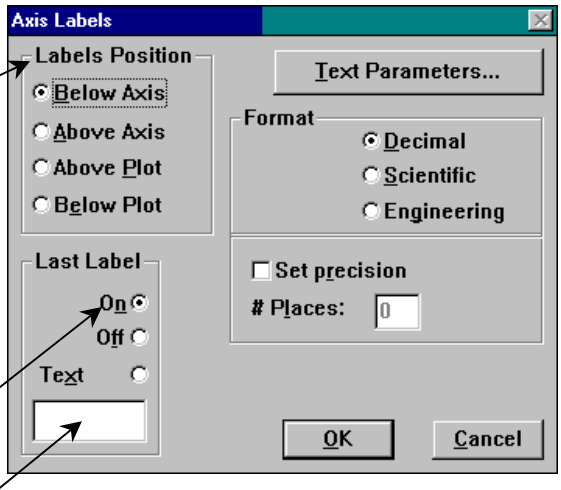
Axis labels are the numbers surrounding the graph. The numerical range of the axis labels can be changed by using the main menu *Scale* choice, where applicable. The appearance of the numbers can be changed by pointing and clicking.



To change the appearance of the numbers

1 Double-click the numbers to be changed.

The ***Axis Labels*** dialog box appears.



2 In *Labels Position*, change the position of the labels.

a Position the label with respect to the plot or the axis, on the right or left of the reference point.

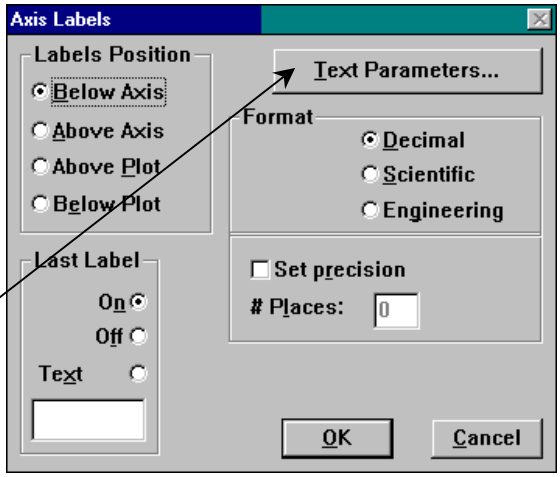
3 In *Last Label*, change the endpoint of the numerical series.

a *On* and *Off* radio buttons show or hide the last number of the series.

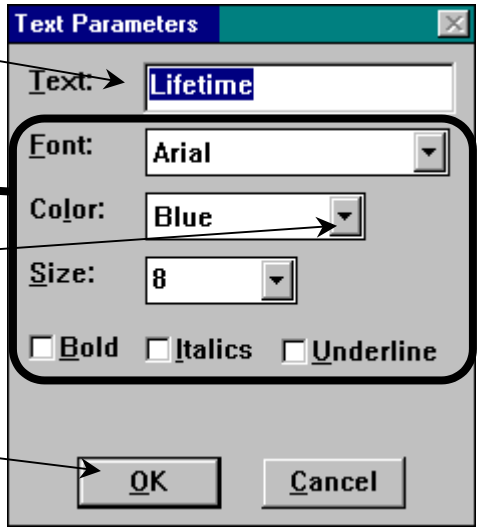
b Enter any *Text* to display at the endpoint.

Note: *Depending on the size of the plot area, numerical precision, etc., all or some of the text is displayed.*

4 With *Text Parameters…*, change the font, color, size, and style of the numbers.



a Click *Text Parameters…*.
The ***Text Parameters*** dialog box appears.



b Enter the new text in the *Text* field.

c Change font, font style, font size, or font color as desired.

d Click on the down-arrow or checkbox next to the option.

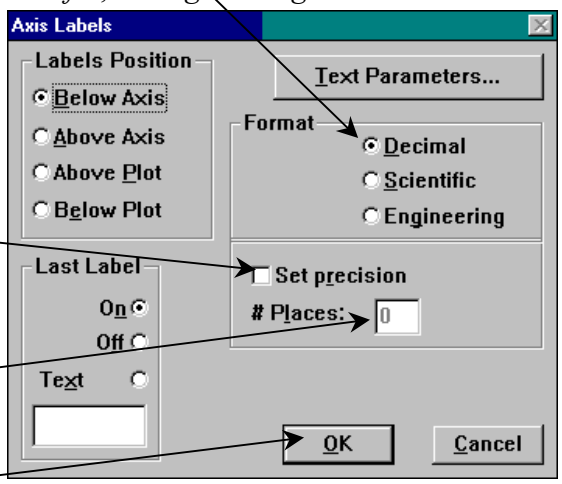
e Select the option.

f Click *OK*.
The ***Text Parameters*** dialog box closes, and the text is changed on the graph. The change is temporary. If the application is re-opened, the text appears with default options.

5 In *Format*, change the format of the numbers:

Choose radio buttons for *Decimal*, *Scientific*, or *Engineering*.

6 Change the numerical precision of the axis labels:



a Check the *Set precision* checkbox, to fix the numerical precision.

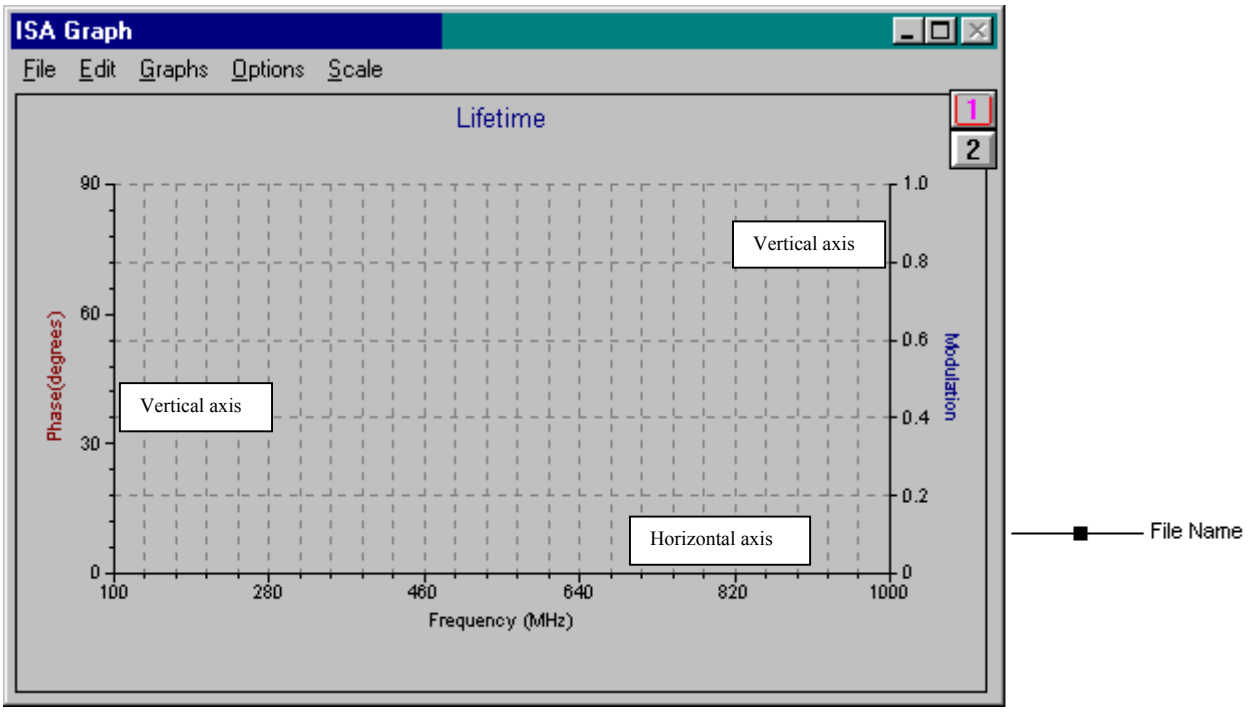
b In *# Places*, enter the number of places after the decimal point.

7 Click *OK*.

The ***Axis Labels*** dialog box closes, and the numbers' appearance changes. When the application is re-opened, the numbers' appearance reverts to default.

Vertical axis and horizontal axis

Each graph contains one or two vertical axes and one horizontal axis. Each axis's parameters are independent of the others. Procedures for modifying all axes are identical.



To modify an axis,

1 Double-click on the desired axis.

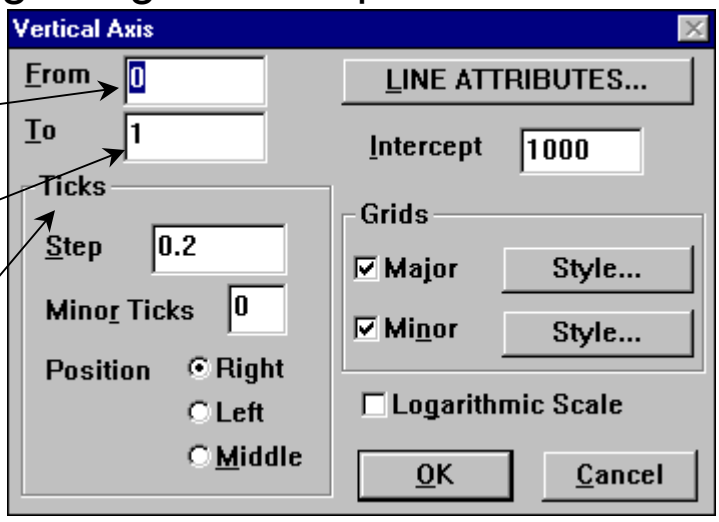
Either the ***Vertical Axis*** or ***Horizontal Axis*** dialog box appears:

2 Change the beginning and end points of the axis.

a Enter a starting value for the axis in *From*.

b Enter an ending value for the axis in *To*.

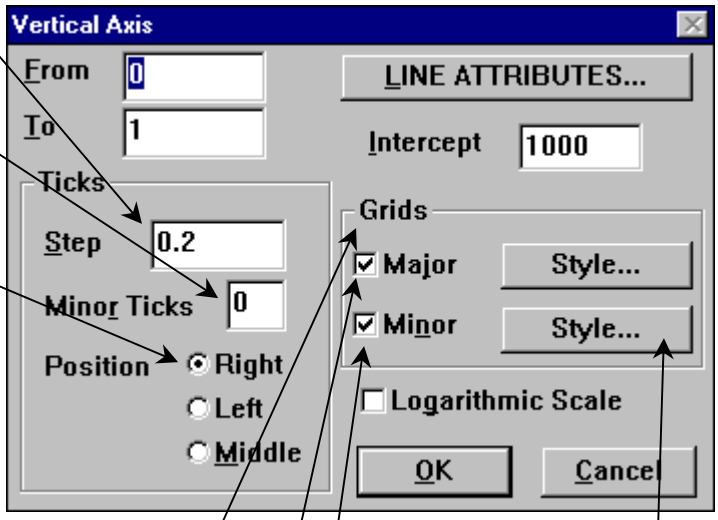
3 In *Ticks*, change the ticks shown on the axis.



a Enter a value to increment the numbers on the axis in *Step*.

b Enter a value to increment the *Minor Ticks* between the numbers.

c Choose the *Position* of the axis using the radio buttons.
Vertical axis: *Right*, *Left*, *Middle*.
Horizontal axis: *Above*, *Below*, *Middle*.



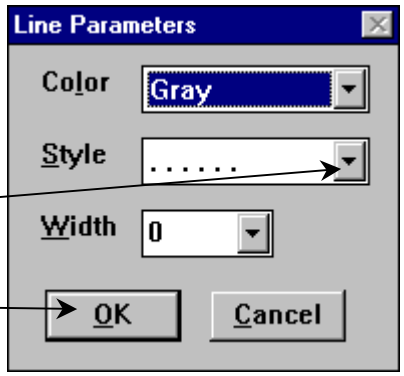
4 In *Grids*, change the appearance of the major and minor grids across the plot.

a To show the major grid lines, click the checkbox next to *Major*.

b To show the minor grid lines, click the checkbox next to *Minor*.

c To change the appearance of a set of grid lines, click the desired *Style...* button.

The ***Line Parameters*** dialog box appears.

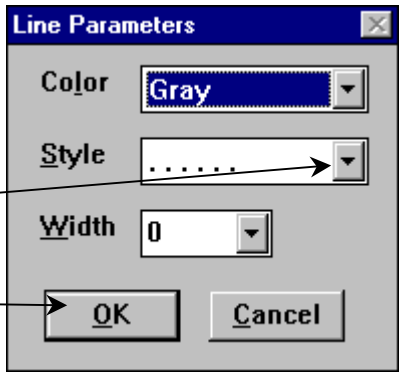


d Adjust the *Color*, *Style*, and *Width* of the grid lines.

- Click the down-arrow next to the desired parameter.
- Choose the attribute from the list.
- Click *OK*.
- The ***Line Parameters*** dialog box disappears.

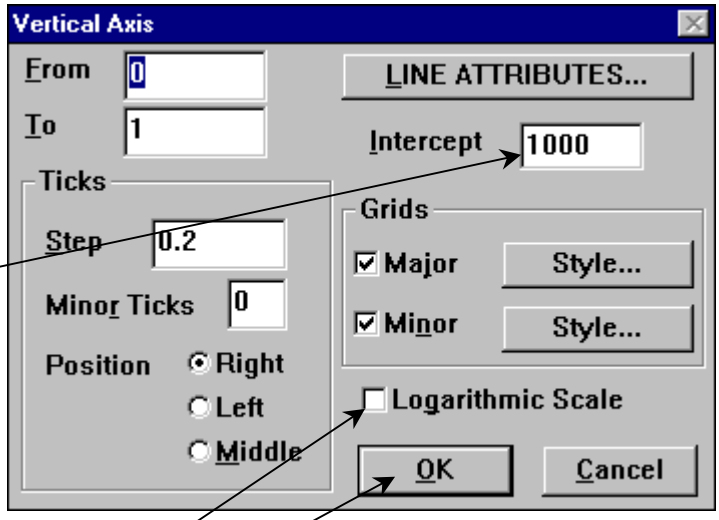
5 In *LINE ATTRIBUTES...*, change the style of the axis line.

a Click *LINE ATTRIBUTES...*.
The ***Line Parameters*** dialog box appears.



b Adjust the *Color*, *Style*, and *Width* of the axis lines.
- Click the down-arrow next to the desired parameter.
- Choose the attribute from the list.
- Click *OK*.
- The ***Line Parameters*** dialog box disappears.

6 Specify an intercept, where the chosen axis crosses the axis perpendicular to it.



a Click on the field next to *Intercept*.

b Enter the value of the intercept.

7 Choose a logarithmic scale, if desired.

a Check the *Logarithmic Scale* checkbox.

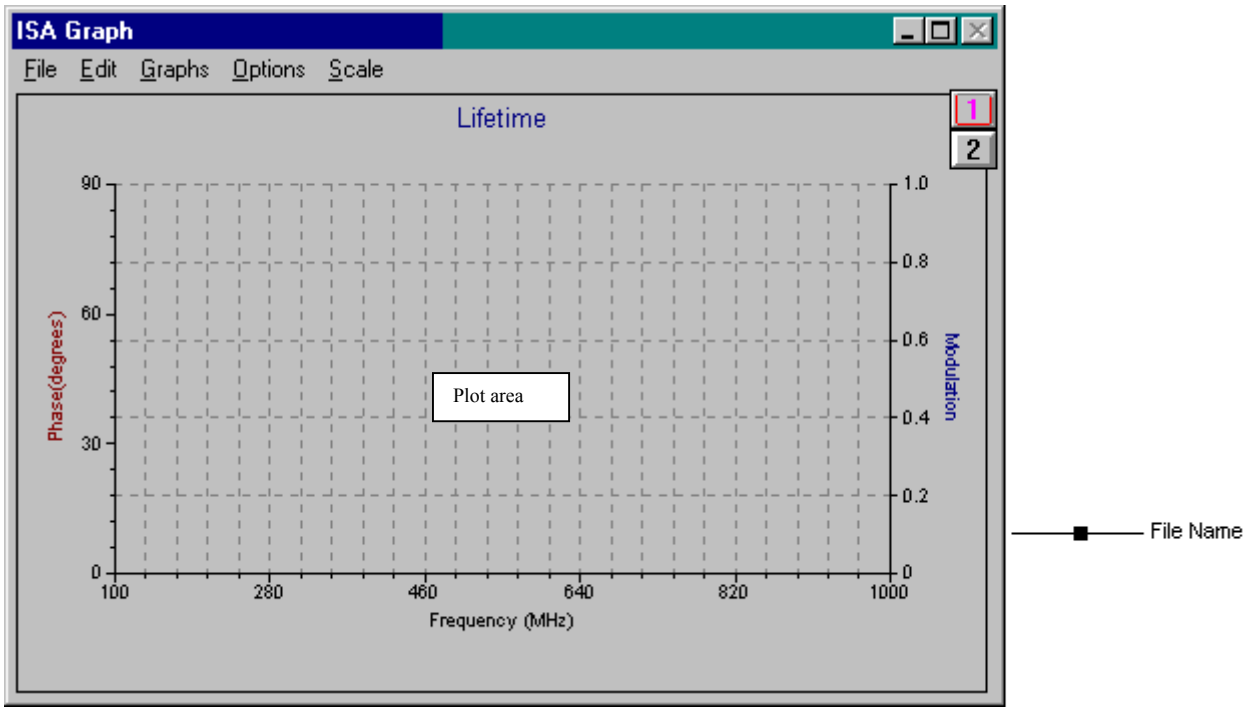
b If the checkbox is empty, the axis's scale is linear.

8 Click *OK*.

The changes take effect, and the axis dialog box closes.

Plot area

To modify parameters for the plot area, an active file must be plotted on the graph.



To modify the appearance of the data points,

1 Click on any data point along the desired trace.

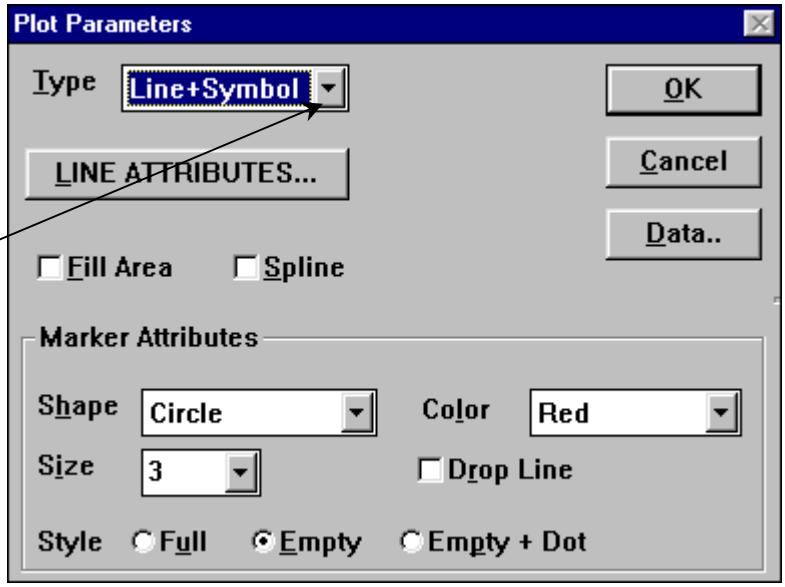
The ***Plot Parameters*** dialog box appears.

Note: *If you fail to click exactly on a data point, a question mark cursor appears. Reposition the mouse cursor and re-click.*

2 Change the line and symbols that represent the data series.

a Click on the down arrow next to the *Type* field. A drop-down menu appears.

b Move the scroll bar up and down to see the



complete list of options:

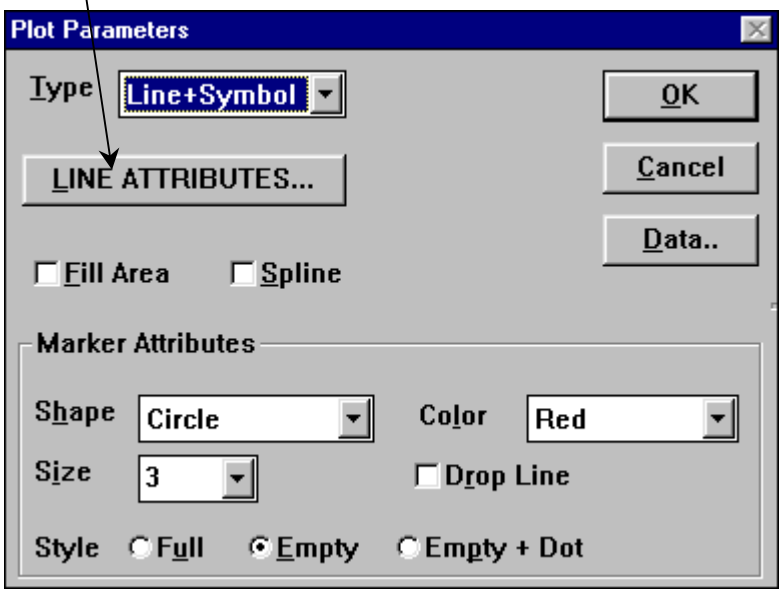
Line	A line segment joins each pair of data points.
Line+Symbol	A line segment joins each pair of data points; each data point is plotted as a symbol.
Scattered	Each data point is plotted as a symbol, but no line segments are joining the symbols.
Vert. Bars	A vertical bar graph appears in place of an *x-y* plot.
Horz. Bars	A horizontal bar graph appears in place of an *x-y* plot.
Vert. 3D Bars	A vertical three-dimensional bar graph appears in place of an *x-y* plot.
Horz. 3D Bars	A horizontal three-dimensional bar graph appears in place of an *x-y* plot.

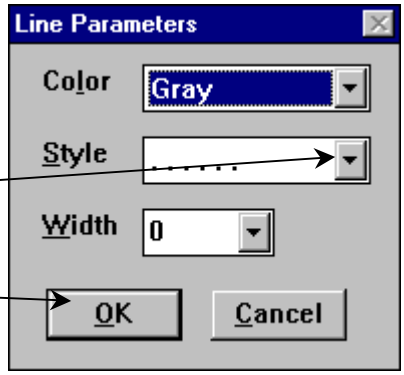
If a bar graph is chosen, the ***Plot Parameters*** dialog box transforms into a ***Bar Graph Parameters*** dialog box. See later in this section about the ***Bar Graph Parameters*** dialog box.

3 Change the style of line segment connecting pairs of data points.

a Click *LINE ATTRIBUTES...*.

b The ***Line Parameters*** dialog box appears.





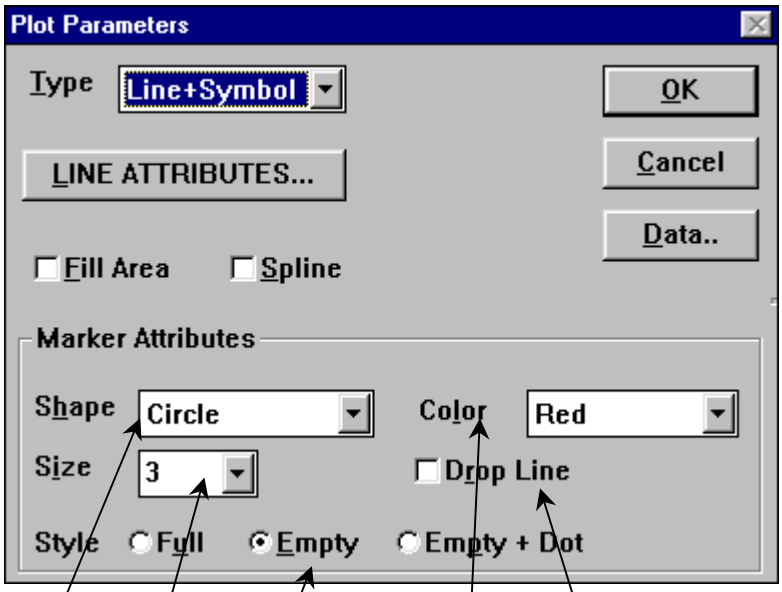
c Adjust the *Color*, *Style*, and *Width* of the axis lines.

- Click the down-arrow next to the desired parameter.
- Choose the attribute from the list.
- Click *OK*.

- The ***Line Parameters*** dialog box disappears.

4 Check the *Fill Area* checkbox to fill the area beneath the desired trace with color.

5 Check the *Spline* checkbox to connect the data points with a smooth spline curve.



6 Change the data-point symbols in the *Marker Attributes* area.

a Choose the *Shape* of the symbol via the drop-down menu:

Circle	○
Plus	+
Asterisk	*
Diamond	◊
Dot	·

b Choose the *Color* of the symbol via the drop-down menu. Colors depend on the monitor, graphics card, and printer.

c Choose the *Size* of the symbol via the drop-down menu.

d Choose a line dropping from the symbol to the *x*-axis with the *Drop Line* checkbox.

e Choose the *Style* of the symbol via the radio buttons:

Full	•
Empty	○
Empty plus dot	⊙

7 View and edit the data points with the *Data..* button.

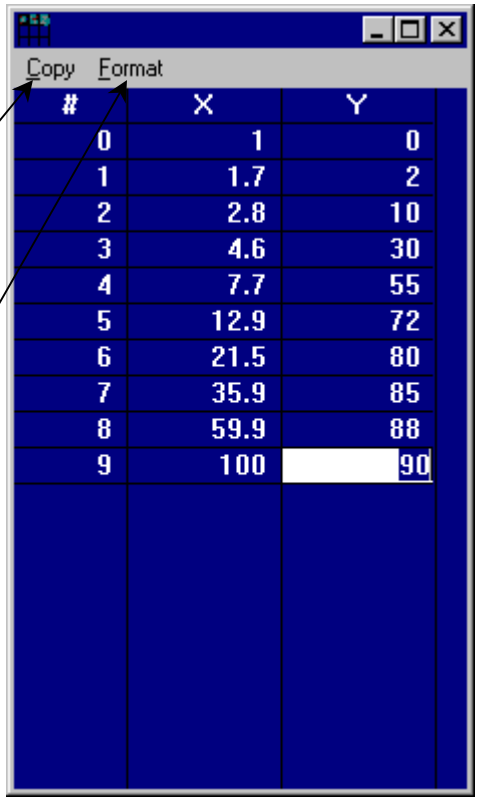
a Click *Data...*

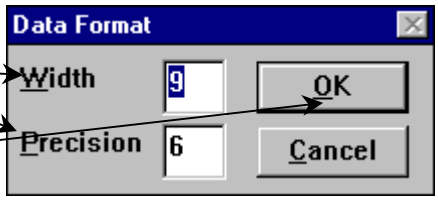
b The data table appears.

c Modify a value:
- Double-click on the value.
- Back-space to remove the contents of the cell.
- Enter a new value.
- Press **ENTER**.
- Click on another cell.

d Copy the data.
- Click *Copy*.
- The data are now on the ***clipboard***.
- Paste the data into any compatible program.

e Re-format the data.
- Click *Format*.
- The ***Data Format*** dialog box appears.
- Enter the *Width* of the column, in characters.
- Enter the *Precision*, i.e., digits after the decimal point.
- Click *OK*.
- The ***Data Format*** dialog box closes, and the columns of data are adjusted.



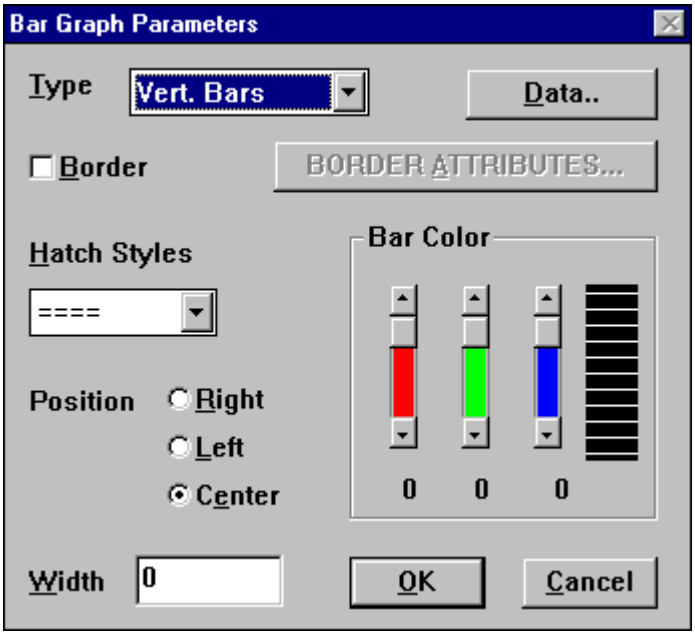


8 Click *OK*.

The ***Plot Parameters*** dialog box closes, and the plot changes according to the new settings.

Bar Graph Parameters

If a graph style such as *Vert. Bars* or *Horz. Bars* is chosen, the ***Plot Parameters*** dialog box changes into the ***Bar Graph Parameters*** dialog box:



1 With *Type*, choose a different type of graph.

2 View and edit the data.

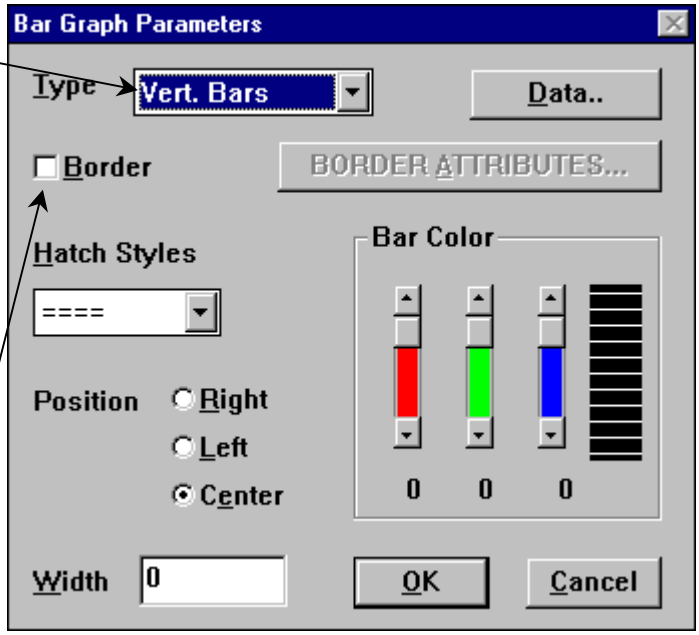
a Click *Data...*

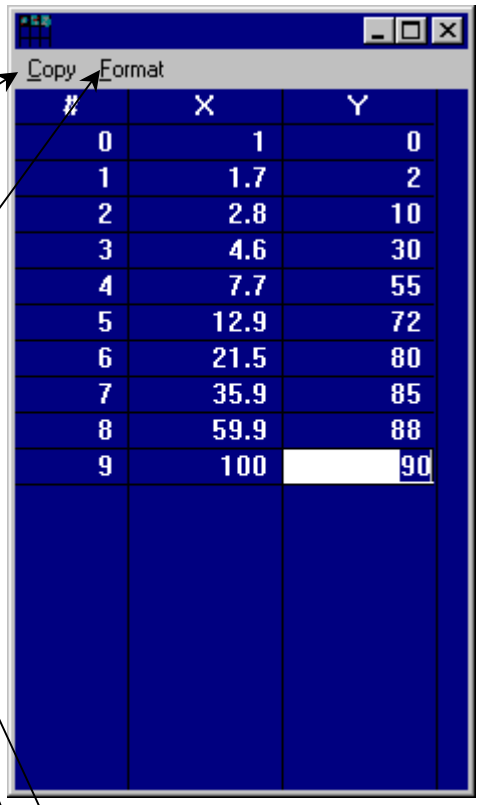
b The data table appears.

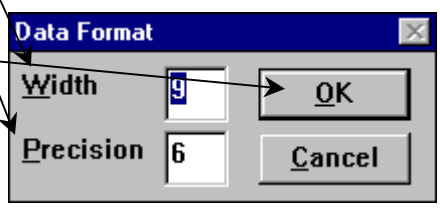
c Modify a value:
- Double-click on the value.
- Back-space to remove the contents of the cell.
- Enter a new value.
- Press **ENTER**.
- Click on another cell.

d Copy the data.
- Click *Copy*.
- The data are now on the ***clipboard***.
- Paste the data into any compatible program.

e Re-format the data.
- Click *Format*.
- The ***Data Format*** dialog box appears.
- Enter the *Width* of the column, in characters.
- Enter the *Precision*, i.e., digits after the decimal point.
- Click *OK*.
- The ***Data Format*** dialog box closes, and the columns of data are adjusted.







3 Check the *Border* check-box to draw an outline around each bar.

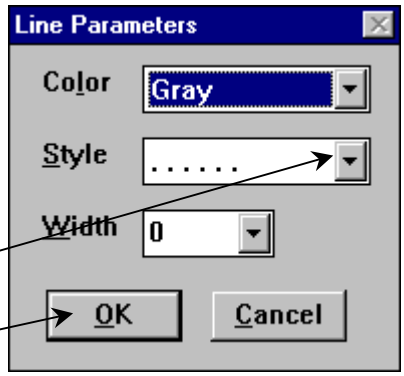
The *BORDER ATTRIBUTES...* button is active when the checkbox is checked.

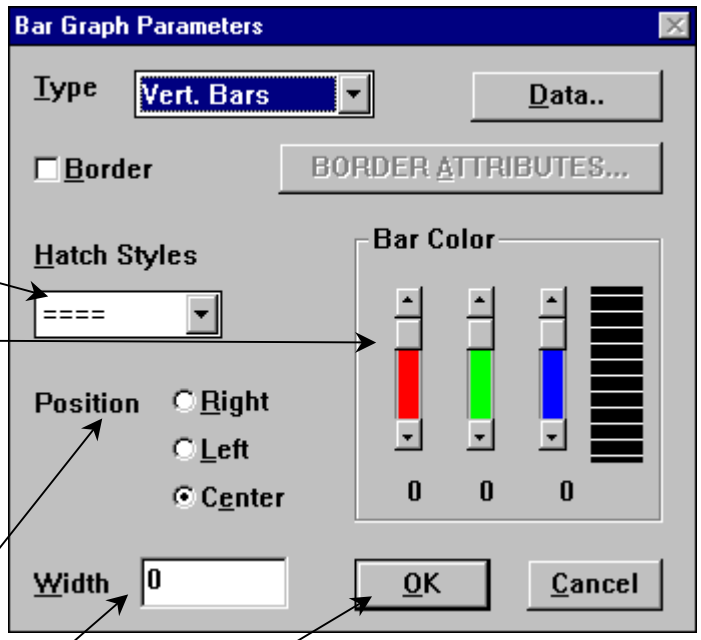
a Click *BORDER ATTRIBUTES...*.

b The ***Line Parameters*** dialog box appears.

c Adjust the *Color*, *Style*, and *Width* of the lines around the bars.

- Click the down-arrow next to the desired parameter.
- Choose the attribute from the list.
- Click *OK*.





4 With *Hatch Styles*, select the kind of fill for each bar.

5 With *Bar Color*, adjust the three sliders to determine the color of the bar.

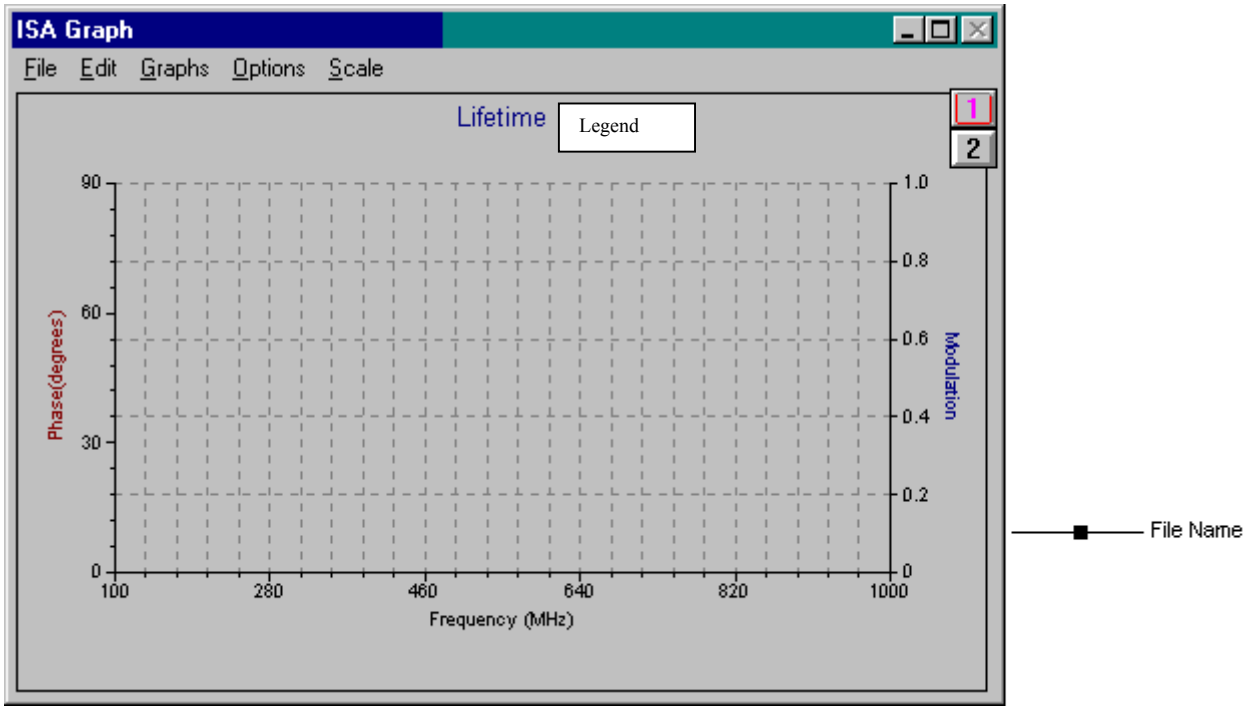
6 With *Position*, choose the position of the fill.

7 With *Width*, set the width of the bars.

8 Click *OK*.

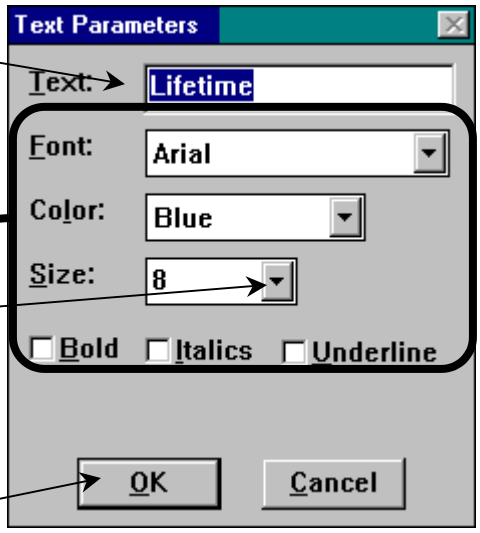
Legend

A graph may have a title, called a legend, whose default text is automatically generated. The size, color, font, and text of the legend all can be modified.



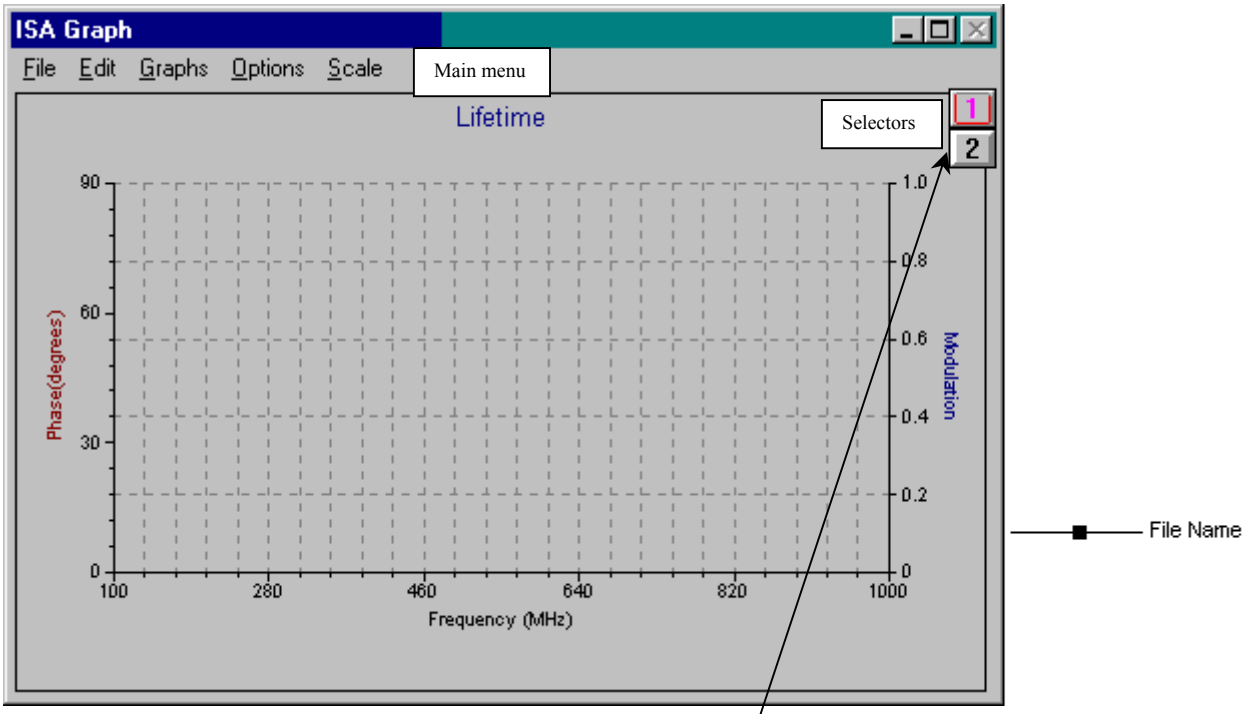
To modify the legend,

1. Double-click on the legend.
   The ***Text Parameters*** dialog box appears.
2. Enter the new text in the *Text* field.
3. Change font, font style, font size, or font color as desired.
4. Click on the down-arrow or checkbox next to the option.
5. Select the option.
6. Click *OK*.
   The ***Text Parameters*** dialog box closes, and the text is changed on the graph. The change is temporary. If the application is re-opened, the text appears with default options.



Modification using menus

Some DataMax graphs and plots contain menus across the top. Certain parameters and functions are executed using these menu items.

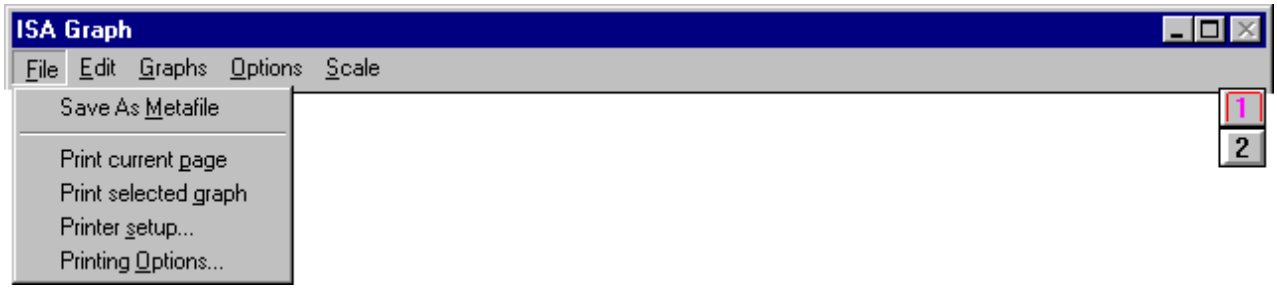


Choosing the active graph

When more than one graph appears on a window, the commands in the menu affect only the active graph.

1 Press a selector button to activate the desired graph.
or
Click on the desired graph.

File

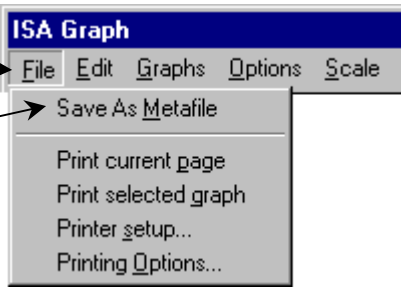


The *File* menu controls saving, printing, and printer setup.

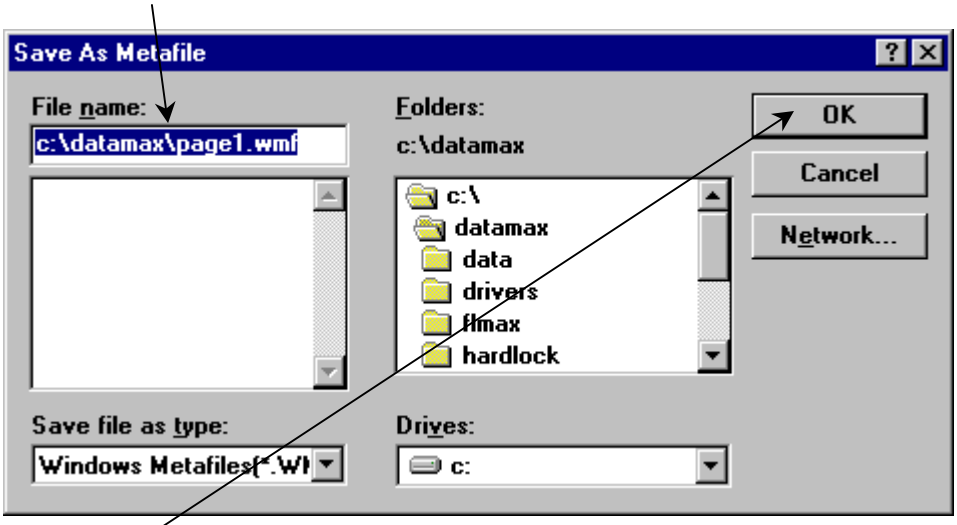
To save the image of the graph as a Windows™ metafile,

1 Click *File*.
A drop-down menu appears.

2 Click *Save As Metafile*.
The ***Save As Metafile*** dialog box appears.



3 Enter the correct path and name for the file.



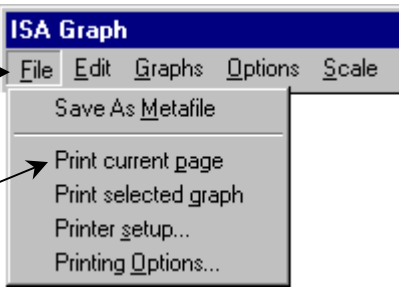
4 Click *OK*.
The file is saved, and the ***Save As Metafile*** dialog box closes.

To print the active window,

1 Click *File*.

2 A drop-down menu appears.

3 Click *Print current page*.



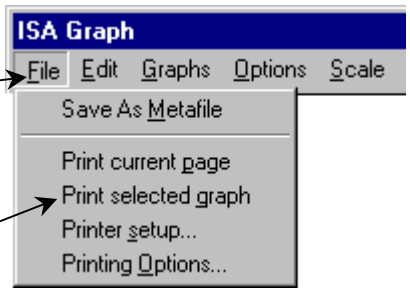
The window is sent to the printer.

To print one of two graphs displayed in a window,

1 Activate the desired graph.
Click on it or press the appropriate selector.

2 Click *File*.
A drop-down menu appears.

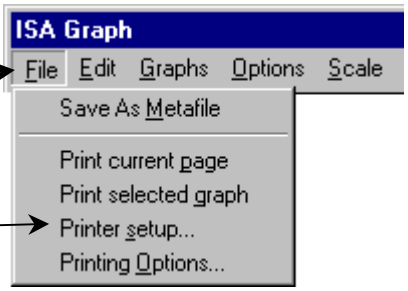
3 Click *Print selected graph*.
The desired graph is sent to the printer.

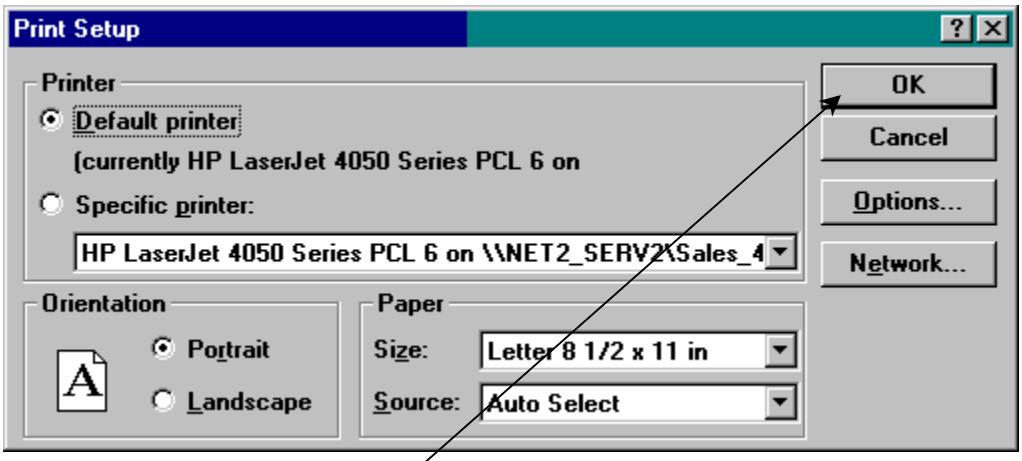


To set up the printer,

1 Click *File*.
A drop-down menu appears.

2 Click *Printer setup…*.
The ***Print Setup*** dialog box appears.





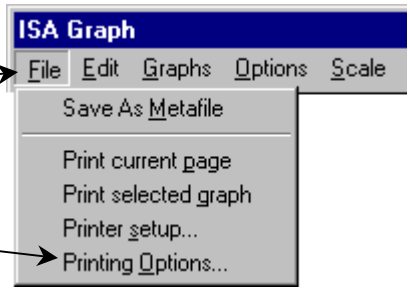
3 Choose the printer's setup parameters.
See the printer operation manual for details concerning the specifications and features of the printer.

4 Click *OK*.
The printer's setup is modified, and the ***Print Setup*** dialog box closes.

To adjust the size, appearance, and position of the overall graph,

1 Click *File*.
A drop-down menu appears.

2 Click *Printer Options…*.
The ***Print Parameters*** dialog box appears.



3 Choose a shaded background for the entire graph.

Check the *Graph Window Background* checkbox.

4 Choose a shaded background only for the area containing the actual data.

Check the *Plotting Area Background* checkbox.

5 Choose the graph's position on the page.

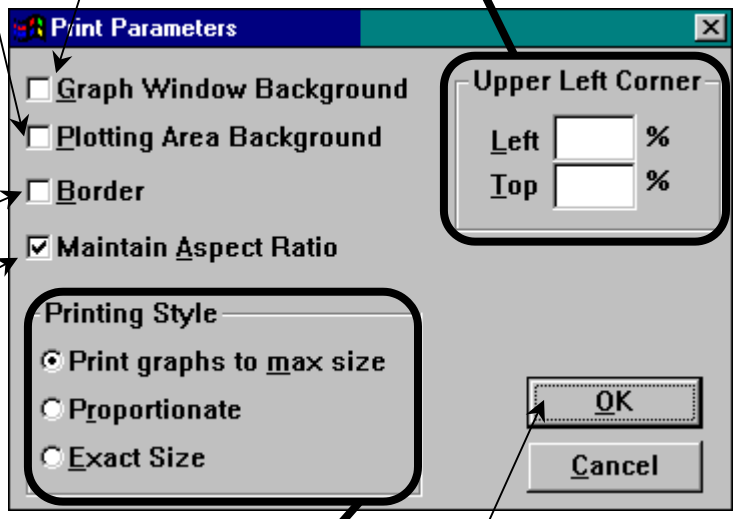
The *Upper Left Corner* area sets the graph's position, using the upper left corner of the page as a reference point. The larger the *Left* and *Top* values are set, the farther away the graph is from the page's upper left corner.

6 Print an outline around the graph.

Check the *Border* checkbox.

7 Print the graph in the same proportion as it appears on the screen.

Check the *Maintain Aspect Ratio* checkbox.



8 Choose a *Printing Style*.

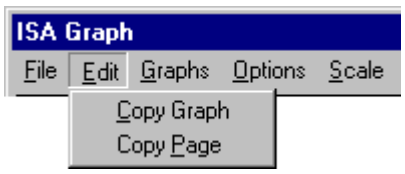
Choose from these radio buttons:

Print graphs to max size	Expands the graphs to the margins of the page.
Proportionate	Prints the graphs in the present proportion, even if the maximum size or exact sizes are chosen.
Exact Size	Prints the graphs a similar to the size on the screen as possible.

9 Click *OK*.

The parameters are set, and the ***Print Parameters*** dialog box closes.

Edit

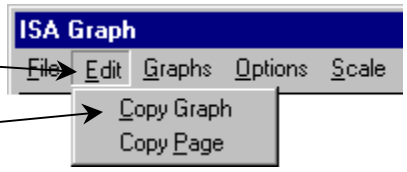


The *Edit* menu controls copying of the graphs.

To copy an image of the active graph,

1 Click *Edit*.
A drop-down menu appears.

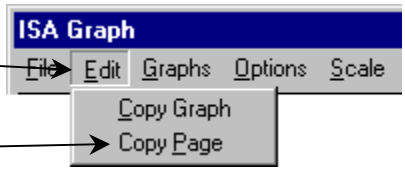
2 Click *Copy Graph*.
The image of the active graph is on the ***Clipboard***. To save an image of the graph onto a disk, use the *Save As Metafile* command.



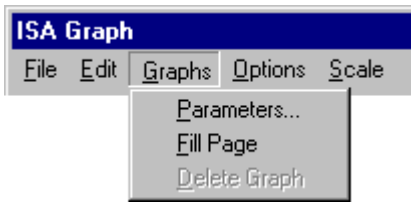
To copy the whole control panel or window,

1 Click *Edit*.
A drop-down menu appears.

2 Click *Copy Page*.
The image of the whole window is on the ***Clipboard***. To save an image onto disk, use the *Save As Metafile* command.



Graphs

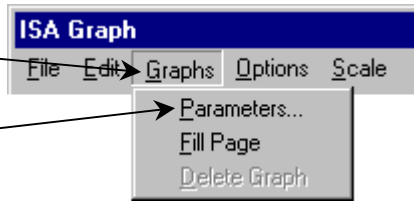


The *Graphs* choice controls the size, border, and overall color of the graph as displayed on the screen, along with displaying one or two graphs on the window, and deleting a particular graph.

To adjust the size, border, and overall color of the graph,

1 Click *Graphs*.
A drop-down menu appears.

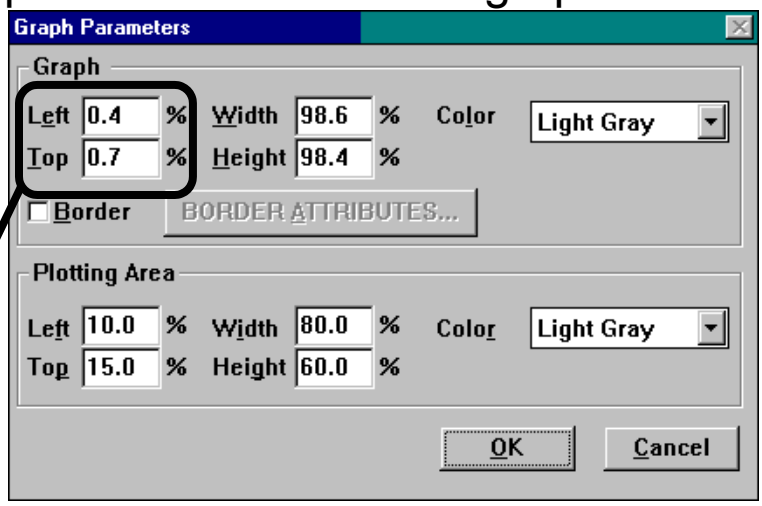
2 Click *Parameters…*.
The ***Graph Parameters*** dialog box opens.



3 Adjust the appearance of the entire graph area.

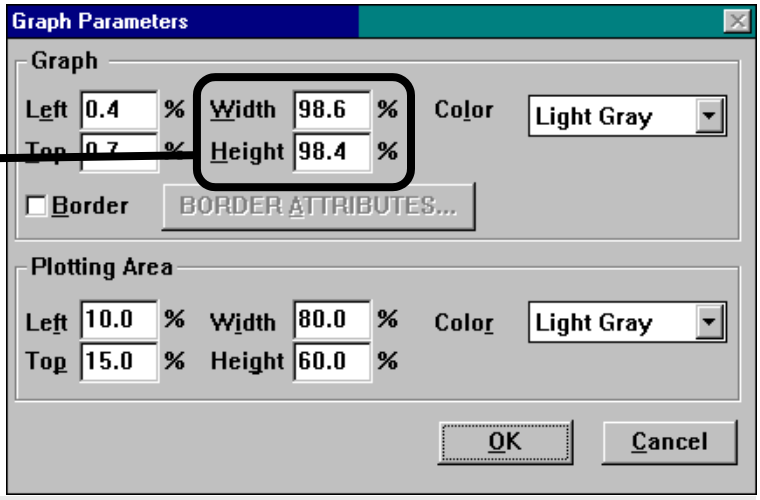
a Choose the position of the plot area with respect to the upper left corner of the entire graph.

Enter the *Left* and *Top* positions of the plot area. *Left* = 0%, *Top* = 0% means the plot starts at the top left of the graph area. To move the plot rightward, increase the *Left* value. To move the plot downward, increase the *Top* value.



b Choose the size of the graph area.

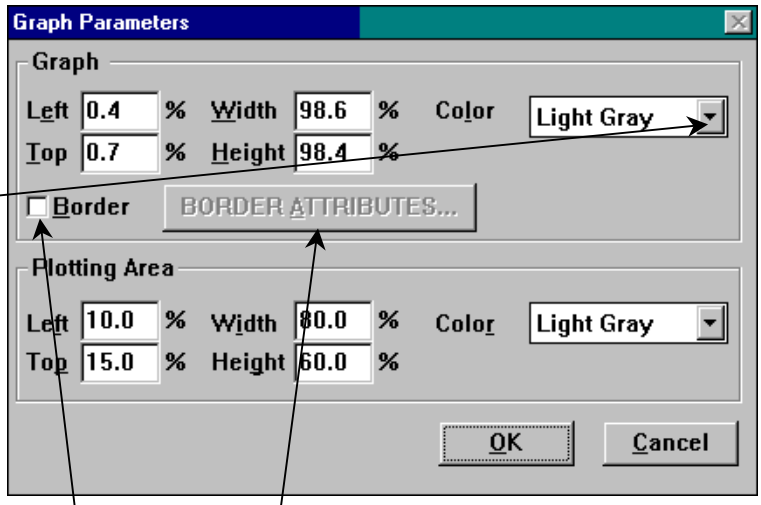
Enter the *Width* and *Height* of the graph area. To enlarge the graph, increase both *Width* and *Height*. To shrink the graph, decrease both *Width* and *Height*.



Note: *The graph area can be shrunken until the actual plot is too large for the screen.*

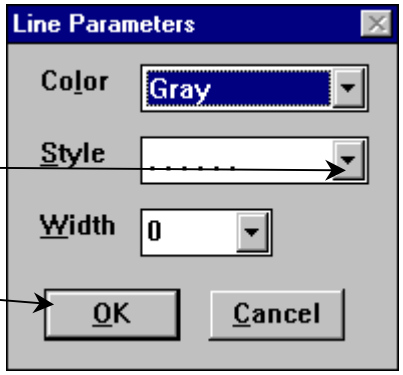
c Choose the color of the graph area.

- Click on the down arrow next to *Color*.
- Select a color.



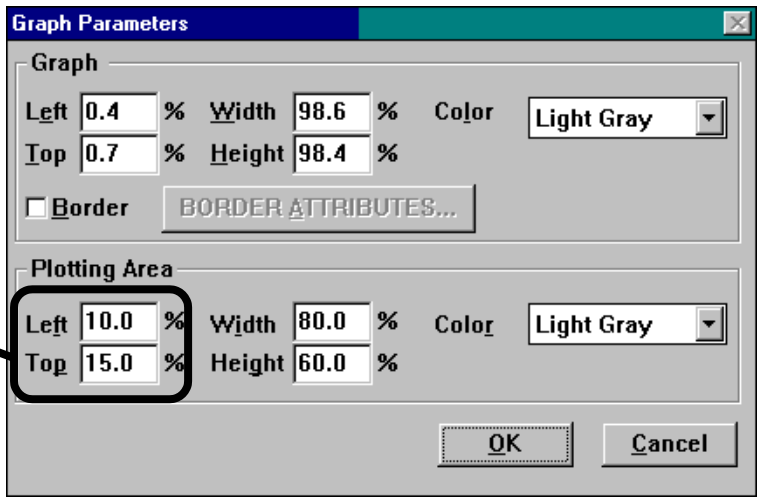
d Choose a type of border around the graph area.

- Check the *Border* checkbox.
- The *BORDER ATTRIBUTES...* button becomes active.
- Click *BORDER ATTRIBUTES...*.
- The ***Line Parameters*** dialog box appears.
  - Adjust the *Color*, *Style*, and *Width* of the lines around the bars.
  - Click the down-arrow next to the desired parameter.
  - Choose the attribute from the list.
  - Click *OK*.
- The ***Line Parameters*** dialog box closes.



4 Adjust the appearance of the plotting area.

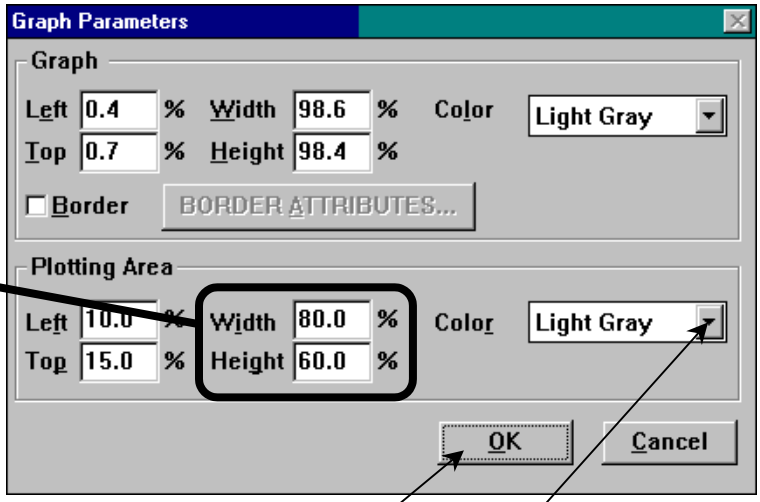
a Choose the position of the plot area with respect to the upper left corner of the whole graph.



Enter the *Left* and *Top* positions of the plot area. *Left* = 0%, *Top* = 0% means the plot starts at the top left of the graph area. To move the plot rightward, increase the *Left* value. To move the plot downward, increase the *Top* value.

b Choose the size of the plot area with respect to the graph area.

Enter the *Width* and *Height* of the plot area. To enlarge the size of the plot, increase both *Width* and *Height*. To shrink the plot area, decrease both *Width* and *Height*.



Note: *The graph area can be shrunken until the actual plot is too large for the screen.*

c Choose the color of the plot area.

- Click on the down arrow next to *Color*.
- Select a color.

5 Click *OK*.

All settings concerning the appearance of the graph are executed, and the ***Graph Parameters*** dialog box closes.

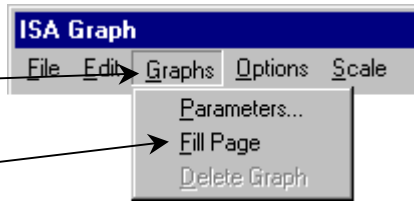
To enlarge the active graph to fill the entire dialog box,

1 Select the desired graph.

2 Click *Graphs*.

A drop-down menu appears.

3 Click *Fill Page*.



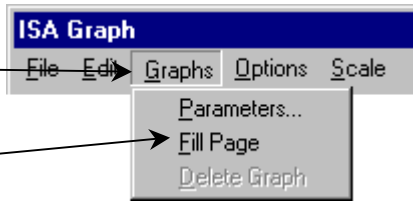
The graph expands to fill the window, while the other graph disappears. A check mark appears next to *Fill Page*.

To return to viewing both graphs on one dialog box,

1 Click *Graphs*.

A drop-down menu appears.

2 Click *Fill Page*.



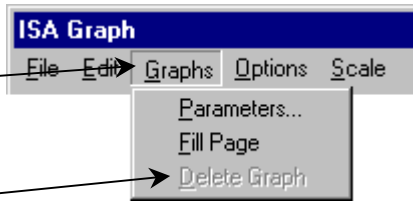
The active graph shrinks, and the other graph appears. The check disappears from next to *Fill Page*.

To delete a graph from view on the screen,

1 Select the active graph.

2 Click *Graphs*.
A drop-down menu appears.

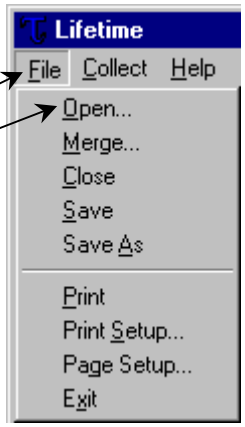
3 Click *Delete Graph*.
The graph disappears from view, but is still in memory.

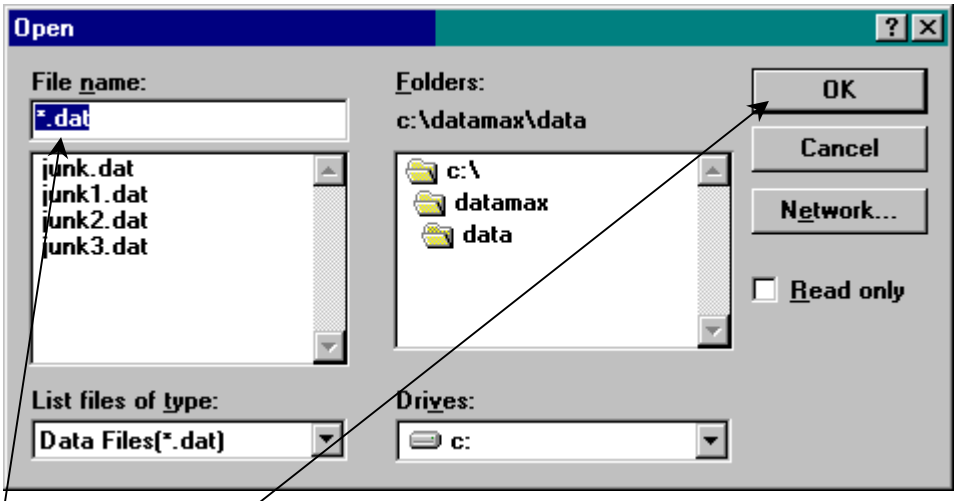


To re-open the graph,

1 Click *File* in the ***Lifetime*** main menu.
A drop-down menu appears.

2 Click *Open*.
The ***Open*** dialog box appears.





3 Choose the desired file.

4 Click *OK*.
The ***Open*** dialog box disappears, and the graph is restored to view.

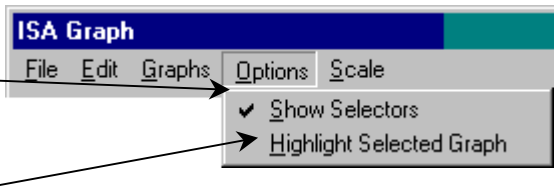
To restore a modeling plot to view,

1 Click the *Modeling* button in the ***Lifetime*** dialog box.
The graphs re-appear.

Options

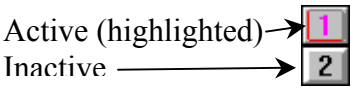
The *Options* function controls which graph is active, and whether to highlight it.

To show the selector buttons,

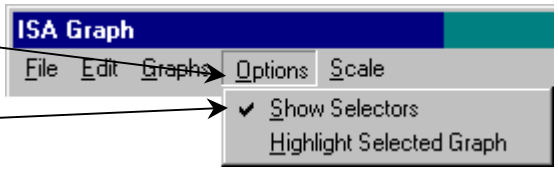


1 Click *Options*.
A drop-down menu appears.

2 Click *Show Selectors*.
The selector buttons appear, and a check appears next to *Show Selectors*. Selectors choose which graph is active. The active graph is indicated by a highlighted selector button.



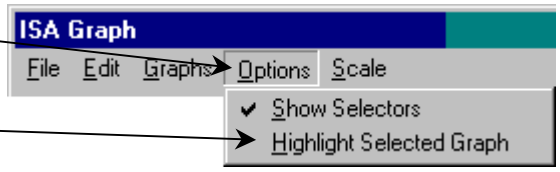
To hide the selector buttons,



1 Click *Options*.
A drop-down menu appears.

2 Click *Show Selectors*.
The selector buttons disappear, and the check mark disappears from next to *Show Selectors*.

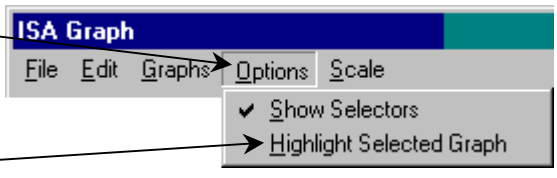
To highlight the active graph itself,



1 Click *Options*.
A drop-down menu appears.

2 Click *Highlight Selected Graph*.
A heavy border appears around the active graph, and a check appears next to *Highlight Selected Graph*. Selecting the other graph moves the border to the other graph.

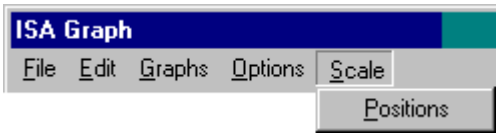
To remove the highlight from the active graph,



1 Click *Options*.
A drop-down menu appears.

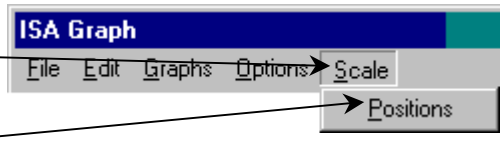
2 Click *Highlight Selected Graph*.
The border disappears from the graph, and the check disappears from next to *Highlight Selected Graph*.

Scale



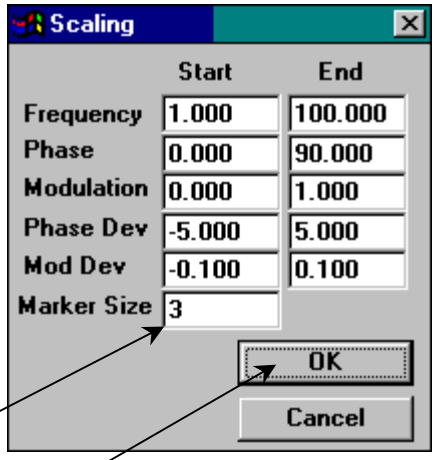
Scale adjusts the graph's scale for the optimum presentation.

To adjust the plot's scale,



1 Click *Scale*.
A drop-down menu appears.

2 Click *Positions*.
The ***Scaling*** dialog box appears. Each row represents one axis, using the current axis label.



3 Click in the desired data-entry field.

4 Enter a new value.

Note: *Frequency uses a logarithmic scale, and plots are adjusted and scaled accordingly.*

5 Change the size of the symbol.

a Click in the data-entry field next to *Marker Size*.

b Enter the desired value.

Larger values represent larger symbols.

6 Click *OK*.
The ***Scaling*** dialog box disappears, and the plot is adjusted.

12: Appendices

Data-acquisition speed keys

DataMax is to be used with a mouse or other pointing device. The keyboard, however, can be used to execute many DataMax commands. The *GRAMS/32® User's Guide* contains a list of keyboard commands applicable to data-manipulation. This section lists keyboard commands applicable to data-collection. Because DataMax is a set of multiple applications, the following list is divided by application.

Key to the list

KEYBOARD COMMAND	*Equivalent Series of Screen Commands*	Effect

Instrument Control Center

CTRL+L	*System/Make User Layout*	Forces the current hardware configuration to be loaded into the default file, to be used when ***Instrument Control Center*** starts the next time.

Run Experiment

CTRL+C	*Collect/Experiment*	Opens a data-acquisition dialog box, in order to enter experiment parameters.

Visual Instrument Setup

CTRL+O	*File/Open*	Opens a setup file stored on disk.
CTRL+S	*File/Save Setup*	Stores an in-memory setup on the disk.

Constant Wavelength Analysis

CTRL+O	*File/Open*	Opens a setup file stored on disk.
CTRL+S	*File/Save Setup*	Stores an in-memory setup on the disk.
CTRL+N	*File/New*	Clears the main screen.

Lifetime

Contains no keyboard commands.

Post-Experiment Modeling

CTRL+P	*File/Print*	Prints the displayed information.

General information about polarizers

Introduction

Among the accessories available for Spex® spectrofluorometers are polarizers. Fluorescence and phosphorescence polarization measurements describe the rotations of molecules between the time of absorption and time of emission. Typically, the excitation beam is passed through a polarizer, and the emitted light is analyzed with a second polarizer oriented parallel or perpendicular to the first polarizer. Polarizer measurements yield information about molecular size, conformation, rigidity, and viscosity.

For detailed information about polarizers, see the polarizer manual.

Brief mathematical discussion

The degree of polarization, ***P***, equals the ratio of the difference between the intensities of the vertical and horizontal components of the luminescence, and the sum of these two intensities. Vertical, ***V***, is parallel to the laboratory vertical axis, and horizontal, ***H***, is perpendicular to vertical. Mathematically, we write this relationship as

$$\boldsymbol{P} = \frac{\boldsymbol{I}_{VV} - \boldsymbol{I}_{VH}}{\boldsymbol{I}_{VV} + \boldsymbol{I}_{VH}} \qquad (1)$$

where $\boldsymbol{I}_{VV}$ and $\boldsymbol{I}_{VH}$ are the measured intensities with the excitation polarizer aligned vertically, while the emission polarizer is oriented vertically or horizontally, respectively.

Corrections for non-uniform response of the monochromator and mirror must be made. Lumping all corrections together into one value, we use the *G* factor, defined as

$$G = \frac{\boldsymbol{I}_{HV}}{\boldsymbol{I}_{HH}} \qquad (2)$$

Therefore, the corrected equation for polarization, ***P***, is

$$\boldsymbol{P} = \frac{\boldsymbol{I}_{VV} - G\boldsymbol{I}_{VH}}{\boldsymbol{I}_{VV} + G\boldsymbol{I}_{VH}} \qquad (3)$$

Anisotropy, ***r***, is another way to express polarized emission. Mathematically, anisotropy is defined as

$$\boldsymbol{r} = \frac{\boldsymbol{I}_{VV} - \boldsymbol{I}_{VH}}{\boldsymbol{I}_{VV} + 2\boldsymbol{I}_{VH}} \qquad (4)$$

Signal types

DataMax collects data with respect to one or more signal types. Spex® spectrofluorometers recognize four types of detectors:

Signal	S
Reference	R
Third (i.e., 2nd signal detector in a Fluorolog®-3)	T
User-defined accessory	A

In the following chart, *X* represents any of these four detector types. When a polarizer is included in a hardware configuration, these options become available:

Polarization, ***P***

The ratio of the difference between intensities of the vertical and horizontal components of the signal detector, *S*, and the sum of these two intensities, divided by the ratio of the difference between intensities of the vertical and horizontal components of the reference detector, *R*, and the sum of these two. Mathematically,

$$\boldsymbol{P} = \frac{\left(\frac{S_{VV} - S_{VH}}{S_{VV} + S_{VH}}\right)}{\left(\frac{R_{VV} - R_{VH}}{R_{VV} + R_{VH}}\right)}$$

Corrected polarization, $\boldsymbol{P}_C$

Polarization, ***P***, multiplied by a user-selected correction-factor file.

Anisotropy, ***r***

The ratio of the difference between intensities of the vertical and horizontal components of the signal detector, S, and the sum of the vertical components of the signal intensity and twice the vertical and horizontal components of the signal intensity, divided by the ratio of the difference between the intensities of the vertical and horizontal components of the reference intensity, R, and twice the vertical and horizontal components of the reference intensity. Mathematically,

$$\boldsymbol{r} = \frac{\left(\frac{S_{VV} - S_{VH}}{S_{VV} + 2S_{VH}}\right)}{\left(\frac{R_{VV} - R_{VH}}{R_{VV} + R_{VH}}\right)}$$

Corrected anisotropy, $\boldsymbol{r}_C$

Anisotropy, ***r***, multiplied by a user-selected correction-factor file.

Raw polarization

A set of four separate spectra consisting of data collected at all four combinations of the two polarizers' orientations, *viz.*, VV, VH, HH, and HV.

X_{VV}	Signal measured on channel *X* when the excitation polarizer is in the V position, and the emission polarizer is in the V position.
X_{VH}	Signal measured on channel *X* when the excitation polarizer is in the V position, and the emission polarizer is in the H position.
X_{HV}	Signal measured on channel *X* when the excitation polarizer is in the H position, and the emission polarizer is in the V position.
X_{HH}	Signal measured on channel *X* when the excitation polarizer is in the H position, and the emission polarizer is in the H position.
X_{VM}	"Magic-angle" condition used to eliminate sample and instrument polarization bias, when both polarizers are in the optical path. Useful for emission and excitation scans. Signal measured on channel *X* when the excitation polarizer is in the V position, and the emission polarizer is in the magic-angle (55°) position.

Information about phosphorimeters

Introduction

Among the accessories available for Spex® spectrofluorometers is a phosphorimeter, which adds a programmable pulsed excitation source, with adjustable gating of the signal from the photomultiplier-tube detector. Phosphorimeter measurements yield information about competing luminescence emissions from the sample, based on different lifetimes. Typically, triplet states (phosphorescence) emit within microseconds to milliseconds, and are distinguishable from singlet states (fluorescence), which occur within nanoseconds.

Because the duration of each exciting pulse from the phosphorimeter is short (~ 5 µs), interference from the lamp during acquisition of decay curves is minimized. Thus, acquisition of decay data with a decay time of ~10 µs and longer is straightforward without reconvolution analysis. The flash-lamp's illumination decays for about 40 µs after a flash, so take care when acquiring and interpreting data with a very short delay after the flash.

System

The system consists of three modules:

- Illuminator (may be a separate module or self contained in the FL-1040 Dual Illuminator accessory)
- Control module
- Reference amplifier module (housed in the control module)

The illuminator housing, or flash lamp, operates at up to 33 Hz. The control module triggers each lamp pulse. When the start of the light output is detected, a signal is sent to the control module for timing purposes. The control module houses the signal-gating circuitry that intercepts the signal from the pulse-counting emission photomultiplier tube, collects a selected, time-delimited portion of the signal, and later passes it to the software. The reference amplifier module houses the fast amplifier for the reference channel. The phosphorimeter also contains a reference channel to monitor the output of the pulsed lamp. When used in ratio mode, the reference signal eliminates variations in lamp intensity, to produce corrected excitation spectra or compensate for time-dependent variations.

A typical sequence of data-acquisition starts with a flash from the pulsed lamp, sensed by the control module as time $t = 0$. The light enters the excitation spectrometer, where it is dispersed. Monochromatic light from the spectrometer passes to the sample. Luminescence emission from the sample then passes through the emission spectrometer to the photomultiplier-tube detector. The control module includes a gate-and-delay generator, allowing the signal at the detector to be integrated only during a specific period after the flash (the *Delay After Flash*), for a pre-determined length of time (the *Sample Window*). Any signal arriving before or after the gating is ignored. This sequence of excitation, delay, and collection is repeated for each lamp pulse. The total signal is accu-

mulated for a pre-determined number of exciting pulses (flashes) and saved to disk. The data then can be manipulated using the *Arithmetic* menu.

For detailed information on the phosphorimeter, see its operation manual. To operate the phosphorimeter, see the spectrofluorometer's operation manual.

Phosphorimeter parameters

Four specific parameters govern experiments involving a phosphorimeter:

- Delay After Flash
- Sample Window
- Time Per Flash
- Number of Flashes

These parameters automatically appear on the phosphorimeter experiment-acquisition dialog box.

Delay After Flash

Delay After Flash sets the time, in ms, between the start of the lamp flash and the onset of data-acquisition (opening of the *Sample Window*). *Delay After Flash* can range from 0–10 000 ms, in increments of 0.001 ms. Accuracy of *Delay After Flash* is better than ±0.001 ms. Set *Delay After Flash* long enough so that fluorescence emission and lamp decay are complete (~ 0.040 ms). Thus, the resulting spectrum represents phosphorescence only.

Delay After Flash can be varied with time, to yield a decay curve. Spectra can be scanned to isolate different phosphorescing components based on the lifetime of the luminescent species in the sample. Together, these two techniques can be used to create three-dimensional plots. For example, successive scans with varying delay times can be plotted. In this example, each contour isolates the species in a particular slice of time.

To record fluorescence and phosphorescence emission, set *Delay After Flash* to zero.

Sample Window

Sample Window sets the duration of signal acquisition, in ms. The *Sample Window* can range from 0.01–10 000 ms, in increments of 0.001 ms. The *Sample Window* opens when *Delay After Flash* ends. When the *Sample Window* opens, the signal that enters the control module is counted and integrated, for later output to the software. At the end of the *Sample Window* time, the "window" closes, and the signal is ignored.

Number of Flashes

Number of Flashes sets the number of lamp pulses per data point. The range for *Number of Flashes* is 1–999. The signal collected during the sampling time is integrated over the number of lamp pulses, before the data are transmitted to the software.

Time Per Flash

Time Per Flash sets the total cycle length per flash, including on time, decay time, and dead time between flashes. The *Time Per Flash* is the reciprocal of the repetition rate of the lamp pulses. The allowable repetition rate is 0.03–33 Hz. The repetition rate must be slow enough to let the *Sample Window* to close completely before another flash be-

gins. Accuracy of the repetition rate is ±1 ms. Mathematically, the maximum repetition rate, in ms, is

$$Rate < \frac{1000}{Delay\ After\ Flash + Sample Window + 10}$$

where *Delay After Flash* and *Sample Window* are also measured in ms.

Information about temperature controllers

Configurations

A temperature bath/circulator is available in three configurations:

F-1000	110-V Temperature bath/circulator with I/O card, probe, and all cables
F-1001	220-V Temperature bath/circulator with I/O card, probe, and all cables
F-1002	I/O card, probe, and all cables, but no temperature bath/circulator

If the software, computer, and temperature bath/circulator were purchased from Jobin Yvon®, the I/O card is already in the computer, and setup and configuration are complete. If these items were purchased separately, perform the setup and configuration as described in this section.

Connections

See the operation manual for the temperature bath/circulator. These directions concern attachment of the external probe, computer hardware, and connections.

Computer hardware (for older host computers)

1 Insert the I/O card in the computer.

2 Configure the I/O card for IRQ 5 and port address 03E8.

In Windows™ 95 or above,

a Click *Start*.
A drop-down menu appears.

b Click *Settings*.
A drop-down menu appears.

c Click *Control Panel*.

d Double-click on *Ports*.

e Choose *COM3*.

Note: *Follow all instructions and precautions included with the card, and if the I/O card was purchased separately and not inserted in the computer.*

f Choose *Settings*.

g Verify (or change) the following (to):

Baud Rate	1200
Data Bits	8
Parity	None
Stop Bits	1
Flow Control	Xon/Xoff

h Select *Advanced*.

i Make sure that *IRQ* is *5* and *Base I/O Port Address* is *03E8*.

j Save all settings.

The I/O card is properly configured to communicate.

Computer hardware (for newer host computers)

Newer PCs have a PCI-based serial card, with two RS-232 ports present on the card.

Note: *Follow all instructions and precautions included with the card, and if the I/O card was purchased separately and not inserted in the computer.*

3 Install the card in an available PCI slot.

4 Restart the PC.

5 Install the drivers for the card according to the instructions that accompanied the card.

6 Using the Windows™ *Control Panel*, identify the new port(s) by locating the new *COMx:* entries that appear.

7 Connect the water-bath serial cable (part #400068) between the water bath's RS-232 port and the new PC's serial port.

8 Using a text editor such as *Notepad*, edit the file `c:\datamax\isa_ini\TCONTROL.ini`.

Select the port added, in the section labeled `[COMMUNICATION]; CHAN_NUM=x`.

The I/O card is properly configured to communicate.

External probe

The external probe is a 100-Ω platinum RTD-type sensor encased in a stainless-steel probe. A three-wire connection is made between the probe element and the water bath; two leads are the temperature-sense leads, and the third is a compensation (Kelvin lead), which corrects for any changes in the resistance of the lead wire with temperature. The probe senses the circulating coolant's temperature at the sample-changer, eliminating temperature errors from heat loss in the connecting tubing.

1 Connect the probe to the *Sensor* connection on the rear of the temperature bath/circulator.

Computer to bath

1 Connect one end of the 9-pin–25-pin cable to the *RS232* jack on the rear of the temperature bath/circulator.

2 Connect the other end of the 9-pin–25-pin cable to the appropriate COM port on the computer.

Note: *Usually the SpectrAcq is connected to COM2, leaving COM1 and COM3 free. When the mouse is connected to COM1, the temperature bath/circulator must be connected to COM3. With a bus-style or PS/2-style mouse, the temperature bath/circulator may be connected to COM1 or COM3.*

k For COM3, set or verify the following, as with the computer hardware:

Baud Rate	1200
Data Bits	8
Parity	None
Stop Bits	1
Flow Control	Xon/Xoff
IRQ	5
Base I/O Port Address	0E38

Note: *Settings other than shown at left result in a conflict between COM1 and COM3. Operation of the temperature bath/circulator becomes impossible.*

l For COM1, no further action is necessary.

3 Attach an end of a hose to each connector at the rear of the temperature bath/circulator.

4 Connect the remaining hose ends to ports on the front of the sample compartment.

Initialization and starting

1 Follow instructions for filling the temperature bath/circulator and verifying operation, as given in the temperature bath/circulator's instruction manual.

2 Turn on the power switch on the side of the temperature bath/circulator.

The front-panel LEDs should indicate *OFF*, and the light near the RS232 option should be on. If the RS232 indicator is not on, see "Resetting the system", below.

Note*: If the temperature bath/circulator is off when DataMax starts, initialization cannot occur.*

3 Test the system.

a Start DataMax.

b Select a layout including the temperature bath/circulator.

c Start ***Visual Instrument Setup***.

The ***Visual Instrument Setup*** window opens.

d Click on the sample-compartment icon.

The ***Sample Compartment Accessories*** dialog box opens.

e Choose *Temperature Bath*.

f Click on the image of the temperature bath/circulator.

g Click *Turn On*.

The front-panel LED should change from OFF, and display the current temperature reading. This verifies the system operation.

h Close the dialog box.

Note*: Always shut off the temperature bath/circulator via the software before closing DataMax.*

Resetting the system

Occasionally the temperature bath/circulator may need to be reset, especially if there is difficulty in control or operation.

Reset the system from the temperature bath/circulator

Perform this if the RS232 indicator light is off.

1. Press and hold the *On/Off* button on the front of the control panel.
   The control panel flashes to show that it is off-line, and ready for additional instructions.
2. Press and release the *RS232* button on the front of the control panel.
3. Within 3 seconds, press the *ENTER* button.
   The panel shows the current temperature, and the RS232 light turns on. Now the front-panel keys no longer respond to key presses.
4. Use the switch on the side of the control panel to turn the system off (for 15 s), and then on again.
   The system now can be initialized through DataMax.

Reset the system from Windows™

To re-establish a communications link between DataMax and the temperature bath/circulator, use Windows™.

1. Open the *Accessories* group.
2. Double-click on *Terminal*.
3. Select *Settings/Communications*.
4. Choose *COM3* (or *COM1*, if the temperature bath/circulator is connected to COM1).
5. Click *OK*.
   The ***Communications*** dialog box closes, but ***Terminals*** remains open.
6. In the *Edit* menu, select *Clear Buffer*.
   The temperature bath/circulator is ready to respond to system commands.
7. Test communication by any one (or all) of:

   a. Issue a *STOP* command:
   Press and hold **SHIFT**, press and release the **S** key, press and release the **O** ("Oh") key, release **SHIFT**, press the **0** ("zero") key, and press **ENTER**.

   b. If the temperature bath/circulator was operating, it is now *OFF*.

c Issue a *START* command:

Press and hold **SHIFT**, press and release the **S** key, press and release the **O** ("Oh") key, release **SHIFT**, press the **1** key, and press **ENTER**.
If the temperature bath/circulator was *OFF*, it is now operating.

d *READ* the current temperature:

Press and hold **SHIFT**, press and release the **R** key, press and release the **T** key, release **SHIFT**, and press **ENTER**.
The current temperature is displayed on the ***Terminals*** window.

8 Close the ***Terminals*** window.

The temperature bath/controller is now ready to operate.

Temperature-monitoring devices

The ThermoNESLAB temperature bath/circulator can monitor

- The bath temperature (DataMax option *Internal*), or
- The sample temperature, using a probe (DataMax option *External*)

Note*: The external probe should remain connected to the system, even when not in use. If the probe is disconnected or broken, an error appears when the External temperature probe is selected: DataMax attempts a scan, but enters an infinite loop because the temperature cannot be monitored.*

To interrupt a scan,

1 Press **Esc**.

2 Choose *Internal*, and re-execute the scan.

Glossary

A — *See* **Signal types**.

Absorption — Transition from the ground state to the excited singlet state. The process occurs within about 10^{-15} s.

Absorbance, A — Extent of absorption, synonymous with optical density, OD.

$A = \mathrm{OD} = \varepsilon c l$,

where ε is the extinction coefficient, in M^{-1} cm^{-1}, c is the sample concentration, in M, and l is the path length, in cm. The wavelength must be specified, because the absorbance is wavelength-dependent.

Acquisition mode — *See* **Signal types**.

Alpha, *α* — The pre-exponential term of a sum of exponentials expression. Usually the pre-exponentials are normalized such that $\Sigma\alpha = 1$, that is, the sum of all alphas must be 1.

Anisotropy-decay acquisition — A scan that examines the rotational properties of fluorescent probes and molecules. As a fluorophore rotates, it depolarizes the emitted light, depending on its size and shape. Analysis of these polarized emission spectra or frequency responses provides information about the rotational properties of the sample. The anisotropy is affected by Brownian motion, energy-transfer, re-absorption, re-emission, and light scattering. Applications include studying asymmetric complex molecules, environmental perturbations, binding, hindered-rotation phase-transitions, and internal viscosities of bilayers.

Bandpass — The wavelength-range of light passing through the excitation and emission spectrometers. The wider the bandpass is, the higher the signal intensity is, with a corresponding decrease in spectral resolution.

Bandpass filter — Optical element that selectively transmits a specified range of wavelengths.

Batch processing — Scanning sample after sample, in order to produce valuable emission information about the fluorescence of each sample at the specified wavelengths.

Bioluminescence — Emission of light via a chemical reaction in a living organism.

Chemiluminescence — Emission of light via a chemical reaction.

Chi-squared, χ^2

A statistical parameter indicating the "goodness of fit" of a model to the data. χ^2 is the ratio between the observed deviation and the expected deviation, and it may include correction for the number of degrees of freedom (the sum is then known as the reduced chi-squared). As χ^2 decreases, the fit better describes the data set. In frequency-domain experiments, the absolute magnitude of the chi-square statistic depends on the selection of errors, which may be fixed for all frequencies measured, or frequency-dependent, in which the standard deviation of a group of replicate determinations is used for the error at that frequency.

Component

One of a number of substances believed to contribute to the fluorescence of the mixture.

Concentration determination

Quantitative analysis of an unknown, using constant-wavelength analysis. DataMax must establish a basis of comparison, by first executing a run using standards, and then executing a run with unknowns. The more standards used, the more accurate the determined concentration is. Various fits are available, based on the minimum number of unknowns: linear, quadratic, or cubic.

Corrected emission scan

An emission scan corrected for the response characteristics of the emission spectrometer and signal detector. To obtain a corrected emission scan, an emission spectrum is multiplied by the emission correction factors. The multiplication is performed automatically when a correction file is selected; the resulting trace represents corrected data. The blank spectrum, or at least the dark signal, must be subtracted before the correction factors are applied.

Corrected excitation scan

An excitation scan corrected for the non-linear emission characteristics of the xenon lamp, the lamp's aging, and the non-linear response of the excitation spectrometer's gratings. Most (~90%) of the correction is obtained when the detector signal is ratioed to the reference signal. For the rest of the correction, the resultant scan is multiplied by correction factors. Select a ratioed-signal type (e.g., S/R) and choose a correction file. The multiplication is performed automatically, and the final trace represents corrected data.

Correction factors

Term files (magnitude versus wavelength) that compensate for the non-ideal response of the system's components (e.g., xenon lamp, gratings, detector), across the UV-visible wavelength range. When an uncorrected (technical) spectrum of an optical source or element is multiplied by the corresponding correction-factor term file, that detector's (and associated optics') response becomes idealized.

Dark counts Inherent background signal produced by the photomultiplier tube when high-voltage is applied. Cooling the detector decreases the dark counts. Red-sensitive detectors generally have higher dark counts because of thermionic emission, and benefit (or even require) cooling to decrease this background noise, depending on the photocathode material.

Data file Stores quantitative information, plus instrument and acquisition parameters. Data files are saved with the extension `.SPC` by default. To run an experiment, a name for a data file must be supplied.

Emission scan Shows the spectral distribution of light emitted by the sample. During an emission scan, the excitation spectrometer remains at a fixed wavelength, while the emission spectrometer scans a selected region.

Equilibration time The length of time that the temperature must stay constant, plus or minus a specified tolerance, before a reading is taken. Whenever the temperature moves outside of the tolerance, the application resets the equilibration time to zero, and restarts.

Excitation scan Shows the spectral distribution of light absorbed by the sample. During an excitation scan, the excitation spectrometer scans a selected region, while the emission spectrometer remains at a fixed wavelength.

Experiment file A file that stores instrument and acquisition parameters about an experiment, but no actual data. An experiment file is assigned, by default, the extension `.EXP`. Experiment files must be specified to save an experiment. When a data file is recalled from disk, experiment parameters are automatically recalled also.

Flash lamp A lamp that provides pulsed-light output. A flash lamp may be free-running or gated.

Fluorescence Emission of light during the transition of electrons from the excited single state to the ground state. Fluorescence typically occurs within ~10^{-9} s.

Fluorescence lifetime The average length of time that a molecule remains in the excited state, before falling back to the ground state.

Fluorolog®-3 Spex® modular spectrofluorometer, for research-grade fluorometry, phosphorimetry, and other luminescence measurements, under control of DataMax.

Fluorolog®-Tau-3 Spex® modular spectrofluorometer, for research-grade fluorometry, phosphorimetry, and other luminescence measurements, under control of DataMax. Additional electronics and an electro-optical modulator are included for lifetime measurements.

FluoroMax®-3 Spex® single-unit spectrofluorometer, for research-grade fluorometry, phosphorimetry, and other luminescence measurements, under control of DataMax.

Fractional amplitude An expression for the fraction of a particular lifetime species contributing to the total steady-state emission, as represented by the relative area under the emission spectrum. It differs from the pre-exponential (alpha) term in that it is weighted to the lifetime of the component and divided by the total intensity. The total fractional amplitudes for a molecular system must equal 1.

Frequency domain A technique in which light is sinusoidally modulated at one or more radio frequencies. This technique offers the fastest measurement with the highest accuracy for short-lived, multi-exponential or nonexponential systems. The Fluorolog®-Tau-3 uses this technique to acquire fluorescence lifetime and anisotropy-decay data.

G factor The correction for instrument response to polarized light at a particular emission wavelength, defined as G = HV/HH.

Grating Optical element in a spectrometer, composed of finely ruled grooves, that disperses multiple wavelengths of light into a spectrum.

Laser A monochromatic light source providing high excitation intensity.

Lifetime The mean length of time between absorption and emission of light by a population of fluorescent molecules. Typical lifetimes range from 1–200 ns for fluorophores, and longer for phosphors.

Lifetime scan Records phase-shift and modulation at specified radio frequencies for an unknown, relative to a standard. From these data, accurate lifetimes can be found. Lifetime data files are assigned a `.DAT` extension by default. The data can be modeled during or after acquisition.

Lifetime-resolved scan Resolves overlapping spectra based on differences in the fluorescence lifetime. Up to three components can be completely resolved. More complex systems can give improved resolution of one or more spectra, but complete resolution requires additional manipulation of the data-acquisition parameters (e.g., excitation wavelength).

Limiting anisotropy, R_0 The steady-state anisotropy in the absence of diffusion.

Matrix scan After the experiment is performed and saved, obtain a three-dimensional view of the results of varying one wavelength by a set increment, while holding the other constant. With a matrix scan, the spectra at the new wavelengths can be readily observed. A matrix scan may be performed on any recalled experiment. The fixed monochromator's wavelength in the original scan is varied in the matrix scan. Each time the wavelength is adjusted, a new spectrum is produced and added to the view.

Modeling Determining the parameters of an actual system that best describe the measured data. Graphically, this is fitting the data to a mathematical model, while adjusting parameters to obtain a minimum chi-square "goodness-of-fit" statistic.

Modulation The excitation source and fluorescent emission are both composed of AC and DC components. Although the fluorescent emission is identical to the excitation in frequency, the emission's amplitude is less than the excitation's. This difference is called modulation.

Monochromator Optical instrument that lets the appropriate range of wavelengths pass.

Multigroup scan Sequentially excites a sample with different wavelengths, and plots the data on one spectral view, as intensity versus time. Uses for multigroup scans include: energy-transfer, and dual-wavelength studies of fluorescent probes for cellular ion-transport (e.g., Ca^{+2}, Mg^{+2}, K^{+}, H^{+}).

Phase shift Time lag in the fluorescent response of a sample to excitation by sinusoidally modulated light. Longer lifetimes produce larger phase-shifts. The lifetime may be recovered by a simple tangent relationship.

Phosphorescence Emission of light during transition of electrons from the triplet state to the ground state. Phosphorescence generally is red-shifted from fluorescence; it occurs within 10^{-6} s to several seconds. To enhance detection of phosphorescence, samples often are chilled to liquid-nitrogen temperature (77 K).

Phosphorimeter emission scan Shows the spectral distribution of phosphorescence emitted by the sample. During an emission scan, the excitation spectrometer remains at a fixed wavelength, while the emission spectrometer scans a selected region.

Phosphorimeter excitation scan Shows the spectral distribution of light absorbed by the sample that emits phosphorescence. During an excitation scan, the excitation spectrometer scans a selected region, while the emission spectrometer remains at a fixed wavelength.

Phosphorimeter synchronous scan Examines phosphorescence instead of fluorescence. *See* **Synchronous scan**.

Quick scan A technique in which the unknown is placed in the optical path, and the entire frequency range is measured. Then the standard is placed in the optical path, and the measurements are repeated. The automatic sample-changer can rotate samples into the light beam quickly.

R *See* **Signal types** *and* **Reference photodiode**.

Real Time Display — A DataMax application that lets the user change instrumental parameters and see the effects on the signal-levels immediately. ***Real Time Display*** does not collect or save data. To transfer the optimized settings found in the ***Real Time Display***, use the *XFER* button.

Reference photodiode — A photodiode detector that monitors the output intensity of the excitation beam's power immediately before it reaches the sample. Useful for excitation spectra, where the excitation beam power is not constant with wavelength, and emission spectra, when lamp power fluctuations may affect the data.

S — *See* **Signal types** *and* **Signal Photomultiplier**.

Setup file — Stores information about preferred units and hardware settings. Selecting a setup file is optional. If no setup file is chosen, the system uses the current or default values. A setup file has the default extension `.SET`.

Signal photomultiplier — A detector used to measure fluorescence from the sample, operating in the photon-counting mode for high sensitivity. Different detectors detect different wavelength bands.

Signal types — Basic signal types are designated by the respective detector: R is the reference detector, recorded in µA; S is the sample signal detector, recorded in counts per second (cps); T is the third detector for a signal on a T-format instrument, recorded in counts per second (cps); and A is a user-defined accessory. By manipulating algebraically the basic detector types, complex signal types are possible. For example, S/R is the sample signal divided by the reference signal, which corrects for lamp-intensity variations. S+R is the sum of sample and reference signals. Both signals are also recorded separately, as file *a* (sample) and file *b* (reference).

Single-point analysis — **See Batch processing** *and* **Concentration determination**.

Singlet state, S_0 or S_1 — Spin-paired ground (S_0) or excited state (S_1) of a molecule or atom. Absorption usually produces the first excited singlet state, which fluoresces, or undergoes intersystem crossing to form a triplet state.

Spectrometer — The component in a spectrofluorometer system that is scanned to provide excitation and emission spectra. A spectrometer is chosen for low stray light, high resolution, and high throughput.

Synchronous scan — A scan type that examines the overlap between excitation and emission. The excitation and emission spectrometers are scanned simultaneously with a constant offset specified in nm (wavelength) or cm^{-1} (energy).

T — *See* **Signal types**.

Tau, τ

The fluorescence or phosphorescence lifetime (in ns or µs) of each unknown in a sample. DataMax derives a τ for each component in a system, and χ^2 indicates its accuracy. Because most solutions are not pure, find a more accurate τ by changing the number of components, the concentration of each component, etc., in the model.

Temperature scan

After an experiment has been performed and saved, re-execute the experiment at a new temperature. With temperature scan, observe spectra produced at different temperatures for up to 4 samples.

Time-based scan

The sample's signal is monitored while both excitation and emission spectrometers are fixed. Use this scan type to examine enzyme kinetics, dual-wavelength experiments, and reaction-rate constants.

Time-resolved scan

Determines the frequency-response of the sample over a specified emission range. Applications involve solvent-relaxation of the excited state, and excimer formation. This scan examines the change in spectral characteristics of a sample during the lifetime of its excited state. The number of scans must be specified. Each scan is run sequentially. The data file's name is truncated to 6 characters, so that each scan can be given the tag 01, 02, 03, …, plus the `.DAT` extension.

Triplet state, T_1

The paired ground or excited state with paired elect formed from the excited singlet state. The triplet state phosphoresces.

Xenon lamp

Light source that produces a continuum of light from the ultraviolet to the near-infrared.

Xenon-lamp scan

A plot of the xenon lamp's output as a function of wavelength. The xenon-lamp scan is acquired using the reference detector, while scanning the excitation spectrometer. The 467-nm peak can be used to verify calibration of the excitation spectrometer.

Technical assistance and support

Preparation

Both Thermo Galactic and Jobin Yvon® offer technical support. Before calling for assistance, answer these questions:

1. Are the computer system's specifications are consistent with DataMax requirements?
2. Are the spectrofluorometer and all its accessories are switched on and properly configured?
3. Is the correct layout file for the current system's configuration chosen?
4. Was the system switched on in the correct sequence, as explained in the spectrofluorometer's operation manual?
5. Is the problem listed in the spectrofluorometer's operation manual? If so, try the solution.

If these steps fail to correct the problem, contact Spex® Fluorescence Service or Thermo Galatic.

Calling for technical assistance

Whom to call	
DataMax and hardware	Spex® Fluorescence Service
Array BASIC™	Thermo Galactic

1. Be near the system while calling, to reach the host computer and spectrofluorometer easily.
2. Have the following information available:
   a. Find the serial number for the core instrument, on the back of the sample compartment. Collect the serial numbers for the host computer and all automated accessories (e.g., temperature bath).
   b. Note the system configuration with all accessories.

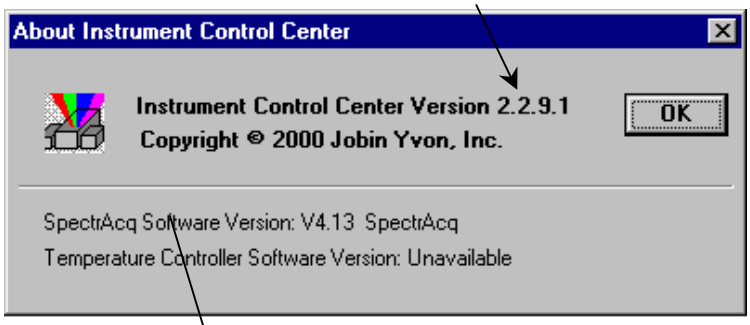
c Determine the brand name and type of host computer, along with processor speed, free RAM, and hard-disk space, and whether the computer was purchased from Jobin Yvon®.

d Write down the experimental configuration and what samples are under study. Be prepared to fully describe the samples and their spectral characteristics, as well as the specific instrument setup and sample geometry.

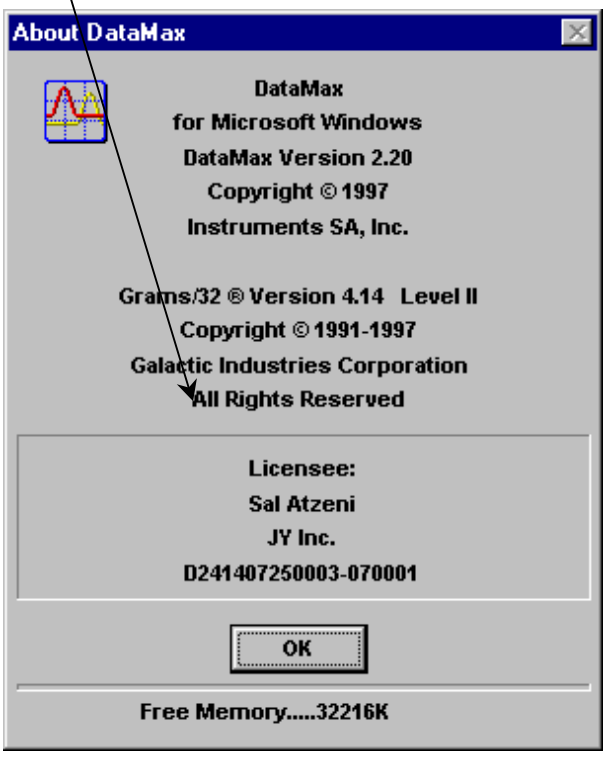
e Try to duplicate the problem and write down the steps required to do so. The service engineers will try to do the same with a test system. Depending on the problem, a service visit may not be required.

f If an error dialog box appears in DataMax, write down the exact error displayed. Note whether the error was in GRAMS/32 or Windows™.

g In DataMax, in the ***Instrument Control Center*** toolbar, choose *Help*. Under *Help*, choose *About Instrument Control Center*. This opens the ***About Instrument Control Center*** window. The version of the software is listed here.



h In ***Run Experiment*** toolbar, open the ***About DataMax*** window. Note the software's and instrument's serial numbers.



3 Call the Spex® Fluorescence Service Department at (732) 494-8660 × 160, fax us at (732) 549-5157, or e-mail us at

fluorescence_service@jyhoriba.com.

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Key to the entries:

regular font	subject or keyword
italic font	*command, menu choice, or data-entry field*
bold italic font	***dialog box***
`Courier New font`	`file name or extension`

B

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